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Spectrophotometric Determination of Lead in Environmental and Biological Samples by Flow Injection Microcolumn Preconcentration and Separation using DBMCSA Chromogenic Agent

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The synthesis and purification procedure of dibromo-p-methyl-chlorosulphonazo (DBMCSA) chromogenic agent have been described. This agent was used for spectrophotometric determination of lead. In 0.16 mol L^{-1} nitric acid solution lead reacts with DBMCSA to form a 1:2 blue-coloured complex of maximum absorbance at 633 nm. Under the optimum conditions Beer's law was obeyed over Pb(II) concentration range 0–1.2 μg mL^{-1} and the apparent molar absorptivity was 9.48×10^4 L mol^{-1} cm^{-1}. Detection limit and variation coefficient were found to be 2.4 ng mL^{-1} and 1.2%, respectively. No interferences from the selected foreign ions were observed except for Sr(II), Ca(II) and Ba(II). However, these interferences were easily eliminated by sorption and separation using microcolumn packed with cigarette filter. The developed method was applied to the determination of lead in environmental and biological samples.

Opisano syntezę i oczyszczanie dibromo-p-metylo-chlorosulfonazolu (DBMCSA). Odczynnik ten zastosowano do oznaczania ołowiu. W 0,16 mol L^{-1} roztworze kwasu azotowego ołów reaguje z DBMCSA tworząc 1:2 niebieski kompleks (maksimum absorpcji 633 nm). W optymalnych warunkach prawo Beer'a jest spełnione w zakresie stężeń Pb(II) 0–1,2 μg mL^{-1}, a molowy współczynnik absorpcji wynosi $9{,}48 \times 10^4$ L mol^{-1} cm^{-1}. Granica wykrywaności i względne odchylenie standardowe wynoszą odpowiednio: 2,4 ng mL^{-1} i 1,2 %. Inne jony nie przeszkadzały w oznaczeniu, z wyjątkiem Sr(II), Ca(II) i Ba(II).

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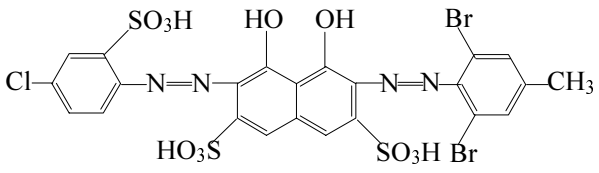
Wpływ ich usunięto przez sorpcję kompleksu Pb(II) z DDTC na mikrokolumnie wypełnionej filtrem papierosowym i oddzielenie od przeszkadzających pierwiastków. Opracowaną metodę zastosowano do oznaczania ołowiu w próbkach środowiskaowych i biologicznych.

Lead is an accumulative toxic element. Combustion of coal and oil, and, especially, wide application of lead and its compounds in industry, has introduced large amounts of lead to the environment [1]. Lead present in plant tissues is one of the easily-absorbed sources of this element by human body. Lead uptake in humans results in its accumulation in bones, liver, kidneys and other parts of the body, which, in turn, affects functioning of hematopoietic, nervous, rennin-angiotensin and reproductive systems [2]. Consequently, the development of reliable methods for the determination of lead in environmental and biological samples is of special interest. Among the already developed methods, including spectrophotometry [3], atomic absorption spectrometry [4], inductively coupled plasma atomic emission spectrometry [5], inductively coupled plasma mass spectrometry [6], and X–ray fluorescence spectrometry [7], spectrophotometric methods are the most important, especially in less-developed countries, due to their simplicity and low operating costs. However, the availability of chromogenic agents for spectrophotometric determination of lead is limited. The following agents are mainly used: dithizone [8], 4-(2-pyridilazo) resorcinol [9], diphenylcarbazone [10], ArsenazoIII [11], 2-(2-thiazolylazo)-p-cresol [12] and porphyrin compounds [3]. The above agents provide different sensitivity, selectivity and rapidity of the determination. Moreover, most of them require extraction with organic solvent, the use of surfactants or highly toxic cyanide as a masking agent to improve the sensitivity or selectivity.

