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MEASUREMENT OF ADSORPTION ISOTHERMS BY LIQUID CHROMATOGRAPHY

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SUMMARY

Adsorption behavior on silica-bound hydrocarbonaceous sorbents used in high-performance liquid chromatography was investigated by frontal chromatography and elution on a plateau, and procedures were developed for data collection, analysis and correlation. Isotherms of selected compounds were measured, including several of biological relevance. The relative merits of various dynamic methods of isotherm measurement were compared and illustrated by experimental findings. The frontal analysis technique was determined to be more accurate and convenient than other methods. A miniaturized liquid chromatographic system was constructed to measure isotherms with milligram quantities of material by frontal development. The performance of this apparatus compared favorably with that of equipment which has dimensions usual in high-performance liquid chromatography and requires orders of magnitude greater amounts of a substance for isotherm measurement. Thus the miniaturized system is eminently suited for applications when the substance of interest is in short supply, as is the case with many biological substances. The effects of operating parameters on adsorption behavior were investigated, both to optimize the procedure, and to further explore the fundamental of the process.

INTRODUCTION

Adsorption isotherms have attracted greater attention in recent years as fundamental tools for investigation of the physical process involved in chromatographic retention. The key role of the isotherm in analysis and design of preparative- or production-scale chromatography has also contributed to the increasing popularity of this topic. The significance of the adsorption isotherm in these roles is that it quantitatively describes the equilibrium distribution of a solute between the two phases involved in the chromatographic process over a wide concentration range. Thus, a comprehensive examination of adsorption isotherms yields information about the solvent, solute and adsorbent as well as the interactions among these species occurring during adsorption. In many instances measurement of adsorption isotherms is the only way of probing these interactions or to obtain the requisite data for process design of a chromatographic purification process. Therefore a need exists for a technique of isotherm measurement that offers speed, accuracy and convenience. As these

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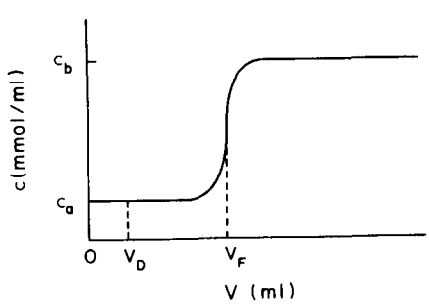
characteristics have made high-performance liquid chromatography (HPLC) a preeminent analytical and preparative separation technique, it follows that HPLC columns and equipment can also be harnessed for the routine gathering of adsorption isotherm data.

Isotherms have been measured traditionally by the static method, in which the change in concentration of a solute upon addition of the adsorbent to the solution is measured and discrete points on the isotherm are calculated from this measurement. The primary drawbacks to this technique are the slowness and uncertainty in reaching equilibrium and the large amounts of solute and adsorbent required for accurate measurements. Non-polar sorbents used in reversed-phase chromatography suffer from the additional difficulty that they are poorly wetted by water-rich solvents and this further exacerbates experimental problems.

Chromatographic methods based on packed columns have been developed in order to circumvent these problems. The two principal chromatographic methods involve changing the concentration of the solute at the column inlet in a stepwise fashion, either up or down. When the concentration is increased, the step develops into a sharp front at the column outlet, as shown in Fig. 1, while when it is decreased, it becomes a tailing boundary at the outlet, shown in Fig. 2. The different shapes of the front and rear boundaries are manifestations of the self-sharpening nature of the former and the diffuse behavior of the latter. As was pointed out by DeVault[1], concave isotherms always exhibit this behavior, and for ideal chromatography without bandspreading the front boundary maintains a square shape as it progresses through the column. In a real system, the front assumes a sigmoidal shape, as shown in Fig. 1, when a quasi-steady state is established between this self-sharpening effect and axial dispersion, a process that tends to broaden the front[2]. The effect of axial dispersion due to mass transfer resistances and maldistribution of flow is less apparent in the rear boundary but also affects the shape of that concentration profile.



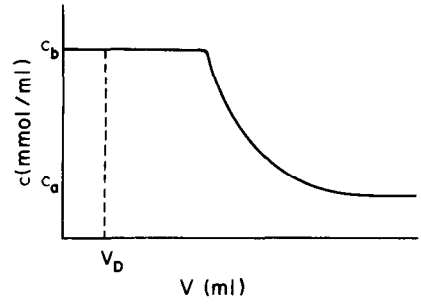


Fig. 1. Self-sharpening boundary in frontal analysis. For symbols, see text.

Fig. 2. Illustration of the diffuse rear boundary used in "frontal analysis by characteristic point" (FACP) measurements.

The method that employs the front boundary, as in Fig. 1, is termed frontal analysis (FA) and by application of a mass balance provides the surface concentration, $q(c)$, in equilibrium with the solute concentration, c. Thus one experiment, as in Fig. 1, yields one point on the isotherm. The second method, frontal analysis by characteristic point (FACP), calculates the entire isotherm from the concentration

profile of the rear boundary shown in Fig. 2. Both calculation procedures are discussed more fully below. A method related to FACP employs the rear boundary of an overloaded peak in lieu of the Fig. 2 profile, and is called elution by characteristic point (ECP). In other respects it is similar to FACP. Other techniques have been introduced that have a narrower scope of application owing to operational difficulties. Thus, FA, FACP and ECP are the natural candidates for a study such as this of the optimum measurement technique. In the experiments carried out in this work these methods were examined in detail. The first goal of this study was to determine the optimum method for isotherm measurement on the basis of precision, accuracy, speed, minimal solute consumption and coverage of the broadest concentration range possible. On the basis of accuracy, precision and speed, De Jong *et al.*[3], utilizing reversed-phase HPLC, found FA to be the best method. Conder and Purnell[4] and Huber and Gerritse[5], however, measured isotherms by using gas chromatography and preferred FACP and ECP as fast efficient methods, provided non-ideal and kinetic effects were minimized.

