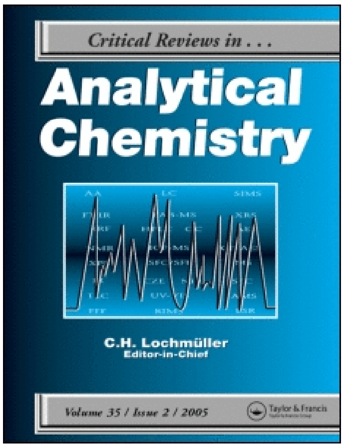


Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Mobile Phase Additives vs. Bonded Phases for HPLC

C. H. Lochmüller[a]; Helen H. Hangac[ab]
[a] Department of Chemistry, Duke University, Durham, NC [b] Pfizer Central Research, Groton, CT

To cite this Article Lochmüller, C. H. and Hangac, Helen H.(1997) 'Mobile Phase Additives vs. Bonded Phases for HPLC', Critical Reviews in Analytical Chemistry, 27: 1, 27 — 48

To link to this Article: DOI: 10.1080/10408349708050579

URL: http://dx.doi.org/10.1080/10408349708050579

Critical Reviews in Analytical Chemistry, 27(1):27–48 (1997)

Mobile Phase Additives Vs. Bonded Phases for HPLC

*C. H. Lochmüller and Helen H. Hangac**
Department of Chemistry, Duke University, Durham, NC 27708

* Current address: Pfizer Central Research, Eastern Point Road, Groton, CT 06340

ABSTRACT: It is likely that 90% of all small-molecule high-performance liquid chromatography (HPLC) separations can be done with a hydrocarbon-bonded phase of C4, C8, or C18 length using various mobile phases. The remaining 10% of the cases are more challenging. One solution is to follow the historical path of gas-liquid chromatography and create more bonded phases. The second and more easily done is to add components to the mobile phase that interact selectively with the molecules of interest and change RPLC selectivity and retention. This paper begins with a generalized discussion of additive strategy and mechanism and then leads to a study of nonionic but coordinatively unsaturated (β-diketonates as additives which serves as an example of the connection between molecular chemistry and chromatographic selectivity and a review of other work done. A system of coordinatively unsaturated metal β-diketonates was investigated as mobile phase additives for HPLC. Unlike previous ionic, metal dopants, these neutral complexes combine reasonable elution times, high chromatographic efficiency, and enhanced chromatographic selectivity for polar compounds in normal and reversed-phase modes. The influences of mobile-phase composition, stationary-phase substrate, and metal on solute retention behavior were examined for the metal-dipivaloylmethane additive systems.

KEY WORDS: mobile phase additives, HPLC, RPLC, metal-dipivaloymethane additive systems.

I. INTRODUCTION

The history of chromatography is one in which the first sense of elation is in the discovery of a new variation, the first separation, and then the attempts by others to apply that to their own unsolved problems. Over the last century, the use of chromatography as a general tool for chemical separations has come from the early work in chemotaxonomy by Tswett at Tartu University in Estonia, through its adoption by the dye industry of Europe, the work of Martin and coworkers on the sequencing of wool, the invention of gas chromatography by Martin and James, to its current application across the interface between chemistry and biology. In the 1960s, by which time column and thin-layer chromatography had become routine, major chemistry departments in the U.S. were still advertising the availability of a single gas chromatograph for general research use. By that time, commercial suppliers of gas-liquid chromatographic columns and phases offered thousands of different stationary phases for use and little booklets advising on their choice. Modern high-performance liquid chromatography (HPLC) awaited the realization that if packings could be made smaller, the resistance to mass trans-

1040-8347/97/$.50

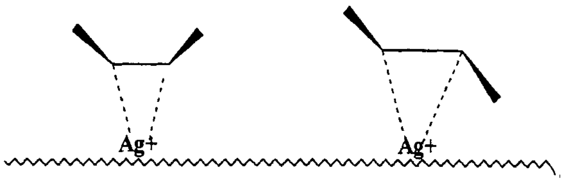
fer arising in the condensed mobile phase would be greatly reduced, and the rest is history.

The earliest HPLC was done with silica as the stationary phase. Later, the same silylation chemistry that had been used for years to treat textile fiber, window glass, etc. was applied to chemical modification of the silica surface. The current dominance of reversed-phase HPLC using water and a modified water mobile phase was not foreseen, and early HPLC instrumentation provided explosion-proof housings for mobile phases. However, the number of mobile phases and stationary phases has not grown as it did in gas chromatography, and today most separations are done using 4-, 8-, or 18-carbon-chain hydrocarbon stationary phases and mixtures of water, methanol, acetonitrile, and tetrahydrofuran. The mobile-phase choices are based on transparency in the ultraviolet (most common detector) and the commercial availability of a relatively pure form. As we discuss mobile-phase additives for RPLC, it will be important to keep in mind the analytical goal that the dynamic range and detection limits of the detector used not be overly compromised by the properties of the additive. Why additives at all and why C4, C8 and C18?

As a first approximation, the stationary phase in water-containing mobile phase, reversed-phase chromatography could be thought of as the surface of graphite. The attachment of a paraffin molecule certainly has that effect. The selectivity is dominated by differences in mobile-phase solubility for the solutes of interest. Unlike the gas chromatographic experiment where retention is only slightly influenced by the mobile-phase character, liquid chromatographic net retention can be infinite in one mobile phase and nonexistent in another. The choice of C4, C8, and C18 derives from the commercial availability of propene, octene, and octadecene — the precursors to the silanes used to make the bonded phases.

II. MOBILE PHASE ADDITIVES

The desire for tailored mobile and stationary phases arises from the need to separate closely related solutes. "Closely related" can have both the chemical sense (e.g., geometrical, stereochemical, or optical isomers) and the chromatographic sense (e.g., same molar volume, dipole moment, polarizability, and degree of ionization). A very early example of a tailored stationary phase is the technique called "argentation" in which a silica stationary phase is treated with silver nitrate. The silver ions coordinate differently with E/Z or *cis/trans* forms, and the effect is believed to be both steric and polarizability dependent. What occurs is the formation of a weak adduct between silver and the olefinic double bond:

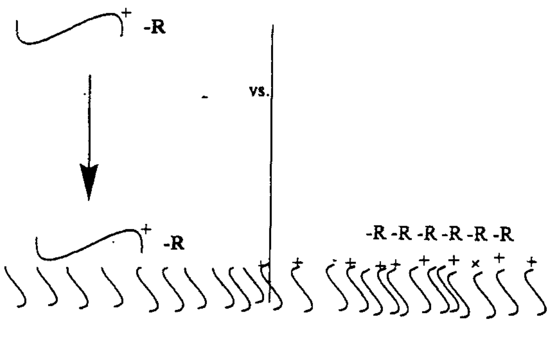


Differences in the stability of the adduct lead to differences in the standard-state free energy change for sorption $\Delta(\Delta G^0)$ and thus the α value for the isomers can be greater than 1.00. Similar effects can be seen if Ag^+ is added to the mobile phase, but strictly speaking the effect here is to produce a chemically tailored stationary phase.

Mobile phase additives can be divided into several classes. Perhaps the best classification scheme would indicate where the additive acts — mobile or stationary phase — and how. This may be important to do in order for designers of new additives to proceed in a logical, chemical fashion. The classification order used in this paper is an arbitrary one and could be changed.

Case 1: Additive reacts with solute — A chemical reaction to form a covalent bond, a loosely associated or strongly bound adduct is one mode of action for a mobile-phase additive. A seemingly trivial example is pH control by acid or base addition, "seemingly trivial" because it is likely one of the most common additive strategies used in RPLC. The goal is to retard ionization and to improve retention as a result. The more hydrophobic, neutral molecule is more strongly sorbed and more retained. Formation of a covalent bond, that is, making a "derivative" in liquid chromatography is akin to doing the same for gas chromatography except that here solubility, not volatility, is being manipulated. Loose aggregates can include ion-pairs deliberately formed by adding the conjugate acid/base to the mobile phase.

Case 2: Additive interacts with stationary phase — "Reacts" includes strong adsorption, and the argentation example given is one case. What is less clear but occurs is a variation on the case 1 additive and the case 3 (*vide infra*) but where the additive sorbs to create a "dynamic" new kind of mobile phase. Dynamic here is used to imply a steady state in which sorbed molecules desorb to be replaced by others of the same kind. An example is the use of alkyl sulfonates as reagents to form ion pairs in solution in hope that the more hydrophobic ion pair will more strongly sorb. The question is whether this strategy involves mobile phase association or a stationary phase that is akin to a conventional ion-exchange support:



In this case, the reagent is adsorbed to form an anion exchanger or forms the ion-pair adduct that sorbs as an entity.

Case 3: Additive changes the solute environment in the mobile — It is possible to add sufficient detergent to create micelles or reverse micelles in the mobile phase. Such entities can either contain the solutes of interest and sorb as the solute-containing micelle or another dynamic equilibrium is set up in which the solutes partition into the micelles in one equilibrium and between the mobile phase and stationary phase in another. The net effect on retention is the averaging of these effects. If the micelle sorbs and releases the solute to the stationary phase in a two-step process, yet another equilibrium is involved.

What follows is the result of a study on the use of nonionic but coordinatively unsaturated metal complexes of β-diketones. It is an example of such additive use and complements the work by others cited in terms of direct coordination to metal sites on bound and free additives.

For more than a decade, the diverse chemistry of metal ions has been used to enhance selectivity in liquid chromatographic systems. It has been well established that metal-ion equilibria can provide unique opportunities for retention manipulation. The simple electrostatic forces utilized in many separation methods (ion exchange, ion pairing) are nonspecific, with no directional or steric requirements. Metal ions, however, incorporate into the separation differences in solute-metal binding as well as electrostatic forces. In addition, metal-ion complexes with fixed coordination sites can produce special steric selectivities because structural and directional effects significantly influence the interaction of a solute with the central metal ion. Secondary chemical equilibria involving metal ions can therefore produce the resolution of molecules with very similar properties such as geometrical isomers, homologs, and optical isomers. Metal ions as mobile-phase additives increase the versatility of a separation method, allowing convenient variation in metal speciation and concentration. Metal ions "doped" into the mobile phase can be used for a specific problem and then removed, returning the column to normal operation. This advantage can eliminate the costly necessity for "tailor-made" metal stationary phases. The efficiency of the column is also improved through the use of mobile phase additives because the poor mass transfer properties associated with ligand-exchange systems is drastically reduced.[1] Finally, the problem of metal loss from the stationary phase is eliminated by the continuous saturation of the column system with the metal additive.

