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HIGH-PERFORMANCE DISPLACEMENT CHROMATOGRAPHY

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SUMMARY

The potential of the displacement mode of liquid chromatography for preparative-scale separations has been investigated by using microparticulate packed columns and instrumentation typically employed in analytical high-performance liquid chromatography (HPLC). Although the physico-chemical basis of the technique has long been established, its development was handicapped by lack of efficient chromatographic systems and suitable tools to monitor column effluent and gather data required for analysis and design of the displacement chromatographic process and for selection of suitable displacers. The availability of novel solid stationary phases with adequate properties to facilitate rapid sorption kinetics, the possibility of monitoring the effluent of the "fractionator" liquid chromatograph by an auxiliary "analyzer" unit as well as the relatively easy evaluation of appropriate adsorption isotherms by using the HPLC equipment, however, provide a basis to advance the displacement mode of liquid chromatography for preparative separations with analytical columns. After a brief review of the principles of the technique the dual chromatographic system used in the experiments for fractionation and analysis is described. Expressions for the efficiency of separation are given and factors affecting the development of the "displacement train" containing the separated feed components are enumerated. The interplay between the nature and concentration of displacer, the column length, amount of feed, flow-rate as well as the nature and concentration of the components of the mixture to be fractionated is discussed qualitatively. Fractionation of mixtures containing phenolic compounds or adenosine and inosine on columns packed with octadecyl-silica bonded phases (reversed-phase chromatography) is used to exemplify the effect of the operating conditions on the afficacy of the separation. The results are related to the adsorption isotherms of the substances that were measured by frontal analysis and found to follow the Langmuir model. The results suggest that upon accumulation of a sufficient amount of data on adsorptivities in chromatographic systems of interest high-performance displacement chromatography offers a promising approach to the use of ubiquitous HPLC equipment for preparative-scale separations with analytical columns.

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INTRODUCTION

Displacement of one sample component by another during the development of a chromatogram in liquid column chromatography was a characteristic feature of the technique prior to the wide acceptance of partition chromatography and the use of linear elution development. The first classification of the different modes of chromatography as frontal, displacement and elution development was made by Tiselius[1]. He, as well as Claesson[2], investigated displacement chromatography with regard to the physico-chemical basis of the separation process with emphasis on potential analytical applications. Therefore, the technique has most commonly been referred to as displacement analysis ("Verdrängungsanalyse" in German) in the literature. The success of Martin and co-workers[3–5] with partition chromatography in the linear elution mode, however, overshadowed further development of displacement chromatography in analytical separations although some of its features are —mainly unwittingly— used in preparative-scale chromatography.

Recent developments in high-performance liquid chromatography (HPLC) with respect to column engineering and instrumentation have prompted us to explore the potential of liquid chromatography in the displacement mode for preparative-scale separations with columns and precision instruments that are presently used in analytical work. Generally, separation of relatively large amounts of material is handicapped in linear elution chromatography by poor utilization of the stationary and mobile phases because of the low permissible eluite concentrations. Furthermore the equipment and the column are only partially occupied by the bands of sample components that travel with different velocities. The inevitable dilution process associated with elution chromatography also hampers product recovery from the effluent.

In contradistinction the feed components in displacement chromatography are separated into adjacent square-wave zones having rather high concentrations and traveling at the same velocity. This difference between the elution and displacement modes of chromatography and its implications for preparative scale separation were already fully appreciated by Tiselius and Hagdahl[6] although the technique has enjoyed popularity neither in analytical nor in preparative chromatography. With the highly efficient columns and precision instrumentation presently available, however, the above features of displacement chromatography offer an opportunity to carry out preparative-scale separations with analytical columns in HPLC.

THEORETICAL

Principles of displacement chromatography

In this mode of chromatography the column packed with a solid adsorbent is first equilibrated with a carrier solvent (mobile phase) that has a low affinity to the stationary phase. Then the feed solution containing the mixture dissolved in the carrier is introduced so that its components are adsorbed in the inlet section of the column.

Subsequently the solution of a displacer substance that has stronger affinity to the stationary phase than any of the feed components is pumped into the column. Provided the column is sufficiently long, the components of the feed arrange them-

selves upon the action of the displacer front moving down the column into a "displacement train" of adjoining square wave concentration pulses of the pure substances, all moving with the same velocity. A displacement diagram is illustrated in Fig. 1. As will be shown later the solute concentrations in the zones are much higher than in linear elution chromatography under comparable conditions[7].

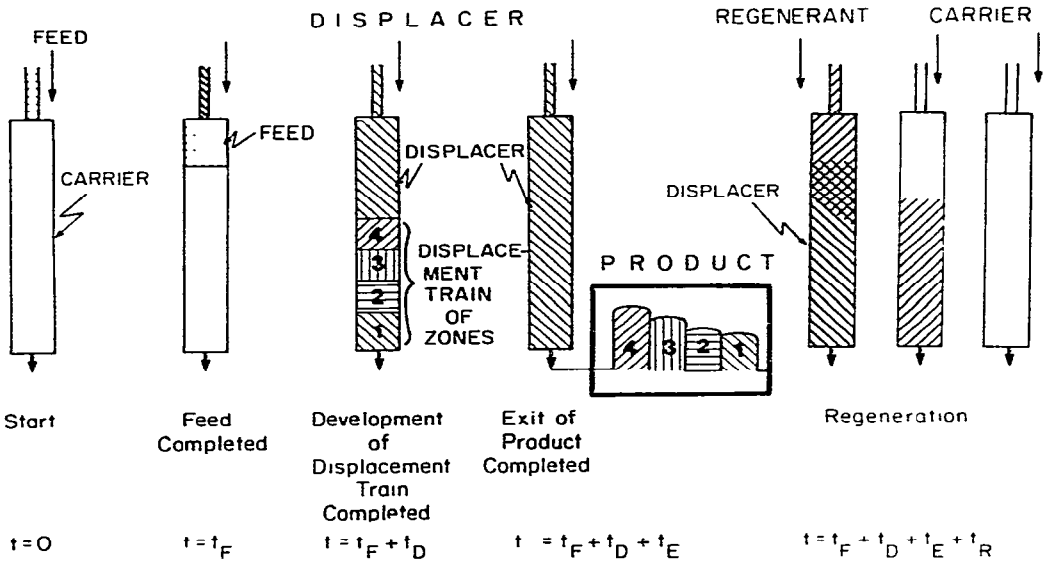

Fig. 1. Stages of operation in displacement chromatography. Initially the column is equilibrated with the carrier. The mixture to be separated is fed into the column and thereafter the displacer solution is introduced. As the displacer front moves down the column the displacer train containing adjacent zones of the separated feed components is developed. After the product zones egress the column it is regenerated by removing the displacer and re-equilibrating with the carrier. The time requirement for the individual steps are as follows: t_F, feed time; t_D, development time of displacement train; t_L, exit time of product; t_R = time of column regeneration.

Fig. 1 shows the sequence of operational steps in displacement chromatography. The process is aimed at obtaining a fully developed displacement train so that the feed components are completely separated and emerge at relatively high concentrations in the effluent at the column outlet where they can be collected as individual fractions. Separation by displacement development is monitored by analyzing the column effluent by a suitable technique. The employment of an "analyzer" HPLC unit as an auxiliary to the "fractionator" liquid chromatograph is probably the most convenient. However, thin-layer chromatography or another analytical device such as one or more selective detectors can be used.

During the introduction of the feed mixture at the top of the column the components saturate the stationary phase and frontal chromatography occurs. The displacer must have greater affinity to the stationary phase than any component of the mixture and the concentration of displacer solution is critical in determining the time and column length required for the development of the displacement train. The velocity of the displacer front moving down the column depends on the adsorptivity

and concentration of the displacer and determines the velocity of the displacement train. The sequence of the individual components from the column outlet toward the displacer front corresponds to increasing affinity of the species for the stationary phase. Thus, in Fig. 1 components 1 and 4 are the most weakly and most strongly adsorbed components, respectively.

After the last component of the feed mixture leaves the column, the displacer has to be removed and the column has to be reequilibrated with the carrier. The need for column regeneration, an operational step that does not contribute directly to separation, is an undesirable feature of the technique.

Upon full development of the displacement train the separation is completed, therefore, further residence in the column does not improve separation. The properties of the fully developed displacement train have a simple relation to the appropriate isotherms of the feed components and the displacer as well as to the concentration of the latter and will be discussed below. On the other hand, a sufficiently general description of the transient part of the process associated with the development of the displacement train in the column has been found mathematically intractable[8]. Consequently we shall restrict ourselves to a qualitative discussion of the effect of various physico-chemical and operational parameters on the transient demixing process that plays a major role in determining the speed and efficiency of separation in displacement chromatography.

Properties of fully developed displacement train

If the column is sufficiently long, successive displacement of the feed components by the displacer and by each other as they move down the column results in a fully developed displacement train. It consists of adjacent square-wave concentration pulses of the individual feed components in the order of increasing affinity to the stationary phase as depicted in Fig. 2. Unlike in elution chromatography where peaks travel at different velocities, at the final stage of displacement development all components move with the same velocity as determined by the adsorption isotherm and concentration of the displacer that "drives" the displacement train. This condition is conveniently termed *isotachic* from τάχοσ, the Greek word for speed.