In this work dibromo-p-methyl-chlorosulfonazo (DBMCSA) agent (I), an asymmetric bis-azo derivative of chromotropic acid with a single o-sulfonic functional group, was synthesised and applied to the spectrophotometric determination of lead in environmental and biological samples by flow-injection microcolumn preconcentration and separation. Conditions required for the formation of coloured Pb-DBMCSA complex and for elimination of interferences from the co-existing ions were investigated in detail.



(I)

EXPERIMENTAL

Synthesis of DBMCSA

p-Chloro-acetylphenylamine. p-Chlorophenylamine (10 g) and glacial acetic acid (20 mL) were placed in a three-necked flask and 24 mL of acetic anhydride were added using a dropping funnel. The contents were stirred for 2 h at the temperature of 50–60°C. After 2 h the reaction mixture was cooled to the room temperature. The formed precipitate was filtered, washed with water and dried applying infrared light. The greyish white solid product was obtained at the yield of 96%, m.p. = 172–174°C.

p-Chlorophenylamine-2-sulfonic acid. p-chloro-acetylphenylamine (10 g) and concentrated sulfuric acid (8.5 g) were placed on evaporating dish and heated using a sand bath. When the temperature reached 140–190°C, acetic acid evaporated and the reactants solidified. Stirring was continued for 2 h at 170–180°C. After that time, the contents were cooled below 100°C. Activated carbon was added and the mixture was re-crystallised with water. p-Chlorophenylamine-2-sulfonic acid was obtained at the yield of 64.6%.

2,6-Dibromo-p-methylphenylamine. p-methylphenylamine (2 g) was dissolved in 25 mL of 1:1 HCl and 2 mL of bromine (previously mixed with 5 mL of acetic acid) were added using a dropping funnel. The mixture was stirred for 3 h at the temperature below 40°C. After that time, the formed precipitate – 2,6-dibromo-p-methylphenylamine – was filtered, washed with water and dried at 80°C. The reaction yield was 88%.

ChlorosulphonazoI. p-Chlorophenylamine-2-sulfonic acid (2.08 g) was dissolved in 15 mL of 1 mol L^{-1} NaOH. 0.7 g of sodium nitrite was added and the contents were mixed thoroughly. Next, the mixture was dropped into 10 mL of 1:1 cold HCl to let diazotization reaction proceed. The reaction temperature was maintained below 5°C. To the obtained mixture 4.0 g of chromotropic acid in 25 mL of 40% (m/m) NaAc solution were added under continuous stirring. The mixture was left for 4 h. After that time, it was acidified with 15 mL of concentrated HCl, left again overnight, and finally filtered. The obtained precipitate was washed with 25 mL of 1:1 HCl and dried under infrared light. One obtained 4.3 g of chlorosulfonazoI at the yield of 82.8%.

Dibromo-p-methyl-chlorosulphonazo agent. Dibromo-p-methylphenylamine (2.65 g) was dissolved in 30 mL of 1:1 HCl. The solution was cooled below 5°C and diazotization reaction was performed by adding sodium nitrite (1.0 g in 5 mL of water) dropwise. Chlorosulfonazol (5.3 g) was dissolved in LiOH solution (8.0 g LiOH in 80 mL of water) and the obtained solution was cooled below 5°C. Next, it was added to the diazotized mixture under constant stirring and pH was adjusted to 9–10 with 10% LiOH solution. After that, the mixture was left for 4 h. After filtering, 50 mL of concentrated HCl were added to the filtrate and the mixture was left overnight to let DBMCSA precipitate. Finally, the precipitate was filtered, washed with 80 mL of 1:1 HCl, and dried using an infrared light. DBMCSA was obtained at the yield of 52%.

Purification of DBMCSA. The obtained DBMCSA (2 g) was soaked in 100 mL of 1 mol L^{-1} HCl for 1 h and then filtered. Concentrated HCl (20 mL) was added to the precipitate and left overnight. Next day, the precipitate was filtered, washed several times with 50 mL of 1:1 HCl, and dried using infrared light.