THEORY

Models of adsorption behavior

The continuing importance of adsorption and chromatographic processes in various areas of science and technology has made them a common focus of study. Numerous theories have been advanced to describe adsorption, based on assumptions about the adsorbate, the physical process of adsorption and/or the shape of the isotherm. A few of the more prominent theories are discussed below.

As noted by Kipling[6], the thermodynamic treatment of adsorption effectively began with the Gibbs equation, which provided a convenient definition of the interfacial region. Gibbs[7] circumvented previous ambiguities by defining an imaginary mathematical surface to which he attributed all the properties of the interfacial region, making it a two-dimensional phase with its own thermodynamic properties. This construct is still the only rigorous method for treating adsorption processes[8,9].

Langmuir[10] proposed a now-classic adsorption model in 1916 for adsorption in a gas–solid system. He assumed a constant heat of adsorption and finite number of surface adsorption sites. With these assumptions maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface.

Experimental data in this study have been correlated to the Langmuir equation for liquid–solid adsorption:

$$q = ac/(1 + bc) \tag{1}$$

where q is the amount of adsorbate on one unit of adsorbent, c is the solute concentration, and b is an empirical constant related to the energy of adsorption. Isotherms in reversed-phase liquid chromatography have been found to follow this general behavior up to the point of saturation of the stationary phase. At higher concentrations, however, this model is no longer valid[9]. Parameter a is the isotherm slope at low solute concentration and within this range

$$a = K \tag{2}$$

where K is the equilibrium constant for the sorption process in the domain of Henry's law and is related to the retention factor, k', by

$$k' = K\varphi \tag{3}$$

where φ represents the phase ratio.

The Langmuir model has been widely used to correlate data obtained in liquid–solid adsorption experiments. However, the model has been criticized because of its restriction to homogeneous surfaces and monolayer coverage[11,12]. Nonetheless, the Langmuir isotherm is convenient for quantitative analysis of adsorption processes[13] and has a physical basis, unlike such empirical equations as that of the Freundlich model[14], which have no significant theoretical underpinnings. For the analysis performed here the Langmuir model offers the greatest simplicity, convenience, and adequate correlation with available data. It should be noted, however, that this use of the Langmuir equations neither assumes a physical model nor assigns physical meaning to the parameters.

Methods of isotherm measurement

Glueckauf[15] was the first to use chromatography to measure isotherms as pointed out by Conder and Young[16], who gave a comprehensive review of methods for gas chromatographic measurement of isotherms. These methods are readily extended to HPLC. The most straightforward chromatographic technique is FA, introduced by James and Phillips[17] and Schay and Székely[18]. It relates the velocity of the concentration front formed by a step increase in concentration of a solute to the value of the solute's adsorption isotherm at the elevated concentration. Fig. 1 depicts the effluent profile after changing the concentration from c_a to c_b, and an integral mass balance shows that the stationary phase concentration of the solute in equilibrium with c_b is given by

$$q(c_b) = q(c_a) + [(c_b - c_a)(V_F - V_D)]/V_{SP} \tag{4}$$

where $q(c_a)$ is the concentration of adsorbed solute in equilibrium with c_a, V_F is the retention volume of the front, V_D is the system dead-volume, including column hold-up volume, and V_{SP} is the volume of adsorbent in the column. Repeating this operation with successively higher concentrated solutions yields additional discrete points on the isotherm.

The diffuse rear boundary is employed by the FACP method, introduced by Glueckauf[15], and permits construction of the entire isotherm from one experiment, in which the concentration of the solution pumped into the column is changed only once. FACP, in contrast to FA, reconstructs the isotherm from measurements on the diffuse rear boundary that arises from a step decrease in concentration. Such a rear boundary is shown in Fig. 2 as a function of the concentration profile recorded at the column outlet, $c(V)$, where V is the volume of effluent. A differential mass balance yields

$$q(c) = 1/V_{SP} \int_{c_a}^{c} (V - V_D)\, dc \tag{5}$$

as the governing equation for determination of the isotherm by FACP. Since the detector signal must be converted into concentration units in order to evaluate eqn. 5, calibration of the detector is necessary. Another potential drawback of FACP is that no correction is made to account for bandspreading, which will broaden and diffuse the boundary depicted in Fig. 2. The FA method, since it depends on accurate measurement of only the retention time of a self-sharpening front, is less prone to error from this source.

FA and FACP are the principal methods for dynamic measurement of adsorption isotherms although other techniques have gained limited currency. Several methods similar to FACP have appeared that are based on the use of eqn. 5 but determine $c(V)$ in a different manner, including ECP from Cremer and Huber[19] and elution on a plateau (EP), introduced by Reilley *et al.*[20]. ECP is identical to FACP, except a limited amount of solute is injected such that an elution peak results, rather than a decrease in concentration from a plateau. Analysis, using eqn. 5, is then carried out on the rear boundary of the peak. The advantage of this technique is the small amount of sample required, but it has the same disadvantages as FACP. In EP the column is equilibrated with a solution at a given concentration, and the retention volume of a perturbation of that concentration is measured to yield $V(c)$, which can be treated in a manner analogous to $c(V)$. Thus, no calibration of the detector is necessary, and the inaccuracy associated with bandspreading for FACP is largely reduced. However, the detection problems with common HPLC equipment at high concentrations can be significant, the size of the perturbation can influence the accuracy of the result, solute consumption is substantial and the experimental complexity is greater than that for the FA technique, all of which argue against EP. One variant of EP employs an isotopically-labelled form of the solute to bring about the perturbation, which simplifies the analysis considerably and eliminates the inaccuracy associated with the magnitude of the concentration perturbation. Still, the problems of procuring an isotopically labelled variant of the solute of interest and of detecting it in the presence of non-labelled forms seriously limit the applicability of this technique.

