Journal of Chromatography, 167 (1968) 253–272

CHROM. 11,055

REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING METAL CHELATE ADDITIVES

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SUMMARY

This paper reports a continuation of earlier work on the addition of metal chelates to the mobile phase in reversed-phase liquid chromatography. Using the relatively hydrophobic chelating agent C_{12}-dien in a 1:1 molar ratio with Zn(II) we have been able to observe high-performance separations with high selectivity as a consequence of complexation of the solute in the outer coordination sphere of the metal-chelate system. We have studied a series of 24 dansyl amino acids by this approach and found unusual structural selectivities. In particular, the retention of the diacids (Asp, $CySO_3H$) suggests bidentate attachment to the atoms of the inner-coordination sphere. Bidentate attachment is also suggested for Asn, where stabilizing electrostatic and hydrogen-bonding interactions are assumed. We have studied the effect of acetate and trifluoroacetate concentration, as well as acetonitrile composition, on retention. Use of these results has led to the achievement of rapid separations of these substances under gradient elution conditions. In the second section of the paper we report the effect of substituting Cd(II) for Zn(II) in the C_{12}-dien system. In general, Cd(II) gives lower absolute retention as a result of the larger size of the C_{12}-dien–metal complex. Selectivity differences are generally observed for the dansyl amino acids. The weaker binding Cd(II) chelate permits separation of nucleotide monophosphates with good peak symmetry. Finally, the Cd(II) chelate appears to be able to bind sulfa drugs more strongly than the Zn(II) chelate.

INTRODUCTION

We have recently explored a variety of approaches in which metal ions can be used to enhance selectivity in high-performance liquid chromatography[1]. A particularly promising approach was found to be the addition of a metal chelate to the mobile phase in reversed-phase liquid chromatography (LC) using *n*-alkyl bonded stationary phases. The addition of 4-dodecyldiethylenetriamine (C_{12}-dien)

$$CH_3(CH_2)_{11}–N(CH_2CH_2NH_2)_2$$

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in a stoichiometric amount with Zn(II) yielded high-performance separations of anionic species such as peptides, sulfa drugs and aromatic acids. This ligand-exchange procedure differs markedly from more classical approaches, which generally exhibit poor efficiency and peak symmetry[2].

We explored in detail the reasons for the high performance and found that the ligand exchange occurred in the outer coordination sphere. Outer-sphere complexes are generally weaker than inner-sphere complexes, and the kinetics of dissociation are hence frequently more rapid in the former case[3]. The rapid kinetics lead to fast mass transfer, which is necessary for modern small particle diameter LC supports. The potential of outer-sphere complexation has recently also been mentioned in a review of ligand exchange chromatography[4].

Metal chelate addition to the mobile phase is equivalent to the addition of a counterion (or soap)[5], since the ratio of metal to chelate is maintained constant. However, using aromatic acids, we showed that functional group selectivity (*e.g.* $RCOO^-$ *vs.* RSO_3^-) and steric or isomeric selectivity was much more pronounced with C_{12}-dien-Zn(II), relative to the usual counterions employed in reversed-phase ion-pair chromatography (*e.g.* quaternary ammonium ions). Moreover, no loss in hydrophobic (*i.e.* alkyl group) selectivity was found. Thus, high selectivity with good column performance is possible in reversed-phase LC using metal chelate additives.

In this paper we will describe a continuation of work in this area. We first explore the retention and performance of a series of 24 dansyl amino acids. Special selectivities are observed relative to reversed-phase and reversed-phase ion-pair chromatography. In particular, the conformational rigidity of the inner coordination sphere results in the possibility of bidendate binding for diacids and ε-amides. After examining the effects of salt and acetonitrile composition, rapid gradient elution separations of a number of dansyl amino acids are obtained. We will then turn to an examination of the substitution of Cd(II) for Zn(II) with C_{12}-dien. It will be shown that, in general, Cd(II) leads to weaker binding of ligands relative to Zn(II). This behavior then leads to the separation of nucleotide monophosphates. The results of this paper illustrate the combination of high selectivity and high performance possible with metal-chelate additives to the mobile phase.

EXPERIMENTAL

Chromatographic systems

The modular LC systems used for all experiments consisted of the following components in various combinations: Altex (Berkeley, Calif., U.S.A.) Model 100 and Waters Assoc. (Milford, Mass., U.S.A.) M6000A pumps; Rheodyne (Berkeley, Calif., U.S.A.) Model 7105 and 7120 valve injectors; a Laboratory Data Control (Riviera Beach, Fla., U.S.A.) Model 1206V UV detector and a Waters Assoc. Model 660 solvent programmer. The columns were thermostated with water jackets using a Haake (Evanston, Ill., U.S.A.) type NBE water circulator and a Neslab (Durham, N.H., U.S.A.) cold finger.

Chemicals

The 4-dodecyldiethylenetriamine chelating agent (C_{12}-dien) was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). The sources of the sulfa drugs have been

previously listed[6]; the dansyl amino acids and nucleotide monophosphates were of reagent grade and were obtained from Sigma (St. Louis, Mo., U.S.A.) and Pierce (Rockford, Ill., U.S.A.). The other solutes and inorganic salts were purchased from various sources. The UV grade organic solvents were from Burdick & Jackson (Muskegon, Mich., U.S.A.). The LiChrospher (Si-100) 10-μm silica particles were obtained from Rainin (Brighton, Mass., U.S.A.) and Hypersil 5-μm silica particles from Shandon Southern (Sewickley, Pa., U.S.A.). The octyldimethylchlorosilane and hexamethyldisilazane were purchased from Silar Laboratory (Scotia, N.Y., U.S.A.).

For gradient elution purposes, water was deionized, distilled and then redistilled from alkaline permanganate; the ammonium acetate salt was recrystallized once from acetic acid.

Packings and columns

Both the LiChrospher 10-μm and Hypersil 5-μm particles were bonded with octyldimethylchlorosilane and then exhaustively silanized with hexamethyldisilazane to minimize accessible silanol groups on the bonded silica surface. The columns consisted of Analabs (North Haven, Conn., U.S.A.) "Anakro I.D." precision-bore stainless-steel tubing (4.6 mm I.D.) with bored-out Swagelok end fittings and Whatman (Clifton, N.J., U.S.A.) 2-μm stainless-steel frits. The C_8 bonded particles were slurried in a balanced-density solvent and forced into the column blank at 5000 p.s.i. using a Haskel (Burbank, Calif., U.S.A.) Model 27486 pump. The columns were then compacted at a higher pressure in order to obtain a more stable bed[7].

RESULTS AND DISCUSSION

Whereas in our previous paper[1] we employed commercial bonded phase packings, we decided to synthesize our own bonded phases in this work in order to have a better control of the quality and reproducibility of the stationary phase. For the most part our retention measurements were performed on LiChrospher 10 μm with *n*-octyl as the alkyl chain. In order to minimize unreacted silanols, we selected a monochlorosilane[8] for which we obtained a coverage of 3.2 μmoles/m^2, based on elemental analysis. A reduction in unreacted silanols was achieved by exhaustive silanization of the bonded phase. For the gradient elution study of the separation of dansyl amino acids we used 5-μm Hypersil and again performed chemical bonding. Coverages of 3.2 μmoles/m^2 were obtained for *n*-octyl and exhaustive silanization was also employed.

