Journal of Chromatography, 513 (1990) 145–158
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 395

Investigation of the reversed-phase high-performance liquid chromatographic ligand-exchange retention mechanism on a triamine stationary phase

THEODORE R. KOZIOL[a] and MARY LYNN GRAYESKI*
Department of Chemistry, Seton Hall University, South Orange, NJ 07079-2694 (U.S.A.)
(First received July 18th, 1988; revised manuscript received February 7th, 1990)

ABSTRACT

A tridentate ligand, trimethoxysilylpropyldiethylenetriamine, was bound to silica and studied as a stationary phase for reversed-phase ligand-exchange chromatography. The tridentate stationary phase "triamine" yielded more stable metal complexes, *i.e.*, better metal loading efficiencies than previously reported diamine and monoamine stationary phases. This triamine packing with its enhanced metal loading properties was evaluated to identify the key factors that affect solute retention and selectivity. Mobile phase properties like buffer ionic strength and metal concentration greatly affected retention, while type of metal, *i.e.*, Zn^{II}, Cd^{II}, Hg^{II}, Cu^{II} or Ni^{II} greatly affected selectivity. The choice of mobile phase organic modifier significantly affected both retention and selectivity. Methanol–water mobile phases elicited relatively little metal binding per triamine site, with Hg^{II}, Zn^{II}, Ni^{II} and Cd^{II} occupying 32, 9.5, 7.8 and 5.7%, respectively. Changing the organic modifier from methanol to acetonitrile provoked a dramatically different but consistent pattern of metal loading to the triamine. The ratio of loaded metal with the acetonitrile modifier was 2.3 ± 0.1 fold higher than that observed for the methanol modifier in each of the cases tested. Factors found to affect metal loading to the stationary phase were the type of metal and the mobile phase solvent. The loading of metals onto the silica-bound triamine correlated directly to metal basicity. The variety of solute selectivities obtained in this study indicate that the key solute–metal interactions are a function of metal and solute functional group basicities and the number and spacing of solute functional groups possessing lone-pair electrons capable of interacting with the metal. Retention can be greatly enhanced when multiple functional groups possessing donatable electrons are present: functionalities containing nitrogen, sulfur or oxygen. Ring-substituted nitrogen, sulfur or oxygen atoms *i.e.* piperdines, pyrimidines, purines, xanthines and triazoles, enhance retention more than non-ring-substituted functionalities, such as carboxylic and sulfonic acids. The presence of non-electron-

[a] Present address: Berlex Laboratories Inc., 110 East Hanover Avenue, Cedar Knolls, NJ 07927 U.S.A.

0021-9673/90/$03.50

donating functionalities in close proximity to strong interacting groups resulted in significant decreases in retention due to steric hindrance of solute lone-pair electrons to the metal coordination sphere. It is suggested that the mechanism of ligand-exchange retention on this triamine phase involves the reversible binding of a metal to the stationary phase followed by outer-sphere solute–metal complexation.

INTRODUCTION

In 1961, Helfferich[1] first introduced the term "ligand exchange" illustrating how metal ions (Cu^{I}, Cu^{II}, Ni^{II}, Ag^{I} and Co^{III}) when loaded onto an ion exchanger could separate or isolate ammonia, organic amines, polyhydric alcohols, olefins and anions of organic acids and amino acids by forming complexes of varying strengths with these metals. In ligand-exchange chromatography (LEC) the interaction between solute and stationary phase is accomplished primarily via the coordination sphere of the complex-forming metal ion. Reviews of early work in LEC have been published by Davankov and Semechkin[2], Walton[3], and Chow and Grushka[4].

Compounds amenable to separation by LEC include those which contain lone-pair electrons or π-orbitals, *e.g.*, isomers or homologues of organic compounds containing nitrogen, oxygen and sulfur. Examples of LEC separations of amino acids and peptides[5–10], nucleosides and nucleotides[11], olefins[12–15], heterocyclics[16,17] and aromatic amines[18,19] now appear in the literature.

The purpose of this work was first to identify an amine-based packing that could give enhanced ligand-exchange retention and selectivity over previously reported mono- and diamine packings; second, to study those parameters and conditions that affect retention and selectivity in reversed-phase LEC using a silica-bonded triamine stationary phase; and last to characterize the stationary ligand (triamine)–metal interaction and the stationary ligand–metal–solute interaction as influenced by metal type and mobile phase organic modifier.

The metal loading of the triamine stationary phase, expressed as the moles/gram of Cu^{II} loaded divided by the molar quantity of ligand per gram, was superior to either the mono- of diamine packings. The metal loading of Cu^{II} to each packing was approximately 0.1, 0.2 and 0.5 for the mono-, di- and triamine stationary phases, respectively. Chromatographic utility of the triamine was investigated by evaluating retention characteristics of compounds with various functional groups. Substituted naphthalenes, benzoic acids and compounds containing one or more heterocyclic nitrogen were chosen as study solutes. All solutes could be detected by UV without metal interference. The substituted naphthalenes were used to quantify the magnitude of the effect of different exocyclic and heterocyclic functionalities on LEC retention, the substituted benzoic acids were used to determine the effect of substitution of a non-ligand-exchanging group, and, finally, the substituted xanthines were used to elucidate the LEC retention mechanism.

