Research Article **Open Access**

Thermodynamic and Kinetic Studies of Tiopronin Gold Nanoparticles Binding With Extracted DNA of Rheumatoid Arthritis

Sura A. AbdulSattar[1*], Noora B. Rashid[2], Dhia H.Hussain[1]
[1]Department of Chemistry, College of Medicine, Mustansiriyah University, IRAQ.
[2]Department of Chemistry, College of Science, University of Baghdad, IRAQ.
*Correspondent email: surasci@uomustansiriyah.edu.iq

A r t i c l e I n f o

Received
09/July/2017

Accepted
15/Sep./2017

Abstract

Nanomaterial can interact with biological systems in different way, using their surface functionalities and depending on the particle size, shape, and aggregation. The present work aims to characterize the binding of tiopronin gold nanoparticles with extracted DNA from blood patients with rheumatoid arthritis kinetically and thermodynamically. A total of 19 patients with rheumatoid arthritis attending Baghdad Teaching Hospital / Medical City were included in this study. Tiopronin monolayer-protected gold nanoparticles) were prepared by chemical methods and a partial purification of DNA extracted was preceded using negative ions exchange column chromatography. Characterization of Tio Au NPs binding with DNA was investigated by using TEM, SEM, FTIR, EDX, and UV-VIS techniques. The results indicated that the average size of Tio Au NPs was 48nm., and there is a different binding affinity of DNA to tiopronin gold nanoparticles depending on pH. Also the melting temperature of DNA was decreased $5C^0$ when binding with tiopronin gold nanoparticles. Thermodynamic studies indicated that the reaction was endothermic, less order, and non-spontaneous.

Keywords: DNA; Tiopronin; gold nanoparticles; SEM, and EDX.

الخلاصة

تتفاعل المواد النانوية مع النظم البيولوجية بطرق مختلفة، وذلك باستخدام وظائفها السطحية واعتمادا على حجم الجسيمات ،الشكل والتجميع. يهدف العمل الحالي الى توصيف ارتباط دقائق الذهب النانوية تيوبرونين مع الحمض النووي المستخلص من الدم الكلي لمرضى التهاب المفاصل الروثوي حركيا وثرموديناميكيا تضمنت هذه الدراسة 19 عينة لمصابين بالتهاب المفاصل الرثوي من مستشفى بغداد التعليمي / مدينة الطب. تم تحضير تيوبرونين أحادي الطبقة الذهب النانوية المحميةبلطريقة كيميائية وتنقية الحمض النووي المستخلص جزئيا باستخدام عمود المبادل الأيونات السالبة. تم توصيف ارتباط دقائق الذهب النانوية المغلفة بالتيوبرونين مع الحمض النووي باستخدام TEM, SEM, FTIR, EDX والأشعة فوق البنفسجية. وأظهرت النتائج أن متوسط حجم الدقائق النانوية كان 48 نانومتر، وهناك تقارب ملزم مختلف من الحمض النووي لتيوبرونين الذهب النانوية اعتمادا على الرقم الهيدروجيني. كما انخفضت درجة حرارة انصهار الحمض النووي 5م^{o} عند الارتباط مع الجسيمات النانوية تيوبرونين الذهب. وأشارت النتائج ان الارتباط كان ماص للحرارة، وأقل رتبا وغير تلقائي.

Introduction

Nanotechnology allows scientists, engineers, chemists, and physicians to produce important advances in life sciences and health care through working at the molecular and cellular levels [1]. One of these is nanomedicine, which includes new interventions in disease detection, treatment and prevention [2]. Nanomaterial can interact with biological systems in different ways: by using their surface functionalities and depending on the particle size, shape, and aggregation. A few types of nanoparticles, such as liposomes, polymeric (albumin) and metallic (Au) have been approved for clinical trial as nanoparticle-based therapeutics [3]. Characterization of gold nanoparticles is significant to appreciate and control nanoparticles synthesis and applications. It is executed using a range of diverse techniques like scanning electron microscopy (SEM),

transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and UltraViolet–Visible spectroscopy (UV–Vis). These techniques are helpful to resolve diverse parameters such as: particle size, pore size, shape, crystallinity, surface area, and fractal dimensions. In addition, orientation, intercalation, and dispersion of nanoparticles are also considered [4].

In the last years, the studies of noncovalent interactions of DNA with ligands have received considerable attention caused by the large number of applications being derived from these interactions. One of these applications is the development of new diagnostic and therapeutic agents [5] [6].

Arthritis is a joints disease. The physiology of joints makes them very difficult targets for drug delivery in a manner that is specific and selective. Gene transfer is the only technology that can solve the delivery problem in a clinically reasonable fashion. There is an abundance of preclinical data confirming that genes can be efficiently transferred to tissues within joints by intra-articular injection using a variety of different vectors in conjunction with ex-vivo and in-vivo strategies [7]. Several different approaches are hopeful in this regard, including gene transfer to the synovial lining cells of individual joints and to extra-articular locations [8]. This work aims to study the effect of DNA concentration, pH, and temperature on binding of tiopronin gold nanoparticles with DNA extracted from whole blood of rheumatoid arthritis patients.