The velocity of a concentration step, u_i, of species i from mobile phase concentration to C_i, in a chromatographic column is given by

$$u_i = \frac{u_0}{1 + [\Phi q_i/C_i]} \tag{1}$$

where u_0 is the carrier velocity, Φ is the phase ratio in the column and q_i is the amount of solute i adsorbed by unit volume of stationary phase in equilibrium with a mobile phase having solute concentration C_i. As seen in Fig. 2, q_i/C_i is the chord to the isotherm at concentration C_i and determines the species velocities according to eqn. 1. Therefore equality of the velocities of the zones in the fully developed displacement train, which can be considered a series of truncated concentration steps rising from zero to C_i, means that the chords of the isotherms of the displacer and the feed components present in the displacement train fall on the same straight line termed the operating or, occasionally, speed line[9]. This is shown in Fig. 2 and can be expressed by the following relationships:

$$u_D = u_1 = u_2 = \ldots = u_i \ldots = u_n \tag{2a}$$

$$\frac{q_D}{C_D} = \frac{q_1}{C_1} = \frac{q_2}{C_2} = \ldots = \frac{q_i}{C_i} = \ldots \frac{q_n}{C_n} \tag{2b}$$

where the subscript D refers to the displacer. From Fig. 2 and eqn. 2b it follows that the concentrations of fully developed zones of the components are determined by the intersections of the individual adsorption isotherms with the operating line. Thus the solute concentration in each zone moving in the fully developed displacement train is

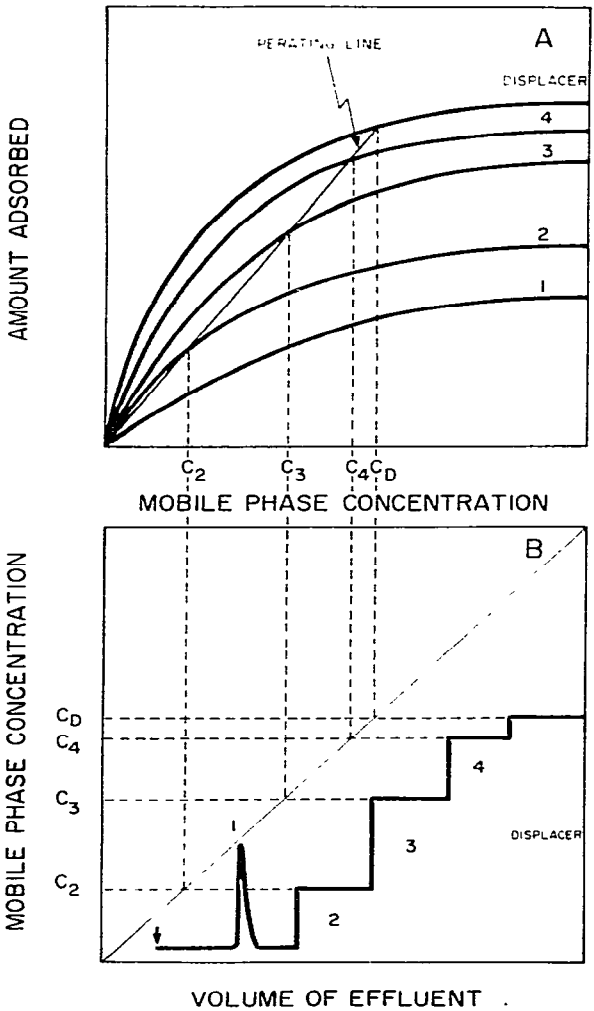

Fig. 2. Graphical representation of the isotherms of the feed components and the operating line (A) as well as the corresponding fully developed displacement train (B). Concentrations of the component zones, determined by the intersections of the operating line and the adsorption isotherms of the components are projected from A to B with the aid of a 45° line. The isotherm of the first component lies below the operating line at all concentrations, hence it elutes from the column.

determined both by the speed line and the isotherm, therefore the height of the zone is characteristic for that solute. On the other hand, conservation of mass requires that the length of each zone be proportional to the amount of solute because concentration is expected to be uniform throughout the zone, with the exception of the boundaries. These features of displacement chromatography were considered very attractive for analytical separation as measurements of zone height and length offer simple means for identification and quantitative determination of the individual components, respectively. Fig. 2 also illustrates that zone heights must increase with retention as the operating line intersects the isotherm at higher and higher concentrations. It also follows that when the concentration in the feed is smaller than this characteristic concentration for a component it will become more concentrated in the course of displacement chromatography.

The requirement for complete displacement development to occur is that the isotherms be convex and the operating line drawn as the chord of the displacer isotherm intersect the isotherms of all feed components. If the affinity of a substance to the stationary phase is so low that its isotherm lies below the operating line, that component will elute as a "peak" as also illustrated in Fig. 2. Adsorbents used as stationary phases in HPLC frequently yield isotherms that can be approximated by the Langmuir model for most substances. Therefore, our description is restricted to chromatographic systems in which Langmuir isotherms prevail and, as a further constraint, the isotherms do not intersect each other.

Factors affecting displacement development

The goal in displacement chromatography is to develop adjacent concentration zones of the components with sharp boundaries in the shortest possible time and with the highest possible load for a particular chromatographic system. High efficiency columns packed with microparticulate stationary phases having mean particle diameter in the range from 3 to 10 μm and appropriate accessories are available today to construct a liquid chromatograph for displacement development and minimize the deleterious effects on the separation process of axial dispersion[10] that plagued early efforts in this field[11]. Furthermore, adsorbents employed in HPLC as stationary phases exhibit a relatively homogeneous surface. As a result, not only are the adsorption isotherms expected to be nearly Langmuirian but also the sorption kinetics are believed to be sufficiently rapid to obtain favorable dynamic behavior.

It has already been mentioned that successful displacement chromatography calls for conditions under which the isotherms of the components are concave downwards such as those of the Langmuir type. The requirement arises from the need for self-sharpening boundaries to develop the square wave pattern and accomplish the separation of the mixture with a minimum of cross-contamination of the individual components. With such isotherms, if they do not intersect each other, that is, they have similar shape, the corresponding boundaries are self-sharpening. As such conditions occur in a given chromatographic system with solutes having similar chemical structure, displacement chromatography appears to be most effective for the separation of closely related compounds such as homologues.

On the other hand, the time or column length required for full development of the displacement train decreases with increasing difference in the affinities of two consecutive components to the stationary phase, *i.e.*, with increasing potency of the

stronger binding component to displace the weaker adsorbing component from the surface. Therefore the more the competitive isotherm of the latter is supressed in the presence of the former, the faster can a sharp boundary develop between the zones. Thus, the efficacy of boundary sharpening in displacement chromatography increases with the difference in the adsorptivity of two components. Of course, the effect is enhanced if the stronger retarded species is present at higher concentrations.

Langmuir isotherms are described by the equation

$$q = \frac{b_i K_i C}{1 + K_i C} \tag{3}$$

where q is the amount of surface bound solute per unit amount of sorbent, b_i is the saturation concentration at the surface and K_i is the binding constant of the solute to the sorbent.

It can be shown easily[12] that when the competitive isotherms of the feed components remain Langmuirian in the presence of the other components the criterion for displacement chromatography is expressed by the inequality

$$b_1 K_1 < b_2 K_2 < \ldots\ldots < b_i K_i < \ldots\ldots < b_D K_D \tag{4}$$

where subscript D refers to the displacer and the numbers increase with the magnitude of retention. In view of the above discussion the development of the displacement train is facilitated by large absolute and relative values of $b_i K_i$. As can be inferred from the definition of the operating line in Fig. 2 a sufficiently high displacer concentration is also required to accomplish rapid displacement development.

Efficacy of separation

In preparative work the amount of product recovered per unit time and the cost involved ultimately determine the efficiency of the chromatographic separation. In view of the various steps involved in displacement development as shown in Fig. 1, the definition of an overall efficiency measure would include a wide range of system parameters and operational variables. The goal of this section is more modest, we wish to measure the degree of separation and sample purity on the basis of the displacement diagram, in a way similar to that given by Partridge and Westall[13].

Fig. 3 illustrates a typical displacement diagram as obtained by measuring separately the concentrations of the individual components in the product stream. It is seen that the zones overlap somewhat and the fraction of the components present in the mixed boundary region cannot be recovered in pure form. In other words, the purity of the product decreases with the fraction of that component lost to the overlapping boundary region, and the efficiency of separation increases with the sharpness of the boundaries that determine the recovery of product in pure form.

As shown in Fig. 3, the front and rear boundaries of the ith component are contaminated by the $(i - 1)$th and $(i + 1)$th components, respectively. The lengths of the plateau region and the height of zone i are given by $l_{i,p}$ and h_i, respectively. The lengths of the corresponding mixed front and rear boundaries are $l_{i,f}$ and $l_{i,r}$, thus, the total zone length of component i, $l_{i,tot}$, is given by

$$l_{i,tot} = l_{i,f} + l_{i,p} + l_{i,r} \tag{5}$$

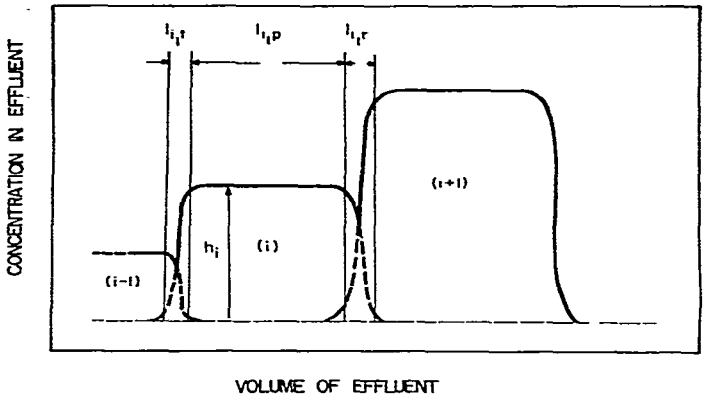

Fig. 3. Diagramatical representation of zone overlap in the displacement train.

The fraction of the ith component that can be recovered in pure form in the product stream, P_i, is given by

$$P_i = \frac{m_{i,\text{out}} - m_{i,\text{f}} - m_{i,\text{r}}}{m_{i,\text{out}}} \tag{6}$$

where $m_{i,\text{out}}$ is the total amount of the ith component in the effluent, and $m_{i,\text{f}}$ and $m_{i,\text{r}}$ are the amounts of the ith component present in the overlapping regions in the front and rear boundaries, respectively. In Fig. 3 quantities $m_{i,\text{f}}$, $m_{i,\text{r}}$ and ($m_{i,\text{out}} - m_{i,\text{f}} - m_{i,\text{r}}$) are represented by areas $h_i l_{i,\text{f}}$, $h_i l_{i,\text{p}}$ and $h_i l_{i,\text{p}}$, respectively. It is also indicated that in the fully displacement train the extent of overlap for adjacent zones is expected to be nearly equal.

