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Research Article

A Simple Spectrophotometric Evaluation Method of Allura Red (E129) in Several Food Samples Using Cloud Point Extraction

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Abstract

Cloud point extraction (CPE) method was successfully used for estimation and preconcentration of Allura red by spectrophotometry. The procedure was depended on the extraction of Allura red as an ion pair with cetylpyridinium chloride from aqueous solution using a nonionic surfactant TX-114 or Brij-35. The absorbance of the extracted surfactant-rich phase was measured at 507 nm after dilution with water. The influence of different factors on cloud point extraction (CPE) of Allura red such as pH media, equilibrium temperature, incubation time, concentration of surfactant and electrolyte concentration were investigated. Calibration curve was linear in a concentration range between 2-40 μgml^{-1} of Allura red with correlation coefficient r= 0.9998 for TX-114 and 0.9992 for Brij-35.The developed procedure was applied for estimation and preconcentration of Allura red in different food samples.

Keywords: AlluraRed,Spectrophotometric,Cloud Point Extraction, Food Samples.

الخلاصـة

تعتبر طريقة الاستخلاص بواسطة نقطة الغيمة من الطرق المهمة و المستخدمة في تقدير العديد من الصبغات الغذائية , حيث استخدمت هذه الطريقة في تقدير صبغة (Allura red) طيفيا في العديد من المنتجات الموجودة في الاسواق المحلية وذلك باستخدام مزيج من المنظفات اللاايونية والايونية الموجبة. تعتمد الطريقة على تكوين زوج ايوني(معقد) بين صبغة Allura red والمنظف الايوني الموجب في المحلول المائي . تم قياس الامتصاصية عند الطول الموجي 507 نانومتر، وتمت دراسة الظروف المثلى لهذه الطريقة مثل الزمن , درجة الحرارة , تركيز الملح , تركيز TX-114, تركيز CPC للحصول على افضل استخلاص . وجد ان استخدام TX-114 يعطي نتائج افضل من استخدامBrij-35 . تم رسم منحني المعايرة لصبغة Allura red ضمن مدى تركيز بين 2-40 مايكروغرام / مل ومعامل أرتباط 0.9998 مع TX-114 و 0.9992 مع Brij-35, وطبقت الطريقة لتقدير وتركيز صبغة Allura red في العينات المختلفة.

Introduction

Allura red (E129) and azo dye food have been widely used in soft drinks, beverages, ham, sausage and jelly. Allura reds include genetic toxicity and cause health risks [1] [2] [3] [4]. Allura red structure is shown in Fig.1. It is not recommended for consumption by children, therefore it is banned in Belgium, France, Denmark, Switzerland, Germany, Austria and Norway [3]. Generally, azo food contains azo group (N=N) and an aromatic ring which are harmful to human health [5]. For these reasons, new methods were found for the determination of azo food such as fluorimetry [6], ion pair HPLC [7], high-performance liquid chromatography (HPLC) [8], capillary electrophoresis [9] and spectrophotometry [10]. Cloud point extraction (CPE) has been used as one of the estimation methods in analytical chemistry due to its advantages, such as safety, fast and low cost. As well as, it is a simple method with high preconcentration and high recoveries factors. The CPE is an environment-friendly method which has received attention because the surfactants used are biodegradable, non-toxic and compatible with different analytical instruments [11] [12] [13]. In this work spectrophotometry analysis was chosen as the method to the evaluation of Allura red. Spectrophotometry analysis is inexpensive,

simple and fast [14]. A new CPE-Spectrophotometry method was used for the evaluation and preconcentration of Allura red using mixed of the nonionic surfactant (TritonX-114 or Brij-35) and cationic surfactant (cetylpyridinium chloride).

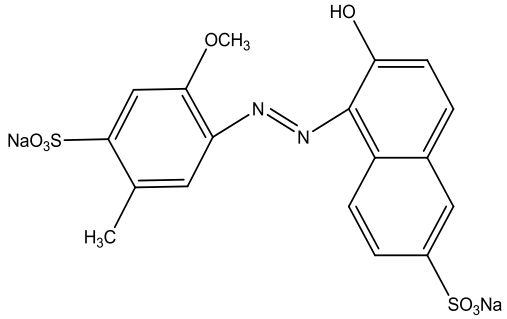

Figure 1: Chemical Structure of Allura red.