It is interesting to note that the relative retention values on LiChrospher C_8 using C_{12}-dien–Zn(II) for the separation of sulfa drugs and carboxylic acids agreed within 10% with the values obtained on the Merck RP-8 column used in our previous paper. The absolute retention values were roughly 10% lower on our synthesized LiChrospher C_8 phase. This small change can be easily explained by a difference in surface area between the silica gel used in this work and the previous Merck RP-8 column. The relative retention values again closely agreed for the LiChrospher and Hypersil bonded phases using the C_{12}-dien–Zn(II) system. The absolute retention values were roughly 30% lower on the Hypersil *vs.* the LiChrospher, and this could again be explained in part by the surface area differences of the two silica gels.

After column packing, the C_{12}-dien–Zn(II) (10^{-3} *M*) mobile phase solution

was equilibrated with the stationary phase. The loading of the metal chelate soap required roughly 50 column volumes (or 1–1.5 h) for constant k' values to be obtained. The slow initial equilibration of the column is a result of the adsorption of significant quantities of chelate on the stationary phase, as already pointed out by Knox and Jurand[9]. We should emphasize, however, that small variations in salt concentration (*e.g.* ammonium acetate) or percent organic modifier (*e.g.* acetonitrile) only require very short equilibration times (*e.g.* 10–15 column volumes or *ca.* 10 min). As a result, gradient elution with high-speed separations and rapid reequilibration to starting conditions is possible (see later).

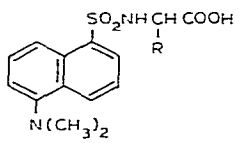
We found roughly 5 mg of C_{12}-dien–Zn(II) per gram of support loaded on the stationary phase with 10^{-3} *M* metal chelate in the mobile phase of 0.13 *M* ammonium acetate and acetonitrile–water (35:65) (35/65 AN/H_2O), the conditions used in our previous paper. Breakthrough volume analysis of soap (two-phase titration) and metal (atomic absorption) were in agreement with one another.

The sample capacity for selected sulfa drugs and aromatic acids was measured as the point at which a 0.1-mm increase in plate height occurred[10]. By this measure, the column sample capacity was found to be *ca.* 35 μg/g of packing with the mobile phase cited above [35/65 AN/H_2O, pH = 7.1, 0.13 *M* NH_4Ac, 10^{-3} *M* C_{12}-dien–Zn(II)]. This low capacity is expected, given the loading of the C_{12}-dien–Zn(II) on the stationary phase.

The sample capacity can be increased in the metal-chelate system by increasing the C_{12}-dien–Zn(II) concentration. At 5×10^{-3} *M* C_{12}-dien–Zn(II), roughly 23 mg of soap was loaded per gram of packing, and sample capacities were roughly 150 μg/g. Nevertheless, for analytical-scale separations the capacities cited above (*ca.* 35 μg/g) are adequate, and so we have used 10^{-3} *M* C_{12}-dien–Zn(II) concentrations for this work.

Dansyl amino acids

Our interest was to investigate the selectivity possible through outer-sphere complexation with the metal-chelate system. Towards this end we have examined a series of 24 amino acids as their dansyl derivatives (see Table I). This solute series provides a wide variation in steric, hydrophobic and ionic properties. The basic structure of the dansyl amino acids is as follows



where R is dependent on the particular amino acid.

While amino acids have been conventionally separated on ion-exchange resins[11], recent work has focused on high speed using silica-based small particle diameter supports. Thus, Kraak *et al.*[12] have been developing reversed-phase ion-pair LC ("dynamic ion exchange") employing an anionic soap along with post-column detection by ninhydrin reaction at high temperatures. Reversed-phase separations of PTH[13] or dansyl amino acids[14] have also been studied.

TABLE I

RETENTION OF DANSYL AMINO ACIDS BY REVERSED-PHASE, ION-PAIR AND METAL CHELATE CHROMATOGRAPHY (30°, pH 7.1)

Dansyl amino acid	*k' values*		
	*Reversed-phase**	*Ion pair***	*Metal chelate****
$CySO_3H$	0.36	0.90	4.95
Glu	0.50	0.66	1.95
Asp	0.53	0.71	7.89
Asn	1.12	0.85	2.75
HO-Pro	1.12	0.87	0.98
Gln	1.23	0.86	0.99
Arg	1.44	0.48	0.49
Ser	1.61	1.27	3.60
Thr	2.03	1.57	2.48
Gly	2.24	1.72	9.05
Ala	2.64	2.05	4.21
γ-NH_2But	3.01	1.78	1.91
Sar	3.33	2.18	2.12
ε-dan Lys	3.37	0.85	1.71
Pro	3.43	2.17	3.49
α-NH_2But	3.71	2.63	3.87
Val	5.34	3.46	4.93
Norval	6.44	3.93	7.22
Met	6.47	3.98	7.30
Ileu	9.24	5.27	8.83
Leu	9.93	5.24	9.97
Trp	12.28	6.29	15.89
Norleu	12.57	6.38	12.49
Phe	13.80	6.75	16.00

* Acetonitrile–water (25:75), 0.13 *M* NH_4Ac.
** Acetonitrile–water (35:65), 0.13 *M* NH_4Ac, 10^{-3} *M* $C_{12}H_{25}\overset{+}{N}(CH_3)_3Br^-$.
*** Acetonitrile–water (40:60), 0.15 *M* NH_4Ac, 10^{-3} *M* C_{12}-dien–Zn(II).

Table I shows the retention (*i.e.* capacity factors) of the 24 dansyl amino acids at pH 7.1 and 0.13 *M* ammonium acetate by (1) reversed-phase chromatography, (2) reversed-phase ion-pair chromatography using 10^{-3} *M* $C_{12}H_{25}\overset{+}{N}(CH_3)_3$ and (3) reversed-phase chromatography using 10^{-3} *M* C_{12}-dien–Zn(II). Different compositions of acetonitrile–water were necessary for reasonable retentions in the three systems. Clearly, significant differences exist in these three approaches.

It is instructive to examine some of these differences in order to illustrate the selectivity possible with the metal-chelate system. We first note that retention in general increases with hydrophobicity in the case of reversed-phase and ion-pair chromatography. Thus *k'* for

$$Gly(R{=}H) < Ala(R{=}CH_3) < \alpha\text{-}NH_2But(R{=}CH_2CH_3)$$

and

$$Ser(R{=}CH_2OH) < Thr(R{=}CH(CH_3)OH)$$

In the case of the C_{12}-dien–Zn(II) system, the elution order is reversed

$$\alpha\text{-}NH_2But < Ala \ll Gly$$

and

$$Thr < Ser$$

This change in elution order can be explained in terms of the steric selectivity afforded by the metal-chelate system. Thus, even though Ala and Thr are more hydrophobic by the addition of a methyl group in the α and β positions of Gly and Ser, respectively, retention is less in the former compounds with the C_{12}-dien–Zn(II) system as a consequence of the weaker outer-sphere complex formation. (We have already presented evidence in our previous paper that monovalent ions would be expected to form outer-sphere complexes with C_{12}-dien–Zn(II) with the mobile phase conditions in Table I[1]). Steric selectivity should be influenced by the substitution position. For the conditions of Table I, methyl substitution on the α-carbon leads to a relative retention value of 2.15 (Gly *vs.* Ala) and 1.45 (Ser *vs.* Thr) for β substitution. This factor evidently overcomes the increased hydrophobicity, leading to lower retention for Ala and Thr, relative to Gly and Ser, respectively.

