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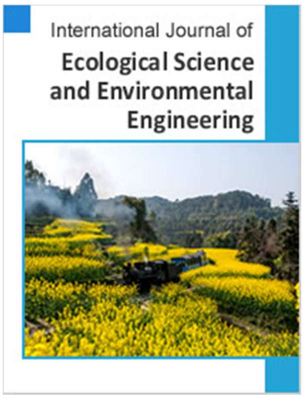

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Analysis of the Possibility of Using Azodyes to Estimation of the Cadmium and Lead Content in Agricultural Soils

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Abstract

The study of regularities in the formation of dissimilar associates is promising for the development of methods for the quantitative analysis of natural objects as agricultural soils. A heterogeneous association between multiply charged anions arsenazo II (ARII) and congo red (CR) and cation of cyanine dyes pinacyanol (PNC) is considered. The spectral changes that occur when increasing amounts of anions are added to the PNC have been considered. The optical density of the mixture of dyes is much lower than the sum of the optical density of the dyes at a certain concentration. Such changes in the absorption spectrum of PNC indicate the intense interaction of various dyes and the formation of an ion associate. The stoichiometric ratios of the particles in the PNC+AR II and PNC+CR associates are 4:1 and 2:1. We have examined the effect of Cd^{2+} or Pb^{2+} on the spectral characteristics of ARII, CR and associates $(PNC)_4 \cdot$AR II, $(PNC)_2 \cdot$CR in a wide range of metal concentrations ($2 \cdot 10^{-6}$ M – $2 \cdot 10^{-4}$ M). The addition of very small concentrations of heavy metals to the associate causes a change in the light absorption. Such effects open up new possibilities in increasing the sensitivity of quantitative determinations of small amounts of heavy metals (10^{-6} M) in soils with the help of aqueous extracts.

1. Introduction

Heavy metals participate in oxidation-reduction processes of biological systems and can migrate in soils. Accumulation of heavy metals violates the natural ecological balance. For example, it has been defined that the reproduction, growth and endurance of earthworms *Eisenia fetida* significantly depends on the content of lead and cadmium in the surface layers of the soil [1].

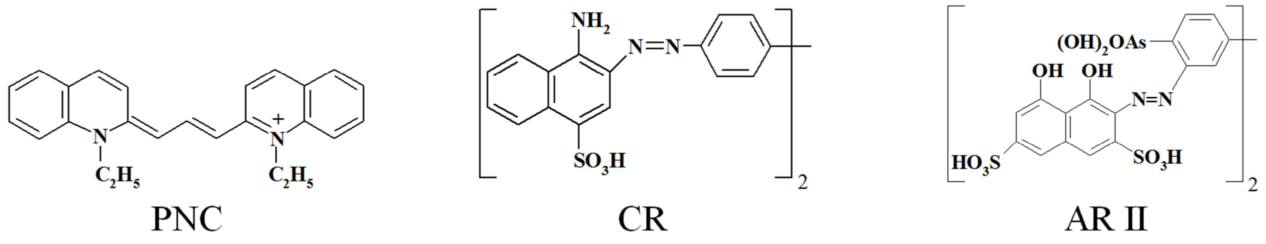
Among heavy metals, lead and cadmium are not vital and pose a serious danger to living organisms. However, the ground urease activity significantly changes, as well as the nitrification of the soil is reduced at their concentrations of 0.5 mg/kg [2]. These and similar facts encourage the development and improvement of methods for determining these elements in the soils and environment [3, 4].

Spectrophotometric methods are characterized by high sensitivity of the determination of metal cations, a wide range of detectable concentrations and are the basis for the development of rapid analysis techniques. Techniques using dyes are especially promising. At present, azo dyes are successfully used for the concentration and separation of a number of chemical elements, as well as metal-indicators [5]. Arsenazo III is the most widely used for the analysis of natural objects. Methods for analyzing heavy metals, alkaline earth, and rare earth elements in an aqueous medium have been developed [6] in the presence of surfactants [7], in a solid phase medium [8]. The possibility of creating chromogenic sensory systems based on gelatinous massifs is shown [9]. Arsenazo II (AR II) is also able to form complex compounds with metal cations over a wide pH range, but methods using arsenazo III are characterized by higher selectivity and sensitivity (detection limits are 0.01 – 0.1 µg/ml) [10]. Nevertheless, the study of ARI is motivated by a more diverse assortment of protolytic forms in comparison with ARIII. In this respect, congo red (CR) also forms a series of ions, each of which can bind to the determined ions of a particular metal [11]. For these reasons, the study of systems involving dye reagents is promising in terms of analyzing natural objects and quantifying cadmium and lead in soils.

