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Adsorption isotherms of phenylalanine in a chromatographic column measured simultaneously by system peaks analysis and frontal analysis

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ABSTRACT

Adsorption isotherms of phenylalanine dissolved in acetate buffer of three concentrations, 0.001, 0.01 and 0.1 *M*, were measured using frontal analysis and system peaks analysis simultaneously. The adsorption isotherms measured by the two methods were identical. System peaks were induced by injecting a small vacancy, pure water, just after the plateau was reached in each step of the frontal analysis. The capacity factor of the system peak corresponding to phenylalanine was used in the calculation of the adsorption isotherm. The use of system peaks for the measurement of adsorption isotherms is promising, especially for multi-component systems where mixed isotherms are needed for rational formulation of a preparative separation.

INTRODUCTION

Often when there is a need for a preparative separation, optimization is based on the conditions used in the analytical separation and amounts are increased up to overloading concentrations thereafter. However, the procedure for optimization of the separation should be formulated using a rational design and prediction of the prospects of a good separation rather than by a trial and error sequence. An extensive rational approach to preparative separations in chromatography was described by Guiochon and co-workers [1–9], Snyder and co-workers [10–12] and Knox and Pyper [13].

A good preparative separation can be rationally formulated by using adsorption isotherms of the sample components. The sample is run through the column as a mobile phase solution, and the concentration of each solute i in the stationary phase, $C_{s,i}$, is measured as a function of the concentrations of the sample constituents in the mobile phase, $C_{m,i}$. The dependence is described by the adsorption isotherm. When there is more than one component in the mobile phase, the system is referred to as a multi-component system. In a multi-component system there is a need for more than one adsorption isotherm for each solute to describe the mutual dependence on the various components, $C_{m,i}$.

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Adsorption isotherms can be implemented in the prediction of peak shape and retention at overloading concentrations [1–13]. There are several methods for the measurement of adsorption isotherms [14]. Frequently, the method of choice for the measurement of an adsorption isotherm is frontal analysis. We suggest here another method, a system peaks analysis.

System peaks have been discussed during the last few years by Levin and Grushka [15–17], Levin and Abu-Lafi [18] and Golshan-Shirazi and Guiochon [19–23] and by Westerlund and co-workers [24–27]. An excellent theoretical study of system peaks in linear chromatography was presented by Golshan-Shirazi and Guiochon [23]. With a multi-component mobile phase where one or more components are adsorbed on the stationary phase, injection of a sample different in any sense from the mobile phase itself induces system peaks. Identification and quantitative treatment of these peaks allowed the calculation of capacity factors of mobile phase components and column void volumes using the appropriate detection [15,16]. It has also been shown that the adsorption isotherm of a particular mobile phase component can be calculated from the corresponding system peak [15–18].

A detailed explanation of the principle behind the use of system peaks for the measurement of adsorption isotherms was given previously [18]. A system peak in the vacancy mode is the outcome of a small equilibrium perturbation at the column head when an additive-free mobile phase solution is injected. The equilibrium of each component of the mobile phase is disturbed and thereby manifested by at least one system peak using the appropriate detection conditions. The small perturbation in the concentration of a mobile phase component i moves down the column at a constant velocity u_i, dictated by the adsorption properties of component i, according to the expression

$$u_i = \frac{u_0}{1 + \mathrm{d}C_{\mathrm{s},i}/\mathrm{d}C_{\mathrm{m},i}} \tag{1}$$

where u_0 is the mobile phase velocity and $\mathrm{d}C_{\mathrm{s},i}$ and $\mathrm{d}C_{\mathrm{m},i}$ are the infinitesimal disturbances in the concentrations at the stationary and mobile phases, respectively, or the slope of the adsorption isotherm of component i.

Theoretical and experimental treatments of system peaks in non-linear chromatography were made by Golshan-Shirazi and Guiochon [19–22] using conditions under which competition between the solute and mobile phase components occurs. It was shown that injection of a solute into a binary mobile phase was accompanied by two system peaks in addition to the solute peak. One of the two system peaks was eluted according to the capacity factor of a corresponding mobile phase component, and the other accompanied the solute peak. Therefore, in principle, a mobile phase component can show up in more than one peak. However, there is always one system peak that elutes according to a particular corresponding component's capacity factor k'_i, and can serve for the measurement of this component's adsorption isotherms. We used such a peak for the measurement of the adsorption isotherm of phenylalanine at various buffer concentrations. Identical adsorption isotherms were obtained using system peaks analysis and frontal analysis.