According to eqn. 6 when $m_{i,\text{tot}} = m_{i,\text{f}} + m_{i,\text{r}}$ the purity is zero because the solute zone consists solely of the two boundaries, *i.e.*, $l_{i,\text{p}} = 0$. In other words, if the amount of component i in the feed is sufficiently small it resides solely in the boundary regions and the zone takes the form of a peak as depicted in Fig. 4A. Upon increasing the amount of c in the feed $m_{i,\text{tot}}$ becomes greater than the sum of $m_{i,\text{f}} + m_{i,\text{r}}$ and a plateau region of constant concentration is formed *vide* Figs. 4B and 4C. The length of the plateau region as well as the purity of the product increase with the amount of component c as illustrated in Figs. 4B and 4C. As a result, upon increasing the amount of that component in the feed, its purity increases as shown by the values of the purity parameter in Figs. 4A to 4B as long as the displacement train is fully developed and the lengths of mixed boundaries remain invariant.

For the case of displacement chromatography of homologous substances when difficulties in product analysis make it impossible to construct a displacement diagram another graphical construction can be used to illustrate the separation effectiveness. Product fractions are chromatographed by using the analyzer, and the peak heights, h_n and h_{n+1}, of the nth and (n + 1)th members of the homologous series are

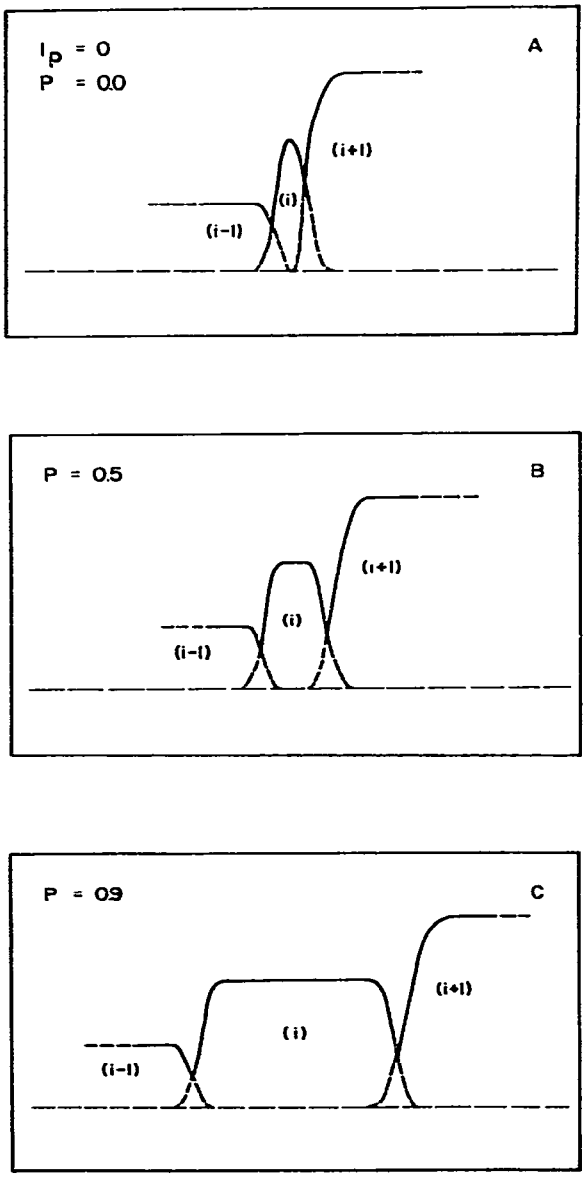

Fig. 4. Schematic illustration of the effect of increasing the amount of component i in the feed on the shape of its zone and the purity.

measured. The sharpness of the boundary between the two zones is measured by the volume dependence of the G function defined by

$$G = \frac{h_n - h_{n+1}}{\alpha h_n + \beta h_{n+1}} \tag{7}$$

with

$$\alpha = 1, \beta = 0 \text{ for } h_n > h_{n+1} \tag{8}$$

and

$$\alpha = 0, \beta = 1 \text{ for } h_n < h_{n+1} \tag{9}$$

Evidently the value of G is $+1$ or -1 when the fraction contains only the pure species n or $n + 1$, respectively, and $G = 0$ when the concentrations of both components are the same in that fraction. As discussed later the G function was used to study the effect of operational conditions on displacement separation of polyethylene glycol oligomers.

EXPERIMENTAL

Materials

Phenol, 2- and 4-hydroxyphenylacetic acids, and 3,4-dihydroxyphenylacetic acid were purchased from Aldrich (Milwaukee, WI, U.S.A.), adenosine, inosine and L-phenylalanine from Sigma (St. Louis, MO, U.S.A.), resorcinol from Baker (Phillipsburg, NJ, U.S.A.), catechol from Matheson, Coleman & Bell (Norwood, OH, U.S.A.), *n*-butanol and methanol were HPLC grade from Fisher Scientific (Fair Lawn, NJ, U.S.A.), *n*-propanol was obtained from Mallinckrodt Chemical Works (St. Louis, MO, U.S.A.) and acetonitrile and isopropanol were "distilled in glass" from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Distilled water was prepared with a Barnstead distilling unit.

Commercial 10-μm Partisil ODS-2 (Whatman, Clifton, NJ, U.S.A.) and 5-μm Zorbax ODS (DuPont, Wilmington, DE, U.S.A.), columns as well as home-made columns packed with 10-μm LiChrosorb RP-18 (Merck, Cincinnati, OH, U.S.A.) and 5-μm Spherisorb (Phase Sep, Hauppage, NY, U.S.A.) treated with octadecyldimethylchlorosilane (Petrarch Systems, Levittown, PA, U.S.A.) were used for fractionation by displacement chromatography. Columns were 250 × 4.6 mm but a 500 × 4.6 mm column packed with octadecyl-Spherisorb was also used. In analytical HPLC a 10-μm Partisil C_8 column (250 × 4.6 mm) from Whatman and a home-made 5-μm octadecyl-Spherisorb column (150 × 4.6 mm) were used.

Instruments

Fractionator liquid chromatograph. A modified Model 601 liquid chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.) was used for separations by displacement chromatography. The two syringe pumps of the instrument were individually connected to the feed loading system as shown schematically in Fig. 5. Pump A, which delivered the carrier solvent and the regenerant to the column before and after a chromatographic run respectively, was connected to a Rheodyne (Berkeley, CA, U.S.A.) Model 7030 switching valve. Pump B, which delivered the displacer, was connected to the Rheodyne Model 7010 feed introduction valve, which in turn was

connected to the switching valve. Drain valves (Scientific Systems, State College, PA, U.S.A.) were connected to each line upstream of the feed loading system. The feed valve was fitted with a loop of the appropriate size made from a length of 1 mm I.D. × 1/16 in. O.D. 316 stainless-steel tubing. The volume of the feed loop varied in the range from 0.5 to 6 ml. Connections to the switching valve were arranged in such a way that in one position pump A was connected to the column and flow from pump B was interrupted, and *vice versa* for the other position. The column effluent was monitored by a Perkin-Elmer Model LC-55 variable-wavelength detector and was collected with a Model 7000 fraction collector (LKB, Rockville, MD, U.S.A.). The detector signal was recorded on a Perkin-Elmer Model 123 strip-chart recorder.

Analyzer. The analytical chromatograph was assembled from a Model 725 automatic injector (Micromeritics, Norcross, GA, U.S.A.), a Model 1220 liquid chromatograph and a Model LC-65T variable wavelength detector, both from Perkin-Elmer, and a Model Electronik 194 (Honeywell, Ft. Washington, PA, U.S.A.) strip chart recorder.

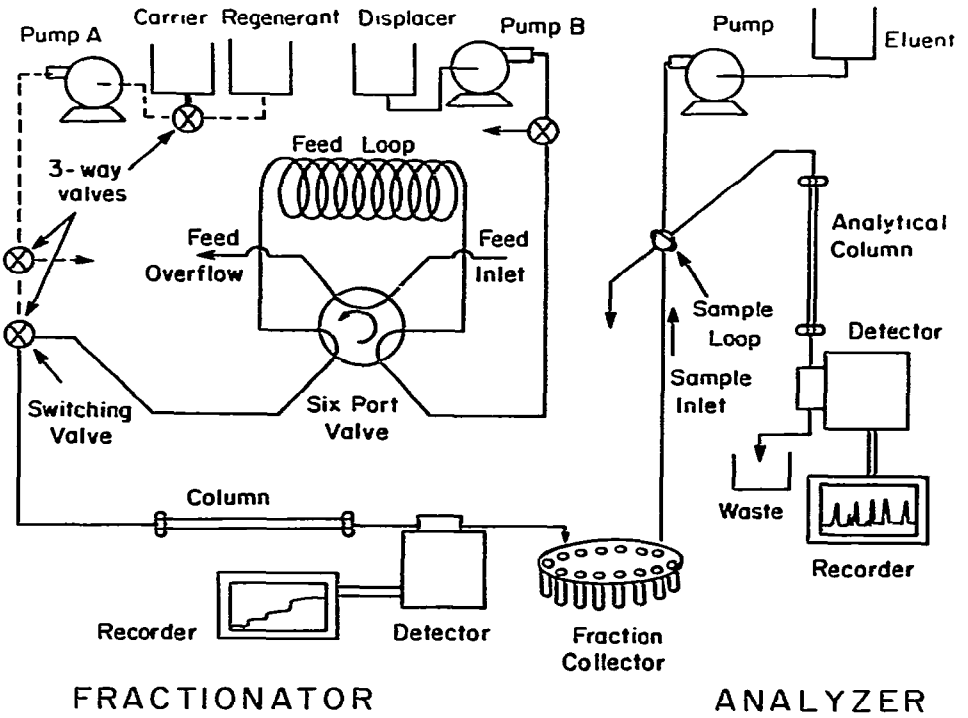

Fig. 5. Diagram of the dual liquid chromatographic system. Preparative-scale displacement chromatography is performed by the "fractionator" and the concentration of the components in the product stream present in the fraction collector is determined by the "analyzer" HPLC unit.

