Cloud Point Extraction Procedure for the Determination of Mercury by Spectrophotometry Using a New Synthesized Ligand

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Abstract

A new thiazolylazo reagent was prepared and exploited for the cloud point extraction (CPE) methodology in the preconcentration of micro amount of Hg (II) as a prior step to its determination by UV-Vis spectrophotometry. The extraction and determination processes of Hg(II) involved the complexation of Hg (II) with synthesized 7-(6-Bromo 2-benzothiazolyl azo)-8-Hydroxyquinoline (7- (6-BrBTA8HQ) at specific pH which is extracted by micelles of the non-ionic surfactant octylphenoxypolyethoxyethanol (Triton X-114) and subsequently detected spectrophotometrically at specific 640 nm. The optimal reaction and extraction conditions (e.g. pH, reagent concentration, surfactant concentration, equilibrium temperature and the incubation time) were studied and the analytical figures of merit of the method (e.g. linearity, limit of detection, sensitivity, enrichment factor etc) were obtained. The interferences effect of the foreigner metal ions was also considered. Under the optimum established conditions, the enrichment factor of 123 folds was achieved which led to a detection limit of 7.4 ng mL^{-1} of Hg (II) and concentration range of 8-500 ng mL^{-1} with percent recovery of 100.43±1.23. The precision for seven replicate measurements of 200 ng mL^{-1} Hg (II) was of 0.67%. The method was applied to the determination of mercury in some fish samples with satisfactory results.

Key Words: 7-(6-Bromo 2-benzothiazolyl azo)-8-Hydroxyquinoline, Mercury, Fish samples, Cloud-point extraction, Spectrophotometry

الخلاصة

تم في هذه الدراسة تحضير كاشف صبغة الثايزو ليل ازو الجديدة واستخدامها في تقنية الاستخلاص بنقطة الغيمة لغرض فصل واستخلاص الكميات الميكروية لايون الزئبق الثنائي كخطوة اولية قبل تقديره بالمطيافية الجزيئية.

تضمنت عمليات الاستخلاص والتقدير ، اجراء عملية التعقيد لايون الزئبق مع الكاشف المحضر 2- 7-(6-Bromo benzothiazolyl azo)-8-Hydroxyquinoline عند دالة حامضية محددة ومن ثم استخلاصه كميا الى Triton X-114 وبالتالي تقديره طيفيا عند الطول الموجى الاقصى (640 نم) . تم دراسة الظروف الفضلى للتفاعل والاستخلاص مثل الدالة الحامضية وتركيز الكاشف وتركيز المادة الفعالة سطحيا ودرجة حرارة ووقت الاستخلاص وتم الحصول على المعطيات التحليلية للطريقة المقترحة مثل الخطية وحد الكشف والحساسية ومعامل الاغناء فضلا عن دارسة تاثير تداخل بعض ايونات الفلزات الدخيلة المتوقع وجودها في منشأ العينات او التي قد تنافس ايون الزئبق في تكوين معقدات مع الكاشف المحضر. لقد وجد تحت الظروف الفضلى ، ان معامل الاغناء كان 123 مرة الذي ادى الى الحصول على حد كشف 7.4 نغم / مل والمدى الخطى للمعايرة 8–500 نغم/ غم مع مدى استردادية مئوية لايون الزئبق في العينات قدرها 100 ±1.23. كما كانت الدقة محسوبة على اساس الانحراف القياسي النسبي المئوي عند تركيز 200 نغم/ مل هي 0.67% في المحلول المائي . طبقت الطريقة المقترحة على تقدير الزئبق في عينات السمك المختلفة المحلية منها والمستوردة .

الكلمات المفتاحية :كاشف 7–(6–برومو–2– بنزوثايزوليل ايزو) –8– هيدروكسي كيونولين ، الزئبق ، عينات السمك ، استخلاص نقطة– الغيمة ، المطيافية الجزيئية .

Introduction

Mercury in fishes comes most probably through the polluted water in which mercury can be transformed into methyl mercury due to bacteria found in the water and can then be consumed by fish or penetrate into fish directly through skin and gill later [1]. Since mercury is highly toxic to human health and then fishes are extremely consumed by humans, FAO/WHO established a tolerable intake of 3.5 µg/kg bodyweight per week for methyl mercury for an adult, but still no limiting figures have been reported for children (up to about 17 years) whom are more sensitive than adults [2]. Thus, the concentration of mercury exceeding a tolerable intake may cause serious health problems such as loss of vision, hearing and mental retardation and finally death occurs [3] . The power of Toxicity depends on its chemical species and it is found that organomercurials are more toxic than inorganic mercury compounds [4] . Consequently, the determination of mercury present in food or food supplements at extremely low concentration necessities the establishment of an accurate, rapid, sensitive , and reliable method that is free from matrix interferences.

