PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

The Current Chromatographic Scene [and Discussion]

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The current chromatographic scene

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Four areas of current activity in chromatography are selected as examples to illustrate the continuing growth and improvement of chromatographic techniques. Illustrations are provided in the areas of high-performance liquid chromatography, solid surface modification, open-tube microcolumns and, finally, total system optimization. Promising research directions are indicated and continuing advance in theory and practice is projected in these and other chromatographic areas.

Introduction

Four milestones are universally recognized on the high road to chromatographic development: first the invention of the technique as a practicable laboratory proposition by Tswett (1906), secondly, the rediscovery and extension of this work by Kuhn *et al.* (1931), then the introduction of liquid–liquid (paper) chromatography by Martin & Synge (1941) and, finally, the first demonstration of the elegance and simplicity of the gas–liquid elution method by James & Martin (1952). While it is the last of these that is widely seen as having triggered the explosive growth in acceptance of chromatographic techniques in general, it seems indisputable, in retrospect, that the seminal moment in the history was the appearance of the work of Martin & Synge. Not only did their paper describe a technique of such elegant simplicity and power that it inaugurated a new era in organic and biochemical studies, but in addition it explicitly predicted the catholicity of gas–liquid elution chromatography, as well as introducing the distillation theory concept of the equilibrium stage, or theoretical plate, an innovation that subsequently dominated theoretical progress and led eventually to remarkable practical gains in all variants of the chromatographic technique.

In the decade after James & Martin's pioneering work, the gas chromatograph entered every laboratory. Meanwhile, chromatographic rate theory, and the associated thermodynamic partition theory, developed hand in hand, and with great rapidity; so much so that by 1965 columns of remarkable efficiency of separation were freely commercially available and the predictions of the several aspects of theory had been realized to the point where mixtures of the utmost complexity were routinely analysed in laboratories all over the world.

At about that time, the implications of rate theory with respect to the moribund technique of liquid column chromatography had become apparent and as a result the most intensive development of the past 15 years has been that of high-performance liquid chromatography (h.p.l.c.). This technique has now for several years formed an indispensible part of our repertoire of chemical techniques, and a massive proliferation of its use over the next decade can be confidently predicted.

The foregoing historical outline is very widely known and appreciated. What is far less familiar to the scientific community is the steady growth, since 1956, of the use of the chromatographic method for non-analytical purposes, i.e. for physico-chemical measurement. Over the

years, the technique has provided information relating to vapour pressures and boiling points of scarce and impure compounds, on molecular masses and organic structures, in addition to activity coefficients and excess solution quantities, adsorption isotherms and even diffusion coefficients. Indeed, many of the published data of the last 20 years on gas–solid adsorption, and most of those relating to gas–liquid equilibria and gas phase diffusion, have been derived in this way.

The rapidity of acceptance and development outlined above inevitably leads to the view that future technical advance must be slower and the gains accruing smaller. Those not active in the field seem to believe that progress is already confined to the area of data acquisition and handling in that it is mainly to be seen in the rapidly increasing use of microprocessors rather than in the more basic aspects of the technique. But in terms of application and approach there are still, in fact, fertile fields to be ploughed, and I attempt here to illustrate this belief with examples drawn from a few of the areas where recent advance has been notable.

High-performance liquid chromatography

Chromatographic rate theory predicts continuously diminishing theoretical plate height (improving column efficiency) with diminishing column solid particle size. This, of course, is achieved at the price of correspondingly increasing column back-pressures which, on account of the ready compressibility of gases and the consequent deleterious effects on sample component retention, has been seen as a considerable limitation on extension of the use of packed, and even open-tube, gas columns. In contrast, liquids, being of very low compressibility, present no particular problem from this point of view. Thus the fact that the theory allows the prediction of theoretical plate heights corresponding to *ca.* 1000 plates per centimetre of column length, with particles of *ca.* 10 μm diameter, i.e. from 30 to 100 times more unit length efficiency than is usual in gas chromatography, provided the early incentive for study of liquid–solid systems in recent years. That the predicted high efficiencies per unit column length are attainable, in both the forward and reverse phase modes of operation, has been amply demonstrated over the past decade and, for suitable analyses, columns 20 cm long that deliver 20 000 theoretical plates can be purchased. However, much remains to be learned of the technology of production of such fine solids, of their performance as partitioning agents in the presence of solvents, and of column packing procedures. In addition, although the high back-pressures have little effect on retention, they make great demands on the pumping systems, which are already immensely expensive. A major consequence of these facts is that very long (say 1 m or longer) highly efficient liquid–solid columns are as yet, and perhaps always will be, denied us and so total column efficiences greater than about 20 000 theoretical plates are only rarely attained in practice. Since very much larger total efficiencies can be readily achieved with packed or open-tube gas columns, which can be fabricated in the laboratory where they are to be used, gas chromatography remains the method of choice, where there is an option.

