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Su Örneklerindeki Pb(II)-Vitamin B6 Kompleksinin Bulutlanma Noktası Ekstraksiyonu

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Öz

Bu çalışma esas olarak kurşun iyonlarının bulutlanma noktası ekstraksiyonu (CPE) prosedürü ile zenginleştirme ve alevli atomik absorpsiyon spektrometrik tayinine odaklanmıştır. Yöntemde, Pb^{2+} ve B6 vitamininin, önderiştirilmesinden önce, bulutlanma noktası ekstraksiyonu ile kompleksleştirilmesi sağlanmıştır. Çözelti pH'sı, yüzey aktif madde ve B6 vitamini konsantrasyonları, girişim yapan iyonlar, inkübasyon sıcaklığı ve numune hacmi optimize edilmiştir. Yöntemin gözlenebilme sınırı (LOQ) ve tayin sınırı (LOD) değerleri sırasıyla 7,88 ve 25,99 olarak bulunmuştur. CPE yönteminin validasyonu, doğal kaynak suları ve maden suları gibi su örneklerine analit eklenmesi (bilinen Pb^{2+} konsantrasyonu) ile sağlanmıştır.

Anahtar Kelimeler: Bulutlanma noktası ekstraksiyonu; Pb^{2+}; seçicilik; doğal sular; B6 vitamini

Cloud Point Extraction Of Pb (II)-Vitamin B6 Complex In Aqueous Samples[1]

Abstract

This study mainly centered on the preconcentration and flame atomic absorption spectrometric determination of lead ions by the cloud point extraction (CPE) procedure. Complexation of Pb^{2+} and vitamin B6 prior to its preconcentration by cloud point extraction was achieved in the method. The solution pH, surfactant, and vitamin B6 concentrations, interfering ions, incubation temperature, and sample volume were optimized. The limit of quantification (LOQ) and limit of detection (LOD) values of the method were found as 7.88 and 25.99 respectively. The validation of the CPE method was achieved by the analyte adding (known concentration of Pb^{2+}) to water samples such as natural spring waters and mineral waters.

Keywords: Cloud point extraction; Pb^{2+}; selectivity; natural water; vitamin B6

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1. Introduction

The rapid increase in human population and fast industrialization causes environmental pollution by vast variety of metal ions releasing from industrial processes into the nature.

Another source of heavy metals is the natural processes such as volcanic eruptions. Cadmium, arsenic, mercury, chromium, antimony and lead can be considered as toxic metals released from both sources to the environment. These metal ions have significant negative effects on the human, animals, plants and aquatic life. The lower concentration of heavy metal ions –even at trace concentrations- can pollute the soil, air and water [1].

The accumulative characteristics of Pb^{2+}, causes its pollution as an environmental priority because Pb^{2+} is one of the most dangerous toxic elements [2].

Some of the known adverse health effects of lead are anaemia, abdominal pains and headaches. The further effects are the disorders of central nervous system, kidney nephritis and brain damage. The concentration of lead even at the trace levels may cause chronic exposure of lead [3].

Consequently, the maximum tolerable limit of lead in drinking waters is 10 ng mL^{-1}. This level is determined by the world health organization (WHO) in 1996. Therefore, it is of prime importance to monitor and determine the lead concentrations in aquatic environmental samples which are the main indicators of drinking waters [2].

The removal, pre-concentration and separation of the analytes in aqueous medium can be achieved by various procedures such as solid phase extraction (SPE), co-precipitation, liquid-liquid extraction (LLE), ion exchange and electrochemical methods. All of these methods represent an increase in sensitivity and selectivity for the methodology in analytical chemistry. In recent years, a greener preconcentration method was developed called CPE. This separation method is an environmentally friendly method for many analytes from organic molecules to metal ions and has evident benefits over the elder extraction methods. The CPE procedure is simple, inexpensive, accurate, fast, precise and in some circumstances selective and also consuming of toxic organic chemicals is minimal for procedure [4].

The CPE technique is relied on the complexing of a metal ion by using an appropriate chelating agent and its extraction into a micelle medium. The overall process is depending on the basic characteristics of surfactant molecules. These are becoming cloudy to form organized assemblies called micelles over a certain concentration and temperature. The volume of the micelle phase is much lower than the aqueous phase after separation that phenomena allows the preconcentration of analytes.

