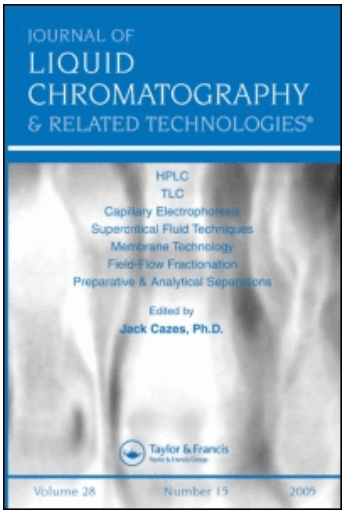

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Preparative Liquid Chromatography. II. Approaches On Non-Compressed Beds

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To cite this Article Nettleton Jr., Donald E.(1981) 'Preparative Liquid Chromatography. II. Approaches On Non-Compressed Beds', Journal of Liquid Chromatography & Related Technologies, 4: 11, 359 — 398
To link to this Article: DOI: 10.1080/01483918108064788
URL: http://dx.doi.org/10.1080/01483918108064788

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(Suppl. 2), 359-398 (1981)

PREPARATIVE LIQUID CHROMATOGRAPHY. II. APPROACHES ON NON-COMPRESSED BEDS.

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A. INTRODUCTION

Part I of this review covered preparative LC on mechanically compressed beds (1). This part will conclude the review with consideration of other systems. Much of the theory and mechanics of this valuable tool were discussed in the first part, but some additional thoughts will be presented here.

In considering preparative LC on mechanically compressed beds there are no problems in deciding what should be included. The systems used have unique features and are clearly products of modern chromatographic technology. However, such is not the case when one comes to other types of preparative LC. Even the abbreviated name makes no distinctions since preparative liquid chromatography has been practised since the days of Michael Tswett.

Actually this term, where LC is understood to mean HPLC, is of modern derivation and, by usage, implies the incorporation of certain of the latter's analytical strengths into the preparative mode. Without going into the many possible conditions or sentiments for which the letter 'P' may stand, this does allow one to set certain limitations for the subject at hand. I recognize that

my choice of these limits may not entirely agree with what others perceive, but I do hope that the reader would agree on the column and its contents as the key feature.

The column is where the great efficiency of modern HPLC is realized. One can plumb any or all of the external LC features into the most ancient of glass columns, sometimes with great effect, but the separation itself will proceed no differently. The external features supply the needs of the column and monitor its output, but it must do the work. Accordingly the scope of this review will be limited to systems where the columns have, at least to a large extent, the features originally designed for analytical HPLC. Some borderline examples are included because of unusual or interesting features.

There are essentially two kinds of preparative LC. One involves the preparation of quantities of material for some use and is, therefore, product oriented. The other is investigative in nature, usually on a much smaller scale, and dedicated to some sort of informational purpose such as providing an analytical reference standard or structural studies. Generally the product oriented efforts, with potential needs of hundreds of grams and using processes which may be run for years, have been carried out on compressed bed systems with greater capacities. New technologies may soon change this as larger, non compressed systems are now appearing. On the other hand the smaller scale work has involved a greater variety of systems which may influence compressed bed systems provided cost factors can be mitigated.

Several excellent reviews and one book chapter on preparative LC should be noted. As cited in Part I, the 1975 two-part review by DeStephano and Kirkland (2) is still timely, and no chromatographer should be without the second edition of Snyder and Kirkland's fine treatise on HPLC (3) which includes a chapter on preparative applications (pp. 615 - 661). In the September and October (1980) issues of The Journal of Chromatographic Science a series

of reviews appeared covering many aspects of HPLC. Preparative LC is dealt with in one by Verzele and Geeraert (4). Two others by, respectively, Rabel (5) and Majors (6) deal with columns and packings and have much application to preparative work. A review by Henry and Smith (7) deals specifically with the small scale and trace collection aspects of preparative LC.

My purpose in both parts of this review has been to provide the reader with a ready reference to the subject from a chemical point of view. While trying to present a comprehensive overview of the things one can do with it, I have attempted to avoid rehashing material already adequately covered in other reviews. Some repetition is unavoidable. Since I consider both parts of this review to constitute a single entity, there will be a number of references to Part I in the following text.

B. EQUIPMENT & MATERIALS

The order of presentation in this section mainly follows the sequence of components in a LC system with one exception. The column and its contents, in light of its key role, is considered first.

1. Columns and Column Packings.

Virtually all preparative and semi-preparative LC columns are made of stainless steel. Occasionally, in somewhat borderline systems, glass is used, but this material at best can sustain 150 psi. Stainless steel has the necessary strength and is corrosion resistant. It does pit in the presence of halide ions, particularly in acid media. This is rarely a problem since a substitute ion can usually be found.

Early preparative columns were generally scaled up from analytical columns. In fact the earliest work involved the use of the latter for what today would be called semi-preparative LC. The mastering of overload techniques has allowed the isolation of milligram quantities on analytical systems. For larger loads

wider diameter columns soon found popularity as larger size packings (20 - 40 μm) were developed. These packings could be used without excessive back pressures and proved less expensive to produce. Also those with particle sizes $\geq$ 30 μm could be dry packed by hand and still deliver the desired efficiencies.

For some years the use of smaller particles in preparative LC had been considered impractical, not just from considerations of cost but also because of the excessively high back pressures anticipated. However, for some time chromatographers had known that the more uniform a particle size could be made the better the flow characteristics would be through it. In 1976-7 Whatman introduced its Magnum 9 series of prepacked stainless steel columns (25 or 50 cm x 9 mm ID) containing its microporous Partisil 10 (10 μm) series of analytical packings. These columns were found to operate with high efficiencies at remarkably low pressures, properties attributable to the very narrow range of particle size. Soon after the Magnum 20 line appeared featuring columns of the same lengths with 2 cm ID.

Last March at the Pittsburgh Conference the Magnum 40 line was introduced. These columns, 50 cm x 48 mm ID, are supplied with very narrowly sized 40 μm packings which the user packs by hand. Thus once a column is acquired only replacement packings need be purchased afterwards. Each column holds about 650 g of material and can be used at pressures up to 2000 psi.

Although it has taken a while many other companies have come out with columns equivalent to the Magnum 9 series filled with their own packings. A few larger columns have appeared as well. The delay was probably due to the time required to develop the narrowly sized particles required for these. In the past the particle sizes given by many suppliers for their packings have actually been average sizes for a fairly broad range. Since particle size ranges are not usually given, anyone packing his own preparative columns in house with analytical packings should keep

this point in mind. If there is any doubt, it would be best to contact the manufacturer's representative.

The question of whether or not to pack one's own columns must occur to many chromatographers at some time or other. It is, once the initial outlay for equipment has been made, certainly less expensive. However, it does take up time which could be spent running chromatograms and, by and large, the commercially produced columns tend to have higher efficiencies and be more uniform. For those who do choose to pack their own columns, Verzele and Geeraert (4) offer some useful tips, particularly concerning the lower limits of particle size for dry packing. Some 20 μm packings (specifically certain spherical ones) are amenable to such use. "Weighting" or "wetting" with solvents improves the process and even allows the dry packing of smaller particles. Cited as an example is 10 m silica wetted with diethyl ether.

DuPont, one of the first to follow the Whatman lead, offers similar columns, the largest one having a 21.2 mm ID. A paper from its German division (8) describes the properties and use of 25 cm x 25 mm columns containing 7 μm and 10 μm packings (silica and octadecylsilyl bonded silica). Majors' review (6) and Snyder and Kirkland's book (3) both have particularly extensive listings of available column packings and their suppliers.

Pirkle (9) has made some interesting comments on commercially available columns. He notes that these may exhibit very high efficiencies (up to 40,000 theoretical plates/meter), but rarely achieve the same in actual usage. However, these efficiencies are generally measured with rather ideal systems, especially in the case of silica where plates are measured in anhydrous systems while many separations are run in systems containing alcohols, water or other even more polar modifiers, all of which greatly alter the surface characteristics. In the long run the only efficiency needed is that which will effect the separation.

Certain of the previous citations (3, 4, 9) discuss packing techniques and concepts. Two German authors have described in detail a preparative LC system utilizing columns up to 50 cm in length with 20 - 50 mm ID's (10). Phase Separations (Hauppauge, N.Y.) offers a relatively affordable modular packing system with a Stansted pump. One can susbstitute a Haskell pump but must purchase this elsewhere. A brochure from the company (11) describes the system and offers what I consider to be the clearest and most complete description of a packing procedure yet to appear in print.