Although the use of metal ions in the mobile phase is a relatively new technique, two successful approaches have been demonstrated. The first employs simple metal ions as additives in the mobile phase for selective complexation with specific solutes. Cadmium and zinc ions have been used in this mode for the separation of amino sugars and peptides on an amino phase,[2] and magnesium ions have been used in reversed-phase separations of nucleotides and nucleosides.[3] Metal ion complexes have also been doped into the mobile phase for the resolution of anionic solutes. Silver (I) octanate has been used successfully for the separation of hydrophilic peptides in reverse-phase high-performance liquid chromatography (RP-HPLC).[4] Other ionic chelates, such as L-2-isopropyl-4-octyldiethylenetriamine Zn(II), L-aspartylcyclohexylamide Cu(II), and L-proline Cu(II) have been used for selective chiral separations.[5–7]

Lochmüller and Hangac reported the use of a coordinatively unsaturated metal complex as a mobile phase additive for RP-HPLC.[8] Unlike previous ionic metal dopants, *bis*-(2,2,6,6-tetramethylheptane-3,5-dionato) Ni(II), Ni(DPM)z, is a neutral square planar complex that interacts with additional ligands to gain stabilization energy from crystal field effects rather than a need to neutralize a formal charge. The formation constants for these adducts are smaller than for ionic species, resulting in more rapid dissociation kinetics. The result is improved chromatographic efficiency and shorter elution times than typical of many ionic metal additives. Enhanced chromatographic selectivity was also demonstrated by the Ni(DPM)$_2$ complex. The bulky tertiary butyl groups in the DPM moiety introduced significant steric requirements for solute-metal interaction (Figure 1), producing the separation of structurally similar pyrimidine bases and pyridine derivatives.[9] Trace analytes could be detected without interference because the nickel complex is transparent in the 210- to 290-nm region of the spectrum. In addition, Ni(DPM)$_2$ was found to be soluble in a wide range of LC solvents, column equilibration was achieved in less than

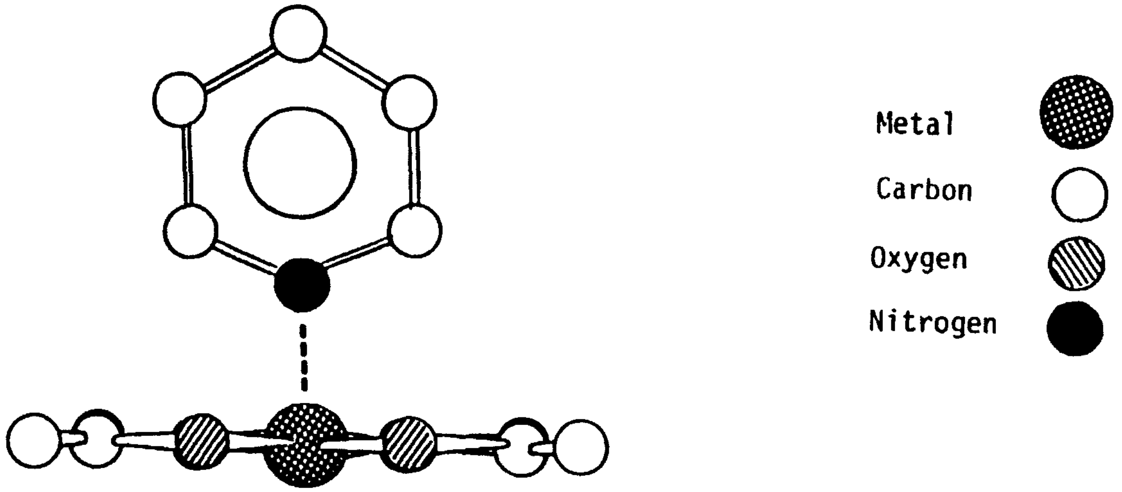


FIGURE 1. Base-metal interaction in Ni(DPM)$_2$ complex.

60 min, and the complex was easily removed from the chromatographic system after use. Changes in the capacity factors of various small probe solutes were studied as a function of the quantity of nickel complex adsorbed on reversed-phase substrates. It was found that a solute's accessibility to the complex's axial sites, and the subsequent increase in retention, was dependent on its dipole moment, pK steric, and solvation properties.

We have continued the examination of coordinatively unsaturated metal complexes as mobile-phase additives in HPLC. A series of pyridine derivatives, nitrogen bases, and sulfur compounds was used to evaluate the influence the mobile-phase composition and stationary-phase substrate have on the selectivity of the $Ni(DPM)_2$ additive. The hydrophobicity of a solute was an important factor in the magnitude of the metal-solute interaction on reversed-phase systems. In addition, increased selectivity for polar compounds was observed *using the* $Ni(DPM)_2$ additive in non-coordinating solvents on amine substrates. The influence of metal was examined by studying the chromatographic properties of the analogous Cu, Zn, and Eu complexes. The square planar copper complex showed similar selectivity but weaker solute-binding strength relative to that found for the nickel complex. The tetrahedral zinc chelate exhibited unique steric selectivity and preferentially retained strongly basic solutes. The Eu(DPM)3 chelate was shown to be ineffective as a mobile-phase additive in aqueous reversed-phase systems. Additional chromatographic and fluorescence spectroscopy studies were performed for the *tris*-(4,4,4-trifluoro-1-phenyl-1,3 butanedionato)aquo Eu(III) chelate. The results suggest that solute-europium complex interactions are reduced in aqueous environments.

III. EXPERIMENTAL DETAILS

A liquid chromatograph equipped with autosampler, 10-μl loop injector, and digital data system was used. Solute elution was monitored with tandem UV and refractive index detectors. Fluorescence measurements were performed on a fluorimeter with variable excitation and emission capabilities. The excitation wavelength was set at 365 nm, and the emission spectrum was scanned from 500 to 700 nm. All spectra were digitized and stored as disk files.

The 2,2,6,6-tetramethylheptane-3,5-dione (dipivaloylmethane) was obtained from Aldrich Chemical Company. The 4,4,4-trifluoro-1-phenyl-1,3-butanedione (benzoyltrifluoroacetone) was obtained from Alpha Chemical Corporation. The nickel acetate tetrahydrate, copper acetate, and zinc acetate were all purchased from Fluka Chemical, while the europium lorate and europium perchlorate were obtained from Alpha Chemical Corporation.

The pyridine derivatives, nitrogen bases, sulfur, oxygen, and phosphorous compounds used as probe solutes were purchased from Aldrich Chemical Company. The pyrimidine bases were obtained from Sigma Chemical Company (St. Louis, MO).

The acetonitrile, methanol, water, methylene chloride, chloroform, and n-hexane used as chromatographic solvents were Omnisolv-HPLC grade purchased from MCB (Cincinnati, OH).

Whatman Chemical Separations 10 μm reversed-phase substrates (ODS-2 and ODS-3 C_{18} bonded phases) as well as the 10 μm silica material (Partisil-10) were used to pack the chromatographic columns in this study. Other columns used were a Dupont Chemicals Zorbax-NH_2 prepacked column, 10 μm particles, 25 × 0.4 cm particles, and a Whatman ODS-3 prepacked microbore column (10 μm particles, 25 × 0.4 cm).

$Ni(DPM)_2$ was prepared by the stoichiometric reaction of 2,2,6,6-tetramethylheptane-3,5-dione and nickel acetate tetrahydrate, using the procedure of Cotton and Wise described elsewhere.[8] The $Cu(DPM)_2$ complex was prepared by reaction of the dipivaloylmethane ligand with copper acetate, using a modified version of a method developed by Cotton.[10] A saturated aqueous solution of copper acetate (6 mmol in 20 ml of water) was added slowly to a methanol solution containing 15 mmol of distilled ligand. A light purple material precipitated, which was vacuum filtered and recrystallized from n-heptane. The melting point of the navy blue crystals was sharp at 198°C; elemental analysis of the purified sample gave the predicted 61% carbon, 8.8% hydrogen, and 14.7% copper. IR and UV-VIS spectral analysis confirmed the identity and purity. The $Zn(DPM)_2$ chelate was prepared using a modification of a method by Hammond.[11] Zinc acetate (6 mmol) was dissolved in warm aqueous ethanol. Freshly distilled ligand (15 mmol) was slowly added to the zinc solution with constant stirring. Concentrated aqueous ammonia was then added dropwise to the mixture, the white precipitate separated, was removed by filtration, and sublimed at 90°C and 0.02 Torr. The melting point of the white crystals was 155°C; elemental analysis gave 61.2% carbon, 8.8% hydrogen, and 15.5% zinc. IR, UV-VIS, and proton nuclear magnetic resonance (NMR) confirmed the identity. The $Eu(DPM)_2$ complex was purchased commercially from Aldrich Chemical Company, recrystallized from n-hexane, and dried under vacuum. The $Eu(BTA)_3{\cdot}H_20$ chelate was prepared by adding an aqueous solution of europium chloride (5.5 mmoles) to a solution of 4,4,4-trifluoro-1-phenyl-1,3-butanedione (16.8 mmoles) in ethanol/NaOH (5:1 v/v). Several recrystallizations from a chloroform/petroleum ether mixture were necessary to produce pure white crystals, which were vacuum dried for 2 h. The melting point was 123 to 130°C; elemental analysis gave the expected 44% carbon, 2.8% hydrogen, and 18.4% europium. Infrared, UV-visible, and emission spectroscopy verified the crystals as $Eu(BTA)_3{\cdot}H20$.[12]

All mobile phases containing metal additive were prepared by pipetting the appropriate dilutions of dopant stock solution. The mobile phases were filtered and degassed. The columns were equilibrated by recycling the mobile phase at 1 µl/min until constant k′ values were obtained for pyridine and benzene. Standard solutions of the distilled compounds used as probe solutes were prepared as $10^{-4}\,M$ concentrations in the appropriate organic modifier. The standards were then diluted to $10^{-5}\,M$ concentrations with the undoped chromatographic solvent and 10 µl injected onto the columns. Measured retention times were the average of duplicate injections, and the void time values were measured by injection of 100% organic modifier or a sample of the mobile phase enriched in D_20. The quantity of metal additive absorbed onto the chromatographic substrate was determined by the "breakthrough" adsorption isotherm method of Knox and Hartwick previously described.[8]