Purity of DBMCSA was checked by paper chromatography using the mixture of 5% sodium citrate and 25% ammonia (v:v = 5:2) as an eluent. The presence of a single blue spot in the chromatogram con-

firmed purity of DBMCSA. The content of water in DBMCSA was estimated to 10.34% by thermogravimetric analysis. The result of elemental analysis of DBMCSA (C 30.55%; H 2.54%; N 5.93%; S 10.28%) corresponded well to its theoretical stoichiometry: C 30.23%; H 2.86%; N 6.12%; S 10.50%.

Pure DBMCSA is a black-purple powder, well-soluble in water. Aqueous solution of DBMCSA is stable for at least half a year at the room temperature.

Apparatus

Absorption spectra and absorbance measurements were carried out using a LAMBDA–35 spectrophotometer (America PE Co.) equipped with 1 cm-in-width cuvettes. For comparison of the results, a Hitachi atomic absorption spectrometer, Model 180–80 equipped with a high-intensity Pb hollow cathode was used to determine the concentration of lead in the investigated samples under flame conditions recommended by the manufacturer.

A FIA–3100 flow injection system (Vital Instruments Co. Ltd., Beijing, China) was used for preconcentration and separation of lead. The system comprised two peristaltic pumps and a standard rotary injection valve (eight ports on both the rotor and the stator). Rotation speed of two peristaltic pumps, their stop-and-go intervals, and actuation of the injection valve were programmed (Tab. 1). Ismaprene pump tubes were used to deliver the samples and the reagents. Small bore PTFE tubings (0.35 mm-in-I.D.) served as connections to keep all the paths possibly the shortest and to minimise dead volumes.

A PTFE microcolumn (20 mm × 2 mm I.D.,) used for preconcentration and separation was packed with a cigarette filter sorbent.

Table 1. The sequence of operational parameters for the on-line FI microcolumn separation of lead required for the elimination of interferences

Step	Function	Time, s	Pumped	Flow rate, mL min^{-1}		Valve position
				Pump 1	Pump 2	
1 (Figure 1-1A)	sample loading	15	sample	off	4.0	fill
			0.1% m/v DDTC		4.0	
2 (Figure 1-1A)	remove residual	15	air	4.0	off	fill
3 (Figure 1-1B)	analyte elution	60	HNO_3 (1 mol L^{-1})	4.0	off	inject

Reagents

All reagents were of the highest available purity (at least of analytical grade). Doubly deionised water (DDW) was used throughout.

Standard stock solution of lead (1 mg mL^{-1}) was prepared by dissolving 0.1077 g of lead oxide (specpure) in 2 mol L^{-1} HNO_3 solution. Excessive acid was evaporated and the solution was diluted with HNO_3 (v:v = 1:100) up to 100 mL. Working standard solution (10 μg mL^{-1}) was prepared by the stepwise dilution of the standard stock solution with DDW.

0.1% (m/v) DBMCSA solution was prepared by dissolving 0.100 g of the compound in 100 mL of DDW.

0.1% (m/v) solution of diethyldithiocarbamate chelating agent (DDTC, Beijing Chemicals Co., Beijing, China) was prepared by dissolving 0.100 g of the compound in 100 mL of DDW.

Sample preparation

Two certified reference materials (CRMs): GBW 08501 (peach leaf) and GBW 08502 (rice) were analysed to investigate the accuracy of the developed method. River water, wastewater and biological samples were collected locally. River water and wastewater samples were taken from local chemical manufacture, and filtered. 30–60 mL of the filtrates were concentrated on a hot plate, acidified with 1 mL of 0.1 mol L^{-1} HNO_3 solution, transferred into a 10 mL calibrated flask, and diluted to the mark with DDW. Biological samples, after collection, were dried at 105°C, ground in an agate mill, and homogenised. 5–10 g of the samples were weighed, placed on evaporating dishes, and burned to ash at 500–550°C in a muffle furnace. After that, the ash was dissolved in 1–2 mL of 0.1 mol L^{-1} HNO_3 solution. The obtained mixture was transferred into a 10 mL calibrated flask and diluted to the mark with DDW.

