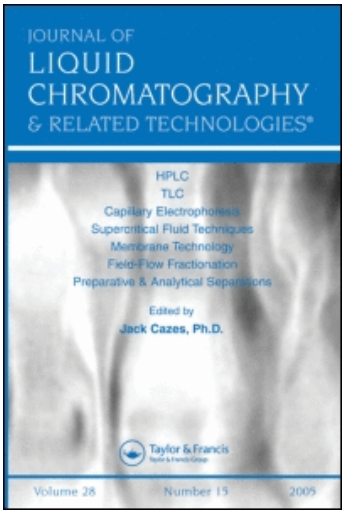

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THEORY AND APPLICATION OF REVERSED PHASE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Reversed phase thin layer chromatography (RPTLC) is reviewed for the period 1970 to present. Both physically coated plate and chemically bonded stationary phase techniques are examined. Background information on RPTLC is presented as well as an exhaustive review of the literature for analytical applications. Coated plate techniques are tabulated by stationary phase and solute, and sources for currently available bonded phase plates are listed.

INTRODUCTION

Thin layer chromatography is a simple, rapid, and inexpensive technique that can be applied to a wide variety of separation problems. Besides extensive use in research, thin layer chromatography is relied upon heavily by the more applied fields such as the health sciences, environmental analysis, and industry. Development of TLC technology has been rapid. Improvements in sample application, elution techniques and new methods of detection are complementary adjuncts to advances being made in the

TLC plate itself. Moreover, TLC has benefited from the rapid growth in high performance liquid chromatography (HPLC) column technology e.g., with introduction of small uniform packing materials and a variety of chemically bonded phases.

Most TLC separations are performed in the normal phase mode on silica beds using relatively nonpolar solvents for chromatographic development. This mode of elution suffices for many applications, and will always be important. However, recent introduction of chemically bonded reversed phase TLC plates by several manufacturers has made reversed phase thin layer chromatography (RPTLC) an attractive alternative to normal phase separations, in instances where polar silica gel layers are unsuited. More importantly, RPTLC expands the scope of thin layer separations to include compounds traditionally not separable by normal phase TLC because of solubility problems associated with the solute. This review will examine some of the advantages, applications, and potentials of reversed phase thin layer chromatography for both physically coated and chemically bonded layers. A period of approximately ten years is reviewed, and the most pertinent references are included in the bibliography.

PHYSICALLY COATED VS CHEMICALLY BONDED PHASES

The term "reversed phase" was coined in 1941 in the classic paper by Martin and Synge (1), and refers to the use of nonpolar

stationary phases and polar eluents in chromatographic systems. This mode of operation is opposite that of a normal phase separation in that the mobile phase is more polar than the stationary phase. Reversed phase thin layer chromatography can best be subdivided into two categories: physically coated stationary phases and chemically bonded phases. Although each utilize nonpolar stationary phases, substantial differences exist between the two approaches.

Physically coated plates consist of conventionally prepared normal phase TLC plates that are coated with a nonpolar liquid phase. The coating process is simple. The TLC plate is dipped into a hexane or benzene solution containing the nonpolar liquid, removed, and then allowed to dry. Alternatively, the nonpolar phase can be applied by "chromatographing" the liquid phase solution onto the TLC plate. As with the preparation of gas chromatographic columns, the percent loading is controlled by the concentration of the solution used to coat the chromatographic support. Concentrations range from 5% to 20%, but 10% is typical.

A wide variety of compounds and mixtures have been used to coat TLC plates (see table I). Most commonly used are paraffin oil (2), silicone oil (3), octanol (4), and oleyl alcohol (5). In a systematic study by Breyer et al. (6), the effects of varying the chain length of the coated stationary phase was examined for a homologous series of alcohols. Alcohols with chain lengths below 12 and above 16 gave unsatisfactory separations or irreproducible

TABLE I
Reagents For Coated Plate RPTLC

REAGENT	REFERENCES	REAGENT	REFERENCES
AMINES		OILS	
Aliquot 336	9, 84	Castor oil	2, 7
Alamine 336S	9,84	Corn oil	8
Amberlite LA-1	9	Paraffin oil	2,5,69,73,74 81,82,83,88
Primene JM-T	9, 84		
Amberlite LA-2	84	Peanut oil	8
		Pump oil	90
		Silicon oil	3,5,24,69,70 72,76,77,78, 91
ALCOHOLS		MISC	
C_8	4,6,72	Fatty acids	68, 5
C_9	6	Pyrozol	85
C_{10}	6	Squalane	69
C_{12}	6	Triolein	2
C_{14}	6	Hexadecane	80
C_{16}	6	Undecane	69,75,89
C_{18}	6		
oleyl alcohol	5,66,67,79		

results. Several naturally occurring oils, including castor oil (7), corn oil (8), and peanut oil (8) have also been used, although the differences between these phases has not been addressed. RPTLC separations have been performed on plates coated with amines or organophosphorous compounds, particularly in extraction chromatography (a separation technique based on complex formation or ion exchange). Commercially available amine preparations such as Aliquot 336 (9), Alamine 336S (10), Amberlite LA-1 (11), and Primene JM-T (9) are commonly used to make these plates.