IV. RESULTS

Table 1 lists the pK_a, molecular dipole moment, and trends in capacity (dk′) for the solutes studied. As expected from the previous investigation of the interaction of similar solutes with the $Ni(DPM)_2$ additive,[8] dk′ does not correlate solely with solute basicity but depends upon the interplay of pK_a, dipole-dipole interactions, steric hindrance, and solvation effects. The $Ni(DPM)_2$ complex has a significant dipole moment in the presence of coordinating polar compounds. It was previously demonstrated that the induced dipole-dipole interactions between the "excited" nickel complex and polar solutes played a significant role in the overall net retention. As expected, solutes not previously studied that show an increase in retention with the $Ni(DPM)_2$ additive are those with dipole moments greater than 1.10 D and excessive bases, such as caffeine, that do not interact despite their large molecular dipole moments. The metal complex can be considered an intermediate Lewis acid that interacts with

TABLE 1
Physical Properties and dk′ Trends for Solutes Studied

Solute	pK_a	μ_{EXP}[a]	μ_{CALC}[b]	dk′[c]
4-Picoline	6.02	2.57	2.57	+[d]
3-Picoline	5.70	2.40	2.38	+
2-Picoline	5.97	2.04	2.02	0
3-Benzylpyridine	5.20	2.18	2.17	
+				
3-Phenylpyridine	4.80	2.28	2.26	+
2-(2-Thienyl)pyridine	—			0
2-(3-Thienyl) pyridine	—			0
2,4 Dipyridyl	4.44	3.84	2.20[e]	+
2,3 Dipyridyl	4.47	3.90	2.20[e]	+
2,6-Di-*t*-butylpyridine				0[f]
Piperidine	11.12	1.20	1.20	+
Pyrrolidine	11.27	1.57	1.57	+
p-Toluidine	5.08	2.10	1.38	+
Aniline	4.60	1.50	1.01	+
Benzoquinone				0
Benzene	—	—		0
Caffeine	0.61	3.70		0
Pyridine	5.26	2.20		0
2-(4-Tolyl)pyridine	—	1.78		0
Thiophene	—	0.54		0
2-Phenylpyridine	4.48	1.94		0
Propylamine	10.70	1.35		0
Triethylamine	11.01	0.92		0

[a] Molecular dipole moment.[33]
[b] Calculated collinear dipole (active solutes only).[34]
[c] k′ solute with $Ni(DPM)_2$ additive − k′ solute without $Ni(DPM)_2$ additive.
[d] Change in solute capacity factor with addition of $Ni(DPM)_2$ additive.
[e] Approximate dipole along y axis of 3′ and 4′ nitrogen on second pyridyl ring.
[f] No change in solute capacity factor observed with additive.

borderline Lewis bases in the affinity order N>O>S. The sulfur compounds listed in Table 1 did not interact with the $Ni(DPM)_2$ additive as evidenced by no net increase in retention on the ODS-3 phase. Structural features that prevent the optimum orientation for the nickel-solute interaction also restrict dk′. Both 2-picoline and 2-phenylpyridine fail to interact with $Ni(DPM)_2$, while 3-picoline and 3-phenylpyridine show increases in retention. Steric requirements also prevent the nitrogen lone-pair of 2-(4-tolyl)pyridine, 2-(2-thienyl)pyridine, 2-(3-thienyl)pyridine, and 2,6-di-*t*-butylpyridine from interacting with the nickel complex. Chromatograms in Figure 2 illustrate the improved resolution of these pyridine derivatives in the $Ni(DPM)_2$ additive system. Table 2 lists the results of the least-squares analysis for the relationship between the increase in the capacity factor of each active solute and the μmole quantity of Ni(DPM)2 sorbed onto the re-

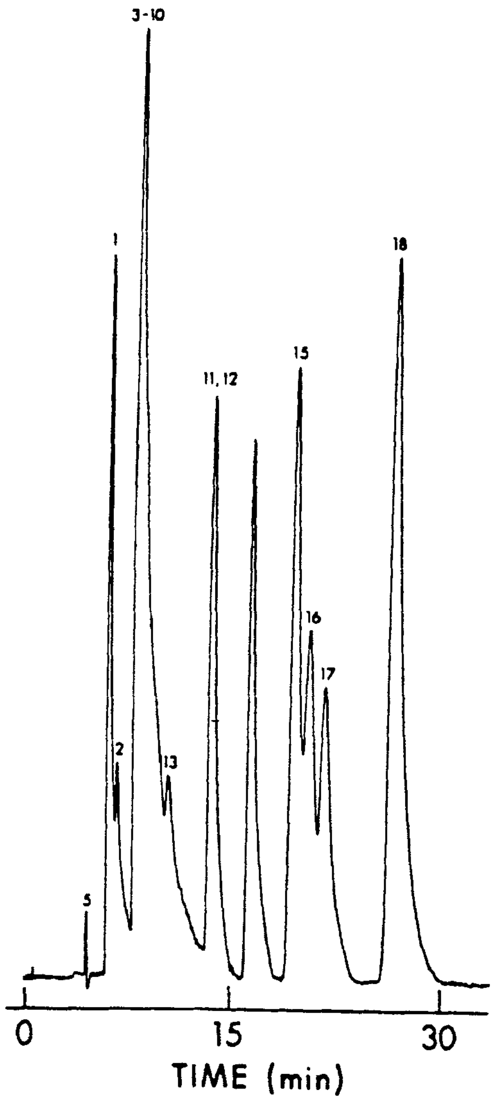


FIGURE 2. Separation of pyridine derivatives on an octadecyl microbore column without and with the Ni(DPM)$_2$ mobile phase additive. (A) 60/40 methanol water, 33 μl/min; (B) 60/40 methanol water + 3.0×10^{-4} *M* Ni(DPM)$_2$, 33 μl/min. Peak identification: 1, caffeine; 2, aniline; 3, 3-picoline; 4, 2-toluidine; 5, 4-toluidine; 6, 3-picoline; 7, 4-picoline; 8, 2,3-dipyridyl; 9, 2,4-dipyridyl; 10, thiophene; 11, 2-(3-thienylpyridine) ; 12, 2-(2-thienylpyridine); 13, nicoline; 14, 2-phenylpyridine; 15, 3-phenylpridine; 16, 2-(3-tolyl)pyridine; 17, 3-benzylpyridine; 18: 2-(4-tolyl)pyridine.

versed-phase substrate. Good linear correlations are observed at each of the methanol-water compositions, suggesting that the observed increase in retention is directly related to the interaction with the Ni(DPM)$_2$ complex. The slope value, dk'/q_{ads}, provides a qualitative estimate of the stability of the solute-Ni(DPM)$_2$ complex under specific mobile- and stationary-phase conditions. The magnitude of a solute's slope value was previously demonstrated to correlate with increasing dipole and was also dependent on basicity, solvation, and steric factors. Similar trends are evident in Table 2. Equivalently sized solutes at a given mobile-phase composition exhibit larger slope values with increased dipole strength.

A. Influence of Mobile Phase on Retention

As the percentage of water in the mobile phase is varied, other factors begin to influence the interaction of a solute with the nickel complex, and this is reflected in the magnitude of the solute's k'/q_{ads} slope value. The plot shown in Figure 4 illustrates the opposing effect the increasing aqueous mobile-phase composition has on the slope values of two differently sized solutes. The behavior of 3-picoline is typical of the other small solutes listed in Table 2. As the percentage of water is lowered, the dk'/q_{ads} value increases, indicating an increase in the stability of the 3-picoline-nickel complex interaction. Since water is a coordinating solvent, this increase in dk'/q_{ads} can be attributed to a reduced competition for axial sites on the nickel complex in a less aqueous environment. When the water content is increased, dk'/q_{ads} values of small solutes gradually decline as the water molecules compete more effectively for interaction with the metal. As shown in Figure 4, the larger, less-polar solutes such as 3-phenylpyridine are influenced dramatically by variations in the water content of the mobile phase (an increase in water produces a rapid increase in the k'/q_{ads} value for 3-phenylpyridine). This suggests that a solute's "solvophobicity" may be an additional influence on the stability of the solute-metal interaction on reversed-phase substrates. Lowering the water content decreases

TABLE 2
Influence of Aqueous Mobile-Phase Composition on dk'/q_{ads} Values of Active Solutes in $Ni(DPM)_2$ System

Active solute	10% H_2O dk'/q_{ads}		25% H_2O dk'/q_{ads}		40% H_2O dk'/q_{ads}	
	Slope	Correlation coefficient	Slope	Correlation coefficient	Slope	Correlation coefficient
4-Picoline	0.149	0.93	0.120	0.996	0.123	0.965
3-Picoline	0.130	0.910	0.110	0.998	0.10	0.99
Pyridine	0.111	0.92	0.08	0.998	0.075	0.975
3-Benzylpyridine	0.092	0.88	0.105	0.920	0.358	0.930
3-Phenylpyridine	0.06	0.89	0.09	0.940	0.344	0.891
2,3-Dipyridyl	0.07	0.90	0.065	0.990	0.134	0.92
2,4-Dipyridyl	0.069	0.83	0.062	0.990	0.124	0.95
Pyrrolidine	0.096	0.98	0.061	0.993	0.070	0.966
Piperidine	0.09	0.97	0.053	0.999	0.065	0.89
p-Toluidine	0.048	0.95	0.01	0.930	0.007	0.95
Aniline	0.005	0.94	0.004	0.980	0.003	0.94

Note: Slope values calculated from retention data at three separate loadings of metal at each mobile phase composition.

the dk'/q_{ads} values for the larger solutes. The solvophobic influence is less dominant in low concentrations of water. The result is that the dipole and basicity of the solute are the major contributing factors in the magnitude of the solute-metal interaction, that is, at decreased levels of water, the magnitude of all the solutes dk'/q_{ads} values are correlated better with trends in pK_a and collinear dipole moments, despite size differentials.

