CHROMSYMP. 454

RAPID OPTIMIZATION OF THE CONCENTRATION OF THE ION-PAIRING REAGENT IN ION-PAIRING REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

   An iterative procedure is applied to the optimization of the concentration of sodium octanesulphonate for the separation of a sample containing cations and anions. The procedure starts with retention data taken at the extreme limits of the concentration range considered and approximates the true retention behaviour by a succession of linear segments. It works best when the actual retention curves do not deviate strongly from linearity, as is the case when the sodium octanesulphonate is expressed as the concentration.adsorbed on the stationary phase or as the logarithm of the concentration in the mobile phase. The correct optimum composition can be found in four to seven chromatographic analyses.

INTRODUCTION

   In previous publications[1,2] we have described a rapid procedure for the optimization of the mobile phase composition in liquid chromatography. It seeks to combine the advantages of the simultaneous experiment-design technique[3] with those of the sequential Simplex approach[4]. Similar to the Simplex, our procedure starts with a limited number of initial compositions, at least one more than the number of parameters to be optimized. At this point the Simplex uses the values calculated for the optimization criteria at each composition to formulate the next mobile phase composition. The procedure is repeated and the optimum is reached through a usually large series of successive approximations.
   In our approach, the initial chromatograms are used to calculate a response surface for the optimization criterion over the entire parameter space. This is similar to the experiment-design technique and permits a direct prediction of the optimum mobile phase composition. However, whereas the experiment-design technique stops at this point, we use the results measured for the predicted optimum composition to refine the response surface and improve the prediction of the optimum composition. This is repeated till no further improvement is obtained.
   On closer examination, our procedure resembles either the experiment design or the Simplex technique, depending upon the shape of the response surface used. If

the response surface is smooth or can be expressed by a simple mathematical expression, the experiment design needs only a few input data to calculate the response surface accurately and to predict the optimum mobile phase composition reliably. Correspondingly, our procedure will then find the optimum in one or two iterations. If, on the other hand, the response surface is very erratic, the experiment design requires a prohibitive number of input data and in our procedure the number of iterations increases rapidly to the point where it comes close to the Simplex approach.

Because in reality the response surface is not known beforehand, our procedure has the advantage of flexibility. Moreover, it maintains an overview over the complete parameter space and permits a verification of the final result. If the response surface is indeed smooth, the iterations will converge rapidly to the true result. If the response surface is irregular, the danger of focusing upon a false, secondary optimum is less than in the Simplex design.

In the liquid chromatography the usual optimization criterion is the resolution of adjacent peak points or some related quantity[3-6]. Especially for a complex sample, the variation of the resolution can be very erratic. A direct use of the optimization criterion, as is required in the Simplex procedure, can then easily lead to erroneous results. An example will be presented in this study. However, a more efficient approach is possible, when we realize that the logarithmic retention of individual solutes behaves much more regularly than the resolution criterion.

Thus, the experiment-design optimization of a quaternary mixture from three binary solvents described by Glajch and Kirkland[3] starts with quadratic surfaces fitted through seven data points for the logarithmic capacity factor. The quadratic dependence upon the organic modifier content not only follows from theoretical considerations[7], but also conforms to analytical practice. In the same way, our procedure for optimizing a ternary mixture from two binary solvents starts by assuming a linear dependence for the logarithmic capacity factor and corrects for experimental curvature in a few iterations[1,2]. Obviously, in both procedures, resolution criteria are readily calculated from the capacity-factor surfaces.

The linear approximation used by us for ternary solvent optimization can readily be extended to quaternary solvent optimization by a successively refined set of planes drawn through three triangular data points. Although the procedure is slightly more complicated than the straightforward quadratic used by Glajch and Kirkland[3], its greater flexibility offers an advantage in situations where a quadratic relationship does not apply. An example is provided by the optimization of pH in the separation of organic acids[8]. In the one-dimensional case where only the pH is varied, the S-shaped retention curves could be adequately approximated by four linear segments. In the two-dimensional case of the simultaneous optimization of pH and organic modifier, a division into four triangles was found to be sufficient to find the optimum composition[8].