So, this technique is very useful for pre-concentration and separation of analytes exist in aqueous and other environmental samples [5].

In this work, Pb^{2+} ions were complexed with vitamin B6 prior to separation and preconcentration by CPE from aqueous solutions utilizing a Tergitol NP-7 (TNP-7) as an anionic surfactant, followed by its determination by flame atomic absorption spectrometer (FAAS). Effect of solution pH, the concentrations of TNP-7 and vitamin B6, temperature and incubation time were optimized. The optimization of these parameters is important for determining the efficiency of CPE method. The advanced CPE method has been victoriously practised for the determination and separation of lead ions in water samples.

2. Material and method

2.1. Apparatus

The concentration values of Pb^{2+} ions were measured by Shimadzu AA 7000 FAAS (equipped an air-acetylene burner and combined a lead hollow cathode lamp). The hollow cathode lamp of lead was utilized as the radiation source. The signal of lead was measured as absorbance of it according to Beer's Law. The cloud point temperature of the method was obtained by using a thermostatic bath (Nuve model BM 402, Nuve Ltd. Türkiye). The centrifuge (Nuve model NF-

400, Nuve Ltd. Türkiye) was utilized for the phase separations. The pH values of solution were measured by a WTW level 1 model pH meter.

2.2. Reagents

Non-ionic Tergitol NP-7 surfactant was bought from Sigma-Aldrich. The chelating agent solution ($1.0x10^{-3}$ mol L^{-1} of VitB6) was prepared by dissolving appropriate amount of VitB6 in distilled water. An appropriate amount of $Pb(NO_3)_2$ was dissolved in distilled water and a 1000 mg L^{-1} stock Pb^{2+} solution was prepared. Different concentrations of Pb^{2+} solutions were prepared by diluting from stock solution. Various citrate and phosphate buffer solutions were prepared and used to keep the solution pH stable in the experiments. The correlation coefficient of calibration curve was 0.999. Fig. 1 shows a sample of the calibration curve used in the CPE experiments. The working conditions of FAAS for Pb^{2+} measurements are given in Table.1 and chemical structure of VitB6 is given in Fig. 2.

Table 1. The operation conditions of the FAAS for lead measurement

Current	10mA	**Burner height**	7mm
Wavelength	283.3 nm	**Burner angle**	0 deg
Slit width	0.5 nm	**Fuel gas and flow rate**	Acetylene/2.0 $Lmin^{-1}$
Lighting mode	BCG-D_2	**Type of oxidant**	Air

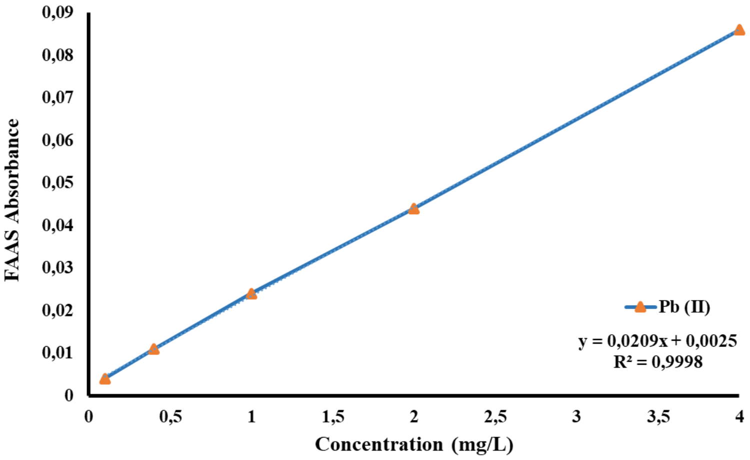

Figure 1. The calibration curve of Pb (II) ions used in experiments

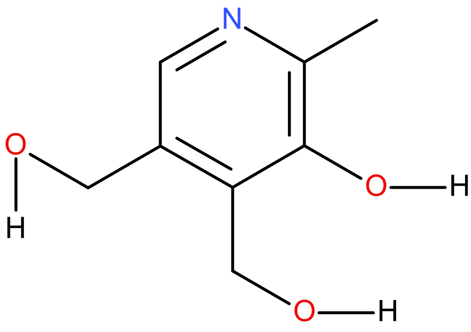

Figure 2. Chemical structure of Vitamin B6 molecule

The ligand and its Pb^{2+} complex must be soluble and highly stable and also should be nontoxic and selective for Pb^{2+}. The vitamin B6 molecules are suitable for all these aspects.