Owing to the extremely low levels of mercury in food matrices, the separation and enrichment steps are a must before its determination. Recently, cloud-point extraction (CPE) methodology has become well known as the best of choice for preconcentration step for most metal ions in various matrices and as an alternative to conventional solvent extraction because of

its excellent enrichment factors, lower cost, higher safety and simplicity, and it does not need to handle a great volume of organic solvent that is generally toxic which reflects the principles of the "Green Chemistry" [5-6] . The recent trends for the principle and applications of CPE have been well reviewed [7] .In last decade, the CPE procedure coupled with various instrumental techniques have been developed for preconcentration and determination of Hg(II) in different matrices using commercial chelating agents, including inductively-coupled plasma-optical emission spectrometry (ICP-OES) [8] ,flow injection cold vapor inductively-coupled plasma-optical emission spectrometry (FI-CV-ICP-OES)[9] , cold vapor atomic absorption sspectrometry (CV-AAS) [10] , flow injection cold vapor atomic absorption spectrometry (FI-CV-AAS) [11], electrothermal atomic spectrometry (ETAAS) [12], cold vapor atomic fluorescence spectrometry (CV-AFS) [13] , flow injection analysis(FIA) [14] , flourimetry [15], high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) [16] , capillary electrophoresis (CE) [17] and spectrophotometry [18-21] .

Due to the attractive features incorporated with chemical structure of thiazolylazo dyes and their derivatives, analytical chemists have paid most of their interest in using these compounds as chelating agents for the determination of metal ions by spectrophotometry in combination , exclusively, with several separation procedures such as liquid-liquid , solid-phase and cloud-point extraction. Thiazolylazo dye derivatives such as, 1-(2-thiazolylazo)-2-naphthol (TAN), 1-(2-Pyridylazo)-2-naphthol (PAN) , 4-(-pyridylazo)-resorcinol monosodium (PAR) and 2-(5-bromo-2pyridylazo)-5-diethylaminophenol(5-Br-PADAP) are among various commercially produced reagents used commonly in extraction procedures, including cloud point extraction because their metal complexes are highly stable and have rather limited solubility in aqueous solution but much greater solubility in organic and micellar sys- tems [22] .The solubilization properties of micellar systems provide additional advantages over existing analytical methods using extraction with toxic organic solvents for determination in aqueous media and for their significant increase in sensitivity and selectivity [23] . This encouraged a few of authors to open new routes for synthesis of thiazolylazo derivatives as chelating agents instead of commercial analogues in cloud point extraction procedure coupled, for example, with atomic absorption spectrometric determination of Cr speciation in water samples [24] and with spectrophotometry for the detection of Pb(II) and Cd(II) in honey samples [25] , in an attempt to improve the selectivity and elaboration the analytical characteristics of CPE methodology in combination with instrumental analytical techniques.

In this piece of work, we have prepared a new thiazolylazo reagent and exploited for the cloud point extraction (CPE) procedure in the preconcentration of micro amount of Hg (II) as a prior step for its determination by UV-Vis spectrophotometry .The method is based on the micelle mediated extraction of the complex of Hg^{2+} with7- (6BrBTA8HQ) (Figure 1). A nonionic surfactant, Triton X-114, was selected as the extraction agent to facilitate phase separation. The developed method was carried out for the determination of trace mercury in various fish samples.

Experimental

Apparatus

UV-Visible Spectrophotometer T 80 (England) equipped with 10- mm optical path cell were used for the scanning study of absorption spectra of the complexes formed, while absorbance measurements were carried out with spectrophotometer SUNNY UV-7804C (China) . Measurement of NMR Spectra of the proton ^{1}HNMR recorded spectra using a type of Bruker , Ultra , Shiel 300 MHZ , SWISS and using (DMSO-d^{6}) as solvent at the University s home in the Hashemite Kingdom of Jordan , FTIR-8400S, Shimadzu, Japan , Elemental Analysis 3764, Carlo Erba, Europ , Shimadzu GSA-4B (Japan). The effect of temperature was investigated by using a water bath WB 710 model (OPTIMA, Japan). A Microprocessor pH meter 211 model (Triup International Corp, Italy) with a combined electrode was used for pH measurements.