The metals used in this study, Zn^{II}, Cd^{II}, Hg^{II}, $Ni^{II}$and Cu^{II}, were chosen based on their properties. The former three metals are water-soluble, have a wide range of basicities and represent a homologous series with filled *d*-shells. Consequently, the stereochemistry of the complexes of these metals will be based solely on considerations of size, electrostatic forces and covalent binding forces. Ni^{II} was chosen for study as it is

a water-soluble *d*-8 metal that contains a large crystal field component. The choice of these metals allowed the use of UV detection in the range 240–290 nm. An experiment was also conducted utilizing Cu^{II} because its use has been described extensively in the LEC literature.

EXPERIMENTAL

Chromatographic apparatus

A modular chromatographic system consisting of a WISP 710B (Waters Assoc., Milford, MA, U.S.A.) autosampler, Model 400 solvent-delivery system (Kratos Analytical, Ramsey, NJ, U.S.A.), Model 773 variable-wavelength detector (Kratos), normally at 254 nm, and an HP 3388 integrator–computer (Hewlett-Packard, Palo Alto, CA, U.S.A.) was used. The pH of aqueous solutions was determined using a digital Orion pH meter. To avoid baseline instability from variation in temperature columns were jacketed at 30 ± 1°C using a column oven (Jones Chromatography, U.K.).

Reagents and chemicals

Reagent-grade trimethoxysilylpropyldiethylenetriamine was obtained from Petrarch (Bristol, PA, U.S.A.). The 10-μm irregular silica gel of 60 Å pore diameter and 500 m^2/g surface area, Polygosil 60-10 (Macherey-Nagel), was obtained from Ace Scientific (East Brunswick, NJ, U.S.A.). Metal salts and buffers were obtained from Aldrich (Milwaukee, WI, U.S.A.). The solvents used for HPLC were obtained from Burdick & Jackson (Muskegon, MI, U.S.A.). All xanthines were kindly supplied by Berlex Labs. (Cedar Knolls, NJ, U.S.A.). The remaining solutes were obtained from Aldrich or Sigma (St. Louis, MO, U.S.A.).

Preparation of bonded phase

A 250-ml aliquot of toluene was added to a 500-ml round-bottom flask and heated with slow stirring. Once the temperature of the toluene reached 50°C, 50.00 g of Polygosil 60-10 silica gel were slowly added to the flask. Following the addition of the silica gel, a 1.00-ml aliquot of distilled water was added to the flask. The temperature was increased to allow azeotropic distillation of all water and *ca.* 50 ml of toluene thereby obtaining dry toluene and silica.

The reaction mixture was allowed to cool to 60°C with constant stirring. A 25.8-ml aliquot of trimethoxypropyldiethylenetriamine was added to the flask. The reaction mixture was refluxed for 20 h with constant stirring. At the conclusion of the reaction the mixture was cooled to room temperature and the toluene decanted. The silica-bonded triamine was washed with *ca.* 1 l of chromatographic-grade methanol and dried for 12 h at 70°C in a vacuum oven. The phase was slurry-packed into 15 cm × 4.6 mm I.D. stainless-steel columns.

Measurement of metal loading

The measurement of metal breakthrough volume was determined colorimetrically using a dye solution containing a 0.2 mg/ml solution of pyridine-2-azo-*p*-dimethylanaline (Sigma). The mobile phase was concomitantly monitored at 210 nm (290 nm for nickel) during metal loading.

TABLE I

METAL LOADING ON THE TRIAMINE PHASE

% = mole% of triamine sites occupied by metal.

Mobile phase	*Loading*							
	Ni^{II}		Zn^{II}		Cd^{II}		Hg^{II}	
	mol/g × 10^{-5}	*%*	*mol/g* × 10^{-5}	*%*	*mol/g* × 10^{-5}	*%*	*mol/g* × 10^{-5}	*%*
Water–methanol (65:35, v/v)	5.1	7.8	6.3	9.5	3.8	5.7	15	23
Water–acetonitrile (1:1, v/v) containing 0.1 *M* ammonium acetate	15	23	34	52	1.5	2.5	–	–

Mobile phase metal content and detection sensitivity

Solute sensitivity using UV detection at 254 nm was affected only when either Cu^{II} or Hg^{II} was present in the mobile phase. Hg^{II} mobile phases were operated at 268 nm where the metal has a lower absorption band. Because of the strength of the Trien–Cu^{II} complex, columns loaded with Cu^{II} could be operated without metal in the mobile phase for up to 4 h.

RESULTS AND DISCUSSION

Characterization of the triamine stationary phase

Based on C, H and N analysis, the surface coverage of the triamine to the Polygosil 60-10 was calculated to be 1.3 μmol/m^2, with $6.57 \cdot 10^{-4}$ mol triamine per g packing or a 13.4% triamine loading.

