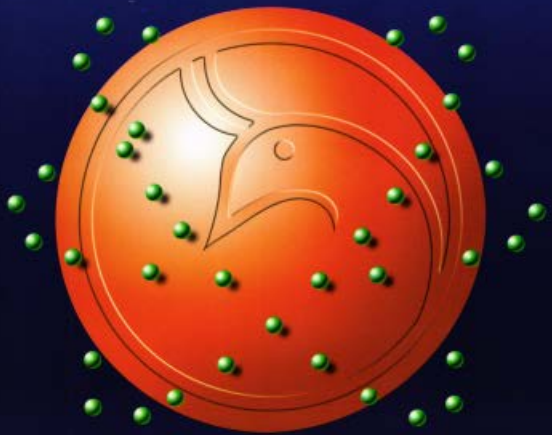
Al-Mustansiriyah Journal of Science

ISSN 1814 - 635X

Vol. 23, No. 5, 2012



Issued by College of Science - Mustansiriyah University

Vol. 23
No. 5
2012

Al-Mustansiriyah Journal of Science

Issued by College of Science, Al-Mustansiriyah University, Baghdad, Iraq

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CONTENTS

ITEM	Page No.
Isolation and identification of some fungi frome Al-Sader Water Treatment Plant,Baghdad,Iraq Bushra K. Shaker and Fayadh M. Sharif	1-12
Distribution of aminoglycoside resistance mediated by 16S rRNA Methylation between locally isolated of *Escherichia coli* and *Pseudomonas aeruginosa* Israa Mohamed safi al- kadmy and Sawsan sajid Al-jubori	13-24
An Efficient Method for DNA Isolation and Purification from *Vibrio fluvialis* Iyad J. Qassar and Jaladet M.S. Jubrael	25-32
Toxoplasma gondii, HCV, and HBV Seroprevalence in Haemodialysis Patients with Chronic Renal Failure in Al-Kindy Hospital Baghdad, Iraqi Sura Basil Kamal Al-Dulaimi, Alia Essam AL-Ubadi, Aysar Essam.Al-Ubadi, Emaan Natiq AL-Bayatti, Sana'a D. K .Al-Saday	33-38
Antibacterial Activity of *Mentha Piperita and Allium Sativum* Against Some of Gram-ve Bacteria Suhad A. Ahmed	39-48
Assessment of Correlations between Sperm Chromatin Structure Assay and Seminal fluid analysis for both Fertile and Infertile men in Iraq Majid Hameed Jaffer AL-Dabbagh, Abd Al-Ameer Nasser Ghaloub and Muhammed-Baqir Muhammed Rashad Fakhrildin	49-62
Study of Some Chemical & Physical and Bacteriological Properties of Yuosifiya River Water احمد جاسم محمد ، بيداء عبد القادر مهدي ، سارة عبد القادر مهدي	63-78
Study of Interleukin-6 ,Testosterone and Zinc in Different Clinicopathological Stages of Malignant Prostate Cancer Patients Ebtehal S. Mohammed, Wafa F. Al-taie and Intesar T. Numan	79-88
Theoretical studies of mixed ligand complexes of mannich base derived from 2-mercaptobenzothiazole with [Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)]ions Rehab A.M.Al-Hasani, Eiman S.Shaheen and Rasha W.Al-Saedi	89-104
Synthesis and Characterization of Some New 2-Aminobenzothiazole Derivatives Souad J. Lafta and Suzanne J. Abass	105-118
Polyurethane Foam Waste for Removal of Ni(II) From Aqueous Solutions Suhad A.Yassin	119-128
Synthesis and Characterization of New Compounds Derived from (1,3-Oxazol-5-(4*H*)-one and Study Their Effect on ALP Activity Nagham M. Al-Janabi	129-138
ABET and QAA based Framework proposal for Higher Education in Iraq Saad Najim Al Saad	139-150

E-shopping Perceptions Assessment Zahraa Abd alsalam saed and Muzhir Shaban	151-176
Strange Attractor and Chaotic Motions of Two-Dimensional Nonlinear Invertable Map Saba Noori Majeed	177-182
Semi-Analytic Method For Solving High Order Ordinary Differential Equations With Initial Condition Luma. N. M. Tawfiq and Heba. A. Abd - Al-Razak	183-194
On S^{**}-PROPER FUNCTIONS Laheeb Muhsen No'man	195-200
On The Completeness Of The Cartesian Product Of Two Complete fuzzy metric spaces Jehad R. kider	201-208
A modified variational Iteration method for solving higher dimensional initial boundary value problems Abeer Majeed Jassim	209-226
Theoretical Study 0f Quality Factor for TM_{mn0}- Modes in Gyrotron Tube Luma Y. Abbas	227-232
Effect of Acetic Acid on Dezincification Process for Brass Alloy and Their Effect on Mechanical Properties of Alloy Samir Bahjat	233-240

Isolation and identification of some fungi frome Al-Sader Water Treatment Plant,Baghdad,Iraq

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Received 12/12/2011 – Accepted 18/4/2012

الخلاصة

تم حساب مجموع الوحدات المكونة للمستعمرة (cfu) للفطريات في نماذج المياه المأخوذة من جميع مراحل المعالجة في مشروع ماء الصدر ، بغداد ، العراق على فترة 6 أشهر متتالية بإستخدام طريقة ورقة الترشيح البكتيري. متوسط عدد 100 / cfu مل للفطريات الخيطية و الخمائر في ماء النهر الداخل للمشروع كان 41.27 و 8.69 على التوالي. إنخفضت هذه الأعداد في ماء الحنفية في المنازل الى 4.88 و 1.36 / cfu 100مل على التوالي. الفطريات الخيطية السائدة التي تم عزلها كانت Aspergillus (A. niger, A. flavus , A. fumigatus) 34.40% و Penicillium spp. 28.34% و Cladosporium.spp. 16.54%في حين كانت الخمائر السائدة Cryptococcus curvatus 33.94% و Candida spp. (C. albicans , C. glabrata and C. parapsilosis)) 32.56% و Rhodotorula mucilaginosa 21.05%و Sacccharomyces cerevisiae 11.96%. التلوث الفطري المنخفض نسبيا المسجل في هذا المشروع المشيد حديثا يمكن أن يعود الى بعد مصدر تجهيز ماء النهر و الإجراءات الصحية الدورية المناسبة المتبعة في هذا المشروع.

ABSTRACT

Total colony forming units (cfu) of fungi in all stages of water treatment in Al-Sader Water Treatment Plant (AWTP) in Baghdad, Iraq was determined using bacterial membrane filter paper over a period of six months. Filamentous fungi and yeasts in raw water were 41.27 and 8.69 cfu / 100 ml respectively. These numbers were dropped to 4.88 and 1.36 cfu / 100 ml respectively in tap water reaching houses. The dominant isolated filamentous fungi in raw water were *Aspergillus* (*A. niger, A. flavus , A. fumigatus*) 34.40% , *Penicillium* spp. 28.34% and *Cladosporium* spp. 16.54% , yeasts were *Cryptococcus curvatus* 33.94% , *Candida* (*albicans , C. glabrata , C. parapsilosis*) 32.56% , *Rhodotorula mucilaginosa* 21.05% and *Sacccharomyces cerevisiae* 11.96%. Relatively low fungal contamination in this new built plant might due to the distant raw water supply and careful periodically done sanitation measures.

INTRODUCTION

Drinking water subjected to contamination by different microorganisms including bacteria, fungi, algae , viruses and protozoa[1]. These microorganisms include saprophytes that may induce undesirable odor or taste and pathogenic species that may cause sporadic or epidemic diseases. Accordingly, control measures including coagulation ,filtration and disinfection are routinely done in municipal drinking water distribution stations all over the world. A longwise , monitoring of microbial contamination is mainly restricted to coliform bacteria being the mean threat for public health. Fungi are widely distributed in nature inhabiting almost all environmental niches including water and air. Fungi are heterotrophic microorganisms including those that cause disease in plants , animals and man. Human

pathogens , account of about more than 300 species most of them oppportunistic pathogens causing disease in immunocompressed persons[2]. Most of the pathogenic fungi are yeasts or filamentous fungi belonging to *Ascomycota* , *Basidiomycota* , imperfect fungi with a few *Zygomycota*[3]. Although earlier studies have raised fungal contamination of drinking water as a possible hygienic problem[4 ; 5], research was intensified during 1980s – 1990s[6; 7; 8 ; 9 ; 10 ; 11] and serious interest was began in the last decade in different countries[12 ; 13 ; 14 ; 15 ; 16 ; 17 ; 18]. As to our knowledge, this issue is not studied in Iraq. This study aimed at evaluation of fungal contamination in drinking water through different stages in Al-Sader Water Plant (AWTP), the biggest water treatment plant in Baghdad.

MATERIALS AND METHODS

Site of Study

This study was conducted at AWTP ,Northeast of Baghdad. The plant is feed by raw water from Military Canal through a pipe 1500 m long. It supply 4000 m^3 / h (650 000 m^3 / day) potable water which cover many municipal sectors that represents about 51 % of potable water production in Baghdad. Treatment procedure used by AWTP include precipitation treatment with addition of a coagulant and sedimentation , filter , chlorine mixing then pumping through distribution pipes.

Sample Collection

Water samples (200 ml each) were taken monthly over six months from October 2010 to March 2011 from raw water (River water supplying the AWTP) , flocculated water (precipitation tanks) , sand filtered water, chlorine treatment tank , tap water from AWTP pipes and tap water from house pipes. Tap water from pipes were collected after sterilization of the tap with alcohol ,flamed and left to flash for 5 minutes.Water samples were kept in 250 ml clean,sterilized bottles with screw caps that were sealed , transferred to the laboratory for isolation procedure to be done in the same day. Sodium thiosulphate 4.7 % w/w was added to water samples to inactivate residual free chlorine[17 ; 12].

Physico-chemical Parameters

Physico-chemical properties of the water was recorded monthly. Variables recorded included temperature (°C), pH (pHmeter Jenway , Denmark), turbidity (NTU) (HACH 2100 USA) and free chlorine (ppm) that was measured with Super Chloro Meter (Lovibond 2000,England).

Isolation of Fungi

Total fungal viable counts in water samples were recorded in all samples as indicated above. In each turn, 100 ml of each sample was filtered through a sterile 0.45 µm membrane cellulose nitrate filters(47mm diameter). With the aid of a sterile forceps,the filter is transferred to a petri dish containing steriliezed culture medium. Two types of culture media were used. Neopeptone Glucose Rose Bengal Aureomycin (NGRBA). This medium composed of : neopeptone 5 g , glucose 10 g , 0.67% (w/v) aureomycin solution 5.0 ml , 1% (w/v) rose bengal solution 3.5 ml , agar 15 g , distilled water 1000 ml , pH 6.5[12]. Sabouraud Dextrose Agar (SDA) supplemented with Choloramphenicol 50 mg/l and Gentamycine 25 mg/l after autoclaving[19]. Three replicates were used for each water sample.
Plates were incubated at 25 – 28 °C for (5-7) days through which they were monitored daily for appearance of fungal colonies. Number of colonies was recorded and fungal isolates were subcultured separately for identification.

Identification of Fungi

Filamentous fungi were identified to generic or species level according to morphological features with the aid of suitable references[20 ; 21].

Yeasts were identified by Polymerase Chain Reaction (PCR) analysis of the 5.8s rRNA gene and the two ribosomal internal transcribed spacers according to the method described by [22]. Briefly, fresh culture (48 h) of yeasts on SDA were obtained from stock cultures. Cells were directly collected from the colony using yellow tip and suspended in 25 µl PCR reaction mix containing 2.5 µM primer ITSI (5'TCCGTAGGTGAACCTGCGG 3') , 2.5 µM primer ITS4 (5' TCCTCCGCTTATTGATATGC 3'). One unit of DNA Polymerase Master Mix (Promega USA) was added to each tube. PCR conditions were as follows : initial denaturation at 95 °C for 5 min. ; 35 cycles of denaturation at 94 °C for 1 min., annealing at 55.5 °C for 2 min. and extension at 72 °C for 10 min. The PCR products were separated on 1.8 % agarose gels with 1 X TBE buffer. After electrophoresis, gels were stained with ethidium bromide , visualized under UV light and photographed. Sizes were estimated by comparison against a DNA length standard (1000 bp ladder, Promega USA). Yeasts showing the same DNA sizes were further seperated using cultural and morphological characteristics(Yeast Morphology Flow Chart , Dalmau Morphology Flow Chart)[3;23]. Identified fungi were cultured on Carrot Potato Agar slants and kept in the refrigerator as stock culture for further work.

RESULTS AND DISCUSSION

Some of the physicochemical properties of water at different stages of treatments in AWTP and tap water at houses are listed in Table 1. The temperature ranged between 17.16 and 18.91 . This range of temperature is near the maximum for psychrophilic fungi (~ 15 °C) , somewhat lower than the optimum of mesophilic fungi (25 – 28°C) and slightly below the minimum for thermophilic fungi (20 °C)[24]. Also, the fluctuation in temperature during different treatment stages is very slight. Accordingly, the prevalent temperature is not a limiting factor for growth of a large number of fungi.

The pH ranged between 7.95 and 7.58 which is slightly higher (Optimum 6.5 – 4.5) for growth of most fungi. These results is in line with that reported by [25] about the role of some physicochemical properties of water and incidence of fungi.

On the other hand , the turbidity is maximum at raw water because of suspended soil and organic particles. It is dropped to about one third during sedimentation/chemical coagulation stage and minimized further after sand filtration. Expectidly , number of fungal cfu is directly related to this factor, where cfu is dropped about one third during coagulation and sedimentation stage and still

Table-1 : The physicochemical properties of water at different stages at AWTP and houses

Specifications	Raw Water	Sedimentation/Chemical coagulation	Sand Filter	Tap Water AWTP	Tap Water House
Temperature °C	17.33	18.25	17.16	18.91	18.83
Turbidity	41.83	14.00	03.03	02.38	02.61
pH	07.79	07.74	07.95	07.77	07.58
Free chlorine	-	-	-	02.70	02.38
Odor	-	-	-	-	ordinary
Taste	-	-	-	-	ordinary

- not present or not recorded

further reduced after sand filtration(Table 2 and 4). These results is in line with that of [7] with respect to the relation between turbidity and fungal cfu numbers. Also, our results confirm those reported by [8] and [17] about efficiency of these physicochemical treatments.

Fungi withstanded physicochemical treatments in descending order are *Aspergillus* spp., *Penicillium* spp. , *Cladosporium* spp , *Fusarium* sp. , *Rhizopus stolonifer*, *Acremonium* sp. *Alternaria* spp., and *Chaetomium* sp.(Table 2). These results are in line with that of [17] who found that the most isolated fungi from raw and treated water were

Cladosporium, *Penicillium*, *Aspergillus* and *Fusarium*. Similarly, *Penicillium* and *Aspergillus* dominated fungi isolated from tap water of

Table-2 : Filamentous fungi isolated from water at different stages at AWTP

Fungi	Raw Water		Sedimentation/ Chemical coagulation		Sand Filter	
	C f u* Rate	Frequency %	C f u Rate	Frequency %	C f u Rate	Frequency %
Acremonium sp.	01.54	03.73	00.72	04.90	00.06	00.89
Alternaria spp.	01.35	03.27	00.52	03.54	00.00	00.00
*Aspergillus***	14.20	34.40	03.21	21.88	02.09	31.24
Botrytis cinerea	00.00	00.00	00.00	00.00	00.12	01.79
Cephalosporium sp.	00.00	00.00	00.26	01.77	00.00	00.00
Chaetomium sp.	00.04	00.09	00.00	00.00	00.04	00.59
Cladosporium spp.	06.83	16.54	03.91	26.95	01.22	18.23
Chrysoporium sp.	00.02	00.04	00.02	00.13	00.10	01.49
Cylindrocarpon sp.	00.12	00.29	00.22	01.49	00.00	00.00
Fusarium sp.	02.81	06.80	00.77	05.24	00.31	04.63
Mucor sp.	00.18	00.43	00.06	00.40	00.20	02.98
Paecilomyces sp.	00.04	00.09	00.00	00.00	00.04	00.59
Penicillium spp.	11.70	28.34	04.06	27.67	02.12	31.68
Pythium sp.	00.00	00.00	00.04	00.27	00.00	00.00
Rhizopus stolonifer	01.72	04.16	00.31	02.11	00.18	02.69
Trichoderma harzianum	00.50	01.21	00.26	01.77	00.00	00.00
Unidentified	00.22	00.53	00.31	02.11	00.21	03.13
Total	41.27	~100	14.67	~100	06.69	~100

* Cfu / 100 ml water

** Among others, *Aspergillus* species included : *A. candidus* , *A. flavus* , *A. fumigatus* and *A. niger*.

Al-Riyadh schools in Saudi Arabia[25] In Portugal , beside other fungi , *Chaetomium* and *Rhizopus* were isolated from tap water [12]. In Norway , although many species were isolated , the dominant genera were *Penicillium* , *Trichoderma* and *Aspergillus*[13]. In a recent review , *Penicillium* and *Aspergillus* were the most prevalent fungi in tap water from seven countries belong to five continents. In some countries more common associated fungi included in addition *Acremonium* , *Cladosporium* , *Exophiala* , *Phialophora* , *Phoma* or *Trichoderma*[18].

Table-3 : Filamentous fungi isolated from tap water at AWTP , houses and stored water

Fungi	Tap Water AWTP*		Tap Water House		Tap Water Stored	
	C f u Rate	Frequency %	C f u Rate	Frequency%	C f u Rate	Frequency%
Acremonium sp.	00.00	00.00	00.04	00.82	00.00	00.00
Alternaria spp.	00.20	05.63	00.31	06.35	01.25	07.17
Aspergillus spp.	01.37	38.59	02.64	54.10	06.50	37.31
Chaetomium sp.	00.06	01.69	00.02	00.41	00.00	00.00
Cladosporium spp.	00.43	12.11	00.39	07.99	02.33	13.37
Fusarium sp.	00.06	01.69	00.04	00.82	00.00	00.00
Penicillium spp.	01.31	36.90	01.08	22.13	03.67	21.06
Rhizopus stolonifer	00.12	03.38	00.28	05.73	03.67	21.06
Trichoderma harzianum	00.00	00.00	00.08	01.64	00.00	00.00
Total	03.55	~100	04.88	~100	17.42	~100

* chlorine treated and delivered through distribution system

Despite relatively higher concentration of free chlorine (Table 3) ,fungi survived chlorine treatment in descending order were *Aspergillus* spp. , *Penicillium* spp. and *Cladosporium* spp. which were recovered with higher frequncy, *Alternaria* spp., *Rhizopus stolonifer* , *Chaetomium* sp. and *Fusarium* sp.with very low frequency. Total fungal cfu in tap water in houses was 4.88 cfu / 100 ml(Table 3). In similar studies , [26] in France recorded 0.2 – 6.5 cfu / 100 ml , [15] in Austria recorded 9.1 cfu / 100 ml.

In tap water reaching houses , all fungi recorded in raw water were reappeared , although in very few numbers. Some of these fungi could be liberated from fungal biofilms that found in the lining surface of pipes[18].

In stored tap water, total number of fungal cfu is increased over the six month of storage with all fungal genera except *Acremonium* sp.,*Chaetomium* sp.,*Fusarium* sp. and *Trichoderma harzianum*.

Table -4 : Yeasts isolated from water at different stages at AWTP

Fungi	Raw Water		Sedimentation/Chemical coagulation		Sand Filter	
	C f u Rate	Frequency%	C f u Rate	Frequency%	C f u Rate	Frequency%
Cryptococcus curvatus	02.95	33.94	03.69	52.86	01.08	48.21
Candida *C. albicans* *C. glabrata* *C. parapsilosis*	02.83	32.56	02.22	31.80	00.50	22.32
Rhodotorula mucilaginosa	01.83	21.05	00.45	06.45	00.66	29.46
Saccharomyces cerevisiae	01.04	11.96	00.54	07.73	00.00	00.00
Geotrichum candidum	00.04	00.46	00.08	01.14	00.00	00.00
Total	08.69	~100	06.98	~100	02.24	~100

Number of recovered yeast cfu in raw water is less (20%) than that of filamentous fungi . Identified yeasts as indicated by PCR results (fig. 1and appendix 1) were *Cryptococcus curvatus*, *Candida* spp. (where C. *albicans* ,*C.glabrata* and *C. parapsilosis* showed similar bp size), *Rhodotorula mucilaginosa*, *Sacccharomyces cerevisiae* and *Geotrichum candidum*. In a similar work [17] in Australia found *Aureobasidium pullulans*, *Rhodotorula* spp., *Lecythophora* spp. and *Cryptococcus laurentii* in almost similar numbers . Yeasts showed similar trend as filamentous fungi regarding the effect of physicochemical treatment where their number is reduced to the forth (Table 4).

Yeasts withstanded chlorine treatment were *Rhodotorula mucilaginosa*, *C. parapsilosis*, *Cryptococcus curvatus* and *Aureobasidium pollulans* .However they were recovered by very low number . Those reaching house tap water were *Cryptococcus curvatus* ,*Rhodotorula mucilaginosa* and *C. parapsilosis* . The same two yeasts *Rhodotorula mucilaginosa* and *C. parapsilosis* were recovered from stored water too (Table 5). In France , yeasts recovered from drinking water were in the range of 0.1 to 2.8 cfu /100 ml[26]. In bottled mineral water and tap water in Brazil , using molecular methods ,[27] found *C. parapsilosis* , *C. glabrata* and *C. albicans* but in higher numbers.

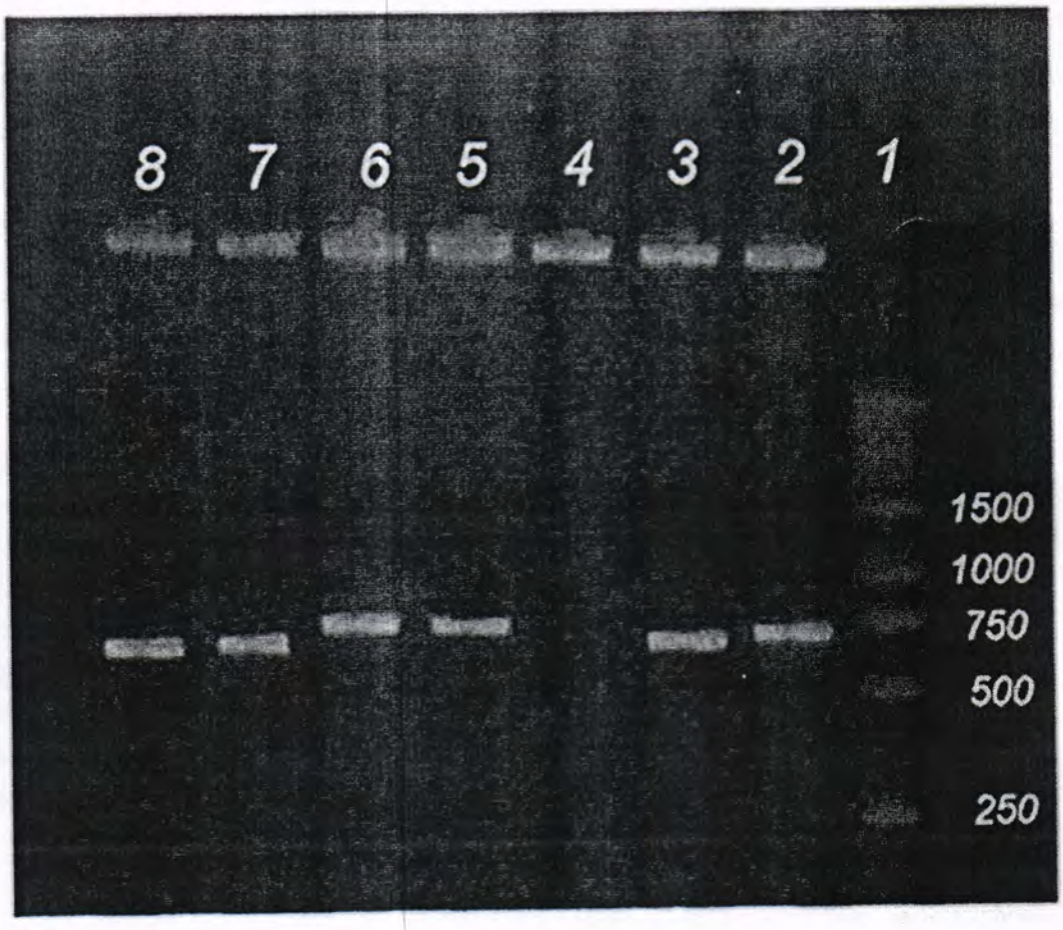

Figure 1: Gel electrophoresis profile of isolated yeasts.
Path1-DNA Ladder (1kb), path 2- *Cryptococcus curvatus* (700)bp ,path3--*Rhodotorula mucilaginosa* (640)bp , path 4- *Geotrichum candidum* (negative resulte) , path 5- *Saccharomyces cerviesiae* (880)bp, path 6- *Candida glabrata* (800)bp , path7- *Candida parapislosis* (550)bp , path 8- *Candida albicans* (550)bp (100)Volt-1hour

Table-5 : Yeasts isolated from tap water at different stages at AWTP, houses and stored water

Fungi	Tap Water AWTP*		Tap Water House		Stored Tap Water	
	C f u Rate	Frequency %	C f u Rate	Frequency%	C f u Rate	Frequency %
Cryptococcus curvatus	00.27	18.88	00.41	30.14	00.00	00.00
C. parapsilosis	00.29	20.28	00.75	55.14	01.50	47.47
Rhodotorula mucilaginosa	00.79	55.24	00.20	14.70	01.66	52.53
Aurobasidium pullulans	00.08	05.60	00.00	00.00	00.00	00.00
Scacharomyces cerevisiae	00.00	00.00	00.00	00.00	00.00	00.00
Geotrichum candidum	00.00	00.00	00.00	00.00	00.00	0.00
Total	01.43	100	01.36	100	03.16	~100

* chlorine treated and delivered for distribution

The observed low fungal load in raw water may due to sedimentation of particles (clearing) since water pass through a pipe for about 1500 m from the source. Also, the plant is new and tanks being sterilized periodically by chlorine at different stages. This may contributes , in addition to treatments for low fungal load during different stages of purification and sterilization.

There is no critical global standard for fungal load in potable water that is considered risky as in the case of coliform bacteria. However , Swed is the only country that implemented fungal analysis in drinking water since 1993. According to the Swedish regulation authority , the limit for the occurrence of fungi in drinking water is 100 cfu per 100 ml[1]. Accordingly , our results show that the fungal contamination in potable water from AWTP is too low to perform a problem. Despite the presence of potentially pathogenic fungi such as species of *Aspergillus* spp. , *Penicillium* spp. , *Cladosporium* spp. and *Candida* spp., their numbers in tap water were shown to be low even when compared to their density in air. In air , although there is no standard limit for fungal contamination, concentration of indoor fungi that exceeds 500 cfu / m^3 is considered risky[28 ; 29]. However , the case in other old,directly supplied drinking water plants might be different. Therefore , more water treatment plants have to be investigated in order to get conclusive generalization.

Appendics: 1 Size in bp of the PCRproducts of yeasts

حجم ناتج التضاعف bp	الخمائر	ت
400	*Candida agrestis*	1
550	*Candida albicans*	2
750	*Candida apicola*	3
450	*Candida auringiensis*	4
650	*Candida beechii*	5
750	*Candida boidinii*	6
700	*Candida cacaoi*	7
700	*Candida cantarellii*	8
450	*Candida diversa*	9
650	*Candida ergatensis*	10
800	*Candida glabrata*	11
425	*Candida incommunis*	12
425	*Candida intermedia var. intermedia*	13
425	*Candida magnoliae*	14
550	*Candida maltose*	15
650	*Candida mesenterica*	16
525	*Candida Montana*	17
580	*Candida norvegica*	18
550	*Candida parapsilosis*	19
450	*Candida sake*	20
450	*Candida salmanticensis*	21
500	*Candida sorbosa*	22

475	*Candida stellata*	23
650	*Candida terebra*	24
550	*Candida tropicalis*	25
480	*Candida wandervaltii*	26
560	*Candida vinaria*	27
500	*Candida vini*	28
660	*Candida wickerhamii*	29
620	*Candida zeylanoides*	30
880	*Saccharomyces bayanus*	31
880	*Saccharomyces cerevisiae*	32
675	*Saccharomyces exiguus*	33
880	*Saccharomyces paradoxus*	34
880	*Saccharomyces pastorianus*	35
750	*Saccharomyces ludwigii*	36
675	*Saccharomyces capsularis*	37
630	*Cryptococcus albicans*	38
630	*Cryptococcus diffluens var. uruguaiensis)*	39
650	*Cryptococcus ater*	40
630	*Cryptococcus bhutanesis*	41
700	*Cryptococcus curvatus*	42
500	*Cryptococcus dimennae*	43
540	*Cryptococcus flavus*	44
630	*Cryptococcus gastricus*	45
660	*Cryptococcus himalayensis*	46
520	*Cryptococcus humicola*	47
500	*Cryptococcus hungaricus*	48
630	*Cryptococcus kuetzingii*	49
600	*Cryptococcus laurentii*	50
490	*Cryptococcus luteolus*	51
650	*Cryptococcus macerans*	52
650	*Cryptococcus magnus*	53
500	*Cryptococcus marinus*	54
565	*Cryptococcus skinneri*	55
900	*Cryptococcus terreus*	56
650	*Cryptococcus uniguttulatus*	57
575	*Rhodotorula acuta*	58
640	*Rhodotorula glutinis*	59
660	*Rhodotorula graminis*	60
660	*Rhodotorula minuta*	61
640	*Rhodotorula muciaginosa*	62

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Distribution of aminoglycoside resistance mediated by 16S rRNA Methylation between locally isolated of *Escherichia coli* and *Pseudomonas aeruginosa*

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Received 19/3/2012 – Accepted 18/4/2012

الخلاصة

جمعت 100 عزلة من (58) Escherichia coli ,(42) Pseudomonas aeruginosa (من مرضى باصابت مختلفة في مستشفى الكندي التعليمي ومستشفى الكاظمية التعليمي ومستشفى ابن البلدي ومستشفى الأمام علي .شخصت العزلات اعتمادا على عدد من الفحوص المظهرية والكيموحيوية المتبوعة بالتشخيص بعدةAPI 20 . اظهرت نتائج الفحص المسحي الاولي لمقاومة مضادات الامينوكلايكوسيدية باستخدام طريقة انتشار الأقراص بان جميع العزلات كانت تحمل صفة المقاومة المتعددة ،واعلى نسبة مقاومة كانت موجه ضد مضاد الكاناميسن واقلها للاميكاسين .اجري فحص التحري عن أنتاج أنزيمات البيتالاكتاميز واسعة الطيف (ESBLs)وبينت النتائج ان 84%من العزلات اعطت نتيجة موجبة .اختيرت 20 عزلة من كلا النوعين ذات المقاومة العالية لاجراء الدراسة الوراثية باستخدام التفاعل التضاعفي لسلسة الدنا (PCR) وذلك لتحديد جينات مقاومة المجوعة الامينوكلايكوسيدية المتواسطة بمثيلة S r RNA 16فضلا عن تحيد جين M-blaCTXالمسؤل عن أنتاج انزيمات . ESBLs تم اكثار جيني مثيلة الاول بحجم armA (846 bp) والثاني بحجمrmtD (500 bp) الى جانب مضاعفة blaCTX –M بحجم (550 ,(bpمنها 6(80%) نتيجة موجبة لجين armA في الوقت الذي لم تمتلك اي من عزلات P.aeruginosa لهذا الجين .ام بانسبة لجين rmtDفتبين ان عزلة واحدة (5%) من E. coli كانت موجبة له في الوقت الذي امتلكت 3 عزلات (15%)من بكتريياP.aeruginosa للجين الثاني .ام نتائج التحري عن جين blaCTX –M وجد في جميع عزلات(100%) E.coli فيما وجد في 17 عزلة من P.aeruginosa..85 (%انها المرة الأولى في العراق التي يتم فيها تسجيل المقاومة عن طريق مثيلة S rRNA 16بين E.coli و P.aeruginosa بالترابط مع أنتاج أنزيمات. ESβLs

ABSTRACT

Clinical isolates (100) of (58) *Escherichia coli* and (42) *Pseudomonas aeruginosa* were collected from patients with different infections at Al-Kindey teaching hospital , Al-Kadhymia teaching hospital, Ibn-Albalady hospital and Al-Imam-Ali hospital in Baghdad. These isolates were diagnosed using different morphological and biochemical test followed by the complementary API 20E. Results of primary screening test for aminoglycoside resistance using disk diffusion method revealed that all the isolates conferring multidrug resistance and the highest resistance was against kanamycin, while the lowest was against amikacin .Detection of Extended spectrum β–lactamase(ESβLs) was also preformed and the results showed that 84% of the isolates gave positive result. Highly resistant isolates (20 for each) were selected for the genetic study using polymerase chain reaction techniqe (PCR) to determine aminoglycoside resistance mediated by methylation 16S r RNA beside detection bla_{CTX-M} gene responsible for ESBLs production .Two 16S rRNA methylase genes were amplified ,the *armA* (846 bp)and *rmtD* (500 bp) beside amplifying bla_{CTX-M} gene (550bp) .Out of 20 *E.coli* isolates ,16(80%)gave positive results for *armA* gene, while non of *P.aeruginosa* harboured this gene .For *rmtD* gene, only one isolates of *E. coli* (5%) was positive, while 3 isolates of *P.aeruginosa* (15%) possess this gene . For bla_{CTX-M} gene ,it was detected in all *E.coli* isolates (100%) and were detected in 17(85%) for *P.aeruginosa.*

This is the first report in Iraq for the emergence of 16S rRNA methylases among *E.coli* and *P.aeruginosa* in correlation with ESβLs production .

Key words: Aminoglycoside resistance, 16S rRNA methylation, *armA* ,*rmtD* and ESβLs bla_{CTX-M} genes.

INTRODUCTION

Aminoglycoside antibiotics are currently used for the treatment of a broad range of life-threatening infections caused by both Gram-positive and Gram-negative bacteria (1). They inhibit bacterial protein synthesis by irreversibly binding to 30S subunit of bacterial ribosome leading to cell death (2) . Recently, resistance to these antibiotics in pathogenic bacteria were either to production of aminoglycoside-modifying enzymes , impaired uptake of the antibiotics or decreasing intracellular antibiotic accumulation and a mutation may occurs in the target ribosomal site (3) . In 2005, a new type of mechanism had been emerged represented by ribosomal protection by methylation 16S rRNA A site (3,4) . Usually this mechanism involved methylation step occur via a methylase enzyme at specific nucleotides in 16S rRNA A sit causing limitation in binding between antibiotics to its target due to loss the affinity of binding thus causing high-level resistance to aminoglycosides (5; 6). Methylation of 16S rRNA A sit conferred by a single gene(*armA*) had been described in a human *E. coli* isolates (7, 8) , now ,most of methylation is mediated by a set of genes designated as *ArmA, RmtA, RmtB, RmtC, RmtD,* and *NpmA* (9,10,11).Usually *armA* gene is most predominant between Enterobacteriaceae and *Acinetobacter* spp. while *rmtD type* had been identified in Enterobacteriaceae and *P.auroginosa* especially in Asia and Europe (11, 12,13) . The six methylase enzymes expressed from the six genes have been described to be carried on plasmid and the pathogenic bacteria producing such enzymes have the ability to resist all aminoglycoside group(10; 11) . Associations between 16S rRNA methylase and extended-spectrum β-lactamase (ESBLs) production specially bla_{CTX-M} group have been reported (10; 11,13) . The aims of this study were to evaluate the prevalence of two 16S rRNA methylase genes(*armA* and *rmtB*) among locally isolated *E.coli* and *P.auroginosa* using polymerase chain reaction (PCR) and studying the correlation between the presence of these genes with bla_{CTX-M} genes responsible for ESBLs production.

MATERIALS AND METHODS

Collection and diagnosis of Bacterial isolates

One-Hundred clinical isolates of *E.coli*, *P.auroginosa* were isolated from patients with different infections at Al-Kindey teaching hospital, Al-Kadhymia teaching hospital, Ibn-Albalady hospital and Alemam-Ali hospital in Baghdad during a period between July 2011 and December 2011. They were obtained from midstream urine from patients suffering from urinary tract infections (68 isolates),

Sputum from patients suffering from respiratory tract infection (8 isolates), wounds infections (6 isolates) and from bacteraemia (18) isolates . Bacterial diagnosis including morphological and biochemical tests were done according to Atlas *et al.* (14) followed by the complementary API 20E test.

Antibiotic susceptibility tests:

All isolates were tested for antimicrobial susceptibility by disk diffusion method according to the CLSI (15) using six type of aminoglycoside discs including: amikacin, kanamycin, gentamicin, neomycin, tobramycin, netilmicin and Sisomicin. The diameter of inhibition zone were measured after 18 hrs and were compared with the control strains; *Escherichia coli* ATCC 35218.

Detection of β-lactamases production

The detection of β-lactamases production was performed using Rapid ESβL Detection kit (MAST Group, UK). This kit includes four tests: preliminary screening kit, Metallo-β-lactamases, ESβLs confirmation and Amp C detection. The test was performed according to the procedure suggested by the manufacturing company: Primary screening test to detect β-lactamas resistance was performed by culturing bacterial isolates on appropriate medium with cefotaxime 30μg disc. The resisted isolates were submitted to extended-spectrum β-lactamases production test . One drop of test substrate (approximately 20μl) was dispensed onto the filter pad of the strip. The test substrate was added to the strip immediately before testing. Using a loop, one colony was picked up and were spread on the filter pad of the test strip any change in color observed around the streaked line was considered a positive result. The test strip was observed after 2 to 15 minutes at room temperature, and the result was read after 15 minutes.

Plasmid DNA extraction :

Plasmid DNA were extracted according to alkaline lysis method from overnight bacterial growth following the procedure of Crosa *et al.*(16) . The plasmid were used as a DNA template for the PCR technique.

Detection of methylase and ESBLs genes using PCR.

The *armA*(amplified size 846bp) ,*rmtD* (amplified size 500 bp) and bla_{CTX-M} gene (amplified size 550 bp) were detected by PCR using specific primers listed in table(1). The reaction mixture was prepared according to the procedure that suggested by the manufacture company (KAPA, south Afriqa). For amplification of *armA* gene and *rmtD* gene, PCR mixture was composed of 5 microliters of template DNA (from

plasmid preparation) while the quantity was reduced to 3 microliters in case of bla_{CTX-M} gene. The template DNA was mixed with PCR mixture which composed from 12.5 µl of GoTaq®Green Master Mix (2x) , 1.5 µl from forward and reverse primers (10pmol) for each gene, then the volume was complete to 25 µl of nuclease free water using 4.5 µl in case of *armA*, *rmtD*, and 6.5 µl for bla_{CTX-M}.

PCR was run under the following conditions: for *armA* gene, primary denaturation step at 95°C for 5 min; 35 repeated cycles of denaturation step at 94°C for 45sec, annealing at 53°C for 45sec, and 1 min at 72°C as extension step followed by final extension step at 72°C for 7 min . Conditions for *rmtD* gene were: 95°C for 5 min; 30 repeated cycles of 94°C for 30sec, 51°C for 30sec, 1 min at 72°C; and final extension step at 72°C for 6 min, while the condition for bla_{CTX-M} gene were : 95°Cfor 5 min; 30 repeated cycles of 94°C for 30sec , 55°C for 30sec and 72°C for 1 min then final extension step at 72 °C for 6 min . PCR products were electrophoresed in 1.5% agarose gels and visualized under UV light according to Sambrook and Russell (17).

RESULTS AND DISCUSSION

Aminoglycoside antibiotics are widely used in clinical settings, especially for treatment of life-threatening infections caused by Gram-negative bacteria. They bind to the highly conserved A-site of the 16S rRNA of the prokaryotic 30S ribosomal subunits, interfering with the protein synthesis with subsequent bacterial death (7,19).

Table-1: Primers used for detection specific genes.

Name of primers	primers sequence 5'--------→3'	Target site	Product size (bp)	Reference
armA	F-CCGAAATGACAGTTCCTATC	*armA*	846	Wassef *et al.*, (18)
	R-GAAAATGAGTGCCTTGGAGG			
rmtD	F- TCAAAAAGGAAAAGGACGTG	*rmtD*	500	Tijet *et al.*, (19)
	R –CGATGCGACGATCCATTC			
bla_{CTX-M}	F- CGCTTTGCGATGTGCAG	*blaCTX*	550	Nasehi *et al*, (20)
	R- ACCGCGATATCGTTGGT			

The most frequently encountered mechanism of resistance to aminoglycosides is their structural modification by specific enzymes produced by resistant organisms. The three classes of such enzymes are aminoglycoside acetyltransferases (AAC), aminoglycoside nucleotidyltransferases (ANT or AAD), and aminoglycoside phosphotransferases (APH) (19). The ribosomal protection by methylation of 16S rRNA in aminoglycoside-producing actinomycetes

gives high level resistance to intrinsic aminoglycosides(7), Since 2003, methylation of 16S rRNA has emerged as a serious threat to the class through the action of plasmid mediated methyltransferase enzymes(5) . Results of antibiotic susceptibility were variable for the two isolated species (figure 1), in general amikacin was the most effective one since the percentage of resistance were 5.023% and 26.1% for *E.coli* and *P.aeruginosa* respectively , followed by netilmicin (used for the first time in Iraq) and neomycin when the resistance rate reached 40% , 31.6% for *E.coli* and 31.2% , 52.3% for *P.aeruginosa*. From the other hand, the highest resistance were toward kanamycin and streptomycin when 65.5% and 67.2% of *E.coli* were able to resist these two antibiotics while the resistance rates for *P.aeruginosa* reached 90.4% and 71.4% respectively .For gentamicin and tobramycin , results of the current study revealed that *P. aeruginosa* isolates showed more resistance percentage as compared with *E.coli* when they were 40.4% , 50% and 56%, 60.35% respectively . In the study carried out by Risberg, (21) he reported that 5% of the *E.coli* were resistant to amikacin and this result agree with the result of the current study, while wassef *et al* ,(18) was agreed with the result of this study in case of gentamicin in which the rate of resistance reached 54.8%. By comparing the result of tobramycin with AL-Kaabi, (22) it can be said that there is similarity with the result reaching 67.85%. For netlimicin, the result of the current study relatively agreed with the result of Wassef *et al.(*18) which they found that 44% of the *E.coli* isolates were resistance. The result of this study was close to the result of AL-Kaabi, (22) in which she reported that 32.14% were resistance to neomycin, as for kanamycin the result relatively agreed with Gad *et al*, (23)were reported 60%. In the study of Haldorsen, (24) which was carried out on *P.aeruginosa* isolats he reported that these isolates showed the same resistance rate reached to 20% against amikacin and this result concur with the result of the current study. For gentamicin the result of the current study relatively agreed with the result of Haldorsen, (24) which he found that 38% of *P.aeruginosa* isolates were resistance while for netilmicin ,Wassef *et al.* (18) reported that 17% were resisted isolates and this results relatively closed with the current study. For kanamycin Gad *et al.*, (23) from Egypt illustrated that resistance rates for this antibiotics was 91.1% and it is a good agreement with the current study but not agreed with their results for neomycin which was higher and reached 77.8% .

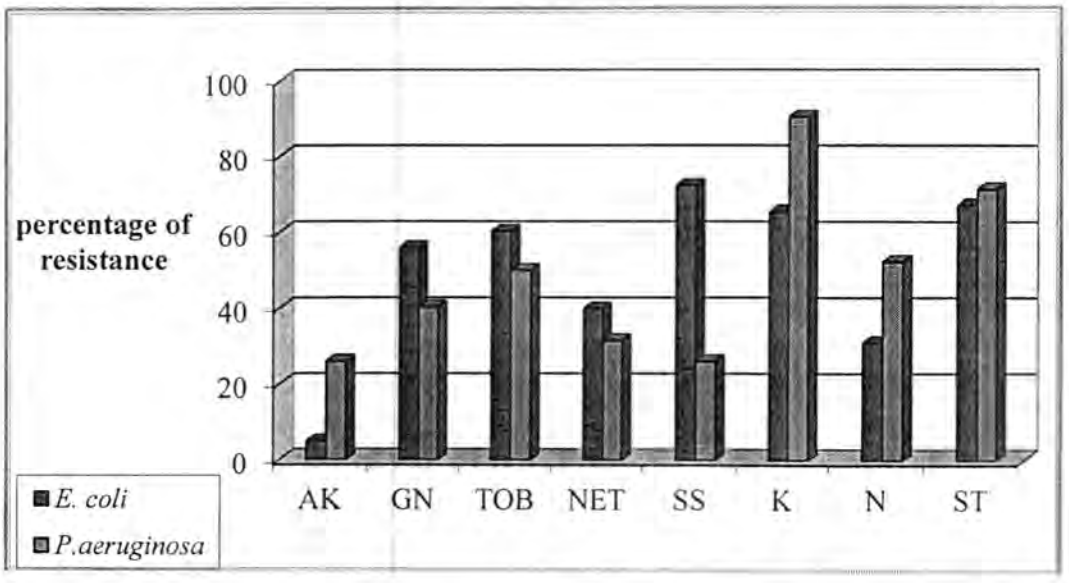

Figure-1: The percentage of aminoglycoside resistance for *E.coli* and *P.aeruginosa* .
AK. Amikacin, **GN.**Gentamicin, **TOB.**Tobramycin, **NET.**Netlimicin, **SS.**Sisomycin, **K.**Kanamycin, **N.**Neomycin, **ST.**Streptomycin.

The individual resistance mechanisms are identified by use of specific inhibitors on the colour coded test strips i.e. ESβL (Clavulanic Acid), MβL (EDTA and Mercaptoacetic Acid) and AmpC (Boronic Acid). The result of this study showed that 84 isolate of total 100 isolates(84%) were β-lactamases producer (ESβL). This result was compatible with Ogbolu *et al.* (25) in which they reported that 82.8% of the Gram negative isolates were producer of this enzyme. In the current study these 84 β-lactamases producer isolates were distributed as 50(86.2) of *E.coli* and 34 (80.9%) isolates for *P.aeruginosa* .

Detection of 16S rRNA methylase, ESβLs genes

The presence of *armA, rmtD* and bla_{CTX-M} were investigated by PCR amplification technique using primer sets as described previously. Twenty isolates from each species were selected depending on their ability to resist amikacin and gentamicin beside their ability to produce ESBLs. Among these 40 isolates, *armA* gene was detected in 16 *E.coli* isolates (Figure 2) which show agarose gel electrophoresis of *armA* PCR products for *E.coli* isolates, As it clear .the positive results in lines 1,2,3,4,6,7 and the amplified segment was 846bp while lane 5 showes negative result. In contrast to *E.coli* non of *P. aeruginosa* isolates gave positive result with this gene ,while *rmtD* was detected only in one *E.coli* isolate and(3) *P.aeruginosa* isolates. Thus, among the 20 *E.coli* isolates and 20 *P.aeruginosa* isolates, the prevalence rates of *armA*

were 80% in *E.coli*, and the prevalenace rates of *rmtD* were 5% in *E. coli* and 15% in *P.aeruginosa* isolates. In the study of Zhou *et al.* (26) showed prevalence rate of *armA* in *E.coli* was 67.2 and this result is relatively closed with the current study while Yan *et al.*, (27) and Wassef *et al.*, (18) reported lower percentage of *armA* prevalence when the rate reaches 28% and 21.2%respectively.

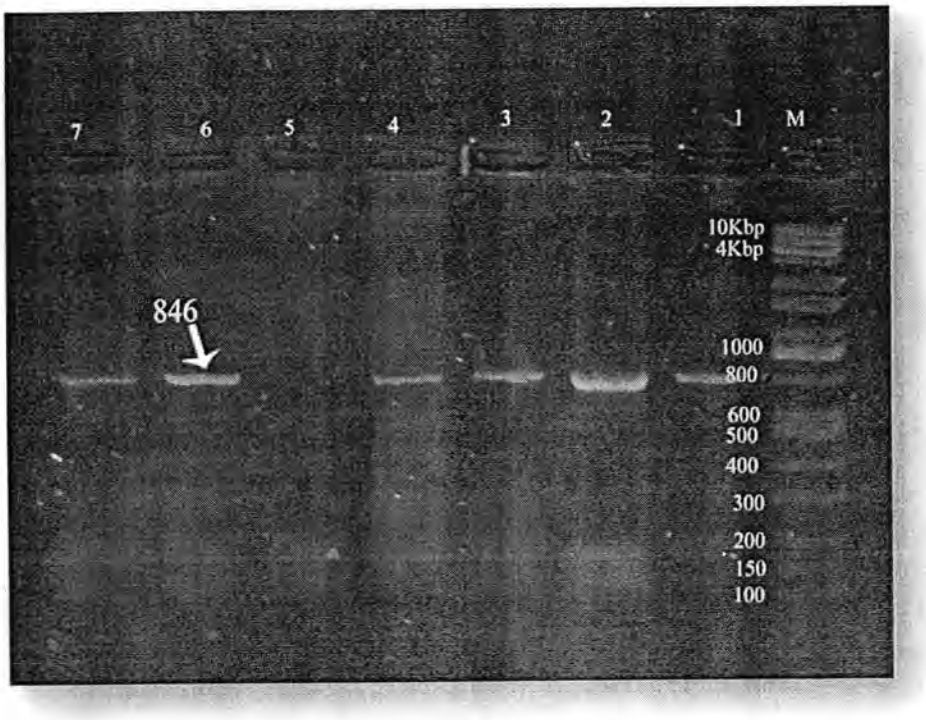

Figure-2:Agarose gel electrophoresis of *armA* PCR products in *E.coli* isolates, lane M (DNA ladder) 100bp molecular marker, lines 1-7armA PCR products of 7 isolates, 1,2,3,4,6,7 lines are positive result 846bp amplicon, line 5 shows negative result.

The results of the current study for *rmtD* gene relatively agreed with the results of Tijet *et al.*(19) which they found that the ratio of prevalence of *rmtD* reached 0.7% in *E.coli* in Argentina, also only one isolate harbouring *rmtD* gene was detected in Norway by Haldorsen (28) from *E.coli*, while Castanheira *et al.*(29) illustrated in their study carried in Barazil that 4 isolates of *P.aeruginosa* out of 26 were positive to this gene. Figure (3) shows agarose gel electrophoresis of *rmtD* in *E.coli* and *P.aeruginosa* and product size 500bp. While figure (4) show bla_{CTX-M} in *P.aeruginosa* and product size 550bp, and it was detected in all *E.coli* isolates (100%) which was resistant to aminoglycoside group and were detected in 17(85%) for *P.aeruginosa* isolates.

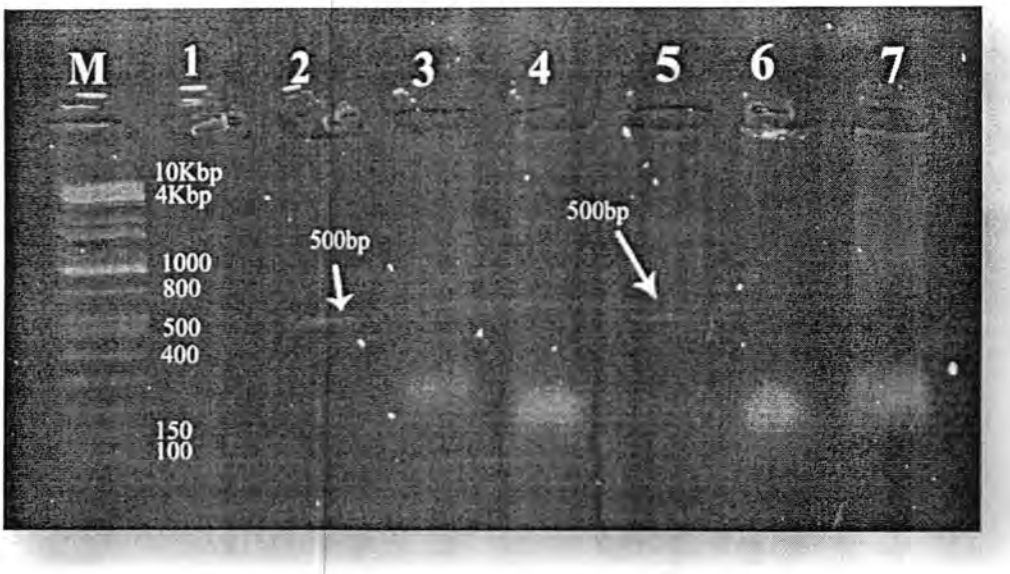

Figure-3: Agarose gel electrophoresis for *rmtD* PCR product in *E.coli* and *P.aeruginosa* lane M (DNA ladder) 100bp molecular marker, lanes 2-7 *rmtD* PCR products of 7 isolates, 2,5 lanes are positive result 500bp amplicon in *E.coli* and *P.aeruginosa* respectively , while lane 3,4,6,7 shows negative result, and lane 1 negative control with out template.

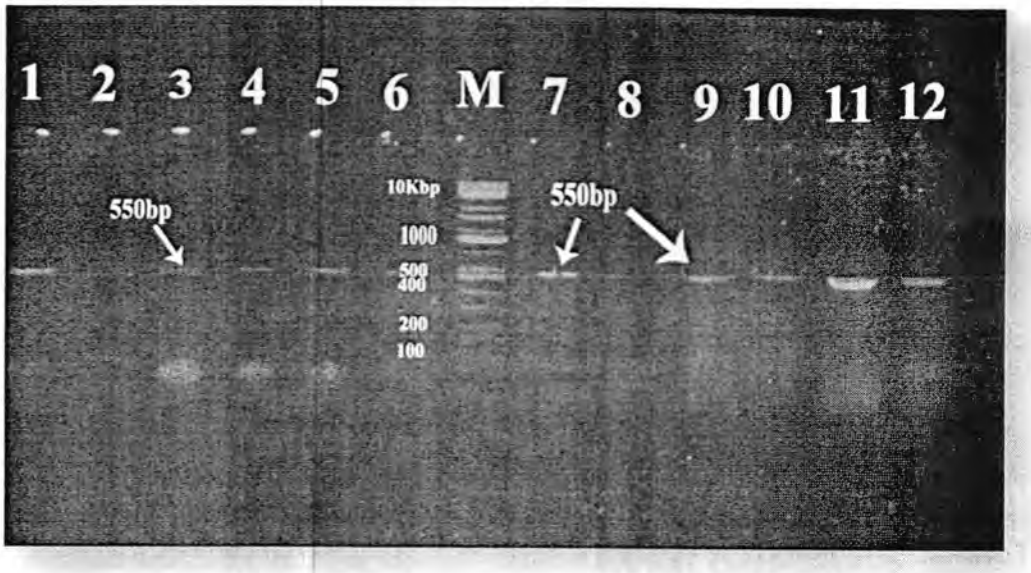

Figure-4: Agarose gel electrophoresis for bla_{CTX-M} gene in *P.aeruginosa*. lane M (DNA ladder) 100bp molecular marker, lanes 1-12armA PCR products of 12 isolates, 1,3,4,6,7,9,10,11,12 lanes are positive results 550bp ampicon, while lane 2 and 8 show negative result.

In conclusion, *armA* was found to be more prevalent than *rmtD* gene among *E. coli* isolates. A high rate of ESBL production among the isolates that exhibited high-level aminoglycoside resistance may result

in part from the spread of closely related plasmids containing both bla_{CTX-M} and *armA*. *armA* and *rmtD* which spread mostly by horizontal transfer that is to say by conjugative plasmids . This is the first report of the occurrence of plasmid- mediated 16S rRNA methylases that confer high-level aminoglycoside resistance in human pathogens isolated in Iraq .Farther genes responsible for 16S methylation recommended detected in another research since this type of resistance is not being studied in Iraq.

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An Efficient Method for DNA Isolation and Purification from *Vibrio fluvialis*

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Received 22/11/2011 – Accepted 18/4/2012

الخلاصة

تم تطوير بروتوكول فعال لاستخلاص وتنقية الدنا من بكتريا Vibrio fluvialis –السالبة لصبغة كرام-وذلك لصعوبة استخلاصه بالطرق الإعتياديه الشائعه.تتلخص هذه الطريقه بالقيام بحضن المعلق البكتيري في خليط انزيمي مكون من ثلاث انزيمات هي اللايزوزايم ، والار ان ايز ، وبروتينيز ك اذ يعمل كل من هذه الانزيمات مستقلا عن الاخر وذلك بتغيير درجات الحرارة بذلك تم تجنب مسببات تكسير خيوط الدنا الناتجة عن المعاملات المطولة للمستخلص.

ان كمية الدنا التي تم الحصول عليها بهذه الطريقة كانت بمعدل ملغرام واحد لكل عشرة ملليترات من المعلق البكتيري وبنقاوة عالية بلغت 1,8- 1,9. بينت نتائج فحص الدنا باستخدام طريقة هلام الاكاروزظهور حزمة واحدة واضحة لاتحوي على الرنا او الدنا المتكسر او المحطم، وتبين ايضا انه كان صالحا للتقطيع واللحم بواسطة انزيمات التقييد والانزيمات اللاحمة.

ABSTRACT

An efficient procedure for the isolation and purification of total DNA has been developed for the gram-negative bacterium *Vibrio fluvialis*, which was found to be difficult to be isolated using the published methods. According to this procedure the cell suspension was incubated with an enzyme mixture containing lysozyme, RNase, and proteinase K, allowing each enzyme to work independently of the other by varying the incubation temperature. Therefore, shearing and degradation of the DNA as a result of extended manipulation and possible inadequate handling of the lysate was avoided.

The average DNA yields obtained were in the range of 1mg per 10ml of cell suspension, with a high level of purity of about 1.8-1.9. Examination of the DNA preparations using agarose gel electrophoresis revealed a well defined single band free of contaminating RNA and residual DNA. Finally, this DNA was found to be a good substrate for restriction enzymes tested and ligation experiments.

INTRODUCTION

Among other pathogens, *V. fluvialis* has been reported to cause infections and outbreaks of diarrhea in humans [1,2]. Different potential toxins have been isolated from this bacterium however; their role in pathogenesis is not well established [3,4]. Information regarding virulence genes and standard gene markers for the identification of this organism are not fully exploited.

DNA isolation and purification is a major task in any gene cloning experiments. It is therefore; necessary to prepare a high molecular and a good quality DNA suitable for restriction enzyme digestions, ligations, nick translation experiments etc. This obviously requires low level of contaminants such as, residual DNA, RNA fragments, proteins and/or polysaccharides. All these contaminants may interfere with cloning and subsequently will affect the transformation frequency of competent cells

[5]. The formation of DNA fragments which result from shearing and degradation of DNA molecule may be minimized substantially by avoiding extended manipulation steps such as, repeated mixing and shaking of lysate, whereas, contaminants may be eliminated by precipitation and/or enzymatic digestions. Attempts in our laboratories were carried out to isolate DNA from the amylolytic, chitinolytic bacterium *V. fluvialis* [6,7] using the standard procedures [8,9] yielded minute quantities of a low quality DNA. The reason for that may be attributed to the high rigidity of lysate obtained following cell lyses. Such a property renders DNA separation from proteins and other contaminants extremely difficult since, the gelatinous lysate does not mix well with phenol [10], thus making complete deproteinization extremely difficult.

In the present work we describe the development of a simple and very efficient method for DNA isolation from *V. fluvialis* that can provide sufficient amount of highly-purified DNA for use in molecular biology experiments.

MATERIALS AND METHODS

Isolation and identification bacterial isolates :

The bacterial samples were isolated from soil, utilizing the Thiosulfate-Citrate-Bile-Sucrose-Agar (TCBS Agar) and prepared according to [11]. After 18-48 h sucrose-fermenting Vibrio appeared as smooth, opaque, thin edged, yellow colonies on TCBS AGAR. To avoid confusion, further tests were necessary to perform since a variety of Vibrio show resemblance to *Aeromonas hydrophilus* [12] *Plesiomonas shigelloides* [13] and *Pseudomonas spp* [14]. Staining a sample from an overnight growth by Gram stain demonstrated typical small, curved Gram-negative rods. Additionally, examination of a live suspension of the suspected isolate of *V. fluvialis* using a phase contrast microscope indicated the presence of an organism with typical small, curved rods and darting motility. The String test was also applied for ruling out non-*Vibrio spp*, particularly, *Aeromonas spp*. It was carried out on a glass microscope slide by suspending a bacterial sample from a 24 h fresh culture grown in nutrient agar [15] in a drop of 0.5% aqueous solution of sodium deoxycholate. The cell suspension lost turbidity and the DNA released from the lysed cells caused the mixture to become viscous. According to this test, *Vibrio fluvialis* show positive results whereas, *Aeromonas spp* do not [3]. Finally, salt tolerance was determined by growing the susceptible Vibrio culture at 37 ^{0}C in 1% peptone broth without NaCl or supplemented with 7% NaCl [16].

Bacterial growth conditions:

Fifty ml of LB medium (Tryptone 10g/L, yeast extract 5g/L, NaCl 10g/L) was inoculated with a single colony and grown with vigorous shaking at 37 ^{0}C to the log phase [17].

DNA isolation and purification:

Cells were collected by centrifugation at1350g for 5min at 4 ^{0}C, and then resuspended in 2ml of NET buffer (50 mM Tris-HCl pH 8.0, 100mM NaCl, 50 mM EDTA). To this, ice cold freshly prepared NET buffer containing 8mg lysozyme, 600µg RNase and 1.45mg proteinase K were added. This suspension was mixed briefly by vortexing and then incubated on ice for 15min. The tubes were then transferred to a water bath at 65 ^{0}C for 5min or complete lyses as indicated by a clear lysate. Incubation at 65 ^{0}C was continued for an additional 60min to allow complete digestion of the released cellular proteins. After the crude lysate was cooled to room temperature, a phenol extraction procedure was performed by chloroform and then several ether extractions. The recovered elute was mixed with 2 volumes of ice cold 96% ethanol to precipitate the DNA. The pellet was dried under low vacuum pressure and redissolved in sterile distilled water. DNA concentration and purity were measured spectrophtometrically as in the following procedure: DNA concentration was estimated by adjusting the A_{260} measurements for turbidity (measured by absorbance at A_{320}), multiplying the dilution factor, and using the relationship that an A_{260} of 1 = 50µg/ml pure DNA. Concentration (µg/ml) = (A_{260} reading – A_{320} reading) x dilution factor x 50µg/ml

An absorbance of A_{260}/A_{280} ratio between 1.7 and 2.0 is generally accepted as a representative of a high-quality DNA sample. The ratio was calculated after subtracting the non-nucleic acid absorbance at A_{320} [18].

DNA purity (A_{260}/A_{280}) = (A_{260} reading –A_{320} reading) ÷ (A_{280} reading – A_{320} reading)

Restriction endonucleases analysis:

DNA was digested with the following restriction enzymes: Apa I, Eco RI, or Pst I, Hind III, Xho I, and Sal I.

Restriction digests were performed using the KGB restriction buffer [19]and all enzymatic reactions were carried out under the conditions recommended by the supplier. The reaction mixtures were prepared each in a thin-wall tube at room temperature; mixed thoroughly, span briefly, and incubated at 37 ^{0}C for 1hr. Reactions were stopped by heating at 80 ^{0}C for 10 min. Gel electrophoresis was carried out on

0.7% agarose in TBE buffer (89mM Tris-HCl pH 7.9, 89mM Boric acid, 2mM EDTA) at a constant voltage of 1-2V/cm for about 16 h [17].

RESULTS AND DISCUSSION

Due to the lack of simple and reliable diagnostic tests, this pathogen has not been studied in great detail, in terms of public health significance [20].

The DNA preparations obtained using the standard procedures mentioned earlier were found to be of poor yields and quality (Figure.1), in addition to the extended manipulation Steps.

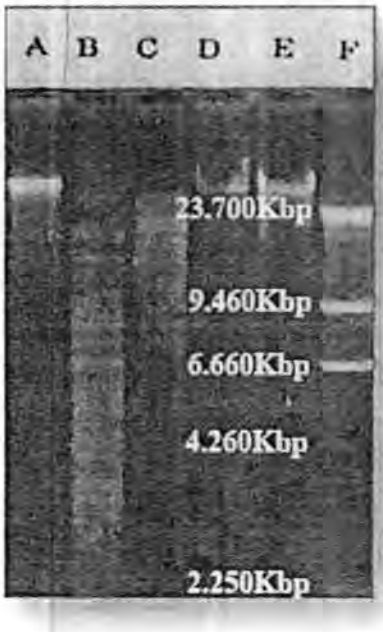

Figure-1: Gel electrophoresis of *V. fluvialis* DNA isolated using the standard procedures. A: DNA band prepared by our method, notice sharpness of the band and the minimal smearing as compared to the DNA obtained by the standard procedures (D and E). B and C represent DNA prepared using our procedure, digested with Eco RI and Pst I respectively. (F): Hind III lambda DNA ladder.

The method described here however, was found to be simple, rapid and very efficient for DNA isolation from *V. fluvialis*. From repeated tests the average DNA yields obtained were 1mg per 10ml of bacterial cell culture in the log phase. The purity determined was 1.8-1.9 as indicated by the A_{260}: A_{280} ratio. The high yields and purity obtained was attributed to, substantial change in the lysate texture following incubation in the enzyme mixture. The lysate was found to mix easily with the phenol/chloroform thus, did not require lengthy centrifugation times to separate phases. Incubation of the cell suspension with the

three different enzymes simultaneously at the different temperatures reduced the number of unnecessary handling steps thus, minimizing possible shearing and also ensured homogenous distribution of enzymes. Somewhat similar results were reported as with the fungus *Phanerochaete chrysoporium* where the number and/or time of such steps were brought down in order to reduce DNA degradation [21]. The reason for the incubation of the bacterial suspension on ice was meant to provide best conditions for the lysozyme activity while, proteinase K remains inactive when incubated at such a low temperature. Raising the temperature to 65 ^{0}C activates proteinase K which destroys spheroplasts thus, leading to the release of cellular contents. The additional 60min incubation at 65 ^{0}C allows complete digestion of proteins. Additionally, RNase is activated at these temperatures, a fact which is revealed by the disappearance of RNA from DNA preparation (Figure.2). digestion with the endonucleases, Eco RI, Hind III, Xho I, Sal I, or Apa I followed by agarose gel electrophoresis analysis showed that the DNA was nearly completely digested (Figure.2) suggesting that this DNA is of a good quality and is therefore, an excellent material for gene cloning experiments.

Figure-2: Electrophoresis of the DNA preparation of *Vibrio fluvialis*. A: DR I digest DNA ladder. B: undigested DNA (0.5µg/slot), notice the absence of shearing and the disappearance of RNA. C: Apa I digest (4.8µg/slot). Electrophoresis conditions: Submarine gel electrophoresis, 0.7% agarose, Tris-borate buffer, 16 h at 20 V.

The current procedure described was also applied successfully as a minipreparation of Vibrio DNA (from 1ml culture) and other bacterial strains suggesting that it may be useful for the isolation of DNA from any gram-negative bacteria. Finally ,the advance researches for studying this species mainly depend on molecular technique such like DuPont Qualicon BAX® System Real-Time PCR assay for detection of *V. parahaemolyticus* and *V. vulnificus* and beside using the multiplex real-time PCR and DNA microarray [22].

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Toxoplasma gondii, HCV, and HBV Seroprevalence in Haemodialysis Patients with Chronic Renal Failure in Al-Kindy Hospital Baghdad, Iraqi

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Received 22/11/2011 – Accepted 18/4/2012

الخلاصة

تهدف الدراسة تحديد الأنتشار المصلي لداء المقوسات والتهاب الكبد الفيروسي C,B في مرضى الفشل الكلوي المزمن قبل وبعد عملية الغسل الكلوي الدموي.
حيث اجريت الدراسة على 152 مريض مصاب بالفشل الكلوي المزمن وتم مقارنتهم ب 28 شخص من الأصحاء كمجموعة سيطرة وتم تقسيم المرضى إلى مجموعتين الأولى تضمنت 68 مريض مصاب بالفشل الكلوي المزمن وغير الخاضعين لعملية الغسل الكلوي الدموي والثانية شملت 84 مريض مصاب بالفشل الكلوي والخاضعين لعملية الغسل الكلوي الدموي المنتظم.
اظهرت النتائج ان اعلى نسبة مئوية للأصابة بداء المقوسات وكل من فايروس C,B المسبب لألتهاب الكبد الفيروسي في المجموعة الثانية كانت 80.9% لداء المقوسات و14.3% لكل من فايروس C,B.
يستنتج من الدراسة انه يجب إجراء الفحص المستمر والمنتظم لداء المقوسات والتهاب الكبد الفايروس C,B لمرضى الفشل الكلوي المزمن قبل اجراء عملية الغسل الكلوي وتنظيم الأجهزة الخاصة بعملية الغسل الكلوي قبل وبعد الأستخدام وذلك لتقليل خطر الأصابة بهذا النوع من الأمراض.

