CHROM. 12,621

ISOTHERM LINEARITY AND SAMPLE CAPACITY IN LIQUID CHROMATOGRAPHY[*]

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(First received July 6th, 1979; revised manuscript received December 13th, 1979)

SUMMARY

The significance of distribution isotherm measurements for preparative liquid chromatography is discussed. Experimental methods for the determination of isotherms on liquid chromatographic phase systems are compared on the basis of accuracy, precision and speed. In this respect the break-through method constitutes a favourable compromise. Using this method, various normal-phase adsorption systems with dichloromethane as the mobile phase, phenol as the solute and porous silicas from several sources with various particle sizes and surface areas were compared. Significant differences in the linear range of the various silicas were found. Particle size has only a small influence, while larger surface areas, as expected, give longer linear ranges.

For adsorption systems using alkyl-modified silicas, phenols as solutes and methanol–water mixtures as the mobile phase, the source of the material was found to be of less importance. Chain length has an adverse effect on the isotherm linearity; the modified silica with the shortest chain length (C-2) has a significantly larger linear range.

INTRODUCTION

In chromatography, a knowledge of the shape of the distribution isotherms of solutes is necessary for establishing optimal conditions for analytical or preparative separations. From a theoretical point of view, a linear distribution isotherm is advantageous for interpreting the chromatographic results. However, in practice, a certain non-linearity of the distribution isotherm can be accepted when performing analytical separations, as was shown by Snyder[1].

Owing to the development of very sensitive detectors, usually solute concentrations can be injected that almost fall in the linear part of the distribution isotherms.

A different situation exists when as large amounts of solutes as possible are injected, as occurs in preparative liquid chromatography, and then one reaches that part of the distribution isotherm where a strong deviation from linearity occurs. This will lead to asymmetric peak shapes, which, apart from the normal dispersion effect, will hamper the collection of pure substances.

In a previous paper[2] we considered the dispersion effect in preparative liquid chromatography, and the optimal choice of geometrical conditions contributing to the dispersion was discussed. The non-linearity of the isotherm was not discussed, but was taken into account by assuming a maximum allowable concentration of a component in the mobile phase at the column outlet, $c_{i, \, max}$, which should not be exceeded if peak overlap due to isotherm effects is to be avoided. The value of $c_{i, \, max}$ will obviously depend on the isotherm shape and on the relative positions of the neighbouring peaks. The farther away an interfering peak j is, the more broadening due to non-linearity can be accepted.

This same relative position of two peaks i and j also determines the required number of theoretical plates, N_r, which will effect the separation at infinite dilution according to the equation

$$N_r = \frac{1}{(r_{ji} - 1)^2} \cdot \left(\frac{\kappa_i + 1}{\kappa_i} \right)^2 \cdot R_{ji}{}^2 \tag{1}$$

where r_{ij} is the selectivity factor, κ_i the capacity factor and R_{ji} the required resolution.

It follows that at high N_r values (difficult separations) $c_{i, \, max}$ will be low. We can try to put this in a quantitative form, although we do not know how non-linearity broadening and dispersion broadening will interact. Also, we know very little about the isotherm effect when various compounds are sorbed into the stationary phase simultaneously, which will certainly occur in the first part of the column in an actual preparative separation.

However, in order to obtain at least some insight into the problem, it is useful to simplify the picture considerably. We shall neglect all higher order effects and all cross-effects between different solutes. Suppose the isotherm can be described in the first order by

$$\kappa_i(c_{i, \, m}) = \kappa_i(0) + [1 + \kappa_i(0)] \cdot \lambda_i c_{i, \, m} \tag{2}$$

where κ_i is related to the derivative of the isotherm, $\kappa_i = q \cdot dc_{i, \, s}/dc_{i, \, m}$ (where q is the phase ratio), $\kappa_i(0)$ is the same parameter at infinite dilution, $c_{i, \, m}$ is the solute concentration in the mobile phase, $c_{i, \, s}$ is the solute concentration in the stationary phase and λ_i is a non-linearity parameter, defined in accordance with the work of Buys and De Clerk[3].

This description of the isotherm may seem peculiar. The constants q and $1 + \kappa_i(0)$ were included in order to simplify the following derivations. The isotherm is then explicitly described by

$$c_{i, \, s}(c_{i, \, m}) = \frac{1}{q} \{ \kappa_i(0) + \tfrac{1}{2}\lambda_i[1 + \kappa_i(0)] \cdot c_{i, \, m} \} \cdot c_{i, \, m} \tag{3}$$

Making a further simplification by neglecting dispersion, we can apply the theory of

non-linear dispersionless chromatography, as described for example by Huber and Gerritse[4], which gives an expression for the elution time at a concentration $c_{i,m}$:

$$t_R(c_{i,m}) = t_0\left(1 + q \cdot \frac{dc_{i,s}}{dc_{i,m}}\right)$$
$$= t_0[1 + \kappa_i(0)] \cdot (1 + \lambda_i c_{i,m}) \tag{4}$$

The peak shape is a rectangular triangle with the hypotenuse having a slope of $\{t_0[1 + \kappa_i(0)] \cdot \lambda_i\}^{-1}$. Whether the vertical side is the front or the rear of the peak is determined by the sign of λ_i; for the most common case of a negative λ_i the front is vertical.