The triamine stationary phase dissociation constants were determined by titration. Two pK_a values were observed in the 9.5–10 range while the third was observed in the acidic range of 4.2. For steric reasons the latter pK_a was attributed to the central amine of the triamine. These numbers are in rough agreement with those reported for a similar compound, 1-amino-2-(2-aminoethyl)amino ethane: 9.9, 9.1 and 4.3 (ref. 20).

Metal loading on triamine stationary phase

The extent of loading of the triamine sites at equilibrium with a given metal were calculated for the mobile phases and are presented in Table I. The methanol–water mobile phases elicited relatively little metal binding per triamine site, with Hg^{II}, Zn^{II}, $Ni^{II}$and Cd^{II} occupying 23, 9.5, 7.8 and 5.7%, respectively. The loading of the above metals onto the triamine from methanolic mobile phase was proportional to the basicity of the metal.

Changing the organic modifier from methanol to acetonitrile provoked a dramatically different but consistent pattern of metal loading to the triamine. Again, the extent of metal loading onto triamine sites followed the metal basicity in all cases. Interestingly, the ratio of metal loaded with the acetonitrile modifier was 2.3 $\pm$ 0.1 fold higher than that observed for the methanol modifier in each of the cases tested.

The volume of mobile phase for metal breakthrough was determined by appearance of metal in the effluent. At that point, each metal was at equilibrium for a given mobile phase except for Ni^{II} in methanol which continued to bind for 48 ml.

The triamine–metal complexes, except in the case of Cu^{II} where a fairly stable complex was formed, were fairly weak and easily reversed by passing through a mobile phase without metal. All the *d*-10 metals tested eluted from the triamine stationary phase at essentially the same rate. The metals possessing unfilled *d*-orbitals and more rigid coordination formed the more stable complexes owing to lower exchange rate of metal–triamine ligands with mobile phase ligands.

Following the use of an LEC mobile phase, a column was washed with approximately fifty column volumes of an acidic mobile phase, 35:65, (v/v) acetonitrile–water containing 5 ml glacial acetic acid.

Triamine as an ion exchanger

For proper assessment of the solute functional group effect in LEC, solute–

TABLE II

CAPACITY FACTORS OF SUBSTITUTED NAPHTHALENES AND OTHER COMPOUNDS ON THE TRIAMINE PHASE OPERATED IN THE ION-EXCHANGE AND LIGAND-EXCHANGE MODES

Chromatographic conditions: 15 cm × 4.6 mm I.D. triamine column; mobile phase, methanol–water (35:65, v/v) containing 0.004 *M* ammonium acetate or 0.004 *M* metal (as acetate or chloride), pH 6.9; flow-rate, 2.0 ml/min; UV detection, 254 nm.

Solute	*Abbreviation*	*No metal (II)*	Cd^{II}		Zn^{II}		Ni^{II}		Hg^{II}	
			k'	*k''*	*k'*	*k''*	*k'*	*k''*	*k'*	*k''*
1-Naphthylamine	N-AM	1.09	1.18	0.08	1.15	0.06	1.15	0.06	1.50	0.38
1-Naphthoic acid	N-COOM	5.18	11.6	1.2	7.0	0.35	12.1	1.3	4.83	0.00
1-Naphthalenesulfonic acid	NSA	4.91	8.0	0.63	5.07	0.03	9.73	0.98	3.10	0.00
8-Aminonaphthalenesulfonic acid	ANSA	7.43	17.9	1.4	8.63	0.16	34.5	3.6	2.75	0.00
Quinoline	QIN	1.00	1.13	0.13	1.85	0.85	1.15	0.15	1.72	0.72
Cinnoline	CIN	0.94	1.10	0.17	1.22	0.30	1.37	0.46	1.30	0.38
Quinazoline	QAZ	0.88	1.03	0.17	1.47	0.67	1.05	0.19	1.42	0.61
Quinoxaline	QOX	0.88	1.00	0.14	1.08	0.23	1.03	0.17	1.02	0.16
8-Hydroxyquinoline	HQIN	1.75	7.80[a]	3.5	17.6[a]	9.1	6.57[a]	2.8	Inf.	Inf.
p-Aminobenzoic acid	PABA	4.33	10.9	1.6	11.9	1.7	13.3	2.1	6.5	0.50

[a] Peak was very broad but symmetric.

stationary phase interaction in the ion-exchange mode (mobile phase minus metal) was established first. A series of substituted naphthalenes were injected into the HPLC system operated in the ion-exchange mode as presented in Table II.

Introduction of the functionalities hydroxy, amino, cyano and aldehyde to the naphthalene backbone contributed little to retention on the triamine when operated in the anion-exchange mode. Furthermore, naphthalenes with single or multiple heterocyclic nitrogen substitutions were generally less retained than naphthalene mainly because the heterocyclic naphthalenes and the triamine possess common functionalities. Predictably, significant increases in retention were observed when anions such as carboxylic acids and sulfonic acids are substituted onto naphthalene.