Horvath and Melander have shown that the effective surface areas of a solute and the solute-complex species can be influential in the retention characteristics of reversed-phase mobile-additive systems.[13] The dk′ parameter used in this study eliminates the effect on solute retention of the *generalized* hydrophobic interaction in the reversed-phase system. However, the dk′ value does not isolate the potential influence of a large solute's hydrophobicity on the stability of a solute-metal complex on a reversed-phase substrate. In an attempt to compensate for the hydrophobic factor in the stability of the solute-metal interaction, dk'/q_{ads} values were normalized by the molecular connectivity[14] of the solute. The connectivity indices listed in Table 3 provide a topological index representative of a solute's surface area and proportional to the hydrophobicity of the solute. The plot shown in Figure 5 illustrates the improved correlation obtained between the dk'/q_{ads} values and pK_a/collinear dipole trends when the size of the solute is considered. The initial interaction with the nickel complex is therefore dependent on collinear the dipole, pK_a, and steric effects; however, the final magnitude of the dk'/q_{ads} value is also influenced by the hydrophobicity of large solutes in reversed-phase systems.

Other solvent systems were studied to evaluate the influence of the mobile-phase composition on the chromatographic properties of the $Ni(DPM)_2$ additive. Table 4 presents the dk'/q_{ads} slope values for several solutes measured under polar and nonpolar mobile phase conditions.

Acetonitrile has a higher dielectric constant than methanol and is considered a stronger coordinating solvent.[15] In an attempt to

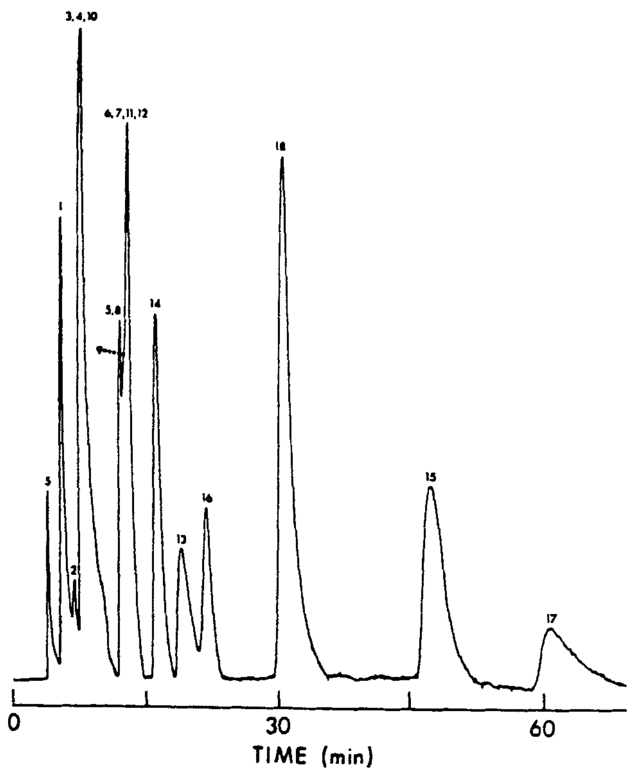


FIGURE 3. Graph of $\Delta k'/q_{ads}$ as a function of solute collinear dipole moment and basicity. 1, 4-Picoline; 2, 3-Picoline; 3, Pyridine; 4, 3-Benzylpyridine; 5, 3-Phenylpyridine; 6, 2,3-Dipyridyl; 7, 2,4-Dipyridyl.

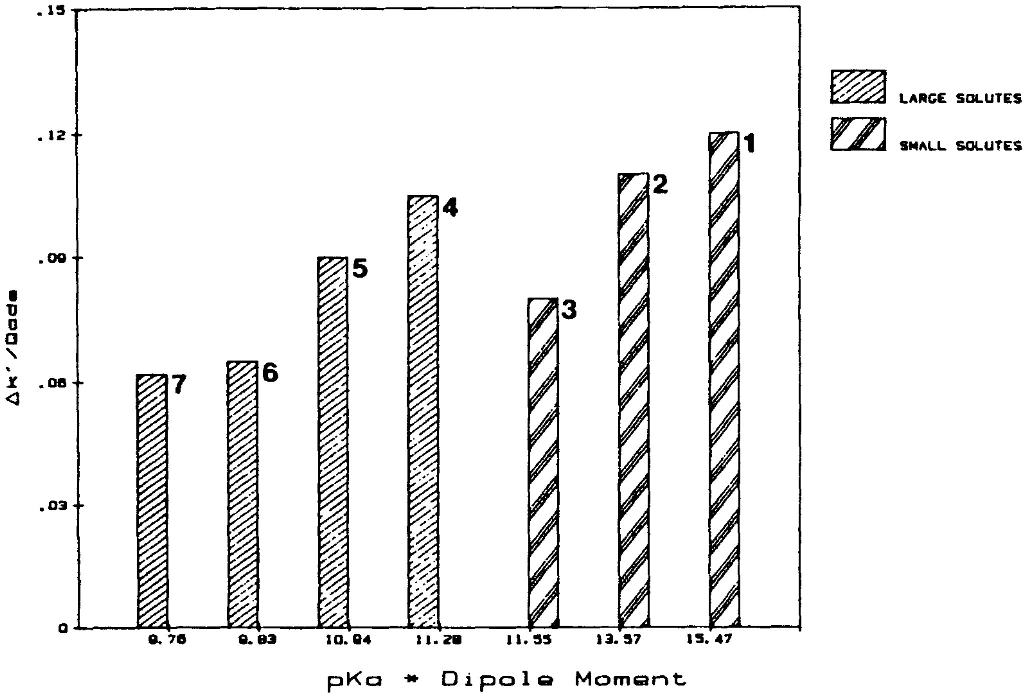


FIGURE 4. Influence of aqueous mobile phase composition on $\Delta k'/q_{ads}$ for differently sized solutes. Conditions: aqueous-methanol mobile phases, ODS-3 stationary-phase substrate.

TABLE 3
Solute dk′/q$_{ads}$ Values Normalized by Molecular Connectivity Indices

Solute	Molecular connectivity index x	dk′/q$_{ads}$ · x^{a}
4-picoline	2.41	0.05
3-picoline	2.41	0.046
Pyridine	1.99	0.04
3-Benzylpyridine	4.528	0.023
3-Phenylpyridine	4.07	0.022
2,3-Dipyridyl	4.07	0.016
2,4-Dipyridyl	4.07	0.015

[a] Conditions: 75/25 MeOH/H^{2}0, ODS-3 Whatman column (25 x 0.4 cm).

produce similar “solvophobic environments”, the composition of the acetonitrile/water mixture was adjusted to the same-solvent strength[16] as the methanol/water mobile phase. An equivalent capacity for benzene in both systems verifies the similar hydrophobic retention characteristics of the solvents. The dk′/q$_{ads}$ value for each solute decreased in the acetonitrile system. In addition, weak solutes such as *p*-toluidine and aniline show no net increase in capacity with the Ni(DPM)$_2$ additive in the acetonitrile containing mobile phase. The hydrophobic factor is still influential in the acetonitrile system, as indicated by the similar order of the solutes’ dk′/q$_{ads}$ values. However, the large solutes show the greatest relative decrease in slope value on switching to an acetonitrile-containing mobile phase. This large reduction is the result of less effective competition by the solutes for axial sites on the metal, and as a result, any influence of hydrophobic stabilization on the solute-metal complex is greatly diminished.

In the nonpolar methylene chloride solvent system, the dk′/q$_{ads}$ slope values for each

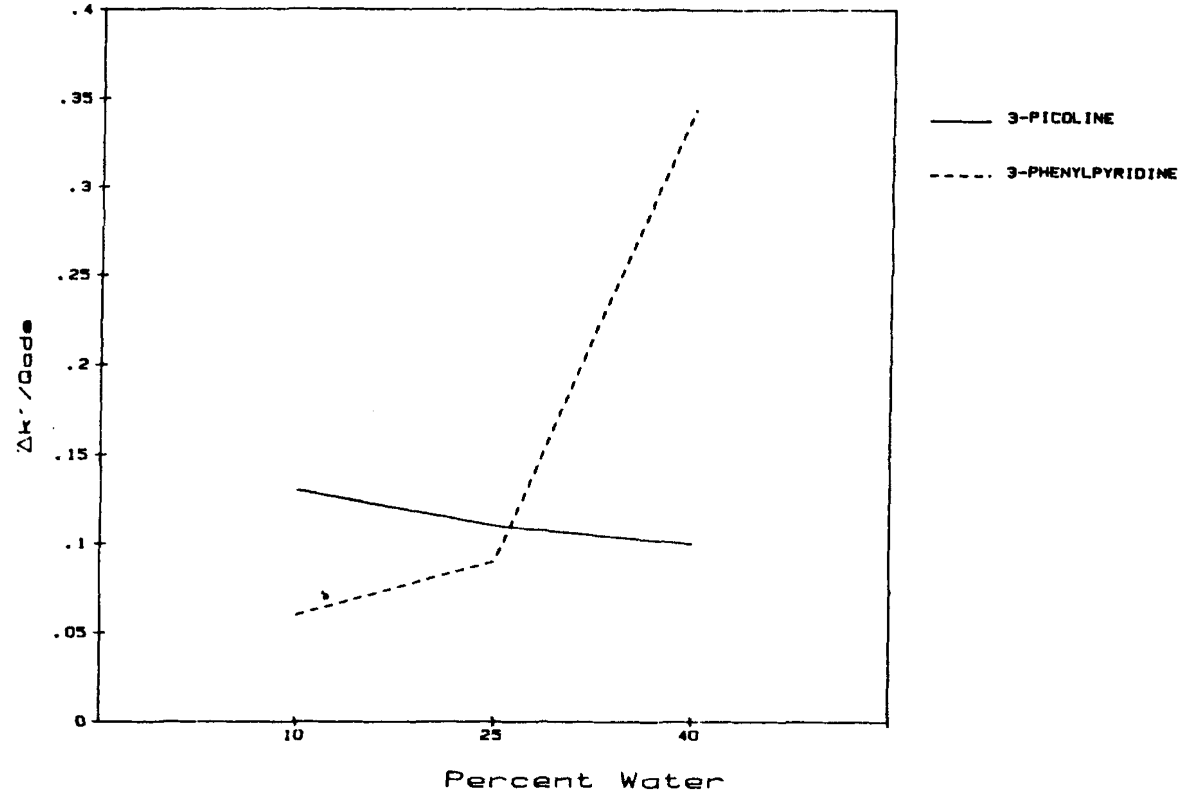


FIGURE 5. Graph of dk′/q$_{ads}$ normalized by molecular connectivity as a function of solute collinear dipole moment and basicity. 1, 4-picoline; 2, 3-picoline; 3, ; 4, 3-; 5, 3-; 6, 2,3-dipyridy; 7, 2,4-dipyridyl.