Given that, at least for the foreseeable future, there is a quantifiable limitation on practically attainable total column efficiencies in h.p.l.c., we must clearly attempt to assess its implications. To do this we should turn our attention to events within the column. In the gas chromatograph there is essentially no competition for sorption sites between the carrier fluid and the sample components. To a reasonable approximation we can also assume the absence of molecular interactions in the gas phase. In contrast, competition for sorption sites and interaction in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

solution lie at the very heart of the separation process in a liquid column. As a result, single eluant solvents are the exception and mixtures the rule, the nature of the solid sorption sites is even more crucial than in the gas column, and pH and other ionic effects can, for certain mixtures, be totally dominating. And, of course, since the end result is a composite of all these things, none of which can be assumed to be independent of any other, our lack of any secure basis for theoretical interpretation, and hence prediction, makes it remarkably difficult to specify, except qualitatively, the means to attain a desired analytical objective. Clearly, therefore, we must look for fundamental studies of sorbate–sorbent–solvent interactions to clarify our ideas and allow advances in h.p.l.c. comparable with those that followed the fundamental g.c. studies of the 1950s and 1960s. This is quite widely recognized and so there can be no doubt that we shall see many such advances over the next decade.

Currently most solvent and column combinations are selected by experimental precedent and, in many cases, often ingenious and frequently laborious programming techniques, such as solvent or pH (or both) gradient elution, are necessary to provide an adequate analytical solution. Even in the empirical approach, opportunity may be missed because with the mechanistic complexity involved, separations may be achievable only in very precisely controlled conditions. This immediately introduces the important proposition that in h.p.l.c., even more than in g.c., we need some approach that allows firm and easy decisions regarding the choice of optimum conditions of operation even where some understanding of the operative processes exists. I shall illustrate this point with an experimental example in the next section.

Analytical optimization

Laub & Purnell (1975) introduced a new point of departure in the area when they pointed out that for a gas chromatographic column packing comprising support plus liquid A mixed mechanically with support plus liquid B, the total retention volume (V_r) of any eluted component must be the sum

$$V_r = w_A V_{gA} + w_B V_{gB}, \tag{1}$$

where w_A and V_{gA} represent, respectively, the mass of A and the specific retention volume of the sample component measured with a column of pure A at the same temperature, and w_B and V_{gB} are the corresponding quantities for pure liquid B. Thus

$$V_r/(w_A + w_B) = V_{gM} = W_A V_{gA} + W_B V_{gB}, \tag{2}$$

where V_{gM} is the specific retention for elution of the sample component from the mixture and W represents a sorbent mass fraction.

Since, in elution theory,

$$V_r = KV_l, \tag{3}$$

where K is the stoichiometric equilibrium constant (partition coefficient), equation (2) can be rewritten

$$K_r = \phi_A K_A + \phi_B K_B, \tag{4}$$

where ϕ represents a volume fraction of a solvent component.

Given the basic information for pure A and pure B, values of V_{gM} or of K_r can be calculated for a mixture of any composition. If we have such information for two eluted components (solutes 1 and 2) the relative retention (α) is calculable from

$$\alpha = K_{r2}/K_{r1} = V_{gM2}/V_{gM1}. \tag{5}$$

Thus, given data for the two pure solvents, for all components in a mixture we can construct a diagram in which, for all pairs, α is plotted against either W_A or ϕ_A. The lower envelope of this curve represents the lowest value of α (most difficult separation) for all sample pairs at any mixture composition. The highest such value identifies the lowest theoretical plate requirement for complete separation of all components in the sample mixture. Figure 1 illustrates the principle. In figure 1*a* I show hypothetical K_r/ϕ_A plots for a four-component mixture