Triamine as a ligand exchanger

Study of substituted naphthalenes and similar compounds. The capacity factors (k') of the substituted naphthalenes on the triamine phase, operated in the ligand-exchange mode, were determined with the mobile phase containing Cd^{II}, Zn^{II}, Ni^{II} or Hg^{II} (Table II). In order to separate the pure solute ligand-exchange retention effects from those inherent in the silica-bonded triamine alone (*i.e.* no metal present) a so-called ligand-exchange capacity factor, k'', was calculated for each solute as follows:

$$k'' = \frac{k'(\mathrm{LE}) - k'(\mathrm{IE})}{k'(\mathrm{IE})} \tag{1}$$

where k'(LE) is the capacity factor of a solute *versus* a non-retained species when a metal is present in the mobile phase and k'(IE) is the capacity factor of a solute *versus* a non-retained species when no metal is present in the mobile phase. Using eqn. 1 the "pure" ligand-exchange effects on a solute can be evaluated. If ligand-exchange retention was less than ion-exchange retention, k'' was defined as zero. Solutes with $k'' \leqslant 0.2$ for all metals (naphthalene, 1-naphthol, 1-cyanonaphthalene, 1-naphthaldehyde and 1,4-naphthoquinone) have been omitted from Table II.

Where single functional groups are concerned, the difference in basicity of solute and metal was the key determinant to retention. In the case of molecules containing an alcohol, aldehyde or quinone functionality, the basic metals Ni^{II}, Cd^{II} and Zn^{II} had essentially no effect on ligand-exchange retention, and cyano and amino functionalities show very minor effects. Conversely, these "basic" functional groups displayed a much greater affinity for, and were retained in the presence of acidic Hg^{II}.

Modest retention increases were noted for solutes with various heterocyclic nitrogen ring substitutions. The difference in the basicity of solute and metal appears to be the controlling factor to each ligand-exchange interaction. The role of metal coordination sphere size and "fit", however, also appear to influence retention. In the case of Zn^{II} and Hg^{II} complexation, the fit also appears better for quinazoline while quinoline appears better for Cd^{II}.

Study of substituted benzoic acids. The ligand-exchange retention properties of benzoic acids substituted at the *p*-position were studied on the triamine phase loaded with Cd^{II} in order to quantify the effect on retention of substitution of a non-ligand exchanging group away from the exchanging functionality. Alkyl substitution at the *p*-position of C_1–C_5 had no significant effect on retention. Also, mono-, di- or

trimethyl substitution close to the ligand-exchanging carboxyl group (2,4-, 2,5-, 2,6- or 2,4,6-methyl substitution) had little effect on retention. Steric hindrances to the carboxy functionality were not sufficiently established by methyl substitutions near the acidic carboxyl group.

Study of substituted xanthines. A series of substituted xanthines were studied to further elucidate the retention mechanism in LEC (Table III). Essentially no retention was observed for these solutes when injected into the system operated in the ion-exchange mode; therefore, retentions observed in LEC could only be attributed to metal–solute interactions.

The xanthine backbone is rigid and essentially planar[21–23], allowing the effect of the solute in metal–solute interactions to be more easily interpreted. Also, deprotonated xanthine bases exhibit a variety of nucleophilic sites which do not differ significantly in their electronic properties[24]. The literature[25–30] illustrates that metal–xanthine binding, via inner-sphere complexation, takes place mainly in the imidizole ring, at atoms N-7 and N-9, under basic conditions where the heterocyclic nitrogens are not protonated. Stability studies of theophylline–Zn^{II} complexes[31] have shown that the complex rapidly breaks down below a pH of 9.0 where atom N-7 is protonated.

The LEC retention of xanthines within each metal studied appears to be controlled by electrostatic effects between multiple functional group interactions and the metal. Xanthine was the most retained solute in all cases; in fact, the interaction was so strong that it only eluted in the ligand-exchange mode on the lightly loaded Ni^{II}–triamine. Based on molecular distances between functionalities of xanthine possessing lone-pair electrons, the molecule is capable of ten metal interactions at atoms N-7 O-6–N-7, N-7–N-9, N-9, N-3–N9, N3, N-1, O-6–N-1, O-2–N-1 and O-2–N-3 (ranked in order of importance).

TABLE III

RETENTION OF SUBSTITUTED XANTHINES ON THE TRIAMINE PHASE USING ACETONITRILE–BUFFER MOBILE PHASES CONTAINING VARIOUS METALS

Chromatographic conditions: 15 cm × 4.6 mm I.D. triamine column; mobile phase, acetonitrile–water (3:7, v/v) containing 0.0667 *M* ammonium acetate, 0.133 *M* sodium chloride and 0.004 *M* metal acetate, pH 6.9; flow-rate, 2.0 ml/min, detection, UV at 254 or 268 nm (for Hg). In the study of Hg^{II} the chloride salt was used. The Cu^{II} data were collected by first pre-loading the column with metal then switching to a mobile phase minus metal; the system was stable for several hours.

	k'				
Solute	*No metal(II)*	Ni^{II}	Zn^{II}	Hg^{II}	Cu^{II}
Xanthine	0.19	5.0	DNE[a]	DNE	DNE
1-Methylxanthine	0.12	3.2	DNE	16.0	DNE
3-Methylxanthine	0.01	1.2	21.0	7.30	DNE
3,7-Dimethylxanthine	0.01	0.34	0.54	1.80	1.10
1,3-Dimethylxanthine	0.01	0.87	14.7	2.40	17.6
1,7-Dimethylxanthine	0.01	0.60	DNE	1.86	2.09
1,3,7-Trimethylxanthine	0.00	0.14	0.22	0.61	0.46

[a] DNE: solute did not elute in 60 min.