EXPERIMENTAL

Materials

The mobile phase was prepared by dissolving the appropriate amount of phenylalanine (Merck, Darmstadt, Germany) in acetate buffer (pH 3.7), prepared from analytical-reagent grade sodium acetate (Merck) and acetic acid (Frutarom, Haifa, Israel) (1:9).

Instrumentation

The chromatographic system, which was used in all of the experiments, was an HP1050 (Hewlett-Packard, Palo Alto, CA, U.S.A.) modular system with a diode-array UV detector and an HPCHEM IBM-compatible data system with a ThinkJet or LaserJet Series II printer when needed. A Rheodyne (Cotati, CA, U.S.A.) injection valve was used, equipped with a 20- or 2000-μl loop as needed. The temperature was kept constant within ± 0.5°C using a circulating water-bath.

Procedure

All the chromatographic runs were done with a LiChrosorb RP-18 cartridge, 125 × 4 mm I.D. (Merck). The temperature was kept constant at 30°C throughout the experiments. The flow-rate was 1.5 ml/min in all the experiments except for the injection of phenylalanine at overload, where it was 1 ml/min. The system was thoroughly washed with acetonitrile–water between changes in the mobile phase composition.

RESULTS AND DISCUSSION

The chromatographic system consisted of a reversed-phase column and aqueous acetate buffer and phenylalanine in the mobile phase, as described previously [18]. A chromatogram of pure water injected into 0.025 *M* phenylalanine dissolved in 0.1 *M* acetate buffer is shown in Fig. 1. The wavelength of detection was 254 nm, so phenylalanine could serve for the visualization of all three system peaks, marked A, B and C in Fig. 1. Peak A corresponds to an unretained species, whereas B and C can both be related to species adsorbed on the stationary phase. The retention times of peaks A and B can be related to acetate salt and acetic acid, respectively, in agreement with previously described sytems which included acetate buffer [15–18].

Peak C eluted at a capacity factor corresponding to phenylalanine. The system peaks here were negative. A peak appears negative, when the detector response to the sample zone is lower than that to the mobile phase. At low acetate concentrations peaks A and B were small, because interference of phenylalanine with the other components was low [18].

Measurement of adsorption isotherms

System peaks analysis. The adsorption isotherm of phenyalanine in 0.001, 0.01 and 0.1 *M* acetate buffer (pH 3.7) was measured from system peak C. The column was equilibrated with phenylalanine and acetate buffer, then a small vacancy (pure water) was injected. Three system peaks appeared as demonstrated in Fig. 1. The capacity factor of peak C was characteristic of phenylalanine (k'_p) and was used for the

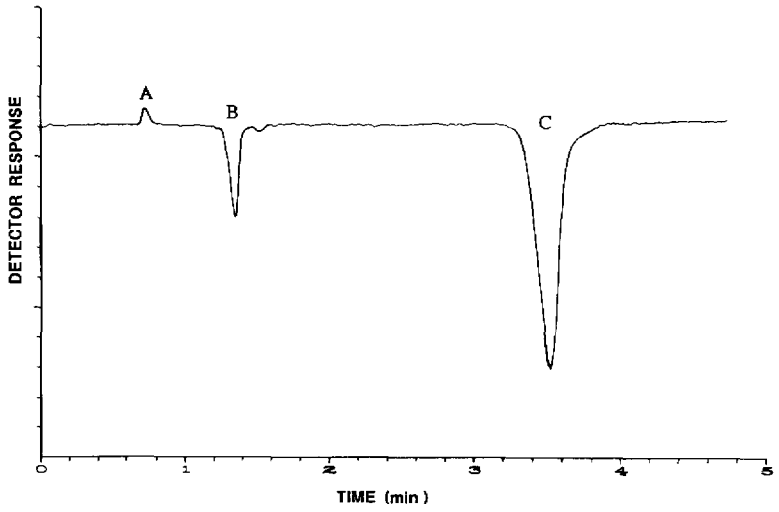

Fig. 1. System peaks A, B and C detected at 254 nm when 20 μl of water were injected into a mobile phase of 0.025 *M* phenylalanine in 0.1 *M* acetate buffer (pH 3.7).

calculation of the adsorption isotherm. The concentration of phenylalanine in the mobile phase, $C_{m,p}$, was changed stepwise, and k'_p (peak C) was measured at each step. The concentration of phenylalanine in the stationary phase was calculated at every step using the following equation [17]:

$$C_{s,p} = \frac{1}{\varphi} \int_0^{C_{m,p}} k'_p \, dC_{m,p} \tag{2}$$

where φ is the phase ratio and $dC_{m,p}$ is the difference in concentration between every two consecutive steps, *i.e.*, at each step k'_p is multiplied by $dC_{m,p}$ and summed over the range of concentrations from 0 to $C_{m,p}$.