Reagents and standard solutions

All the chemicals used were of analytical reagent grade, and used without further purification. Distilled water was used for diluting the samples and reagents. The reagents 2-amino-6-bromo benzothiazole, 8-Hydroxyquinoline (RIEDEL-DE HAEN AGSEELZE-Germany), Sodium nitrite (B.D.H), hydrochloric acid (BDH), and ethanol were purchased from(GCC, England). Triton X-114(Acros Organics, New Jersey, USA). The aqueous solution (10% v/v) of Triton X-114 was prepared by dissolving 10 mL of concentrated solution in 100 mL water. A stock solutions of Hg^{2+} (1000 mg L^{-1}) was prepared by dissolving 0.1353 g of mercury(II) chloride (Merck, Darmstadt, Germany) in appropriate amount of water and made to 100 mL with water. Working standard solutions of metal ion were freshly prepared by appropriate dilutions of the stock solution. Acetate buffer solution was prepared from acetic acid and ammonium acetate.

Synthesis and characterization of reagent

The synthesis of 7-(6-BrBTA8HQ) was accomplished according to general procedure described elsewhere [26] , with some modification. A 2-amino-6-bromobenzothiazole (2.2909 g, 0.01 mol) was dissolved in 25 mL of distilled water and 5 mL of concentrated hydrochloric acid and diazotized below 5ºC with (0.75 g , 0.01 mol) of sodium nitrite. The resulting diazonium chloride solution was added drop wise with cooling to solution of (1.4516 g,0.01 mol) of 8-Hydroxyquinoline dissolved in 50 mL of alkaline ethanol and the mixture kept in the refrigerator overnight. The mixture was then neutralized with dilute hydrochloric acid to (pH=5-3). The solid product was filtered off, washed with cold water, crystallized twice from hot ethanol and dried over $CaCl_2$ to give a dark red crystals of compound as shown in Fig.1. Yield 66%; mp 188-190˚C; anal.calcd.for $C_{16}H_9BrSN_4O$ (385.24 g mol^{-1}) C,49.88; H,2.35; N,14.54; S,8.32; found C,48.78; H,2.23; N,14.03; S,8.16; IR(KBr) ν_{max}/cm^{-1}3139 ,3047(ν, Ar-OH), 2877,2808(ν,Ar-H), 1674 , 1643(ν, C=N), 1596, 1550(ν, C=C), 1404(ν, N=N), 1265(ν,C-N), 1134(ν, C-O), 1010(ν, C-S), 879,817, 709(δ, Ar-H)), 517(δ, C-Br); ^{1}H-NMR(DMSO-d_6, 298 K,) d/ppm) 4.89 (m, OH), 7.20(s,3H, pyridyl, phenoyl), 7.30(s,H, phenyl)7.40(s,H, pyridyl),7.80-8.20 (m,2H, phenyl) 8.99(s,H, pyridyl). The chemical structure of 7-(6-Bromo 2-benzothiazolyl azo)-8Hydroxyquinoline abbreviated as 7-(6-MBTA8HQ) is shown in Figure 1. The reagent is insoluble in water but very soluble in some organic solvent like methanol, ethanol, acetone, DMS and DMF. The sulfur was determined gravimetrically by

mineralization of 7-(6-MBTA8HQ) with concentrated mineral acids (HNO_3,HCl) and sulfate was precipitated as $BaSO_4$, dried, filtered and weighed.

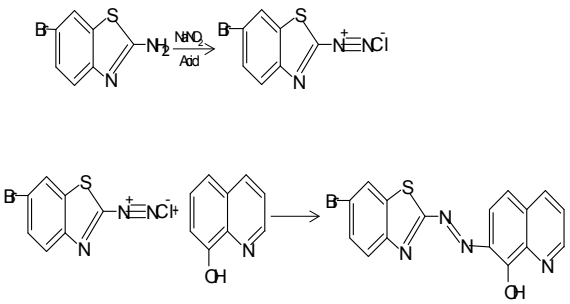

Figure 1. Synthesis route of reagent 7- (6- BrBTA8HQ)

General procedure for CPE

For cloud point extraction, 0.2 mL of 10%(v/v) Triton X-114 , 0.45 mL of 7- (6-BrBTA8HQ) 3×10^{-5} M and 1.5mL of buffer acetate (pH 5.0), in a 5 mL flask were added and a proper amount of mercury was (8-500 ng mL) added to it, and diluted to 5 mL with distilled water. Then, the whole of solution transferred into 10 mL centrifuging tubes. The tubes were kept for 15 min in the thermostatic bath at 70 ◦C. Subsequently, separation of the phases was achieved by centrifugation for 15 min at 3500 rpm. The phases were cooled down in an ice bath in order to increase the viscosity of the surfactant-rich phase. The bulk aqueous phase was easily decanted by tilting the tube. The surfactant-rich phase in the tube was made up to 0.5 mL by adding ethanol. The absorbance of the complex was measured at 640 nm against the corresponding reagent blank prepared under identical conditions.