EXPERIMENTAL

Materials

Stationary phases. Isotherm measurements were carried out on 5-μm Spherisorb (Phase Separations, Hauppage, NY, U.S.A.) octadecyl-silica particles. The adsorbent was prepared by mixing 5-μm Spherisorb silica gel with octadecyl-dimethylchlorosilane (Petrarch Systems, Levittown, PA, U.S.A.) in refluxing toluene (Fisher Scientific, Fair Lawn, NJ, U.S.A.) for at least 96 h. Thereafter, the material was treated with trimethylchlorosilane (Petrarch Systems) and, subsequently, columns were slurry-packed[21] at 10,000 p.s.i. The columns were constructed of stainless-steel tubing with internal dimensions of 59.3 × 4.6, 150 × 1.18, 50 × 1.18 and 40.5 × 1.18 mm. Analysis of the material by Barron Consulting Co. (Orange, CT, U.S.A.) revealed a carbon content of 7.87% (w/w).

Chemicals and solvents. *p*-Cresol was purchased from Aldrich (Milwaukee, WI, U.S.A.) and resorcinol, hydroquinone and benzoic acid from J. T. Baker (Phillipsburg, NJ, U.S.A.). *m*-Cresol, *p*-toluidine, *o*-toluidine, *p*-aminobenzoic acid, *m*-nitro-

benzoic acid, *o*-aminophenol and 2-amino-4-nitrophenol were obtained from Chem Service (West Chester, PA, U.S.A.). Fisher Scientific supplied the phenol and catechol as well as the monobasic and dibasic sodium phosphate. The amino acids were purchased from Sigma (St. Louis, MO, U.S.A.). A Barnstead distilling unit produced the distilled water.

Equipment

Apparatus for isotherm measurement. Fig. 3 schematically depicts the apparatus used for isotherm measurement. A Model 100A solvent metering pump (Altex, Berkeley, CA, U.S.A.) was connected to two Model 7010 (Rheodyne, Berkeley, CA, U.S.A.) sampling valves. Tubing to the valves was connected to permit switching from loop A to loop B, back to loop A and so on without a significant system dead volume. The loop volumes were adjusted from 1 to 10 ml by substitution. The valves were connected to the 59.3 × 4.6 mm column. The column effluent is fed to a Model LC-55 (Perkin-Elmer, Norwalk, CT, U.S.A.) variable-wavelength spectrophotometer set at a suitable wavelength and the detector signal was recorded by a Model 123 (Perkin-Elmer) dual-pen strip-chart recorder. The column and sample loops were thermostatted to the appropriate temperature in a Model K-2/R Messgeraetewerke (Lauda, F.R.G.) circulating water bath.

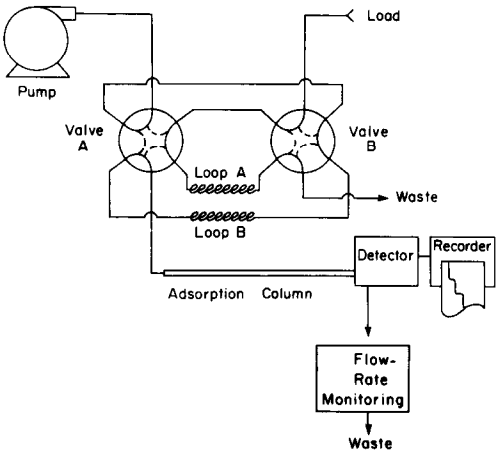


Fig. 3. Diagram of the chromatograph used for isotherm measurement.

Miniaturized apparatus for frontal analysis. Fig. 3 represents a schematic diagram of this system, although many parts of the instrument were different from those employed in the regular system. Initially, a Model A-30-S (Eldex Labs., Menlo park, CA, U.S.A.) pump was used for low flow-rate deliveries. Later measurements were carried out with a Model 302 (Gilson, Villiers Le Bel, France) pump. Feed loop volumes from 100 μl to 1.0 ml were used. Each of the three narrow-bore columns, 150 × 1.18, 50 × 1.18 and 40.5 × 1.18 mm, was fitted at the outlet side with a Valco (Houston, TX, U.S.A.) inverted nut which connected directly to the 0.5-μl flow cell of the Model 770 (Kratos, Ramsey, NJ, U.S.A.) variable-wavelength spectrophotometer. Model 7001 Rheodyne pneumatic actuators connected to the Model 7010 Rheo-

dyne injectors and a Model 7163 Rheodyne solenoid valve were used to switch from one loop to another. In addition, the flow-rate was measured periodically at the detector outlet by using a buret and an electronic timer.

Procedures

Measurement of column hold-up volume. The gravimetric method[8], was used to determine the hold-up volumes of the columns, V_0, for plain water or sodium phosphate buffer as the mobile phase. This is an accurate technique for such eluents[9]. Methanol and carbon tetrachloride were the liquids used for this determination. Adsorbed solute molecules did not significantly change the hold-up volume measurement, since the maximum solute weight in all cases was less than 3% of the total solution weight. Hold-up volumes for the two experiments with methanol–water, as measured by sodium nitrate in methanol–phosphate buffer, were within 2% of the hold-up volume for a neat aqueous mobile phase.

