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USE OF METAL IONS FOR SELECTIVE SEPARATIONS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The use of transition metals for the achievement of selective separations is well known, but the column performance that has been achieved through such ligand association processes has often been poor, with asymmetrical peaks. Two approaches to the establishment of high-performance conditions using transition metals are described. In the first, a chemically bonded diamine phase was used to chelate Cd(II). A number of parameters were examined, for example, the concentration of Cd(II) in the mobile phase, the volume percentage of acetonitrile and the concentration of the buffer ammonium acetate, in order to achieve optimized separations. It is concluded that this mode is normal phase, as the retention decreases with decreasing acetonitrile concentration. Separations of sulfa drugs and dipeptides are demonstrated. In the second approach, a relatively hydrophobic chelating agent, C_{12}-dien, was added to an acetonitrile–water mobile phase containing Zn(II) with a chemically bonded *n*-alkyl stationary reversed phase. Excellent performance with good peak symmetry was observed even with k' values up to 25. This mode has been shown to be reversed phase, as retention significantly increases with decreasing acetonitrile concentration. Evidence is presented to show that the chromatographic process occurs by outer-sphere complexation. As such complexes are generally weaker than are inner-sphere complexes, the rates of dissociation are rapid, an important factor in determining high performance. Finally, some general observations are presented concerning the effects of direct addition of metal ions to the mobile phase.

INTRODUCTION

The use of transition metal ions, such as Cu(II), Ni(II), Zn(II) and Cd(II), for the achievement of high selectivity has been popular in classical column liquid chromatography (LC). For example, Helfferich[1,2] introduced the procedure of ligand-exchange chromatography, which, as the name implies, involves separation based on exchange of solutes (ligands) with metal ions immobilized on a stationary phase.

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Extensive reviews of this subject by Walton have appeared[3,4]. Guha and Janák have more generally reviewed the use of metals in chromatographic separations[5]. It should be noted that argentation chromatography is not considered in this paper, although the use of silver for the separation of olefinic substances is very popular[6,7].

In a typical ligand-exchange experiment, an ion-exchange or chelating resin (*e.g.*, iminodiacetate) is employed for metal binding. Separations of nitrogenous bases, amino acids[8], amino sugars[9] and carboxylic acids[10] have been successfully achieved. Most commonly, ammonia is selected as the competing ligand via use of aqueous ammonia solutions of high pH (> 12). These conditions, of course, are not satisfactory for silica-based materials.

Selectivity, based on differences of ligand binding to metals, is found to be high. Thus, functional group and steric differences lead to good separation (*e.g.*, resolution of amino acid racemates[11,12]). However, the separations are characterized by poor efficiency and frequently by severe band asymmetry. The latter arises from the slow rates of desorption of ligands strongly bound to metals[13], and the former from not only the desorption kinetics problem but also the slow rate of diffusion in the resin.

Attempts have been made to transfer the chemistries developed for classical ligand-exchange chromatography to silica-based materials for potential high performance liquid chromatography (HPLC) (with appropriate modifications, *e.g.*, pH < 8). Thus, Frei and co-workers[14,15] and others[16,17] have deposited metal on to or incorporated metal into the silica matrix. In addition, Chow and Grushka[18] have recently employed a propylamine-bonded phase for adsorbed Cu(II). These studies, while interesting, suffer from poor efficiency and peak asymmetry and the stability of the phases, particularly in aqueous media, may not be high.

Another promising approach is simply to add the metal to the mobile phase for selective complexation with particular entities. Thus, nickel has been used to complex with *o*-aminophenol, the complex species then eluting more rapidly[19]. It should be noted that Bengtsson and Samuelson[20] also used complexation with transition metal ions to control retention in ion-exchange chromatography. We shall comment on this approach later.

The aim of this work was to develop HPLC using transition metal ions for high selectivity. We have attempted to increase the kinetics of mass transfer to the point where supports with small particle diameters ($d_p = 5$–$10\ \mu$m) can be employed. In this paper, we shall explore two successful approaches towards the above aim. Firstly, we describe the use of a chelating bonded phase, namely *n*-propylethylenediamine, as a support matrix for Cd(II) ions. In the second approach, we shall explore the use of a relatively hydrophobic chelating agent, namely 4-dodecyldiethylenetriamine (C_{12}-dien), $CH_3(CH_2)_{11}N(CH_2CH_2NH_2)_2$, which is added to an acetonitrile–water mobile phase containing Zn(II) in reversed-phase chromatography.

Several general comments need to be made. Firstly, in both approaches positively charged entities are formed, so that the work described in this paper deals solely with the separation of anionic species. However, the extension to positively charged species and possibly even to neutral substances is clear. Secondly, as test substances we selected mainly sulfa drugs, summarized in Table I, as we have previously used these components in ion-pair chromatographic studies[21]. These drugs provide a wide variation in pK_a, hydrophobicity and, especially for this work, heterocyclic nitrogen

and sulfur ring systems. Thirdly, we used ammonium acetate both as a buffer at pH $\approx$ 7 and as a masking agent to prevent hydrolysis of the metals at this pH. Fourthly, we selected Zn(II) and Cd(II) for most of these studies in the belief that these weaker binding metals [relative to Ni(II) and Cu(II)] would provide enhanced ligand desorption rates[13]. Later, we shall see that, under some conditions, it does not follow that metals such as Ni(II) cannot be used.

As we shall show, an important principle to result from this work is that outer-sphere complexation equilibria[22] can be used to achieve high selectivity (*e.g.*, steric), while maintaining rapid adsorption–desorption kinetics. We believe that this approach has great potential in HPLC.

EXPERIMENTAL

Chromatographic apparatus

Modular liquid chromatographic systems were used for all experiments. The systems consisted of the following components in various combinations: Altex Model 100 (Berkeley, Calif., U.S.A.) and Waters Assoc. (Milford, Mass., U.S.A.) M 6000 pumps, Rheodyne (Berkeley, Calif., U.S.A.) Models 7105 and 7120 valve injectors, and Laboratory Data Control (Riviera Beach, Fla., U.S.A.) Model 1206 V (254 nm) and Altex Model 100-40 spectrophotometric detectors. The columns were thermostated at 30 $\pm$ 0.1° using a water-jacket system similar to that described elsewhere[23], with a Haake (Evanston, Ill., U.S.A.). Type NBE water circulator in combination with a Neslab Instruments (Durham, N.H., U.S.A.) cold finger.

Chemicals and packings

The C_{12}-dien chelating agent was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). The sources of the sulfa drugs shown in Table I have been listed previously[21]. The peptides and dansylamino acids were of reagent grade (Sigma, St. Louis, Mo., U.S.A.); the other solutes and inorganic salts were obtained from various sources. The samples for all the chromatograms consisted of 10-μl injection volumes of approximately 1 μg for each standard substance. Reagent-grade 3(-2-aminoethylamino)propyltrimethoxysilane was obtained from Silar Labs. (Scotia, N.Y., U.S.A.). The UV-grade organic solvents were obtained from Burdick & Jackson (Muskegan, Mich., U.S.A.). Nucleosil 5- and 10-μm silica (surface area 300 m^2/g) was obtained from Rainin Instruments (Brighton, Mass., U.S.A.). The 5-μm particles were sedimented in methanol prior to packing to remove fines.

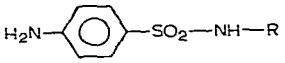
Columns

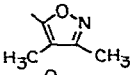
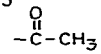
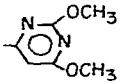
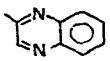
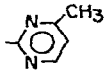
Commercial μBondapak C_{18} (30 cm $\times$ 4.0 mm I.D.; Waters Assoc.) and Merck LiChrosorb C_8 (25 cm $\times$ 4.6 mm I.D.; Altex) columns were employed.

Home-made high-performance columns consisted of Analabs (North Haven, Conn., U.S.A.) Anakro I.D. precision-bore stainless-steel tubing (15 cm $\times$ 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 columns were packed in the conventional manner[24] by carefully preparing a slurry (10%, w/v) in reagent-grade tetrachloroethylene, which had been previously purified by passage through an activated silica column. The slurry was forced into the column blank at 7000 p.s.i. using a Haskel (Burbank, Calif., U.S.A.) Model 27486 pump which is set up specifically for this purpose.

