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Ion-interaction chromatographic studies on metal ions complexed with Plasmocorinth B dye

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ABSTRACT

The ion-interaction chromatographic behaviour of Plasmocorinth B (a disulphonated azo dye) and of its complexes formed with metal ions in oxidation states +2 and +3 (Cu^{2+}, Ni^{2+}, Al^{3+}, Co^{3+}, Fe^{3+}, Ga^{3+}) was investigated. The effect of cationic ion-pairing agents (tetramethyl-, benzyltrimethyl- and tetrabutylammonium) was also evaluated in the presence of alkali metal ions competitors (Li^+ and Na^+). The effects of organic modifier and of pH were also considered. The remarkably different behaviour of the ligand and the complexes suggests the formation of $M(II)L^-$ and $M(III)L_2^{2-}$. The stoichiometry of complexes was confirmed by applying the electrostatic theory derived from Gouy–Chapman and a modified Langmuir adsorption isotherm to the experimental data.

INTRODUCTION

Ion-interaction chromatography is an efficient and sensitive technique for the separation of heavy and transition metal ions [1–5]. These species may be separated as simple, hydrated ions or as anionic complexes, using suitable ligands. The ion-interaction reagent added to the eluent mixture is usually a strong base cation, such as the tetraalkylammonium ion, which is considered to give a dynamic equilibrium between the eluent and the stationary phase surface [6,7]. An increase in the organic solvent in the eluent decreases the interaction with the stationary phase [8], but pH and ionic strength also affect the equilibria involved in the separation [9–11]. Many studies have examined the retention mechanism [12–18] and, for a quantitative evaluation of the proposed models, the Gouy-Chapman theory associated with a modified Langmuir isotherm is a useful tool [17].

In this work the chromatographic behaviour of 3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (Plasmocorinth B, a disulphonated azo dye) and its metal ion complexes has been studied. PC acts as a planar tridentate ligand with a metal, forming five- and six-membered or two six-membered ring systems [18,19]. The evaluation of capacity factors (k') for Plasmocorinth B and its chelates with metal ions in different oxidation states (*e.g.* Cu^{2+} and Fe^{3+}), for different concentrations of ion interaction reagent, enabled us to determine or confirm the charge and the stoichiometry of the metal chelates.

The efficiency of different eluent compositions was also evaluated in order to optimize the separation of considered metal ions.

EXPERIMENTAL

Apparatus and reagents

The chromatographic system was a Varian LC 5000 liquid chromatograph (Varian, Walnut Creek, CA,USA) equipped with a Reodyne injection valve, a Varian spectrophotometric detector and a Vista 401 Data System. The analytical column was a LiChrospher 100 RP-18 (10 μm) (250 × 4 mm I.D.) column, coupled with a LiChroCART

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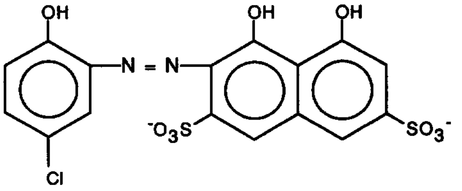
100 RP-18 (5 μm) guard column (4 × 4 mm I.D.), both obtained from Merck (Darmstadt, Germany). Columns and tubings were cleaned daily with a methanol–water (50:50, v/v) solution for 1 h at a flow-rate of 1.0 ml/min. Absorption measurements and spectra were performed with a Hitachi 150-200 double-beam spectrophotometer. An Orion digital pH-meter (Orion, Cambridge, MA, USA) was used for pH measurements.

All solutions were prepared with high-purity water obtained from a Milli-Q System (Millipore, Bedford, MA, USA). Acetic acid, sodium hydroxide, methanol for chromatography and standard metal solutions (Cu^{2+}, Ni^{2+}, Al^{3+}, Co^{3+}, Fe^{3+} and Ga^{3+} chlorides at 1000 mg/l) were Merck analytical-grade products. Lithium nitrate, Plasmocorinth B (see Fig. 1), tetramethylammonium hydroxide (TMAOH), tetrabutylammonium hydroxide (TBAOH) and benzyltrimethylammonium hydroxide (BTMAOH) were obtained from Aldrich (Milwaukee, WI, USA).

