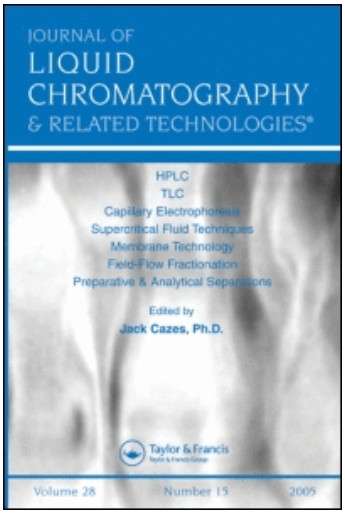

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Scott, Ronald M.(1981) 'The Stationary Phase in Thin Layer Chromatography', Journal of Liquid Chromatography & Related Technologies, 4: 12, 2147 — 2174
To link to this Article: DOI: 10.1080/01483918108066850
URL: http://dx.doi.org/10.1080/01483918108066850

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(12), 2147-2174 (1981)

THE STATIONARY PHASE IN THIN LAYER CHROMATOGRAPHY

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ABSTRACT

This review provides an overview of the stationary phases used in thin layer chromatography organized according to the classes of attractive forces between the solute and the stationary phase. Specific examples of recent innovations are included.

INTRODUCTION

Rather than being a unique chromatographic principle, the thin layer approach is really a special technique for applying several different principles. Samples are applied to a stationary phase coated on a support rather than one packed in a column. The solvent then either rises up the stationary phase by capillary action or runs down by gravity. Thin layer chromatography has sometimes been referred to as an "open column" technique, sharing this classification with paper chromatography. The first thin layer separations applied the principles of adsorption chromatography.

Since then the thin layer technique has been extended to partition chromatogrpahy, ion exchange separation, electrophoresis, and gel filtration.

The advantages of thin layer chromatogrpahy lie in its requirement for only very small samples and, more significantly, in the ease with which compounds on the plate may be detected visually and be quantitated by relatively simple scanning techniques. However when compared with a column procedure the method imposes the constraint on the stationary phase that it must adhere to the surface of the support. This constraint eliminates some stationary phases from consideration, while others that might better be used as pure as possible must have binders added, compounds like calcium sulfate, polyvinyl alcohol, or starch, whose function is to cause the stationary phase to adhere to the support. The binders alter the character of the separation, may prevent desired heating of the plate for activation or solute detection, or may otherwise interfere with solute detection.

Reviewing the character of the thin layer chromatography stationary phase has been done previously in books and articles (1-4). It was decided in this article to focus on the attractive forces between the solute and the stationary phase that are responsible for retarding the movement of the solute. Thin layer electrophoresis and gel filtration are omitted on the basis that the attractive forces between the solute and the stationary phase are of secondary importance, while ion exchange separations are included in spite of arguments that these are not by definition classical chromatography.

ADSORPTION CHROMATOGRAPHY

Adsorption is a complex process with several classes of forces making contributions. These include dispersion forces and electrostatic attraction between ions and ionic charges, dipoles, and induced dipoles. Hydrogen bonding and charge transfer may also take place. Between the solutes and an adsorbing stationary phase several of these forces may be acting concurrently.

Adsorption occurs in a monolayer on the surface of the stationary phase and should be viewed as displacement of solvent rather than as simple attraction to an empty site. In the usual case the surface includes numerous binding sites at which the chemical structures necessary for adsorption are available. These sites vary in terms of binding effectiveness or activity. The first solute molecules tend to bind to the most active site available and once attached will spend a relatively long time there. The more nearly the surface approaches saturation the broader is the range of activities being utilized, and the likelihood of solute binding readily to one of the remaining sites is statistically decreased. Solute is more likely to move on to a new section of stationary phase. These occurrences alter the dissolved/adsorbed equilibrium of the solute. As a result the quality of separations is altered by heavy loading of the adsorbent surface.