Some mention might be made here of packings designed for gel permeation chromatography (GPC), sometimes called size exclusion. Their use in the past has been largely analytical or semi-preparative, but fully preparative needs are increasing in this area. Many of the analytical GPC supports are softer than most packings and less tolerant of higher pressures and flow rates. The macroporous packings have shown size exclusion properties and some silica based GPC supports have been developed, but these do not seem to have great capacity. There is also the problem in working with size exclusion systems that it takes longer to establish reasonable equilibrium at each stage due to the mechanics of larger molecules moving in and out of pores where they can be accomodated. This creates a need for slow flow rates if any efficiency is to be attained. LKB has recently introduced a GPC system utilizing what they refer to as an organic-inorganic compostion in spherical microparticles (10 ± 2 μm or 13 ± 3 μm) and of a rigid nature. The columns are steel (Blue Columns), 30 cm or 60 cm × 7.5 mm ID, with a top load of 10 mg. Unfortunately, to maintain efficiency flow rates must be kept at or below 50 μl/min, hence the preparative capabilities of this system are severly limited. A recent book has been published on GPC and, while primarily concerned with analytical applications, does have something to offer the investigator with preparative needs (12).

The Sephadex gels, produced by Pharmacia, Inc. and long used in low pressure GPC, have never been suitable for HPLC. However, a recent paper (13) describes the use of Sephasorb HP Ultrafine in glass columns to isolate 3,8-dihydroxy-2-methylchromone, a degradation product of D-xylose. In a 3:2 water:ethanol system the separation appears to have proceeded by aromatic affinity and/or hydrophobic effects. Use of this material is limited to low molecular weight compounds, the upper exclusion limit being 500 daltons. It can be used in a wide variety of solvents, however, and is amenable to gradient systems.

Carbon black has long been used as a strong adsorbent but usually only for batch processes since its column characteristics have proven distinctly unappealing. Its potential and its success in gas chromatography have prompted some efforts to overcome the problems. One approach has been to pyrolize benzene and form a modified carbon black with a pyrocarbon surface. Another tried to coat silica with pyrocarbon, but the heat (~900° C) involved tended to alter the packing surface with adverse effects. Chemical approaches have been more promising in achieving the latter goal, but as yet the only commercially available packing, Spherosil XOB 75 from Rhone-Poulenc, has not been available in fine particles. An excellent review of the history and status of carbon black HPLC appeared two years ago in a trade publication (14). This may be difficult to find now, but it is worth the effort for those interested in the subject.

Very recently a group of Italian investigators reported on the modification and fractionation of a commercially available graphitized carbon black, Carbopack B, 80 - 100 mesh, from Supelco (15). This support was dry packed in glass columns and evaluated for a variety of separations. No preparative work was done, but the potential is of interest.

The porous but rigid macroreticular styrene-divinylbenzene copolymer resins show chromatographic propeties analagous to

alkyl bonded reverse phase packings. The origin of these materials goes back some years when scientists at Rohm & Haas noted that non-ionic species, particularly aromatics, were adsorbing onto some of its ion-exchange resins from aqueous systems. Recognizing the hydrophobic effect, they developed a series of Amberlite XAD resins in which the lower numbered members (1, 2 and 4) were simply the resin matrix with no functional groups. Originally the resins were used to concentrate organic impurities from water and proved very useful in pollutant analysis. More recently they have proven very attractive as HPLC supports, particularly for their stability at higher pH ranges. The recently announced PRP-1 column from Rainan Instrument Co. contains just such a packing. Today the only fine particle materials of this type available in other than in a prepacked form appear to be from Japanese sources. Table 1 lists those available.

There are several drawbacks to the resins of this class. When new they tend to bleed off plasticizers in organic solvents and require extensive pretreatment if the manufacturer has not already provided this. Also some compounds have a tendency to disappear onto the support and not elute. Some feel this is due to a layering effect where other compounds adsorb on top of the first adsorbed substances. The top layers may elute, but it seems difficult to get down to the buried one.

TABLE 1

Rigid and Porous Styrene-Divinylbenzene Copolymer HPLC Packings

Amberlite XAD resins	100-200 mesh	Rohm & Haas, Phila., Pa.,USA
Diaion CHP-3C	10 μm	Mitsubishi Chem., Tokyo
Hitachi gel 3010	25 μm	Hitachi, Tokyo
Hitachi gel 3011	10 μm	Hitachi, Tokyo
Jasco HP-01	10 μm	Japan Spectroscopic.

Pirkle has done a great deal of work with chiral stationary phases and recently has reported on the synthesis and use of several of these (16, 17). The paper describes the developement of a chiral recognition rationale based on the observation that a minimum of three reference points are needed to distinguish the handedness of any chiral system. The authors conclude that a chiral stationary phase must undergo at least three simultaneous interactions with a preferentially retained enantiomer. Using NMR data they then proceeded to design chiral bonded phases for specific enantiomer separations.

2. Solvent Delivery and Sample Injection Systems.

For non-compressed beds, most preparative LC systems have been able to utilize the same pumps and injectors used for analytical systems. Generally maximum flow rates of 10 ml/min and injection limits of 2 ml (in some cases 5 ml) have been sufficient. Now, however, with 2 inch diameter columns holding over half a kilogram of silica one approaches the proportions of the compressed bed systems which can require literally torrents of solvent at flow rates up to 500 ml/min.

Pumping systems to handle this demand are appearing. DuPont recently introduced its 8800 series preparative pump with a maximal flow capability of 40 ml/min and pressure limits of 2900 psi. Glenco's Model HPLPS systems are rated to 5000 psi and can deliver 0.25 - 15 ml/min, although the exact range depends on the particular pump one purchases. Whatman is developing means to feed its new Magnum 40 columns. Given the competitive nature of the HPLC market one can expect more advances in this area.

Meanwhile many chromatographers have been able to make use of pumps not specifically designed for HPLC such as metering or single piston units. In preparative work the needs are not as critical, base line fluctuations and other aggrevations to the analytical chromatographer tending to become lost or swamped at

the higher attenuations used. In any case, with no need for quantitation one can put up with a considerably messier situation. Pirkle (9) offers some comments on high flow pumps with regard to their availability and adaptability to preparative LC.

Sample injection systems may present more of a problem, especially with regard to the larger columns. Even with 1 cm diameter columns, loops of 2 ml (in some cases 5 ml) have usually been adequate to load a sample. One might consider building larger loops which is possible with most injectors, but this would not solve the problem of the overall system which will require modification in all modules. DuPont has designed an integrated preparative system and more in this line can be expected.

3. Solvents and Solvent Considerations.

Except with compressed beds, most preparative LC today is still run on analytical systems. Accordingly, chromatographers tend to use the same HPLC solvents for this work that they use for analytical efforts. Since analytical packings are appearing more and more in preparative columns this has some rationale. However, one should keep in mind that HPLC grades of solvents are developed more with analytical uses in mind. Therefore the presence of non-UV adsorbing residues may not seem as important to a manufacturer as adsorbing volatiles. Given the competition today in the solvent market, all impurities are assuming greater importance. Most HPLC solvents now give residue content in their specs, and one can hope that this will become the purest solvent grade of all.

Rabel's review on columns (5) offers a wealth of information concerning solvents. Ethers are discussed at some length, especially with regard to the problems of flammability and peroxide formation. Despite having useful polarities and other solvent properties, most alkyl ethers are simply too dangerous to use for preparative LC purposes. Burdick and Jackson offer methyl tert-

butyl ether, an ether which does not accumulate peroxides due to the immediate breakdown of these as they form. Presumably this occurs through some free radical dissociation of the tert-butyl group. This solvent is volatile and, despite a mildly unpleasant odor, quite useful in its solvent properties.

Rabel also makes the point that chloroform usually contains ethanol (generally one percent) as an inhibitor against phosgene accumulation, a situation which he notes some chromatographers forget to take into consideration. The alcohol can be removed but the processes take time and effort. One manufacturer (MCB) offers HPLC grade chloroform containing a volatile olefin as inhibitor. This additive is transparent to UV light above the solvent's cut off. If one switches to this product, however, the lack of alcohol should be kept in mind while designing systems.

