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Research Article

Sorption-based **Spectrophotometric Assay for** Lead(II) with Immobilized Azo Ligand

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Abstract

This study introduces a novel sorption-spectrophotometric method for the determination of lead(II) ions in water, addressing the critical need for sensitive and selective environmental monitoring. The method utilizes Amido Black (AB), a diazo dye, immobilized on a hexamethylenediamine-modified polyacrylonitrile fibrous support (PPD-1). The resulting immobilized reagent system (Pb(II)-AB/PPD-1) exhibits a significant bathochromic shift upon complexation with Pb(II), enabling spectrophotometric determination at 490 nm.

Optimal conditions for immobilization and complexation were established, including a pH range of 6-7 and an AB concentration of 8.2·10⁻⁵ M on the support. The method demonstrates a linear response to Pb(II) concentrations between 5 - 50 μ g/ml (R² = 0.9938), with a low limit of detection (0.141) and quantification (0.47). Interference from common metal ions was minimal or mitigated with citric acid. Application to natural water samples showed excellent accuracy and precision (RSD ≤ 2.9).

The immobilized system offers a simplified procedure by eliminating elution steps, enhancing throughput, and achieving a ten-fold improvement in the limit of detection compared to solution-based methods. This rapid, sensitive, and selective method presents a promising alternative for lead(II) determination, offering comparable or superior performance with added benefits of simplicity and enhanced sensitivity.

Introduction

Anthropogenic activities are a major contributor to environmental pollution, with the contamination of natural water sources being of particular concern. In Central Asia, characterized by limited water resources, the availability of potable water is a critical issue. Fresh water reserves in Uzbekistan's rivers and lakes are severely constrained, and groundwater and glacial sources, often considered a solution, are also susceptible to the accumulation of toxic pollutants. Thus, access to clean drinking water constitutes a strategic challenge for the 21st century. The World Health Organization has designated Heavy Toxic Metals (HTMs) - including cadmium, lead, mercury, arsenic, chromium, copper, and zinc - as priority pollutants requiring rigorous monitoring. Consequently, the detection and quantification of HTM ions in water are of paramount importance. Soil contamination by HTMs is also a significant concern due to their widespread anthropogenic discharge [1-3]. The relevance of this study lies in the established pathway of ecotoxicant entry into the human body: soil-plant-human-environment [4]. Effective environmental pollution monitoring is essential to mitigate anthropogenic impacts, thus highlighting the need for improved and comprehensive monitoring methods Sorption-spectrophotometric methods solid-phase immobilized organic reagents have emerged as promising alternatives to conventional solution-based spectrophotometry, exhibiting enhanced sensitivity and selectivity [6,7]. Lead, a ubiquitous environmental contaminant from natural sources, such as galena, anglesite, and cerussite, is widely used in various industrial applications including batteries, cables, solder, and radiation shielding [8]. These large-scale industries, often associated with significant waste generation and emissions, contribute to both

atmospheric and waterborne lead pollution. Given the low permissible concentration limit of lead in drinking water (30 μ g/L), continuous monitoring of lead contamination in various environmental matrices and water is imperative [1,2,6].

Various optical techniques are employed for the analytical determination of lead in environmental matrices, including Atomic Absorption Spectrometry (AAS) and spectrophotometric methods utilizing diverse classes of organic reagents. Diazo reagents are among the most extensively studied in this context [3-5]. However, sorptionspectrophotometric methods for lead quantification remain relatively limited, with a scarcity of highly effective organic reagents specific for lead [5]. Given the inherent advantages of sorption-spectrophotometry, particularly its enhanced sensitivity for trace analysis of various toxicants, the development of robust and sensitive sorptionspectrophotometric methods for lead is a critical area of research. Recent advancements in improving the analytical performance of luminescent reagents have focused on the immobilization of organic ligands onto solid supports. This approach combines analyte preconcentration with in situ determination directly on the solid matrix [8-13].

The objective of this study was to establish a rapid and sensitive sorption-spectrophotometric method for the determination of lead(II) ions. This was achieved through the use of sodium 4-amino-5-hydroxy-3-((E)-(4-nitrophenyl)diazenyl)-6-((E)-phenyldiazenyl)naphthalene-2,7-disulfonate (AC), a diazoazo compound, immobilized on a fibrous support. The inherent selectivity of this reagent has enabled its application in lead analysis, even in the presence of numerous other metal ions. This article demonstrates the superior performance of sorption-spectrophotometric lead(II) determination using the immobilized organic reagent compared to conventional solution-based spectrophotometry.