Preparation of fish samples

Fish sample solution was prepared according to the procedure adopted by Prester and Blanusa [27] using acid digestion in closed tubes . About 1 g of homogenized fish was first digested with 2 mL of concentrated nitric acid in closed borosilicate glass tube at room temperature and then the next day at 80 ºC for five hours in a programmed water bath WB 710 model (OPTIMA, Japan). After digestion samples were cooled at room temperature (25±5ºC) and the volume adjusted to 10 mL with water. The aliquots of the final solution were extracted and analyzed for mercury according to the prescribed general procedure for CPE.

Statistical analysis

All mathematical and statistical computations were made using Excel 2007 (Microsoft Office) and Minitab version 14 (Minitab Inc., State College, PA, USA).

Results and Discussion

Absorption spectra

Figure 2 shows the absorption spectra for the reagent 7- (6-BrBTA8HQ) and its complex with mercury (II) in surfactant-rich phase against reagent blank prepared under similar conditions. It was appeared that at pH 5 the absorption maxima of mercury(II) chelate occurs in visible region of 640 nm with molar absorpitivity of $5.02x10^5$ L mol^{-1} cm^{-1} while the reagent 7-(6-MBTA8HQ displays an absorption maxima of 448 nm.

The Reagent 7-(6-BrBTA8HQ) reacts immediately with mercury forming an (greenish-blue) complex in aqueous medium (pH 5) and the absorbance reached its maximum within 5 min and remained stable, for at least 24 h. The stoichiometry of the Hg (II)-7-(6-BrBTA8HQ) complex was studied, under the established experimental conditions, by Job's and mole ratio methods. The obtained results indicated that the composition of the complex was (1: 2). In additions, the Hg (II)-7-(6-BrBTA8HQ) complex was characterized previously on the basis of spectroscopic techniques and

the suggested related chemical structure is shown in Figure 3.

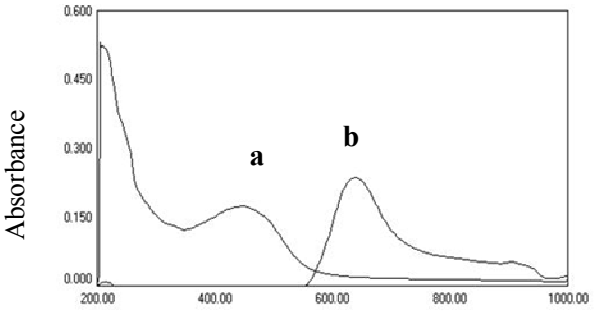

Figure 2. Absorption spectra (a) Reagent 7-(6-BrBTA8HQ) = 3 x 10^{-5} M (b) Hg(II)- 7-(6-BrBTA8HQ) complex , Hg(II) = 0.13 µg mL^{-1} , 0.45 ml of 7-(6-BrBTA8HQ) = 3 x 10^{-5} M , Buffer pH= 5(1.5 mL) , 0.2 mL of 10 % (v/v)Triton X-114 .

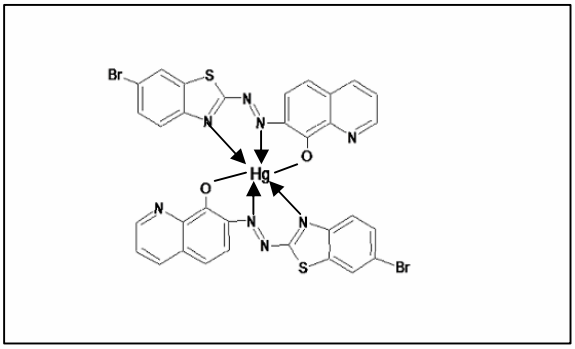

Figure 3. The suggested chemical structure of Hg (II) 7-(6-MBTA8HQ) complex.

Optimization of CPE procedure

Since the extraction efficiency in the CPE depends on paired factors, one regarding the prior formation of a complex with sufficient hydrophobicity and the other for the formation of micelles to obtain the desired separation and preconcentration. Consequently, the optimization for several variables versus absorption signals including pH, concentration of ligand, nonionic surfactant Triton X-114 concentration and equilibration temperature, incubation time and centrifugation time were performed using the traditional one-variable-at-time (OVAT) optimization . Although this method does not ensure at all that the real optimum will be conformed (i.e. no strong interactive effects among the variables), but it leads certainly to an improvement of the analytical procedure.