Another example of this steric effect can be seen in the separation of Ileu from Leu. For reversed-phase ion-pair and reversed-phase LC chromatography the α values for this pair are *ca.* 1.0 and 1.07, respectively. For the C_{12}-dien–Zn(II) system α is found to be 1.13, in spite of the highest percent organic modifier. For these two isomers, the alkyl group structure is

$$R = -CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_3 \qquad \text{Leu}$$

$$R = -\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-CH_3 \qquad \text{Ileu}$$

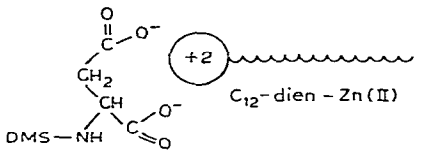
We again see that a methyl group attached to the β carbon (Ileu) leads to lower retention due to the weaker outer-sphere complexation caused by steric hindrance. Indeed, a lower ammonium acetate concentration (such as 0.065 *M*) causes the relative retention of this isomeric pair to increase to 1.25 with the C_{12}-dien–Zn(II) system.

We turn next to the diacids, which are of particular interest because of multi-attachment to the C_{12}-dien–Zn(II) system.

$R = -CH_2COOH$	Asp
$R = -CH_2CH_2COOH$	Glu
$R = -CH_2SO_3H$	$CySO_3H$

Dansyl glutamic and aspartic acids have relative retention values close to one on the reversed-phase and ion-pair systems. On the other hand the α value in the metal chelate system is close to 4. The selectivity arises mainly from a *ca.* tenfold retention change for Asp from the reversed-phase ion-pair to the metal-chelate system, the largest change for any dansyl amino acid in Table I.

We attribute this strong interaction of Asp with C_{12}-dien–Zn(II) to chelate formation of the following form:



Here the circle represents the boundary of the rigid inner-coordination sphere of the metal–soap complex. The geometry and size of the inner-sphere is determined by the metal, and the amine groups of dien are thus arranged in a specific orientation; this creates the possibility of selective interactions with particular solutes in the outer-sphere. Thus, the metal chelate serves as a "template". Evidently, Asp in the outer-sphere is able to satisfy the structural requirements of the template. A similar effect leads to unusually strong and specific interaction between outer-sphere PO_4^{3-}, SeO_3^{2-} and (+)-tartrate and the inner-sphere amine groups of conformationally compatible CoN_6^{3+} complexes[15]. It appears that solutes with 6 atoms in a conformationally compatible arrangement and two or more groups capable of binding to the inner coordination sphere will exhibit unexpectedly high relative retention in a C_{12}-dien–metal system. It is well known that 5 and 6 membered chelate rings are most stable in the inner sphere, when considering bidentate attachment. This means that the ligands that contribute 4 or 5 atoms to the chelate ring are preferred. Spectrophotometric results with PADA*[1] indicate the Asp is bound to the outer-coordination sphere of C_{12}-dien–Zn(II) under the chromatographic mobile phase conditions (40/60 AN/H_2O). Because of the much larger size of C_{12}-dien–Zn(II) compared to Zn^{2+}, it is logical to anticipate that some increase of the number of atoms in an outer-sphere chelate ring would occur. A stable complex can evidently form when the ligand itself contributes 6 atoms to the ring system. This result constitutes an important and special selectivity characteristic for this outer-sphere metal ligand-exchange site.

Dansyl glutamic acid may also form a chelated ring structure in the outer coordination sphere of C_{12}-dien–Zn(II), but from the retention change in Table I from the ion-pair to the metal-chelate system, this complex must be significantly weaker than that for Asp. Evidently, the extra methylene group in the chelate ring (*i.e.* the ligand contributing 7 atoms to the outer-sphere structure of the metal complex) results in a less stable interaction between the solute and the exchange site.

Dansyl cysteric acid is also significantly retained in the metal chelate system, relative to reversed-phase ion-pair and reversed-phase LC. $CySO_3H$ is a bidentate ligand analogous to Asp, except that a carboxylate is substituted by a sulfonate. We would then anticipate bidentate attachment of $CySO_3H$ to C_{12}-dien–Zn(II) as is found with Asp. However, $-SO_3^-$ is known to be a weaker ligand than $-CO_2^-$ (ref. 16), so that $CySO_3H$ elutes before Asp on the C_{12}-dien–Zn(II) system, whereas the two dansyl amino acids elute together on reversed-phase ion-pair chromatography. Note, however, that $CySO_3H$ elutes after Glu on the metal chelate system, which indicates that the former binds more strongly in the outer sphere of C_{12}-dien–Zn(II).

* PADA = pyridine-2-azo-*p*-dimethylaniline.

The ε-amides of the diacids Asp and Glu

$R = -CH_2CONH_2$ Asn
$R = -CH_2CH_2CONH_2$ Gln

are also interesting to compare. We observe no separation of these two substances on reversed-phase ion-pair chromatography and only a small selectivity ($\alpha = 1.10$) on the reversed-phase LC column; however, a very large selectivity ($\alpha = 2.75$) with a reversal in elution order (compared to reversed phase) is found with the C_{12}-dien–Zn(II). Evidently, the amide group is able to interact with the complex cation sufficiently (probably by hydrogen bonding) to cause a bidentate chelate ring complex to form as in the case of Asp. This important result means that bidentate ligands need not contain two negatively charged groups for enhanced outer-sphere binding. Complexation (and enhanced retention) can occur when one group is charged and the second can interact with the outer sphere (*e.g.* by hydrogen bonding), provided the correct ring size is formed. We note that Gln is not significantly more retained on the C_{12}-dien–Zn(II) system relative to the reversed-phase ion-pair system, undoubtedly as a result of the much weaker complex formation, in agreement with the comparison of Asp and Glu.

Since the three phase systems in Table I are different, we should not be surprised to find some pairs of dansyl amino acids which are best resolved by one system. In particular, we note that dansyl tryptophan is not separated from dansyl phenylalanine in the C_{12}-dien–Zn(II) system, whereas an α value of 1.12 is achieved in reversed-phase

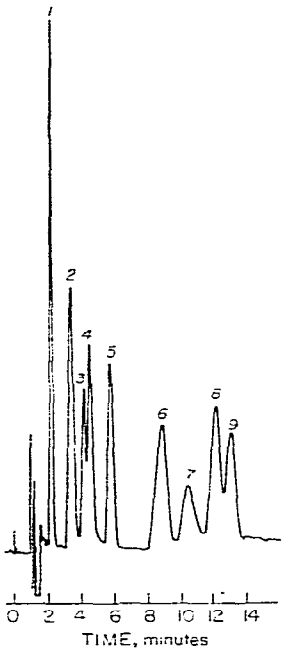


Fig. 1. Separation of dansyl amino acids by C_{12}-dien–Zn(II) chromatography. Conditions are 10^{-3} *M* C_{12}-dien–Zn(II), 0.15 *M* NH_4Ac, 40/60 AN/H_2O. Temperature, 30°; flow-rate, 2 ml/min. Solutes are (1–10 μg): 1 = Gln; 2 = Glu; 3 = Thr; 4 = Asn; 5 = α-NH_2But; 6 = $CySO_3H$; 7 = Asp; 8 = Gly; 9 = Leu.

LC using 25/75 AN/H_2O. A shorter chain dien soap might be used for the resolution of this pair by ligand exchange.

Fig. 1 shows the isocratic separation of 9 dansyl amino acids on a 20-cm 10-μm LiChrospher C_8 column. We note that the diacids, which bind more strongly to the C_{12}-dien–Zn(II) chelate than the monoacids, are roughly 50% as efficient. For the monoacids we find a plate count of roughly 2500 whereas for the diacids approximately 1000. It is expected that an increase in column temperature would significantly improve column performance, but the relationship of this improvement to the loss in selectivity would need to be evaluated. However, when it is considered that the diacids are involved in outer-sphere chelation, the performances for these substances are surprisingly good. This result is, of course, a consequence of the formation of the chelate in the outer coordination sphere. If the chelate were to form in the inner coordination sphere, extremely poor column performances would be anticipated.

