Separation and Simultaneous Determination of Pb(II) and UO₂(II) Using a Ligand Containing Mobile Phase in Reversed-Phase High Performance Liquid Chromatography

MAJID SOLEIMANI[1,*], ALI MORSALI[2] and MAJID GHAHREMAN AFSHAR[1]

[1]Department of Chemistry, Imam Khomeini International University, Qazvin, Iran
[2]Department of Chemistry, Tarbiat Modarres University, Tehran, Iran

*Corresponding author: Fax: +98 2813780040; E-mail: m-soleimani@hotmail.com

Separation and determination of Pb(II) and UO₂(II) was performed by a simple, isocratic, reversed phase high performance liquid chromatography using mobile phase containing 1,4-*bis*-(4-pyridyl)-2,3-diazo-1,3-butadiene (4-bpdb) as a ligand. The resolution was determined with various ligand concentrations as well as the ratio of methanol in aqueous mobile phase at different pH values. The effect of ionic salts, type and concentration of buffer on the separation is also discussed. The results showed that a good separation was obtained at 25 ℃, using a mobile phase containing 0.05 mM (4-bpdb), 30 mM acetate buffer and 20 % methanol at pH 6.5. The response was linear from 0.1 to 0.5 mM with a detection limit of 5.8916×10^{-3} mM for Pb(II) and 15 to 25 mM with a detection limit of 0.2626 m M for UO₂(II), respectively.

Key Words: Complexation, 1,4-*Bis*-(4-pyridil)-2,3-diazo-1,3-butadiene, Uranyl, Lead, HPLC.

INTRODUCTION

Because of toxicity of trace heavy metal ions in human health, environmental pollutions by heavy metal are most important for public health[1]. Among these, uranium and lead are the most toxic metal ions. The toxicology of uranium is complicated by the dual nature of the biological effects of uranium on organisms. Biological deficiency produce by uranium are the result of its toxicity, whereas the effects are due to the radioactivity of delayed uranium[2]. But lead is proved to be a carcinogenic agent and to cause disturbances to several organs[3]. The development of nuclear science and technology in the peaceful uses of nuclear energy has been ongoing for a long period. The reduction of radioactive waste and further improvement of nuclear safety and reliable environment management system are the tasks that remain to be solved by exploring the wider application of nuclear technology. Uranium and lead are the elements that contribute to the long-term radiotoxicity and toxicity of nuclear waste. Then, the necessity of separation and determination of these metal ions in environmental samples are more importance in recent years. The trace analysis of uranium and lead in environmental sample is particularly difficult because of the complex matrix, the low concentration of these elements and the presence of interfering matrix, which requires sensitive instrumental techniques and frequently a separation or pre-concentration step.

Several methods have been described for the separation and determination of uranyl and lead ions, *e.g.* complexation[4], organic sorbents and adsorbents[5-9], solvent extraction[10,11], cloud point extraction[3], membranes[12,13], coprecipitation[14], interaction with resins[15,16], molecularly imprinted ion exchange resin[2] and solid phase extraction[17].

Although the capability and sensitivity of analytical instrumentations have increased in recent years, the routine determination of trace heavy metals in complex media is still difficult. In fact, most analytical techniques do not permit direct analysis of heavy metal ions in such matrix samples. Hence, recent research reveals the spread of variously pretreated methods. In every case, the aim is to concentrate or separate of each analyte in order to improve the detection limit and reduce the matrix effect, thus enhancing the analytical conditions.

High performance liquid chromatography (HPLC) has been mainly used as a technique for separation of organic and inorganic solutes and its leading position in this role remains unchallenged. Some of the attractive features of this technique are fast and high-efficiency separation, sensitive detection capability, low instrumentation and operating costs. The present paper investigate the application of 1,4-*bis*-(4-pyridyl)-2,3-diazo-1,3-butadiene (4-bpdb) as a new ligand for separation and determination of Pb(II) and UO₂(II) by using HPLC (Fig. 1).
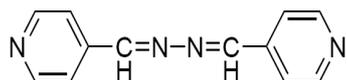
Fig. 1. Structure of 1,4-*bis*-(4-pyridyl)-2,3-diazo-1,3-butadiene (4-bpdb)

EXPERIMENTAL

HPLC was performed by using KNAUER-HPLC (Germany) equipped with a K2500 UV-Visible detector and a Mod.7125 Rheodyne injector with a 20 µL external loop. The column was vertex (KNAUER Co.) 250 × 4 mm I.D packed with Eurospher-100 C18, 5 µm. The elution was carried out under isocratic condition at a flow-rate of 1 mL min⁻¹. A B2000 pH meter equipped with a GCFC 11 combination glass electrode was used for pH measurements. All glassware containers were carefully treated with 2.0 M nitric acid (guaranteed reagent) and rinsed with HPLC grade water.