Procedures

Measurement of adsorption isotherms. Frontal analysis by characteristic

point[14] was used to estimate the adsorption of various solutes from water at 25°C. The injector valve of the liquid chromatograph was fitted with a 6-ml sampling loop prepared from a 764 cm long, No. 316 stainless-steel tubing having 1.0 mm I.D. and 1/16 in. O.D. First the sampling valve was connected directly to the detector and about 5 ml of sample solutions at five different concentrations were successively pumped through the flow cell in order to calibrate the detector at one or two appropriate wavelengths. Thereafter a 6 cm long, No. 316 stainless-steel column (4.6 mm I.D., 1/4 in. O.D.) packed with the stationary phase of interest was installed and washed with isopropanol, methanol and exhaustively with water. At a water flow-rate of 0.5 ml/min the introduction of the sample solution began and was marked on the recorder chart paper. After the detector response reached a plateau on the chromatogram, sample introduction was terminated by returning the sampling valve to its original position and this point was marked on the chromatogram. Subsequently the pumping of water continued until the recorder pen returned to baseline. The diffuse rear portion of the chromatogram obtained with the "square-wave" concentration input was used to evaluate the isotherm. For known system dead volume, flow-rate and chart speed, retention times particular to certain solute concentrations in the effluent, $t_R(C)$, were calculated from the recorder trace and the calibration data. The isotherm was evaluated from $t_R(C)$ by using the following equation

$$t_R(C) = t_0 + (t_{s'} - t_0)\frac{dq}{dC} \tag{10}$$

where t_0 is the hold-up time of an unsorbed solute in the column, $t_{s'}$ is the residence time corresponding to the superficial (empty tube) velocity and q the amount of solute adsorbed by unit volume of the stationary phase. Thus, dq/dC is the slope of the isotherm at the characteristic mobile phase concentration. Evaluating dq/dC over the experimental range of $t_R(C)$ and integrating, we obtain q as a function of the concentration, *i.e.*, the adsorption isotherm of the solute. Due to the inaccuracy associated with the measurement of $t_R(C)$ at $C = 0$, that is, when the recorder pen returns to baseline, the boundary condition required for integration is given by frontal analysis[14] of the self-sharpening front boundary of the chromatogram. From this method of analysis, the amount of solute adsorbed onto the stationary phase at the concentration corresponding to the plateau of the chromatogram, $q_{max.}$, is given by

$$q_{max.} = (t_F - t_0)\,FC_{max.} \tag{11}$$

where t_F is the breakthrough time of the sharp front, and F is the flow-rate.

Evaluation of the parameters

Assuming that the dependence of the adsorbed solute on the concentration in solution follows the Langmuirian model linear regression analysis was performed by using eqn. 3 in the rearranged form as follows

$$\frac{1}{q} = \frac{1}{bK}\frac{1}{C} + \frac{1}{b} \tag{12}$$

Correlation coefficients greater than 0.99 indicated an adequate fit.

Fractionation

The flow sheet of the two liquid chromatographs, the fractionator and analyzer, is depicted in Fig. 5 in which the main components of the system are labelled. The fractionation of the feed by displacement chromatography is carried out in the fractionator and the column effluent is collected as discrete fractions that were sampled and analyzed by the other HPLC unit operated in the elution mode.

To begin a displacement run, the switching valve was turned so that pump A delivered the carrier to the column at the desired flow-rate, with the drain valve closed. The feed introduction valve was turned to the load position and the loop filled with the feed solution. Pump B was then started at the same flow-rate setting as pump A and the feed valve was turned to pressurize the feed up to the column inlet pressure. When the pressure on the B side of the system equalled that on the A side, the switching valve was turned so that the feed, followed by the displacer, entered the column. The feed valve was returned to its original position after the desired volume of feed was introduced and the displacer solution flowed into the column. The progress of the run was monitored by the detector at an appropriate wavelength setting determined in advance. Following the run, pump A was started pumping the regenerant solvent, and when its pressure equalled that of the system the switching valve was returned to its original position for regeneration of the displacer-saturated column. Following the regenerant the column was perfused with the carrier solvent, delivered to the column by pump A, and the system was ready for another run.

Analysis of fractions

The concentration of the product in each of the fractions obtained from the displacement development run was determined by using the internal standard technique. The peak heights of the feed components injected in a solution of either phenylalanine or phenol were divided by the height of the internal standard peak, and this ratio was correlated with the known concentration of the feed component. Aliquots of each fraction were then diluted with the internal standard solution, and the retention times and peak height ratios measured in order to determine the presence and concentration of feed components in the fraction. The mobile phase for the analysis of the phenyl acetic acid derivatives was 50 m*M* phosphate buffer, pH 2.12, containing 10% (v/v) acetonitrile and for the dihydroxybenzenes an aqueous solution of 1% triethylamine phosphate, pH 3.0, containing 25% (v/v) methanol was used.

RESULTS AND DISCUSSION

Adsorption isotherms on octadecyl-silica

As seen in Fig. 6 the shape of the isotherms of hydroxyphenylacetic acids resemble the rectangular hyperbolas characteristic for Langmuir isotherms, *cf.* eqn. 3. Similar observations were made also with other aromatic substances upon investigating their adsorption properties from aqueous solutions on hydrocarbonaceous silica-bonded phases. Table I shows the parameters obtained by fitting experimental data to eqn. 3 from isotherm measurement by frontal analysis on the different kinds of octadecyl-silica columns. The product of the saturation concentration, b, and the binding constant, K, is expected to be proportional to the retention (capacity) factor of the solute in linear elution chromatography on the same column when the eluent

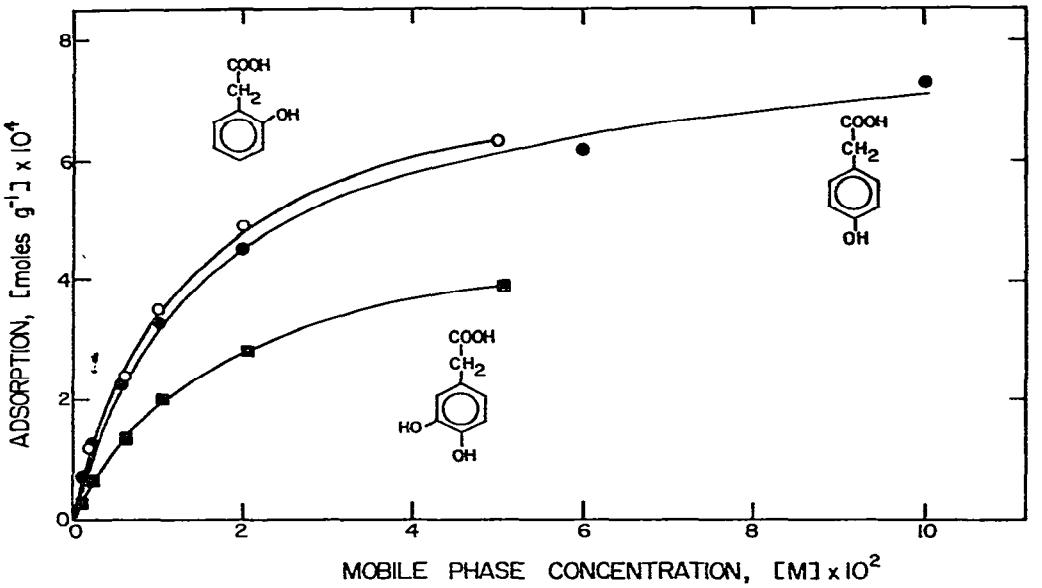

Fig. 6. Adsorption isotherms as measured by the frontal analysis of 3,4-dihydroxyphenyl (■), 2-hydroxyphenyl (○) and 4-hydroxyphenyl (●) acetic acids on 10-μm Partisil ODS-2 from 0.1 *M* phosphate buffer, pH 2.12, at 25°C. Solid lines were drawn from values calculated by linear least square analysis of the data points to the Langmuir isotherm according to eqn. 12.

composition is the same as that of the carrier used in the determination of the isotherm. The proportionality factor is the phase ratio of the column and therefore, the analysis of a series of Langmuir isotherms and the corresponding chromatographic retention factors may be used to evaluate the phase ratio of the column.

TABLE I

PARAMETERS OF LANGMUIR ISOTHERMS FOR ADSORPTION ON OCTADECYLSILICA FROM WATER

The isotherms were evaluated by frontal analysis and the parameters from eqn. 3.

	*b**** *(mol/g × 10⁴)*	*K**** *(M^{-1})*	*bK* *(ml/g)*
Resorcinol*	3.297	19.20	6.33
Catechol*	7.228	10.15	7.33
3,4-Dihydroxyphenyl acetic acid**	5.068	63.41	32.1
4-Hydroxyphenyl acetic acid**	7.804	74.00	57.7
2-Hydroxyphenyl acetic acid**	7.972	76.62	61.1

* Column: Spherisorb ODS; carrier: water.
** Column: Partisil ODS-2; carrier: 0.1 *M* phosphate buffer, pH 2.12.
*** See eqn. 3.

Present development in displacement chromatography with bonded phases have been encouraging as the isotherms that we investigated so far were of the concave downwards type, *i.e.* suitable to meet the requirement for displacement chromatography. Nonetheless, further knowledge of the isotherms will be required for the design of the fractionation system without a great deal of trial and error that may be associated with the selection of a suitable displacer and its concentration. It is hoped that further studies on the relationship between chemical structure and the parameters of the isotherm will facilitate the prediction of isotherms from structural increments of the solute molecules[15–17].