Frontal analysis. The adsorption isotherms of phenylalanine measured by system peaks analysis were compared with the most commonly used method, frontal analysis. The column was equilibrated with phenylalanine-free mobile phase (0.001, 0.01 and 0.1 *M* acetate buffer, pH 3.7), then the mobile phase composition was changed abruptly and buffer containing a new concentration of phenylalanine, $C_{m,p}$, entered the column. A front that had a retention volume $V_{R,p}$ characteristic of the concentration of phenylalanine in the new mobile phase, $C_{m,p}$, appeared as shown in Fig. 2. The concentration of phenyalanine in the stationary phase can be calculated according to the equation normally used in frontal analysis:

$$C_{s,p} = \frac{(V_{R,p} - V_0 - V_h)}{V_s} \cdot C_{m,p} \tag{3}$$

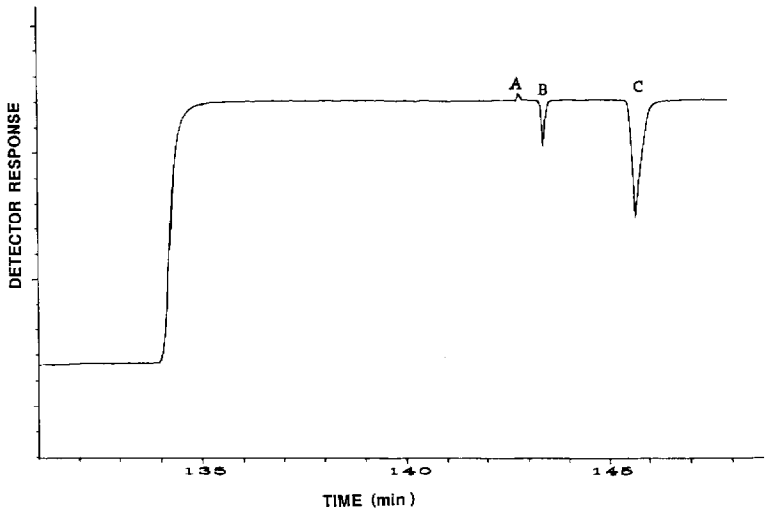

Fig. 2. Front obtained during one step in the frontal analysis of phenylalanine dissolved in 0.1 *M* acetate buffer (pH 3.7). Water (20 μl) was injected after the plateau was reached and induced the three system peaks.

where V_0 is the column void volume, V_s is the stationary phase volume (calculated by subtracting the void volume from the volume of the empty column) and V_h is the hold-up volume from the pump to the detector (measured by placing a zero dead volume union instead of the column). The concentration was increased stepwise in our experiment rather than beginning from the phenylalanine-free solution in every step, and therefore the following expression was used rather than eqn. 3:

$$C_{s,p} = \int_0^{C_{m,p}} \frac{(V_{R,p} - V_0 - V_h)}{V_s} \cdot dC_{m,p} \quad (4)$$

where $dC_{m,p}$ is the difference in concentrations between two consecutive steps.

Combination of frontal analysis and system peaks analysis. Formation of system peaks can be associated with frontal analysis by injecting a small vacancy just after the plateau has been reached. At this point the column is equilibrated with the mobile phase containing the new composition. Normally, in an independent measurement of the adsorption isotherm using system peaks analysis the detector is zeroed on the plateau. Consequently, the plateau becomes the baseline and then a vacancy is injected [15 18].

A 20-μl volume of water was injected over the plateau using 0.1 *M* acetate buffer containing the new concentration of phenylalanine in each step and the corresponding system peaks appeared superimposed on the plateau as shown in Fig. 2. The sequence of steps obtained at 0.1 *M* acetate buffer is shown in Fig. 3. A similar sequence was obtained using 0.01 *M* acetate buffer. However, the combination of both the frontal analysis and system peaks analysis methods was difficult to achieve with 0.001 *M* buffer, relatively small system peaks being obtained. As a rule, the large fronts were