TABLE 4
Influence of Mobile-Phase Composition on dk'/q_{ads} Values

Solute	$Ni(DPM)_2$ dk'/q_{ads}	$Cu(DPM)_2$ dk'/q_{ads}	$Zn(DPM)_2$ dk'/q_{ads}
4-Picoline	1.20	0.110	0.164
3-Picoline	0.110	0.096	0.132
Pyridine	0.08	0.065	0.112
3-Benzylpyridine	0.105	0.088	0.085
3-Phenylpyridine	0.09	0.065	0.057
p-Toluidine	0.010	0.05	
Benzene	0	0	0
2-Phenylpyridine	0	0	0
Aniline	0.004	0	0.009
2-(3-Thienyl)pyridine	0	0	0
2-(2-Thienyl)pyridine	0	0	0
2-Picoline	0	0	0
Propylamine	0	0	0.08

[a] ODS-3 Whatman Column, 75/25 $MeOH/H_2O$
[b] ODS-3 Whatman column, 73/27 ACN/H_2O
[c] NH_2-ZORAX column, 99/1 $CH_2Cl_2/MeOH$

of the small solutes increased, indicating the absence of competition by solvent molecules for $Ni(DPM)_2$ coordination sites. The large solutes exhibit a decrease in the dk'/q_{ads} value due to the loss of solvophobic influences in the nonaqueous solvent system. The extent of interaction of a solute with the nickel complex is now solely dependent on dipole, pK_a and steric effects. Propylamine and other aliphatic amine compounds were previously shown not to interact with the $Ni(DPM)_2$ complex in aqueous solvent systems. It was proposed that the solvation sheath formed by water molecules around aliphatic amines served to reduce the extent of interaction with the nickel. In the absence of water, propylamine demonstrates a significant attraction to the nickel complex with a comparatively large dk'/q_{ads} value. All the solutes' slope values correlate with increasing dipole and pK_a in this noncoordinating solvent.

B. Influence of Substrate on Retention

The graphs in Figure 6 illustrate the influence reversed- and normal-phase substrates have on the quantity of $Ni(DPM)_2$ sorbed to the surface. In the reversed-phase system, ODS-2 sorbs approximately twice the quantity of nickel complex as does ODS-3. The higher percentage of carbon on the ODS-2 stationary-phase material (15% vs. 11%) may account for the phenomenon. In the normal phase system, the nickel complex demonstrates little affinity for either of the two substrates under the conditions studied. The Lewis acidity of the $Ni(DPM)_2$ species limits the interaction with the acidic silica surface, while the nickel complex does exhibit some retention on the basic amino phase.

Table 5 lists the slope values obtained for several solutes on each of the four substrates. The similar slope values measured on the ODS-2 and ODS-3 columns suggest equivalent retention mechanisms. Therefore, under identical mobile-phase conditions, the increase in capacity of a small solute with the appropriate characteristics for interaction with the $Ni(DPM)_2$ complex is linearly dependent on the quantity of nickel sorbed on the substrate.

The $Ni(DPM)_2$ complex is not retained in the silica normal-phase system and as a result

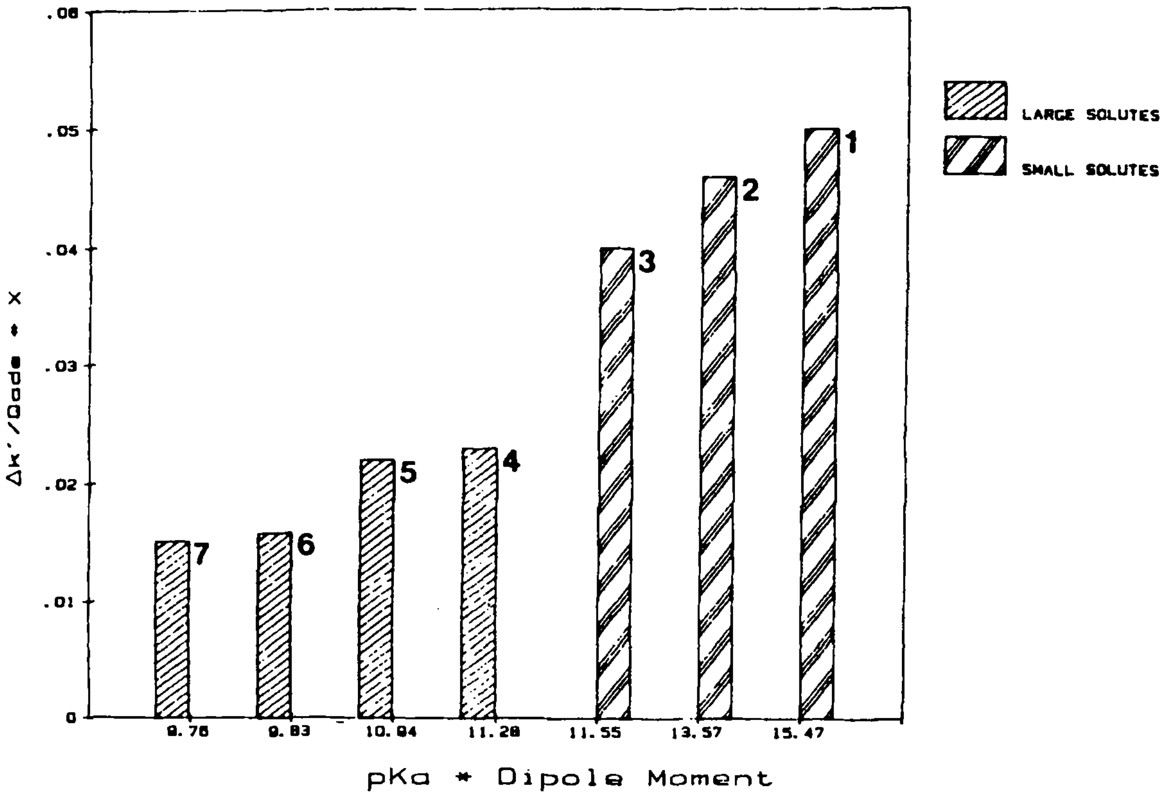


FIGURE 6. Influence of substrate on q_{ads}, Ni(DPM)$_2$. Reversed-phase conditions: 75/25 MeOH/H$_2$0, 2.5 × 10^{-4} *M* Ni(DPM)$_2$, 25 × 0.4 cm column, 10 μm derivatized silica. Normal-phase conditions: 99/1 CH$_2$Cl$_2$/MeOH, 2.5 × 10^{-4} *M* Ni(DPM)$_2$, 25 × 0.4 cm column, 10 μm silica.

TABLE 5
Influence of Stationary-Phase Substrate on dk'/q_{ads} Values

Solute	ODS-2[a] dk'/q_{ads}	ODS-3 dk'/q_{ads}	ZORBAX-NH[b] dk'/q_{ads}	SILI[c] dk'
4-Picoline	0.127	0.120	0.164	−0.14
3-Picoline	0.113	0.110	0.132	−0.10
Pyridine	0.079	0.08	0.112	−0.03
3-Benzylpyridine	—	0.105	0.085	−0.01
3-Phenylpyridine	—	0.09	0.057	
p-Toluidine	0.01	0.01	0.05	
Benzene	0	0	0	
2-Phenylpyridine	—	0	0	
Aniline	0.0037	0.004	0.009	
2-Picoline	0	0	0	
Propylamine	0	0	0.08	

Note: Ni(DPM)$_2$ did not absorb on the silica surface. Only dk' values are available.

Concentration of Ni(DPM)$_2$ in all mobile phases = 2.5 × 10^{-4} *M*.

[a] 75/25 MeOH/H$_2$O.
[b] 99/1 CH$_2$Cl$_2$/Me.
[c] 90/10 CH$_2$.

increases in capacity factors are not observed. However, preferential complexation with the Ni(DPM$_2$) molecules in the mobile phase may account for the decrease in the retention of several solutes. Retention of the nickel complex is achieved on the propylamine phase, and, in general, better δ-values are found in the non-coordinating solvent system, where nickel interactions predominate and hydrophobic factors are eliminated.

C. Influence of the Metal on Retention

The influence of metal substitution on solute retention was evaluated by studying the chromatographic properties of the Cu, Zn, and Eu dipivolylmethane complexes. Each of the metal species shown in Figure 7 is a neutral, coordinatively unsaturated metal complex. The rapid dissociation kinetics associated with these complexes produce the same high efficiencies found with the Ni(DPM)$_2$ analog. In addition, the changes in geometry and electronic configuration generate differences in the magnitude of the solute-metal interaction as well as variation in solute selectivity.