ABSTRACT

This study aimed to determine the seroprevalence of *Toxoplasma gondii* and anti HCV antibodies , HBVs Ag in patients with chronic renal failure (CRF) before and after hemodialysis, Serum samples were taken from 152 patients with CRF in addition to 28 healthy controls. patients were classified into two groups, The first group comprised 68 patients with CRF who haven't any hemodialysis session, The second group comprised 84 patients with CRF undergoing regular hemodialysis . *T.gondii* antibodies were detected in(44.1%) of the first group and (80.9%) in the second group and (57.1%) in the healthy control.From the present results it was noticed high percentage of positivity of toxoplasma antibodies in patients with CRF undergoing hemodialysis also we determined that out of 84 CRF patients undergoing hemodialysis (14.3%) were positive for Anti-HCV the same for Anti HBVs Ag. So it can be concluded that CRF patients undergoing hemodialysis shoud be screened for toxoplasma ,HCV and HBVs Ag before dialysis to prevent the dissemination of these infections through dialysis procedure.

INTRODUCTION

Toxoplasma is a globally distributed pathogen for humans and animals. Toxoplasmosis is an infectious disease caused by the on-celled protozoan parasite *Toxoplasma gondii* (*T. gondii*).Although most individuals do not experience any symptoms, the disease can be very serious and even fatal, in individuals with weakened immune system.In situations of immunodeficiency, *T. gondii* emerges as a life-threatening infection.*T. gondii* is transmitted parenterally, flourish in state immunosuppression [1]. *T.gondii* is one of the major opportunistic

infectious agent in immunocompromised individuals such as hemodialysis patients [2].Chronic renal failure (CRF) patients are under risk from a variety of infections [3]. Patients under immunocompromised therapy or hemodialysis with CRF have deficient cellular immunity and this makes them susceptible to infection [4].The presence of infections caused either by hepatitis B virus (HBV) or hepatitis C virus (HCV) or both of them complicates the evolution of CRF considerably.The liver disease caused by HBV and HCV has become the important cause of morbidity and mortality in patients with chronic renal failure [5].In the present study we aimed to investigate the prevalence of anti -*T.gondii* antibodies IgG in patient with CRF before and after hemodialysis with or without HCV or HBV infection.

MATERIALS AND METHODS:-

This study was carried out on patients with CRF from out clinic of the kindy hospital between November 2010 to March 2011.In the present study 152 patients with CRF aged between14and76 years ,24 healthy controls were investigated who were aged between 20and70 years. Two groups of patients of both sexes were examined , the first group comprised 68 patients with CRF who haven't any hemodialysis sessions ,the second group comprised 84 patients with CRF undergoing regular hemodialysis in hemodialysis unit of al kindy hospital. The blood samples were taken from patients and healthy control centrifuged at 1500 rpm for 5min to obtain serum samples and preserved at -20c in deep freeze until tested. Seroprevalence of *T.gondii* IgG antibodies in the serum samples were tested using the enzyme linked immunosorbent assay (ELISA) KITS purchased from commercial manufacturer biocheck ,Inc which were performed following the manufacturers instructions. hepatitis B surfase antigen (HBsAg) were detected by bioelisa HBsAg bio kit (Barcelona-Spain),anti HCV antibodies were detected by bioelisa HCV biokit (Barcelona-Spain).

Statistics analyses were performed using a chi-square test using minitab under windows.

RESULTS AND DISCUSSION

One hundred and fifty two patients with CRF were included in this study their mean age was 46.37±15.76 years, The gender distribution of patients was 86(56.6%) males and 66(43.4%) females. 24 healthy controls were also included their mean age was 43.36±16.47 years,10(41.7%) males and 18(75%) females. on the basis of ELISA, samples were diagnosed as either positive (yellow greenish well) or negative (white well) for specific antibodies to *T.gondii* . In this study we investigated the incidence of *T.gondii* infection in CRF patients

before and after undergoing hemodialysis sessions.The prevalance of *T.gondii* IgG antibodies was 44.1% (30/68) in CRF patient before hemodialysis,While in healthy control 57.1% (16/28) as shown in Table1 .The protozoa that most frequently cause disease in immunocompromised patients is *Toxoplasma gondii* beside other parasites[6].Patients on HD suffer from general immune incompetence resulting in a high incidence of infectious complications. Various abnormalities in T-cell function of HD patients have been described [7].which may have been due to the changes in immunological status of patients with CRF which make them at a high risk of contracting bacterial, parasitical and viral infections [8].

Table-1: The percentage of Anti-*T.gondii* IgG in CRF patients before and after hemodialysis (HD) and in healthy control

Anti-T.gondii antibodies	Group1 before HD (n=68)	Healthy control	p-value	Group 2 after HD (n=84)	Healthy control	p-value	Group 1 before HD (n=68)	Group 2 after HD (n=84)	p-value
IgG +ve	30(44.1%)	16(57.1%)	P =0.246*	68(80.9%)	16(57.1%)	P =0.012**	30(44.1%)	68(80.9%)	P =0.000***
IgG -ve	38(55.9%)	12(42.9%)		16(29.0%)	12(42.9%)		38(55.9%)	16(29.0%)	

*No significant correlation**significant correlation***high significant correlation

Group 1shows no significant correlation with healthy control ($p>0.05$), probably because that serological evidence indicates a high rate of human exposure to *T.gondii* organisim [4]and that the prevalance of toxoplasmosis is related to several factors including culture, nutritional habits, age and rural or urban setting (9).While a statistical significance ($p<0.05$)was found in group2 with healthy control,These findings may due to the hemodialysis patients having immunocompromised and susceptible to infections ,this findings is similar to other study [1]. And the percentage found positive for the anti-*T.gondii* IgG of group 2 patients was (80.9%) which was significantly greater than(44.1%) in group 1 patients before hemodialysis ($p<0.001$).In this study we also investigated the relationship between HCV,HBV infection in CRF patients before and after hemodialysis. Hepatitis C virus antibody and hepatitis B surface antigen HBsAg were detected in 24(28.6%) in group 2 patients undergoing hemodialysis, 12(14.3%) were seropositive to HCV and 12(14.3%) were seropositive to HBsAg. while group 1and healthy control were seronegative to HCV antibodies and HBsAg significant difference was found among patients in group 2 undergoining hemodialysis and healthy control group ($p<o.o5$) as shown in Table 2.also there was high significant difference between patients undergoing hemodialysis and patients before hemodialysis session($p<o.oo1$) Table 2.

Table-2:HCV&HBV percentage among CRF patient before and after hemodialysis (HD) and in healthy control

	Group 2 after HD (n=84)	Healthy control	p-value	Group1 before HD (n=68)	Group 2 after HD (n=84)	p-value
HCV +ve	12(14.3%)	0	P=0.034*	0	12(14.3%)	P=0.001**
HCV- ve	72(85.7%)	28(100%)		68(100%)	72(85.7%)	
HBsAg +ve	12(14.3%)	0	P=0.034*	0	12(14.3%)	P=0.001**
HBsAg -ve	72(85.7%)	28(100%)		68(100%)	72(85.7%)	

*significant difference**high significant difference

Hemodialysis patients are at high risk of viral hepatitis infection [10]. It has been reported that viral hepatitis infection rates are in proportion to blood transfusion sessions, protracted vascular access, and the probability of exposure to infected patients, and contamination of equipment [11][12].The range of HCV in hemodialysis patients varies from 4% to 70% in different countries (11) and the HBV infection in 13.3% of hemodialysis patients similar to other researchers around the world [13]. The prevalence of hepatitis B virus (HBV) varies from 20–45% and that of hepatitis C virus (HCV) from 7–60% in dialysis patients (14). In addition 10.7% (9/84) of each HCV,HBVsAg infected patients were seropositive for anti-*T.gondii* antibodies.These results show a likley association between *T.gondii* and some other diseases ,and that patients with chronic hepatitis B are at a high risk of contracting other infectious diseases such as toxoplasmosis [15].

CONCLUSION

the results of the present study confirm a high prevalence of *T.gondii* infection among CRF patients undergoing HD in Iraq, and that they can be exposed to hepatitis C virus, hepatitis B virus, or both so they shoud be secreened for T.gondii and HCV,HBV infection regulary to prevent the dissemination of these infections through hemodialysis procedure.

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Antibacterial Activity of *Mentha Piperita and Allium Sativum* Against Some of Gram-ve Bacteria

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Received 31/5/2011 – Accepted 17/1/2012

الخلاصة

تمت دراسة الفعالية التثبيطية للمستخلص المائي لنباتي النعناع Mentha piperita والثوم Allium sativumوبتراكيز (0.1 ، 0.2 ، 0.3 ، 0.4 ، 0.5) (ملغم/ملليلتر) تجاه أنواع من البكتريا المرضية شملت : Escherichia coli, Klebsiella pneumoniae , Pseudomonas aeruginosa, Proteus vulgaris، وذلك باستخدام طريقة الانتشار في الحفر (Well Diffusion Method). أظهر كلا المستخلصين لنباتي النعناع والثوم فعالية تثبيطية عالية ضد أنواع البكتريا قيد الدراسة وقد تزايدت الفعالية بازدياد التراكيز لكليهما حيث أعطى التركيز الأخير (0.5 ملغم/ملليلتر)، أعلى قدرة تثبيطية مما شجعنا لعمل خليط لكلا المستخلصين بنسبة 1 : 1 لغرض معرفة تأثيرهما معا ضد البكتريا حيث تم الحصول على نتيجة مشجعة للغاية بمعدل قطر تثبيط 27 ملم. ومن ثم مقارنة هذه الفعالية التثبيطية بقدرة عدد من المضادات الحيوية هي Ampicillin ، Nalidixic acid ، Trimethoprim ، Cephalexin ،Erythromycin ،Amoxicilin Gentamicin ،Tetracyclin Chloromphenicol، على تثبيط نمو البكتريا المرضية وذلك باستخدام اختبار الحساسية للمضادات الحيوية.(Antibiolgram test)

ABSTRACT

The aqueous extracts of Peppermint (*Mentha piperita)* and garlic (*Allium sativum*) with five concentrations (0.1, 0.2, 0.3 , 0.4 and 0.5mg/ml) of each one were tested for antibacterial activities against pathogenic bacteria like: *Escherichia coli* , *Klebsiella pneumoniae* , *Pseudomonas aeruginosa and Proteus vulgaris* . The testing was performed by well diffusion method. Both extracts of (peppermint, garlic) showed clear antimicrobial activity against pathogenic bacteria, and this activity was enhanced with the increasing of concentrations belongs to them. The concentration (0.5mg/ml) of both extracts gave highest activity against these bacteria; therefore we screened a mixture of equivalent ratio 1:1 of both extracts in this concentration which has exhibited a significant result (average zone of inhibition 27 mm). Antimicrobial activity of both extracts was compared with that for a number of antibiotics that include: ampicilin, cephalexin, erythromycin, amoxicillin, gentamicin , tetracyclin, chloromphenicol, nalidixic acid and trimethoprim by using antibiogram test.

INTRODUCTION

The use of plants in medicine goes as far back as thousands of years and still continues today, many plants are used for the treatment of different diseases and many possess antimicrobial activities [1].

Mentha piperita (peppermint) is a medicinally important plant that belongs to the family Labiate, an aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [2]. Peppermint has a high menthol content, and is often used as tea and for flavouring ice cream, confectionery, chewing gum, and toothpaste[1]. The oil also

contains menthone and menthyl esters, particularly menthyl acetate [3]. Peppermint extracts are bacteriostatic against *Staphylococcus aureus*, *Salmonella typhimuium*, *E.coli* and *Listeria monocytogenes* [4]. Peppermint is also found to have antiviral and fungicidal activity [5]. Menthol and peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and Dermatophytic fungi [6].

Allium sativum, (garlic) plant's bulb is the most commonly used part of the plant, with the exception of the single clove types; the bulb is divided into numerous fleshy sections called cloves[7]. The cloves are used for consumption (raw or cooked), or for medicinal purposes, and have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking [7]. *Allium sativum* has been consumed as a spice and medicine for thousands of years, ancient Egyptians were known to use it for the treatment of diarrhoea; in ancient Greece it was used for intestinal and lung disorders [8].

The antimicrobial activity is, however, diminished upon boiling, which is attributed to its key component allicin, which is denatured at high temperature [9]. It has been proposed that the development of resistance to betalactam antibiotics is 1000 fold easier than the development of resistance to allicin making garlic a prime candidate for therapeutic use [10]. Even today, garlic is popular in use as an alternative remedy in infectious diseases such as otitis media [11]. Garlic extracts are also known to be effective against *Helicobacter pylori*, the cause of gastric ulcers [12]. The aim of study to determine the antibacterial activity of aqueous extract of both Garlic and Peppermint against some pathogenic bacteria.

MATERIALS AND METHODS

PREPARATION OF EXTRACTS

Apparently healthy plants were collected, washed thoroughly in tap water and dried at dark room temperature for 15 days. The garlic and peppermint were powdered and extracted separately following the published procedure [13]. The powdered material was soaked in Distilled water by keeping it in a shaker for 3 days at 30 °C. The extracts were filtered through Whatman No.1 filter paper and the filtrate was concentrated in vacuum using a rotary evaporator in order to reduce the crude extract and then autoclaved at 121°C and 15 lb pressure for 20 min. The extract was cooled and immediately assayed for antibacterial activity. It is a known fact that the loss of antibacterial activity of natural products by heating may be due to volatilization and/or the physical and chemical changes that take place during heating.

Antibacterial activity of garlic extracts at 80°C to 90 °C for 5 minutes completely destroyed [12].

PREPARATION OF BACTERIAL SOLUTION

The microorganisms were tested including: *Escherichia coli* , *Klebsiella pneumoniae* , *Pseudomonas aeruginosa and Proteus vulgaris* which stored in Nutrient agar slant at 4°C obtained from Biotechnology department – Applied sciences - University of technology . Each organisms (*Escherichia coli , Klebsiella pneumoniae , Pseudomonas aeruginosa and Proteus vulgaris)* were inoculated separately onto Nutrient Broth (Hi-media) at 37 °C for overnight and were stored in Nutrient agar slant at 4°C and sub-cultured fortnightly. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline) and (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer *(neubauer counting chamber).* The bacterial cells were diluted to approximately 10^5 CFU/ml before use [14].

WELL DIFFUSION TECHNIQUE:

Screening of antibacterial activity was performed by well diffusion technique [15]. The Mueller Hinton Agar (MHA) (Hi-media) plates were seeded with 0.1 ml of the standardized inoculums of each testing organism. The inoculums were spread evenly over plate with sterile an L-shaped rod glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 100 µl of each fresh vegetable extracts were placed in the well. The inoculated plates were incubated at 37°C for 24 hours and the inhibition zones were measured to the nearest millimeter (mm).

A mixture of equivalent ratio 1:1 of both extracts (peppermint and garlic) in the concentration (0.5mg/ml) which has exhibited a significant result by mixing equivalent ratio 1:1 in sterile beaker then placed 100µl in the wells. The inoculated plates were incubated at 37°C for 24 hours and the inhibition zones were measured to the nearest millimeter (mm).

Antibiogram test in which small discs containing different antibiotics are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible

RESULTS AND DISCUSSION

The aqueous extract of peppermint (*Mentha piperita)* with five concentrations (0.1, 0.2, 0.3 , 0.4 and 0.5mg/ml) was actively against the strains of the bacteria that are common cause of infections. The

antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent (Table-1). We observed maximum activity at 0.5mg/ml concentration against *Escherichia coli* , *Klebsiella pneumoniae* , *Pseudomonas aeruginosa and Proteus vulgaris* (Figure-1).

Table-1: The mean of inhibition zone of the aqueous extract of peppermint (M) against *Escherichia coli* , *Klebsiella pneumoniae* , *Proteus vulgaris and Pseudomonas aeruginosa*

Concentration(mg/ml) / bacteria species	0.1	0.2	0.3	0,4	0.5
	Zone of inhibition(mm)				
E. coli	-	4	6	9	12
Klebsiella pneumonia	2	5	6	8	11
Proteus vulgaris	4	6	7	10	12
Pseudomonas aeruginosa	2	4	7	10	14

The cold equates extract of *Mentha piperita*(M) shows significant activity (Figure -1) Upon chemical analysis, the extracts were found to possess glycosides and alkaloids. In addition, plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and flavonoides which have been found *in vitro* to have antimicrobial properties [16]. In addition to these properties, it has also been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported[17] (Derrida, 2003).

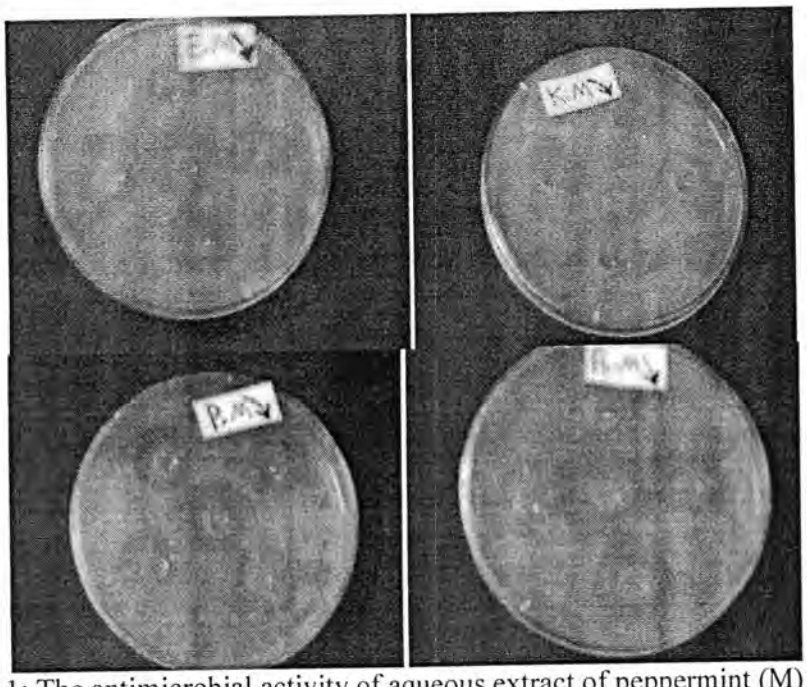

Figure-1: The antimicrobial activity of aqueous extract of peppermint (M) against *Escherichia coli*, *Klebsiella pneumoniae* , *Proteus vulgaris and Pseudomonas aeruginosa*

The present work was similar to [16], shows that the compounds from *Mentha piperita* possess potent antimicrobial activity. Besides, extract of the entire plant has shown antiprotozoal activity against *Entamoeba histolytica* and has demonstrated antibacterial properties against *Helicobacter pylori*, the bacteria causing stomach ulcer.[18].

The water extract of garlic (G) with five concentrations tested against pathogenic bacteria, all bacterial strains showed promising sensitivity to water extract of garlic (Table-2). When crushed, *Allium sativum* yields allicin, a powerful antibiotic and antifungal compound (phytoncide) [19]. *Allium sativum* may have other beneficial properties, such as preventing and fighting the common cold.[20]

Table-2: The mean of inhibition zone of The aqueous extract of garlic (G) against *Escherichia coli* , *Klebsiella pneumoniae* , *Proteus vulgaris and Pseudomonas aeruginosa*

Concentration(mg/ml) / bacteria species	0.1	0.2	0.3	0.4	0.5
	Zone of inhibition(mm)				
E. coli	4	6	9	12	14
Klebsiella pneumonia	4	5	6	8	10
Proteus vulgaris	-	4	8	10	12
Pseudomonas aeruginosa	-	4	7	9	12

The main antimicrobial effect of allicin is due to its oxidative interaction with important thiol containing enzymes [21]. The active allicin molecule also has a very short half-life, keeping the defence mechanism rapid and much localized, preserving the rest of the alliin in the clove for future attacks [21].

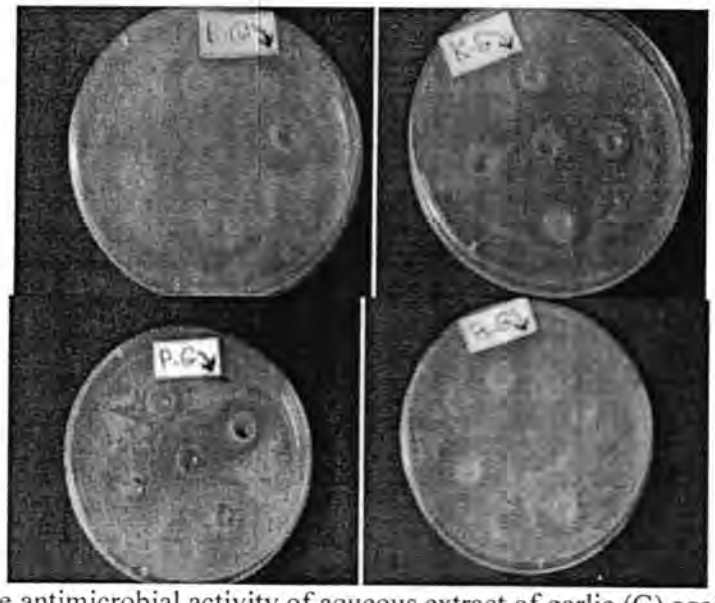

Figure-2: The antimicrobial activity of aqueous extract of garlic (G) against *Escherichia coli, Klebsiella pneumoniae , Proteus vulgaris and Pseudomonas aeruginosa*

allicin also contains the sulfr containing compounds alliin, ajoene, diallylsulfide, dithiin, S-allylcysteine, and enzymes, vitamin B, proteins, minerals, saponins, flavonoids, and maillard reaction products, which are non-sulfur containing compounds[22]. The antibacterial property of garlic has been tested in many studies and *in vitro* experiments have shown inhibition of 14 species of bacteria including *Escherichia coli, Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* [15]. A striking aspect of the activity of garlic is the apparent inability of most bacteria to develop resistance to it because its mode of action is completely different from that of other antibiotics[10].

These results encourage us to screen a mixture of equivalent ratio 1:1 of both extracts (peppermint and garlic) in the concentration (0.5mg/ml) which has exhibited a significant result by mixing equivalent ratio 1:1 in sterile beaker then placed 100µl in the wells. The inoculated plates were incubated at 37°C for 24 hours and the inhibition zones were measured to the nearest millimeter (mm). (Table-3). As we mentioned earlier peppermint and garlic has a many effective compound and the key component allicin in garlic which is denatured at high

temperature, so when we got a mix of the two extractions perhaps an interaction between the components and the formation of more efficient vehicles, which led to greater inhibition of bacterial growth.

Table-3: The mean of inhibition zone of the aqueous extract of peppermint and garlic (GM) against *Escherichia coli, Klebsiella pneumoniae , Proteus vulgaris and Pseudomonas aeruginosa*

peppermint and garlic concentration (0.5mg/ml)	Zone of inhibition (mm)			
	E. coli	*Klebsiella pneumonia*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
	27	23	20	21

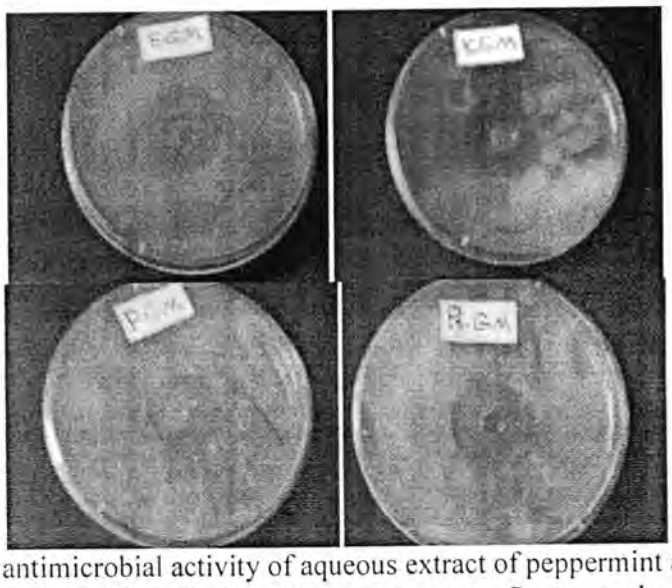

Figure-3: The antimicrobial activity of aqueous extract of peppermint and garlic (GM) against *Escherichia coli, Klebsiella pneumoniae , Proteus vulgaris and Pseudomonas aeruginosa*

Antimicrobial activity of both (peppermint and garlic) extracts was compared with that for a number of antibiotics (A*) that known for their ability of inhibition pathogenic bacteria which causes injuries inflammation and infections include ampicilin, cephalexin, erythromycin, amoxicillin, gentamicin , tetracyclin, chloromphenicol, nalidixic acid and trimethoprim by using antibiogram test.(Table-4).
A* = antibiotic disc.

Table-4: The mean of inhibition zone of antibiotics (A) against *Escherichia coli*, *Klebsiella pneumoniae* , *Proteus vulgaris and Pseudomonas aeruginosa*

Bacteria sp.	Inhibition Zone (mm)								
	Amoxicillin	Tetracycline	Erythromycin	Cephalexin	Ampicilin	Gentamicin	Chloramphenicol	Trimethoprim	Nalidixic acid
E. coli	R*	4	R	R	R	10	14	R	R
Klebsiella pneumonia	R	12	R	R	R	12	18	R	R
Proteus vulgaris	R	10	R	10	R	12	18	4	4
Pseudomonas aeruginosa	R	R	R	8	R	14	20	R	R

R* = Resistance

An antimicrobial activity of aqueous extract of peppermint was better than of tetracyclin and gentamicin against *E. coli* while the antimicrobial activity of latest concentration of garlic extract against *E. coli* was better than the effect of same antibiotic. The effect of chloramphenicol against *Pseudomonas aeruginosa*, was better than that of both extracts concentrations, while the mixture of (with 0.5mg/ml concentration) both extracts had significant antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris and Pseudomonas aeruginosa*, which affected by chloromphenicol, gentamicin and tetracyclin. The resistance of pathogenic bacteria to amoxicillin,erythromycin and ampicilin was noticed.(Figure-4)

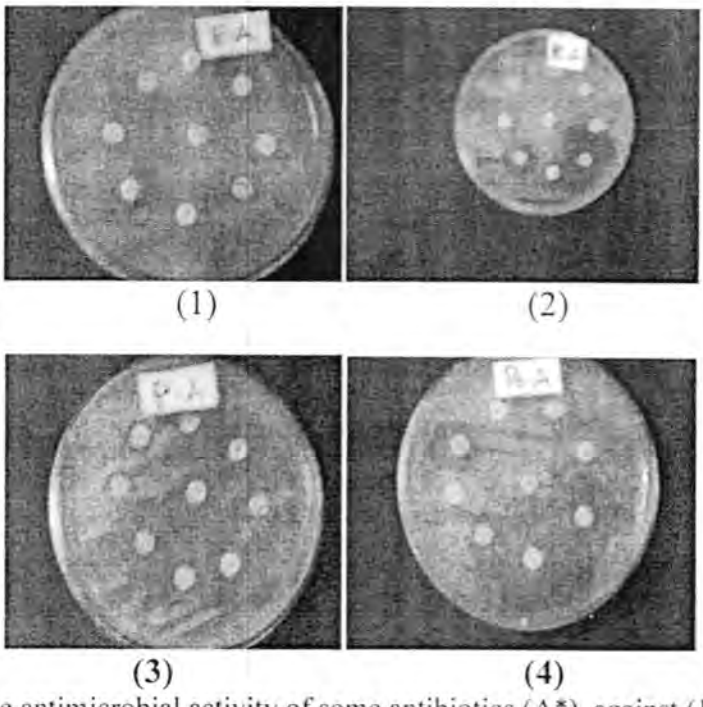

Figure- 4: The antimicrobial activity of some antibiotics (A*) against (1) *Escherichia coli*, (2)*Klebsiella pneumoniae* , (3)*Proteus vulgaris and* (4)*Pseudomonas aeruginosa*

Because garlic and peppermint are known to act synergistically with antibiotics, and resistance has not been reported for them (garlic and peppermint), more dose-response preclinical studies and eventually clinical studies should be done to assess the use of garlic /peppermint combination for bacteria that are difficult to eradicate. In view of the strong antibiotic properties and the complete absence of development of resistance

Finally, it can be concluded that the active chemical compounds present in *Mentha piperita and Allium sativum* should certainly use in treatment of various bacterial infections. The results from the present study are very encouraging and indicate this herb should be studied more extensively to explore its potential in the treatment of infectious diseases as well, besides, the same may also be used for self medication in domestic settings.

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Assessment of Correlations between Sperm Chromatin Structure Assay and Seminal fluid analysis for both Fertile and Infertile men in Iraq

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Received 7/6/2011 – Accepted 18/4/2012

الخلاصة

تم فحص السائل المنوي ل (39) من الذكور السليمين و متوسط أعمارهم (33.61±5.23) سنة في حين كان عدد الذكور العقيمين الذين تم أجراء فحص السائل المنوي لهم هو(111) وكان متوسط أعمارهم (40.121 ±6.708) سنة. كذلك اجري فحص (AO test) لكل من الذكور السليمين و العقيمين من أجل معرفة سلامة المادة الوراثية للنطف. اجري فيما بعد فحص (AB test) لكل من الرجال السليمين والعقيمين لقياس مدى تكثيف المادة الوراثية (Chromatin condensation) التي تحصل أثناء تكوين الحيامن في الخصى والمهمة في المحافظة على سلامة المادة الوراثية.

أظهرت نتائج الدراسة الحالية وجود فرق معنوي (P< 0.05) بين الضرر في (DNA) في نطف المرضى العقيمين مقارنة بالذكور السليمين باستعمال فحص (AO test)،في حين لم يسجل أي فرق معنوي بين مجموعتي الذكور السليمين والعقيمين باستعمال فحص (AB test).

كذلك أظهرت نتائج الدراسة علاقة سالبة (r = - 0.511 ; P = 0.001) بين (AO test) ونشاط النطف الصنف (D) ، وكذلك بين (AO test) وشكل النطف (r = - 0.380 ; P = 0.017) في مجموعة الرجال الخصبين .

كذلك أظهرت مجموعة السليمين علاقة موجبة (r = 0.425 ; P = 0.007) بين فحص (AO test) وحركة النطف وكذلك مع حجم السائل المنوي (r = 0.454 ; P = 0.004). بالمقابل ظهرت علاقة موجبة بين فحص (AB test) وكل من حركة النطف (r = 0.336 ; P = 0.037) وكذلك مع نشاط النطف الصنف (C) (r = 0.398 ; P = 0.012) في مجموعة الخصبين .

في حين أظهرت مجموعة الرجال العقيمين علاقة سالبة بين (AB test) وزمن التميع (السيولة) (r= - 0.242 ; P= 0.010).

من ناحية أخرى أظهرت النتائج أن نسبة الضرر في المادة الوراثية لفحص (AO test) كانت أكبر من نسبة فحص (AB test).

بينما لوحظت علاقة موجبة بين الفحصين (AO ,AB) لكل من مجموعتي الرجال العقيمين والخصبين فأنه لم يسجل وجود فروق معنوية بين الفحصين (AO , AB) لكلا المجموعتين.

ومن النتائج التي ظهرت في هذه الدراسة نجد أن فحص السائل المنوي كان غير كافيا لتحديد خصوبة الرجل من عدمها بمعزل عن فحص المادة الوراثية والتي أظهرت كفاءتها في الكشف عن شظايا DNA النطف . وكلا الفحصين يمكن اجرائهما بدون تعقيد ولا يكلفان كثيرا.

ABSTRACT

Seminal fluid analysis was performed for thirty nine fertile men with average age (33.61 ± 5.23) years, and one hundred eleven infertile patients with average age (40.121 ± 6.708) years. The AO test was performed also for fertile and infertile men, then the chromatin condensation was measured by using AB test for fertile and infertile men.

The results of the present study showed a significant (P< 0.05) difference in DNA fragmentation percentage between fertile and infertile men using AO test, but no significant difference (P>0.05) assessed when using AB test between both fertile and infertile men.

Also a negative correlation was recorded between AO test and sperm activity grade D (r = - 0.511; P= 0.001), and sperm morphology (r = - 0.380 ; P = 0.017) in the fertile group.

There was a positive correlation assessed between AO test and both sperm motility (r = 0.425 ; P = 0.007) , and seminal fluid volume (r = 0.454 ; P = 0.004) in the fertile group.

On the other hand, a positive correlation was noticed between AB test and both sperm activity grade C (r = 0.398; P = 0.0012), and sperm motility percentage (r = 0.336 ; P = 0.037) in the fertile group.

While the infertile group appears a negative correlation between AB test and liquefaction time of seminal fluid (r = -0.242 ; P = 0.010).

Furthermore, results of DNA fragmentation percentage using AO test was higher than those using AB test with no significant difference.

While there was a positive correlation between AO test and AB test in both fertile and infertility groups, but no significant difference was assessed between the percentage of AO test and AB test in the same groups.

From the results of this study, it was concluded that the seminal fluid analysis was not enough to obtain the fertility potential, hence the sperm DNA integrity test was essential to detect DNA fragmentation in fertility clinics and laboratories. Both AO and AB test were easy and not expensive to perform for integrity test.

INTRODUCTION

It is common to recommend an infertility evaluation in couple with a history of unprotected intercourse for at least 12 months with attempts to time intercourse with ovulation. Problems of male infertility can be seen like minor issues within the larger realm of urology. But much male infertility diagnosis can be successfully treated [1].

Semen analysis is the cornerstone of the evaluation of infertile men, semen volume and pH levels are an indication of seminal vesicle and prostate function. Sperm concentration, motility and morphology are determined by testicular function and to a lesser extent, by postesticular (e.g. epididymal) genital tract function. Some patients presenting with male infertility can have more significant disease [2].

The integrity of sperm DNA may be tested to predict pregnancy outcomes in couples who do not know their fertility potential (first pregnancy). Couples in whom the man has a high percentage of spermatozoa with DNA damage have very low potential for natural fertility, and will have to wait a long time before conceiving, whose pregnancy resulted in miscarriage demonstrate a tread toward poorer sperm DNA integrity compared with highly fertile couples [3].

In natural conception, a DNA-damage sperm would likely be unsuccessful in fertilizing ova. Human spermatozoa that bind to oviduct cells have better DNA integrity than spermatozoa that do not bind to these cells, which suggests that nature can select spermatozoa with enhanced DNA integrity during natural fertilization [4].

Different agents that act on germ cells at various stages of development usually showed sperm DNA fragmentation when that germ cell fraction arrived in the epididymis or ejaculate. Some of these treated samples capable of success *in vitro* fertilization but with frequent embryo failure [5].

Sperm DNA damage can be measured by AO test and AB test, both tests were dependent on acidic stains (low pH level). Acridine orange is DNA intercalating dyes which are practical alternatives to AO test [6]. These indirect methods are based on principle that damaged DNA denatures much faster than undamaged DNA when subjected to stresses such as heat and pH changes [7].

Acridine orange binds to denatured DNA (single stranded) and emits red fluorescence, hence AO binds to double stranded DNA and emits green fluorescence, DNA that is associated with disulphide rich protamines is resistant to denaturation procedures [8].

Also, AB test was performed to detect the integrity of chromatin condensation (15% histone protein) by staining lysine rich histone and sperm seem colorless. While, the sperm head staining with blue color when the percentage of histone protein was more than 15% and the integrity of chromatin condensation was diminished [9].

The aims of this study was to evaluate the sperm DNA integrity for fertile men and infertile patients by using acridine orange (AO) test and aniline blue (AB) test, then to assess correlation between semen parameters with both AO test and AB test. Also to study the correlation between results of AO test and of AB test.

MATERIALS AND METHODS

Thirty nine fertile and one hundred and eleven infertile men were involved in the present study. The samples of semen from fertile and infertile men were collected at Institute of Embryo Research and Infertility Treatment/AL-Nahrain University at AL-kadhumia city. The study was conducted through the period from 1st of November 2009 to the 25thof April 2010. In addition, the mean age of fertile men was (33.61 ± 5.23) years, while the mean age of infertile men was (40.125 ± 6.708), and the mean duration of infertility was (7.55 ± 2.181) years.

The clinical assessment was evaluated by a specialist andrologist or urologist for presence or absence of varicocele, hydrocele, hernia, mumps and other congenital abnormalities. Additionally, infertile patients with azoospermia were excluded from this study.

Seminal fluid analysis (SFA):

All samples of seminal fluid were collected after (3-5) days of abstinence directly in a clean, dry and sterile disposable Petri- dish by

masturbation. The container obtained the sample of seminal fluid must be labeled with the information about name, age, abstinence period and time of sample collection.

The specimens were placed in an incubator at 37°C for 30 minutes to allow liquefaction. The liquefied semen was then carefully mixed for few seconds, and the specimen was examined in details by macroscopic and microscopic examination. However, the viscous samples are difficult to pipette, so repeated aspiration through pipette can help break down the threads of semen, and the results of examination were recorded, such as liquefaction time, pH, appearance, viscosity, sperm concentration, sperm motility, sperm morphology and any agglutination between sperms or any round cells (RBC, WBC, germ cells). The standard form of [10] was used to assess the results of seminal fluid analysis as standard values.

Preparation of Tyrode's Solution (for sperm wash):

Prepared by adding small amount of warm distilled water to ($MgCl_2$), followed by adding all of the components (NaCl 120.7 mM, KCl 5.9 mM, $CaCl_2$. $2H_2O$ 2.5mM, $MgCl_2$ 1.2mM, $NaH_2PO_4.2H_2O$ 0.2mM, $NaHCo_3$ 15.5mM) together. Then after preparing the mix, the volume was complete to (1L) and (pH) adjusted to 7.3 [11].

Preparation of AO fixative (Carnoy's solution).

Carnoy's fixative provides a better predictive value for DNA damage to semen using acridine orange (AO.) stain. Carnoy's solution consists of (3) parts methanol / (1) part glacial acitic acid [11].

Preparation of acridin orange (AO) stain.

Acridine orange was used to prepare staining solution from a stock solution consisting of one gm acridine orange (AO.) in (1000 ml) of distilled water and stored in the dark at 4 °C. Then (10 mL) of stock solution was added to (40 mL) of 0.1M Citric acid and (2.5. mL) of 0.3M $Na_2Hpo_4.7H_2o$ and the pH was adjusted to (2.5) before staining. All solutions were maintained at room temperature [11].

Staining technique by AO stain.

After washing all fresh semen samples twice by add equal amount of tyrode's solution to the semen sample into the eppendrof tube and run in a centrifuge at 3000 rpm for 10 minute, then the supernatant discarded and resuspension the pellet with Tyrode's solution and one drop separate on slide and leaving to dry, then put the slides in the fixative solution overnight in (3) parts of methanol to (1) part of glacial acetic acid (3:1) at room temperature. The slides were removed from the fixative and allowed to dry for few minutes before

staining with (AO. pH=2.5) for (5) minutes at room temperature. After staining the slides were gently rinsed in a stream of distilled water and sealed under a cover slip with nail polish.

All slides were examinated under fluorescence microscope to score the samples within one hour after staining, sperm cell heads with good DNA integrity had green fluorescence, and those with diminished DNA integrity had orange-red staining [11].

Preparation of AB. fixative

The fixative solution was prepared by added (3 mL) glutaraldehyde to (97mL) PBS, to obtain (3%) concentration of glutaraldehyde in PBS [9].

Preparation of Aniline Blue (AB) Stain.

Staining solution was prepared with (5g) AB. per (100 ml) phosphate Buffer Saline (PBS), then boiled for a short while, filtered, and adjusted to a pH of (3.5) with glacial acetic acid [9].

Staining Technique by (AB.) Stain.

After washing the semen, air dried smears were fixed in (3%) glutaraldehyde in PBS for thirty minutes, dipped twice in PBS for five minutes, stained with (AB. pH=3.5) for seven minutes, washed with PBS, and air-dried, and all slides were exanimate under light microscope to score the samples. Sperm cell heads with good chromatin integrity were nearly colorless, and those with diminished integrity were blue [9].

RESULTS AND DISCUSSION

Macroscopic and microscopic parameters of seminal fluid analysis (SFA) were recorded and shown in (table 1). Most parameters of SFA for both groups assessed according to the criteria of WHO (1999).

Fertile men group recorded normal values for most of the seminal fluid analysis (SFA) including semen liquefaction time, semen volume, semen pH, sperm concentration, sperm motility, normal sperm morphology and progressive sperm motility, except grade (A) of sperm activity which was decreased from the normal criteria of [10] as shown in (table 1).

In the group of fertile men the AO test has a negative significant correlation with both of grade (D) of sperm activity ($r=-0.511$; $P=0.001$) and normal sperm morphology ($r=-0.38$; $P=0.017$). In contrast, a positive significant correlation had been recorded between AO test and both of sperm motility ($r=0.425$; $P=0.007$) and semen volume ($r=0.454$; $P=0.004$).

Also, the percentage of DNA fragmentation using AB test has a positive significant correlation with grade (C) of sperm activity ($r=0.398$; $P=0.012$), and sperm motility percentage ($r=0.336$; $P=0.037$). There was Also a positive and significant correlation ($r=0.574$;

P=0.01) assessed between AO test and AB test in fertile group as shown in Table (2).

Finally there was no significant difference (P>0.05) observed between AO test and AB test in fertile group (Figure 1).

The infertile men group showed an increase in the semen liquefaction time up to the normal criteria of [10], and the semen pH in the maximum value was observed, but the semen volume and sperm concentration reported normal values as well as in sperm agglutination. In contrast, the remainder parameters like sperm motility, progressive sperm motility and normal sperm morphology are reduced. Furthermore, the grade (A) of sperm activity was highly deviated from the normal criteria of [10], as presented in (Table 1),and affect the fertility of men.

In general, non significant correlation was noticed between percentages of DNA damage using AO test with most parameters of seminal fluid analysis parameters of infertile patients, but a negative significant correlation ($r = - 0.242$; $P = 0.01$) was assessed between AB test and liquefaction time. Furthermore, a highly significant positive correlation ($r = 0.589$; $P = 0.0001$) was observed between AO test and AB test for infertile group but no significant difference (P>0.05) was observed between AO test and AB test in the infertile group as shown in Table (2).

In the present study, infertile men have the highest percentage of sperm DNA fragmentation using AO test when compared with fertile men assessed in this study. Also, infertile men showed the large percentage of DNA fragmentation using AB test as presented in (Figure 1). However, percentage of DNA fragmentation using AO test for infertile patients was higher than that in healthy fertile men with a significant difference ($P < 0.05$). But there was no significant difference in the percentage of DNA fragmentation using AB test between fertile and infertile men (Figure 1).

Table-1: Parameters of seminal fluid analysis for fertile and infertile men reported in the present study.

Parameters of semen fluid analysis		Fertile men (No.=39)	Infertile men (No.=111)	WHO criteria (1999)
Semen liquefaction time (min)		27.307± 0.85	41.87± 1.887	within ≤30 minutes
Semen volume (mL)		2.207± 0.160	2.087 ± 9.16	(2-6) mL
Semen (pH)		8.00 ± 3.967	8.057 ± 2.027	(7.2-8)
Sperm Concentration ($X10^6$ sperm/mL)		69.48 ± 5.27	36.61 ± 2.84	≥ $20X10^6$ Sperm/mL
Sperm motility (%)		73.23 ± 1.60	49.64 ± 1.957	≥ 50%
Sperm grade activity (%)	A	11.33 ± 0.998	1.513 ± 0.26	Grade A≥25% or A+B≥50% within 60 minutes
	B	47.179 ± 1.208	28.864± 1.44	
	C	16.128 ± 0.75	22.10 ± 1.174	
	D	24.205 ± 1.47	48.05 ± 1.967	
Progressive sperm motility (%)		58.51± 1.21	30.528 ± 1.56	≥ 50%
Normal sperm normality (%)		47.48 ± 1.360	28.387 ± 1.21	> 30%
Sperm agglutination (%)		1.569 ± 0.578	3.905 ± 0.880	< 10%

*Data are Mean ± S.E.

Table-2: show the correlations between AO test and AB test with semen parameters of both fertile and infertile men.

Parameters of semen fluid analysis		Fertile men (No.=39)	Infertile men (No.=111)
Semen liquefaction time (min)		27.307± 0.85	41.87± 1.887 B
Semen volume (mL)		2.207± 0.160 O	2.087 ± 9.16
Semen (pH)		8.00 ± 3.967	8.057 ± 2.027
Sperm Concentration ($X10^6$ sperm/mL)		69.48 ± 5.27	36.61 ± 2.84
Sperm motility (%)		73.23 ± 1.60 B,O	49.64 ± 1.957
Sperm grade activity (%)	A	11.33 ± 0.998	1.513 ± 0.26
	B	47.179 ± 1.208	28.864± 1.44
	C	16.128 ± 0.75 B	22.10 ± 1.174
	D	24.205 ± 1.47 O	48.05 ± 1.967
Progressive sperm motility (%)		58.51± 1.21	30.528 ± 1.56
Normal sperm normality (%)		47.48 ± 1.360 O	28.387 ± 1.21
Sperm agglutination (%)		1.569 ± 0.578	3.905 ± 0.880
Acridine orange test (AO)		48.74± 4.07 C,O	64.54±2.63 C,O
Aniline blue test		45.43±4.58 C,B	58.9±2.53 C,B

❖ In field fertile men: letter O means correlation between AO test and semen parameters, and letter B means correlation between AB test and semen parameters, while the letter C means correlation between AO test and AB test.

❖ In field infertile men: letter B means correlation between AB test semen parameters, and letter C means correlation between AO test and AB test.

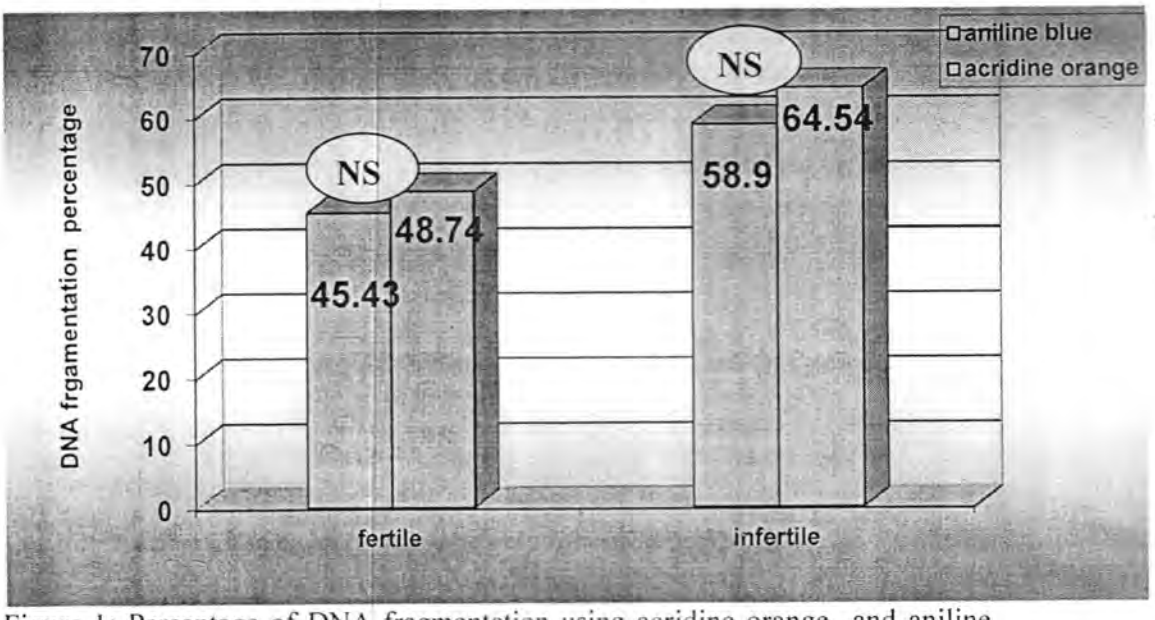

Figure-1: Percentage of DNA fragmentation using acridine orange and aniline blue for fertile and infertile men.

*Number of fertile men (no. = 39).

*Number of infertile men (no. =111).

NS= Non significant difference (P>0.05) between AO test and AB test.

All infertility groups in the present study registered increase in the percentage of DNA fragmentation measured by using both AO test and AB test as compared to the healthy fertile (control) group this result may be due to the Sperm fixation in Carnoy's solution showed

significantly more damage than (2%) gluteraldehyde,(4%) paraformaldehyde, or no fixation [12].

In general, non significant correlation was noticed between percentages of DNA damage using AO test with most parameters of seminal fluid analysis parameters of infertile patients specially sperm concentration, this agree with [13].

In general, chromatin condensation is vital for the function of the spermatozoon as the motile carrier of paternal genome. The degree of condensation can be shown with the aid of acidic acridine orange (AO) called intercalation stains to the DNA strands, or aniline blue (AB) stain in which is able to discriminate between lysine- rich histone and arginine and cystine rich protamines [14]. They also demonstrated that the defect in condensation through spermatogenesis would impair not only malformed but also normal spermatozoa.

Furthermore, the DNA packaged in the mammalian sperm cell has important implications for DNA integrity and human infertility as well as for the cell biology. Furthermore, the nuclear matrix organization is essential for DNA replication, and the histone bound chromatin identifies genes that are important for embryonic development [15].

In the present study it was noticed that the pH level was increased this may indicated un infection in the genital tract, and sometimes up to the normal criteria of [10], and there was a significant correlation with AO test. Susceptibility of mammalian sperm DNA to low pH or heat-induced denaturation in DNA and reduced the fertility potential [16]. In addition, the sperm activity increase in the case of mediate or high pH, while the activity decrease in the case of low pH.

Semen liquefaction time also witnessed an increase in the time up to the normal value. This increased viscosity of ejaculate was reported to occur more frequently among infertile couples than in fertile males [17]. Several conditions, such as concentrations of prostate-specific antigen, zinc and calcium, and activity of neutral-glucosidase in seminal plasma, were found to be correlated with changed semen viscosity [18].

On the other hand, it is important to understand that the seminal fluid contains non- enzyme antioxidants such as vitamin C and E, pyruvate, glutathione and carnitine [19]. Therefore, some authors reported that ageing, diet, smoking and life style tend to reduce immune defenses and antioxidant activity, lowering semen quality, volume and impairing fertilizing capacity [20].

Generally speaking, there are many causes for DNA damage or spermatogenesis defecte, such causes are the genital tract inflammation, post-testicular genital tract infection and inflammation

(combine inflammation of epididymis and testis or of the prostate gland), resulting leukocytospermia and have been associated with increased levels of reactive oxygen species (ROS) and subsequently DNA damage [21].

On another hand, varicocele has been associated with sperm DNA damage [22], and they noticed that the level of sperm DNA damage is related to the high levels of oxidative stress found in semen of infertile men.

Furthermore, lipid peroxidation by ROS causes structural damage of the acrosome, head and neck, as well as triggering apoptosis and inducing DNA breakage [23].

In the present study noticed the correlation between grade (C) and AB test is associated with abnormal condensation of mitochondrial DNA with histone and protamine proteins, while the correlation between grade (D) and AO test in the fertile group associated with DNA fragmentation in mitochondria, and these were the same reasons result in reducing the values of grades (A) and (B) and progressive motility in group infertile men, The sperm mitochondria-associated cysteine-rich protein (SMCP) is a cysteine- and proline-rich structural protein that is closely associated with the keratinous capsules of sperm mitochondria in the mitochondrial sheath surrounding the outer dense fibers and axoneme [24]. In vivo experiments with homozygous mutant (*Smcp*$^{-/-}$) on the genetic background 129/Sv in sperm mice revealed that the migration of spermatozoa from the uterus into the oviduct is reduced [25].

In vitro fertilization assays showed that *Smcp*-deficient spermatozoa are able to bind to the oocyte but that the number of fertilized eggs is reduced, and that the infertility of the male *Smcp*$^{-/-}$ mice on the 129/Sv background is due to reduced motility of the spermatozoa and decreased capability of the spermatozoa to penetrate oocytes [26].

While [27] revealed that ROS induces DNA breakage and reduces nicotine amide adenine dinucleotide phosphate (NADPH) or activates white cells.

Young men with cancer like testicular cancer typically have poor semen quality and sperm DNA damage, even before cancer therapy. They then experience cumulative dose- related damage during therapy, which often renders them completely sterile [28].The rapidly dividing germinal epithelium of the testis is a natural target for cytotoxic medications; radiation therapy and chemotherapy inflict similar damage and are dependent on both duration and dose of exposure.The recovery of spermatogenesis may occur for months to years after

therapy, but evidence of sperm DNA damage may often persist beyond that period [29].

Sperm DNA damage may be due to apoptosis during normal spermatogenesis results in destruction of up to75% of potential spermatozoa, the selective apoptosis of these early germ cells prevents over proliferation of cells and selectively aborts abnormal sperms forms [30].

In addition, a febrile illness has been also shown to cause an increase in the histone: protamine ratio and DNA damage in ejaculated spermatozoa [31]. Direct testicular hyperthermia has also the same effect on sperm DNA.

Furthermore, certain behaviors have been associated with increased scrotal temperatures (e.g., use of hot bath, down-filled blankets, laptop computers and prolonged periods of driving), [32].

Varicocele has long been implicated as a major cause of maleinfertility, but the pathophysiology remains unclear. Clinical varicocele is found in about 15% of the general population including adolescents and adults: in 35% of men with primary infertility and in up to 80% of men with secondary infertility [33].

Moreover increased apoptosis has been associated with varicocele. Recent studies have demonstrated that varicocele is associated with abnormal retention of sperm cytoplasmic droplets (a morphological feature associated with high levels of reactive oxygen species) and that these retained droplets are correlated with sperm DNA damage in the infertile men [34].

Sperm DNA damage has been associated with high levels of reactive oxygen species, high levels of which have been detected in the semen of 25% of infertile men, although low levels of (ROS) are necessary for normal sperm function, high levels are generated by defective spermatozoa and semen leukocytes, which results in sperm dysfunction, and ROS suggested to be a cause of DNA damage through defect in spermatogenesis [35]. Exposure to organophosphates and air pollution has also been associated with increased levels of sperm DNA damage [36]. In addition, cigarette smoking is associated with a decrease in sperm counts and motility and increase the abnormality sperm forms and sperm DNA damage [37].

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Study of Some Chemical & Physical and Bacteriological Properties of Yuosifiya River Water

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Received 24/11/2011 – Accepted 18/4/2012

الخلاصة

أجريت هذه الدراسة على مياه نهر اليوسفية لمسافة تمتد الى 68 كم ، بواقع ثلاث محطات تمتد على طول النهر ، ولمدة سنة كاملة بداية من فصل الخريف 2010 الى فصل الصيف 2011 وبمعدل فصلي للمحطة الواحدة . شملت الدراسة قياس بعض العوامل الفيزيائية والكيميائية والبكتريولوجية.

تراوحت قيم الكدرة بين (1.5- 20.3) NTU ، اما قيم المواد الصلبة الكلية فقد تراوحت بين (520- 1395) ملغم/ لتر أعلى القيم سجلت خلال فصل الربيع وادنى القيم سجلت خلال فصل الخريف 2010 . وقد تراوحت قيم الرصاص (0.02-0.035) ملغم/ لتر أذ سجلت أعلى القيم خلال فصل الشتاء وأدناها خلال فصلي الخريف والصيف أذ سجلت نتائج غير محسوسة على عكس مالوحظ للنحاس أذ سجلت اعلى القيم خلال فصل الربيع وكانت (0.045) ملغم / لتر وادناها خلال فصلي الخريف والصيف . اما قيم الحديد فقد تراوحت بين (0.04 -0.44)ملغم/ لتر، وتراوحت قيم العدد الكلي للبكتريا الهوائية بين ($1.3 * 10^2 - 6.5 * 10^6$) خلية/ مل إما بكتريا القولون فقد تراوحت قيمها بين ($1.7 * 10^2 - 8 * 10^5$) خلية/ مل واخيرا تراوحت قيم بكتريا القولون البرازية بين ($1.3 * 10^2 - 2.15 * 10^5$) خلية/ مل.

ABSTRACT

This study was performed on Al-Yousifiya River waters for a distance extending to 68 Km at the rate of three stations extending along the river, and for a period of one complete year starting from the autumn season of 2010 to the Summer season of 2011 and at seasonal rate for the one station. The study included measuring some of the Physical, Chemical and Bacteriological Factors.

The values of turbidity ranged between (1.5 – 20.3) NTU, as for the total solid substance they ranged between (520 – 1395) mg/L, the highest values were recorded during the Spring season and the lowest values were recorded during the autumn season of 2010. The values of lead ranged between (0.02 – 0.035) mg/L where the highest values were recorded during the winter season and the lowest during the autumn and summer season which intangible results , contrary to what was noticed for copper where it recorded the highest values during the spring season which was (0.045) mg/L and the lowest during autumn and summer season which was intangible results , as for the value of iron which ranged between (0.04-0.44) mg/L, the values of the total aerobic bacterial count ranged between ($1.3\times 10^2 - 6.5\times 10^6$) cell / ml , as for the coliform bacteria , their values ranged ($1.7\times 10^2 - 8\times 10^5$) cell/ml, and lastly the value of fecal coliform bacteria ranged between ($1.3\times 10^2 - 2.15\times 10^5$) cell / ml.

INTRODUCTION

The water forms the main sinew for the existent of creatures on earth where it covers 71% 0f the earth and forms 65% - 70% of the human body, thus authorities and states paid attention to the awareness in the importance of water pollution through conferences seminars and issuing legislations. The world health organization (WHO) paid attention to the conservation of water wealth in all continents through its conferences and legislating (1,2,3) and the American Public Health Assertion (4) who participated in making (2nd March) an international

day for waters to make to people aware of their importance and preserving them pollution.

The bacterial pollution is considered of the important pollutants which cause damage to various water surfaces and which include various kinds of bacteria with various health effects, the nature intestinal creatures are considered indicators of fecal pollution instead of the sickening creatures themselves (5,6,7).

The aims of this research :-

study the physical and chemical properties of Al- Yousifiya River water in the district of study.

estimating the volume of microbic pollution of the river water through estimating the microbic indications represented by the total number of aerobic bacteria and the total coliform and the total fecal coliform.

MATERIALS AND METHODS

Description of Study District

The study included Al- Yousifiya river which length is approximately 68 Km extending from western Al-Radhuoaneya (south west of Baghdad) to fallows it to the south east district of Baghdad approaching Tigris river (picture No.1). Al-Yousifiya river is considered one of the projects of the Euphrates river which was dugged in 1918 to feed extensive areas of fertile agricultural lands and situated on it the townships of Al-Yousifiya and Al-Rashid. The district is famous in the horticulture (palm trees, fruits and citrus) as well as being famous in all kinds of vegetables and strategic crops (wheat, barley and maize) and their inhabitants depend on animal breeding and the poultry farms are spread in it and in the last years fish basins have spread in it noticibly and extensively.

The study was performed from the beginning of the river until its end where 3 sites were taken as follows:

Site No. (1) is situated before Al-Yousifya township in 3 Km.

Site No. (2) is situated after Al-Yousifya township in 2 Km.

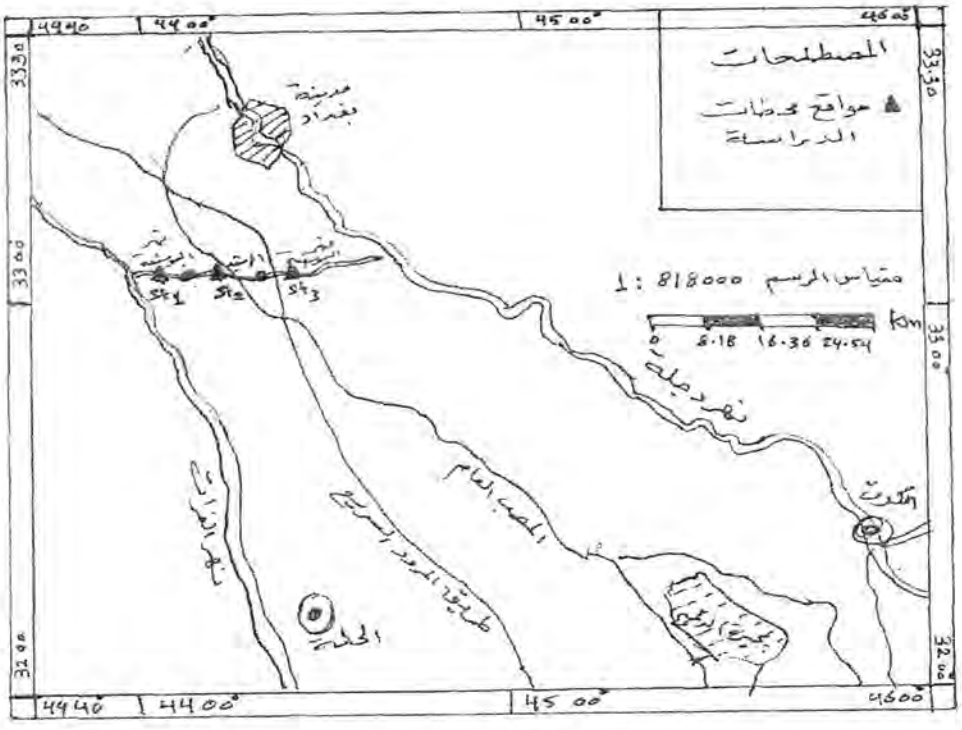
Site No. (3) is situated after Al-Rashid township.

Gathering Samples

The samples pertaining the physical and chemical factors were taken in plastic bottles of volume (2-3.5 liters). The bottles were filled with samples of water, closed tightly and were taken to the laboratory by refrigerated containers of styropore to preserve the properties of the samples. The temperature of the water was measured in the field directly. As for the microbiological tests, they were performed immediately upon arrival to the laboratory where they were gathered in glass bottles already sterilized in oven at temperature of 118C^{0} for 30

minutes and cooled after that the samples were gathered in these sterilized bottles.

Where the samples were gathered monthly and seasonally from September 2010 up to August 2011 and the three stations were tasted located within Al-Yousifya river depending on the availability of the sample and existence of the population gatherings according to the native of the studied district and the aims of the research.The turbidity of the water was measured by using turbidity meter. Then the total dissolved substances (TDS) were measured by using millipore fitter paper mentioned in (8) as for the iron element the phenathroline method was used according to American Public Health Authority (9), and for the tests of heavy elements, the method described in (10) was used. And for the bacteriological tests the samples were gathered and the total bacterial count performed by plate count method as well as testing the most probable number (MPN) (11) and (12). As well as investigating the coliform bacteria and fecal coliform bacteria (1). Then the results were analyzed statistically by using the analysis of variance (ANOVA), Duncan Test and the correlation coefficient.



Picture (1)

RESULTS AND DISCUSSION

The Physical Factors

The Turbidity

The water becomes turbid due to the presence of organic and inorganic materials and the turbidity is considered to the degree of water purity because the existence of solid particles lead to obscuring part of the light from its water path. These particles may be motile of small size reaching the water from the sand as for the large particles they are plant and animal remains and agglomerations of bits of organic materials. Thge waters differ in the intensity of their turbidity the rivers become turbid and wherever the turbidity increases the number of germs in the water increase (13).The results of this study showed that the highest values were recorded in the first station reached to (20.3) NTU during the season of spring and the other stations coincided with the first station in the time of recording the highest values. The reason of rising turbidity in spring is due to the rising of water levels which lead to an increase in the speed of water currents which cause agitating and mixing the deposited materials and making them rise thus increasing the suspended materials (14)and this is what was noticed in moral linkage of the turbidity and the total solid materials (0.70 at the level of $p \leq 0.01$) and the total number of bacteria (0.41 at the level of $p \leq 0.01$) and the coliform (0.56 at the level of $p \leq 0.01$) and the fecal coliform (0.40 at the level of $p \leq 0.01$), the results of statistical analysis showed the existence of moral differences between the four seasons and between the stations and as Showed in figure (1)and table (1) (at the level of $p \leq 0.05$).

The Total Solid Substances TSS, TSD

They are the solid suspended substances insoluble in water and include mud, silt and sand. They remain on the filter paper after passing sample of river water through it, as for the solid substances soluble in water as a result of dissolving the compounds producing ions of negative and positive elements (10).

The results of statistical analysis showed of moral differences between the four seasons and the stations figure (2) table (2). The results of the present study showed increase in the concentrations of total solid materials in the seasons of winter and spring where the highest value of the total solid substances was recorded during the spring season and amounted 1395 mg/ L and all the stations coincided with it at the time of recording the highest values. It was retied that there is direct relationship between the total solid substances and turbidity (0.88 at the level of $p \leq 0.01$). The increase in the concentrations of the total solid substances of the river in the rainy seasons is due to the increase in water levels and their motion in the seasons of winter and spring which lead to not precipitating the suspended solid substances in it as well as due to the fall of rains to drifting soils, muds and other substances which cause increase in the

concentrations of solid substances (15). The results of the present study coincided with what was mentioned by (16).

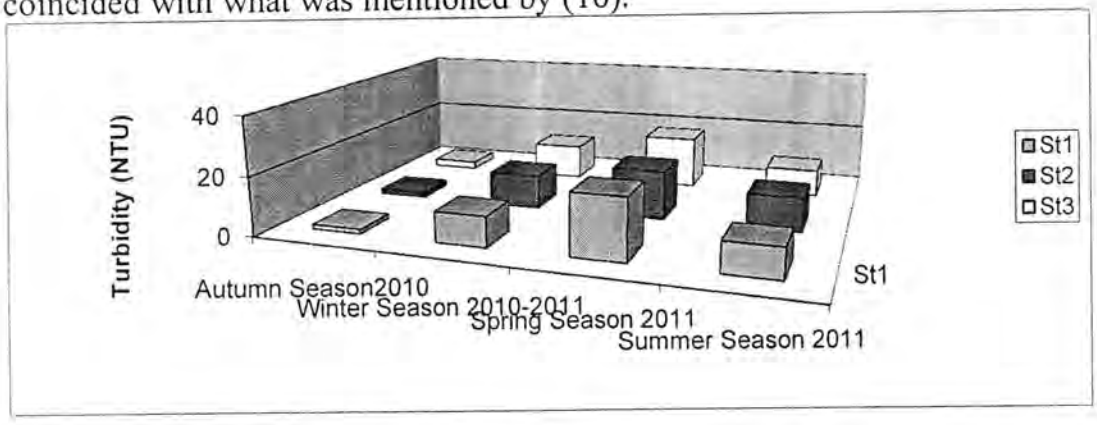


Figure -1: The turbidity value according to seasons and satations

Table-1: Turbidity value (Unit naphthalene turbidity) in three stations through study time.

ST3	ST2	ST1	Station / Date
±1.55 2.5 aD	±1.06 1.85 bC	±0.49 1.55 cC*	**Autumn Season2010**
±1.55 11.9 aB	±1.90 11.5 aB	±0.84 10.2 bB	**winter Season 2010-2011**
±10.4 17.7 bA	±7.91 16.4 bA	±10.11 20.3 aA	**Spring Season 2011**
±5.44 9.05 bC	±6.92 11.4 aB	±7.49 9.9 bA	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

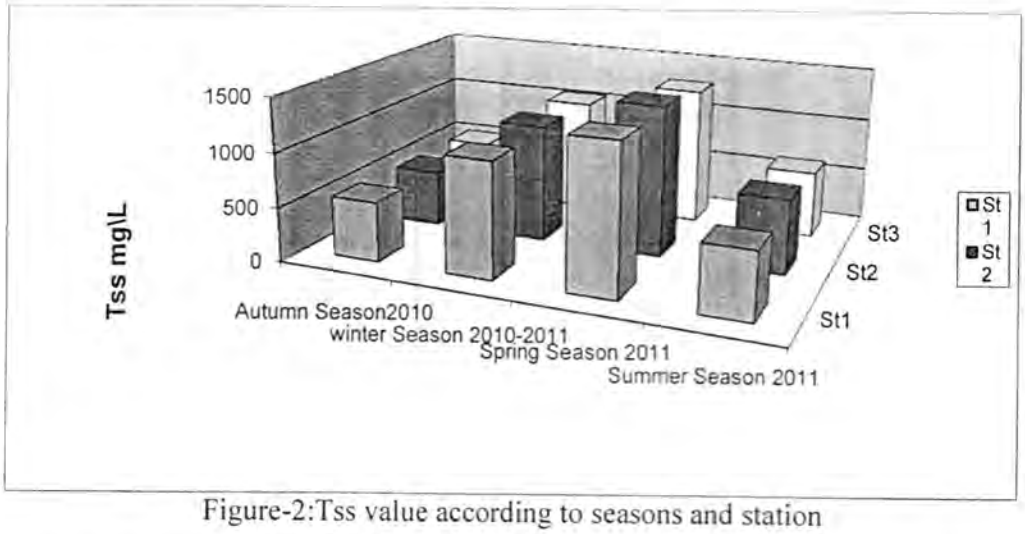


Figure-2:Tss value according to seasons and station

Table-2: Total solid substances value (mg\L) in the three stations through study time.