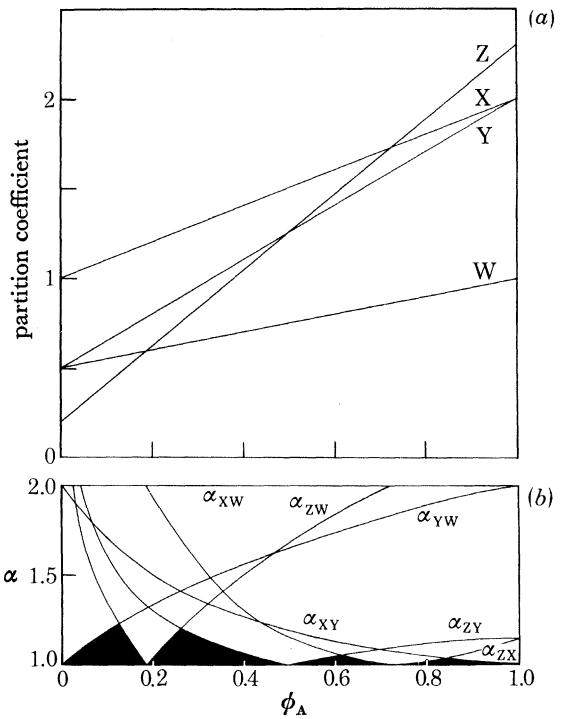

FIGURE 1. (*a*) Hypothetical data for solutes W, X, Y, Z, with solvents A and B plotted as K_r against ϕ_A. X and Y cannot be separated with A; W and Y cannot be separated with B. (*b*) Values of α for all solute pairs corresponding to data of (*a*). The dark region represents areas allowing separation of all four solutes. The highest window (easiest separation) is at $\phi_A \approx 0.15$, which is thus the optimum solvent composition.

(W, X, Y, Z), and in figure 1*b* the corresponding α/ϕ_A diagram, the 'windows' in which complete mixture separation can be achieved being shown in black. The highest window occurs at about $\phi_A = 0.15$ with $\alpha \approx 1.2$. We can now ascertain the number of theoretical plates required to achieve this separation by use of the well known equation

$$N_r = 36(\alpha/\alpha - 1)^2 (1 + k_1'/k')^2, \tag{6}$$

where k' is the capacity factor ($= KV_1/V_d$, where V_d is the column dead volume) for the second eluted of the most difficult pair to separate. Knowing values of N per unit length attainable with the test columns containing A and B that were used to generate the original retention data, the length of column required for complete separation can now be calculated. Since the most difficult pair is separated, so should all others, i.e. complete resolution should be achieved.

Figure 2 illustrates the complex set of windows that characterize the separation of a 30 component C_5–C_8 hydrocarbon mixture with columns containing squalane (B) and di-nonyl phthalate (A). Figure 3 then shows the actual first-time chromatogram obtained with a column of $\phi_A = 0.09$, which is clearly a remarkable justification of the very simple theory advanced.

This window analysis approach is obviously attractive and requires no more than a recognition of the variable determining the extent of resolution that can be achieved, and its dependence upon other experimental variables. Indeed, if this dependence is known to be linear, by determining values of, for example, α as a function of ϕ_A, the analysis of mixtures of components of unknown identity can be achieved since the datum for a given component in each chromatogram must lie on a straight line with the others on a K_r/ϕ_A plot. Thus an α/ϕ_A plot can be constructed and optimization realized in total ignorance of the identity of the mixture components. Figure 4 illustrates an example of this in the separation of some major, and over 30 minor, components of a distillation residue, the composition of which is still unknown to us.

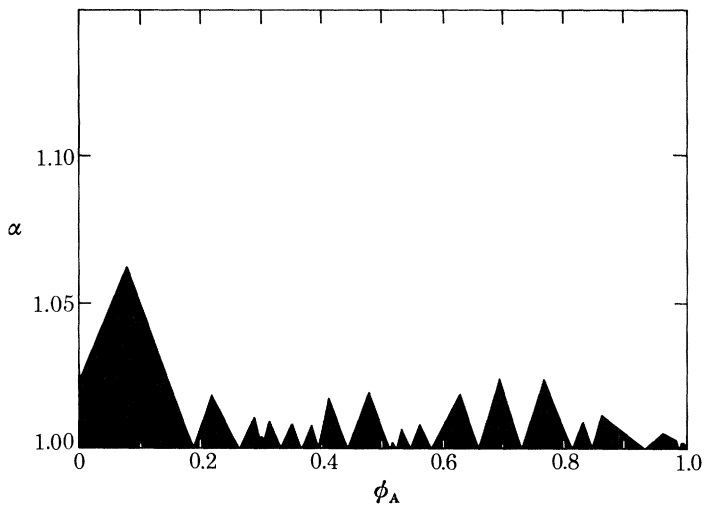

FIGURE 2. Window diagram for a 30-component C_5–C_8 hydrocarbon mixture eluted from columns of squalane (B) and di-nonyl phthalate (A).