4. Detectors.

Probably the biggest problem area in preparative LC, including compressed bed systems, has concerned detectors. Even at the top attenuations most analytical UV detectors black out with any kind of load. Even with excellent separations there may be enough adsorption between peaks that they can not be differentiated on the recorder trace. Off-peak settings with variable wave length detectors sometimes helps, but other components with different spectral properties may then produce some very peculiar and misleading results. Refractive index detectors may be somewhat less sensitive, but with large loads they can still black out and they also may not differentiate peaks as well. They can not be used with gradients.

There are UV detectors specifically designed for preparative LC. DuPont's 8800 series includes a variable wave length (195 - 350 nm) unit with easily exchanged 10 mm and 1 mm path lengths. As noted in Part I of this review, Pharmacia offers a UV-2 detector with both analytical and preparative capabilities. Gow-Mac

Instrument Co. has developed a preparative LC detector having a unique arrangement in place of a cell (18). Column effluent is directed onto a quartz plate tilted at an angle, typically 60°, from the horizontal. The light beam, perpendicular to the plate, passes through it at a short distance directly below the delivery tube. Film thickness for the effluent flowing down the plate can be calculated from the angle of tilt, flow rate, solvent density, and width of film spread. This value, equivalent to the optical path, generally falls in the range of 0.1 - 0.3 mm, considerably less than one could expect to achieve with an enclosed cell. The detector can accomodate high flow rates.

Combined Sciences Corp. offers a combination UV detector and peak selecting fraction collector. While apparently designed for standard column chromatography, this unit should have some application to preparative LC. Codina has given a fairly detailed description of its components and usage (19). The optical path is 3 mm, but the sensitivity appears a bit high and the attenuation range (0.03 - 1.0 OD) rather narrow. It is not clear from the article or from company literature whether the electronics of the system can utilize off-scale readings. Fraction selection is made by positive and negative slope detection, but this will not help if there is no slope to detect between peaks.

For several years Vestal at the University of Houston has been studying ways to couple LC systems to a mass spectrometer, an approach which has had great success in gas chromatography. This work has resulted in considerable progress for both highly volatile (20) and relatively nonvolatile (21) solutes. Last Fall at the 15th International Symposium on Advances in Chromatography, held in conjunction with Expochem '80 in Houston, Texas, Vestal reported on his somewhat serendipitous discovery that the energy adsorbed in the ionization system of a mass spectrometer could be measured and related to solute concentration (22). He noted that, while not inexpensive, the ionizing portion of the apparatus has

a considerably lower cost than the whole unit. Modified, it has the potential of being almost a universal detector which might well be worth the price.

Two investigators from the Institute for Physical Chemistry in Bonn, Germany have reported on the interfacing of HPLC systems with a mass spectrometer (23). They use a moving wire or belt to transport eluate to the ion source with appropriate solvent evaporation en route. The system has some rather severe flow limitations which would have to be overcome for preparative use. The design of the apparatus reminds one of the Pye Universal Detector of the early and mid '70's, an apparatus which also employed a moving wire transport but followed in this case by some rather complex chemical steps.

Preparative application of a modified mass spectrometer detector seems likely in the next decade. Vestal's discovery is particularly significant. There will be molecular weight limits, but these may be higher than expected.

5. General Considerations.

Clearly there have been great advances in columns and supports, particularly with regard to microparticulate packings. Improvements are needed in solvent delivery systems, injectors, and detectors, particularly the last. However, present systems are adequate for much of the preparative LC work now being done. The mechanically compressed bed systems enjoy an advantage in processing large amounts of material due to their greater capacities. Large bore columns are now appearing and may cut into this edge.

As to other features which might be incorporated into preparative systems, microprocessor or computer interfacing is the main one that comes to mind. Almost five years ago Bristow (24) described one such system. There are a number of commercially available systems now on the market, but their primary use is

analytical. They can be applied to preparative uses, especially for smaller scale repetitive runs.

C. PRACTISE

1. Theoretical Considerations.

Much of the theory presented with regard to compressed beds in Part I of this review is pertinant to other types of preparative LC. The incorporation of microparticulate packings into preparative columns provides the same degree of intimate contact between mobile and stationary phases that mechanical compression has already established for coarser packings.

Theoretically all the practises of high flow rate, overload, and recycle are possible on both compressed and non-compressed beds. This is true to the extent that any two are equivalent. To date no real comparisons seem to have been made, although there are models to work with. Waters Associates' microparticulate preparative columns and its radial compression modules for the RCSS unit employ the same packings in beds with nearly equivalent ID's. Calculations can be made to adjust for the differences in length. Another comparison might be made between Whatman's Magnum 40 columns and Waters' Prep LC/System 500A loaded with two PrepPaks. Here the actual weight of support would be essentially the same in both systems.

It is also interesting to speculate on how the finer, closely sized packings might perform in mechanically compressed beds. Presumably there would be limitations in smallness of particle size, but where these might lie is certainly not clear. The differences between compressed and non-compressed systems should decrease with particle size. Analytically the Waters radial compression modules seem very analogous to their prepacked columns. With larger particles the closely sized packings should be superior. Quite possibly Whatman's 40 μm silica would

require equivalent or less pressure and perform better than the coarser and non-uniform silica now used. In any case cost will be a serious deterrent to the use of the finer packings in compressed bed systems. This may change eventually, but at least now one can enjoy better preparative LC systems than have ever been available before.

The same calculations and measurements discussed in Part I apply to non-compressed systems. With microparticulate supports one advantage of the latter is that the column and system can be pretested with an analytical shot of the mixture being fractionated. In fact the larger bore columns are very efficient analytically due to the infinite diameter conditions with small loads. They do require more solvent and running time and cost more, so generally this usage is not economically attractive.

There are several interesting and useful papers on preparative LC based on work with non-compressed beds. B. Coq et al have published a paper entitled "How to Approach Preparative Liquid Chromatography" (25). Using overload the authors demonstrate mathematically that best results are obtained by using the maximum injection volume consistent with separation. Means of determining the latter are discussed as well as factors which can introduce uncertainties. With overloaded columns, in increasing the amount of sample being loaded it is better to use increased injection volumes at the same concentration than to keep the volume constant. For those trained in classical methods, the tendency is to do the opposite.

Another earlier paper (26) also concerned this subject as it demonstrated the relation between peak shape and sample volume. The authors discuss ways to determine where to collect fractions or initiate recycle. In the same light Wehrli (27) has noted that feed volumes should be increased to the point where resolution starts to decrease. Beck and Halasz (28) have studied sample volume effects in preparative LC chromatography of crude

polystyrenes (M_n 1100) on silica (solvent system, 14:1 n-heptane: methylene chloride) and steroidal cardiac glycosides on octadecyl bonded silica (solvent system, 2:1 or 3:2 methanol:water).

The surfaces of packings often display complex chemistries which vary according to the solvents being used. For silica, Scott has published an intriguing paper on the most ubiquitous surface modifier of all, namely water (29). He has been able to identify up to three layers of water and feels confident that there can be a fourth under the right conditions. The two outer layers are removed by dry solvents or relatively mild heating, less heat being needed for the outermost one. The innermost layer is removed totally only by strong heating to 650° C. The removal of these layers is reversible. The elimination of water from the silica itself requires drastic heating and leads to changes in and break down of the gel matrix. The last is not reversible.

One can readily appreciate how much chromatographic properties may be affected by such differences in surface chemistry. Each variation actually represents a different packing, a bonded phase system, so to speak, which, while not stable enough for bottling and selling, is chromatographically real and unique.

Other polar adsorbents such as alumina presumably will have similar interactions with water. Hydrogen bonding effects with non-aqueous components can also be expected. Reverse phase supports may have no such affinities (unless inadequately covered) but do attract and adsorb relatively non-polar components, thus altering their surface chemistries. In overload, solute components themselves may act as surface modifiers. In short, the surface of an LC support is a dynamic area, sensitive to every nuance of the solvent system.

It is very important that a chromatographer keep these considerations of surface chemistry in mind. Tailing peaks and poor resolution may indicate a need for column repair or replacement.

Or they may indicate a mixed mechanism where one surface adsorption is out of balance with another. For example, there are the problems experienced in fractionating amine bases on silica where acidic silanols interact indiscriminately with the latter. This was discussed along with other less drastic problems among the examples cited in Part I. While consideration of surface chemistry will not eliminate the trial and error process of systems searching, it can expedite it and make it less confusing.

2. Scope and Limitations.

Preparative LC now encompasses loads in a range from less than a milligram to tens of grams, possibly more. The determination of whether a chromatogram is preparative or not depends on the purpose for which it was run. Even when a sample is loaded in microgram quantities, if the goal is to collect one or more components and do something with them, the chromatogram may be said to be preparative, or at least semi-preparative.