ST3	ST2	ST1	Station / Date
±2.61 520.1 aD	±2.33 520.3 aD	±3.32 550.6 aD*	**Autumn Season2010**
±371.9 1057 aB	±365.5 1088.5 aB	±414.3 1067 aB	**Winter Season 2010-2011**
±14.14 1290 aA	±7.07 1395 aA	±14.14 1370 aA	**Spring Season 2011**
±238.6 619.2 bC	±266.2 691.7 aC	±243.2 616 bC	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

The Chemical Factors

Lead Pb and Copper Cu

Lead and copper are heavy elements, they are called insignificant because they exist in small quantities in the earth surface not exceeding 0.1% and they are called heavy according to the classification of elements according to density. The heavy elements are those which density exceed (5 gm/ cm^3)(17). The results of the present study showed intangible results of lead element during the seasons of autumn 2010, and summer season 2011 except the first station where it recorded the highest value during winter season2010-2011 and reached 0.035 mg/L. As for the second and third stations they showed tangible results during

the season of spring 2011, as shown in figure (3) and table (3). The statistical analysis results showed the existence of moral differences between the stations and between the seasons. As for copper element, it showed intangible results during the seasons of autumn and summer, while recorded the highest value during the spring season in the first station which was 0.045 mg/L.

The statistical analysis results showed the existence of moral differences between the seasons and the stations, as shown in figure (4) and table (4). These results may be attributed to the high ability of each of lead and copper to form complexes of high stability with the organic substances existing in water cause decrease in their concentration and this was confirmed by (18).

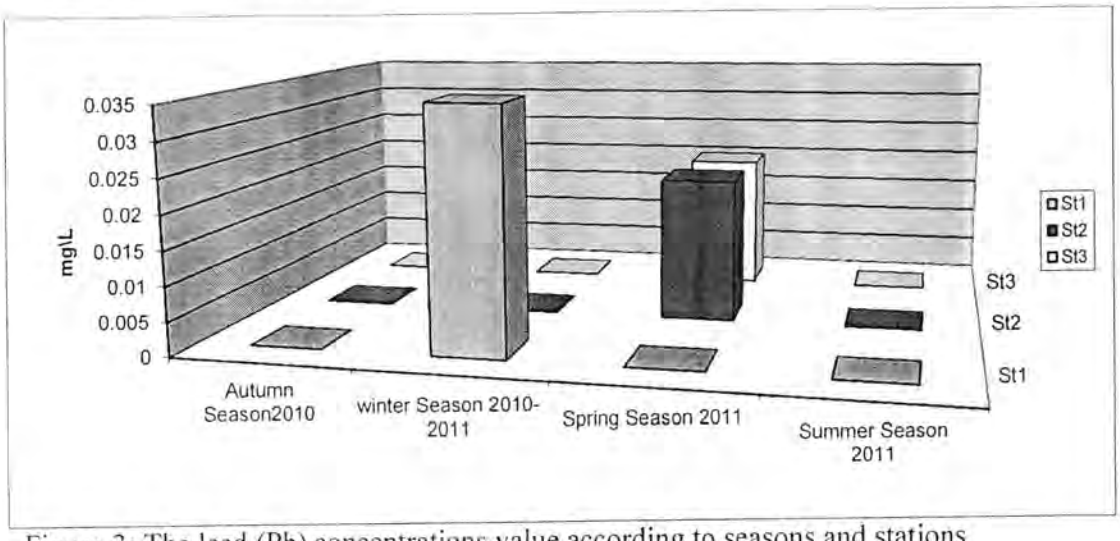


Figure-3: The lead (Pb) concentrations value according to seasons and stations

Table-3: Total Pb value (mg\L) in the three stations through study time

ST3	ST2	ST1	Station / Date
$\bar{a}B$	$\bar{a}B$	$\bar{a}B$*	**Autumn Season2010**
$\bar{b}B$	$\bar{b}B$	±0.049 0.035 aA	**Winter Season 2010-2011**
±0.01 0.02 aA	±0.02 0.021 aA	$\bar{b}B$	**Spring Season 2011**
$\bar{a}B$	$\bar{a}B$	$\bar{a}B$	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05)

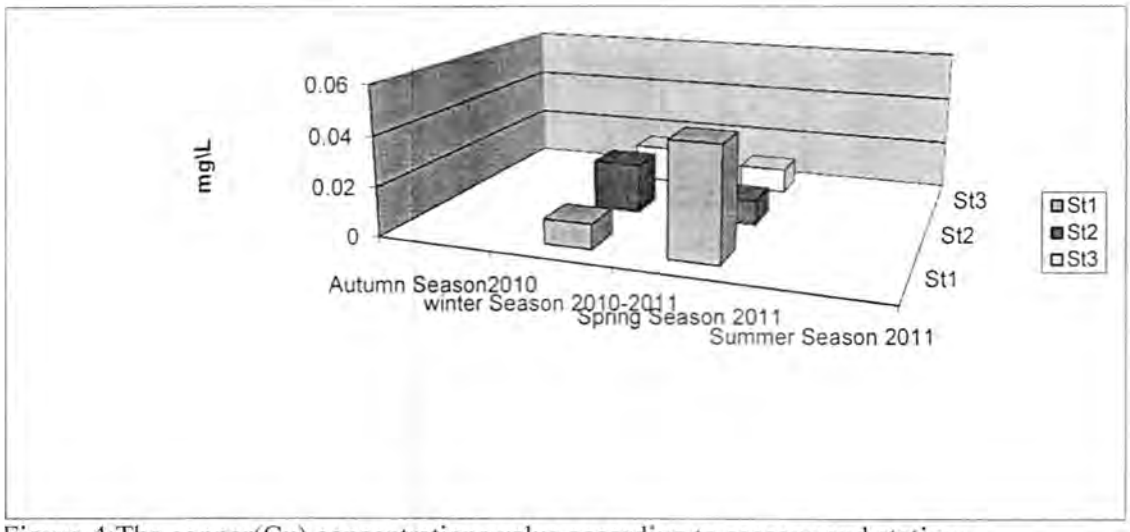


Figure-4:The copper(Cu) concentrations value according to seasons and stations

Table 4: Total Cu value (mg\L) in the three stations through study time

ST3	ST2	ST1	Station / Date
$\bar{a}B$	$\bar{a}B$	$\bar{a}C$*	**Autumn Season2010**
±0.02 0.015 bA	±0.02 0.02 aB	±0.014 0.01 cB	**Winter Season 2010-2011**
±0 0.01 bA	±0 0.01 bA	±0.063 0.045 aA	**Spring Season 2011**
$\bar{a}B$	$\bar{a}B$	$\bar{a}C$	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

Iron Fe

The results of the statistical analysis showed the existence of moral differences between the four seasons and stations studied as shown in figure (5) and table (5). The highest value of iron concentration recorded during the winter season which was 0.44 mg/L in the second and third stations while it recorded the least value of iron concentration during the summer season in the first and second stations which recorded 0.04 mg/L. The results showed the existence of direct relationship (0.45 at the level of $p \leq 0.05$) between the concentration of iron and the turbidity. This may be attributed to the fall of rains in winter which washing the soil and drifting mud into the river due to the reduction of iron into dissolved ferrous ion causes increasing in the

concentration of iron (19,20), however our study do not agree with what found by (21) in her study in nonexistence of moral differences between the iron concentrations in the seasons of winter and summer.

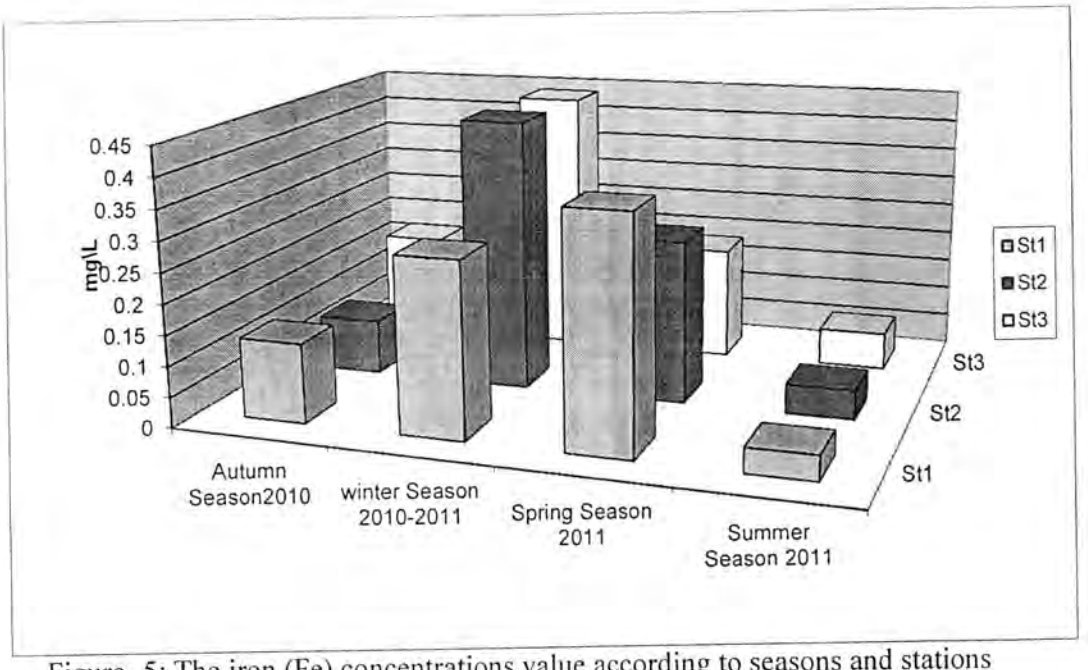


Figure- 5: The iron (Fe) concentrations value according to seasons and stations

Table-5: Total Fe value (mg\L) in the three stations through study time

ST3	ST2	ST1	Station / Date
±0.1 0.17 aB	±0.08 0.09 cC	±0.09 0.13 bC*	**Autumn Season2010**
±0.1 0.44 aA	±0.1 0.445 aA	±0.06 0.28 bB	**Winter Season 2010-2011**
±0.04 0.185 cB	±0.09 0.265 bB	±0.04 0.375 aA	**Spring Season 2011**
±0.01 0.06 aC	±0.03 0.047 bD	±0.02 0.04 bD	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

The Bacteriological Factors

The Total Aerobic Bacterial Count

The highest total aerobic bacterial count during the four seasons in the river water recorded during the spring season in the second station which was 6.5×10^{6} cell / ml and coincided with it each of the first and

third stations at the time of recording the highest count, while the lowest count, was recorded in the three stations during the autumn season which was 1.35×10^2 cell/ml as shown in figure (6) and table(6) .

The rise in aerobic bacterial count during the spring season may be attributed to several factors such as the suitability of temperatures for the growth and activity of microorganisms (22) and the increase of food tiff concentrations of organic and inorganic substances and salts in the surface of water sources by the effect of sand storms which the district witnessed during the spring and summer seasons which encouraged the increase of the living mass in the water , where the results of the present study showed the existence of moral linkage between the total aerobic bacterial count and the turbidity (0.41 at the level of $p \leq 0.01$) in agreement with what was found by each of (20,23,24,25). The results of the statistical analysis showed the existence of moral differences between the seasons and the stations (at the level of $p \leq 0.05$).

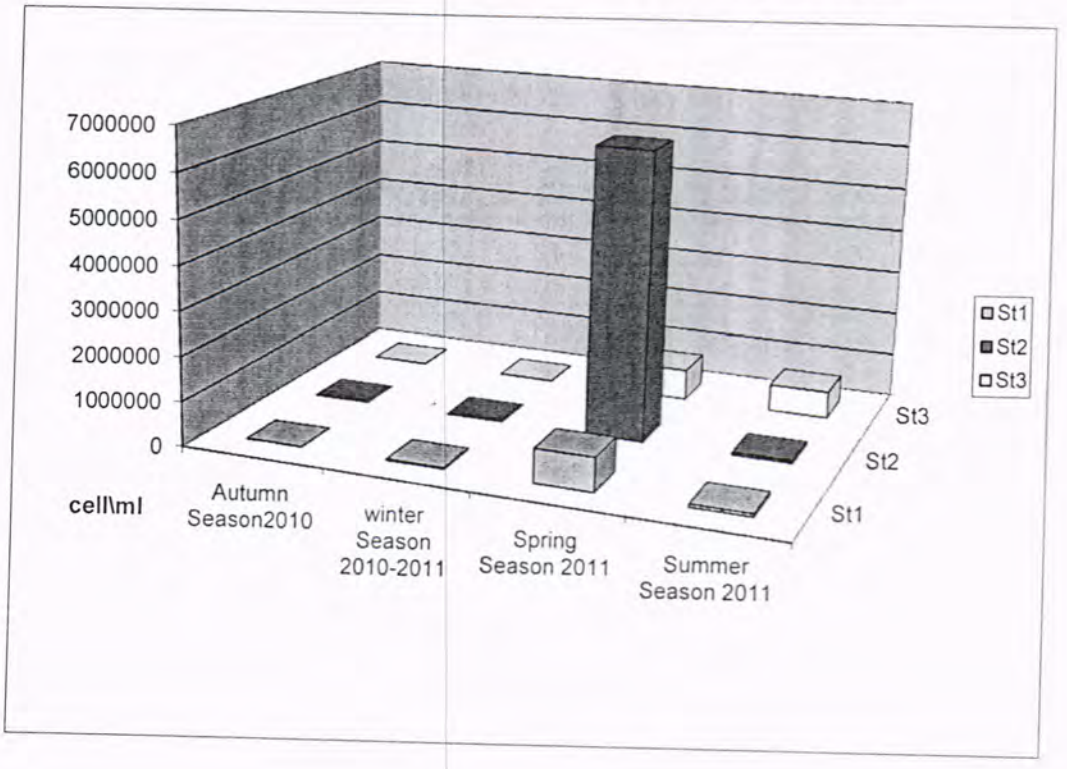


Figure-6:The total aerobic bacterial count according to saesons and stations

Table-6: The value of total aerobic bacterial count in the three stations through study time

ST3	ST2	ST1	Station / Date
±0.3 1.35 x10^2 bC	±0.21 1.35 x10^2 bD	±0.28 1.8 x10^2 aD*	**Autumn Season2010**
±0.84 3.7 x10^3 bC	±0.56 3.6 x10^3 bC	±0.63 2.45 x10^4 aC	**Winter Season 2010-2011**
±0.77 6.95 x10^5 cA	±0.56 6.5 x10^6 aA	±0.70 7.5 x10^5 bA	**Spring Season 2011**
±2.12 6.1 x10^5 aB	±2.61 6.15 x10^4 bB	±2.75 7.05 x10^4 bB	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

Coliform Bacteria & Fecal Coliform Bacteria

Their existence in the water environments indicates the unsuitability of those water for human consumption and their existence is a proof on the contamination of those waters (26). The coliform bacterial count in the river water has increased noticeably during the spring season and the highest count has been recorded in the second station during the spring season which recorded 8×10^5 cell / ml, while noticed a decrease in coliform bacterial count during the autumn season , where the three stations recorded the lowest coliform bacterial count which was 1.7×10^2 cell/ ml as shown in figure (7) and table(7) , it was noticed an increase in the coliform bacterial count during the spring season and a decrease in the autumn season , and this agrees with what was mentioned by (20,23) through their studies on Tigris River water . As for the reason of their increasing growth during the summer season may belong to the suitability of temperature for microbic growth (22,27) , in addition to the increase of turbidity and this is what was noticed through the moral linkage (0.56 at the level of ≤ 0.01) , and the statistical analysis results showed the existence of moral differences between the four seasons and the study stations at the level of $p \leq 0.05$.

The fecal coliform bacterial count has witnessed a noticeable increase during the spring season with decrease in its count during autumn season , and this agrees with many of the studies among them (28,29,30,31) , where the fecal coliform bacterial count ranged between ($1.3\times 10^2 - 2.15\times 10^5$) cell /ml where they recorded the highest count during the spring season in the third station,as for the lowest count which recorded in the three stations during the autumn season as shown

in figure(8) and table (8). As for the fecal coliform bacteria it was noticed that there is moral linkage between the coliform bacteria and the fecal coliform bacteria (0.15 at the level of $p \leq 0.01$).

The increase of counts during the spring season belongs to the drifting of soil due to rains which contain and population wastes because the district is famous in agriculture and the suitability of temperatures for microbic growth and activity (32).

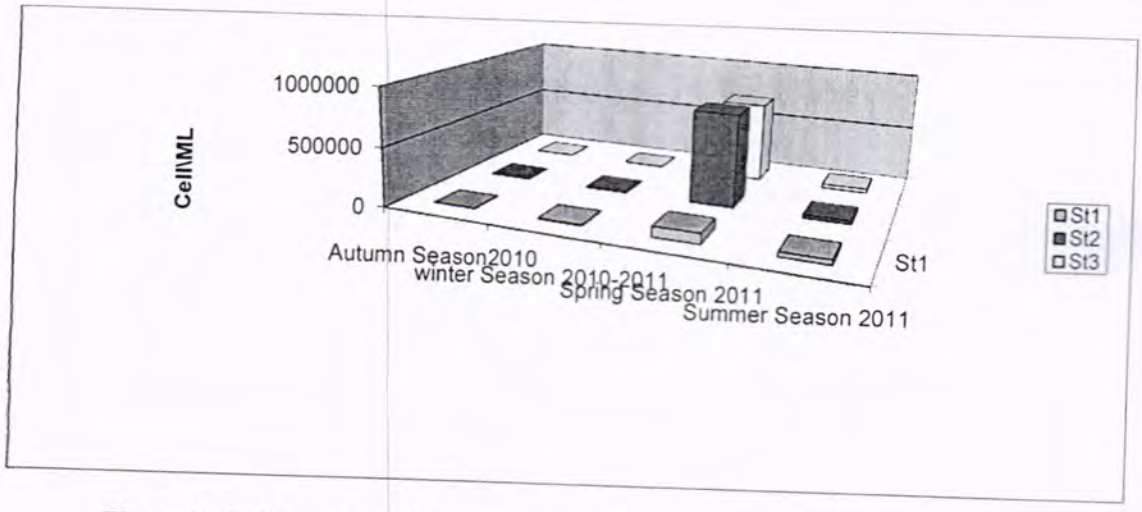


Figure-7: Coliform bacterial count according to seasons and stations

Table -7: The value of coliform bacterial count (cell\ml) in the three stations through study time.

ST3	ST2	ST1	Station / Date
±0.70 1.6 x10^2 aC	±0.56 1.7 x10^2 bD	±0.70 1.7 x10^2 bD*	**Autumn Season2010**
±1.13 7.2 x10^3 cD	±1.06 7.75 x10^3 aC	±1.27 7.1 x10^3 bC	**Winter Season 2010-2011**
±1.34 7.05 x10^5 bA	±1.41 8 x10^5 aA	±1.06 8.75 x10^4 cA	**Spring Season 2011**
±0.28 3.04 x10^4 bB	±0.21 2.94 x10^4 cB	±0.35 4.05 x10^4 aB	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

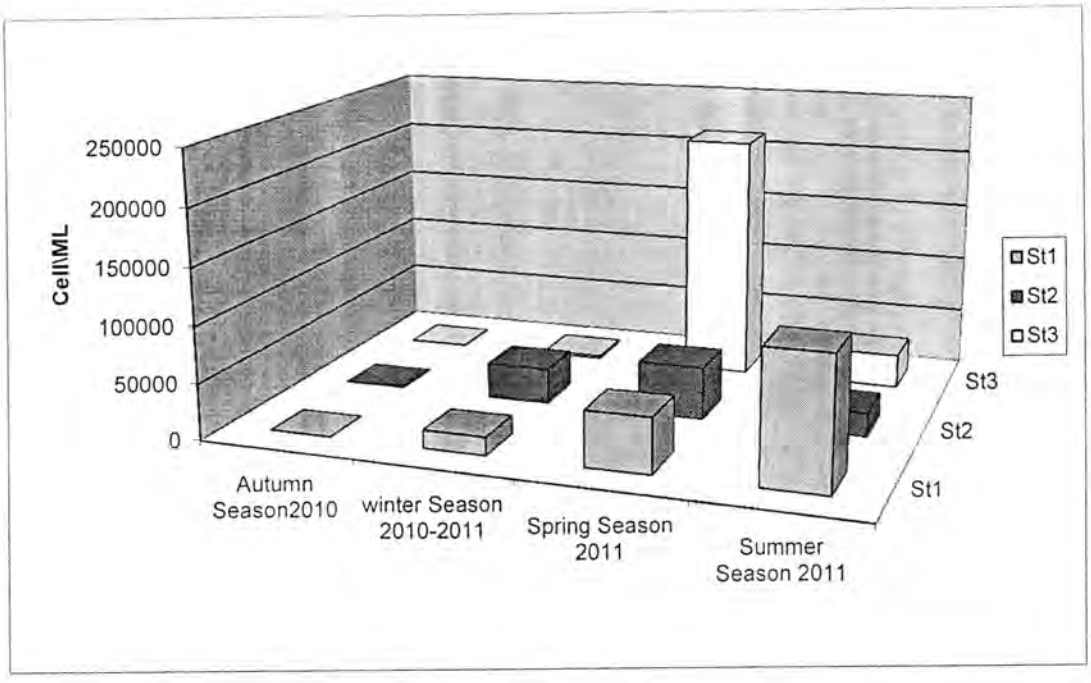


Figure-8: Fecal coliformbacterial count according to seasons and staions

Table- 8: The value of fecal coliform bacterial count (cell\ml) in the three stations through study time

ST3	ST2	ST1	Station / Date
±0.21 1.35×10^2 aD	±0.28 1.3×10^2 aD	±0.14 1.3×10^2 aD*	**Autumn Season2010**
±0.35 2.25×10^3 cC	±0.42 3.15×10^4 aB	±0.56 1.6×10^4 bC	**Winter Season 2010-2011**
±1.48 2.15×10^5 bB	±2.05 4.65×10^4 aA	±1.06 4.85×10^4 aB	**Spring Season 2011**
±0.07 2.95×10^4 bA	±0.07 2.05×10^4 cC	±0.07 1.15×10^5 aA	**Summer Season 2011**

*The different big letters refer to present significant differences between the stations, and the different small letters refer to present significant differences between the seasons (at level P>0.05).

CONCLUSION

The present study results showed that the Al Yousifiya River water is contaminated and it is wrong use river water for drinking without treatment fundamentalism.

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Study of Interleukin-6 ,Testosterone and Zinc in Different Clinicopathological Stages of Malignant Prostate Cancer Patients

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Received 2/10/2011 – Accepted 18/4/2012

الخلاصة

تقدم سرطان البروستات الى حالة الانتشار هي عملية متعددة الخطوات تتضمن السايتوكنات،الهرمونات والبايوكيميائيات. في هذه الدراسة تم قياس سلسلة من مستويات الانترلوكين-6 كعلامة التهابية، التستوستيرون كعلامة هرمونية والزنك كعلامة بايوكيميائية لمرضى سرطان البروستات الخبيث خلال المراحل المختلفة للمرض .تم قياس العلامات وارتباطها بمختلف المراحل المرضية و تم تقييم هذه التغيرات وعلاقتها بتقدم المرض. اظهرت النتائج اختلاف مهم بين المراحل الاربعة لمرضى سرطان البروستات الخبيث وعلى اية حال اظهرت المرحلة الرابعة من المرض اختلاف عالي الاهمية مقارنة بمجاميع السيطرة وتنسج البروستات الحميد وبمعنوية واضحة ($P<0.05$).

ABSTRACT

The progression of prostate cancer to a metastatic state and to hormone independence is thought of as a multi-step process involving cytokines , hormones and some biochemicals . In this study, was measured serial levels of serum IL-6 as inflammatory marker , testosterone as hormonal marker and zinc as biochemical marker in patients with malignant PCa at different clinicopathological stages of the disease. All markers were measured and correlated with clinicopathological variables and patient survival. Serial changes in these markers were also assessed and related to disease progression. The results showed a significant difference observed between the four stages of PCa patients; however, highly significant difference was observed in stage IV compared to control and BPH ($P<0.05$) .

Keywords: Prostate cancer,Inflammation ,IL-6 , Testosterone,Znic

INTRODUCTION

Inflammation of prostate area caused a novel putative PCa precursor lesion called proliferative inflammatory atrophy, which shares some molecular traits with prostate intraepithelial neoplasia and PCa [1]. During theses transitions to PCa, a number of cytokines and and cytokine receptors display variations in their expression. It is most important to notice that cytokines are produced by a variety of cells such as tumor-associated fibroblasts, endothelial cells, or tumor infiltrating cells such as macrophages and or lymphocytes have selected advantage of progression of prostate disease. Interleukin-6 plays a major role in pathogenesis and development of malignancies. It helps tumor to grow through inhibiting cancer cell apoptosis and the induction of tumor angiogenesis [2]. IL-6 may be involved in the regulation of solid tumor growth in paracrine and autocrine ways [(2)]. Interleukin-6 concentrations can depend on the tumor stage and can correlate with

survival. Current investigations have focused on the use of IL-6 as a prognostic factor for cancer. In the adult, prostate gland size and function is maintained through a homeostatic balance between the process of cell renewal (proliferation) and cell death (apoptosis). This balance is regulated by hormones secreted by the endocrine system, mainly androgens. After diffusing into the prostatic epithelium, testosterone is rapidly and irreversibly converted into dihydrotestosterone (DHT), which is the major activator of the androgen receptor (AR) [3]. DHT bound AR then translocates to the nucleus, where it mediates transcriptional activation (or repression) of target genes [4] . These androgen-stimulated changes in gene expression promote cell survival, growth arrest, and the synthesis of tissue-specific proteins such as PSA [5]. Trace elements status also affects the synthesis and secretion of cytokines and chemokines that modulate the activities of immune and other cells. Beck et al demonstrated that mild zinc deficiency in humans induces an imbalance in vivo cytokine secretion by peripheral blood mononuclear cells despite maintenance of normal number of total leukocytes and B- and T-lymphocytes [6].

Although zinc is essential for proper maintenance of all cells, it is particularly important in the prostate which secretes high levels of citrate and proteins that contains zinc. There is compelling evidence that zinc is involved in the pathogenesis of prostate cancer [7]. Physiologic concentrations of zinc inhibit growth of androgen-sensitive and androgen-independent prostate cancer cell lines via cell cycle arrest, programmed cell death, and necrosis [8], which may be initiated in mitochondria [9].

MATERIALS AND METHODS

This study was carried on 100 patients with prostate cancer (PCa) at the Radiotherapy and Nuclear Medicine Teaching Hospital in Baghdad. Their ages range was 58-72 years , and all of them are newly diagnosed with the disease. After careful clinical, biochemical and histopathological evaluation by specialist in oncology, they are classified into four groups as follow:

Group I: Include 25 patients, who are newly diagnosed with stage I PCa.

Group II: Include 25 patients, who are newly diagnosed with stage II PCa.

Group III: Include 25 patients, who are newly diagnosed with stage III PCa.

Group IV: Include 25 patients, who are newly diagnosed with stage IV PCa.

Other 25 patients, with the same age ranges, diagnosed for benign prostatic hyperplasia, were included and served as comparator with BPH. Twenty five healthy subjects, with the same age ranges, were selected and served as controls for comparison of the studied parameters, at the Department of Urology-Baquba Teaching Hospital. From all subjects, blood samples were collected by vein puncture . IL-6 enzyme linked immunosorbent assay (ELISA) was utilized depending on a technique called quantitative sandwich immunoassay using a kit supplied by Anogen , testosterone level was measured by (ELFA) technique (Enzyme Linked Fluorescent Assay) using a kit supplied by bioMerieux .While zinc level was carried out by flame atomic absorption spectrophotometer method . To compare the significance of the difference in the mean values of any two groups, Student's t-test was applied and P value less than 0.05 was considered statistically significant. The correlation coefficient (r) test is used to describe the association between the different studied parameters.

RESULTS AND DISCUSSIONS

Table (1) indicated that IL-6 levels in all stages of malignant prostate patients were significantly higher compared to control and BPH (57.46,169.84,330.54,679.95 respectively, $P<0.05$) ; there were significant differences observed between the four stages of PCa; however, highly significant difference was observed in stage IV of malignant PCa patients compared to control and BPH patients ($P<0.05$) (Figure 1).

Table (2) demonstrated that testosterone levels in serum of all stages of malignant prostate patients were significantly lower than that of control and BPH (3.75 ,3.53 ,3.19 ,2.7 , respectively) ; significant differences were observed between the four stages of PCa; however, lower significant difference was observed in stage IV of malignant PCa patients compared to control and BPH patients ($P<0.05$) (Figure 2).

The data presented in table (3) showed that serum zinc levels in all stages of PCa patients were significantly lower than those in control and BPH (78.46, 69.96 ,65.6,58.12,respectively) ; significant difference were reported between the four stages of PCa; however, a lower significant difference was observed in stage IV of malignant PCa patients compared to control and BPH ($P<0.05$) (Figures 3).

Table-1. Serum levels of the interleukins -6 in healthy subjects, BPH patients and patients with different stages of malignant prostate cancer.

Patients groups	S. IL-6 Pg/ml
Healthy subjects	7.01 ± 2.8^{a}
BPH patients	17.0 ± 33^{b}
Stage I P. Cancer	57.46 ± 19.3^{c}
Stage II P. Cancer	169.84 ± 40.9^{d}
Stage III P. Cancer	330.54 ± 61.3^{e}
Stage IV P. Cancer	679.95 ± 152.0^{f}

Values represent mean ±SD; values with non-identical superscripts (a,b,c,d,e,f) indicated significant differences between groups ($P<0.05$).

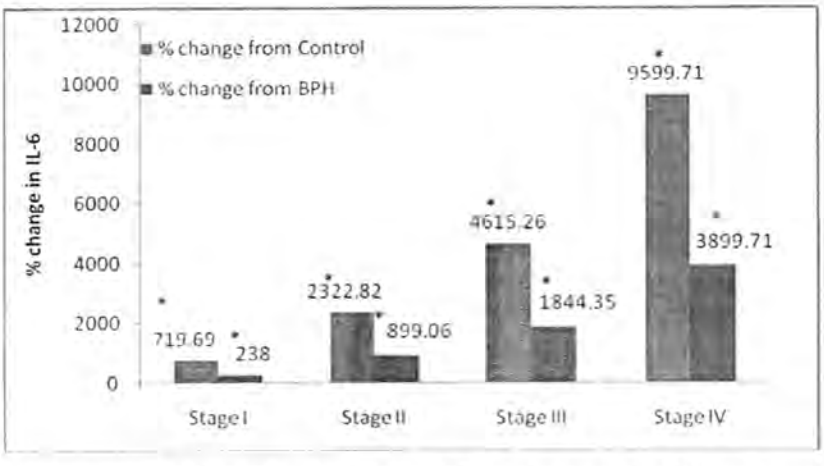

Figure-1: Percentage (%) differences in IL-6 in PCa patients with different stages of the disease compared to BPH patients and controls; * $P<0.05$.

Table-2: Serum levels of Testosterone in healthy subjects, BPH patients and patients with different stages of malignant prostate cancer.

Patients groups	S. Testosterone (ng/ml)
Healthy subjects	4.73 ± 0.9^{a}
BPH patients	4.07 ± 0.7^{b}
Stage I PCa	3.75 ± 0.5^{c}
Stage II PCa	3.53 ± 0.4^{d}
Stage III PCa	3.19 ± 0.3^{e}
Stage IV PCa	2.7 ± 0.2^{f}

Values represent mean ±SD; values with non-identical superscripts (a,b,c,d,e,f) indicated significant differences between groups ($P<0.05$).

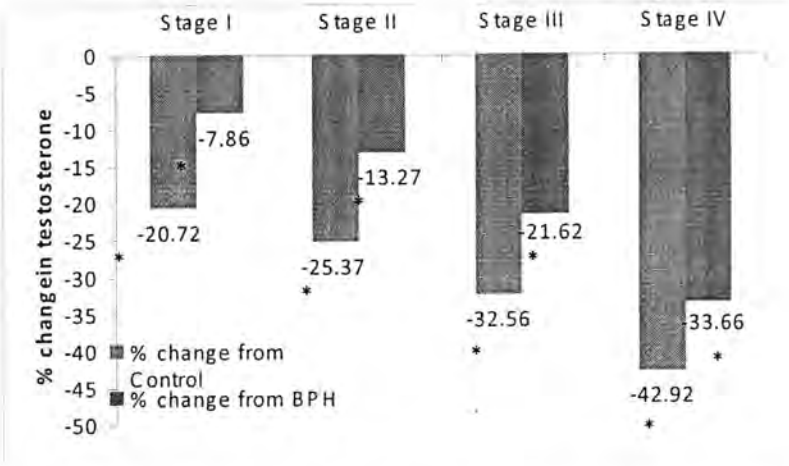

Figure -2: Percentage (%) differences in serum testosterone levels in PCa patients with different stages of the disease compared to BPH patients and controls; * P<0.05.

Table -3: Serum levels of Zinc in healthy subjects, BPH patients and patients with different stages of malignant prostate cancer.

Patients groups	S. Zinc (µg/dl)
Healthy subjects	86.25 ± 2.6^{a}
BPH patients	80.19 ± 2.5^{b}
Stage I PCa	78.46 ± 3.5^{c}
Stage II PCa	69.96 ± 4.1^{d}
Stage III PCa	65.6 ± 6.0^{e}
Stage IV PCa	58.12 ± 3.7^{f}

Values represent mean±SD; values with non-identical superscripts (a,b,c,d,e,f) indicated significant differences between groups (P<0.05).

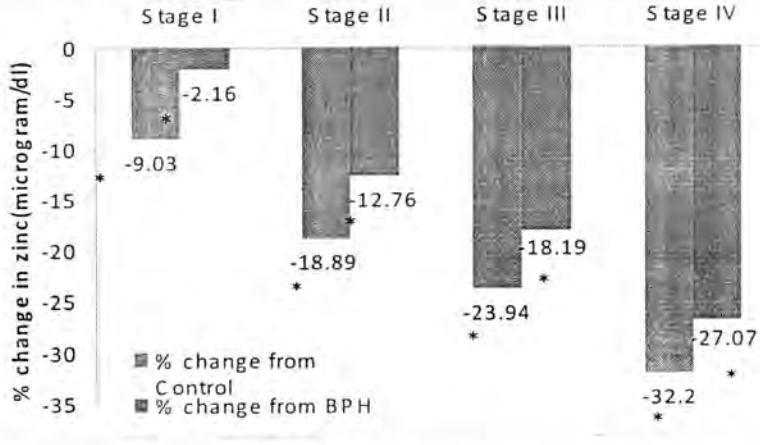

Figure -3: Percentage (%)differences in serum zinc levels in PCa patients with different stages of the disease compared to BPH patients and controls; * P<0.05.

Although there are clear description for the relationship of cytokines to the development and progression of many cancers, there are few published observations relevant to PCa. Surprisingly perhaps for a tumor where there is such clear evidence of a primary hormonal basis to its development, there is emerging evidence of cytokine involvement. Shariat et al. and smith et al. reported that IL-6 and TNF-α, two cytokines with multiple and overlapping biological properties, are involved in PCa development [10,11], While Twilie et al. found that human PCa cell lines secrete IL-6 [12]. Okamato et al. also reported that IL-6 acts as a paracrine growth factor for PCa cells [13].

Aside from PCa, it has been reported that IL-6 induced lymphoblastoid tumorigenicity is due possibly to the inhibitory effect on tumor immunity of very high concentrations of this cytokine [14], and that natural killer cell dysfunction induced by IL-6 production from tumor cells is a novel mechanism of tumor escape from immune surveillance [14]. On the other hand, Alder et al. and Nakashima et al. showed that in cancer patients, IL-6 may reflect prognosis and tumor load, and elevated IL-6 levels have associated with advanced stages and metastasis-related morbidity [15,16]. In the present study, serum IL-6 levels were significantly higher in patients with stage IV than in patients with stage I, II, and III (Table 1, figure 1), which support previous reports which indicate that patients with metastatic PCa had significantly elevated serum IL-6 levels compared with other PCa patients [14,17]. Therefore, it seems possible that IL-6 stimulates the growth of PCa cells via autocrine and/or paracrine mechanisms and might contribute to tumor escape from immure surveillance, resulting in disease progression through the increased production of IL-6.

The prostate requires hormones for growth and development but hormones are also essential for cancer maintenance and growth; however, their role in the initiation of PCa is not clearly defined [18]. Circulating androgens, particularly testosterone, are known to play a fundamental role in prostate growth. The role of testosterone in the pathophysiology of prostate cancer remains elusive and the conundrum is yet to be solved, especially after Huggins and Hodges proved that reduction in testosterone production by castration or estrogen treatment caused regression of metastatic disease [19]. The results presented in table (2) indicated that testosterone concentrations were significantly lower in patients with PCa than in those with BPH and were also significantly lower in patients with advanced stage disease than in patients with organ-confined disease (Figure 2), this means that testosterone promotes normal prostate epithelium differentiation; therefore, it is possible that lower testosterone activity may also affect tumor differentiation.

On the other hand, Severi et al. and Platz et al. concluded that although no association was observed between testosterone levels and PCa risk, lower pre-diagnostic serum testosterone levels were associated with increased risk of future diagnosis of high-grade PCa [20,21], and it has even been suggested that maintaining normal serum testosterone levels may prevent PCa [22]. There are several hypotheses that correlate the advanced pathological stage in men with lower testosterone levels, and it might be lower secondary to chronic disease or as a consequence of advanced disease [23]. Others have suggested that PCa inhibits androgens through negative feedback of inhibin, PSA or dihydrotestosterone. This was recognized as a possible mechanism in a study, where testosterone was found to be higher in patients after prostate removed [24]. Prehn also reported that with low testosterone the normal milieu might be varied enough to disrupt the normal growth and maintenance of prostatic tissue, while compensatory hyperplasia, arises when the prostate atrophies, might lead to cell mutations and consequent selection of androgen-independent aggressive prostate cell growth [25]; he also found that decreased testosterone levels was associated with increased cholesterol and triglycerides, with consequent increase in arterial plague and coronary vasoconstriction, which can predispose to elevated blood pressure and other heart diseases.

Zinc may play an anti-carcinogenic role by stabilizing the structure of DNA, RNA, and ribosome [26]. Stevens and Vallwarf reported that zinc is essential to the function of several transcription factors and proteins that recognize certain DNA sequences and regulator of gene transcription [27]. Merk et al. also reported that zinc protect against free radical injury [28], while Swauger et al. and Nelson et al. suggested that zinc may affect the immune response [29,30]. In case of normal prostate, higher concentration of zinc present in the tissue causes a block in Krebs cycle and accumulation of citrate in the prostatic fluid; thus, normal prostate glandular epithelial cell have low respiration causing low terminal oxidation, energy inefficient and presumably generate less ROS [31]. Unlike normal prostate, malignant transformation is associated with an early metabolic switch leading to decreased zinc accumulation, and increased citrate oxidation [26]. Thus malignant prostate is energy efficient, capable of higher role of respiration and therefore, generates more ROS.

RECOMMENDATIONS FOR FUTURE WORK

1- Consider sub-grouping the enrolled patients according to the type of medical interventions they practiced to exclude the interference of drug treatment with the measured parameters.

2- More specific clinico-pathological markers are required for more precise ranking, follow up and investigation of the pathophysiology of the disease.

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Theoretical studies of mixed ligand complexes of mannich base derived from 2-mercaptobenzothiazole with [Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)]ions

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Received 27/2/2012 – Accepted 18/4/2012

الخلاصة

يتضمن هذا البحث دراسة تكوين المعقدات [احادية الليكاند لقاعدة مانخ (3-dicyclohexyl amino methyl -2-mercaptobenzothiazole)(E) ومختلطة الليكاند الحاوية على قاعدة مانخ (E) كاليكاند اولي والحامض الاميني التربتوفان (Trp.) كاليكاند ثانوي] نظريا في الطور الغازي باستعمال برنامج (HyperChem.8) وبتطبيق الميكانيك الجزيئي والشبه التجريبي في الحساب، وباستعمال الدالة (PM3)، لحساب حرارة التكوين(ΔH^{o}_{f}) وطاقة التآصر(ΔE_b) لكل من الليكاندات(E و.Trp) والمعقدات الأحادية والمختلطة الليكاند، وبدرجة حرارة (298 كلفن). كذلك تم حساب الجهد الكهروستاتيكي لليكاندات (E و.Trp) لتحديد المواقع الفعالة ضمن الجزيئة. وجرى حساب التردد الإهتزازي نظرياً وباستعمال الدالة (PM3) لليكاندات ومعقداتها الأحادية والمختلطة الليكاند، ومقارنة النتائج المستحصلة مع القيم المقاسة عملياً، ووجد أن هناك توافقاً كبيراً بين القيم العملية والمحسوبة نظرياً مع زيادة إمكانية تشخيص الحزم بشكلٍ أدق،كذلك تم حساب أطوال الأواصر باستعمال الدالة (PM3).

ABSTACT

This work include a theoretical studies of the formed complexes[mono ligand of mannich base (3-dicyclohexyl amino methyl-2-mercaptobenzothiazole) **(E)** and mixed ligand which contain of mannich base **(E)** as a primary ligand and amino acid tryptophan **(Trp.)** as a secondary ligand] in gas phase by using (HyperChem.8) program for the molecular mechanics and semi-empirical calculations. The heat of formation (ΔH^{o}_{f}) and binding energy (ΔE_b) for all free ligands**(E and Trp.)** and their mono and mixed ligand complexes were calculated by **PM3** method at **298 K°.** Furthermore the electrostatic potential of the free ligands **(E and Trp.)** were calculated to investigate the reactive sites of the molecules. PM3 was used to evaluate the vibration spectra of the free ligands (**E** and **Trp.**) and their mono and mixed ligand complexes, and comparing the theoretically calculated wave numbers with the experimented values. The theoretically obtained frequencies agreed well with those found experimentally, in addition, calculations helped to assign unambiguously the most diagnostic bands. and bond length were calculated by using **PM3** method.

INTRODUCTION

Mannich reaction is a three component, condensation reaction consisting of active hydrogen containing compound ,formaldehyde and a secondary amine[1-4].In previous work[5] the mannich base **(E)** and their mono and mixed ligand metal complexes(S_1-S_{10}) have been prepared and investigated using different chemical techniques, this paper reports here the theoretical studies in the gas phase was done by using semi-empirical method in order to show the most stable conformation. The study aims to calculating the heat of formation and binding energy for all the probable geometries and to find the most active sites of the ligands (**E** and **Trp.**) by using the electro static potential calculations. Calculation of the vibrational frequencies of the(2-Mercaptobenzothiazole)(2-MBT) and free ligands [**E** and **Trp.**]

have also been carried out in order to compare the results with the experimental vibrational frequencies to make a certain assignment of the most diagnostic bands.

MATERIALS AND METHODS

Hyperchem is a sophisticated molecular modeler, editor and powerful computational package, that is known for its' quality, flexibility and ease of use [6,7]. It can plot orbital wave functions resulting from semi-empirical quantum mechanical calculations, as well as the electrostatic potential, the total charge density or the total spin density can also be determined during semi-empirical calculation, this information is useful in determining reactivity and correlating calculation results with experimental data.

Computational methods:

a) Semi -empirical quantum mechanical
b) Molecular mechanics
c) Mopac 2000

Types of calculations:

The types[8,9] of prediction possible of Molecules are:

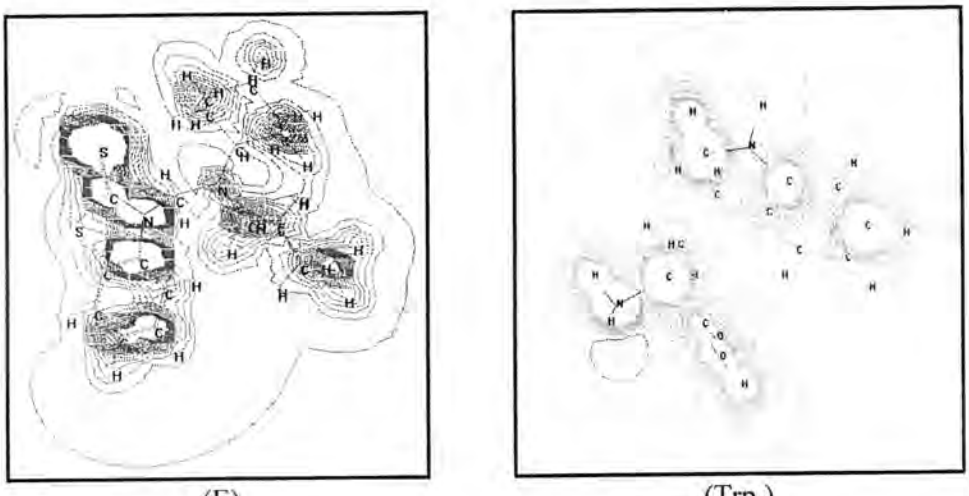
a) Geometry optimization calculations employ energy minimization algorithms to locate stable structures.
b) Bond distances
c) Molecular dynamics which provide the thermodymemic calculations and dynamic behavior of molecules
d) Plot the electrostatic potential field (HOMO and LUMO).
e) Vibrational spectrum (I.R and Raman spectra).

RESULTS AND DISCUSSION

A) Electrostatic Potentials:

Electron distribution governs the electrostatic potential of the molecules. The electrostatic potential (E.P) describes the interaction of energy of the molecular system with a positive point charge. (E.P) is useful for finding sites of reaction in a molecule; positively charged species tend to attack a molecule where the electro static potential is strongly negative (electrophonic attack) [6]. The (E.P) of the free ligands [**E** and **Trp.**]were calculated and plotted as 2D contour to investigate the reactive sites of the molecules, Fig (1). Also one can interpret the stereochemistry and rates of many reactions involving "soft" electrophiles and nucleophiles in terms of the properties of frontier orbital (HOMO, highest occupied molecular orbital) and (LUMO, lowest unoccupied molecules). The results of calculations show that the LUMO of transition metal ions prefer to react with the

HOMO of sulfur and nitrogen atoms of Mannich base free ligands [**E** and **Trp.**].



(E) (Trp.)

Fig.-1: Electrostatic potential (HOMO and LUMO) as 2D contours for (E and Trp.)

B) Optimized energies:

The program Hyperchem-8 was used for the semi-empirical and molecular mechanics calculations. The heat of formation (ΔH_f^o) and binding energy (ΔE_b) for free ligands(**E** and **Trp.**) and their mono and mixed ligand complexes(S_1-S_{10}) were calculated , Table (1).

Table :1- Conformation energetic (in KJ. mol^{-1}) for Mannich bases and their mono and mixed ligand complexes

Com.No.	ΔE_{tot}	ΔH^o_f	ΔE_b
2-MBT	-33156.46	104.98	-1597.55
E	-80673.95	35.07	-5200.37
S_1	-185054.96	-84.88	-10681.47
S_2	-113605.75	-266.77	-5662.60
S_3	-119290.09	-85.25	-5481.49
S_4	-122833.58	-98.94	-5607.85
S_5	-96003.98	-36.79	-5361.40
Trp.	-55905.2	100.09	-2854.2
S_6	-160413.87	-218.00	-8750.92
S_7	-169537.74	-405.47	-8838.31
S_8	-175289.15	-291.03	-8724.27
S_9	-178572.51	-186.11	-8597.26
S_{10}	-137008.36	15.17	-8153.68

C) Optimized vibrational spectra for ligands [E and Trp.]:

The vibrational spectra of the free ligands **[E and Trp.]** and their metal complexes (S_1-S_{10}) have been calculated, Tables (2)and(3) , Fig's (6) , (7) and (8) . The theoretically calculated wave numbers for these ligands showed that some deviations from the experimental values, these deviations are generally acceptable in theoretical calculations[10,11]. The most diagnostic calculated vibrational frequencies were chosen for the assignment of ligands(E,Trp.) and metal complexes(S_1-S_{10}) which are included in Tables [(2),(3)] and their respective experimental vibrational modes are shown in the same Table, Fig's (2) , (3),(4) and (5) .

The results obtained from the theoretical calculated wave numbers for the ligands were compared successfully with the calculated frequencies of **[(2-Mercaptobenzothiazole)] (2-MBT),** as authentic sample to predict the deviation or error caused from the difference between the experimental measurements and theoretically calculated results of the vibrational spectra, Fig (6). The results obtained for the theoretical calculations of the frequencies agreed well with those obtained for the experimental values, tables (2) and (3).

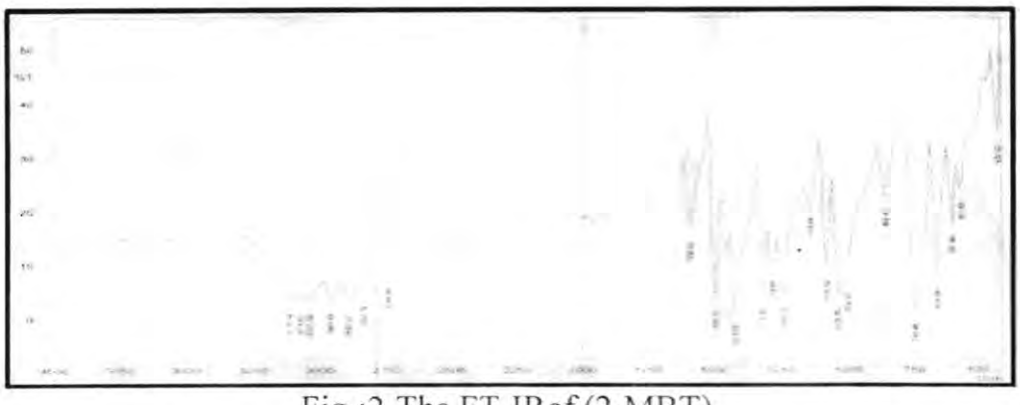


Fig.:2-The FT-IRof (2-MBT)

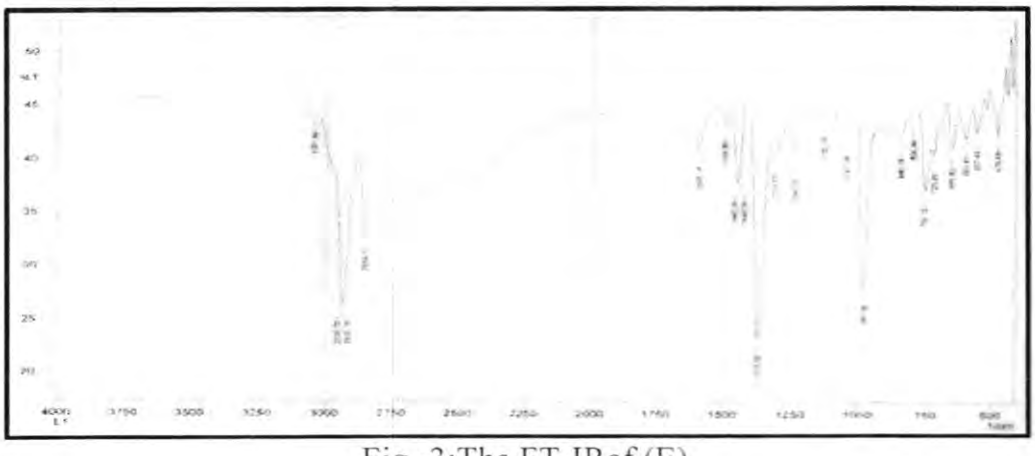


Fig.-3:The FT-IRof (E)

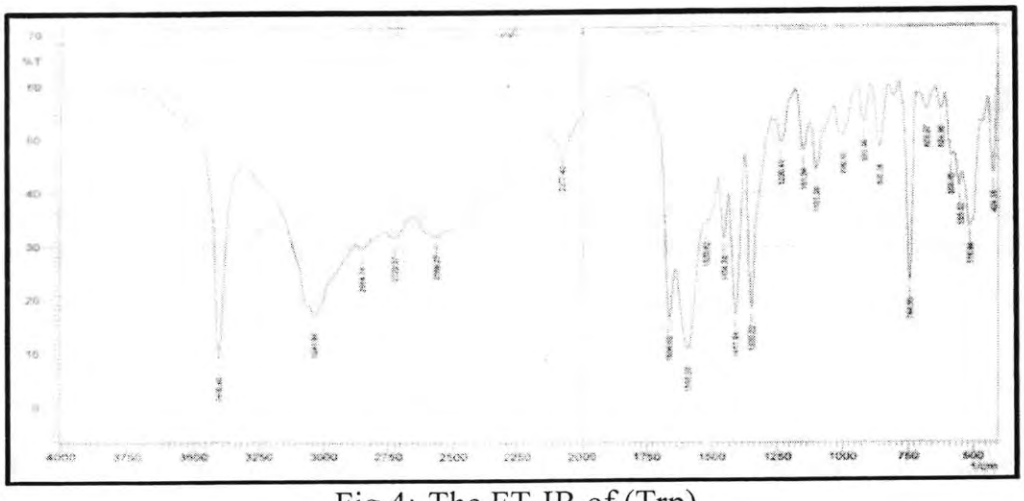


Fig.4:-The FT-IR of (Trp)

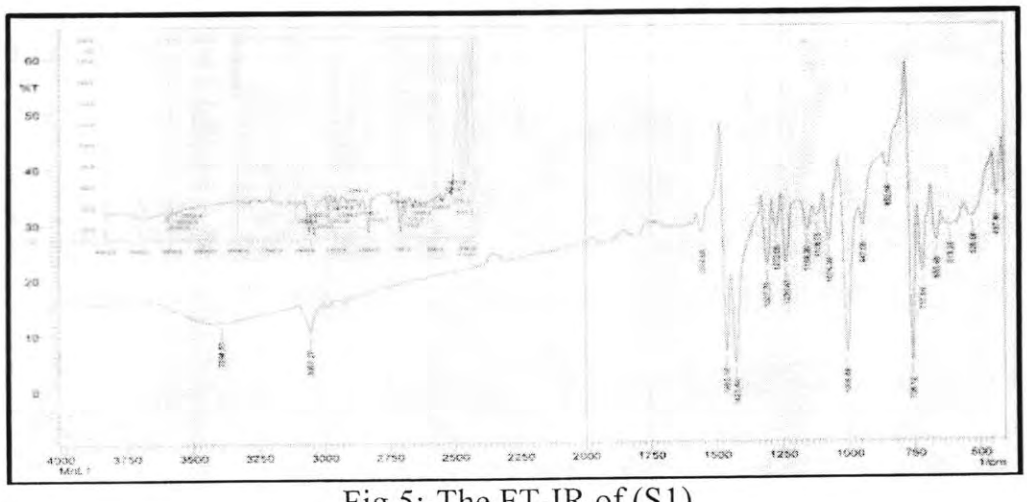
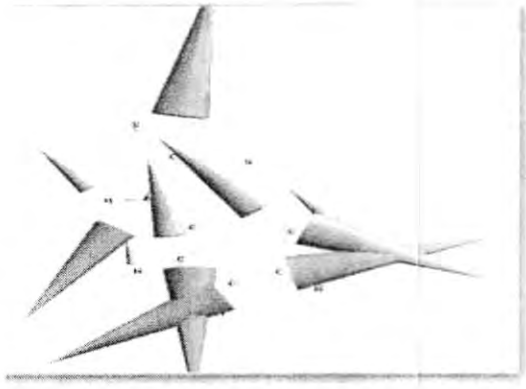
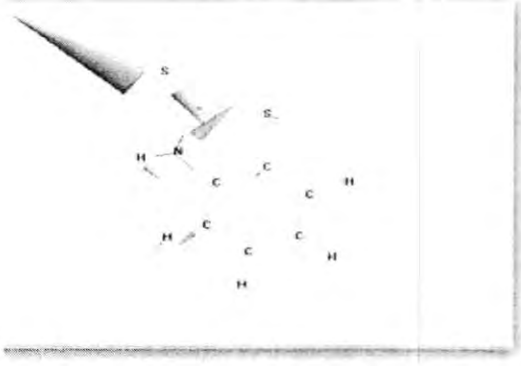


Fig.5:-The FT-IR of (S1)

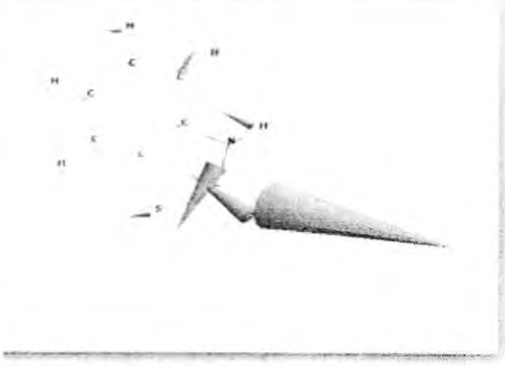


$\upsilon_{NH+C=S}$

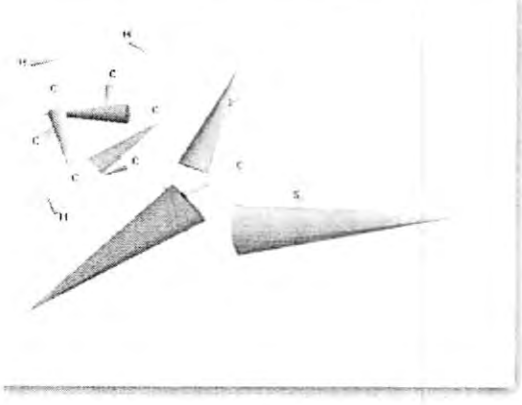
$\upsilon_{C\cdots N\cdots S}$



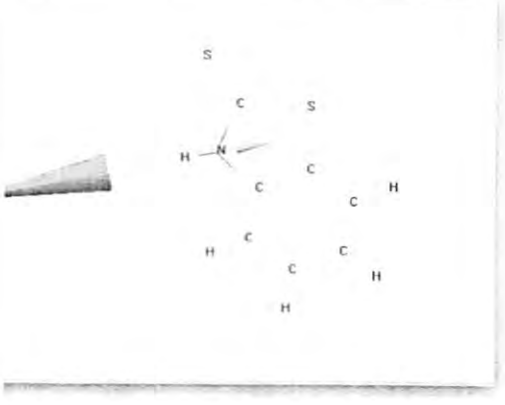
υ_{C-S}



$\upsilon_{C=S}$

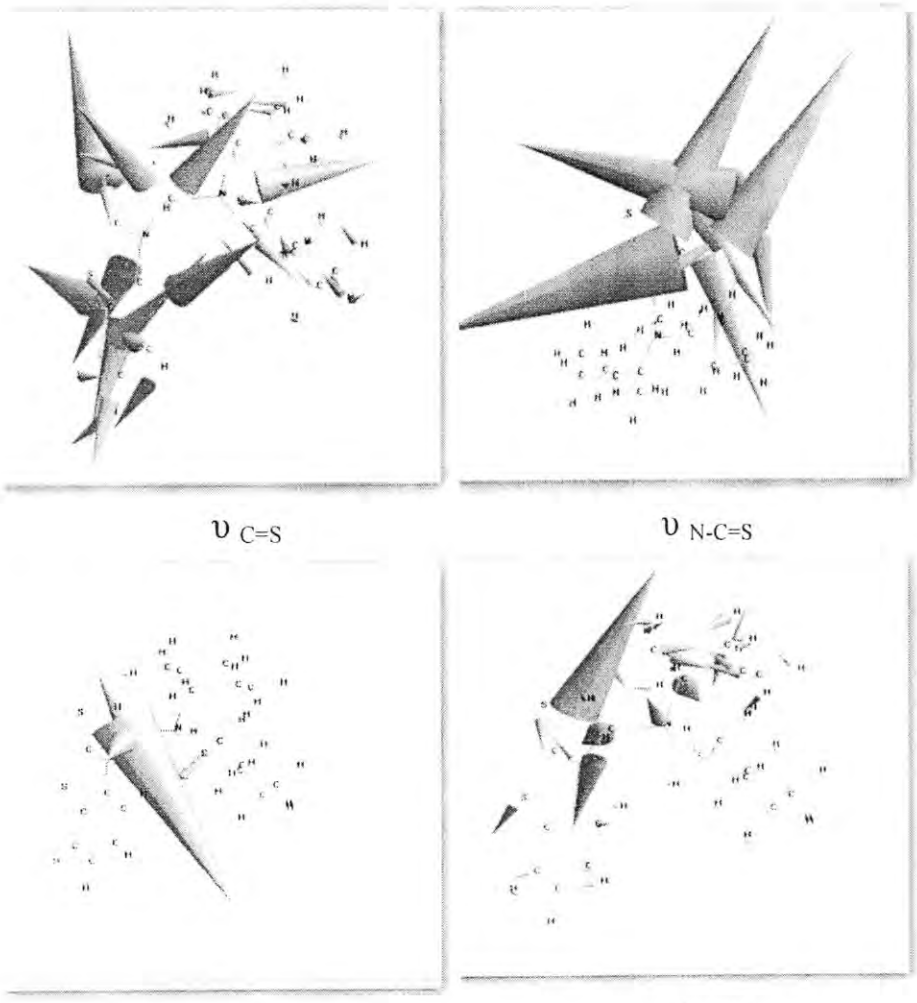


$\upsilon_{N-C=S}$



υ_{NH}

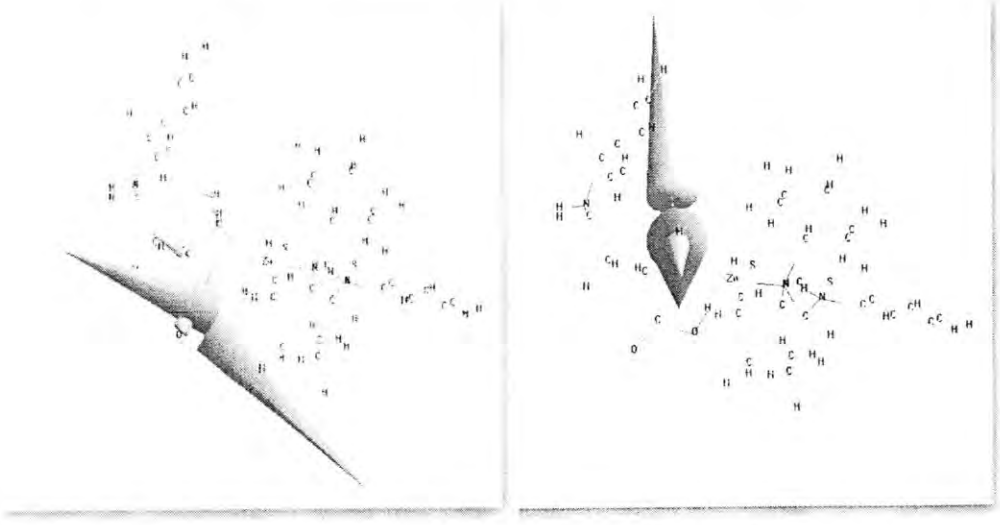
Fig.-6:The calculated vibrational frequencies of (2-MBT)



$\upsilon_{C=S}$ $\upsilon_{N-C=S}$

$\upsilon_{(C-H)\ of\ CH2}$ υ_{C-N}

Fig.-7:The calculated vibrational frequencies of (E)



υ_{COO^-} υ_{NH_2}

Fig.-8: The calculated vibrational frequencies of (Trp.)

Table-2: Comparison between the experimental and theoretical vibational frequencies (cm^{-1}) for free ligand (E) and mono ligand complexes

Com. No.	$\upsilon_{C=S}$	υ_{C-N}	υ_{M-S}	υ_{M-N}	υ_{M-Cl}	υ_{M-O}	υ_{O-H}, ρ_{O-H} *of* $_{H2O}$
E	(1045,1030)* (1056, 1047)** (1.04, 1.62)***	975* 981** 0.61***	-	-	-	-	-
S_1	1080* 1074** 0.55***	984* 1006** 2.18***	455* 435** 4,59***	545* 530** 2.83***	431* 407** 5.89***	-	3550* 3394** 4.59***
S_2	1058* 1087** 2.66***	1040* 1020** 1.96***	435* 428** 1.63***	517* 505** 2.37***	389* 372** 4.56***		3637* 3427** 6.12***
S_3	1068* 1082** 1.29***	961* 1028** 6.51***	444* 428** 3.73***	505* 540** 6.48***	370* 391** 5.37***	-	3617* 3450** 4.84***
S_4	1057* 1080** 2.12***	1032* 1006** 2.58***	419* 434** 3,45***	512* 500** 2.4***	379* 372** 1.88***	470* 480** 2.08***	(3704, 972)* (3452, 947)** (7.3, 2.63)***
S_5	1052* 1085** 3.04***	1041* 1024** 1.66***	409* 430** 4.88***	554* 542** 2.21***	375* 393** 4.58***	-	-

Table-3:Comparison between the experimental and theoretical vibational frequencies (cm^{-1}) for free ligand (Trp.) and mixed ligand complexes

Com. No.	$\upsilon_{C=S}$	υ_{C-N}	υ_{COO} *asy.*	υ_{NH2} *Asy. Sym.*	υ_{M-N}	υ_{M-Cl}	υ_{M-S}	υ_{M-O}	υ_{O-H}, ρ_{O-H} *of* $_{H2O}$
Trp.	-	-	1656* 1665** 0.54***	3394* (3079, 3039)** 10.23***	-	-	-	-	-
S_6	1054* 1093** 3.70***	1041* 1037** 0.38***	1585* 1665** 4.80***	(3329,3274)* (3342, 3277)** (0.38, 0.15)***	579* 590** 1.86***	-	422* 426** 0.93***	463* 511** 9.39***	(3713, 1012)* (3489, 988)** (6.42, 2.42)***
S_7	1031* 1093** 5.67***	990* 1056** 6.25***	1570* 1635** 3.97***	(3352,3244)* (3340,3282)** (0.351.15)***	585* 536** 9.14***	372* 372** 0.0***	415* 426** 2.58***	447* 462** 12.55***	(3669, 1108)* (3530, 997)** (3.93, 11.13)***
S_8	1049* 1095** 4.20***	1033* 1053** 1.89***	1591* 1593** 0.12***	(3314, 3093)* (3342, 3284)** (0.83, 5.81)***	591* 545** 8.44***	354* 347** 2.01***	416* 422** 1.42***	446* 462** 3.46***	(3710, 1113)* (3525, 997)** (5.24, 11.63)***
S_9	1042* 1098** 5.10***	997* 1008** 1.09***	1582* 1624** 2.58***	(3312, 3078)* (3338, 3271)** (0.71, 5.90)***	584* 534** 10.29***	358* 370** 3.24***	415* 396** 4.79***	450* 469** 4.05***	(3735, 976)* (3530, 940)** (5.80, 3.82)***
S_{10}	1032* 1087** 5.05***	975* 1003** 2.79***	1586* 1618** 1.97***	(3348, 3077)* (3323, 3281)** (0.75, 6.21)***	540* 536** 0.74***	-	384* 424** 9.43***	444* 462** 3.89***	-

Where:-

*Theoretical frequency.

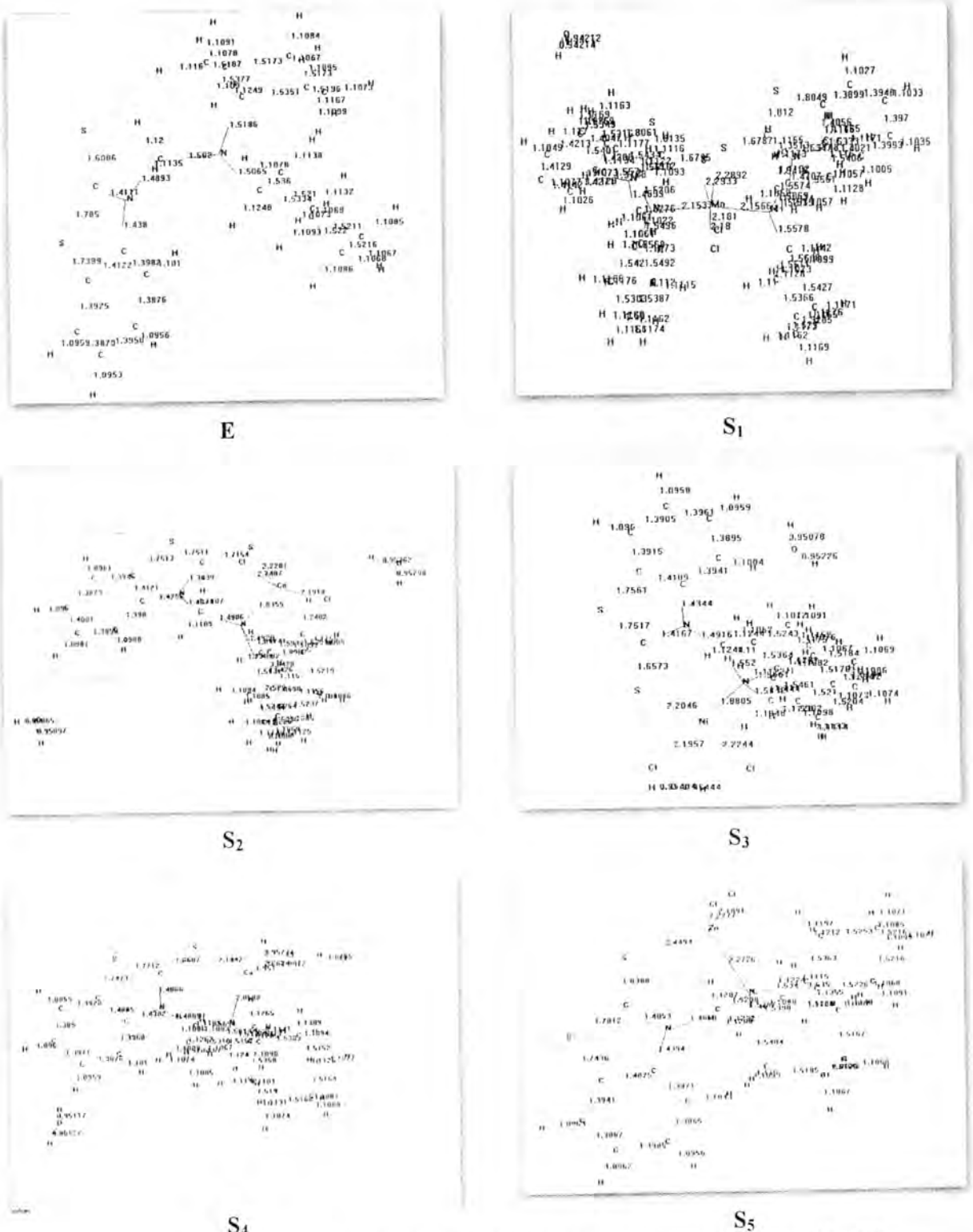
**Experimental frequency.