The isotherm non-linearity effect, in the absence of dispersion, will therefore lead to a relative peak width of $\lambda_i \bar{c}_{i,m}$, where $\bar{c}_{i,m}$ is the concentration at the maximum. When the isotherm is linear and dispersion is present, a relative peak width of $4/\sqrt{N}$ occurs, where N is the plate number and taking the total peak width at four times the standard deviation. When both peak broadening effects are operative, we cannot predict the resulting peak width, but it is improbable that it can be smaller than the peak width obtained when either effect is present alone. It follows, then, that the relative peak width is larger than $4/\sqrt{N}$ and larger than $\lambda_i \bar{c}_{i,m}$; the largest of the two will determine to a great extent the effective resolution that can be obtained. In order to obtain a high throughput, we want to make $\bar{c}_{i,m}$ as large and N as small as is allowed with respect to the required resolution. An optimal compromise is to be expected when both broadening mechanisms contribute significantly to the final peak width. For this situation, the peak widths caused by the two mechanisms in the absence of each other would be of comparable magnitude; apart from an unknown numerical factor we can state, therefore:

$$\frac{4}{\sqrt{N}} = \lambda_i \bar{c}_{i,m} \tag{5}$$

Table I illustrates the deviations in the isotherm linearity that can be tolerated as a function of the plate number. If an actual $\lambda_i \bar{c}_{i,m}$ value is higher than those indicated, one could obtain a higher throughput at essentially the same resolution with

TABLE I

RELATIONSHIP BETWEEN PLATE NUMBER AND FRACTIONAL DEVIATION FROM LINEARITY IN THE ISOTHERM AT THE PEAK MAXIMUM CONCENTRATION, $\bar{c}_{i,m}$, IN THE CASE OF AN OPTIMAL COMPROMISE BETWEEN DISPERSION AND ISOTHERM NON-LINEARITY BROADENING (TENTATIVE VALUES)

Theoretical plate number, N	Maximum deviation in the isotherm linearity at the column outlet, $\lambda_i \bar{c}_{i,m}$ (%)
100	40
300	23
1000	13
3000	7
10,000	4
30,000	2
100,000	1

a column with a lower plate number; if the $\lambda_i \bar{c}_{l,m}$ value is lower, then dispersion still determines peak broadening and the throughput can be increased via larger $\bar{c}_{l,m}$ values.

It is interesting to consider the absolute amount of solute i, Q_l, which has to be put on the column in order to obtain a particular $\bar{c}_{l,m}$ value. The amount of solute, Q_l, must be related to the peak time integral of the concentration in the mobile phase. This fixes the value of the peak maximum and the position of the sharp edge. By changing the integration axis, it can be shown[4] that

$$\frac{Q_l}{w} = \int_{\text{peak}} c_{l,m} \cdot \mathrm{d}t = \int_0 \bar{c}_{l,m} \pm \{t_R(c_{l,m}) - t_R(\bar{c}_{l,m})\}\mathrm{d}c_{l,m} \tag{6}$$

and substitution yields the following relationship between the amount of solute, Q_l, and parameters of the column, isotherm and peak maximum:

$$\frac{Q_l}{w} = t_0[1 + \kappa_i(0)] \cdot \frac{|\lambda_i|}{2} \cdot \bar{c}_{l,m}^{\,2} \tag{7}$$

where w is the volume flow-rate. This leads to

$$\begin{aligned}
\bar{c}_{l,m} &= \left\{ \frac{2Q_l}{|\lambda_i| \cdot w\, t_0[1 + \kappa_i(0)]} \right\}^{\frac{1}{2}} \\
&= \left\{ \frac{2Q_l}{|\lambda_i| \cdot V_c[1 + \kappa_i(0)]} \right\}^{\frac{1}{2}}
\end{aligned} \tag{8}$$

where V_c is the column mobile phase volume.

As the choice of $\bar{c}_{l,m}$ is determined by the acceptable relative peak broadening, we can arrive at an expression for Q_l which has to be injected in order to make dispersion and isotherm non-linearity broadening identical:

$$\frac{Q_l}{V_c[1 + \kappa_i(0)]} = \frac{8}{N|\lambda_i|} \tag{9}$$

The expression on the left-hand side is, for a particular solute and phase system and also apart from a constant, equal to the specific load (grams of solute per gram of packing material). As can be seen, the value of the specific load which brings about a certain percentage of extra peak broadening depends not only on the phase system parameter λ_i, but also on the plate number. Therefore, studies relating the relative plate height increase to the specific load do not find support in these considerations; at least, the results are only valid for the particular column used and not for the phase system in general.