Displacement diagrams

Concentration profiles of the individual feed components in the effluent of the fractionator column are depicted on displacement diagrams such as those shown in Figs. 7 and 8. Fractions of column effluent were analyzed by HPLC in order to determine accurately the concentrations of both components in the mixed boundary region. On the other hand, when the effluent of the fractionator is monitored by a flow-through detector that cannot distinguish between the two species, a displace-

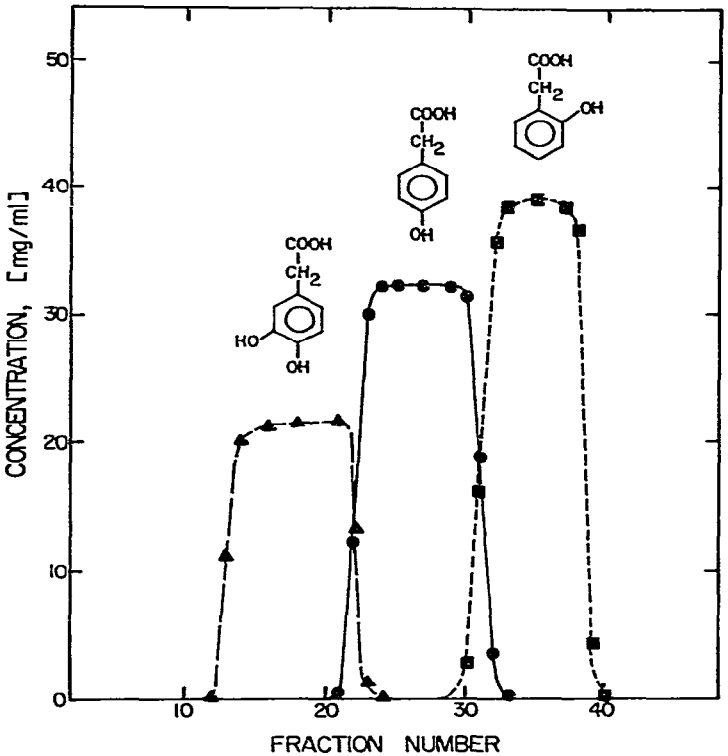

Fig. 7. Separation of hydroxyphenylacetic acids by displacement chromatography on Partisil ODS-2 column (250 × 4.6 mm). The carrier was 0.1 *M* phosphate buffer, pH 2.12, and the displacer was *n*-butanol at a concentration of 0.87 *M*. Flow-rate and temperature were 0.05 ml/min and 25°C, respectively. The feed had a volume of 1.5 ml and contained 30, 35 and 45 mg of 3,4-dihydroxy-, 4-hydroxy and 2-hydroxyphenylacetic acids, respectively. Fraction size was 0.15 ml and fraction number 40 marks an effluent volume of 12 ml.

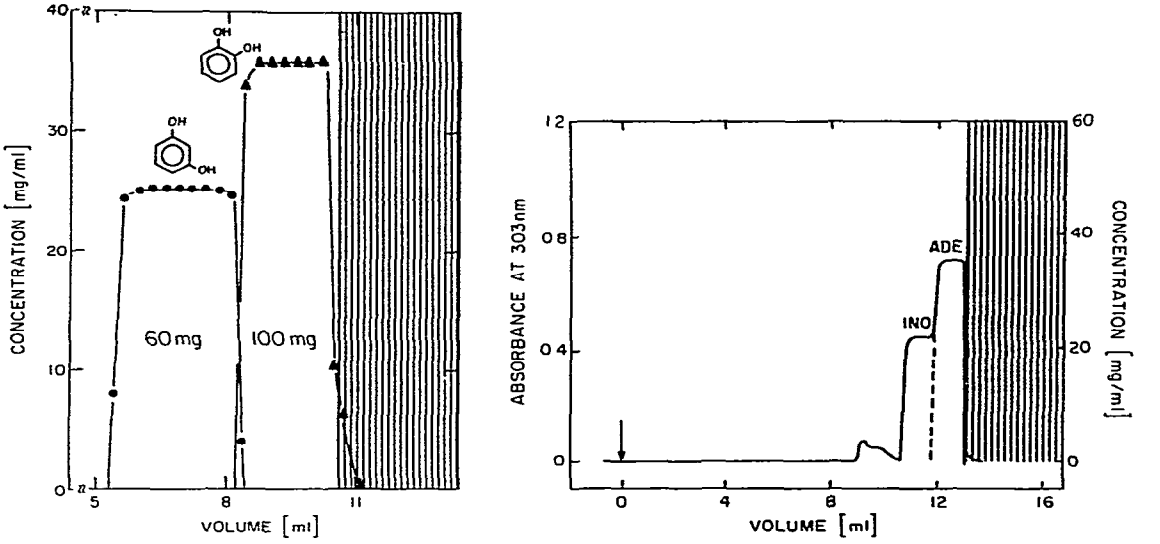

Fig. 8. Displacement separation of resorcinol (●) and catechol (▲), with water as carrier and 0.8 *M* *n*-propanol in water as the displacer. The flow-rate was 0.15 ml/min and the feed contained 60 mg of resorcinol and 100 mg of catechol dissolved in 1.0 ml of water. The purity, *P*, of resorcinol and catechol fractions in the product was 0.97 and 0.94, respectively. The column was packed with 5-μm octadecyl-Spherisorb and its dimensions were 500 × 4.6 mm. The displacer is indicated by the shaded region.

Fig. 9. Displacement chromatogram of adenosine and inosine obtained by monitoring the fractionator effluent. The displacer was 0.66 *M* *n*-butanol in 0.01 *M* phosphate buffer, pH 3.5 and the temperature was 22°C. The flow-rate was 0.20 ml/min and the feed contained 24 mg of each component in 1.5 ml of the neat aqueous phosphate buffer used as the carrier. The purity, P, of the adenosine and inosine fractions in the product was 0.92 and 0.83, respectively. The dimensions of the 10-μm LiChrosorb RP-18 column were 250 × 4.6 mm. The displacer is indicated by the shaded region.

ment chromatogram is obtained that depicts only the contour of the concentration profiles as shown in Fig. 9. In fact, the employment of an "analyzer" liquid chromatograph to monitor the concentrations of all components in the effluent of the fractionator column greatly facilitate not only the assessment of the purity of the product but also examination of the course of the displacer process and its optimization. Thin-layer chromatography has also been found to be suitable for this purpose but due to its superior accuracy, convenience and speed the analysis of the fractions was carried out in this study by using HPLC only.

The analyzer liquid chromatograph acts as a selective detector, and it can be used to determine zone boundaries accurately, a feat which is impossible by using only the trace from the fractionator detector alone. All separations depicted above are believed to reflect fully developed displacement trains although the boundaries between the zones are not completely sharp due to the effect of axial dispersion.

Purity of the product

Parameter P. That fraction of a component that is recovered in pure form has been defined as its purity, *P*, according to eqn. 6. This parameter has been used to quantitatively measure the efficiency of separation as shown in Tables II and III.

TABLE II

THE VARIATION OF PURITY WITH OPERATING CONDITIONS FOR THE SEPARATION OF PHENOLIC COMPOUNDS

The carrier was a 0.1 *M* phosphate buffer, pH 2.12, and the column was a 10-μm Partisil ODS-2 (250 × 4.6 mm).

Purity, P			*Displacer*	*Feed (mg)*	*Flow-rate (ml/min)*
3,4-Dihydroxy-phenyl acetic acid	*4-Hydroxy-phenyl acetic acid*	*2-Hydroxy-phenyl acetic acid*			
0.95	0.83	0.73	0.87 *M* *n*-butanol	110	0.05
0.87	0.71		0.64 *M* phenol	52.5	0.30
0.84	0.84		0.74 *M* phenol	75	0.05
0.86	0.84		0.74 *M* phenol	75	0.15
0.87	0.80		0.74 *M* phenol	75	0.50

Some of the figure captions also contain *P* values in order to quantify the degree of separation shown by the displacement diagram.

It is seen that for separations which are considered good the value of *P* is higher than 0.9, that is, after removing the contaminated boundary regions of a given zone more than 90% of the component is obtained in pure form. From the definition of *P* it follows that its value increases with the amount of component when the width of the mixed region remains constant. Such a situation may arise when the displacement train is already isotachic with the largest feed under investigation. The first emerging component of the displacement train is expected to yield a relatively high

TABLE III

THE EFFECT OF OPERATING CONDITIONS ON THE MAGNITUDE OF THE PURITY, *P*, DEFINED BY EQN. 6, OF INOSINE AND ADENOSINE

The carrier was 0.1 *M* phosphate buffer, pH 3.5, and the displacer contained 0.66 *M* *n*-butanol. The 10-μm LiChrosorb RP-18 column (250 × 4.6 mm) was maintained at 22°C.

Purity, P		*Feed*		*Flow-rate (ml/min)*
Inosine	*Adenosine*	*Volume (ml)*	*Amount (mg)*	
0.76	0.86	1.5	25	0.05
0.74	0.84	1.5	25	0.15
0.53	0.61	1.5	25	0.50
0.83	0.92	1.5	48	0.20
0.73	0.85	4.5	48	0.20
0.47	0.54	6.0	48	0.20

value of P because it does not contain a contaminated front boundary. Despite its shortcomings the purity parameter can conveniently be used to express the effect of changing operating conditions on the afficacy of separation as it is shown in Tables II and III.

G Function. For measuring the degree of separation of homologous series the G function has been defined in eqns. 7–9. Fig. 10 shows a late of the G function