The surface area of adsorbent available is an important characteristic. The size of the particles obviously directly effects the the amount of surface area available. Beyond this the roughness of the particle surface is a factor. The more rough the surface, the

greater is the total surface area. A more irregular surface includes more varied binding sites because of the increase in edges and corners. Some portions of the surface, usually described as pores, are infolded and more difficult to reach.

An outstanding reference by L. R. Snyder (5) deals with all aspects of solute-stationary phase interaction in adsorption chromatography.

Electrostatic Interactions

Perhaps the simplest model for a polar adsorbing stationary phase is that of a crystalline array of inorganic ions. The surface has a mix of positive and negative ions arising from the local character of the crystal structure. Molecules with a permanent or inducible dipole orient so as to bind to the surface by electrostatic attraction with the largest dipole producing the strongest interaction. In practice the operation of such a chromatographic system is likely to be more complicated than the model suggests.

a. Alumina

Alumina represents a good example of the ionic crystal type of stationary phase. Alumina is prepared by removal of water by heating from hydrated aluminum hydroxide preparations. A variety of crystalline forms result depending on the starting material and the dehydration process used, and these differ in their chromatographic properties. Broadly we can differentiate mixed oxide-hydroxide preparations, low temperature (200-600^{o}C) aluminas, high temperature (900-1000^{o}C) aluminas, and very high temperature (1100^{o}C)

preparations. The various aluminas differ in their surface area and pore size (5). In general surface area is reduced as the temperature of dehydration is increased. For example the very high temperature product has a relatively low surface area, and is in fact not useful chromatographically. Alumina for thin layer chromatography is generally low temperature with a surface area of 100-250 m^2/g and a grain size of about 60 μm (1). Mixing it with water, spreading it, and activating (drying) it at 110°C leaves some adsorbed water and hydroxide ions still clinging to the surface. Water may be present as capillary water in the pores. Both water and hydroxide ions are adsorbed to the surface in a variety of fashions through dipolar attraction or hydrogen bonding as indicated by infrared studies. Thus there exists a complex variety of binding sites including Al^{+3}, OH^-, and O^{-2}. Heating to 300°C is required to strip off the water, but at this temperature hydroxide ion remains. The oxide and hydroxide sites can be classed as basic or proton accepting sites, while the aluminum ion provides an acidic site. The geometry of the crystal lattice provides further variety in terms of the availability of any specific ion to solute and the nearness and character of the neighbors to that ion. Having inferred this degree of complexity, it is necessary next to say that adsorption on alumina seems to depend primarily on the aluminum and the oxide ions. Activity, hence the energy of adsorption, increases as water is removed from the alumina, and increases further upon heating to temperatures above 800°C, which corresponds to the removal of the hydroxide ions.

Studies by Snyder (6) on the contribution of solute functional groups to adsorption indicate that for most groups the strength of adsorption relates to the basicity, in the sense of hard or electrostatic basicity, of the group. The fact that the energies of adsorption of such groups as -OH, -NH- or -SH are not excessively high infers that hydrogen bonding is not making a significant contribution to adsorption.

The surface of alumina because of the oxide ions is quite basic, being estimated to be approximately pH 12. Acids of pK_a lower than about 13 transfer protons to this surface producing charged conjugate bases that are strongly adsorbed. The use of calcium sulfate as a binder neutralizes the alumina layer eliminating the selective adsorption of such acids.

Finally charge transfer makes a contribution to adsorption on alumina in the case of easily ionizable aromatic molecules, and some small amount of ion exchange has been observed in the separations of inorganic ions.

Hydrogen Bonding

Hydrogen bonds form and break very rapidly yet are of sufficient strength to be ideal contributors to the adsorption process. Since molecules capable of hydrogen bonding are polar, electrostatic attraction of solutes to the stationary phase could be used as the sole explanation of adsorption. The evidence that hydrogen bonding does contribute is based on the observation that molecules capable of such bonding are in fact retained more strongly than would otherwise be anticipated.

a. Polyamide

Probably the best example of hydrogen bonding as a major contributor to the bonding forces between solute and stationary phase is provided by separations on polyamide. Polycaprolactam is generally used, although nylon 6,6 has also been employed. Chromatographic applications on polyamide have been reviewed extensively by Wang with various coauthors (7,8). Polyamide was used in columns as early as 1955 but difficulties experienced in bonding polyamides successfully to a glass plate or any other support held back the use of polyamide for thin layer chromatography. The use of loose layers or binding with starch, polyvinyl alcohol, or cellulose was attempted. However in 1961 the evaporation of a fomic acid solution of polyamide resin successfully deposited the material onto glass (9) providing a useful layer. Later a technique for coating polyamide on a polyester support was also developed (10).