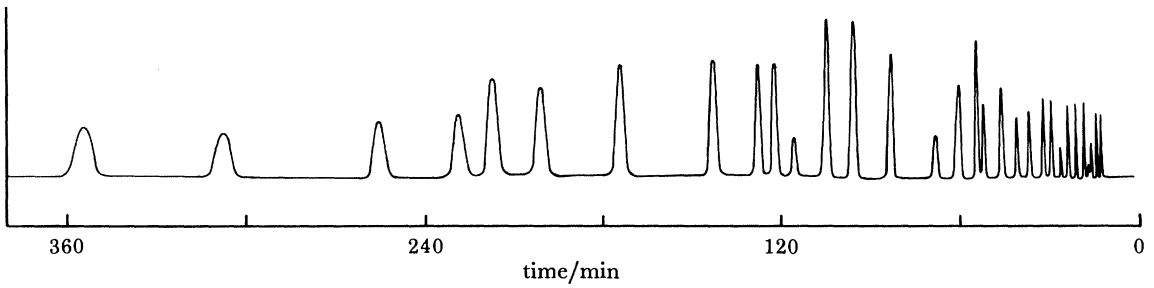

FIGURE 3. Chromatogram of the 30-component mixture of figure 2 with a column of predicted length and optimum solvent composition, A:B = 0.09: 0.91, by volume.

Self-evidently, the method is of wider applicability than simply to facilitate the choice of column substrate composition and I have shown how analysis temperature, for instance, can be optimized in this way, and even how fully resolved spectra in lanthanide shift n.m.r. analysis can be obtained.

Most recently the theory has been extended to bring overall analysis time into the calculation: figure 5 illustrates an optimized minimum-time analysis of a mixture of the seven halocarbon vapours of greatest probable environmental significance. The method is sufficiently successful in this instance to have allowed prior calculation of all operating parameters and to yield an experimental analysis time within 0.2% of prediction.

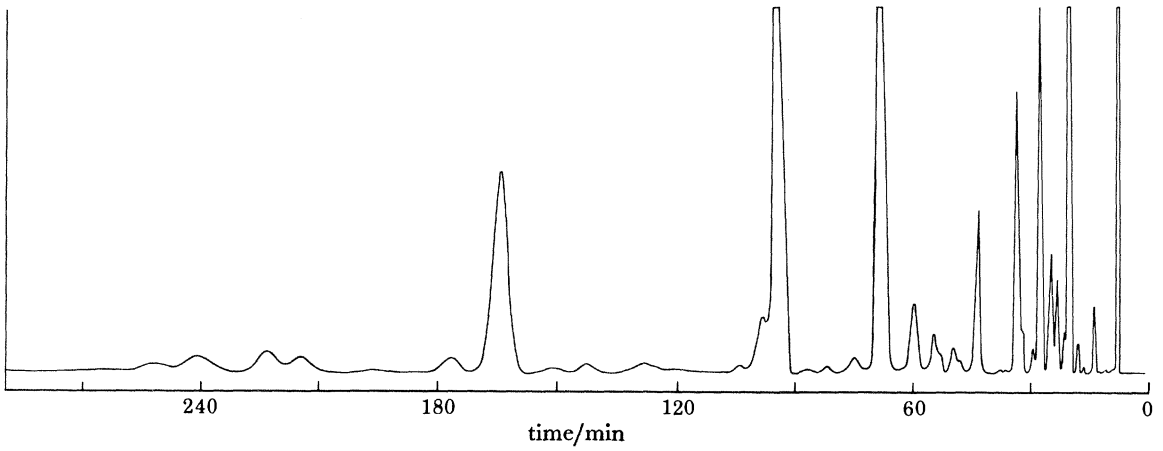

FIGURE 4. Chromatogram showing optimized separation of a mixture of components of unknown identity.