Experimental Subject

A total of 19 patients with rheumatoid arthritis attending Baghdad Teaching Hospital/Medical City are included in the study. General information of each patient such as: age, sex, duration of illness, and therapy were recorded. Sixteen matches of healthy individuals with age and sex were included in the present study as a control.

Blood Samples:

Five milliliters from venous blood were collected for patients and healthy controls, then immediately transferred into EDTA tube and kept in a cool place until used.

DNA Extraction

A simple, rapid method for extraction of human DNA has been used. One milliliter of EDTA-treated blood was mixed with Tris buffer and centrifuged to yield nuclear fraction. The nuclear pellet is treated with sodium dodecyl sulfate/urea and phenol/ chloroform to remove contamination protein [9].

DNA Purification by Ion Exchange Chromatography

A partial purification of DNA extracted from rheumatoid arthritis patients and control was proceeded using negative ions exchange Colum chromatography (1.3 × 6.5 cm) (Di Ethyl Amino Ethyl (DEAE) Sephadex G-25 [10].

Preparation of Tiopronin Gold Nanoparticles

Tiopronin monolayer-protected gold nano-particles (Tio Au Nps) were prepared by chemical methods using the procedure of Temple *et al* [11].

DNA Binding With Tiopronin Gold Nanoparticles

The binding of gold nanoparticles capped with N-(2-mercaptopropionyl) glycine (Tio Au Nps) with double-stranded DNA extracted from blood of patient with rheumatoid arthritis was carried out by mixed them for at least 3 min.

Kinetic Studies of DNA Binding with Tiopronin Gold Nanoparticles

Effect of DNA Concentration: A different concentrations of DNA were used in order to study the effect of DNA concentration on binding with tiopronin gold nanoparticles (Tio Au NPs) by using different volume ratios of DNA (26.83×10-4M) /TioAuNPs (5.734×10-3M) [(1/30), (2/30), (4/30), (10/30)and (30/30)] v/v [12].

Effect of pH: A different pH values (2, 4, 6, 8, and10) of DNA/TioAuNPs solution were tested with increase concentration of DNA, and then the absorbance spectra by UV-Vis was measured to get a point intersection [13].

Effect of temperature: A different temperature (30, 40, 50, 60, 70, 80, and 90^{o}C) was used to study the effects of the temperature on DNA melting in presence and absence of tiopronin gold nanoparticles (Tio Au NPs) [14].

Characterization of DNA binding with Tiopronin Gold Nanoparticles

Scanning Electron Microscopy (SEM): A droplet of solution (TioAuNPs) was deposited on glass cover slid (2×2) cm2 to dry the sample before scanning, it should be used furnace (at low temperature) to accelerate the drying process [15].

Energy Dispersive X-Ray Analysis (EDX): Energy Dispersive X-Ray Analysis (EDX) is generally coupled with either an SEM or a TEM. During EDX analysis, the sample is bombarded with an electron beam inside the electron microscope and the bombarding electrons collide with the sample atom, freeing them in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher energy electron from an outer shell but for this to happen; the transferring outer electron must give up some of its energy by emitting an X-ray,EDX was carried out using a Hitachi SU70 Analytical SEM. It is equipped with an Oxford Instruments 50mm2 X-Max silicon drift EDS detector [16].

Fourier Transform Infrared spectroscopy (FTIR): A droplet of solution is deposited on glass cover to dry sample before measurement; it should be left over night in dust protected environment [17].

UV-VIS: UV-Visible plasma absorption measurements were carried out at room temperature on Shimadzu 1800 UV-Visible spectrophotometer using a quartz cell with 1cm path length and Lambda 40, (Perkin Elmer, USA) in the wavelength range of 200-800 nm. The deionized water was used as the blank [18].

Fluorescence Measurements: Fluorescence characterization of the (AuNPs/DNA, Au NPs) solutions was performed using a FluoroMax-2 JOBAN YVON SPEX, Instruments S.A., Inc., France. The fluorescence measurements were also made over the wavelength range of 250-900 nm using 1 cm path length quartz cuvettes, which were cleaned before each use by sonicating them for 5 min in distill water (DW) and then rinsing with distill water [4].