Effect of pH

The influence of pH in CPE procedure is deemed the crucial step to ensure the reaction between metal ions and chelating molecules with sufficient hydrophobicity and subsequent extraction into the small volume of surfactant-rich phase. So, pH plays an incomparable role in complex formation and its extraction into micelle-mediated solvent to obtain the desired preconcentration by CPE. The effect of pH on absorbance signals at 640 nm for the formation of the Hg (II) -(6-MBTA8HQ) complex in Triton X-114 medium was studied by varying the pH from (3.0-9.0) using different pH buffer solutions. As shown in Figure 4 that the absorbance first increased sharply with increasing pH and reached a maximum at pH 5.0 indicating extraction efficiency was achieved. The absorbance was gradually decreased because of partial dissociation of the complexes at higher pH, which may result in an incomplete extraction of complex. Therefore, pH 5.0 was chosen as the optimum working pH for complete formation of Hg(II)-7-(6-BrBTA8HQ) complex and hence a good extractability.

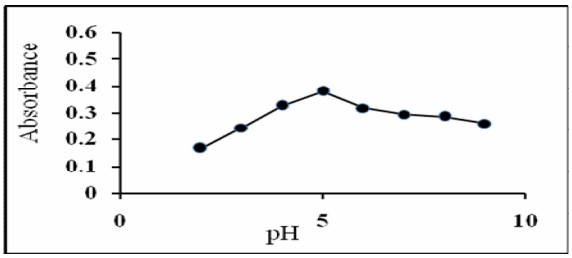

Figure 4. Effect of pH on the formation of 7-(6-BrBTA8HQ)- Hg(II) complex . [Conditions : Hg(II) = 0.2 µg mL^{-1} , 0.45 ml of 7-(6-BrBTA8HQ) = 3 x 10^{-5} M , 0.2 mL of 10 % (v/v)Triton X-114)]

Effect of 7-(6-BrBTA8HQ) concentration

The concentration effect of chelating agent 7-(6-BrBTA8HQ) on absorption signal was studied and the results are depicted in Figure 5. A solution containing 0.2 µg Hg (II) mL^{-1} was subjected to CPE procedure by varying the volume of reagent from 0.1 to 0.6 mL of 3 x 10^{-5} M of 7-(6-BrBTA8HQ) and keeping other factors constant. As it is seen for mercury complex, the signal increases up to 0.4 mL volume of 3 x 10^{-5} M 7-(6-BrBTA8HQ) , then reaching a plateau, which is considered as complete chelate formation and efficient extraction. Consequently, 0.45 mL of 3 x10 $^{-5}$ mol L^{-1} of 7-(6-BrBTA8HQ) was selected as the optimum for further experiments.

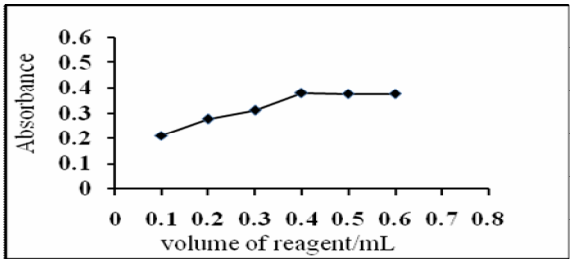

Figure 5. Effect of concentration o f 7-(6-BrBTA8HQ) on the Hg(II) analytical signal. [conditions : Hg(II) = 0.2 µg mL^{-1} , X mL of 7-(6-BrBTA8HQ) = 3 x 10^{-5} M , 0.2 mL of 10 % (v/v)Triton X-114), pH 5.0)] .

Effect Triton X-114 amount

Figure 6 illustrates the effect of variation of Triton X-114 amount on the absorbance signal for the determination of Hg (II) ion. Different volumes of 10%(v/v) Triton X-114 ranging from 0.1-0.6 mL were used in this study at previously optimum conditions. As shown in Figure 6, the absorbance was highest at 0.2 mL of 10% Triton X-114, but decreased suddenly thereafter. This may be attributed to the presence of an excessive amount of surfactant leading to increase the volume surfactant-rich phase and accordingly its high viscosity resulting in poor sensitivity. While below 0.2% of surfactant, the absorbance was slightly low because the assembles may be insufficient to quantitatively entrap the hydrophobic complex. Therefore, 0.2 mL of 10% (v/v) Triton X-114 was used as optimal.

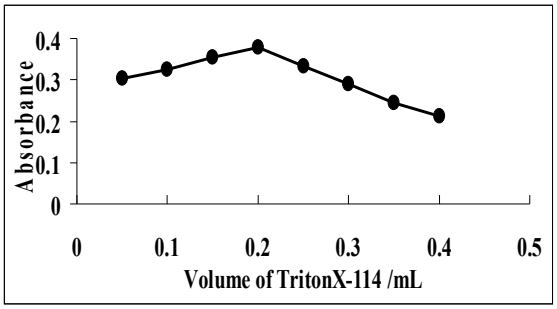

Figure 6. Effect of Triton X-114 concentration on the Hg(II) analytical signal. [conditions : Hg(II) = 0.2 µg mL^{-1} , 0.45 mL of 7-(6-BrBTA8HQ) = 3 x 10^{-5} M , X mL of 10 % (v/v)Triton X-114), pH 5.0)] .