After examining the dansyl amino acids under one set of elution conditions we next decided to explore retention as a function of salt concentration and organic modifier composition. Fig. 2 shows the change in k' that occurs when the ammonium acetate concentration is increased from 0.065 M to 0.13 M, while maintaining all other mobile phase conditions constant, *i.e.* 40/60 AN/H_2O, pH 7.1, 10^{-3} M C_{12}-dien–Zn(II). For purposes of clarity we have changed the k' axis by a factor of 2 from the left hand to the right hand side of Fig. 2. As is well known in ion-exchange chromatography[16], the retentions of the diacids (Asp, Glu, $CySO_3H$) are much more sensitive to salt concentration than are the monoanionic dansyl amino acids. The slope of the plot of log k' *vs.* log [Ac^-] is also found to be the same for the diacids,

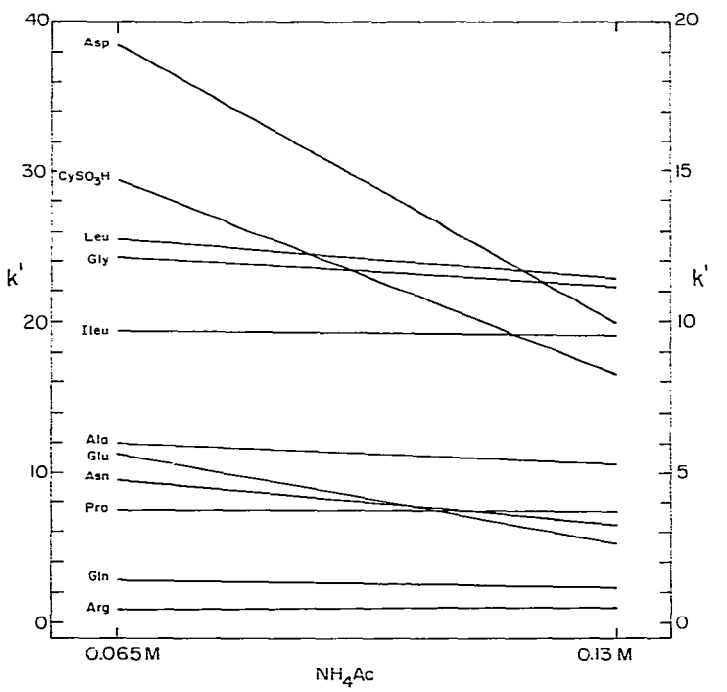


Fig. 2. Influence of NH_4Ac concentration on the retention of dansyl amino acids. Common conditions are 10^{-3} M C_{12}-dien–Zn(II), 40/60 AN/H_2O, pH 7.1, 30°.

indicative of general electrostatic effects[16]. As the stability of the dansyl Asn-C_{12}-dien–Zn(II) complex is governed by electrostatic attachment at one end and hydrogen bond formation at the other end to complete ring formation, the slope of the Asn plot in Fig. 2 lies between those of the diacids and monoacids.

Fig. 2 reveals that selectivity does not change greatly with acetate concentration when monoanionic dansyl amino acids are chromatographed. This result is in agreement with our previous work with monoanionic sulfa drugs[1]. However, selectivity becomes strongly dependent on acetate concentration when the diacids are included. It is easy to see that peak reversals are possible as the acetate concentration is varied.

A further examination of the effect of salt type and concentration on retention is shown in Fig. 3. Here, the ionic strength is maintained constant and varying ratios of acetate to trifluoroacetate concentrations are employed. Since retention decreases with decreasing trifluoroacetate concentration (and corresponding increasing acetate concentration), trifluoroacetate is seen to be a weaker displacing anion than acetate. As in Fig. 2, we observe that the retention of the diacids is more sensitive to acetate concentration than are those of the monoacids.

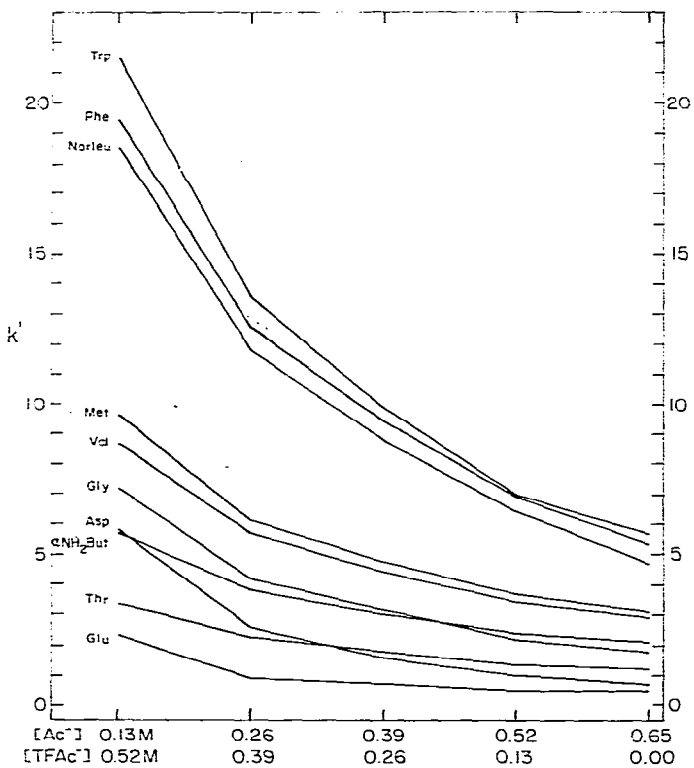


Fig. 3. Influence of displacing ion as NH_4^+ salt on the retention of dansyl amino acids. Common conditions are 10^{-3} *M* C_{12}-dien–Zn(II), 35/65 AN/H_2O, pH 7.1, 30°.

We next examined the influence of percent (v/v) of acetonitrile on retention for the dansyl amino acids, and the results are shown in Fig. 4. In this case we varied the AN/H_2O ratio from 30/70 to 35/65 while maintaining all other conditions con-

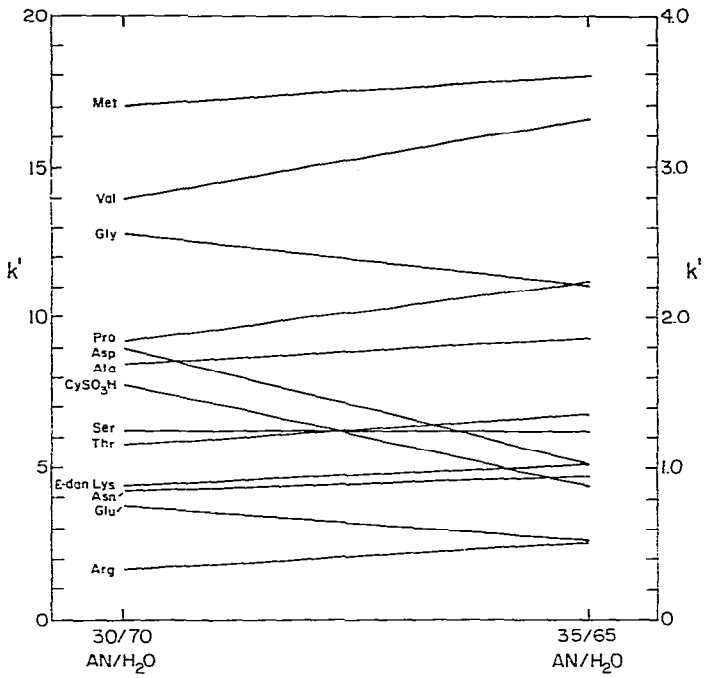


Fig. 4. Influence of acetonitrile-water percent composition on the retention of dansyl amino acids Common conditions are 10^{-3} *M* C_{12}-dien–Zn(II), 0.52 *M* NH_4Ac, 0.13 *M* NH_4TFA, pH 7.1, 30°.

stant, *i.e.* pH 7.1, 0.52 *M* ammonium acetate, 0.13 *M* ammonium trifluoroacetate, 10^{-3} *M* C_{12}-dien–Zn(II). A high salt concentration was selected in order to obtain reasonable retention of the solutes at 30/70 AN/H_2O composition. For purposes of clarity the left hand axis differs by a factor of 5 from the right hand axis of Fig. 4.