Rearranging the equations, we can arrive at another relationship, that also describes the situation where dispersion and isotherm non-linearity effects cause the identical peak broadening:

$$\frac{Q_l}{A_m H[1 + \kappa_i(0)]} = \frac{8}{|\lambda_i|} \tag{10}$$

Here, the expression on the left-hand side is equivalent to the load per plate volume, or, if the plate height is independent of the column cross-section and the particle size

is constant, equivalent to the load per cm^2 cross-section. Studies carried out in this way would be independent of the particular length of the column used.

It is probable that the results given above also have an approximate validity when only a slight decrease of apparent efficiency is caused by an isotherm non-linearity effect. Buys and De Clerk[3], in deriving the influence of isotherm non-linearity in a first-order perturbation treatment, while taking dispersion into account and starting from a Gaussian profile, arrived at the conclusion that at $H = 0.14 \cdot |\lambda_i| \cdot Q_t/A_m$ $[1 + \kappa_t(0)]$, peak broadening due to dispersion is as large as that due to non-linearity of the isotherm. The numerical factor was estimated on the basis of computer simulation studies. The value of 0.14 is not too far from our factor of 0.125 obtained by crudely equating the total peak width to four standard deviations, while treating the dispersionless case.

Finally, it is interesting to compare the influence of isotherm non-linearity and dispersion on the peak width for different column lengths. For a particular Q_t we find a relative broadening of

$$\left\{ \frac{2Q_t \, |\lambda_t|}{V_c[1 + \kappa_t(0)]} \right\}^{\pm} = \left\{ \frac{2Q_t \, |\lambda_t|}{A_m L[1 + \kappa_t(0)]} \right\}^{\pm}$$

due to the isotherm non-linearity. The dispersion effect causes a relative broadening (4σ) of $4/\sqrt{N} = 4(H/L)^{\pm}$. It follows that the length of the column has no influence on the relative importance of these effects, at least in this first-order treatment, as both relative peak widths decrease with the square root of the length. The same holds, of course, for the various positions of a peak during its migration through the column.

The treatment above is a rather coarse simplification and optimization of preparative liquid chromatography will invariably require a lot of trial-and-error procedures with the particular solute mixture, phase system and packing material to be used owing to the complexity of the problem. However, the treatment shows the limited value of overload (H increase) studies with one column and stresses the importance of the study of isotherms. In particular, the comparison of different phase systems and different packing materials in this respect will have a more general significance than that for only the particular compound and column with which the measurements are carried out.

Knowledge of the shapes of distribution isotherms of solutes opens the possibility of adjusting the amount of the mixture injected in such a way that an optimal compromise such as is indicated in Table I is reached to obtain maximal throughput and purity of the separated components. Therefore, a method for the determination of the shape of the distribution isotherm is of great value.

In this paper we report the results of an investigation on the applicability to liquid chromatography of known methods for distribution isotherm measurements as described for gas chromatography by Huber and Gerritse[4]. The selected method was applied to the determination of the distribution isotherms of phenols on different types of normal-phase and alkyl-modified silicas.

EXPERIMENTAL

Apparatus

The equipment for the different methods was built from the following commer-

cial parts: a single- or double-headed reciprocating pump (Orlita Type DMP 1515 or AE 10-4.4; Orlita, Giessen, G.F.R.); a Bourdon-type flow-through manometer, in some experiments in series with a 6 m × 0.15 mm I.D. stainless-steel capillary tube as resistance, acting as a pulse damper; a variable-wavelength detector (Pye Unicam LC-UV; Pye Unicam, Cambridge, Great Britain); a high-pressure injection valve (Valco CV-6-UHPa-C20; Valco, Houston, TX, U.S.A.), fitted with either a 50-μl loop or a loop system with an appropriate larger volume (up to 150 ml), was used as such or as a switching valve; a potentiometric recorder with integrating unit (Goerz RE 542; Goerz, Vienna, Austria); a calibrated burette was used to measure the flow-rates. Stainless-steel columns of dimensions 250 × 4.6, 250 × 10, 250 × 16 and 150 × 4.6 mm I.D. were used.

Materials

All organic solvents were of analytical-reagent grade and were used as delivered. The water used was de-ionized and distilled. The column packings used were the porous silicas Si 40, Si 60 and Si 100 (Merck), ground and classified by means of an air-classifier (Alpine M.Z.R., Augsburg, G.F.R.) to appropriate particle sizes, Zorbax-Sil (DuPont, Wilmington, DE, U.S.A.) and Partisil-5 (Reeve Angel, Clifton, NJ, U.S.A.) and the alkyl-modified silicas LiChrosorb RP-2, RP-8 and RP-18 (Merck), Zorbax-ODS (DuPont), Hypersil ODS (Shandon, Runcorn, Great Britain) and Nucleosil C8 and C18 (Macherey, Nagel & Co., Düren, G.F.R.).