Previously, we have experimentally established that cations of cyanine dyes (for example, pinacyanol [12] (PNC) can interact with organic anions and form ionic associates at very low concentrations in water ($5\cdot10^{-7}$ M and more). The fact of association at such concentrations opens up new possibilities in increasing the sensitivity of quantitative determinations. It was of interest to find out to what extent it is possible to use the properties of associates such as «arsenazo + PNC», «Congo red + PNC» for the quantitative determination of cadmium or lead in aqueous extract of soils. Proceeding from this, the following stages of research were determined: experimental study between ARII and PNC, CR and PNC; analysis of changes in the absorption spectra of ArII with the addition of cations Cd^{2+} or Pb^{2+} at concentrations of $2\cdot10^{-7}$ – $2\cdot10^{-3}$ M; determination of the influence of metal cations on dye associates.

2. Materials and Methods

The sodium salts were used for CR, ARII and chloride of PNC.



(1) (2) (3)

Figure 1. *Structural formulas of: (1) – pinacyanol, (2) – congo red, (3) – arsenazo II.*

The preparation of PNC and CR were of the trade mark «Sigma» and the AR II preparation was of «Reanal Labor». The contents of the main substance were not less than 95%. The initial solutions of AR II and CR were prepared by dissolving the exact samples with a concentration of 10^{-3} M. Dilutions of dye solutions with concentrations at 10^{-4} – 10^{-5} M were used for the measurements. The light absorption was measured under room temperature immediately after the preparation of the solutions in order to avoid the influence of a perceptible adsorption of the reagents on the surfaces of the photometric cuvettes. To study the effect of Cd^{2+} or Pb^{2+} cation we used solutions of $CdSO_4$ and $Pb(CH_3COO)_2$ at a concentration level of 0.01 M.

In the aqueous medium, the PNC exists in cationic form and at concentrations $3\cdot10^{-7}$ – $2\cdot10^{-6}$ M does not change its spectral characteristics in the pH range from 3 to 10. The presence of inorganic ions at the concentration level 10^{-5} – 10^{-2} M also does not affect the light absorption of the PNC. However, in the alkaline medium (pH≥10) the dye is hydrolyzed. As a result, partial or complete discoloration of the solutions takes place [13]. The formation of PNC dimers is noticeable at $7\cdot10^{-6}$ M and water-insoluble aggregates at $5\cdot10^{-5}$ M or more.

AR II has in its composition two hydroxyarsenic groups, four sulfo- groups and four gydroxy- groups. Protolytic properties AR II can be estimated by analyzing data on the dissociation constants and the distribution of mole fractions AR I and AR III as a function of pH [14]. Thus, as a result of dissociation, AR II can form quite a lot of anions depending on the pH of the solution. The dissociation of oxonaphthol fragments occurs in a strongly alkaline medium (at pH 10 or more), and AR II predominantly exists in the form of a multiply charged anion H_4R^{8-} at pH 9.2 [13I] due to dissociated of hydroxyarsenic and sulfo-groups. CR forms a doubly charged anion in an alkaline medium (pH>5.2), the molar fraction of which is close to 100%. So, buffer solutions with pH 9.2 were used in investigations.

Association between cation (PNC) and anion (AP II or CR) was interpreted as an equilibrium process:

$$j\mathrm{Ct}^{+} + \mathrm{An}^{j-} \rightleftharpoons (\mathrm{Ct}^{+})_{j}\cdot\mathrm{An}^{j-}$$

The association constants, K_{as}, were determined by the law of the effective masses:

$$K_{\text{as}} = \frac{[(\text{Ct}^+)_j \cdot \text{An}^{j-}]}{[\text{Ct}^+]^j \times [\text{An}^{j-}]} = $$

$$= \frac{[(\text{Ct}^+)_j \cdot \text{An}^{j-}]}{(C_{\text{An}} - [(\text{Ct}^+)_j \cdot \text{An}^{j-}]) \times (C_{\text{Ct}} - j \times [(\text{Ct}^+)_j \cdot \text{An}^{j-}])^j}.$$

For the calculation of the association constants, the molar equilibrium concentrations of the associate $[(\text{Ct}^+)_j \cdot \text{An}^{j-}]$ were determined from the spectral data by the equation:

$$[(\text{Ct}^+)_j \cdot \text{An}^{j-}] = \frac{\varepsilon_{\text{Ct}} \times C_{\text{Ct}} \times l - A}{(j \times \varepsilon_{\text{Ct}} - \varepsilon_{\text{As}}) \times l}$$

where C_{Ct} is the analytical concentration of PNC (which does not vary in a single series of measurements); A is the optical density for the current value of the analytical concentration of the anion (C_{An}); l is the length of absorbing layer; ε_{Ct} and ε_{as} are the molar absorption coefficients of the cation and associate, respectively.