The ligand 1,4-*bis*-(4-pyridyl)-2,3-diazo-1,3-butadiene (4-bpdb) ligand was prepared by using method previously reported[18]. HPLC grade methanol was obtained from Merck (Germany). All solutions was prepared by HPLC grade water and then filtered on a 0.45 µ PTFE filter. All other reagents were analytical grade unless stated otherwise.

General procedure: A solution containing 0.05 mM of 4-bpdb in methanol-water (20:80) was prepared as a mobile phase. The pH adjusted at 6.5 using 30 mM of sodium acetate-acetic acid buffer. The other parameters were as follow: flow-rate, 1.0 mL/min; the column temperature, 25 °C; wavelength for UV detection, 228 nm; the analysis time was less than 12 min. The capacity factors were calculated using k = $(t_r-t_0)/t_0$, where t_r is retention time of a sample solute averaged over repeated measurements at the peak of the elution curve and to is that of a non-absorbed substance that nitrate ion was used. Determination of Pb(II) and UO₂(II) in samples were carried out by two calibration curves method using the peak height method.

RESULTS AND DISCUSSION

Chromatographic behaviour of UO₂(II) and Pb(II) chelates: Fig. 2 shows typical chromatograms of standard solution of a: Pb(II) b: UO₂(II) and c: mixture of Pb(II) and UO₂(II) by UV detection at 228 nm. Sample standard solutions were prepared by dissolving suitable amounts of UO₂(CH₃COO)₂· 2H₂O and Pb(NO₃)₂ in the mobile phase. As can be seen from the chromatograms, two peaks appeared due to different in adsorption of complexed ions. Because the retention is mainly based on the partition between non-polar stationary phase and polar mobile phase, the RP-HPLC behaviour of analytes is often estimated from polarity of these compounds.

Effect of 4-bpdb concentration: Some preliminary experiments showed that acetate ion is a suitable ion for forming of UO₂(II) and Pb(II) complexes, because separately chromatographic peaks were obtained for UO₂(II) and Pb(II) ions even in the absent of (4-bpdb). But a long analysis time and wide peak widths were observed.

The dependence of resolution on the concentration of (4-bpdb) was investigated by using mobile phases containing 0.00625-0.3 mM ligand at pH 6.5. In 0.05 mM 4-bpdb, resolution,
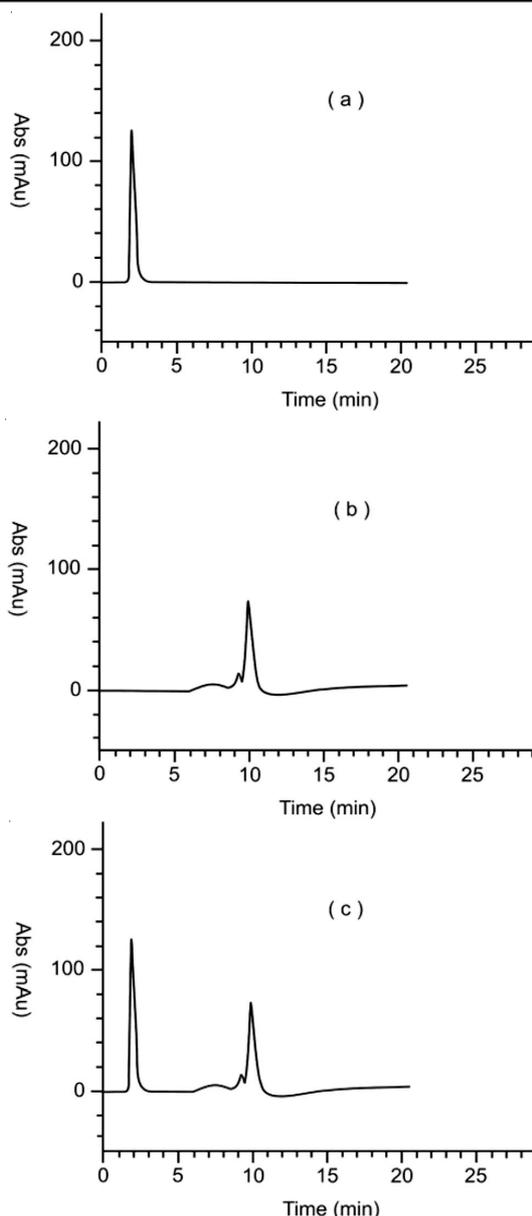