Experimental

Reagents and materials: Analytical reagents used were standard solutions. Amido Black 10B (systematic name: sodium 4-amino-5-hydroxy-3-((E)-(4-nitrophenyl)diazenyl)-6-((E)-phenyldiazenyl)naphthalene-2,7-disulfonate) was selected as the immobilized analytical reagent, obtained from "Ximreaktivinvest 000," Tashkent, Uzbekistan (CAS number: 1064-48-8). The molecular structure of Amido Black is presented in Figure $1.4 \times 10^{-5} \, \mathrm{M}$ standard solution of Amido Black 10B was prepared in distilled water. Working solutions of the organic reagent, Amido Black, were produced by diluting stock solutions with bidistilled water. This standard solution was employed in immobilization procedures.

Lead standard solution: $Pb(NO_3)_2$ was also sourced from "Ximreaktivinvest OOO," Tashkent, Uzbekistan. A total of 1.598 g of $Pb(NO_3)_2$ was dissolved in distilled water and

Figure 1: Structural formula of the Amido Black reagent.

the volume was adjusted to 1 liter in a volumetric flask with distilled water, yielding a standard solution containing 1 mg Pb^{2+} per ml. This solution was utilized in the determination of Pb^{2+} as described in [12].

Buffer solutions: pH values ranging from 1 to 10 were prepared using chemically pure-grade salts and acids, following previously described methodologies [12]. Throughout the study, freshly distilled and purified solvents, bidistilled water, and deionized water were employed, all verified to be non-luminescent.

Infrared (IR) spectra were recorded using both a UR-10 spectrophotometer (Carl Zeiss, Jena) and an Analitrsystem 360 FT-IR spectrometer (Nikolet Instrument Corporation, USA). Samples were analyzed in KBr and LiF pellets, as well as in CHCl₂ solution, within the 500-4000 cm-1 range. pH measurements were conducted using a METTLER TOLEDO pH meter, calibrated with standard buffers in the same solvent used for analysis. Aqueous pH values for the buffer mixtures were referenced [13]. A PP-2-15 peristaltic pump was used for solution handling. Reagents of "chemically pure" and analytical grades were used. 0.1 M solutions of metal salts were prepared by dissolving the appropriate nitrate or chloride salts and further diluting them to obtain the desired concentrations. The organic reagent, Amido Black, was prepared by dissolving a specific amount of the reagent in a 100 mL volumetric flask.

Various fibrous materials, based on polyacrylonitrile, with different functional groups, were evaluated as solid supports. The sorbent was used in the form of disks, 20 mm in diameter and 30 mg - 40 mg in wet mass. These disks were pretreated with 0.1 N hydrochloric acid, washed with distilled water, and stored in Petri dishes.

Spectral analysis: Diffuse reflectance spectra of the solid surfaces were acquired using an X-Rite recording spectrophotocolorimeter. Reflectance (R) and reflectance function F(R) were also analyzed as functions of different variables using a dual-beam UV-Vis SPECORD 50 spectrophotometer.

053

The analytical signal was quantified as the change in diffuse reflectance (ΔR) measured at 610 nm. This value represents the difference in reflectance between the solid supports following analyte sorption and subsequent reaction with the immobilized reagent, and that of a blank solid support. The pH of the medium was monitored using an I-130 ionometer.

Experiments were conducted in both static and dynamic modes. In the static mode, 10.0~mL of the reagent solution was added to a 50.0~mL flask. A support disk was then immersed and stirred for 5-8 minutes. After this time the solution was decanted while retaining the solid support, which was then washed with distilled water and subsequently immersed in the analytical solution. In the dynamic mode, the analytical solution was passed through the immobilized support disk at a flow rate of 10~mL/min.

The retention of Amido Black on the solid support (R%) was quantified using the following equation: R% = $100 * (A / A_0)$, where A represents the absorbance of the immobilized reagent after it was retained on the solid support, and A_0 is the absorbance of the reagent before the immobilization process.

Results and discussion

The diazo dye Amido Black (AB) demonstrates moderate sensitivity in its interaction with lead(II) ions. The complex molecular architecture of AB, illustrated in Figure 1, suggests a multifaceted reaction mechanism with metal ions that remains to be fully elucidated. Current hypotheses propose that this interaction involves a combination of ionic bonding and physical adsorption processes.

The accurate determination of lead is significantly affected by the presence of several interfering metal ions, including iron, aluminum, bismuth, and other metals. These interferences are typically mitigated through the introduction of masking agents and by optimizing the acidity of the reaction medium.

Several fibrous materials, functionalized with various anion-exchange groups, were evaluated as potential solid-phase supports for Amido Black immobilization. Among the materials tested, the fibrous support modified with hexamethylenediamine (designated PPD-1) exhibited superior analytical performance, demonstrating the effective complexation of Amido Black. Optimal immobilization of Amido Black within the PPD-1 polymer matrix was observed within a pH range of 3.0 to 6.0. Due to its effective retention of Amido Black, the PPD-1 support, forming the PPD-1:AB complex (hereafter referred to as IMAB), was selected for subsequent experimentation.