Very dilute solutions of dyes were used, the ionic strength (I) of photometric solutions did not exceed 0.001–0.008 M. So, the concentration association constant is actually thermodynamic at such values of I.

3. Resuts and Discussion

3.1. Properties of the Reagents in Water

The absorption spectra of AR II and CR have been investigated in the $2 \cdot 10^{-7} - 1 \cdot 10^{-4}$ M concentration range. As the concentration of dye increases, a gradual increase in the absorption intensity is observed. In Figure 2 shows the absorption spectra of AR II. The optical density of solutions with increasing concentration at the maximum of the absorption band obeys the basic law of light absorption. A similar picture is observed for CR.

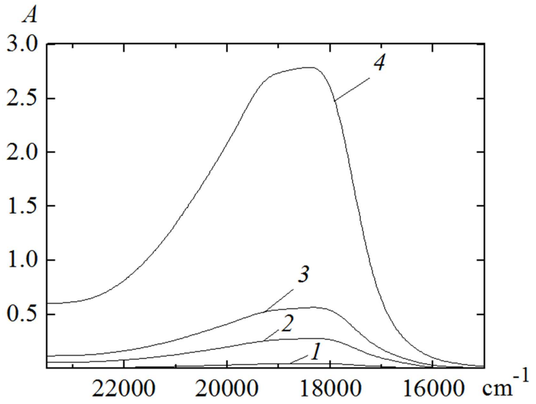


Figure 2. *Absorption spectra of AR II. Concentrations of AR II: 2,04·10^{-6} M (1), 1,02·10^{-5} M (2), 2,04·10^{-5} M (3), 1,02·10^{-4} M(4).*

Regression equations for dyes have the form:

AR II: $A_{545} = -0{,}007_{(0{,}005)} + 27457_{(112)} \cdot C$, correlation coefficient $0{,}99997_{(0{,}0099)}$,

CR: $A_{522} = 0{,}008_{(0{,}003)} + 41868_{(378)} \cdot C$, correlation coefficient $0{,}99997_{(0{,}0099)}$,

where A is the optical density at the indicated wavelength, C is the initial molar concentration of the dye, and the standard deviation of the each parameter is indicated by the brackets.

In fact, $A = k \times C$, since the free term of the regressions is a statistical zero. Thus, it can be considered that the AR II and CR dyes do not dimerize in the indicated concentration ranges. On the contrary, for PNC, the basic law of light absorption is observed in a very narrow range of concentrations ($3 \cdot 10^{-7} - 2 \cdot 10^{-6}$ M), since PNC is prone to self-association (in more detail, the properties of PNC in aqueous solution are considered in [15]).

The absence of a self-association of AR II or CR at pH 9.2 can be explained by the nonplanar structure of molecules (Figure 3, the simulation was made for the molecule using the Hyper 7.0 package, geometry optimization was performed using the MM+ method;, the σ-bond length of the C–C biphenyl (is marked in green) is 1.41 Å; the angle of rotation of these benzene rings relative to each other along the C–C bond is 18°; 1, 2, 3 – are inertial axes), as well as by the presence of charged functional groups in ions that contribute to electrostatic repulsion.

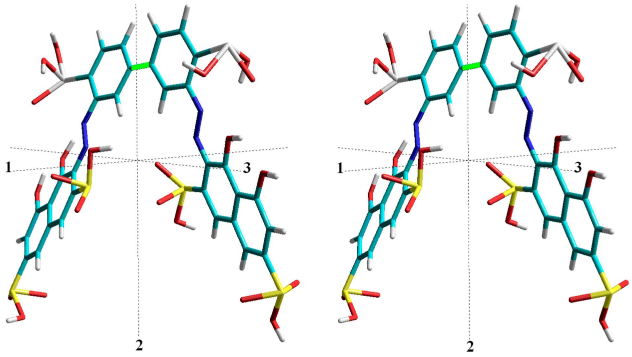


Figure 3. *Structure of AR II (stereo image).*

Unlike AR II and CR, the planar cation of PNC is able to form self-associates. The logarithm of the equilibrium dimerization constant in water is 4.79 ± 0.06 [16]. So, PNC is significantly dimerized at concentrations more than $5 \cdot 10^{-6}$ M.