Effect of the equilibrium temperature and the incubation time

The effects of the equilibrium temperature and the incubation time were examined due to their importance for the reaction completion and efficient separation of the phases, which reflect certainly the magnitude of preconcentration factor of an analyte. The temperature was varied from 30 ºC to 90 ºC in a search of optimum value. It can be

seen from Figure 7 that the highest absorbance signals were obtained when the temperature at 70 ºC to achieve quantitative extraction. Unreasonably high temperatures are not suitable for the CPE procedure because higher temperatures could cause problems to the stability of complex. In this study, 70 °C was chosen as the optimized incubation temperature for subsequent experiments.

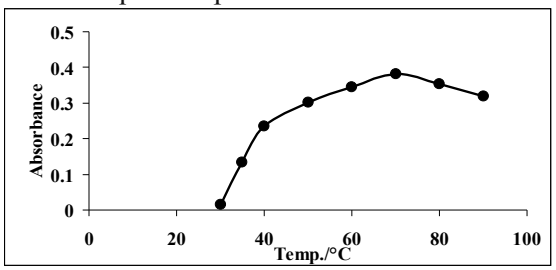

Figure 7. Effect of the temperature on the absorbance for Hg(II) complex. [conditions : Hg(II) = 0.2 µg mL^{-1} , 0.45 mL of 7-(6-BrBTA8HQ) = 3 x 10^{-5} M , 0.2 mL of 10 % (v/v)Triton X-114), pH 5.0)].

It was also observed that the incubation time of 15 min is sufficient for the maximum absorbance of mercury complex (Figure 8). Thus, the temperature of 70 ºC for 15 min was selected to fulfill efficient separation conditions. The effect of centrifugation rate and time also was investigated on extraction efficiency. A centrifuge time of 15 min at 3500 rpm was selected for the entire procedure as being optimum and beyond this time no confirmation was observed for improving extraction efficiency.

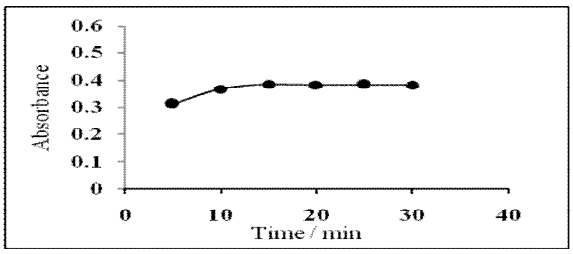

Figure 8. Effect of the incubation time on the absorbance for Hg(II) complex. [conditions : Hg(II) = 0.2 µg mL^{-1} , 0.45 mL of 7-(6-BrBTA8HQ) = 3 x 10^{-5} M , 0.2 mL of 10 % (v/v)Triton X-114), pH 5.0, temp 70 º C].

Analytical Figures of Merit

Under the optimized conditions, the calibration graphs were constructed by plotting the absorbance signal against the concentration of analyte subjected to the CPE. The solutions were transferred into the optical cell of 10-mm for the measurement of metal ion spectrophotometrically at 640 nm against a reagent blank prepared under similar conditions .The statistical analytical figures of merit of the proposed method are summarized in Table 1. The enrichment factor was calculated as the ratio between the slop of a curve obtained using aqueous solutions submitted to the CPE procedure (regression line y=1.850 x+0.009) to that obtained without CPE (regression line y=0.015 x+0.000) and found to be of 123-fold enhancement factor.

In aqueous solution, the limit of detection of 7.4 ng mL^{-1} in the suggested protocol was achieved calculated as three times the standard deviation for blank signal divided by the slope of the calibration curve.

The detection limit obtained for mercury by the proposed method was generally in harmony with and /or better than that obtained by combined CPE-

Spectrophotometric and other methods using commercial reagents reported in the chemical literatures (Table 2). But, it was worse than that obtained by CPE combined with sophisticated techniques such as FI-CV-ICP/OES[9] and HPLC-ICP-MS [16] . However, by considering a limit of detection of 7.4 µg L^{-1} and 1 g of fish sample in 10 mL, the LOD of the method would be 0.074 µg g^{-1}.

Table 1. Analytical figures of merits of the spectrophotometric determination of Hg (II) using CPE methodology.