The amide nitrogen can serve as a hydrogen donor and the amide carbonyl can be a hydrogen acceptor in hydrogen bond formation with solutes. Retention of the solute can also involve adsorption by despersion forces or by attraction to the polar bond of the amide structure. Partitioning with the traces of water retained by the stationary phase is also possible. These may make a contribution, but are established as not being the major attractive force (7). Given the very low basicity of the amide nitrogen, it is unlikely to become protonated to provide the charged structure necessary for ion exchange types of solute retention.

The substances separated most successfully on polyamide layers usually contain an -NH- or an -OH group which can donate a hydrogen

TABLE 1. Sample R_f Values on Polyamide and Silica Gel Layers

Compound	Polyamide	Silica Gel
a. o-Cresol	0.30	0.26
b. m-Cresol	0.21	0.19
c. p-Cresol	0.21	0.20
d. o-nitroaniline	0.92	0.38
e. p-nitroaniline	0.70	0.17

a,b,c solvent: benzene
d,e solvent: hexane/acetane (3:1)v/v
data from reference 7

to the amide linkage. Examples include phenols, sulfonic acids, carbohydrates, anilines, nucleosides, nucleotides, imidazols, and dansyl derivatives of amino acids. Perhaps the most convincing evidence of the involvement of hydrogen bond formation is provided by studies of the effect substituting phenols has on the R_f value on polyamide (11, Table 1). An ortho nitro group which provides an internal and competing hydrogen bond, reduces adherance to the polyamide. Substitution of nitro or halogen groups elsewhere on the phenol, increasing the acid strength of the phenol and consequently its ability to hydrogen bond, increase the phenol-polyamide bonding. Finally and most significantly, substituting the phenol in the ortho position, thus sterically interfering with hydrogen bond formation, reduces attraction to the polyamide layer. The larger the substitution the greater the interference. Further support is provided by studies of nitroanilines. Once again if a nitro group is in the ortho

position where it can form an internal hydrogen bond adsorption is reduced, while no similar effect is shown on palcement of the nitro group meta or para.

There is also evidence that the amide proton interacts with the electrons of a double bond. Ethylene urea for example is more strongly retained than is ethyl urea.

When a very polar partially aqueous solvent system is employed polyamide acts like a non-polar surface. For example, gallic esters with a variable length of hydrocarbon chain display decreasing R_f with increasing chain length. However in solvents wherein acetic acid is the sole polar component the pattern reverses (12). This was explained at first by assuming that the acetic acid bound to the polyamide, creating a polar surface.

Nitro groups on solutes may interact specifically with free $-NH_2$ groups at the ends of polyamide chains. For example DNP-amino acids are retained far less well when the stationary phase is acetylated, a process which eliminates the terminal amine groups.

b. Silica Gel

Properties of silica gel were studied early and have been well reviewed (5, 13). Silica gel has been (4) and still is overwhelmingly the most frequently used stationary phase.

Silica gel is prepared by polymerization and dehydration of aqueous silicic acid which is generated by adding acid to sodium silicate. The product of this process is an amorphous porous solid. The amount of surface area can vary over quite a range (200 to more

than 1000 m^2/g) as can the size of the pores. Pores less than 100A in diameter are generally termed small.

A study of the effects of layer thickness and particle size led to an optimization of silica gel characteristics for the separation of small samples (14). This formulation has been described as high performance thin layer chromatography (HPTLC) and has been commercially marketed as precoated plates by Merck.