In our previous paper we found that k' increased by a factor of about 3 for sulfa drugs on changing from 35/65 AN/H_2O to 30/70 AN/H_2O. In Fig. 4 we find much larger changes in k' for the 5% (v/v) change in AN. This is particularly true for the relatively strongly binding diacids (Asp, $CySO_3H$, Glu) which vary by 8–9-fold over the small change in AN/H_2O composition. This large change is unusual for reversed-phase LC and means that the composition of the mobile phase must be carefully controlled for reproducible conditions with such strongly interacting species. It is anticipated that conditions for weaker binding to the metal chelate complex (*e.g.* higher temperature) would reduce this dependency somewhat, as is the case for the monoacids. The reason for the sensitivity of the diacids to AN/H_2O composition probably arises from the influence that the mobile phase has on the environment in the outer coordination sphere of the metal chelate complex. Changes in that environment ought to affect those solutes which bind most strongly to the inner coordination sphere atoms.

It is interesting to note that strong binding monoanionic dansyl amino acids also follow this trend. For example, Gly varies by 6-fold from 35/65 to 30/70 AN/H_2O. It appears that changes in AN composition can discriminate monoanionic dansyl amino acids better than changes in acetate concentration (*cf.* Fig. 2). Whereas the role of acetate appears to be related directly to the electrostatic interaction of the solute

with the metal chelate, the role of AN/H_2O composition appears to be related more generally to the binding strength of the solute to the metal chelate. An anomaly to this trend is dansyl asparagine (Asn), which would be expected to vary more greatly with AN/H_2O composition, considering our previous arguments of bidentate attachment. It is unclear at present as to why Asn is not more affected by AN/H_2O composition. However, we should point out that the hydrophobic selectivity increases somewhat from 35/65 to 30/70 AN/H_2O (ref. 17).

Given the results of Table I and Figs. 2–4, we decided to examine the possibility of separating the dansyl amino acid standards using gradient elution techniques. This effort was made primarily to indicate the potential of the metal chelate system to rapidly separate complex mixtures. For this work we employed 5-μm Hypersil.

Two gradient systems were developed, based on the above results: (1) a variation in trifluoroacetate concentration while maintaining acetate and acetonitrile

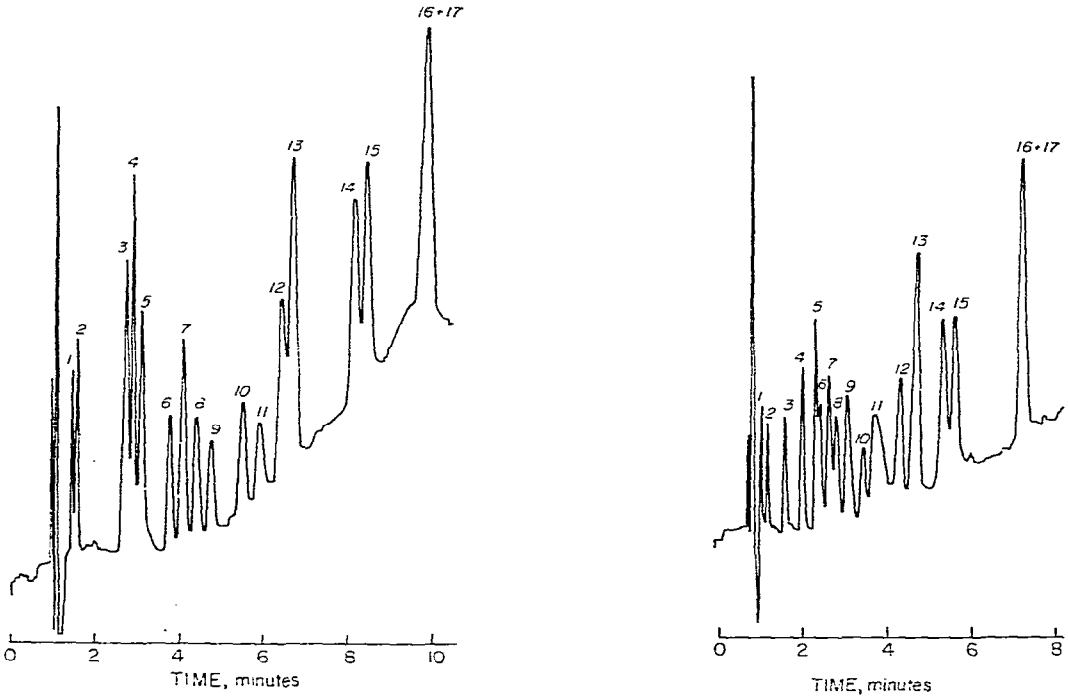


Fig. 5. Gradient elution of dansyl amino acids. Solvent A, 0.0 *M* NH_4TFA; solvent B, 0.52 *M* NH_4TFA. Common conditions are 10^{-3} *M* C_{12}-dien–Zn(II), 0.13 *M* NH_4Ac, 40/60 AN/H_2O, pH 7.1. Gradient conditions are A–B (80:20) for 1 min to 100% B, 5 min linear gradient (program No. 6 on Waters Assoc. 660 Solvent Programmer). Column, 15 cm × 4.6 mm I.D., 5-μm Hypersil C_8; flow-rate: 2.0 ml/min; temperature, 30°. Solutes (1–10 μg): 1 = Arg; 2 = dansyl sulfonic acid; 3 = dansylamide; 4 = Glu; 5 = Thr; 6 = Pro; 7 = Ser; 8 = Ala; 9 = Val; 10 = $CySO_3H$; 11 = Asp; 12 = Gly; 13 = Met; 14 = Ileu; 15 = Leu; 16 = Phe; 17 = Trp.

Fig. 6. Gradient elution of dansyl amino acids. Solvent A, 40/60 AN/H_2O; solvent B, 60/40 AN/H_2O. Common conditions are 10^{-3} *M* C_{12}-dien–Zn(II), 0.15 *M* NH_4Ac, pH 7.1. Gradient conditions are A–B (90:10) to A–B (40:60), 7-min linear gradient (program No. 6 on Waters Assoc. 660 Solvent Programmer). Column, 15 cm × 4.6 mm I.D., 5-μm Hypersil C_8; flow-rate, 2.0 ml/min; temperature, 30°; Solutes (1–10 μg): 1 = Arg; 2 = dansyl sulfonic acid; 3 = Glu; 4 = dansylamide; 5 = Thr; 6 = Pro; 7 = Ser; 8 = Ala; 9 = Val; 10 = Met; 11 = Asp; 12 = $CySO_3H$; 13 = Gly; 14 = Ileu; 15 = Leu; 16 = Phe; 17 = Trp.

compositions constant and (2) a variation in acetonitrile composition while maintaining salt concentration constant. The results for these two gradients are shown in Figs. 5 and 6.