TABLE I

SULFA DRUGS USED



Name	*Abbreviation*	*R*	*pK$_a$*
Sulfabenzamide	S-Bz		4.6
Sulfisoxazole	S-Isox		4.9
Sulfacetamide	S-Acet		5.4
Sulfadimethoxine	S-Dimeth		6.0
Sulfadiazine	S-Dz		6.4
Sulfaquinoxaline	S-Quin		6.5
Sulfamerazine	S-Mer		6.9
Sulfathiazole	S-Thia		7.2
Sulfapyridine	S-Py		8.4
Sulfanilamide	S-Anil	-H	10.4

Preparation of bonded phase

The bonded diamine was prepared by a direct reaction technique similar to that described by Unger *et al.*[25]. Ten-gram batches of silica and an ampule containing a molar excess of 3-(2-aminoethylamino)propyltrimethoxysilane were placed in the reaction vessel[26] and the silica was activated at 250° for 24 h. The ampule was then broken and the silane modifier condensed on to the silica and subjected to reaction in a dry, purified nitrogen atmosphere for 8 h at 190°. After reaction, the particles were washed and extracted in a Soxhlet extractor with dry toluene for 24 h. Finally, the particles were activated under a vacuum of 10^{-3} torr at 100° for 2 h. The surface coverage was calculated to be 2.6 μmole/m^2 by the method of Unger *et al.*[25] based on the elemental C,H,N analysis (Galbraith Labs., Knoxville, Tenn., U.S.A.). The reproducibility in surface coverage between different preparations was $\pm$ 5%.

Preparation of mobile phases

The mobile phases were prepared by carefully adding the constituents to a 500-ml volumetric flask. A stock solution of 2.0 *M* aqueous ammonium acetate was prepared, filtered (5-μm filter; Millipore, Bedford, Mass., U.S.A.) and stored in a tightly sealed bottle. All mobile phases were prepared by pipetting the appropriate

amount of ammonium acetate from the stock solution. The C_{12}-dien and metal salt were carefully weighed on an analytical balance and added to the other constituents. The water (distilled, deionized and filtered) and the acetonitrile were both added by pipet. All mobile phases were kept under a positive pressure of dry helium during use. The preparation of mobile phases in this manner was found to be highly reproducible, as evidenced by chromatographic measurements. The pH of the solutions (6.8 ± 0.05 for the bonded diamine phase work and 7.1 ± 0.05 for the C_{12}-dien work) were measured before the acetonitrile was added. When necessary, the mobile phases were adjusted to the above pH values with either dilute acetic acid or aqueous ammonia.

Coating procedures

Bonded diamine and the C_8 and C_{18} columns were equilibrated by flowing the appropriate mobile phase through the column until constant k' values were obtained for a standard test mixture. The column effluent was then recycled back to the reservoir. For the bonded diamine phase, constant k' values could be achieved within 2–3 h at room temperature. For the C_{12}-dien loading, several hours are required to reach equilibrium. To ensure high reproducibility, the initial equilibration process was generally carried out overnight. However, subsequent changes in mobile phases, such as variation of the ammonium acetate concentration, were found to reach equilibrium rapidly.

The adsorption of metal on the bonded diamine and C_{12}-dien-loaded C_{18} and C_8 columns was measured in the following way. The column was first equilibrated with the desired mobile phase containing no metal and was then switched to the same mobile phase containing a known concentration of metal. The metal breakthrough volume was determined by collecting 2.0-ml fractions and measuring the metal content by flame atomic-absorption spectrophotometry on a Varian (Palo Alto, Calif., U.S.A.) Model AA-6 instrument. The data from the atomic-absorption determination for each fraction were compared with the outputs of the mobile phases with and without metal. In this manner a sigmoidal curve was obtained. The amount of metal ion adsorbed in the stationary phase was determined from the volume at which the effluent metal ion concentration was equal to that of the influent. The difference between this point and the breakthrough volume was approximately 1%.

The adsorption of the C_{12}-dien on the C_8 or C_{18} support was determined in a manner similar to that described by Knox and Laird[27]. In this instance the breakthrough volume was determined by collecting 2.0-ml fractions in vials containing chloroform and indigo carmine dye. The breakthrough volume was taken to be the fraction in which the dye was first seen to partition into the aqueous phase.

RESULTS AND DISCUSSION

Metal-loaded bonded diamine phase

As discussed in the Introduction, our first approach to the development of HPLC using transition metals was to employ a chemically bonded phase that would bind strongly to metal ions. Initial results indicated that a bonded propylamine (a monoamine ligand) did not bind metals sufficiently strongly with aqueous mobile phases. Chow and Grushka[18] have recently reported the use of this phase as a support for copper(II); however, they were forced to use non-aqueous mobile phases. The

well known ethylenediamine functionality was employed in subsequent work. This phase has been used by Majors and Hopper[28] as a polar bonded phase and by Becker and Unger for the separation of biopolymers[29,30]. The metal-binding properties of this phase bonded to silica have been described[31].

It was also found desirable to employ weakly binding metals to improve poor mass transfer characteristics. Initial experiments with zinc(II) showed very long retention with the mobile phase conditions for the solutes of this study. More useful k' values were obtained using cadmium(II) as the metal modifier.

Influence of metal ion on retention. We first measured the effect of Cd(II) concentration in the mobile phase [acetonitrile–water (40:60) containing $5 \cdot 10^{-2}$ *M* ammonium acetate] on the retention of sulfa drugs, and the results are shown in Fig. 1. Up to 10^{-3} *M* of Cd(II) the retention generally increases, after which a dramatic decrease in retention is observed, particularly for the most strongly retained species.

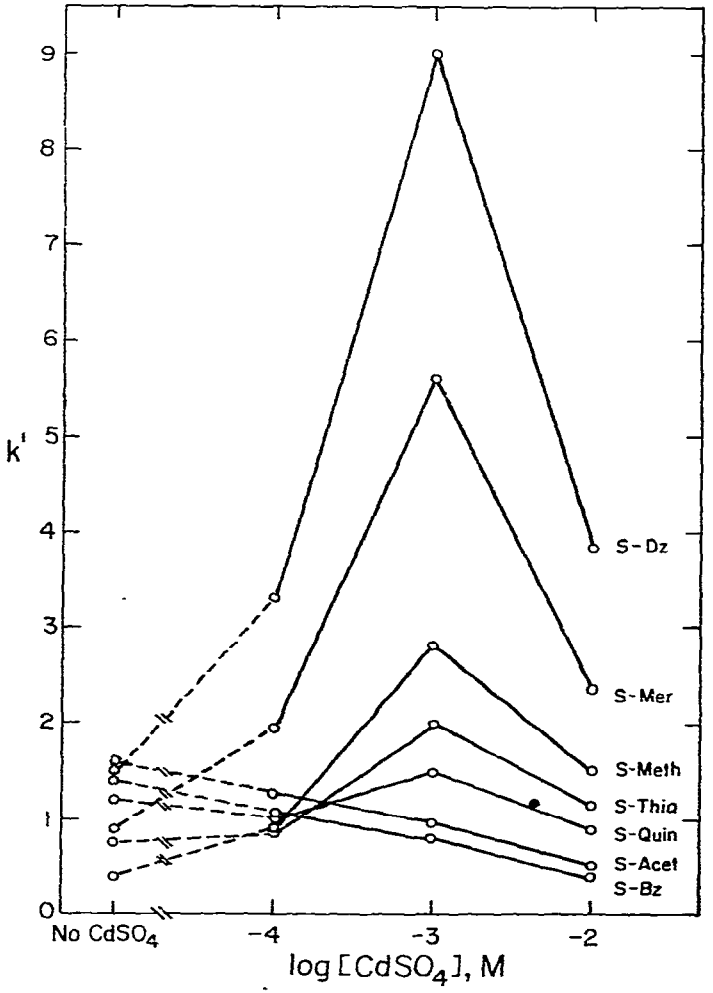


Fig. 1. Influence of $CdSO_4$ concentration on retention of sulfa drugs. Conditions: $5 \cdot 10^{-2}$ *M* ammonium acetate; acetonitrile–water (40:60).

We measured the amount of Cd(II) adsorbed in the bonded diamine in an attempt to understand the behavior in Fig. 1. Using the mobile phase conditions cited above, we found 0.06, 0.24 and 0.51 μmole/m^2 at 10^{-4}, 10^{-3} and 10^{-2} *M* Cd(II), respectively. The increased amount of Cd(II) adsorbed at 10^{-2} *M* relative to 10^{-3} *M* did not agree with the behavior of Fig. 1, so another explanation was sought for the decrease in retention at high Cd(II) concentrations.

At 10^{-2} *M*, the SO_4^{2-} concentration is only 5-fold less than the Ac^- concentration, and we therefore surmised that the SO_4^{2-} may effectively compete (as well as Ac^-) with the negatively charged sulfa drugs for exchange sites on the metal-loaded column. That this is so can be seen in Table II, in which sulfa drug retention at 10^{-2} *M* Cd(II) is compared using sulfate and nitrate salts. We observe that retention is significantly higher with the nitrate salt, indeed even higher than retention at 10^{-3} *M* cadmium sulfate, shown in Fig. 1. This result strongly suggests the importance of electrostatic effects in retention on the Cd(II)-loaded bonded diamine system.