2.3. Procedure for cloud point extraction

1.00 mL Pb^{2+} analyte solution (from10 ppm stock) and 2 ml $1x10^{-3}$ M vitamin B6 solution were mixed and transferred into a 15 ml conical plastic tubes containing 5 mL of buffer. After complexation, 1 mL of 2% (w/v) TNP-7 with the chemical name of nonylphenol ethoxylate was added to the mixture, and diluted to 10 mL with distilled water and shaken. The phase separation was achieved by incubation of the mixture in a thermostatic bath (40 °C, 30 min.) and centrifugation (5 min. at 4100 rpm). The aqueous phase was removed by decantation. The phase of surfactant-rich was dissolved in up to 2 mL of ethanol using 1.0 mL of 1.0 M HNO_3. The concentration of Pb^{2+} was examined by FAAS. Recovery values were determined according to equation 1.

$$R(\%) = \frac{c_t}{c_0} x100 \qquad \text{(Equation 1)}$$

Where c_t and c_0 are the concentrations of Pb^{2+} in sample and reference respectively.

2.4. Sample preparation

In order to validate the proposed CPE method, three different natural spring water and three different brand natural mineral water samples were purchased from the local market, two different tap water samples were gathered from Niğde city centre (37.966135, 34.659311) and Nigde Ömer Halisdemir University (37.942345, 34.626984) and all the samples were subjected for Pb^{2+} analysis. For this purpose, 45 mL from each sample was used in the optimized CPE procedure without an outer purifying process. The pH of the samples studied was determined by utilizing buffer solution at pH 6.

3. Results and Discussion

3.1. Effect of pH

The acidity of the solution acts significant role in cloud point extraction procedure by involving mainly in the complex formation and also micelle aggregation [6]. In our experiments, the Pb^{2+} extraction was examined in various solution pH between pH2-pH8. A suitable complexing is one of the key parameter for separation of Pb^{2+} with a ligand by CPE technique. This phenomenon firstly depends on the pH of the solution at which an appropriate Pb (II)-VitB6 complex formation takes places. For this purpose, experiments were conducted between pH 2 and pH 8. The acquired results were shown in Fig 3.

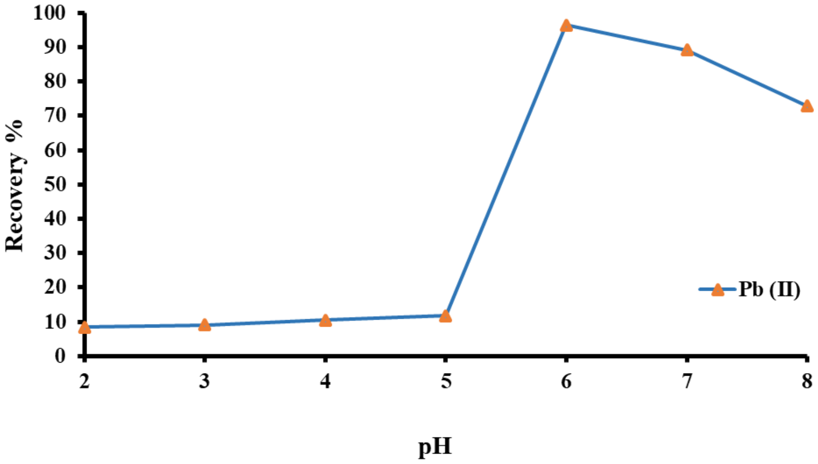

Figure 3. The effect of solution pH on the preconcantration of Pb (II) ions.

According to Fig 3. Pb (II)-VitB6 complexes were quantitatively extracted at pH 6. The extraction values were lower than 20% up to pH 5 and 90%, 78% for pH 7 and pH 8 respectively. This behaviour can be explained with complexation characteristics, thermodynamic interactions and the chemical structure of the VitB6 molecule.