Fig. 5 illustrates a 10-min separation of 14 out of 15 dansyl amino acids (Phe and Trp not resolved) in which the trifluoroacetate concentration is varied linearly from 0.10 to 0.52 *M* over a 5-min time span after a 1-min isocratic elution at starting conditions. The ammonium acetate concentration and the AN/H_2O composition are maintained constant at 0.13 *M* and 40/60 (v/v), respectively. The increase in baseline is probably caused by the refractive index change of the mobile phase upon the addition of the trifluoroacetate. Recycle time to starting conditions was approximately 12 min, which indicates that rapid reequilibration occurs in this system.

The use of the weak ligand trifluoroacetate permitted relatively easy control of retention, even for the diacids. The good performance of the diacids $CySO_3H$ (No. 10) and Asp (No. 11) should be noted. Fig. 5 illustrates therefore that high performance and selectivity can be combined to lead to rapid separation of related solutes.

We also explored a gradient consisting of acetonitrile composition and an 8-min separation of 14 out of 15 dansyl amino acids (Phe and Trp again not resolved) is shown in Fig. 6. A linear gradient is applied between 42/58 AN/H_2O to 52/48 AN/H_2O over a roughly 7-min time span. The concentration of acetate is maintained constant at 0.16 *M* and the pH is 7.1. Recycle time to starting conditions is approximately 10 min, again illustrative of rapid reequilibration.

The retentions of the diacids, particularly Asp, are now quite sensitive to the gradient conditions, as suggested by Fig. 4. Asp (No. 11) exhibits poor peak symmetry and efficiency in the gradient, as a consequence of the slow dissociation kinetics from the strong binding of this diacid to C_{12}-dien–Zn(II).

The variation in trifluoroacetate concentration would appear to be the better approach for these substances, as the retention control and peak performance is better than with an acetonitrile gradient. However, the trifluoroacetate gradient can only cover a limited range of solute hydrophobicities, whereas the acetonitrile gradient can be broader. For example, by extending the linear gradient in Fig. 6 to 9 min, we have been able to elute and easily separate didansyl lysine, histidine and tyrosine in 13 min. More studies are required using gradient elution and the metal-chelate system in order to elucidate the proper gradient type for particular separation problems. Nevertheless, the results in Figs. 5 and 6 reveal that this area holds a great deal of promise for rapid separation of complex mixtures.

Substitution of Cd(II) for Zn(II) in the C_{12}-dien system

An important factor in the chelate system which we have employed is the influence of the metal bound to the C_{12}-dien soap. Many properties of the exchange site might be changed by metal substitution, including (1) the charge on the complex, (2) the size and conformation of the inner-sphere chelate rings and (3) the structure of the inner coordination sphere, *e.g.* a change from a tetracoordinated to a hexacoordinated metal center. In practice, the choice of metal is somewhat limited when using an ultraviolet (UV) detector. This arises from the significant UV absorption of many transition metal complexes through charge transfer interactions in the metal chelate itself. However, Cd(II) can be substituted for Zn(II) while still maintaining a transparent background at 254 nm.

Cd(II) is a particularly good metal to compare with Zn(II) for the following reasons. First, Cd(II) is generally believed to be pentacoordinate when chelated with the dien functionality[18] whereas Zn(II) is tetracoordinate[1]. Second, both Zn(II) and Cd(II) do not undergo crystal field stabilization on complexation. Third, while both have the same charge, the effective ionic radius of tetracoordinated Zn(II) is 0.60 Å, while that of pentacoordinated Cd(II) is 0.78 Å[19]. The resulting size difference of the inner coordination sphere, as we shall see, can have a significant effect on the stability of the complexes formed with different solutes.

Our first efforts with Cd(II) involved the question of whether outer-sphere complexation occurred when the solutes interacted with C_{12}-dien–Cd(II). We followed the same spectrophotometric procedures used in our first paper[1] in order to examine this question. Using a 1:1 stoichiometric ratio of C_{12}-dien to cadmium acetate [in which all the Cd(II) would be bound to the chelate[18]], pH 7.1 and 0.13 *M* ammonium acetate, we found that it was necessary to add 30% (v/v) acetonitrile in order to displace PADA from the inner coordination sphere of C_{12}-dien–Cd(II). This value can be compared with 23% (v/v) acetonitrile needed for C_{12}-dien–Zn(II). The larger percentage in the case of Cd(II) may be a consequence of the pentacoordination of this metal as opposed to the known tetracoordination of Zn(II). Separate experiments revealed that neither dansyl aspartic acid nor nucleotide monophosphates (see later) displaced PADA from the inner coordination sphere. This result means that a minimum of 30% AN will result in outer-sphere complexation. Outer-sphere complexation of solutes can be reasonably assumed to occur down to at least 25% AN[1]. Consequently, in the chromatographic experiments to be discussed we can consider the inner coordination sphere of Cd(II) to consist of the tridentate C_{12}-dien ligand and two acetonitrile molecules.

Since Cd(II) is pentacoordinate in C_{12}-dien–Cd(II), we examined briefly the behavior of a complex consisting of a 2:1 stoichiometric ratio of C_{12}-dien to Cd(II). The spectrophotometric experiment revealed that 17% AN was required to remove PADA from the inner coordination sphere of the chelate. For $(C_{12}\text{-dien})_2$–Cd(II), a significantly lower AN concentration is thus required to displace PADA than is necessary for displacement from the 1:1 complex. However, we were somewhat surprised that as much as 17% AN was required, since we reasoned that two C_{12}-dien ligands might block all the coordination sites on Cd(II). [It was thought that Cd(II) might expand to hexacoordination when two C_{12}-dien molecules were available for chelation[18]]. Our result suggests that at least one nitrogen in the chelate system is reversibly attached to Cd(II), and that either PADA or AN can displace this nitrogen from the metal. It also implies a general tendency towards 5-coordination in the C_{12}-dien–Cd(II) system.

Our first chromatographic studies with 10^{-3} *M* C_{12}-dien–Cd(II) involved the separation of aromatic carboxylic acids. As previously[1], we found high performance of this outer-sphere ligand-exchange system. For example, using a 20 cm × 4.6 mm I.D. column of 10-μm LiChrospher C_8 and a flow-rate of 2 ml/min, we achieved plate counts of at least 3000 with asymmetry factors A_s (ratio of half widths at 10% of peak height[1]) of 1.1 to 1.2 for acids with k' values up to at least 10. Equivalent performances were found on a 10^{-3} *M* C_{12}-dien–Zn(II) column. The high performance arises from the weak binding of the aromatic acids in the outer coordination sphere, and hence the rates of dissociation are rapid.

TABLE II

RETENTION OF AROMATIC CARBOXYLIC ACIDS. COMPARISON BETWEEN C_{12}-DIEN–Zn(II) AND C_{12}-DIEN–Cd(II)

Conditions: acetonitrile–water (35:65), 0.13 *M* NH_4Ac, pH 7.1, 30°.