The present study is devoted to the optimization of the mobile phase composition in ion-pairing chromatography. Lindberg et al.[9] have described a four-parameter factorial design that requires sixteen chromatograms for the optimization of an ion-pairing separation of four alkaloids. In a previous publication we have shown that the number of parameters can be reduced by a judicious selection of the separation conditions[10]. Accordingly, we describe the optimization of the concentration of sodium octanesulphonate for the separation of a mixture of positively and negatively charged solutes (catecholamines and metabolites).

EXPERIMENTAL

The liquid chromatograph comprised a Waters M6000 A pump, a Rheodyne 7125 sample injector with 10-μl loop and a Waters M 440 UV detector. The analytical column was packed with 5-μm ODS-Hypersil (Shandon Southern Products). The nitrogen BET surface area and carbon content were 173 m^2 g^{-1} and 8.8% (w/w)), respectively, according to the manufacturer (Batch No. 8/1017). The mobile phase was always prepared in 250-ml quantities by taking 6.25 ml of 1 M H$_3$PO$_4$, 6.25 ml of 1 M NaH$_2$PO$_4$, 19.78 g methanol (10%, v/v) and weighed (four decimals) amounts of NaBr (Baker, Deventer, The Netherlands) and sodium octanesulphonate (Janssen Chim., Beerse, Belgium). The total sodium concentration was maintained at 175 mM. The solutes were: 3,4-dihydroxymandelic acid, noradrenaline, 3,4-dihydroxyphenyl-acetic acid, adrenaline, octopamine, 5-hydroxyindole-3-acetic acid, homovanillic acid, 1-DOPA (3,4-dihydroxyphenylalanine), dopamine and tyrosine. Aqueous buffered solutions of individual solutes and the mixture were stored in a refrigerator.

The adsorption isotherm was measured by the breakthrough method using a Waters R-401 differential refractometer. The breakthrough and the chromatographic retentions were measured in the same instrument[10]. The residence time in the connecting capillary tubing was taken into account. The flow-rate was 1.50 ml/min, accurately measured during each experiment. Retention times were corrected for small changes in flow. The UV detector was used at 0.01 a.u.f.s.

RESULTS AND DISCUSSION

In reversed-phase ion-pairing chromatography the retention of charged solutes is a complex function of the nature and the concentration of the ion-pairing reagent, and of the organic modifier type and content, the counter ion concentration and possibly the pH of the mobile phase. In a previous publication[10] we have demonstrated that the system is greatly simplified when the counter ion concentration is kept constant and when the ion-pairing reagent is expressed as the concentration adsorbed on the stationary phase, P_s, rather than as the concentration dissolved in the mobile phase, P_m. The change of variable is easily accomplished, once the adsorption isotherm is known. In a separate communication[11] we shall show that for alkanesulphonates the changeover to P_s removes the influence of the alkyl chain length and reduces the effect of the organic modifier to an approximately parallel shift of all logarithmic retention curves. As in ordinary reversed-phase liquid chromatography(RPLC), the type of organic modifier can give rise to specific retention effects. However, in ion-pairing RPLC the concentration of the ion-pairing reagent is the main optimization parameter.