Thermodynamic Studies of DNA binding with Tio Au NPs: Thermodynamic parameters of DNA binding with Tio Au NPs were obtained by plotting (1/T vs. Lin A) using Equation 1 (Arrhenius equation) [19].

$$Lin\ K = \frac{-Ea}{R} * \left(\frac{1}{T}\right) + Lin\ A \qquad (1)$$

From the plot, the slope is equal to Ea and Arrhenius constant is equal to the intercept:

Slope = -Ea/R Intercept = ln K

Enthalpy change ΔH was calculated from Equation 2 and the Gibbs free energy ΔG was calculated from the Equation 3. Change of entropy ΔS was calculated from the Equation 4.

$$\Delta H^* = Ea - RT \qquad (2)$$

$$\Delta G^* = -RT\ Lin\ A + RT\ Lin\ (KT/h) \qquad (3)$$

$$\Delta S^* = (\Delta H^* - \Delta G^*)/T \qquad (4)$$

Results and Discussion

Clinical Characterization

Nineteen patients with a diagnosis of rheumatoid arthritis were initially evaluated. Mean age was 46.2 years (± 12.66), ranging from 20 to 68 years. Female gender (17 patients, 89.47%).The mean duration of illness was 9.84 years (±8.37) ranging from 1 to 31 years. The following laboratorial were diagnosed: ESR (mm/1hr) 72.84 (±22.15), RF (IU/ml) 32.52(±9.46), CRP (mg/l) 49.15(±31.87) and ASOT (IU/ml) 22(±8.19). Table 1 summarizes the characteristics of 19 patients analyzed in the initial evaluation.

Table 1: Clinical characterization of patients with rheumatoid arthritis.

Parameter	Min	Max	Mean	±SD
ESR(mm/1hr)	35	108	72.84	22.15
RF(IU/ml)	21	55	32.52	9.46
CRP (mg/l)	7	110	49.15	31.87
ASOT(IU/ml)	10	43	22	8.18

Deoxyribonucleic acid Extraction and Purification

The Deoxyribonucleic acid was extracted from whole blood of control and patients with rheumatoid arthritis by mixed 5ml of whole blood pool with lysis buffer to lyse the cells then the fraction was suspended in a buffer containing SDS and urea. The DNA was extracted twice with phenol/chloroform to remove most of proteins. The extracted DNA was purified partially by using Sephadex G-25 Colum to remove the contamination proteins. The concentrations of DNA were determined by measuring the absorbance at 260 nm using biodrop spectrophotometer. Meanwhile the ratios of A_{260}/A_{280} were determined to evaluate the protein contamination, see Table 2.

The results of the present study indicated that the purity of DNA of control and patients with rheumatoid arthritis were 1.818 and1.841 respectively these results were in agreement with the general rule that any preparation of DNA with A_{260}/A_{280} greater than 1.7 is called pure [20].

Characterization of tiopronin gold nanoparticles binding with DNA Scanning Electron Microscopy (SEM)

Figures 1 and 2 show the SEM images of Au-tiopronin nanoparticles binding with different amounts of DNA it is very clear that Au-tiopronin has good ability to activate aggregation after binding with DNA for both control and patient for rheumatoid arthritis and it is clear from these images that gold nanoparticles induce a compaction of DNA molecules. It can also be seen in the figure that the degree of compaction increases with the concentration of nanoparticles. As shown in this figures it was found that DNA for control differ than that for patients in their ability to aggregate and that was due to genetic differences between them where can be expected that the tiopronin nanoparticles binding occur principally through the formation of stable hydrogen bonding between the hydrophilic groups of the tiopronin and the DNA bases [12].

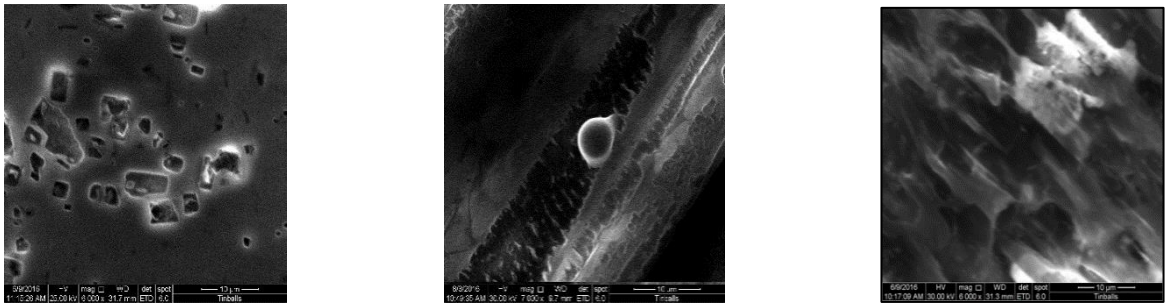
Energy Dispersive X-Ray Analysis (EDX)

In Figure 3, DNA patient with rheumatoid arthritis was found. Wt% for C, N and O were 33.32%, 30.73% and 19.96% respectively. While for control (Figure 2) EDX analysis shows Wt% of O, C, S and Au equal to 16.04%, 2.63%, 0.73% and 0.41%.The gold weight present was decrease after binding with DNA which was a normal result because the surface of the particles was completely coated with DNA and tiopronin [16].