Carboxylic acid	10^{-3} *M* C_{12}-*dien–Zn(II)*		10^{-3} *M* C_{12}-*dien–Cd(II)*	
	k'	α	k'	α
p-Toluenesulfonic	0.52		0.50	
		5.79		5.44
p-Toluic	3.01		2.72	
2,6-Dimethylbenzoic	0.81		0.77	
		6.62		6.18
3,5-Dimethylbenzoic	5.36		4.76	
2,4-Dichlorobenzoic	2.16		1.92	
		1.60		1.59
p-Chlorobenzoic	3.45		3.05	
p-Toluic	3.01		2.72	
		1.95		1.88
p-Ethylbenzoic	5.78		5.11	
		1.71		1.71
p-Isopropylbenzoic	9.88		8.76	
		1.61		1.79
p-tert.-Butylbenzoic	15.89		15.68	

Table II compares retention on the C_{12}-dien–Zn(II) system with that of C_{12}-dien–Cd(II) under otherwise identical mobile phase conditions. We have previously examined retention data for the same aromatic acids under slightly different mobile phase conditions[1], so that we will not repeat that discussion here. However, we note that the retention on C_{12}-dien–Cd(II) is only 10% lower than that on C_{12}-dien–Zn(II), while the selectivity does not vary greatly in the two systems. In order to demonstrate more clearly the differences between Zn(II) and Cd(II) it is necessary to examine solutes such as the dansyl amino acids, which bind more strongly to these metal-chelate systems.

We next studied the retention of the dansyl amino acids on C_{12}-dien–Zn(II) and C_{12}-dien–Cd(II) under constant mobile phase conditions and the results are shown in Table III. Note that the concentration of the ammonium acetate is slightly lower than that employed in Table I; hence, the k' values for the dansyl amino acids are slightly larger on C_{12}-dien–Zn(II) under the conditions of Table III.

Consider first the monoprotic acids Gly, Ala and α-NH_2-But and then Ser and Thr. In both cases the elution order is reversed on Zn(II) compared to Cd(II). As we noted earlier, Cd(II) is a larger ion than Zn(II), and outer-sphere complexes would therefore be expected to be generally weaker in the C_{12}-dien–Cd(II) system. It is evident that the steric selectivity afforded by a methyl group on the α and β carbons when Zn(II) is used is greatly diminished when Cd(II) is employed. Further evidence of this loss in steric selectivity is seen in the decrease in α value for Leu–Ileu from 1.19 to 1.04 [Cd(II)].

The weaker binding with Cd(II) also affects retention and selectivity with the diacids. Thus, the α value for dansyl aspartic acid relative to dansyl glutamic acid is 3.9 on C_{12}-dien–Zn(II) and decreases to 2.7 on C_{12}-dien–Cd(II). Dansyl cysteic acid

TABLE III

THE EFFECT OF METAL ION ON SELECTIVITY OF DANSYL AMINO ACIDS USING C_{12}-DIEN

Conditions: acetonitrile–water (40:60), 0.13 *M* NH_4Ac, 1.0×10^{-3} *M* C_{12}-dien–metal, pH = 7.1.

Dansyl amino acid	*k'*	
	Zn(II)	*Cd(II)*
Gly	11.18	1.91
Ala	5.27	2.51
α-NH_2 But	4.84	3.50
Ser	4.50	1.45
Thr	3.12	2.05
Leu	11.43	9.75
Ileu	9.60	9.37
Glu	2.59	1.82
$CySO_3H$	8.28	2.95
Asp	10.00	4.95
Gln	1.20	0.87
Asn	3.30	1.20

appears anomalous in this regard. That bidentate outer-sphere attachment for the diacids also occurs with C_{12}-dien–Cd(II) can be seen in the much higher *k'* values in this system relative to reversed-phase ion-pair chromatography (see Table I). For aspartic acid, we obtained 1200 plates with an asymmetry factor of *ca.* 1.8 on the C_{12}-dien–Cd(II) system. Undoubtedly, operation at a higher temperature would improve performance. Nevertheless, this result is again surprisingly good, considering that bidentate attachment occurs. Thus, the kinetics of dissociation are again seen to be relatively fast in the outer-sphere compared to the slow desorption expected from the inner-sphere.

Finally, a loss in selectivity in changing from Zn(II) to Cd(II) is also observed when the two amides Gln and Asn are considered. In fact, bidentate outer-sphere attachment of Asn to C_{12}-dien–Cd(II) cannot even be argued, given the small change in *k'* from reversed-phase ion-pair (Table I) to the Cd(II) system (Table III).

The dansyl amino acids therefore show in a striking fashion the loss in selectivity when Cd(II) is substituted for Zn(II). Again, this is undoubtedly a result of the weaker binding of the solutes to C_{12}-dien–Cd(II) as a consequence of the larger size of C_{12}-dien–Cd(II) relative to that of C_{12}-dien–Zn(II).

The generally weaker interaction of ligands with C_{12}-dien–Cd(II) can be valuable when strong binding ligands are considered. In this case the rates of dissociation may be more favorable than those for Zn(II). In this sense, the change from Zn(II) to Cd(II) may be viewed as somewhat similar to the effect of raising the column temperature.

In Table IV we show retention and performance data for relatively weak (sulfabenzamide and 2,6-dimethyl benzoic acid) and strong (nucleotide monophosphates and phthalic acids) ligands. All mobile phase conditions are maintained constant except for variation of the metal chelate additive (10^{-3} *M* C_{12}-dien–Zn(II) or 10^{-3} *M* C_{12}-dien–Cd(II)).

Consider first the results for sulfabenzamide and 2,6-dimethylbenzoic acid. For both chelate systems, equal performances of roughly 2300–2500 plates and A_s

TABLE IV

COMPARISON OF PERFORMANCE AND RETENTION BETWEEN C_{12}-DIEN-Zn(II) AND C_{12}-Dien-Cd(II) WITH WEAKLY AND STRONGLY INTERACTING LIGANDS

Common conditions: 10-μm LiChrospher C_8 (20 cm × 4.6 mm I.D.), acetonitrile–water (30:70), 0.13 *M* NH_4Ac, pH 7.1, 30°, flow-rate 2 ml/min. A_s = asymmetry factor; CMP = cyclic monophosphate; MP = monophosphate.

Solutes	*10^{-3} M C_{12}-dien-Zn(II)*				*10^{-3} M C_{12}-dien-Cd(II)*			
	k'	*α*	*N*	A_s	*k'*	*α*	*N*	A_s
Sulfabenzamide	1.81		2200	1.3	1.60		2500	1.3
		1.18				1.21		
2,5-Dimethylbenzoic acid	2.14		2150	1.4	1.94		2250	1.3
Adenosine-3',5'-CMP	0.17		1900	1.4	0.12		1550	1.8
		12.7				2.33		
Adenosine-5'-MP	2.16		250	4.3	0.28		1250	2.0
Cytidine-2'+3'-MP	1.82	1.38	600	≈1.7	0.97	1.00	—	—
	2.51		500	≈3.0	0.97		—	—
Guanosine-2'+3'-MP	4.10	1.34	700	≈2.5	0.97	1.58	1200	1.4
	5.48		600	≈2.0	1.53		1050	1.5
Uridine-2'+3'-MP	4.68	1.12	—	—	0.52	1.17	—	—
	5.24		—	—	0.61		—	—
Terephthalic acid	1.64		1500	1.2	1.21		1600	1.3
		2.01				1.98		
Isophthalic acid	3.29		1200	1.4	2.40		1400	1.2
		3.47				2.63		
Phthalic acid	11.40		1100	2.0	6.31		1700	1.2

values close to unity are achieved on a 20-cm column of 10-μm LiChrospher C_8 at 30°. These results agree with those found for weakly interacting ligands in Table II (however, see later for a general discussion of sulfa drugs) and can serve as a base upon which to compare the results with more strongly bound ligands.

Consider next the nucleotide monophosphates, which would be expected to be dinegatively charged at pH 7.1 (except the cyclic monophosphate CMP which possesses only a single negative charge). For this reason and from the fact that phosphate is a strong outer-sphere ligand[15,20], we expect a stronger interaction with the metal chelate than that found for the aromatic acids. That this association must be strong can be seen from the fact that these hydrophilic substances are relatively strongly retained at 30/70 AN/H_2O with C_{12}-dien–Zn(II).

We note that retention of adenosine-3',5'-cyclic monophosphate is low in both mobile phase systems, as a consequence of the mononegative charge on the molecule. For all other nucleotide monophosphates retention is significantly greater on C_{12}-dien–Zn(II) than on C_{12}-dien–Cd(II). This result demonstrates the stronger binding of these solutes to the Zn(II) chelate system.

The performance of adenosine-5'-monophosphate is poor on the C_{12}-dien–Zn(II) system and improves significantly on the C_{12}-dien–Cd(II) system, albeit at lower retention. The same trend is seen for guanosine-2' and -3'-monophosphates

i.e. better performance on the C_{12}-dien–Cd(II) phase. For the other nucleotide monophosphates in Table IV, the separation was not sufficient to determine either plate count or peak asymmetry. Unfortunately, we were only able to obtain the isomeric nucleotide monophosphates as commercial mixtures.

The improved performance for the C_{12}-dien–Cd(II) system again results from the weaker interaction of the phosphate group in the outer coordination sphere, relative to the corresponding situation in C_{12}-dien–Zn(II). Fig. 7 shows a rapid separation of 6 nucleotide monophosphates using the C_{12}-dien–Cd(II) system at 25/75 AN/H_2O. The peak symmetry is seen to be quite good ($A_s \approx 1.2$ for uridine-2′- and -3′-monophosphate).

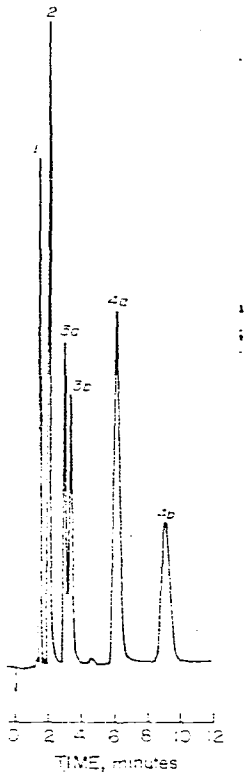


Fig. 7. Separation of monophosphate nucleotides by C_{12}-dien-Cd(II) chromatography. Conditions: 10^{-3} *M* C_{12}-dien-Cd(II), 0.13 *M* NH_4Ac, 25/75 AN/H_2O, pH 7.1, 30°; flow-rate, 2 ml/min. Solutes (1–10 μg): 1 = adenosine-3′,5′-cyclic monophosphate; 2 = adenosine-5′-monophosphate; 3a and 3b = uridine-2′ and 3′-monophosphate; 4a and 4b = guanosine-2′ and 3′-monophosphate.

Table IV shows another example of the differences in retention of the isomeric phthalic acids in the two metal chelate systems. From previous arguments for the dansyl amino acids, we expect significantly weaker interaction of terephthalic (the *p*-isomer) and isophthalic (*m*-isomer) acids, relative to phthalic (*o*-isomer) acid. This arises from the similarity of phthalic acid to dansyl aspartic acid in that it is a 6-membered bidentate ligand, conformationally appropriate for attachment to the boundary of the inner-sphere of the metal chelate. The stronger interaction of phthalic acid is demonstrated in Table IV for both metal-chelate systems in terms of

the relatively long retention of this particular isomer. That phthalic acid interacts more weakly with C_{12}-dien–Cd(II) than with C_{12}-dien–Zn(II) can be seen in the lower asymmetry factor for the Cd(II) chelate. Moreover, the relative retention of phthalic acid to isophthalic acid is lower on the Cd(II) chelate than on the Zn(II) chelate. From this result, we observe a loss in selectivity with an increase in performance when the Cd(II) chelate is substituted for the Zn(II) chelate. Obviously, a balance must ultimately be struck between selectivity and performance, but the change from Zn(II) to Cd(II) is a possible means of achieving this goal in specific separation problems.

Finally, we have compared the chromatographic properties of the sulfa drugs in these two metal-chelate systems. First, as shown in Table IV, good plate counts and asymmetry factors have been obtained for all sulfa drugs tested on the C_{12}-dien–Cd(II) system. Thus, rapid exchange of solute in the outer-sphere of the metal chelate occurs.

However, a surprising result has been obtained for absolute retention. Whereas the aromatic acids exhibited lower retention in the Cd(II) chelate system relative to Zn(II) (see Table III), we now find significant increases in retention for most of the sulfa drugs in the Cd(II) chelate system, as shown in Fig. 8. Indeed, in some cases the retention increase is 2–3-fold and in one case, sulfaquinoxaline, the retention changes by a factor of *ca.* 5.

It is not clear to us at the present time why some sulfa drugs undergo relatively strong interaction with C_{12}-dien–Cd(II). We should note that the sulfa drugs are larger molecules than the simple aromatic acids and contain a number of polar groups which may interact with the inner coordination sphere atoms of a metal chelate. Our work with dansyl asparagine suggested bidentate attachment to the metal chelate via electrostatic interaction (through the carboxylate ion) and hydrogen

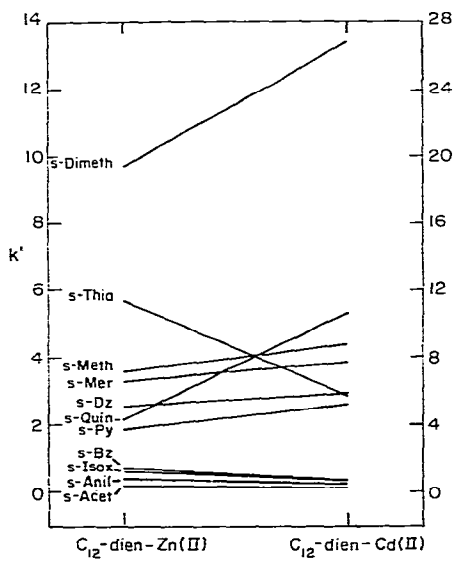


Fig. 8. Comparison of C_{12}-dien–Zn(II) and C_{12}-dien–Cd(II) chromatography for sulfa drugs. Common conditions are 10^{-3} *M* C_{12}-dien–metal, 0.13 *M* NH_4Ac; 35/65 AN/H_2O, pH 7.1.

bonding (through the amide group). It may be, with appropriate molecular geometry of the sulfa drugs, that bidentate attachment also occurs with the sulfonamide group providing electrostatic interaction and some basic nitrogen providing additional hydrogen bonding capability. This geometry could be more favorable in the case of C_{12}-dien–Cd(II) than C_{12}-dien–Zn(II), particularly with sulfaquinoxaline. However, more work will be necessary to unravel the interesting trends in Fig. 8.

CONCLUSION

This work has demonstrated that the metal-chelate additive approach in reversed-phase LC provides a new dimension in selectivity control, with good efficiency and peak symmetry. In our previous paper[1], we found this approach to be highly reproducible for analytical purposes.

Because of the relatively rigid geometry of the inner coordination sphere we have been able to observe unusual structural selectivities for solute molecules interacting with the outer coordination sphere of metal chelates. Bidentate attachment to inner coordination sphere atoms leads to highly selective separations. Moreover, the kinetics of dissociation in the outer-sphere are sufficiently rapid for good column performance.

Much more effort will be necessary to elucidate the structural details of the specific interactions between solutes and different metal chelates that lead to selective separations. Work is continuing in this area.

ACKNOWLEDGEMENTS

The authors wish to thank the National Science Foundation for support of this work. The assistance of Ronald Lee in this work is also acknowledged. Contribution No. 43 from the Institute of Chemical Analysis.

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