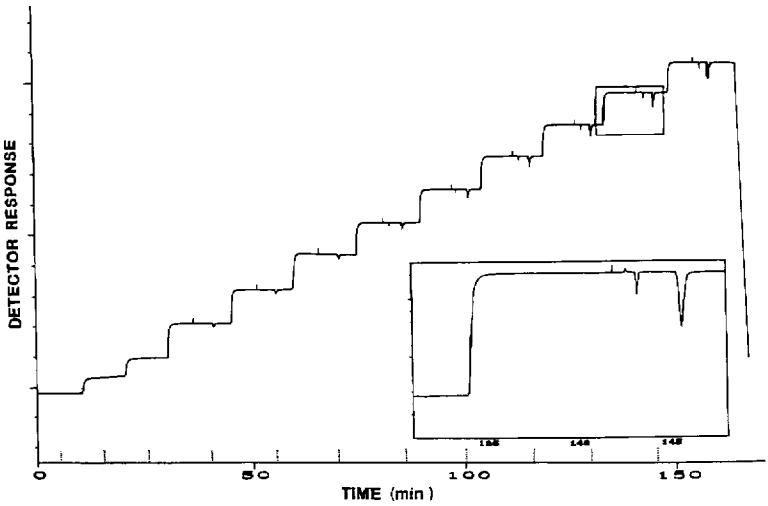

Fig. 3. Sequence of steps in which the adsorption isotherm of phenylalanine was simultaneously measured using both the frontal analysis and system peaks analysis methods. The solute-free mobile phase was 0.1 *M* acetate buffer (pH 3.7). The small vacancy injected on the plateau was 20 μl of pure water. The inset is an enlargement of one step in the combined analysis for illustration. The broken lines on the time axis mark the times when the concentration of phenylalanine in the mobile phase was changed. The injection point is marked on the plateau for each step.

detected using a relatively low sensitivity of the detector, whereas the superimposing system peaks were detected using a relatively high sensitivity. A compromise could not be attained using the 0.001 *M* buffer, so the two measurements were made separately. Generally, the retention volume of the fronts decreased with the concentration of phenylalanine, as well as the capacity factor of system peak C.

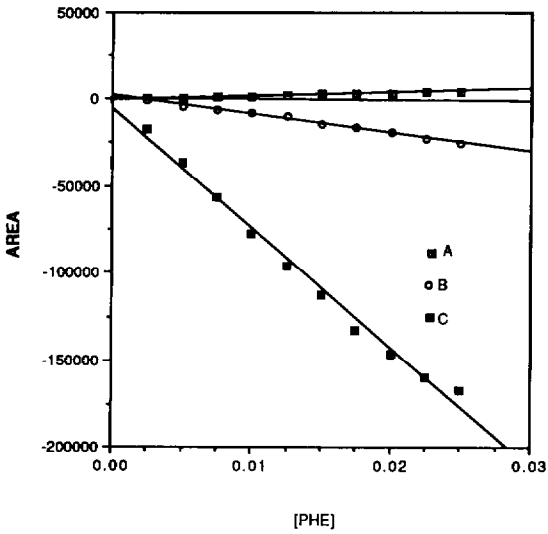

Fig. 4. Areas of peaks A, B and C when 20 μl were injected on the plateau during a stepwise increase in phenylalanine concentration in 0.1 *M* acetate buffer (pH 3.7).

An interesting point in Fig. 3 is the behaviour of the peak areas of the three system peaks with increase in phenylalanine concentration in the mobile phase. All three peaks areas were measured at each step and the results are shown in Fig. 4. Peak A became more and more positive whereas peaks B (with k' of acetic acid) and C (with k' of phenylalanine) became more and more negative as the concentration increased, as was observed in previous work [15–18]. The relationship between the system peaks areas and the distribution equilibria in the column is under study.

The three adsorption isotherms of phenyalanine at three different buffer concentrations measured by both the frontal analysis and system peaks analysis methods, are shown in Fig. 5. The similarity between the adsorption isotherms obtained by the two methods is striking; differences fell within the experimental error.

Peak shape at overload conditions

The relationship between adsorption isotherms and peak shape in non-linear chromatography has frequently been discussed in the literature [1–13]. The chromatograms of phenylalanine at volume and concentration overload (2 ml of 0.025 *M* solution) injected into two different mobile phases are shown in Fig. 6. Points of