Procedures

Procedure for the determination of lead (Procedure A). Sample amount equivalent to 30 μg of lead was transferred into a 25 mL calibrated flask, 4 mL of 1 mol L^{-1} nitric acid and 4 mL of 0.1% DBMCSA solutions were added successively. The obtained solution was diluted to the mark with water, mixed well, and left for 10 min. The absorbance was measured at 633 nm in a 1 cm-in-width cuvette against the reagent blank.

Procedure for the elimination of interferences from foreign ions (Procedure B). To eliminate the interferences from foreign ions in complex matrices, an on-line FI microcolumn sorption and separation was applied. The FI manifold utilised in this study is shown in Figure 1 (for two different valve positions). The details concerning duration and performance of each step of the on-line microcolumn sorption and separation are given in Tab. 1. In step 1 (Fig. 1A), the Pb-DDTC complex was formed on-line and sorbed onto the microcolumn. In step 2 (Fig. 1A), the air flow moved the effluent from the microcolumn to the waste. In step 3 (Fig. 1B), 1 mol L^{-1} HNO_3 solution was introduced into the microcolumn to elute the sorbed analyte and, subsequently, to deliver the eluted analyte into a 25 mL calibrated flask. Finally, the absorbance of the eluted analyte was measured as described in procedure A.

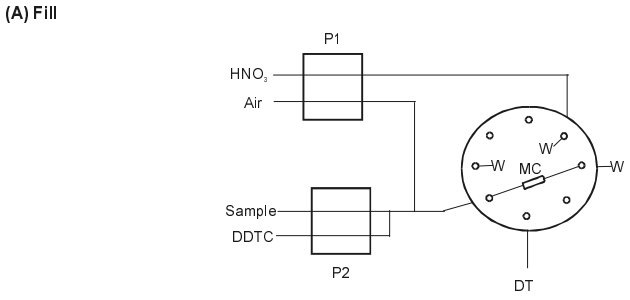


Figure 1. FI manifold for the microcolumn preconcentration and separation of lead: P1 and P2 – peristaltic pumps; MC – microcolumn; DT – delivery tubing; W – waste; (A) fill; (B) inject. Continuation on the next page

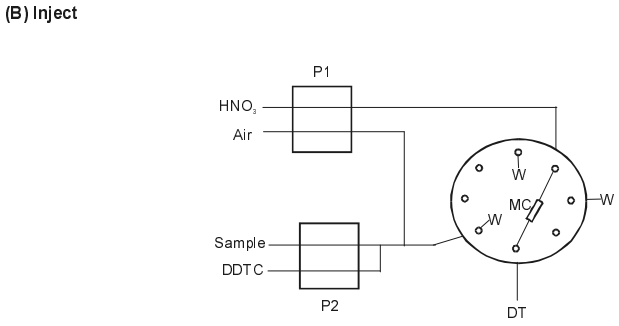


Figure 1. (Continuation)

RESULTS AND DISCUSSION

Absorption spectra

Absorption spectra of DBMCSA and Pb-DBMCSA complex were recorded. Maximum absorbance of DBMCSA and Pb-DBMCSA complex were measured at 533 nm and 633 nm, respectively. Absorption peaks were separated by 100 nm. The wavelength of 633 nm was chosen for further quantitative analysis (Fig. 2).

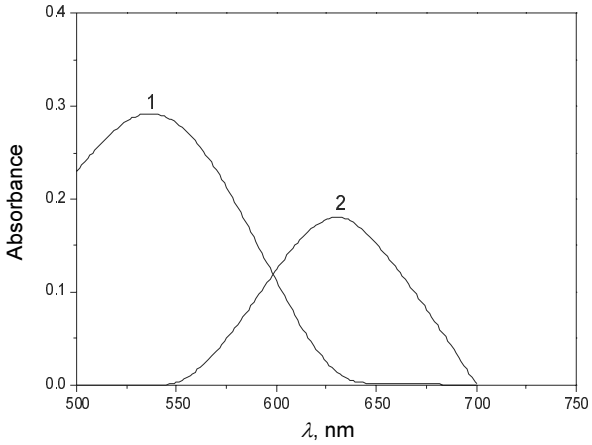


Figure 2. Absorption spectra of Pb (II)-DBMCSA complex and DBMCSA: 1 mL of 10 μg mL^{-1} Pb(II) + 4 mL of 1 mol L^{-1} HNO_3 + 4 mL of 0.1% DBMCSA in 25 mL of the solution. (1) DBMCSA against water; (2) complex against the blank