Fourier Transform Infrared spectroscopy (FTIR): DNA molecule contains two kinds of potential nucleophilic sites which can function as hydrogen receptors: the exocyclic nitrogen or carbonyl oxygen of the purine and pyrimidine bases and the phosphate oxygen atoms. Each of these groups is capable of forming a hydrogen bond with a hydrogen donor molecule under specific chemical conditions. The molecular vibrations arising from these different moieties of DNA are observed in specific region of the IR spectrum [21]. The comparison between Figures 3 and 4 was observed that the band at$1589cm^{1}$, which due to the aromatic ring of DNA, is present on the Au- tiopronin nanoparticles binding with DNA IR spectra with shifting to $1627cm^{-1}$ which means that DNA was bonded with Au-tiopronin. C=O vibrational band ($1730cm^{-1}$) is visible only in the spectrum of Au-tiopronin and disappeared after binding with DNA, this confirm that the ligand make hydrogen bonds with DNA at C=O group.

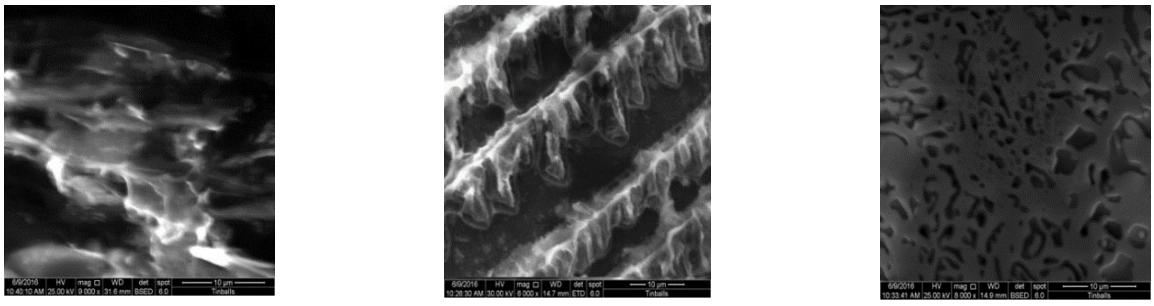
Table 2: Purity and Concentration of DNA extracted from whole blood of control and patients with rheumatoid arthritis.

Group		A_{260} nm	A_{280} nm	A_{260}/A_{280}	Concentration (µg/ml)
Control	Crud DNA	1.207	0.825	1.463	2260.1
	Partially purify DNA	2.651	1.458	1.818	1694.1
Patients with RA	Crud DNA	3.752	2.758	1.360	2303.7
	Partially purify DNA	1.989	1.080	1.841	1402.8



(a) (b) (c)

Figure 1: Scanning Electron Microscopy (SEM) (A) for DNA control =2.1×10-6 M, (B) [DNA] =2.7×10-6 M/[Tio Au NPs] =2.4×10-7 M (C) [DNA] =2.1×10-6 M/ [Tio Au NPs].



(a) (b) (c)

Figure 2: Scanning Electron Microscopy (SEM): (a) for DNA Patient with rheumatoid arthritis =2×10-6 M; (b) [DNA]= 2.5×10-6 M [Tio Au NPs]= 2.4×10-7 M; (c) [DNA]=2×10-6 M/Tio Au NPs]= 1×10-6M.

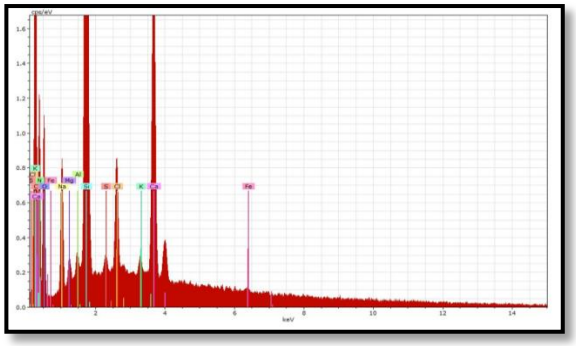


Figure 1: Energy Dispersive X-Ray Analysis (EDX) for DNA patient for rheumatoid arthritis.

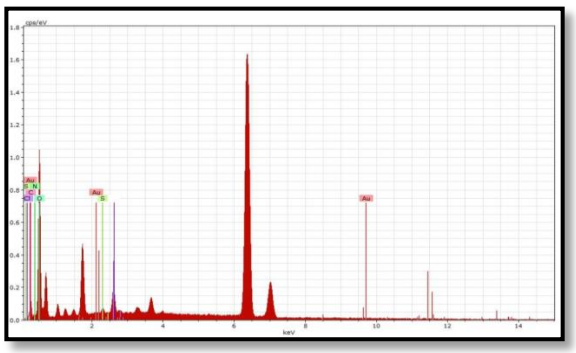


Figure 2: Energy Dispersive X-Ray Analysis (EDX) for DNA binding with Tio Au NPs.

Fluorescence Measurements: It is well known that small gold nanoparticles are able to emit fluorescence [22]. The change on the fluorescence spectra with increasing the amount of DNA which added to a solution containing a fixed gold nanoparticles concentration was observed [12] [22]. In previous studies of the emission from (Tio Au NPs), it was reported that the luminescence was (λ_{em}=770) for crystalline size (1.8nm) and (λ_{em}=656) for crystalline size (1.4nm), also they observed that the luminescence maximum shifts to lower energy with increasing core size [21]. The results of the present study presented. Figure 5 indicates that λ_{em} was (700nm) which means that the effective crystalline size of (Tio Au NPs) was about (1.6nm). Figure 6 and Figure 7 show the intensity of emission which was modified by the presence of DNA where the intensity was increased and shifted to lower energy. That means, the electronic levels of Au were closed to each other with the addition of DNA.