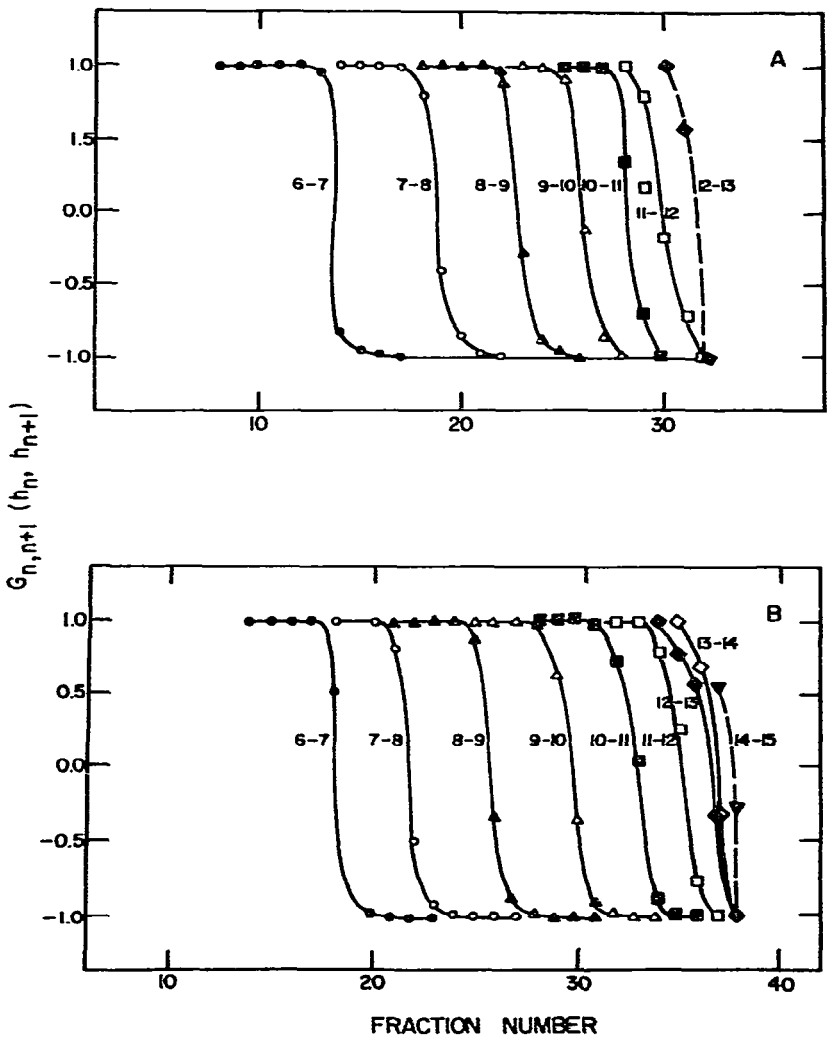

Fig. 10. Illustration of the displacement separation of Carbowax 400 by plotting the G function, eqn. 7 *vs.* fraction number of the effluent. The numbers at each curve indicate the number of ethylene glycol units, n and $n + 1$, in the two oligomers present in the fractions. The flow-rate and fraction volume were 0.15 ml/min and 0.15 ml, respectively. The column temperature was 22°C and the displacer was 0.66 M *n*-butanol in the carrier. A, The carrier was 10% (v/v) acetonitrile in water and the feed was 45 mg of Carbowax 400. At fraction number 30, 11.0 ml of column effluent was collected. B, The carrier was 5% (v/v) acetonitrile in water, the feed was 90 mg of Carbowax 400. Fraction number 40 marks 12.3 ml of column effluent.

against the number of fractions in the case of displacement chromatography of ethylene glycol oligomers. The G function denotes the degree of separation of adjacent zones in the displacement train containing n and $n + 1$ ethylene glycol units. The flat portions of the S-shaped curves, in which $G_{n,n+1}$ has a value of $+1$ or -1, denotes the fractions in which only one of the two oligomers is present, whereas the curved portion indicates the mixed boundary between adjacent zones in the effluent. For instance, in Fig. 10A, heptaethylene glycol appears mixed with hexaethylene glycol in fractions 15–17, and is mixed with octaethylene glycol in fractions 18–21.

The lower homologs were obtained in pure form as depicted in Fig. 10A, but the separation of the larger molecules was less complete, as indicated by the absence of flat portions of the curves corresponding to the 11-, 12- and 13-unit homologues. However, a decrease in the organic modifier content of the carrier results in an increase in the molecular weight of homologues which can be recovered in pure form as shown in Fig. 10B, and also allows the column to accommodate a larger feed. The greater adsorptivity of the feed components as well as the displacer in the presence of the more polar carrier permits the recovery of fractions containing pure nonaethylene glycol as illustrated in Fig. 10B in contrast with the insufficient separation obtained with water lean carrier and depicted in Fig. 10A.

Effect of operational parameters. With presently available microparticulate columns, accurate flow control and precision instrumentation, the ease of separation by displacement chromatography is largely affected by thermodynamic factors such as the shape of isotherms, relative adsorptivity of the components as manifested by the competitive isotherms, solubility, as well as the relative concentration of the components in the feed. Although the fundamental relationships between most of these factors has long been established[18,19] no analytical expressions could be developed due to the mathematical complexity of the non-linear differential equations underlying the model.

From the practical point of view optimization of the process is tantamount to selection of conditions that yield the most rapid transient period for a full development of the displacement train, *i.e.*, that require the shortest column length to bring about a given separation.

The need for the displacer solution increases the number of variables within the system, compared to elution chromatography. Furthermore, the necessity of achieving the isotachic state before optimal separation is obtained requires that the variables have some minimum value such that the sample resides in the column long enough to attain this state. In order to examine the effect of changes in the variables, the feed concentration and volume, column length, mobile phase flow-rate, displacer and species concentrations were varied. Changes in the variables were made concommitantly as well as individually in order to determine their interdependence.

Flow-rate. Fig. 11 illustrates the effect of flow-rate on the separation of adenosine and inosine by displacement chromatography. It is seen and can be verified by the pertinent values of the purity parameter P that the efficacy of the separation even at the relatively low load of the column is strongly dependent on the flow-rate. Efficiency increases with decreasing flow-rate and as a result P_{Ado} increases from 0.61 to 0.86 upon a respective decrease of the flow-rate from 0.50 to 0.05 ml/min. This behavior is attributed to an increase in the time available for development of the displacement train in the column upon reducing the flow velocity. The purity of the

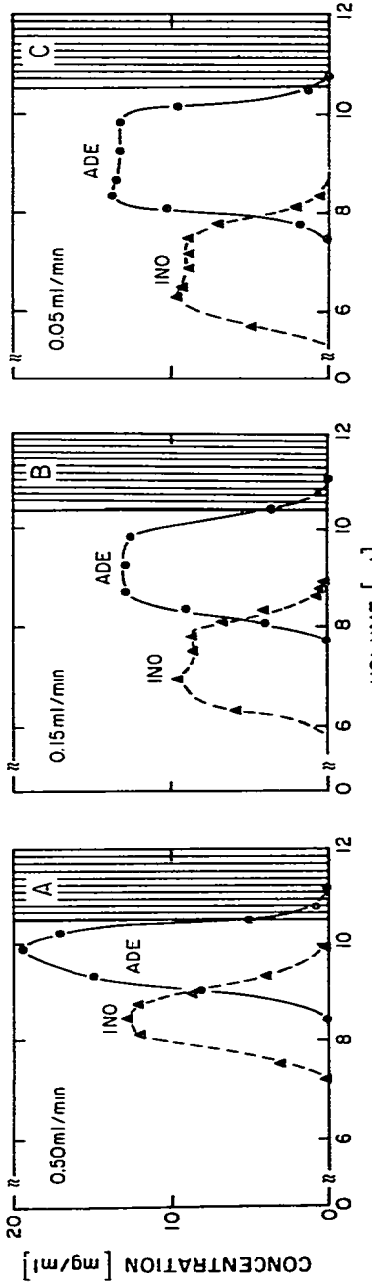

Fig. 11. Effect of flow-rate on the displacement separation of inosine (▲) and adenosine (●). Flow-rates: A, 0.05, B, 0.15; C, 0.50 ml/min. The displacer was 0.66 *M* *n*-butanol in 0.10 *M* phosphate buffer, pH 3.5. The feed solution contained 10 and 15 mg of inosine and adenosine, respectively, and the column was a 250 × 4.6 mm 10-μm LiChrosorb RP-18. The purities of the products are given in Table III.

product increases with decreasing flow-rate in this manner only up to a point, however. Decreasing the flow-rate below that value at which the displacement is fully developed induces no further effect on any characteristics of the displacement train. This fact is illustrated by the purity values of the last three entries in Table II. The constancy of the *P* values with changing flow-rate —while all other conditions were kept constant— indicates that the displacement was fully developed at a flow-rate of 0.5 ml/min, and no further benefits in purity were obtained from the longer column residence times achieved by decreasing the flow-rate to 0.15 or 0.05 ml/min.

Feed: Amount. When the column is overloaded in displacement chromatography no full development can take place for two reasons. First, the length of time required to separate the feed components is longer, so a larger column or slower flow rate may be required to achieve separation. It is because an increase in the amount of feed can extend the development time that is defined in Fig. 1. The second quite obvious reason why feed overloading can prevent full development is that the column length may be insufficient to accommodate the length of the fully developed displacement train, so that full development would require a longer column.

Fig. 12 illustrates the effect of column overload that manifests itself in incompletely developed concentration zones having shapes different from the "square wave" form encountered under ideal conditions in displacement chromatography. In the three cases shown, the column was overloaded in different ways and to a different extent with the first and second components, 3,4-dihydroxyphenylacetic acid and 4-hydroxyphenylacetic acid respectively. It is noted that in other experiments, quasi isotachic conditions were obtained in displacement chromatography under operating conditions identical to those given in Fig. 12 when the feed contained 15 and 37.5 mg of 3,4-dihydroxyphenylacetic acid and 4-hydroxyphenylacetic acid, respectively.

As seen in Fig. 12A the zone of the less retarded component, the amount of which in the feed is relatively low, is substantially separated from the band of the more strongly adsorbed component, 4-hydroxyphenylacetic acid. It shows a nearly developed band and the estimated plateau concentration of the zone is close to that predicted from the isotherm and obtained in other runs performed under the same conditions but with reduced load.

The progressive degeneration of the displacement train on increasing the sample load is illustrated by change in the shape of the zone of the more retained 4-hydroxyphenylacetic acid. When the amount of the second component is doubled the band is much broader as shown in Fig. 12B and the residence time in the column is insufficient to attain isotachic conditions. Finally, it is seen in Fig. 12C that when the amount of 3,4-dihydroxyphenylacetic acid is also doubled in the feed, the shape of the 4-hydroxyphenylacetic acid zone further degenerates. The observed zone shapes are in agreement with the expectations. As the less retained component is present in the zone of the other at relatively high concentrations, the isotherm of the first is suppressed to such and extent that its velocity increases and consequently the band width increases.