Chemically bonded stationary phases have received much more attention in HPLC than in TLC. However, this appears to be changing as researchers become more familiar with these bonded phases. Much of what has been learned about bonded phases in HPLC is directly applicable to TLC. A number of excellent papers have been written on the preparation and properties of bonded phases in HPLC (12-16), and they should be consulted for detailed information. However, a few generalizations can be presented here. The bonded alkyl phase is produced by the reaction of alkylchlorosilane with the silica support (see figure 1). The alkylchlorosilane electrophilicly attacks the silanol (Si-OH) oxygen found on the silica surface with subsequent elimination of HCl. Two types of bonded phases are commonly prepared: (i) Monomeric bonded phases result from the reaction of silica with monofunctional alkylsilane reagents. For example, the reaction of

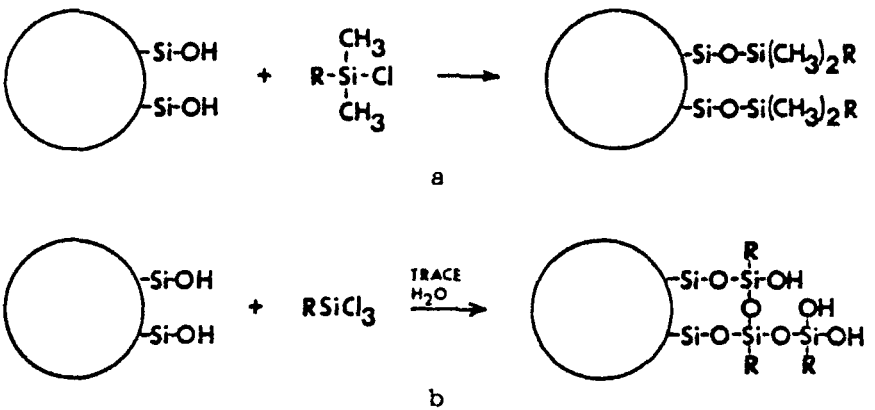

Figure 1.

dimethyloctadecylchlorosilane with silica results in a bonded layer one molecule thick. (ii) Polymeric phases result from the use of di- or tri-functional alkylsilane reagents. Trace amounts of water present in the reaction media cause some self polymerization of the silane reagent in addition to previously discussed electrophillic attack on the silanol groups. Truely monomeric phases can be produced from polyfunctional alkylsilanes if water is rigorously excluded from the reaction media. As might be expected, the reproducibility of polymeric phases is only fair to poor, due to the high dependence of the reaction on trace amounts of water present. The reproducibility of the monomeric bonded surface is excellent (since the reaction is complete, and only limited by steric hindrance of the bonded phase towards itself and unreacted silanol groups). Monofunctional silanes are hydrolyzed by water, but as long as an excess of the reagent is present, this side reaction generally does not affect the bonding efficiency.

Bonded reversed phase TLC plates can be prepared in two ways. In one process, silanized silica gel is mixed with a binder, slurried in a nonpolar solvent, and spread on glass plates to dry. Specialized devices are available for spreading uniform layers of specified thickness. When dry, the plates are ready for use. In the second procedure, commercially prepared silica TLC plates are silanized by an in situ reaction. Gilpin and Sisco have described in detail the apparatus and steps used in this process (17).

Stated simply, the TLC plates are immersed in a silanizing reaction mixture, from which water has been rigorously excluded. In this way, highly reproducible bonded phases are prepared.

THEORY OF SOLUTE RETENTION

Solute retention in thin layer chromtography is traditionally characterized by R_f values, where R_f is defined as (distance traveled by the solute) / (total distance of solvent movement). Thus for an unretained solute, R_f=1 and for a totally retained solute R_f=0 (e.g., $0 \leq R_f \leq 1$). In high performance liquid chromatography, retention is designated by k':

$$k' = t_R - t_0 / t_0 \qquad [1]$$

where t_R is the retention time of the retained solute and t_0 is the retention time of the unretained solute. In an effort to relate these two important chromatographic techniques, it it can easily be shown that R_f and k' obey the following relationship:

$$k' = (1-R_f)/R_f \qquad [2]$$

Consequently, if the same mechanisms of solute retention were to occur on a TLC plate and in an HPLC column, retention data between the two techniques would be transferable. However, this is seldom observed and empirical relationships are often more useful.

