Spectrofluorimetry - Cloud point extraction determination of trace arsenic (III) with 2',7'-dichlorofluorescein

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Abstract. In this work, explains a separation and Preconcentration procedure for the determination of arsenic (III) by spectrofluorimetric method after complexation with the 2',7'-dichlorofluorescein as a fluorogenic reagent presence by the nonionic surfactant Triton X-114 in λ_{ex} 271 nm and λ_{em} 537 nm, after that, the quantitatively isolated into a unimportant volume of the surfactant-rich phase later a centrifugation. The optimum conditions, such as pH, volume of surfactant and reagent (TritonX-114), equilibrium temperature and incubation time on the cloud point extraction procedure, were studied and optimized on the extraction productivity percentage. In this method limits of detection and quantification (LOD and LOQ) of As(III) ion was (0.102) and (0.525) ng/mL respectively, and a linear calibration range of (5.0 - 50.0) ng/mL for As(III). The proposed method has been successfully applied to the determination of As(III) ion in water samples with agreeable results.

Keywords: 2',7'-dichlorofluorescein, Cloud point extraction, TritonX-114, Spectrofluorimetry, Preconcentration, Benesi-Hildebrand.

Introduction:

2',7'-dichlorofluorescein has a chemical formula is known as C_{20}H_{10}Cl_{2}O_{5} it is used for the determination of trace amount of arsenic (III) ion by cloud point extraction (CPE), and the chemical structure of the reagent is shown in figure 1.

![Fig.1. The chemical structure of 2',7'-dichlorofluorescein[1]](image)

Arsenic is one of the ordinary impurity of ground water which has been found to unsympathetically effect on the human health at concentrations as low as (10) ppb [2]. It has a destructive dosage at 50% of the populace of 763 mg, kg\(^{-1}\) of body quantity.
The highest pollutant level recommended by the United States Environment Protection Agency (USEPA) for arsenic is 50 ppb in drinking water. The World Health Organization (WHO) has suggested the maximum concentration of its ion in drinking water as low as 10 ppb [3].

It is well identified that arsenic constrains the strategic metabolic enzyme pyruvate dehydrogenase and arsenate participates with phosphate for the enzyme which interrupts ATP manufacture and eventually uncouples oxidative phosphorylation. This embarrassment results in the decrease of the energy linked NAD+, mitochondrial breathing, and ATP production[4].

In general, the inorganic species of As are more toxic than the organic forms. The toxicity of arsenic (III) is 60 times greater than arsenic (V)[5]. In which case, several various method for the determination of As, such as (HPLC-ICP/MS)[6], LC-HG/AFS[7], (LC–ICP/MS) [8]. Since the past decade, few articles have appeared in the chemical literatures reflect the applications feature of CPE coupled with atomic spectrometers and other techniques for arsenic extraction and preconcentration from water, and food matrices. In the middle of the different analytical applications and separation processes, cloud point extraction (CPE) based on micellar extraction [9-12] is an efficient procedure for synchronized enrichment and separation of objective species from matrices. CPE is considerable supplementary environmental friendly, and is safer for the reason that small volumes of truncated -noxiousness surfactants are used instead of toxic organic solvents. CPE is successfully applied for the extraction of metal chelates previous to quantification using analytical techniques, such as FAAS inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma/mass spectrometry (ICP-MS) [13-15].

Experimental

Instrumentation

Spectrofluorimetir (Shimadzu- Model RF-5301PC) was secondhand for completely the magnitudes in this procedures for the control the attentiveness of the arsenic (III). A numerical pH meter (inolab 720 modele WTW Germany) was charity for all pH quantities. A thermo quantified bath Thermo, balance (Circulater Englands) preserved as the anticipated temperature was consumed for the cloud point extraction investigates, and phase departure was supported using a centrifuge (PLC 03 USA), AA-6300 Shimadzu Atomic Absorption spectrophotometer type GFA-EX71 Graphite Furnace.

Reagents and solutions

Totally the chemicals used were of analytical reagent grade. Stock solutions of As(III) was prepared by dissolving suitable quantities of As₂O₃ in deionized water. Triton X-114 (non-ionic surfactant types), was used deprived of further decontamination. 1 × 10⁻⁵ mol L⁻¹ solution of 2',7'-dichlorofluorescein stock solution was organized via softening suitable quantities of this substance in absolute ethanol. The tubes and receptacles secondhand for indication analysis were reserved in 5% nitric acid for a minimum 24 h and afterward wash away two periods by deionized water.