Column hold-up volumes were used to determine the total column porosity, ε, as follows:

$$\varepsilon = V_0/V_{ET} \tag{6}$$

where V_{ET} is the internal volume of the empty column, as determined by the gravimetric method. The dead-volume in the tubing external to the column was also determined. The sum of this dead-volume plus the column hold-up volume is denoted by V_D.

Isotherm measurement. ECP measurement was carried out by loading loop A in Fig. 3 with a 1.0 M solution of catechol. The pump delivered pure water through loop B to the column. At time $t = 0$, both valves A and B were rotated. The alternate flow path within the injectors is indicated by the dashed lines in Fig. 3. Loop B was filled with mobile phase, and valves A and B were again rotated to terminate injection. The detector was calibrated by direct injection of solutions into the detector flow cell.

FACP experiments were performed with the same loop-switching technique as ECP. Valves A and B were rotated after passage of the concentration step through the detector.

Measurements by frontal analysis were conducted by stepwise increasing the concentration of solute pumped into the column. Mobile phase was passed through loop B to the column and loop A was loaded with the lowest solute concentration solution. Valves A and B were rotated at time $t = 0$. Once the solute plateau was recorded, loop B was loaded with the second lowest concentration solution and the valves were rotated again. This process was repeated until the highest concentration plateau had been recorded at which time the unused loop was filled with mobile phase and the valves were rotated. This technique produced FA plateaus and also allowed FACP measurement on the rear boundary.

The data were fitted to the Langmuir isotherm equation by the non-linear regression routine NLIN in the SAS statistics package[22]. Plotting of the isotherms was carried out with the Tellagraph[23] system.

RESULTS AND DISCUSSION

All isotherms measured were Type I, according to the classification of Brunauer *et al.*[24], as has generally been found with the stationary phases used in HPLC. This isotherm, the slope of which decreases as concentration increases, has the shape expected of a Langmuir isotherm, with an initial linear region of Henry's law adsorption behavior at low concentration and, at high concentration, a plateau region in which the amount of solute adsorbed on the stationary phase remains constant.

Isotherm data were plotted as single-component isotherms, with the assumption that the aqueous mobile phase was unretained. This, rather than composite isotherms, is appropriate, since the solvent is only slightly retained and the solute has a relatively low concentration compared to the solvent. The stationary phase concentrations are reported as mmole per ml of adsorbent, a conveniently measured basis. The use of mass units in place of moles did not improve the correlations described below.

Fig. 4 shows the experimental data obtained for ten compounds together with the curves obtained by correlation of the data with eqn. 1. In all cases the data correlate well with the Langmuir equation. The data do not cover identical ranges of concentration due to limited solubilities. Therefore, extrapolation to high concentration must be interpreted with care. However, the concentration ranges employed for correlation are similar so that comparison among compounds can be made.

Comparison of the methods

ECP was examined initially, since it requires the least amount of solute of the three techniques examined. A method of direct injection into the detector cell was developed to minimize solute consumption during detector calibration. A catechol elution peak used for ECP measurement is shown in Fig. 5. The peak height corresponds to a 0.68 *M* concentration of solute in the effluent. Approximately 0.1 ml of 1.0 *M* catechol solution in water was injected. Injecting a larger volume of solute solution enables calculation of higher concentrations. However, at some point the slug volume approaches that for FACP. Although ECP requires less solute for isotherm measurement, FACP allows measurement of the entire isotherm range and requires about the same amount of time as ECP measurements under the conditions studied, as seen in Table I. Thus, FACP was preferred over ECP.

FACP measurement were thereupon carried out. Detector calibration curves for FACP were measured with the direct cell injection technique developed for ECP. The major drawback of FACP is that no practical means for accounting for band-spreading could be employed. Comparisons of isotherms measured by frontal analysis and FACP illustrate the impact of this shortcoming. The results of four experiments with *p*-cresol by the two methods are shown in Fig. 6. FA is more reproducible than FACP, as shown by standard deviations of 25 and $89 \cdot 10^{-5}$ for parameter *a*, and 17 and $85 \cdot 10^{-5}$ for parameter *b*, for FA and FACP, respectively. The discrepancy between the FACP and FA curves is believed to arise from axial dispersion, which produces higher diffuse rear boundaries and thus error in the FACP calculations. Conder and Purnell[4], Huber and Gerritse[5], and De Jong *et al.*[3] found a similar discrepancy. Static measurements by De Jong *et al.*[3] nearly replicated FA isotherms. Both static and FA measurements, when carried out under appropriate conditions