The flow rate of the mobile phase in TLC depends predominantly on the solvent used and the specific characteristics of the thin layer. Guiochon et al. (18-20) related the migration distance, z, of the solvent front above the tank solvent level to the

development time, t, according the the following equation:

$$z = \sqrt{(2k_o d_p t\gamma/\eta)\cos\theta} \qquad [3]$$

where d_p is particle diameter, γ the surface tension, η the viscosity of the mobile phase, and θ the wetting angle of the solvent on the adsorbent surface. The parameter k_0 is complex in nature and is representative of the bed permeability, the pore distribution around the outside of the particles, and the relative relationship between solvent front and bulk velocities (21). In normal phase TLC, $\cos\theta=1$ with all solvents used. However, with RPTLC, $\cos\theta$ decreases rapidly as the percentage of water in the solvent is increased. This critical parameter is often responsible for long analysis times when the mobile phase contains high percentages of water. For example, it has been reported that it requires 90 minutes (a prohibatively long development time) to achieve a 4 cm rise on an RP-18 plate using (60:40) methanol-water (22).

Another measurement in TLC, used to express the relative lipophilic character of a solute, has been designated R_m, where R_m is defined:

$$R_m=\log(1/R_f-1) \qquad [4]$$

In reversed phase chromatography, small R_m values indicate compounds of low lipophilic character and larger R_m values denote high lipophilicity. The Hansch parameter π (23) is also used as a measure of lipophilicity but is more representative of lipophilic

character contributed by a substituent and is illustrated by the following:

$$\pi = \log(P_X/P_H) \quad [5]$$

where P_H and P_X are the partition coefficients of unsubstituted and substituted compounds, respectivly. Note that the partition coefficient P (P=(concentration solute in stationary phase) / (concentration solute in mobile phase)) is also referred to as K_p or K in the literature.

Mechanisms of solute retention in reversed phase chromatography is a much debated subject and many papers have been published on the topic. Of the coated and bonded reversed phase methods, theory of solute retention in physically coated TLC plates is the least controversial. It is generally agreed that the major contribution to retention in coated plate RPTLC is from partitioning of the solute between two immiscible liquids. The concentration of solute in each phase is described by the distribution coefficient, K_p which in turn can be related to the free energy change for the solution process where $\Delta G° = -RT\ln(K_p)$. This observed partitioning mechanism for coated RPTLC makes this technique valuable for the chromatographic measurement of K_p values for certain eluent systems. For example, several workers have derived relationships between R_f values (from coated RPTLC) and K_p values (obtained by the "shake" method) (24,3,5). Measurement of K_p values for octanol/methanol and other solvent systems is one of the largest current applications of coated plate

RPTLC, because this is a quick and easy technique for determining relative degrees of lipophilicity. It should be noted that although partitioning is probably the major factor in retention, active silanol groups are also present on the silica support, and in some systems, adsorption may occur (3).

Understanding solute retention on chemically bonded phases is a much more difficult problem and no general consensus exists for the retention mechanism. Again, most of the work in the literature is concerned with HPLC, but its applicability to bonded phase RPTLC seems apparent. It is widely believed that the nature of a bonded phase is in someway different from that of the corresponding physically coated liquid. Since bonded phase molecules are attached at one end, the resulting phase is more ordered than a liquid. That is bonded phases have fewer degrees of freedom than liquids, and in the case of monomeric phases, are only one molecule thick. For these reasons and others, the liquid partitioning mechanism credited to coated supports is usually discounted as an explanation of bonded phase solute retention.

One current theory suggests that retention on chemically bonded phases is governed by hydrophobic interactions. The hydrophobic effect can be described as the tendency of a nonpolar solute molecule to reduce its surface area exposed to water -- either through association with other nonpolar molecules, or through removal from the solution by adsorption (25) -- and thereby increase the entropy of the system. Horvath (26), Karger

(27), and Colin (28) have described these interactions in detail. The major limitation of various hydrophobic retention theories is that they do not explain the changes in selectivity observed for different nonpolar bonded phases (e.g., C_{18} vs C_2 and C_8).

A number of approaches have been made in the effort to further understand the bonded phase retention mechanism. Hemetsberger et al. investigated effects of hydrocarbon length (29) and structure (30) for several chemically bonded phases. Unger and Roumeliotis systematically studied several dimethylalkyl phases (31), and Locke related retention to solute solubility in the eluent (32). In addition, Löckmuller presented an interesting theory supporting a pseudopartitioning mechanism with bonded phases by envisioning the phase as "liquid droplets" (33). It is evident that bonded phase retention theory is still evolving and further developments will be forthcoming.