Fig. 1A presents the characteristic increase of cation retention and decrease of anion retention with increasing concentration of sodium octanesulphonate. Comparison with Fig. 7 (top) shows that the change from mobile phase concentration, P_m, to stationary phase concentration, P_s, linearizes the logarithmic retention curves. However, the approximately linear relationship between the capacity factor, k, and P_s reported by Knox and Hartwick[12] over a similar range is not observed in our experiments. Obviously, the non-linear P_m-scale shown in Fig. 1 is derived from the linear P_s-scale through the adsorption isotherm of sodium octanesulphonate for this phase system.
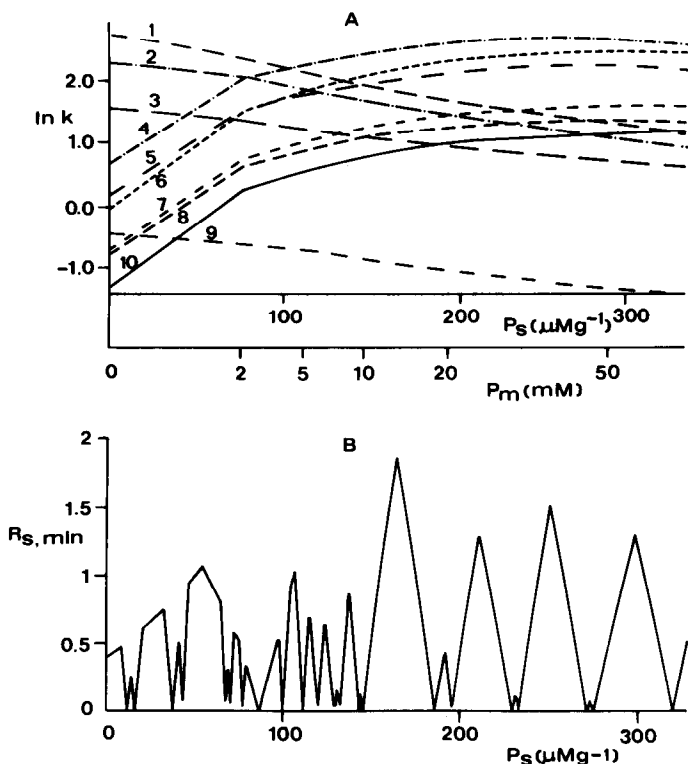
Fig. 1. Variation of solute retention with increasing concentration of sodium octanesulphonate (A) and corresponding variation of the resolution of the least resolved solute pair (B). Solutes: 1 = homovanillic acid; 2 = 5-hydroxyindol-3-acetic acid; 3 = 3,4-dihydroxyphenylacetic acid; 4 = tyrosine; 5 = L-DOPA; 6 = dopamine; 7 = octopamine; 8 = adrenaline; 9 = 3,4-dihydroxymandelic acid; 10 = noradrenaline. Stationary phase: Hypersil ODS. Mobile phase: 10% (v/v) methanol.

Fig. 1B shows the true variation of the minimum resolution of the least resolved solute pair, $R_{s,min}$, with P_s for this particular sample. The need for optimizing the concentration of the ion-pairing reagent and the benefits to be expected from it are manifest from Fig. 1. On the one hand the large and opposite shifts of the capacity factors of cations and anions, respectively, make the optimization of the ion-pairing reagent concentration profitable. On the other hand the many crossings of the retention curves in Fig. 1A indicate a corresponding number of reagent concentrations where at least one solute pair will be completely unresolved. At such concentrations the optimization criterion becomes zero. As a result, the response surface of $R_{s,min}$ with P_s is very complex. Attempts to optimize the system by means of a supermodified Simplex[13] were not successful. Depending on the starting values chosen for P_s, the Simplex stopped at one of the many local optima, using a minimum of ten steps.

At this point we discard knowledge of Fig. 1, but assume the composition of the sample to be known. The first step is the determination of a suitable organic modifier content. This can be achieved with the gradient-elution procedure described previously[14]. Because we expect strong retention increases for cations, the organic

modifier content is chosen such that their capacity factors in the absence of sul-phonate are modest. The opposite conclusion applies to the capacity factors of the anions. Since the extent of the retention shifts is not known beforehand, a tentatively selected modifier content can be verified in a chromatogram run at high P_s.

Fig. 2 shows the chromatograms run in 10% (v/v) methanol at $P_m = P_s = 0$ and at $P_m = 70$ mM, $P_s = 330$ μmol/g, respectively. The correspondence between P_m and P_s follows from the adsorption isotherm and can be read from the two concentration scales in Fig. 1A. Similar overall retentions were obtained with 6% acetonitrile and 6.6% tetrahydrofuran instead of 10% methanol for the modifier, but complete separation of the sample is not achieved.
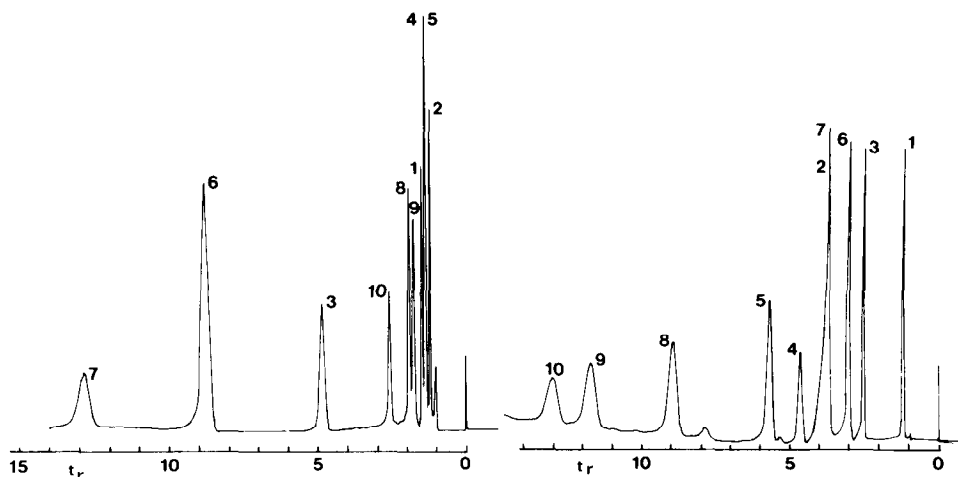