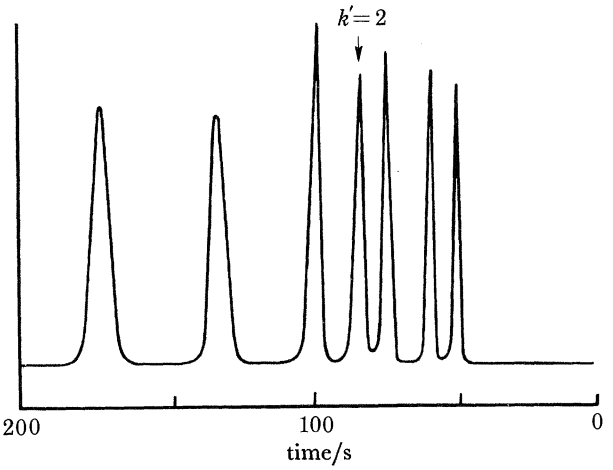

FIGURE 5. Fully optimized gas chromatogram of C_1–C_3 halogenated alkanes and alkenes. The overall analysis time was essentially identical to that predicted by calculation.

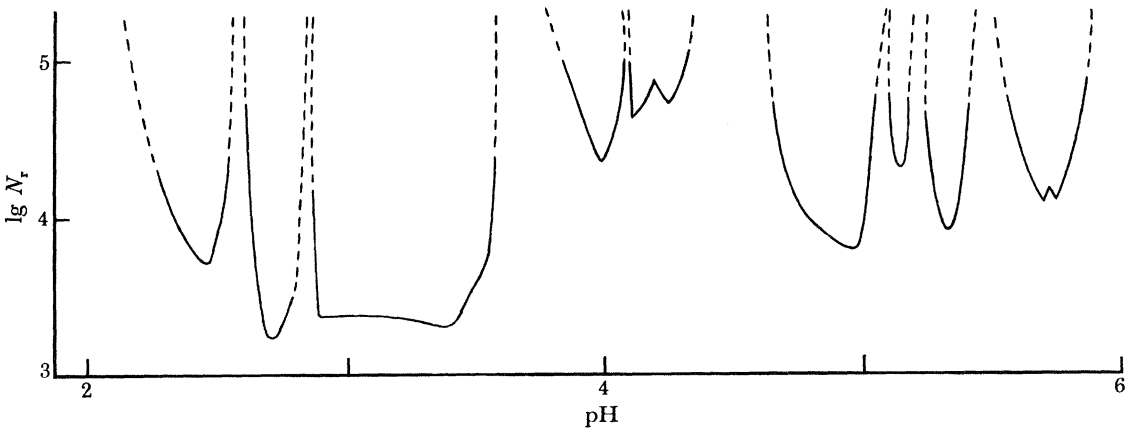

FIGURE 6. Window diagram, plotted as the logarithm of theoretical plates required against solvent pH, for the separation of a mixture of involatile aromatic acids by h.p.l.c. Column, Hypersil; solvent, methanol – aqueous phosphate buffer. The optimum window is at pH 2.70. From unpublished work by Miss B. Patel.

Finally, I show in figure 6 a window diagram in terms of lg N_r against pH for the h.p.l.c separation of a group of aromatic acids. The solvent comprised a 30:70 (by volume) methanol – aqueous phosphate buffer and the column was a 25 cm × 0.4 cm i.d. tube containing 5 μm diameter 'Hypersil' MOS(C_8). The sensitivity of the degree of separation to operating conditions in h.p.l.c. commented upon earlier is now clearly revealed. This particular column could provide up to 16000 theoretical plates for this experimental system so we see that full separation of the mixture could therefore be achieved at any one of four or five pH values. But, significantly, we see regions on the diagram where changes of pH of no more than 0.05 unit take us into a situation where the separation is impossible. An empirical approach would obviously be a very hit-and-miss affair, possibly even totally misleading.

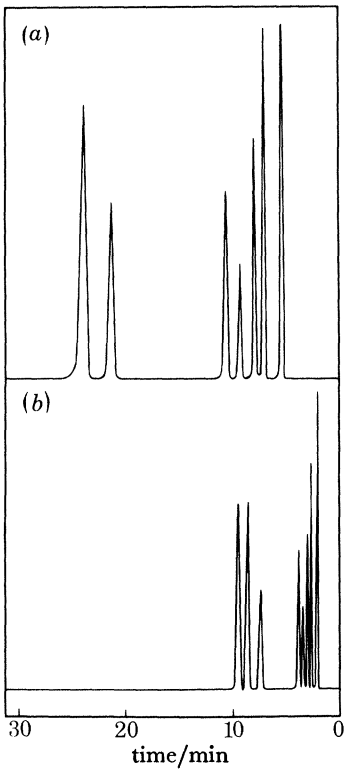

FIGURE 7. (*a*) Separation of seven aromatic acids at a solvent flow rate of 0.94 ml min^{-1} at the optimum pH taken from figure 6 (2.70). (*b*) Illustration of increased speed of analysis by sacrificing column efficiency by operation at a flow rate of 2.50 ml min^{-1}. From unpublished work by Miss B. Patel.