Materials and Methodology

Absorption spectra were recorded by cintra-5, UV-Vis Spectrophotometric (Australia) equipped with 1cm quartz cell. Metler pH meter was used to measure the pH values. A thermostat water bath (Human Lab model DWB-22, Korea) was used for the cloud point temperature.

The chemical reagents were used without further purification as received from various companies and distilled water was used for diluting the samples and reagents. A stock solution of 1000 µg ml^{-1} of allura red (sigma-aldrich) was prepared by dissolving 0.1 g of the allura red and diluting to 100ml in a volumetric flask. Daily, diluted solutions were prepared using stock solution. TritonX-114 or Brij-35 solution o.5M (sigma-aldrich) was prepared by dissolving 26.85 g for TX-114 and 59.5g for Brij-35 in 100 ml volumetric flask and completing to the mark with distilled water. A cetylpyridinium chloride (sigma-aldrich) 0.01 M was prepared by dissolving 0.358 g in water and diluting to 100 ml in the volumetric flask. Buffer solutions were prepared using HCl and sodium hydroxide solution and adjusting to desired pH by a pH meter. A 2 M of sodium chloride NaCl was prepared by dissolving 11.68 g of NaCl in 100 ml water.

An aliquot of Allura red solution was poured in a 10ml volume graduated test tube and mixed with 1.5 mL(0.5M) TX-100 or 2ml(0.5M) Brij-35 also 1 mL buffer solution pH=4, then add 0.5 ml (0.01M) Cetyl pyridinium chloride, 0.75 ml(2M) NaCl and Completed the volume with distilled water. The mixture was heated at (70 ^{0}C) in water bath about 30 min, then cooled in ice bath for 10 min for completely phases separation, then the watery upper layer was removed by decantation, whereas the cloud surfactant layer was diluted with distilled water up to 5 ml constant volume and measured the absorbance at λ_{max} 507 nm.

Results and Discussion

In recent years, a number of researchers have developed cloud point extraction to estimation and preconcentration a number of azo foods, to own several advantages such as inexpensive, safety and fast. In this work, Allura red (E129) using a mixture of the nonionic and cationic surfactant (TX-114+ CPC) and (Brij-35+CPC) respectively was studied. The results showed that using a mixture (TX-114+CPC) gives better extraction efficiency than the mixture (Brij-35+CPC). The absorption spectra of Allura red was recorded at the range of 400-800 nm and the maximum absorption (λ_{max}) obtained at 507 nm with the molar absorptivity of 10447.761 and 5472.636 L $mol^{-1}cm^{-1}$ for TX-114 and Brij-35 respectively. The influence of different factors on performance CPE method such as pH of the medium, salt, CPC concentration; TX-114 or Brij-35 concentration, equilibration temperature and time were studied.

For this study, the influence of the pH on a cloud point extraction of Allura red (E129) was investigated in the range of the pH (1-7) using hydrochloric acid and sodium hydroxide. The best absorbance of solution was obtained at pH 4, Figure 4. Different buffer solutions such as acetate, citrate and NaOH+HCl buffer solutions were examined and selected NaOH+HCl buffer since the absorbance did not changed after cloud point extraction.

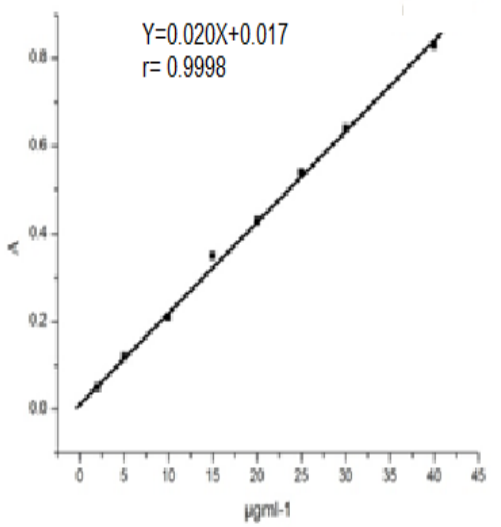

Figure 2: Calibration graph of E129 at concentration range 2-40µgml^{-1} in TX-114.