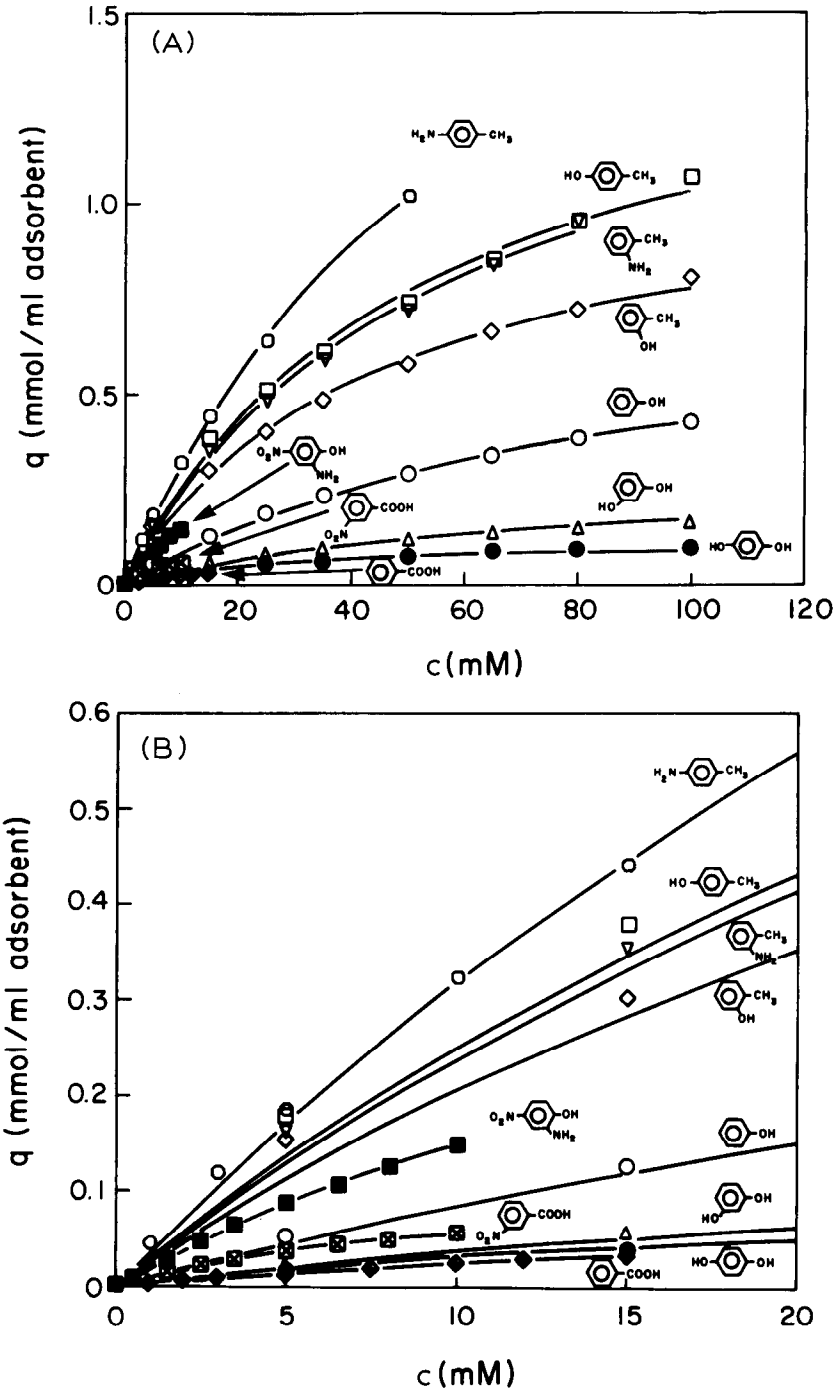


Fig. 4. Single-solute isotherms on octadecyl-silica, as measured by frontal analysis. (A) Entire experimental isotherms. (B) Enlargement of the lower concentration range. Experimental data points and the Langmuir correlations are shown by symbols and curves, respectively. The symbols represent: *p*-toluidine (○), *p*-cresol (□), *o*-toluidine (▽), *o*-cresol (◇), phenol (○), 2-amino-4-nitrophenol (■), resorcinol (△), *m*-nitrobenzoic acid (⊠), hydroquinone (●) and benzoic acid (◈). Conditions: column, 50.0 × 1.18 mm for phenol, resorcinol and hydroquinone, 40.5 × 1.18 mm for the remaining compounds; mobile phase, 0.20 *M* sodium phosphate buffer (pH 6.3) for benzoic acids, water for the remaining compounds; flow-rate, 70 μl/min; temperature, 25°C.

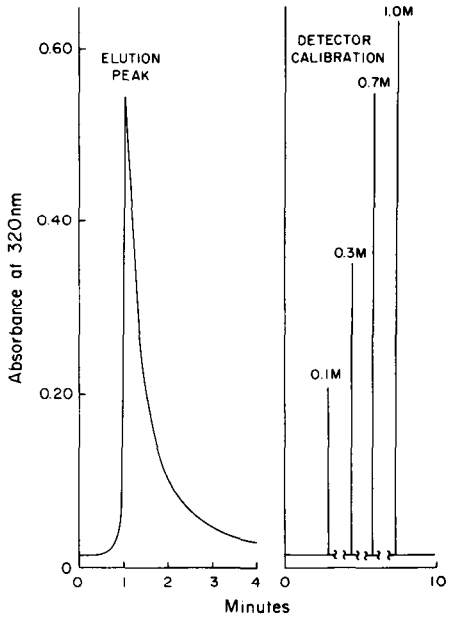


Fig. 5. Elution peak (left) and detector calibration (right) for isotherm determination by ECP on octadecyl-silica. The solute is catechol. Conditions: column, 59.3 × 4.6 mm; flow-rate, 1.0 ml/min; temperature, 25°C.

and for suitable systems, are thought to fairly accurately represent the "true" isotherm, since the above-mentioned effects have no or little influence on the final shape of the isotherm. In the present study, the FACP isotherms differed appreciably from the FA measurements, indicating that the non-ideal effects manifested in the FACP isotherm cannot be ignored. Therefore, FA was selected as the most precise isotherm measurement method that required no more time for isotherm determination than other methods.

TABLE I

HPLC ISOTHERM MEASUREMENTS FOR *p*-CRESOL ON OCTADECYL-SILICA FROM WATER AT 25°C

Method	*Solute requirement (mg)*	*Analysis time (min)**	*Maximum concn. (mM)***	*Langmuir parameters****	
				$\bar{a}$ ± *S.D. (l/ml adsorbent)*	$\bar{b}$ ± *S.D. (mM)*
ECP§	17	42.0	65	–	–
FACP§	55	45.0	100	0.0248 ± 0.00089	0.0082 ± 0.00085
FA§,§§	150	39.0	100	0.0232 ± 0.00025	0.0118 ± 0.00017
FA§§,§§§	9.9	37.0	100	–	–

* Not including sample preparation or column regeneration.
** In the mobile phase for a feed concentration of 100 m*M*.
*** For data in Fig. 6, where $\bar{a}$ and $\bar{b}$ are average parameter values.
§ Regular isotherm system: column, 59.3 × 4.6 mm; flow-rate, 1.0 ml/min.
§§ FA isotherms, measured with eight concentrations steps.
§§§ Miniaturized system: column, 40.5 × 1.18 mm; flow-rate, 0.068 ml/min.

