Graphical Abstract

Voltammetric determination of stability constants of lead complexes with diallyl disulfide, dimethyl disulfide, and diallyl sulfide

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Pb[DAS]₂⁺²

Pb[DMDS]₂⁺²

Pb[DADS]₂⁺²

Polarizable

Non-polarizable

Polarizability of complexation of lead by DADS, DMDS and DAS

Voltammetric determination of stability constants of lead complexes with diallyl disulfide, dimethyl disulfide and diallyl sulfide

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ABSTRACT

The complexation of Pb²⁺ by diallyl disulfide (DADS), dimethyl disulfide (DMDS) and diallyl sulfide (DAS) has been studied by differential pulse voltammetry. Stability constants, (log β), of the 1:1 (PbL) and 1:2 (PbL₂) complexes, where L = ligand, were found to be in the range of *ca.* 3.8-4.2 and 9.4-10.2, respectively. Complex formation is accompanied with a significant decrease in the peak current and the shift of the Pb²⁺/Pb half-wave potential to a higher one upon the addition of the sulfur containing ligands (L). The formation of 1:1 and 1:2 complexes, *i.e.*, PbL²⁺ and PbL₂²⁺ was observed in the case of the three ligands. The consecutive formation constants of labile lead complexes with DADS, DMDS and DAS were determined at 298K by the method of DeFord and Hume. Apparently due to the large size of the lead ion, steric hindrance caused by the greater steric bulk of DADS compared to that of DMDS have little or no effects on the formation constants, so that very similar values were obtained in the case of the two ligands. Also, the possible participation of C=C double bonds in coordination with the metal center in the case of DADS appears to compensate for the steric effects caused by the larger size of this ligand. In agreement with the Jorgensen principle of symbiosis, the second formation constants were found to be approximately six orders of magnitude greater than the first ones.

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1. Introduction

Lead is a highly toxic metal found in the earth's crust. Because of its abundance, low-cost, and physical properties, lead and lead compounds were used in a wide variety of products including paint, ceramics, pipes, solders, gasoline, batteries, and cosmetics. Lead is a serious cumulative body poison and enters our body system through food, water, and air [1], since Pb²⁺ inhibits -SH enzymes, especially by interaction with cysteine residues in proteins [2]. Although the total human exposure to lead has been significantly reduced due to the controls on lead in gasoline and on industrial air emissions, lead poisoning is still a serious public health problem [3,4]. Drinking water is another possible source of lead exposure [5-7] and other main sources of lead exposure are lead-based paints and lead-contaminated dusts or soils [8], plumbing materials and equipment, car batteries, lubricating compounds and foods prepared in lead-glazed ceramics [4]. The human body is only able to excrete lead very slowly, consequently, it can accumulate in the bones and liver where it reacts with cell membranes to alter their permeability, or destroys them completely [9]. Chronic lead poisoning can impair memory and cause headaches, abdominal pain, anemia, aggressive behavior, and learning disabilities. In fact, health experts claim that lead poisoning is an entirely preventable environmental health disease in children [10].

Chemopreventive agents have been used against the toxic effect of many compounds, including heavy metals. A

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range of vegetables, plants and synthetic compounds have been screened in order to ascertain whether they have potential in the prevention and cure of many diseases and conditions [12]. As one plant studied extensively, garlic (*Allium sativum*), has been used since ancient times as a cure for many diseases [13]. It has antifungal, antithrombotic, antibacterial, antihypertensive, anti-carcinogenic and antioxidant properties [14].

Garlic belongs to the family *Liliaceae* and has a higher concentration of sulfur compounds which yield the characteristic flavor and taste and are also responsible for the beneficial effects [15]. In this respect, garlic aids the liver in lowering the levels of heavy metal especially lead, cadmium and mercury [11]. When garlic is minced, or crushed, the allyl sulfur compounds are formed by enzymatic activity. These extracts are composed of allicin, water-soluble *S*-allylmercaptocysteine and *S*-allylcysteine, and oil-soluble diallyl disulfide (DADS), diallyltrisulfide and diallyl sulfide [16,17], and additionally, allicin also consists of DADS, diallyl sulfide (DAS), diallyltrisulfide (DTS) and sulfur dioxide [18]. Organosulfur compounds can adsorb on the surface of gold or silver electrodes through several chemisorption steps. Thiols and disulfides compounds can adsorb by an anodic oxidation process on the surface of Au and Ag [19]. The study of the binding of lead (II) to the organic sulfur compounds of garlic oil and extracts is especially of importance in the modeling of the inhibitory effects of garlic on the lead poisoning. In the Pearson hard and soft acid and base sense, lead ions are classified as soft and, hence, should form complexes with sulfur containing ligands due to the soft character of the sulfur atom [18,20]. Of note, the compound 2,3-dimercaptopropanol (SHCH₂CH(SH)CH₂OH), also known as British anti-Lewisite, is a strong chelating agent which has been used in the treatment of lead poisoning [2].