***Error% due to main difference in the experimental measurements and theoretical treatment of vibration spectrum

D) Bond length measurements for the (E and Trp.) and their mono and Mixed ligand metal complexes:

Calculation of parameters has been optimized bond lengths of the free ligand (E and Trp.) and their metal complexes by applying the

Semi-empirical (PM3) at Geometry Optimization (0.001 K.Cal.mol^{-1}), which to give excellent agreement with the experimental data[12,13], as shown in Tables(4) and (5), Fig's (9)and(10).



E S_1 S_2 S_3 S_4 S_5

Fig.-9: Bond lengths (A°) for (E) ligand and their mono ligand complexes [S_1-S_5]

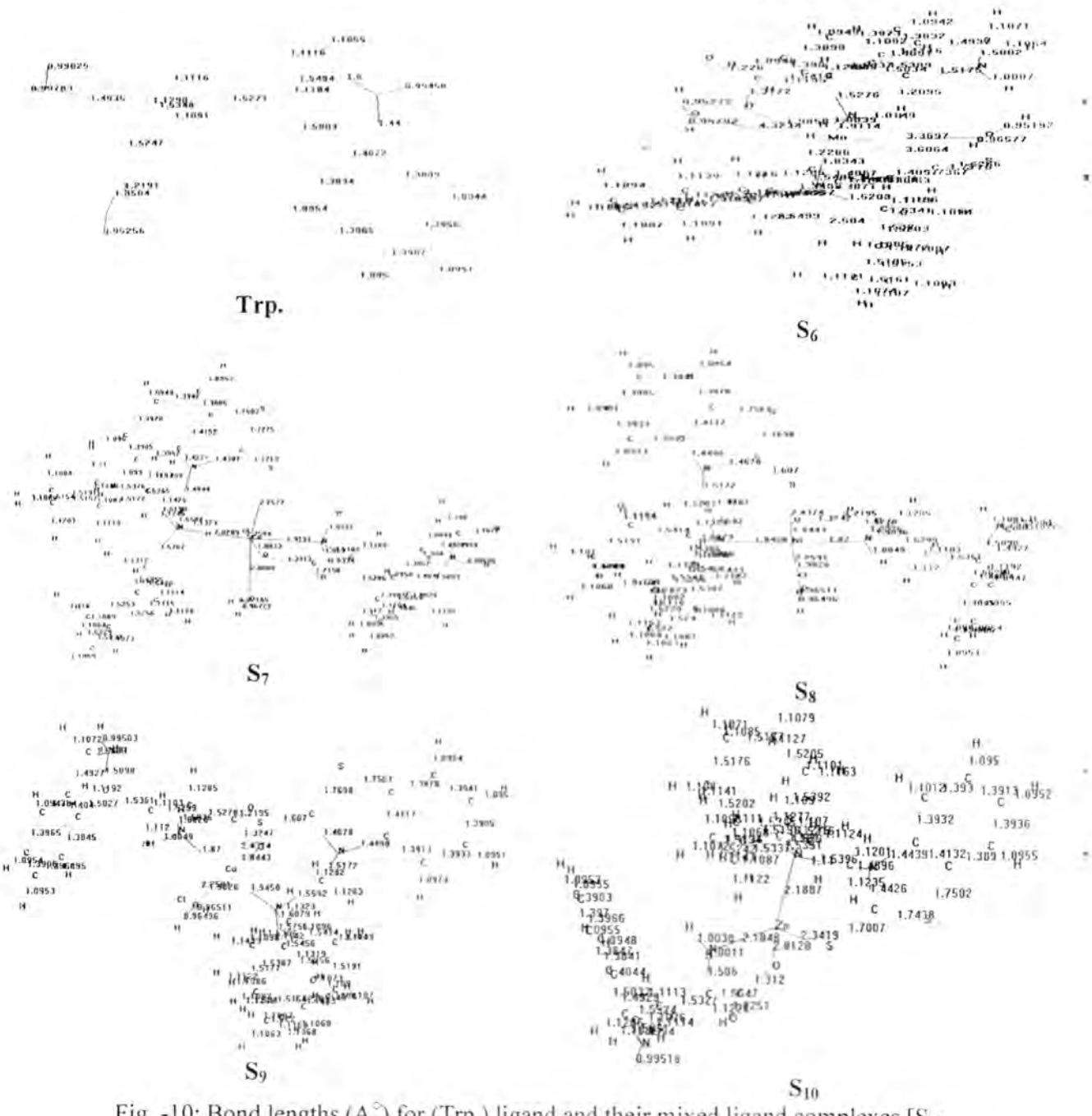


Fig. -10: Bond lengths (A°) for (Trp.) ligand and their mixed ligand complexes [S_6-S_{10}]

Table-4: Selected bond lengths (A°) for (E) ligand and their mono metal complexes [S_1-S_5]

Com. No.	*C=S*	*C-N*	*M-S*	*M-N*	*M-Cl*	*M-O*
E	1.6086	1.502	-	-	-	-
S_1	1.67	1.55	2.28	2.15	2.18	-
S_2	1.71	1.49	2.22	1.83	2.19	-
S_3	1.65	1.5	2.20	.88	2.19	-
S_4	1.66	1.53	2.14	2.051	1.95	2.05
S_5	1.63	1.52	2.44	2.27	2.18	-

Table-5: Selected bond lengths (A°) for (Trp.) ligand and their mixed metal complexes [S_6-S_{10}]

Com. No.	C=S	C-N	N-H of NH_2	C-O of COO^-	M-S	M-N of CH_2N	M-N of NH_2	M-Cl	M-O of COO^-	M-O of H_2O
Trp.	-	-	0.99	0.95	-	-	-	-	-	-
S_6	1.73	1.59	1.01	1.31	3.6	1.83	1.911	-	1.98	4.32
S_7	1.72	1.57	1.01	1.33	2.25	2.02	1.91	2.25	1.88	2.00
S_8	1.60	1.66	1.604	1.32	2.43	1.94	1.87	2.25	1.84	1.98
S_9	1.607	1.55	1.004	1.32	2.43	1.94	1.87	2.25	1.84	1.50
S_{10}	1.70	1.53	1.00	1.31	2.34	2.18	2.10	-	2.01	-

E) Theoretical electronic spectra for the (E,Trp.) and their mono and Mixed ligand metal complexes:

The electronic spectra for the (E,Trp.) and their mono and mixed ligand metal
complexes have been calculated and the wave numbered for these compounds showed some deviations from the experimental values as shown in Table(6 and 7) . These deviations in theoretical calculation are generally acceptable due to couplings between the electronic spectra modes and the approximation that each normal mode of the electronic spectra inter acts independently electronic spectra beam [14,15]. The most diagnostic calculated electronic spectra were chosen for the assignment of the free ligand (E and Trp.) and their mono and mixed metal complexes which their respective experimental electronic modes as shown in Fig.(11 and12).

All the theoretical electronic spectra of the free ligands (E and Trp.) and their mono and mixed metal complexes were calculated by using the semi- empirical (PM3) method at Geometry Optimization (0.01K.Cal. mol-1) was used.

Table-6:Comparison between the experimental and theoretical of the electronic spectra for (E) ligand and their mono metal complexes [S_1-S_{45}]

Com. No.	Band(Experimental)	Band(Theoretical)	Assignment
E	31440 42372	41764 30900	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
S_1	11816 20294 29674	11753 21068 28769	$^6A_1g(S) \rightarrow {}^4T_1g\ (G)$ $^6A_1g(S) \rightarrow {}^4T_2g\ (G)$ $^6A_1g\ (S) \rightarrow {}^4A_1g(G) + {}^4Eg(G)$
S_2	3749 6310 16994	3896 6270 17843	$^4A_2 \rightarrow {}^4A_2\ (F)$ $^4A_2 \rightarrow {}^4T_2\ (F)$ $^4A_2 \rightarrow {}^4T_2(P)$
S_3	16129 23310	16324 23406	$^1A_1g \rightarrow {}^1A_2g$ $^1A_1g \rightarrow {}^1Eg$
S_4	11161 23809	10844 25696	$^2B_1g \rightarrow {}^2Eg$ $^2B_1g \rightarrow {}^2A_1g$

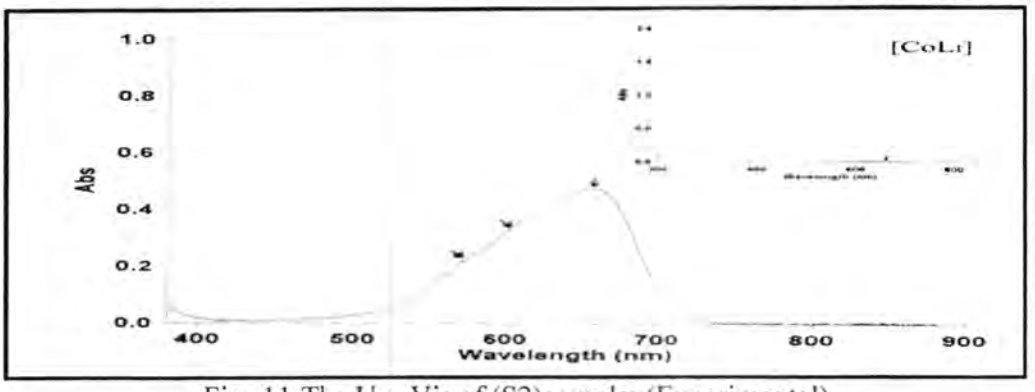


Fig.-11:The Uv.-Vis of (S2)complex(Experimental)

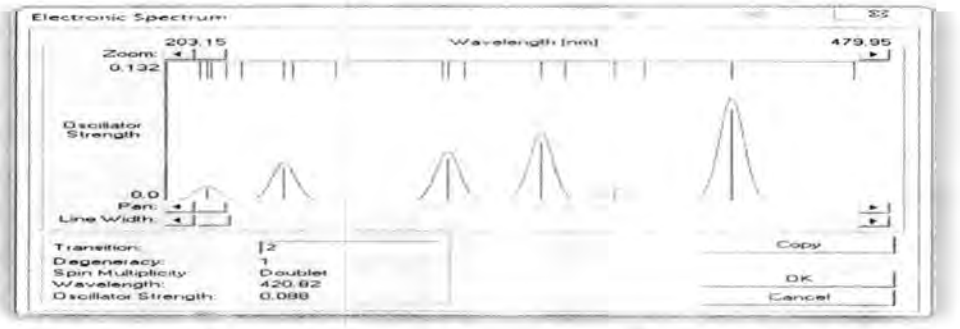


Fig.-12:The Uv.-Vis of (S2)complex(Theoretical(

Table-6:Comparison between the experimental and theoretical of the electronic spectra for (Trp.) ligand and their mixed metal complexes [S_6-S_9]

Com. No.	Band(Experimental)	Band(Theoretical)	Assignment
Trp.	34602 44444	33725 43123	$n\rightarrow\pi^*$ $\pi\rightarrow\pi^*$
S_6	15385 19920 29690	14450 20026 29012	$^6A_1g(S)\rightarrow{}^4T_1g(G)$ $^6A_1g(S)\rightarrow{}^4T_2g(G)$ $^6A_1g(S)\rightarrow{}^4A_1g(G)+{}^4Eg(G)$
S_7	8100 15385 18450	7908 15314 19940	$^4T_1g\rightarrow{}^4T_2g(F)$ $^4T_1g\rightarrow{}^4A_2g(F)$ $^4T_1g\rightarrow{}^4T_1g(P)$
S_8	12987 14425 24525	13736 15991 24208	$^3A_2g\rightarrow{}^3T_2g(F)$ $^3A_2g\rightarrow{}^3T_1g(F)$ $^3A_2g\rightarrow{}^3T_1g(P)$
S_9	16949	16417	$^2Eg\rightarrow{}^2T_2g$

F) Optimized geometries of (E and Trp.) and their mono and mixed ligand complexes (S_1-S_{10}):

All theoretically probable structures of free ligand ***(E and Trp.)*** and their mono and mixed ligand complexes have been calculated by (PM3) method in gas phase to search for the most probable model building stable structure , Fig's(13) and (14).

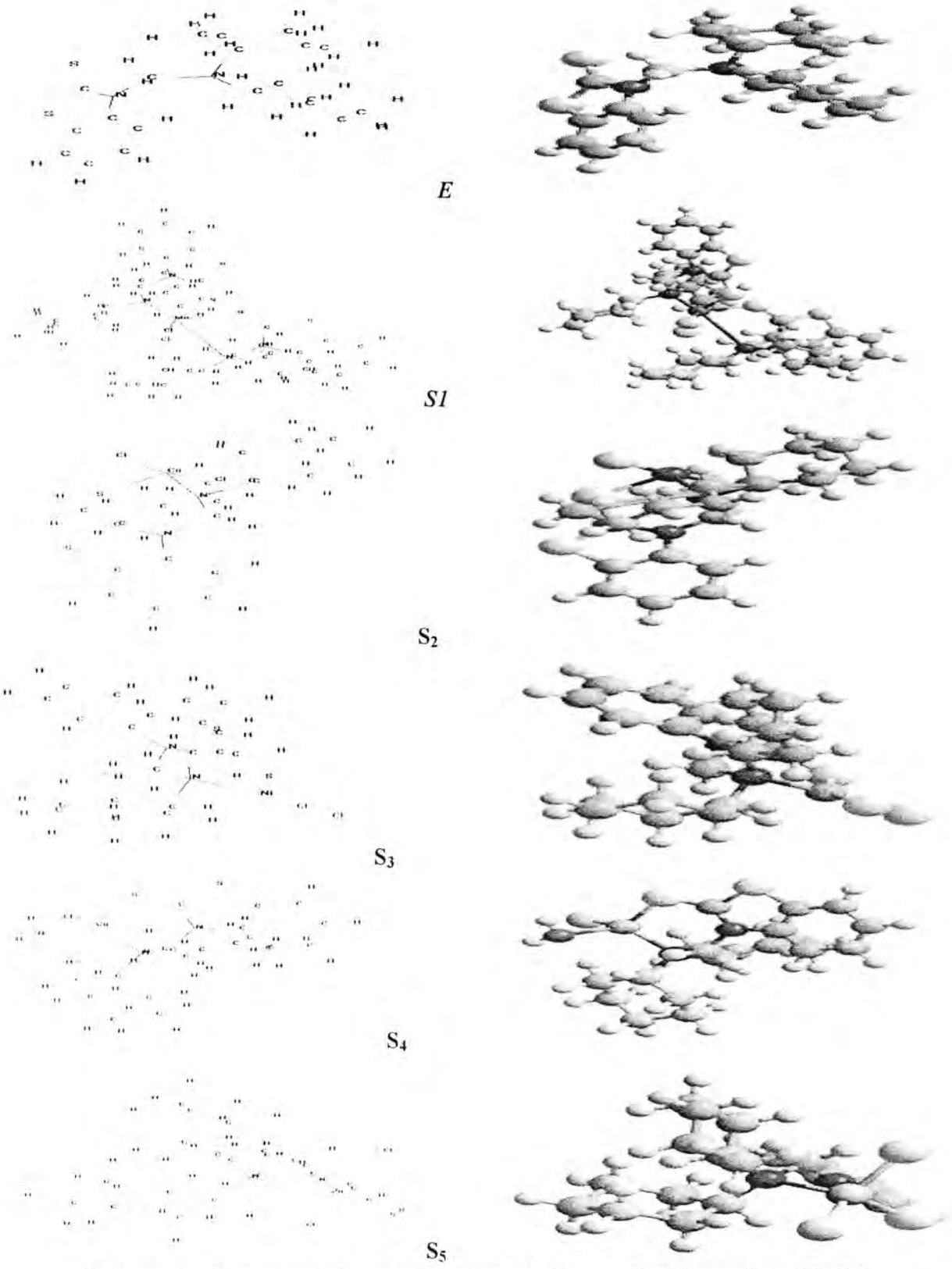


Fig. -13: Conformational Structure of(E) and their mono ligand complexes (S_1-S_5)

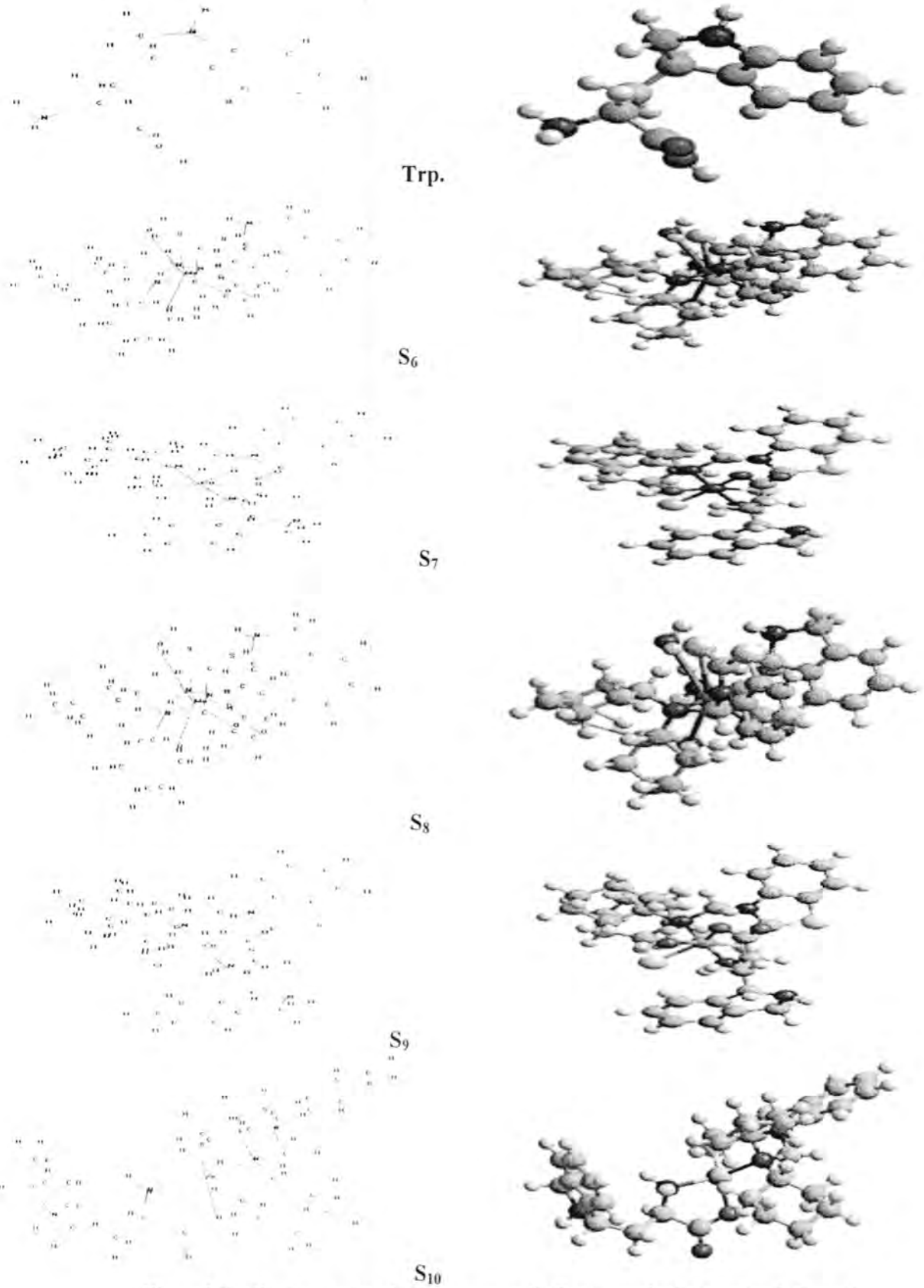


Fig. -14: Conformational Structure of(Trp.) and their mixed ligand complexes (S_6-S_{10})

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Synthesis and Characterization of Some New 2-Aminobenzothiazole Derivatives

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Received 12/12/2011 – Accepted 18/4/2012

الخلاصة

تم تحضير المركب S_1 (2-أمينوبينزوثايوزول) من تكاثف الأنلين مع ثايوسيانات الامونيوم بوجود البروم كعامل مساعد ثم تم استخدامه في تحضير المركبات المطلوبة من خلال الخطوات التالية :

أولاً : تمت معاملة المركب2-أمينوبينزوثايوزول مع *m*- نتروبنزالديهايد في الأيثانول المطلق لتحضير قاعدة شيف S_2 التي عند تفاعلها مع انهدريد السكسنيك في البنزين الجاف أعطت المركب ذو الحلقة الغير المتجانسة السباعية S_3 .

وعند مفاعلة المركب S_2 مع مركبتو حامض الخليك في الأيثانول أعطى المشتق S_4 الذي يحتوي على حلقة خماسية غير متجانسة .

ثانياً : تفاعل المركب 2-أمينوبينزوثايوزول مع *p* – كلورو فنيل ايزوثايوسيانيت أعطى المشتق S_5 الذي عند معاملته مع كلورو حامض الخليك اعطى المركب S_6 الذي يحتوي على حلقة ثايازول إضافية .

ثالثاً : عند معاملة المركب 2-أمينوبينزوثايوزول مع (انهدريد السكسنيك و انهدريد الفثاليك) تكون المركبان S_{7a} وS_{7b} اللذان يحتويان على مجموعة كاربوكسيلية والتي عند معاملتها مع المركب *o*- فنلين ثنائي الأمين أعطت المركبان S_{8a} و S_{8b} اللذان يحتويان على حلقة الأيميدازول .

رابعاً : إن تفاعل المركب 2-أمينوبينزوثايوزول مع كلوريد الكلورو اسيتيل أعطى المركب S_9 الذي عند معاملته مع أمينات أروماتية أولية وثانوية مختلفة أعطى أميدات جديدة مختلفة S_{10a-e}

ABSTRACT

Compound S_1 (2-aminobenzothiazole) was prepared through the condensation of aniline with amoniumthiocyanate in presence of bromine as a catalyst[3],its derivatives were synthesized through four different lines as follows .

1: 2-aminobenzothiazole was treated with *m*-nitro banzaldehyde in absolute ethanol to obtain shiff's base S_2 . Shiff's base was treated with succinic anhydride in dry benzene to obtain a compound with seven membered heterocyclic ring S_3 . Compound S_2 was treated with mercaptoacetic acid in ethanol to obtain a compound with five membered heterocyclic ring S_4.

2: 2-aminobenzothiazole was treated with *p*-chlorophenylisothiocyanate to obtain thiourea derivative compound S_5 and the later was treated with chloroacetic acid to obtain a compound with two thiazole rings compound S_6.

3 : 2-aminobenzothiazole was treated with two different anhydrides (succinic anhydride & phthalic anhydride) to obtain 2-aminobenzothiazole derivatives with carboxylic acid moiety S_{7a-b} and these two compounds were treated with *o*-phenylenediamine to obtain heterocyclic compounds with imidazole ring S_{8a-b}.

4 : 2-aminobenzothiazole was treated with chloroacetyl chloride to obtain compound S_9 which was treated with different primary and secondary aromatic amines to obtain different amide derivatives S_{10a-e} .

INTRODUCTION

2- substituted benzothiazole has immerged in its usage as a core structure in the diverse therapeutical applications . The studies of structure – activity relationship interestingly reveal that change of the structure of substituent group at C-2 position commonly results the change of its bioactivity[1] . Since most of the benzothiazole derivatives were reported for their diversified activity such as antitumor, anti-tubercular, antimalarial , anticonvulsant , anthelmintic , analgesic , anti-

inflammatory and antifungal **[2]** . Recently , several new methods have been reported , some of the most common methods for synthesis of 2-aminobenzothiazole and its derivatives are as follows : 1) Hofmann method 2) Jacobson cyclyzation 3) Using Bromine as catalyst 4) Using Sulfuric acid as a catalyst 5) Using Benzen as a catalyst 6) Copper and palladium catalyzed cyclization and many other methods .

MATERIALS AND METHODS

Materials :

All materials were from BDH ,FLUKA and REDLE –DE HAEN . All other solvents were analar grade .

Instruments :

Melting points were measured on a Gallan Kamp MFB-600 Melting point apparatus and were uncorrected .

FTIR spectra were recorded as potassium bromide (KBr) disk on FTIR -8400S Fourer Transform Infrared Spectrophotometer "SHIMADZU"

UV-Visible spectra were recorded on CARY 100 Conc UV-Visible Spectrophotometer "VARIAN"

H^1 NMR spectra were recorded on Burker DMX- 500 NMR (300-600 \MHz)Spectrophotometer with using DMSO as a solvent in Jordan .University.

Preparation of compound(S_1) [3]

(2-aminobenzothiazole)

Aniline (4.6g ,0.05mol) and ammonium thiocyanat (3.8g ,0.05 mol) were dissolved in absolute ethanol containing 4 ml of con. HCl .To this mixture bromine in glacial acetic acid (6.75ml,0.125 mol) was added and the reaction mixture was refluxed for 1 hr. Then it was cooled in ice bath. The precipitate obtained was filtered, washed with cold water and dried . The crude product was recrystallized from ethanol . The physical properties are listed in table 2.

Preparation of compound(S_2) [4]

***(N*-(3-nitrobenzylidene)-1,3-benzothiazol-2-amine)**

To a mixture of compound (S_1) (1.50g ,0.01mol) and m-nitro benzaldehyde (1.51g ,0.01mol) in (30ml) ethanol , 3 drops of glacial acetic acid were added . The mixture was refluxed for 7 hrs. The solvent was evaporated under reduced pressure and the precipitated solid was washed with petroleum ether (range of B.P =60-80^{0}C)and recrystallized from ethanol. The physical properties are listed in table 2.

Preparation of compound (S_3) [5]

(3-(1,3-benzothiazol-2-yl)-2-(3-nitrophenyl)-1,3-oxazepane-4,7-dione)

Mixture of compound (S_2) (0.28g , 0.001mol) with succinic anhydride (0.1g ,0.001mol) in (10 ml) of dry benzene was refluxed on

water bath for 1hr. The solvent was evaporated and the precipitated solid was recrystallized from tetrahydrofuran (THF). The physical properties are listed in table 2.

Preparation of compounds (S_4) [6]

(3-(1,3-benzothiazol-2-yl)-2-(3-nitrophenyl)-1,3-thiazolidin-4-one) , (*N*-(1,3-benzothiazol-2-yl)-2-{[2-(3-nitrophenyl)-4-oxo-1,3-thiazolidin-3-yl]amino}acetamide)

Compound S_2(0.15g ,0.001mol)and mercaptoacetic acid (0.001mol) was heated under reflux for 8hrs . The reaction mixture was neutralized with 10% sodium carbonate solution .The solid was. filtered off, washed with water, dried and recrystallized from toluene The physical properties are listed in table 2.

Preparation of compound(S_5) [7]

(1-(1,3-benzothiazol-2-yl)-3-(4-chlorophenyl)thiourea)

A mixture of compound S_1 (1.5g , 0.01mol) and p-chloro phenyl isothiocyanate (1.7g ,0.01mol) in (50ml) dry dioxan was refluxed for 7hrs. The reaction mixture was concentrated and the obtained solid was filtered off, dried and recrystallized from ethanol-dioxan mixture . The physical properties are listed in table 2.

Preparation of compound (S_6) [7]

((2*E*)-3-(1,3-benzothiazol-2-yl)-2-[(4-chlorophenyl)imino]-1,3-thiazolidin-4-one)

A mixture of compound (S_5) (0.32g , 0.001mol) and chloro acetic acid (0.094g, 0.001mol) in dioxan was refluxed for 5hrs. After cooling , the formed solid was filtered off ,dried and recrystallized from ethanol. The physical properties are listed in table 2.

2.2.7 Preparation of compounds (S7a-b) [8]

(2-(1,3-benzothiazol-2-ylcarbamoyl)benzoic acid) &(4-(1,3-benzothiazol-2-ylamino)-4-oxobutanoic acid)

A mixture of compound (S_1) (0.76g , 0.005mol) and anhydride (0.005mol) in glacial acetic acid was refluxed for 3hrs and then cooled . The formed solid filtered off, dried and recrystallized from dioxan . The physical properties are listed in table 2.

2.2.8 Preparation of compounds (S_{8a-b}) [9]

(*N*-(1,3-benzothiazol-2-yl)-2-(2,7a-dihydro-1*H*-benzimidazol-2-yl)benzamide) & (*N*-(1,3-benzothiazol-2-yl)-3-(2,7a-dihydro-1*H*-benzimidazol-2-yl)propanamide)

Compounds (S_{7a-b}) (0.005mol) and o-phenylenediamine (0.005mol) were dissolved in absolute ethanol , the reaction mixture was refluxed for 12hrs. It was cooled and poured onto (30ml) ice cold water containing (1ml) of conc. HCl .The precipitate which was allowed to settle down for 1hr at room temperature was filtered off , dried and recrystallized from ethanol . The physical properties are listed in table 2.

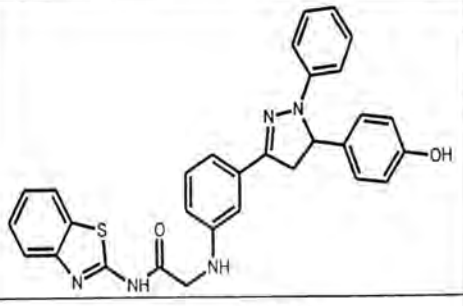
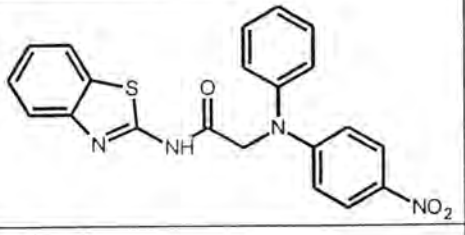
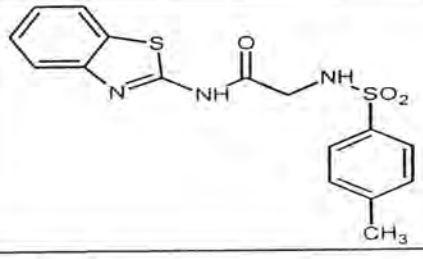
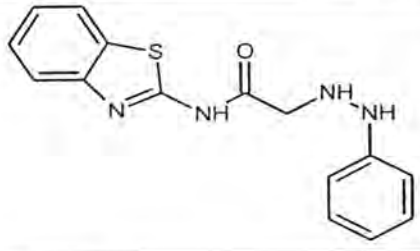
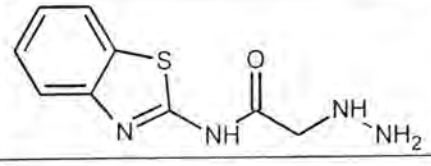
2.2.9 Preparation of compound (S_9) [10]
(N-(1,3-benzothiazol-2-yl)-2-chloroacetamide)

To a solution of compound (S_1) (2.5 g , 0.016mol) in (30ml) glacial acetic acid , chloroacetyl chloride (3.7g, 0.032mol) was added drop wise with constant stirring .The reaction mixture was refluxed for 5 hrs then it was powered onto crushed ice .The precipitated solid that obtained was filtered off , washed with cold water , dried and recrystallized from aqueous ethanol . The physical properties are listed in table 2.

2.2.10 Preparation of compounds (S_{10a-e}) [11]

A mixture of compound (S_9) (0.001mol) and amines (0.001mol)in DMF in presence of (0.2g) sodium carbonate was refluxed for 10hrs, then it was poured onto ice-cold water and stirred for 30 minutes. The reaction mixture was filtered and the precipitated solid was dried and recrystallized from ethanol . The physical properties are listed in table 2.

Table-1: the structure & the scientific name for compounds S_{10a-e}

Comp. No.	Comp. Structure	Comp. Name
S_{10a}		N-(benzo[d]thiazol-2-yl)-2-((3-(5-(4-hydroxyphenyl)-1-phenyl- 4,5-dihydro-1H-pyrazol-3-yl) phenyl)amino) acetamide
S_{10b}		*N*-(1,3-benzothiazol-2-yl)-2-[(4-nitrophenyl)(phenyl)amino] acetamide
S_{10c}		*N*-(1,3-benzothiazol-2-yl)-2-{[(4-methylphenyl)sulfonyl] amino}acetamide
S_{10d}		*N*-(1,3-benzothiazol-2-yl)-2-(2-phenylhydrazinyl)acetamide
S_{10e}		*N*-(1,3-benzothiazol-2-yl)-2-hydrazinylacetamide

RESULTS AND DISCUTION

2-aminobenzothiazolewas prepared by condensation reaction between equimolar quantities of aniline and ammonium thiocyanate in presence of bromine as a catalyst [3] .The structure of this compound is confirmed by its spectral data. FTIR spectrum (Fig. No. 1) shows appearance of doublet band of (NH_2) stretching frequency at 3394-3271 cm^{-1} and (C=N) stretching frequency at 1641 cm-1. UV spectrum (Fig. No. 2) shows appearance of three absorption peaks, at 262 nm for (n -

π^*) transitions, at 222 nm for (π - π^*) of aromatic benzene ring and 205 nm for other (π -π^*) transitions.

Schiff's base (S_2) was prepared by condensation reaction between m-nitro banzaldehyde and 2-aminobenzothiazole . The structure of this compound is confirmed by its spectral data . FTIR spectrum (Fig. No. 3) shows appearance of (C=N) stretching frequency at about 1604 ,beside that it shows disappearance of doublet band of (NH_2) group of 2-aminobenzo- thiazole and disappearance of the stretching frequency that belongs to (C=O) group of *m*-nitro banzaldehyde . H^1NMR spectrum shows peaks at : 7.2-8.5 ppm (8H, Ar-H), 8.6 ppm (1H ,CH=N). UV spectrum shows appearance of three absorption peaks, at 345 nm for (n - π^*) transitions , at 260 nm for (π - π^*) of aromatic benzene ring and at 217 nm for other (π - π^*) .

Reaction of Schiff's base with succinic anhydride leads to prepare compound S_3.The structure of this compound is confirmed by its spectral data. FTIR spectrum shows appearance of (C=O) stretching frequency at 1693 cm^{-1} and disappearance of (C=N) stretching frequency that belongs to Schiff's base .UV spectrum shows appearance of three absorption peaks, at 346 nm for (n - π^*) transitions , at 263 nm for(π - π^*) transitions of aromatic benzene ring and 214 nm for other(π - π^*).

Compound S_4 was prepared through the condensation between 2-aminobenzothiazole and mercaptoacetic acid. The structure of this compound is confirmed by its spectral data. FTIR spectrum shows appearance of (C=O) stretching frequency at 1705 cm^{-1}. UV spectrum shows appearance of two absorption peaks, at 259 nm for (n - π^*) transitions, at 210 nm for (π - π^*) transitions of aromatic benzene ring .

2-aminobenzothiazole reacts with 4-chloro phenyl isothiocyanate in dry dioxan to produce thiourea derivative (compound S_5) . The structure of this compound is confirmed by spectral data . FTIR spectrum (Fig. No. 4) shows appearance of singlet band of (N-H) stretching frequency at 3171 cm^{-1} instead of the doublet band of (NH_2) group , it also shows appearance of (C=S)stretching frequency at 1188 cm^{-1}. UV spectrum shows appearance of three absorption peaks, at 342 nm for (n - π^*) transitions, at 315 nm for (π - π^*) transitions of aromatic benzene ring and at 216 nm for other (π - π^*) transitions .

Condensation reaction between compound S_5 and chloroacetic acid produces compound S_6 through ring closure reaction . The structure of this compound is confirmed by its spectral data . FTIR spectrum shows appearance of (C=O) stretching frequency at 1728 cm^{-1} and (C-S) stretching frequency at 1141 cm^{-1} and disappearance the stretching frequencies for (N-H) group and (C=S) group H^1NMR spectrum (Fig. No. 6) shows : 4.2 ppm (2H,CH_2),7.3-7.9 ppm (8H, Ar-H).UV spectrum

shows appearance of three absorption peaks, at 320 nm for (n - π*) transitions, at 261 nm for (π - π*) transitions of aromatic benzene ring and at 208 nm for other (π - π*) transitions .

2-aminobenzothiazole was acylated by succinic and phthalic anhydride in glacial acetic acid to form compounds S_{7a-b} The structures of these compounds are confirmed by their spectral data . FTIR spectrum shows appearance of (O-H) stretching frequency at 3254 cm^{-1} for compound S_{7a} and at 3259 cm^{-1} for compound S_{7a}. It also shows appearance of the stretching frequencies of (C=O) of carboxylic group at 1693 cm^{1} for compound S_{7a} and at 1734 cm^{-1} for compound S_{7b} while (C=O) stretching frequency of amide group appears at 1630 cm^{-1} for compound S_{7a} and at 1695 cm^{-1} for compound S_{7b} .UV spectrum shows appearance of two absorption peaks for each compound, the first at 273 nm of (n - π*) transitions for compound S_{7a} and at 274 nm for compound S_{7b}. The second at 244nm of (π - π*) transitions for compound S_{7a} and at 220 nm for compound S_{7b}.

The condensation reaction between compounds (S_{7a-b}) and *o*-phenylene diamine produces benzimidazole derivatives (compounds S_{8a-b}) . The structures of these compounds are confirmed by their spectral data. FTIR spectrum shows disappearance of stretching frequencies of each (O-H) group and (C=O) group that belong to carboxylic groups in the precursor compounds, while(C=O) stretching frequencies that belong to amide groups are apparent at 1656 cm^{-1} for compound S_{8a} and at 1648 cm^{-1} for compound S_{8b} .UV spectrum shows appearance of three absorption peaks for each compound, at 297 nm of (n - π*) transitions for compound S_{8a} and at 287 nm for compound S_{8b} , at 274nm for (π - π*) transitions of aromatic benzene ring for compound S_{7a} and at 220 nm for the same moiety for compound S_{8b} and at 206 nm of other (π - π*) transitions for compound S_{8a} and at 205 nm for compound S_{8b} .

2-aminobenzothiazole on reaction with chloro acetylchloride gives amide derivative (compound S_9) through S_N2 mechanism . The structure of this compound is confirmed by its spectral data . FTIR spectrum shows appearance of singlet band of (N-H) stretching frequency at 3178 cm^{-1} instead of the doublet band of (NH_2) group which belongs to (2-aminobenzothiazole),it also shows appearance of (C=O)stretching frequency at 1701cm^{-1}.UV spectrum shows appearance of three absorption peaks, at 297 nm for(n - π*) transitions , at 274 nm for (π-π*) transitions of aromatic benzene ring and at 208 nm for other (π - π*) transitions .

The reaction of primary and secondary amines with chloroacylated derivative of (2-aminobenzothiazole) proceeds through S_N2 mechanism and produces different amide derivatives(S_{10a-e}) .The structures of these compounds are confirmed by their spectral data. FTIR spectrum for

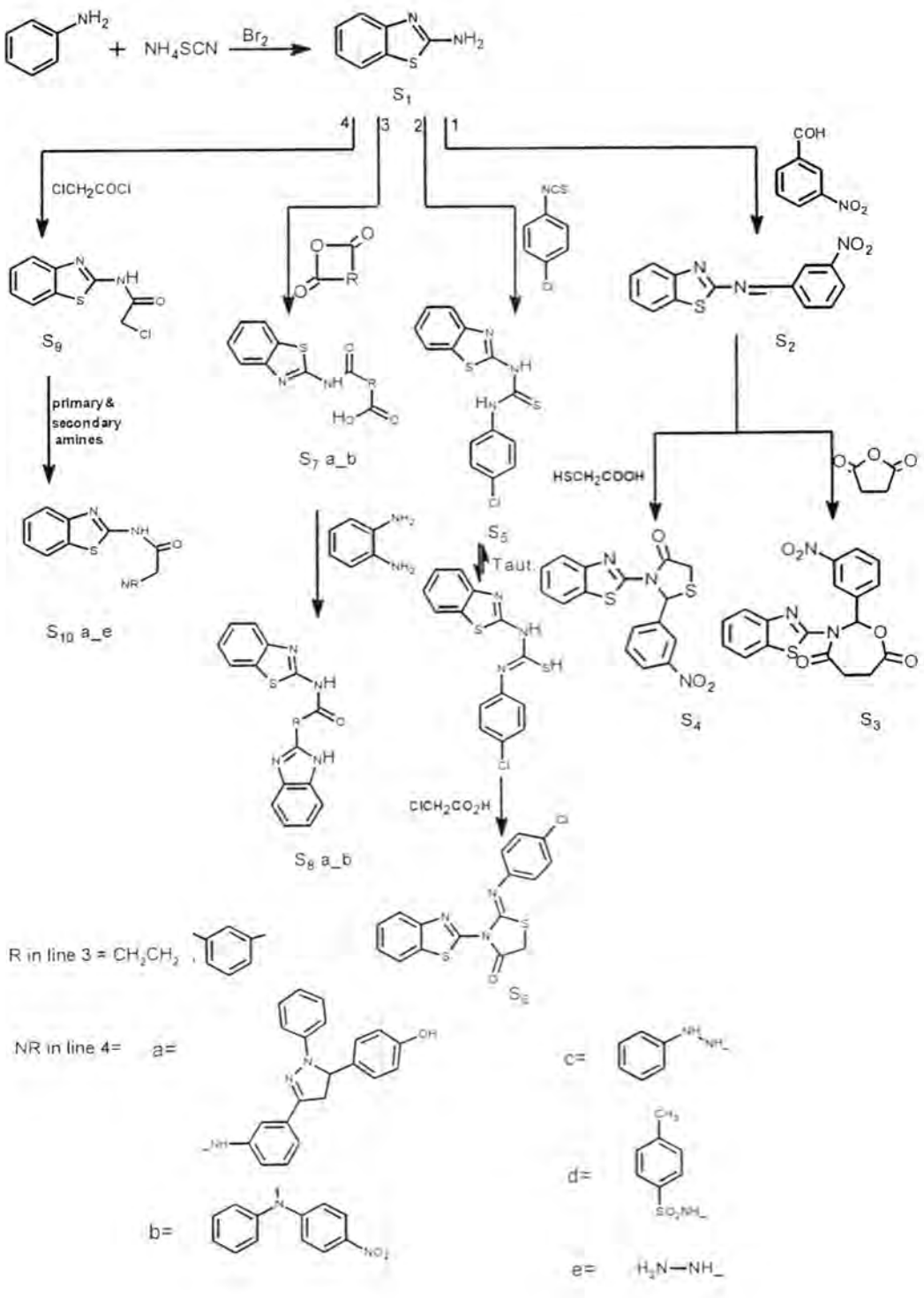
compound S_{10e} (Fig. No. 5) shows appearance of doublet band for (NH_2) stretching frequency at 3201(asymmetric),3128 cm^{-1} (asymmetric) and a singlet band for (NH) stretching frequency at 3317 cm^{-1}, it also shows appearance of (C=O) stretching frequency at 1649 cm^{-1} .UV spectrum for compound S_{10e} shows appearance of two absorption peaks, at 340 nm for (n - π^*) transitions and at 221 nm for (π - π^*) transitions .

Table-2 The physical properties for the synthesized compounds

Comp. No.	Color	m.p^{0}C	Yield%	Mol . Formula
S_1	pale yellow	117-120	55	$C_7H_6N_2S$
S_2	Yellowish green	179-182	77	$C_{14}H_9N_3SO_2$
S_3	Yellow	158-161	56	$C_{18}H_{11}N_3SO_5$
S_4	dark yellow	150-153	60	$C_{16}H_{10}N_3S_2O_3$
S_5	pail white	207-210	53	$C_{14}H_{10}N_3S_2Cl$
S_6	dark yellow	240-243	69	$C_{16}H_{12}N_3S_2OCl$
S_{7a}	White	152-155	40	$C_{11}H_{10}N_2SO_3$
S_{7b}	White	177-180	43	$C_{17}H_{10}N_2SO_3$
S_{8a}	brown light	179-181	35	$C_{17}H_{14}N_4SO$
S_{8b}	light brown	250-252	40	$C_{21}H_{15}N_4SO$
S_9	Pail white	143-145	97	$C_9H_7N_2SOCl$
S_{10a}	Brown	82-83	60%	$C_{27}H_{25}N_5SO_2$
S_{10b}	Brown	120-122	51	$C_{21}H_{16}N_4SO_3$
S_{10c}	Pale yellow	131-133	57	$C_{16}H_{15}N_3S_2O_3$
S_{10d}	Brown	68-69	71	$C_{15}H_{14}N_4O$
S_{10e}	Pale yellow	100-102	60	$C_9H_{10}N_4SO$

Table-3 The spectral data for the synthesized compounds

Comp No.	UV (EtOH)	Characteristic bands of FT-IR (cm^{-1} , KBr disk)			
	λ max	ν (N-H) cm^{-1}	ν (C=O) cm^{-1}	ν (C-H) cm^{-1}	ν (others) cm^{-1}
S_1	262 222 205	(NH_2) =3394-3271	---------	ar. =3055	ν (C=N) = 1641 ν (C-S) =1107
S_2	345 260 217	----------	---------	ar. =3086 al. =2978	ν (NO_2) =1531-1350 ν (C-S) =115 ν (C=N) =1604
S_3	346 263 214	--------	1693	ar. =3080 al. =2978	ν (C-O) =1074 ν (C-N) =1205
S_4	259 210	----------	1705	ar. =3086 3059	ν (C-S) =1091 ν (C-N) =1284
S_5	342 315 216	3171	----------	ar. =3020	ν (C=S) =1309 ν (C-Cl) =653
S_6	320 261 208	----------	1728	ar. =3095	ν (C-S) =1141
S_{7a}	273 244	3134	1693,1630	ar =3059 al=2929	ν (O-H) =3254
S_{7b}	274 220	3213	1734,1695	ar=3059	ν (O-H) =3259
S_{8a}	297 274 206	3186 3373	1648	ar =3053 al=2970	ν (C=N) =1599
S_{8b}	287 220 205	3167 3140	1648	ar=3064	ν (C=N) =1600
S_9	297 274 208	3178	1701	ar =3053 al =2960	ν (C-Cl) =740
S_{10a}	345 228	3218	ν (C=O) =1669	ar =3059 al=2929	ν (O-H) =3259
S_{10b}	344 220	3173	ν (C=O) =1689	ar=3022 al=2968	ν (NO_2) =1552-1305
S_{10c}	335 219	3414	ν (C=O) =1639	ar=3078 al=2947	ν (SO_2) =1411-1128
S_{10d}	333 220	3221	ν (C=O) =1653	ar=3050 al=2933	ν (C=S) =1300
S_{10e}	340 221	3317	ν (C=O) =1649	ar=3059 al=2956	ν(NH_2) = 3201-3128


NH4SCN
Br2
S1
ClCH2COCl
S9
primary & secondary amines
S10 a_e
S7 a_b
S5
Taut.
S8 a_b
ClCH2CO2H
S6
HSCH2COOH
S4
S3
S2
R in line 3 = CH2CH2
NR in line 4=
a=
b=
c=
d=
e=
H2N—NH_

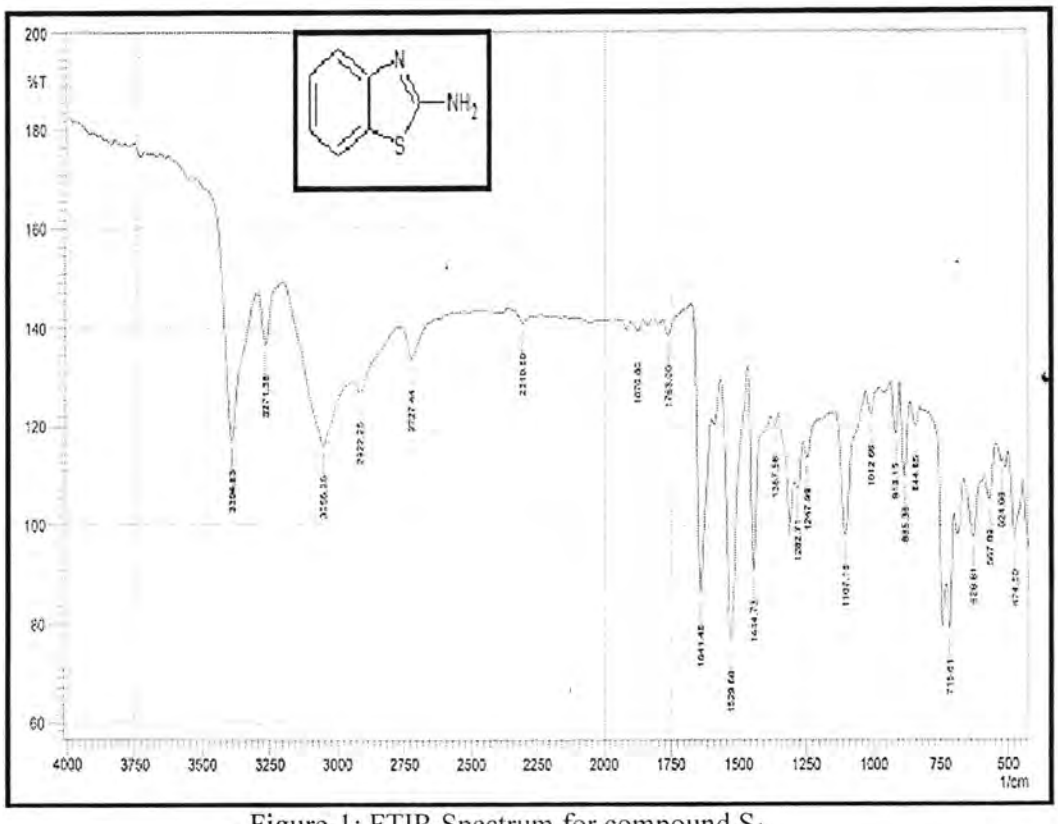


Figure-1: FTIR Spectrum for compound S_1

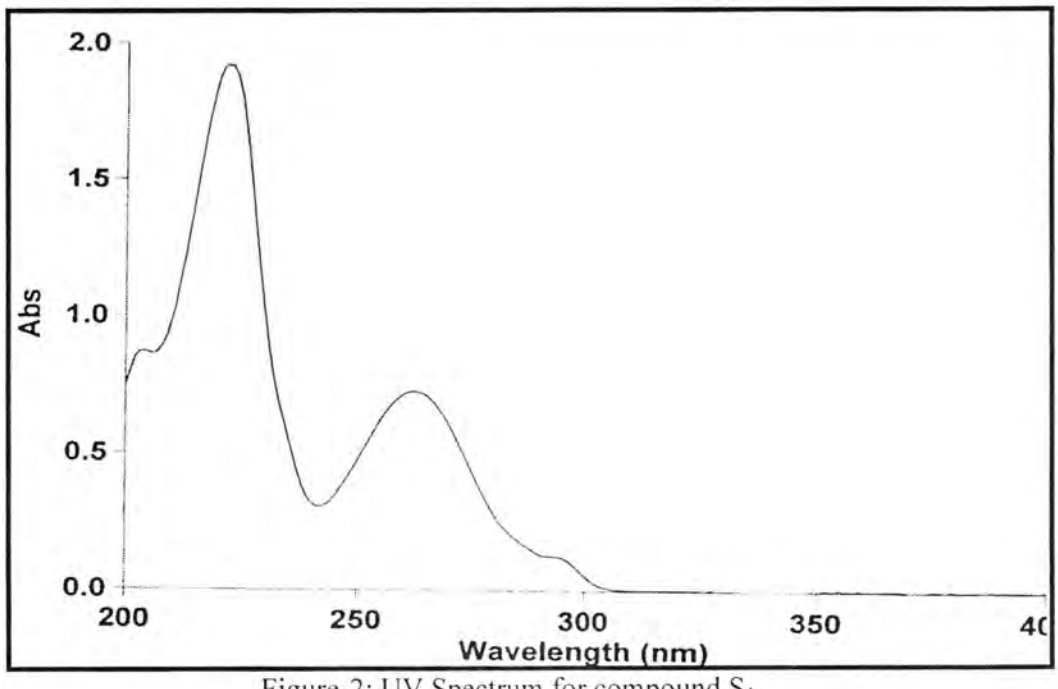


Figure-2: UV Spectrum for compound S_1

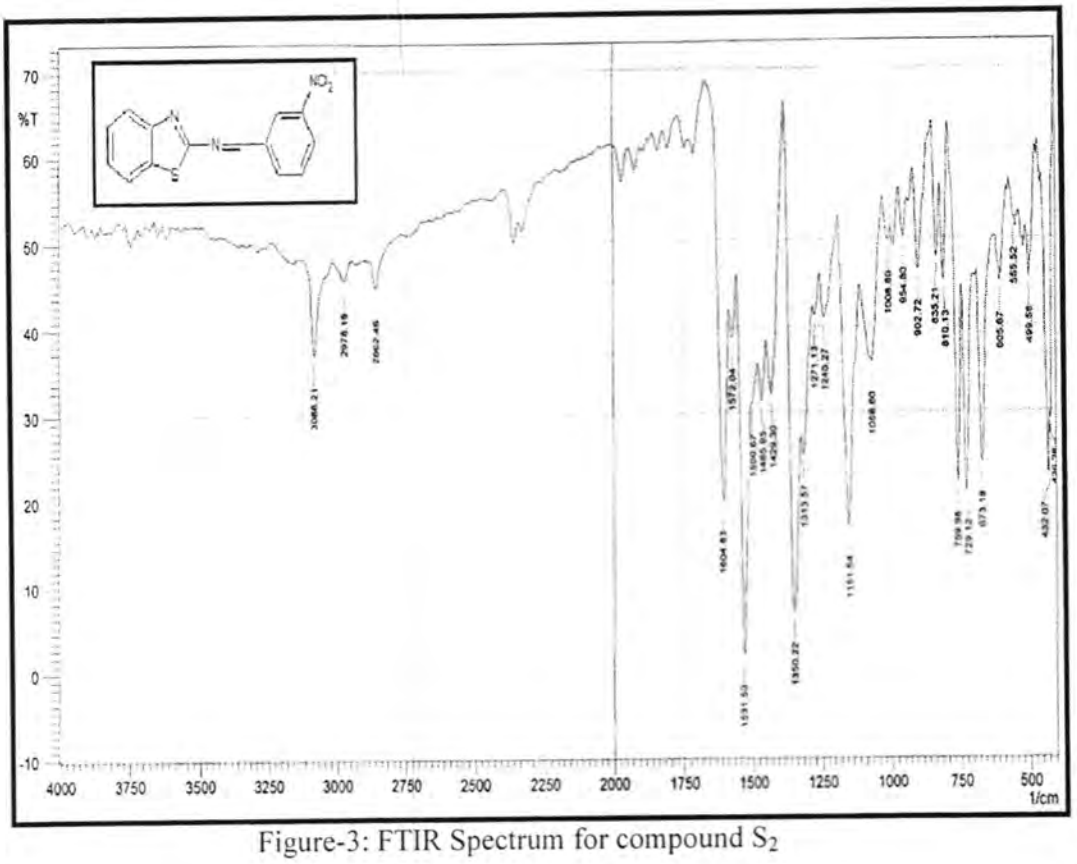


Figure-3: FTIR Spectrum for compound S_2

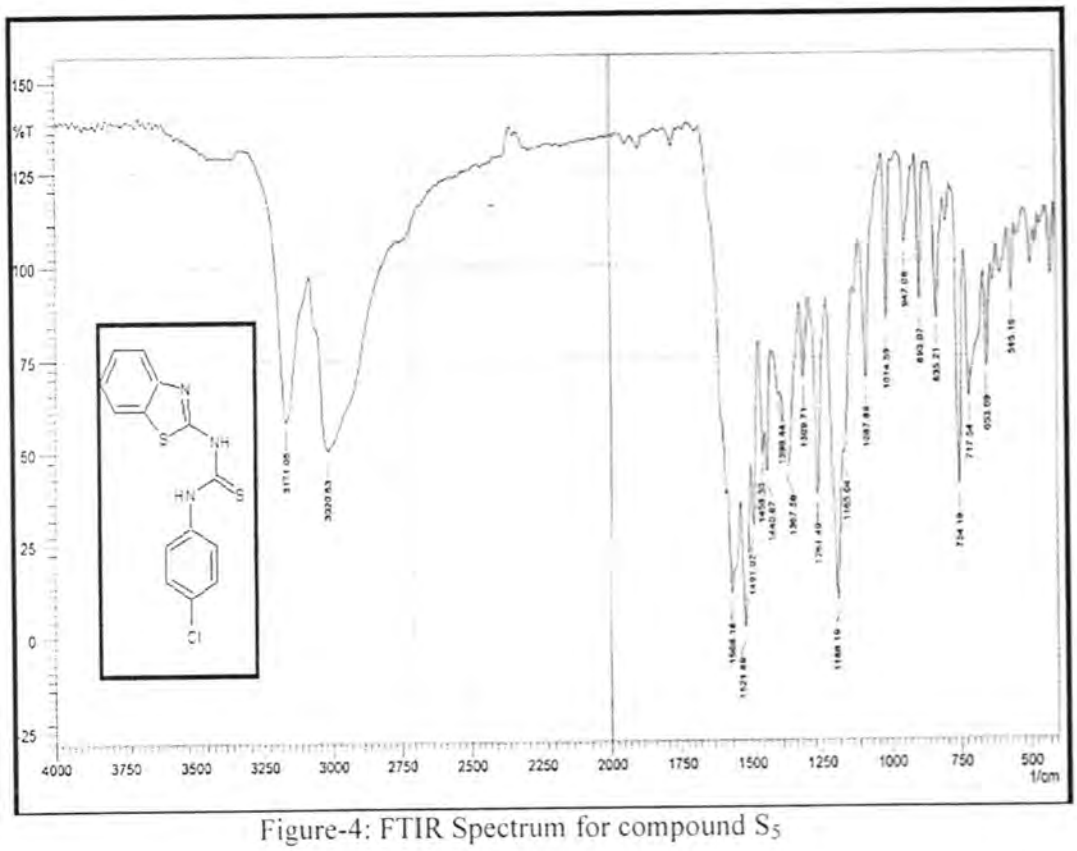


Figure-4: FTIR Spectrum for compound S_5

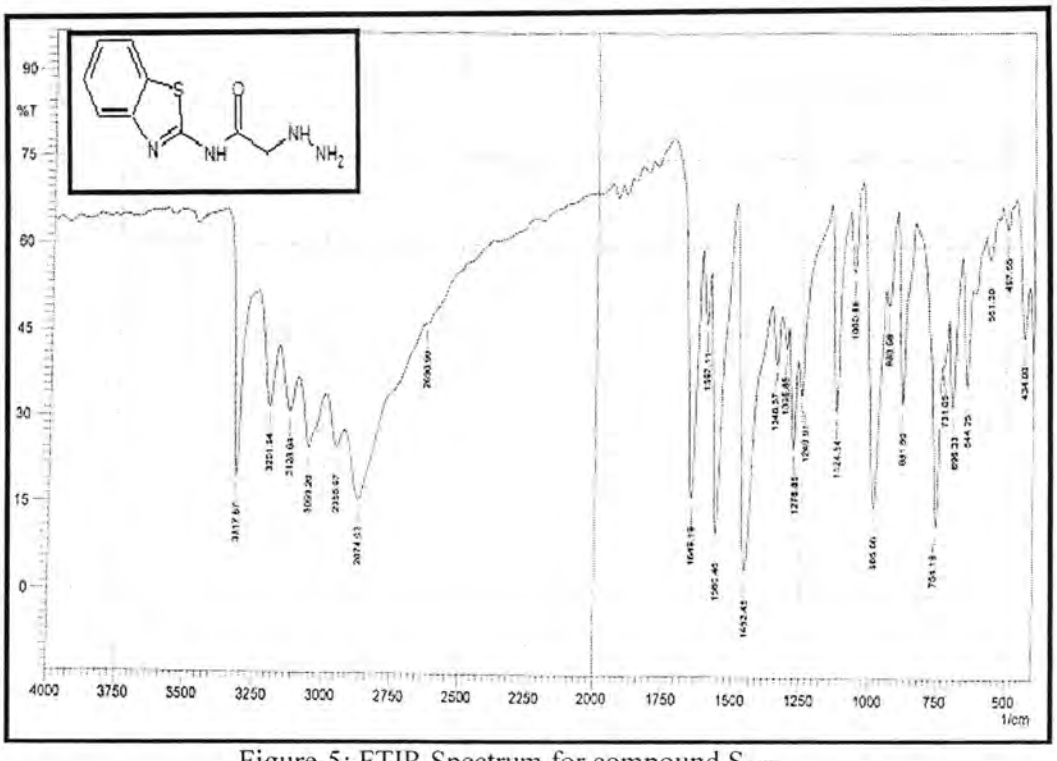


Figure-5: FTIR Spectrum for compound S_{10e}

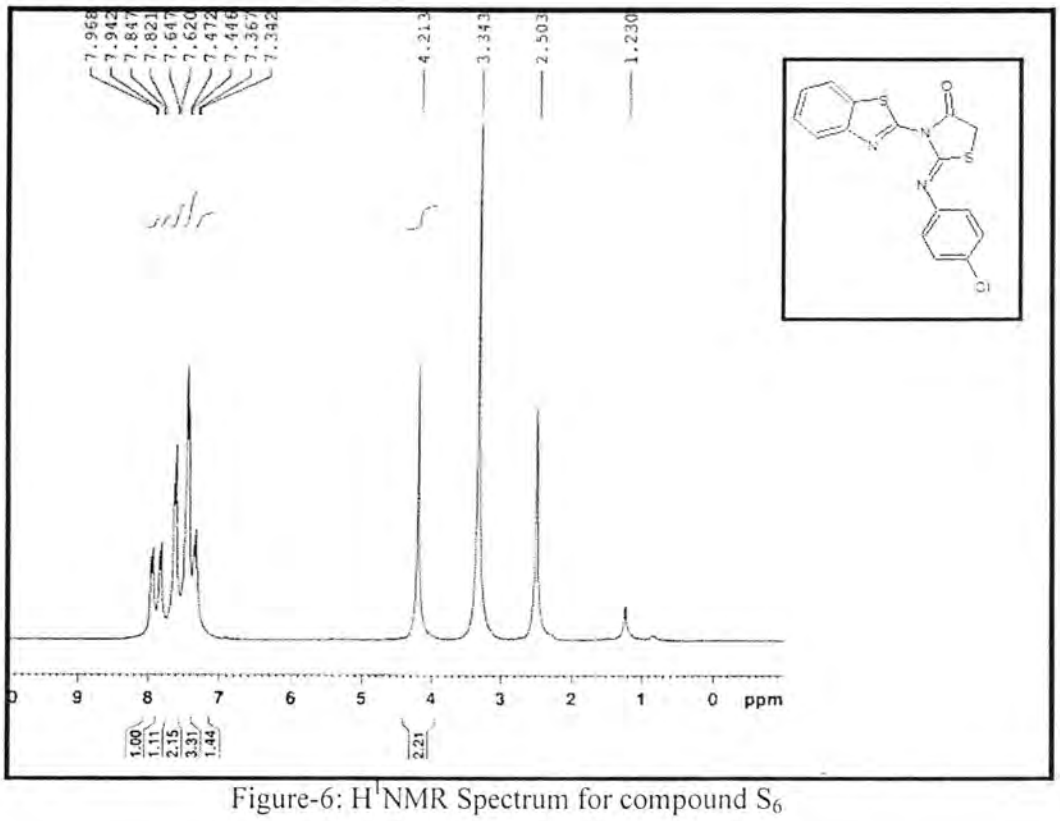


Figure-6: H^1NMR Spectrum for compound S_6

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Polyurethane Foam Waste for Removal of Ni(II) From Aqueous Solutions

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Received 11/2/2011 – Accepted 18/4/2012

الخلاصه

في هذه العمل تمت دراسة سلوك ازالة ايونات النيكل من المحلول المائي باستخدام رغوة البولي يوريثان PUF . وقد وجد ان اكبر كميه من ايونات النيكل تمت ازالتها في الداله الحامضيه 8 . تمت دراسة العوامل المؤثره على الامتزاز وقد كشفت الدراسه بان زيادة تركيز النيكل من 10-200 mg/L ودرجة حراره من 35- 55°C تزيد من عملية ازالة ايونات النيكل. ومن نتائج حركية الامتزاز ظهر ان امتزاز ايونات النيكل على سطح ال PUF تتبع معادلة الحركيه من الدرجه الثانيه . اما نتائج الدراسة الثرموداينميكيه فقد اوضحت بان العمليه ماصه للحراره وان ميكانيكية الامتزاز فيزيائيه.

ABSTRACT

In this study, the uptake behavior of Ni(II) ions from aqueous solution using waste polyurethane foam (PUF) was investigated. It was found that the pH of maximum adsorption is 8. The parametric study reveals that the increase of adsorbate concentrations (10 to 200 mg/L) and temperature (35 – 55°C) enhances the removal of Ni(II) ions, and from application of kinetic data it is inferred that the adsorption of Nickel ions onto PUF follow second order rate equation. The equilibrium adsorption data are tested for Langmuir and Freundlich equations, results indicate that both isotherms are applicable. The thermodynamic study reveals that the process is endothermic in nature and the mechanism of adsorption is physical.

Key words: adsorption, Nickel ions, thermodynamic parameters, Isotherms.

INTRODUCTION

Nowadays, polyurethane products are an important part of everyday life. The variety of polyurethane products reaches from flexible and rigid foams over thermoplastic elastomers to adhesives, paints and varnishes. This variety of usage would result in a huge amount of consumption, causing some environmental problem [1]. Solid waste disposal has been a problem in today's world, and overall trends indicate that the overall amount of solid wastes that we generate continues to increase.

Because polyurethanes are used in so many diverse applications and industrial uses, they enter the municipal solid wastes stream, usually by ways of discarded consumers and industrial products. These products, e.g. upholstered furniture, mattresses and automobile parts, are frequently durable goods with long lifespan. By weight, approximately 1.3 million tons of waste polyurethanes are generated each year only in the US as part of the municipal solid wastes representing 5% of all current plastic waste [2]. Polyurethane foams (PUF) can be defined as plastic materials in which a proportion of the solid phase is replaced by gas in the form of numerous small bubbles (cell) [3].

Usually Polyurethane foams prepared in soft, flexible and rigid forms using a variety of polyesters and polyether polyol. The two most

important reactions in the preparation of urethane foams are those between isocyanate and hydroxyl compounds (polyether and polyester polyols) and those between isocyanate and water [4]. In the literature we have seen that unloaded PUF only adsorbs metal ions after complex formation. Organic and Inorganic ligands, can be used for this purpose, for example, Molybdenum (VI) ions were quantitatively extracted with unloaded PUF after formation of thiocyante complexes [5] loaded PUF offers a wider field of applications than unloaded PUF. polyurethane loaded with dimethylglyoxime was, for example, proposed for the selective extraction of Nickel ions [6].

This paper deals with the use of rigid PUF waste, which has been used for gap filling or insulation purposes, for adsorption of toxic heavy metal ions like nickel from aqueous solution. The prime objective of this work is to exploit the waste materials for removal of pollutants from water.

MATERIALS AND METHODS

Materials

Rigid polyurethane foam was collected from local waste collection yard where washed several time with distilled water and dried, then shreded to small particles which pass through sieve no.35, Then washed with acetone in order to remove most of the plasticizer, then treated with 0.1M Hcl solution and washed repeatedly with deionized water until it was free from acid and dried in vacuum oven at 60°C for 24 hrs. Nickel nitrate of analytical grade was used to prepare a stock solution of 500 mg/L.

Sorption procedure and analysis

The metal adsorption experiments were performed using batch process under optimum conditions. The metal binding experiments were conducted within the pH=8 at 35, 45 and 50°C and different concentration of metal ion (10, 20, 40, 80mg/L).

The effect of temperature on the adsorption of Ni(II) onto waste PUF were determined using 12g/ L of PUF in 50ml of Ni(II) solution. Shaking was carried out using horizontal thermostat shaker model LSB 0155, at 150 rpm for 100 minutes. In all batch experiments and after completion of adsorption time, samples were filtered off and the filtrate was analyzed for residual nickel concentration using atomic absorption spectrophotometer type Analyst 200 Perkin Elmer. The amount of Ni(II) adsorbed onto PUF, qe mmol/g was calculated using the following equation .

$$q_e = \frac{(C_o - C_e)V}{M}$$

Where, C_o and C_e are the initial and equilibrium concentration of Ni(II) in the liquid phase respectively, V is the volume of the solution (L) and M is the weight of PUF used (in gram).