Most smaller scale work is carried out on analytical columns. Where larger amounts are needed overload may be used. Mainly this scale serves to prepare reference standards for analytical HPLC or material for structural studies. With modern instrumentation a great deal of information can be obtained from very small amounts of a compound.

In peptide mapping studies, workers at the Salk Institute used reverse phase preparative LC to separate complex mixtures generated by enzymatic cleavage of proteins (30). The isolation and derivatization of drug metabolites for later analysis and structure analysis is of vital importance to the pharmaceutical industry. Two recent papers reported work on C_{18} - μBondapak with gradient systems involving metabolites of carbamazepine (31) and quinidine and quinine (32). The fractions were derivatized and analyzed by gas chromatography/mass spectrometry. Natural products isolation and structure determination can often be done

at the analytical scale as was the case in a study of porphyrin and chlorophyll mixtures (33). These were separated and analyzed by field desorption mass spectrometry.

At the milligram to gram levels the newer microparticulate columns are probably preferred. Milligram loads can be run on the larger compressed bed systems, but they do tend to get lost. Iron and other impurities in the Waters PrepPAK 500 silica cartridges can totally remove or destroy chelating and sensitive compounds at loads on the order of hundreds of milligrams, while the effect may be scarcely noticeable at multigram loads.

For large loads the compressed bed systems may still provide the most economical approach. The new Whatman Magnum 40 columns should be equal in performance, but the silica to pack one is over $500 (if purchased in quantity) as compared to under $200 for the equivalent in Waters' PrepPAKs.

The examples that follow should provide the reader with a better idea of the scope of preparative LC on non-compressed beds. A real advantage of these systems lies in the wide variety of packings available for them and the broad spectrum of chromatographic properties thus provided. Loads may be more limited, but usually a way can be found to deal with at least gram quantities.

D. EXAMPLES

1. Silica.

Silica is probably the most widely used packing in HPLC. As noted before it does have problems. But it also has great versatility due to large extent to the same complex surface chemistry which creates many of these problems. Thus, while capable of promoting some remarkable separations, it can also deliver some exasperating surprises to the unwary chromatographer.

The examples given here are mostly cited to show the range of separations available on this medium. Unfortunately, while

analytical HPLC has been applied to virtually every chemotype imaginable, this is not the case at the preparative level. It is probably safe to say that, if an analytical example does exist and can be duplicated, it can be scaled up to at least a semi-preparative level.

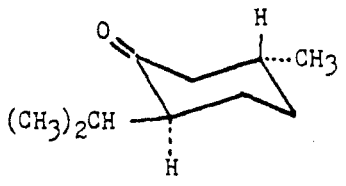
For a general review of work in the natural products area, mainly on silica but also including some reverse phase systems, a publication from Nakanishi's group at Columbia University is recommended (34).

For very non-polar mixtures, where solubility considerations preclude reverse phase anyway, highly active silicas in anhydrous systems will retain components. Workers at Gulf have published two papers on preparative LC class separations of hydrocarbons. In the first a mixture of paraffins, aromatics, and polar non-hydrocarbons from which hexane insolubles has been removed was resolved on Bio-Sil A (20 - 44 μm) (35). The paraffins eluted together with hexane, following which the aromatics were obtained by backflushing the column with hexane. The polar mixture was eluted with 1:1 methylene chloride:acetone. In the later paper the same group separated the olefins in the paraffin fraction from saturated compounds, the former eluting from LiChrosorb Si-60 (10 μm) by backflushing with iso-octane (36).

In the same vein is the fractionation of plant lipids by investigators at the Welsh School of Pharmacy (37). The purpose was to simplify a complex mixture, consisting primarily of hydrocarbons, phytosterols, triterpenes, and triglycerides for analysis by gas-liquid chromatography. Initial fractionation utilized TLC silica packed in 4 ft by 1 in ID steel columns with hexane elution for 1 hr followed by a hexane to 1:1 hexane:ethyl acetate gradient over 5 hr. Since no on-line detectors available could detect these compounds, TLC was used for this purpose. Mixed triterpene and sterol fractions were further resolved isocrati-

cally on Partisil 10 (10 μm) in a 25 cm x 1.0 cm ID column with a 10% ethyl acetate in hexane system.

The separation of isomeric mixtures is particularly suited to preparative LC where the rapid establishment of equilibrium produces large effects from small structural differences. Swedish workers have resolved (-)-menthone, 1, and (+)-isomenthone, 2, using a Partisil 10 (10 μm) column (30 cm x 7.7 mm ID)



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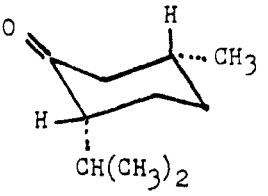
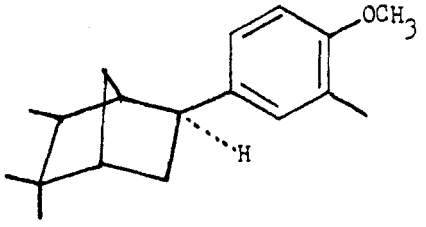


2

with a 30:1 hexane:ethyl acetate system at 1 ml/min and loads of 50 mg (38).

A group at International Flavors & Fragrances synthesizing chiral norbornylcyclohexanones resolved an isomeric pair of anisole derivatives, 3 and 4, made as intermediates (39). Preparative LC was carried out on a Porasil T (37 μm) column (3.3 ft x 3/8 in ID) using hexane at 9.9 ml/min with recycle for a total of four cycles. The shave techniques used were the same as those described for compressed beds in Part I.



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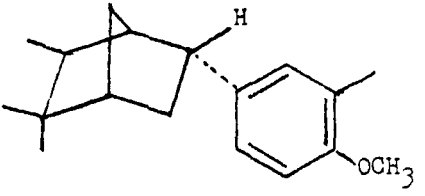
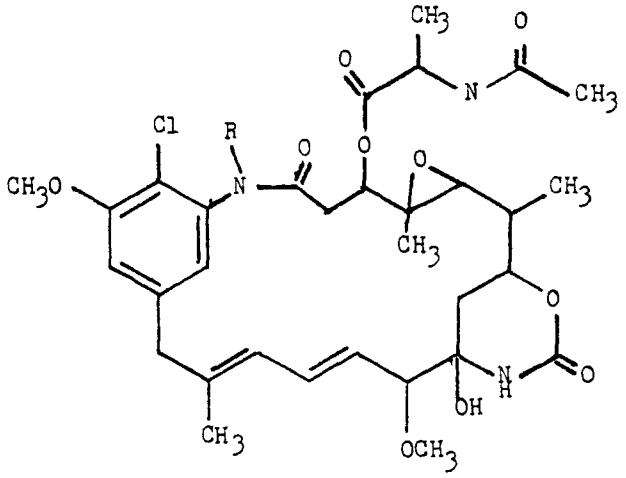


4

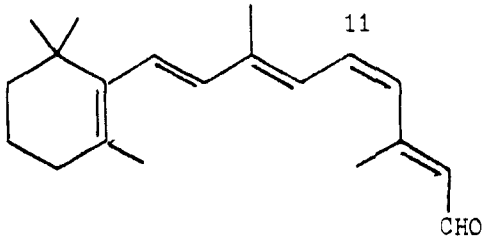
Naturally occurring normaytansine, 5 (R = CH_3), required preparative LC to separate it from maytansine, 5 (R = H) (40).



5

Chromatography was first carried out with 5.2 g of the mixture on a column (1 m x 2.54 cm) developed by Montsanto Research Corp. and packed with EM silica gel (230 - 400 mesh) eluting successively with methylene chloride containing 0, 3, and 4% iso-propanol. Enriched fractions were rechromatogrammed on a Partisil M9 10/50 silica column with a 75:75:2:1000 methylene chloride: methanol:diethyl amine:hexane system. Preparative TLC was still needed for final purification.

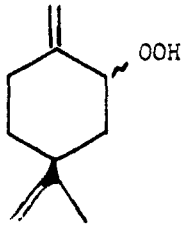
Even more impressive are the preparative separations of unstable or highly sensitive compounds. Nakanishi has done some elegant work in this area with retinals as cited above (34) and in Part I. Another publication from Japan (41) describes the preparative separation of 11-cis-retinal, 6, produced by irradiation of all-trans-retinal in acetonitrile. The work was done on



6

a silica column (25 cm x 7.9 mm ID) using a 88:12 hexane:ether system at 2 ml/min with loads of 20 - 50 mg. Other cis-retinals were also isolated as well as unreacted starting material.