Influence of acids and acidity

The influence of various acids on the formation of Pb(II)-DBMCSA complex was investigated. It has been found that the absorbance of the complex remained almost unchanged in HCl, H_3PO_4, $HClO_4$, H_2SO_4 and HNO_3 solutions in the following concentration ranges: HCl 0.24–1.2 mol L^{-1}; H_3PO_4 0.12–0.30 mol L^{-1}; $HClO_4$ 0.24–1.44 mol L^{-1}; H_2SO_4 0.48–1.2 mol L^{-1} and HNO_3 0.04–0.28 mol L^{-1}. Owing to the fact that nitric acid provides ideal conditions for the sorption of Pb-DDTC complex onto the cigarette filter, HNO_3 solution was used in the analysis. In the solution volume of 25 mL in the presence of 1–7 mL of 1 mol L^{-1} HNO_3 the measured absorbance was maximum and constant. Considering both the selectivity of the determination and the reaction rate, the concentration of HNO_3 of 0.16 mol L^{-1} in a total volume of 25 mL was applied. This corresponded to the addition of 4 mL of 1 mol L^{-1} HNO_3 to the reaction solution.

Effect of the amount of DBMCSA

With the increase of the added volume of 0.1% DBMCSA solution, absorbance increased rapidly, levelled off at larger volumes of DBMCSA solution, and finally slowly decreased. Absorbance of 25 mL of the sample solution containing 10 μg of lead was constant and maximum in the range of 2–7 mL of 0.1% DBMCSA solution. Thus, 4 mL of DBMCSA solution were applied in further studies.

Stability and stoichiometry of Pb-DBMCSA complex

Under optimum conditions complexation reaction was completed after 10 min. After that time absorbance of the sample was stable for at least 24 h at the temperature of 30°C, however it slowly decreased with the increase of the temperature above 30°C. The stoichiometry of the obtained Pb(II)-DBMCSA complex estimated from the continuous variation and the slope-ratio methods was 1:2 [Pb(II):DBMCSA].

Effect of foreign ions

Procedure A was applied to the solutions containing 10 μg of lead and various foreign ions. Tolerance limits (mg) for the foreign ions studied were as follows (relative error < ±5%): K^+, Na^+, NH_4^+ (10); Zn^{2+}, Mn^{2+}, Al^{3+} (2); Fe^{3+} (1.5); Cu^{2+} (1); Ni^{2+}, Co^{2+}, Hg^{2+}, Cd^{2+}, Cr^{3+}, Mg^{2+}, (0.6); Bi^{3+} (0.5); Ca^{2+}, Ba^{2+} (0.002); Sr^{2+} (0.001); F^-, Cl^-, NO_3^-, SO_4^{2-} (10). It was found that except for Sr(II), Ca(II) and Ba(II) the investigated foreign ions did not interfere in the determination of lead.

Elimination of interferences from co-existing ions

Our experiments have demonstrated that DBMCSA reacts with lead, alkaline earth and rare earth metals, and is almost inert towards other elements in acidic media. Thus, only alkaline earth metal ions interfered during determination of lead in environmental and biological samples. To eliminate these interferences, FI microcolumn separation procedure with DDTC as the chelating agent and cigarette filter as the sorbent [13] was employed.

In the proposed procedure, Pb(II) was first complexed on-line with DDTC. Only the formed Pb-DDTC complex was sorbed onto the cigarette filter; alkaline earth metal ions were not sorbed. Subsequently, the retained Pb-DDTC complex was eluted with 4 mL of 1 mol L^{-1} HNO_3 solution and simultaneously decomposed to release Pb(II). 1 mol L^{-1} HNO_3 served not only as an efficient eluent but also as the appropriate solution for the subsequent determination. Other ions that could potentially react with DDTC did not interfere in the determination.