COMMERCIALLY AVAILABLE RPTLC PLATES

Several manufacturers presently offer TLC plates designed for use with reversed phase solvent systems (see TABLE II). A wide variety of chemically bonded hydrocarboneous phases are available, with chain lengths ranging from C_2 to C_{18} and percent surface coverages from 50-100% of accessible reactive sites. Of the RPTLC plates listed, only the RPS Uniplate, manufactured by Analtech (Newark, Delaware) utilizes a physically coated stationary phase. The RPS Uniplate consists of a hard analytical layer impregnated

TABLE II

Commercially Available RPTLC Plates

MANUFACTURER	PLATE	STATIONARY PHASE	MAX % H_2O	INDICATOR	COMMENTS
Analtech	RPS Uniplate	long chain hydrocarbon	100	no	Coated stationary phase; Available with preadsorbent zone.
Nacherey-Nagel	Nano-RP-plates				
	SIL C_{18}-100	C_{18}	-	yes	maximal surface coverage
	SIL C_{18}-75	C_{18}	-	yes	75% maximal coverage
	SIL C_{18}-50	C_{18}	-	yes	50% maximal coverage
Merck	RP-HPTLC plates				
	RP-2	C_2	60*	yes, F_{254s}	Acid resistant indicator
	RP-8	C_8	40*	yes, F_{254s}	
	RP-18	C_{18}	35	yes, F_{254s}	
Tridom	Opti UP C_{12}	C_{12}	100	no	Complete water compatibility
Whatman	KC_{18}	C_{18}	40	no	
	$KC_{18}F$	C_{18}	40	yes, F_{254}	
	$KC_{18}D$	C_{18}	40	no	channeled
	$KC_{18}DF$	C_{18}	40	yes, F_{254}	channeled
	Multi-K type CS5	C_{18}/silica			dual mode plate

* see reference 52

with a long chain hydrocarbon, and is designed to correlate retention behavior observed in RPTLC with that seen in modern HPLC. The hydrocarbon coating is not appreciably soluble in water or alcohol, and the plates are intended for single use only. Uniplates are available with 250, 500, and 1000 Å layer thicknesses for analytical and preparatory applications. Furthermore, the plates may be obtained with preadsorbent or preconcentrating zones for increased resolution of highly retained nonpolar substances. E. Merck (Darmstadt, Germany) produces three chemically bonded RP-HPTLC plates that have different chromatographic selectivities. These plates come with C_2, C_8, and C_{18} bonded hydrocarbon chains and are known commercially as RP-2, RP-8, and RP-18 respectively. Macherey-Nagel also produces three reversed phase plates (Nano-RP-Plates), but these plates use a C_{18} bonded phase but differ in their percent carbon loading. The Nano-RP plates are compatible with highly aqueous eluents and are limited only by their surface wettability. For example, at high water concentrations, SIL C_{18} 50 plates (50% of reactive silanol groups are bonded) work best, and with a 1:3 methanol/water eluent, development occurs at a rate of 4.0 cm/15 minutes. Plates with higher carbon loading develop considerably slower with high percentages of water. The Opti UP C_{12} plate, manufactured by Tridom (Hauppauge, New York) has a C_{12} bonded stationary phase and is indicator free. The plates are reported to be compatible with most reversed phase solvents including 100% water. This is of

importance not only for choice of eluent, but also in detection, since water based visualizing reagents may be used (see LIMITATIONS). Whatman (Clifton, New Jersey) offers two types of reversed phase plates, a bonded C_{18} plate (KC_{18}) and a dual-mode, reversed phase and normal phase plate (Multi-K type CS5). The KC_{18} product is available in several forms, with or without indicator and grooved or ungrooved. The stability of this plate to water (stable up to 40% water) may be increased by the addition of NaCl to the mobile phase. The Multi-K type CS5 plate is a hybrid of reversed and normal phase TLC. The plates consist of a 3 cm wide C_{18} bonded layer contiguous with a silica gel analytical layer on a single 20 x 20 cm plate. In actual use, the sample is spotted on the reversed phase layer and developed under reversed phase conditions. Additional separation is obtained by normal phase development 90 degrees from that of the first. The particularly complex separations that have been obtained on this plate (e.g., see figure 2) indicate the enormous potential of this new technique.