Chemically the surface of silica gel contains these species: siloxane groups (Si-O-Si), free silanol groups (Si-OH), silanol groups hydrogen bonded to one another $\begin{matrix} OH...OH \\ | \quad | \\ Si \quad Si \end{matrix}$, water ($H_2O$...H-O-Si), and "capillary" or bulk water. Siloxane groups are unreactive and do not contribute significantly to binding of solutes. Silanol groups are largely free on the large pore gels, at a population density of approximately 4-5 groups per 100 A^2. Most TLC gels have large pores. In the small pore gels a large population of self hydrogen bonded silanol groups are found. In these gels the structure is less orderly.

The hydrogen bonding of water to the silanol groups competes with solute binding. Activation by heating at 150 - 200°C removes this water thereby improving binding characteristics. Sintering by heating to higher temperatures gradually converts silanol groups to siloxane groups with the release of water. Above 400°C the surface area is decreased, and after heating at 1000°C the ability to rebind water is lost. Such a silica has become a hydrophobic surface of siloxane structures. Similarly when silanol groups are chemically

converted to methoxy or trimethylsiloxy groups polar solutes are no longer well retained.

Silanol groups are weakly acidic (aqueous pK 6-8) making them attractive to basic solutes. This is not sufficiently acidic to encourage the idea that bonding of adsorbates is due to attraction to negatively charged ionized silanol groups. The primary bonding forces must be hydrogen bonding or simple electrostatic attraction to the polar hydroxyl group. Studies of the retention of phenols suggest that as with polyamide layers hydrogen bonding is of primary importance.

c. Other Hydrogen Bonding Phases

Other stationary phases capable of hydrogen bonding are encountered. Silicates are used, particularly magnesium silicates and occasionally calcium silicates. Early successes of magnesium silicates included separations of hydroxylated compounds such as carbohydrates. Florisil is a commercial magnesium silicate, and talc (Mg_3 $[Si_4O_{10}](OH)_2$) is occasionally reported as successful for separations. The silicates are different from silica gel in a few important ways. They possess an ionic matrix rather than a largely covalent one. Some resemblance to alumina type adsorbances is therefore expected. Furthermore, while silica gel is weakly acidic, suspensions on magnesium silicate display a pH ranging from 8 to around 10.

The diatomaceous earth preparation usually referred to as Kieselguhr is largely a highly porous silicic acid preparation with a variety of impurities, some of which are removed by processing.

It has a relatively inactive surface as compared with silca gel. Advantage has been taken of this low activity by coating the first 3 cm of a 20 cm plate with Kieselguler as a "zone of inert application" which shortens the time required for application. The rest of the plate was coated with silica gel, and separation did not begin unitl the solutes reached the silica gel (15).

Chitin is a cellulose-like polysaccharide in which the 2-hydroxyl of the glucose monomer has been replaced with an acetylated amine group. It has been used with phenols, amino acids, nucleic acid derivatives, and metal ions, producing separations comparable with those on silica gel, polyamide, or cellulose (16).

Special effects were noted when plates were impregnated with oxalate salts compared to other salts in the separation of aromatic amines (17). These were attributed to hydrogen bond formation between the amines and the oxalate ion.

Van Der Waals Forces

In chromatographic systems involving polar solutes and stationary phases relatively strong electrostatic forces operate between the solute and the stationary phase. However if the solutes are very non-polar, electrostatic forces cannot assist in adsorption. The weak Van der Waals forces must provide the explanation for such separations. A key consideration in application of these attractive forces is the requirement that atoms must approach one another very closely before significant attraction is present. The distance term in the equation for calculating the magnitude of these forces is raised to the sixth power as contrasted to the corresponding calculation

for electrostatic attractions where it is squared. The solute must therefore be able to fit the stationary phase very closely to generate good adsorption. As a result the success of a particular stationary phase is affected by the presence of surface irregularities, the presence or absense of which relates to the original preparation of the adsorbent, especially in terms of heating and mechanical treatment.