The square planar Cu(DPM)$_2$ complex is a paramagnetic species with a d^9 electronic configuration.[17] As in the case of the nickel compound, Cu(DPM)$_2$ interacts with additional ligands to increase its coordination number, gaining stabilization energy from crystal field effects.[18] To determine the chromatographic utility of the Cu(DPM)$_2$ complex, studies of dk′ as a function of qads were performed for the same series of solutes utilized in the nickel evaluation. Inspection of Table 6 reveals several interesting trends. Similarly sized solutes at a specific mobile-phase composition display relatively larger slope values with increasing collinear dipole moments and pK_a values. This is the same behavior exhibited by the nickel species, and suggests that the interaction of a solute with the Cu(DPM)$_2$ is dependent on a similar interplay of pK_a and dipole influences. In addition, solutes that did not interact with the Ni(DPM)$_2$ complex because of steric restrictions also fail to show an increase in retention with the copper additive. This is expected because both metal complexes have square planar geometries. Table 6 also indi-

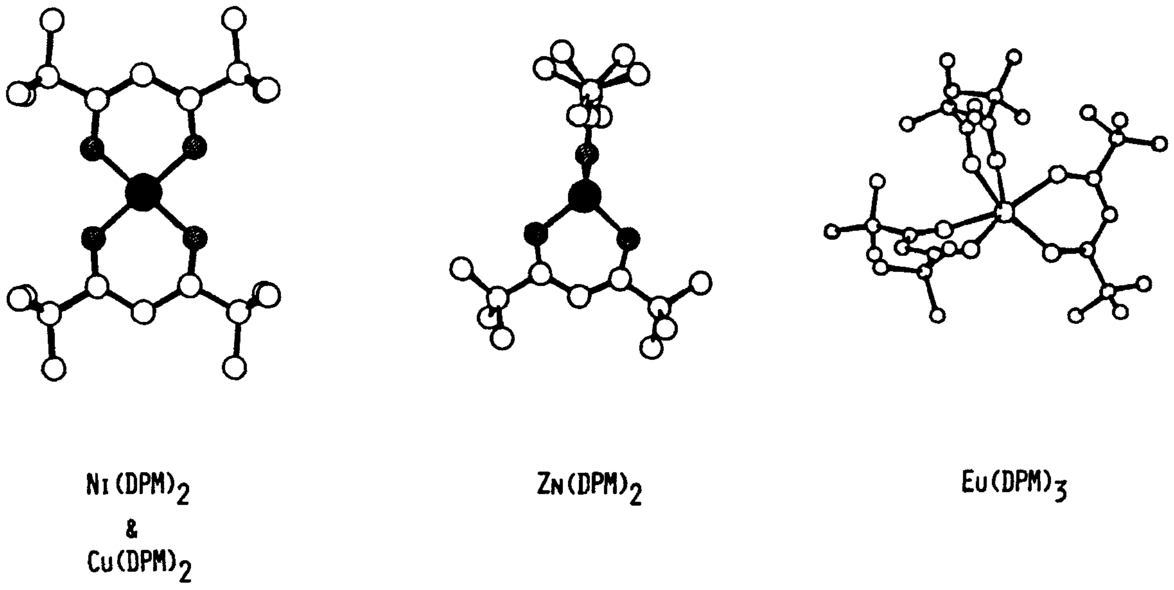


FIGURE 7. Coordinatively unsaturated metal complexes.

TABLE 6
Influence of Mobile-Phase Composition on dk'/q_{ads} Values of Solutes in $Cu(DPM)_2$ System

Solute	10% H_2O dk'/q_{ads}		25% H_2O dk'/q_{ads}		40% H_2O dk'/q_{ads}	
	Slope	Correlation coefficient	Slope	Correlation coefficient	Slope	Correlation coefficient
4-Picoline	0.127	0.997	0.117	0.998	0.107	0.986
Pyridine	0.10	0.983	0.083	0.996	0.062	0.932
3-Benzylpyridine	0.054	0.963	0.045	0.987	0.035	0.978
3-Phenylpyridine	0.046	0.954	0.100	0.986	0.227	0.963
2,3-Dipyridyl	0.023	0.936	0.085	0.952	0.151	0.954
2,4-Dipyridyl	0.049	0.973	0.048	0.998	0.058	0.979
Pyrrolidine	0.025	0.953	0.024	0.998	0.040	0.965
Piperidine	0.05	0.972	0.019	0.903	0.013	0.980
p-Toluidine	0.028	0.989	0.013	0.920	0.01	0.972
Aniline[a]						
Benzoquinone[a]						
2-(2-Thienyl) pyridine[a]						
2-(3-Thienyl) pyridine[a]						
2-Phenylpyridine[a]						
Propylamine[a]						
Caffeine[a]						
Thiophene[a]						
Benzene[a]						
2-Picoline[a]						

Note: Slope values calculated from retention data at three separate loadings of metal at each mobile-phase composition.

[a] No change in k′ observed.

cates that $Cu(DPM)_2$ has the same functional group affinity as the nickel compound.

Variation in the water content of the mobile phase produces behavior similar to that exhibited by solutes in the $Ni(DPM)_2$ additive system. Small solutes show slightly decreased slope values with increasing water content, while large hydrophobic solutes demonstrate a dramatic increase in dk'/q_{ads} with increasing aqueous mobile phases. Apparently, the hydrophobicity of the solute is once again influencing the stability of the solute-metal complexes on the reversed-phase substrate. Normalization of a solute's slope value by its molecular connectivity index helps to eliminate this additional hydrophobic influence, so that again dk'/q_{ads} values correlate with trends in pK_a and dipole moment despite increasing aqueous conditions. Comparative chromatograms illustrating the separation of a mixture by the $Ni(DPM)_2$ and the $Cu(DPM)_2$ mobile phase additives are shown in Figure 8. The $Ni(DPM)_2$ resolves more components and is non-UV absorbing, while the copper complex absorbs significantly in the 210- to 270-nm region of the spectrum and could interfere with the detection of trace components.

Unlike the nickel and copper species, $Zn(DPM)_2$ is a tetrahedral complex with a d^{10} electronic configuration.[19] Because the zinc compound has filled d shells, interaction with additional ligands does not provide stabilization energy from crystal field effects. In-

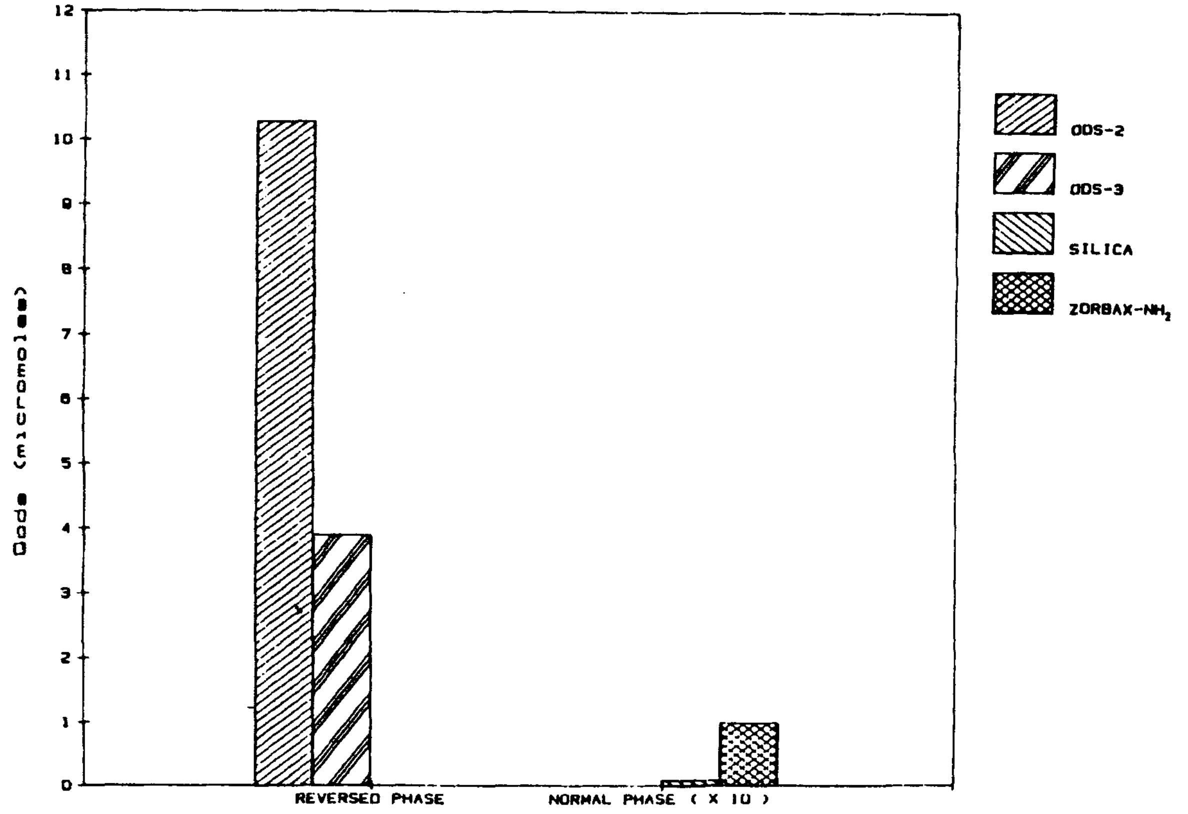


FIGURE 8. Comparative separations of amine compounds using Cu(DPM)$_2$ and Ni(DPM)$_2$ mobile-phase additives. Conditions: (A) 75/25 MeOHπH$_2$O, 1 ml/min, 30°C; (B) 1.25×10^{-4} *M* Ni(DPM)$_2$ in 75/25 MeOH/H$_2$O; (C) 1.25×10^{-4} *M* Cu(DPM)$_2$ in 75/25 MeOH/H$_2$O. Peak: a, aniline; b, pyridine; c, *o*-tuluidine; d, 2-picoline; e, *p*-toluidine; f, 4-picoline.

stead, the formation of five-coordinate zinc complexes is usually attributed to a need for further dispersal of charge at the metal center.[20] The results of the studies of dk′ as a function of q_{ads} for the Zn(DPM)$_2$ complex are listed in Table 7. Comparison of these data with the previous nickel and copper trends shows several unique variations in selectivity produced by the zinc species. Large solutes such as 3-benzyl- and 3-phenylpyridine, which had previously shown increases in retention in the nickel and copper system, fail to interact with the Zn(DPM)$_2$ complex. Only the smaller solutes listed in Table 7 show increases in retention with the zinc additive, indicating stringent size restrictions in the solute-zinc interaction. In addition, 2-picoline exhibits a slight increase in k′ with the zinc complex, illustrating the unique selectivity of the tetrahedral compound compared with the square planar species. Unlike the nickel and copper complexes, Zn(DPM)$_2$ preferentially retains very basic solutes, as exemplified by the large slope values.

As the percentage of water in the mobile phase is increased, several trends unique to the Zn(DPM)$_2$ system are observed. Larger relative decreases in the small solute slope values are displayed as the water content increases. This is the result of the known, greater dissociation of trigonal bipyramidal zinc complexes in polar solvents.[21] Because the large solutes fail to interact with the Zn(DPM)$_2$ complex, the hydrophobic influence so dominant in the nickel and copper systems is absent with the zinc additive.