Fig. 2. Typical chromatograms obtained from standard solutions of the samples; a: Pb(II) b: UO₂(II) c: mixture of Pb(II) and UO₂(II)

analysis time and peak widths are optimum. The increasing of 4-bpdb concentration over than 0.05 mM decreased peaks height and method sensitivity because high matrix absorbance and lower concentration caused a decreasing in the resolution (Fig. 3).

Effect of pH, type and concentration of buffer: The influence of type of buffer in water phase portion of the eluent was investigated by 50 mM acetate, phosphate, citrate and formate buffer at pH 6.5. The results showed, acetate buffer is performed a suitable conditions for UO₂(II) and Pb(II) complexes formation with 4-bpdb. So, the influence of concentration of acetate buffer was studied in the ranging of 10-70 mM (Fig. 4). The obtained results showed that increase of buffer concentration more than 30 mM did not influence on the resolution of ions, while decreasing in concentration of buffer less than 30 mM increased it. Therefore, a good resolution and optimal analysis time was obtained in 30 mM buffer concentration.
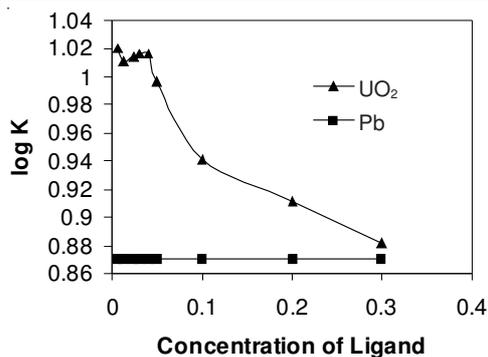
Fig. 3. Dependence of capacity factor(k) of Pb(II) and UO$_2$(II) on (4-bpdb) concentration
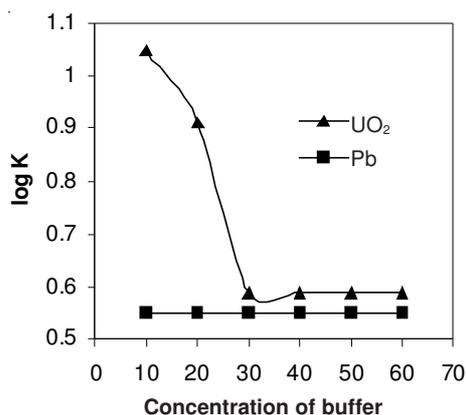

Fig. 4. Dependence of capacity factor of Pb(II) and UO$_2$(II) on acetate buffer concentration

Also, the effect of pH on separation and determination of UO$_2$(II) and Pb(II) ions were studied in pH range of 2.5-7.5, using a acetate buffer solution (30 mM) and HCl (1 M) or NaOH (1 M) for pH adjustment. An increase in the pH values of eluent from pH 2.5-6.5 dramatically increased the resolution of ions but more than pH 6.5 did not improve the resolution of peaks (Fig. 5).
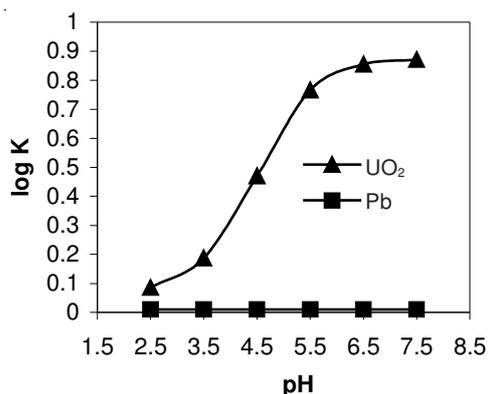