Two papers have described the preparative LC isolation of hydroperoxides of naturally occurring substances, compounds which have been very difficult to obtain in a pure state by other means. One paper (42) describes the separation of 9-hydroperoxy-γ-linoleic, 13-hydroperoxy-γ-linoleic, and 13-hydroperoxyarachidonic acids, as well as their esters, on Porasil columns (8 ft x 3/8 in ID) using 50 - 100 mg loads. The systems were, for the acids 1:10:989 acetic acid:iso-propanol:hexane, and for the esters 1:249 iso-propanol:hexane. Similar work was performed to separate the various chiral hydroperoxides of limonenes of the general structures 7 - 9 (43). Best results ensued from use of two columns (25 cm x 4.6 mm ID) in tandem, the first packed with Partisil 10 (10 μm) and the seconde with Partisil 5 (5 μm). Systems used



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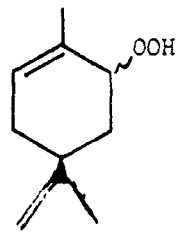

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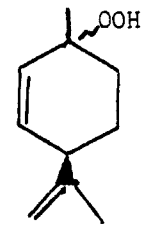
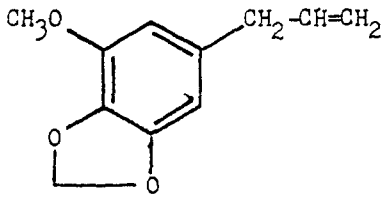


9

were 96:4 hexane:ethyl acetate, 99.5:0.5 toluene:ethyl acetate, and pure methylene chloride, all at 2 ml/min. The analogous hydroxy compounds were also resolved. In both papers the hydroperoxides were generated synthetically.

Various toxins, often present in minute amounts, have been the object of preparative work, both for reference samples and for studies to better understand the nature and properties of the compounds. In an interesting paper, two workers at the Max von Pettenkofer Institute in Berlin have described the use of a LiChrosorb SI 60 (7 μm) column (25 cm x 1.6 cm ID) in three preparative applications to isolate compounds of various biological interests (44). Using 0.5% iso-propanol in iso-octane and 400 mg loads they were able to prepare pure β-tocopherol, needed to establish a calibration curve, from an oily, commercially purchased sample. On the same column they used a 75:25:1 chloroform: iso-octane:methanol mixture to isolate Vitamin D_3 from an instant chocolate drink powder and aflatoxins from peanut extracts.

Myristicin, 10, the major hallucinogen in nutmeg and mace,



10

was isolated on a Partisil 5 (5 μm) column (25 cm x 9 mm ID) with a 1% ethyl acetate in heptane system at 8.0 ml/min. Three related compounds were similarly separated with a 3% ethyl acetate concentration. This work is particularly interesting for its use of a 5 μm packing (45).

Toxins have been isolated from cultures of the dinoflagellate Gymnodinium breve on μ-Porasil (10 μm) with iso-propanol modified hexane systems (46).

Generally the higher capacity compressed bed systems have been used for separations of synthetic products and intermediates. However, Meienhofer's group at Hoffman-La Roche has utilized E. Merck's silica gel 60 in glass columns (43 cm x 3.8 cm ID) with pressures of 50 - 150 psi to resolve a series of protected peptide intermediates ranging from three to eight amino acid units. For peptides esterified at the terminal carboxyl group, mainly chloroform to 5% ethanol in chloroform step sequences were used to develop the chromatograms. In one case the initial elution was with 1-chlorobutane going then in one step to chloroform and the usual sequence. For one free acid heptapeptide, chloroform modified with iso-propanol and acetic acid (91:7:2) was used isocratically. Flow rates were generally 10 ml/min and loads ranged from 0.2 to 5.0 g (47). While some might consider this borderline LC, the excellent separations achieved are of HPLC calibre.

2. Alumina.

Unfortunately there are very few uses of alumina being reported in any of the HPLC literature today, much less preparative ones. Whereas in the classical era this medium was widely used for steroids and alkaloids (the latter no doubt due to the severe and then little understood problems encountered with silica), it now seems unable to compete with silica.

Its use is reported by a British group in the semi-preparative purification of fractions containing the mycotoxins patulin, penicillic acid, and zearalenone for subsequent HPLC analysis (48). Alox 0520 (5 - 20 μm) from Machery-Nagel was used in 25 cm x 4.6 mm ID columns with a 50:50:1 chloroform:hexane:acetic acid system at 4 ml/min and loads of 100 μl. Preparative LC data

was also presented for aflatoxins and ochratoxin A on the same support.

3. Other Non-bonded Adsorbants.

a. Silver nitrate coated silica.

Whether this support belongs here or in the silica category is not easy to say. However the silver ion is certainly responsible for its unique chromatographic properties and I have therefore chosen to treat it separately. Two preparative applications are worth noting.

A Florida based group has isolated a number of olefinic insect sex pheromones, needed in a highly purified state for biological studies (49). A commercially available support, Adsorbosil-2-ADN (20% $AgNO_3$ coated silica, 2 - 11 μm) from Applied Science Laboratories, was evaluated as well as three commercially available silicas which were coated in house. Benzene or benzene-hexane mixtures were used for developement. Columns were 9.3 mm ID by lengths up to 50 cm with maximal loads of 100 - 200 mg.

A Japanese group prepared their own 10% and 20% $AgNO_3$ coated silica from LiChroprep Si-60 (15-25 μm) to separate urushiol diacetate on 60 cm x 2.2 cm ID columns with a 4:1 benzene: chloroform system (50).

b. Clay derived supports.

A rather unique support was devised by workers at Gulf to separate saturates, aromatics, resins, and asphaltenes from various liquid fuels (eg. mixtures derived from tar sands and coal) (51). The packing was prepared from Attapulgus clay by treatment with anhydrous $FeCl_3$ to 3% iron content and sieving to 60 - 80 mesh. The resins are retained on the clay from hexane and eluted with 1:1 methylene chloride:acetone. Eluate is passed through a IRA-904-OH^- column to remove any escaped iron.

c. Styrene-divinyl benzene copolymers.

The origins of these materials were discussed in an earlier section. There are several examples of their preparative use, notably the following. Both Hitachi gel 3010 and Amberlite XAD-2 (cf. Table 1) have been used to separate polymixin antibiotics and colistins, these being macrocyclic polypeptides. Only the Hitachi gel was used in a HPLC sense as it served for the final purifications on a small scale (52). The mechanism of separation appears much the same as on alkyl bonded reverse phase supports.

Swiss workers have studied micronized Amberlite XAD-2 (average size 60 μm) in glass columns (13 in x 0.5 in ID and 23 in x 1 in ID) as a support for the resolution of a variety of polar and non-polar drug metabolites (53). This work is of interest in that good retentions of many solute components are observed in a number of solvent systems containing high proportions of organic components. This is not usually the case with this support in its normal form with classical chromatography.

d. Other systems

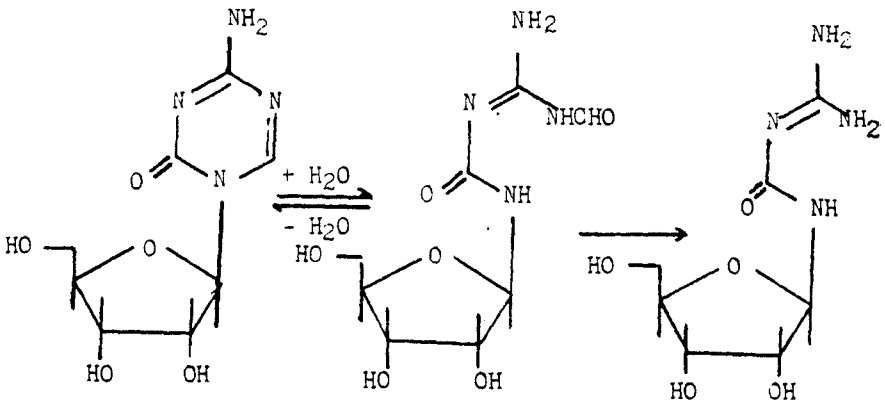
One paper has described the use of liquid crystals, long used in gas chromatography, as an HPLC support (54). While no preparative applications were made, the potential is of interest.

4. Bonded Packings.

a. With aqueous systems, C-18 supports.