Several of the commercially available RPTLC plates have been designed to correlate to HPLC retention. The retention order and magnitude for solute mixtures is generally similar for the two techniques. One use advanced by manufacturers for the reversed phase plates is to scout solvent systems for HPLC separations (34-35). RPTLC plates offer advantages of speed and economy, and permit preliminary evaluation of uncharacterized samples without

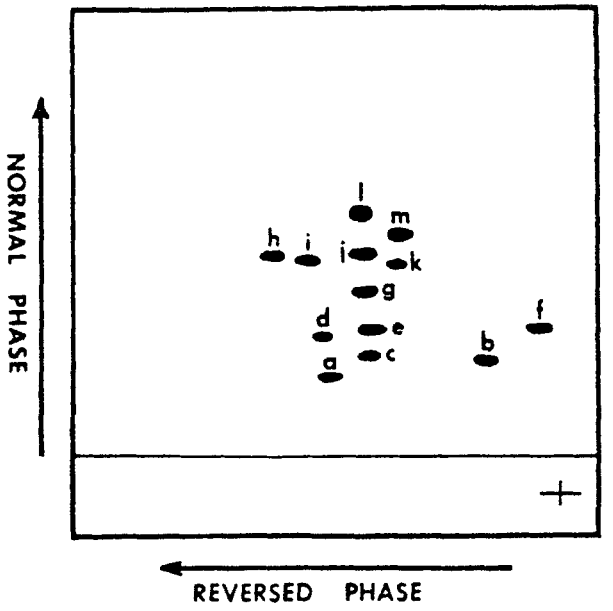

Figure 2.

risking expensive HPLC columns. Suitable solvent systems can often be quickly determined using RPTLC, and then transferred to HPLC.

USE OF RPTLC PLATES

Unlike conventional plates coated with silica gel, modern RPTLC plates do not require activation or other preparation prior to use. In normal phase TLC, the activity of the plate used (i.e., the number and type of silanol groups available to solute interactions) must be carefully controlled to insure reproducible retention. Adjustment of water content of the silica layer is usually accomplished by heating and equilibration under controlled

conditions. In RPTLC, most accessible silanol groups are deactivated by the addition of chemically bonded hydrocarbon chains. Solvent systems used in reversed phase separations consist of polar liquids -- usually solutions of water and methanol, acetonitrile, or acetone which cancel out the effects observed for water in normal phase. Thus, control of the initial water content of RPTLC plates has little effect on solute retention. Sample preparation in the reversed phase separation mode is only slightly different from normal phase TLC. For example, several of the commercial RPTLC products are not compatible with water (due to stability and/or wettability limitations); consequently solute solutions should be made up in organic solvents. Methanol is usually a convenient choice. Siouffi et al. (22) note that to prevent excessive band spreading of the original solute spot, the sample should be dissolved in a solvent of lower elutropic strength than that used for chromatographic development. Plates with preconcentrating zones (e.g., Analtech's RPS Uniplate) help to minimize the effects of an original finite spot size by compressing the spot vertically as development is started. Solvent selection, of course, depends on the separation problem at hand, but Whatman suggests ethanol/water (80:20) as a starting point for separations on KC_{18} plates. Equilibration of the tank prior to development (via a solvent saturated filter paper lining) is recommended. Analysis times vary widely with the solvent system and brand of RPTLC plate used.

TABLE III
Distance of Solute Travel in mm for a 15 Minute Separation Using Machery-Nagel Nano-RP Plates (36)

SOLVENT	SIL C18 100%	SIL 18 75%	SIL 18 50%
CH_3OH/H_2O			
2:1	45	51	57
1:1	21	32	52
1:2	0	11	50
1:3	0	0	40
0:1	0	0	0
CH_3CH/H_2O			
2:1	46	51	62
1:1	30	35	52
1:2	27	33	51
1:3	15	25	48
1:9	0	0	20

However, the rate of development decreases dramatically with increasing water content of the eluent, and stops completely as the wettability limit is reached (see table III). Above 30% water irreproducible results have been observed for certain chromatographic systems (22).

Chemically bonded phases in TLC are in general quite stable to common organic solvents. It should be noted, however, that under acidic or basic conditions these phases may undergo slow hydrolysis, but due to the short term processes of TLC separations (as opposed to column separations in HPLC) separations in these solvent systems are quite feasible. Furthermore, if relatively neutral solvents are used and the coated surface is not physically altered, bonded plates can in many cases be washed with solvents and reused up to five times with no apparent change in

chromatographic properties (37). Normal phase separations are also possible on bonded phase plates, with the plates behaving as deactivated silica gel plates. Normal phase separations using RPTLC plates may be applicable to polar compounds that have traditionally been irreversibly adsorbed on standard silica TLC plates.