Complexation of Pb²⁺ with various ligands, such as Vitamin C [21], 2,5-dimercapto-1,3,4-thiadiazole [22], Dicyclohexano-18-Crown-6 [23] and glutathione[24], has been investigated. Voltammetric techniques are widely used to study the interactions between metal ions and various ligands [25] because of their very high reproducibility and reliability at low concentrations [26] and also due to their ability to detect different chemical forms of the same element [21]. The usual strategy with the use of voltammetric modeling involves: first, the assumption of the physico-chemical phenomena taking place during the measurements, and secondly, the use of fundamental equations (Nernst equation, Fick laws of diffusion, equilibrium constants, kinetic equations, *etc.*) to generate a set of equations which can be analytically or numerically solved [27]. As a result, to obtain quantitative information is through the postulation of an electrochemical model and its mathematical solution followed by the fitting of the parameters of the equations of the model to the experimental data [28].

In this study, the cumulative stability constants (β) of three organic sulfur compounds, DADS, DMDS and DAS (Fig.1), which are compounds of garlic oil [29] that complex with Pb²⁺ have been measured using differential pulse voltammetry (DPV) and a gold electrode. The information about metal complexation is provided from the fitting of the parameters of the model to the experimental data.

Fig. 1. Organic sulfur compounds used in this study

2. Experimental

2.1. Chemicals and instrumentation

Diallyl disulfide, dimethyl disulfide and diallyl sulfide, lead nitrate, sulfuric acid, acetic acid, sodium hydroxide and boric acid were purchased from Merck or Sigma-Aldrich analytical grade. A stock solution of the ligands (0.01

mol/L of DADS, DMDS and DAS) was prepared by dissolving appropriate amounts of each in 40 ml distilled methanol and diluting to volume in a 100 ml calibrated flask with distilled water. Stock lead ion solution (0.02 mol/L) was prepared by dissolving an appropriate amount of $\text{Pb}(\text{NO}_3)_2$ in doubly distilled water.

Electrochemical experiments (differential pulse voltammetry, DPV) were carried out using a Computrace 757 VA from Metrohm. In all cases, a three-electrode system consisting of a gold electrode as the working electrode with the diameter of two mm from Metrohm, a platinum wire counter electrode and an Ag/AgCl, KCl (3 mol/L) reference electrode was used. All electro-chemical measurements were carried out in a 12 mL cell.

The gold electrode was abraded with a fine grade of furriery paper and polished to a mirror-like surface with 0.05 mm Al_2O_3 powder, and immersed in NaOH (0.2 mol/L) then rinsed ultrasonically with water and ethanol for three min.

2.2. Voltammetric measurements

Electrochemical measurements were performed in the solutions of acetate buffer pH = 5.5, with ionic strength 0.10 mol/L. All the measurements were carried out in a glass cell at r.t. (25 °C) and the solutions were purged with nitrogen for at least 30 minutes prior to each experiment and the nitrogen atmosphere was maintained thereafter and DPV for each addition was recorded at a sweep rate of 0.1 V/s. The starting and ending potentials of all the titration experiments were from 0.2 V to -0.6 V vs. Ag/AgCl. After recording each voltammogram (at different ligand to metal molar ratios), the electrode surface was cleaned electrochemically by applying the potential of 1.5 V for 100 s. Each titration experiment was repeated at least four times. The voltammograms were smoothed and converted into data matrices by means of internally written programs implemented in MATLAB.

3. Results and discussion

3.1. Optimization of conditions

The electrochemical study of the complexation of thiol-containing ligands with lead ion is faced with difficulties caused by the high affinity of thiol-containing ligands, or their complexes, onto the surface of electrode. In the present study, the complex formation between Pb^{2+} and DADS, DMDS and DAS has been studied by DPV. A simple voltammetric method was proposed for *in situ* and simultaneous cleaning of the electrode surface. According to the results, applying a high positive potential (+1.5 V for 100 s) can remove the organic sulfur compounds of garlic oil and their complexes from the surface of electrode after each voltammogram.

The complexation of Pb^{2+} with the subject ligands in aqueous solution can be affected by a variety of factors and conditions, such as the type of electrode, type of buffer solution, pH and initial concentration of metal ions, all of which were considered in this study. Although electrodes, such as mercury film, glassy carbon, carbon paste and platinum electrode, were also checked for this purpose, it was experimentally verified that the changes in the voltammograms during the titration were not enough for the evaluation of the stability constants, and therefore a gold disk electrode was used as the working electrode, since gold is widely used in micro fabrication for a variety of applications [1]. In electrochemical experiments, the quality of the active surface will affect the measurements. The interaction between gold surfaces and thiol-terminated molecules has been widely studied and leveraged for biological purposes [2,3]. Furthermore, gold is also used as an electrochemically active surface on which the behaviors of chemical or biological samples are studied [30,31].

The effect of pH on the peak current was investigated by a set of DPV of the electrolyte containing 6.92×10^{-6} mol/L Pb^{2+} , while the solution pH was varied from 3 to 7. The current of signal of the voltammetric peak of Pb^{2+} increased with increasing pH from 3 to 5.5, and then decreased, which is a consequence of the formation of labile, lead hydroxide complexes [24]. Also the peak potential of Pb^{2+} was nearly constant with increasing the pH up to 5.5 and subsequently shifted to lower potentials, (more negative), with increasing pH [21]. Hence, this pH was chosen for further experiments. Fig. 2 shows the voltammograms of 1.36×10^{-5} mol/L Pb^{2+} in different buffer solution (pH 5.5). Three buffers, *i.e.*, acetate, citrate and Britton-Robinson were checked for this system. Among these buffers, acetate buffer was found to be the most suitable for reduction of lead ion. The lower reduction currents in the citrate and Britton-Robinson buffers may be due to the interaction of Pb^{2+} with the buffer ions.

Fig. 2. DP voltammograms for reduction 1.36×10^{-5} mol/L Pb^{2+} in different buffer solutions

The Pb^{2+} reduction signal is split, which seems to indicate a two-step deposition process of Pb^{2+} on the Au-electrode surfaces. Fig. 3 shows the set of voltammograms obtained by the successive addition of Pb^{2+} into the acetate buffer solution. It can be seen that up to a concentration of 1.36×10^{-5} mol/L of added lead there is no evidence of the second peak, but the second peak appears at higher concentration levels. At this high concentration of Pb^{2+} ions, the reduction process can occur more quickly and can cover some parts of the electrode surface. The reduction of lead ions at this new surface may need more over-potentials. Therefore, under this condition, two peaks appears for the reduction process of lead ions [1]. Such a behavior was also observed for Hg^{2+} ions on the surface of gold electrode [32] in DPV experiments. For this purpose, a concentration of 1.36×10^{-5} mol/L Pb^{2+} in which there is only one peak in the voltammogram was chosen as initial concentration of metal ion in titration of the metal ion with the ligands.

Fig. 3. DP voltammograms recorded in 0.1 mol/L acetate buffer (pH 5.5 ± 0.1) with increasing concentrations of Pb^{2+} ions

3.2. Determination of the stability constants by fitting method

Fig. 4 shows a typical set of DPV obtained in the titration of a Pb^{2+} solution with DADS, DMDS and DAS, respectively. As this Figure shows, the increment in ligand concentration produces a shift of the peak potential towards more negative values. The basis for interpreting the DPV data was well-known by the De Ford-Hume relation:

$$\Delta E (C_{\text{Pb}}) = E ([L]) - E ([L] \rightarrow 0) = (RT/2F) \ln \alpha_0 \quad (1)$$

Where E stands for a half-wave, or a peak potential, whichever is appropriate, and α_0 stands for the fraction of non-ligated lead. The ratio of the diffusion currents of a ligated and a non-ligated lead was omitted because it was not readily calculable from the DPV data and because the E_p value has a greater effect on the calculated stability constants than the variation in the height of the lead peak. In terms of Leden's function F_0 , the final mathematical model can be written as:

$$F_0 = 1 / \alpha_0 = 1 + \beta_1[L] + \beta_2[L]^2 + \dots + \beta_n[L]^n \quad (2)$$

If only one labile complex is present, the shift of the peak potential can be expressed as:

$$\Delta E_p = RT \ln(1 + \beta_1[L] + \beta_2[L]^2 + \dots + \beta_n[L]^n) / 2F \quad (3)$$

where L stands for the ligand and β_n are the corresponding cumulative stability constants [33].

Fig 4. DPV obtained for titration system of Pb^{2+} 1.36×10^{-5} mol/L solution with a) DADS, b) DMDS, c) DAS in the presence of 0.1 mol/L acetate buffer (pH 5.5 ± 0.1)

The complex formation equilibrium of the Pb^{2+} ion with DADS, DMDS and DAS in dilute aqueous solution is assumed to be established by the consecutive two-step reactions:

Computations of the stability constants, under the assumption of a series of mononuclear complexes (ML_n ; $n = 1, 2, \dots$) estimated by voltammetry, yield the values of formation constants as follows: (DADS): $\log \beta_1 = 4.1 \pm 0.2$, $\log \beta_2 = 10.2 \pm 0.4$; (DMDS): $\log \beta_1 = 4.2 \pm 0.2$, $\log \beta_2 = 10.0 \pm 0.4$, (DAS): $\log \beta_1 = 3.8 \pm 0.2$, $\log \beta_2 = 9.4 \pm 0.5$. All measurements were performed at pH 5.5, where the formation of Pb^{2+} hydroxide was negligible. Interestingly, the second formation constants are approximately six orders of magnitude greater than the first ones. This observation is in agreement with the Jorgensen principle of symbiosis with respect to hard and soft acid-base behavior [34], which this rule states that the presence of some soft ligands enhances the ability of the central atom to accept other soft ligands. Although the sulfur groups of the subject ligands are most probably involved in coordination to the metal center, the involvement of double bonds in the optimized structure of the complexes of DADS and DAS cannot be excluded; in addition to the well-known Pb^{2+} -(η^5 -cyclopentadienyl) compounds [35], Auneret *al.* reported the unique coordination of C=C bond of two cyclopentene molecules to an electron-deficient Pb^{2+} center [36]. Very similar formation constants obtained in the case of DADS and DMDS suggests that the steric hindrance due to the larger size of DADS relative to DMDS have little effects on the binding of the ligands to the metal center. This behavior may be also attributed to the large size of Pb^{2+} ion which reduces the ligand-ligand repulsion, though the participation of C=C double bonds in coordination to the metal center in the case of DADS cannot be excluded. Also, the comparable consecutive formation constants in the case of DADS and DMDS, in spite of the absence of C=C bonds in DMDS, suggests that the Pb-S bonds are probably the main interactions in the complexes. According to the determined formation constants, the inhibitory and treatment effects of garlic oil [37] on the lead poisoning may be quantitatively described based on the formation of the 1:1 and 1:2 complexes.

Fig.5 shows dependence of the shift of the potential reduction of Pb^{2+} on DADS, DMDS and DAS concentration. This figure shows the shift of the potential obtained from experimental data extreme affinity with shift of the potential obtained from De Ford-Hume equation. Fitting between the experimental and theoretical data were obtained by finding β_1 and β_2 .

Fig.5 Dependence of the shift of the potential reduction of Pb^{2+} on a) DADS, b) DMDS and c) DAS concentration presence of 0.1 mol/L acetate buffer pH = 5.5 ± 0.1)

DADS, DMDS and DAS was titrated by the Pb^{2+} ion solution (see Fig. S1, Supporting information). As this Figure illustrates, DADS alone showed two peaks, (reduction of the ligand), which were named as I and II in Fig. S1.a. These two peaks decreases and a new peak for reduction of the complex of DADS- Pb^{2+} increases by addition of Pb^{2+} ion solution. The DMDS ligand showed three peaks, (Fig. S1.b.), and DAS has only one peak in the absence of Pb^{2+} ions (Fig. S1.c.).

When Pb^{2+} is added to the DADS, DMDS and DAS solutions, the concentration of free ligand decreased continuously with increasing the Pb^{2+} concentrations. The great affinity of sulfur atoms for Pb^{2+} -ions and the inert character of the formed complex suggests that the binding may be very stable.

4. Conclusion

In this study, the formation of 1:1 and 1:2 complexes between Pb^{2+} and diallyl disulfide (DADS), dimethyl disulfide (DMDS) and diallyl sulfide (DAS) has been studied by DPV. The effect of pH on the peak current was investigated by a set of DPV from 3 to 7. The pH 5.5 was chosen for further experiments because at this pH no hydroxide complexes are formed. Three buffers, *i.e.*, acetate, citrate and Britton-Robinson were checked for this

system. Among these buffers, acetate buffer was found to be the most suitable one for reduction of Pb^{2+} .

Accordingly, the inhibitory effects of DADS, DMDS and DAS, since they are compounds of garlic oil, on lead poisoning may be explained on the basis of formation of the 1:1 and 1:2 complexes with stability constants ($\log \beta$) in the range of *ca.* 3.8-4.2 and 9.4-10.2, respectively. Apparently due to the large size of Pb^{2+} , the greater steric bulk of DADS relative to DMDS has little effects on the formation constants, so that very similar formation constant values were obtained in the case of the two ligands. However, the involvement of C=C double bonds in coordination to metal center in the case of DADS may also compensate for the steric effects caused by the larger size of this ligand. In agreement with the Jorgensen principle of symbiosis, the second formation constants are approximately six orders of magnitude greater than the first ones. In addition, DADS and DMDS each have two sulfide groups within the molecule, while DAS has one sulfide that can affect the complexation between lead and these ligands.

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