A number of applications have been made in various areas of nucleotide research. Khorana's group has used it to purify intermediates obtained in the course of synthetic studies (55). Acetonitrile:aqueous buffer (ammonium acetate or triethylammonium acetate) systems in various proportions were used on μ-Bondapak C_{18} in 30 cm x 4 mm ID columns.

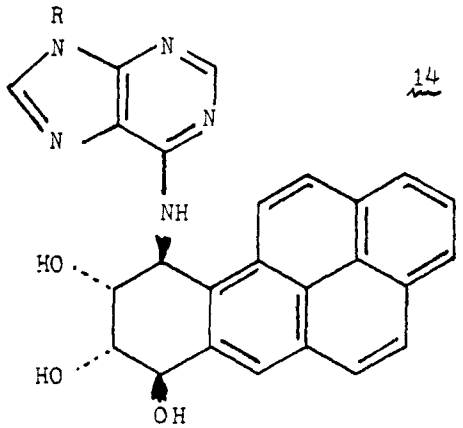
A labile hydrolysis product, 12, formed from the antitumor nucleoside 5-azacytidine, 11, could only be isolated by chromatography on a μ-Bondapak C_{18} column (30 cm x 7 mm ID) using 32 mg



11 12 13

loads and pure water as the solvent (56). A secondary hydrolysis product, 13, was also isolated but only in an impure state.

C_{18}-reverse phase preparative LC has been used to isolate carcinogen adducts such as the adenosine-benzo(a)pyrene complex, 14. Chromatography was carried out on Rainan C-18 columns (25 cm x 6.4 cm ID) using water:methanol systems (57).



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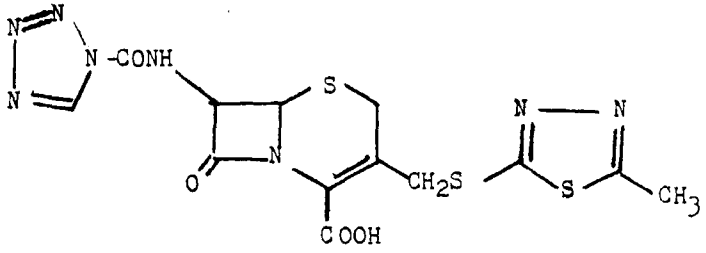
A guanine-aflatoxin adduct was isolated on a C_{18} Corasil B column (70 cm x 8 mm ID) using a 10% to 80% methanol gradient in water (58).

Aflatoxins themselves were preparatively isolated on C_{18}-Porasil B (35 - 75 μm) columns (8 ft x 3/8 in OD) with acetonitrile-water mixtures (59), an alternative to the normal phase systems noted earlier.

Normally chromatographers avoid counter-ions (PIC reagents) in preparative LC due to the difficulties of getting them out of the products. However, in forensic studies, Lurie and Weber have used various alkyl sulfonic acids to isolate ephedrine, phenylpropanolamine, LSD, and iso-LSD on Whatman Magnum 9 columns (60). The free bases were isolated, free of counter ion, by extraction with chloroform at pH $\geq$ 9.

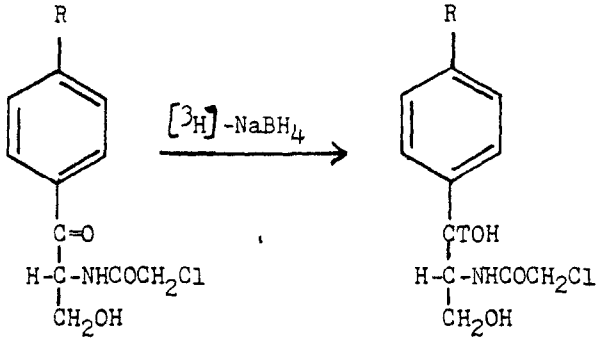
Tritium labelled amphenicols (cf. fig. 1) were synthesized and purified on C_{18}- Bondapak columns (30 cm x 7.8 mm ID) using methanol-water systems, eg. 15:85 for TAP.

The semisynthetic cephalosporin, cephazolin, 15, was purified on C_{18} Porasil B columns (1.22 m x 8 mm ID) using water as eluting solvent (62)



15

The synthetically derived diastereomeric mixtures [3-DL-[2-^{13}C]-leucine]- and [8-DL-[2-^{13}C]-leucine]-oxytocins were resolved on Partisil 10-MD ODS columns (50 cm x 9.4 mm ID) using an 81:19 0.05M ammonium acetate (pH 4) buffer:acetonitrile system at a flow rate of 6 ml/min with 15 - 20 mg loads (63).



Chloramphenicol: R = NO_2 (CAP)

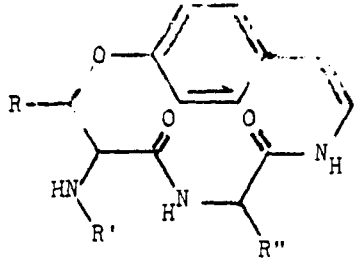
Thiamphenicol: R = SO_2CH_3 (TAP)

FIGURE 1

b. With aqueous systems, other than C_{18} packings.

A generalized approach for the semi-preparative resolution of complex mixtures such as crude plant extracts has been reported by a group from Germany (64). They use programmed solvent gradient sequences on LiChrosorb RP 8 (C_8 bonded phase, 7 μm) columns (25 cm x 8 mm ID). Gentian (Radix gentianae) root extracts are used as a model with a gradient system of 5 - 70% acetonitrile in water. Structural elucidations of isolated components is carried out by UV and mass spectral analysis.

An ethyl bonded support, LiChrosorb C2 (10 μm) in 15 cm and 25 cm x 1 cm ID columns has been used with good success for the semi-preparative fractionation of various phencyclopeptides, 16.



R = iso-propyl or phenyl

R' = iso-butyl, phenylethyl, or -indolylmethyl

R" = N-methyl-isoleucyl or valyl

16

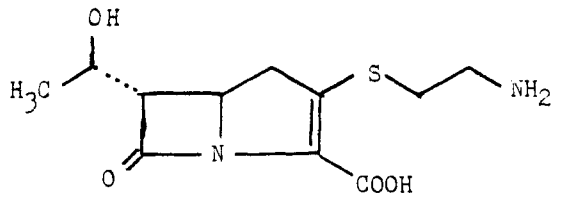
Elution was carried out with acetonitrile containing 10 - 30% of 0.0015% aqueous ammonia, the actual concentration depending on the peptides being fractionated. At loads of less than a milligram, 10 - 20 injections were needed to obtain enough material for structure work (65).

Rubinstein at Roche Institute has explored the preparative LC of proteins on bonded phases (66). While peptides of up to 12,000 daltons are fractionated well on C_{18} reverse phase supports, small proteins tend to be irreversibly bound. Resolution and recovery are poor on C_2 packings. A C_8 support, specifically LiChrosorb RP-8, has proven suitable for the fractionation of proteins up to 30,000 daltons. The paper goes into considerable detail on the chromatographic properties of the proteins with both isocratic and gradient n-propanol:aqueous buffer solvent systems.

Investigators at Johannes Gutenberg University, Mainz, Germany have used LiChrosorb diol, having a dyhydroxyalkyl bonded phase, to fractionate proteins in the molecular weight range of 10,000 - 100,000. Using aqueous buffer systems and columns 25 cm by 0.6 cm or 2.35 cm ID (depending on scale), they find the separations to proceed almost entirely by size exclusion effects. They find this packing superior to flaccid gels such as Sephacryl S-200 Superfine both in terms of time and the volumes of liquid which must be processed (67).

A group at the Chemical Synthesis Laboratories of Dynapol in Palo Alto, Calif. has used Waters' carbohydrate analysis packing in a 30 cm x 7.8 mm ID column with isocratic water:acetonitrile systems to resolve 500 mg samples of synthetic carbohydrates for evaluation as nonnutritive sweeteners (68). According to Kirkland (3) these packings are probably silica based with an aminoalkyl bonded phase.

Derivatives of thienamycin, 17, acetylated at the C-12 amino group and/or lacking the hydroxyl group at the β-position, were produced by mutants of Streptomyces cattleya and purified by



17

preparative LC (69). LiChrosorb NH_2 (10 μm, an aminoalkyl bonded support) in 25 cm x 1 cm ID columns was used with acetonitrile: 0.02 M phosphate (pH 6) or ammonium acetate (pH 6.5) buffers. Proportions were 81:19 and 74:26 respectively.

c. With non-aqueous systems.