RESULTS AND DISCUSSIONS

Characterization of PUF

A fourier transform infrared (FTIR) spectrophotometer from shimadzu model FTIR 8000 was used to analyses the waste PUF adsorbent fig.1 by KBr disk. The characteristic band are: N-H stretching vibration of polyurethane appear at 3282.95cm^{-1}, the peak at 3066.92cm^{-1} is the stretching vibration of C-H of phenyl group of toluene diisocyanate, the absorption peaks at 2980.12 and 2929.97cm^{-1} stretching vibration of aliphatic group of poyol, while the peak at 1639.55cm^{-1} may be for the C=C vibration of phenyl group of diisocyanate.

Figure-1. FTIR spectrum of PUF before adsorption.

Effect of pH on Ni(II) adsorption

pH is an important parameter influencing heavy metal adsorption from aqueous solution. It affects both the surface charge of adsorbent and the degree of ionization of heavy metal in solution [7]. Fig. 2 represents the effect of initial pH of the solution on the adsorption of Ni(II) onto PUF. It was observed that the adsorption of Ni(II) onto PUF increases with increasing the pH values, the optimum pH was found to be pH 8 using 20 mg/L of initial Ni (II) concentration and 12 g/L PUF, beyond pH 8 precipitation of nickel hydroxide is observed. It is believed that the attraction between positive charges of Ni(II) and lone pairs of electrons on the nitrogen atoms of the polyurethane is the main attribution for such process.

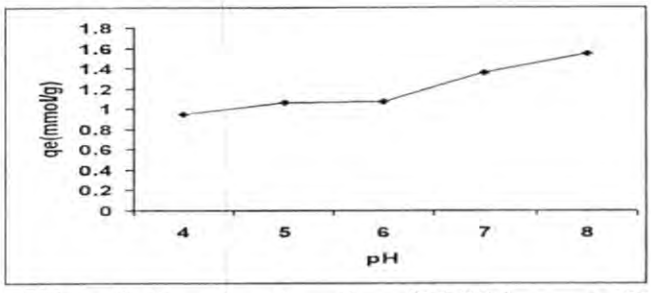

Figure-2. Effect of pH on the adsorption of Ni(II) ions onto PUF waste

Effect of contact time and initial Ni(II) concentration

Effect of contact time for removal of Ni(II) at 12g/L of waste PUF at pH 8 is shown in Fig. 3. There was a rapid removal of Ni(II) from its solution in the first 10 minutes, thereafter little change in the rate of adsorption occurred it was found that 100min contact time is the enough to reach equilibrium. Effect of initial Ni(II) concentration on adsorption was investigated at concentration ranging from 10 to 80 mg/L as shown in Fig. 4. By increasing the Initial Ni(II) concentration the percentage of Ni(II) removal decreased from 66% at 10 mg/L of Ni(II) to 30.2% at 80 mg/L, whereas, the actual amount of Ni(II) adsorbed per unit mass of PUF increased with the increase of initial Ni(II) concentration. This increase is due to the decrease in resistance to the uptake of metal ion from aqueous solution. As, the initial concentration provides an important driving force to overcome the mass transfer resistance of Nickel between the aqueous and solid phases. The same trends was also found by other researchers [8].

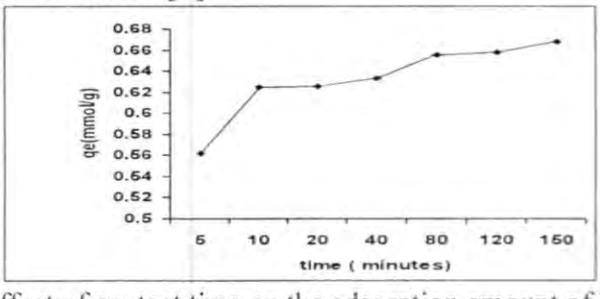

Figure-3. Effect of contact time on the adsorption amount of Ni(II) ions onto PUF waste at pH 8.

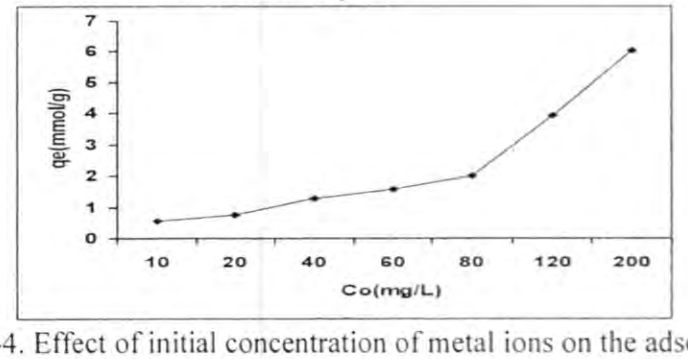

Figure-4. Effect of initial concentration of metal ions on the adsorption amount of Ni(II) ions onto PUF waste at pH 8.

Effect of temperature on adsorption

The effect of the increasing temperature on the adsorption amount of Ni(II) ions onto waste PUF was studied by performing adsorption experiments in a temperature range of 35 to 55°C. The results are shown in Fig. 5, which clearly implies that a rise temperature causes the adsoption amount of Ni(II) ion gradually increases. To predict the adsorption nature of Ni(II) ion on waste PUF the thermodynamic parameters were calculated such as enthalpy (ΔH^o), entropy (ΔS^o) and free energy (ΔG^o) of adsorptions. The values of these parameters were calculated as follows [9-14]:

$$[Ni(II)]_{liquid} \underset{K_2}{\overset{K_1}{\leftrightarrow}} [Ni(II)_{solid}$$

$$K_e = \frac{[Ni(II)]_{solid}}{[Ni(II)]_{liquid}} \quad \text{.....(2)}$$

$$\Delta G^o = -RT \ln K_e \quad \text{.....(3)}$$

$$\log K_e = \frac{\Delta S^o}{2.303R} - \frac{\Delta H^o}{2.303RT} \quad \text{.....(4)}$$

Where K_e is the equilibrium constant, $[Ni(II)]_{solid}$ is the quantity of Ni(II), onto PUF, and $[Ni(II)]_{liquid}$ is the quantity of Ni(II) in solution. T is the temperature (in Kelvin) and R is the gas constant (8.314J / mol. K). The relationship between log K_e and 1/T for adsorption of Ni(II) on PUF is shown in Fig. 6, according to equation 4.

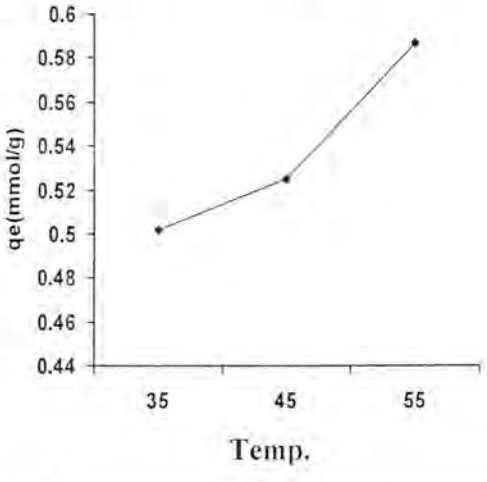

Figure-5. Effect of temperature on the adsorption amount of Ni(II) ions onto PUF waste.

From the slope and intercept of the linear line in figure 6, the thermodynamic parameters of Ni(II) adsoption onto waste PUF were

calculated and were shown in table 1. The results revealed that the adsorption process was endothermic at all temperatures studied because of the positive values of ΔH^o and spontaneous process at 10 mg/ L of Ni(II) due to negative values of ΔG^o. The positive and weak values of ΔG^o at 20, 40 and 80 mg/L of Ni(II) ions indicates that the process is feasible but nonspontaneous. The range of ΔH^o values table 1, indicate that the mechanism of adsorption is physical and positive values of ΔS^o table 1 indicate that the adsorption lead to randomness at the solid / liquid interface .

Table-1. Thermodynamic parameter for adsorption of Ni(II) onto PUF waste 12g/L, for 100 minutes, pH 8 and agitation speed 150 rpm.

C_i mg/L	ΔH^o KJ mol^{-1}	ΔS^o J mol K^{-1}	ΔG^o KJ mol^{-1}		
			35	45	55
10	18.82	64.56	-1.064	-1.72	-2.37
20	8.995	24.33	1.511	1.25	1.02
40	9.957	26.46	1.833	1.50	1.30
80	9.50	28.60	0.678	0.436	0.095

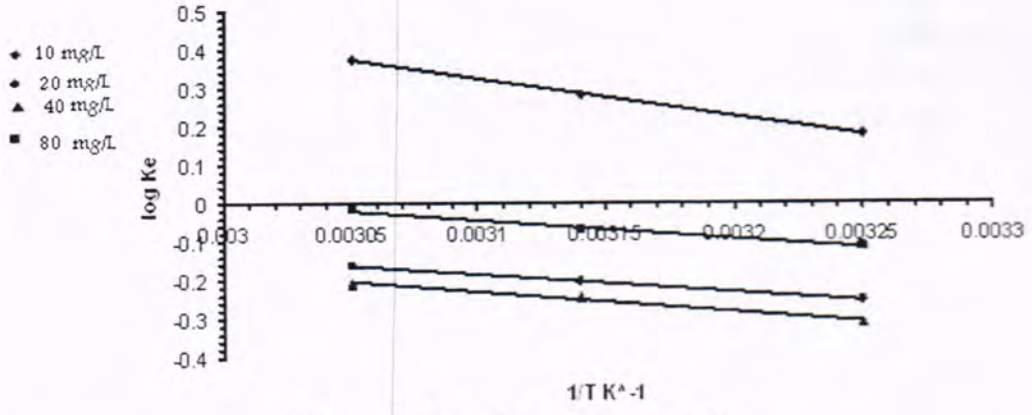

Figure-6. Plot of log K_e versus 1/T

The other parameters that can be calculated from the experimental data are, the activation energy (Ea) and sticking probability (S*), these two parameters can give further support for the involvement of physical adsorption mechanism in the removal of Ni(II) by waste PUF. They were calculated using modified Arrhenius type equation related to surface coverage (θ) as follows [15, 16].

$$\theta = 1 - \frac{C_e}{C_o} \qquad or \qquad \frac{C_e}{C_o} = 1 - \theta \text{.....(5)}$$

$$S^* = (1-\theta)e\frac{-Ea}{RT} \quad \text{.....(6)}$$

$$\ln S^* = \ln(1-\theta) - \frac{Ea}{RT} \quad \text{.....(7)}$$

$$\ln(1-\theta) = \ln s^* + \frac{Ea}{RT} \quad \text{.....(8)}$$

The Sticking probability (S*), is a function of the adsorbent / absorbate system under investigation, its values lie in the range $0 < S^* < 1$ for preferable process and is dependent on the temperature of the system [15, 16]. The plot of the ln (1- θ) against 1/T, Fig.7 should give a straight line with slope of Ea/R and an intercept of lnS*. The values of Ea were comparable with values of ΔH^o table 2, and their values lie in the range of physisorption. The values of sticking probability are less than one , table 2 , which indicate that the probability of Ni(II) ions to stick on surface of PUF is high as $S^* < 1$ these values confirm that , the sorption process is physisorption.

Table-2. Sticking probability for adsorption of Ni(II) onto PUF waste, 12g/L, for 100 minutes, pH 8 and agitation speed 150 rpm.

C_i mg/L	S*	E_a kJ/mole	R^2
10	0.0033	12.26	0.9967
20	0.167	3.45	0.9995
40	0.168	3.53	0.9941
80	0.101	4.414	0.9778

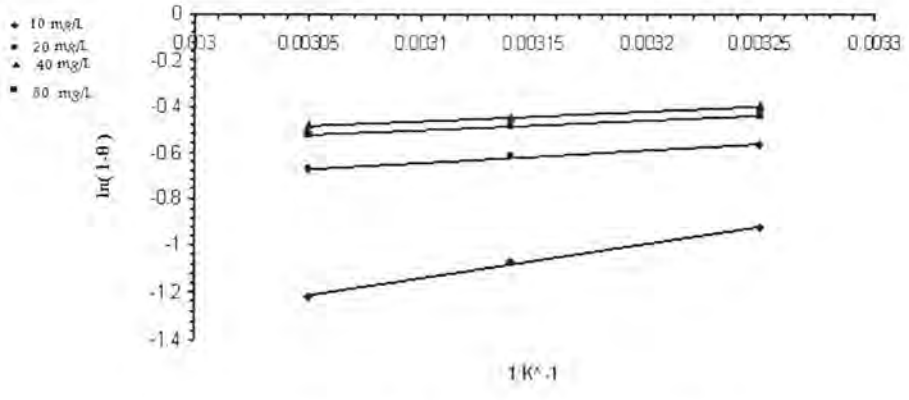

Figure-7. Plot of (1- θ) versus 1/T

Sorption kinetics of Ni(II) onto PUF

Two models have been suggested to express the kinetics of adsorption of solute molecules onto adsorbent. These models were pseudo-first-order and pseudo-second-order models.

The pseudo-first- order kinetic model used was [17]:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad \text{.....(9)}$$

The pseudo-second-order kinetic model used was [18]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad \text{.....(10)}$$

In these models, k_1 is the rate constant for the pseudo-first-order model, k_2 is the rate constant for the pseudo-second-order model, q_e is the amount of solute adsorbed at equilibrium and q_t is the amount of solute adsorbed at time t. The initial adsorption rate in the pseudo-second-order kinetic model is $h = k_2 q_e^2$.

The kinetic constants for the two kinetic models are summarized in table 3. The correlation coefficient (r^2) for the pseudo-first-order kinetic model was relatively low compared with those for pseudo-second-order kinetic model, and the value of q_e calculated from the pseudo-first-order kinetic model is far away from the experimental data table 3. Therefore, the pseudo-first-order kinetic model does not adequately describe the adsorption process of Ni(II) onto PUF waste .

In the application of pseudo-second-order kinetic model, the r^2 value of the linear plot of t/q_t vs. t Fig. 8 was 0.9999 this was greater than r^2 value of the first-order kinetic model table 3. Moreover the adsorption capacity values q_e from the pseudo-second order kinetic model agreed well with the experimentally determined adsorptive capacity value. This result indicates that the adsorption of Ni(II) ions on PUF waste follows a pseudo-second-order reaction. Thus the adsorption of Ni(II) onto PUF waste appear to occur by chemical processes involving valence forces due to sharing or exchange of electrons between Nickel and free lone pair of electrons on nitrogen atoms of PUF .

Table-3. First-order and second-order parameters for the adsorption of Ni(II) 0.17 mmol/L onto PUF waste 12g/L at 35°C.

First – order mode				Second – order model			
q_e (exp.)	q_e (cal)	k_1	r^2	q_e (cal)	k_2	r^2	h
0.668	0.07	0.0175	0.8662	0.663	1.387	0.9999	0.619

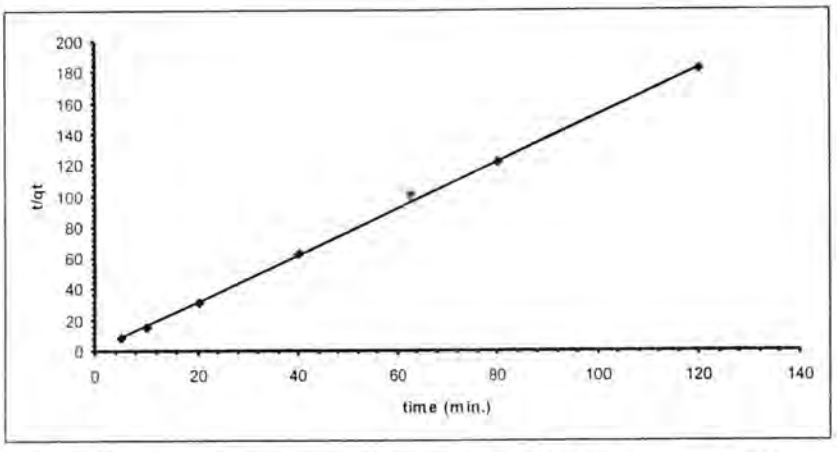

Figure-8. Pseudo-second order plot of adsorption of Ni(II) ions onto PUF waste .

CONCLUSION

The PUF waste was used as adsorbent for separation of Ni(II) ions from aqueous solution. It was found that the kinetic data are well fitted to the pseudo-second order equation this was deduced from the correlation coefficient and the calculated adsorption capacity which is more consistent with the experimental value than in the pseudo-first order equation. From the thermodynamic parameters, it was found that the adsorption process is endothermic and spontaneous at low concentration of Ni(II) and accopanied with the randomeness at liquid/solid interface.

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Synthesis and Characterization of New Compounds Derived from (1,3-Oxazol-5-(4*H*)-one and Study Their Effect on ALP Activity

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Received 15/2/2012 – Accepted 18/4/2012

الخلاصة

تم في هذا البحث تحضير مشتقات جديدة للمركب الأساس (3) (1،3-أوكسازول-5-(4*H*)اون) الذي حضر بحولقة الكلايسين مع النيكونيويك كلورايد وبمفاعلة المركب (3) مع الهيدرازين المائي يتكون مشتق الإيميدازول (4)، وبمفاعلة الأخير مع ألديهايدات مختلفة (a ،b ،c) تكونت قواعد شف جديدة (5a-c) وعند إضافة البنزويل كلورايد إلى مجموعة الآزومثين أعطى المشتقات (6a-c) والتي أعطت بوجود الثايويوريا المشتقات (7a-c)، والتي تم حولقتها باستعمال الداي أثيل مالونيت (8).

تم إجراء دراسة كيموحياتية لمعرفة تأثير المشتقات المحضرة على الإنزيم المختار لألكلاين فوسفاتيز القاعدي؛ حيث وجد أن كل هذه المركبات لها تأثير تثبيطي عند التركيز (10^{-2}) مولاري، ولوحظ أن التركيز (10^{-4}M) لم يظهر أي تثبيط أو تنشيط للأنزيم.

ABSTRACT

Synthesis of new derivatives compound of 1,3-oxazol-5(4*H*)-one) (3) by cyclization of (Glycin) with nicotinoyl chloride, reacting compound (3) with hydrazine hydrate 99% to give the imidazole derivative (4), when treating compound (4) with a, b and c different aldyhades to form Schiff bases compound (5a-c). Schiff bases converted into heterocyclic compound by reacting it with benzoyl chloride to give derivatives of compound (6a-c). Reacting thiourea with compounds (6a-c) will give derivatives of compound (7a-c), the last reaction of compounds are cyclized with diethyl malonat giving the derivatives of compound (8).

Also, the biochemical study has been done to show the effect of these derivatives on the chosen enzyme total ALP. It was found that all these compound have inhibitory effect at both concentration (10^{-2}, 10^{-4})M and observed that (10^{-4} M) does not have any inhibition or activation on enzyme activity.

INTRODUCTION

Heterocyclic compounds acquire more recent years due to the pharmacological activities. Five member heterocyclic compound which containing N, S and O has occupied enormous significance in the field of drugs discovery process [1]. Oxazole have been used as a various biological activities such as antibacterial [2] antifungal [3] antilabrecular [4] such as anti-inflammatory activities [5].

Imidazole derivatives are an important class of heterocyclic compound and many naturally occurring imidazole are known to possess biological activity [6]. The imidazole nucleus is also, a major component of a variety of drugs such as angiotensin II receptor anlagonisist [7], oral anti-inflammatory agents, protein kinase inhibitors and fungicides [8].

Alkaline phosphates ALP are widely distributed throughout the body, but clinically important one for diagnostic reasons is in bone, liver, placenta and intestine.

Growing bone is associated with the release of ALP and so in Childhood the level of ALP is around 3 times of that of adults. During

pregnancy in 2nd and 3rd trimester the enzyme rises considerably due to placenta releasing ALP. It can be used to examine placental function. Elevated levels are seen in bone diseases, e. g., pargets disease, rickets, osteoplastic metastatic and in obstructive disease of biliary tract. Decreased levels are rarely seen, e. g., in vitamin A resistant rickets [10-12].

The known inhibitors of ALP are: ureate, oxzalyte, cynide ions, L-phenylalauine, L-tryptophan, L-leucin, L-homoarginine and urea [13].

MATERIALS AND METHODS

Melting points were determined in open capillary tubes on a Gallen kamp melting point apparatus and are uncorrected. The IR Spectra were recorded by KBr discs using a perkin-elmer 1600 seies FT-IR spectrometer. ^{1}HNMR spectra were recorded on a Varian-Mercury 200 MHZ spectrometer.

Synthesis of Nicotinoyl Chloride (1)

To a solution of nicotinic acid (1.23g, 0.01mol) in dry benzene (20ml), thionyl chloride (1.19g, 0.01mol) was added. Then, the reaction mixture was refluxed for 7hrs. After evaporation, the product was collected without recrystallization. (yield 86%), m. p. (200-203) °C, IR (KBr), (υ, cm^{-1}) 3066 (C-H ar.), 1799 (C=O, Acid Chloride), (C=N) 1336, 768 (C-Cl).

Synthesis of [(Pyridyl-3-yl-carbonyl amino] acetic acid (2)

To stirring solution of glycine (0.75g, 0.01mol) and sodium hydroxide (10ml, 10% solution), compound (1) (1.41g, 0.01mol) was added. Then, the reaction mixture was shacked vigorously for 1hr., a few grams of crushed ice were added with stirring. After that, the solution was acidified with conc. HCl and the product was collected and recrystallized from ethanol. (yield 67%), m. p. (250-253) °C, IR (KBr) (υ, cm^{-1}) 3180 (NH). 3178 (acid OH), 2976-2888 (C-H alph.), 1741 (acid C=O), 1690 (amide C=O), (C=N) 1310, ^{1}HNMR (DMSO-d_6), ppm 3.31, (s, NH), 4.42 (s, CO-CH_2-NH), 7.15-8.20 (m, Aromatic Protons).

Synthesis of 4-(arylidene)-2-(pyridine-3-yl)-1,3-oxazol-5(4*H*)-one (3)

To stirring mixture of compound 2 (1.8g, 0.01mol) acetic acid (5 ml) acetic anhydride (20ml), aromatic aldehyde (0.01mol) was added. The temperature of reaction was reached to 70°C for 10min., Then, the mixture was poured into crushed ice and stirred for 30mins. The product was collected and recrystallized from ethanol to afford the desired compound.

3. (yield 71%), m.p. (120-123) °C, IR (KBr), (υ, cm^{-1}) 3050 (C-H ar.) 3123 (C-H olifen), 1755 (Oxazole C=O), 1656 (oxazole C=N), 1600-1511 (C=C ar.), 1280 (C-O) 848 (para substitution), ^{1}HNMR (DMSO-d_6) ppm 8.90, (s, C=CH-), 6.91-8.1 (m, Aromatic Protons).

Synthesis of 3-amino-5-(arylidene)-2-(pyridine-3-yl)-3,5-dihydro-4*H*-imidazol-4-one (4)

To mixture of compound (3) (0.01 mol) in dry pyridine (5ml) hydrazine hydrate (99%) (10ml) was added. The reaction mixture was refluxed for 20hrs. Then, the mixture was allowed to cool to room temperature and pyridine was removed. The product was recrystallized from ethanol to afford the desired compound.

4. (yield 53%), m.p. (230-233) °C, IR (KBr) (υ, cm^{-1}) 3424-3227 (NH_2), 3065 (C-H ar), 3190 (C-H olifen), 1606 (imidazole C=O), 1600-1511 (C=C ar.), 1232 (C-N), (N-NH) 1100, 855 (para substitution), ^{1}HNMR (DMSO-d_6), ppm. 8.75, (s, C=CH-), 8.51 (s, NH_2), 6.91-8.1 (m, aromatic protons).

Synthesis of 5-(arylidene)-3-[(arylidene)amino]-2-(pyridine-3-yl)-3,5-dihydro-4*H*-imidazol-4-one (5a-d)

The corresponding aryl aldehyde (0.01 mol) was added to stirred solution of compound (4) (0.01 mol) in absolute ethanol (20ml) and the mixture was refluxed for 2hrs. After cooling, the mixture was filtered and the solid recrystallized from ethanol to afford the desired compound.

5a. (yield 83%), m.p.°C 242-245, IR (KBr) (υ, cm^{-1}) 3028 (C-H ar), 3284 (C-H olifen), 1628 (imidazole C=O), 1654 (C=N, Schiff's base), 1604-1519 (C=C ar.), 1267 (C-N) 850 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.65 (s, C=CH-), 8.91 (s, CH-N), 6.54-8.12 (m, aromatic protons).

5b. (yield 87%), m.p.°C 192-194, IR (KBr) (υ, cm^{-1}) 3062 (C-H ar), 3190 (C-H olifen), 1631 (imidazole C=O), 1663 (C=N, Schiff's base), 1600-1523 (C=C ar.), 1278 (C-N) 841 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.71 (s, C=CH-), 8.95 (s, CH=N), 6.43-8.22 (m, aromatic protons).

5c. (yield 74%), m.p.°C 143-146, IR (KBr) (υ, cm^{-1}) 3080 (C-H ar), 3122 (C-H olifen), 1632 (imidazole C=O), 1645 (C=N, Schiff's base), 1611-1500 (C=C ar.), 1230 (C-N) 813 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.72 (s, C=CH-), 8.98 (s, CH=N), 6.32-7.98 (m, aromatic protons).

Synthesis of *N*-[chloro(aryl)methyl]-*N*-[arylidene-5-oxo-2-(pyridine-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl]benzamide (6a-b)

To stirring solution of compound (5a) (0.003 mol) in dry benzene (15ml), benzoyl chloride (0.003 mole, 0.35 g) in benzene (10ml) was added dropwise, then the mixture was refluxed for 4hrs. with stirring, after cooling, the precipitated crystals were filtered and recrystallized from ethanol.

6a. (yield 62%), m.p.°C 170-173, IR (KBr) (υ, cm^{-1}) 3036 (C-H ar), 3123 (C-H olifen), 1642 (imidazole C=O), 1643 (C=O amide), 1588-

1481 (C=C ar.), 1205 (C-N) 858 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.52 (s, C=CH-), 4.76 (s, N-CH-Cl), 6.79-8.42 (m, aromatic protons).

6b. (yield 71%), m.p.°C 190-192, IR (KBr) (υ, cm^{-1}) 3044 (C-H ar), 3149 (C-H olifen), 1634 (imidazole C=O), 1649 (C=O, amide), 1603-1524 (C=C ar.), 1267 (C-N) 879 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.75 (s, C=CH-), 5.21 (s, N-CH-Cl), 7.15-8.26 (m, aromatic protons).

6c. (yield 70%), m. p. (200-202) °C, IR KBr (25 cm^{-1}),3010 (C-H ar.), (C-H Olifen), 1645 (imidazole C=O), 1665 (C=O, amide), 1600-1500 (C=C ar.), 1270 (C-N) 885 (parasubstitution) ^{1}HNMR (DMSO-d_6)ppm, 8.6 (s,C=CH-), 4.9 (s, N-CH-Cl), 7.3-8.5 (m, aromatic protons).

Synthesis of aryl{[4-arylidene-5-oxo-2-(pyridine-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl](benzoyl)amino}methyl carbamimidothioate (7a-c)

Mixture of compounds (6a-b) (0.005 mole), thiourea (0.005 mole, 0.44 g) and anhydrous sodium carbonate (0.005 mole) in absolute ethanol (20ml) was refluxed for 5hrs. with stirring and the precipitated crystals was filtered and recrystallized from appropriate solvent.

7a. (yield 58%), m.p.°C 134-136, IR (KBr) (υ, cm^{-1}) 3385-3277 (NH_2), 3182 (NH), 3049 (C-H ar.), 3112 (C-H olifen), 1627 (imidazole C=O), 1666 (C=O,amide), 1608-1527 (C=C ar.), 1297 (C-N) 858 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.65 (s, NH_2), 8.43 (s, C=CH-), 4.21 (s, N-CH-S), 6.11-7.86 (m, aromatic protons).

7b. (yield 65%), m.p.°C 165-167, IR (KBr) (υ, cm^{-1}) 3362-3269 (NH_2), 3169 (NH), 3060 (C-H ar.), 3102 (C-H olifen), 1628 (imidazole C=O), 1643 (C=O, amide), 1589-1473 (C=C ar.), 1294 (C-N) 823 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.75 (s, NH_2), 8.52 (s, C=CH-), 3.86 (s, N-CH-S), 6.46-8.12 (m, aromatic protons).

7c. yield (70%), m. p. (180-182) °C, IR (KBr), (υ, cm^{-1}), 3330-3250 (NH_2), 3148 (NH), 3010 (C-H ar.), 3110 (C-H olifen), 1635 (imidazole C=O), 1660 (C=O, amide), 1610-1500 (C=C ar.), 1290 (C-N), 875 (para substitution).

Synthesis of N-[1-[(4,6-dioxo-1,4,5,6-tetrahydropyrimidin-2-yl) sulfanyl] benzyl]-N-[arylidene-5-oxo-2-(pyridine-3-yl)-4,5-dihydro-1*H*-imidazol-1-yl-benzamide (8)

Mixture of compounds (7a) (0.005 mole), diethylmalonate (0.005 mole, 0.8g) and anhydrous sodium carbonate (0.005 mole) in dry benzene (20ml) was refluxed for 7hrs. with stirring, the product was collected as oily, it is purified by column chromatography with silica gel and mixture of EtOH : Benzene as eluent.

8. (yield 55%), m.p.°C 297-299, IR (KBr) (υ, cm^{-1}), 3248 (NH), 3044 (C-H ar.), 3176 (C-H olifen), 2978-2845 (C-H aliph.), 1701 (pyrimidine

C=O), 1639 (imidazole C=O), 1665 (C=O, amide), 1600-1501 (C=C ar.), 1241 (C-N) 825 (para substitution), ^{1}HNMR (DMSO-d_6) ppm. 8.57 (s, NH), 8.13 (s, C=CH-), 3.56 (s, N-CH-S), 4.42 (s, NH-CH_2-NH), 6.11-7.94 (m, aromatic protons).

Biochemical Study

The study addresses the effects of some new prepared 1,3-oxazole-5-(4*H*)-one derivatives on serum Alkaline Phosphatase activity, steps of the work are as follows:

1- Five milliliters of blood were drown from the same subject from vein directly more than one time, and then centrifuged at 4000 rpm for 10 mints. The serum sample was separated and used immediately as a source of enzyme.

2- The Kinetic determination of ALP was determined in human serum using method of Belfled a kind [10, 11, 12], according to the following reaction :

$$\textbf{Paranitrophenyl phosphate} + H_2O \xrightarrow{ALP} \textbf{p-nitrophenol + inorganic phosphate}$$

Reagents :

A) Disodiumphenyl phosphate ($C_6H_5OPO_3Na_2$) (5mmole/L) and buffer solution carbonate-bicarbonate (Na_2CO_3-$NaHCO_3$) (50mmole/L), pH=10.

B) Standard phenyl solution (C_6H_5OH) (20kind and king U/100ml).

C) 4-Amino antipyrine ($C_{11}H_{13}Na_3O$) (60mmole/L) and Sodium arsenate ($Na_2HASO_4.7H_2O$)

D) Potassium fercyanide ($K_3[Fe(CN)_6]$) (150 mmole/L).

Alkaline phosphatase activity was measured by these laboratory procedure :

Reagent	Sample Serum	Serum Blank	Standard	Reagent Blank
2ml	2ml	2ml	2ml	A
Incubate for 5mints. at 37°C				
-	-	-	50µl	Serum
-	50 µL	-	-	B
Incubate for 15mints. at 37°C				
0.5	0.5ml	0.5ml	0.5ml	C
Good Mix				
0.5	0.5ml	0.5ml	0.5ml	D
-	-	50 µL	-	Blood Serum
50 µ L	-	-	-	D.W

Mix and incubate for 10 mints and measured the absorbance at 510nm. Enzyme activity was calculated as follows:

$$ALP\ Activity(KAU) = \frac{A_{sample\ serum} - A_{serum\ blank}}{A_{standard}} * 20$$

3- A stock solution (10^{-2}) concentration of each compound at table 1, prepared by diluting with DMSO as solvent, ALP activity is measured in human serum in presence of these derivatives by adding (0.05ml) from each compound to 1.95ml of reagent A[14]. The activity without inhibitor was measured with 2ml of reagent A without adding the inhibitor.

These results show that the inhibition effect of our compound on ALP, the inhibition percentage is calculated by comparing the activity with and without inhibitor and under the same conditions according to the following equation:

$$\%Inhibition = 100 - \frac{the\ activity\ with\ inhibitor}{100 - the\ activity\ without\ inhibitor} * 100$$

$$\%Retrieve\ Ratio = 100 - Inhibition\ Ratio$$

RESULTS AND DISCUSSION

Synthesis of new compound of nicotionyl chloride by reacting nicotinic acid with thionyl chloride was carried out, (Scheme -1) IR spectrum of compound (1) showed band at (1799cm^{-1}) C=O of acid chloride. Compound (1) reacted with Glycin in the presence of sodium hydroxide to give compound (2) [(pyridyl-3-yl-carbonyl amide] acetic acid.

The IR spectra showed the band, of C=O acid chloride decreasing of absorption at (1690 cm^{-1}) and (NH) band appearance at 3180 cm^{-1}, ^{1}HNMR spectra, DMSO-d_6 CO-CH_2-NH was recorded at 4.42ppm for two protons. Compound (2) reacted with aryl aldehyde in presence of acetic acid and acetic anhydride to give compound (3) 4-(arylidene)-2-pyridin-3-yl)-1,3-oxazole-5-(4*H*)-one. The IR spectrum showed the appearance of the oxazole ring at 1755cm^{-1}, NH band at 34448.84cm-1 CH aromatic at 2906.82cm^{-1}, μNH-CH_2 at 2794.95cm^{-1}, 813.99 due to parasubstitution. ^{1}HNMR spectrum showed signals at 7.72 ppm due to C-H, and at 6.7-9.6 aromatic protons.

Condensation of hydrazinhydrate (99%) with compound (3) gave compound (4). The IR spectrum showed band at (1606.76cm^{-1}) for imidazole C=O and NH_2 3442-3227cm^{-1}, CH aromatic 3065cm^{-1}, ^{1}HNMR (8.212-8.027ppm) due to NH_2, 6.997 ppm CH-olefinic, 7.988 belonged to aromatic protons.

Refluxing compound (4) with different aryl aldehyde gave Schiff bases compounds (5a-c) revealed the IR spectra band of (C=N) at 1645-1663cm^{-1} with disappearance of NH_2 bond at 3322-3435 cm^{-1}, ^{1}HNMR

spectra of C=C 1550-1537cm^{-1}, compound (5a-c) show signal absorbed at 8.629-8.136 ppm for protons as signal to aryl groups.

Reaction of benzoyl chloride with Schiff base gave compounds (6a-c), the IR spectra showed υCON (1643, 1649)cm^{-1} and υCH (3171-3186)cm^{-1}, ^{1}HNMR spectra signals at (8.86ppm) CH olefinic at 6.80 ppm aromatic proton and at 6.75 (N-CH-Cl).

Reaction of thiourea with compounds (6a-c) gave compounds (7a-c), the IR spectra indicated the presence of υNH_2 (3269-3385)cm^{-1}, ^{1}HNMR spectra showed signals at 8.33-8.60ppm due to (NH_2), at 6.97 ppm, which belongs to aromatic protons and at 3.17ppm (N-CH-S).

Cyclization of compound (7a) with diethylmalonate, by oxidative gave compound (8a). The IR spectra revealed υCH aliphat 299748cm^{-1} and NH 3362 cm^{-1}. ^{1}HNMR spectra shows signals at 4.30 ppm due to (NH-CH_2-NH) and at 6.959-8.142ppm, which belongs to aromatic protons.

ALP inhibitory is a chemical that inhibits the ALP enzyme from hydrolysed p-nitrophenyl phosphate to its products. ALP activity in the present study was assayed in the absence and presence of new derivatives compound of (1,3-oxazol-5(4*H*)-one) under 10^{-2} concentration of each one.

The biochemical tests revealed that all compounds caused good inhibitory effects on enzyme activity, table 1. Activity of enzyme without compounds was 12.5 KAS in this subject.

Table-1: Enzyme Activity, Inhibition% and Retrieve % of the Prepared Compounds

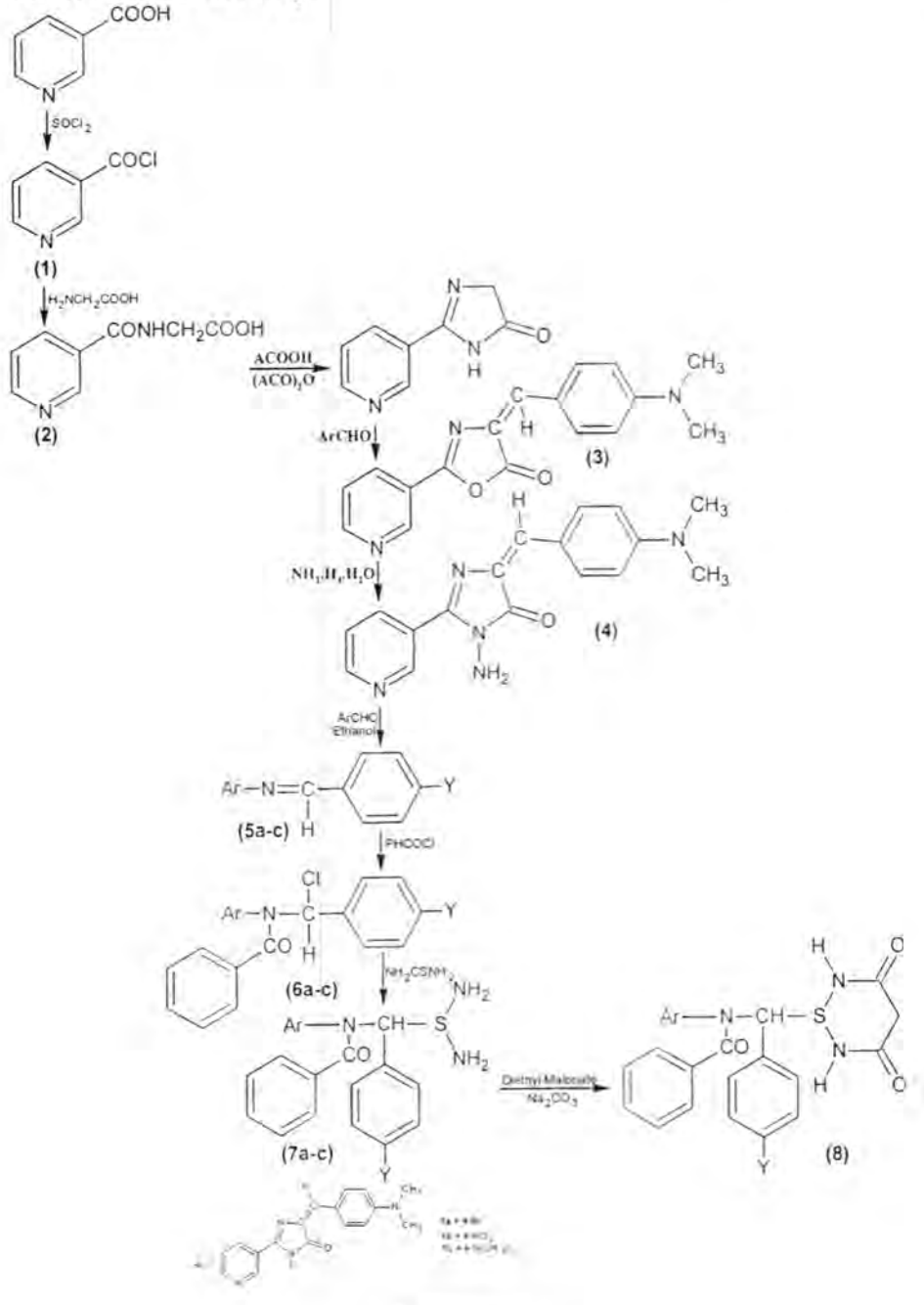
Compound No.	Enzyme Activity (KAS)	Inhibition%*	Retrieve%**
4	4	68	32
5c	3.8	69	31
7a	3.3	73	27
5c	1.5	88	12
6a	3.6	71	29
7c	2	84	16
5a	4.5	64	36
5b	1.1	91	9

That mean the greater inhibition of all compounds is demonstrated at concentration (10^{-2})M, and the highest inhibition was noticed by compound (5b) which may be contributed in the free pair of electrons of nitrogen in amine group (-$\ddot{N}H_2$), so the compound (5b) can easily bond with enzyme via the positively charged of active site. We noticed that 10^{-4} concentration doesn't have any inhibition or activation to enzyme activity for all compounds.

Also, Schiff base of nicotinic acid derivatives compounds (5a) and (5b) show higher inhibition compared to other compounds, it can be

explained by saying that the presence of many denoting groups in their structure may increase the electron density of nitrogen atom, hence, increase its ability to be bound by the positively charged active site of the enzyme.

Steric hindrance is another possible explanation of the greater inhibition of compounds (5a) and (5b), these compounds have large structure resulting in limited access to active site of ALP. Our results was in good agreement with (15) study, which found that nicotinic derivatives had inhibition on ALP activity.



Scheme 1

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ABET and QAA based Framework proposal for Higher Education in Iraq

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Received 11/11/2010 – Accepted 2/3/2011

الخلاصة

أقترحت وزارة التعليم العالي والبحث العلمي في العراق مرجعيتين عالمتين تختصان بضمان الجوده لكي يتم تبنيها من قبل الكليات. المرجعية الأولى هي معايير الأعتماد الأكاديمي للهندسة والتكنولوجيا والتي تُخصص الى كليات الهندسة والعلوم والمرجعية الثانية هي معايير وكالة ضبط الجودة والتي تُخصص الى كليات الأداب. ان البحث يقترح مقاييس وأجراءات تنفيذية موحدة تتطابق مع معايير المرجعيتين أعلاه. أن هذه المقاييس يمكن أعتبارها كأطار أساسي يمكن تبنيه في جميع الكليات في العراق. أن هذا سوف يقلل الجهد والوقت و سيقضي على التضارب الذي يمكن ان يحصل أثناء التنفيذ.

ABSTRACT

The Ministry of Higher Education and Scientific Research in Iraq suggests two international quality assurance references to be adopted by all the colleges. The first one is the **A**ccreditation **B**oard for **E**ngineering and **T**echnology **(ABET)** criteria for science and engineering colleges. The second is **Quality Assurance A**gency **(QAA)** aspects for art colleges. This paper proposed a unified standards and implementation procedures framework that conform to the above criteria and aspects. This unified standards can be considered as basic framework that can be adopted by all the colleges in Iraq. This will save time and effort and eliminate the conflictions that may occur during the implementation.

1. Introduction

Teaching quality is a major requirement for higher education. Despite the large number of colleges in Iraqi higher education (HE) system, the teaching quality assurance system has not been a significant focus by most of the colleges. This effect negatively on the quality of the output (graduates students). Most of the graduate students have no confidence with the skills that they have. There is an urgent need for establishing a teaching quality assurance system for universities (private and governments) in order to drive the improvement of the quality of teaching. Currently there are trends in the Ministry of Higher Education and Scientific Research in Iraq to improve the higher education system throw working to attain the accreditation from **A**ccreditation **B**oard for **E**ngineering and **T**echnology **(ABET)** or the assessment from **Q**uality **A**ssurance **A**gency of Higher education **(QAA)**. This paper focuses on the criteria that are dependent by these two organizations and unified these aspects in one proposal framework. The paper is structured as follows: section 2 describes quality and accreditation concept. Section 3 discusses ABET criteria. Section 4 discusses QQA aspects. Section 5 presents the proposal and section 6 is the conclusion.

2. Quality and Accreditation

Quality in higher education means the educational process is such that it ensures students achieve their goals and thereby satisfies the needs of the society and help in national development [1]. Quality assurance aims to give stakeholders confidence about the management of quality and the outcomes achieved.

The term accreditation expresses the abstract notion of a formal authorizing power, acting through official decisions on the approval of institutions [2]. The aims of accreditation in higher education can be summarized as [3]:

- To assure that the institutions meet their responsibility for the quality of the programs offered.
- To guarantee students, and employers that the program has to undergo a quality assurance.

The accreditation is a binary judgment (pass – not pass, positive – negative, meeting certain standards – not meeting)

3. ABET

It is a non-governmental organization in the United States. It is the recognized accreditation for college and university programs in applied science, computing, engineering, and technology. ABET does not rank programs but it declares that the programs are either accredited or not .The purpose of ABET are [4]:

a) Organize and carry out a comprehensive process of accreditation of pertinent programs leading to degrees, and assist academic institutions in planning their educational programs.

b) Promote the intellectual development of those interested in applied science, computing, engineering, and technology professions, and provide technical assistance to agencies having professional regulatory authority applicable to accreditation.

ABET accomplishes its purposes through standing committees and commissions. The commissions are Applied Science Accreditation Commission (ASAC), Computing Accreditation Commission (CAC), Engineering Accreditation Commission (EAC), and Technology Accreditation Commission (TAC). The more details for ABET accreditation polices in [4].

ABET annually has been issuing four criteria reports for accreditation. Each report is related to one of discipline (applied science, computing, engineering, and technology). These reports demonstrate two types of criteria that a program seeking for accreditation must meet. The two types of criteria are

3.1 General Criteria: consists of eight criteria all the programs seeking for accreditation should adhere. The criteria are Students, Program Education Objectives, Program Outcomes, Continuous Improvements, Curriculum, Faculty, Facilities and Support. These criteria are same in all the four reports but the content are slightly different because each report explains these criteria according to its perspective. For example, in the Program Outcomes criterion, the number of outcomes in computing report is 11 started form a and ended to k while in engineering report, they are from a to i. On other hand the content of continuous improvement criterion is same in all reports. The criteria are listed below with abstract details [5,6,7,8].

3.1.1 Students

The program must evaluate student performance, and monitor student's progress to foster their success in achieving program outcomes, thereby enabling them as graduates to attain program objectives.

The program must have and enforce procedures to assure that all students meet all program requirements.

3.1.2 Program Educational Objectives

The program has documented measurable educational objectives that are based on the needs of the program's constituencies. Each program must have in place an educational program, including a curriculum that enables graduates to achieve the program educational objectives.

3.1.3 Program Outcomes

The program has documented measurable outcomes that are based on the needs of the program's constituencies.

3.1.4 Continuous Improvement

The program uses a documented process incorporating relevant data to regularly assess its program educational objectives and program outcomes, and to evaluate the extent to which they are being met. The results of the evaluations are documented and used to effect continuous improvement of the program through a documented plan.

3.1.5 Curriculum

The program's requirements are consistent with its educational objectives and are designed in such a way that each of the program outcomes can be achieved. The curriculum combines technical and professional requirements with general education requirements and electives to prepare students for a professional career.

3.1.6 Faculty

a) Faculty Qualifications

Faculty members teaching in the program have the educational backgrounds or expertise consistent with their expected contributions to the program. Each has a level of competence that normally would be obtained through graduate work in the discipline, relevant experience, or relevant scholarship. Collectively, they have the technical breadth and depth necessary to support the program.

b) Faculty Size and Workload

There are enough full-time faculty members to provide continuity, oversight, and stability, to cover the curriculum reasonably, and to allow an appropriate mix of teaching, professional development, scholarly activities, and service for each faculty member. The program must have sufficient responsibility and authority to define, revise, implement, and achieve program educational objectives.

3.1.7 Facilities

Facilities include:

a) suitable library, classrooms, laboratory, computer networks, and offices are adequate to support the educational objectives and outcomes of the program.

b) Internet and information infrastructures, including electronic information repositories, equipment catalogs, professional technical publications, and manuals of industrial processes and practices adequate to support the educational objectives of the program and related scholarly activities of students and faculty

3.1.8 Support

Support includes administration, institutions that can be provided to assure the quality and the continuity of the program.

3.2. Program Criteria

They are specific criteria related to the program itself. Each program must satisfy program criteria that amplify the general criteria and provide the specifics needed for a given discipline. It is the responsibility of the program seeking accreditation to demonstrate clearly that the program meets the above criteria.

Programs wish to demonstrate that they meet the standard criteria should undergo an ABET accreditation general review. General reviewers are conducted once every six years, and include preparation of a self-study and a visit by an ABET evaluation team [9].

4. QAA

In United Kingdom (UK), QAA is one of the most important independent bodies that carry out HE evaluations. QAA was established in 1997 by subscriptions from UK universities and colleges of HE, and through contracts with the main UK funding bodies. Its mission is to safeguard the public interest in standards of HE qualifications and to inform and promote continuous improvement in the management of the quality of HE [10].

QAA also carries out external reviews and audits by visiting the universities and colleges and reporting on how well they meet their responsibilities according to six aspects. The review will examine the extent to which the student learning experience and the student achievement in each of the six aspects of provision contribute to meeting the objectives and the aims set.

The six core aspects of provision are:

- Curriculum Design, Content and Organization
- Teaching, Learning and Assessment
- Student Progression and Achievement
- Student Support and Guidance
- Learning Resources
- Quality Management and Enhancement

4.1 Curriculum Design, Content and Organization.

This aspect defines the curriculum development and design of study program, identifies the relevant aims and objectives. The curriculum should be effective in enabling students to achieve the intended learning outcomes for the program. The curriculum content is appropriate to each stage of the program. One of the best available guides for the formulation of learning outcomes is provided in the "Benchmarking Statements" by the QAA. This document could perhaps be adopted as the starting point for the definition of educational objectives, in terms of contents and levels [2].

4.2 Teaching, Learning and Assessment

This aspect is divided into two parts:

4.2.1 Teaching and learning:

It identifies the strategy for teaching and learning so that meet clearly with aims and objectives and outcomes of the program and the intended learning outcomes and curriculum content. It also contains: [11]

- The range of teaching methods employed in relation to curriculum content and programs aims.
- How the quality of teaching is maintained and enhanced through staff development.

- How the materials provided support learning and how students independent learning is encouraged.

4.2.2 Assessments [11]

It describes any processes that appraise an individual's knowledge, understanding, abilities or skills. There are many different forms of assessment, serving a variety of purposes. The assessment should be effective in measuring the achievement of the intended learning outcomes of programs. The following should be considered:

- the assessment methods selected and their appropriateness to the nature of the intended learning outcomes.
- the security, integrity and consistency of the assessment procedures, the setting, marking, and the return of student work with feedback;
- the assessment strategy and the adequate formative function in developing student abilities, assists them in the development of their intellectual skills and enables them to demonstrate achievement of the intended learning outcomes.

There are ten principles for assessments are listed in [12] are used as guidance for college or institution.

4.3 Student Progression and Achievement

Identify effective strategies for recruitment, admission and guidance. The strategies facilitate students' progression and completion of the program.

4.4 Student Support and Guidance

Identifies the strategy for academic support, including written guidance, and the extent to which it is consistent with the student profile and the overall aims of the program and support to facilitate student progression

4.5 Learning Resources

This aspect demonstrates a strategic approach to linking physical and human resources to intended learning outcomes at program level. The learning resources should be available successfully underpin the programs and the staff should be effectively contributed to the achievement of the intended learning outcomes.

4.6 Quality Management and Enhancements

Effective internal arrangement for monitoring, revisions, measuring has been taken to maintain and enhance academic standards and the quality of learning opportunities provided for the programs.

5. Framework Proposal

One of the aims of the ministry of HE in Iraq is to implement the quality assurance standardization on all the universities. And one of the plans is to adopt the QAA aspects for Art colleges and to adopt ABET criteria for Engineering and scientific colleges.

In this section an attempt to combine the two above approaches and find out a proposal approach appropriates for all the universities in Iraq and can inspire the principles of both approaches. The aim is to follow a unified approach and to avoid the conflict that may occur during the understanding the both. The unified can be considered as a basic framework to be adopted by all universities in Iraq. Table 1 depicts the proposal. It consists of three columns. The first one are the ABET criteria. The third is QAA aspects and the middle is the proposed standards. The total standards are five. Each one is combination of aspect(s) and / or criteria. It important to mention that these standards should be performed in parallel not sequential and that requites several committees work together. The following describe briefly the practical outlines for each standard

Table 1

ABET Criteria	Proposal	QAA Aspects
Program Outcomes	Program and curriculum Design	Curriculum Design, Content and Organization
Program Educational Objectives		
Curriculum		
Students	Students management	Student Support and Guidance
		Student Progression and Achievement
	Teaching and Learning Management	Teaching, Learning and Assessments
Continuous Improvement	Quality improvement	Quality Management and Enhancements
Facilities	Resources managements	Learning Resources
Faculty		
Support		

5.1 Program and curriculum design management

This is the first standard that should be considered in a new program. It is divided into two parts: program design and curriculum design. A useful guide for this standard is available in [13, 14]

5.1.1 Program Design:

a. Program should identify clear missions, objectives and outcomes that the student should achieve.
b. A study plane should be available for each discipline.
c. The program should have modules classifies as department requirement (core), college requirement and university requirement.
d. Prepare a program specification book.

5.1.2 Curriculum Design

a. The broad structure of curriculum should be based on international reference. There are many organization give outline advices and recommendation for designing curriculum. For example several developments that have assisted computer science education in its pursuit of quality include the creation of widely accepted curriculum standard such as the ACM/IEEE standards [15] and the benchmark statements [16].
b. The outcome, objectives should be indentified clearly in each course or module and consistence with the objective programs.
c. The aim of the core modules is to prepare students for more complex and specialist work which could be studied at a later stage.
d. E-learning is another strategy that is recently should be added to develop the curriculum.
e. The curriculum of all modules should be available on Web sites

5.2 Teaching and Learning Management

5.2.1 Teaching and learning methods

a. Teaching and learning methods should be designed to achieve program mission, objectives and outcomes.
b. Teaching methods aims to improve student's skills (communication, problem solving and social).
c. Staff members may communicate with students through email. This enables students to communicate easily in coursework supplement. Each staff member writes his/her email address in the course descriptor sheet that should be distributed to students at the beginning of the course.

5.2.2 Teaching Assessments

a. Teaching assessments strategy should be specified (quizzes, exams, classroom interactivity, seminars, laboratories, etc)
b. Assessment can be diagnostic, formative or summative.
c. The program should make sure that the methods of student assessment are fit for purpose and work well.

5.3 Student management

a. Data is collected and statistical report are issued about the data movements related to the number of students (new, dropped, failure and complete)
b. Statistical report is issued for the graduate students and grade they attain (Excellent, very good, good and pass)
c. Designing and implementing a strategy for student's guidance. All students should have academic tutors to give the advice the student needs. The students remain with the same tutor until their graduation.
d. Providing students with a complete undergraduate handbook, which contains all the relevant information, required by students through their academic year. This handbook is available on the Web.
e. University Students Affairs Deanship is available.

5-4 Resources Managements

a. Convenient resources handbook describing the resources available and how to obtain and make use of these resources (laboratories, CDs, books, libraries and E-learning resources)
b. A web site for university, college and the department should be available.
c. Suitable training for staff in operational matters
d. Networking facilities used in teaching, learning, and communicating with students through Web-based exercises and assignments.
e. Standard number of students per classroom and laboratory and staff/students ratio
f. Suitable library, classrooms, laboratory, computer networks, and offices
g. The academic staff members are well qualified and experienced in teaching the material at undergraduate and postgraduate levels

5-5 Quality improvements

This standard is the hart of quality assurance process. It consists of two important processes: Quality Managements and quality Monitoring (Action Plan)

5.5.1 Quality Management

Manage the reports issued relating to ratio of poor, good, excellent students in each module.
a. Mange the problems the students face in each module.
b. Revision and updating of the aims and objectives of the program
c. Revision of the curriculum design and content.
d. Revision of the syllabus content.

e. Quality Management system should be build to analyze data. Data is collected from the academic activities.

5.5.2 Quality Monitoring

a. Reports are collected from the previous process. The reports are analyzed carefully to diagnose the weakness and strength points.
b. Action plan report is issued per semester to present new suitable actions to avoid the mistakes and improve the academic operation. The report should cover the all the above criteria. This criterion is a spiral movement like towards the goals.

It is important to mention that these two pocesses play the role of evolution process. And each process completes the others.

6. Conclusion

This paper has investigated the criteria and the aspects of the ABET and the QAA respectively. Also the paper has combined the criteria and aspects into uniform proposed quality assurance standards. The proposal presents practical outline for each standard. The proposal is presented to avoid the misunderstanding and the confliction that may occur during the implementation and to ensure that all required tasks are completed without unnecessary demands.

It is important to mention that the most important things to remember in preparing for getting accreditation is that the administration leader should have the full convincing about the importance of the quality assurance.

Although the standards described above need more details but we are believed it is important to start the first step. The start could be with one specific department in order to minimize the effect of any mistake. Then we can generalize the expertise to other departments.

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E-shopping Perceptions Assessment

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Received 8/2/2012 – Accepted 18/4/2012

الخلاصة

لقد أصبح إجراء الأعمال التجارية عبر الإنترنت الشيء المؤكد لكثير من الناس والشركات في النظام الدولي. هذا البحث يهدف الى التحقيق في المواقف والسلوكيات للمتسوقين عبر الإنترنت في هذا التطور السريع للتجارة. وقد تم تصميم استبيان لهذا الغرض وايضا أجريت دراسة استقصائية. وجمعت بعد ذلك الردود على الاستبيان وتم تحليلها. ومنها تم الحصول على نتائج وتوصيات في هذا المشروع. ويمكن النظر في تطبيق التجارة الإلكترونية في الأردن منذ البداية، والتنفيذ البسيط نوعا ما. وكيف يتأثر ممارسة التجارة الإلكترونية في الأردن من قبل المقاومة الثقافية، والثقة، وانعدام الوعي، وعدم وجود إطار تنظيمي. بناء على دراستنا، المتسوقين الالكترونيين تمثل حوالي 14٪ من أفراد العينة عموما، ويتميز المتسوقين بارتفاع دخل الأسرة الشهري، دراية وتجربة بالعمل على شبكة الانترنت ومعرفة القراءة والكتابة على شبكة الإنترنت مقارنة مع غيرهم من المتسوقين. وكانت دوافع المشاركين الرئيسية لممارسة التسوق الالكتروني الراحة، وبأسعار تنافسية والخيارات المتنوعة من الشراء. وأظهر المشاركون تدني مستوى الثقة في أمن المعاملات على الانترنت وأعلنوا أن وقت التسليم أطول ، وعدم وجود اتصال جسدي يمثل صعوبة في التعامل مع العوائد والمبالغ المستردة هذه بعض من عيوب كبيرة في التسوق الإلكتروني. ووجد أيضا أن معظم الشركات التي يتعامل معها المتسوقين الالكترونين مع شركات أجنبية مثل الأمازون، والعناصر التي يتم شراؤها في معظم الأحيان على الانترنت هي الكتب تليها الملابس والأحذية.

ABSTRACT

Conducting business online has become the sure thing for many people and companies in the international system. This research investigates shoppers-online attitudes and behaviors in this fast evolving business landscape. A questionnaire was designed for this purpose and a survey was conducted. The responses to the questionnaire were then compiled and analyzed. The results and recommendations are reported in this project. E-commerce application in Jordan could be considered as in the beginning, and what implemented is rather simple. Practicing e-commerce in Jordan is influenced by cultural resistance, trust, lack of awareness, and absence of regulatory framework. Based on our study, e-shoppers represent about 14% of overall sample individuals, those shoppers are characterized by higher household's monthly income, longer internet experience and higher household's internet literacy compared to non-shoppers. The participants' main motives of practicing e-shopping were convenience, competitive prices and variety of purchase choices. The participants showed a low level of trust in security of transactions online and they declared that longer delivery time, lack of physical contact and difficulty in handling returns and refunds are some of the significant disadvantages of e-shopping. It was also found that most of the companies that e-shoppers' deal with are foreign companies like Amazon, and the most frequently purchased items online were found to be books followed by clothes and shoes.

INTRODUCTION

Since the late 1990's e-business has been the most revolutionary aspect of the new digital world around us. Jordan as a part of this dynamic changing world has known the importance of catching up with this new global trend toward digitalizing the new life for the good of the

community. Digitalizing commerce is a major part of this digital revolution.

In spite of all the difficulties surrounding this sensitive country cause of it's unstable parameters due to it's location and different relations with adjacent countries, in spite all of that Jordan has made great steps towards digitalizing life aspects that surround us, those steps were made possible by his majesty king Abdullah II who issued his royal decree to integrate all efforts to set Jordan as the leading country in the electronic industry among all other countries in the region.

His royal decree was translated in to several projects in all related fields that insure the achievement of Jordan's vision of being recognized as an information technology center in the region that serves all nearby countries by human as well as technological facilities [11, 12].

Jordan is witnessing many rapid developments in industrial modernization and liberalization of trade. The successful penetration of the Internet services into Jordanian business offices, manufacturing plants, schools and universities, government departments and ministries, and households is becoming a reality. Many Jordanian businessmen are becoming more convinced every day that the Internet is The Place to do business. Those who are learning the benefits of the Internet are looking for ways and means to improve their business operations, upgrade their resources, expand their marketing networks, and achieve successful local and foreign sales. Due to the lack of specialized studies in the field of e-shopping perceptions in Jordan. This study will be of great help for decision-makers in different community sectors that include both private and governmental sector [9, 10].

ONLINE SHOPPING VERSUS OFFLINE SHOPPING

Online shopping is convenient, accessible, allows comparison of pricing and products, and in general, gives buyers a great deal of freedom and control. On the other hand, offline shopping gives consumers the ability to touch and try on merchandise, and it provides them the opportunity to take possession of goods immediately.

Off line shopping is more impulsive than online shopping The general lack of impulsivity online is due to the inability to take possession of goods immediately, Visual inspection is not nearly as easy as in a store, Buyers cannot touch products or try on clothing, and the trouble of having to mail back unwanted items. Moreover, slow download speed makes browsing several products online a somewhat unsatisfying experience [1,2,3].

The fact that there is no immediate gratification from online purchases is probably the most frustrating inconvenience regarding e-shopping. Matter of speed of delivery remains a disadvantage of e-shopping.

Nevertheless shopping online can result in instant delivery when it comes to Digital products such as software and songs. When shopping online, the primary relationship is not between the seller and buyer, but rather between buyer and the mediated environment. The primary relationship while shopping offline is between the seller and the buyer. this situation constructs the potential for competitiveness between online and offline vendors to get the customer satisfied either buy friendly interface and facilities offered online, or buy high level of service offered offline. Shopping offline is characterized by higher level of commitment felt by shoppers' offline. Different from shoppers online who experience less level of commitment while shopping online. Visiting an offline store and coming back home empty-handed results in disappointment. Online however, coming away empty-handed is not a problem because of the more limited investment required as compared to offline shopping. The lack of sales help online is also associated with lower commitment since sales workers will not be there to watch you and affect your decision of buying in any means. Online buyers can much more freely abandon shopping carts because a sales person is not watching them and because they have decided that they just don't want to spend the money right at the moment. Shopping offline is simply more alive than shopping online. Shopping offline is described as resulting in many more opportunities to have fun and be diverted, In addition to having access to help when needed; shopping offline is enjoyable in means of shopping with friends and family. Factors such as ambiance, smells, sounds and people-watching are enjoyable elements of offline shopping. While offline shopping is more likely to be associated with these experiential benefits, some online buyers nevertheless describe online shopping as being enjoyable, fun, and even sociable [4,5,6].

E-COMMERCE IN JORDAN

E-commerce application in Jordan could be considered as in the beginning, and what implemented is rather simple. Internet and PC's diffusion in Jordan are still limited and at low levels compared to international levels.

Awareness is hindering the understanding of e-commerce concept, besides the absence of trust about on-line payment and security, in addition to the lack of legal protection against deceiving.

Most of e-transactions are undertaken between a few Jordanian institutions with non-Jordanian partners. Most of these applications take place over websites that are hosted outside Jordan, in countries where Internet services and telecommunications facilities are more developed. The transactions are also joined with methods of payment that go

through foreign banks that offer payment gateway usually located outside Jordan [7,8].

The Jordanian government has shown a great concern regarding the issue of ICT sector .this was translated in to several programs that aim at improving and promoting ICT sector in Jordan. Encountering low internet-use where the proportion of those who uses internet is no more than 12% of Jordanians. the government has promoted PC and internet penetration by canceling out custom duties concerning PC's and it had increased level of awareness of computer literacy by spreading out PC's in schools and universities as well encouraging people to participate in the digital community.

The following table shows some statistical figures regarding ICT sector in Jordan [11,12]:

Table -1: Statistical figures regarding ICT sector in Jordan

No. of internet subscribers	197,000 subscriber	(2005)
Proportion of internet users	12%	(2006)
No. of ISP's (internet service providers)	12 company	(2005)
No. of telephone lines	862,000 line	(2006)
No. of software and web design companies	200-250 company	(2006)

Regarding Jordan's SME's status, ESCWA (Economic and Social Commission for Western Asia) has carried out a survey on 30 companies in Jordan and found that most of Jordanian companies lack the clear understanding of e-commerce either for consumers or companies as well. The study also showed that most internet activities in Jordan are limited to e-mail, chatting, researching and rarely used for e-commerce.

As for Jordanian companies experience, it was found that most relatively large companies have a website for sake of promotional campaigns and advertising and not for conducting commerce online. Jordan enjoys a numerous human resource facilities that are comprised of highly educated and trained workforce which gave Jordan an advantage of being one of the major suppliers of skilled and trained workforces among neighboring countries.

Barriers hindering e-commerce application

- The lack of coordination between the public and the private sectors in order to form and advance e-commerce market in Jordan.
- Absence of payment solutions in addition to the small international credit cards.
- Awareness is still hindering the understanding of e-commerce concept, besides the absence of trust about on-line payment and

security, in addition to the lack of legal protection against deceiving.

- Moreover, insufficient infrastructure such as computer and Internet literacy, Internet services, law Internet links and expensive telephone rates.
- Other obstacles such as lack of legislation concern regulatory framework in addition to the insufficient legal manners of e-commerce application in Jordan.
- High rates of wired and wireless communication facilities which in turn restrains internet diffusion.
- Low level of e- readiness of banks sector to conduct monetary transactions online.
- Cultural resistance where people prefer to shop offline.
- No existence of a clear strategy for setting e-commerce in to practice.
- Lack of convenient standard physical addresses. Where Postal system services are negatively affected by the current address location system, a new system of marking the address location is required in order to help e-commerce application [13, 14, 15].

QUESTIONNAIRE DESIGN AND SAMPLING PROCEDURE

The questionnaire questions were selected from several questionnaires developed by GVU's User Survey team.

Those questions were arranged into several main groups as follows:

- Financial capability.
- Awareness of e-shopping.
- Privacy and security via the web.
- Attitudes and cultural perceptions towards e-shopping.
- E-shoppers preferences.
- E-shopping experience.
- Personal information: name, gender, job, education level and monthly income.

Questions formats were as follows:

- Multiple choice questions, where answers were chosen from alternatives.
- Questions that requires written answers.
- Question requires Yes or No answers.

Population under interest: Internet users who are interested in e-shopping in general or likely to having experienced e-shopping in particular.

Sampling technique: With respect to sampling techniques, convenience sampling was adopted in this survey. Convenience sampling is defined as selecting individuals that are the easiest to reach. According to Hair et al, and Kinnear and Taylor , convenience sampling is suitable for the requirements of exploratory research designs like the present one Convenience Sampling was adopted here due to limited funds and small number of researchers namely (2).

Sampling procedure: Approaching population under interest, two samples were selected from the students sector and the public community sector. It is worth mentioning that target participants were limited to those who are internet literate.
Attempting to cover students sector, university of Jordan students were approached conveniently. And the following procedure was undertaken:

- Two visits were made to the Jordan University's library on randomly selected days and times.
- Two visits were made to elective courses classes which usually encompass randomly distributed students from different faculties.
- Two visits were made to the square at randomly selected days and times.

Above locations were chosen for convenience. Since each of them is a place where randomly distributed students are expected to be present. For each location the available students were covered on voluntary basis.
Approaching public community sector, the following institutions were also visited conveniently:

- Abdulahameed Shouman public library.
- Addustour newspaper.
- Al-Arab Al-Yawm newspaper.
- The Arabic Academic.
- The Arab bank.
- Aramex.
- University of Jordan.

Sample size: The selected sample included 315 individuals from which 43 persons had experienced shopping online before.
Sample individuals classification: Analyzing participants results, participants were classified in to two groups:

- Those who have experienced e-shopping before. This group results are of major concern since they represent real experience regarding shopping online.

- Those who have never experienced e-shopping before. This group comprises the majority of the sample individuals but comes after the first group in significance since the results of this category represent only attitudes of non-shoppers online towards e-shopping as they lack the real experience of shopping online.