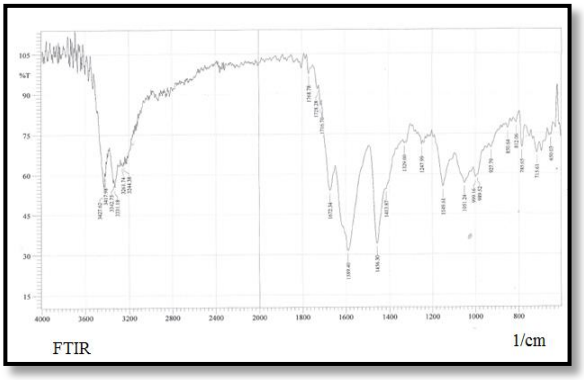


Figure 3: Fourier Transform Infrared spectroscopy (FTIR) for DNA.

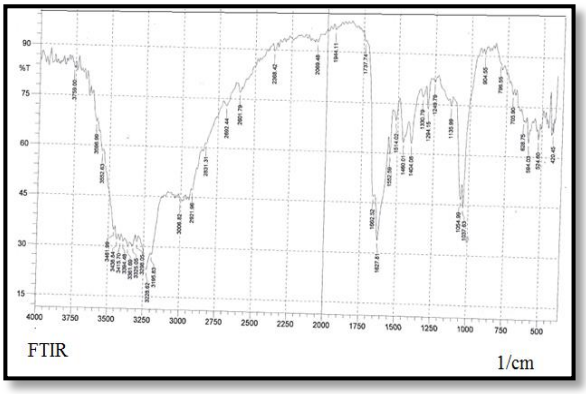


Figure 4: Fourier Transform Infrared spectroscopy (FTIR) for DNA binding with Tio Au NPs.

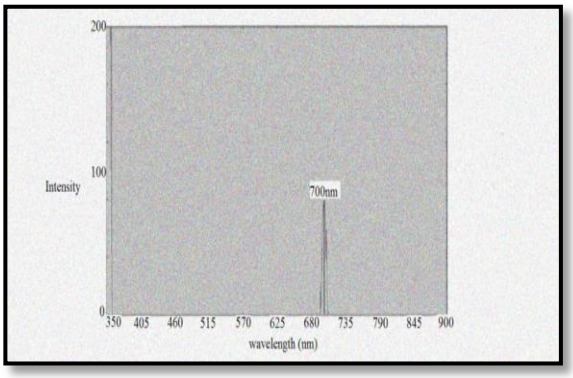


Figure 5: Fluorescence spectra of Tio Au NPs.

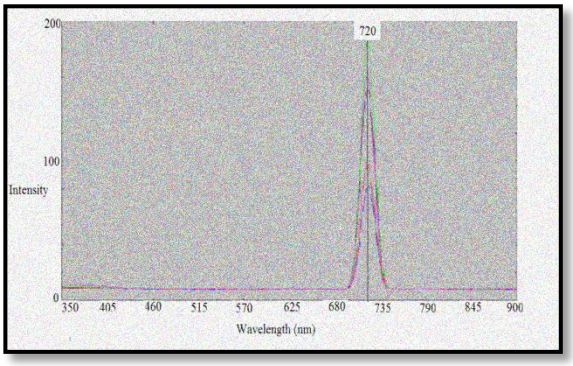


Figure 6: Fluorescence spectra of Tio Au NPs/DNA for Control.

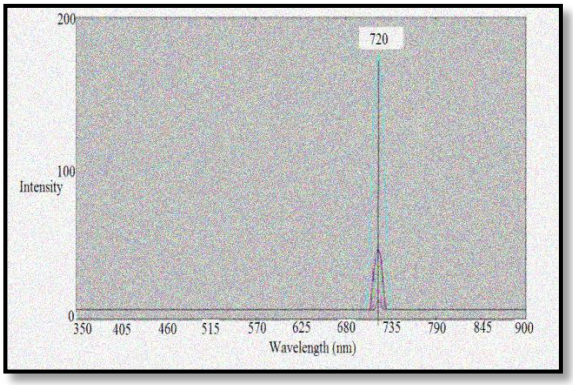


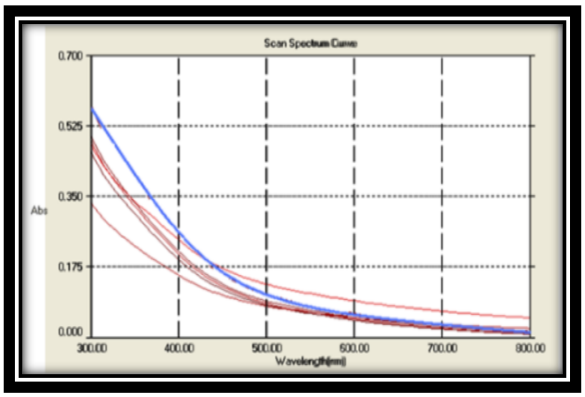
Figure 7: Fluorescence spectra of Tio Au NPs/DNA for Patient of rheumatoid arthritis.