TABLE II

INFLUENCE OF METAL COUNTER ION ON RETENTION OF SULFA DRUGS ON BONDED DIAMINE PHASE

Mobile phase: acetonitrile–water (40:60) containing $5 \cdot 10^{-2}$ *M* ammonium acetate.

Solute	*k'*	
	10^{-2} *M* $CdSO_4$	10^{-2} *M* $Cd(NO_3)_2$
S-Bz	0.39	1.40
S-Acet	0.59	1.36
S-Quin	0.90	3.40
S-Thia	1.15	3.60
S-Meth	1.51	3.80
S-Mer	2.34	6.50
S-Dz	3.84	10.10

Returning to the Cd(II) loading, we note that even at the highest mobile phase concentration of 10^{-2} *M*, the metal surface coverage is low by a factor of almost 5 compared with that which would be expected on the basis of a 1:1 stoichiometric ratio of metal to diamine (bonded diamine coverage = 2.6 μmole/m^2). Most of our subsequent work was performed with a mobile phase metal concentration of 10^{-3} *M*, where good retention was obtained. At this level, the loading is a factor of 10 lower than the expected stoichiometric ratio. These low values not only reflect the high solubility of the cadmium salt in the mobile phase, but probably also the inaccessibility of some of the diamine sites due to attraction of the positively charged amino groups to the negatively charged surface[32]. In this respect we should also note that the amount of Cd(II) retained by exposed silanol groups on the bonded diamine surface is believed to be small. A separate experiment on pure silica gel using the above mobile phase and 10^{-3} *M* cadmium sulfate revealed an adsorption of 0.04 μmole/m^2. This value will, of course, be significantly lower on the bonded diamine phase, as the concentration of accessible silanol groups is much smaller than on pure silica gel. Thus, the bonded phase is the major adsorption site for Cd(II).

Influence of metal ion on selectivity. Fig. 2 illustrates the significant changes in

elution order for the sulfa drugs which occur when 10^{-3} *M* Cd(II) is added to the mobile phase. Note that the ordinate scale on the right-hand side is 5 times greater than that on the left-hand side (the ion-exchange mode) .We first point out that S-Anil maintains a constant and low k' value both with and without added metal (not shown in Fig. 2). S-Anil is un-ionized at the pH used in this work and therefore is expected to be unaffected by added Cd(II). However, the elution order for the Cd(II) bonded diamine phase does not follow directly the relative degree of ionization. Thus, S-Py ($pK_a = 8.4$) elutes after S-Acet ($pK_a = 5.4$). The largest changes in retention upon addition of Cd(II) to the system appear for those substances in which R in Table I consists of pyridine or pyrimidine rings. In any event, selectivity is much different in the metal mode than in the ion-exchange mode.

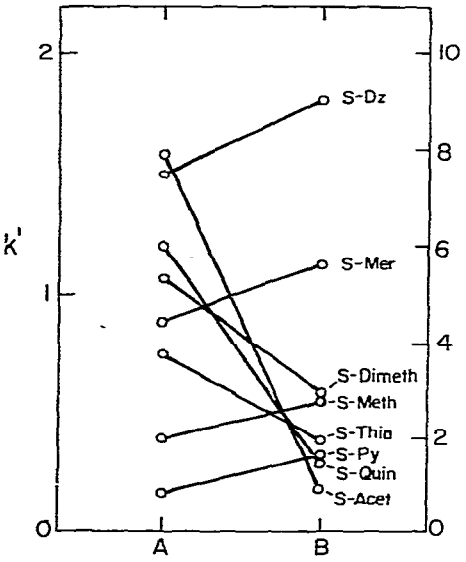


Fig. 2. Comparison of k' for sulfa drugs on the bonded-phase diamine in the absence (A) and presence (B) of 10^{-3} *M* $CdSO_4$. Common conditions: $5 \cdot 10^{-2}$ *M* ammonium acetate; acetonitrile–water (40:60).

In a second study, not presented here, we briefly examined a series of aromatic carboxylic acids. In general, the retention changes upon addition of Cd(II) were much smaller than those in Fig. 2, except for the acids that contain nitrogen heterocyclic groups, *e.g.*, for quinaldic acid $k' = 2.30$ on the bonded diamine phase alone and $k' = 6.63$ upon the addition of 10^{-3} *M* Cd(II). In addition, a small steric effect on selectivity is observed. Thus, the α value for 3,5-dimethylbenzoic acid *versus* 2,5-dimethylbenzoic acid is found to be 0.94 on the bonded diamine acid and 1.15 upon the addition of 10^{-3} *M* Cd(II). We shall contrast this result with the steric selectivity found upon the addition of C_{12}-dien in reversed-phase LC.

Influence of ammonium acetate and acetonitrile concentrations. Fig. 3 illustrates the effects of ammonium acetate concentration on sulfa drug retention with 10^{-3} *M* Cd(II) added to the mobile phase. A general decrease in retention is observed with increasing ammonium acetate concentration; however, there are only minor changes in

elution order and relative retention. Similar behavior has been observed in ion-exchange chromatography[33]. The behavior in Fig. 3 is in agreement with the results in Table II, indicating again the importance of electrostatic interactions. The type and concentration of counter ion evidently allows a convenient control of absolute retention.

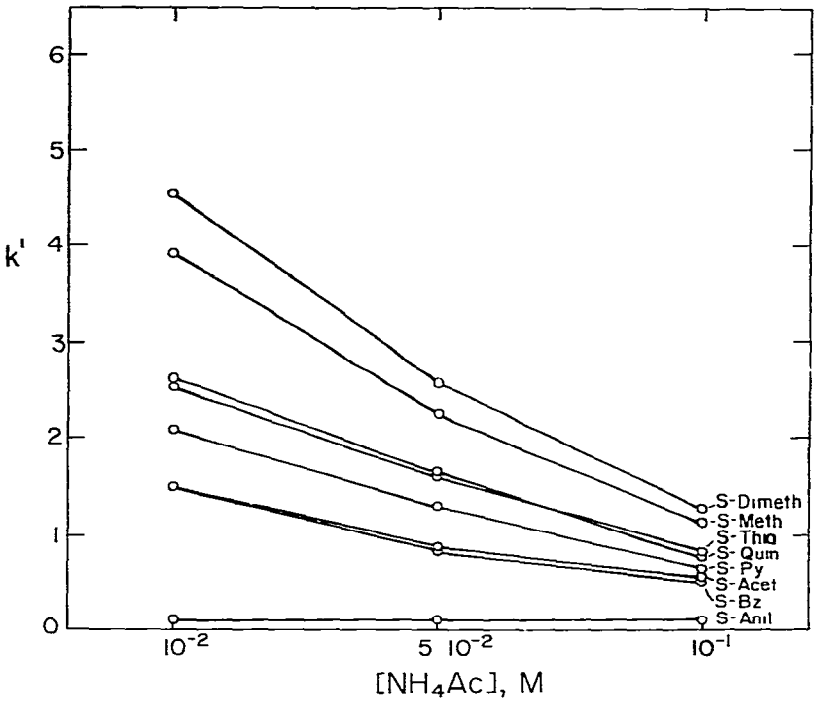


Fig. 3. Influence of ammonium acetate concentration on the retention of sulfa drugs. Conditions: 10^{-3} *M* $CdSO_4$; acetonitrile–water (35:65).

Fig. 4 illustrates some typical results obtained for the influence of acetonitrile concentration on retention for the sulfa drugs. It is interesting that, from 35% down to 5% of acetonitrile in water, a normal-phase retention pattern is generally followed, with decreasing retention with the use of less acetonitrile. Thus, the Cd(II) bonded diamine phase acts as a polar (and/or ionic) bonded phase. Below 5% of acetonitrile there is a marked increase in retention, accompanied by a severe decrease in efficiency.

Stability and reproducibility. We have found that the day-to-day reproducibility of retention is *ca.* 2% under normal operating conditions. With careful attention to the make-up of the mobile phase, the relative standard deviation in retention decreases to *ca.* 1%. Also of interest are the data in Table III, which shows the reproducibility of two bonded diamine preparations using the same batch of porous silica (Nucleosil). As can be seen, excellent reproducibility is achieved. It needs to be pointed out, however, that care must be exercised in maintaining a constant coverage of bonded phase, as the amino phase is known, for example, to be reactive and unstable[34].