Parameter	Value
λ_{max} (nm)	640
Regression equation with CPE	A= 1.850 conc. + 0.009
Correlation coefficient(r)	0.9998
C.L. for the slope (b±tsb) at 95%	1.850± 0.0180
C.L. for the intercept (a±tsb) at 95%	0.009± 0.00372
Concentration range/ (ng mL^{-1})	8-500
Limit of Detection /(ng mL^{-1})	7.4
Limit of Quantitation / (ng mL^{-1})	25.4
Sandell's sensitivity / (µg.cm^{-2})	0.00039
Molar absorptivity / (L.mol^{-1}.cm^{-1})	$5.02x10^5$
Composition of complex (M: L)*	1:2
RSD% (n=7) at 200 ng mL^{-1}	0.67%
Preconcentration factor	58.82
Enrichment factor	123

*Job's and mole ratio methods used

Table 2. Previous studies using cloud point extraction prior mercury detection in different Matrices.

Sample	Reagent	Surfactant	LOD (ng mL^{-1})	PF	Detection	Reference
CRM biological origin	DDTP	TritonX-114	0.4	10	CV-AAS	10
River and drinking water	Dithizone	TritonX-100	14	6	On-line FIA	13
Tap, river and mineral water	TPPP	TEGII (Ionic liquid)	80	45	Fluorimetry	15
Natural water and tilapia muscle	PAN	TritonX-114	10.0	52	CE	17
No application	DDTC	TritonX-100	0.53	-	UV/Vis spectrophotometry	18
Water	ThioMichler's Ketone	Triton X-114	Up to 0.83	11	UV–Vis spectrophotometry	19
Environmental water	(PAN and TAR)	TritonX-114	1.65 and 14.5	33.3	UV-Vis spectrophotometry	20
Speciation, environmental water	TAC	CPC	6	-	UV-Vis spectrophotometry	21
Honey	ADDTP	Triton X-114	2.2	13	CV-ICP-OES	28
Water	Iodide media	TritonX-114	3.0 /0.1	10	UV/vis and CV-AAS	29
Fish	7-(6-BrBTA-8HQ)	TritonX-114	7.4	58.82	UV-Vis spectrophotometry	This work

LOD; limit of detetion ,PF; preconcentration. factor , CV-AAS: Cold Vapor- Atomic Absorption Spectrometry , FIA; flow injection analysis, CV- ICP-AES: Cold Vapor- inductively coupled plasma- atomic emission spectrometry , CE: capillary electrophoresis , DDTP:*O*,*O*-Diethyldithiophosphate , TPPP:5,10,15,20-tetra-(4-phenoxyphenyl)porphyrin , DDTC: sodium diethyldithiocarbamate , PAN: 1-(2-pyridylazo)-2-naphthol, TAR: 4-(2-thiazolylazo) resorcinol), TAC : 2-(2-thiazolylazo)-p-cresol , ADDTP ammonium diethyldithiophosphate , 7-(6-BrBTA-8HQ) : 7-(6-Methoxy 2-benzothiazolyl azo)-8-Hydroxyquinoline, CPC :cetylpyridinium chloride, TEGII:tetraethyleneglycol-bis(3-methylimidazolium) diiodide CPC .

Precision and Accuracy

Seven replicate analyses of 200 ng ml^{-1} mercury solution following the general CPE procedure gave repeatability in term of relative standard deviation (RSD) of 0.67% in aqueous solution (Table 1). The precision of the method for fish sample solution spiked with Hg at three different concentrations as shown in Table 3 was in range from 2.13 to 3.94%.

Since the certified reference material (CRM) for fish sample is not available, accuracy has been determined through the recovery percent evaluation. Three portions of fish sample were taken and increasing aliquots of standard Hg (II) solution were added to give sample solution with 50, 100 and 150 ng mL^{-1} standard additions and the same steps were followed with the CPE procedure. The recovery percentage of these additions was estimated and the results are seen in the Table 3.

Interference Study

The effect of most concomitant ions (at concentration of 100 fold) which can be expected in the fish matrix on the determination of 200 ng mL^{-1} Hg (II) solution was studied and the same general CPE procedure was performed. It is agreed that an extraneous ion deemed to interfere seriously when it gives a relative error percent of more than ± 5%.

Table 3. Accuracy of the proposed method

Sample brand	Amount Hg(II) taken (ng mL^{-1})	Amount Hg(II) found(ng mL^{-1})	Recovery (%)	E_{rel} (%)	Mean Rec% ±s.t/√n
Iraqi (4)	-	241			
	50	292	102	2.0	100.43±1.23
	100	339	98	-2.0	
	150	393	101.3	1.3	
Iranian	-	181			
	50	229	96	-4.0	100.57±2.28
	100	283	103	2.0	
	150	335	102.7	-2.7	

It was shown that all the monovalent ions (M^{+}) have no effects on analytical response or percent recovery, while the divalent ions (M^{2+}) have exceeded the allowable limits of interferences on mercury absorbance signal as shown in Table 4. Therefore, several masking agents such oxalic acid, citric acid, tartaric acid, 5- sulphosalicylic acid, 1-10 phenathroline, sodium fluoride and ascorbic acid individually or mixture were tested to control the interferences of Mg(II), As(III) Sb(II), Co(II) , Ni(II), Cu(II), Cd(II) and Pb(II) .