The impact of both the Amido Black concentration and the immobilization time was examined over a range of $1 \cdot 10^{-6}$

to $1 \cdot 10^{-3}$ M Amido Black solution and 3 to 30 minutes, respectively (Table 1).

Table 1 details the optimal conditions for the determination of Pb(II) utilizing the immobilized Amido Black organic reagent. The maximum reflectance spectrum (λ_{MeR}) of the immobilized Amido Black organic reagent with Pb(II) is observed at 490 nm, while the reflectance spectrum (λ_{R}) of the immobilized Amido Black reagent occurs at 610 nm. The optimal pH range for complex formation is determined to be between 6 and 7. The immobilization of Amido Black on the fiber is achieved within 7 minutes. The concentration of Amido Black immobilized on 0.2 g PPD-1 fiber is quantified as $8.2 \cdot 10^{-5}$ M. Figure 2 depicts the reflectance spectra of the complex formed by Amido Black immobilized on PPD-1 fiber and the immobilized Amido Black with Pb(II).

Upon immobilization on the PPD-1 support, the complexation of Amido Black (AB) with Pb(II) resulted in a substantial bathochromic shift of 120 nm ($\Delta\lambda$ = 120 nm), indicating a significant alteration in the electronic structure compared to the reaction in solution. The maximum reflectance wavelength (λ_{MeR}) of the resulting Pb(II)-AB complex on the PPD-1 was observed at 490 nm. Comparatively, other reagents reported for Pb (II) determination, including 4-(Pyridyl-2-azo)-resorcinol (520 nm) [14], 4-(Thiazolyl-2-azo)-resorcinol (540 nm) [15,16], Dithizone (520 nm) [17,18], Arsenazo III (605 nm) [19], Xylenol Orange (580 nm) [20], Bromopyrogallol Red (630 nm) [21], Glycine Thymol Blue (574 nm) [22], Methylthymol Blue (600 nm) [23], Sulfonazo III (650 nm) [24], KI + Methyl Green (642 nm) [25], and Sulfarsazene (510 nm)

 Table 1: Spectrophotometric characteristics of amido black fixed on PPD-1 sorbent.

 $\lambda_{MeR'}$ nm
 $\lambda_{R'}$ nm
 pH
 Time, min
 Concentration of reagent on carrier, M

 490
 610
 6-7
 7
 8.210^{-5}

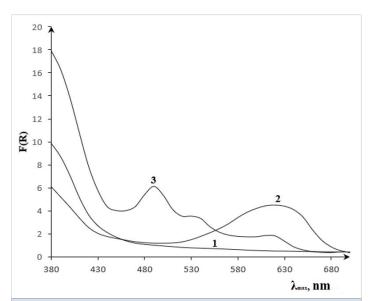


Figure 2: Reflectance spectra of the complex formed by fiber (1), immobilized reagent (2) with lead (II) ion (3) in a universal buffer mixture.

[26], exhibit λ_{max} values generally higher than that of the Pb(II)-AB/PPD-1 complex. However, the observed λ_{MeR} for the immobilized system falls within a similar spectral region. This suggests that while the immobilized AB system exhibits a unique spectral response upon interaction with Pb(II), its performance, in terms of maximum wavelength, is comparable to established reagents.

The dependence of the analytical signal on Pb(II) concentration for the complex formed between Pb(II) and Amido Black immobilized on the PPD-1 matrix (Pb(II)-AB/PPD-1) is depicted in Figure 3. Experimental conditions were maintained as follows: V = 100 mL, m = 0.2 g AB/PPD-1, pH = 6.5 \pm 0.5, T = 20 \pm 2 °C, and t = 20 min. The investigation spanned a Pb(II) concentration range of 5-50 µg/ml A strong linear correlation was observed within this range, as indicated by a regression coefficient (R²) of 0.9938. This linearity validates the accurate quantification of Pb(II) ions using the Amido Black reagent immobilized on the PPD-1 support and suggests the suitability of this immobilized reagent system for quantitative analysis within the specified 5-50 µg/ml Pb(II) concentration range.

