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Reversed-phase high-performance liquid chromatography of iron(II) and copper(II) chelates with 4,7-diphenyl-1,10-phenanthroline disulfonate

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Abstract

The chromatographic behaviour of negatively-charged iron(II) and copper(II) chelates with 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) disulfonate (BPS) on a reversed-phase Inertsil ODS-2 column was studied by using methanol–water (60:40, v/v) containing 0.02 *M* tetramethylammonium bromide (TMAB) and 0.025 *M* tris(hydroxymethyl)aminomethane (Tris)–HCl at pH 7.5 as a mobile phase. The possibility that the hydrophilic Tris–HCl buffer also serves as a counter ion together with TMAB was briefly discussed. Chromatographic parameters influencing the separation of the chelates were optimized. The method was successfully applied to indirectly determine iron(II) and iron(III) contents in natural water. Nickel(II) ion and possibly organic pollutants interfere with the present method. © 1998 Elsevier Science B.V.

Keywords: Iron; Copper; 4,7-Diphenyl-1,10-phenanthroline disulfonate; Metal chelates

1. Introduction

The development of analytical methods for the determination of trace metal ions as their metal chelate complexes by reversed-phase ion-pair chromatography (RP-IPC) has received notable attention in recent years [1–3]. Several reviews regarding this subject have appeared in the literature either for normal-phase [4] or both normal-phase and reversed-phase [5,6].

Since the introduction of high-performance liquid chromatography (HPLC) to the determination of metal ions, there have been many chelating reagents used for derivatizing metal ions prior to their separation by HPLC. Among these chelating reagents, some sensitive derivatives such as PAR [7], PAN [1,8,9], ferrozine [10] and 1,10-phenanthroline [11,12] are of considerable interest because they form either stable neutral or ionic chelates with a number of metal ions and their metal chelates in the eluate can be readily detected by spectrophotometry.

Recently, we have succeeded to separate iron(II) complexes of 1,10 phenanthroline and its derivatives by ion-pair chromatography [13] and developed a separation method for sensitive HPLC–spectrophotometric detection of metal ions using 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) as a de-

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rivatizing agent [14]. In the present work, we report the reversed-phase HPLC of iron(II) and copper(II) chelates using a sulfonate of 4,7-diphenyl-1,10-phenanthroline, i.e. bathophenanthroline disulfonate (BPS: Fig. 1) as a derivatizing agent. This reagent has been widely used for the spectrophotometric determination of iron(II) [15,16] because of their large solubility in water compared to the unsulfonated one. By using this reagent, a time-consuming step of extraction of iron(II) chelate from the aqueous solution becomes unnecessary. H. Saitoh et. al. [17] reported the use of this reagent as a colouring agent for the post-column determination of iron(II) and iron(III) after separating these cations on an ion-exchange column and reducing iron(III) to iron(II) with ascorbic acid. Flow-injection analysis of iron(II) using 1,10-phenanthroline [18] as a colouring agent and of copper(II) using 4,7-diphenyl-3,9-dimethyl-1,10-phenanthroline [19] have been also reported recently. However the sensitivity and/or selectivity of the methods are still poor and thus they are still inadequate for the purpose of metal-ion determination at the level of sub part per billion (ppb). In the proposed method, the chelates were detected with a wavelength in the UV region because metal chelates are well separated from their free ligand. Hence the sensitivity is improved and the detection limit of the present method goes down to the subnanogram level. In addition, the reported method seems promising because it can be applied to the determination of iron(II) and iron(III) at the ppb level in samples such as river water and tap water.

2. Experimental

2.1. Apparatus

The absorption spectra were recorded on a V-550 double beam UV–VIS spectrophotometer (Jasco, Tokyo, Japan). A model HM-12P pH-meter from TOA Electronics (Japan) was used for pH measurements. The HPLC setup used, consisted of a model BIP-I HPLC pump (Jasco), a model M-315 variable wavelength UV spectrophotometric detector (Shodex, Japan) together with a model 807-IT integrator (Jasco) and a Rheodyne Model-7120 sample injection valve with a 100-mm^3 loop. The analytical column used was an Inertsil ODS-2 from GL Sciences (Tokyo, Japan; 4.6 mm bore, 250 mm in length, packed with 5-μm particle). The column temperature was adjusted to various temperatures by dipping the column into a thermostat bath (model UC-65 from Eyela, Tokyo Rikakikai, Japan) containing water. The mobile phase was degassed every time before use by ultrasonically vibrating their containers. All the sample solutions of chelate compounds were filtered prior to injection into the HPLC with a Toyo Roshi DISMIC-25 JP filter (0.45 μm).

2.2. Reagents

The sulphonate of bathophenanthroline (bathophenanthroline sulfonic acid, disodium salt) obtained from Dojindo Laboratories was dissolved in doubly distilled water to give the concentration of

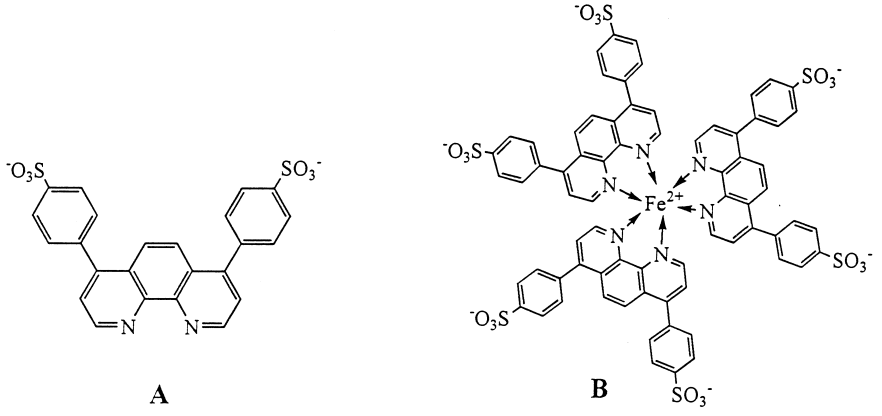

Fig. 1. Structure of bathophenanthroline disulfonate (A) and its iron (II) chelate (B).

5.0×10^{-4} *M*. Methanol (analytical-reagent grade) and distilled water (HPLC grade) were purchased from Katayama Chemical Industries. As an ion pairing reagent, tetramethyl- and tetrabutyl-ammonium bromides (TMAB and TBAB) obtained from Kanto Chemical were used. The pH buffer solutions of tris(hydroxymethyl)aminomethane (Tris)–HCl, 3-morpholinopropane–sulfonic acid (MOPS)–KOH, citrate, phosphate and acetate were used for the pH range of 3.5–7.5. A typical mobile phase solution was methanol–water (60:40, v/v) containing 0.020 *M* TMAB and 0.025 *M* Tris–HCl at pH 7.5. The flow-rate of the mobile phase was 0.5 cm^3 min^{-1} and the detector was operated at 280 nm. Stock solutions of iron(II) (freshly prepared) and copper(II) were prepared from their corresponding salts which were obtained from the following sources: $FeSO_4(NH_4)_2SO_4\cdot6H_2O$ was from Wako Pure Chemical Industries and $CuSO_4\cdot5H_2O$ was from Junsei Chemical. All other reagents and solvents used were of guaranteed reagent grade.

2.3. Procedures

2.3.1. General HPLC procedure

The required quantity of the stock solution (100 $\mu g\ cm^{-3}$) of each metal ion was transferred into a 25-cm^3 volumetric flask. After diluting the solution to about 10 cm^3 with the mobile phase containing an ion pair reagent and buffer solution, a sufficient amount (ca. 2 cm^3) of 5.0×10^{-4} *M* BPS was added. The solution was then shaken for several minutes and finally made up to 25 cm^3 with the mobile phase. The resulting solution was loaded into the sample loop (100 mm^3) and injected into HPLC. The capacity factor (k') of BPS and its chelates were calculated using the retention time of the unretained peak of water (4.0 min) as a measure of the void volume.

2.3.2. Determination of iron(II) in samples

An aliquot (5 cm^3) of slightly acidified river water (the Asakawa river) or tap water (in Hiyoshi, Yokohama) samples was transferred to a 25-cm^3 volumetric flask and treated as described in the general procedure.

2.3.3. Determination of total iron (Fe^{II} + Fe^{III}) in samples

To an aliquot of river water or tap water in 25-cm^3 volumetric flasks was added, 2 cm^3 of 5% hydroxylamine hydrochloride solution and diluted to about 10 cm^3 with the mobile phase. The solution was then heated to 80°C for about 10 min to completely reduce iron(III) to iron(II), cooled to room temperature and finally treated as described in the general procedure.

3. Results and discussion

3.1. Complexation and absorption spectra

Bathophenanthroline disulfonate (BPS) reacts with various metal ions in a similar manner to its parent ligand, 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline). With iron(II) ion, this reagent readily forms a relatively stable octahedral chelate, $[Fe(BPS)_3]^{4-}$ (log $\beta_3=22.3$), over a wide range of pH 2–9, while copper(II) ion forms a tetrahedral chelate, $[Cu(BPS)_2]^{2-}$, in slightly acidic solution [16]. These differences in structure are the basis of separation by RP-HPLC. Under the present experimental conditions, it was also confirmed by the spectrophotometric method that the molar ratio of iron(II) to ligand in the solution is 1:3. Both metal chelates and the ligand showed a characteristic absorption in the ultraviolet region between 250–300 nm which corresponds to intraligand transition (ILT), $\pi-\pi^*$. The maximum wavelength (λ_{max}) and molar absorptivity (ε: in parenthesis$\times10^4$) of the ligand and its chelate are 277(4.11), 284(8.02) and 280(11.7) nm for free ligand, copper(II) and iron(II) respectively. In the case of iron(II) chelate, another absorption in the visible region of wavelength, $\lambda_{max}=533(2.13)$ nm, was also observed due to metal-to-ligand charge transfer (MLCT), $d-\pi^*$ [20]. It is obvious that the chelates have a maximum of detection sensitivity near 280 nm and that the sensitivity of iron(II) chelate at this wavelength is at least five-fold higher than that at 533 nm. For this reason, the detection wavelength of 280 nm was selected for the further optimization of HPLC conditions.

3.2. Chromatographic behaviour of metal-chelates

Preliminary attempts to separate the chelates by classical ion-pair chromatography using alkylammonium (R_4N^+) as counter ions in methanol/acetonitrile–aqueous mobile phase containing buffer solution such as acetate and phosphate were not successful. The peak of the ligand on the chromatogram was very broad and both $[Fe(BPS)_3]^{4-}$ and $[Cu(BPS)_2]^{2-}$ chelates were almost unretained and eluted at the same time just behind the void volume. An attempt to alter either concentration of counter ion, pH of the mobile phase, organic composition in the mobile phase or the column packed with nonpolar poly(sterenedivinylbenzene) gel failed to yield a good retention of the chelates. This suggests that the formation of ion-pair species between anionic chelates and cationic R_4N^+ is incomplete due to the highly negative charge and/or the bulky structure of the chelates. Consequently, the chelates must be in anionic forms and thus still very polar. At this stage, it seemed that the classical ion-pair technique was not able to be used for the separation of the chelates.

The best separation of iron(II), copper(II) and the ligand was finally obtained when methanol–water (60:40, v/v) containing 0.020 *M* TMAB and 0.025 *M* Tris–HCl at pH 7.5 was used as the mobile phase. This result indicates that the buffer must play a significant role other than just maintaining pH of the mobile phase solution, i.e. as a counter ion. This conclusion is in good agreement with what has been reported most recently by E. Kaneko et al. [3]. In their paper, they reported that a remarkable improvement in separation and sensitivity was attained when a hydrophilic Tris–HCl (instead of the hydrophobic one such as R_4N^+) was employed as the counter ion and the pH buffer agent for the separation of anionic metal chelates with 2,2′-hydroxyazobenzene (DAHB). However our experimental results are somewhat different from what has been reported by Kaneko, et al., in which Tris–HCl can completely replace R_4N^+ in the mobile phase. In our case, both TMAB and Tris–HCl should exist together in the mobile phase. For instance, removing TMAB from the mobile phase can cause poor separation of the metal chelates, while replacing Tris–HCl by other buffer solutions results in no separation of them. A typical chromatogram of the chelates eluted in this composition of the mobile phase is shown in Fig. 2. The retention time of the three species was 4.6, 5.8 and 6.6 min for $[Fe(BPS)_3]^{4-}$, $[Cu(BPS)_3]^{2-}$ and BPS respectively.

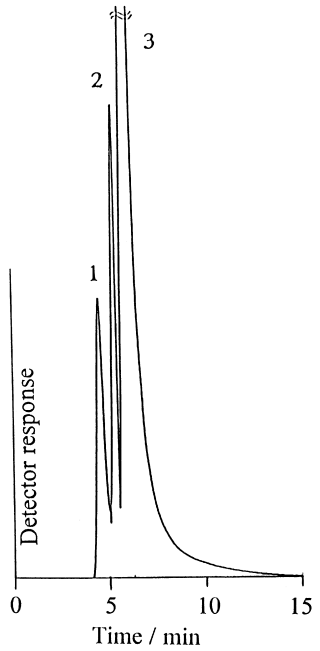

Fig. 2. Typical chromatogram of metal–BPS chelates. Mobile phase: methanol–water (60:40, v/v) containing 0.020 M TMAB and 0.025 *M* Tris–HCl (pH=7.5). Other conditions: see Section 2. 1=$[Fe(BPS)_4]^{4-}$; 2=$[Cu(BPS)_2]^{2-}$; and 3=BPS.

It is noteworthy that the shorter alkyl chain of R_4N^+ was likely more favourable for the retention of metal chelates than the longer one. For example, replacing TMAB with TBAB resulted in no separation. This might be explained in regard to the structure of the chelates which were relatively bulky due to the phenyl substituent containing sulfonic groups at the 4 and 7 position of 1,10-phenanthroline. The ion-paired formation of anionic chelates with the longer alkyl chain of R_4N^+ tends to make the chelates extremely large (see Fig. 1). It was assumed that the association strength of the longer alkyl chain of R_4N^+ with the chelates is weaker compared to that of the shorter one. Moreover, if one R_4N^+ forms an ion-pair with the chelates, it likely becomes difficult for the second R_4N^+ or Tris–HCl species to associate with the adjacent sulfonic group due to the steric hindrance effect. As a result, either

R_4N^+ or Tris–HCl cannot associate completely with the chelates and thus the separation of the chelates is not achieved.

The capacity factor (k') behaviour of the chelates with methanol-based solvent was investigated as a function of the concentration of organic components and the results are presented in Fig. 3. As expected, the retention time of the chelate are only slightly influenced by the water content in the mobile phase as indicated by low capacity factor (k') even for a high composition of water (>70%). This is obviously due to the nature of analytes which are a highly charged species. The optimum composition of the mobile phase was between 50 and 60% of methanol in water but the methanol content of 60% was chosen because it gave sharper and more symmetrical peaks.

3.3. Effect of the pH of the mobile phase and column temperature

The effect of the pH of the mobile phase on the capacity factor (k') of the ligand and its chelates was examined in the pH range of 2–8. In general, the capacity factor of all species decreases as the pH of the mobile phase increases, while the resolution between iron(II) and copper(II) chelates slightly increases. At pH>7.0, the resolution of all species seemed to increase significantly but the use of a mobile phase solution at higher pH (pH>8.0) was not feasible because of possible damage to the ODS column. Therefore, pH 7.5 was used for the analytical purposes. The effect of column temperature on capacity factor (log k') values for BPS and its chelates was also studied in the temperature range of 10–50°C. By increasing column temperature, elution time was reduced, while the resolution among the species was sustained. The best separation was obtained when column temperature was maintained at 20°C which was the same as room temperature.

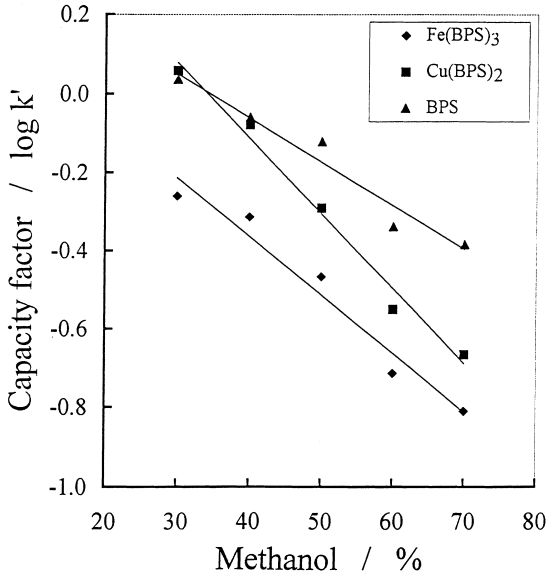

Fig. 3. Effect of methanol concentration (%, v/v) in the mobile phase on the capacity factor (log k') of metal–BPS chelates.

3.4. Determination of iron(II) and iron(III) ions

For the purpose of the determination of iron(II) and iron(III) ions in natural water, the calibration graph of peak area (mV min^{-1}) vs. iron(II) ion concentration ($\mu g\ cm^{-3}$) was constructed according to the general HPLC procedure described in the experimental section. The calibration graph obtained was linear in the concentration range of 0.0–1.0 $\mu g\ cm^{-3}$. The linear regression equation was $y=(25.76\pm1.00)\times10^6x+(0.03\pm1.25)$; where y=peak area and x=iron(II) concentration, with a correlation coefficient (r) of 0.997 for four replications (n=4) of each point. The repeatability for the determination of iron(II) ion was examined with five injections at a concentration level of 0.5 ng cm^{-3}. The relative standard deviation (R.S.D.) at this concentration was found to be 4.2% and the detection limit of 0.25 ng cm^{-3} was obtained for a signal-to-noise ratio (S/N) equal to 3. From the above data, it is clear that the analytical performance of the present method is quite competitive.

Nickel(II) ion interferes with the determination of iron(II) by the present method because this cation also forms a chelate with very similar charge and structure to iron(II). In the case that the sample to be analyzed contains a large amount of nickel(II) ion, it is advisable to determine iron(II) ion at 533 nm. Indeed no absorption is observed for nickel(II) chelate at this wavelength, but the sensitivity of iron(II) ion is somewhat lower than that at 280 nm. Some organic pollutants possibly also interfere with

the present method due to the low retention and short wavelength employed. Therefore, precaution must be taken when samples containing organic pollutants are analyzed, but for common natural water, such interference was not encountered.

The applicability of the present method to routine analysis was checked for the determination of iron(II) and iron(III) ions in tap water and river water as model samples. Iron(II) and total iron ($Fe^{II}+Fe^{III}$) were determined separately by reducing iron(III) to iron(II) with hydoxylamine hydrochloride as described in the experimental section for the total iron determination because their simultaneous determination was not possible due to the instability of iron(III) chelate with BPS. Iron(III) content was then calculated by subtracting iron(II) content from the total iron. The analytical results of iron(II) and iron(III) (average values for four replications) are respectively 191 ± 15 and 340 ± 35 ng cm^{-3} in tap water, and 72 ± 9 and 26 ± 1 ng cm^{-3} in river water. The somewhat higher value of total iron content in tap water is attributed probably to the contamination of iron resulting from the corrosion of the pipeline.

As a conclusion, it was demonstrated that the combination of tetramethylammonium bromide (TMAB) and the hydrophilic buffer of tris(hydroxymethyl)aminomethane (Tris)–HCl in the mobile phase is useful for the separation of the highly negatively-charged chelates which are normally difficult to be separated by classical ion-pair chromatography. The applicability of the proposed HPLC method to routine analysis was checked for the determination of iron(II) and iron(III) in natural water. A further study was needed to better understand the effect of the combination of TMAB and Tris–HCl on the separation of anionic chelates.

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