1. E-SHOPPERS PERCEPTIONS ASSESSMENT

In our analysis e-shoppers results were under focus for the following reasons:

- E-shoppers' results represent a real experience regarding shopping online. So it enjoys higher significance than that of non-shoppers.
- Inspecting non-shoppers' results ,there was found some sort of uncertainty and lack of obvious or common trends between non-shoppers in general .this unstable results are indicated by high proportions of those who neither agree nor disagree answering related questions. I have also noted that the proportions of those who agree or disagree are close to each other which emphasize the hesitance of non-shoppers towards those questions. Nevertheless non-shoppers results were employed to compare their personal profile with that of e-shoppers.

2. ANALYSIS PROCEDURE

E-shoppers perceptions were sought considering following aspects:

- Internet experience.
- Financial capability.
- Awareness of e-shopping.
- Privacy and security via the web.
- Attitudes towards e-shopping.
- E-shoppers preferences.
- E-shopping experience.

Each aspect of those mentioned above was measured by a set of questions.

3. E-SHOPPERS PERSONAL PROFILE

Figure (1) shows the gender distribution of e-shoppers.

As shown from figure (1) we can see that males' constructs about 70% of e-shoppers based on our sample. This larger share for males may be explained under the fact that males have a broader access to the web than females in our community; as well more chances to shop online cause working males have their own monthly income. This also could be related to the larger share of males in the labor force. where the

females in the labor force constructs 15% of the total labor force according to DOS /2002 statistical indicators booklet,

Figure (**2**) shows the age distribution for e-shoppers based on the selected sample. As shown from the figure we can see that the ages of e-shoppers are clustered between 25 and 40 years old .having on a very rare occasion ages that exceeds 40 years old.

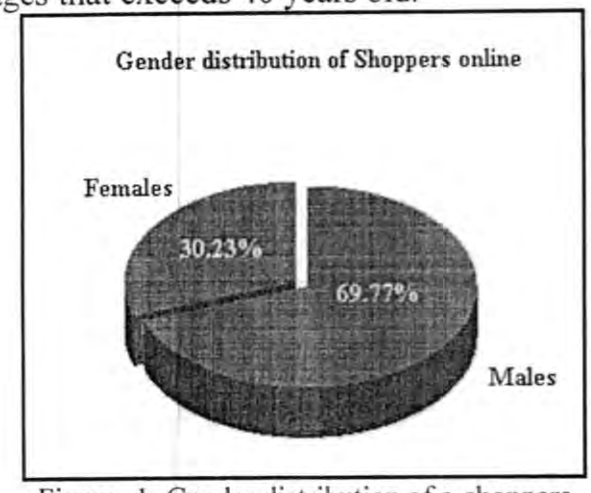

Figure -1: Gender distribution of e-shoppers

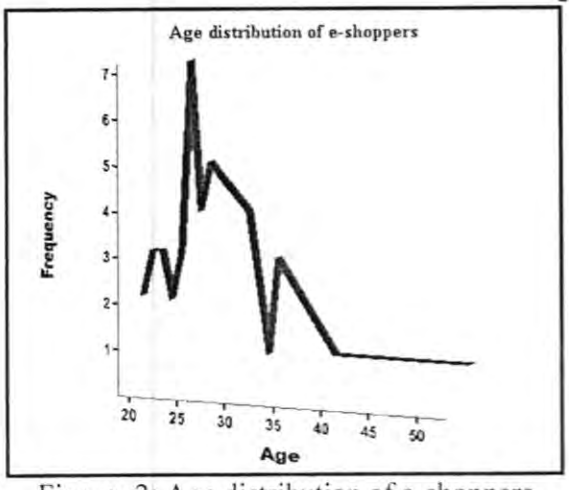

Figure -2: Age distribution of e-shoppers

Figure (**3**) shows the occupation distribution for e-shoppers based on the selected sample. As shown from the figure we can see that the academic sector occupies the largest share of the e-shoppers which constructs about 40% of the total .followed by medical and employees sectors with equal shares namely 15.79% for each. IT related and (Accounting and marketing) sectors comes in the third place with equal shares for each that is about 8%.

Actually I can not make much of the occupation distribution since the sample was selected conveniently and it did not cover all occupations in different sectors. But this will not affect this study since I was interested in E-shoppers perceptions regarding e-shopping experience and that is

the common thing between all those 43 e-shoppers that has nothing to do with the occupation of a certain individual. So here I just present the occupational profile of e-shoppers based on our sample.

Figure (**4**) shows the educational level for e-shoppers based on the selected sample. One can see from the figure that almost 60% of e-shoppers covered in the sample are bachelor degree carriers, followed by about 33% who are higher educated. What is interesting about figure (**4**) which represents educational level of those e-shoppers covered in our sample is that it contains about 10% who are below the bachelor. Half of those who are under bachelor are only high school graduates the other half are intermediate college graduates. This notice is of a significant importance .since it reveals the fact that e-shopping is not limited on those who enjoys higher educational level.

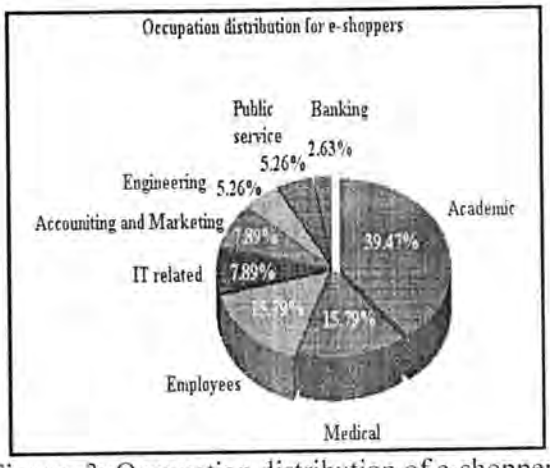

Figure -3: Occupation distribution of e-shoppers

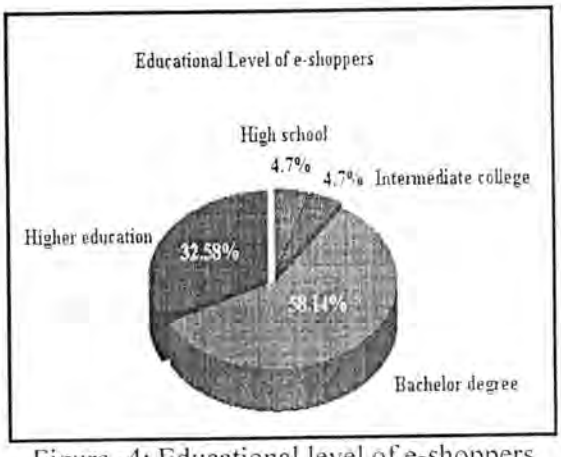

Figure -4: Educational level of e-shoppers

Figure (**5**) shows the household's monthly income for e-shoppers based on the selected sample. Observing figure (**5**) we can see that about 43% of e-shoppers enjoy a household's monthly income of greater than JD 500.

35% of them enjoy an income of less than JD 300 and the rest of them namely 22% enjoy a monthly income of JD 300- JD 500.
From obtained results we can see a significant notice over here that is relatively low monthly income did not construct an obstacle for a noticeable group of e-shoppers which constructed 35% of e-shoppers covered. This phenomenon is highly positive and promising for e-shopping diffusion as it reveals the fact that e-shopping can serve all financial levels of people.

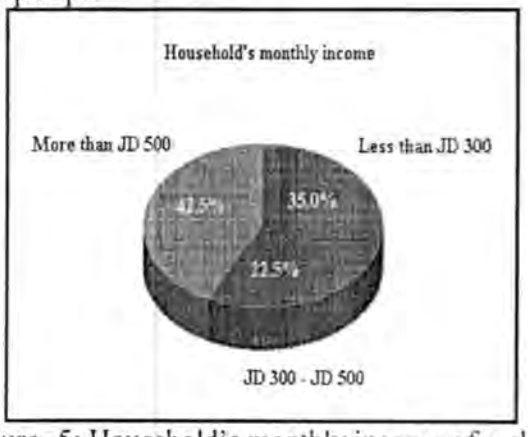

Figure -5: Household's monthly income of e-shoppers

4. E-SHOPPERS RESULTS

Figure (6) shows the internet experience for e-shoppers based on selected sample. As the figure shows there is about 63% of those who have experienced e-shopping before who enjoy an internet experience of more than 4 years. This percentage drops gradually as internet experience declines. This indicates that most of e-shoppers-covered in our sample- enjoy a relatively long experience of internet.
Figure (7) shows e-shoppers opinions regarding affordability to buy a pc and subscribe on the internet. Observing figure (7) we notice that about 40% of e-shoppers covered in our sample were hesitant about the question of whether buying a pc and subscribing on the internet were beyond their financial capabilities. An almost equal proportion of them namely 38% declared that buying a pc and subscribing on the internet are within their financial capabilities.
A considerable proportion from e-shoppers - namely 21.4% - thinks that it is beyond their financial capabilities to buy a pc and subscribe on the internet .what's significant about those results is that there is a noticeable proportion of those who have experienced e-shopping in spite of their inability to buy a pc and subscribe on the web -i.e. having access to the web from home.

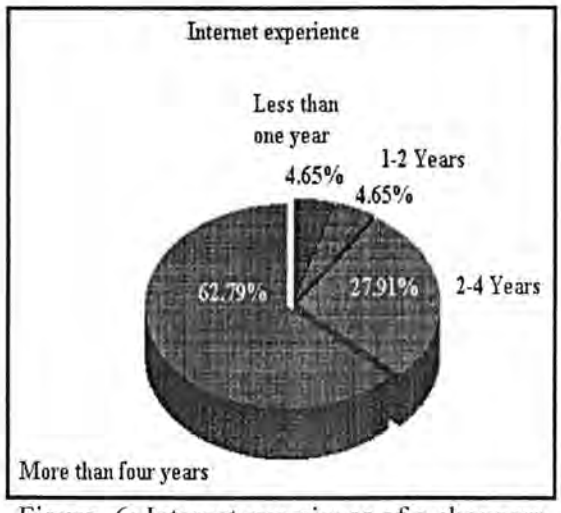

Figure -6: Internet experience of e-shoppers

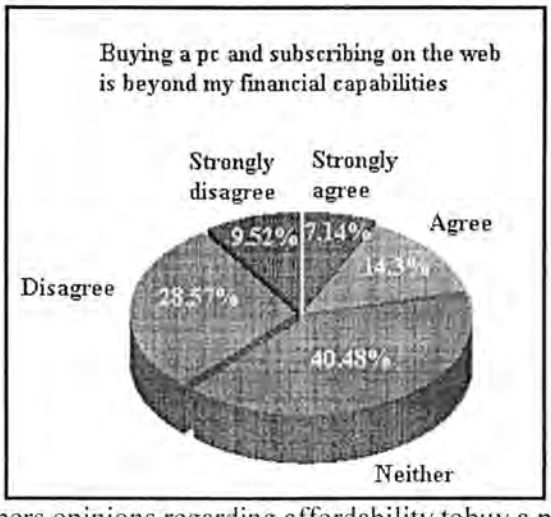

Figure -7: E-shoppers opinions regarding affordability tobuy a pc and subscribe on .the internet

In addition to that the ones who are hesitant about their financial capabilities can be considered as not much sure of their affordability to buy a pc and gain access to the web from home. If we sum up those two categories namely –the ones who think that it's beyond their financial capabilities to have a pc and gain access to the web from home and the ones who are not sure about that the resulting percentage will be about 62% which is enough significant to assure that every body has the opportunity to shop online even if he does not have access to the web from home or even if he does not have a pc at home!

Previous records shows that personal computer per 100 inhabitants amounted to 1.4, comparing to the world figure which was 6.8 in year 1999, and increased to about 4% in year 2002 . So we're talking about a relatively small diffusion of pc's in Jordan although this constructs a barrier in front of internet penetration rates it did not prevent 21.4% of

e-shoppers from conducting shopping online based on our sample. As to analyze level of awareness of e-shopping between e-shoppers, the covered e-shoppers were asked whether they can explain if e-shopping is advantageous or not. The results obtained were as shown in figure (**8**).

Figure (**8**) shows that about 45% of e-shoppers think that advantages of e-shopping are clear for them.19% of covered e-shoppers declared that it's hard for them to explain if shopping online is beneficial or not. And the rest of them-namely 35% are hesitant about that issue.

Obtained results indicate that there is a significant percentage of those who have experienced e-shopping before-namely 55% but yet are either unaware of e-shopping advantages or not sure if they can explain why e-shopping may be beneficial.

This situation illustrates the need for more efforts as to increase awareness level of e-shopping in Jordan. As to analyze security and privacy aspect three questions were set to discover e-shoppers attitudes towards the issue of privacy and security over the web. These questions discover the following:

- E-shopping level of security
- Possibility of capturing credit card number and misuse it in an illegal way.
- Possibility of being monitored while using the web.

Figures (**9**), (**10**), and (**11**) show the overall results obtained for those three questions.

Figure (**9**) shows that about 33% of e-shoppers covered in our sample think that e-shopping is not a secure method of shopping compared with 26% who believe that e-shopping is a secure way of shopping. Leaving more than 40% who are hesitant about e-shopping security.

These results can be explained in the light of the different criteria adopted by each category of e-shoppers regarding the issue of security via the web. Where different opinions obtained can be related to different considerations adopted by e-shoppers. Or in other words security issue depends on several factors like reliability and reputation of e-vendors and payment method.

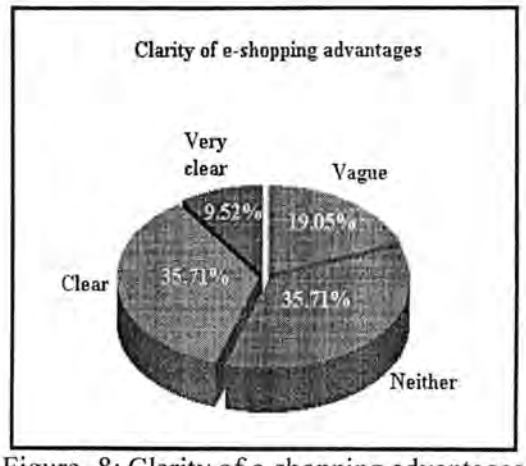

Figure -8: Clarity of e-shopping advantages

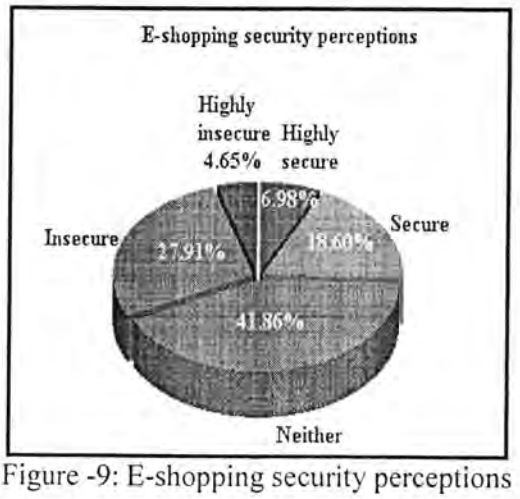

Figure -9: E-shopping security perceptions

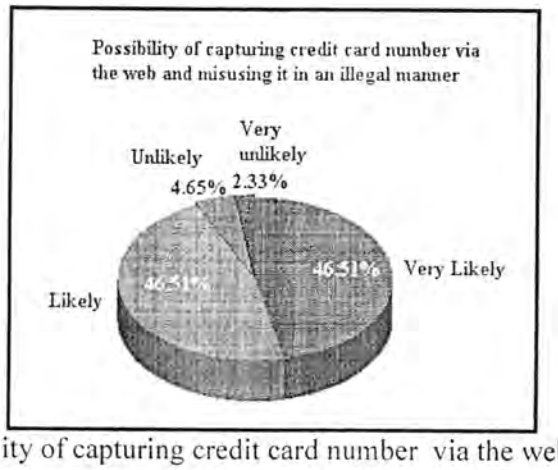

Figure -10: Possibility of capturing credit card number via the web and misusing in an illegal manner

Observing figure (**10**) which shows e-shoppers' opinions about the possibility of capturing credit card number via the web and misusing it in an illegal way, 47% of e-shoppers think that it's highly possible to thieve and misuse credit card number. Another 47% agree about the

possibility of credit card theft making a total of 93% of e-shoppers who believes about the possibility of capturing credit card number and misuse it in an illegal manner.

Looking at figures(**9**) and (**10**) we note that e-shoppers showed much clearer response when they were asked about a specific topic that is (possibility of credit card theft).different from their response for previous question that was about security via the web in general.

This fear from the possibility of being a victim of a credit card theft is reasonable since there is no guarantee for the victim to be covered by the law when his credit card is misused.

Investigating e-shoppers attitudes towards level of privacy they feel while using the web, e-shoppers were asked about the possibility of being monitored while using the web and the results obtained are shown by figure (**11**).

As shown from the figure about 72% of e-shoppers covered in our sample believe that it is possible to be monitored while online. About 35% thinks that it is very likely to be monitored while on line. Only a small proportion of them namely 14% thinks that it is unlikely to be monitored while using the web.

Obtained results indicate that e-shoppers think that the level of privacy via the web is poor in general.

Examining e-shoppers' attitudes towards e-shopping several issues were considered, these issues include:

- Easiness of learning how to shop online.
- Perceptions about Kind of people who shop online.

Obtained results are shown below.

Figure (**12**) shows e-shoppers opinions regarding the easiness of learning how to shop online. As the figure shows there are about 88% of e-shoppers who thinks that it's easy to learn how to shop online. Only a small portion of them-namely 2% think not.9.3% are not sure. This attitude shows that it's not of a great deal to learn how to shop online from e-shoppers point of view.

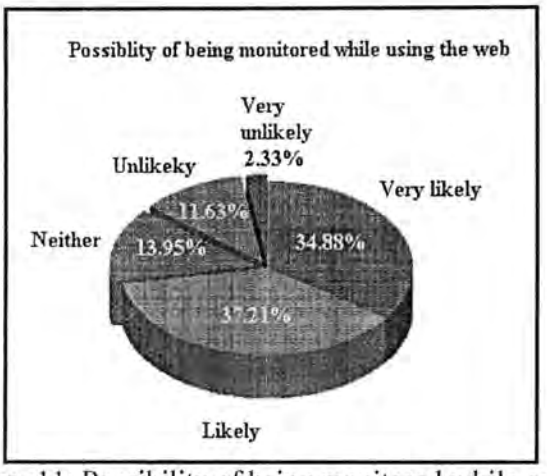

Figure -11: Possibility of being monitored while online

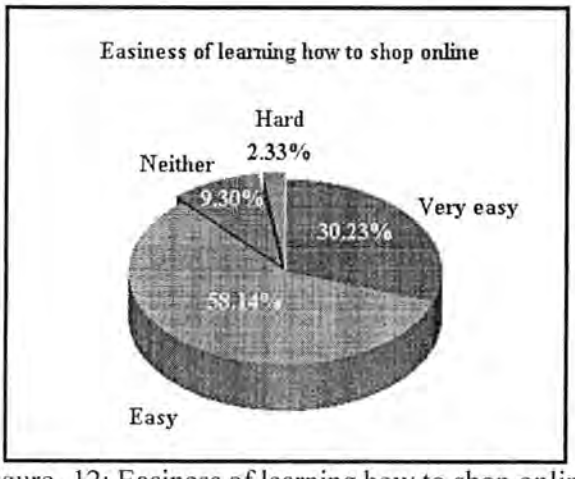

Figure -12: Easiness of learning how to shop online

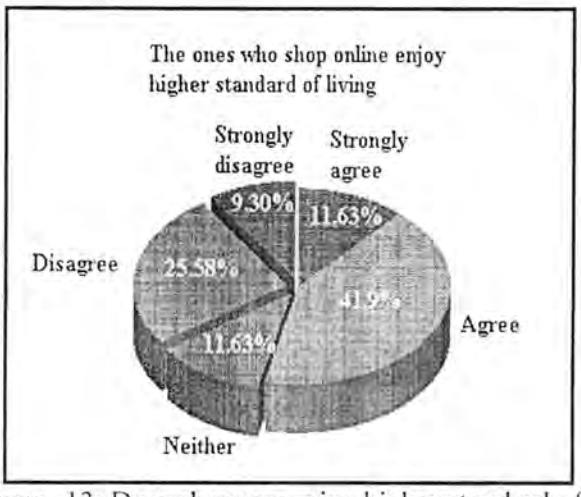

Figure -13: Do e-shoppers enjoy higher standard of living

Figure (**13**) identifies e-shoppers' attitudes regarding the lifestyle of those who do shop online. The results show that 53% thinks that those who do shop online enjoy a higher standard of living.35% of them think

not, and the rest don't know. Comparing these results with that of non-shoppers online regarding this question it was found that 56% of non-shoppers online think that e-shoppers enjoy higher standard of living, 24% of them think not. And 19% don't know. The closeness of obtained results regarding this question for e-shopper and non-shoppers online suggests that it's some kind of common attitude between people that e-shoppers enjoy higher standard of living, and this contradicts what we found previously that there is about 35% of e-shoppers who enjoy a monthly income of less than JD 300!

E-shoppers preferences were investigated considering two matters that are

- Preferred intermediate (third) party.
- Importance of various features that qualifies a certain site as a potential one to do e-shopping with.

E-shoppers were asked about the third intermediate party they prefer where this third party is characterized by the following:

Instead of dealing separately with each vendor, the buyer would set up an account with a central company (the issuer), then when making a purchase from any participating vendor; the buyer would provide this account number to the vendor. In turn, the vendor would get approval from the issuer, and ship/provide the product/service to the buyer. Finally the issuer would charge the purchase amount from buyer's account.

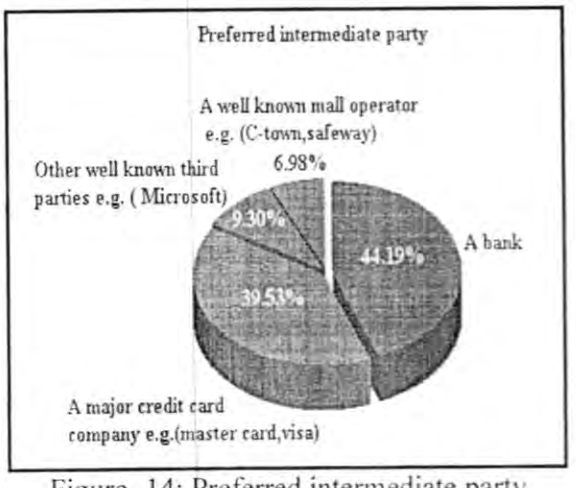

Figure -14: Preferred intermediate party

As observed from figure(**14**) which shows e-shoppers' preferred intermediate party we can see a general comfort of e-shoppers to have a bank as a third intermediate party, this comfort is indicated by a proportion of 44% of e-shoppers who preferred the bank choice. Major credit card companies come in the second place of e-shoppers' preferences with a proportion of about 40% of e-shoppers who preferred a major credit card company as a third intermediate party. Other well

known companies like Microsoft come in the third place followed by major mall operators' choice that come at last. With supporters of 9.3% and 7% of e-shoppers respectively.

E-shoppers experience regarding actual practice of shopping on line was sought through the following issues:

- Number of purchases made last year (figure 15).
- E-shopping advantages.
- Order, payment and delivery features of e-shopping.
- E-vendors characteristics.
- Most known e-companies.
- Frequently purchased items.

Following figures summarize the results obtained.

- **Number of purchases made online last year**
- **E-shopping advantages**

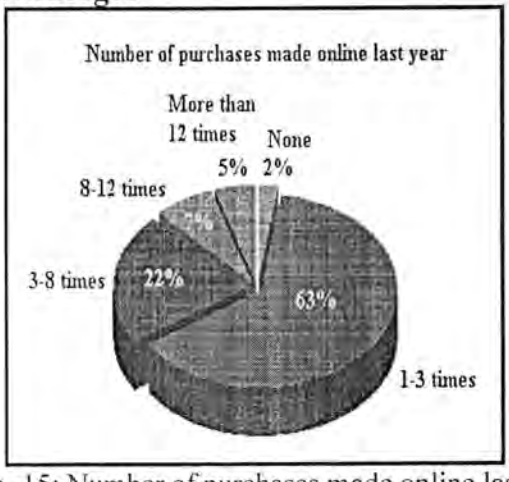

Figure -15: Number of purchases made online last year

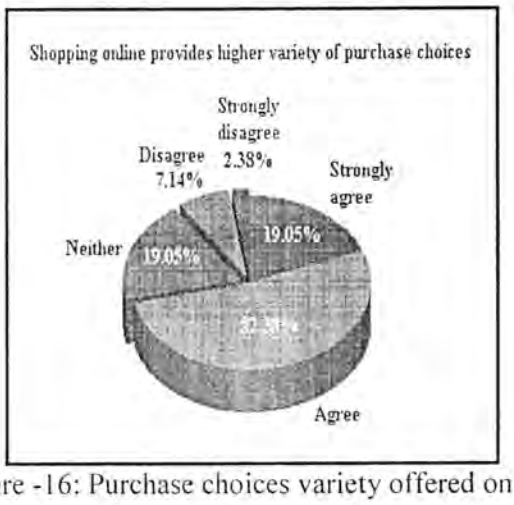

Figure -16: Purchase choices variety offered online

Figure (**16**) shows e-shoppers' opinions about the variety of purchase choices offered online. As obvious from the figure about 71% of e-shoppers covered in our sample believe that shopping online provides higher variety of purchase choices concerning a certain good. Only 9% don't think so. And 19% are not sure.
This feature is one of the most important characteristics of e-shopping since the amount of purchase choices for a certain good is considerably high.

Order, payment and delivery features of e-shopping

Figure (**17**) shows e-shoppers opinions about time taken by e-vendors to deliver a certain product to the target customer. As observed from the figure there is a considerable proportion of e-shoppers covered in this study namely 51% who believe that e-vendors take longer time to deliver goods to their destinations than that time taken by traditional vendors. A small proportion of e-shoppers-namely 10% disagree and 38% of them are not sure about the answer of this question.
Discussing matter of delivery times it differs based on several factors like location of e-vendor, type of purchased product and delivery mechanism. Where farther e-vendors are expected to take longer time to deliver a certain product. And a digital product differs from a physical one in terms of time of delivery where digital product like songs, movies, software takes virtually no time for delivery except that needed for downloading.
It is worth mentioning that physical post addresses in Jordan are one of the major problems that limit that diffusion of e-shopping and e-commerce in general since the lack of a standard globally recognized and efficient physical address establishes a major obstacle regarding delivery.

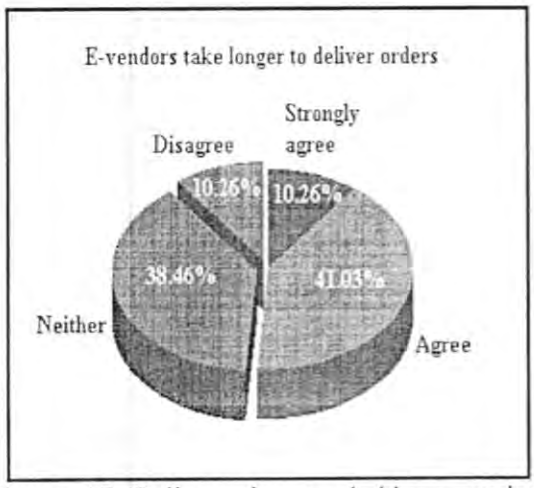

Figure -17: Delivery time needed by e-vendors

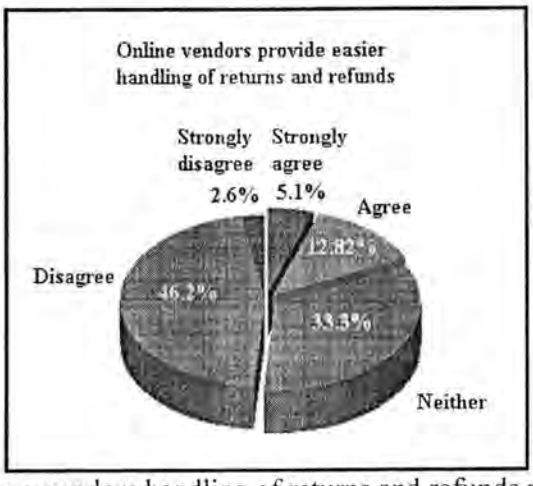

Figure -18-: e-vendors handling of returns and refunds perceptions

A significant proportion of e-shoppers covered in our sample namely 49% declare that it's not easy to handle returns and refunds with e-vendors as shown from figure (**18**). Only 18% think that it's easy to do so and 33% are not sure of their answers. This issue can be considered as disadvantage of e-shopping especially when e-vendor is located faraway from the customer, which makes it hard to physically contact e-vendor.

Figure (**19**) investigates e-shoppers opinions regarding e-payment facilities offered by e-vendors. About 46% of e-shoppers covered in selected sample think that e-vendors provide easier payment procedures.13% disagree and 41% are not sure. These results indicate that the matter of payment online is questionable. Since a significant proportion of 41% are hesitant or not sure if e-payment procedures online is easier than traditional ones.

It is worth mentioning that e-payment procedures and settlement still have some problems in Jordan and the main reason is the lack of regulation which is the real environment under which companies and organizations can conduct e-commerce effectively. The law is there but the regulations needed to set the law in practice are still under debate.

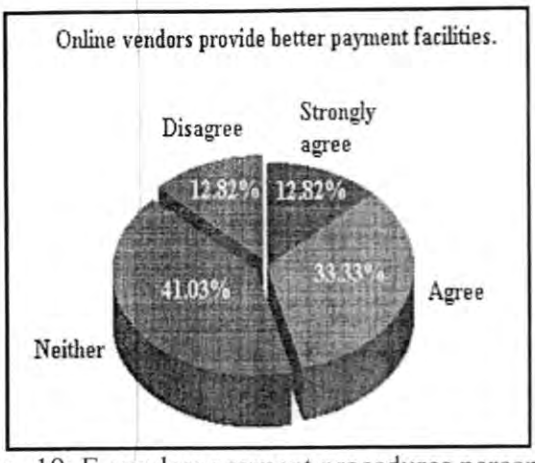

Figure -19: E-vendors payment procedures perceptions

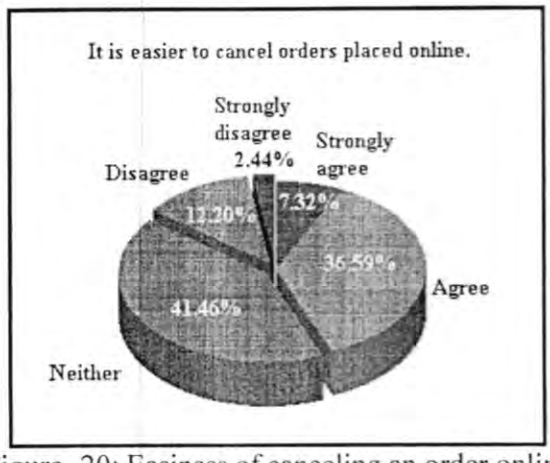

Figure -20: Easiness of canceling an order online

Figure (**20**) shows 44% of e-shoppers covered in our sample think that it's easier to cancel orders placed online than those made traditionally. Only 15% disagree and 41% are not sure.

E-vendors characteristics

Answering the question whether online vendors provide better customer services and after –sale support 27% of e-shopper don't think so as seen from figure (**21**). About 23% agree and around 50% neither agree nor disagree. These percentages are some how reasonable since e-shopping lacks physical contact that enables for effective customer service and after sale support.

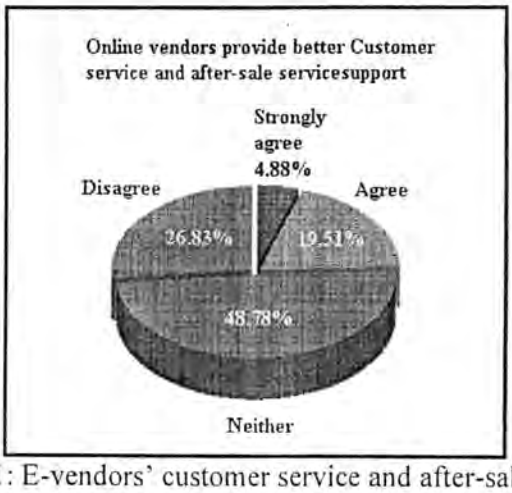

Figure -21: E-vendors' customer service and after-sale support

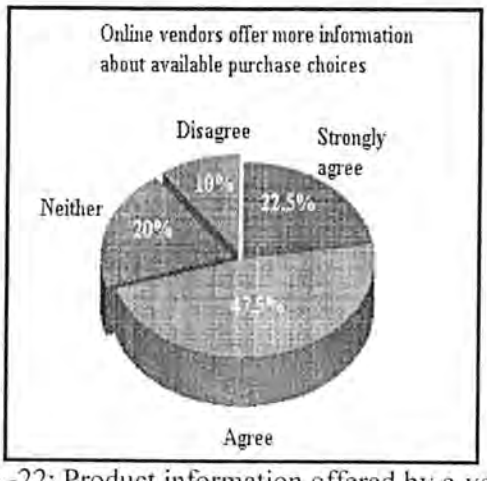

Figure -22: Product information offered by e-vendors

70% of e-shoppers covered in selected sample believe that e-vendors provide more information about available purchase choices as clear from figure (**22**).only 10% of them disagree and 20% are hesitant. Obtained results make sense, since available facilities of presentation and exhibition for products' features and specifications via the web can be more detailed and condense. Where customers can gain more detailed information about the things they look for attempting to discover their shopping potentials.

Figure (**23**) displays e-shoppers opinions regarding reliability of e-vendors. As shown from the figure about 48% of them think that e-vendors are not reliable, 20% think that e-vendors are reliable and 33% are not sure. Differences in e-shoppers opinions regarding reliability of e-vendors can be due their different experiences or different kinds of e-vendors they deal with. But in general there is low level of trust in e-vendors which constructs a major obstacle that faces e-shopping as well as e-commerce diffusion. This low level of trust is explained by the fact that there is no regulatory frame work that guarantees consumer rights if

subjected to an electronic crime. Accounting for this problem Jordan government makes it efforts as develop necessary regulatory framework aiming at boosting e-conversion.

Based on our sample 54% of covered e-shoppers believe that e-vendors offer better prices than that of traditional vendors as figure (**24**) shows. Only 7% do not think so and a proportion of about 40% are not sure. Results of those who think that e-vendors offer better prices can be explained by high competition between e-vendors via the web which in turn allows for better prices for a certain product online.

On the other hand a certain product may be competent price-wise nevertheless delivery and shipment costs may add extra cost that alter the advantage of low cost of the product it self.

Considering the situation in Jordan, the shipping cost is relatively high due to lack of physical post addresses which in turn forces the consumer to employ an intermediate party – Aramex for example – to deliver him involved product.

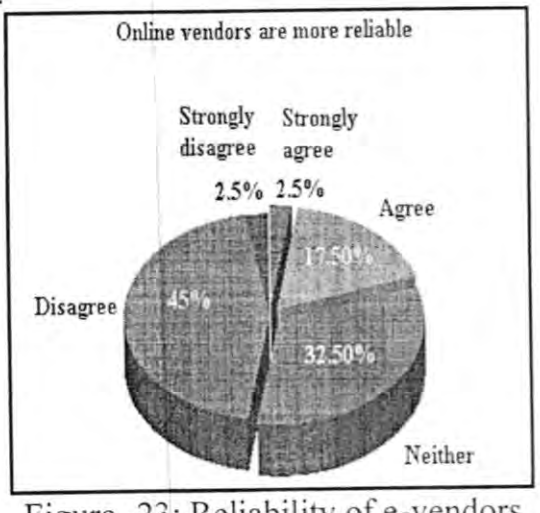

Figure -23: Reliability of e-vendors

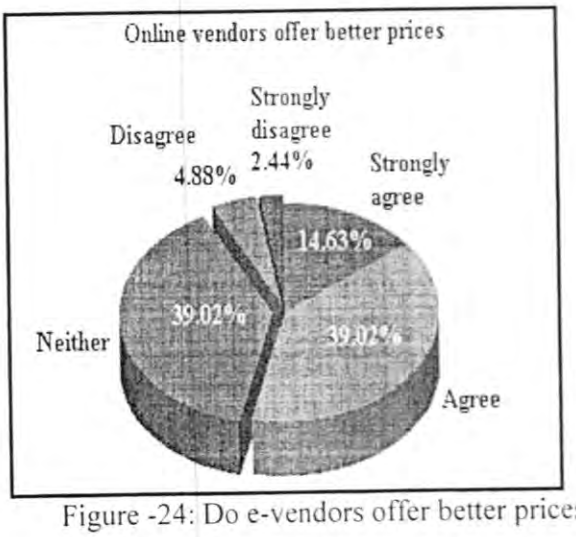

Figure -24: Do e-vendors offer better prices

Most known e-companies

Amazon ranked in the first place of most known companies e-shoppers deal with ,with a share of 29%,Aramex and GAP ranked in the second place with equal shares of 6% followed by Microsoft, Old nany and Yahoo who ranked third with equal shares of 4%.all shown in figure(**25**)

Frequently purchased items

As shown from figure(**26**) books ranked in the first place as the most purchased items through the web with a share of 29% followed by clothes and shoes which ranked second with a share of 16%.Softwares come in the third place with a share of 6% followed by CD's and movies with equal shares of 5%.

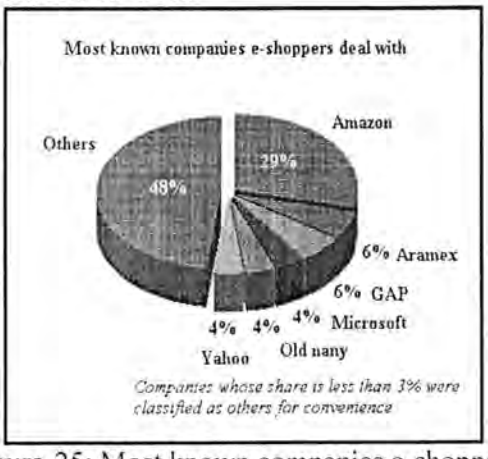

Figure-25: Most known companies e-shoppers

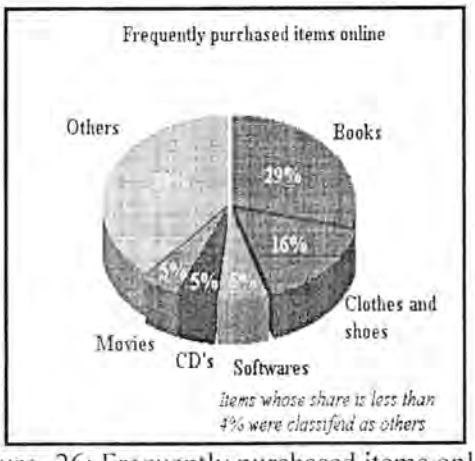

Figure -26: Frequently purchased items online

Investigating the clothes share it was found that about 80% of those who buy clothes via the web are females. And 33% of those who buy books online are from the academic sector- i.e. either students or

teachers with higher student's share that is as much as twice of that for teachers.

CONCLUSIONS

Based on selected sample the following conclusions are developed:

- Most of e-shoppers are males with a share of about 70% against about 30% for females.
- About 40% of e-shoppers' occupations were academic related i.e. (students and teachers).
- E-shopping is not limited on those who enjoy higher educational level, since about 10% of e-shoppers are carriers of scientific degrees below bachelor. Half of those who are under bachelor are only high school graduates and the other half are intermediate college graduates.
- E-shopping is not limited on those who enjoy higher household's monthly income since there are about 35% of e-shoppers who enjoy relatively low monthly income of less than JD 300 yet they have practiced e-shopping. This phenomenon is highly positive and promising for e-shopping diffusion as it reveals the fact that e-shopping can serve all levels of people.
- Most of e-shoppers-namely 63% enjoy an internet experience of more than 4 years.
- There is a noticeable proportion- namely 21.4% - of those who have experienced e-shopping in spite of their inability to buy a pc and subscribe on the web -i.e. having access to the web from home.
- There is a significant percentage of those who have experienced e-shopping before-namely 55% but yet are either unable to explain whether e-shopping is beneficial, or not sure if they can explain why e-shopping may be beneficial.
- There is a low level of trust regarding e-shopping as a method of shopping, also there is a common belief that it's highly likely to be a victim of a credit theft via the web.
- 53% of e-shoppers and corresponding 56% of non-shoppers think that e-shoppers enjoy higher standard of living. This common belief constructs an obstacle facing e-shopping diffusion since it suggests that e-shopping may be limited to a certain group of people.
- There is a general comfort of e-shoppers to have a bank as a third intermediate party; this comfort is indicated by a proportion of 44% of e-shoppers who preferred the bank choice. Major credit card companies come in the second place of e-shoppers' preferences with supporters' proportion of about 40%.a minor

proportion of e-shoppers' namely 9% and 7% prefer to have another well known company like(Microsoft) or a well known mall operator like(C-town or Safeway) as an intermediate party respectively.

- Longer delivery time is one of the disadvantages of e-vendors as perceived by 51% of e-shoppers.
- Handling of returns and refunds is a disadvantage of e-vendors as declared by 50% of e-shoppers.
- Lack of physical contact is another disadvantage of e-shopping that decreases the level of effective customer service and after sale support.
- Offering more information about available purchase choices is an advantage of e-shopping over traditional shopping as perceived by 70% of e-shoppers.
- Amazon ranked in the first place of most known companies e-shoppers deal with ,with a share of 29%,Aramex and GAP ranked in the second place with equal shares of 6% followed by Microsoft, Yahoo and (Old nany) which ranked third with equal shares of 4%.
- Books ranked in the first place as the most purchased items through the web with a share of 29% followed by clothes and shoes which ranked second with a share of 16%.Softwares come in the third place with a share of 6% followed by CD's and movies with equal shares of 5%.
- 80% of those who buy clothes via the web are females.

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Strange Attractor and Chaotic Motions of Two-Dimensional Nonlinear Invertable Map

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Received 11/2/2011 – Accepted 17/1/2012

الخلاصة

هذا البحث يستعرض التطور المصاحب لجاذب غريب مع الحركة الفوضوية لتطبيق ثنائي البعد في نظام ديناميكي. تطبيق هينون سيكون مثال للنظام أعلاه مع طريقة مبسطة لحساب البعد الكسري، والذي هو صفه اساسية لما يسمى "الجاذب الغريب".

ABSTRACT

In this work, a review will be presented of development related to strange attractor and motions of 2-dimensional dynamical system. Hénon map will be an example to the above system with simple method to calculate it's fractal dimension (capacity dimension), which it one of the basic property of "strange attractor".

INTRODUCTION

A general two-dimensional map can be written as:

$$\left.\begin{aligned} x_{n+1} &= f_1(x_n, y_n) \\ y_{n+1} &= f_2(x_n, y_n) \end{aligned}\right\} \qquad [1]$$

The map is invertable if equation [1] can be solved uniquely for x_n and y_n as a function of x_{n+1} and y_{n+1}; $x_n = g_1(x_{n+1}, y_{n+1})$ and $y_n = g_2(x_{n+1}, y_{n+1})$. i.e. it's possible to go either back wards or for words in time, see (1).

The theory of dynamical systems was put in shape by Poincaré and his followers, who considered only finite dimensional spaces, the first system studied were, quite naturally, the simplest ones, for instance in the plan, were the only limit sets and attractors are points of equilibrium (rest point), periodic solutions (closed orbits) or other things not much more complicated.

In studying tridimensional systems there appeared limite sets of structure not so simple, for these systems, there is no theorem telling us what the limite sets or the attractors should look like, see (2).
i.e. of one consider a system and it's phase space the initial conditions may be attracted to some subset of the phase space (the attractor) as time $t \longrightarrow \infty$. For example for a damped harmonic oscillator (Figure 1, a) the attractors is the point at rest (in this case the origin) for periodically driven oscillator in its limit cycle the limit set is a closed curve in the phase space (Figure 1, b).

In the above two examples the attractors were point (Figure 1, a) which is a set of dimension (zero), and a closed curve (Figure 1, b), which is a set of dimension (one), for many other attractors, the

attracting set can be much more irregular (some would say pathological and in fact can have a dimension that is not an integer, such sets have been called (fractal) and, when they are attractors, they are called "strange attractors", see (3).

Strange Attractors

There can be attractors which have non-integer dimension (at least according to the definition of dimension that we will use). Such attractors would be termed "strange" the relevant definition of dimension is that due to Housdorf (1), (called the box-counting or capacity dimension (3), and defined as follows:

$$d = \lim_{\varepsilon \to 0} \frac{\ln(N(\varepsilon))}{\ln(1/\varepsilon)} \qquad \ldots[2]$$

Examples: (2)

1. As an example of equation [2], if the set in equation is a point, then $N(\varepsilon) = 1 = \varepsilon^0$, and according to equation [2] the Housedorf dimension (capacity dimension) is zero, i.e. $d = 0$.

2. If the set in equation [2] is the section of the xy-plane given by $0 < x < 1$ and $0 < y < 1$, then $N(\varepsilon) = \varepsilon^{-2}$, and the Housdorf dimension is two, i.e. $d = 2$.

3. If the set is a straight line joining (0,0) and (1,0) then $N(\varepsilon) = \varepsilon^{-1}$ and the Housdorf dimension is one, i.e. $d = 1$.

4. As an example of a set with non-integer dimension, consider the following construction of a (Cantor set) (illustrated in figure 2), take a line of unit length, $0 \leq x \leq 1$ and remove the middle third $1/3 < x < 2/3$, then take the two remaining intervals between 0 and 1/3, and between 2/3 and 1, divide them in third, in the limite as this process is repeated and infinite number of time, what is left is a set that has zero net length and an uncountable number of elements, to apply equation 2 to this sets, we note the following which is evedent from (figure 2).

5. Thus from equation [2]

$d = \ln(2) / \ln(3) = 0.630$.

Note that the Cantor set just constructed has the property of scale invariance. i.e., by the nature of the construction, the set between 0 and 1 will look precisely, the same as that part of it between 0 and 1/3, if the latter is examined under magnifying glass which magnifies by a factor of three.

Hénon Attractor

About 35 years ago the French astronomer-mathematician Michel Hénon was searching for simple two properties of more complicated systems, the result was a family of functions denoted by H_{ab} and given by

$$H_{ab}\begin{pmatrix} x \\ y \end{pmatrix} = \begin{bmatrix} 1 - ax^2 + y \\ bx \end{bmatrix} \quad \ldots[3]$$

where a and b are real numbers the maps defined in equation 3 is called Hénon map. Hénon map was one to one, invertable map with two fixed points:

$$p = \begin{bmatrix} 1/2a\left(b - 1 + \sqrt{(1-b)^2 + 4a}\right) \\ b/2a\left(b - 1 + \sqrt{(1-b)^2 + 4a}\right) \end{bmatrix} \text{ and } q = \begin{bmatrix} 1/2a\left(b - 1 - \sqrt{(1-b)^2 + 4a}\right) \\ b/2a\left(b - 1 - \sqrt{(1-b)^2 + 4a}\right) \end{bmatrix}.$$

Where p is an attracting fixed point when a is a non zero number lying in the interval $J = (-1/4(1-b)^2, 3/4(1-b)^2)$, and q is a saddle fixed point when a is a non-zero number and $a > -(1-b)^2/4$ where $0 < b < 1$, see (4).

Analytic Look

In this section we will analyze the chaotic iterated motion of Hénon map in equation [3].

As a increases further, H_{ab} undergoes a period doubling cascade, for b = 0.3 the bifurcation values of a is as follows see (4).

Period n-cycle Appears	Bifurcation of a
1	- 0.1225
2	0.3675
4	0.9125
8	1.0260
16	1.0510.....
32	1.0565.....

For a > 1.06 the iterates of virtually any initial point would be sprinkled unpredictably throughout a region in the plane for example let a = 1.4, b = 0.3, if we neglect the first few iterates of 0 and plote the next 10.000 then we obtain that the shape A_H appearing in (figure 3) the set A_H is called Hénon attractor, because the iterates of every point in a certain quadrateral Q surrounding A_H approach the attractor. (Note, take initial value near one of the unstable fixed points such as x = 0.631355, y = 0.18941 these values yield in the xy-plane the strange attractor with its (basin of attraction in black); initial condition in the white regions outside the basin are (attracted to infinity).

Although A_H may appear to consist of a few fairly simple curves (figure 4) when zoom in on a small rectangle containing the fixed point p, we see that there are several strands (figure 4(a)) no matter how much we magnify the region, nearly identical new sets of strands appear in (figures 4(b) and 4(c)). It turns out that there are reality an infinite

number of such stands that make the region near to p look like a product of a line and "Cantor set:

Capacity Dimension of Hénon Map:

The Author in (1) p. 661 tried to approximate the capacity dimension to the strange attractor of Hénon map gives $d \cong 1.26$.

To do so see figure 5, notice that the number of rectangles that intersect A_H are 140 if we let ε equal to the high of each rectangle, then $1 / \varepsilon = 12.8$.

S o that

$$d = \frac{\ln(N(\varepsilon))}{\ln(1/\varepsilon)} = 1.89700099 \square 1.897$$

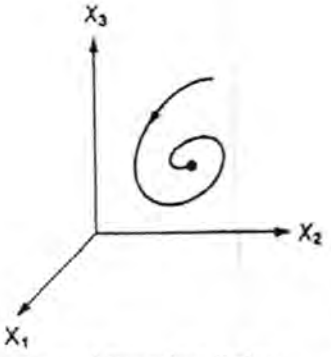

Figure-1-a: Attracting Point

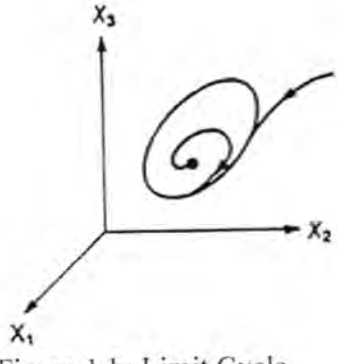

Figure-1-b: Limit Cycle

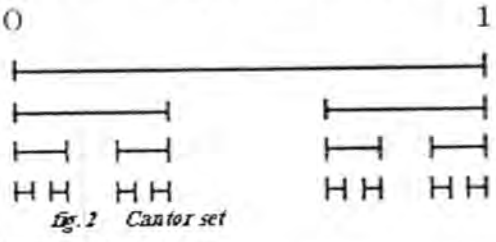

fig. 2 Cantor set

Figure-2: Cantor Set

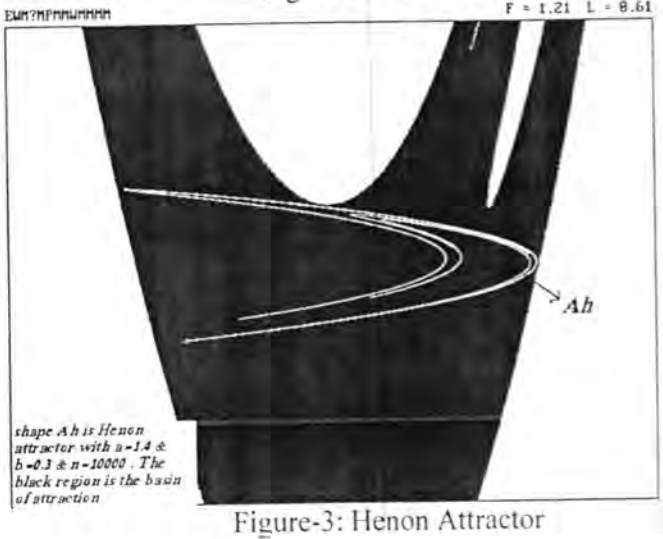

Figure-3: Henon Attractor

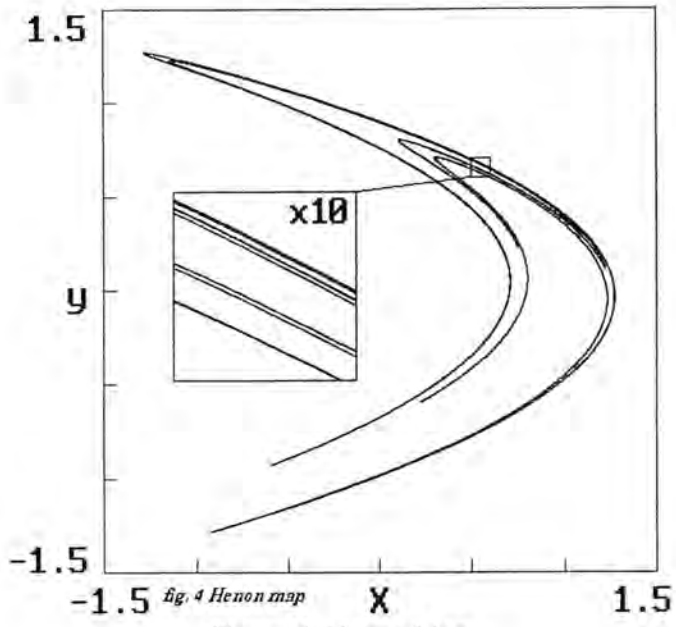

Figure-4: Henon Map

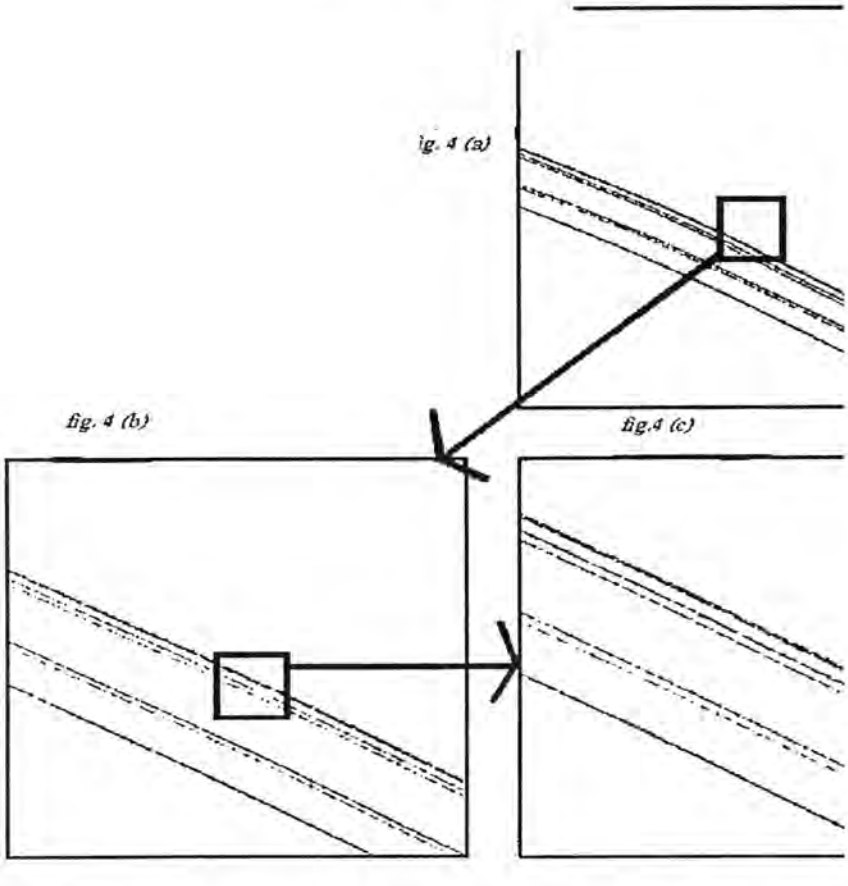

Figure-4: After Zooming in on Small Rectangle from figure 4 containing the Fixed Point at (a) to (b) then (c)

Note:

Figure 5 gained by using basic statement language, the program executed on a Pentium pro computer.

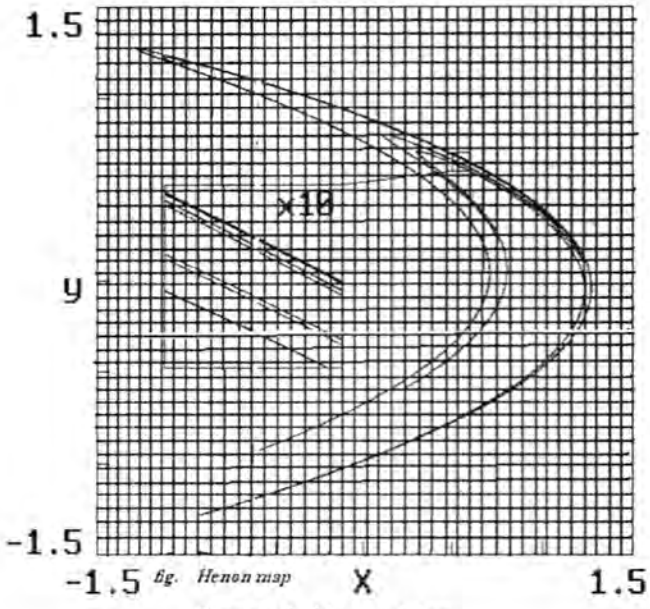

Figure-5: Henon Map

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Semi-Analytic Method For Solving High Order Ordinary Differential Equations With Initial Condition

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Received 7/9/2010 – Accepted 2/3/2011

الخلاصة

الهدف من هذا البحث عرض طريقة لحل معادلات تفاضلية اعتيادية ذات رتب عالية لمسائل القيم الابتدائية باستخدام التقنية شبه التحليلية مع تكوين الحل كمتعددة حدود ، أصل المسالة يتعلق باستخدام الاندراج التماسي ذو النقطتين والذي يتفق مع الدالة ومشتقاتها عند نقطتي نهاية الفترة [1 , 0] المعرفة عليها .
كذلك تم مناقشة التحسس للمعادلات التفاضلية الاعتيادية .

ABSTRACT

The aim of this paper is to present method for solution high order ordinary differential equations with initial condition using semi-analytic technique with constructing polynomial solutions. The original problem is concerned using two-point osculatory interpolation with the fit equal numbers of derivatives at the end points of an interval [0 , 1] and give example illustrate suggested method and accuracy, easily implemented . The accuracy of the method is confirmed by compared with conventional methods (Runga-Kutta (RK4), RK-Butcher .Differential Transformation method (DTM)) .
The sensitivity of solutions high order ordinary differential equations with initial condition is discussed.

1. INTRODUCTION

The ordinary differential equation (ODE) problems are encountered in many practical applications such as physics, engineering design, fluid dynamics and other scientific applications. The exact solutions of ODE are practically difficult due to its dynamical nature, so the need to approximate the solution arises. In this regard we have numerical algorithms like Euler , Improved Euler , Runge – kutta, Adams Bashforth ,Finite Difference [1] ,Differential Transform Methods ,shooting methods [2] and collocation method [3] .

Today some of the most interesting methods are introduce in [4]. Since in various application use the analytic and approximation methods together so, these methods is said to be a semi-analytic method. In 2003, R.E.Grundy investigate the feasibility of using Hermite interpolation as a practical tool for constructing polynomial approximations to initial - boundary value problems for partial differential equations, also in 2005[5] he examine the feasibility of using two points Hermite interpolation as a systematic tool in the analysis of initial-boundary value problems for nonlinear diffusion equations. In 2006 R.E.Grundy analyses initial - boundary value problems involving nonlocal nonlinearities using two points Hermite interpolation, also, in 2006

show how two-points Hermite interpolation can be used to construct polynomial representations of solutions to some initial-boundary value problems for the inviscid Proudman-Johnson equation. In 2009, Mohammed [6] investigate the feasibility of using osculatory interpolation to solve two points second order boundary value problems. In this paper a semi-analytic method used which mixed the analytic and approximation methods for constructing polynomial solutions of high order ordinary differential equations with initial condition using two-points osculatory interpolation, in the present paper we concentrate on the development of the application to ordinary differential equations with the advent of modern symbolic computational facilities it has become possible to implement many techniques which were hitherto computationally and algebraically inaccessible. Thus an important feature of the paper is the use of the symbolic computational package MATLAB in the process of implementation together with its IVP and BVP library codes as a checking device. The main purpose is to demonstrate the general superiority of our preferred method vis-à-vis conventional methods. We note that Grundy in 2005[5] say such methods by semi-analytic method since, solving the problems by analytic method but the solution of problems contain the error : truncation error (local truncation error mean error made in advancing one step ,and global truncation error mean maximum error in the interval [a ,b]) and rounding error .

2. Interpolation Theory

In this paper, we shall consider the interpolatory approximation .From Weierstrass Approximation Theorem[7] ,it follows that one can always find a polynomial that is arbitrarily close to a given function on some finite interval. This means that the approximation error is bounded and can be reduced by the choice of the adequate polynomial. Unfortunately Weierstrass Approximation Theorem is not a constructive one, i.e. it does not present a way how to obtain such a polynomial. i.e. the interpolation problem can also be formulated in another way, viz. as the answer to the following question: How to find a .good. representative of a function that is not known explicitly, but only at some points of the domain of interest .In this paper we use Osculatory Interpolation since has high order with the same given points in the domain .

2.1. Osculatory Interpolation [7]

Given the data $\{x_i\}$, $i = 0,1, \ldots,n$ and values $f_i^{(0)}, \ldots, f_i^{(mi)}$,where m_i are nonnegative integers and $f_i = f(x_i)$ .We want to construct a polynomial P(x) such that :

$$P^{(j)}(x_i) = f_i^{(j)} \qquad \ldots\ldots\ldots\ldots \quad (1)$$

For each $i = 0, 1, \ldots, n$ and $j = 0, \ldots, m_i$.

. , n . Such a polynomial is said to be an osculatory interpolating polynomial of a function f .

Remark [7]

The degree of P(x) is at most $M = \sum_{i=1}^{n} m_i + n$, since the number of conditions to be satisfied is $\sum_{i=1}^{n} m_i + (n + 1)$, and a polynomial of degree M has M + 1 coefficients that can be used to satisfy these conditions .

There exist various form for osculatory interpolation , but all of these differed only in formula ,the following theorem illustrate this :

Theorem 1 [8] ,[9]

Given the nodes $\{x_i\}$, i = 0, . . . , n and values $\{ f_i^{(j)} \}$, j = 0, . . . , m_i , there exists a unique polynomial satisfying (1).

In this paper we use two-point osculatory interpolation [10]. The idea is to approximate a function y(x) by a polynomial P(x) in which values of y(x) and any number of its derivatives at given points are fitted by the corresponding function values and derivatives of P(x) ,we are particularly concerned with fitting function values and derivatives at the two end points of a finite interval, say [0 , 1] , a useful and succinct way of writing osculatory interpolant $P_{2n+1}(x)$ of degree 2n + 1 was given for example by Phillips [11] as :

$$P_{2n+1}(x) = \sum_{j=0}^{n} \{ y^{(j)}(0)\, q_j(x) + (-1)^j\, y^{(j)}(1)\, q_j(1-x) \} \qquad \ldots\ldots\ldots\ldots(2)$$

$$q_j(x) = (x^j / j!)(1-x)^{n+1} \sum_{s=0}^{n-j} \binom{n+s}{s} x^s = Q_j(x) / j! \qquad \ldots\ldots\ldots\ldots(3)$$

so that (2) with (3) satisfies :

$y^{(j)}(0) = P_{2n+1}^{(j)}(0)$, $y^{(j)}(1) = P_{2n+1}^{(j)}(1)$, j = 0, 1, 2,..., n .

implying that $P_{2n+1}(x)$ agrees with the appropriately truncated Taylor series for y(x) about x = 0 and x = 1. The error on [0, 1] is given by :

$$R_{2n+1} = y(x)-P_{2n+1}(x) = \frac{(-1)^{n+1} x^{(n+1)} (1-x)^{n+1} y^{(2n+2)}(\varepsilon)}{(2n+2)!}$$ where $\varepsilon \in (0, 1)$ and $y^{(2n+2)}$ is assumed to be continuous.

The osculatory interpolant for $P_{2n+1}(x)$ may converge to y(x) in [0 , 1] irrespective of whether the intervals of convergence of the constituent series intersect or are disjoint .The important consideration here is whether $R_{2n+1} \to 0$ as $n \to \infty$ for all x in [0 ,1]. We observe that (2) fits an equal number of derivatives at each end point but it is possible and indeed sometimes desirable to use polynomials which fit different numbers of derivatives at the end points of an interval .

Finally we observe that (2) can be written directly in terms of the Taylor coefficients a_i and b_i about x = 0 and x = 1 respectively, as :

$$P_{2n+1}(x) = \sum_{j=0}^{n} \{ a_j Q_j(x) + (-1)^j b_j Q_j(1-x) \} \quad \ldots \quad (4)$$

3. Solution Of Higher-Order Equation With Initial Condition

A general mth-order initial value problem :

$$y^{(m)} = f(x , y, y', y'', \ldots , y^{(m-1)}), \quad a \le x \le b \qquad \ldots\ldots(5)$$

With initial conditions :

$$y(a) = \alpha_1, y'(a) = \alpha_2, \ldots, y^{(m-1)}(a) = \alpha_m \qquad \ldots\ldots\ldots (6)$$

The system of m first-order differential equations (meaning that only the first derivative of y appears in the equation and no higher derivatives) have the form :

$$y'_1 = f_1 (x , y_1 , \ldots, y_m)$$
$$y'_2 = f_2 (x , y_1 , \ldots, y_m)$$
$$\vdots$$
$$y'_m = f_m(x, y_1, y_2, \ldots, y_m) \qquad (7\ a)$$

with initial conditions :

$$y_1(a) = \alpha_1 , y_2(a) = \alpha_2 , \ldots , y_m(a) = \alpha_m \qquad \ldots\ldots(7b)$$

It is easy to see that (7) can represent either an mth-order differential equation, a system of equations of mixed order but with total order of m , or system of m first -order equations.

This section contains an introduction to the semi - analytic solution of higher-order differential equations subject to initial conditions (equations (5) and (6)) The techniques we discuss are limited to those that transform a higher-order equation into system of first-order differential equations in the form equation (7) . The object is to find m functions $y_1, \ldots, y_m$ that satisfy each of the differential equations together with all the initial conditions .

New techniques are not required for solving these problems ; by relabeling the variables, can reduce a higher-order differential equation into a system of first-order differential equations and then apply semi-analytic technique .

A general mth-order initial value problem (5) with initial conditions (6) can be converted into a system of equations in form (7) by the following :

Let $u_1(x) = y(x), u_2(x) = y'(x), \ldots$, and $u_m(x) = y^{(m-1)}(x)$. This produces the first-order system :

$$\frac{du_1}{dx} = \frac{dy}{dx} = u_2$$
$$\frac{du_2}{dx} = \frac{dy'}{dx} = u_3$$
$$\vdots$$
$$\frac{du_{m-1}}{dx} = \frac{dy^{(m-2)}}{dx} = u_m$$

And

$$\frac{du_m}{dx} = \frac{dy^{(m-1)}}{dx} = y^{(m)} = f(x, y, y', ..., y^{(m-1)}) = f(x, u_1, u_2, ..., u_m),$$

With initial conditions :

$u_1(a) = y(a) = \alpha_1$, $u_2(a) = y'(a) = \alpha_2$,..., $u_m(a) = y^{(m-1)}(a) = \alpha_m$

then solving by apply semi-analytic technique as the following :

first we discuss the method where m = 2 , i.e. :

$y_1' = dy_1 / dx = f_1(x, y_1, y_2)$

$y_2' = dy_2 / dx = f_2(x, y_1, y_2)$, ... (8a)

For $0 \le x \le 1$, with the initial conditions :

$y_1(0) = a_0$, $y_2(0) = b_0$, ... (8b)

where f_i , i = 1, 2 are in general nonlinear functions of their arguments .

The simple idea behind the use of two-point polynomials is to replace y(x) in problem (8a) – (8b), or an alternative formulation of it, by P_{2n+1} which enables any unknown derivatives of y(x) to be computed . The first step therefore is to construct the P_{2n+1} . To do this we need the Taylor coefficients of $y_1(x)$ and $y_2(x)$ respectively about x = 0 :

$$y_1 = a_0 + a_1 x + \sum_{i=2}^{\infty} a_i x^i \quad ..... \quad (9a)$$

$$y_2 = b_0 + b_1 x + \sum_{i=2}^{\infty} b_i x^i \quad ..... \quad (9b)$$

where $y_1(0) = a_0$, $y_1'(0) = a_1$, ... , $y_1^{(i)}(0) / i! = a_i$, i = 2, 3, ...

and $y_2(0) = b_0$, $y_2'(0) = b_1$, ... , $y_2^{(i)}(0) / i! = b_i$, i = 2, 3, ...

then insert the series forms (9a) and (9b) respectively into (8a) and equate coefficients of powers of x .

Also ,we need Taylor coefficients of $y_1(x)$ and $y_2(x)$ about x = 1, respectively :

$$y_1 = c_0 + c_1(x-1) + \sum_{i=2}^{\infty} c_i (x-1)^i \quad ..... \quad (10a)$$

$$y_2 = d_0 + d_1(x-1) + \sum_{i=2}^{\infty} d_i (x-1)^i \quad ..... \quad (10b)$$

where $y_1(1) = c_0$, $y_1'(1) = c_1$, ... , $y_1^{(i)}(1) / i! = c_i$, i = 2, 3, ...

and $y_2(1) = d_0$, $y_2'(1) = d_1$, ... , $y_2^{(i)}(1) / i! = d_i$, i = 2, 3, ...

then insert the series forms (10a) and (10b) respectively into (8a) and equate coefficients of powers of (x – 1) .