Volume. In Figs. 9, 13A and 13B three displacement diagrams for adenosine and inosine are shown under identical feed loads and chromatographic conditions but with feed volumes of 1.5, 4.5 and 6.0 ml, respectively. Whereas at the smallest feed volume a fully developed displacement diagram is obtained as shown in Fig. 9, at a larger feed volume a frontal zone is observed for inosine as seen in Fig. 13A. The

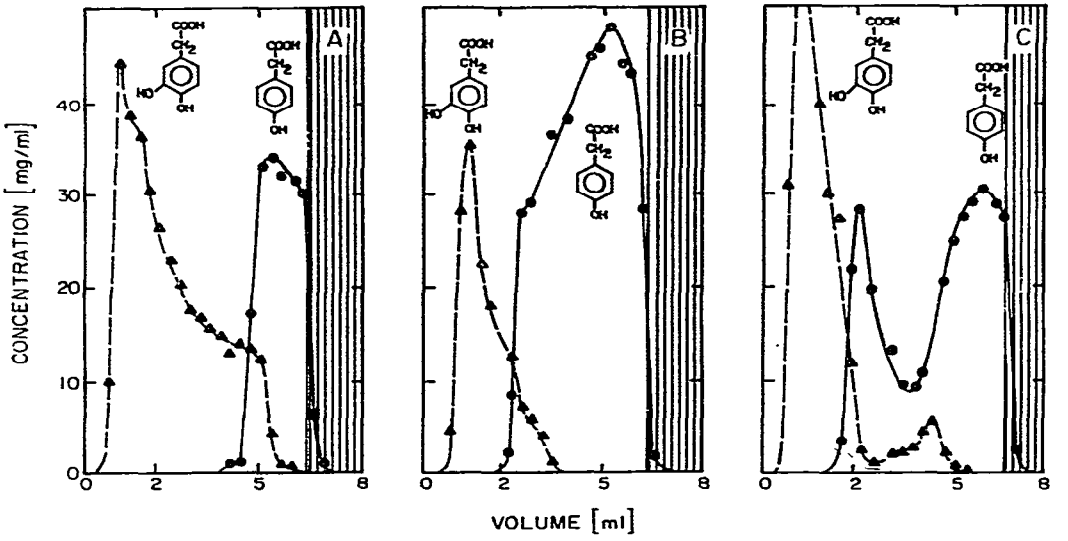

Fig. 12. Effect of feed size on the separation of 3,4-dihydroxyphenylacetic acid (▲) and 4-hydroxyphenyl acetic acid (●) by displacement chromatography. The displacer was 0.64 *M* phenol in 0.10 *M* phosphate buffer, pH 2.12, and the temperature was 25°C. The mobile phase flow-rate was 0.3 ml/min. The respective amounts of 3,4-dihydroxyphenyl acetic acid and 4-hydroxyphenyl acetic acid in the feed were: A, 114 and 57 mg; B, 57 and 114 mg; C, 114 and 114 mg. The column was a 250 × 4.6 mm 10-μm Partisil ODS-2.

degree of separation declines as well, as seen from the purity values listed in Table III. The displacement diagram obtained with a feed volume of 6.0 ml and shown in Fig. 13B exhibits further deterioration of the separation as seen from the low purity values calculated for this case and the irregular zone shapes as shown in the figure.

The feed volume effect noted here is a manifestation of the mixed frontal and displacement chromatography mechanisms which occur at the beginning of every displacement run. Frontal chromatography takes place when a solution of constant composition is introduced as a step function feed into the column. The feed stage of displacement chromatography is essentially a frontal chromatographic process, only the step function is truncated when the displacer is fed to the column. Therefore, the larger the feed volume, the more advanced this frontal chromatographic development is, and at some limiting value of the feed volume the first feed components emerge from the column before the displacer enters, and essentially a type of frontal chromatography is performed. A mixed mechanism is always operative at the beginning of any displacement run, but with a column long enough, the process of displacement development becomes dominant and prevails until the isotachic stage is reached.

In practice the limits on feed volume are important when the solubility of the feed components in the carrier is low. Therefore, at fixed feed volume the amount of sample that can be separated in a single chromatographic run is limited, among other factors, the solubility of the sample components in the carrier used for the preparation of the feed solution.

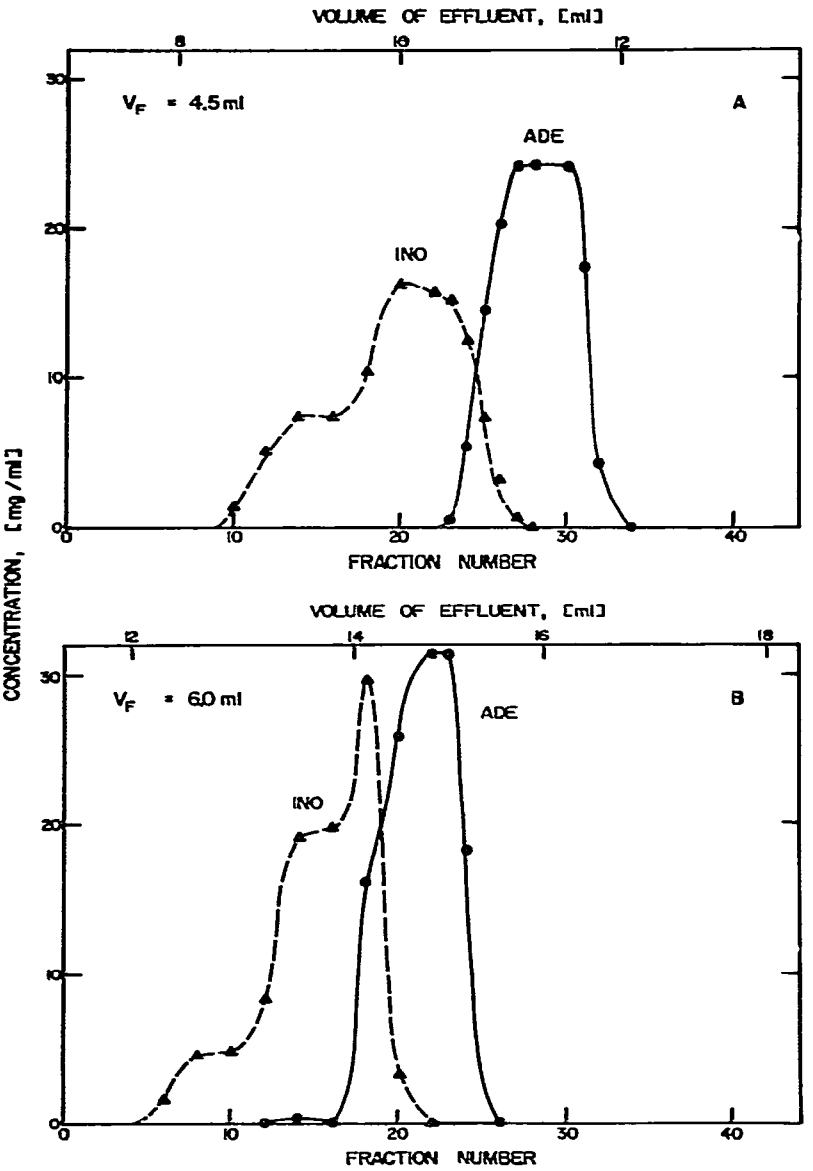

Fig. 13. Effect of feed volume on the separations of fixed amounts of adenosine (●) and inosine (▲) by displacement chromatography. The chromatographic conditions were as specified in Fig. 8. The feed volumes were A, 4.5 ml and B, 6.0 ml.

Column length

Fig. 14A shows the results of fractionation of the two phenolic compounds under conditions identical to those in Fig. 8, except the column was 25 cm instead of 50 cm. It is evident from the figure that with the shorter column the displacement is not completely developed, whereas isotachic conditions were approached with the

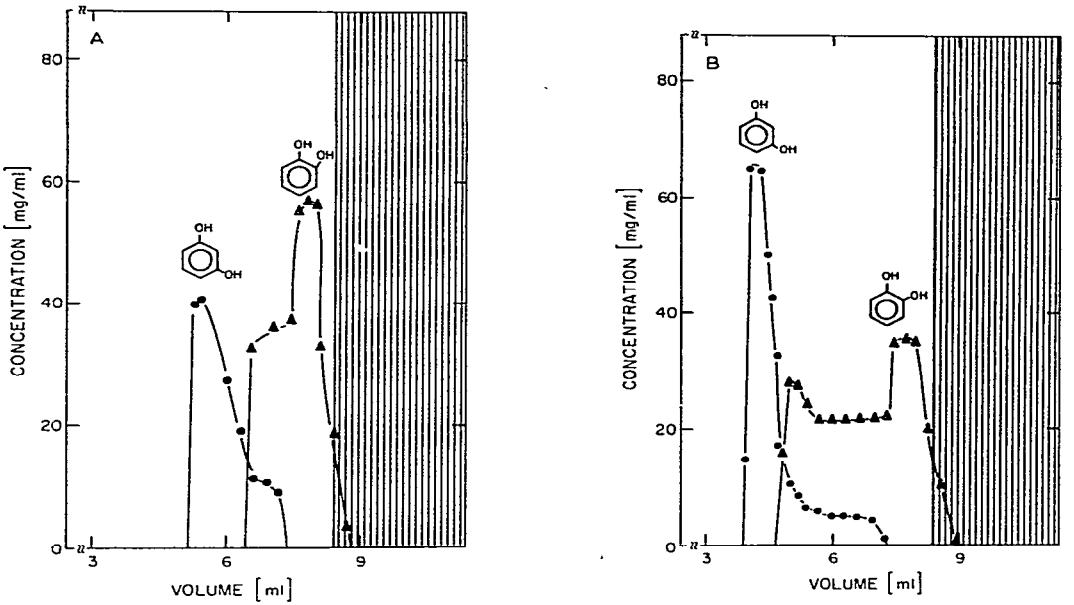

Fig. 14. Effect of flow-rate on the separation of phenolic compounds by displacement chromatography with a 250 × 4.6 mm I.D. 5-μm octadecyl-Spherisorb column. The flow-rate was 0.15 ml/min in A and 0.075 ml/min in B, whereas other conditions were the same as those in Fig. 8.

longer column. As pointed out above, the column length is a critical parameter that must have some minimum value under a given set of operating conditions. In Fig. 1 a hypothetical dissection of the column during the stages of displacement development was indicated, and the three parts required for the minimum column length given as the feed length, the development length and the length of the fully developed displacement train. The column length when it is minimum consists of length increments necessary for these three stages. The incomplete separation depicted in Fig. 14A demonstrates that the 25 cm long column is not long enough to accomodate the three constituent lengths. The result obtained with the 25 cm long column are representative to those that occur half-way down in the 50 cm long column that just encompasses the length of the three stages or is even longer then necessary.