The experiments have shown that the interference effect of the above ions on Hg(II) absorbance signal was held efficiently by adding of 1 mL of 0.01M mixture of (citric acid +sodium florid +tartaric acid +1,10,phenanthroline) without any appreciable masking of Hg ions. Whilst ascorbic acid, 5- sulphosalicylic acid and oxalic acid have substantially decreased the absorbance of Hg due to its competing with chelating agent used thereby it ruled out.

Table 4. Effect of divers ions on the absorption signal of Hg (II) (200 ng mL^{-1}an 0.388 absorbance unit) by CPE-spectrophotometry

Interferent	A unit	AΔ	E_{rel} (%)
Mg(II)	0.420	0.032	8.2
As(III)	0.424	0.036	9.2
Sb(II)	0.420	0.032	8.2
Co(II)	0.422	0.034	8.7
Ni(II)	0.436	0.048	12.3
Cu(II)	0.348	-0.04	-10.2
Cd(II)	0.430	0.042	10.8
Pb(II)	0.434	0.046	11.8
K(I)	0.385	0.003	0.7
Na(I)	0.386	0.002	0.5

Determination of Hg in fish samples

The developed method was applied to the mercury determination in five Iraqi and four foreign fish samples randomly purchased from local markets. The results of the combined CPE-Spectrophotometry were compared with standard electrothermal atomic absorption spectrometric method done in our laboratory under the optimized conditions outlined by company's manual as displayed in Table 5.

Table 5. Spectrophotometric determination of mercury (II) in fish sample using CPE.

Sample No.	Fish sample	Concentration of Hg (µg g^{-1})	
		Present method [a]	ETAAS[b]
1	Iraqi 1*	0.291±0.0034	0.289±0.0017
2	Iraqi 2*	0.275±0.0020	0.278±0.0017
3	Iraqi 3*	0.264±0.0043	0.268±0.0036
4	Iraqi4*	0.241±0.0045	0.248±0.0030
5	Iraqi5*	0.253±0.0034	0.251±0.002
6	Iranian	0.181±0.0043	0.178±0.0034
7	Morocco	0.287±0.002	0.289±0.0016
8	Jordanian	0.298±0.0036	0.294±0.0017
9	Indonesian	0.298 ±0.0047	0.296±0.0170

[a] mean ± standard deviation; n = 5
[b]mean ± standard deviation; n = 3
*from Al-Diwaniyah city, Al-Qadyisia Governorate

The statistical analysis performed by the paired t- test for comparison of means between the proposed and standard ETAAS methods for the selected samples (Table 7) have revealed that the p value [P(T<t; 0.05< 0.798) two tailed] based on the 5% critical value of 2.31 was more than the t calculated value(0.26), indicating acceptance of null hypothesis (Ho) which specify there is insufficient evidence to suggest the accuracy (i.e. systematic errors) of the proposed method differs to that of ETAAS method. On the other hand, Fisher F-test for the comparison of precision (variances) between the two methods has also shown that the p value [P (F<f; 0.05<0.997) two-tailed] based on the 5% significance level of 4.33 was much more than the f-calculated (1.0) .It would be therefore conclude that there is no significant difference between variances (precision) of the two methods at the 5% level.

The results in Table 5 has revealed that mean values obtained for Hg in all the selected samples were ranged from 0.181 to 0.298 µg g^{-1} which considerably below the maximum limit permitted for human consumption by the European Communities Commission regulation (EC) 466/2001 of 0.5 µg g^{-1} for Hg.[30] and the Food and Drug Administration (FDA) of 1 µg g^{-1} for methyl mercury in seafood [31] .

Conclusions

In this piece of work, a new synthesized ligand used for complexation of mercury prior to its extraction and determination by combined CPE/ spectrophotometry , has resulted in an increase the sensitivity, limit of detection, selectivity and enhance the popularity of UV-Vis spectrophtometry beside the solvent-free extraction of toxic metals from complex matrices. The proposed method provides simple, reliable and accurate determination of Hg (II) ion with no significant difference compared to ETAAS technique and it can be considered as an alternative to the other spectrometric and separation techniques such as, ETAAS, FI-CV-ICP/OES, HPLC-ICP-MS and electrophoresis.

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