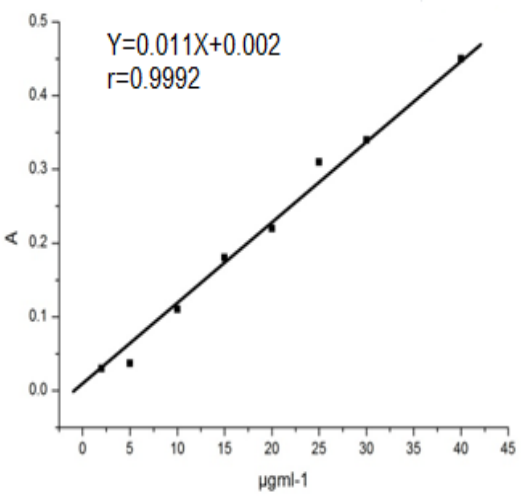

Figure 3: Calibration graph of E129 at concentration range 2-40µgml^{-1} in Brij-35.

Therefore, (1ml) of this pH4 buffer was added to the solutions for subsequent experiments.

The influence of nonionic surfactant (TX-114 or Brij-35) in the concentration range of 5×10^{-3} – 2×10^{-1} M on the CPE method was investigated. The difference of absorbance solution as a function of TX-114 and Brij-35 concentration is shown in Fig.5. It is found that the absorbance of the surfactant-rich phase was increased with the increasing of TX-114 and Brij-35 concentration. The results showed that the maximum efficiency was achieved when the concentration of TX-114 and Brij-35 was 7×10^{-2} and 1×10^{-1} M respectively, also using TX-114 in CPE give good extraction efficiency than Brij-35.

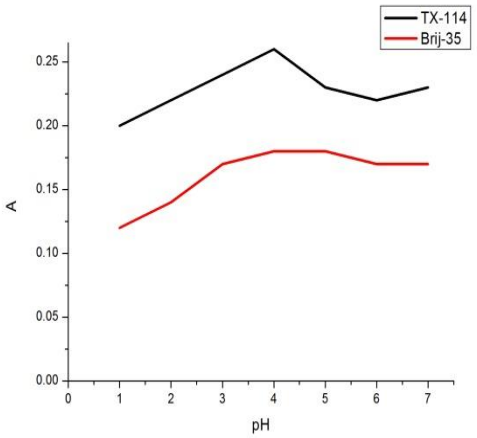

Figure 4: Effect of PH on the cloud point extraction of Allura red in the TX-114 and Brij-35.

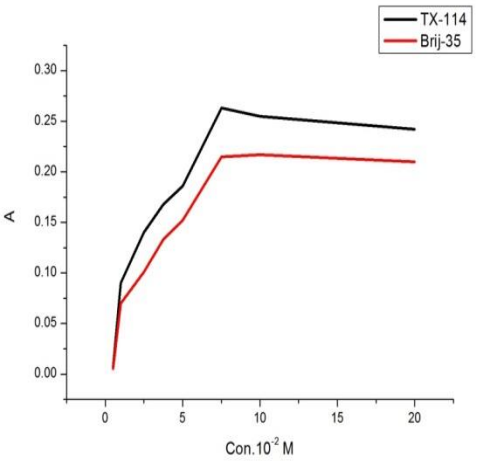

Figure 5: Effect of TX-114 and Brij-35 concentration on the CPE of Allura red.

A cetylpyridinium chloride (CPC) associated with Allura red (anionic dye) to form ion-associate complex and used in the CPE [15] . Therefore, the influence of CPC on the performance of CPE system was investigated in the range of concentration between 2×10^{-5}-1×10^{-3} M. Figure 6 was showed that the absorbance of the solution was increased up to CPC concentration of 5×10^{-4} M and will remain constant. Therefore, a 5×10^{-4} M of CPC was chosen as optimal concentration.