The resulting system of equations can be solved using MATLAB version 7.9 to obtain a_i , b_i , c_i and d_i for all $i \geq 2$, we see that c_i's and d_i's coefficients depend on indicated unknowns c_0 and d_0 .

The algebraic manipulations needed for this process .We are now in a position to construct a $P_{2n+1}(x)$ and $\widetilde{P}_{2n+1}(x)$ from (9) and (10) of the form (2) by the following :

$$P_{2n+1}(x) = \sum_{i=0}^{n} \{ a_i Q_i(x) + (-1)^i c_i Q_i(1-x) \} \quad \text{.....(11a)}$$

and

$$\widetilde{P}_{2n+1}(x) = \sum_{i=0}^{n} \{ b_i Q_i(x) + (-1)^i d_i Q_i(1-x) \} \quad \text{.....(11b)}$$

Where $Q_i(x)$ defined in (3) ,

We see that (11) have only two unknowns c_0 and d_0 .

Now, integrate equation (8a) to obtain :

$$c_0 - a_0 = \int_0^1 f_1(x, y_1, y_2) dx \quad \text{.....} \quad (12a)$$

$$d_0 - b_0 = \int_0^1 f_2(x, y_1, y_2) dx \quad \text{.....} \quad (12b)$$

use P_{2n+1} and $\widetilde{P}_{2n+1}$ as a replacement of y_1 and y_2 respectively in (12) .

Since we have only the two unknowns c_0 and d_0 to compute for any n we only need to generate two equations from this procedure as two equations are already supplied by (12) and initial condition (8b). Then solve this system of algebraic equations using MATLAB version 7.9 to obtain c_0 and d_0 ,so insert it into (11) thus (11) represent the solution of (8) .

Extensive computations have shown that this generally provides a more accurate polynomial representation for a given n .

Use the same manner to solve in general the system of more than two equation .

Now consider the following example illustrate suggested method where the results are presented in tables and figures for comparison solutions and errors between P_9 and exact, also, between P_9 , exact , RK4 , RK-Butcher , DTM (Differential Transformation method) to assign the effectiveness and accuracy of the suggested method .

Example

Consider the following I.V.P of 4 rth order linear ODE's :

$y^{(4)} = y''(x) + y(x) + \exp(x)(x-3)$, $0 \leq x \leq 1$,

with I .C's : $y(0) = 1$, $y'(0) = 0$, $y''(0) = -1$, $y'''(0) = -2$

The exact solution given in [12] : $y = (1-x)\exp(x)$

Rewrite the four order IVP as a system of first order differential equations :

$y'_1 = y_2$ $\quad y_1(0) = 1$

$y'_2 = y_3$ $\quad y_2(0) = 0$

$y'_3 = y_4$ $\quad y_3(0) = -1$

$y'_4 = y_3 + y_1 + e^x (x - 3)$ $\quad y_4(0) = -2$

Then from equations (5) and (6) , we have :

$P_9 = -0.0000392857\, x^9 - 0.00013532401\, x^8 - 0.0012310308\, x^7 - 0.0069233067\, x^6 - 0.03333772256\, x^5 - 0.125\, x^4 - 0.33333333335\, x^3 - 0.5\, x^2 + 1.0$

$\widetilde{P}_9 = -0.000043892\, x^9 - 0.0001560564\, x^8 - 0.0014337298\, x^7 - 0.0083099704\, x^6 - 0.0416715166\, x^5 - 0.1666666667\, x^4 - 0.5\, x^3 - 1.0\, x^2 - 1.0\, x$

$T_9 = -0.000048499\, x^9 - 0.0001767843 x^8 - 0.00163644324\, x^7 - 0.00969661557\, x^6 - 0.0500053193 x^5 - 0.2083333333\, x^4 - 0.66666666667\, x^3 - 1.5\, x^2 - 2.0\, x - 1.0$

$\widetilde{T}_9 = -0.0000531926\, x^9 - 0.000197125\, x^8 - 0.00183981549\, x^7 - 0.0110827546\, x^6 - 0.0583392712\, x^5 - 0.25\, x^4 - 0.833333333\, x^3 - 2.0\, x^2 - 3.0\, x - 2.0$

The results for n = 4 are presented in table (1) ,figures for comparison solutions : errors between P_9 and exact given in figure (1) and between P_9 , exact , RK4 , RK-Butcher and DTM given in figure (2) to assign the effectiveness and accuracy .

Results are summarized in table (2) that represents the comparison between solution and errors for using the above methods where, the results of each other methods are given in [12] .

Table-1 : The result of the method for n = 4 of example

		P_9	$\widetilde{P}_9$	T_9	$\widetilde{T}_9$
b_{10}		-0.0000000309880672	-0.0000000309880672	-0.000000003098806	-0.000000003098806
b_{20}		-2.718281832018528	-2.718281832018528	-2.718281832018528	-2.718281832018528
b_{30}		-5.436563661441717	-5.436563661441717	-5.436563661441717	-5.436563661441717
b_{40}		-8.154845491722615	-8.154845491722615	-8.154845491722615	-8.154845491722615
X	Y_1:exaxt	P_9	$\widetilde{P}_9$	T_9	$\widetilde{T}_9$
0.1	0.9946538	0.994653826241637	-0.110517091836780	-1.21568800991527	-2.32085892799480
0.2	0.9771222	0.977122206046732	-0.244280552163793	-1.46568331037614	-2.68708606861122
0.3	0.9449012	0.944901163365262	-0.404957644413171	-1.75481645220224	-3.10467526010497
0.4	0.8950948	0.895094814618110	-0.596729883437111	-2.08855458152660	-3.58037927991827
0.5	0.8243606	0.824360630068972	-0.824360641182930	-2.47308191251370	-4.12180318439947
0.6	0.7288475	0.728847515080301	-1.09327128584539	-2.91539008691674	-4.73750888877810
0.7	0.6041257	0.604125808368537	-1.40962689952503	-3.42337960764866	-5.43713231670254
0.8	0.4451081	0.445108182787217	-1.78043274605366	-4.00597367521804	-6.23151460533784
0.9	0.2459601	0.245960308323947	-2.21364280320798	-4.67324591515718	-7.13284902801495

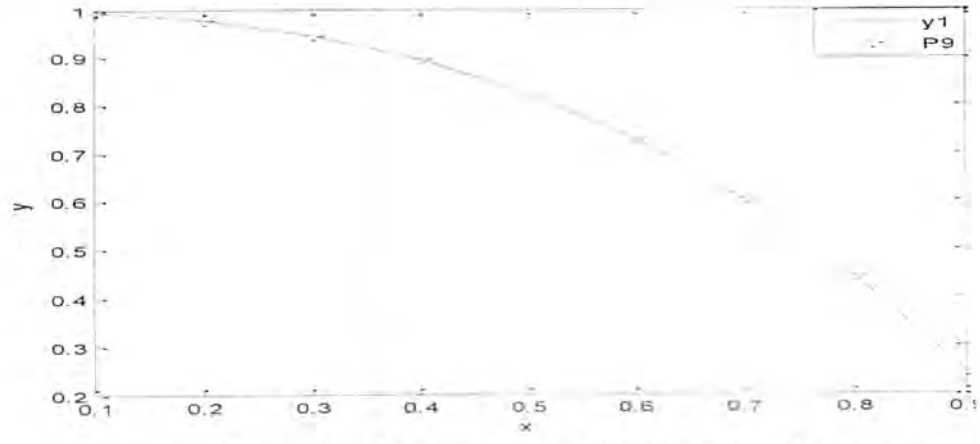

Figure 1 : Comparison between the exact solution y_1 and the solution obtained from semi-analytic method p_9.

Table-2 : A comparison between semi-analytic method P_9 and other methods .

X_i	Y_i:exact	RK4 solution	RK_Butcher solution	DTM solution	P_9 by using Osculatory
0.1	0.9946538	0.9946542	0.9946581	0.9946536	0.994653826241637
0.2	0.9771222	0.977123	0.9771916	0.9771218	0.977122206046732
0.3	0.9449012	0.9449024	0.94526	0.9449	0.944901163365262
0.4	0.8950948	0.8950967	0.8962532	0.895093	0.895094814618110
0.5	0.8243606	0.8243633	0.8272503	0.8243582	0.824360630068972
0.6	0.7288475	0.7288511	0.7349727	0.7288443	0.728847515080301
0.7	0.6041257	0.6041306	0.6157312	0.6041222	0.604125808368537
0.8	0.4451081	0.4451143	0.4653665	0.4451046	0.445108182787217
0.9	0.2459601	0.2459681	0.279182	0.2459573	0.245960308323947

X_i	Y_i:exact	RK4 error	RK-Butcher error	DTM error	P_9 by using Osculatory error
0.1	0.9946538	3.576E-07	4.351E-06	1.788139 E-07	2.624163697451110e-008
0.2	0.9771222	7.748E-07	6.967E-05	4.172325 E-07	6.046731892972446e-009
0.3	0.9449012	1.251E-06	3.592E-04	1.132488 E-06	3.663473802095751e-008
0.4	0.8950948	1.907E06	1.158E-03	1.8477 44E-06	1.461811005576408e-008
0.5	0.8243606	2.682E0-6	2.890E-03	2.384186 E-06	3.006897197899150e-008
0.6	0.7288475	3.635E-06	6.126 E-03	3.159046 E-06	1.508030100794144e-008
0.7	0.6041257	4.827E-06	1.160 E-02	3.516674 E-06	1.083685370328880e-007
0.8	0.4451081	6.258E-06	2.026 E-02	4.457069 E-06	8.278721697063673e-008
0.9	0.2459601	8.016E-06	3.322 E-02	2.846122 E-06	2.0832394001524e-007
$S.S.E_i$=6.540886821358780e-014					

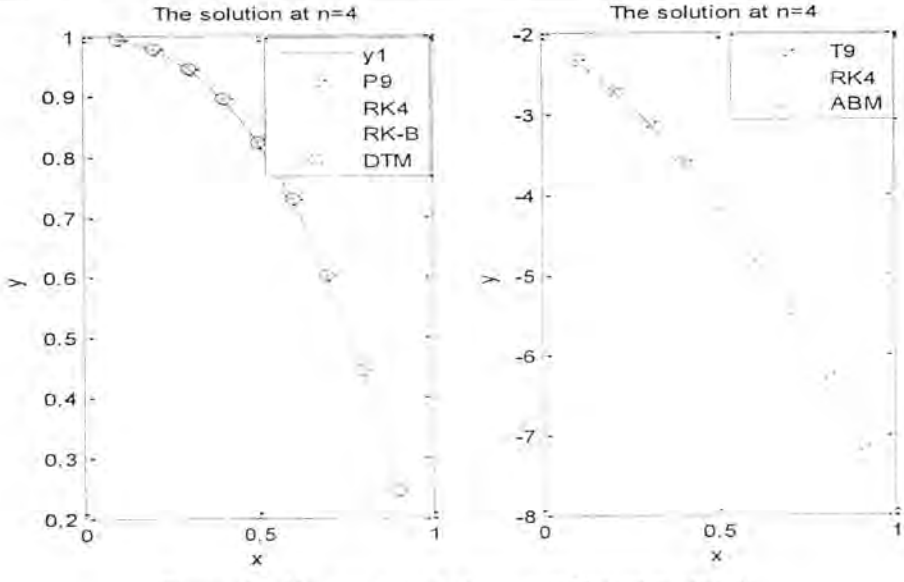

Figure-2 : Comparison between different methods

4. Sensitivity Of Solution To The Data

In our study of IVP (5), we have been guided by the four questions : Does IVP (5) have any solution ? how many ? What are they ? How do solutions respond to changes in the data ? If the function f and $\partial f / \partial y$

are continuous in some region R in the xy-plane and (x_0, y_0) is a point of R , we gave satisfactory answers to the first, second, and third questions .

In this section we show that these same simple conditions on the data also lead to a satisfactory answer to the last question .

Loosely phrased, the question amounts to this: Is it always possible to find bands on the determination of the data $\vec{f}(x, y)$ and $\vec{y}_0$ in IVP (5) which will guarantee that the corresponding solution will be within prescribed error bounds over a given x-interval ?

If this question can be answered in the affirmative, one consequence is that any " small " enough change in the data of an IVP produces only a "small " change in the solution .

In addressing the last question, it would be extremely helpful to have a formula for the solution of IVP (5) in which the data appear explicitly .

But for general nonlinear differential equations, there rarely is a solution formula for IVP(5) in which the data appear explicitly .

To estimate the change in the solution to IVP(5)as the data $\vec{f}(x, y)$ and $\vec{y}_0$ are modified, we give the following theorem about perturbation estimate :

Theorem 2 [13]

Let the function f in IVP(5) be continuous with $\partial f / \partial y$ in a rectangle R described by the inequalities :

$$x_0 \le x \le x_0 + a, |y - y_0| \le b .$$

Suppose that $g(x, y)$ and $\partial g(x, y) / \partial y$ are also continuous functions on R and that on some common interval : $x_0 \le x \le x_0 + C$, which $C \le a$, the solution y(x) of IVP(5) and the solution y(x) of the " perturbed " IVP

$$y' = f(x, y) + g(x, y),\ y(x_0) = \tilde{y}_0 \qquad \ldots . \quad (13)$$

Both have solution curves which lie in R, then we have the estimate :

$$|y(x) - \tilde{y}(x)| \le |y_0 - \tilde{y}_0| e^{L(x - x_0)} + \frac{M}{L}\left(e^{L(x - x_0)} - 1\right),\ x_0 \le x \le x_0 + C \ldots (14)$$

Where L and M are any numbers such that :

$$M \le |g(x, y)|,\ L \le \left|\frac{\partial f}{\partial y}\right|,\ all\ (x, y)\ in\ R$$

Now, in a position to answer that last of the basic questions , we give the following theorem about continuity in the data .

Theorem 3 [13]

let f , $\partial f / \partial y$,g and $\partial g / \partial y$ be continuous functions of x and y on the rectangle R defined by : $x_0 \le x \le x_0 + a, |y - y_0| \le b$.

Let $\varepsilon > 0$ be a given error tolerance. Then there exist positive constants $H < b$ and C $\leq$ a such that the respective solution y(x) and $\tilde{y}$(x) of the system of IVP :

$$(a)\ y' = f(x,y)\ , \qquad y(x_o) = y_0 \ldots$$
$$(b)\ y' = f(x,y) + g(x,y)\ \ y(x_0) = \tilde{y}_o \qquad \ldots\ldots\ldots\ldots(15)$$

Satisfy the inequality :

$$|y(x) - \tilde{y}(x)| \leq \varepsilon\ ,\ x_0 \leq x \leq x_0 + c \ldots\ldots\ldots\ldots(16)$$

For any choice of $\tilde{y}o$ for which $|yo - \tilde{y}o| \leq H$

5. Conclusions

A remarkable advantage of the semi-analytic technique for solving high order ordinary IVP is that it is easily implemented and gives a result with high accuracy. The high accuracy of the method is confirmed by example and the suggested method compared with conventional methods via example and is shown to be that seems to converge faster and more accurately than the conventional methods .

Another advantage of suggested method is that it gives the approximate solution on the continuous finite domain whereas other numerical techniques provide the solution on discrete only.

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On S^{**}-PROPER FUNCTIONS

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Received 20/12/2010 – Accepted 25/5/2011

الخلاصة

ان الهدف من هذا البحث هو ادخال ودراسة صنف جديد من الدوال الفعلية في الفضاءات التبولوجية سميت بالدوال الفعلية – s^{**} وكدلك ايجاد وكشف ميزات وصفات ومقارنة بين هده الدالة مع انواع اخرى من الدوال الفعلية .هذا النوع من الدوال اقوى من الدوال الفعلية – s واضعف من الدوال الفعلية – s^{*} .

ABSTRACT

The purpose of the present is study s**-proper functions , and investigate it is characterizations , properties and comparisons among this function with other types of proper functions .This type of function is stronger than s-proper function and weaker than s^{*}-proper function .

Remark:-In this paper we replace X_T , Y_T and Z_T by X, Yand Z respectively

INTRODUCTION

In this paper, we study another type of proper namely s^{**}-proper function ,this function depend on semi-closed sets (written s-closed),the class of s-closed set was first introduced by Das P. in (1966) and he defined the s-closed set as (a set A of a topological space X is said to be a semi-closed set if there exists a closed subset F of X, such that $F^{o} \subseteq A \subseteq F$, where F^{o} is the interior of F in X.)

Many scientists and researches studied the proper function, such as Vainstein (1947), Leray (1950), Bourbaki (1951), Henrisken and Isbell (1958), Frolik (1960), Arhange'skil (1967), Michel (1971), Chaber (1972), Pareek and Hedeib (1977), Jungck (1992) and Vermeulen (1994).

In [9], the notion of semi-proper (breifly s-proper) functions is given as a generalization of proper functions.

In this work we used s^{**}-proper function to prove some propositions and we get some results as well as we give some examples on this subject.

Definition (1.1) [1]:
A set A of a topological space X is said to be a semi-closed set (written s-closed) if there exists a closed subset F of X, such that $F^{o} \subseteq A \subseteq F$, where F^{o} is the interior of F in X.Clearly every closed set is s-closed, but the converse is not true in general see[1].

Definition (1.2)[2],[3]:

Let X and Y be topological spaces. A function $f : X \longrightarrow Y$ is said to be

(i) An s-closed function if the image of every closed subset of X is s-closed in Y.

(ii)An s^*-closed function if the image of every s-closed subset of X is closed in Y.

(iii) An s**-closed function if the image of every s-closed subset of X is s-closed in Y.

Proposition (1.3):

Let X, Y and Z be topological spaces and let $f : X \longrightarrow Y$ be s^*-closed function and $g : Y \longrightarrow Z$ be s-closed function, then $gof : X \longrightarrow Z$ is s^{**}-closed function.

Proof:

Let F be s-closed in X, since f is s^*-closed function, then f(F) is closed in Y

But g is s-closed function, then g(f(F)) is s-closed in Z.But g(f(F)) = (gof)(F), then gof is s^{**}-closed. ■

Definition(1.4)[4]:

Let X,Y and Z be topological spaces and let $f:X\to Y$ and $g:X\to Z$ be functions,then

the diagonal function $f \Delta g = X\to Y\times Z$ is defined as follows : $(f \Delta g)(x)=(f(x),g(x))$. Notice that:$f \Delta g=(f\times g)od$ where $d:X\to X\times X$ is the diagonal function $d(x)=(x,x)$.

Proposition(1.5)[4]:

Let X be a topological space ,then X is a T_2 space if and only if $d=\{(x,x) \mid x \in X\}$ is closed in $X\times X$.

Remark(1.6):

Let X be any topological space ,then $d=\{(x,x)|x\in X\}$ is one-one ,since if $d(x_1)=d(x_2)$,then $(x_1,x_1)=(x_2,x_2)$,this implies that $x_1=x_2$.

Definition(1.7)[5]:

Let X and Y be topological spaces and let $f:X\to Y$ be any function and A be a subset of X ,then $f \mid A:A\to Y$ is said to be restriction f on a subset A and we will define $(f \mid A)(a)=f(a)$ for each $a \in A$.

Definition (1.8) [6]:

A space X is said to be a semi-compact space (written s-compact) if and only if for each s-open cover of X, there exists a finite subcover of X.

Remark (1.9)[7]:

Every s-compact space is compact, But the converse is not true in general. For example Let X = [−1, 1] with the relative usual topology on R, then X is compact but it is not s-compact.

Proposition (1.10)[8]:

Every s-closed subset of an s-compact space is s-compact.

Chapter two:-

In this section we state and prove the main results of this paper first we recall the following definition:

Definitions(2.1) [4],[9]:

Let X and Y be topological spaces. A continuous function $f : X \longrightarrow Y$ is said to be

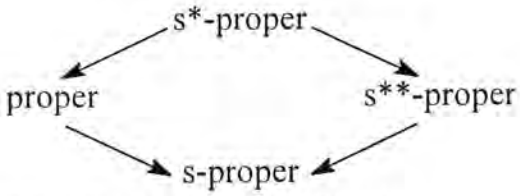
(i) A proper if and only if $f\times I_Z : X\times Z \longrightarrow Y\times Z$ is closed for any topological space Z.

(ii) An s-proper if and only if $f\times I_Z : X\times Z \longrightarrow Y\times Z$ is s-closed for any topological space Z.

(iii) An s^*-proper if and only if $f\times I_Z : X\times Z \longrightarrow Y\times Z$ is s^*-closed for any topological space Z.

(iv) An s^{**}-proper if and only if $f\times I_Z : X\times Z \longrightarrow Y\times Z$ is s^{**}-closed for any topological space Z.

Remark(2.2)



The following propositions appeared in [9],[8].

proposition (2.3) [4]:

Let X be a topological space and let F be a subset of X, then the inclusion function i: $F \longrightarrow X$ is proper if and only if F is closed in X.

Proposition (2.4)[4]:

Let X and Y be topological spaces and let $f : X \longrightarrow Y$ be an injective continuous function, then the following are equivalent:

(i) f is proper.

(ii) f is closed.

(iii) f is a homeomorphism from X onto a closed subset of Y.

Proposition (2.5)[8]:

The constant function $f : X \longrightarrow p$ is s^*-proper if and only if X is s-compact.

Proposition (2.6)[10]:

Let $f_1 : X_1 \longrightarrow Y_1$ be s^{**}-proper and let $f_2 : X_2 \longrightarrow Y_2$ be s^{**}-proper, then $f_1\times f_2$ is s^{**}-proper.

Proposition (2.7)[9]:

Let X, Y and Z be topological spaces and let $f : X \longrightarrow Y$, be a proper and $g : Y \longrightarrow Z$ be an s^{**}-proper, then $gof : X \longrightarrow Z$ is s-proper.

.Remark (2.8):

Let X, Y and Z be topological spaces, and let f:X$\longrightarrow$Y, g:Y$\longrightarrow$Z, be any functions. Put

$(f\times I_W) = f_1$ and $(g\times I_W) = g_1$, for any topological space W, and put $(f\times I_W)(F) = f_1(F) = G_1$, for any subset F of X.

Now, $(gof)\times I_W = (g\times I_W)o(f\times I_W) = g_1of_1$

We shall use the above remark to prove the following propositions:

Proposition (2.9) [9]:

Let X, Y and Z be topological spaces and let $f : X \longrightarrow Y$, $g : Y \longrightarrow Z$ be functions,

then $gof : X \longrightarrow Z$ is s^{**}-proper if:

(i) f is s^{**}-proper and g is -s^{**}proper.

(ii) f is s^{*}-proper and g is s-proper.

Proposition (2.10):

Let $f_1 : X_1 \longrightarrow Y_1$ be s^{*}-proper and let $f_2 : X_2 \longrightarrow Y_2$ be s-proper, then $f_1\times f_2$ is s^{**}-proper.

Proof:

Since f_1 and f_2 are continuous, then $f_1\times f_2$ is continuous

Now, to prove that $f_1\times f_2\times I_Z : X_1\times X_2\times Z \longrightarrow Y_1\times Y_2\times Z$ is s^{**}-closed for any topological space Z.

Since $f_1\times f_2\times I_Z = I_{Y1}\times f_2\times I_Z of_1\times I_{X2}\times I_Z$, and f_1 is s^{*}-proper, then $f_1\times I_{X2}\times I_Z$ is s^{*}-closed by (2.1)(iii).

Also, f_2 is s-proper , then $I_{Y1}\times f_2\times I_Z$ is (s-closed) by (2.1)(ii).

This implies that $I_{Y1}\times f_2\times I_Z of_1\times I_{X2}\times I_Z$ is s^{**}-closed (see[3])

Hence $f_1\times f_2\times I_Z$ is s^{**}-closed. Thus $f_1\times f_2$ is s^{**}-proper (by (2.1)(iv)). ■

Proposition (2.11)[9]:

Let X and Y be topological spaces and let $f : X \longrightarrow Y$ be a continuous function, then the following are equivalent:

(i) f is s^{**}-proper.

(ii) f is s^{**}-closed and $f^{-1}(y)$ is s-compact, for each $y \in Y$.

Proposition (2.12):

Any continuous function f from an s-compact space X into a T_2-space Y is s^{**}-proper.

Proof:

To prove f is s^{**}-closed, let F be s-closed in X.

Since X is s-compact, then F is s-compact in X (by (1.10).From(1.9) F is compact,but f is continuous, then f(F) is compact in Y.

Since Y is a T_2-space, then f(F) is closed in Y .

Hence f(F) is s-closed in Y (by (1.1)), then f is s^{**}-closed .

Now, to prove for each $y \in Y$, $f^{-1}(y)$ is s-compact.

For each $y \in Y$, $\{y\}$ is compact in Y. But Y is a T_2-space, then $\{y\}$ is closed in Y, and since f is continuous, then $f^{-1}(y)$ is closed in X. From(1.1)$f^{-1}(y)$ is s-closed in X
But X is s-compact, then $f^{-1}(y)$ is s-compact in X (by (1.10)).
Hence f is s^{**}-proper (by (2.11)). ■

Proposition (2.13)[9]:

Let X, Y and Z be topological spaces and let $f : X \longrightarrow Y$ be proper, $g : Y \longrightarrow Z$ be s^{**}-proper. Then $gof : X \longrightarrow Z$ is s-proper.

Proposition (2.14):

Let X and Y be topological spaces and let Abe a closed subset of X .If $f X \rightarrow Y$ is s^{**}-proper ,then $f \mid A : A \rightarrow Y$ is s-proper.

Proof:

Since A closed in X , then the inclusion function $i : A \rightarrow X$ is proper (by(2.3)).But
$f : X \rightarrow Y$ is s^{**}-proper , then $foi : A \rightarrow Y$ is s-proper (by(2.13)).
Not that, $foi = f \mid A$ .hence $f \mid A$ is s-proper.

Proposition(2.15):

Let X,Y and Z be topological spaces and let $f : X \rightarrow Y$, $g : X \rightarrow Z$ be s^{**}-proper
functions and X is a T_2-space , then $f \Delta g$ is s-proper .

Proof:

First we have $f \Delta g = (f \times g)od$ such that $d : X \rightarrow X \times X$ defined by $d(x)=(x,x)$ and since X is aT_2-space ,then d is a closed in $X \times X$ (by(1.5)). From (2.4) d is a homeomorphism of X on to a closed subset of $X \times X$,then d is proper (by(2.4)).
Also since f and g are s^{**}-proper ,then $f \times g$ is s^{**}-proper (by(2.6)).Thus $(f \times g)od$ is s-proper (by(2.7)).
Hence $f \Delta g$ is s-proper.

Proposition(2.16):

If X is s-compact and Y is any topological space , then $Pr_2 : X \times Y \rightarrow Y$ is s^{**}-proper .

Proof:

Since $Y \times \{p\} \cong Y$. ,then we can write $Pr_2 : X \times Y \rightarrow \{p\} \times Y$,this implies that $Pr_2 = f \times I_Y$ where $f : X \rightarrow p$. Since X is s-compact , then f is s^{*}-proper (by(2.5)) and it is clear that $I_Y : Y \rightarrow Y$ is proper.
From(2.2) I_Y is s-proper .Hence $Pr_2 = f \times I_Y$ is s^{**}-proper (by(2.10)).

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On The Completeness Of The Cartesian Product Of Two Complete fuzzy metric spaces

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Received 14/9/2011 – Accepted 18/4/2012

الخلاصة

في هذا البحث برهنا الضرب الديكارتي لفضائيين متريين ضبابيين متكاملين هو فضاء متري ضبابي متكامل.

ABSTRACT

In this paper we prove that the Cartesian product of two complete fuzzy metric spaces is again a complete fuzzy metric space .

Key Words : Fuzzy metric space , Cartesian product , Cauchy sequence , Complete fuzzy metric space .

INTRODUCTION

In this paper we recall the definition of fuzzy metric space in [1] and the definition
of complete fuzzy metric spaces from [2] . The aim of this work is to prove that
completeness of $X\times Y$ comes from completeness of X and Y also if $X\times Y$ is
complete then it will inherit it to X and Y .

S1:Preliminaries

Definition 1.1:[3]

Let X and Y be any two sets ,the Cartesian product is denoted by $X\times Y$ and is defined by $X\times Y = \{ (x,y) : x \in X , y \in Y \}$.

Definition 1.2:[4] ,[2] ,[1]

Let M be a fuzzy subset of $X\times X\times \mathbb{R}$ is said to be a fuzzy metric on X incase for each x , y and z in X :

(M_1) $M(x,y,t) = 0$ for each $t \leq 0$.

(M_2) $M(x,y,t) = 1$ for each $t > 0 \Leftrightarrow x = y$

(M_3) $M(x,y,t) = M(y,x,t)$ for each t in $\mathbb{R}$.

(M_4) $M(x,y,s+t) \geq M(x,z,s) \wedge M(z,y,t)$ for each t,s in $\mathbb{R}$, where $\wedge$ is the minimum of M(x,z,s) and M(z,y,t) .

(M_5) $M(x,y,\bullet)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M(x,y,t) = 1$.

The pair (X,M) is called a fuzzy metric space .

Definition 1.3 :[4]

Let (X,M) be a fuzzy metric space . A sequence $\{ x_n \}$ in X is said to be convergent if there exists x in X such that $\lim_{n\to\infty} M(x_n , x , t) = 1$,for each $t > 0$.

Definition 1.4 :[5]

Let (X,M) be a fuzzy metric space . A sequence $\{ x_n \}$ is said to be Cauchy sequence if $\lim_{n\to\infty} M(x_{n+p}, x_n, t) = 1$, for each $t > 0$ and $p = 1,2,3,\ldots$.

Definition 1.5 :[6]

A fuzzy metric space (X,M) is said to be complete if every Cauchy sequence is convergent .

S2:Completeness of X× Y with M = $M_1.M_2$

Theorem 2.1 :

Let (X,M_1) and (Y,M_2) be two fuzzy metric spaces .Then $(X\times Y,M)$ is a fuzzy metric space by defining $M((x_1,y_1),(x_2,y_2),t) = M_1(x_1,x_2,t).M_2(y_1,y_2,t)$.

Proof :

For each $(x_1,y_1),(x_2,y_2),(x_3,y_3)$ in $X\times Y$

$(M_1)$for each $t \leq 0$, we have $M_1(x_1,x_2,t) = 0$ and $M_2(y_1,y_2,t) = 0$ .Hence $M((x_1,y_1),(x_2,y_2),t) = 0$

$(M_2)M_1(x_1,x_2,t) = 1$ for each $t > 0 \Leftrightarrow x_1 = x_2$,also $M_2(y_1,y_2,t) = 1$, for each $t > 0 \Leftrightarrow y_1=y_2$.Together $M_1(x_1,x_2,t) . M_2(y_1,y_2,t) = 1$, for each $t > 0 \Leftrightarrow (x_1,y_1) = (x_2,y_2)$. Hence $M((x_1,y_1),(x_2,y_2),t) = 1$ for each $t > 0 \Leftrightarrow (x_1,y_1) = (x_2,y_2)$.

$(M_3)M_1(x_1,x_2,t) = M_1(x_2,x_1,t)$ for each t in $\mathbb{R}$,also $M_2(y_1,y_2,t) = M_2(y_2,y_1,t)$ for each t in $\mathbb{R}$. Now for each t in $\mathbb{R}$

$M((x_1,y_1),(x_2,y_2),t) = M_1(x_1,x_2,t) . M_2(y_1,y_2,t) = M_1(x_2,x_1,t) . M_2(y_2,y_1,t) = M((x_2,y_2),(x_1,y_1),t)$.

$(M_4)M_1(x_1,x_2,s+t) \geq M_1(x_1,x_3,s) \wedge M_1(x_3,x_2,t)$ for each s,t in $\mathbb{R}$. Also $M_2(y_1,y_2,s+t) \geq M_2(y_1,y_3,s) \wedge M_2(y_3,y_2,t)$ for each s,t in $\mathbb{R}$. Now for each s,t in $\mathbb{R}$

$M((x_1,y_1),(x_2,y_2),s+t) = M_1(x_1,x_2,s+t) .M_2(y_1,y_2,s+t) \geq [M_1(x_1,x_3,s) \wedge M_1(x_3,x_2,t)].[M_2(y_1,y_3,s) \wedge M_2(y_3,y_2,t)] \geq [M_1(x_1,x_3,s) .M_2(y_1,y_3,s)] \wedge [M_1(x_3,x_2,t) .M_2(y_3,y_2,t)] = M((x_1,y_1),(x_3,y_3),s) \wedge M((x_3,y_3),(x_2,y_2),t)$.

$(M_5)M_1(x_1,x_2,\bullet)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M_1(x_1,x_2,t) = 1$ .Also $M_2(y_1,y_2,\bullet)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M_2(y_1,y_2,t) = 1$.Now if $t_1 < t_2$ then $M_1(x_1,x_2,t_1) \leq M_1(x_1,x_2,t_2)$ and $M_2(y_1,y_2,t_1) \leq M_2(y_1,y_2,t_2)$. Hence $M((x_1,y_1),(x_2,y_2),t_1) = M_1(x_1,x_2,t_1) .M_2(y_1,y_2,t_1) \leq M_1(x_1,x_2,t_2) . M_2(y_1,y_2,t_2) = M((x_1,y_1),(x_2,y_2),t_2)$ .This implies that $M((x_1,y_1),(x_2,y_2),\bullet)$ is a non decreasing function of $\mathbb{R}$ .Also $\lim_{t\to\infty} M((x_1,y_1),(x_2,y_2),t) = [\lim_{t\to\infty} M_1(x_1,x_2,t)].[\lim_{t\to\infty} M_2(y_1,y_2,t)] = 1$. Thus $(X\times Y,M)$ is a fuzzy metric space .

Proposition 2.2 :

If $\{ x_n \}$ is a sequence in fuzzy metric space (X,M_1) converges to x in X and $\{ y_n \}$is a sequence in the fuzzy metric space (Y,M_2) converge to y in Y then $\{ (x_n,y_n) \}$ is a sequence in the fuzzy metric space $(X\times Y,M)$ converge to (x,y) in $X\times Y$, where $M = M_1.M_2$.

Proof:

By Theorem 2.1 , $(X\times Y,M)$ is a fuzzy metric space . Now for each $t > 0$,

$\lim_{n\to\infty} M((x_n,y_n),(x,y),t) = [\lim_{n\to\infty} M_1(x_n,x,t)].[\lim_{n\to\infty} M_2(y_n,y,t)] = 1$.

Hence $\{ (x_n,y_n) \}$ converge to (x,y) .

Proposition 2.3 :

If $\{ x_n \}$ is a Cauchy sequence in the fuzzy metric space (X,M_1) and $\{y_n\}$ is a Cauchy sequence in the fuzzy metric space (Y,M_2) then $\{ (x_n,y_n) \}$ is a Cauchy in the fuzzy metric space $(X\times Y,M)$ where $M = M_1.M_2$.

Proof:

By Theorem 2.1 , $(X\times Y,M)$ is a fuzzy metric space . For each $t > 0$ and $p = 1,2,3,\ldots$ $\lim_{n\to\infty} M((x_{n+p},y_{n+p}),(x_n,y_n),t) = [\lim_{n\to\infty} M_1(x_{n+p},x_n,t)].[\lim_{n\to\infty} M_2(y_{n+p},y_n,t)] = 1$.

Thus $\{(x_n,y_n) \}$ is a Cauchy sequence in $(X\times Y,M)$

Theorem 2.4 :

If (X,M_1) and (Y,M_2) are complete fuzzy metric spaces then $(X\times Y,M)$ is a complete fuzzy metric space where $M = M_1.M_2$.

Proof:

By Theorem 2.1 , $(X\times Y,M)$ is a fuzzy metric space .Let $\{ (x_n,y_n) \}$ be a Cauchy sequence in $X\times Y$, that is for each $t > 0$,and $p = 1,2,3,\ldots$ $1 = \lim_{n\to\infty} M((x_{n+p},y_{n+p}),(x_n,y_n)t) = [\lim_{n\to\infty} M_1(x_{n+p},x_n,t)].[\lim_{n\to\infty} M_2(y_{n+p},y_n,t)]$. Hence $\lim_{n\to\infty} M_1(x_{n+p},x_n,t) = 1$ and $\lim_{n\to\infty} M_2(y_{n+p},y_n,t) = 1$. Therefore $\{x_n\}$ is a Cauchy sequence in (X,M_1) and $\{y_n\}$ is a Cauchy sequence in (Y,M_2) . But (X,M_1) and (Y,M_2) are complete fuzzy metric spaces , hence there is x in X and y in Y such that for each $t > 0$ $\lim_{n\to\infty} M_1(x_n,x,t) = 1$ and $\lim_{n\to\infty} M_2(y_n,y,t) = 1$. so that $\lim_{n\to\infty} M((x_n,y_n),(x,y),t) = [\lim_{n\to\infty} M_1(x_n,x,t)].[\lim_{n\to\infty} M_2(y_n,y,t)] = 1$.

Hence $\{(x_n,y_n) \}$ converges to (x,y) in $X\times Y$. Thus $(X\times Y,M)$ is a complete fuzzy metric space .

Theorem 2.5 :

If $(X\times Y,M)$ is a fuzzy metric space then (X,M_1) and (Y,M_2) are fuzzy metric spaces by defining $M_1(x_1,x_2,t) = M((x_1,y_0),(x_2,y_0),t)$ and $M_2(y_1,y_2,t) = M((x_0,y_1),(x_0,y_2),t)$,

For fix $x_0 \in X$ and fix $y_0 \in Y$.

Proof:

For each x_1,x_2,x_3 in X

(M_1) $M_1(x_1,x_2,t) = M((x_1,y_0),(x_2,y_0),t) = 0$, for each $t \leq 0$.

(M_2) For each $t > 0$, $1 = M_1(x_1,x_2,t) = M((x_1,y_0),(x_2,y_0),t) \Leftrightarrow x_1 = x_2$.

$(M_3)M_1(x_1,x_2,t) = M((x_1,y_0),(x_2,y_0),t) = M((x_2,y_0),(x_1,y_0),t) = M_1(x_2,x_1,t)$,for each t in $\mathbb{R}$ (M_4) $M_1(x_1,x_2,s+t) = M((x_1,y_0),(x_2,y_0),s+t) \geq M((x_1,y_0),(x_3,y_0),s) \wedge M((x_3,y_0),(x_2,y_0),t) = M_1(x_1,x_3,s) \wedge M_1(x_3,x_2,t)$

$(M_5)M_1(x_1,x_2,\bullet) = M((x_1,y_0),(x_2,y_0),\bullet)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M_1(x_1,x_2,t) = \lim_{t\to\infty} M((x_1,y_0),(x_2,y_0),t) = 1$. Thus (X,M_1) is a fuzzy metric space . Similarly (Y,M_2) is a fuzzy metric space .

Theorem 2.6;

If $(X\times Y,M)$ is a complete fuzzy metric space then (X,M_1) and (Y,M_2) are complete fuzzy metric spaces where $M_1(x_1,x_2,t) = M((x_1,y_0),(x_2,y_0),t)$ and $M_2(y_1,y_2,t) = M((x_0,y_1),(x_0,y_2),t)$, for fix $x_0 \in X$ and fix $y_0 \in Y$.

Proof:

By Theorem 2.5 , (X,M_1) and (Y,M_2) are fuzzy metric spaces .Let $\{ x_n \}$ be Cauchy sequence in (X,M_1) that is for $t > 0$ and $p = 1,2,\ldots\ldots$ $\lim_{n\to\infty} M_1(x_{n+p},x_n,t) = 1$.

Now for $t > 0$ and $p = 1,2,\ldots\ldots$ $\lim_{n\to\infty} M((x_{n+p},y_0),(x_n,y_0),t) = \lim_{n\to\infty} M_1(x_{n+p},x_n,t) = 1$,this implies that $\{ (x_n,y_0) \}$ is Cauchy sequence in $X\times Y$. But $(X\times Y,M)$ is complete so $\{ (x_n,y_0) \}$ converge to (x,y_0) in $X\times Y$, that is for $t > 0$, $\lim_{n\to\infty} M((x_n,y_0),(x,y_0),t) = 1$. Hence $\lim_{n\to\infty} M_1(x_n,x,t) = \lim_{n\to\infty} M((x_n,y_0),(x,y_0),t) = 1$ for each $t > 0$, that is $\{ x_n \}$ converge to x in X . Thus (X,M_1) is complete . Similarly we can prove that (Y,M_2) is complete .

S3:Completeness of $X\times Y$ with $M = M_1 \wedge M_2$

Theorem 3.1 :

If (X,M_1) and (Y,M_2) are fuzzy metric spaces then $(X\times Y,M)$ is a fuzzy metric space by defining $M((x_1,y_1),(x_2,y_2),t) = M_1(x_1,x_2,t) \wedge M_2(y_1,y_2,t)$.

Proof:

For each (x_1,y_1) , (x_2,y_2) , (x_3,y_3) in $X\times Y$

(M_1) For each $t \leq 0$, we have $M_1(x_1,x_2,t) = 0$, and $M_2(y_1,y_2,t) = 0$. Hence $M((x_1,y_1),(x_2,y_2),t) = 0$.

(M_2) $M_1(x_1,x_2,t) = 1$, for each $t > 0 \Leftrightarrow x_1 = x_2$, also $M_2(y_1,y_2,t) = 1$, for each $t > 0 \Leftrightarrow y_1 = y_2$. Together $[M_1(x_1,x_2,t) \wedge M_2(y_1,y_2,t)] = 1$, for each $t > 0 \Leftrightarrow x_1 = x_2$ and $y_1 = y_2$. That is $M((x_1,y_1),(x_2,y_2),t) = 1$, for each $t > 0 \Leftrightarrow (x_1,y_1) = (x_2,y_2)$.

(M_3) $M_1(x_1,x_2,t) = M_1(x_2,x_1,t)$, for each t in $\mathbb{R}$ and $M_2(y_1,y_2,t) = M_2(y_2,y_1,t)$, for each t in $\mathbb{R}$. Now for each t in $\mathbb{R}$

$M((x_1,y_1),(x_2,y_2),t) = [M_1(x_1,x_2,t) \wedge M_2(y_1,y_2,t)] = [M_1(x_2,x_1,t) \wedge M_2(y_2,y_1,t)] = M((x_2,y_2),(x_1,y_1),t)$.

(M_4) $M_1(x_1,x_2,s+t) \geq [M_1(x_1,x_3,s) \wedge M_1(x_3,x_2,t)]$, for each s,t in $\mathbb{R}$ .Also $M_2(y_1,y_2,s+t) \geq [M_2(y_1,y_3,s) \wedge M_2(y_3,y_2,t)]$, for each s,t in $\mathbb{R}$. Now for each t in $\mathbb{R}$ $M((x_1,y_1),(x_2,y_2),s+t) = [M_1(x_1,x_2,s+t) \wedge M_2(y_1,y_2,s+t)] \geq [M_1(x_1,x_3,s) \wedge M_1(x_3,x_2,t) \wedge M_2(y_1,y_3,s) \wedge M_2(y_3,y_2,t)] \geq [M_1(x_1,x_3,s) \wedge M_2(y_1,y_3,s)] \wedge [M_1(x_3,x_2,t) \wedge M_2(y_3,y_2,t)] \geq [M((x_1,y_1),(x_3,y_3),s) \wedge M((x_3,y_3),(x_2,y_2),t)]$.

(M_5) $M_1(x_1,x_2, \bullet)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M_1(x_1,x_2,t) = 1$, and $M_2(y_1,y_2, \bullet)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M_2(y_1,y_2,t) = 1$, that is if $t_1 < t_2$ then $M_1(x_1,x_2,t_1) \leq M_1(x_1,x_2,t_2)$ and $M_2(y_1,y_2,t_1) \leq M_2(y_1,y_2,t_2)$. Now $M((x_1,y_1),(x_2,y_2),t_1) = [M_1(x_1,x_2,t_1) \wedge M_2(y_1,y_2,t_1)] \leq [M_1(x_1,x_2,t_2) \wedge M_2(y_1,y_2,t_2)] \leq M((x_1,y_1),(x_2,y_2),t_2)$.

Hence $M((x_1,y_1),(x_2,y_2,)$ is a nondecreasing function of $\mathbb{R}$ and $\lim_{t\to\infty} M((x_1,y_1),(x_2,y_2),t) = [\lim_{t\to\infty} M_1(x_1,x_2,t)] \wedge [\lim_{t\to\infty} M_2(y_1,y_2,t)] = 1$. Thus $(X\times Y,M)$ is a fuzzy metric space .

Proposition 3.2 :

If $\{ x_n \}$ is a sequence in the fuzzy metric space (X,M_1) converge to x in X and $\{ y_n \}$ is a sequence in the fuzzy metric space (Y,M_2) converge to y in Y . Then $\{ (x_n,y_n) \}$ is a sequence in the fuzzy metric space $(X\times Y,M)$ converge to (x,y) ,where $M = M_1 \wedge M_2$.

Proof:

By Theorem 3.1 , $(X\times Y,M)$ is fuzzy metric space . Now for each $t > 0$ $\lim_{n\to\infty} M((x_n,y_n),(x,y),t) = [\lim_{n\to\infty} M_1(x_n,x,t)] \wedge [\lim_{n\to\infty} M_2(y_n,y,t)] = 1$

Hence $\{ (x_n,y_n) \}$ converge to (x,y) .

Proposition 3.3 :

If $\{ x_n \}$ is a Cauchy sequence in the fuzzy metric space (X,M_1) and $\{ y_n \}$ is a Cauchy sequence in the fuzzy metric space (Y,M_2) then $\{ (x_n,y_n) \}$ is a Cauchy sequence in the fuzzy metric space $(X\times Y,M)$, where $M = M_1 \wedge M_2$.

Proof:

By Theorem 3.1 , $(X\times Y,M)$ is fuzzy metric space . Now for $t > 0$ and $p = 1,2....$ $\lim_{n\to\infty} M((x_{n+p},y_{n+p}),(x_n,y_n)t) = [\lim_{n\to\infty} M_1(x_{n+p},x_n,t)] \wedge [\lim_{n\to\infty} M_2(y_{n+p},y_n,t)] = 1$.Hence $\{ (x_n,y_n)\}$ is Cauchy sequence .

Theorem 3.4:

If (X,M_1) and (Y,M_2) are complete fuzzy metric space then $(X\times Y,M)$ is a complete fuzzy metric space . where $M = M_1 \wedge M_2$.

Proof:
By Theorem 3.1, (X×Y,M) is a fuzzy metric space .Let { (x_n,y_n) } be a Cauchy sequence in X×Y, that is for each $t > 0$ and $p = 1,2,\ldots\ldots$ $1 = \lim_{n\to\infty} M((x_{n+p}, y_{n+p}), (x_n, y_n), t) = [\lim_{n\to\infty} M_1(x_{n+p}, x_n, t)] \wedge [\lim_{n\to\infty} M_2(y_{n+p}, y_n, t)]$. Hence $\lim_{n\to\infty} M_1(x_{n+p}, x_n, t) = 1$ and $\lim_{n\to\infty} M_2(y_{n+p}, y_n, t) = 1$. Therefore { x_n } is Cauchy sequence in (X,M_1) and { y_n } is Cauchy in (Y,M_2) . But (X,M_1) and (Y,M_2) are complete fuzzy metric spaces so there is x in X and y in Y such that for each $t > 0$, $\lim_{n\to\infty} M_1(x_n, x, t) = 1$, and $\lim_{n\to\infty} M_2(y_n, y, t) = 1$. Hence $\lim_{n\to\infty} M((x_n, y_n), (x, y), t) = [\lim_{n\to\infty} M_1(x_n, x, t)] \wedge [\lim_{n\to\infty} M_2(y_n, y, t)] = 1$. Therefore { (x_n,y_n) } converge to (x,y) in X×Y .Thus (X× Y,M) is a complete fuzzy metric space .

S4:α-Completeness of X×Y where $M = M_1 \wedge M_2$
In this section based on the idea that appeared in [7] we introduce the following definitions
Definition 4.1 :
Let (X,M) be a fuzzy metric space and $\alpha \in (0,1)$. A sequence { x_n } in X is said to be
convergent in X if there is x in X such that for each $t > 0$, $\lim_{n\to\infty} M(x_n, x, t) \geq \alpha$ α-
and x is called the limit of { x_n } .
Definition 4.2 :
Let (X,M) be a fuzzy metric space and $\alpha \in (0,1)$. A sequence { x_n } is said to be α-
Cauchy if $\lim_{n\to\infty} M(x_{n+p}, x_n, t) \geq \alpha$, for each $t > 0$ and $p = 1,2,\ldots$.
Definition 4.3 :
Let (X,M) be a fuzzy metric space and $\alpha \in (0,1)$ .Then (X,M) is said to be α-complete
If each α-Cauchy sequence in X is α-converge to a point in X .
Theorem 4.4 :
Every α–convergent sequence is α-Cauchy .
Proof :
Let (X,M) be a fuzzy metric space and let { x_n } α-convergent sequence to an
element x in X so for $\alpha \in (0,1)$, $\lim_{n\to\infty} M(x_n, x, \frac{t}{2}) \geq \alpha$, for all $t > 0$.
Now for $t > 0$, $M(x_{n+p},x_n,t) \geq M(x_{n+p},x,\frac{t}{2}) \wedge M(x,x_n,\frac{t}{2}) \geq \alpha \wedge \alpha = \alpha$.
Thus { x_n } is α–Cauchy in (X,M) .
Proposition 4.5 :
If { x_n } is a sequence in the fuzzy metric space (X,M_1) is α-converge to x in X and

$\{ y_n \}$ is a sequence in the fuzzy metric space (Y,M_2) is α-converge to y in Y, then
the sequence $\{ (x_n,y_n) \}$ is a sequence in the fuzzy metric space $(X\times Y,M)$ which is
α-converge to (x,y) in $X\times Y$ where $M = M_1 \wedge M_2$.

Proof :

By Theorem 3.1,$(X\times Y,M)$ is a fuzzy metric space .Now for $\alpha\in (0,1)$ and for each $t> 0$

$\lim_{n\to\infty} M((x_n,y_n),(x,y),t) = [\lim_{n\to\infty} M_1(x_n,x,t)] \wedge [\lim_{n\to\infty} M_2(y_n,y,t)] \geq \alpha.$

Thus $\{ (x_n,y_n) \}$ is α-converge to (x,y) .

Proposition 4.6 :

If $\{ x_n \}$ is α-Cauchy sequence in the fuzzy metric space (X,M_1) and $\{ y_n \}$ is α-
Cauchy sequence in the fuzzy metric space (Y,M_2) ,then $\{ (x_n,y_n) \}$ is α-Cauchy
sequence in the fuzzy metric space $(X\times Y,M)$ where $M = M_1 \wedge M_2$.

Proof :

By Theorem 3.1,$(X\times Y,M)$ is a fuzzy metric space ,Now for $\alpha\in (0,1)$ and for each
$t>0$, $p = 1,2,\ldots$

$\lim_{n\to\infty} M((x_{n+p},y_{n+p}),(x_n,y_n),t) = [\lim_{n\to\infty} M_1(x_{n+p},x_n,t)] \wedge [\lim_{n\to\infty} M_2(y_{n+p},y_n,t)] \geq \alpha$.Thus $\{ (x_n,y_n) \}$ is α-Cauchy in $X\times Y$.

Theorem 4.7 :

If (X,M_1) and (Y,M_2) are α-complete fuzzy metric spaces,then $(X\times Y,M)$ is α-
Complete fuzzy metric space where $M = M_1 \wedge M_2$.

Proof ;

By Theorem 3.1,$(X\times Y,M)$ is a fuzzy metric space ,let $\{ (x_n,y_n) \}$ be α-Cauchy
sequence in $X\times Y$,that is for $\alpha \in (0,1)$ and for each $t \geq 0$, $p = 1,2,\ldots$
$\lim_{n\to\infty} M((x_{n+p},y_{n+p}),(x_n,y_n),t) \geq \alpha$ .This implies that
$[\lim_{n\to\infty} M_1(x_{n+p},x_n,t)] \wedge [\lim_{n\to\infty} M_2(y_{n+p},y_n,t)] \geq \alpha$,Then
$\lim_{n\to\infty} M_1(x_{n+p},x_n,t) \geq \alpha$,and $\lim_{n\to\infty} M_2(y_{n+p},y_n,t) \geq \alpha$ .This means that $\{ x_n \}$ is α-Cauchy in (X,M_1) and $\{ y_n \}$ is α-Cauchy in (Y,M_2).

But (X,M_1) and (Y,M_2) are α-complete that is there is x in X and y in Y such
That for each $t> 0$, $\lim_{n\to\infty} M_1(x_n,x,t) \geq \alpha$ and $\lim_{n\to\infty} M_2(y_n,y,t) \geq \alpha$.

Now for each $t> 0$, $\lim_{n\to\infty} M((x_n,y_n),(x,y),t) = [\lim_{n\to\infty} M_1(x_n,x,t)] \wedge$

$[\lim_{n\to\infty} M_2(y_n, y, t)] \geq \alpha$ .Thus (X×Y,M) is α-complete.

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A modified variational Iteration method for solving higher dimensional initial boundary value problems

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Received 2/10/2011 – Accepted 18/4/2012

الخلاصة

في هذه البحث، نقدم تطوير طريقة التغاير التكراري لحل مسائل ابتدائية وحدودية القيمة بأبعاد عليا .هذه الطريقة هي اختيار معامل خطي لمسائل ابتدائية وحدودية القيمة، بحيث مضروب لاكرانج يكون مميز عمليا. عدة امثلة بينت كفاءة وقدرة هذه الطريقة.

ABSTRAC

In this paper, we introduce a modified variational iteration method for solving higher dimensional initial boundary value problems. This method choose linear operator for initial boundary value problems. So that the Lagrange multiplier can be effectively identified. Several numerical examples are presented to show the ability and efficiency of this method.

INTRODUCTION

The numerical and analytical solutions of higher dimensional initial boundary value problems of variable coefficients, linear and nonlinear, are of considerable significance for applied sciences. Several numerical and analytical techniques including the spectral methods, characteristics method, and Adomian's decomposition method have been developed for solving these problems.. For implementation of the Adomian decomposition method, one has to find the so-called the Adomian polynomial, which is itself a difficult problem. To overcome these difficulties and drawbacks. He.J.H [1-5] developed the variational iteration method for solving linear and nonlinear problems, which a risen various branches of pure and applied sciences. It is worth mentioning that the origin of variational iteration method can be traced back to .Inokuti.M and Sekine.H [6].

The variational iteration method, has been widely applied to solve nonlinear problems, more and more merits have been discovered and some modifications are suggested to overcome the demerits arising in the solution procedure. For example Noor M.A,et al[7] applied a modified $H's$ variational iteration method for solving singular fourth order parabolic partial differential equations. Noor M.A. and Mohyud-Din S.T. [8] introduced the variational homotopy perturbation method. This method was suggested by combining the variational iteration technique and the homotopy perturbation technique. Ghorbani.A and Saberi-Nadjafi.J[9]modified the VIM by constructing an initial trial function without unknown parameters.Abessy.T.A,et al[10,11] proposed a modification of the variational iteration method and used it to give an approximate power series solutions for some well-known

nonlinear problems.Abassy.T.A,et al [12,13] also proposed further treatments of these modification results by using pade approximation and the laplace transform.

Soltani.L.A, Shirzadi.A[14] applied anew modifition of Variational iteration method, which provides great freedom in choosing linear operators for various nonlinear equations.

In this paper, we consider the Modified Variational Iteration Method (MVIM) and the Variational Iteration Method(VIM) for solving higher dimensional initial boundary value problems. The results are computed by using Maple 13 and compared to exact solution .

THE VARIATION ITERATION METHOD[6]

In this section, we introduce the basic idea underlying the variational iteration method for solving equations. Consider the general equation

$$L[u(x,t)]+N[u(x,t)]=g(x,t) \qquad (2.1)$$

where L is a linear differential operator, N is a nonlinear operator, and g is a given analytical function. The essence of the method is to construct a correction functional of the form

$$u_{n+1}(x,t)=u_n(x,t)+\int_0^t \lambda(t,s)(Lu_n(x,s)+N\tilde{u}_n(x,s)-g(x,s))ds \qquad (2.2)$$

where λ is a Lagrange multiplier which can be identified optimally via the variational theory M.Inokuti and H.Sekine,et al, $u_n(x,t)$ is the approximate solution and $\tilde{u}_n$ denotes the restricted variation, i.e. $\delta\tilde{u}_n=0$. After determining the Lagrange multiplier λ and selecting an appropriate initial function u_0, the successive approximations $u_n(x,t)$ of the solution $u(x,t)$ can be readily obtained. Consequently, the solution of Eq. (2.1) is given by $u(x,t)=\lim_{n\to\infty}u_n(x,t)$..

For convergence of the sequence obtained via the VIM and its rate, we recall Banach s theorem:

Theorem .1.(Banach s Fixed point Theorem)[14]:

Assume that X is a Banach space,

$B: X\to X$

is a linear mapping, and suppose that

$$\|B[u]-B[\bar{u}]\|\le\gamma\|u-\bar{u}\|,\quad \forall u,\bar{u}\in X \qquad (2.3)$$

for some constant $\gamma<1$ .Then B has a unique Fixed point .Furthermore, the sequence

$$u_{n+1}=B[u_n] \qquad (2.4)$$

with an arbitrary choice of $u_0 \in X$ converges to the fixed point of B and

$$\|u_k - u_l\| \le \|u_1 - u_0\| \sum_{j=l-1}^{k-2} \gamma^j$$

According to the above theorem, for the nonlinear mapping

$$B[u_n(x,t)] = u_n(x,t) + \int_0^t \lambda(s,t)(Lu_n(x,s) + N\widetilde{u}_n(x,s) - g(x,s))ds$$

A sufficient condition for the convergence of the variational iteration method is strictly contraction of B.Furthermore, sequence (2.4) converges to the fixed method of B, which is also the solution of the equation (2.1). In the above theorem, the rate of convergence depends on γ and therefore, in the variation iteration method, the rate of convergence depends on λ.

A MODIFIED VARIATION ITERATION METHOD

A modified variational iteration method can be identified by the following, eq.(2.1) can be rewritten in the following form:

$$L[u(x,t)] - g_1[u(x,t)] + g_1[u(x,t)] + N[u(x,t)] = g(x,t) \qquad (3.1)$$

Where $g_1[u(x,t)]$ is an arbitrary linear operator of $u(x,t)$. Now we can construct

a correction functional based on the new linear operator which is:

$$u_{n+1}(x,t) = u_n(x,t) + \int_0^t \lambda(s,t)(Lu_n(x,s) - g_1[u_n(x,s)] + g_1[u_n(x,s)] + N\widetilde{u}_n(x,s) - g(x,s))ds \qquad 3.2)$$

Where $\widetilde{u}_n$ is considered as a restricted variation i-e, $,\delta\widetilde{u}_n = 0$ .The Lagrange multiplier, λ, obtained from the correction functional (3.2) is different from (2.2). we can choose the auxiliary linear operator. This provides great freedom in applying the variational iteration method to higher dimensional initial boundary value problems

APPLICATIONS

In this section we shall illustrate the VIM and MVIM by following examples:

Example(4-1)[7,15]: Consider the two–dimensional initial boundary value problem:

$$\frac{\partial^2 u}{\partial t^2} = \frac{y^2}{2}\frac{\partial^2 u}{\partial x^2} + \frac{x^2}{2}\frac{\partial^2 u}{\partial y^2}, \quad 0 < x, y < 1 \quad , t > 0 \qquad (4.1)$$

subject to the initial conditions

$$u(x,y,z,0) = x^2 + y^2, \qquad u_t(x,y,z,0) = -(x^2 + y^2)$$

and the boundary conditions

$u(0,y) = y^2 e^{-t}, \qquad u(\pi,y) = (1+y^2)e^{-t}$

$u(x,0,) = y^2 e^{-t}, \qquad u(x,\pi) = (1+x^2)e^{-t}$

which has the exact solution $u(x,y,t) = (x^2+y^2)e^{-t}$.

The solution by VIM:

To solve eq.(4.1), according to the variational iteration method, we derive a correction functional as follows

$$u_{n+1}(x,y,t) = u_n(x,y,t) + \int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{2}(y^2\frac{\partial^2 \tilde{u}_n}{\partial x^2} + x^2\frac{\partial^2 \tilde{u}_n}{\partial y^2})\right]ds, \quad (4.2)$$

Where $\tilde{u}_n$ is considered as a restricted variation. Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda = s-t$,which yield the following iteration formula:

$$u_{n+1}(x,y,t) = u_n(x,y,t) + \int_0^t (s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{2}(y^2\frac{\partial^2 u_n}{\partial x^2} + x^2\frac{\partial^2 u_n}{\partial y^2})\right]ds, \quad (4.3)$$

now, we begin with the initial approximation:

$u_0(x,y,t) = (x^2+y^2) - (x^2+y^2)t$

by the variational iteration formula(4.3), we get

$$u_1(x,y,t) = (x^2+y^2) - (x^2+y^2)t + (x^2+y^2)\frac{t^2}{2!} - (x^2+y^2)\frac{t^3}{3!}$$

$$u_2(x,y,t) = (x^2+y^2) - (x^2+y^2)t + (x^2+y^2)\frac{t^2}{2!} - (x^2+y^2)\frac{t^3}{3!} + (x^2+y^2)\frac{t^4}{4!} - (x^2+y^2)\frac{t^5}{5!}$$

$$u_3(x,y,t) = (x^2+y^2) - (x^2+y^2)t + (x^2+y^2)\frac{t^2}{2!} - (x^2+y^2)\frac{t^3}{3!} + (x^2+y^2)\frac{t^4}{4!} - (x^2+y^2) - \frac{t^5}{5!} + (x^2+y^2)\frac{t^6}{6!} - (x^2+y^2)\frac{t^7}{7!}$$

$$u_n(x,y,t) = (x^2+y^2)(1-t+\frac{t^2}{2!} + \ldots\ldots + (-1)^n\frac{t^n}{n!}),$$

$u(x,y,t) = (x^2+y^2)e^{-t}$, when $n \to \infty$.

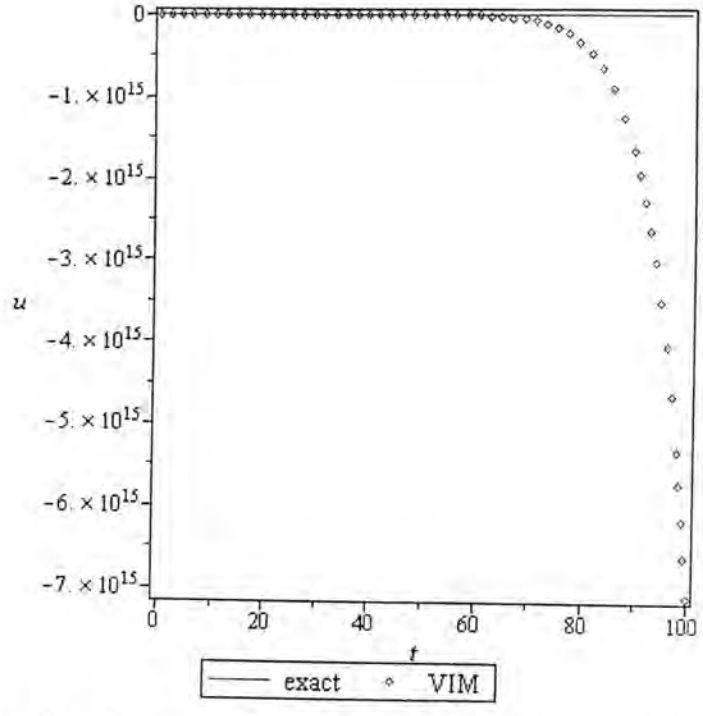

Figure -1: The plot of approximation solution (VIM)and exact solution of example (1).

The solution by MVIM:

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1}(x,y,t) = u_n(x,y,t) + \int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2} - u_n + \tilde{u}_n - \frac{1}{2}(y^2\frac{\partial^2 \tilde{u}_n}{\partial x^2} + x^2\frac{\partial^2 \tilde{u}_n}{\partial y^2})\right]ds, \quad (4.4)$$

and the stationary condition of the above correction functional can be expressed as :

$$\frac{\partial^2 \lambda(s,t)}{\partial s^2}\Big|_{s=t} - \lambda(s,t)\Big|_{s=t} = 0$$

$$1 - \frac{\partial \lambda(s,t)}{\partial s}\Big|_{s=t} = 0$$

$$\lambda(s,t)\Big|_{s=t} = 0$$

the Lagrange multiplier, therefore, can be identified as follows:

$$\lambda = \sinh(s-t) \quad (4.5)$$

substituting (4.5) for correction functional (4.4), we have the following iteration formula:

$$u_{n+1}(x,y,t) = u_n(x,y,t) + \int_0^t \sinh(s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{2}(y^2\frac{\partial^2 u_n}{\partial x^2} + x^2\frac{\partial^2 u_n}{\partial y^2})\right]ds, \quad (4.6)$$

by the variational iteration formula (4.6)and initial approximation, we get

$$u_1(x,y,t) = (x^2+y^2)e^{-t}.$$

Which means that $u_1(x,y,t) = u(x,y,t) = (x^2 + y^2)e^{-t}$. Is the exact solution.

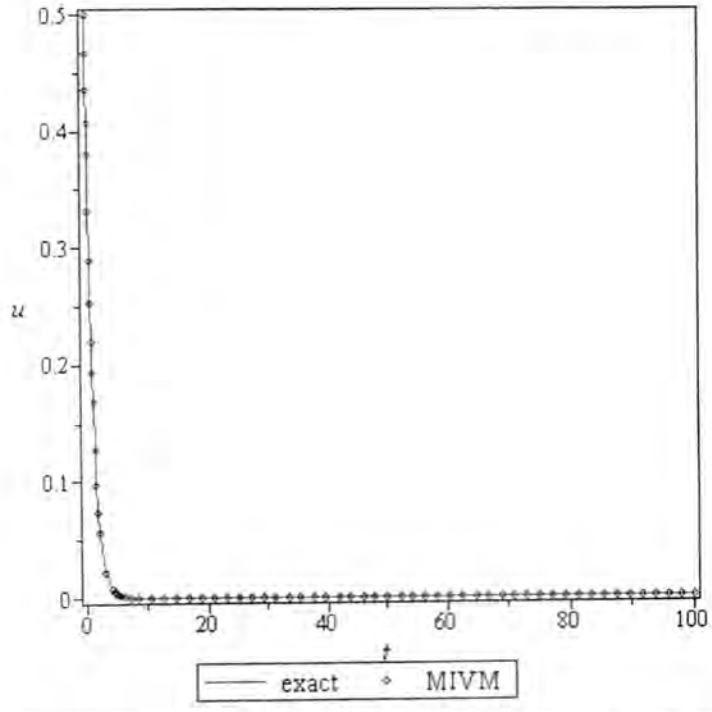

Figure-2. The plot of approximation solution (MVIM)and exact solution of example (1).

In Figure1, Approximate solution $u(x,y,t) = u_8(x,y,t)$ of (4.1) using VIM and the exact solution have been for $x = 0.5, y = 0.5$. In Figure2, Approximate solution $u(x,y,t) = u_1(x,y,t)$ of (4.1) using MVIM and the exact solution have been for $x = 0.5, y = 0.5$.

Example4-2[15,16]Consider the three –dimensional initial boundary value problem

$$\frac{\partial^2 u}{\partial t^2} = \frac{1}{45}x^2\frac{\partial^2 u}{\partial x^2} + \frac{1}{45}y^2\frac{\partial^2 u}{\partial y^2} + \frac{1}{45}z^2\frac{\partial^2 u}{\partial z^2} - u, \qquad 0 < x, y < 1, t > 0 \qquad (4.7)$$

subject to the initial conditions

$$u(x,y,z,0) = 0, \qquad u_t(x,y,z,0) = x^6 y^6 z^6$$

and the Neumann boundary conditions

$$u_x(0,y,z,t) = 0, \qquad u_x(1,y,z,t) = 6y^6 z^6 \sinh t,$$

$$u_y(x,0,z,t) = 0, \qquad u_y(x,1,z,t) = 6x^6 z^6 \sinh t,$$

$$u_z(x,y,0,t) = 0 \qquad u_z(x,y,1,t) = 6x^6 y^6 \sinh t,$$

which has the exact solution $u(x,y,z,t) = x^6 y^6 z^6 \sinh(t)$.