a. Reversed Phase Chromatography

A decade or more ago separations dependent on Van der Waals forces were generally performed on silica gel or alumina using solvent systems composed of such non polar liquids as hydrocarbons, ethers, or esters. Recently it has become increasingly popular to use an adsorbent which has been chemically altered to reduce its polarity. Somewhat more polar solvents are then employed and the result is a reversal of the normal chromatographic pattern so that the less polar solutes are more strongly adsorbed. This is termed reversed phase chromatography.

b. Modifying Traditional Stationary Phases

Silica gel can be made less polar by reacting the silanol groups by a process called silanization. Reagents such as alkyltrichlorosilane (18) or dimethyl dichlorosilane (19) convert the hydroxyl groups to ether structures, thus completely deactivating the silica gel with respect to polar or hydrogen bonding adsorption sites. Such phases are commercially available and have been studied for their relative effectiveness (20).

Similarly cellulose has been modified by acetylation of the hydroxyl groups. Such acetyl cellulose is a satisfactory non polar stationary phase, and can be used either by itself or in combination with other supports such as kieselguhr.

Traditionally reversed phased svstems have been created by impregnating a solid support with a nonpolar liquid. This is discussed later under "Partition Chromatography". The partition approach has the disadvantage that an additional component is now part of the system, introducing problems of saturation and reproducibility in impregnating the layer, solution or evaporation of the inert liquid during chromatography, and extraction of the inert liquid with solute in a preparative procedure.

c. New Stationary Phases

Nonpolar plastics have been utilized for reversed phase stationary phases. Amberlite XAD-2, a polystyrene-divinylbenzene copolymer, and XAD-7, a polymethacrylate,have been employed. These were applied as crushed solid and required large amounts of binder. Of the binders tried, calcium sulfate had the least effect on separations (21). Porapak Q, P, and N, ethylvinyl benzene polymers crosslinked with divinyl benzene, have also been employed. These polymers are porous enough to have a high surface area.(22)

Charge Transfer

The transfer of electrons between solute and adsorbent accounts for some attractive forces observed. Most frequently unsaturated organic compounds have been involved, particularly aromatic molecules.

The need to separate a large variety of polycyclic aromatic structures in environmental and toxicological studies has stimulated efforts to improve chromatographic techniques by incorporating electron acceptors into the stationary phase. Silver nitrate when added to silica gel was observed to retain structures with pi electrons more strongly. More recently organic electron acceptors were employed, including 2, 4, 7-trinitrofluorenone (23,24), 1, 3, 5-trinitrobenzene (23), and picric acid (23,25). Positive results were also attributed to this type of bonding when nitrobenzene and chlorobenzene were added to the stationary phase (26).

Other examples of charge transfer or complexation influencing separations are abundant (3,27). Layers impregnated with boric acid or borate salts have long been used in the separations of carbohydrates and other polyols (23,29). Tungstate complexes have recently also been reported to be useful (30). Dihydroxybile salts have been separated on silica gel impregnated with KH_2PO_4 (31). The addition of $NaHSO_3$ promotes stronger retention of carbonyl compounds. Salts of several metal ions which complex readily including copper, cadmium, manganese, and zinc have been employed to modify chromatographic separations of nitrogenous compounds (32-34).

Chelating agents have been added to stationary phases to analyze mixtures of metal ions. Recent reports mention the use of EDTA (35) and nitrilotriacetic acid (36, 37).

Ligand Exchange

Ligand exchange chromatography is defined in a recent comprehensive review (38) as "a process in which the interaction between

the stationary phase and the molecules to be separated occurs during the the formation of coordination bonds inside the coordination sphere of the complex forming ion". Two classes of separation are described. In the first the complex-forming metal is firmly bound to the stationary phase with the coordination sites probably occupied by solvent molecules. The solute displaces these solvent molecules and becomes a ligand of the metal. The order of elution of solutes then depends on the tightness of the coordinate bond formed. In the second class of separation the solutes are already bound to a metal ion, all solutes complexed to the same metal species. The stationary phase is composed of potential ligands which are free of metal until the solute complex is bound by them. Metals employed include Cu, Co, Ni, Fe, Zn, Cd, Mn, Hg, Ag, UO_2^{+2}, and VO_2^{+2}. These are bound by cation exchangers with carboxylic, phosphoric, iminoacetate, and α-aminoacid groups.