Kinetic Studies

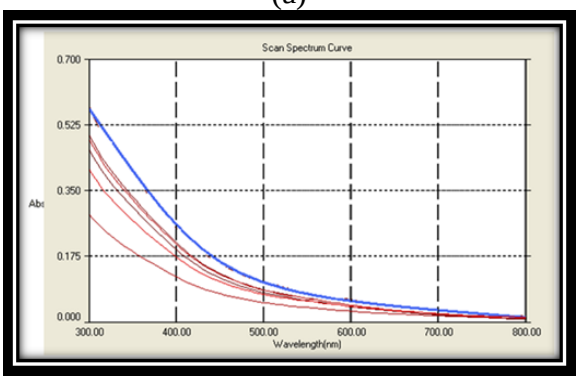
Effect of pH: The strength of the DNA interaction with Tio Au NPs could be modified as a function of the media pH [14].UV-Visible spectra was used to characterize the binding of Tio Au NPs with DNA in different ratios of their volume (v/v) [(1/30), (2/30), (4/30), (10/30) and (30/30)] at different pH values (2, 4, 6, 8, and 10). Figure 8a) shows that spectra are modified upon the binding of DNA and the isobestic range are (590-700) which give a support to the DNA binding with Tio Au NPs. The isosbestic range of DNA binding with Tio Au NPs at pH= 2, 4, 6, 8, and 10 for both patients and control were presented in Table 3.

Table 3: pH region binding of DNA with TioAuNPs for control and patients with rheumatoid arthritis.

pH Value	Control	Patients
2	500-700	500-600
4	400-680	380-700
6	400-710	460-700
8	480-680	470-680
10	415-730	480-710



(a)



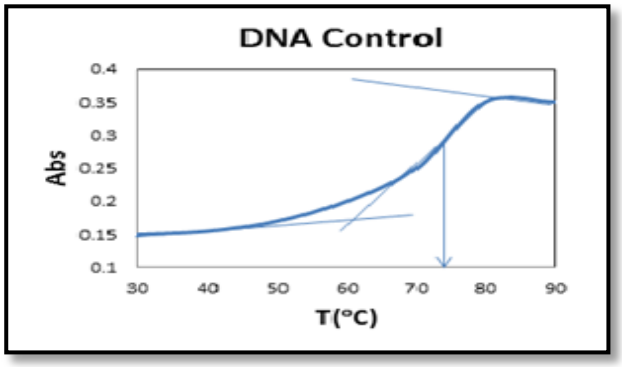
(b)

Figure 8: UV-Visible spectra for DNA binding with Tio Au NPs at pH=2. (a) Patient with rheumatoid arthritis. (b) Control.

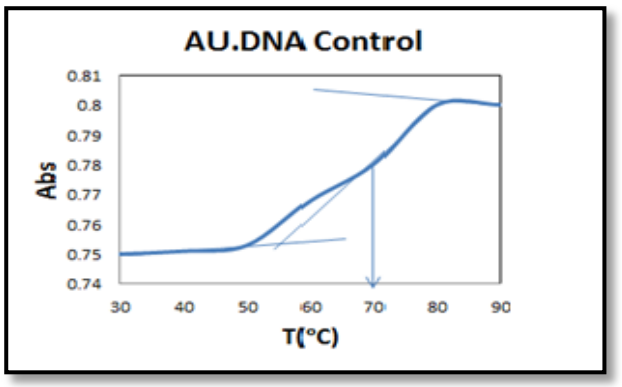
Effect of temperature: DNA melting is an important factor that can be used to distinguish between the molecules depending on their binding type, where the intercalation of small molecules into the double helix of DNA is caused an increase of the helix melting temperature which stabilizing the double helix structure of DNA, while there is no increase of $T_{m\ in}$ non-intercalative binding [23]. Figure 8 shows the melting curve of DNA for both control and patient with rheumatoid arthritis in the absence and presence of AuNPs. DNA in aqueous solution has a melting temperature of $75^{O}C$ but DNA patient of rheumatoid arthritis is $70^{O}C$, (Figure 9A and C). From (Figure 9B and D), it is obvious that DNA became unstable in the presence of gold nanoparticle where, T_m decreased $5^{O}C$. These decreases are presumably due to the binding of gold nanoparticles with DNA and the high affinity of mononucleotides and polynucleotides for small gold nanoparticles, the result is agreement with [24].