Chromatographic separations. Figs. 5 and 6 show some representative separa-

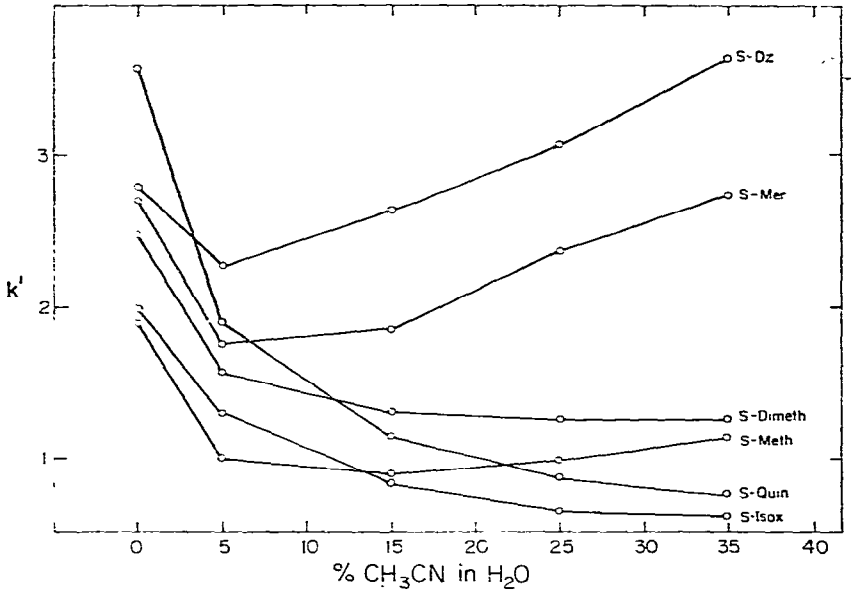


Fig. 4. Influence of acetonitrile concentration on the retention of sulfa drugs. Conditions: 10^{-3} *M* $CdSO_4$; 10^{-1} *M* ammonium acetate.

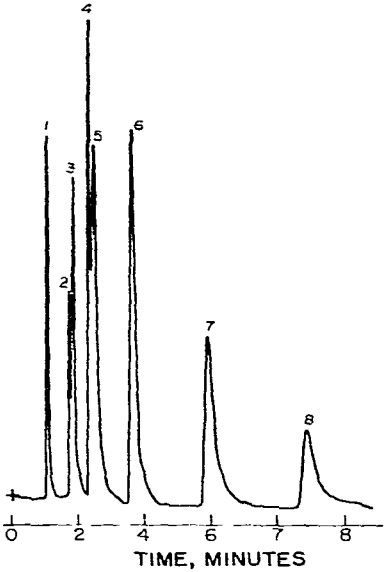
tions on 5-μm bonded diamine phase in the presence of Cd(II), using a column length of 15 cm. Good performance and selectivity are observed in both instances. For example, in Fig. 5 an apparent plate count of 2650 is obtained for S-Mer ($k' = 5.6$) as measured from the peak width at half-height. The true plate count is somewhat lower owing to the tailing of the peaks ($A_s = 2.0$)[35]; however, satisfactory separations are obtained. We believe from separate studies that the tailing results from both thermodynamic and kinetic effects. Finally, the separations were performed at 30°; undoubtedly, increasing the temperature to 50–70° would improve performance and band symmetry.

TABLE III

REPRODUCIBILITY OF COLUMN CONDITIONS: COMPARISON OF TWO DIFFERENT BONDED DIAMINE PHASE PREPARATIONS

Mobile phase: acetonitrile–water (40:60) containing 10^{-3} *M* cadmium sulfate and $5 \cdot 10^{-2}$ *M* ammonium acetate.

Solute	*k'*	
	Prep. 1	*Prep. 2*
S-Anil	0.05	0.06
S-Acet	1.05	0.98
S-Py	1.86	1.88
S-Meth	3.15	3.23
S-Mer	6.31	6.21
S-Dz	10.29	10.10



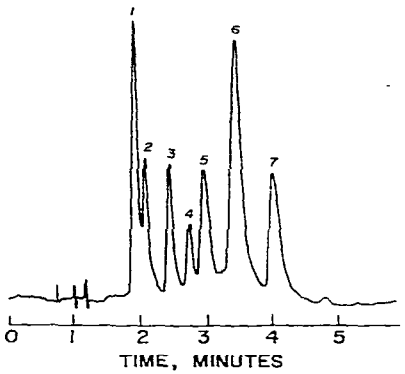


Fig. 5. Separation of sulfa drugs on bonded diamine phase. Conditions: 10^{-3} *M* $CdSO_4$; $5 \cdot 10^{-2}$ *M* ammonium acetate; acetonitrile–water (40:60). Solutes: 1, S-Anil; 2, S-Bz; 3, S-Acet; 4, S-Quin; 5, S-Py; 6, S-Dimeth; 7, S-Mer; 8, S-Dz.

Fig. 6. Separation of dipeptides on bonded diamine phase. Conditions: 10^{-3} *M* $CdSO_4$; $5 \cdot 10^{-2}$ *M* ammonium acetate; acetonitrile –water (35:65). Solutes: 1, Pro–Phe; 2, Pro–Tyr; 3, Phe–Phe; 4, Phe–Tyr; 5, Tyr–Tyr; 6, Trp–Trp; 7, Gly–Tyr.

Metal–C_{12}-dien chelating agent

In recent years there has been great interest in the use of reversed-phase chromatography and it is estimated that at least 60–70% of separations are now performed by reversed-phase methods[36]. This development has resulted from the use of various mobile phase additives (*e.g.*, counter ions for ion-pair chromatography), with one stationary phase thus providing a broad range of separations. With this in mind, we have combined the high selectivity of metal ions with the bonded hydrocarbon phases by using a relatively hydrophobic species that is capable of acting as a chelating agent for metal ions.

We believe this approach may offer certain advantages for analytical separations over that discussed in the previous section (*i.e.*, bonded diamine phase). Firstly, the synthesis of tailor-made binding groups necessary for the control of the environment around a metal is generally found to be easier in bulk solution compared with modification on the silica surface. Secondly, as we have noted, the stability of the polar amino-bonded phases can in some instances be of concern. Thirdly, it is simple to change from straight reversed-phase chromatography to the use of metal-loaded systems.

Initial attempts with long-chain primary amines indicated that their metal-binding properties were not sufficiently strong for successful chromatographic operation. We next tested the C_{12}-dien chelating agent, which is known to bind metals very strongly[37] and is commercially available. Use of this additive was found to be remarkably successful, as evidenced by the separation of sulfa drugs illustrated in Fig. 7 using

a commercial reversed-phase column. This separation gives a reduced plate height of *ca.* 7 at a reduced velocity of *ca.* 12 with an asymmetry factor of 1.2[35] for S-Meth with a k' of 4.90. We now examine the characteristics of this approach and the reasons that high performances are achieved. In all instances to date, we have used Zn(II) as the metal ion chelated to the C_{12}-dien.

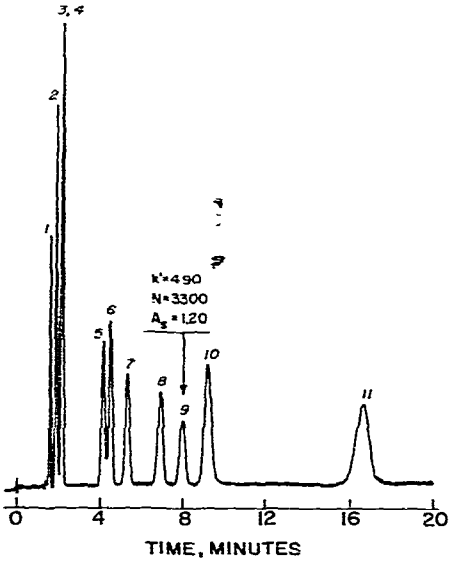


Fig. 7. Separation of sulfa drugs by C_{12}-dien–Zn(II) chromatography. Conditions: 10^{-3} *M* $ZnSO_4$; 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65); Merck C_8 column. Solutes: 1, S-Acet; 2, S-Anil; 3, S-Bz; 4, S-Isox; 5, S-Quin; 6, S-Py; 7, S-Dz; 8, S-Mer; 9, S-Meth; 10, S-Thia; 11, S-Dimeth.

Influence of metal ion on retention and selectivity. That the metal ion plays a significant role on selectivity can be seen in Fig. 8, which compares sulfa drug retention on the C_{12}-dien system with and without Zn(II). All conditions in both experiments were the same, except that an approximately equimolar amount of Zn(II) (to C_{12}-dien, 10^{-3} *M*) was added in one instance. Note that the ordinate scale on the right-hand side of Fig. 8 is 20-fold greater than that on the left-hand side.

We first note the remarkable changes in elution order that occur upon the addition of the metal to the mobile phase. As a general rule, those substances with heterocyclic nitrogen or sulfur atoms in the aromatic ring attached to the sulfonamide group are most influenced by the Zn(II) complex species. Thus, S-Dz, S-Mer, S-Thia and S-Dimeth show significant increases in retention upon addition of Zn(II) to the mobile phase. On the other hand, the retentions of S-Bz, S-Acet are not significantly affected by the metal. As with the bonded diamine phase, S-Anil, which is uncharged at pH 7, has a retention independent of the added Zn(II). We also note that the elution order is remarkably different from that obtained on the Cd(II) bonded diamine phase, as shown in Fig. 2. As we shall see later, this is undoubtedly related in part to the hydrophobic nature of the stationary phase in the C_{12}-dien case relative to the hydrophilic phase in the bonded diamine case.