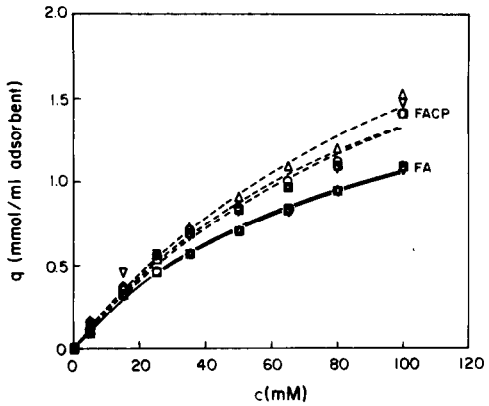


Fig. 6. Comparison of *p*-cresol isotherms, measured by FACP and FA on octadecyl-silica. Experimental data (symbols) are correlated to the Langmuir equation (curves). Dashed curves correspond to FACP measurements and solid to FA measurements. Conditions as given in Fig. 5.

Miniaturized system for frontal analysis

Minimizing solute consumption was the prime target in optimizing the FA method. Narrow-bore columns and a miniaturized system were employed to reduce the capacity of the system. Results for isotherm measurement of *p*-cresol on three sizes of columns are seen in Fig. 7. The good match between the individual isotherms prompted us to take advantage of the smallest column size with resulting savings in material. This reduction, along with volume reductions in the loops, flow cell, and tubing lines for the miniaturized system, results in a drastic decrease in the overall solute requirements, as seen in Table I. The solute requirement of 9.9 mg for complete isotherm determination of *p*-cresol is far less than the requirement of De Jong *et al.*[3], estimated to be about 400 mg for *p*-cresol. The miniaturized system for frontal analysis thus allows isotherm measurements with rare and/or expensive substances.

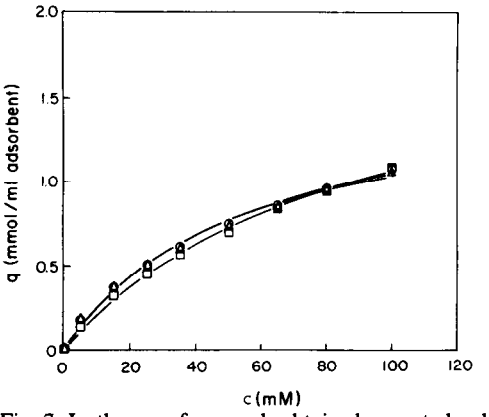


Fig. 7. Isotherms of *p*-cresol, obtained on octadecyl-silica with different sizes of columns. The symbols represent the following column dimensions: 59.3 × 4.6 (□), 150.0 × 1.18 (○) and 50.0 × 1.18 mm (△). Solid curves represent Langmuir correlations to the experimental data. Other conditions as given in Fig. 4.

The effect of flow-rate for the system was investigated in order to avoid non-idealities arising from kinetic effects. Fig. 8 shows isotherms of *p*-cresol at five different flow-rates, ranging from 11 to 165 μl/min. There is no apparent change in the isotherm shape over this flow-rate range. Therefore, no appreciable mass transfer ressistances were encountered. Subsequent isotherm measurements were carried out at flow-rates of approximately 70 μl/min by frontal analysis, and results obtained under such conditions are shown in Fig. 4. The Langmuir parameters determined for these compounds are listed in Table II. The data obey the general pattern of retention behavior expected in reversed-phase chromatography, *e.g.*, cresol is more strongly retained than phenol, phenol is retained more than benzoic acid or resorcinol, and the *para*-isomers of toluidine and cresol are more strongly retained than the *meta*-isomers. In general, the behavior obeys the prediction of the solvophobic theory[25,26] that adsorption increases with the non-polar surface area of the solute molecule.

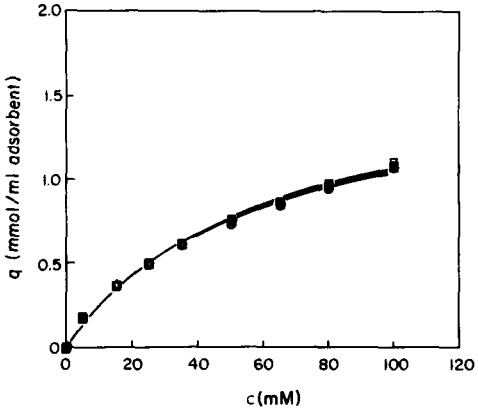


Fig. 8. Adsorption isotherms, measured at different flow-rates for *p*-cresol on octadecyl-silica with the miniaturized system. Flow-rates correspond to the following symbols: 11 (◇), 34 (○), 42 (△), 74 (□) and 165 μl/min (▽). Langmuir correlations to the experimental data points are given by the solid curves. Other conditions as in Fig. 4, except the column dimension is 50.0 × 1.18 mm.