A specific example is provided by the use of layers of cellulose and chelex resin saturated with Co^{+3}, Ni^{+2}, Cu^{+2}, or Zn^{+2}. Separations of diamines, amino acids, and carboxylic acids were performed (39).

PARTITION CHROMATOGRAPHY

In partition techniques the relative rates of movement of the solute in the chromatographic medium result from the partition coefficients between the solvent and a liquid coated onto a stationary support. This presents the system in a convenient fashion, but may allow some oversimplification to mislead the user.

Cellulose

Oversimplification is a clear danger when the stationary phase is cellulose. Cellulose is viewed as serving as the support for water, and partition occurs between the solvent and the water layer. Given the relatively small amount of water taken up by the cellulose, one would be badly misled to view the stationary phase as bearing a close resemblance to the contents of a mountain stream, but would be more accurate to liken it to a container of syrup. Water molecules hydrogen bond to the cellulose alcohol groups or ring oxygen as well as to one another. A solute in such a system might interact either with water or with cellulose, and because of the similarity of the chemical groups it is difficult to distinguish which is actually involved. The system is most effective with polar solutes, particularly those which can form hydrogen bonds, using a relatively polar solvent system. A very non-polar solvent system might well not wet, hence interact with, the stationary phase. It is desirable to include water in the solvent to insure that the water layer remains intact. Anhydrous cellulose serves as an adsorption stationary phase which also has a small ion excahnge capacity (see Ion Exchange-Celluose). As an adsorption stationary phase it is composed of layers of polymer strands tightly hydrogen bonded together. Approached perpendicular to the surface of the glucose rings it can hydrogen bond to free alcohol groups and ring oxygens. However parallel to the rings only Van der Waals attractive forces are possible.

Cellulose was early used in the form of paper sheets. The adaptation of cellulose to thin layer systems was an easy transition, al-

though given the simplicity and low cost of paper chromatography, such a transition may at first seem pointless. However in practice separations on cellulose thin layers often are superior to the corresponding analysis on paper, especially when the plate size is small. Part of the credit for this is due to the uniformity of the coated layers, but the character of the cellulose itself may be of greater significance. Cellulose fibers for thin layer use are generally shorter than would be found in chromatography grade paper sheets, especially in the case of microcrystalline cellulose which has been deliberately hydrolyzed to lower molecular weight structures. It is thought that solute moves rapidly along the fibers resulting in more rapid diffusion of the spots than would occur otherwise, and the shorter fibers mimimize the problem.

Cellulose adheres to a glass surface, so that the problems inherent to introducing a binder into the stationary phase are avoided. In fact, cellulose has occasionally been mixed with other stationary phases to help bind them to the plate. Other advantages are sometimes claimed for such combination. A silica gel - cellulose mixed layer is described, for example, as allowing the employment of inexpensive solvents and permitting a convenient method for stripping for liquid scintillation counting.

Partition chromatography on cellulose may involve other liquid coatings than water. Glycols also adhere to the cellulose to produce a polar stationary liquid phase.

Starch is chemically similar to cellulose and is occasionally used as a stationary phase for the same types of separations as

cellulose. The author has not been impressed with it as offering any advantages over cellulose, and it suffers the disadvantage of not being widely commercially available either for spreading or as a precoated sheet.

Reversed Phase Chromatography

The non-polar character of the stationary phase is achieved here by coating a support with a liquid of very low polarity and volatility. In principle the support does not enter into the solute - stationary phase equilibrium, and in practice silica gel and kieselguhr are most often used. As largely uncharged covalent structures they accept the liquid coating in a reasonable fashion. Silicone or paraffin oils are common examples of the non-polar liquid. The moving phase is usually a mixture of polar liquids. Solvents rich in water often produce very slow runs since the water does not wet the plate. Similarly spray reagents conventionally prepared in water solution may have to be adapted to solvents like methanol or acetone to be effective.