In the xanthine molecule, the most important binding site, atom N-7, is only partially ionized as compared to the other substituted xanthines. This is advantageous to stronger binding at the N-7 atom with concomitant O-6 hydrogen bonding to other ligands, probably water or solvent, in the metal coordination sphere. The N-9 atom can be considered to moderately affect retention, while binding influences from atoms N-1, N-3 and O-2 are considered to be small. The N-1 position is protonated and sterically hindered by the two neighboring oxo functionalities, while N-3 is also protonated.

The effect of changing mobile phase metal on the retention of substituted xanthines was also studied in a methanol–water mobile phase (Table IV). Substituted xanthines were slightly retained on the triamine phase operated in the ion-exchange mode. The slight retention by ion exchange was caused by the poor solubilities of the xanthines in this mobile phase. Retention was, therefore, achieved by the aversion of the solute for the mobile phase which allowed longer interaction with the stationary phase.

TABLE IV

RETENTION OF SUBSTITUTED XANTHINES ON THE TRIAMINE PHASE USING METHANOL–WATER MOBILE PHASES CONTAINING VARIOUS METALS

Chromatographic conditions: 15 cm × 4.6 mm I.D. triamine column; mobile phase, methanol–water (35:65, v/v) containing either no metal or 0.005 *M* metal acetate, pH 6.9; flow-rate, 2.0 ml/min; detection, UV at 254 or 268 nm (for Hg). In the study of Hg^{II} the chloride salt was used.

	k'				
Solute	*No metal(II)*	Zn^{II}	Cd^{II}	Ni^{II}	Hg^{II}
Xanthine	1.26	DNE[a]	DNE	DNE	DNE
1-Methylxanthine	0.85	DNE	DNE	24.2	DNE
3-Methylxanthine	0.61	DNE	2.31	4.45	DNE
3,7-Dimethylxanthine	0.32	0.55	0.39	0.43	5.87
1,7-Dimethylxanthine	0.37	DNE	1.00	1.88	14.4
1,3-Dimethylxanthine	0.50	9.86	1.51	3.07	0.43
1,3,7-Trimethylxanthine	0.32	0.32	0.33	0.36	0.89
1,3,7,9-Tetramethylxanthine	0.32	0.32	0.36	0.36	0.44

[a] DNE: solute did not elute in 60 min.

Spectroscopic studies of xanthine and metals were carried out in the mobile phases tested in order to determine the mechanism of complexation between the various metals and the xanthine at pH 6.9. Based on the experimental results, the possibility of solute–metal inner-sphere complexation was ruled out. It can then be assumed that the electron transfer process in reversed-phase LEC on the triamine phase involves intact coordination spheres. The reactant ions do not come into intimate contact but are separated by ligands at the time of electron transfer, *i.e.* outer-sphere complexation. This result would be expected based on the previously reported xanthine–metal complexation data and because almost all the nitrogens are ionized.

Study of other chromatographic parameters affecting LEC retention on the triamine phase

Mobile phase metal concentration. The effect of varying mobile phase Zn^{II} concentration from 0.0002 to 0.02 *M* on a set of test solutes was determined using Zn^{II} as the acetate salt in the mobile phase. The ionic strength of the mobile phase was kept constant using ammonium acetate. Dramatic increases in solute retention were observed accompanied by changes in separation factor (Fig. 1). Over a limited range of Zn^{II} concentrations, *e.g.* 2–8 m*M*, a linear relationship was observed between the molar concentration of metal and the *k'* of a solute.

Ionic strength. The effect of mobile phase ionic strength on retention of solutes on the triamine phase was evaluated by varying the amount of ammonium acetate buffer in acetonitrile–water (1:1, v/v) containing 0.004 *M* Zn^{II} at pH 6.9. Increasing ionic strength of the mobile phase from 0.13 to 0.23 and then to 0.33 resulted in approximately a 2.5-fold loss in capacity factor per increment for the solutes 8-chlorobenzotriazole, 1,7-dimethylxanthine and theophylline.

To evaluate the effect of counter-ion type on retention, the ionic strength of a mobile phase containing Zn^{II} was held constant while a portion of the ammonium acetate buffer was replaced with sodium chloride. Significantly increased retention was observed with the weaker chloride anion.

Mobile Phase pH. pH 6.8–6.9 was chosen for these comparative ligand-exchange studies based on the criteria that a common mobile phase had to be found that could solubilize all metals under investigation. Studies on the effect of mobile phase pH on the ligand-exchange retention based on extent of solute ionization have been conducted by Lindner *et al.*[32].