It has been found that Sr(II), Ca(II) and Ba(II) present at as large amounts as 100 mg (Sr(II)) and 200 mg (Ca(II) and Ba(II)) were not interfering in the determination of 10 μg Pb(II) in 25 mL solution when microcolumn separation was applied. This clearly demonstrates high selectivity of the proposed on-line FI separation for spectrophotometric determination of lead.

In order to check the accuracy of the proposed method, various amounts of lead were separated and determined. Relative difference between the absorbance measured in the presence and in the absence of sorption and separation did not exceed ± 0.5%.

Analytical characteristics

Calibration plot was constructed using the results obtained in procedure A. Beer's law was obeyed in the range: 0–30 μg of lead in 25 mL solution. Absorbance was measured at the wavelength of 633 nm. Regression equation of the calibration line is: $A = 0.0183\ C - 0.0127$; $r = 0.9998$ (C: μg per 25 mL)

Apparent molar absorptivity was 9.48×10^4 L mol^{-1} cm^{-1}. Detection limit (definition given by IUPAC [14]) equalled 2.4 ng mL^{-1}. Ten replicate determinations of 10 μg of lead in test solution were performed applying procedure A. One obtained variation coefficient of 1.2%.

Applying procedure A, another calibration plot was constructed for various concentrations of lead in the presence of 200 mg Ca(II). Microcolumn packed with the cigarette filter was used for the separation. The obtained results were almost the same as previously. This indicated that the separation step did not influence the results.

According to procedure B, the effects of sample loading time and concentration were investigated at the sample loading flow rate of 4 mL min^{-1}. It was found that the measured absorbance values of the analytes were the same for: 10 μg mL^{-1} Pb(II) after

15 s and 1 μg mL^{-1} Pb(II) after 150 s, and 1 μg mL^{-1} Pb(II) after 15 s and 0.1 μg mL^{-1} Pb(II) after 150 s. Apparently, it is possible to determine trace Pb(II) applying preconcentration.

To evaluate the accuracy of the developed method two certified reference materials (CRMs): GBW 08501 (peach leaf) and GBW 08502 (rice) were analysed. The results presented in Table 2 show a good agreement between the determined and certified concentrations of lead in CRMs. The developed method was also applied to the analysis of six real samples. The results of this analysis are given in Table 3. The determined concentrations of lead in real samples were consistent with those found by atomic absorption spectrometry.

Table 2. Determination results of trace lead in certified reference materials (CRMs)

Sample	Certified, μg g^{-1}	Found by the proposed method, μg g^{-1} (mean ± σ, n = 5)
Peach leaf (GBW08501)	0.99 ± 0.04	0.97 ± 0.03
Rice (GBW08502)	0.75 ± 0.10	0.71 ± 0.04

Table 3. Determination results of trace lead in real samples

Samples	Found by the proposed method, μg g^{-1} (mean ± σ, n = 5)	Found by atomic absorption spectroscopy, μg g^{-1} (mean ± σ, n = 5)
Waste water	2.45 ± 0.02	2.51± 0.02
River water	nd[a]	nd[a]
Rice	0.30 ± 0.05	0.34 ± 0.03
Tea	0.56 ± 0.07	0.59 ± 0.03
Tomato leaves	0.48 ± 0.03	0.47 ± 0.08
Spinach leaves	0.077 ± 0.05	0.079 ± 0.01

[a] Not detectable.

CONCLUSIONS

The synthesis and purification of dibromo-p-methyl-chlorosulfonazo chromogenic agent are very simple. Purification requires only the reagent to be soaked in HCl solution without extraction.

The proposed procedure is uncomplicated and requires the use of such simple reaction medium as 0.16 mol L^{-1} HNO_3, in contrast to other methods, which require more complex media [8–12].

The proposed method is sensitive. Molar absorptivity was improved compared to the values reported in references [8–11].

The proposed method provides much higher selectivity than other methods based on other chromogenic agents for the determination of lead. Nearly all the anions and cations do not affect the course of the colour reaction. Interferences from Sr(II), Ca(II) and Ba(II) can be easily eliminated by sorption and separation using microcolumn packed with the cigarette filter; extraction or masking agents are not needed.

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