In CPE, adding of sodium chloride and surfactant to the mixture of aqueous solution has many advantages such as; change the

density of aqueous solutions, assist the phases separation, also increase the transfer of Allura red from the aqueous phase to surfactant phase due to the salting-out agent.

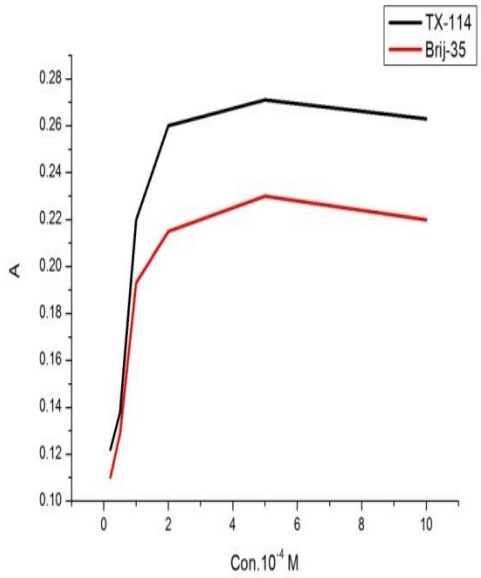

Figure.6: Effect of CPC on the cloud point extraction of Allura red.

However, the increasing in NaCl concentration showed high increasein absorbance which assists the extraction. The effect of NaCl concentration was showed that the absorbance was increased up to 0.15 M of NaCl, Figure 7, therefore, the 0.15M NaCl was chosen as optimal concentration.

The influence of equilibrium temperature on the performance of CPE was studied in the range between 10-80 ^{0}C .The results showed that the extraction of Allura red reaches maximum at above 65 ^{0}C. Also, an equilibrium temperature of 70^{0}C was selected for the analysis. As well as, the effect of an incubation time on performance CPE was investigated in the range 10-50 min. Fig. 8 showed the incubation time 30 min was chosen as optimum time.

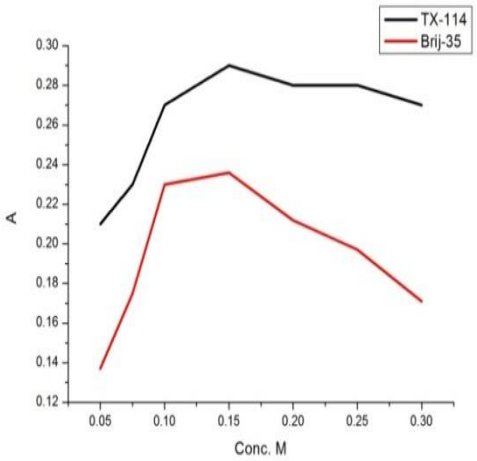

Figure 7: Effect of NaCl concentration on the CPE of Allura red.In CPE two very important factors are equilibration temperature and incubation time.

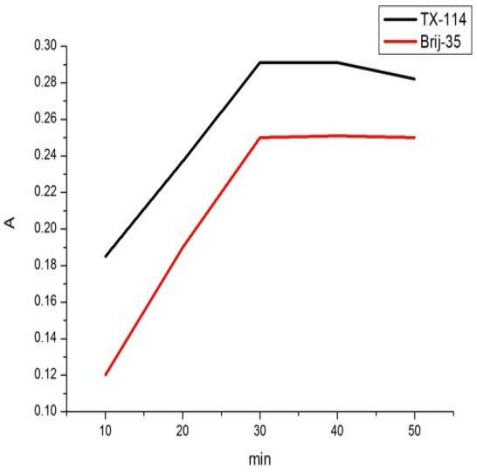

Figure 8: Effect of incubation time on the CPE of Allura red.