The solution by VIM:

The correct functional is given as

$$u_{n+1} = u_n + \int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{45}(x^2\frac{\partial^2 \widetilde{u}_n}{\partial x^2} + y^2\frac{\partial^2 \widetilde{u}_n}{\partial y^2} + z^2\frac{\partial^2 \widetilde{u}_n}{\partial z^2}) + \widetilde{u}_n\right]ds \qquad (4.8)$$

Where $\widetilde{u}_n$ is considered as a restricted variation. Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda = s - t$, which yield the followin iteration formula:

$$u_{n+1} = u_n + \int_0^t (s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{45}(x^2\frac{\partial^2 \widetilde{u}_n}{\partial x^2} + y^2\frac{\partial^2 \widetilde{u}_n}{\partial y^2} + z^2\frac{\partial^2 \widetilde{u}_n}{\partial z^2}) + \widetilde{u}_n\right]ds \qquad (4.9)$$

now, we begin with the initial approximation,

$u_0(x,y,z,t) = x^6 y^6 z^6\ t$

by the variational iteration formula(4.9), we get

$$u_1(x,y,z,t) = x^6 y^6 z^6\ t + x^6 y^6 z^6 \frac{t^3}{3!}$$

$$u_2(x,y,z,t) = x^6 y^6 z^6\ t + x^6 y^6 z^6 \frac{t^3}{3!} + x^6 y^6 z^6 \frac{t^5}{5!}$$

$$u_3(x,y,z,t) = x^6 y^6 z^6\ t + x^6 y^6 z^6 \frac{t^3}{3!} + x^6 y^6 z^6 \frac{t^5}{5!} + x^6 y^6 z^6 \frac{t^7}{7!}$$

$$u_n(x,y,z,t) = x^6 y^6 z^6 (t + \frac{t^3}{3!} + ... + \frac{t^{2n+1}}{(2n+1)!}),$$

$u(x,y,z,t) = x^6 y^6 z^6 \sinh(t)$, when $n \to \infty$.

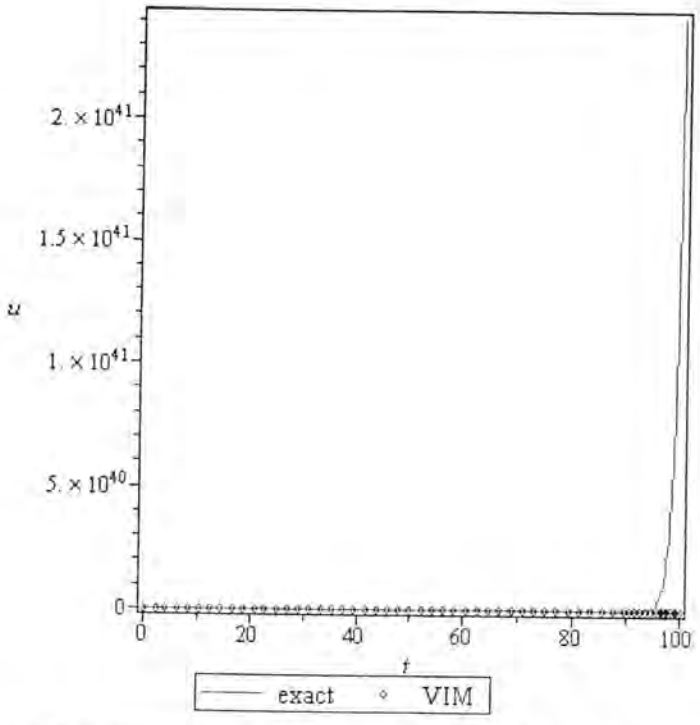

Figure -3: The plot of approximation solution (VIM)and exact solution of example (2).

The solution by MVIM:

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1} = u_n + \int_0^t \lambda \left[\frac{\partial^2 u_n}{\partial s^2} - u_n + \tilde{u}_n - \frac{1}{45}(x^2 \frac{\partial^2 \tilde{u}_n}{\partial x^2} + y^2 \frac{\partial^2 \tilde{u}_n}{\partial y^2} + z^2 \frac{\partial^2 \tilde{u}_n}{\partial z^2}) + \tilde{u}_n \right] ds \quad (4.10)$$

and the stationary condition of the above correction functional can be expressed as :

$$\frac{\partial^2 \lambda(s,t)}{\partial s^2}\Big|_{s=t} - \lambda(s,t)\Big|_{s=t} = 0$$

$$1 - \frac{\partial \lambda(s,t)}{\partial s}\Big|_{s=t} = 0$$

$$\lambda(s,t)\Big|_{s=t} = 0$$

the Lagrange multiplier, therefore, can be identified as follows:

$$\lambda = \sinh(s-t) \quad (4.11)$$

substituting (4.11) for correction functional (4.10), we have the following iteration formula:

$$u_{n+1} = u_n + \int_0^t \sinh(s-t) \left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{45}(x^2 \frac{\partial^2 u_n}{\partial x^2} + y^2 \frac{\partial^2 u_n}{\partial y^2} + z^2 \frac{\partial^2 u_n}{\partial z^2}) + u_n \right] ds \quad (4.12)$$

by the variational iteration formula (4.12), we get

$u_1(x,y,z,t) = (x^6 y^6 z^6)\sinh(t)$,

which means that $u_1(x,y,z,t) = u(x,y,z,t) = (x^6 y^6 z^6)\sinh(t)$, is the exact solution .

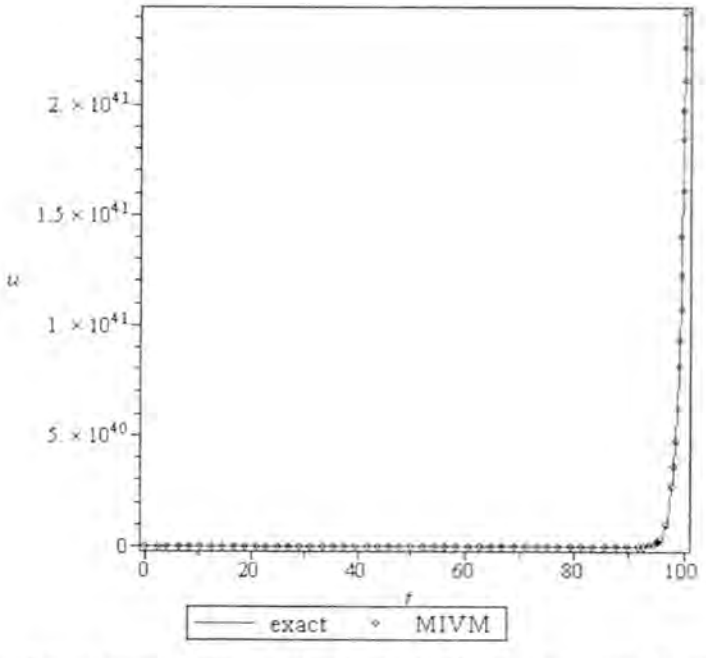

Figure-4: The plot of approximation solution (MVIM)and exact solution of example (2).

In Figure3, approximate solution $u(x,y,z,t)=u_8(x,y,z,t)$ of (4.7) using VIM and the exact solution have been for $x=0.8, y=0.8, z=0.8$. In Figure 4, approximate solution $u(x,y,z,t)=u_1(x,y,z,t)$ of (4.7) using MVIM and the exact solution for $x=0.8, y=0.8, z=0.8$..

Example(4-3)[17,18]: Consider the three –dimensional initial boundary value problem

$$\frac{\partial^2 u}{\partial t^2}+(\frac{y+z}{2\cos x}-1)\frac{\partial^4 u}{\partial x^4}+(\frac{z+x}{2\cos y}-1)\frac{\partial^4 u}{\partial y^4}+(\frac{x+y}{2\cos z}-1)\frac{\partial^4 u}{\partial z^4}=0, \qquad (4.13)$$

$$0<x,y,z,<\frac{\pi}{3}, 0\le t\le 1,$$

subject to the initial conditions

$$u(x,y,z,0)=-\frac{\partial u}{\partial t}(x,y,z,0)=x+y+z-(\cos x+\cos y+\cos z),$$

and the boundary conditions

$$u(0,y,z,t)=e^{-t}(-1+y+z-\cos y-\cos z),$$

$$u(\frac{\pi}{3},y,z,t)=e^{-t}(\frac{2\pi-3}{6}+y+z-\cos y-\cos z),$$

$$u(x,0,z,t)=e^{-t}(-1+x+z-\cos x-\cos z),$$

$$u(x,\frac{\pi}{3},z,t)=e^{-t}(\frac{2\pi-3}{6}+x+z-\cos x-\cos z),$$

$$u(x,y,0,t)=e^{-t}(-1+x+y-\cos x-\cos y),$$

$$u(x,y,\frac{\pi}{3},t)=e^{-t}(\frac{2\pi-3}{6}+x+y-\cos x-\cos y),$$

$$\frac{\partial u}{\partial x}(0,y,z,t)=\frac{\partial u}{\partial y}(x,0,z,t)=\frac{\partial u}{\partial z}(x,y,0,t)=e^{-t}$$

$$\frac{\partial u}{\partial x}(\frac{\pi}{3},y,z,t)=\frac{\partial u}{\partial y}(x,\frac{\pi}{3},z,t)=\frac{\partial u}{\partial z}(x,y,\frac{\pi}{3},t)=\frac{\sqrt{3}+2}{2}e^{-t}$$

which has the exact solution $u(x,y,z,t)=(x+y+z-\cos x-\cos y-\cos z)e^{-t}$.

The solution by VIM:

The correct functional is given as:

$$u_{n+1}=u_n+\int_0^t \lambda(\frac{\partial^2 u_n}{\partial s^2}+(\frac{y+z}{2\cos x}-1)\frac{\partial^4 \tilde{u}_n}{\partial x^4}+(\frac{z+x}{2\cos y}-1)\frac{\partial^4 \tilde{u}_n}{\partial y^4}+(\frac{x+y}{2\cos z}-1)\frac{\partial^4 \tilde{u}_n}{\partial z^4})ds \qquad (4.14)$$

Where $\tilde{u}_n$ is considered as a restricted variation .Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda=s-t$,which yield the following iteration formula

$$u_{n+1}(x,y,z,t)=u_n(x,y,z,t)+\int_0^t(s-t)(\frac{\partial^2 u_n}{\partial s^2}+(\frac{y+z}{2\cos x}-1)\frac{\partial^4 u_n}{\partial x^4} + (\frac{z+x}{2\cos y}-1)\frac{\partial^4 u_n}{\partial y^4}+(\frac{x+y}{2\cos z}-1)\frac{\partial^4 u_n}{\partial z^4})ds \quad (4.15)$$

now, we begin with the initial approximation,

$u_0(x,y,z,t)=(x+y+z-(\cos x+\cos y+\cos z))(1-t)$

by the variational iteration formula(4.15), we get

$u_1(x,y,z,t)=(x+y+z-(\cos x+\cos y+\cos z))(1-t+\frac{t^2}{2!}-\frac{t^3}{3!})$

$u_2(x,y,z,t)=(x+y+z-(\cos x+\cos y+\cos z))(1-t+\frac{t^2}{2!}-\frac{t^3}{3!}+\frac{t^4}{4!}-\frac{t^5}{5!})$

$u_3(x,y,z,t)=(x+y+z-(\cos x+\cos y+\cos z))(1-t+\frac{t^2}{2!}-\frac{t^3}{3!}+\frac{t^4}{4!}-\frac{t^5}{5!}+\frac{t^6}{6!}-\frac{t^7}{7!})$.

$u_n(x,y,z,t)=(x+y+z-\cos(x)-\cos(y)-\cos(z))(1-t+\frac{t^2}{2!}+.....+(-1)^n\frac{t^n}{n!})$,

$u(x,y,z,t)=(x+y+z-\cos x-\cos y-\cos z)e^{-t}$, when $n\to\infty$.

The solution by MVIM

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1}(x,y,z,t)=u_n(x,y,z,t)+\int_0^t\lambda(t,s)(\frac{\partial^2 u_n}{\partial s^2}-u_n+\tilde{u}_n+(\frac{y+z}{2\cos x}-1)\frac{\partial^4 \tilde{u}_n}{\partial x^4} + (\frac{z+x}{2\cos y}-1)\frac{\partial^4 \tilde{u}_n}{\partial y^4}+(\frac{x+y}{2\cos z}-1)\frac{\partial^4 \tilde{u}_n}{\partial z^4})ds \quad (4.16)$$

and the stationary condition of the above correction functional can be expressed as :

$\frac{\partial^2\lambda(s,t)}{\partial s^2}\Big|_{s=t}-\lambda(s,t)\Big|_{s=t}=0$

$1-\frac{\partial\lambda(s,t)}{\partial s}\Big|_{s=t}=0$

$\lambda(s,t)\Big|_{s=t}=0$

the Lagrange multiplier ,therefore, can be identified as follows:

$\lambda=\sinh(s-t)$ (4.17)

substituting (4.17) for correction functional (4.16), we have the following iteration formula:

$$u_{n+1}(x,y,z,t)=u_n(x,y,z,t)+\int_0^t\sinh(s-t)(\frac{\partial^2 u_n}{\partial s^2}+(\frac{y+z}{2\cos x}-1)\frac{\partial^4 u_n}{\partial x^4} + (\frac{z+x}{2\cos y}-1)\frac{\partial^4 u_n}{\partial y^4}+(\frac{x+y}{2\cos z}-1)\frac{\partial^4 u_n}{\partial z^4})ds \quad (4.18)$$

by the variational iteration formula (4.18), we get

$u_1(x,y,z,t)=(x+y+z-(\cos x+\cos y+\cos z))e^{-t}$

which means that $u_1(x,y,z,t) = u(x,y,z,t) = (x+y+z-\cos x-\cos y-\cos z)e^{-t}$, is the exact solution .

Example4-4[19] Consider the three –dimensional initial boundary value problem

$$\frac{\partial^2 u}{\partial t^2} = \frac{1}{6}x^2\frac{\partial^2 u}{\partial x^2} + \frac{1}{6}y^2\frac{\partial^2 u}{\partial y^2} + \frac{1}{6}z^2\frac{\partial^2 u}{\partial z^2}, \quad 0 < x,y,z < 1, t > 0 \qquad (4.19)$$

subject to the initial conditions

$$u(x,y,z,0) = x^2y^2z^2, \qquad u_t(x,y,z,0) = 0,$$

and the boundary conditions

$$u(0,y,z,t) = 0, \qquad u(1,y,z,t) = y^2z^2\cosh t,$$

$$u(x,0,z,t) = 0, \qquad u(x,1,z,t) = x^2z^2\cosh t,$$

$$u(x,y,0,t) = 0 \qquad u(x,y,1,t) = x^2y^2\cosh t,$$

which has the exact solution $u(x,y,z,t) = x^2y^2z^2\cosh(t)$.

The solution by VIM:

The correct functional is given as

$$u_{n+1}(x,y,z,t) = u_n(x,y,z,t) + \int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{6}(x^2\frac{\partial^2 \tilde{u}_n}{\partial x^2} + y^2\frac{\partial^2 \tilde{u}_n}{\partial y^2} + z^2\frac{\partial^2 \tilde{u}_n}{\partial z^2})\right]ds \quad (4.20)$$

Where $\tilde{u}_n$ is considered as a restricted variation. Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda = s - t$, which yield the following iteration formula:

$$u_{n+1} = u_n + \int_0^t (s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{6}(x^2\frac{\partial^2 u_n}{\partial x^2} + y^2\frac{\partial^2 u_n}{\partial y^2} + z^2\frac{\partial^2 u_n}{\partial z^2})\right]ds \qquad (4.21)$$

now, we begin with the initial approximation,

$u_0(x,y,z,t) = x^2y^2z^2$

by the variational iteration formula(4.21),we get

$$u_1(x,y,z,t) = x^2y^2z^2 + x^2y^2z^2\frac{t^2}{2!}$$

$$u_2(x,y,z,t) = x^2y^2z^2 + x^2y^2z^2\frac{t^2}{2!} + x^2y^2z^2\frac{t^4}{4!}$$

$$u_3(x,y,z,t) = x^2y^2z^2 + x^2y^2z^2\frac{t^2}{2!} + x^2y^2z^2\frac{t^4}{4!} + x^2y^2z^2\frac{t^6}{6!}$$

$$u_n(x,y,z,t) = x^2y^2z^2(1 + \frac{t^2}{2!} + \ldots.. + \frac{t^{2n}}{2n!}),$$

$u(x,y,z,t) = (x^2y^2z^2)\cosh(t)$, when $n \to \infty$.

The solution by MVIM:

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1} = u_n + \int_0^t \lambda\left[\frac{\partial^2 u_n}{\partial s^2} - u_n + \tilde{u}_n - \frac{1}{6}(x^2\frac{\partial^2 \tilde{u}_n}{\partial x^2} + y^2\frac{\partial^2 \tilde{u}_n}{\partial y^2} + z^2\frac{\partial^2 \tilde{u}_n}{\partial z^2})\right]ds \qquad (4.22)$$

and the stationary condition of the above correction functional can be expressed as :

$$\frac{\partial^2 \lambda(s,t)}{\partial s^2}\Big|_{s=t} - \lambda(s,t)\Big|_{s=t} = 0$$

$$1 - \frac{\partial \lambda(s,t)}{\partial s}\Big|_{s=t} = 0$$

$$\lambda(s,t)\Big|_{s=t} = 0$$

the Lagrange multiplier, therefore, can be identified as follows:

$$\lambda = \sinh(s-t) \qquad (4.23)$$

substituting (4.23) for correction functional (4.22), we have the following iteration formula:

$$u_{n+1} = u_n + \int_0^t \sinh(s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - \frac{1}{6}(x^2\frac{\partial^2 u_n}{\partial x^2} + y^2\frac{\partial^2 u_n}{\partial y^2} + z^2\frac{\partial^2 u_n}{\partial z^2})_n\right]ds \qquad (4.24)$$

by the variational iteration formula (4.24), we get

$$u_1(x,y,z,t) = (x^2y^2z^2)\cosh(t)$$

which means that $u_1(x,y,z,t) = u(x,y,z,t) = (x^2y^2z^2)\cosh(t)$, is the exact solution.

Example4-5[20]Consider the two –dimensional initial boundary value problem

$$\frac{\partial^2 u}{\partial t^2} + \frac{x^2}{2}\frac{\partial^2 u}{\partial x^2} + \frac{y^2}{2}\frac{\partial^2 u}{\partial y^2} = k_1x^2 - k_2y^2, \qquad (4.25)$$

subject to the initial conditions

$$u(x,y,0) = 0, \qquad u_t(x,y,z,0) = x^2 - y^2,$$

where $x, y \in [0,1], t \in [0,2\pi]$

which has the exact solution

$$u(x,y,t) = -k_1x^2(\cos t - 1) + k_2y^2(\cos t - 1) + (x^2 - y^2)\sin t$$

The solution by VIM:

The correct functional is given as

$$u_{n+1}(x,y,z,t) = u_n(x,y,z,t) + \int_0^t \lambda(t,s)(\frac{\partial^2 u_n}{\partial s^2} + \frac{x^2}{2}\frac{\partial^2 \tilde{u}_n}{\partial x^2} + \frac{y^2}{2}\frac{\partial^2 \tilde{u}_n}{\partial y^2} - k_1\tilde{x}^2 + k_2\tilde{y}^2)ds \quad (4.26)$$

where $\tilde{u}_n$ is considered as a restricted variation .Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda = s - t$, which yield the following iteration formula:

$$u_{n+1}(x,y,z,t) = u_n(x,y,z,t) + \int_0^t (s-t)(\frac{\partial^2 u_n}{\partial s^2} + \frac{x^2}{2}\frac{\partial^2 u_n}{\partial x^2} + \frac{y^2}{2}\frac{\partial^2 u_n}{\partial y^2} - k_1x^2 + k_2y^2)ds \qquad (4.27)$$

now, we begin with the initial approximation,

$$u_0(x,y,t) = (x^2 - y^2)t$$

by the variational iteration formula(4.27), we get

$$u_1(x,y,z,t) = k_1x^2\frac{t^2}{2!} - k_2y^2\frac{t^2}{2!} - x^2\frac{t^3}{3!} + y^2\frac{t^3}{3!} + t(x^2 - y^2)$$

$$u_2(x,y,z,t) = -k_1x^2(-\frac{t^2}{2!}+\frac{t^4}{4!}) + k_2y^2(-\frac{t^2}{2!}+\frac{t^4}{4!}) + x^2(-\frac{t^3}{3!}+\frac{t^5}{5!}) - y^2(-\frac{t^3}{3!}+\frac{t^5}{5!}) + t(x^2-y^2)$$

$$u_n(x,y,z,t) = -k_1x^2(-\frac{t^2}{2!}+.....+(-1)^n\frac{t^{2n}}{(2n)!}) + k_2y^2(-\frac{t^2}{2!}+....+(-1)^n\frac{t^{2n}}{(2n)!})$$
$$+(x^2-y^2)(-\frac{t^3}{3!}+.....+(-1)^n\frac{t^{2n+1}}{(2n+1)!}) + t(x^2-y^2)$$

$u(x,y,t) = -k_1x^2(\cos t-1) + k_2y^2(\cos t-1) + (x^2-y^2)\sin t$, when $n\to\infty$.

The solution by MVIM:

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1} = u_n + \int_0^t \lambda(t,s)(\frac{\partial^2 u_n}{\partial s^2} + u_n - \widetilde{u}_n + \frac{x^2}{2}\frac{\partial^2\widetilde{u}_n}{\partial x^2} + \frac{y^2}{2}\frac{\partial^2\widetilde{u}_n}{\partial y^2} - k_1\widetilde{x}^2 + k_2\widetilde{y}^2)ds \quad (4.28)$$

and the stationary condition of the above correction functional can be expressed as :

$$\frac{\partial^2\lambda(s,t)}{\partial s^2}\Big|_{s=t} + \lambda(s,t)\Big|_{s=t} = 0$$

$$1 - \frac{\partial\lambda(s,t)}{\partial s}\Big|_{s=t} = 0$$

$$\lambda(s,t)\Big|_{s=t} = 0$$

the Lagrange multiplier ,therefore, can be identified as follows:

$$\lambda = \sin(s-t) \quad (4.29)$$

substituting (4.29) for correction functional (4.28), we have the following iteration formula:

$$u_{n+1} = u_n + \int_0^t \sin(s-t)(\frac{\partial^2 u_n}{\partial s^2} + \frac{x^2}{2}\frac{\partial^2 u_n}{\partial x^2} + \frac{y^2}{2}\frac{\partial^2 u_n}{\partial y^2} - k_1x^2 + k_2y^2)ds \quad (4.30)$$

by the variational iteration formula (4.30), we get

$u_1(x,y,t) = -k_1x^2(\cos t-1) + k_2y^2(\cos t-1) + (x^2-y^2)\sin t$

which means that $u_1(x,y,t) = -k_1x^2(\cos t-1) + k_2y^2(\cos t-1) + (x^2-y^2)\sin t$ is the exact solution.

Example4-6[19] Consider the three –dimensional initial boundary value problem

$$\frac{\partial^2 u}{\partial t^2} = 3(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2}), \quad 0 < x,y,z < \pi, t > 0 \quad (4.31)$$

subject to the initial conditions

$u(x,y,z,0) = 0, \qquad u_t(x,y,z,0) = 3\sin x\sin y\sin z,$

and the boundary conditions

$u(0,y,z,t)=0, \quad u(\pi,y,z,t)=0,$

$u(x,0,z,t)=0, \quad u(x,\pi,z,t)=0,$

$u(x,y,0,t)=0 \quad u(x,y,\pi,t)=0.$

The exact solution is $u(x,y,z,t)=\sin x \sin y \sin z \sin(3t)$.

The solution by VIM:

The correct functional is given as

$$u_{n+1}=u_n+\int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2}-3\left(\frac{\partial^2 \widetilde{u}_n}{\partial x^2}+\frac{\partial^2 \widetilde{u}_n}{\partial y^2}+\frac{\partial^2 \widetilde{u}_n}{\partial z^2}\right)\right]ds \qquad (4.32)$$

Where $\widetilde{u}_n$ is considered as a restricted variation .Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda=s-t$, which yield the following iteration formula:

$$u_{n+1}=u_n+\int_0^t (s-t)\left[\frac{\partial^2 u_n}{\partial s^2}-3\left(\frac{\partial^2 u_n}{\partial x^2}+\frac{\partial^2 u_n}{\partial y^2}+\frac{\partial^2 u_n}{\partial z^2}\right)\right]ds \qquad (4.33)$$

now, we begin with the initial approximation,

$u_0(x,y,z,t)=3\sin(x)\sin(y)\sin(z)t$

by the variational iteration formula(4.33), we get:

$$u_1(x,y,z,t)=\sin(x)\sin(y)\sin(z)\left(3t-\frac{(3t)^3}{3!}\right)$$

$$u_2(x,y,z,t)=\sin(x)\sin(y)\sin(z)\left(3t-\frac{(3t)^3}{3!}+\frac{(3t)^5}{5!}\right)$$

$$u_n(x,y,z,t)=\sin(x)\sin(y)\sin(z)\left(3t-\frac{(3t)^3}{3!}+\frac{(3t)^5}{5!}-\ldots\ldots+(-1)^{2n+1}\frac{(3t)^{2n+1}}{(2n+1)!}\right)$$

$u(x,y,z,t)=\sin x \sin y \sin z \sin(3t)$, when $n\to\infty$.

The solution by MVIM:

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1}=u_n+\int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2}+9u_n-9\widetilde{u}_n-3\left(\frac{\partial^2 \widetilde{u}_n}{\partial x^2}+\frac{\partial^2 \widetilde{u}_n}{\partial y^2}+\frac{\partial^2 \widetilde{u}_n}{\partial z^2}\right)\right]ds \qquad (4.34)$$

and the stationary condition of the above correction functional can be expressed as :

$$\frac{\partial^2 \lambda(s,t)}{\partial s^2}\Big|_{s=t}+9\lambda(s,t)\Big|_{s=t}=0$$

$$1-\frac{\partial \lambda(s,t)}{\partial s}\Big|_{s=t}=0$$

$$\lambda(s,t)\Big|_{s=t}=0$$

the Lagrange multiplier ,therefore, can be identified as follows:

$$\lambda=\frac{1}{3}\sin 3(s-t) \qquad (4.35)$$

substituting (4.35) for correction functional (4.34), we have the following iteration formula:

$$u_{n+1} = u_n + \int_0^t \frac{1}{3}\sin 3(s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - 3(\frac{\partial^2 u_n}{\partial x^2} + \frac{\partial^2 u_n}{\partial y^2} + \frac{\partial^2 u_n}{\partial z^2})\right]ds \qquad (4.36)$$

by the variational iteration formula (4.36), we get

$u_1(x,y,z,t) = \sin(x)\sin(y)\sin(z)\sin(3t)$,

which means that $u_1(x,y,z,t) = \sin(x)\sin(y)\sin(z)\sin(3t)$, is the exact solution.

Example4-7[19] Consider the three –dimensional initial boundary value problem:

$$\frac{\partial^2 u}{\partial t^2} = \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} - u + 1, \quad 0 < x,y,z < \pi, t > 0 \qquad (4.37)$$

subject to the initial conditions

$u(x,y,z,0) = 1 + \sin(x)\sin(y)\sin(z)$, $\qquad u_t(x,y,z,0) = 0$,

and the boundary conditions

$u(0,y,z,t) = u(\pi,y,z,t) = 1$,

$u(x,0,z,t) = u(x,\pi,z,t) = 1$,

$u(x,y,0,t) = u(x,y,\pi,t) = 1$,

the solution exact is $u(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)\cos(2t)$,

The solution by VIM:

The correct functional is given as

$$u_{n+1} = u_n + \int_0^t \lambda(t,s)\left[\frac{\partial^2 u_n}{\partial s^2} - (\frac{\partial^2 \tilde{u}_n}{\partial x^2} + \frac{\partial^2 \tilde{u}_n}{\partial y^2} + \frac{\partial^2 \tilde{u}_n}{\partial z^2}) + \tilde{u}_n - \tilde{1}\right]ds \qquad (4.38)$$

Where $\tilde{u}_n$ is considered as a restricted variation. Making the above functional stationary, the Lagrange multiplier can be determined as $\lambda = s - t$, which yield the following iteration formula:

$$u_{n+1} = u_n + \int_0^t (s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - (\frac{\partial^2 u_n}{\partial x^2} + \frac{\partial^2 u_n}{\partial y^2} + \frac{\partial^2 u_n}{\partial z^2}) + u_n - 1\right]ds \qquad (4.39)$$

now, we begin with the initial approximation,

$u_0(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)$

by the variational iteration formula(4.39), we get

$$u_1(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)(1 - \frac{(2t)^2}{2!})$$

$$u_2(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)(1 - \frac{(2t)^2}{2!} + \frac{(2t)^4}{4!})$$

$$u_n(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)(1 - \frac{(2t)^2}{2!} + \frac{(2t)^4}{4!} - \ldots\ldots + (-1)^{2n}\frac{(2t)^{2n}}{(2n)!})$$

$$u(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)\cos(2t), \quad \text{when } n \to \infty.$$

The solution by MVIM:

According to the modified variational iteration method, we derive a correction functional as follows:

$$u_{n+1} = u_n + \int_0^t \lambda \left[\frac{\partial^2 u_n}{\partial s^2} + 4u_n - 4\widetilde{u}_n - \left(\frac{\partial^2 \widetilde{u}_n}{\partial x^2} + \frac{\partial^2 \widetilde{u}_n}{\partial y^2} + \frac{\partial^2 \widetilde{u}_n}{\partial z^2}\right) + \widetilde{u}_n - 1 \right] ds \qquad (4.40)$$

and the stationary condition of the above correction functional can be expressed as :

$$\frac{\partial^2 \lambda(s,t)}{\partial s^2}\Big|_{s=t} + 4\lambda(s,t)\Big|_{s=t} = 0$$

$$1 - \frac{\partial \lambda(s,t)}{\partial s}\Big|_{s=t} = 0$$

$$\lambda(s,t)\Big|_{s=t} = 0$$

the Lagrange multiplier ,therefore, can be identified as follows:

$$\lambda = \frac{1}{2}\sin 2(s-t) \qquad (4.41)$$

substituting (4.41) for correction functional (4.40), we have the following iteration formula:

$$u_{n+1} = u_n + \int_0^t \frac{1}{2}\sin 2(s-t)\left[\frac{\partial^2 u_n}{\partial s^2} - \left(\frac{\partial^2 u_n}{\partial x^2} + \frac{\partial^2 u_n}{\partial y^2} + \frac{\partial^2 u_n}{\partial z^2}\right) + u_n - 1 \right] ds \qquad (4.42)$$

by the variational iteration formula (4.42), we get

$u_1(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)\cos(2t)$,

which mean that $u_1(x,y,z,t) = 1 + \sin(x)\sin(y)\sin(z)\cos(2t)$, is the exact solution.

CONCLUSION

In the work, we applied the modified variational iteration method for solving higher dimensional initial boundary value problems. The method is very powerful and efficient in finding the analytical solutions for a wide class of boundary value problems. And through comparison between The MVIM and VIM, we see the modified variational method shows that the convergence is fast from the VIM to the closed from solution, only one iteration leads to exact solutions. The obtained solution shows that MVIM is a very convenient and effective.

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Theoretical Study 0f Quality Factor for TM_{mn0}- Modes in Gyrotron Tube

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Received 19/9/2011 – Accepted 18/4/2012

الخلاصة

تضمن هذا البحث دراسة عامل الجودة (Q) كدالة لابعاد التجويف الاسطوانى(D/L) لانبوب الجايروترون حيث D قطر التجويف و L طوله (بوحدات cm). ومن خلال تلك الدراسة ، تم تصميم برنامج MODE_3 لحساب عامل الجودة (Q) للانماط المغناطيسية $TM_{mn\ell}$ فقط لـ ℓ=0 .

ABSTRACT

This paper involves study of quality factor as a function of (D/L) where D is the cavity diameter and L is the cavity length (cm unit) of cylindrical cavity in gyrotron tube. In this study we written the program MODE_3 for calculate the quality factor of the $TM_{mn\ell}$ modes only for ℓ= 0, resonant mode chart for a cylindrical cavity, and shows the relation between applied magnetic field and dimension of the cavity, and

INTRODUCTION

Gyrotrons are microwave sources operation is based on the stimulated cyclotron radiation of electrons oscillating in a static magnetic field. Gyrotron devices are now able to generate several orders of magnitude as much power at millimeter wave length as classical microwave tubes, and can operate at frequencies higher than are conveniently available from other types of tubes. [1]

Which are based on cyclotron resonance maser (CRM) instability. In a Gyrotron, a hollow beam from a special kind of electron Gun (magnetic injection Gun) is injected in to a region with very strong axial magnetic field. Flux densities of the order of several Tesla are normally required and this usually necessitates the use of cyrogentic magnets. The magnetic field(B_0)makes the beam gyrate in a number of small orbits (of radius equal to larmor radius) know as beam lets in a frequency equal to the cyclotron frequency ($\omega_c = \frac{eB_0}{m}$).

The gyrating beam is allowed to enter in a RF cavity region .The shape of the cavity is dependent on the mode of the electromagnetic field with which the beam is intended to interact and also the harmonic number of interaction . The required diffractive quality factor of the cavity is achieved by proper fine tuning of the cavity shape. [2]

Quality factor is a measure of the selectivity of a cavity; that is a high Q indicates that the cavity has a sharp resonance. Energy is stored in the time –varying electromagnetic fields with in the cavity and can be found by integrating field intensities over the volume of the cavity. Losses in a cavity are primarily the resistive losses on the inner surfaces of the cavity. [3, 4]

The quality factor Q of a real cavity resonator is determined from the field configuration for the ideal resonator .all the energy is stored either in electric or magnetic form so that:

$\varepsilon = ½\mu H^2 + ½\varepsilon E^2 \quad \cdots\cdots\cdots (1)$

If we choose the magnetic field at resonance in the cavity in equation (2) for H, we find [5]

$$w = \frac{1}{2}\mu \int_v H^2\, dv \quad \cdots\cdots\cdots (2)$$

The normal modes in the right circular cylinder are also divided into TE-and TM-classes, where the axis of reference is also divided into TE-and TE classes, where the axis of reference is along the cylinder axis. See Fig.1.

They are further specified in terms of three integers m, n, and ℓ, which are defined by.[6]

m≡ number of full-period variations of E_r with respect to θ.

n≡ number of half –period variations of E_θ with respect to r,

ℓ≡ number of half – period variations of E_r with respect to z.

For TM-modes, the integers are corresponding defined in term, of the components of H [5].

The problems of exciting waves in waves guides and absorbing their energy in a receiver are usually not simple field problems.

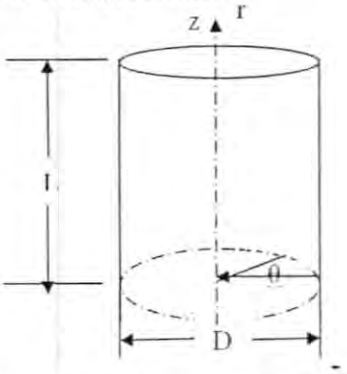


Fig.-1: Show the dimension and cylindrical.

For the very short millimeter or sub millimeter wave lengths, single mode guides of any shape are small and difficult to construct and have high attenuation thus for other shapes, and modes , also one may wish to go to oversize guides in order to obtain larger sizes. These have the capability of propagating more than the mode

+/to be utilized, the mode is usually excited by flaring gradually from a single –mode guide of the desired form mode filters may be introduced according to the fore going principles.

The guide if large compared with wave length, is coupled little to the have and acts largely as a shield. The axial components of field are small so that the wave is essentially a TEM-mode.

For either TE or TM-modes, the resonant frequencies are given by [4].

$$f_r = \frac{1}{2\sqrt{\mu\varepsilon}}\sqrt{\left(\frac{m}{a}\right)^2 + \left(\frac{n}{b}\right)^2 + \left(\frac{\ell}{d}\right)^2} \quad \text{--------} (3)$$

The resonant frequency can also be written as:

$$(\text{fD})^2 = (\frac{CXmn}{\pi})^2 + (\frac{C\ell}{2})^2(\frac{D}{L})^2 - - - (4)$$

The quantities X_{mn} are:

$X_{mn} = m^{th}$ Root of J'_m (x)= 0 for the TE-modes.

$X_{mn} = m^{th}$ Root of $J_m(x) = 0$ for the TM-modes.

Where μ and ε are the permeability and permittivity of the dielectric material fields for some of the lower-order TM_{mnl} modes are shown. We has been studied the quality- factor of TM-modes if $\ell > 0$ [3]. In the present work, we studied the quality- factor of TM-modes if $\ell = 0$.

Numerical Method:

For the cylindrical cavity resonator, as shown in Fig.(1). We choose cylindrical co-ordinates r, θ, z.

The wave equations are obtained with the aid of Hertz vectors and separation into TE and TM components fields for some of the lower-order TM_{mnl} .We use the method of variable separation to get Bessel's equation with Bessel function $J_m(r)$ as the solution. J_m (r) represents a Bessel function of the first sort, order m and argument r.

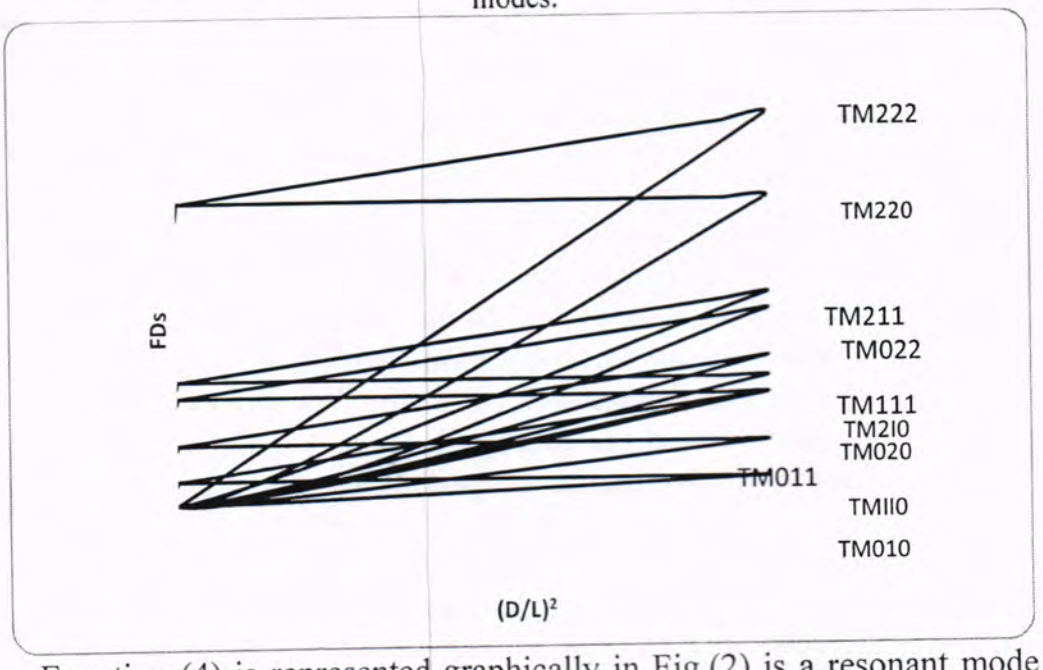
The results were obtained of solving the cylindrical wave equations. The field in a cylindrical cavity for TM waves may be written. [3]

$$\left.\begin{aligned}
E_r &= -\sqrt{\frac{\mu}{\varepsilon}} A_o \frac{K_z}{K} J'_m(K_c r).\cos m\theta \cdot \sin K_z \cdot e^{j\omega t} \\
E_\theta &= \sqrt{\frac{\mu}{\varepsilon}} A_o \frac{K_z J_m(K_c}{K_c r} \sin m\theta \cdot \sin KZ. e^{j\omega t} \\
E_z &= \sqrt{\frac{\mu}{\varepsilon}} A_o \frac{K_c}{K} J'_m(K_c r).\cos m\theta \cdot \cos K_z Z \cdot e^{j\omega t} \\
H_r &= -jA_o m \frac{J_m(K_c r)}{K_c r} \sin m\theta. \cos K_z Z. e^{j\omega t} \\
H_\theta &= -jA_o m J_m(K_c r) \sin m\theta. \cos K_z Z. e^{j\omega t} \\
H_z &= 0
\end{aligned}\right\} \quad (5)$$

RESULT AND DISCUSSION:

We need to know the roots X_{mnl} of $J_m(r)=0$ and $J'_m(r)=0$ to determine the resonance frequencies and quality factors of the TM and TE modes in the resonator. These roots, i.e. the zero of $J_m(r)$ and $J'_m(r)$ for TM-wave. We designed a Fortran program MODE-3 to evaluate the Q – factor.

Fig.-2: Shows a graphical relation between $(fD)^2$ and $(D/L)^2$ for different $TM_{mn\ell}$ modes.



Equation (4) is represented graphically in Fig.(2) is a resonant mode chart for a cylindrical cavity, which shows the resonant frequencies of the lowest order modes as a function of the cylinder radius to length ratio. Here, it is seen that the TM_{010}mode has the lowest resonant frequency when D/L>2. In important characteristic of a resonant mode is its quality factor Q, defined as

$$Q = 2\pi f \frac{average\ energy\ stored}{power\ loss} \quad \text{---------- (6)}$$

The Q-factors are given by the equations for the TM- modes [3, 4]

$$Q\frac{\delta}{\lambda} = \frac{x_{mn}}{\pi(2+R)} \quad \text{for } \ell=0 \text{ --------- (7)}$$

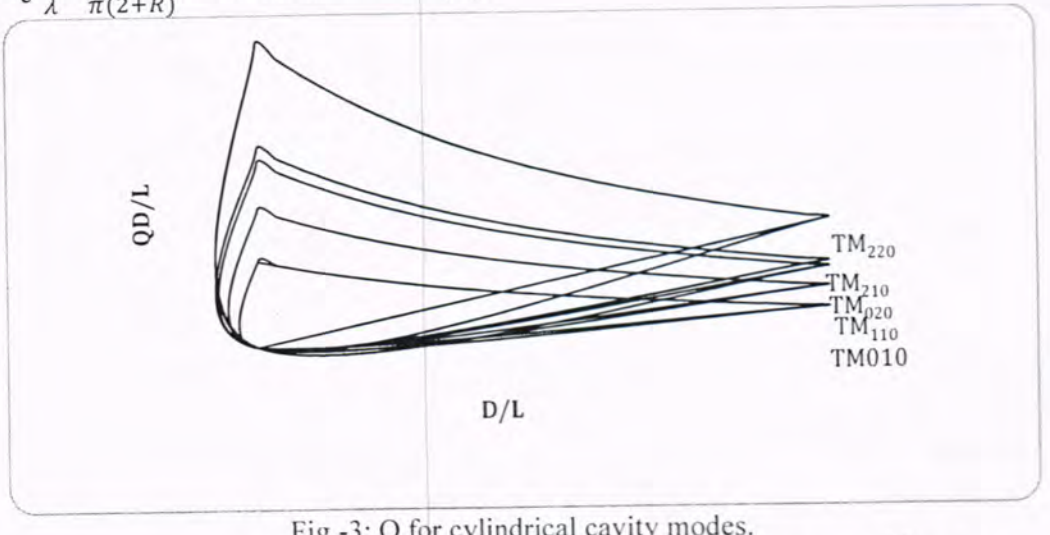


Fig.-3: Q for cylindrical cavity modes.

These equation is represented graphically in Fig.(3).Where $\frac{Q\delta}{\lambda}$ values are plotted for several TM modes as a function of D/ L , where R=D/L

.The quantity, $\frac{Q\delta}{\lambda}$ is commonly tabulated instead of Q ,since this quantity is a function of only the mode and shape of the cavity. This figure shows the Q values of some of the lowest order modes as a function of the of the cylinder radius –to-length ratio. Here it is seen that the TM_{010} has the lowest Q, which makes it no useful for applications where a sharp resonance is needed. from eq.(4) seen that$(fD)^2$versus$(D/L)^2$ is a straight line with intercept $(CX/\pi)^2$ slope $(C\ell/2)^2$.Plots of this type are called mode chart .such a chart indicate that competing modes include TE and TM interacting at the third harmonic and higher order. By using this chart cavity and to estimate the magnetic field required to excite the mode of interest. Also this chart is very useful in studying the mode competition with desired mode .Fig. (4) shows a relation

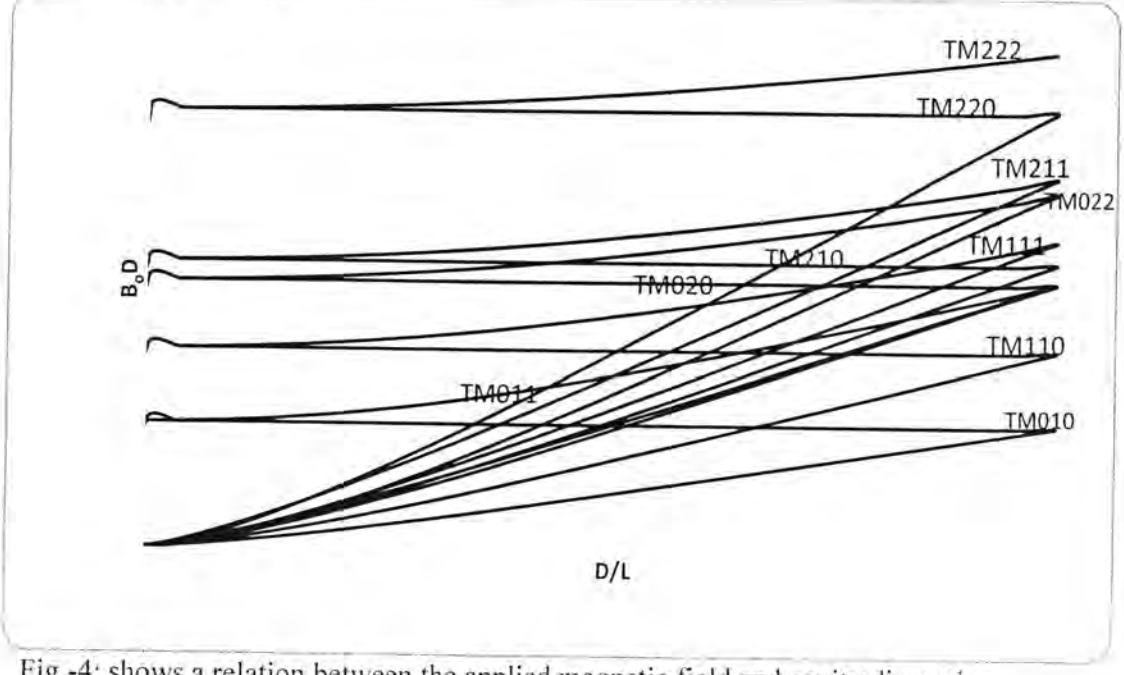


Fig.-4: shows a relation between the applied magnetic field and cavity dimension.

between the applied magnetic field and the cavity dimension for each TM_{mnl}-mode at the resonance condition, one may follow the approximation:[7]

$$\omega = \omega_c = eB / \gamma m$$

Or $f = eB / 2\pi\gamma m$ ---------- (8)

In this figure the parasitic mode competition increased when the magnetic field increased and as increased (D/L).

CONCLUSIONS

Where ℓ=0, 1, ---- Each value of ℓ corresponds to a unique frequency, called a resonant frequency. The quality factor is proportional to volume of the cavity and the axial eigen number (ℓ) gives the lowest quality factor when ℓ=0 .the relation between the applied magnetic field and the

cavity dimensions for TM_{mnl} –modes shown behavior similar to TE_{mnl} – modes that had been study by J. w. Salman [8].

Appendix1:

```
PROGRAM MODE-3
PARAMETER (IMAX=15,JMAX=15)
COMMON/MES/X(0:IMAX+1,0:JAMX+1)
OPEN (UNIT=2,FILE='XM.DAT')
OPEN (UNIT=8,FILE ='OUTAM.OUT')
OPEN (UNIT=1,FILE='DATA1.OUT')
OPEN (UNIT=3, FILE='DATA2.OUT')
OPEN (UNIT=5, FILE='DIL.OUT')
OPEN (UNIT=6, FILE='DATA3.OUT')
X(0,1)=2.465
X(1,1)=3.832
X(2,1)=5.136
X(0,2)=5.520
X(2,2)=8.417
WRITE (6,*)
X(3,1), X(4,1), X(5,1), X(6,1), X(7,1), X(8,1)
DO 1 I=1, 13
READ (2,*) M, N
XMN1=X(M,N)
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
 CALL Q factor (M1, N1, XMN1, FDS1, BD1, DOL1, QDOL1)
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
1 CONTINUE
STOP
END
```

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Effect of Acetic Acid on Dezincification Process for Brass Alloy and Their Effect on Mechanical Properties of Alloy

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Received 3/7/2011 – Accepted 18/4/2012

الخلاصة

تم تحضير عينات من سبيكة البراص- ألفا (70-30) . بقطر(1) سم و(2) سم سمكا،ومن أجل معرفة التركيب الكيمياني للبراص المستخدم تم أجراء فحص XRF للعينات، وبعد اجراء عمليات التنعيم والصقل وغسل العينات، تم وزنها ومن ثم غمرها في محلول حامض الخليك بتراكيز مختلفة(0.5-2.5) (Mولمدة عشرة أيام ، ووزنها من جديد لحساب معدل التآكل أضافة الى ذلك تم حساب تركيز النحاس في المحلول باستخدام جهاز مطيافية الامتصاص الذري ,(AAS)تم فحص العينات مجهرياً وبقوة تكبير (300) ومن ثم تم أخذ فحص الشد للعينات وفحص البلى قبل وبعد الغمر . ان هذا البحث يمثل دراسة تجريبية تبين تأثير حامض الخليك على التآكل الحادث للسبائك التي يكون معدن النحاس أساسها عند درجة تصل الى (100درجة مئوية) . وجد ان معدل التآكل للبراص تزداد بزيادة تركيز حامض الخليك وصولاً الى نسبة , (M 2.5) اضافة الى ذلك تكون الزيادة نتيجة ازدياد درجة الحرارة وزمن التعريض . وان أزدياد معدل التآكل يؤدي الى ضعف في الخصائص الميكانيكة .

ABSTRACT

Using α-brass (70-30) to prepare specimens with dimensions of 1 cm diameter and 2 cm length . XRF test was performed for specimens to ensure the chemical construction of used brass. After grinding, polishing, washing the specimens, they were weighted and immersed in different concentrations of acetic acid (0.5-2.5 M) for ten days. Determination of corrosion rate was calculated, moreover copper concentration in the solution provided estimation of copper release rate from the brass surface using AAS device. Optical microscopy test, the tensile test, and wear rate test were performed before and after immersing. This paper presents results of experimental study aimed to investigate effect of acetic acid on corrosion products of copper-based alloys at temperatures up to 100°C. It was found that the corrosion rate of brass was higher with increase in concentration of acetic acid up to 2.5 M. Also it increased with increasing temperature and exposure time. More corrosion rate caused more worse in mechanical properties.

INTRODUCTION

Copper and copper alloys are well known for a combination of good corrosion resistance in a variety of environments, excellent workability, high thermal and electrical conductivities, and attractive mechanical properties at low, normal and moderately elevated temperatures. Copper and copper alloys thus are widely used in the electronic application and bearing materials.[1-3].

Presently copper-based alloys are widely used at power plants for manufacture of condensers and low-pressure heaters. These alloys have higher thermal conductivity as compared to steel that reduces size of heat transfer surface. In addition, copper-based alloys (e.g. brass) are more technologically effective than steel [4].

Corrosion failures of brass condenser tubes are mainly in a form of dezincing and .corrosion cracking [5-7].

Brass corrosion products (copper and zinc oxides) go to water. This intensifies corrosion of carbon steel. Corrosion of copper-based alloys is

influenced by different factors: temperature, oxygen concentration, chloride, sulfate, organics etc. Recently a lot of attention in utility industry has been attributed to the effect of organic impurities (acetic, formic, and lactic acids, in particular) on corrosion of construction materials. Of abovementioned acids, concentration of acetic acid in the power plant cycle is significantly higher. Therefore, it is important to study the effect of acetic acid on brass corrosion rate and release of copper corrosion products from metal surface.

Recently a lot of attention in utility industry has been attributed to the effect of organic impurities on corrosion of construction materials. Negative impact of organic acids (acetic and formic acids, in particular) on corrosion of water-steam cycle at power plants is mentioned [8-10]. Elevated levels of these acids in the cycle could result in intensification of corrosion processes in condensers, turbines, and other power plant equipment.

There is a limited amount of data on the effect of organic acids on brass corrosion rate. Corrosion depends not only on composition of impurities in water, but also on its temperature. Maximum corrosion rate at different flow velocities of water to be at temperature of about 80°C [11]. It was also found that flow velocity of water being in contact with copper also exerts an effect on corrosion. Increase in flow velocity from 0.5 to 4.0 m/s at temperature of 10-27°C resulted in about. 6 times increase in copper corrosion rate. In the temperature range of 10-43°C the effect of flow velocity on corrosion rate was insignificant[12] .Therefore, the basic aim of this work was to study the effect of acetic acid on corrosion of brass at different temperatures up to 100°C. This temperature range from 25 to 100°C is typical for operation of condensers and LP heaters equipped with copper-based tubes.

MATERIALS AND METHODS

Specimens of α-brass(70-30), was prepared from cutting a rode by dimensions of 2 cm length and diameter of 1 cm. they have been grinded and polished with SiC papers and washed with water and alcohol . XRF test was performed for specimens as in table (1), after that they were weighed with a balance of four digits. They were immersed in beakers containing acetic acid with different concentrations (0.5-2.5 M) for ten days. Specimens were removed from the beakers for optical microstructure test were performed as shown in figure (1), and after that they weighed. The corrosion rate (Wcorr), (g/m^2.day), was calculated with the following equation [13]:

$$W_{corr} = \frac{\Delta m}{S \cdot \tau}$$

..... (1)

where Δm is the difference in specimen's weight before and after the test, g; S is the area of the specimen, m^2. τ is the time of specimen exposure in the test solution, days. Figure(2) illustrates this relation. During the tests portion of copper corrosion products was released from the specimens to the test solution. Determination of copper concentration in the solution provided estimation of copper release rate from the brass surface in (25 ^{o} C) using AAS device as shown in figure (3). To find the effect of temperature on corrosion rate, the corrosion rate of (2.5 M) specimen was tested in different temperatures (25,50,75,100 ^{o} C) as in figure (4). The tensile tests were performed using instron universal machine made in germen. The experiment results can be seen in table (2), and figure (5). Dry sliding wear tests of brass specimens were carried out using pin on disc apparatus. The pins were slid against a hardened steel disc with a hardness of HRC60, within a load range of (5,10,15N) and at a constant sliding velocity of (2m/s).The results were prepared in figure (6), and within variation of speeds with (10N) load as illustrated in figure (7).

Table -1: XRF analysis for used brass

The element	Cu	Zn	Fe	Si	C	Ca
The percentage	69.2	27.4	0.12	2.2	1.54	0.5

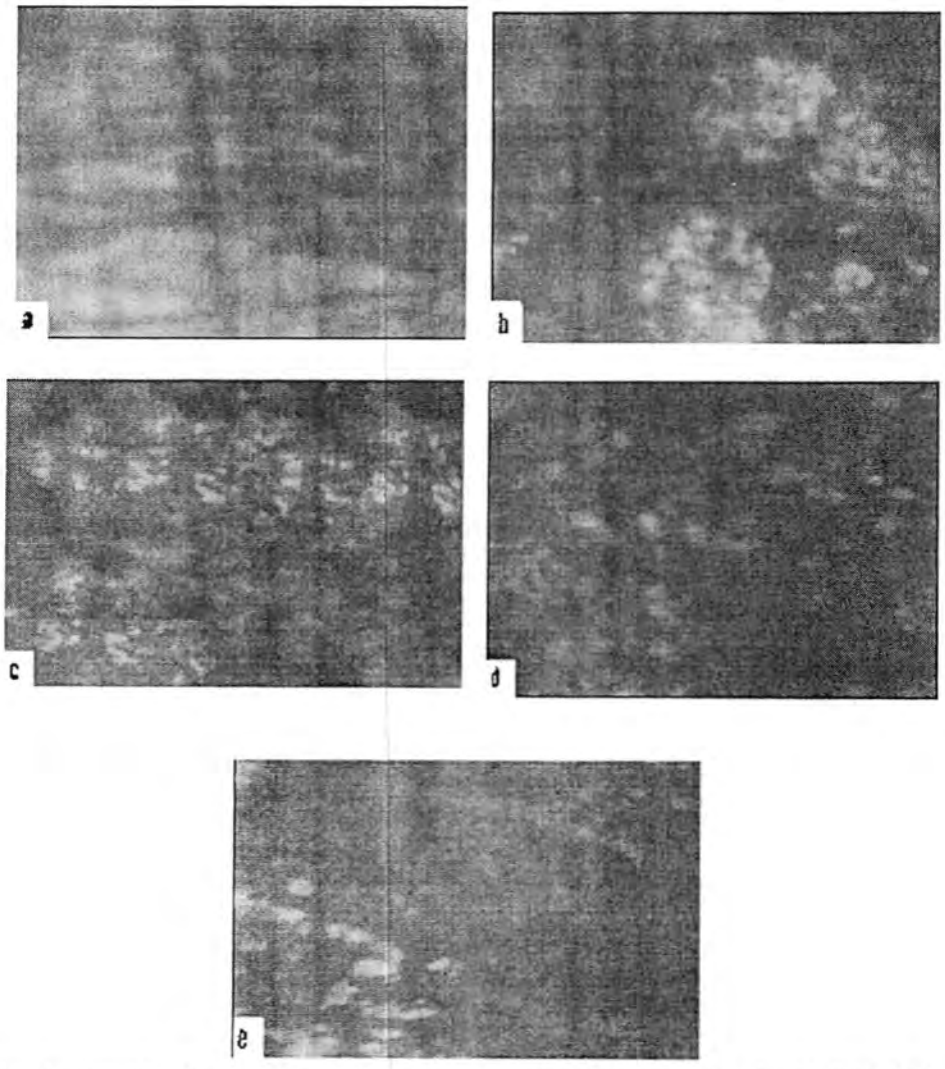

Fig -1: samples that treated with acetic acid (300 X) (a) As Received, (b)0.5M, (c)1M, (d)1.5M, (e)2M

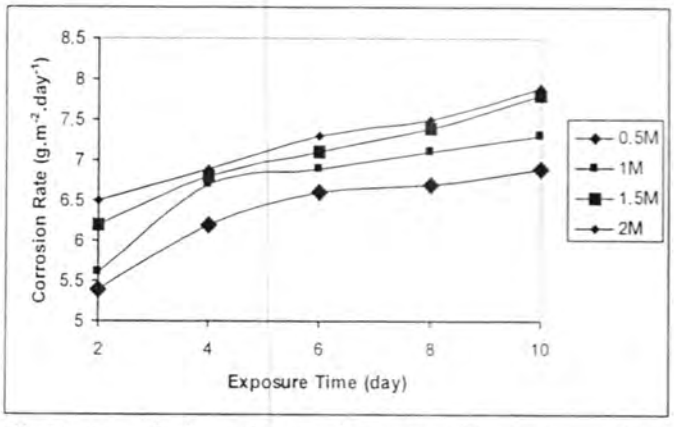

Fig -2: illustrate variation of corrosion rate with exposure time.

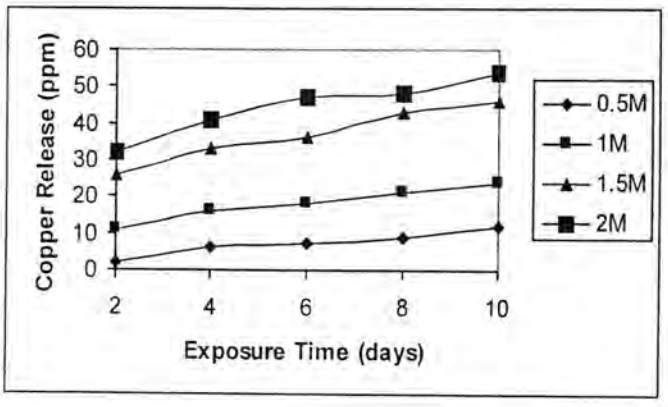

Fig-3 : variation of copper release versus exposure time with different acetic concentrations.

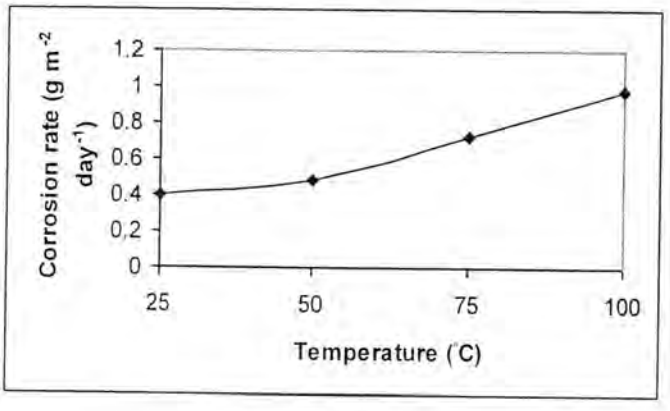

Fig -4: illustrate variation of corrosion rate with temperature for (2M) concentration

Table -2: Mechanical properties values for samples treated with acetic acid for ten days

Concentration (M)	U.T.S (MPa)	Yield Strength (MPa)	Fracture Toughness (MPa)	Elongation (%)	Elasticity (MPa)
Sample as Received	353	322	305	42	1065.62
0.5	305	303	295	47.7	1046.76
1	307	292	286	47.6	1018.05
1.5	291	284	271	47	974.45
2	279	268.5	260	43	1019.92

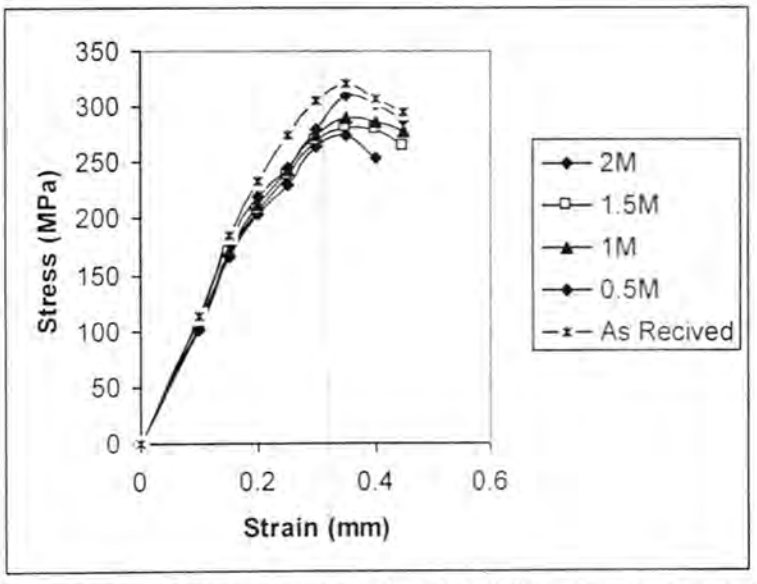

Fig-5 : variation of stress versus strain with different acetic concentrations

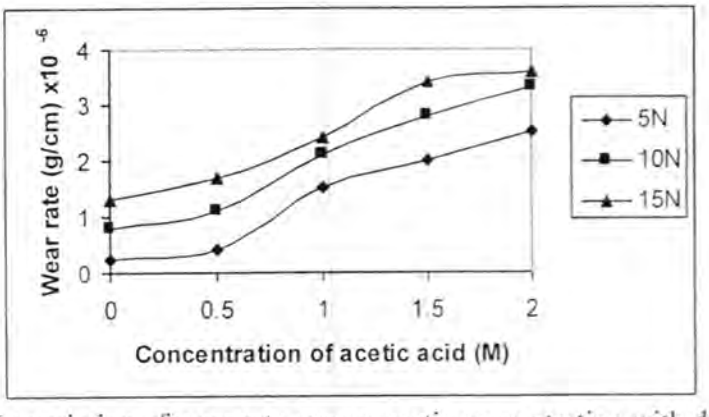

Fig.-6 : variation of wear rate versus acetic concentration with different loads, at (2m/s) speed.

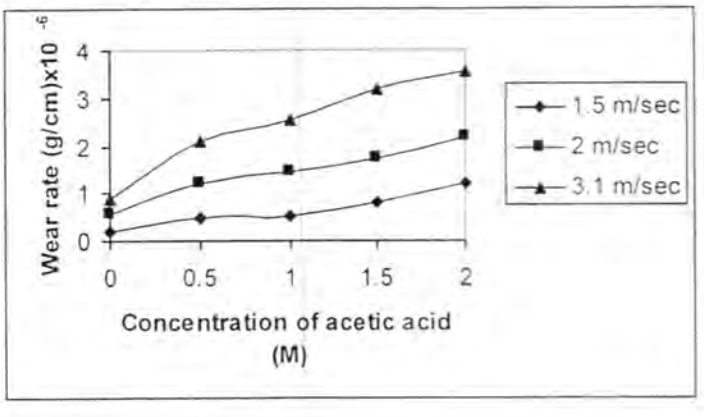

Fig.(7) : variation of wear rate versus acetic concentration with different wearing speeds at (10N) load.

CONCLUSIONS

1- Higher levels of acetic acid increased corrosion rate and release rate of copper from brass surface over studied temperature (25 o C).
2- Brass corrosion rate increases with increasing the temperature rate and the exposure time.
3- The microstructure patterns illustrates different composition of copper and zinc oxides formed on surface obviously at concentrations > 1 M .
4- It is noted that a decreasing of ultimate tensile stress for specimens which immersed in acid medium compared with as received specimen, and will be more decreased when the concentration be in high levels.
5- The wear rate for specimens which immersed in acid medium is higher compared with as received specimen, and will increased gradually with acid concentrations.
6- Increasing sliding speed will increase wear rate for specimens which immersed in acid medium compared with as received specimen, and this effect will be more obvious when the concentration will be higher.

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تعليمات النشر لمجلة علوم المستنصرية

مجلة علوم المستنصرية

هي مجلة علمية رصينة تصدر عن عمادة كلية العلوم في الجامعة المستنصرية في تخصصات الكيمياء والفيزياء وعلوم الحياة وعلوم الحاسبات وعلوم الجو. تقوم المجلة بنشر البحوث العلمية التي لم يسبق نشرها في مكان آخر بعد إخضاعها للتقويم العلمي من قبل مختصين وباللغتين العربية او الانكليزية وتُصدر المجلة عددين سنوياً بكلا اللغتين.

تعليمات النشر في المجلة

1. يقدم الباحث طلبا تحريريا لنشر البحث في المجلة ويكون مرفقا بأربع نسخ من البحث مطبوعة على ورق ابيض قياس (A4, 21.6×27.9 cm) مع ترك حاشية بمسافة انج واحد لكل اطراف الصفحة ومطبوعة بأستخدام برنامج (Microsoft Word, 97-2003) بصيغة (.doc).
2. يرفق مع البحث ملخص باللغة العربية وآخر باللغة الإنجليزية على ان لاتزيد كلمات الملخص عن (150) كلمة.
3. عدد صفحات البحث لاتتجاوز 10 صفحة بضمنها الاشكال والجداول على ان تكون الاحرف بقياس 14 نوع (Time New Roman) وبمسافة مزدوجة بين الاسطر. وينبغي ترتيب اجزاء البحث دون ترقيم وبالخط العريض (Bold) كالاتي: صفحة العنوان، الخلاصة باللغة العربية، الخلاصة باللغة الإنجليزية، مقدمة، المواد وطرائق العمل (الجزء العملي)، النتائج والمناقشة، الاستنتاجات وقائمة المراجع.
4. يطبع عنوان البحث واسماء الباحثين (كاملة) وعناوينهم باللغتين العربية والانكليزية على ورقة منفصلة شرط ان لاتكتب اسماء الباحثين وعناوينهم في أي مكان اخر من البحث ، وتعاد كتابة عنوان البحث فقط على الصفحة الاولى من البحث.
5. ترقم الجداول والأشكال على التوالي حسب ورودها في المخطوط، وتزود بعناوين، ويشار إلى كل منها بالتسلسل نفسه في متن البحث.
6. يشار الى المصدر برقم يوضع بين قوسين بمستوى السطر نفسه بعد الجملة مباشرة [1]، [2]، [3] وهكذا. تطبع المصادر على ورقة منفصلة ، ويستخدم الاسلوب الدولي المتعارف عليه عند ذكر مختصرات اسماء المجلات.
7. يتبع الاسلوب الاتي عند كتابة قائمة المصادر على الصفحة الاخيرة كالاتي: ترقيم المصادر حسب تسلسل ورودها في البحث ، يكتب الاسم الاخير (اللقب) للباحث او الباحثين ثم مختصر الاسمين الاولين فعنوان البحث ، مختصر اسم المجلة ، المجلد ، العدد ، الصفحات الاولى والاخيرة ، سنة نشر البحث. وفي حالة كون المصدر كتابا يكتب بعد اسم المؤلف او المؤلفين عنوان الكتاب ، الطبعة ، الصفحات ، سنة النشر ، المؤسسة الناشرة، الدولة مكان الطبع.
8. بخصوص اجور النشر يتم دفع مبلغ (50000) خمسون الف دينار عند تقديم البحث للنشر وهي غير قابلة للرد ومن ثم يدفع الباحث (25000) عشرون الف دينار اخرى عند قبول البحث للنشر.

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Vol. 23
No. 5
2012

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