Thermodynamic study of Tio Au NPs binding with DNA

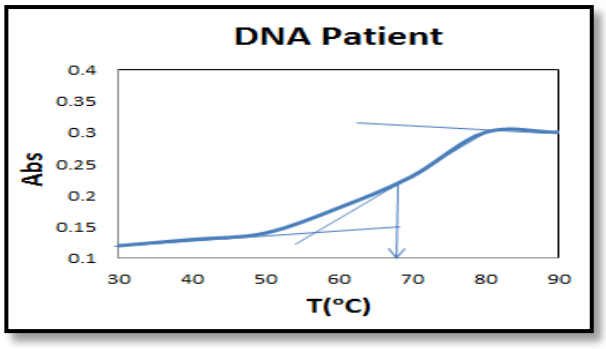
The thermodynamic parameters of the transitional state were determined by using Arrhenius equation, which connects between speeds constant K and temperature inverse in Kelvin. The resulted plot of Ln K against 1/T gives a straight line with slope equals (-Ea/R) as shown in Figure 10. The ΔH*, ΔG*, and ΔS* were calculated. The thermodynamic parameters of the transitional state are illustrated. It is noticed from Table 4 that (ΔH*) value in patient of rheumatoid arthritis is a positive value, which indicates that formation of DNA is an endothermic reaction. The positive value of (ΔS*) refers to the inactive complex structure, which is less order than the reactors structure. (ΔG*) have positive value in the studied group which indicates that the formation of activated complex is a non-spontaneous process.



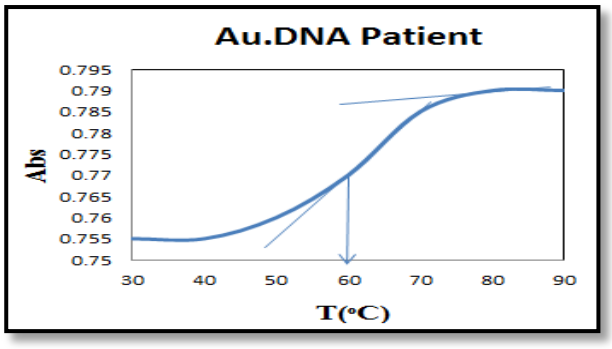
(a)



(b)



(c)



(d)

Figure 9: The melting curves of calf thymus DNA, [DNA] =5×10-5M. (A) and (C) in the absence of gold nanoparticles (B) and (D) in the presence of gold nanoparticles, [Au NPs] =1×10-6M

Table 4: Thermodynamic parameter of patient with rheumatoid Arthritis.

(t) ^{o}C	T(K)	Ea (kJ/mol.)	ΔH*(kJ/mol.)	ΔG*(kJ/mol.)	ΔS*(j/mol.K)
40	313	62.578	59.975	23.629	116.12
50	323	62.578	59.892	24.468	109.67
60	333	62.578	59.809	25.310	103.60
70	343	62.578	59.726	26.155	97.87
80	353	62.578	59.643	27.002	92.46

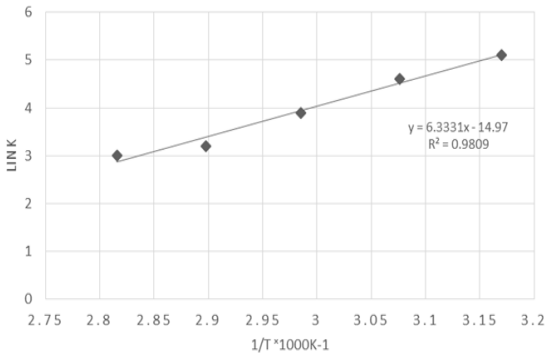


Figure 10: Arrhenius plot of patient with rheumatoid arthritis.

Conclusions

The interaction of tiopronin gold nanoparticles with DNA must be investigated by using different techniques such as FTIR-spectroscopy, TEM, SEM, UV-visible and EDX. The reaction was endothermic, less order and non-spontaneous.

References

[1] Dubchak S., Ogar A., Mietelski J. W. and Turnau K., "Influence of silver and titanium nanoparticles on arbuscular mycorrhiza colonization and accumulation of radiocaesium in Helianthus annuus.," *Spanish Journal of Agricultural Research.*, vol. 8, no. 51, 2010.

[2] Rees M., and Moghimi SM., "Nanotechnology: From fundamental concepts to clinical applications for healthy aging," *NanomedNanotechnolBiol Med*, vol. 8, no. Suppl 1, pp. S1-4., 2012.

[3] Sanhai WR., Sakamoto JH., Canady R., Ferrari M., "Seven challenges for nanomedicine,"*Nature nanotechnology*, vol. 3, no. 242-244, 2008.

[4] Huang X., Jain P.K., El-Sayed I.H., El-Sayed M.A., "Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy.Nanomed,," *Nanomed.*, vol. 2, no. 5, pp. 681-693, 2007.

[5] Scott Vandiver M., Page Bridges E., Koon RL. et al., "Effect of ancillary ligands on the DNA interaction of [Cr (diimine) 3] 3+ complexes containing the intercalating dipyridophenazine ligand.," *Inorganic Chemistry*, vol. 49, no. 3, p. 839–848., 2010.