Fig. 2. Sample chromatograms recorded at the extreme concentrations of the ion-pairing reagent, sodium octanesulphonate. Solutes: 1 = 3,4-dihydroxymandelic acid; 2 = noradrenaline; 3 = 3,4-dihydroxyphen-ylacetic acid; 4 = adrenaline; 5 = octopamine; 6 = 5-hydroxyindol-3-acetic acid; 7 = homovanillic acid; 8 = L-DOPA; 9 = dopamine; 10 = tyrosine. The column (15 cm × 4.6 mm I.D.) is packed with Hypersil ODS. Mobile phase: 10% (v/v) methanol.

We are now ready to start the optimization. By separate injection of the in-dividual solutes their capacity factors at the extreme limits $P_s = 0$ and $P_s = 330$ μmol/g are determined. Fig. 3 presents as a first approximation linear plots of ln k and the corresponding variation of the resolution criterion. Comparison with the true behaviour in Fig. 1 shows the crude nature of this first approximation. Never-theless, a clear optimum at $P_s = 214$ μmol/g is predicted. To gain as much infor-mation as possible from the next chromatogram, it is actually carried out at a shifted value[2] of $P_s = 195$ μmol/g. Since the expected positions of the solute peaks are known, there is no need to inject the solutes separately and we can analyse the actual sample. In agreement with Fig. 3, the resulting chromatogram is rather poor, but it provides new retention data with which to refine the ln k plots. The results shown in Fig. 4 demonstrate that for cations the variation of ln k is indeed not linear over the whole range of P_s. The new data points are again connected by straight lines and this leads to changes in the pattern of the optimization criterion.

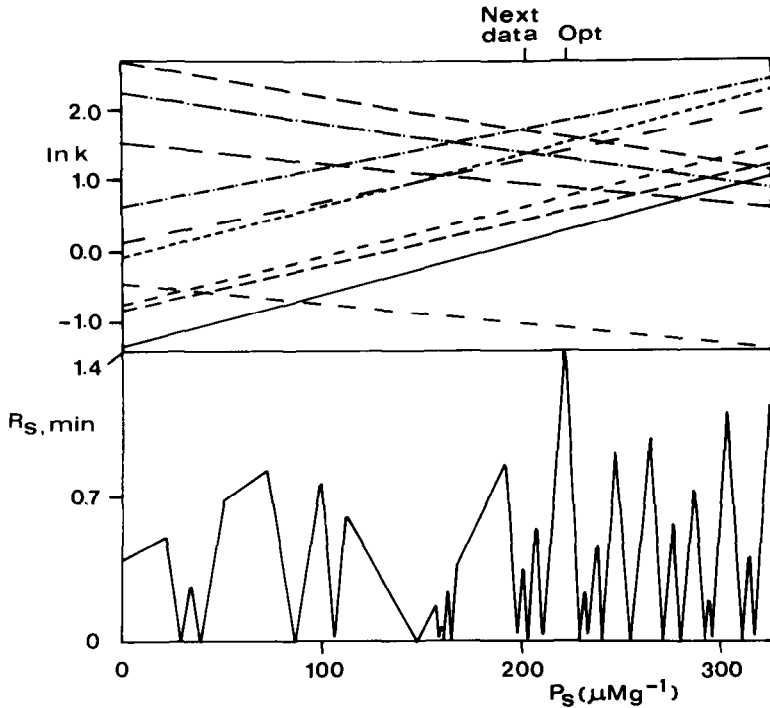Although the minimum resolution at $P_s = 214$ μmol/g (the optimum concen-