Allura red used in different candy and jelly samples that purchased from the local market. The results and analytical data in table1and 3 showed a good precision, accuracy and recoveries of Allura red after adding the known amount of standard solution of Allura red to the samples.

The calibration graph was obtained by using the general method for various concentrations of Allura red under the optimization conditions.The calibration graph was linear in the range between 2-40 µg ml^{-1} of Allura red in the aqueous solution.The equation of a regression was Y=0.02X+0.017 for TX-114 and Y=0.011X-0.002 for Brij-35, respectively . X is the concentration of Allura red in µgml^{-1} .

The correlation coefficient(r) was 0.9998 for TX-114 and 0.9992 for Brij-35.The detection limit of the procedure depend on the three times the standard deviation of the blank (3S) was 1.9 μgml^{-1} for TX-114 and 1.2 μgml^{-1} for Brij-35 of Allura red.

Table 1: Analytical data of the determination of Allura red by proposed cloud point extraction method.

Brij-35	**TritonX-114**	**Parameter**
507		λ_{max} nm
Y=0.011x-0.002	Y=0.020x+0.017	Equation
0.9992	0.9998	Correlation Coefficient(r)
2-40	2-40	Concentration range(μgml^{-1})
0.011	0.020	Slope
0.002	0.017	Intercept
5472.636	10447.761	Molar absorpitivity $L.mol^{-1}.cm^{-1}$
0.0907	0.0475	Sandells sensitvity
0.004	0.01	Standared deviation (S)
1.2	1.9	LOD(μgml^{-1})
3.6	5.0	LOQ(μgml^{-1})

Table 2: Comparison the values of LOD of the proposed method with different methods reported in literature.

Ref.	LOD µg ml^{-1}	Method
[3]	7.8	CPE
[2]	0.01	CPE
[16]	1.2	SPE
[17]	2.4	SPE
[18]	1.2	SPE
[19]	32	HPLC
This work	1.9,1.2	CPE

Table 3: Applicationof the proposed CPE method for the determination Allura red.

Sample	**TritonX-114**				**Brij-35**			
	E129 add (μgml^{-1})	E129 found (μgml^{-1})	RSD%	Recovery%	E129 add (μgml^{-1})	E129 found (μgml^{-1})	RSD%	Recovery%
Candy 1	-	35.06±0.87	2.1	-	-	30.17±0.62	1.8	-
	10	44.78±0.80	1.5	99	10	35.40±1.22	3.0	88
	20	54.48±0.74	1.2	98	15	40.22±0.69	1.5	89
Candy 1 Without CPC	-	30.01±0.64	1.8	-	-	23.80±0.44	1.6	-
Candy 2	-	22.97±0.52	2.0	-	-	18.89±0.45	2.1	-
	10	31.27±1.01	2.8	94	5	19.94±0.41	1.8	83
	15	36.67±0.79	1.9	96	10	25.51±0.69	2.3	88
Candy 2 Without CPC	-	17.95±0.75	3.7	-	-	13.01±0.65	4.4	-
Jelly 1	-	26.23±1.76	5.7	-	-	23.22±0.26	0.9	-
	10	35.92±0.34	0.8	97	10	29.71±0.46	1.3	89
	15	40.79±0.59	1.2	98	15	33.67±0.88	2.3	88
Jelly 1 Without CPC	-	20.90±0.46	1.9	-	-	16.73±0.48	2.5	-
Jelly 2	-	41.65±0.58	1.2	-	-	38.07±0.48	1.1	-
	10	50.57±0.75	1.3	97	10	43.76±0.24	0.5	91
	20	59.86±0.29	0.43	97	15	48.71±0.49	0.9	91
Jelly 2 Without CPC	-	36.91±0.62	1.4	-	-	31.92±0.61	1.6	-

Conclusions

In this study, sensitive cloud point extraction method was developed for estimation and preconcentration of Allura red .It is found that using a mixture of nonionic surfactant (TX-114 or Brij-35) with cationic surfactant CPC gives a good extraction compared with the extraction without adding cationic surfactant which is poor efficiency. The application of this method was investigated by determination of Allura red in several commercial samples.

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