3.2. Interactions Between PNC and AR II or CR

Let us consider the spectral changes that occur when increasing amounts of AR II are added to the PNC (Figure 4). Even with insignificant additions of APII (at a concentration ten times lower than the concentration of PNC, spectrum *2*), the intensity of light absorption decreases, and at a concentration $5.1 \cdot 10^{-7}$ M) the optical density decreases by half compared to the initial light absorption of the PNC. The optical density of the mixture is much lower than the sum of the optical density of the dyes at a certain concentration. Such changes in the absorption spectrum of PNC indicate the intense interaction of various dyes and the formation of an ion associate. In the spectral series, regions of «saturation» are observed when the optical density remains almost unchanged with increasing concentrations of the anion (spectra *6*, *7*). Analogous spectral effects are also indicated when CR is added to PNC, but at higher concentrations.

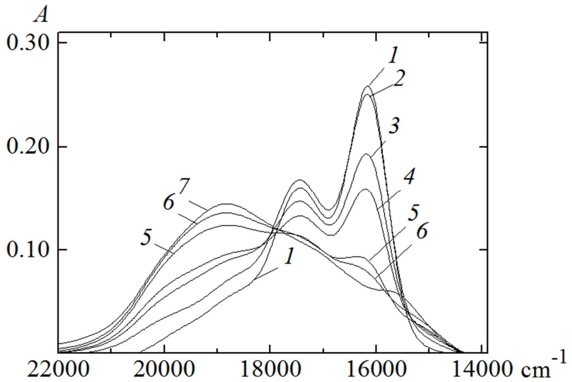


Figure 4. *Absorption spectra in «PNC + AR II» system. Concentration of PNC: 3.18·10^{-6} M (1–7). Concentration of AR II: 0 M (1), 3.06·10^{-7} M (2), 4.08·10^{-7} M (3), 5.1·10^{-7} M (4), 6.12·10^{-7} M (5), 8.16·10^{-7} M (6), 1.43·10^{-6} M (7). pH 9.2.*

Similar changes were also earlier observed when sulfonephthalein or oxyxanthene anions were added to the PNC solution [12].

The stoichiometric ratios of the particles in the PNC+AR II and PNC + CR associates are 4: 1 and 2: 1, respectively. Thus, for one AR II ion there are only four cations of PNC. It can be assumed that four PNC cations can be located near the multiply charged anion AR II in this way: two cations are near the arsonic groups of the biphenyl fragment, and the other two are next to the sulfo groups on the outside of the naphthol fragments. The addition of more cations of PNC is unlikely due to significant steric hindrances.

The value of log K_{as} is 2.15 ± 0.01 for the $(PNC)_4$·AR II associate and 4.09 ± 0.02 for the PNC_2·CR associate. These values are in 2–4 times lower than for associates with doubly charged anions, and in 1.5 times less for PNC associates with singly charged anions of sulfonephthaleins or oxyxantenes. For example, log K_{as} = 5.83 and log K_{as} = 11.81 for associate of the single- or double-charged of phenol red and log K_{as} = 13.46 for the double-charged Bengal rose associate [17]. A significant decrease in the association constants of AR II or CR is probably due to the already mentioned non-planarity of azo-dyes, which weakens the cation-anion interactions.

3.3. Interactions Between AR II, CR or Associates with Metal Cations of Agricultural Soils

A specific technique for the isolation or analysis of metals in soils takes into account the presence of reaction centers coordinating some of the protolytic forms of these metals in the soils. For example, one of the methods for determining the content of heavy metals directly related to organic matter is the preliminary treatment of soil samples with a NaOH solution. In this case, the metals are extracted together with the humic acids [18]. The acidity of such extracts is at least pH 11. Increasing the acidity of soil extracts reduces the solubility of humic acids and precipitations are formed [19]. In this connection, it makes sense to investigate the interactions between cadmium or lead ions with AR II, CR in an alkaline medium, in particular at pH 9.2.

The basis of effective techniques for determining the content of cadmium and lead in soils is the formation of complex compounds of these metals with colored organic ions [20]. We have examined in more detail the effect of additions of Cd^{2+} or Pb^{2+} with concentrations 2.0·10^{-7} – 9.7·10^{-3} M on the absorption spectra of AR II. The range of metal concentrations corresponded to their possible content in the soil [21].

Our investigations allow us to state that the reagents AR II, CR have proved to be sensitive to the content of the metals at 1·10^{-4} M level. For example, as follows from Figure 5, changes in the absorption spectrum of the AR II begin to be observed with the addition of solutions with a 1·10^{-4} M concentration of lead.