Fig. 3. First result of the optimization procedure. The top part presents linearly connected retention data taken from Fig. 2. The bottom part presents the calculated variation of the optimization criterion, $R_{s,min}$, with the stationary phase concentration of ion-pair reagent. The optimum composition (Opt) is indicated together with the composition (next data) selected for the next measurement.

tration predicted in Fig. 3) changes very little from its previous value of 1.4, a better value of $R_{s,min}$ = 1.5 is now predicted at P_s = 180 μmol/g. To gain a better insight in the lower P_s range, the procedure instructs us to carry out the next chromatogram at P_s = 124 μmol/g. The predicted optimum now changes to $R_{s,min}$ = 1.6 at P_s = 165 μmol/g or P_m = 13 mM (Fig. 5). Since this value lies in a range welll covered by previous experiments there is no need to test another concentration. Consequently, the optimization is terminated and the chromatogram at this composition is indeed satisfactory (Fig. 6). The final result has been obtained in a total of five chromatograms: two initial experiments, two iterations and one final verification. The agreement between Figs. 1 and 5 is remarkable.

However, some potential problems might be pointed out. First, the speed of the procedure is enhanced by the fact that the variations of ln k with P_s can be closely approximated by a few linear segments. In practice, of course, P_s is changed by a suitable change in P_m read from the adsorption isotherm. Although the isotherm can be stored in the computer memory, it must first be determined for the particular mobile phase composition chosen. It is, therefore, of interest to consider the possibility of optimization directly in terms of P_m. To this end the retention curves of Fig. 1 are replotted in Figs. 7 and 8 as a function of P_m and log P_m, respectively. When P_m is scaled linearly, as in Fig. 7, the retention curves of anions become weakly
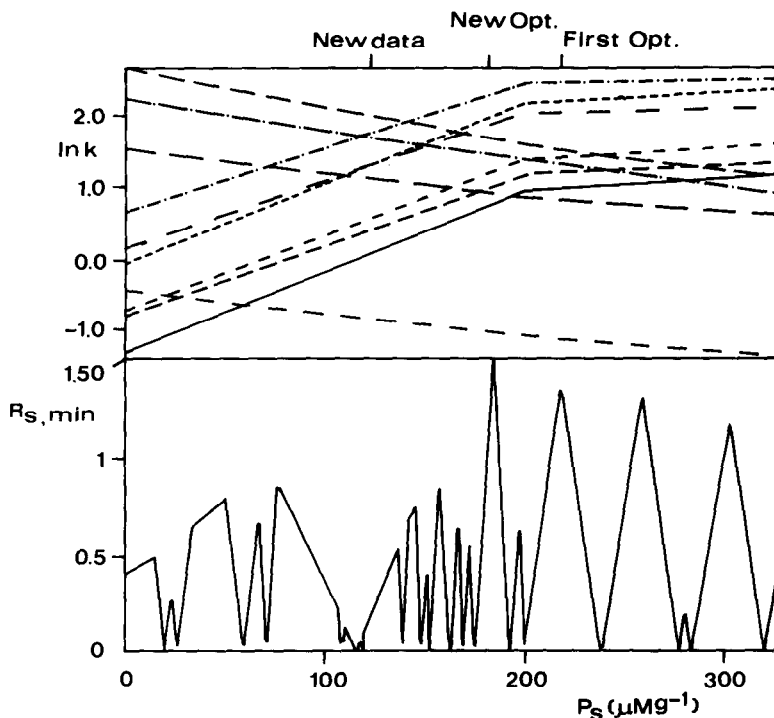
Fig. 4. Second result of the optimization procedure. Explanation as in Fig. 3. Second predictions of the optimum composition and the next measurement composition are indicated.

curved and those of cations very strongly curved at low P_m. The optimization procedure must now approximate these curves by a succession of linear segments running from one predicted composition to the next one. The consecutively predicted optimum compositions are indicated by numbers in Fig. 6. The result is that the optimization procedure stops after three consecutive steps and predicts an optimum P_m of 37 mM. Not only is this value different from the result found in Fig. 4, but the resulting chromatogram is poorer ($R_{s,min}$ = 1.2) than the one shown in Fig. 6 ($R_{s,min}$ = 1.6). Apparently, with this procedure one fails to investigate the region between P_m = 0 and P_m = 25 mM, because the strong non-linearity that exists in reality is not predicted. When the procedure is restarted with P_m = 10 mM (number 4 in Fig. 6) one finds the true optimum in one more step. The warning that emerges from this failure is not unique for our approach, but applies to all optimization procedures: a result is to be mistrusted if a large region of the parameter space is left unsearched.