ION EXCHANGE

In the ion exchange technique the stationary phase has charged groups, usually titrable groups maintained in the charged form by appropriate adjustment of the solvent pH. Ions of opposite charge are attracted to these groups and adhere with degrees of firmness that vary primarily with the size and the charge of the ion in simple inorganic examples. When the technique is extended to charged organic molecules, as has been done extensively in the cases of amino

acids, peptides, proteins, nucleotides, and oligonucleotides, adsorption of the molecule to the stationary phase also occurs, requiring that the character of the uncharged portion of the stationary phase also be considered. In the straightforward application of ion exchange, ions charged oppositely to the stationary phase attach at the charged site of the stationary phase by displacing an ion that is already there, but is held less strongly. Later these ions are displaced in order of the tightness of their binding either by low concentrations of ions bound more tightly yet or by high concentrations of ions bound less tightly.

Homochromatography, a technique that has been applied extensively to nucleotide studies, is an interesting variation of the method (40). The ion exchange stationary phase is saturated before analysis with a mixture of non-radioactive nucleotides which array themselves in order of tightness of binding on the plate, generating a series of fronts. Mixtures of radioactive nucleotides under analysis then move to appropriate zones on the plate. Recent papers report a number of variations of the method combining it with electrophoresic analysis and using DEAE cellulose or polyethyleneimine cellulose (41-43).

Cellulose Based Methods

Cellulose itself has been used as an ion exchanger. Its ability to perform in this fashion depends on the presence of carboxyl groups on about 1% of the glucose monomer units (44). As such, however, the capacity is small and in an aqueous solvent the attractive forces are not strong. The glucose primary alcohols may be converted to

TABLE 2 - Cellulose Based Ion Exhangers

Name	Structure substituted on alcohol groups
oxycellulose	-COOH (primary alcohol only)
cellulose phosphate	$-OPO_3H_2$
carboxymethyl cellulose	$-OCH_2\ COOH$
sulfoethyl cellulose	$-OCH_2CH_2SO_3H$
cellulose citrate	$-OOC-C(OH)(CH_2COOH)_2$
diethylaminoethyl cellulose	$-OCH_2CH_2N(CH_2CH_3)_2$
aminoethyl cellulose	$-OCH_2CH_2NH_2$
triethylaminoethyl cellulose	$-OCH_2CH_2N^+\ (CH_2CH_3)_3$
ECTEOLA cellulose	$-O(CH_2\text{-}CHOH\text{-}CH_2)_n\ OCH_2\ CH_2N(HOCH_2CH_2)_2$
guanidinoethyl cellulose	$-OCH_2CH_2NH\ C(=NH)NH_2$
p-aminobenzyl cellulose	$-OCH_2C_6H_4NH_2$
QAE cellulose	$-OCH_2CH_2N^+\ (CH_2CH_3)_2CH_2CHOHCH_3$

carboxyl groups by oxidizing agents, generating a much higher capacity product called oxycellulose.

A variety of other chemical derivatives of cellulose with exchange properties are produced (45) as is summarized on table 2. These involve replacement of more than one alcohol group with charged groups. The interchain hydrogen bonds involving the alcohol groups that maintain the cellulose in a crystalline, water-soluble state are replaced by electrostatic repulsive forces, To prevent the polymer from dispersing in the solvent as the result of these changes, it is necessary to crosslink the polymer before modifying the side chains.

In order to react groups in the interior of the cellulose, the matrix may be swollen with alkali (Mercerized) before reacting the alcohol groups. As the cellulose dries unaltered alcohol groups reform hydrogen bonds and attractive interactions made possible by the new substituents occur. The matrix shrinks, and a portion of the new ion exchange sites became unavailable unless the substituted cellulose is reswollen before use by treatment with acid or base. Microgranular cellulose avoids some of these difficulties. The noncrystalline regions of cellulose are more susceptible to hydrolysis and can be removed to form pores. The crystalline regions are then crosslinked. Substitution of alcohol groups then occurs primarily at the borders of the pores, and a product that may be used without reswelling results.