[6] Dykman L.A., Khlebtsov N.G., "Gold nanoparticles in biology and medicine: recent advances and prospects. Acta Naturae," *Acta Naturae*, vol. 3, no. 2, pp.

34-55, 2011.

[7] Evans CH, Ghivizzani SC., Robbins PD., "Arthritis gene therapy and its tortuous path into the clinic," *Trans Res.*, vol. 161, no. 4, pp. 205-216., 2013.

[8] Gouze E., Ghivizzani SC., Palmer GD., Gouze JN., Robbins PD., Evans CH., "Gene therapy for rheumatoid arthritis," *Expert OpinBiolTher.*, vol. 1, no. 6, pp. 971-978, 2001.

[9] Adell K.& Ogbonna G., "Rapid Purification Of Human DNA From Whole Blood For Potential Application InClinical Chemistry Laboratories.," *Clin.Chem.*, vol. 36, no. 2, pp. 261-264, 1990.

[10] M. DW., "Separation of tissue and serum acid phosphatase isoenzymes by ion-exchange column chromatography.," *Clin Chem.*, vol. 23, no. 4, pp. 653-8, 1977.

[11] Templeton AC;Chen S; Gross SM;Murray RW., "Water-soluble, isolable gold clusters protected by tiopronin and coenzyme amonolayers.," *Langmuir*, vol. 15, pp. 66-76, 1999.

[12] Prado-Gotor R. & Grueso E., "Noncovalent Interactions of Tiopronin-Protected Gold Nanoparticles with DNA: Two Methods to Quantify Free Energy of Binding," *ScientificWorldJourna*, vol. 22, p. 143645., 2014.

[13] Sun L.,.Zhang Z., Wang S., Zhang J., Li H.,Ren L., Weng J., Zhang Q., "Effect of pH on the Interaction of Gold Nanoparticles with DNA and Application in the Detection of Human p53 Gene Mutation.," *Nanoscale Res Lett.*, vol. 4, no. 3, pp. :216-220., 2008.

[14] Prado-Gotor R.& Grueso E., "A kinetic study of the interaction of DNA with gold nanoparticles: mechanistic aspects of the interaction.," *Phys Chem Chem Phys.*, vol. 13, p. 1479–1489., 2011.

[15] Shanahan AE.,Sullivan JA;McNamara M;Byrne HJ., "Preparation and characterization of a composite of gold nanoparticles and single-walled carbon nanotubes and its potential for heterogeneous catalysis.," *New Carbon Materials,*, vol. 26, no. 5, pp. 347-355, 2011.

[16] Xu P., Xu J., He M., Song L., Chen D., Guo G., Dai H., "Morphology and chemical characteristics of micro- and Nano-particles in the haze in Beijing studied by XPS and TEM/EDX," *Total Environ*, vol. 15, no. 565, pp. 827-32, 2016.

[17] Ojea-Jimenez I.,Garcia-Fernandez L., Lorenzo J.,Puntes VF., "Facile Preparation of Cationic Gold Nanoparticle-Bioconjugates for Cell Penetration and Nuclear Targeting ACS," *Nano*, vol. 6, no. 9, p. 7692–7702, 2012.

[18] Behzadi S., Ghasemi F., Ghalkhani M.,Ashkarran AA., Akbari SM.,Pakpour S., Hormozi-Nezhad MR.,Jamshidi Z., Mirsadeghi S., Dinarvand R.,Atyabi F., Mahmoudi M., "Determination of nanoparticles using UV-Vis spectra," *Nanoscale*, vol. 17, no. 12, p. 5134, 2015.

[19] Rodriguez-Correa D., &Dahlberg AE., "Kinetic and thermodynamic studies of peptidyltransferase in ribosomes from the extreme thermophile Thermus thermophiles.," *RNA*, vol. 14, pp. 2314-2318, 2008.

[20] John AM., Brandon SC., Wayne RC., "Improving accuracy of cell and chromophore concentration measurements using optical density.," *BMC Biophys*, vol. 6, p. 4, 2013.

[21] Gustavo Jesus VZ.,Monica Maribel MM., Virginia SM., Raul Jacobo DM.,David Guillermo pI., Marlon RL., "FTIR Spectroscopic and Molecular Analysis during Differentiation of Pluripotent Stem Cells to Pancreatic Cells," Stem Cells Int., 2016.

[22] Anderson BJ.,LarkinC., Guja K., Schildbach JF., " Using fluorophore-labeled oligonucleotides to measure affinities of protein-DNAinteractions.,"

Methods Enzymol, vol. 450, pp. 253-72, 2008.

[23] Sun Y., Bi SH.,Song D., Qiao Ch., Mu D.,Zhang H., "Study on the interaction mechanism between DNA and the main active components in Scutellaria baicalensis Georgi," *Sensors and Actuators B: Chemical,* vol. 129, no. 2, p. 799–810, 2008.

[24] Yang J., Pong BK., .Lee JY &Too HP., "Dissociation of double-stranded DNA by small metal nanoparticles," *J. Inorg. Biochem.*, vol. 101, p. 824, 2007.