Although relatively little preparative LC has been reported to date where bonded packings were used with normal phase solvent systems, this is a situation which is likely to change. For now two examples might be cited.

In addition to the isolation of myristicin and related natural products on Partisil 5, Wulf et al (44) used a Partisil PAC (cyanoalkyl bonded packing) column (25 cm x 4.6 mm ID) to further purify falcarinol, 18, on a semi-preparative scale. The solvent

$$CH_3\text{-}(CH_2)_5\text{-}CH{=}CH\text{-}CH_2\text{-}C{\equiv}C\text{-}C{\equiv}C\text{-}CH_2\text{-}CHOH\text{-}CH{=}CH_2$$

18 (believed structure) .

system was 7% tetrahydrofuran in heptane, run at 3 ml/min with recycle.

Rubinstein, along with his work on C_8 reverse phase systems, has studied the fractionation of hydrophobic proteins on LiChrosorb diol (66). As the systems are 50 - 80% n-propanol (mixed with aqueous buffer), the author considers them normal phase. The most severe limitation is that the proteins must be soluble in the system. General comment is made concerning the need for

slower flow rates in working with such large molecules to allow ample time for equilibrium since the latter diffuse much more slowly than do small molecules.

5. Gel Permeation Media.

Because of the macroporous nature of most HPLC column supports, molecular sieving effects are often encountered when chromatogramming larger molecules. One such case on LiChrosorb diol was cited in the previous section (67). The limitations are, as already noted, the slow flows required and the problems of scaling up.

One recent paper has dealt with this area in general (70). A variety of gel permeation media were evaluated including some suited only to low pressures. The maximum capacity for the systems investigated was 1 mg, but the author notes that preparative columns are now available with capacities for up to 100 mg of protein.

Gel permeation columns have long been used to clean up samples, primarily by removing high molecular weight impurities. This usage has been at both the analytical and semi-preparative levels.

6. Ion-Exchange Packings.

A wide variety of ion-exchange packings are now in use analytically and can be expected to make their mark in the preparative area. As long ago as 1971, Pearson et al had developed a reversed phase ion-exchange system, the RPC-5 system. The support is a polychlorotrifluoroethylene resin coated with a C_8 - C_{10} trialkylmethylammonium chloride adsorbent called Adogen 464. It was designed to separate individual tRNA species (71).

A group at Brown University has adapted this material to the preparative fractionation of DNA restriction fragments (72). They found some fractionation patterns different from those observed on gel electrophoresis. Although not a high resolution system,

it does have a much greater capacity, allowing loads of up to 5 - 10 mg. The authors found it ideal for first stage fractionation.

7. Other Systems.

Earlier reference was made to the work of Pirkle (16,17) in developing chiral bonded phases. A unique approach to chiral packings has been reported by a French group (73). These were obtained in two steps. A porous beaded gel (10 - 20 μm) was first prepared by copolymerization of acrylamide and methylene bisacrylamide. An amino acid was then grafted to the resin by condensation with formaldehyde. Finally the beads were shaken with an aqueous solution of a complexing metal salt, eg. copper. The beads have a porosity of 0.4 ml/ml wet resin and amino acid content was up to 3 meq/g. Columns used were 30 cm x 4.8 mm ID. A variety of amino acids were grafted and studied. The authors present various structures possibly formed as complexes during chromatography of DL-amino acid mixtures to give chiral entities. Although only divalent copper was used in this study reference is made to another in which a dozen or so complexing metal ions were studied.

The support itself has some unusual properties. As expected high flow rates cause loss of resolution due to compression. But at low flow rates resolutions becomes poor also with peak tailing. Ideal flow rates appear to vary with the particle size from about 2 ml/min for those 20 - 28 μm to about 5.5 ml/min for those 10 μm. Examples given, however, were run at much slower flow, namely 0.4 - 0.5 ml/min.

E. FUTURE POTENTIALS

Today the competition among the suppliers of HPLC equipment and materials is enormous. It is particularly intense in two major areas, solvents and microprocessors. The end point for the first is not too difficult to predict. Logistically it will come

when everyone produces an absolutely pure, particle free product. From then on it's all a matter of price and perhaps a little snake oil salesmanship.

There is no logical end point for microprocessors. Many of those available today have potentials to do things for which no interfacing has as yet been made available. They probably have potentials to do things no one has even thought of.

Except possibly for large scale work, the applications to preparative LC should be many and varied. Waters now sells the only unit (the WISP) capable of loading different volumes from varied sizes of vials, but they probably will not enjoy this advantage much longer. The area of sample preparation and injection is just now being developed for purposes other than repetitive analysis.

With the coming decade, the chromatographer will find himself able to place any liquid sample into a container and program it to be injected onto columns with and/or without dilutions. Interfacing with the detector will be available so that the injector may be informed as to whether to go on or to repeat the sample, possibly at a different concentration or level. Microprocessors will prepare samples with capabilities of weighing, dissolving and diluting anything that will pour while being in a reasonably uniform state. Interfacing with fraction collectors should allow cuts in tubes to be sampled and evaluated by analytical HPLC.

The potentials are limitless. With banks of solvent reservoirs and solenoid or other switching devices, integrated pumps may be directed to run systems searches through a variety of solvent systems with multicomponent and gradient capabilities. By using high-pressure switching devices to go from one column to another, a variety of supports in both preparative and analytical modes could be explored and utilized. I suspect that eventually

the entire HPLC process will become almost completely automated and able to run virtually unattended for 24 hours a day.

For the truly dedicated who may have home computer centers, there will be the option of tying in the lab microprocessor over the phone lines. When something should go amiss in the night such as a power failure, equipment problems, or a slapdash janitor's mop handle, all it will take is a few rings. Clad in bathrobe and slippers the intrepid investigator can rush to his console and, with a few deft manipulations after consulting its screen, set all right again. From this one can go on to conjure up pictures of scientists sitting comfortably at home beside their home computer center peacefully dreaming up new experiments, while at the lab work progresses at breakneck speed with nothing to be heard but the quiet humming of the pumps interspersed with occasional clicks as switches go and reels advance.

In reality, the investigator will be working harder than ever. Someone will have to write the programs, evaluate the results, decide what fractions to pool, work them up, and then do whatever it was they wanted the stuff for in the first place. Considering that the system can run 168 hours a week, an easy computation shows that it has capabilities for running better than four times the number of experiments that could be done in a forty hour week. This adds up to a sizeable amount of evaluation, writing, planning, deciding, work up, and other kinds of doing. As for staying home with the domestic unit to do all this, this is possible to a certain extent. However, it's hardly likely to become popular with bosses or home bound spouses.

While it is fun to speculate, some time will probably pass before very many labs will have a total system anything like the one described above. There are cost factors. And there are human factors. It will require considerable training and experience just to approach some kind of maximal use of such a complex

arrangement of apparatus. Most workers will probably prefer a less frenetic level of activity. Sharing the system between different groups and persons will ease individual strain. Isolation work will still be fun. Productivity will be increasing and bosses will be smiling.

In another area, column supports or packings will become even more varied. Three micrometers is now considered the lower limit for particle size insofar as one may gain efficiency by reducing particle size. However, this may not be hard and fast. Bonding will introduce even more properties to surfaces. Various polymers with rigid matrices will appear in fine macroporous forms. Hopefully better terminology and instructions will appear to help the non-mathematically inclined to choose the best porosity for a given purpose. Actually many company representatives are very good at helping one in this area.

Fine packings in large columns, compressed or otherwise, will bring even greater efficiency to large scale preparative work. Microprocessors will probably have the least direct effect on this kind of effort. The torrential solvent flows and rapidity with which things happen simply don't allow such interfacing to be worth the effort.

One thing will not change. Sticky preparative problems will still be sticky. But they probably will get solved faster just because one can try so many more things in a given amount of time. And that is the primary commodity, the only commodity that money can not really buy, which modern preparative LC is, and will be, giving us, namely time.

REFERENCES.

1. Nettleton, D. E., Jr., J. Liq. Chromatogr., 4 (Suppl. 1), 141 (1981).
2. DeStefano, J. J. and Kirkland, J. J., Anal. Chem., 47, 1103A, 1193A (1975).

3. Snyder, L. R. and Kirkland, J. J., Introduction to Modern Liquid Chromatography, second edition, John Wiley & Sons (1979).