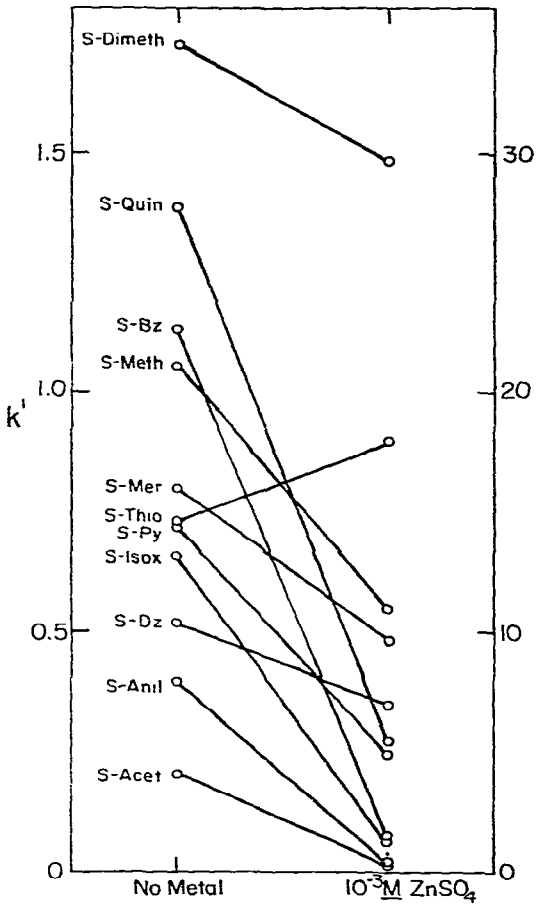


Fig. 8. Comparison of k' for sulfa drugs in the absence and presence of 10^{-3} M $ZnSO_4$. Common conditions: 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65).

A question may arise as to whether C_{12}-dien can be considered a typical cationic counter ion for reversed-phase ion-pair chromatography, as the potential for hydrogen bonding, as well as simple electrostatic attraction, may exist. Consequently, we selected a more typical counter ion for reversed-phase ion-pair chromatography, namely tetrapentylammonium (TPA), for selectivity comparisons with C_{12}-dien–Zn(II), and the results are shown in Fig. 9.

We again find remarkable selectivity differences between typical reversed-phase ion-pair chromatography and the C_{12}-dien–Zn(II) mode. Thus, the C_{12}-dien–Zn(II) clearly provides new phenomena for control of relative retention beyond the electrostatic effects of ion pairing and hydrophobic–hydrophilic characteristics of the sample substances to be separated.

Table IV presents some relative retention results on monofunctional acids, which provide insight into the selectivity possible with the C_{12}-dien–Zn(II) system. We first note that for the same mobile phase, the selectivity differences between C_{12}-dien and TPA are in general not great, relative to those found on C_{12}-dien–Zn(II).

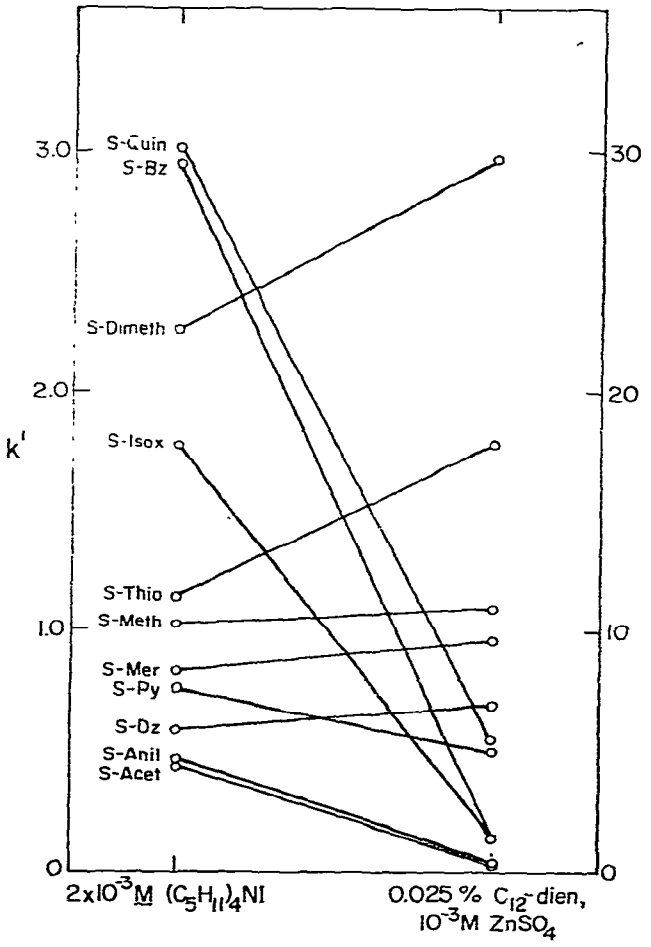


Fig. 9. Comparison of ion-pair with C_{12}-dien–Zn(II) chromatography. Common conditions: 1% ammonium acetate; acetonitrile–water (30:70).

The large differences in functional group selectivity are clearly seen in the relative retention of *p*-toluic acid *versus p*-toluenesulfonic acid, where an α value of 6.45 is obtained on the C_{12}-dien–Zn(II) system compared with *ca.* 1.2 for TPA and C_{12}-dien. Sulfonic acids are known to be weaker ligands for metals than carboxylic acids[38] and this may in part explain the behavior. We also find steric effects to be more significant for C_{12}-dien–Zn(II) relative to reversed-phase ion-pair chromatography, as shown in Table IV, particularly for 2,6- *versus* 3,5-dimethylbenzoic acid. This result can be contrasted with the poor steric selectivity found in the Cd(II) bonded diamine system. Finally, hydrophobic selectivity appears to be similar for reversed-phase ion-pair chromatography and C_{12}-dien–Zn(II), as can be seen in the relative retention of the carboxylic acids containing *p*-CH_3, *p*-C_2H_5, *p*-iso-C_3H_7 and *p-tert.*-C_4H_9 groups. Obviously, other structure–selectivity studies are necessary, but the C_{12}-dien–Zn(II) appears to provide good steric and functional group selectivity without loss of hydrophobic selectivity.

TABLE IV

RETENTION OF AROMATIC CARBOXYLIC ACIDS: COMPARISON OF ION PAIR WITH C_{12}-dien–Zn(II) CHROMATOGRAPHY

Mobile phase: acetonitrile–water (30:70) containing 1% ammonium acetate. Merck C_8 column.

Solute	*0.025% C_{12}-dien*		*$2 \cdot 10^{-3}$ M TPA*		*0.025% C_{12}-dien–10^{-3} M Zn(II)*	
	k'	*α*	*k'*	*α*	*k'*	*α*
p-Toluenesulfonic acid	0.87		1.28		1.24	
		1.24		1.20		6.45
p-Toluic acid	1.08		1.53		8.00	
2,6-Dimethylbenzoic acid	0.54		0.90		2.02	
		3.52		2.78		7.53
3,5-Dimethylbenzoic acid	1.90		2.50		15.22	
2,4-Dichlorobenzoic acid	1.58		2.90		6.21	
		1.04		1.02		1.55
p-Chlorobenzoic acid	1.64		2.97		9.62	
p-Toluic acid	1.08		1.53		8.00	
		1.93		1.84		2.01
p-Ethylbenzoic acid	2.08		2.82		16.10	
		1.78		1.74		2.13
p-Isopropylbenzoic acid	3.71		4.90		34.0	
		1.57		1.56		1.61
p-tert.-Butylbenzoic acid	5.83		7.66		54.6	

Reproducibility of the C_{12}-dien–Zn(II) system. We examined the reproducibility of this system under several sets of conditions. When thermostating the columns and taking care in the make-up of the mobile phase, the relative standard deviation in *k'* is *ca.* 1% on the Merck C_8 column. This value is normally found in HPLC and thus indicates that there are no fundamental problems in the make-up of the mobile phase.

Another important reproducibility study deals with the question of washing and re-loading of the reversed-phase column with the mobile phase containing C_{12}-dien–Zn(II). A C_8 column was first equilibrated with the mobile phase and the retentions of the sulfa drugs were measured. The results are shown in the *Before wash* column in Table V. The C_{12}-dien–Zn(II) was removed from the column by washing successively with 100 ml each of 1% aqueous acetate buffer (pH 4), methanol and acetonitrile. Normal reversed-phase chromatography could then be performed. Subsequently, the column was re-loaded with the C_{12}-dien–Zn(II) mobile phase and the retentions of the sulfa drug mixture were measured. The results are given in the *After wash* column in Table V. As can be seen, excellent reproducibility is achieved. These results indicate that the bonded reversed-phase column can be used successfully in several modes.