Column preparation

The normal-phase silica columns were filled by means of a balanced-density slurry technique using mixtures of tetrabromoethane and chloroform as the slurry liquid. 2,2,4-Trimethylpentane was used to press the slurry into the column at 400 bar. The columns were washed with dichloromethane (50–100 column volumes) until constant retention was achieved. The void volume (V_c) of each column was determined with an unretained solute (ethylbenzene).

The reversed-phase silica columns were filled by means of a slurry method using tetrachloromethane as the slurry liquid. The slurry was pumped into the column with methanol at 400 bar. The void volumes (V_c) of the reversed-phase columns were determined with 1,3-benzenedisulphonic acid as an unretained solute.

Distribution isotherm measurements

The equipment for the recycling method was the same as that described by Kraak[5] with the addition of a UV detector to monitor the equilibration of the system. The single-headed pump was used to recycle 20 ml of a solution of known concentration of the solute through a 10 mm I.D. column filled with Si 60 (mean particle size 25 μm). The amount of solute adsorbed on the surface was determined from the hold-up volume of the equipment, the initial solute concentration and the concentration of the solute in the eluent after equilibration. For the determination of the latter concentration, use was made of high-performance liquid chromatography (HPLC) with peak-area quantitation, using the 250 × 16 mm I.D. column filled with 6-μm Si 60 and flow surrounding injection[2].

In the batch method, 5 ml of a solution of the solute of known concentration was added to 0.5 g of 25-μm Si 60 in a PVC-stoppered vessel. The solution was well

stirred by means of a glass-coated magnetic stirrer (PTFE-coated stirrers were found to adsorb the solutes). The amount of solute adsorbed was determined from the initial solute concentration and the concentration in the supernatant after equilibration. The determination of the latter concentration was carried out by HPLC and peak-area quantitation as described above.

In the peak maxima method, 50-μl samples of solutions of the solute of known concentration were injected on to a 10 mm I.D. column filled with 25-μm Si 60. The $c_{i,m}^{max}$ values of the eluted peaks were calculated from the deflections observed on the recorder after suitable calibration. Integration of the $dc_{i,s}/dc_{i,m}$ versus $c_{i,m}$ curve to obtain the distribution isotherm was carried out with a pocket calculator using trapezoidal numerical integration.

In the minor disturbance method, the eluents consisted of the solvent with known concentrations of the solute. After equilibration a small solute sample was injected, which caused a minor disturbance in the recorder signal. From the retention time observed at the different solute concentration levels in the eluent the value of $dc_{i,s}/dc_{i,m}$ could be calculated[4]. From these values the distribution isotherm was obtained by means of trapezoidal numerical integration using a pocket calculator.

In the break-through method, the column was equilibrated with solvent. Then a sufficient volume of a solution of known solute concentration was pumped into the column via a loop system (between 30 and 80 ml, depending on the inner diameter of the column) to produce a stepwise change in the recorder signal. From the deflection of the recorder and from the void volume of the column the amount of solute adsorbed could be calculated as described by Huber and Gerritse[4]. Fig. 1 shows schematically the breakthrough arrangement. The double-headed pump delivered the pure solvent, while the single-headed pump was used to fill the loop system with the various
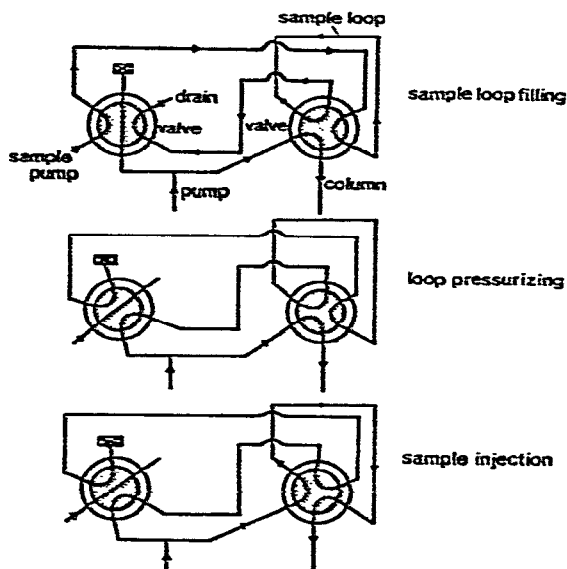

Fig. 1. Schematic representation of the configuration and operation of the loop system and switching valves in the break-through method.

solutions of known solute concentration. Before switching in line, the loop system had to be pre-pressurized in order to avoid a disturbance of the flow-rate after switching the injection valve, due to the compressibility of the solvent in the loop.

In the desorption method, the same arrangement as for the break-through method could be used. After each break-through measurement a desorption curve could be measured by switching back to the pure solvent.