As shown in Fig. 8 such a problem does not occur when the optimization is performed in terms of log P_m, because then all retention curves are nearly linear over the whole range. As a result, the data collected with the next run (at P_m = 14 mM) agree so closely with those predicted from the two initial chromatograms that the procedure is stopped. Indeed, the correct optimum has been found in one step.

Another problem is the recognition of corresponding solutes in successive chromatograms. It is a decisive advantages of the Simplex approach that this need is
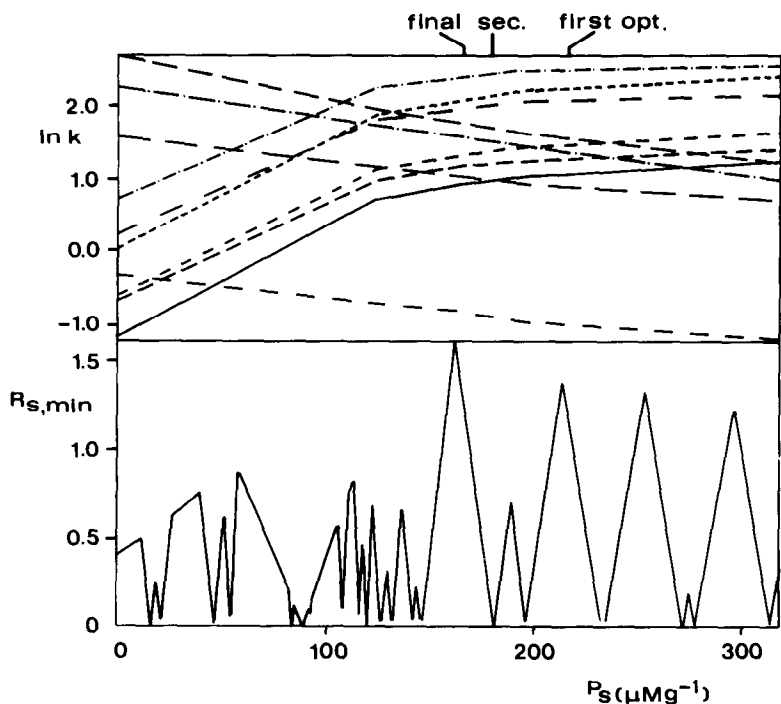
Fig. 5. Final result of the optimization procedure. The composition for the first, second and final optimum is indicated in the top part.
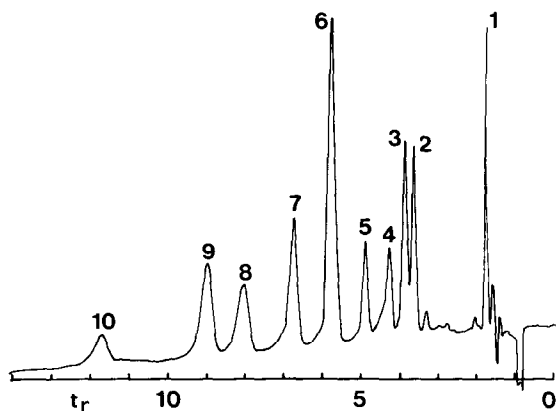
Fig. 6. Final chromatogram recorded at the predicted optimum concentration of sodium octanesulpho-nate: P_s = 165 μmol/g corresponding to P_m = 13 mM. The solute numbering is the same as in Fig. 2.