The next question deals with column-to-column reproducibility. Table VI shows the results obtained when two Merck C_8 columns were loaded with the C_{12}-dien–Zn(II) mobile phase and the retentions of sulfa drugs were measured. As can be seen, the column-to-column reproducibility is acceptable.

As might be expected, the retention results are a function of the bonded stationary phase selected. Table VII presents a comparison of *k'* values on the Merck C_8 column with those of a Waters μBondapak C_{18} (coverage *ca.* 11%). In general, reten-

TABLE V

WASH AND RELOAD REPRODUCIBILITY

Mobile phase: acetonitrile–water (35:65) containing 10^{-3} *M* zinc sulfate, 0.025% C_{12}-dien and 1% ammonium acetate. Merck C_8 column.

Solute	*k'*	
	Before wash	*After wash*
S-Acet	0.19	0.18
S-Isox	0.58	0.58
S-Bz	0.62	0.62
S-Quin	2.16	2.18
S-Py	2.40	2.39
S-Dz	3.00	3.01
S-Mer	4.21	4.21
S-Meth	4.96	4.95
S-Thia	6.54	6.55
S-Dimeth	11.67	11.61

tion is 1.5–2-fold higher on the C_{18} column, with the elution order remaining roughly the same on the two columns. One striking variation, however, is S-Thia, which has roughly a 3-fold greater retention on the C_{18} column. Metal–C_{12}-dien loading experiments revealed that a significantly greater amount of the chelating agent was adsorbed on the μBondapak column, *viz.*, 5.5 mg/g on Merck C_8 and 22 mg/g on μBondapak under the mobile phase conditions in Table VII. We note that equimolar amounts of Zn(II) and C_{12}-dien were found to be adsorbed on both stationary phases at equilibrium, indicating that the zinc is fully complexed with the chelating agent. We shall consider this point later.

Finally, we examined briefly the stability of the C_{12}-dien–Zn(II) system to reaction with carbonyl substances (*e.g.*, formation of Schiff base products). We injected relatively large amounts (200 μg) of benzaldehyde and acetophenone into the

TABLE VI

RETENTION REPRODUCIBILITY FROM COLUMN TO COLUMN (C_{12}-dien–METAL)

Mobile phase: acetonitrile–water (35:65) containing 10^{-3} *M* zinc sulfate,[1] 0.025% C_{12}-dien and 1% ammonium acetate. Merck C_8 column.

Solute	*k'*	
	Column 1	*Column 2*
S-Acet	0.19	0.18
S-Anil	0.39	0.36
S-Isox	0.58	0.54
S-Bz	0.62	0.58
S-Quin	2.16	2.10
S-Py	2.40	2.19
S-Dz	3.00	2.82
S-Mer	4.21	3.89
S-Meth	4.96	4.00
S-Thia	6.54	6.26
S-Dimeth	11.67	10.70

TABLE VII

COMPARISON OF C_8 AND C_{18} BONDED-PHASE COLUMNS

Mobile phase: acetonitrile–water (35:65) containing 10^{-3} *M* zinc sulfate, 0.025% C_{12}-dien and 1% ammonium acetate. Merck C_8 and Waters C_{18} columns.

Solute	*k'*	
	C_8	C_{18}
S-Acet	0.15	0.22
S-Anil	0.35	0.44
S-Bz	0.51	0.98
S-Isox	0.53	0.91
S-Quin	1.95	3.34
S-Py	2.23	3.90
S-Dz	2.81	5.40
S-Mer	3.87	6.90
S-Meth	4.60	6.12
S-Thia	5.62	17.93
S-Dimeth	10.86	13.60

column. While both substances can react with C_{12}-dien, we found no change in *k'* and α values for the sulfa drug test mixture. This result means that even if the additive to the mobile phase is reactive, the column is able to regenerate itself fairly rapidly. This is a clear advantage over the bonded diamine phase.

In summary, we have seen that the C_{12}-dien–Zn(II) phase is a reproducible and

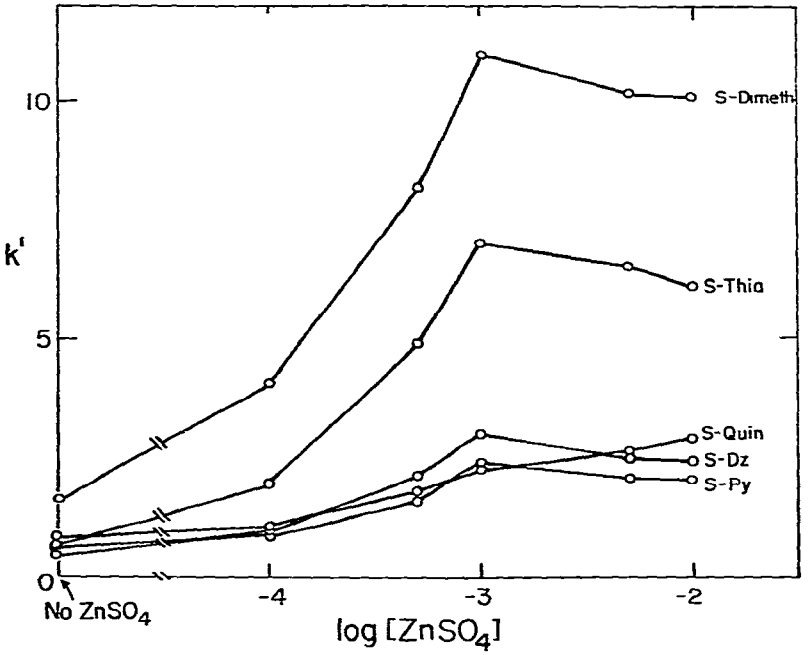


Fig. 10. Influence of $ZnSO_4$ concentration (*M*) on retention of sulfa drugs. Conditions: 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65).

stable chromatographic system. We next examined some of the parameters controlling retention, in order to be able to optimize separations.

Influence of Zn(II) concentration at constant C_{12}-dien concentration. In Fig. 10, k' values for the sulfa drugs are plotted against concentration of Zn(II) while maintaining the C_{12}-dien concentration at 0.025% with a mobile phase consisting of acetonitrile–water (35:65) containing 1% of ammonium acetate. Generally, retention increases up to a 1:1 stoichiometric ratio, at which point small decreases in k' occur. Poor efficiency was also observed when the Zn(II) concentration exceeded a 1:1 ratio to the chelating agent. For all of this work, we consequently operated at roughly a 1:1 stoichiometric ratio, 0.025% C_{12}-dien and 10^{-3} *M* Zn(II).

Influence of mobile phase composition on retention. Fig. 11 shows the effect of ammonium acetate concentration on retention of the sulfa drugs. The general effect, as with the bonded diamine phase, is a decrease in retention with increasing ammonium acetate concentration, with little or no effect on selectivity. Hence the ammonium acetate concentration can be used to control retention, *e.g.*, in gradient elution. The results in Fig. 11 again reveal the importance of electrostatic effects on retention.

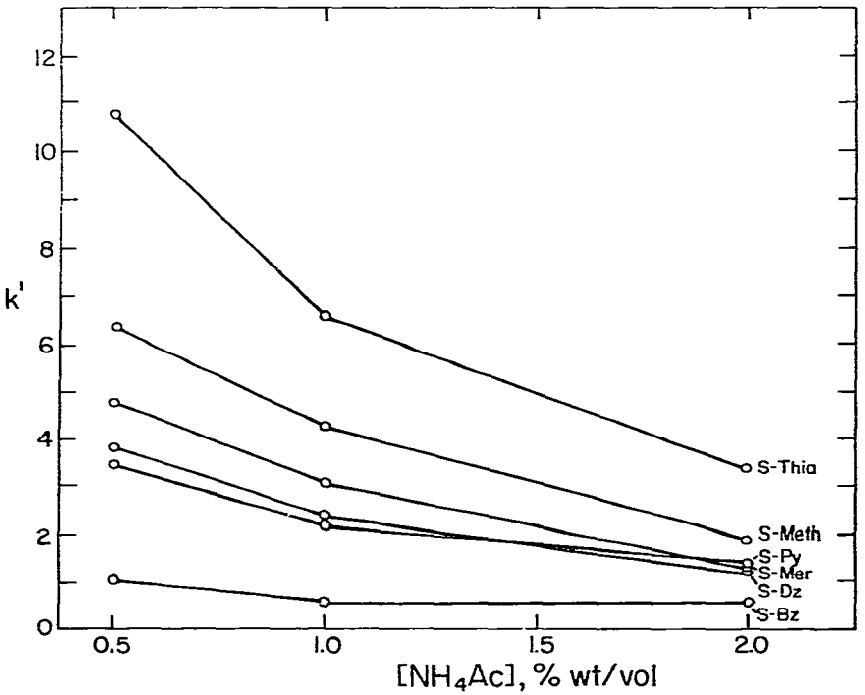


Fig. 11. Influence of ammonium acetate concentration on retention of sulfa drugs. Conditions: 10^{-3} *M* $ZnSO_4$; 0.025% C_{12}-dien; acetonitrile–water (35:65).