The optimum among the several choices clearly lies at pH 2.70 in the sense that much the smallest number of theoretical plates is then required. Figure 7*a* illustrates the chromatogram obtained in these conditions, the flow rate being 0.94 ml min^{-1} and the overall analysis time at the predicted value of close to 30 min. Although the separation objective has been realized it is, in fact, now possible to do better. Theoretical plate heights in h.p.l.c. vary very little with fluid velocity in the range *ca.* 1–10 ml min^{-1}. Thus, having optimized an analysis to the point where all the column efficiency is not fully used we are in a position to sacrifice efficiency for speed of analysis. The lower figure shows the same analysis run at around 2.5 ml min^{-1}, the pumping limit of the system, and we see a totally acceptable result achieved in only around 10 min.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Had the optimum among the choices not been uniquely identifiable there would have been no spare efficiency and so little prospect of capitalizing on the available improvement in analysis time. H.p.l.c. is, in general, characterized by rather lengthy analysis times and, as indicated above, I believe that a marked improvement in this facet of the technique can be looked for in the near future provided that a coherent and rational approach to system choice is adopted. But this will also require a move to columns of low dead volume, i.e. of much reduced diameter, and in addition access to pumping rates far higher than are now available. There are clear signs that such systems will be commercially available very soon.

Solid surface modification

Chemically modified solids are used very widely in h.p.l.c. Almost all involve silica, and the inorganic reactive group is the surface hydroxyl through which a range of organic groups can be bonded to the solid surface. A substantial selection of such column materials is already commercially available and we must expect fundamental study to fluorish in the 1980s and hope that it will provide the information necessary for a quantitative evaluation and comparison.

Gas chromatographic supports have long been subject to chemical modification with the aim not of inducing selective sorption but of eliminating as far as possible all adsorptive surface effects with respect to sample, while retaining or improving wettability by the solvent. There is in general little current interest in further improvement in this direction with packed columns. In contrast, a very large proportion of the recent literature on capillary column construction and operation is concerned with surface modification.

Open-tube columns offer the attraction of a unit length efficiency comparable with or greater than that of packed columns and such low pressure drop that very considerable lengths may be used. Further, they occupy little physical space and so these substantial lengths can be readily accommodated in conventional thermostatic ovens. Such columns can therefore provide very large total theoretical plate efficiencies and so provide a route to handling extremely complex mixtures.

For many years such columns have been constructed of glass but as a result of technological advance the recent trend has been towards the use of fused silica. Indeed, the advantages of these latter columns in terms of performance, and more particularly physical strength and pliability, are such that we can confidently expect fused silica columns to replace glass columns totally in the very near future.

Despite the large efficiencies attainable with open tubes in favourable cases, problems of wettability of the surface by solvent or adsorptive effects involving solute, or both, lead in many situations to substantial efficiency reductions and marked peak distortion. It is this, allied with the considerable popularity of such columns, that has attracted the very considerable recent interest and effort in the area of surface modification.

The elimination of solute adsorption effects in packed columns has long been brought about by treatment of the siliceous support by hexamethyldisilazane, trimethylchlorosilane or some similar reagent. This treatment eliminates surface –OH groups and introduces non-polar ether moieties. Non-polar solvents such as squalane then spread well on the support and this confers improved efficiency. However, polar liquids, as might be expected, spread less well and poor columns then result. For example, the unit length efficiency of a tricresyl phosphate column on untreated support is commonly double that obtained when a silazaned support is used. Thus

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

this kind of pretreatment is not universally beneficial. Similar behaviour is noted with open-tube columns subjected to such chemical pretreatment. As a result, we have seen in recent years a move towards pretreatment of a more drastic kind, usually directed towards an inorganic conversion of the surface by heat treatment, acid etching or attack by reagents, as for example in the widely advocated barium carbonate treatment. As a complete alternative, attempts have been made to lay down alternative surfaces, mainly inorganic salt films that are expected to be inert. All these methods have something to be said for them and some notable improvements have from time to time been reported. None, however, have met with universal approval since each has demonstrable drawbacks.