It was determined that the presence of Cd(II), Mn(II), Ni(II), Co(II), Zn(II), and Mg(II) at concentrations up to 100 mg/L does not interfere with the quantification of lead(II). The interference caused by Fe(III), Bi(III), and Al(III) at concentrations of 50 mg/L was successfully mitigated through the addition of 0.1 M citric acid solution to the sample. A method for the determination of lead(II) in natural water samples was developed. The accuracy of this method was verified using a spike-recovery assay, as presented in Table 2. The analysis time was determined to be 10-15 minutes. The pH range

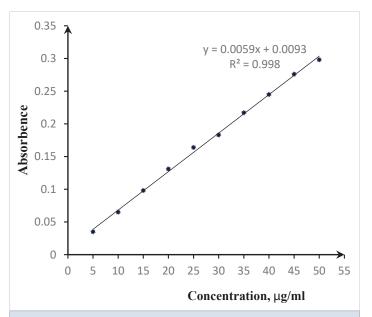


Figure 3: Dependence of the optical density of the Pb(II) complex with the AB/PPD-1 matrix on the Pb2+ concentration (V = 100 ml, m = 0.2 g AB/PPD-1 matrix, pH = 6.5 ± 0.5 , T = 20 ± 2 °C), t = 20 min).

Table 2: Lead(II) determination results in water samples ($n = 5$, $P = 0.95$).						
Sample No.	Lead introduced, µg/ml	Lead found, μg/ml	Std. (standard deviation)	RSD		
1.	5.00	5.24 + 0.389	0.151	2.892		
2.	10.00	10.18 + 0.309	0.121	1.184		
3.	15.00	15.26 + 0.385	0.150	0.982		
4.	20.00	20.25 + 0.423	0.165	0.814		
5.	25.00	25.24 + 0.453	0.176	0.699		
6.	30.00	30.26 + 0.495	0.193	0.637		
7.	35.00	35.29 + 0.658	0.256	0.726		
8.	40.00	40.31 + 0.760	0.296	0.734		
9.	45.00	45.372 + 0.859	0.334	0.737		
10.	50.00	50.374 + 0.872	0.339	0.674		

for the reaction is similar to that reported for 4-(Thiazolyl-2-azo)-resorcinol (6.5-7) [15,16] and Bromopyrogallol Red (4-7) [21].

The analytical method demonstrated high precision, with a Relative Standard Deviation (RSD) consistently \leq 2.9. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.141 and 0.47, respectively, while method reproducibility was 1.67. Notably, the use of Amido Black immobilized on fibrous supports enabled selective lead analysis without the need for an elution step. This streamlined procedure enhanced the analytical throughput and resulted in a ten-fold improvement in the LOD compared to the presented method. Furthermore, this approach offers advantages over existing methods utilizing chromogenic reagents such as 4-(Pyridyl-2-azo)-resorcinol [14], Dithizone [17,18], and Sulfarsazene [26], which require higher pH conditions for optimal performance.

Conclusion

This study demonstrates the successful immobilization of Amido Black (AB) on a hexamethylenediamine-modified polyacrylonitrile fibrous support (PPD-1) for the sensitive and selective determination of lead(II) ions in aqueous solutions. The resulting immobilized reagent system, denoted as Pb(II)-AB/PPD-1, exhibited a significant bathochromic shift upon complexation with Pb(II), allowing for its spectrophotometric determination at a λ MeR of 490 nm. Optimal immobilization and complexation were achieved within a pH range of 6-7, with an immobilization time of 7 minutes and an AB concentration of 8.2·10-5 M on the PPD-1 support.

The method exhibited a strong linear response to Pb(II) concentrations between 5-50 μ g/ml, with a regression coefficient (R²) of 0.9938, demonstrating its suitability for quantitative analysis. The presence of potentially interfering ions such as Cd(II), Mn(II), Ni(II), Co(II), Zn(II), and Mg(II) did not affect the determination of Pb(II) at concentrations up to 100 mg/L. The interference of Fe(III), Bi(III), and Al(III) was effectively eliminated using citric acid as a masking agent.

The developed method was successfully applied to the determination of Pb(II) in natural water samples, showing excellent accuracy and precision, with a Relative Standard Deviation (RSD) consistently \leq 2.9. The method exhibited a low limit of detection (LOD) of 0.141 and a limit of quantification (LOQ) of 0.47, demonstrating its high sensitivity. The reproducibility was determined to be 1.67.

Importantly, the immobilized system offers a simplified analytical procedure by eliminating the need for an elution step, thereby enhancing throughput and achieving a tenfold improvement in the LOD compared to the standard solution-based method. Furthermore, the optimized pH range for complexation (6-7) offers an advantage over other established chromogenic reagents, which often require higher pH conditions.

In conclusion, the developed method utilizing Amido Black immobilized on PPD-1 presents a rapid, sensitive, selective, and reliable approach for the determination of lead(II) in environmental samples. This novel immobilized reagent system offers a promising alternative to existing methods, providing comparable or superior performance with the added benefits of simplicity, speed, and enhanced sensitivity. Further research could explore the application of this system to other matrices and the immobilization of other analytical reagents for diverse analytical applications.

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