In conclusion, the optimum pH was found as pH 6 and this value was utilized for all experiments.

3.2. *Effect of temperature*

The temperature of the extraction medium is a tool parameter in CPE systems [7]. This behaviour is directly related with the micelle formation by the surfactant used in experiments. Because any surfactant that forms micelles requires a temperature above its specific CPT (Cloud Point Temperature). So, the effect of equilibrium temperature was studied between 20 °C to 60 °C. The obtained results were indicated in Fig 4.

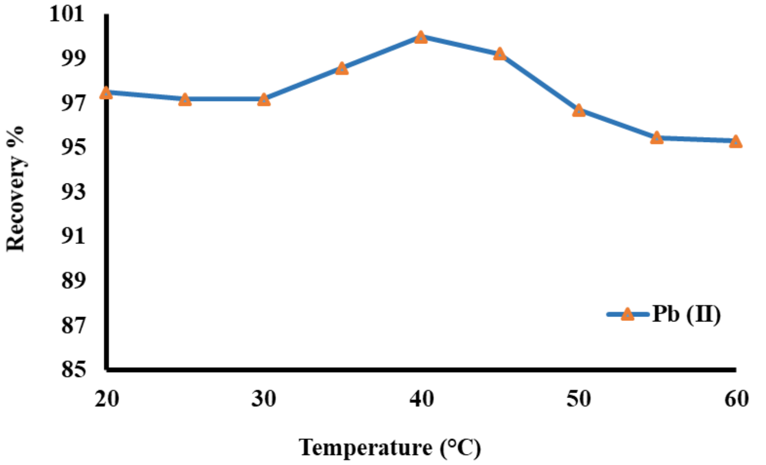

Figure 4. The effect of solution temperature on the recovery of Pb (II) ions.

According to Fig. 4, the recovery values were quantitative for all studied temperatures. But the highest recovery value was gained at 40 °C. This can be explained by the chemical property of the surfactant TNP-7 of which has a very low CPT (20 °C) [8].

The phase separation is correlated with micelle formation in a cloud point extraction so it also depends on the temperature. The phase separation was not complete at temperatures lower than 20 °C. The obtained results revealed that the 40 °C of temperature was optimum and the experiments were completed at this value.

3.3. *Effect of TNP-7 concentration*

The optimization of surfactant concentration in CPE experiments is of prime important [9]. The reason is that the technique is rest on the characteristics of surfactants. In this method, surfactant molecules become cloudy at a certain concentration which is called as critical micelle concentration.

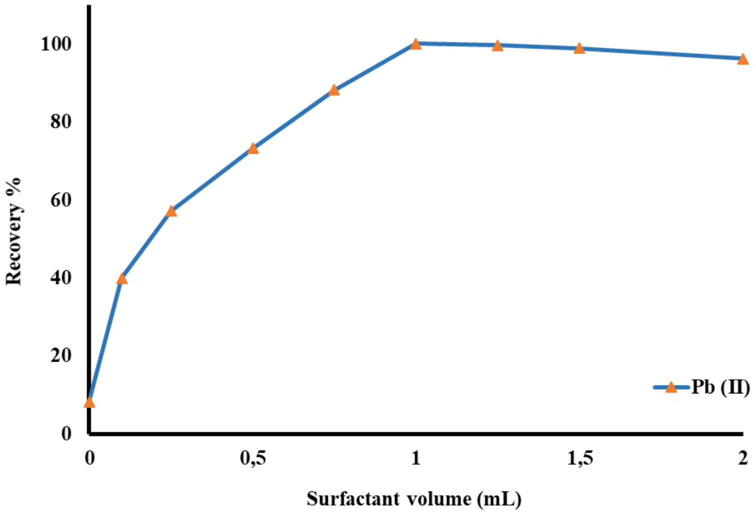

Figure 5. The effect of surfactant volume on the recovery of lead ions.