ANALYTICAL APPLICATIONS: BONDED PHASE RPTLC

Bonded stationary phases for TLC have only recently become commercially available. As a result, it has only been in the last two years that applications using these plates have appeared in the literature. A number of papers have dealt with the separation of biogenic substances. Ryu and MacCoss chromatographed phospholipid derivatives using a buffered eluent on Whatman KC_{18} plates (38). A modified Dittmer-Lester reagent was used for visualization, and the detection limit was reported to be 1-5 μg. Vidrine and Nicholas separated 8 nonpolar lipids using a mixed mode plate of their own design (39). In their two dimensional chromatographic approach, the lipids were first separated by normal phase TLC with a hexane/ethyl acetate eluent, and then by reversed phase TLC with a p-dioxane/water system. The retention behavior of cholesterol has also been studied, on home-made plates by Halpaap et al. (40). Adsorption and reversed phase partition mechanisms were identified, depending upon the eluent used. Separation of saturated and unsaturated cholesteryl esters has

been performed on K_{18} plates (41). Raedsch et al. (42) separated individual sulfated bile acid conjugates as calcium complexes using KC_{18} plates . Free fatty acids of chain length C_2 through C_9 have been resolved using RPTLC (43). The acids were radioactively labeled, and detection was by autoradiography. Pheyacyl esters of fatty acids (44), and free amino acids (45) have been separated on KC_{18} plates. In a novel separation of nucleoside residues, micellar solutions produced by addition of surfactants to organic solvents were used as the eluents on Merck RPTLC plates (46). The mechanism of interaction is believed to involve both electrostatic interaction with the polar head group and stabilization in the water pool of the hydrophilic cored micelle. Hsiung et al. (47) examined the feasibility of RPTLC separation of several nucleotide fragments. In this work, eight deoxyribooligonucleotide fragments of human insulin A DNA were resolved using two dimensional chromatography on KC_{18} plates.

Many papers concerning the RPTLC separation of various aromatic compounds have also appeared. Brinkman and de Vries measured the retention of aromatic oxy compounds, aminophenols and aromatic acids on a series of reversed phase plates (48). The performance of KC_{18} (Whatman), RP-2, RP-8, RP-18 (E. Merck) as well as RPS Uniplate (Analtech) products were evaluated. For the samples run, KC_{18} and (Merck RP plates were found to perform equally well. However, development on the KC_{18} plates was found to be 3 to 4 times faster than the RP plates. Solute retention

behavior between the KC_{18} and RP-8 were essentially identical. RPS Uniplates were not directly compared due to the different composition of this plate. In a separate study, Brinkman and de Vries chromatographed chrysene, coronene, fluorene, perylene, phenanthrene, and pyrene, and were able to resolve all but chrysene and fluorene (49). Polynuclear aromatic hydrocarbons were also separated by Dzido and Soczewinski (50), Janchen and Schmutz (51), and Halpaap et al. (40) with varying degrees of success. Sander and Field used gradient elution TLC to separate hydroquinone and catechol from pyrine and 2-methylnaphthalene (37). A correlation between k' (column) and R_f (plate) was demonstrated for barbituates and n-alkylbenzenes on both Merck and Whatman products ($k'=const(1/R_f - 1)$) (52). In addition, water limitations for each plate used were reported.

Other applications have included flavanoids, phenolic and related compounds (53), cobalamins (including vitamin B_{12}) (54), unsaturated fatty acids (55), and separation of the diastereoisomers of Zeranol (56). Using Multi-K type CS5 plates, sulphonamides (57), lubricating oil extract (58), and human bile acids (59) have been chromatographed. Each sample was chromatographed by two dimensional chromatography using reversed phase and normal phase elution. The use of RPTLC in the analysis of pharmaceutical preparations has been demonstrated. Sherma and Beim separated the three components of APC tablets and determined caffeine quantitatively by densitometry (60). Tricyclic

neuroleptics (tranquilizers useful in the treatment of schizophrenia) were chromatographed, and their geometric isomers were resolved (61). Penicillin N and Cephalosporin C were separated using a 100% water eluent with OPTI-UP C_{12} plates (62). Volkman used ion pair chromatography in conjunction with (Merck) RP layers to separate phenothiazine bases and sulphoxides (63). Becker et al. separated quercetin and related compounds (64), and Sherma and Latta chromatographed a complex mixture of chloroplast pigments (65). Because of the recognized lability of these pigments, chlorophylls and carotenoids were found to make excellent probes for testing bonded TLC plates for silica surface activity. No reaction or change due to interaction with the silica surface was regarded as a negative response for surface activity.

ANALYTICAL APPLICATIONS: COATED PHASE RPTLC

Coated plate techniques have been used for many years in TLC separations for a broad spectrum of applications. Because a true liquid is used for the stationary phase, (and assuming silanol interactions are minimal for the solvent system in question), solute retention can be described by a partitioning model. Hulshoff and Perrin developed an RPTLC method for the determination of partition coefficients for a series of phenothiazines (5). Twenty six different phenothiazine drugs were characterized (66), and R_m values were related to albumin binding