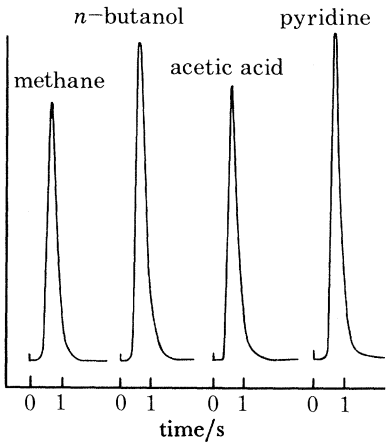

FIGURE 8. Chromatograms for the elution of methane, *n*-butanol, acetic acid and pyridine, respectively, from a 20 m glass capillary coated with silicon and containing no retentive liquid.

I have recently made a proposal that seems to offer real advance in this area. During the period 1966–75 my group presented comprehensive kinetic data relating to the formation of silicon films in silane pyrolysis (see, for example, Purnell & Walsh 1966; Cox & Purnell 1975). One notable feature of such films was their remarkable chemical inertness to pyrolysing gas rich in free radicals. But in addition, silicon is thermally stable, chemically inert and, most important of all, has an excess surface energy that allows it to be readily wetted by most liquids. On this basis, deposition of silicon was thought to offer a very promising route to the production of an open-tube column surface of very desirable general properties. This, it appears, is borne out in practice (Pretorius *et al.* 1981).

Figure 8 illustrates chromatograms obtained with a 20 m × 0.3 mm i.d. borosilicate glass column, silicon-coated by monosilane pyrolysis, containing no retentive liquid at all. The four samples were chosen to represent the widest possible range of polarity and are seen not only to be essentially co-eluted but to give peaks that are superimposable. Further, the retention volume for each is that corresponding to the column dead volume. Thus the silicon surface clearly verges on total inertness in the adsorptive sense.

Figure 9 then shows two chromatograms for a well known standard mixture recommended for 'polarity testing'. The first was obtained with a siliconized column coated with the non-polar silicone liquid substrate, SE-30, the second with a similar column coated with the widely used polar phase FFAP. Although the former is slightly superior in terms of column efficiency, both are excellent and it is clear that the silicon surface provides a more or less equally good

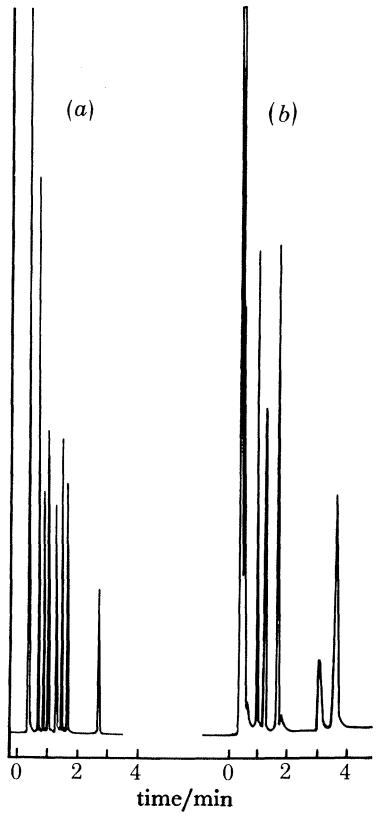

FIGURE 9. (*a*) Gas chromatogram of 'polarity' test mixture with a 20 m silicon-coated glass capillary containing SE-30. Order of elution: sample diluent, octan-2-one, octan-1-ol, naphthalene, 2,6-dimethyl aniline. 2,4-dimethyl phenol, *n*-dodecane, *n*-tridecane. (*b*) Gas chromatogram of 'polarity' test mixture with a 20 m silicon-coated glass capillary column containing FFAP. Order of elution: sample diluent + dodecane + tridecane, octan-2-one, octan-1-ol, naphthalene, 2,6-dimethyl aniline, 2,4-dimethyl phenol.