Cloud point extraction technique (CPE)

For the CPE method, aliquots of 5 mL of a solution comprehending of the As(III), Triton X-114, 2',7'-dichlorofluorescein and a buffered solution at a fit pH was possessed in the thermostatic bath continued at 60 °C at old-fashioned 15 min. For the reason that the surfactant density, its 1.052 gm.L⁻¹, the surfactant rich phase canister become peaceful concluded the aqueous phase, then the centrifuge for 15 minute at 5000 route per minute quicker the phase separation, at that time construct a make cold in an ice, its small organic phase enhanced thick and was still in the lowest of the tube centrifuge. So as to lessening the glueyness and simplify sample furnishing previous to the
spectrofluorimeter tool, suitable volume of ethanol was additional to the organic phase. The last solution was familiarized into the spectrofluorimetric visual to comprehensive its dimension of CPE.

**Fluorescence magnitudes**

A spectrofluorimetric technique was used to development the 2',7'-dichlorofluorescein fluorescence spectrum intensity (2',7'-dichlorofluorescein – As(III)) intended in place of the magnitude of its arsine ion in all the untried. The signal concentration of its complex was measured in the intensity solution at an excitation wave length of 271 Nano meter and an emission wavelength of 537 Nano meter then standardization straighten was assembled. The fluorescence signal of its complex is liquefied during C₂H₅OH next abstraction was calculated and the As(III) was determined. All capacities stayed agreed out at room temperature after the optimization for the temperature of its cloud point extraction for its ion, Fig.(2) show the excitation and the emission spectrum of As(III) – 2',7'-dichlorofluorescein complex.

![Fig. 2. Spectrum of 2',7'-dichlorofluorescein and its As(III) complex.](image)

**Results and discussion**

**Effect of pH**

Cloud point extraction pro the arsenic species was organized at dissimilar buffered pH emulsions. Disconnection of arsenic ion by cloud point extraction encompasses previous development for an complex with satisfactory hydrophobic and the 2',7'-dichlorofluorescein to be extracted addicted to the little quantity of the organic phase. The pH of the solution was augmented for the non-ionic CPE in order to acquire the optimum signal for the nominated emission spectrofluorimetry, the pH was accustomed to the anticipated value by addition of buffer solution. Subsequent the experimental procedure described in the procedure segment it was found that the extraction efficiency
is almost dependent on pH circumstances for the pH range of (5-7) for its complex. (Fig. 3) giving a tableland in the area of its value. This performance is projected payable to the hydrophobic, non ionizable kind of the investigates.

Fig. 3. Effect of pH on the extraction signal and percent of As(III) - 2',7'-dichlorofluorescein complex.

Effect of 2',7'-dichlorofluorescein volume on its extraction

The extraction method through CPE style depends on furthermore significant step of complexation between organic reagent 2',7'-dichlorofluorescein and As(III), that can be transferred to surfactant after establishment cloud point phase. In interpretation of the practicality that the complexation step was also be contingent on the countryside of organic reagent which specify the extraction competency through the formation new stable complexes. For that purpose, the effect of 2',7'-dichlorofluorescein concentration was carried out by taking 5 mL solution containing 5.00 ng mL⁻¹ As (III), different quantity since (0.1-1.0) mL of 1.0×10⁻⁵ mol L⁻¹ 2',7'-dichlorofluorescein, 0.5 mL of 10% TritonX-114 at optimum (pH=6.0) value. Each experimentation was and subjected to the CPE development. The results are shown in Figures (4) for each enhancement signal and extraction percent respectively.
Fig. 4. Effect the volume of 2',7'-dichlorofluorescein on the extraction percent of As(III) - 2',7'-dichlorofluorescein complex.

The volume of Triton X-114 Effect

In which case the volume of TritonX-114 whose employed in CPE classification for the separation and extraction of some metal or organic type was actual critical factor for the reason that its factor foremost to accomplished intensification the preconcentration factor and lowest phase volume nonetheless this is should be by the all-out extraction efficiency. The effect of TritonX-114 volume was dissected via engaged a 5.0 mL volumetric flask counting of 5.00 ng.mL$^{-1}$ from As(III) and 0.4 mL of 1.0 $\times 10^{-5}$ mol L$^{-1}$ from (2',7'-dichlorofluorescein) at the optimization pH value equal to (6) and different volume from a nonionic surfactant TritonX-114 corresponding to the volume from (0.05-0.7) mL from its 10.0 % (v/v) nonionic surfactant. Figure (5) it can be understood that the optimum measure was extracted at the volume 0.5 mL of 10.0% (v/v) Triton X-114 and therefore chosen for supplementary experiments.

Fig. 5. Effect the volume of TritonX-114 on the extraction percent, signal of As(III) - 2',7'-dichlorofluorescein complex.
Effect of the temperature and time equipment

In the system of cloud point extraction procedure, temperature of extraction is identical significant limit intended for micelle by way of arising. The effect of equilibration temperature and time incubation in the CPE technique was reconnoitered at 30 to 70 °C. At temperatures inferior than 30 °C the split-up of the binary phases on the cloud point temperature was not perfect. Subsequently 60 °C, the indication intensity was almost same value or reduction for the reason that this complex resolve be dissociated at extraordinary temperature (Fig. 6) and (Fig.7). The incubation time in the water bath was kept at 15 min. for As(III) complex which is sufficient for the completion separation into two phase aqueous and organic phase of these progressions.

Fig. 6. Effect of time incubation on the extraction signal, percent of As(III) - 2',7'-dichlorofluorescein complex.

Fig. 7. Effect of temperature on the extraction signal, percent of As(III) - 2',7'-dichlorofluorescein complex.
The effect of the equilibrium temperature was reviewed by taking 5 mL aqueous phase comprising of 5.00 ng mL\(^{-1}\) As (III) and then keeping other factors at optimum circumstances. The temperature was different from 40 to 80 °C at 15 min in an examination of optimum significance. It was shown that the highest intensity signals and thereby the highest extraction percent were obtained when the temperature at 60 °C realizing quantitative extraction that shown in Figure (7) in above dailies.

**Calibration Graph for its complex**

Afterwards complete all the optimization complaints to obtained an extraordinary sensitivity and low detection limit for the determination of As(III) in different samples then, a linear calibration curve were attained by trickery the intensity signal in contradiction of the concentrations of As(III). The calibration material are shown in Figure (8) and the selected of analytical restrictions are summarized in table (1). The subsequent regression equation was found: \( y = (17.667x - 3.3333) \), and \( r = 0.9906 \) and \( n = 10 \), where \( y \) is the intensity, and \( x \) is the arsenic (III) concentration in ng mL\(^{-1}\). This regression line had a coefficient of determination \( (R^2) \) of 99.6% which recommends it is statistically valid. Analysis of variance (ANOVA) for the regression line was also carried out as shown in Table (2). ANOVA analysis provisions that there is a strong significant relationship amongst the concentration of the analytic and intensity units as F critical, \( F_{1.9} = 23.258 \ll 2170.609 \).

**Table 1 Analysis of Variance of regression line of As(III)**

| Regression statistics |
|-----------------------|
| Multiple R            | 0.9906 |
| R square              | 99.06% |
| Adjusted R square     | 0.998  |
| Standard error        | 0.0042 |
| Observations          | 10     |

**ANOVA**

| Model | Df | SS  | MS  | F    | Significance F |
|-------|----|-----|-----|------|----------------|

![Fig. 8. Calibration graph , As(III) - 2’,7’-dichlorofluorescein complex.](image)
Table 2 Method validation of the spectroflurimetric determination of As (III) using CPE procedure.

| Parameter | Value |
|-----------|-------|
| Excitation wave length $\lambda_{ex}$ (nm) | 271 |
| Emission wave length $\lambda_{em}$ (nm) | 537 |
| Regression equation with CPE procedure | $y=17.667x-3.3333$ |
| Correlation coefficient (r) | 0.9906 |
| Concentration range (ng mL$^{-1}$) | 5.00-50.00 |
| Limit of Detection (ng mL$^{-1}$) | 0.102 |
| Limit of Quantitation (ng mL$^{-1}$) | 0.521 |
| RSD% (n=7) at 5.00 ng mL$^{-1}$ | 2.01 |
| Preconcentration factor | 48 |
| Enrichment factor | 96 |

Precision and Accuracy

While the determination of arsenic ion in water samples by anticipated method was completed by application the standard additions method for the avoided the matrix interferences and to make a high precision, but to safeguard that made a seven reproduce analyses of 10.00 ng mL$^{-1}$ arsenic solution following the general CPE system gave repeatability in term of relative standard deviation (RSD) of 2.01% (table 2). The results were tabularized in Table (3), indicating that there is no highly important systematic error and can be able to application this method for the determination of its ion by high accuracy.

Table 3 Accuracy of the proposed method.

| Amount metal ion taken (ng mL$^{-1}$) | Amount metal ion found (ng mL$^{-1}$) | Rec (%) | $E_{rel}$ (%) | Mean Rec% ± t.s/$\sqrt{n}$ |
|-------------------------------------|-------------------------------------|---------|--------------|--------------------------|
| 10.00 | 10.050 | 97.50 | -2.50 | 96.25±3.75 |
| 15.00 | 15.100 | 96.25 | -3.75 | |
| 20.00 | 20.105 | 95.00 | 5.00 | |

Thermodynamic Parameters

The requirement of the stability constant significance on the temperature, a thermodynamic systems was considered to be accountable for this interaction. The thermodynamic parameters: enthalpy changes ($H$), entropy changes ($S$), and free energy changes ($G$) are the main evidences to
determine the binding mode. The free energy change \( (G) \) can be estimated from the following equation, based on the binding constant at different temperatures.

\[
G = -2.303RT \log K
\]

where \( R \) is the gas constant, \( T \) is the experimental temperature, \( K \) is the stability constant at the corresponding temperature. From the value of stability constant at different temperatures, the enthalpy changes can be calculated by using equation (2):

\[
\log K_2/K_1 = 2.303R \left( H \left( 1/T_1 - 1/T_2 \right) \right)
\]

The entropy changes can be calculated by using equation (3):

\[
G = H - T S.
\]

The stability constant of As(III) complex and other thermodynamic parameters are shown in Table (4) [16].

**Table 4. Stability constant and other thermodynamic parameter**

| Metal -complex | Log K | \( \Delta G \) KJ/mol | \( \Delta H \) KJ/mol | \( \Delta S \) KJ/mol |
|----------------|-------|------------------------|-----------------------|----------------------|
| As \( 3^+ \)   | 50 °C | 60 °C | 50 °C | 60 °C | 50 °C | 60 °C |
| 8.46           | 8.89  | 47.41                  | 52.42                 | 37.10                | 0.185 | 0.185 |

From the results in Table (4) the \( H \) indicating that the extraction reactions are endothermic and the negative values of \( G \) dictates that the solubilization are spontaneous phenomena.

**Determination of As(III) in water samples**

After calculated all the above parameter for found the optimal conditions, this proposed method was applied to the determination of arsenic (III) ion at pH=6.0 in the 15 water samples were taken from Iraqi river other were R.O and tap water samples. For negligible the interferences effect that found in water samples, this determination of its ion was applied by using standard additions method to ensure that determination of trace level of its ion without the other ions that will be found. The results of the proposed method (CPE – spectrofluorimetric) were compared with hydride generator atomic absorption spectrometer method in our laboratory it has also been illustrated in Table (5). The calculated experimental values \( |t| \) was 0.521 for As(III) results. This value is less than the critical \( t_{0.05,12} = 2.18 \) (two-tailed) indicating that no difference between the two means (Ho, \( \mu_1=\mu_2 \)) lead to the same statistical conclusion. For the assessment, the precision of the measurements by both methods, F-test analysis provided evidence that the variability in the proposed method and HGAAS were not significantly different at 95% confidence level as the test statistic \( F \) of 3.21 in As measurements obtained by both methods were less than the critical \( F_{0.05, 6, 6} = 5.82 \) two-tailed.

**Table 5 Spectrofluorimetric determination of As(III) in water samples**

| NO. water samples* | Concentration of As ng mL\(^{-1}\) Introduce method \( ^\pm SD \) | Concentration of As ng mL\(^{-1}\) HG-AAS \( ^\pm SD \) |
|---------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| .1                  | 9.035±0.026                                                   | 9.040±0.027                                                   |
| .2                  | 13.042±0.013                                                  | 13.419±0.042                                                  |
| .3                  | 5.012±0.002                                                   | 5.019±0.050                                                   |
| .4                  | 7.048±0.003                                                   | 7.065±0.062                                                   |
|   | 7.024±0.018 | 7.040±0.024 |
|---|-------------|-------------|
| .6| 11.076±0.029| 11.281±0.012|
| .7| 10.051±0.062| 10.358±0.032|
| .8| 6.036±0.026 | 6.056±0.006 |
| .9| 4.042±0.013 | 4.012±0.003 |
| .10|12.012±0.032| 12.112±0.002|
| .11|13.068±0.043| 13.468±0.003|
| .12|5.024±0.008 | 5.034±0.001 |
| .13|7.026±0.09  | 7.076±0.029 |
| .14|8.031±0.062 | 8.351±0.062 |
| .15|11.056±0.026|11.356±0.026 |

*a*from Al-Hilla river, Babylon Governorate

a mean ± standard deviation; n = 5

b mean ± standard deviation; n = 3

**Conclusions**

A easy spectrofluorimeteric development for the strength of character of arsenic (III) was industrialized. Optimization of the limitations fashioned a constant and exceedingly fluorescence signal for As – 2',7'-dichlorofluorescein complex. This organization developed in a little significance of LOD and LOQ its presented in above your head table for its As(III). Straightforward and quick technique by sensitive usefulness of spectrofluorimeteric determination of its ion.

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