Isotherms of *p*-cresol at 25, 40 and 60°C are shown in Fig. 9, and the corresponding Langmuir parameters are given in Table II. These isotherms follow the pattern observed with simple, low-molecular-weight solutes[6]: a decrease in adsorption occurs with increasing temperature. A plot of the logarithmic Langmuir parameters against $1/T$, where T is the temperature in °K, results in a good linear fit, $R^2 = 0.986$, for log *a versus* $1/T$, and a good linear fit for log *b versus* $1/T$, $R^2 = 0.961$, as shown in Fig. 10. Since *a* is equivalent to Henry's law constant for the adsorption process, Fig. 10 for parameter *a* is analogous to a Van 't Hoff plot. The process is exothermic, $\Delta H = -4.91$ kcal/mol, and shows a negative change in entropy.

Isotherms of *p*-cresol in 100% water, methanol–water (5:95), and methanol–water (10:90) are shown in Fig. 11, and their Langmuir parameters are given in Table II. As predicted by the solvophobic theory, sorption is weaker with increasing concentrations of the organic solvent components, *i.e.* the solute is less excluded from the solution. Fig. 12 shows that the logarithms of both *a* and *b* are roughly linear

TABLE II

ISOTHERM DATA ON OCTADECYL-SILICA, MEASURED BY FRONTAL ANALYSIS AND CORRELATED TO THE LANGMUIR EQUATION

Conditions as in Fig. 4, except as noted.

Compound	*Retention factor, k'**	*Parameter a (1/ml adsorbent)*	*Parameter b (mM q)*
Phenol	6	0.00935	0.0116
Resorcinol	1	0.00401	0.0126
Hydroquinone	1.1	0.00441	0.0377
m-Cresol	11.6	0.0255	0.0227
p-Cresol	16	0.0297	0.0187
o-Toluidine	9.7	0.0282	0.0180
p-Toluidine	14.7	0.0373	0.0168
Benzoic acid	1.1	0.00326	0.0293
p-Aminobenzoic acid	0.3	0.00029	0.03
m-Nitrobenzoic acid	9	0.0117	0.107
2-Amino-4-nitropohenol	7.2	0.0213	0.0432
p-Cresol**	–	0.0180	0.0144
p-Cresol***	–	0.0149	0.0124
p-Cresol§	–	0.0199	0.0167
p-Cresol§§	–	0.0123	0.0117

* Independently measured under otherwise identical conditions.
** Mobile phase: methanol–water (5:95).
*** Mobile phase: methanol–water (10:90).
§ Temperature: 40°C.
§§ Temperature: 60°C.

with the volume percent of methanol in the mobile pohase, $R^2 = 0.926$ and $R^2 = 0.959$, respectively.

Fig. 13 depicts the plot of parameter *a versus k'* for the first 11 entries in Table II. Good correlation is shown, $R^2 = 0.983$, between the two values. The slope of the

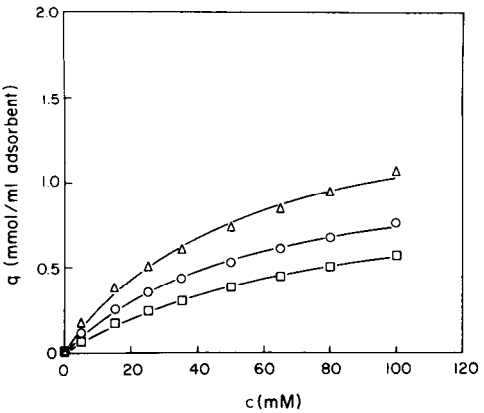


Fig. 9. Isotherms illustrating the effect of temperature on the adsorption of *p*-cresol on octadecyl-silica. The symbols represent the following temperaturess: 25 (△), 40 (○) and 60°C (□). Best fits of the experimental data to the Langmuir equation are given by the solid lines. Conditions as in Fig. 4, except the column dimension is 50.0 × 1.18 mm.

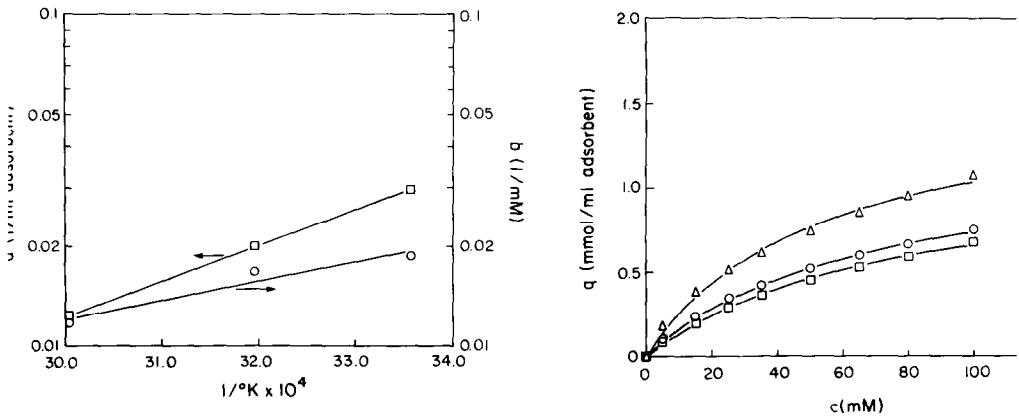


Fig. 10. Plot illustrating relationship of logarithmic Langmuir parameters and inverse temperature for *p*-cresol on octadecyl-silica. Experimental data for parameters *a* and *b* are given by the symbols (□) and (○), respectively, and the best linear fits are shown by solid lines. Conditions as in Fig. 4, except the column dimension is 50.0 × 1.18 mm.