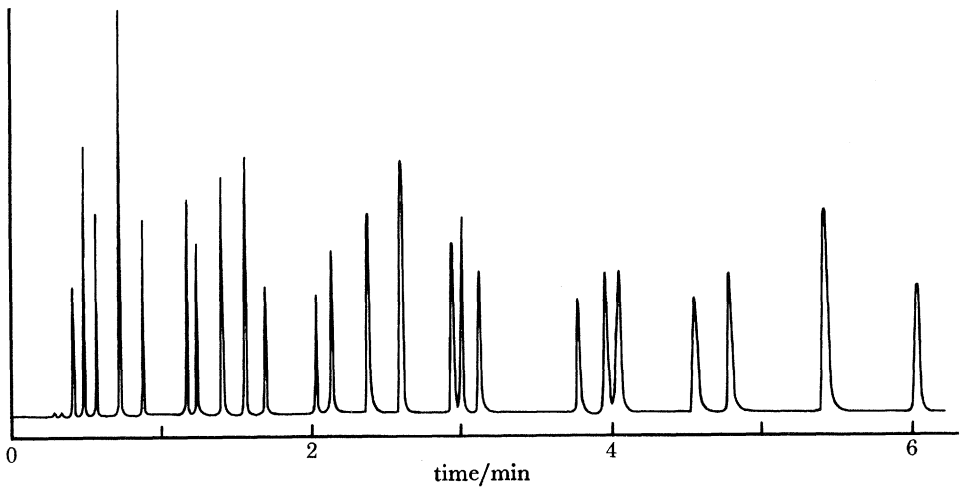

FIGURE 10. Gas chromatogram of a 24-component alkane – alkyl benzene mixture achieved with 8.5 m of micro-bore glass capillary (Schutjes *et al.* 1981). Maximum efficiency (with respect to the last peak) *ca.* 120000 theoretical plates.

base for liquid substrates of widely different type, as shown by the fact that the FFAP column does not retain the alkanes at all.

Studies in progress will undoubtedly reveal whether or not silicon deposition represents the universal approach that is being sought. Even if this is not so, the relative ease with which a clear advance has been achieved must give us confidence in attainment of an eventual solution when the theoretically calculable ultimate efficiencies may be reached.

What this last statement implies may be judged from figure 10 taken from the recent work of Schutjes *et al.* (1981). This chromatogram was achieved with a micro open-tube column of only 50 μm internal diameter, constructed of untreated borosilicate glass and coated with SE-30. The theoretical plate height achieved was 0.005 cm and is thus comparable with h.p.l.c. systems. Put alternatively, the 8.5 m column used provided 120000 theoretical plates, a remarkable result by any standard, and certainly the best known to me. But there is significant peak asymmetry in the chromatogram and I thus expect a still more effective performance with appropriate column pretreatment. We may not, in the short term, find such microcolumns in routine use in the laboratory, but recognizing first the rapid adaptation that has characterized the technique, and secondly that Rijks and his colleagues are currently developing techniques for the construction of micro-packed columns, rapid acceptance and widespread use seem quite possible.

Conclusion

I have chosen here to illustrate a few areas in which advances in understanding and technique are continuing. These represent only the tip of the iceberg and can give no more than a suggestion of what can be expected to emerge over the decade. What can be said with conviction is that, wide though the use of chromatographic techniques is now, it will expand ever more widely in the future.

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Discussion

J. D. R. Thomas (*Chemistry Department, U.W.I.S.T., Cardiff, U.K.*). Although *ca.* 10^5 plate columns are mentioned as about the maximum for convenient separations, others (notably Scott and Kucera) have mentioned that columns of much greater efficiencies are desirable, indeed a necessity, for some instances, such as in the field of biological materials. Columns of 10^6 plates have been shown to be possible, and even higher efficiencies have been mentioned. Is there a rationale favouring the high-efficiency columns with *ca.* 10^6 plates?

J. H. Purnell. As a general rule, the more theoretical plates one can generate the better, because one can not only achieve better separation but in addition attain much faster analysis. This is a

matter of particular importance in h.p.l.c. where column lengths are necessarily quite short and unlike gas chromatography cannot be indefinitely extended. It is essentially this point of view that has led me to the opinion that capillary and microbore systems represent the most probable direction in h.p.l.c. developments in the immediate future. However, there is a price to be paid for higher efficiency. First, sample sizes must be reduced because the effective plate column is smaller and with sharper peaks smaller detector volumes are required: this presents, particularly again in h.p.l.c., a significant technological challenge.

A. F. Fell (*Heriot-Watt University, Edinburgh, U.K.*). What does Professor Purnell consider to be the prospects for simplex optimization procedures in the context of method development in high-performance liquid chromatography? Recent experience in industry indicates that for eluents of up to three components, computer-controlled h.p.l.c. systems can be used for the optimization of these variables routinely, as described recently by Berridge (*Analyt. Proc.*, in the press).

J. H. Purnell. I am not aware of any published work on the simplex procedure although I have heard that it is in use in some laboratories. I look forward to seeing the paper by Berridge.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES