Abstract A solid-phase reagent based on 1-(4-adamantyl-2-thiasolylazo)-2-naphthol adsorbed onto silica gel was prepared for Co(II) recovery and preconcentration prior to its sorption-spectroscopic detection. The immobilized reagent was applied to the determination of free cobalt ions in natural water. The solid-phase reagent and chemiluminescent method coupled with membrane filtration, gel-permeation and ion-exchange chromatography were applied to the study of the speciation of iron and cobalt in water from the Dnieper reservoirs and lakes of Kyiv City; their predominant forms were complexes of Fe(III) and Co(II) with dissolved organic matter and fulvic acids play a main role in their complexation.

Keywords Speciation · Iron · Cobalt · Humic substances · Solid-phase reagent · 1-(4-Adamantyl-2-thiasolylazo)-2-naphthol

Introduction

Toxicity and bioavailability of heavy metals (HMs) depends not only on their total content in water, but also on the concentration and ratio of the various species [1, 2, 3]. Free metal ions are regarded as the most toxic form, as complexation of HMs with dissolved organic matter (DOM) decreases their toxicity in most cases [4, 5, 6]. Estimation of the degree of the toxic effect of HMs on hydrobionts and water quality may be carried on the basis of data about their distribution among coexisting forms in aquatic environment. However, the low concentration of individual metal species and their wide variety creates additional drawbacks in sample handling, species separation, and their detection. Moreover, during the fractionation of existing forms of metal, for example, by means of gel-permeation chromatography (GPC), its total content is split into separate fractions. Consequently, it is necessary to be able to determine very low concentrations of the metal. Chemiluminescent (CL) methods are the most suitable for these purposes. CL methods are characterized by a low detection limit and wide calibration ranges. It is reasonably cheap, and it does not require preliminary sample concentrating (extraction, evaporation, freezing out, etc.). The short time of analytical signal registration is a very important factor, as the equilibrium between the particular HM forms in the water sample is not disturbed during the detection. CL methods are suitable for the reliable determination of the concentration of free metal ions, which are reactive in catalytic CL reactions. However, identification of the various metal species in natural waters using CL methods coupled with different methods of separation can only be carried out in the laboratory. The samples must be analyzed as soon as possible, but it is rather very difficult to carry out in situ CL analysis. Thus, the samples may sometimes be preserved, transported, and analyzed in the laboratory after a couple of days. The distribution of metals among their coexisting forms under these conditions is naturally changed [3]. Therefore, the development of analytical techniques for the determination of individual HM species at the sampling place is an important task. Solid-phase reagents based on modified silica gels, in particular, may be applied for chemical species determination or their fast preconcentration. Concentrates may be analyzed in the laboratory if necessary [7]. Silicas modified by adsorbed analytical reagents are found to be environmentally friendly and cost effective tools for analytical sampling and sensitive detection of various compounds in complicated objects [7, 8, 9, 10, 11, 12]. These kinds of sorbent are suitable for the preconcentration of metal ions due to their high adsorptive and kinetic properties [11]. Moreover, the adsorption is known to be one of the effective and simple methods for silica modification.

Therefore, the present study focused on the development of a solid-phase reagent based on highly dispersed...
silica for the rapid in situ detection of labile HM species. This paper describes the properties of a new chelating sorbent, obtained by adsorption of 1-(4-adamantyl-2-thiasolylazo)-2-naphthol (ATAN) from hexane solution, and its sorption behavior towards Co(II). In addition, iron and cobalt speciation in natural water was investigated by means of membrane filtration, GPC, ion-exchange chromatography, and a CL method.

**Experimental**

**Chemicals and reagents**

All chemicals were of analytical reagent grade and doubly distilled water was used throughout the study. The aqueous solution of 4-(2-pyridylazo)resorcinol (PAR) (Chemapol, Prague, Czech Republic), hexane, and acetone (LIChrsolv, Merck, Germany) solutions of ATAN were obtained by dissolving appropriate substances. Silica gel 60 (SG) for column chromatography (Merck, Germany) was used without preliminary treatment. Stock solutions of humic acids (Works of chemical reagents, Olaine, Latvia) were prepared by dissolving of a weighed material.

**Apparatus**

The absorbance spectra of solutions and solid-phase reagent were recorded with a Specord M-40 UV/Vis spectrophotometer (Carl Zeiss Jena, Germany) and KFK-3 spectrophotometer (Russia), respectively. A potentiometer model EV-74 with glass electrode (Gomel, Belarus) was used for pH measurements.

UV-photolysis was carried out in quartz glasses (≈40-mL volume) with a DRT-1000 mercury lamp (Ekaterinburg, Russia) for 2.5–3.0 h. CL measurements were performed with a CL photometer (Kyiv, Ukraine) equipped with a photomultiplier tube PMT-19A to allow measurement of the CL intensity and sum of CL for the fixed time [13].

The ion-exchange chromatography was used for the fractionation of metal complexes with DOM. The glass columns (24×300 mm) with cellulose ion-exchangers DEAE and CM (Reanal, Budapest, Hungary) were used for this purpose.

The molecular weight distributions were studied using GPC. The GPC system has a packed column, peristaltic pump P-1 (BioMark, Inc., Lviv, Ukraine), and fraction collector Dombifrac D-002 (Diagnostykum, Lviv, Ukraine).

**Procedures**

**Adsorption of ATAN onto SG and metal ions onto modified SG**

The batch technique was used for the study of ATAN adsorption onto unloaded silica gel. SG (0.02 g) was stirred with 5 mL of 1–50 μmol L⁻¹ ATAN hexane solution for 1–40 min. The ATAN residue in the solution after sorbent separation was controlled spectrophotometrically at λₘₐₓ=490 nm by the absorbance of ATAN.

The equation for the calibration graph was 

\[ A_{490} = (15.2 ± 0.1) C_{\text{ATAN}} \text{ (mmol L}^{-1} \text{)}, \ r = 0.999. \]

The adsorption of Co(II) onto SG loaded with ATAN (ATAN-SG) from aqueous solution at pH 2–7 was performed by stirring 0.02–0.20 g of modified sorbent with 5–50 mL of solution containing 1–100 μmol L⁻¹ Co(II). The pH of the solution was adjusted to the desired value with HNO₃ or KOH. After 5–30 min, the residual concentration of the metal ions was measured spectrophotometrically with PAR [14]. The equation for the calibration graph was 

\[ A_{510} = (0.083±0.001) C_{\text{Co(II)}} \text{ (μmol L}^{-1} \text{)}, \ r = 0.999. \]

The amount of compound adsorbed on the surface (a, mol g⁻¹) and the distribution coefficient (D, L g⁻¹) were calculated according to the equations: 

\[ a = (C_0 – C) V m^{-1}; D = (C_0 – C) V C^{-1} m^{-1}, \]

where \( C_0 \) and \( C \) are the initial and equilibrium concentrations of adsorbate in the solution (mol L⁻¹), \( V \) is the volume of solution (L), and \( m \) is the sorbent mass (g).

The reagent desorption from the surface into aqueous solutions at different pH was also studied by the batch technique.

**Sampling**

The water samples were collected from Dnieper reservoirs (Fig. 1) and some lakes from areas of Kyiv and placed in polyethylene bottles. The main physicochemical characteristic of these water bodies are given in Table 1 and Table 2. Dissolved and suspended forms of iron and cobalt were separated by pressure filtration through a 0.40-μm-membrane filter (Synpor, Prague, Czech Republic). If the transportation of samples took no more than 1 h, filtration was carried out in the laboratory. Otherwise water samples were filtered at the sample place.

**Fractionation of metal complexes by ion-exchange chromatography**

The DEAE-cellulose and CM-cellulose were cleaned and prepared as described by Sirotkina et al. [19]. Sample filtrates were fractionated into three fractions (Fig. 2a): anionic complexes (mainly with humic substances), cationic complexes (mainly with protein-like compounds), and neutral complexes (mainly with carbohydrates). The steps were as follows: (1) pass 1,000 mL of the filtrate of water sample through the DEAE-cellulose column (void volume 13 mL) and rinse the column with 10–15 mL of doubly distilled water.
water; (2) elute the anionic complexes with 25 mL of 0.1 mol L\(^{-1}\) NaOH (concentration factor 40 times); (3) pass filtrate from the DEAE-cellulose column through the CM-cellulose column (void volume 6 mL) and rinse with 10–15 mL of doubly distilled water; (4) elute the cationic complexes with 28 mL of 0.1 mol L\(^{-1}\) HCl (concentration factor 35 times). The filtrate after DEAE- and CM-cellulose columns contained the neutral metal complexes. This filtrate was concentrated by freezing-out (concentration factor 8–10 times) \[2\].

**Fractionation of metal complexes by GPC**

The experimental set-up for carrying out the molecular weight separation of metal complexes with DOM is depicted in Fig. 2b. The glass column (800\(\times\)26 mm) with the neutral gel Molselect G-75 (Reanal, Budapest, Hungary) was applied. KNO\(_3\) (0.02 mol L\(^{-1}\)) was used as mobile phase at a flow rate of 1 mL min\(^{-1}\). The GPC column was calibrated by the set of molecular weight standards composed of glucose (0.18 kDa), poly(ethylene glycol) (1.0 and 2.0 kDa), trypsin (23.8 kDa), carbonic anhydrase (29.0 kDa), and dextran (70 kDa). The column exclusion volume \(V_0\) (87 mL) was determined with Dextrane blue (2,000 kDa). The distribution coefficient \((R)\) was calculated for each marker according to the following equation \[20\]:

\[
R = \left(\frac{V_e - V_0}{V_{ex}}\right) / \left(\frac{V_{ex} - V_0}{V_{tot}}\right)
\]

where \(V_e\) is the elution volume, \(V_0\) is the exclusion volume, and \(V_{tot}\) is the total volume of the GPC column. The calibration curve was obtained by plotting the observed \(R\)-value of the marker against the logarithm of its molecular weight. Molecular weights of metal complexes with DOM were determined by comparing their \(R\)-values with the calibration curve.

Filtered water (200 mL) was concentrated to approximately 25 mL (concentration factor 8 times) using the freezing-out technique. The concentrated water sample (6 mL) was introduced onto a column and 15–mL fractions were collected using a collector.
Metal determination

Concentration of free metal ions was determined by a CL method based on the catalytic effect of Fe³⁺/Co²⁺ in the reaction between luminol or 4-diethylaminophthalhydrazide (4-DEAPH) and hydrogen peroxide [21, 22]. Total dissolved metal concentration was determined after UV-irradiation of ≈30-mL aliquots acidified to pH=1 by addition of purified sulfuric acid. Concentrations of free metal ions were determined without UV treatment.

Results and discussion

The speciation of iron and cobalt in water from the Dnieper reservoirs and some lakes of Kyiv City was studied by means of membrane filtration, GPC, and ion-exchange chromatography. The CL method was used for the detection of the concentration of free iron(III) and cobalt(II) ions. For the determination of free Co(II) ion in situ the solid-phase reagent was also prepared. For these purposes the adsorption of ATAN onto SG from acetone, hexane, and their mixtures was studied. The best results were observed when hexane was used and therefore this solution was used for ATAN adsorptions. The kinetics experiments showed that the equilibrium of reagent adsorption onto SG under these conditions was reached in 5 min. ATAN adsorption was studied as a function of reagent concentration in solution. The isotherm of ATAN adsorption onto SG is represented in Fig. 3 (curve 1); the initial part of the isotherm is vertical. Its shape can be attributed to the H-type in accordance with ref. [23], indicating that there is a strong reagent bond to the SG surface. The maximum value of reagent adsorption was 4.9 µmol g⁻¹.

The procedure of SG modification was therefore simple and rapid (5 min). These properties combined with stability (the desorption of reagent from the ATAN-SG in pH range 1–8 was found to be less than 1%) testified to the suitability of solid-phase ATAN for analytical practice.

The sorption of cobalt ions on modified sorbent is dependent on the pH of solution due to the competitive reaction between chelate-forming groups of ATAN and hydrogen ions in the solutions. The recovery of Co(II) with solid-phase reagent at pH 5–6 was found to be quantitative. Kinetic experiments showed that the cobalt adsorption equilibrium was reached in 20 min. The recovery as a function of sorbent mass and solution volume was studied. It was found that the maximum distribution coefficient of Co(II) was 1.85 L g⁻¹ at a solution volume of 25 mL and sorbent mass of 0.1 g. The isotherm of Co(II) adsorption is presented in Fig. 3 (curve 2); the L-type isotherm testifies to the complexity between the Co(II) and immobilized ATAN. The absorbance spectra of ATAN-SG treated with Co(II) solution of various concentrations are presented in Fig. 4. The intensity of signal at 570 nm increased with increasing cobalt concentration in solution. The equation of the calibration graph was $A_{570}=(0.065±0.005)C_{\text{Co(II)}}$ (µmol L⁻¹), $r=0.990$. The detection limit (blank+3σ) was 0.5 µmol L⁻¹.

Macro- and microcomponents of natural water did not influence Co(II) determination using ATAN-SG. This testifies to the applicability of ATAN-SG to Co(II) determination in natural water. To examine the usefulness of the solid-phase reagent for the determination of Co free ions in natural water, the model solution containing humic acids was analyzed. The determination of Co(II) was per-

<table>
<thead>
<tr>
<th>$N$</th>
<th>Added Co(II) (mg L⁻¹)</th>
<th>Found Co(II) (mg L⁻¹)</th>
<th>$S_r$</th>
<th>Sorption-spectroscopic method $S_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.13±0.01</td>
<td>0.03</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>0.16±0.03</td>
<td>0.08</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0.30±0.03</td>
<td>0.04</td>
<td>0.35±0.03</td>
</tr>
</tbody>
</table>

$^{}\text{a}n=3, P=0.95$; concentration of humic acids was 10 mg L⁻¹; pH=7.1
formed after establishing complexation equilibrium (one month) between free ions and complexes with humic acids. The concentration of free cobalt ions in the model solution is lower than its total content due to the binding of Co(II) by humic acids. A comparison of the results of Co(II) determination using CL and sorption-spectroscopic methods was carried out (Table 3). It is possible to conclude that the data obtained by both methods are in good agreement and ATAN-SG is suitable for free cobalt ion determination.

Our research has also focused on the study of the dissolved forms of iron(III) and cobalt(II), in particular the ratio of free metal ions and complexes with DOM. The data on total content of their dissolved forms in the water bodies investigated are summarized in Table 4. The results indicate that the concentration of complexes of Fe(III) and Co(II) with DOM reaches 93–97% of the total content of dissolved forms in natural water. Humic substances, chiefly fulvic acids, play a main role in complexation. These ligands bind 42–93% of iron and cobalt ions (anionic complexes in Fig. 5). The cationic fraction was 6.5–14.3%, indicating that protein-like substances do not contribute significantly to the complexation of Fe(III) and Co(II) in the studied water bodies. Neutral complexes of iron and cobalt make up 15–36% of bound metal ions. Such distribution is possible because humic substances are dominated in DOM composition from the Dnieper reservoirs (Table 1). It is also known [2, 4, 24, 25, 26, 27] that humic substances form stable complexes with metal ions in aquatic environments. On the other hand, the protein-like substances and carbohydrates cannot be present in significant quantities as their free forms in water because of their rather rapid decomposition. Therefore, the complexes of iron and cobalt with humic substances, as well as in the case of other heavy metals, are the main class of their species in natural water. The influence of

Table 4 Concentration of iron(III) and cobalt(II) dissolved forms in water from the Dnieper reservoirs and lakes of Kyiv City

<table>
<thead>
<tr>
<th>Water bodies, sampling location and date</th>
<th>Total content of metal dissolved (µg L⁻¹)</th>
<th>Free ions and complexes with inorganic ligands (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaniv reservoir, upper part</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 2001</td>
<td>132±3</td>
<td>9.1±0.9</td>
</tr>
<tr>
<td>October 2001</td>
<td>323±18</td>
<td>13.3±0.7</td>
</tr>
<tr>
<td>Lake Tel’bin</td>
<td>99±6</td>
<td>5.1±0.6</td>
</tr>
<tr>
<td>August 2001</td>
<td>214±9</td>
<td>9.4±0.8</td>
</tr>
<tr>
<td>October 2001</td>
<td>331±7</td>
<td>17±1</td>
</tr>
<tr>
<td>Lake Verbne</td>
<td>130±10</td>
<td>6.0±0.7</td>
</tr>
<tr>
<td>August 2001</td>
<td>331±7</td>
<td>17±1</td>
</tr>
<tr>
<td>Lake Red’kino</td>
<td>169±13</td>
<td>8.4±0.8</td>
</tr>
<tr>
<td>October 2001</td>
<td>364±23</td>
<td>10±1</td>
</tr>
<tr>
<td>Co(II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyiv reservoir, lower part</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 1998</td>
<td>6.7±0.4</td>
<td>0.70±0.09</td>
</tr>
<tr>
<td>February 1999</td>
<td>3.9±0.3</td>
<td>0.60±0.08</td>
</tr>
<tr>
<td>Kyiv reservoir, upper part, September 1998</td>
<td>5.9±0.5</td>
<td>0.63±0.08</td>
</tr>
<tr>
<td>Kremenchug reservoir, central part, June 1998</td>
<td>7.9±0.3</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Kakhovka reservoir, lower part, June 1998</td>
<td>8.4±0.3</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Kaniv reservoir, upper part, January 1999</td>
<td>3.2±0.4</td>
<td>0.39±0.09</td>
</tr>
<tr>
<td>March 2001</td>
<td>0.62±0.08</td>
<td>&lt;LOD[^a]</td>
</tr>
<tr>
<td>April 2001</td>
<td>2.3±0.3</td>
<td>0.20±0.03</td>
</tr>
</tbody>
</table>

[^a] Limit of detection

Fig. 5a,b Distribution of iron (a) and cobalt (b) among their complexes with DOM of different chemical nature in the Kaniv (I) and the Kyiv (II) reservoirs in different seasons
seasonal dynamics of DOM composition on the molecular weight distribution of the complexes of metals was also studied. On the whole, the tendency toward the domination of the complexes with molecular weight of less than 5 kDa is observed. These complexes include 38–65% of the bound iron and 27–69% of cobalt (Fig. 6).

Conclusion

Thus, in the investigated surface waters the complexation of metal ions with natural organic substances plays the priority role in the fate and transport of iron and cobalt. Anionic complexes (mainly with fulvic acids) were found to be the main forms of bound Fe and Co. Metal complexes with relatively low molecular weight (<5 kDa) are dominant. The content of free Fe\(^{3+}\) and Co\(^{2+}\) ions was not more than 3–5 and 10–17% of their dissolved forms, respectively.

ATAN-SG is found to be a sensitive solid-phase reagent for the determination of free Co(II) ions in natural water at pH 5.0–7.0. The procedure of ATAN immobilization onto SG is simple and rapid.

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References