Column quality under LEC conditions

Column stability and reproducibility. In order to test column stability and column reproducibility, a column was selected from each of three different triamine reaction

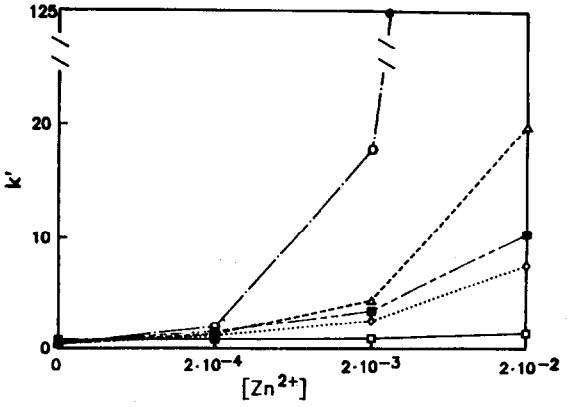

Fig. 1. Effect of metal concentration (wide range). Chromatographic conditions: 15 cm × 4.6 mm I.D. triamine column; mobile phase, acetonitrile–water (35:65, v/v) containing 0.2 *M* ammonium acetate and various concentrations of zinc acetate, pH 6.9; flow-rate, 2.0 ml/min; detection, UV at 254 nm. ■ = 3-Methylxanthine; ○ = 1,7-dimethylxantine; △ = 5-chlorobenzotriazole; ◇ = theophylline; □ = *p*-aminobenzoic acid.

batches and operated with various mobile phases and metals. A test solute, *p*-aminobenzoic acid, was injected into a mobile phase of acetonitrile–water (1:1, v/v) containing 0.1 *M* ammonium acetate at pH 6.9 onto each column before and after use with each mobile phase containing metal. The k' of the test solute did not change (range 6.87–6.97), illustrating that not only could each metal be removed, but also no column degradation took place.

Chromatographic quality. Chromatograms of *p*-aminobenzoic acid under the ion-exchange and ligand-exchange chromatographic conditions illustrated in Table II are shown in Fig. 2. The peak shapes shown for *p*-aminobenzoic acid are typical and represent a compound that had moderate increased capacity over the ion-exchange mode when chromatographed in the LEC mode. The number of theoretical plates (N), calculated by

$$N = 5.54\ [\text{retention time (s)}]/\text{peak width at half-height (s)}]^2,$$

observed for the solutes tested in Table II ranged from 1000 to 2000. Asymetry, assessed as tailing factor (calculated at 10% peak height: peak width front/peak width tail, generated from construction of perpendicular line from peak maximum), averaged approximately 0.8. The latest eluted solute, 8-aminonaphthalene sulfonic acid with $k' = 34.5$, exhibited a tailing factor of 0.34. The solute, 8-hydroxyquinaline, exhibited the poorest mass transfer characteristics of all compounds tested ($n = 10$). It

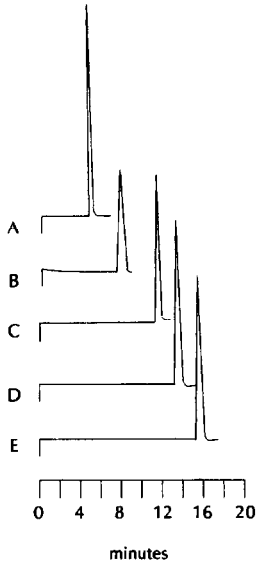

Fig. 2. Chromatograms of *p*-aminobenzoic acid when chromatographed in a common mobile phase containing different metals. Chromatographic conditions: 15 cm × 4.6 mm I.D. triamine column; mobile phase, methanol–water (35:65. v/v) containing 0.004 *M* ammonium acetate or 0.004 *M* metal (as acetate or chloride), pH 6.9; flow-rate, 2.0 ml/min; detection, UV at 254 nm. (A) No metal added to mobile phase; (B) mobile phase containing Hg: (C) mobile phase containing Cd; (D) mobile phase containing Zn, (E) mobile phase containing Ni.

is postulated that this compound can inner-sphere coordinate with the metals tested making it a poor candidate for LEC.

Application of LEC

The separation of xanthines has historically been a difficult task. In order to study the metabolism and pharmacokinetics of theophylline (1,3-dimethylxanthine) one must first chromatographically separate it from its metabolites as well as caffeine (1,3,7-trimethylxanthine). It is particularly diffficult to separate the metabolite 1,7-dimethylxanthine from its parent compound[31,33].