Considering the pK_a values of the sulfa drugs in Table I, we would expect that pH could sharply influence retention, and Fig. 12 indicates that this is so. Thus, S-Thia, with a pK_a of 7.6, has the greatest change in retention from pH 6.8 to 7.8, whereas the retentions of substances such as S-Py (pK_a = 8.4) do not change greatly

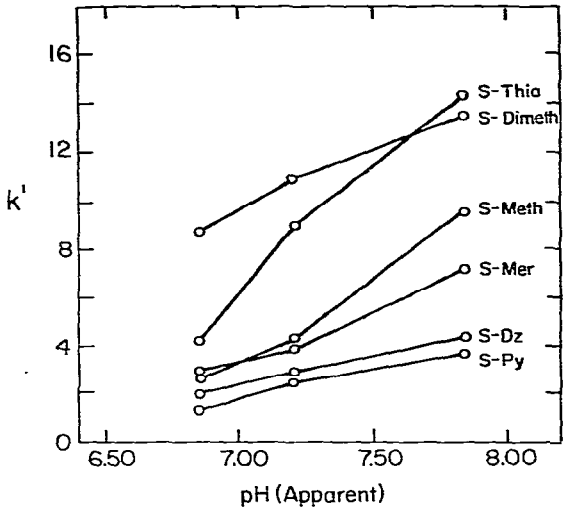


Fig. 12. Influence of pH on retention of sulfa drugs. Conditions: 10^{-3} *M* $ZnSO_4$; 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65).

over this range. Thus, as expected, pH can be used to control relative retention through the extent to which a substance is ionized under the experimental conditions.

Finally, Table VIII shows the influence of acetonitrile concentration on retention. In contrast to the bonded diamine phase, we now find that k' increases with decrease in acetonitrile concentration, as expected in reversed-phase chromatography. We find an increase of roughly 3-fold for only a 5% decrease in acetonitrile concentration. Knox and Laird[27] found a similar dependence of k' on organic modifier concentration in soap chromatography.

Chromatographic separations. Fig. 7 has already demonstrated the high performances that are possible with the C_{12}-dien–Zn(II) system. Other examples of aro-

TABLE VIII

INFLUENCE OF ACETONITRILE CONCENTRATION ON RETENTION

Mobile phase: acetonitrile–water containing 10^{-3} *M* zinc sulfate, 0.025% C_{12}-dien and 1% ammonium acetate. Merck C_8 column.

Solute	*Acetonitrile concentration (%, v/v)*	
	30	*35*
S-Acet	0.35	0.18
S-Anil	0.43	0.36
S-Isox	1.40	0.54
S-Bz	1.47	0.58
S-Py	5.04	2.19
S-Quin	5.59	2.10
S-Dz	7.01	2.82
S-Mer	9.63	3.89
S-Meth	10.96	4.00
S-Thia	17.91	6.26
S-Dimeth	29.7	10.70

matic acids, dansylamino acids and dipeptides are shown in Figs. 13, 14 and 15, respectively. In all instances, excellent efficiency and band symmetry are observed. For example, in Fig. 14, the k' value for dansyltryptophan is 24 with a plate count of 2500 and an asymmetry factor of 1.2. Note also that 2% of ammonium acetate is used to obtain reasonable retention. For all 20 common amino acids, a gradient in ammonium acetate concentration would be necessary. With respect to the dipeptides (Fig. 15), the band asymmetry of tryptophanylphenylalanine and tryptophanyltryptophan may result from impurities. Alternatively, a higher temperature may improve peak performance. Further study of these substances is therefore desirable. In summary, the C_{12}-dien–Zn(II) system generally leads to high selectivity and high performance.

Outer-sphere complexation. In this study, the viability of HPLC utilizing metal ion centers complexed to chelating agents has been demonstrated. The high selectivity of metal ions can be exploited without large decreases in chromatographic performance. An important question is the origin of the high performance that has been observed.

In utilizing a metal center as a chromatographic exchange site, good column efficiencies can be achieved only if the rates of formation and dissociation of metal–solute complexes are rapid.

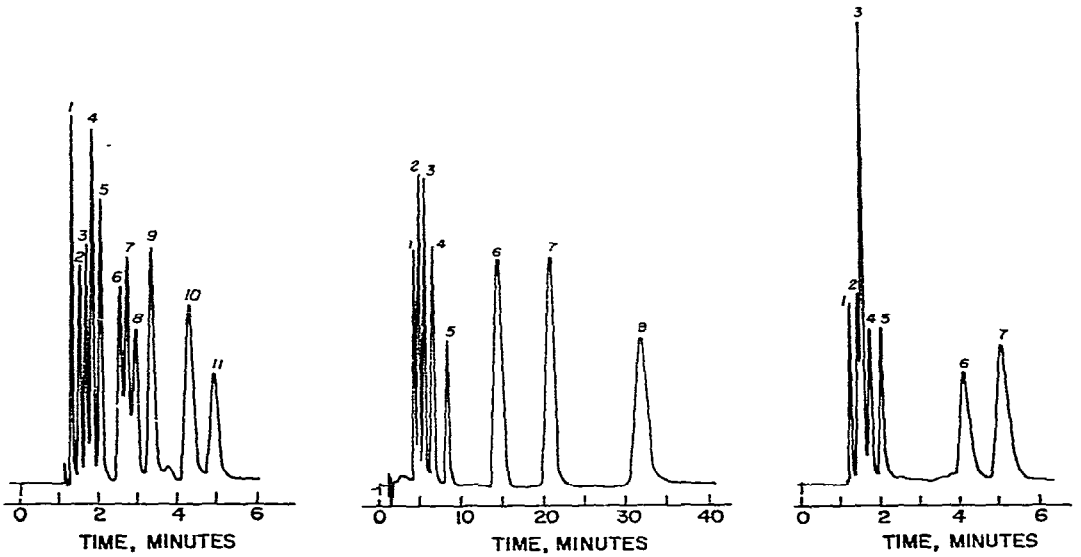


Fig. 13. Separation of aromatic acids by C_{12}-dien–Zn(II) chromatography. Conditions: 10^{-3} *M* $ZnSO_4$; 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65). Solutes: 1, sulfanilic; 2, *p*-aminophenylacetic; 3, *p*-toluenesulfonic; 4, *p*-aminobenzoic; 5, 2,6-dimethylbenzoic; 6, phenylacetic; 7, benzoic; 8, *o*-toluic; 9, 2,4-dichlorobenzoic; 10, *p*-toluic; 11, *p*-naphthoic acid.

Fig. 14. Separation of dansylamino acids by C_{12}-dien–Zn(II) chromatography. Conditions: 10^{-3} *M* $ZnSO_4$; 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65). Solutes: 1, glutamic acid; 2, γ-aminobutyric acid; 3, threonine; 4, serine; 5, α-aminobutyric acid; 6, norvaline; 7, leucine; 8, tryptophan.

Fig. 15. Separation of dipeptides by C_{12}-dien–Zn(II) chromatography. Conditions: 10^{-3} *M* $ZnSO_4$; 0.025% C_{12}-dien; 1% ammonium acetate; acetonitrile–water (35:65). Solutes: 1, Pro–Tyr; 2, Pro–Phe; 3, Pro–Trp; 4, Phe–Pro; 5, Tyr–Phe; 6, Trp–Phe; 7, Trp–Trp.

It is well known that two classes of metal–solute complexes exist. In inner-sphere complexes, the metal and solute are directly linked, whereas in outer-sphere complexes the metal–solute association occurs without disruption of the inner co-ordination sphere of the metal center. As outer-sphere complex formation and dissociation are invariably fast processes[13,39,40], conditions which lead to the existence of such species are desirable for the achievement of high performance.