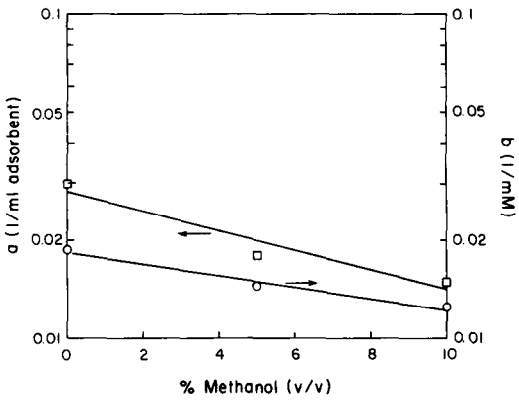
Fig. 11. Isotherms of *p*-cresol on octadecyl-silica from mobile phases of different compositions. The following compositions were investigated: water (△), methanol–water (5:95) (○) and methanol–water (10:90) (□). The solid lines represent the Langmuir correlations with the experimental data. The column was 50.0 × 1.18 mm and the other conditions are as in Fig. 4.

resulting line is 2.59 ml of solution/ml of adsorbent and, therefore, the value of the phase ratio can be calculated from eqns. 2 and 3. The ressulting value is 0.387, which compares quite favorably with the value of 0.374, obtained by the gravimetric dead-volume determination method, and gives confidence in this value. This plot also illustrates the utility of predicting parameter *a* from *k'* values. Compared to other techniques which relate physical properties to parameter *a*[27], it appears to be a better or at least as good a correlation. Moreover, large data bases of *k'* values are available or else, they are easily measured.

The techniques developed here are particularly well suited for isotherm measurement of biochemicals. The small solute requirement for isotherm determination via the miniaturized apparatus makes adsorption measurements of such species feasible. Since, the surface of octadecyl-silica resembles that of biological membranes, the measurement of isotherms on this sorbent may provide insight into adsorption on membranes in the living system. As examples, isotherms of L-phenylalanyl-L-valine, L-phenylalanine, and L-leucine, determined under various conditions, are shown in Fig. 14.

CONCLUSIONS

Distribution isotherms were studied in order to better understand, measure and quantify the adsorption process. The techniques for measurement of single-solute isotherms were reviewed and compared to develop a fast, accurate and precise method that delineates as much of the isotherm as possible and consumes little solute. Frontal analysis with a high-performance liquid chromatographic system, incorporating a narrow bore column, 50.0 × 1.18 or 40.5 × 1.18 mm, deliveres the most



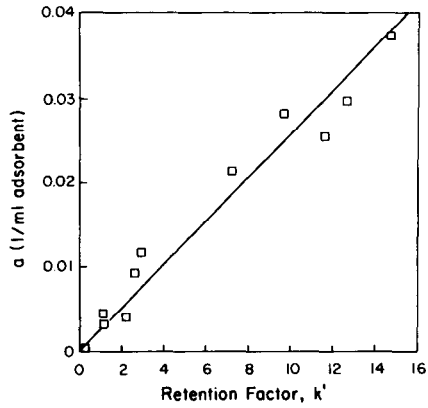


Fig. 12. Graph of logarithmic Langmuir parameters as a function of mobile phase composition at low concentrations of the organic solvent component. Parameters are for adsorption of *p*-cresol on octadecyl-silica. The symbols represent parameter *a* (□) and parameter *b* (○). The solid lines are the best linear fit to the experimental data. The conditions are as given in Fig. 4, but the column has dimensions of 50.0 × 1.18 mm.

Fig. 13. Graph illustrating the correlation between Langmuir parameter *a* and retention factor. The data points are given by the first 11 entries in Table II. A linear regression is given by the solid line.

rapid, precise, and accurate measurement. Small sample consumption for isotherm measurement with this system allows isotherm determination of rare and expensive compounds.

Isotherms were measured with this system for frontal analysis and found to be Langmuirian or quasi-Langmuirian in shape. The relative adsorption behavior of different solutes concurred, in qualitative terms, with predictions of the solvophobic theory[26]. For example, isomers were found to have different adsorption character-

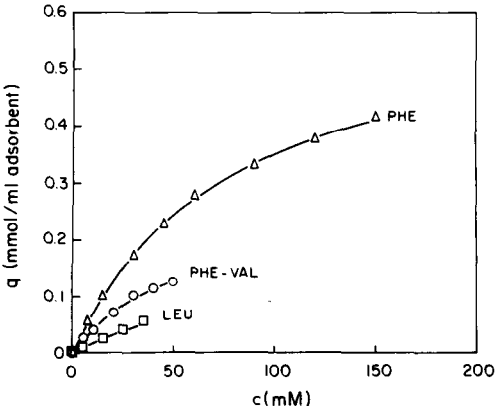


Fig. 14. Isotherms of amino acids on octadecyl-silica. L-Phenylalanyl-L-valine isotherm conditions: column, 59.3 × 4.6 mm; mobile phase, 0.050 *M* sodium phosphate buffer pH 6.0; flow-rate, 1.0 ml/min; temperature, 30°C. L-Phenylalanine isotherm conditions as above, except: mobile phase, pH 2.; temperature, 50°C. L-Leucine isotherm conditions: column, 50.0 × 1.18 mm; mobile phase, 0.50 *M* sodium phosphate buffer (pH 5.0); flow-rate, 70 μl/min; temperature, 25°C.

istics that could be roughly correlated with the size of the contact surface of the molecules upon adsorption. The effects of temperature and presence of 5 and 10% methanol in the aqueous mobile phase were also studied. The Langmuir parameter a yielded a linear Van 't Hoff relationship when plotted as log a *versus* $1/T$. The relationship between parameter a and the retention factor, k', was found to be linear, as expected. This relationship can be utilized to determine the phase ratio or to predict parameter a from measured retention factors. Isotherm measurement of biological compounds with the miniaturized system was found to be particularly convenient due to the small amount of solute required for isotherm determination.

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