The distribution isotherm was evaluated from the desorption curve[4] either by using the integration unit of the recorder or by digitizing (with a home-made system) the detector output and subsequent use of a computer.

RESULTS AND DISCUSSION

Comparison of methods for distribution isotherm measurement

Six known methods[4] for the measurement of distribution isotherms were compared for an adsorption system consisting of porous silica (Si 60, $d_p = 25 \mu m$) as adsorbent, dichloromethane as eluent and phenol as solute. The results of the distribution isotherm measurement by the different methods are given in Fig. 2. Three points can be made: (1) for the direct measurement of the distribution isotherm (*i.e.*, break-through, recycling and batch method) the different points coincide fairly well; (2) for the indirect methods, where the derivative of the distribution isotherm is measured (*i.e.*, minor disturbance, peak maxima) and for the desorption method also a good mutual agreement is found; and (3) the indirect methods give systematically higher $c_{i,s}$ values compared with the direct methods, particularly at very high $c_{i,m}$ values. The
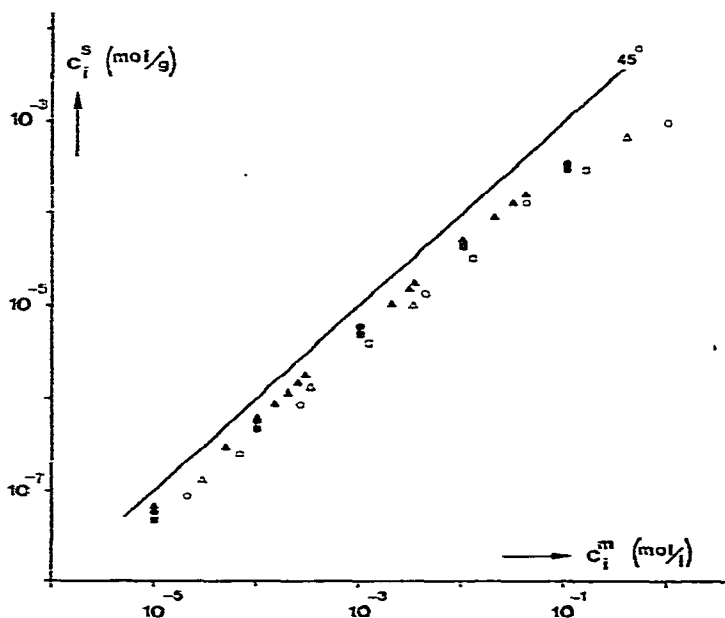

Fig. 2. Distribution isotherm of phenol in the phase system consisting of 25-μm Si 60 and dichloromethane, determined with the different methods: O, break-through; □, recycling; △, batch; ●, minor disturbance; ■, peak maxima; ▲, desorption.

significant difference between the direct and indirect methods might be attributed to a number of reasons, such as systematically inaccurate measurement of the derivative of the distribution isotherm, dispersion or the effect of baseline noise on peak-area measurement in the indirect methods. Apart from these differences in the absolute value of the distribution isotherm, which needs a more detailed investigation, all methods show very well the shape of the distribution isotherm, which is of value for the selection of the optimal chromatographic conditions in analytical and preparative liquid chromatography. When comparing the methods on the basis of aspects related to speed, precision, effect of dispersion and instrumentation, the following points, summarized in Table II, can be made. The detector quality, especially its linearity, is crucial in those methods where the signal intensity is used directly for the calculation of $c_{i,s}$ or $c_{i,m}$, i.e., in the batch, the recycle, the desorption and the peak maxima method. Therefore, the minor disturbance method has an advantage and to a large extent this also holds for the break-through method, because detector distortion of the sharp step generally affects its mean position to only an insignificant extent. It should be noted that in the case of convex isotherms a sharp break-through profile can be obtained by switching from a high to a lower concentration[6,7].

Dispersion in columns obviously does not affect the measurements with the batch and recycling methods, where a final equilibrium state is reached. In the desorption and peak maxima methods the influence of dispersion on the accuracy of the results can be significant and unpredictable. In the minor disturbance method the dispersion will only affect the precision with which the position of the disturbance can be determined. Also for the break-through method a small influence of dispersion can be expected: for measurements within the linear part of the isotherm a symmetrical step function will be obtained and in the non-linear part the step will be self-sharpening in the case of a concave isotherm shape.

The speed of the desorption method, where a complete isotherm is obtained in one run, is obviously superior, provided that automatic data handling is available. However, the influence of dispersion in this method cannot be corrected for or estimated. Moreover, with present detectors for liquid chromatography the detection linearity is insufficient for covering several orders of magnitude in concentration in one run. A series of experiments, each covering a certain concentration range is then necessary and the speed advantage is lost to a great extent. In two methods, viz., the batch method and the recycling method, a lower phase ratio exists in comparison with a column filled with the material at hand. Therefore, the precision of the results for compounds with a capacity factor of 1 or lower (in a column) will be less. Finally, a drawback of the batch method should be mentioned. When using mixed solvents as the "mobile" phase, selective uptake of one of the solvents (the moderator) by the stationary phase changes its composition. This results in systematically higher values for the distribution coefficients of the solutes.

Based on these experiments and the considerations above, it was decided that the break-through method was the most convenient and accurate way of determining distribution isotherms and was used for the comparison of different solid supports.

Comparison of phase systems

Normal-phase adsorption. Fig. 3 shows the distribution isotherms of phenol on some commercially available porous silicas with comparable surface areas. It can be

TABLE II

COMPARISON OF METHODS FOR DISTRIBUTION ISOTHERM MEASUREMENT USED IN THIS WORK

Advantage, +; disadvantage, −.

Method	Independence of detector quality	Influence of dispersion	Single point vs. whole curve	Phase ratio	Other aspects
Recycling	−	+	−	−	Time consuming due to slow equilibration
Batch	−	+	−	−	Abrasion of stationary phase possible; formation of emulsions possible
Peak maxima	−	−	−	+	Integration necessary
Minor disturbance	+	+	−	+	Integration necessary
Break-through	+	+	−	+	Can be speeded up by cumulative measurements
Desorption	−	−	(+)	+	Calculations without a computer are time consuming
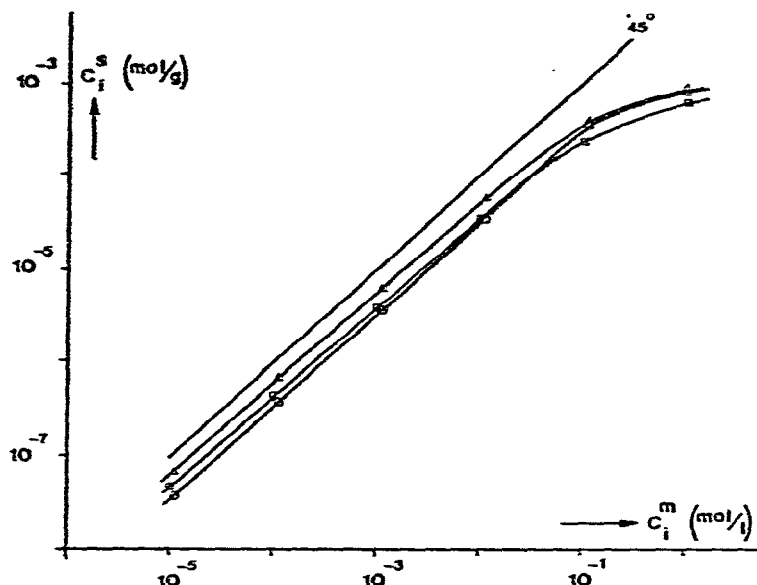
Fig. 3. Distribution isotherms of phenol in dichloromethane–silica systems. Silicas used: $\triangle$, Partisil ($\lambda = -6$ l/mol); $\square$, Si 100 ($\lambda = -7$ l/mol); $\bigcirc$, Zorbax ($\lambda = -1.5$ l/mol). Values for λ give an approximate first-order description of the isotherm non-linearity and were found by least-squares treatment of the data.

seen that significant differences in the linear range can occur, although the maximum amount that can be adsorbed seems to be about the same for all of the materials investigated. Under our experimental conditions and from the viewpoint of isotherm linearity, Zorbax-Sil appears to be superior.

Fig. 4 shows the isotherms obtained for different particle sizes of the same material (Si 60). If in the grinding and classifying process adsorbent characteristics such as pore volume, pore diameter, surface area and activity would be unaffected, identical isotherms would be expected. Apparently some changes in these respects occur, as Fig. 4 shows. However, the changes are not dramatic and can be neglected in a first attempt to optimize preparative liquid chromatography.

The effect of the surface area (pore diameter) is shown in Fig. 5. As expected, the linear range is larger for a larger surface area. The data in Figs. 4 and 5 were obtained with a lower water content in the mobile phase than for the data in Fig. 3. Careful comparison of the shape of the Si 100 graph in these figures shows the increased linearity with increased water deactivation, as emphasized by Snyder[1].

Reversed phase adsorption. Fig. 6a shows the distribution isotherms of phenol on alkyl-modified silicas with different alkyl chain lengths. The linearity decreases with increasing chain length, which is an unexpected result if one describes these distributions as partitioning processes. Probably the difference in surface area plays a role in this effect. Comparison of Fig. 6a and b does not reveal a dependence of this effect on the mobile phase composition. The conclusion that the silica with a shorter chain length (RP-2) is superior should be treated with caution. In order to obtain sufficiently high capacity ratios the organic modifier content should be lower with RP-2; this might yield solute solubility problems in some instances.
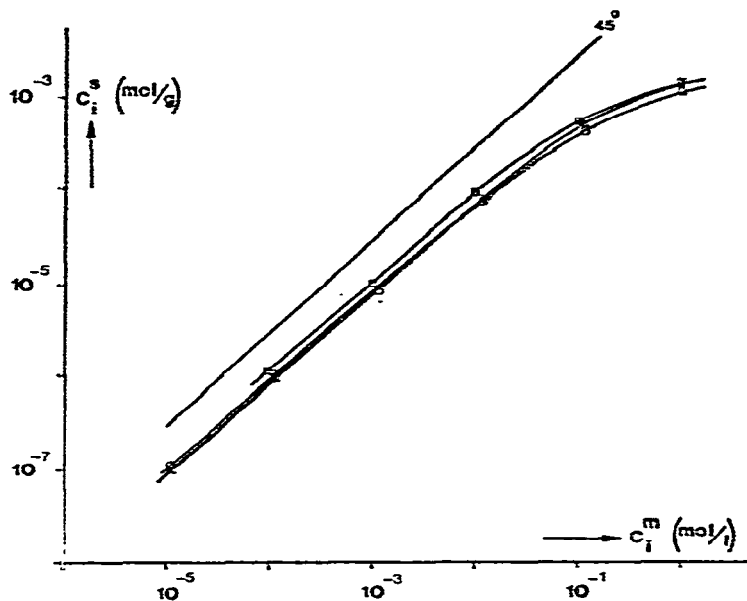
Fig. 4. Distribution isotherms of phenol in phase systems consisting of Si 60 and dichloromethane with different particle size: □, 50; ○, 25; △, 10 μm.
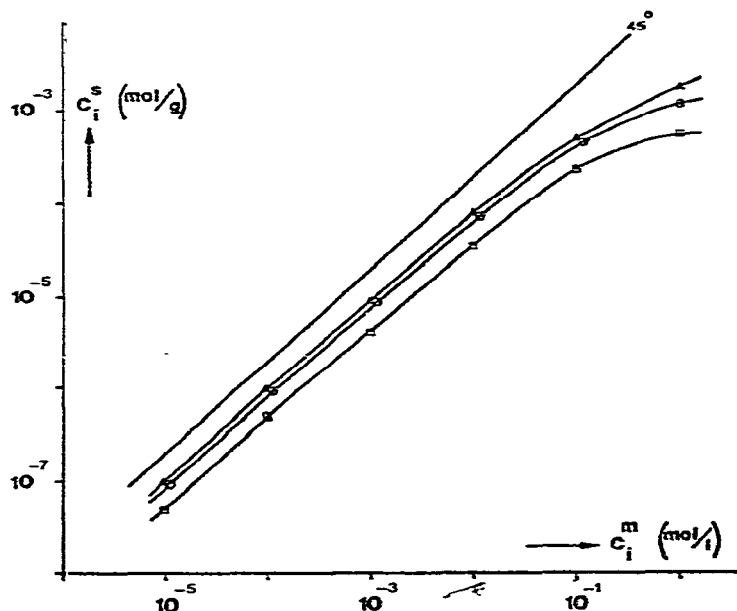

Fig. 5. Distribution isotherms of phenol in phase systems consisting of silica and dichloromethane with silicas of different surface area: △, Si 40, 650 m^2/g; ○, Si 60, 500 m^2/g; □, Si 100, 400 m^2/g. For all silicas $d_p = 25$ μm.
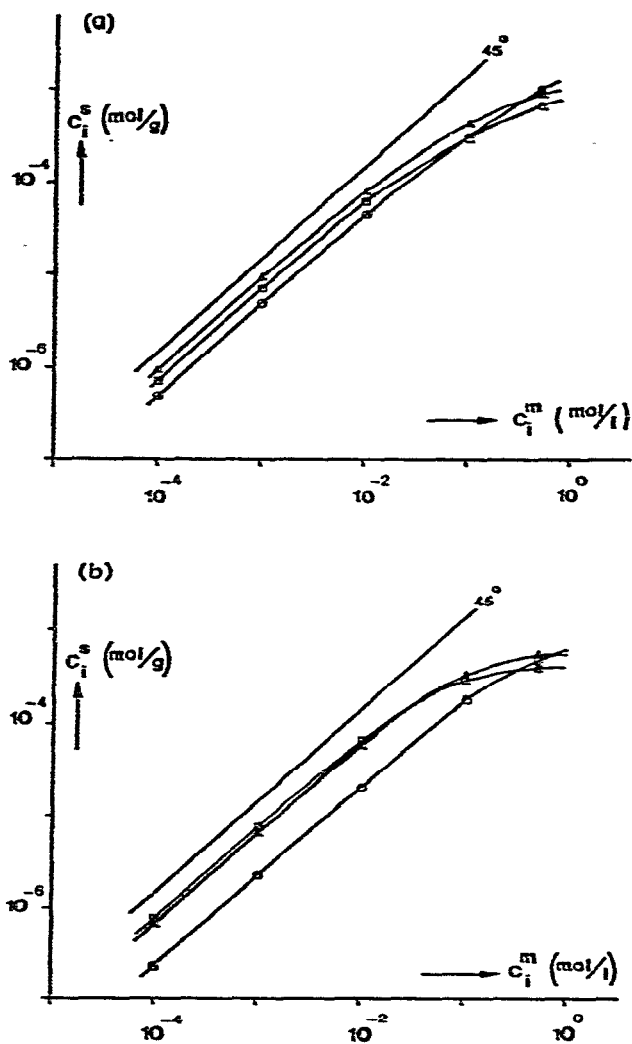
Fig. 6. Distribution isotherms on alkyl-modified silicas ($d_p = 10 \mu m$) with different alkyl chain lengths: (a) solute, phenol; mobile phase, methanol–water (25:75); (b) solute, 4-*tert.*-octylphenol; mobile phase, methanol–water (75:25). Packings used: △, RP-8 ($\lambda = -9$ l/mol for phenol, -8 l/mol for 4-*tert.*-octylphenol); □, RP-18 ($\lambda = -10$ l/mol for phenol, -11 l/mol for 4-*tert.*-octylphenol); ○, RP-2 ($\lambda = -6$ l/mol for phenol, -3 l/mol for 4-*tert.*-octylphenol. Values for λ give an approximate first-order description of the isotherm non-linearity and were found by least-squares treatment of the data.