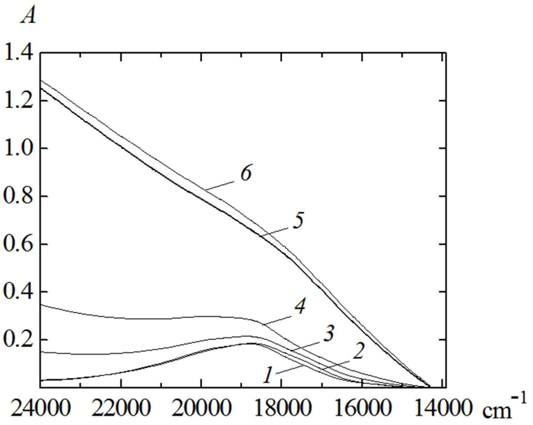


Figure 5. *Absorption spectra in «AR II + Pb^{2+}» system. Concentration of AR II: 6.5·10^{-6} M (1–6). Concentration of Pb^{2+}: 0 M (1), 1.0·10^{-6} M (2), 1·10^{-4} M (3), 2.0·10^{-4} M (4), 1.0·10^{-3} M (5), 2.0·10^{-3} M (6).*

At the same time, the intensity of light absorption increases. In the presence of cadmium, such increase in light absorption occurs at a lower cation concentration (1.9·10^{-5} M). The unequal effect of cations can be explained by the different degree of their hydrolysis. At pH 9, the mole fraction of Cd^{2+} is approximately 95%, whereas for Pb^{2+} the mole fraction is equal to 2.5% [22]. Due to hydrolysis, more than 90% of the lead is in the form PbOH and 5% is in the form $Pb(OH)_2$. This prevents the interaction between the dye and the metal, and such interaction occurs only at a high concentration of cation in an alkaline medium.

Based on the results of a number of investigations [3-6], it can be assumed that the more sensitive to metal ions are not the dyes, but their ionic associates. However, spectrophotometric studies of the effect of heavy metal cations on dye associates have not been investigated previously. Therefore, we have examined the effect of Cd^{2+} or Pb^{2+} on the spectral characteristics of the associate $(PNC)_4$·AR II in a wide range of metal concentrations, starting from 2·10^{-6} M. As can be seen from Figure 6, the addition of very small concentrations of Cd^{2+} to the associate causes a change in the light absorption.

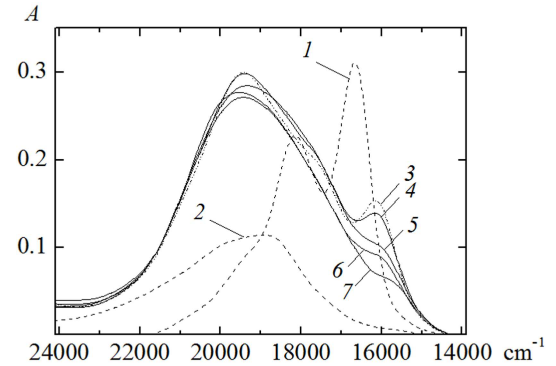


Figure 6. *Absorption spectra in «PNC + AR II + Cd^{2+}» system. Concentration of PNC: $3.2 \cdot 10^{-6}$ M (1, 3–7). Concentration of AR II: 0 M (1), $3.06 \cdot 10^{-6}$ M (2–7).*

Concentration of Cd^{2+}: 0 M (1–3), $1.94 \cdot 10^{-6}$ M (4), $9.7 \cdot 10^{-6}$ M (5), $1.94 \cdot 10^{-5}$ M (6), $1.94 \cdot 10^{-4}$ M (7).

A gradual decrease in the intensity for a long-wave absorption band takes place. Similar spectral changes are observed with the addition of Pb^{2+} with similar concentrations. We note that the interaction occurs at low values of the cation concentrations and in a wide range, which corresponds to the level of their content in the soil. Comparing the interactions in the systems "dye + metal" and "associate of dyes + metal" it can be argued that associate is a more sensitive reagent for both lead and cadmium. In addition, unlike the dye, the associate is not sensitive to the form of the cation (the cation is hydrolysed or unhydrolyzed) in an aqueous medium.

4. Conclusions

Spectral and equilibrium properties have been considered of systems «dye + metal», «dye associate + metal» for pinacyanol, congo red, arsenazo II with participant of lead or cadmium. The formation of dye associates and the interaction of associates with cations of lead and cadmium are accompanied by noticeable spectral effects at very low concentrations. Such effects open up new possibilities in increasing the sensitivity of quantitative determinations and can find application in the search for new efficient methods for the quantitative determination of small amounts of heavy metals (10^{-6} M) in soils with the help of aqueous extracts. The peculiarity of such methods is the high sensitivity of the determination under the condition of a successful choice of the equilibrium and spectral characteristics of the dye associate.

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