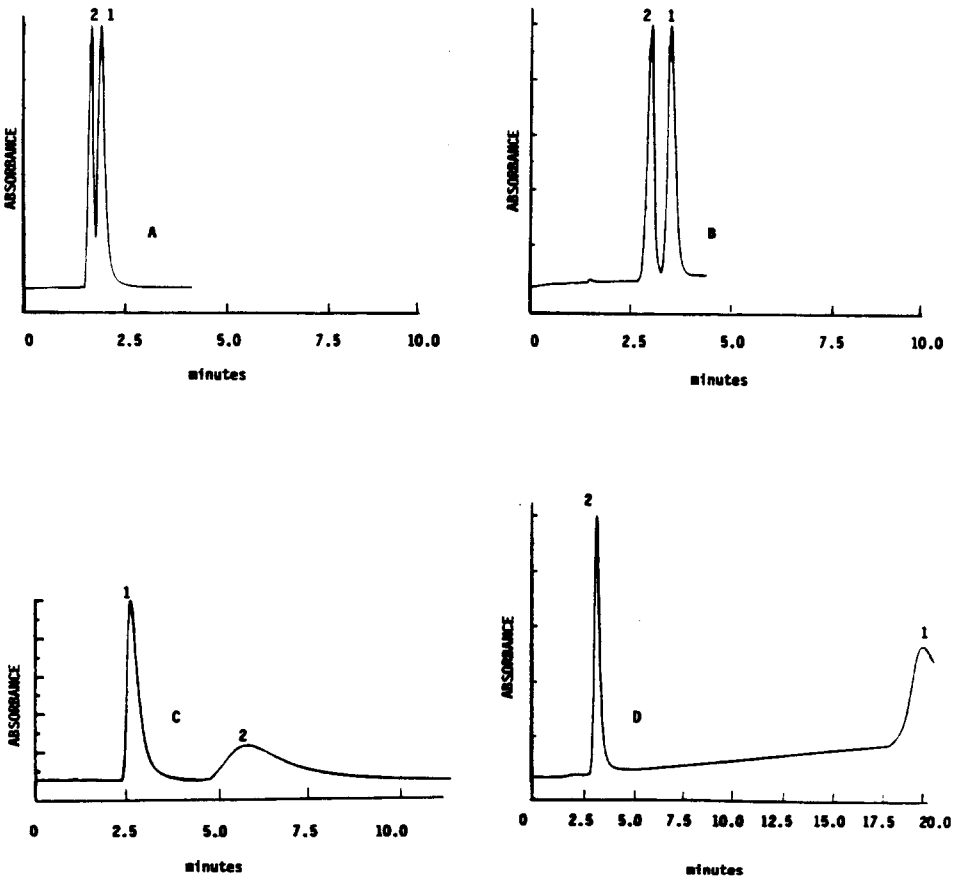

Fig. 3. Examples of ligand-exchange separations of 1,3-dimethylxanthine from 1,7-dimethylxanthine. Chromatographic conditions: 15 cm × 4.6 mm I.D. triamine column; mobile phase, acetonitrile–water (3:7, v/v) containing ammonium acetate and/or sodium chloride and 0.005 *M* metal acetate, pH 6.9; flow-rate, 2.0 ml/min; detection, UV at 254 or 268 nm (for Hg). Solutes, 1,3-dimethylxanthine (1) and 1,7-dimethylxanthine (2). (A) Mobile phase containing Ni, 0.1333 *M* sodium chloride and 0.0667 *M* ammonium acetate; (B) mobile phase containing Hg, 0.1333 *M* sodium chloride and 0.0667 *M* ammonium acetate; (C) mobile phase containing Zn and 0.2 *M* ammonium acetate; (D) mobile phase equilibrated with Cu, 0.1333 *M* sodium chloride and 0.0667 *M* ammonium acetate.

Using the LEC systems described in this paper, a wide range of selectivities can be attained for xanthines. Separation factors between theophylline and 1,7-dimethylxanthine in the methanol–water mobile phase were 1.51, 1.63, 33.5 and infinity for Cd^{II}, Ni^{II}, Hg^{II} and Zn^{II}, respectively, while separation factors in the acetonitrile–buffer mobile phase were 1.45, infinity, 1.3 and 8.4, for Ni^{II}, Zn^{II}, Hg^{II} and Cu^{II}, respectively.

Comparison of the separation factors obtained for the theophylline/1,7-dimethylxanthine separation for Ni^{II} and Hg^{II} loaded onto triamine when mobile phase organic solvent was changed from acetonitrile to methanol resulted in selectivity changes of 100 and 600%, respectively. These changes again illustrate the effect of solvent and the wide range of separations that are possible (Fig. 3).

CONCLUSION

The silica-bonded tridentate amine stationary ligand yields more stable metal complexes, resulting in better metal loading efficiencies, than previously reported bidentate and monodentate amine silica-bonded ligands studied in reversed-phase LEC.

LEC can be applied to routine, yet specific chromatographic separation problems encountered in the analytical laboratory. Compounds amenable to separation by LEC include those which contain lone-pair electrons or π-orbitals, *e.g.*, isomers or homologues of organic compounds containing nitrogen, oxygen and sulfur.

Solute–metal interactions in ligand-exchange chromatography are determined by factors such as the number of sites capable of donating electron pairs, the ionizability of each site, steric hindrances imposed by bound and/or neighboring functionalities, the hardness of a metal, the intermolecular distances between key functionalities and the ability of other ligands in the metal sphere to form hydrogen bonds to solute functionalities creating a more stable chelated outer-sphere complex.

Retention can be greatly enhanced when multiple functional groups possessing donatable electrons are present: N-, S- and O-containing functionalities. Ring-substituted N, S and O atoms, *i.e.*, piperidines, pyrimidines, purines and triazoles, enhance retention more than non-ring -substituted functionalities, such as carboxylic and sulfonic acids.