Table 8 lists the dk′/q_{ads} slope values for each of the metal complexes determined under identical mobile-phase conditions. The magnitude of the solute-metal interaction fol-

TABLE 7
Influence of Aqueous Mobile-Phase Composition on dk'/q_{ads} Values of Solutes in $Zn(DPM)_2$ System

Solute	10% H_2O dk'/q_{ads}		25% H_2O dk'/q_{ads}		40% H_2O dk'/q_{ads}	
	Slope	Correlation coefficient	Slope	Correlation coefficient	Slope	Correlation coefficient
4-Picoline	0.060	0.958	0.117	0.998	0.107	0.986
Pyridine	0.23	0.985	0.083	0.996	0.062	0.932
3-Benzylpyridine	0.30	0.976	0.045	0.987	0.035	0.978
3-Phenylpyridine[a]						
2,3-Dipyridyl[a]						
2,4-Dipyridyl[a]						
Pyrrolidine						
Piperidine	0.070	0.953	0.068	0.988	0.050	0.993
p-Toluidine[a]	0.031	0.972	0.027	0.989	0.015	0.945
Aniline[a]						
Benzoquinone[a]						
2-(2-Thienyl) pyridine[a]						
2-(3-Thienyl) pyridine[a]						
2-Phenylpyridine[a]						
Propylamine[a]						
Caffeine[a]						
Thiophene[a]						
Benzene[a]						
2-Picoline						

Note: Slope values calculated from retention data at three separate loadings of metal at each mobile-phase composition.

[a] No change in k′ observed.

lows in the general order: Ni > Cu > Zn. This indicates that the $Ni(DPM)_2$ complex forms the strongest, most stable adducts. An examination of the stability constants for the dipivaloylmethane ligand complexes predicts this behavior (see Table 9).[22] The $Cu(DPM)_2$ is less inclined to interact with additional ligands because of its already large stability constant. The $Zn(DPM)_2$ complex has the lowest stability constant but lacks stabilization from crystal field effects, which, in turn, do not favor adduct formation. The $Ni(DPM)_2$, however, has a comparatively low stability constant and gains from crystal field influences, favoring adduct formation, and thereby increasing solute retention. The chromatographic equilibration periods for each of the metal complexes are illustrated in Figure 9. The tetrahedral zinc complex exhibits a faster equilibration on the ODS-3 substrate compared with the more strongly retained nickel and copper square planar species. In addition, the longer breakthrough volume for the $Cu(DPM)_2$ complex indicates a slightly larger hydrophobicity than the $Ni(DPM)_2$ compound. The equilibration periods of these complexes on reversed-phase materials are short compared with the excessive time required for the chromatographic stability with long alkyl-chain, ionic metal additives.

The europium dipivaloylmethane complex is six coordinate with a distorted octa-

TABLE 8
Comparison of Solute dk'/q_{ads} Values Obtained in Each Metal Complex Additive System

Solute	$Ni(DPM)_2$ dk'/q_{ads}	$Cu(DPM)_2$ dk'/q_{ads}	$Zn(DPM)_2$ dk'/q_{ads}
4-Picoline	0.120	0.117	0.044
3-Picoline	0.110	0.083	0.0138
Pyridine	0.08	0.045	0.024
3-Benzylpyridine	0.105	0.100	—
3-Phenylpyridine	0.09	0.085	—
2,3-Dipyridyl	0.065	0.048	—
2,4-Dipyridyl	0.062	0.024	—
Pyrrolidin	0.061	0.019	0.068
Piperidine	0.053	0.013	0.027
p-Toluidine	0.01	—	—
Aniline	0.004	—	—
2-Picoline	—	—	0.005

Note: Conditions were 75.25 MeOH/H_2O, ODS-3 substrate.

TABLE 9
Stability Constants for Metal β-Diketonates

	$Ni(DPM)_2$	$Cu(DPM)_2$	$Zn(DPM)_2$
Stability constants for metal complexes	$lgK_1 = 9.90$ $lgK_2 = 9.10$	$lgK_1 = 13.91$ $lgK_2 = 11.55$	$lgK_1 = 9.3$ $lgK_2 = 8.9$

hedral geometry and a $4f^7\ 6s^2\ 5d^0$ electron configuration.[23] Numerous studies have indicated that europium β-diketones in the presence of Lewis bases form complexes with coordination numbers of eight and nine.[24–26] The 4f orbitals of europium (III) complexes are partially shielded from interaction with ligand orbitals by the outlying 5s 5p shells. This prevents extensive interaction with the 4f orbitals, minimizing increases in stabilization energy from crystal field effects; however, the large ionic radius of the lanthanide metal readily accommodates additional adduct formation.[27] It was therefore anticipated that the $Eu(DPM)_3$ complex, a harder Lewis acid than the previous transition metal complexes, would coordinate additional "solute-ligands", thus increasing the retention of specific hard bases and enhancing their selectivity on the reversed-phase substrate.

Slight increases in retention are observed for the piperidine, phosphorus, and pyrolidine solutes using a 100% methanol mobile phase. However, as the percentage of water is increased, no net increase in retention is exhibited for any of the basic solutes despite heavier loadings of $Eu(DPM)_3$ on the ODS-3 substrate. This suggests that the acidic $Eu(DPM)_3$ chelate promotes additional coordination with specific solutes, thus increasing their retention on the reversed-phase under nonaqueous conditions. But as the water content is increased, competition for coordination sites on the europium is heightened and adduct formation with the nitrogen and phosphorous molecules is precluded (Table 10).

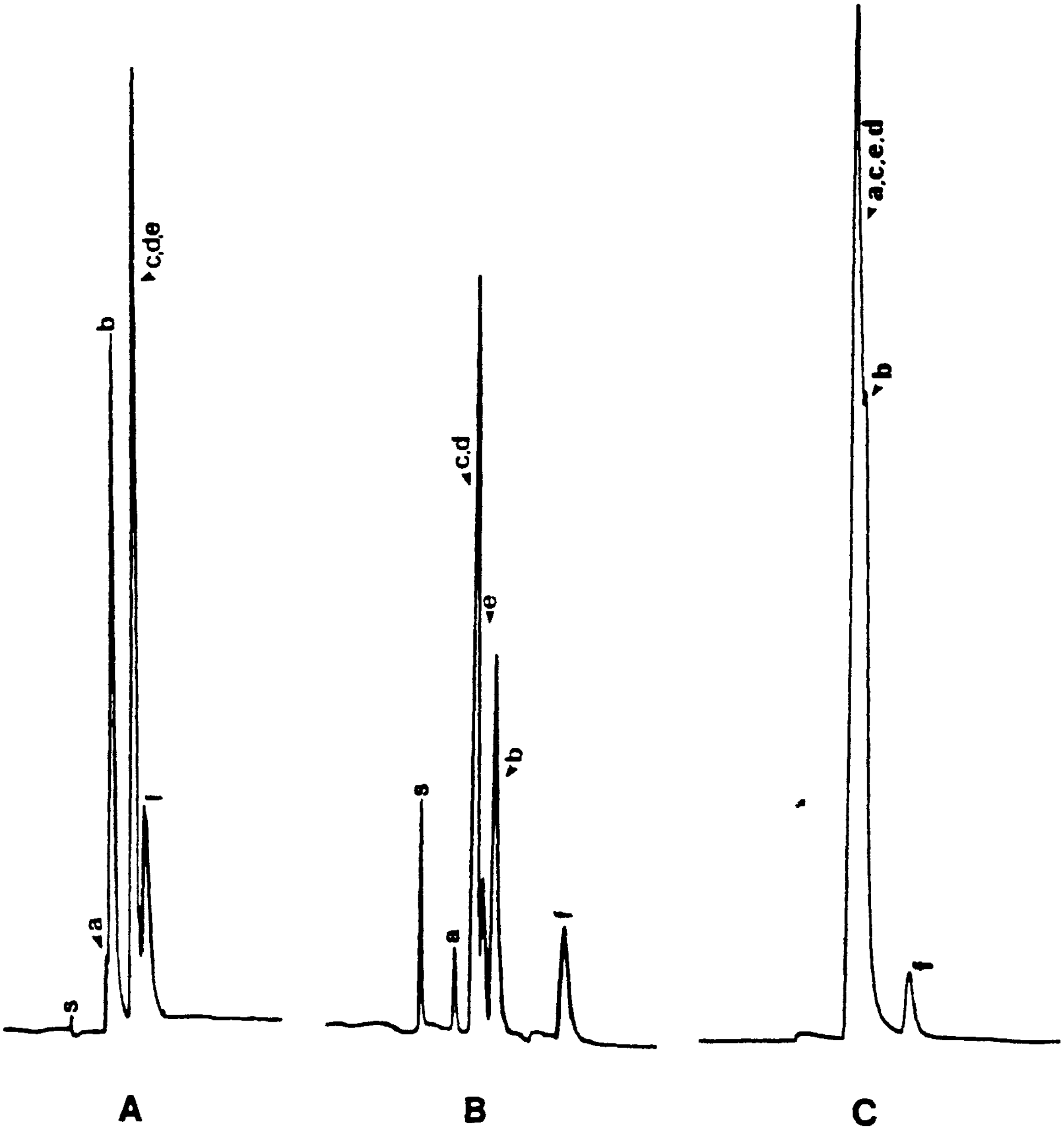


FIGURE 9. Comparison of equilibration periods of the metal β-diketonates in reversed-phase systems. Conditions: ODS-3 reversed-phase substrate, 75/25 MeOH/H_2O, 1.0 × 10^{-4} *M* metal additive in mobile phase, flow rate of 1 ml/min, 30°C.

Additional chromatographic studies were performed on the *tris*(4,4,4-trifluoro-2-phenyl-1,3-butandione)aquo Eu(III) chelate, $Eu(BTA)_3$-H_2O. The adduct formation constants for this fluorinated complex are generally higher than those produced by $Eu(DPM)_3$.[29–30] Despite the increased acidity of the $Eu(BTA)_3$-H_2O complex, trends similar to those exhibited by solutes in the $Cu(DPM)_3$ additive system were again observed. The basic solutes, piperidine and hexamethylphosphoramide, showed increased retention with the europium additive in nonaqueous solvent systems, but as the water content was increased in the mobile phase, no net increase in retention was observed for any of the solutes.