Decreasing the flow velocity does not decrease the minimum column length by a proportionate amount, as one might expect on a residence-time basis, as shown in Fig. 14B. There the fractionation was run in the 25 cm at a flow-rate of 0.075 ml/min, *i.e.*, half the value in both Figs. 8 and 14A. The chromatogram appears to be nearer to fully developed, but again the isotachic state has not been reached, even though the residence time of the feed in the column was the same as in Fig. 8. The reason for this is that decreasing the flow-rate increases the development time for the train, and so effectively increases the development length whithin the column, but it has no effect on the length occupied by the fully developed train. The length of the isotachic train is determined by the amount of feed and the concentrations of the isotachic zones, and so is unaffected by the velocity of the carrier. Therefore, changes in the column length are not fully compensated by proportionate changes in flow velocity that primarily effects the length of the feed and development zones in the column.

The discussion of Fig. 12 pointed out similar constraints on column length. There the feed loading was increased and it was argued that this had the effect of increasing both the development time required for the separation and the length of the column required to accomodate the isotachic train. The net effect of an increase in feed loading is, therefore, that a longer column is required for complete separation. This conclusion can be contrasted to the practice in preparative chromatography in the elution mode, where the diameter of the column has to be increased in order to accomodate a larger feed, and still maintain pulse-shaped peaks.

Concentrations at full displacement development

According to eqn. 2b the concentrations of the individual components in the isotachic displacement train are given by the intersection of their isotherms with the appropriate chord of the displacer isotherm.

In order to test the theoretical predictions we compared plateau concentrations of the zones in the displacement diagrams such as those depicted in Figs. 7 and 8 to the values obtained from the isotherms described in Table I. Both kinds of data are listed in Table IV. It is seen that a reasonably good agreement exists between the concentrations obtained from displacement development and those predicted from the isotherms for different displacer strengths, *i.e.*, speed lines. The results strongly suggest that under the experimental conditions employed here the displacer train was fully developed, *i.e.*, the limiting isotachic condition was closely approached. This conclusion is also supported by the observation that the width of the mixed boundary regions between the zones was approximately the same, a feature that is characteristic for full displacement development.

TABLE IV

COMPARISON OF THE CONCENTRATIONS AT PLATEAU IN DISPLACEMENT CHROMATOGRAPHY OF PHENOLIC COMPOUNDS ON OCTADECYLSILICA BY USING ALKYL ALCOHOLS AS DISPLACER TO VALUES CALCULATED FROM THE ISOTHERMS OF THE SUBSTANCES EVALUATED FROM INDEPENDENT MEASUREMENTS

Component	*Plateau concentrations (mg/ml)*	
	Experimental	*Predicted*
Resorcinol	25*	21*
Catechol	36*	39*
3,4-Dihydroxyphenyl acetic acid	20**, 21***	22**, 23***
4-Hydroxyphenyl acetic acid	32**, 33***	31**, 34***
2-Hydroxyphenyl acetic acid	38***	35***

* 0.8 *M* *n*-propanol.
** 0.76 *M* *n*-butanol
*** 0.87 *M* *n*-butanol.

Advantages of relatively narrow-bore columns

Preliminary results suggest that in the displacement mode the column diameter can be smaller by at least one order of magnitude than in the elution mode to obtain

the same throughput. The actual factor, of course, depends on the particular chromatographic system employed even if all requirements for complete displacement are fulfilled.

The use of small diameter columns is associated with a number of advantages[20]. From the operational point of view, the narrowness of the inside diameter relaxes the stringent requirements such as thick wall and bulky connections for operating the column at high inlet pressures. Then the use of small particles having 3- to 5-μm diameter for high efficiency column packing is facilitated. Moreover, the regeneration step can be expedited by using high flow velocities. Uniform radial and flow temperature profiles are essential in attenuating axial dispersion. In narrow-bore columns the occurence of deleterious temperature non-uniformities is greatly reduced even when heat associated with adsorption and desorption at the high solute concentrations employed in displacement chromatography is significant. Concommitantly fingering and other flow instabilities arising from density and viscosity differences are expected to play a lesser role when the column inner diameter is reduced. Furthermore, the significance of channeling and other packing non-uniformities in determining axial dispersion may decrease with column diameter with the exception of "a wall effect" which generally increases to some limit. Low solvent consumption associated with the use of narrow bore columns has obvious felicitous economical and environmental effects and the compactness of column and instrumentation afforded by employing narrow-bore columns may also be a preferred feature.

Comparison of elution and displacement chromatography

A number of features facilitate analysis by using the linear elution mode of chromatography, including that it yields Gaussian or quasi-Gaussian peaks, the relationship between the migration velocity and thermodynamic equilibrium constant is simple and the role of transport and kinetic phenomena in determining band spreading is well understood. The wide popularity of the technique in analytical separations is most likely due to the relative simplicity of operation and the ease of detection of distinct concentration pulses. In addition its detailed theoretical framework has also contributed to the almost exclusive use of elution chromatography.

The key features of displacement chromatography were clearly recognized even before the rapid development of elution chromatography commenced with the introduction of partition chromatography and the plate height theory. Yet difficulties associated with the detection of the individual sample components, the surface heterogeneity of the adsorbents and the excessive band spreading in columns available at that time thwarted displacement chromatography's becoming an accepted analytical method. Although displacement may occur in the practice of preparative chromatography with overloaded columns, analytical applications involving the conscientious employment of displacement development with a judiciously chosen displacer[21] or other means such as temperature[22] have been largely confined to the scientific literature.

On the other hand, the results of this work suggest that fully developed displacement chromatography may be eminently suitable to carry out preparative scale chromatography with columns packed with microparticulate stationary phases, the use of which is prohibited in large diameter columns because of mechanical problems associated with operation at high column inlet pressures. The method is further

recommended for its characteristic recovery of the products at concentrations significantly higher than obtained in elution chromatography, accompanied by the concommitant economic and environmental benefits of relatively low solvent consumption. In fact, both the stationary and mobile phases as well as the equipment are better utilized in the displacement than in the elution mode of chromatography. As a consequence, the amount of pure product per unit column volume should be greater in displacement chromatography than in linear elution chromatography. Nevertheless the need to regenerate the column by removing the displacer is a significant disadvantage of displacement development in comparison to isocratic elution, although it may vanish if gradient elution is required to carry out a preparative separation. In any case the regeneration of the column is associated with a general cleaning of the system and removal of contaminants that otherwise may accumulate.

Usually zone boundaries are not perfectly sharp in displacement chromatography even when columns are used that are highly efficient in terms of low axial dispersion. Therefore in the recovery of products the boundary regions of the zones are preferentially collected separately and rechromatographed directly without concentrating.

An important difference between elution and displacement chromatography is that an increase in sample size in the former usually mandates an increase in column diameter in order to maintain the efficiency of separation constant, whereas in displacement chromatography the increase in both the diameter and length of column can be used very efficiently to increase throughput.

Selection of the column, carrier and displacer

All results to illustrate displacement chromatography presented in this report were obtained by reversed-phase chromatography using silica-bonded hydrocarbonaceous sorbents and a neat aqueous or a water-rich hydro-organic carrier. The main reason for this was our interest in studying the potential of such bonded phases for displacement chromatography as far as the isotherm shape and column loading capacity are concerned. It should be noted that the use of charcoal[23], ion-exchange resins[24] and silica gel[25] for displacement chromatography is well documented in the literature but to our best knowledge the employment of bonded phases for such purposes has not yet been described. Other results from our laboratory (to be published) have shown that columns packed with microparticulate silica gel also yield very good results in displacement chromatography when a less polar solvent is used as the carrier. On the other hand, the use of aqueous carriers as described in this study facilitated a strong binding of the feed components as well as that of the displacer to the hydrocarbonaceous stationary phase.

Lack of data on adsorption isotherms is the major impediment to optimization of displacement chromatography because it precludes the *a priori* determination of the nature and concentration of a suitable displacer. Therefore, the selection of the displacer by trial and error appears to be inevitable unless the isotherms of the potential displacers and preferably those of the feed components as well are known. The following guidelines may be useful in choosing the displacer. It should: (i) be adsorbed more strongly from the carrier onto the stationary phase than any of the feed components; (ii) not complex with any of the components; (iii) be inert to the stationary phase; (iv) be readily removed from the column by a suitable regenerant; (v) be highly soluble in the carrier; and (vi) be non-hazardous and inexpensive.

CONCLUSIONS

In displacement chromatography a certain amount of the feed mixture is first adsorbed at the inlet section of the column and thereafter the solution of a suitable displacer that binds more strongly to the adsorbent than any of the feed components is introduced in a continuous fashion. As the displacer front moves down the column the feed components gradually become separated as more strongly sorbed substances displace weakly sorbed components from the surface of the adsorbent. Finally a "displacement train" containing square-wave concentration zones of the individual components is developed in which each zone moves with the same velocity. Successful separation by displacement chromatography largely depends upon the sharpness of the boundaries between the adjacent zones. Therefore the requirements for high efficiency columns are very exacting, however, they can be met by columns presently available.

Indeed the potential of the HPLC columns and instrumentation used in analytical work can be exploited for preparative scale separations by using the displacement mode of chromatography, as illustrated by the separation of binary mixtures, for higher system productivity and product concentration in the effluent than generally encountered in elution chromatography. Knowledge of pertinent adsorption isotherms and solubilities of the feed components and potential displacers greatly facilitates the selection of operating conditions on the basis of the theory describing the properties of the fully developed displacement train and a qualitative understanding of the factors involved in the transient development process leading to separation. In agreement with recent findings[26], results presented here indicate that with a packed microparticulate column having the usual length of 25 cm and inside diameter of 0.4 to 0.5 cm binary mixtures containing 50 to 100 mg of each component mentioned above can readily be separated by displacement chromatography within one hour. This mode of chromatography may also be the method of choice in micropreparative separations of precious substances with narrow bore (I.D. $\leqslant 1$ mm) columns. Furthermore, its potential in special analytical applications such as liquid chromatography–mass spectrometry may also warrant serious consideration.

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