Cellulosic ion exchangers have been of particular value with biochemical separations, most frequently on columns, but also on thin layer plates. The fact that the stationary phase is hydrophilic rather than hydrophobic, as in the case of more traditional polystyrene based ion exchangers, has avoided problems with denaturation and overly strong retention of the proteins be separated.

Resins

The traditional ion exchange resins used for so long in columns are not inherently well suited to open column methods. Typical polystyrene based beads do not adhere at all to glass. The beads are very large by camparison with the typical particles coated on plates, the expectation being that ions will penetrate into the very porous bead to interact at interior binding sites. Using the usual binders

to attach the beads to the plate usually inhibits good interaction between ions and the resin. However because so much ion exchange work has been done with resins the temptation to find some means of using them in the open column mode is irresistable. Examples of successful applications are provided by the comparative studies of phenol separations by L. Lepri and coworkers (46) using a variety of ion exchange stationary phases. Cellulose derivates (DEAE-, PEI-, and benzoylated DEAE-) were used, but in addition they employed Dowex 50-X4, Rexyn 102, BioRad AG 3-X4A, and BioRad AG 1-X4. Crushed resin was bound to the plates by mixing 3g resin with 9g microcrystalline cellulose.

Liquid Ion Exchangers

In the mid-sixities the idea of impregnating a support with a liquid ion exchanger for inorganic analysis was explored extensively. Silica gel was usually the support of choice, although other media were tried including a polyvinyl chloride/vinyl acetate copolymer (47). Most interest centered on di-(2-ethylhexyl) hydrogen phosphate (47,48), and on a series of amimes including Amberlite LA-1, N-dodecenyl-(trialkylmethyl)- amine, Primene JM-T, triisooctylamine and Alamine 336 (47,49,50). Cellulose impregnated with polyethyleneimine, called PEI cellulose, has been used by biochemists in a variety of ways.(51)

Other Ion Exchange Media

A wide range of other approaches have attempted to provide an ion exchange stationary phase. For example silanized silica gel has

been impregnated with detergents. The non-polar portions of the detergent associates with the very non-polar support, leaving the charged group available to attract solute ions. Such detergents as sodium laurysletherosulfate, triethanolamine dodecylbenzene sulfonate, and sodium dodecylhydrogen sulfate were studied (52).

Spreading insoluble inorganic ionic material in which a large negative ion attaches cations in an exchangable fashion is also possible . Tungstate, molybdate, or their heteropolyacid anions are useful in this fashion, metal ions having been separated on layers of ammonium molybdophosphate (55) and thorium tungstate (56). Silica gel impregnated with these salts is also successful for ion exchange separations of metal ions. The use of ceric molybdate (57) and lanthanum tungstate (58) have been reported recently. Separations of cations on hydrous ZrO_2 has also been reported (59).

Deacetylating chitin generates a polysaccharide with a primary amine group on each carbohydrate unit. This has been used for the separation of nucleosides and nucleotides (60).

SINTERED THIN LAYER CHROMATOGRAPHY

As a final section, a promising technique for binding the stationary phase to the plate should be mentioned. Mixing the coating with a binder which melts and flows on heating, if carefully done, produces a very tightly bound layer. For example, silica gel is mixed with sodalime or borosilicate glass powder, spread on a plate, and heated from 470-770°C. The resulting plate survives rough handling and can be recycled. Reuse of the plate permits greater reproducibility, especially after recycing a few

times. Problems with visualization reagents that char or corrode organic binders are eliminated.

The use of a fluorescent glass such as zinc silicate allows detection by fluorescence quenching.

The method has been extended to alumina, kieselguhr, florisil, ZnO, MgO, and titania. Using polyolefin in place of powdered glass, one may bind organic and cellulose ion exchangers and other organic phases.

The techniques have been reviewed by Okumura .(61)

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