avoided. However, as we have seen, Simplex offers no solution to the optimization problem addressed in this study. Experiment designs, such as ours and that of Glajch and Kirkland[3], that calculate full retention curves from a few data points must take care to connect corresponding data points. In the present example the problem was solved by injecting all solutes separately, which is possible only when all constituents
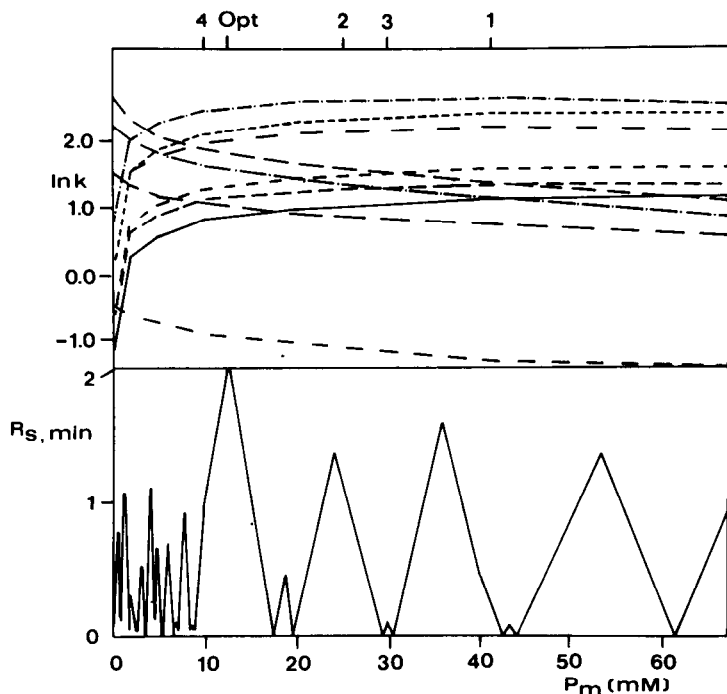
Fig. 7. Retention data and criterion variation of Fig. 1, replotted as a function of the mobile phase concentration of the sodium octanesulphonate, P_m, scaled linearly. The numbers indicate the progression of the optimum reagent concentration predicted during the optimization procedure.
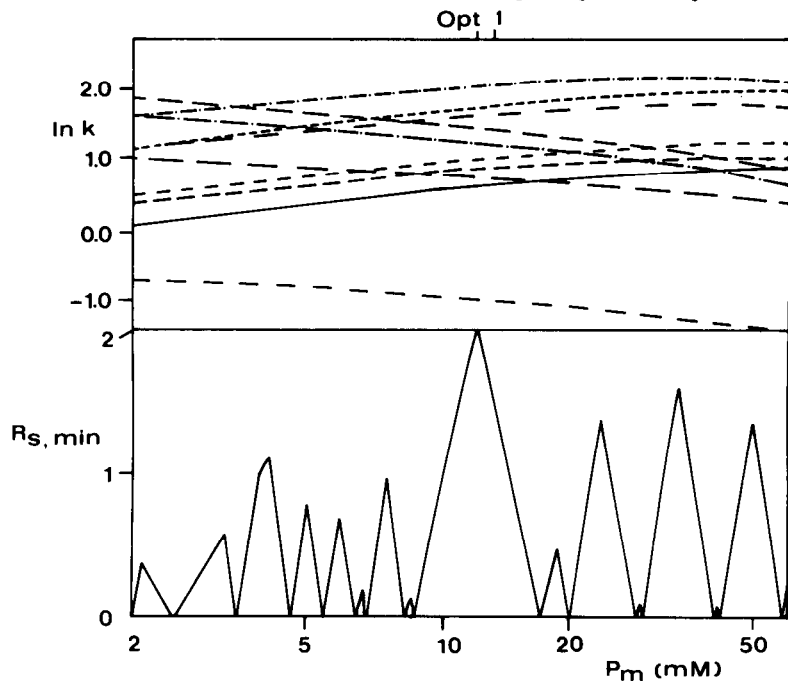
Fig. 8. Retention data and criterion variation of Fig. 1, replotted as a function of the mobile phase concentration of sodium octanesulphonate, P_m, scaled logarithmically. The optimum is found in one step.

of the sample are known and available. If the sample composition is unknown, the recognition of corresponding solutes pressents a challenge, especially in the initial chromatograms recorded under widely different conditions. Attempts to identify solutes by recording the ratio of two absorbances measured at different wavelengths have not been successful[15]. Better perspectives are offered by full UV-absorption or fluorescence spectra, as will be reported in future publications.

A final problem is the choice of the modifier content. In the present example a methanol concentration of 10% was adequate at either extreme of the ion-pairing reagent concentration. For other samples the situation might be less fortunate. Especially at high P_m, the modifier content could be too high for the anions or too low for the cations. Such a case would call for the simultaneous optimization of modifier content and ion-pairing reagent concentration. An example of a similar two-parameter optimization has been reported previously[8].

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