There is a means of establishing the existence of outer-sphere complexes of the solutes with C_{12}-dien–Zn(II) under the chromatographic conditions, as follows. The formation of an inner-sphere complex between pyridine-2-azo-*p*-dimethylaniline (PADA) and a zinc center (ZnL_n) is accompanied by a 90-nm shift in the absorption maximum (Fig. 16), which corresponds to a color change from yellow to pink[41]. PADA (final concentration $1.4 \cdot 10^{-5}$ *M*) was added to each of the solutions listed in Table IX. The C_{12}-dien and ammonium acetate buffer do not prevent PADA complexation. However, in the presence of the C_{12}-dien, PADA is displaced from the inner coordination sphere by acetonitrile at a concentration $>$ *ca.* 23% (v/v). Thus, under the chromatographic conditions, acetonitrile blocks inner-sphere coordination of PADA. It is interesting that a higher methanol content (*ca.* 38%) is required in order to displace PADA from the C_{12}-dien–Zn(II) center. This result is in the expected direction, as methanol is a weaker ligand than acetonitrile[38]. We also note that two strongly retained solutes, Trp–Trp and S-Dimeth, are not able to displace PADA at higher concentrations than are normally encountered in chromatography.

Consider now the results in Table IX in the context of the chromatographic experiments. Firstly, the association constant for complexation between Zn(II) and the dien functionality is sufficiently high[37] that, at a 1:1 stoichiometric ratio, we can

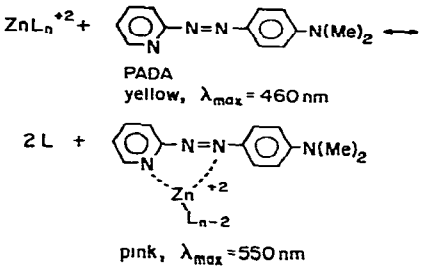


Fig. 16. Inner-sphere complex formation of PADA with Zn(II) complexes.

TABLE IX

ANALYSIS OF INNER- AND OUTER-SPHERE COMPLEXES USING PADA IN THE PRESENCE OF C_{12}-dien

Common conditions: PADA = $1.4 \cdot 10^{-5}$ *M*; 1% (w/v) ammonium acetate.

Solution	*Zn(II) (M)*	*C_{12}-dien (%, w/v)*	*CH_3CN (%, v/v)*	*CH_3OH (%, v/v)*	*Color*
A	—	0.025	—	—	Yellow
B	$1.0 \cdot 10^{-3}$	0.025	—	—	Pink
C	$1.0 \cdot 10^{-3}$	0.025	23*	—	Pink → yellow
D	$1.0 \cdot 10^{-3}$	0.025	—	38*	Pink → yellow

* Minimum amount of organic modifier needed to displace PADA from metal.

consider each Zn(II) ion bonded to the chelating agent. It is further known that, in this complex, Zn(II) is tetracoordinated, forming a distorted tetrahedral configuration[42]. Under the conditions of our experiments (*i.e.*, 35% acetonitrile), we can conclude that the fourth coordination site is occupied by an acetonitrile molecule; the inner-sphere structure of the complex is shown in Fig. 17.

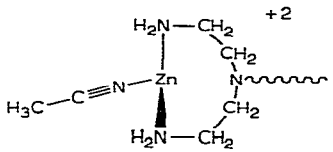


Fig. 17. Four-coordinate $Zn(C_{12}\text{-dien})(CH_3CN)^{2+}$ complex.

Association must occur in the outer-coordination sphere for the substances we have studied, as they are unable to displace PADA from association with Zn(II). As we have seen, outer-sphere complexation offers not only rapid exchange, but also high selectivity. Outer-sphere complexes are known to have significantly different association constants[43,44]. We believe that the use of outer-sphere complexation for the achievement of high performance and high selectivity (*e.g.*, functional group, steric) can be important in HPLC.

Direct addition of metal to the mobile phase. As we have already mentioned, another promising approach to the use of transition metals in HPLC is via their direct addition to the mobile phase. In the work reported to date[18], the metal ion has formed a complex with a solute species, thus enhancing its hydrophilicity and reducing its retention in reversed-phase LC. The metal ion may also act as a species in which ion-pair or ligand association can occur with solute substances, thus enhancing hydrophobicity (by charge neutralization) and retention in reversed-phase LC.

In a very real sense this latter approach is simply an extension of the work on C_{12}-dien–Zn(II). The basic difference is that the environment around the metal ion is fully controlled by the components in the mobile phase, rather than by a chelating agent. From this point of view, the chelation approach represents a more powerful technique in that selectivity can be tailor-made. Nevertheless, addition of metal ion to the mobile phase can be a useful approach, as it represents a fundamentally simple and rapid means of changing selectivity. We have carried out a few studies using this approach, as described below.

Table X presents results using PADA as a means of determining inner- *versus* outer-sphere complexation when no C_{12}-dien is present. When no organic modifier is present, the color of the solution is pink indicating that PADA is in the inner coordination sphere of Zn(II). In this instance we need 30% acetonitrile or 55% methanol to displace the PADA. The larger values compared with when C_{12}-dien is present (*cf.*, Table IX) are to be expected for the reason that at least four and perhaps up to six sites[42] are available for coordination of acetonitrile or methanol to Zn(II). Again, long-retained sulfa drugs and dipeptides were unable to displace PADA from the inner coordination sphere.

In the work we have performed, we have found 20% acetonitrile to be necessary for successful retention when a metal is added to the mobile phase. From the ratio of

TABLE X

ANALYSIS OF INNER- AND OUTER-SPHERE COMPLEXES USING PADA IN THE ABSENCE OF C_{12}-dien

Common conditions: PADA = $1.4 \cdot 10^{-5}$ *M*; 1% (w/v) ammonium acetate; [Zn(II)] = $1 \cdot 10^{-3}$ *M*.

Solution	*CH_3CN (%, v/v)*	*CH_3OH (%, v/v)*	*Color*
A	—	—	Pink
B	30*	—	Pink → yellow
C	—	55*	Pink → yellow

* Minimum amount of organic modifier needed to displace PADA from metal.

concentrations of, for example, sulfa drugs to PADA which did not displace the PADA from the inner coordination sphere, we estimate that the complexation constants for the sulfa drugs are at least 10^{-2} times lower than that for PADA[41]. As a consequence, it is likely that, at 20% acetonitrile, outer-sphere complexation occurs for the sulfa drugs and dipeptides interacting with Zn(II), with the major portion of the inner coordination sphere taken up by acetonitrile molecules.

Table XI presents retention results for sulfa drugs and dipeptides under various modes of operation. Addition of metal to the mobile phase was effected with zinc nitrate and nickel nitrate at the 10^{-3} *M* level.

TABLE XI

COMPARISON OF REVERSED-PHASE, METAL ION IN THE MOBILE PHASE, AND C_{12}-dien–Zn(II) CHROMATOGRAPHY

Common conditions: 1% ammonium acetate; Merck C_8 column.

Solute	*CH_3CN–H_2O (20:80)*	*10^{-3} M $Zn(NO_3)_2$, CH_3CN–H_2O (20:80)*	*10^{-3} M $Ni(NO_3)_2$, CH_3CN–H_2O (20:80)*	*10^{-3} M $ZnSO_4$, 0.025% C_{12}-dien, CH_3CN–H_2O (30:70)*
S-Anil	0.53	0.55	0.54	0.43
S-Dz	0.54	0.64	1.74	7.01
S-Bz	0.69	0.63	0.71	1.47
S-Mer	1.17	1.47	3.30	9.63
S-Py	1.38	1.61	2.71	5.04
S-Meth	2.20	2.32	4.75	10.96
S-Dimeth	2.56	3.48	11.14	29.70
Tyr–Phe	0.61	0.65	0.93	2.57
Trp–Phe	3.17	3.53	5.85	10.38
Trp–Trp	4.12	4.54	7.60	15.09

We first note that, when metal is added to the mobile phase, retention for both classes of substances increases relative to the straight reversed-phase system. As previously, S-Anil, which is uncharged, is unaffected by the metal. We also find that retention is greater with Ni(II). What is most interesting is that, especially for the sulfa drugs, the selectivity is very different when the C_{12}-dien–Zn(II) system is used relative to direct addition of metal in the mobile phase. Clearly, the coordination environment around the metals affects retention.

We have found column performances to be similar when the metal is added

directly to the mobile phase to those found for the straight reversed-phase system. We consider that outer-sphere complexation is occurring, resulting in rapid mass transfer kinetics. Thus, we can conclude that direct addition of a metal to the mobile phase is a useful and simple technique. For specific and highly selective separations, the addition of chelating agents would appear to be the more effective approach, as the environment around the metal can then be directly controlled.

ADDENDUM

It has been pointed out that Cd(II) readily forms complexes with chloride in aqueous media and that chloride may thus constitute a potential interference in the Cd(II)-bonded diamine case. Of course, one must always be aware of potential interferences, for example, SO_4^{2-}, as illustrated in our paper. It is likely that interferences based on complex formation with the metal ion will be less prevalent in the case of outer sphere complexation, as the metal will most likely be preferentially complexed with the chelating species. For example, the complexation constant for C_{12}-dien with Zn(II) $> 10^8$. This represents a further advantage of the metal chelate approach relative to simple addition of metal to the mobile phase.

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