Fig. 5. Dependence of capacity factor of Pb(II) and UO$_2$(II) on the pH of mobile phase

Choice of eluent: The solvent effect on resolution was studied using water-methanol (90:10 to 50:50 v/v) mobile phase containing 0.05 mM (4-bpdb) at pH 6.5. The increasing methanol content in the eluent caused a decrement in peak widths, resolution and analysis times. A perfect separation (Rs = 5.3) was obtained by 20 % methanol content (Fig. 6).
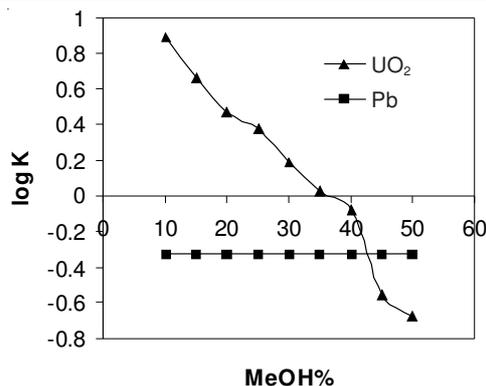

Fig. 6. Dependence of capacity factor of Pb(II) and UO$_2$(II) complexes on MeOH %

Calibration graph and limit of detection: The peak height of UO$_2$(II) complex was directly proportional to UO$_2$(II) concentration in the range from 15 to 25 mM and the regression equation (three-point calibration) was h(cm) = 5.3298C$_{(UO_2)}$ - 43.1123 with a correlation coefficient of 0.9992. The RSD of peak height for three replicate determination of 1.0×10^{-3} M UO$_2$(II) were 1.8 % and the peak height of the Pb(II) complex was directly proportional to Pb(II) concentration in the range from 0.1 to 0.5 mM and the regression equation (three-point calibration) obtained h (cm) = 237.5404C$_{(Pb)}$ + 57.4382 with a correlation coefficient of 0.9999. The RSD of peak height for three replicate determination of 1.0×10^{-3} M Pb(II) were 0.8 %. The limit of detection (LOD) of the method for the determination of UO$_2$(II) and Pb(II) was studied under the optimal conditions. The LOD obtained from C$_{LOD}$ = 3 S$_b$ m^{-1}, where S$_b$ is standard deviation of blank for ten replicate determination and m is the slope in the regression equations. The LOD was calculated 0.26 mM for UO$_2$(II) and 5.89×10^{-3} mM for Pb(II), respectively.

Analytical application: We have explored the feasibility of the proposed method for the determination of UO$_2$(II) and Pb(II) in different environmental matrices by standard addition method. To investigate the matrix effect, the regression equations of standard samples were used for calculating UO$_2$(II) and Pb(II) concentrations in different water samples. The results for this study are presented in Table-1 for three different water samples. The recovery of samples was satisfactorily reasonable and was confirmed using addition method, which indicated the capability of the system in the determination of UO$_2$(II) and Pb(II). The recovery values calculated for the added standards were normally more than 91 and 99 % for UO$_2$(II) and Pb(II) respectively, which confirm the accuracy of the procedure and its independence from the matrix effects.

TABLE-1
DETERMINATION OF Pb(II) AND UO$_2$(II) IN
DIFFERENT ENVIRONMENTAL MATRICES

Matrix	Sample	Added (Mm)	Recovery (%)	RSD (%)
Tap water	UO$_2$(II)	17.50	92.00	1.41
(Mehrshahr-Iran)	Pb(II)	0.20	101.00	2.05
Damavand mineral	UO$_2$(II)	21.00	94.07	5.97
water (Iran)	Pb(II)	0.35	99.37	0.58
Chaloos river water	UO$_2$(II)	20.00	91.07	1.97
(Iran)	Pb(II)	0.40	99.21	0.92

Conclusion

The presented RP-HPLC method had the following advantages: simple, rapid, sensitive, reproducible and low analysis costs. The recoveries of analyte ions were more than 91 and 99 % for $UO_2(II)$ and $Pb(II)$ respectively at the optimal working conditions. The time required for the separation and determination of analytes was about 10 min. The analyte ions can be sensitively determined without any influence of matrix ions. The RP-HPLC method can be applied for the separation and determination of $UO_2(II)$ and $Pb(II)$ in different environmental matrices.

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