Mobile phase

The mobile phases were methanol–water (50:50, v/v) mixtures unless otherwise stated. The optimized eluent composition was: 2.0 *μM* Plasmocorinth B (ligand), 1.6 m*M* TBAOH (ion-interaction reagent), 6.2 m*M* sodium nitrate (ionic strength modifier), 10 m*M* acetic acid and sodium hydroxide up to pH 6.3. During the optimization the concentrations of TMAOH, TBAOH and BTMAOH ranged from 0 to 25.0 m*M*. The concentrations of sodium nitrate or lithium nitrate were from 0 to 0.25 *M* for studying the influence of ionic strength on capacity factors. The eluents were prepared daily, filtered on a 0.45-μm filter (Millipore HAWP 04700) and degassed under vacuum. In all experiments the flow-rate was 1.0 ml/min. The void volume of the chromatographic system was measured for every composition of mobile phase (without ion-interaction reagent present) by injection of a 100-μl sample of 0.5 m*M* sodium nitrate.



PLASMOCORINTH B

Fig. 1. Structure of Plasmocorinth B.

Samples

Appropriate quantities of metal stock solution (1000 mg/l) were added to an aqueous solution of 0.80 m*M* Plasmocorinth B (pH 5.3) to obtain the metal chelates in concentrations from 1 to 6 mg/l. pH was adjusted (1.0 *M* sodium hydroxide or lithium hydroxide) up to pH 6.3 in order to match that of the eluent; 100-μl sample volumes were analysed.

Detection

Before the chromatographic studies a detailed spectrophotometric study was performed on metal chelates. Plasmocorinth B exhibits two analytical peaks with maximum absorbance values at 270 and 527 nm. The wavelength of detection for chromatographic determinations of metal chelates was 270 nm because at this wavelength the maximum absorbance difference between metal complexes and Plasmocorinth B occurs.

RESULTS AND DISCUSSION

The parameters governing the separation of anion metal complexes by ion-interaction chromatography can be summarized as follows.

The pH affects the complex stability as well as the chromatographic separation. In this study the eluent was buffered to pH 6.3 with acetic acid and sodium or lithium hydroxide.

The ligand, added to the eluent, improves the complex stability. Experiments (0–4 *μM* Plasmocorinth B) also showed that there is an improvement in the stability of the chromatographic baseline when the ligand is added. A concentration of 2.0 *μM* Plasmocorinth B resulted in the best compromise, and this concentration was therefore used since an increase of ligand concentration results in higher background adsorbance.

The optimization of ion-interaction reagent and organic modifier concentrations such as ionic strength are detailed below.

Effect of ion-interaction reagents

The dependence of capacity factors (k') on the concentration and type of ion-interaction reagent was evaluated for Plasmocorinth B and its complexes with Cu^{2+} and Fe^{3+}, chosen as reference metal ions. The effect of TMAOH, TBAOH and BTMAOH on the chromatographic elutions is shown in Fig. 2. It is shown that only TBA enables metal separation through a partition equilibrium between the solid and the mobile phase. The TBAOH was selected as the hydrophobic ion-interaction reagent. A more detailed study and a large number of experiments were performed to determine the difference in retention times, for Plasmocorinth B, Cu and Fe, as a function of TBAOH concentration. Fig. 3 shows that the magnitude of the retention increases with increasing TBAOH ion concentration. Each anion after a maximum shows a dramatic decrease in k', for TBAOH concentrations between 10 and 15 m*M*, followed by a subsequent increase until a plateau is reached. Increasing amounts of TBAOH in the mobile phase result in increased competition between the counter ion (*e.g.* nitrate) and the sample ion, as well as competition between free TBAOH and ion pairs for the surface of the stationary phase. Micelle formation could be advanced as an explanation for the reduced capacity factors at high TBAOH concentrations, but light-scattering experiments did not provide evidence of any detectable micelle formation.

It seems of interest to point out that divalent cations (Cu^{2+}, Ni^{2+}) have, at all TBAOH concentra-

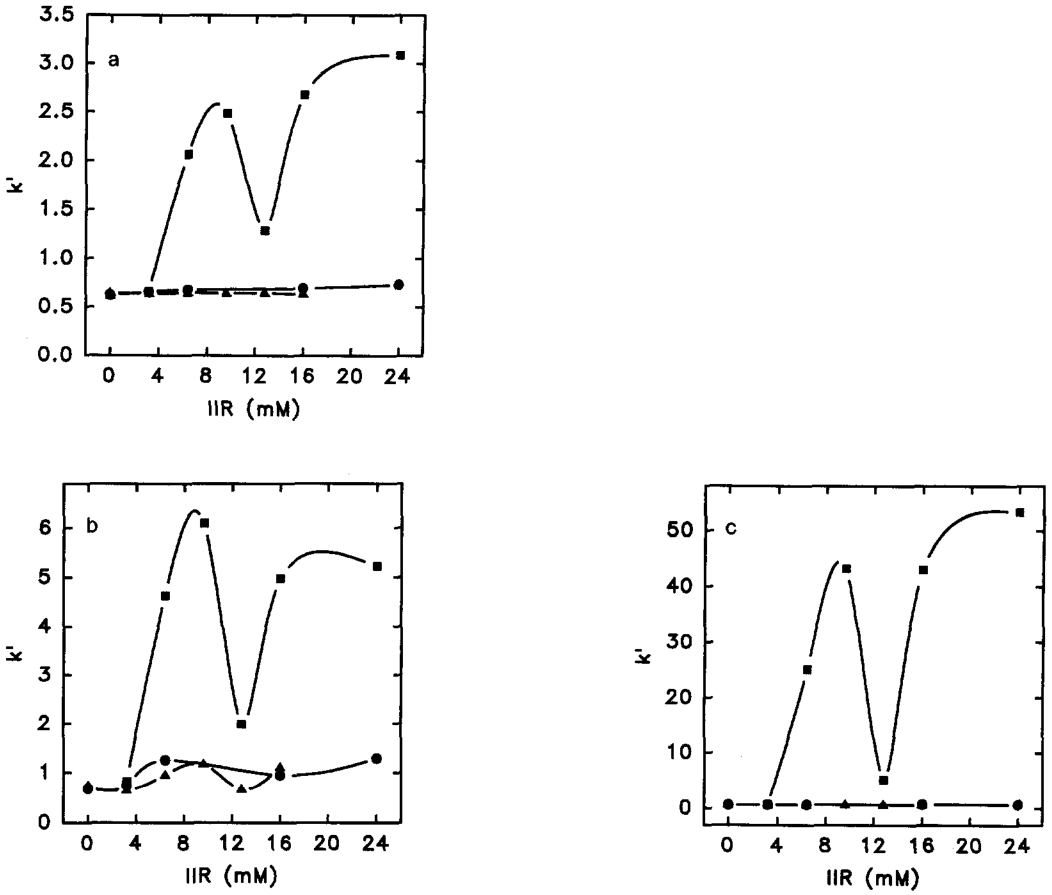


Fig. 2. Effect of ion-interaction reagents (IIR) on capacity factor (k') of (a) Cu, (b) Plasmocorinth B and (c) Fe. Chromatographic conditions: mobile phase, methanol–water (60:40, v/v) containing 19 m*M* acetic acid, 2.0 μ*M* Plasmocorinth B, 15 m*M* sodium nitrate, sodium hydroxide up to pH 6.3; IIR: ■ = Tetrabutylammonium; ▲ = tetramethylammonium; ● = benzyltrimethylammonium, as shown. Injection volume, 100 μl; 2.0 mg/l Cu, 0.80 m*M* Plasmocorinth B, 6.0 mg/l Fe.

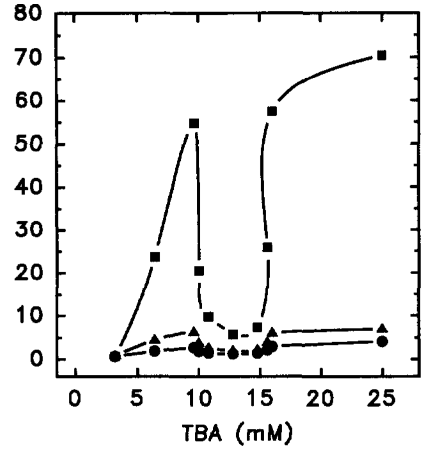


Fig. 3. Dependence of capacity factor (k') on tetrabutylammonium (TBA) concentration. Chromatographic conditions: mobile phase, methanol–water (60:40, v/v) containing 17 m*M* acetic acid, 2.0 μM Plasmocorinth B, 15 m*M* sodium nitrate, sodium hydroxide up to pH 6.3; TBA as shown. Injection volume, 100 μl; ● = 2.0 mg/l Cu; ▲ = 0.80 m*M* Plasmocorinth B; ■ = 6.0 mg/l Fe.

TABLE II

EFFECT OF IONIC STRENGTH (LITHIUM NITRATE) ON CAPACITY FACTOR (k')

Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10.0 m*M* acetic acid, 2.0 μM Plasmocorinth B (PC), 1.6 m*M* TBA, lithium hydroxide up to pH 6.3; flow-rate 1.0 ml/min; injection volume 100 μl; 0.80 m*M* Plasmocorinth B; 2.0 mg/l Cu; 6.0 mg/l Fe.

Lithium nitrate conc. (m*M*)	Capacity factor (k')			
	PC1	PC2	Cu(PC)	Fe(PC)$_2$
0	–	4.12	1.32	11.7
5	–	4.65	1.44	14.6
25	2.93	3.89	1,28	9.23
50	2.54	3.61	1.27	3.96
75	2.51	–	1.25	1.65
100	2.43	–	1.22	1.40
175	2.59	–	1.18	1.38
250	2.46	–	1.11	1.11

tions, a capacity factor lower than Plasmocorinth B, and trivalent cations (Al^{3+}, Co^{3+}, Fe^{3+}, Ga^{3+}) show the opposite behaviour. This may be explained by considering that trivalent cations are coordinated by two molecules of ligand, and the complexes have four sulphonato groups able to form ion pairs. This situation enables the formation of 1:2:2 (metal–ligand–ion-interaction reagent molar ratio) metal complex ion pairs, which show greater adsorption onto the stationary phase than 1:1:1 metal complex ion pairs of divalent metal ions. This explanation is supported also by the variation in k' with Plasmocorinth B concentration as a function of ionic strength (see below).

TABLE I

EFFECT OF IONIC STRENGTH (SODIUM NITRATE) ON CAPACITY FACTOR (k')

Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10.0 m*M* acetic acid, 2.0 μM Plasmocorinth B (PC), 1.6 m*M* TBAOH, sodium hydroxide up to pH 6.3; flow-rate 1.0 ml/min; injection volume 100 μl; 0.80 m*M* Plasmocorinth B; 2.0 mg/l Cu; 6.0 mg/l Fe.

Sodium nitrate conc. (m*M*)	Capacity factor (k')			
	PC1	PC2	Cu(PC)	Fe(PC)$_2$
0	–	4.12	1.32	11.7
5	–	5.18	1.54	14.8
25	3.10	4.17	1.29	6.73
50	2.79	3.64	1.11	2.38
75	2.75	–	1.04	1.85
100	2.74	–	1.01	1.57
175	2.62	–	1.03	1.39
250	2.30	–	1.05	1.05

Effect of ionic strength

In order to evaluate the effect of ionic strength (I) and the counter cation of analytes, I was modified by using salts with the same anion and different cations (sodium nitrate or lithium nitrate). Tables I and II show the capacity factors for Plasmocorinth B and its complexes with Cu^{2+} and Fe^{3+} as a function of the ionic strength of eluent expressed as sodium nitrate or lithium nitrate molarity. The variation in k' is not affected by the presence of different cations, and this confirms that there is great competition between the nitrate counter anion and the analytes for ion-pairing TBA. Tables I and II include two species (PC1 and PC2) corresponding to two peaks that can be ascribed to the ligand. Fig. 4 shows the disappearance of one species and the ap-

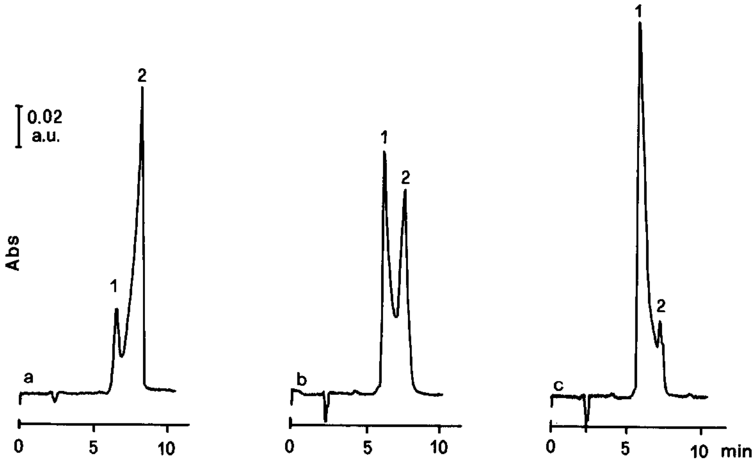


Fig. 4. Effect of sodium nitrate concentration on Plasmocorinth B (PC) chromatographic behaviour: 1 = PC(TBA); 2 = PC(TBA)$_2$. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 m*M* acetic acid, 2.0 μM Plasmocorinth B, 1.6 m*M* TBA and sodium hydroxide up to pH 6.3; (a) 25 m*M* sodium nitrate; (b) 40 m*M* sodium nitrate; (3) 50 m*M* sodium nitrate; flow-rate 1.0 ml/min. Injection volume, 100 μl; 0.80 m*M* Plasmocorinth B; detection 270 nm.

pearance of another at lower retenton times by increasing *I*. This fact agrees with the point made above about the formation of two species with different stoichiometry, the first, 1:2 (Plasmocorinth B–TBA molar ratio), existing at low concentrations of anion competitor and the second, 1:1, due to the competition of nitrate for TBA counter ion.

Effect of organic modifier

The effect of organic modifier (methanol) concentrations was studied with reference to the chromatographic performance. Fig. 5 shows the behaviour of k' for Plasmocorinth B and metal complexes as a function of methanol concentration (v/v) in the eluent. In order to obtain reduced retention times with well resolved peaks as well as to reduce W/2 (width of the peak at half height), the best compromise for the next experiments required the use of a 50% methanol (v/v) concentrations.

Evaluation of complex stoichiometry

Experimental k' values obtained as a function of ion-interaction reagent concentration can give useful information on the charge of the complexes and on their stoichiometry.

An electrostatic theory for ion-pair chromatography has been formulated from the Gouy–Chapman equation coupled with a modified Langmuir adsorption isotherm [17,20]. This theory is summarized by the general expression:

$$\left(\frac{k'_c}{k'_{0c}}\right)^{-z_i/z} = f(C) \qquad (1)$$

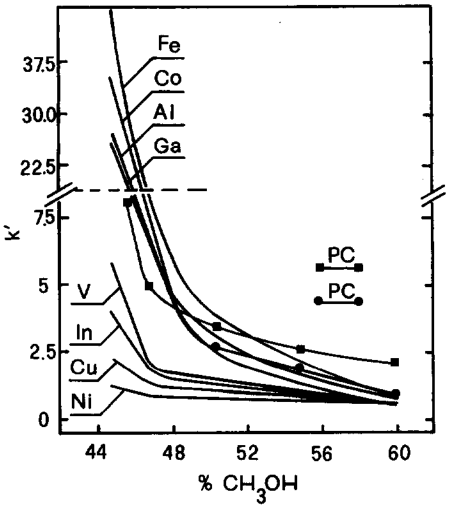


Fig. 5. Effect of methanol on capacity factor (k'). Chromatographic conditions: mobile phase, methanol–water, as shown, containing 10 m*M* acetic acid, 2.0 μM Plasmocorinth B (PC), 15 m*M* sodium nitrate 1.6 m*M* TBA and sodium hydroxide up to pH 6.3; methanol as shown. Injection volume, 100 μl; 0.80 m*M* Plasmocorinth B, 2–6 mg/l metals. ● = PC(TBA); ■ = PC (TBA)$_2$.

where k'_c and k'_{0c} are the capacity factors for an analyte at the concentrations c and zero of ion-interaction reagent; z_i is the charge of the ion-interaction reagent and z is the charge of the analyte. It must be pointed out that when no amphiphilic modifer is present in the mobile phase the electrostatic potential is set to zero. In addition, eqn. 1 is the same for different analytes and it is a function of the ion-interaction reagent concentration [20].

The higher retention time for complexes of trivalent metals in comparison with Plasmocorinth B is attributed to the 1:2 (metal to ligand ratio) stoichiometry of the complexes. We will consider now, for example, the iron–Plasmocorinth B (PC) complex: if the charge, z, for PC is -2, for $Fe(PC)_2$ it will be -4, and we can write:

$$\left(\frac{k'_{Fe}}{k'_{0Fe}}\right)^{1/4} = \left(\frac{k'_{PC}}{k'_{0PC}}\right)^{1/2} \qquad (2)$$

by squaring the eqn. 2 and by collecting the constant terms in a, one obtains

$$k'_{Fe} = a(k'_{PC})^2 \qquad (3)$$

Eqn. 3 becomes for 1:1 metal chelates [metal (M) to ligand ratio], *i.e.* Cu^{2+}:

$$k'_{Cu} = a(k'_{PC}) \qquad (4)$$

Linear regression coefficients (R) for plots of k'_M *vs.* (k'_{PC}) or $(k'_{PC})^2$ were within 1.00 and 0.994 (three replicates for six points for each element). Copper and nickel complexes have a 1:1 metal to ligand molar ratio and a molecule of water is also coordinated [3,21]. This fact explains their reduced affinity for the stationary phase and their lower retention times compared with Plasmocorinth B molecules.

In conclusion, Fig. 6 shows the chromatogram, after optimization of the method, of a sample containing Co^{3+}, Cu^{2+}, Fe^{3+} and Ni^{2+} (2.0, 1.5, 3.0 and 1.0 mg/l, respectively). The metal ions are well separated and behaviour typical of divalent metals, ligand and tervalent metals is shown by their retention times. The procedure has been optimized in terms of preconcentration, separation and determination of metal ions at trace levels [22].

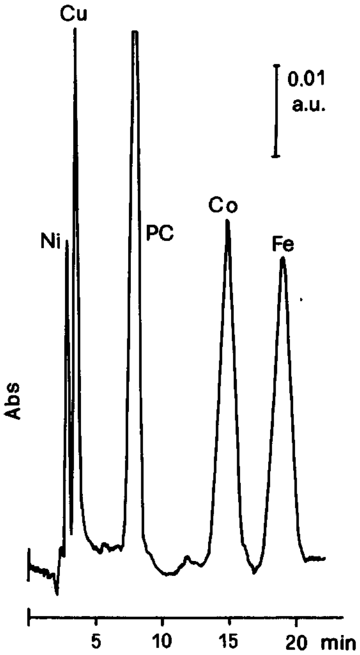


Fig. 6. Chromatogram of metal complexes. Chromatographic conditions: mobile phase, methanol–water (50:50, v/v) containing 10 m*M* acetic acid, 2.0 μM Plasmocorinth B (PC), 6.2 m*M* sodium nitrate, 1.6 m*M* TBA and sodium hydroxide up to pH 6.3; flow-rate 1.0 ml/min; injection volume, 100 μl; detection 270 nm. Peaks: 1.0 mg/l Ni; 1.5 mg/l Cu; 0.8 m*M* PC, 2.0 mg/l Co; 3.0 mg/l Fe.

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