TABLE IV
RPTLC APPLICATIONS

SOLUTE	BONDED PHASE REFERENCES	COATED PHASE REFERENCES	MIXED PHASE REFERENCES
AROMATIC			
amines	48		94, 95
acids	48	3	
flavonoids	53		92
phenolic compounds	48, 53	70	
oxy compounds	48		
PNA's	40,49,50,51	82	
quinones		81	
BIOGENIC SUBSTANCES			
amino acids	45		
bile acids	42, 59		
plant pigments	65	2, 8	
cholesterol	40, 41	75	
capsaicenoids (pepper)		74	
fatty acids	43, 46, 55		
gliotoxin analogs			90
lipids	38, 39		
nucleotides	46, 47		
peptides	62		
phosphatidyl cholines			89
steroids		71, 73	
PHARMACEUTICALS			
APC (analgesic)	60		
barbituates	52	7	
benzodiazepines		72, 79	
cobalamins	54		
cephalasparins	64	77	
neuroleptics	61		
penicillin compounds	62	4,24,76	91
phenothaiazine	63	5, 66, 67	
probiotics		68	
rifamycin		78	
sulphonamides	57, 61	69	95
vitamin K2		80	
zeronol isomers	56		
MISCELLANEOUS			
amines			88,93
food dyes			96
lubricating oil	58		
mercury compounds		83	
metal ions		87	
surfactants		6	

constants (67). Bird and Marshall obtained partition coefficients for penicillines using an n-octanol stationary phase (4). Fujii et al. (68) examined chromatographic retention of 10 probiotics (ω-amino acids and L-histadine dipeptides) on several coated liquid phases. A series of C_{16}, C_{18}, and C_{20} fatty acids, ethyl esters, and alcohols were investigated as possible stationary phases. A correlation was made between reversed phase retention, solute structure, and biological response. Such "structure-activity relationships" have been studied in detail by Biagi et al. The relationship between lipophilic character and R_m values have been examined for sulfonamides (69), phenols (70), steroids (71), and benzodiazepines (72). In these studies, R_m values were used as an indication of lipophilic character of the selected solute molecules.

Complex biogenic mixtures have been separated with some success using coated plate techniques. Silica layers have traditionally been unsuited for the separation of chlorophyll pigments and their derivatives due to the previously discussed lability of these compounds on the silica surface. Jones et al. (8) chromatographed a series of chlorophylls on silica gel G coated with corn or peanut oil and no artifacts were observed. In another study, 26 chlorophyll derivatives were separated using castor oil and paraffin oil (2). Other biogenic substances analyzed by coated plate RPTLC have included sterols (73) capsaicenoids (ingredient in hot peppers) (74), and cholesterol

and related compounds (75). A number of pharmeceuticals have been chromatographed with success, using coated plates. Herbst detected penicillin G and ampicillin in the presence of tetracylines and penicillamine by separation on a silicone oil stationary phase (76). Penicillines and cephalosporins were chromatographed by Biagi et al. (24,77). Tischler et al. (78) chromatographed rifamycin derivatives and related the R_f values (and Hansch parameter π) to lipophilicity. Hulshoff and Perrin determined partition coefficients for 1,4 benzodiazepimes (79) and Pla-Delfino et al. (7) examined the retention behavior of 5 substituted barbituates. Vitamin K_2 isoprenologues were chromatographed using 5% hexadecane stationary phase (80). In other unrelated but scientifically interesting work, isoprenoid quinones (81), polychlorinated biphenyls (82), alkyl mercury compounds (83) and arylacetic acids (3) were separated. Finally, in a study by Breyer, Fischl and Seltzer (6) twelve alkyl sulfate surfactants were studied using a variety of stationary phase--eluent systems.

Another application of coated plate RPTLC has been termed extraction chromatography. This technique has been used with some success in the separation of transition and rare earth metal ions. Silica gel or cellulose layers are impregnated with an organic phase such as a high molecular weight amine, substituted ammonium salt, or organophosphorous compound. Eluents are usually

water/organic buffers, or salt solutions. The R_f value of a given ion and system is often dependent upon the concentration of the counter ion in the eluent, and published values are reported as R_f values versus concentration profiles. Analysis with two or more stationary phases and/or eluents, or two dimensional analyses, can sometimes make feasible difficult or otherwise impossible separations.

An important characteristic of the stationary phase is sorption strength (or extraction efficiency). For substituted amine stationary phases, sorption strength has been found to decrease in the order quaternary (Aliquot), tertiary (Alamine), secondary (Amberlite) and primary (Primene) (84). Brinkman et al., (9) however, observed variation in this ordering for certain ions. The sorption strength for phenylpyrazol and methylpyrazole phases was found to be intermediate between secondary and primary amines (85). Cardaci et al. (86) used ion exchange resins, notably Polygram Ionex 25-SB (strongly basic) and Polygram Ionex 25-SA (strongly acidic) in conjunction with a nitrite eluent for metal ion separations. Numerous other examples exist in the literature. Brinkman, de Vries and Kuroda have compiled an authoritative review on inorganic metal separations by TLC and RPTLC (87). Suitable combinations of stationary and mobile phases exist for the separation of almost all metal ion mixtures.