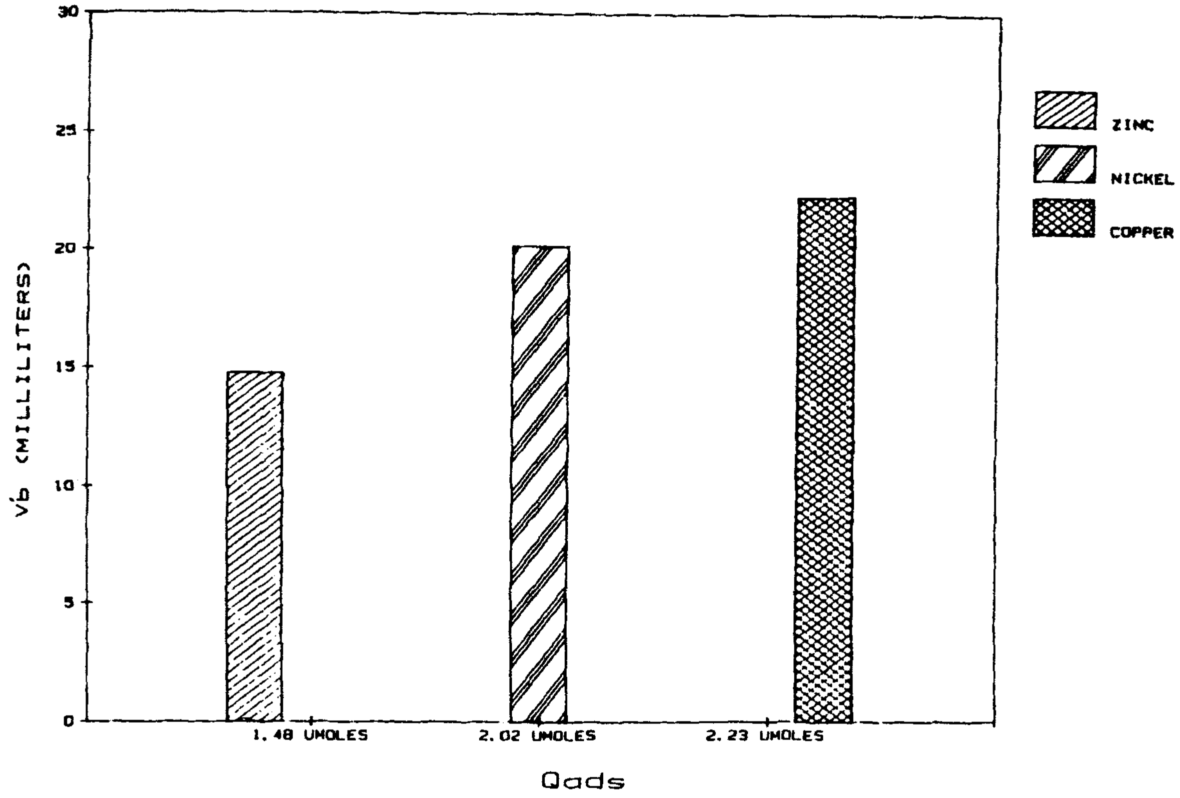


FIGURE 10. Comparison of equilibration periods of the metal β-diketonates

TABLE 10
Influence of Aqueous Mobile-Phase Composition on Solute Retention in Eu(DPM)$_3$ System

Solutes	100% MeOH			80/20 MeOH/H$_2$O			75/25 MeOH/H$_2$O		
	k′	k′	dk′	k′	k′	dk′	k′	k′	dk′
4-Picoline	0.11	0.10	[a]	0.41	0.41	[a]	1.1	1.13	[a]
3-Picoline	0.10	0.10	[a]	0.39	0.39	[a]	1.04	1.05	[a]
2-Picoline	0.10	0.09	[a]	—	—	[b]	0.97	0.96	[a]
Benzene	0.095	0.19	[a]	0.66	0.65	[a]	2.42	2.43	[a]
3-Phenylpyridine	0.13	0.13	[a]	0.77	0.78	[a]	4.30	4.26	[a]
Dimethylforamide	0.10	0.098	[a]	0.14	0.14	[a]	0.17	0.17	[a]
Dimethylsulfoxide	0.10	0.098	[a]	0.118	0.12	[a]	0.11	0.11	[a]
Piperdine	0.17	0.12	0.05	0.42	0.43	[a]	1.03	1.05	[a]
Pyrrolidine	0.12	0.09	0.03	0.19	0.19	[a]	0.97	0.98	[a]
Benzoquinone	—	—	[b]	0.62	0.62	[a]	—	—	[b]
2,2-Bipyridyl	0.14	0.14	[a]	—	—	[b]	—	—	[a]
Glutaric Acid	—	—	[b]	0.44	0.45	[a]	1.57	1.58	[b]
Hexamethyl-phosphoramide	0.092	0.06	0.032	0.30	0.31	[a]	0.34	0.35	[a]
	q_{ads} = 0.80 μmoles [Eu(DPM)$_3$] = 1.0 × 10^{-4} *M*			q_{ads} = 2.20 μmoles [Eu(DPM)$_3$] = 1.0 × 10^{-4} *M*			q_{ads} = 4.6 μmoles [Eu(DPM)$_3$] = 1.0 × 10^{-4} *M*		

[a] No net increase observed.
[b] Not used in run.
[c] Concentration of complex in mobile phase.

In order to evaluate the influence of water on the solute-europium complex interaction, fluorescence solution studies were performed. It has been shown that a Lewis base interaction with fluorescent europium β-diketonates, such as $EU(BTA)_3$-H_20 generally enhances the ionic fluorescence intensity.[31] This fluorescence increase is attributed to the additional perturbation of the molecular field surrounding the metal by further adduct formation.[32] Monitoring the fluorescence intensity fluctuations produced by the addition of Lewis base in varying aqueous environments qualitatively estimates the influence of water on these interactions.

When the Lewis base piperidine interacts with the $Eu(BTA)_3$-H_2O complex, the overall fluorescence intensity is increased. However, as the water content of the solvent is increased, the normalized change in fluorescence intensity declines. This may suggest that water molecules are interfering with solute-europium complex interactions and are thus preventing an increase in fluorescent intensities. These spectral studies are meant only to provide a possible qualitative explanation for the ineffectiveness of europium β-diketonates as mobile-phase additives in aqueous reversed-phase systems. The chromatographic and fluorescence solution studies show that interaction of a solute with $Eu(DPM)_3$ and $Eu(BTA)_3$-H_2O does occur to some extent and that steric hindrance from the bulky diketone ligands does not completely prevent additional solute coordination. Further studies using nonaqueous mobile phases where sufficient quantities of europium complex can be sorbed to the stationary phase are warranted.

REFERENCES

1. **Leyden D. E. and Luttrell, G.,** *Anal. Chem.*, **1975,** *47*, 1161.
2. **Dua, V. and Bush, C.,** *J. Chromatogr.*, **1982,** *244*, 128.
3. **Chow, F. K. and Grushka, E.,** *J. Chromatogr.*, **1979,** 185.
4. **Naleway, J. and Hoffmann, N.,** *Anal. Lett.*, **1981,** *14*, 1711.
5. **Lindner, W., LePage, J., and Karger, B.,** *J. Chromatogr.*, **1979,** *185*, 323.
6. **Hare, P. E. and Gil-Av, E.,** *Science*, **1979,** *204*, 1226.
7. **Gilon, C. and Grushka, E.,** *Anal. Chem.*, **1980,** *52*, 1206.
8. **Lochmüller, C. H. and Hangac, H. H.,** *J. Chromatogr. Sci.*, **1982,** *20*, 171.
9. **Lochmüller, C. H., Hill, W. B., Jr., Porter, R. M., Hangac, H. H., Culberson, C. F., and Ryall, R. R.,** *J. Chromatogr. Sci.*, **1983,** *21*, 70.
10. **Cotton, F. A. and Wise, J. J.,** *Inorg. Chem.*, **1966,** *2*, 1200.
11. **Hammond, G.,** *Inorg. Chem.*, **1963,** *2*, 57.
12. **Gmelin.** *Coordination Compounds*, 1982, 207–209.
13. **Horvath, C., Melander, W., and Nahum, A.,** *J. Chromatogr.*, **1979,** *186*,371.
14. **Karger, B. L., Grant, J., Hartkopf, A., and Weiner, P. H.,** *J. Chromatogr.*, **1976,** *128*, 65.
15. **Huheey, J.,** *Inorganic Chemistry*, Harper and Row, London, 1978.
16. **Snyder, L. R. and Kirkland, J.,** *Introduction to Modern Liquid Chromatography*, John Wiley & Sons, New York, 1979, chap. 6.
17. **Fackler, J., Cotton, F. A., and Barum, D. W.,** *Inorg. Chem.*, **1963,** 2, 97.
18. **Partheimer, W. and Drago, R. S.,** *Inorg. Chem.*, **1970,** *9*, 47.
19. **Cotton, F. A. and Wood, J. S.,** *Inorg. Chem.*, **1964,** *3*, 245.
20. **Madden, D. P., Mota, M., and Nelson, S. N.,** *J. Chem. Soc. A*, **1970,** 790.
21. **Graddon, D. P. and Weeden, D. G.,** *Austr. J. Chem.*, **1964,** *17*, 607.
22. **Gmelin.** *Handbook of Inorganic Chemistry*.
23. **Ryba, M.,** *J. Chromatogr. Sci.*, **1981,** *219*, 245.

24. **Ohlmann, R. and Charles, R.,** *J. Inorg. Chem.*, **1964,** 131.

25. **Ohlmann, R. and Charles, R.,** *J. Inorg. Chem.*, **1965,** 2.

26. **Selbin, R. and Bhacca, N.,** *Inorg. Chem.*, **1971,** *10*, 1383.

27. **Cotton, F. A.,** *Advanced Inorganic Chemistry*, John Wiley & Sons, New York, 1972.

28. **Shepherd, T. M.,** *J. Inorg. Nucl. Chem.*, **1967,** *29*, 2551.

29. **Shigamatsu, T. and Honjyo, T.,** *Bull. Chem. Soc. Jpn.*, **1967,** *29*, 2551.

30. **Shigamatsu, T. and Honjyo, T.,** *Bull. Chem. Soc. Jpn.*, **1967,** *45*, 282.

31. **Saoki, Y. and Okaxaki, Y.,** *Chem. Pharm. Bull.*, **1978,** *26*, 1066.

32. **Kleinerman, M. and Hove, R.,** *J. Chem. Phys.*, **1964,** *41*, 4009.