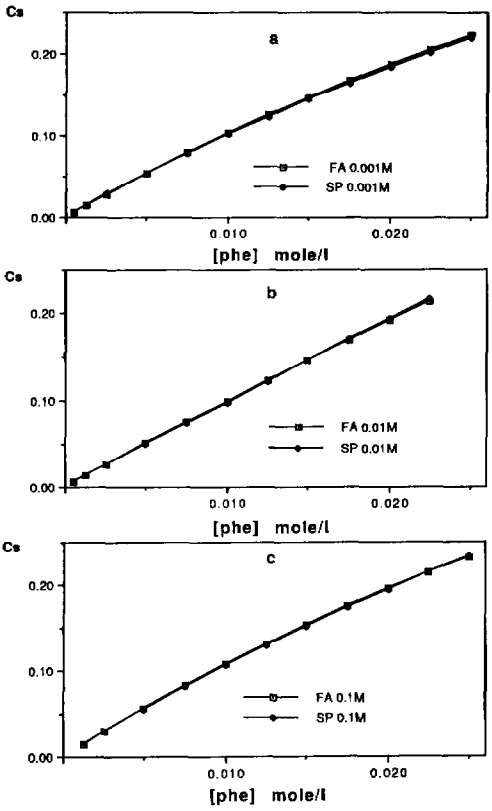

Fig. 5. Adsorption isotherms of phenylalanine measured by (□) frontal analysis and (◆) system peaks analysis. Phenylalanine was dissolved in (a) 0.001, (b) 0.01 and (c) 0.1 *M* acetate buffer (pH 3.7).

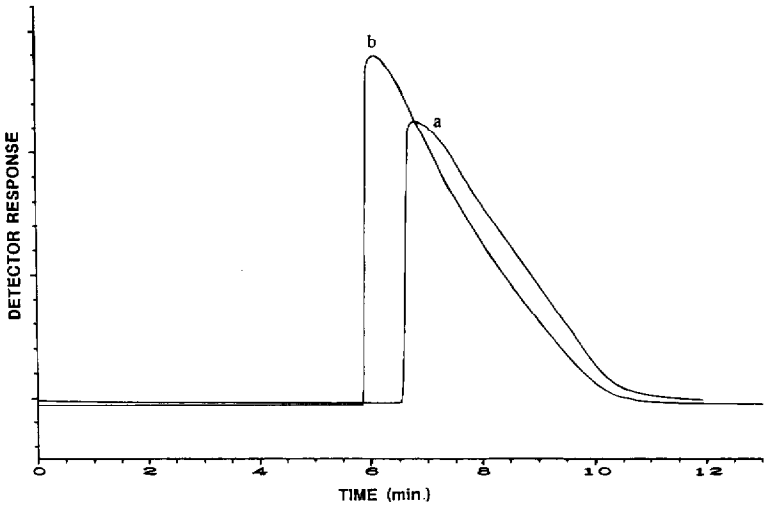

Fig. 6. Injection of 0.025 *M* phenylalanine using a 2-μl loop. The mobile phase was (a) 0.001 and (b) 0.1 *M* acetate buffer (pH 3.7).

interest are the peaks shapes and retention. The peaks have a sharp front and diffuse rear, as expected from their slightly concave adsorption isotherms. Generally, retention was smaller than the linear range. The retention at a 0.1 *M* buffer concentration was smaller than that at 0.001 *M*, as predicted by the adsorption isotherm.

In principle, one peak at overloading concentrations can be used for measurements of adsorption isotherms by elution of a characteristic point (ECP) [28]. In this method, retention volumes of various points on the rear boundary of the peak are taken for the analysis using eqn. 4. The detector response is calibrated to actual concentrations, so the rear boundary can be represented as a series of points with characteristic concentrations in the stationary and mobile phases, *i.e.*, it can represent a whole adsorption isotherm in one injection.

The peaks shown in Fig. 6 were used for the measurement of adsorption isotherms by ECP. The heights of the fronts obtained by frontal analysis were used for detector response calibration. The concentrations of the solute in the stationary phase calculated from ECP were far above the values obtained by frontal analysis and system peaks analysis. The discrepancy between the isotherms obtained by frontal analysis and ECP is well documented in the literature (see ref. 28 and references cited therein). It is believed to arise from axial dispersion, which produces more diffuse rear boundaries and hence the higher results for the concentrations in the stationary phase.

CONCLUSION

Adsorption isotherms can be measured using system peaks analysis as they are identical with the adsorption isotherms obtained by frontal analysis. The use of system peaks for the measurement of adsorption isotherms is especially favourable for multi-component systems. Mixed adsorption isotherms cannot be readily measured

experimentally by current methods. We believe that system peaks analysis can be used where mixed isotherms are needed for a rational formulation of a preparative separation. There is a need for a definite assignment of each of the peaks obtained in a multi-component system. Once the system peaks have been defined, their capacity factors can be used for the measurement of the adsorption isotherm of each component in the mobile phase.

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