Fig. 7 shows the distribution isotherms of phenol on commercially available octadecyl-modified silicas. Also here differences between materials from various sources can be observed.

Finally, Fig. 8 shows that RP-2 has a superior linearity to the most linear octyl- and octadecyl-modified materials (Nucleosil C8 and Zorbax ODS).
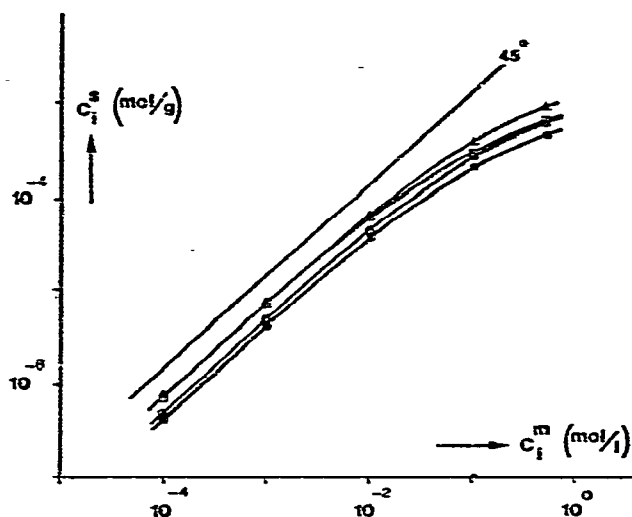
Fig. 7. Distribution isotherms of phenol in phase systems consisting of C18-modified silica and methanol–water (25:75) as the mobile phase with commercially available stationary phases: △, Nucleosil C18; □, LiChrosorb RP-18; ○, Zorbax-ODS; ●, Hypersil ODS.
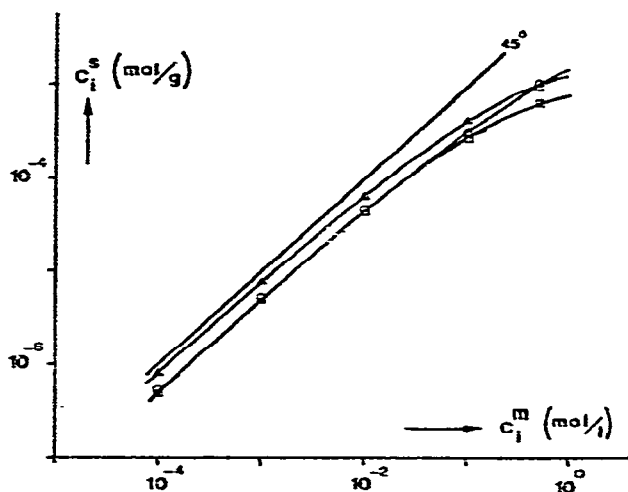

Fig. 8. Comparison of distribution isotherms of phenol with different packings ($d_p = 10 \mu m$) and methanol–water (25:75) as the mobile phase. Packings: ○, LiChrosorb RP-2 ($\lambda = -6$ l/mol); △, Nucleosil C8 ($\lambda = -7$ l/mol); □, Zorbax-ODS ($\lambda = -7$ l/mol). Values for λ give an approximate first-order description of the isotherm non-linearity and were found by least-squares treatment of the data.

ACKNOWLEDGEMENTS

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