Key mobile phase factors affecting solute retention include the metal concentration, buffer ionic strength and percentage organic modifier, while solute selectivity is mainly governed by the choice of metal and organic modifier.

The mechanism of chromatographically useful ligand-exchange retention on the triamine phase is postulated to involve a process in which metal is reversibly "bound" to the stationary triamine phase through chelation of the metal coordination sphere to the amine functionalities of the triamine. This triamine–metal(II) complex can then interact with sterically unhindered functional groups containing lone-pair electrons according to the scheme

triamine ---- metal(II) **** solute,

where **** represents an outer-sphere attraction and ---- represents an inner-sphere coordination.

The limitations of reversed-phase LEC are related to the requirement that

a metal be present in the mobile phase. Consequently, practical considerations can include metal precipitation, limited UV detection and waste disposal.

REFERENCES

1 F. G. Helfferich, *Nature (London)*, 189 (1961) 1001.
2 V. A. Davankov and A. V. Semechkin, *J. Chromatogr.*, 141 (1977) 313.
3 H. F. Walton, *Sep. Purif. Methods*, 4 (1975) 189.
4 F. K. Chow and E. Grushka, *Anal. Chem.*, 50 (1978) 1346.
5 S. K. Lam and F. K. Grushka, *J. Liq. Chromatogr.*, 3 (10) (1980) 1579.
6 J. N. LePage, W. Lindner, G. Davies, D. Seitz and B. L. Karger, *Anal. Chem.*, 51 (1979) 433.
7 Y. A. Zolotarev, N. F. Myasoedov, V. I. Penkina, I. N. Dostovalov, O. V. Petrenik and V. A. Davankov, *J. Chromatogr.*, 207 (1981) 231.
8 A. Foucault, M. Claude and L. Oliveros, *J. Chromatogr.*, 185 (1979) 345.
9 V. A. Davankov, A. S. Bochkov, A. A. Kurganov, P. Roumeliotis and K. K. Unger, *Chromatographia*, 13 (1980) 677.
10 G. Gubitz, W. Jellenz and W. Santi, *J. Liq. Chromatogr.*, 4 (4) (1981) 701.
11 E. Grushka and F. Chow, *J. Chromatogr.*, 199 (1980) 283.
12 E. A. Emken, J. C. Hartman and C. R. Turner, *J. Am. Oil Chem. Soc.*, 55 (1978) 561.
13 B. Vonach and G. Schomburg, *J. Chromatogr.*, 149 (1978) 417.
14 D. Tabak and M. N. Dos Santos, *J. Chromatogr.*, 106 (1975) 471.
15 R. R. Heath, J. H. Tumlinson, R. E. Doolittle and A. T. Proveaux, *J. Chromatogr. Sci.*, 13 (1975) 380.
16 N. H. C. Cooke, R. L. Viavattene, R. Eksteen, W. S. Wong, G. Davies and B. L. Karger, *J. Chromatogr.*, 149 (1978) 391.
17 R. Vivilecchia, M. Thiebauld and R. W. Frei, *J. Chromatogr. Sci.*, 10 (1972) 411.
18 D. Kunzru and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 191.
19 F. K. Chow and E. Grushka, *Anal. Chem.*, 49 (1977) 1756.
20 D. D. Perrin (Editors), *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965.
21 M. J. Gardner, F. X. Smith and E. Shefter, *J. Pharm. Sci.*, 72 (1983) 346.
22 E. Shefter and P. Sackman, *J. Pharm. Sci.*, 60 (1971) 282.
23 E. Schefter, *J. Pharm. Sci.*, 58 (1969) 710.
24 R. Bonaccoral, A. Pullman, E. Scrocco and J. Tomasi, *Theor. Chim. Acta*, 24 (1972) 51.
25 G. M. Blackburn and A. W. Johnson, *J. Chem. Soc.*, (1960) 4347.
26 A. R. Norris, R. Kumar, E. Buncel and A. L. Beauchamp, *J. Inorg. Biochem.*, 21 (1984) 277.
27 R. Weiss and H. Venner, *Hoppe-Seyler's Z. Physiol. Chem.*, 340 (1965) 138.
28 D. J. Salda, T. J. Kistenmacher and L. G. Marzilli, *Inorg. Chem.*, 15 (1976) 2783.
29 A. T. Tu and M. J. Heller, *Metal Ions in Biological Systems*, Vol. I, Marcel Dekker, New York, 1974.
30 F. Allaire and A. L. Beauchamp, *Can. J. Chem.*, 62 (1984) 2249.
31 K. T. Muir, J. H. G. Jonkman, D. Tang, M. Kunitan and S. Riegelman, *J. Chromatogr.*, 221 (1980) 85.
32 W. Lindner, J. N. LePage, G. Davies, D. E. Seitz and B. L. Karger, *J. Chromatogr.*, 185 (1979) 323.
33 J. J. Grygiel, M. W. Lindon, M. B. Wing, J. Farkas and D. J. Burkett, *Clin. Pharmacol. Ther.*, 26 (1979) 660.