Thus, volume of TNP7 (from 2%, v/v stock solution) was investigated between 0 and 2,0 mL to determine the concentration of maximum recovery value. The obtained results were given in Fig. 5. The recovery of Pb^{2+} ions were raised up to 1,0 mL of surfactant volume and reached to a maximum value of 100% and after this point recovery values remained quantitative although small changes. First manner can be explained as reaching the CMC and latter is a result of equilibrium between formed micelles and TNP7 monomers in aqueous solution [10]. In conclusion the optimum surfactant was chosen as 1,0 mL and applied for all the experiments.

3.4. *Effect of sample volume*

Sample volume is a substantial analytical parameter in pre-concentration studies. In this work, the sample volume effect was examined in the range between 10–50 mL. This can be a small value for pre-concentration but same limitations seem as a drawback for CPE studies. According to obtained results all recovery values for Pb^{2+} were quantitative (10–50 mL). According to sample volume results the maximum preconcentration factor is obtained as 25.

3.5. *Interferences*

The VitB6 ligand was used as a chelating agent to bind the Pb^{2+} ions to extract. So, the possibility of complexation with several transition metals, alkaline, alkaline-earth and other ions in aqueous media was investigated by using proposed method at pH 6. It is obvious from the chemical structure of VitB6 (Figure 2) that the molecule can bind the Pb^{2+} ions from –O and/or –N atoms. The interference experiments were performed by replacing each variable matrix ion separately at a time. Obtained results were shown in Table 2.

Table 2. Effect of matrix ions on recovery of lead ions

Ion	Added as	Concentration (mg l^{-1})	Pb^{2+} recovery (%)
K^+	KNO_3	1000	102 ± 7*
Cl^-	NaCl	1000	98 ± 4
SO_4^{2-}	Na_2SO_4	1000	97 ± 3
Mg^{2+}	$Mg(NO_3)_2$	1000	97 ± 1
Ca^{2+}	$Ca(NO_3)_2$	50	98 ± 2
Al^{3+}	$Al(NO_3)_3$	50	99 ± 4
Co^{2+}	$Co(NO_3)_2$	50	142 ± 9
Ni^{2+}	$Ni(NO_3)_2$	50	138 ± 2
Cu^{2+}	$Cu(NO_3)_2$	50	136 ± 4
Cd^{2+}	$Cd(NO_3)_2$	50	130 ± 9
Cr^{3+}	$Cr(NO_3)_3$	50	135 ± 9

*Mean ± standard deviation

Table 2 shows that the recovery of Pb^{2+} did not significantly change in the existence of selected interfering ions in isolated solution. The table 2 also proved there is no possible competitive chelating effect.

3.6. Analysis of real samples

The real sample analyses were performed on three different natural mineral waters and five different drinking water samples. These tests were applied by analyte addition of Pb^{2+} in to the samples. Obtained data were given in Table 3.

Table 3. Analyte addition-recovery tests by optimized CPE method.

Pb^{2+}			
Samples	**Added (µg)**	**Found (µg)**	**Recovery (%)**
Natural Spring Water I	5.0	4.9	98
Natural Spring Water II	5.0	4.8	96
Natural Spring Water III	5.0	4.9	98
Tap Water I (City centre)	5.0	5.1	102
Tap Water II (City centre)	5.0	5.0	100
Natural Mineral Water I	5.0	5.2	104
Natural Mineral Water II	5.0	4.9	98
Natural Mineral Water III	5.0	5.1	102

*Mean ± standard division

The recovery values given in table 3 clearly indicates that the CPE technique could succesfully be practised to analyze the Pb^{2+} in water samples.

4. Conclusions

A selective and efficient CPE procedure was optimized for isolation, determination and pre-concentration of Pb^{2+} in aqueous samples prior to their FAAS determination. Obtained results showed that the VitB6 ligand was highly selective for Pb^{2+} at pH 6. The matrix studies revealed that the method is successful to isolate the Pb^{2+} in the existence of earth, earth-alkaline and major common ions. The developed method successfully applied to analyse of Pb^{2+} ions in real samples. The optimized CPE method is inexpensive, eco-friendly and time saving to operate. In conclusion, the method may be considered as a useful tool for definition of Pb^{2+} ions in water samples.

5. Statement of contribution

Graduate student N.B: Collecting real samples, conducting extraction studies, discussing results and writing article. Y.S: Development of methodology, design and execution of optimization studies, discussion of results and article writing.

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