ANALYTICAL APPLICATIONS: MIXED RPTLC

A few studies have appeared in the literature in which the TLC plates used are both bonded and coated with nonpolar materials. In most cases, a one or two carbon silane reagent is bonded to the silica surface. The purpose of this phase is to tie up free silanol groups and thus reduce the possiblility of silanol-solute interactions. The liquid used to coat the silanized plates depends on the type of separation or kind of compound to be chromatographed. Prandi chromatographed 56 aliphatic amines, using paraffin oil and silanized silica gel H (88). In a separate study, rat liver lecithins were separated to give 8 different phosphatidyl cholines (89). Using silicone oil on silanized silica, Ottenheijm et al. (90) obtained lipophilicity measurements of gliotoxin analogues from R_f values , and Thijssen (91) separated isoxazolylpenicillins and active metabolites. The retention behavior of phenolic acid derivatives of cinnaminic acid was examined and compared to HPLC data (92). From the results, it was concluded that in certain cases, RPTLC correlates to HPLC and is thus useful in the optimization of an HPLC separation.

Several recent studies have made use of silanized silica gel layers impregnated with anionic and cationic detergents (sometimes termed "soap chromatography"). Commonly used detergents are triethanolamine dodecylbenzenesulponate (DBS), sodium laurylethylsulphonate (LES), sodium dodecylhydrogen sulphate (DHS)

and n-dodecylpyridinium (N-DPC). Lepri et al. (93) used DBS and LES to separate some primary aliphatic amines and DBS, LES, and DHS to separate primary aromatic amines (94). DBS and N-DPC were used to chromatograph sulphonamides and primary aromatic amines (95). DBS was found to retain amines more but with an identical sequence of affinities and to have stronger retention than N-DPC. DBS and DHS were found to have similar chromatographic behavior. In a notable separation by Lepri et al. (95), isomers of diaminotoluene and toluidine, previously unresolvable by anion exchange techniques, were successfully resolved. Water soluble food dyes were less successfully separated using this approach (96).

LIMITATIONS

Because of the presence of coated or chemically bonded organic stationary phases characteristic of RPTLC, certain limitations are imposed that are not normally of concern in normal phase TLC. For example, on coated phase plates, it is necessary that immiscible or nearly immiscible stationary phase/eluent combinations be chosen. Miscible combinations result in the stationary phase being stripped from the plate and lead to irreproducible solute retention. To minimize this problem, the eluent is often presaturated with the stationary phase. The constraint of immiscibility greatly limits the range of solutes that can be chromatographed by limiting solvent choice for the eluent.

Bonded phases of course do not share this problem and are stable in most solvents. However, water introduces a number of different problems for some of the commercially available RPTLC plates. In high concentrations, water often destabilizes the binder used to hold the reversed phase particles to the plate and the layers swell up and flake off (see TABLE II for water limitations). Aside from the problem of layer stability to high percentages of water is the question of bonded phase wettability. Wettability is a function of the makeup of the bonded phase (i.e., length of the carbon chain and percent surface coverage). At a given eluent composition, poorly wetted bonded phase surfaces result in long development times, tailing, and a general loss in separation efficiency. Halpaap et al. (40) studied in detail the effects of solvent wettability for numerous bonded phases and eluents.

The problem of water instability introduces yet another difficulty. For compounds which are not strong UV absorbers, visualization of the TLC separation is often accomplished by spraying with an appropriate reagent. Many such visualizing reagents have been tabulated for various classes of compounds (97), but the solutions are largely aqueous. The use of water based reagents for solute detection is limited to water compatible RPTLC products. Visualization by heating or charring is also limited in chemically bonded plates due to the presence of the organic phase (Merck notes for their product that discoloration of

the adsorbent may result from use of aggressive agents or heating).

CONCLUDING REMARKS

Reversed phase thin layer chromatography is an important analytical technique that has demonstrated great utility and flexibility in chemical separations. Recent introduction of chemically bonded phases for TLC has made RPTLC an attractive choice for some separation problems, due to excellent selectivity, efficiency, reproducibility, and speed of analyses. Coated plate RPTLC is still useful for specialized applications (e.g., determination of partition coefficients), but routine reversed phase separations will soon (if not already) be dominated by bonded phases. Problems associated with recently introduced RPTLC plates are certain to be solved as the technology progresses. Use of RPTLC to preview separations in HPLC is expected to be among the major applications of the technique, but its use as a primary separations method will remain of highest importance. Thus, technology and applications of reversed phase thin layer chromatography can be expected to grow rapidly as the power of this separations technique is discovered.

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