4. Verzele, M. and Geeraert, E., J. Chromatogr. Sci., 18, 559 (1980).

5. Rabel, F. M., J. Chromatogr. Sci., 18, 394 (1980).

6. Majors, R. E., J. Chromatogr. Sci., 18, 488 (1980).

7. Henry, R. A. and Smith, V. M., Chromatogr. Sci., Chapter 6, 303 (1978).

8. Krusche, J. U., Chimia, 33, 93 (1979).

9. Pirkle, W. H., Chromatogr. Sci., 9, 331 (1978).

10. Beck, W. and Halász, I., Fresenius Z. Anal. Chem., 291, 340 (1978).

11. Packing HPLC Columns with the Spherisorb Range of Column Packing Materials, brochure available from Phase Separations, 255 Oser Ave., Hauppauge, N.Y., 11787 (Phase Separations, Ltd. in the United Kingdom).

12. Yau, W. W., Kirkland, J. J. and Bly, D. D., Modern Size-Exclusion Liquid Chromatography, John Wiley & Sons (1979).

13. Lindgren, G. and Pernemalm, P.-A., J. Liq. Chromatogr., 3, 1737 (1980).

14. Guiochon, G. and Colin, H., Spectra-Physics Chromatography Review, 4 (#2), 3 (1978).

15. Ciccioli, P., Tappa, R., DiCorcia, A. and Liberti, A., J. Chromatogr., 206, 35 (1981).

16. Pirkle, W. H. and House, D. W., J. Org. Chem., 44, 1957 (1979).

17. Pirkle, W. H., House, D. W. and Finn, J. M., J. Chromatogr., 192, 143 (1980).

18. Miller, J. M. and Strusz, R., Amer. Laboratory, 12, 29, (1980).

19. Codina, J., Amer. Laboratory, 11, 93 (1979).

20. Blakley, C. R., Carmody, J. J. and Vestal, M. L., Anal. Chem., 52, 1636 (1980).

21. Blakley, C. R., McAdams, M. J. and Vestal, M. L., Adv. Mass Spectrom., 1980, 8B, 1616 (1980).

22. Vestal, M. L., A New Universal Detector for Liquid Chromatography, paper #117, Expochem '80 (1980).

23 Schäfer, K. H. and Levsen, K., J. Chromatogr., 206, 245 (1981).

24. Bristow, P. A., J. Chromatogr., 122, 277 (1976).

25. Coq, B., Cretier, G., Gonnet, G. and Rocca, J. L., Chromatographia, 12, 139 (1979).

26. Barford, R. A., McGraw, R. and Rothbart, H. L., J. Chromatogr., 166, 365 (1978).

27. Wehrli, A., Hermann, U. and Huber, J. F. K., J. Chromatogr., 125, 59 (1976).

28. Beck, W. and Halasz, I., Fresenius Z. Anal. Chem., 291, 312 (1978).

29. Scott, R. P. W. and Traiman, S., J. Chromatogr., 196, 193 (1980).

30. Böhlen, P. and Kleeman, G., J. Chromatogr., 205, 65 (1981).

31. Horning, M. G. and Lertratanangkoon, K., J. Chromatogr., 181, 59 (1980).

32. Barrow, S. E., Taylor, A. A., Horning, E. C. and Horning, M. G., J. Chromatogr., 181, 219 (1980).

33. Evans, N., Games, D. E., Jackson, A. H. and Matlin, S. A., J. Chromatogr., 115, 325 (1975).

34. Adams, M. A. and Nakanishi, K., J. Liq. Chromatogr., 2, 1097 (1979).

35. Suatoni, J. C. and Swab, R. E., J. Chromatogr. Sci., 14, 535 (1976).

36. Suatoni, J. C. and Swab, R. E., J. Chromatogr. Sci., 18, 375 (1980).

37. Anderson, L. A., Doggett, N. S. and Ross, M. S. F., J. Liq. Chromatogr., 2, 455 (1979).

38. Bergman, N.-A. and Hall, B., Acta. Chem. Scand., B33, 148 (1979).

39. Yoshida, T., Shu, C.-K. and Theimer, E. T., J. Chromatogr., 137, 461 (1977).

40. Sneden, A. T. and Beemsterboer, G. L., J. Nat. Prod., 43, 637 (1980).

41. Tsukida, K., Kodama, A. and Ito, M., J. Nutr. Sci. Vitaminol., 24, 593 (1978).

42. Funk, M. O., Isacc, R. and Porter, N. A., Lipids, 11, 113 (1976).

43. Jones, B. B., Clark, B. C., Jr. and Iacobucci, G. A., J. Chromatogr., 202, 127 (1980).

44. Tiebach, R. K. D. and Schramm, M., Chromatographia, 13, 403 (1980).

45. Wulf, L. W., Nagel, C. W. and Branen, A. L., J. Chromatogr., 161, 271 (1978).

46. Risk, M., Lin, Y. Y., MacFarlane, R. D., Ramanujam, V. S., Smith, L. L. and Trieff, N. M., Dev. Mar. Biol., 1, 335 (1979).

47. Gabriel, T. F., Michalewsky, J. and Meienhofer, J., J. Chromatogr., 129, 287 (1976).

48. Hunt, D. C., Bourdon, A. T. and Crosby, N. T., J. Sci. Food Agric., 29, 239 (1978).

49. Heath, R. R., Tumlinson, J. H., Doolittle, R. E. and Proveaux, A. T., J. Chromatogr. Sci., 13, 380 (1975).

50. Yamauchi, Y., Oshima, R. and Kumanotani, J., J. Chromatogr., 198, 49 (1980).

51. Galya, L. G. and Suatoni, J. C., J. Liq. Chromatogr., 3, 229 (1980).

52. Kimura, Y., Kitamura, H., Araki, T., Noguchi, K. and Baba. M., J. Chromatogr. 206, 563 (1981).

53. Dieterle, W., Faigle, J. W. and Mory, H., J. Chromatogr., 168, 27 (1979).

54. Taylor, P.J. and Sherman, P. L., J. Liq. Chromatogr., 3, 21 (1980).

55. Fritz, H. J., Belagaje, R., Brown, E. L., Fritz, R. H., Jones, R. A., Lees, R. G. and Khorana, H. G., Biochemistry, 17, 1257 (1978).

56. Beisler, J. A., J. Med. Chem., 21, 204 (1978).

57. Jeffrey, A. M., Grzeskowiak, K., Weinstein, I. B., Nakanishi, K., Roller, P. and Harvey, R. G., Science, 206, 1311 (1979).

58. Essigmann, J. M., Croy, R. G., Nadzan, A. M., Busby, W. F., Jr., Reinhold, V. N., Buchi, G. and Wogan, G. N., Proc. Natl. Acad. Sci. U.S.A., 74, 1870 (1977).

59. Stubblefield, R. D. and Shotwell, O. L., J. Assoc. Off. Anal. Chem., 60, 784 (1977).

60. Lurie, I. S. and Weber, J. M., J. Liq. Chromatogr., 1, 587 (1978).

61. Martin, J. L., Taburet, A. M. and Pohl, L. R., Anal. Biochem., 96, 215 (1979).

62. White, E. R., Carroll, M. A., Zaremba, J. E. and Bender, A. D., J. Antibiot., 28, 205 (1975).

63. Viswanatha, V., Larsen, B. and Hruby, V. J., Tetrahedron, 35 1575 (1979).

64. Hupe, K.-P., Lauer, H. H. and Zech, K., Chromatographia, 13, 413 (1980).

65. Lagarias, J. C., Goff, D., Klein, F. K. and Rapoport, H., J. Nat. Prod., 42, 220 (1979).

66. Rubinstein, M., Anal. Biochem., 98, 1 (1979).

67. Roumeliotis, P. and Unger, K. K., J. Chromatogr., 185, 445 (1979).

68. Wingard, R. E., Jr., Ng, S., Dale, J. A. and Wang, P. C., J. Liq. Chromatogr., 1, 775 (1978).

69. Rosi, D., Drozd, M. L., Kuhrt, M. F., Terminiello, L., Came, P. E. and Daum, S. J., J. Antibiot., 24, 341 (1981).

70. Welinder, B. S., J. Liq. Chromatogr., 3, 1399 (1980).

71. Pearson, R. L., Weiss, J. F. and Kelmers, A. D., Biochim. Biophys. Acta., 228, 770 (1971).

72. Landy, A., Foeller, C., Reszelbach, R. and Dudock, B., Nucl. Acids Res., 3, 2575 (1976).

73. Lefebure, B., Audebert, R. and Quivoron, C., J. Liq. Chromatogr., 1, 761 (1978).