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COMPARISON OF VARIOUS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF ADSORPTION ISOTHERMS IN SOLUTIONS

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SUMMARY

The thermodynamic basis of the minor disturbance method in elution chromatography is considered and the theories of integrated and differential variants of frontal chromatography are described. The excess adsorption isotherms of acetonitrile from aqueous solutions on silica gel modified with octadecylsilane were measured by various chromatographic methods and compared.

INTRODUCTION

One of the major problems with the physico-chemical applications of molecular liquid chromatography is the determination of excess adsorption isotherms of individual components in multi-component systems and the calculation of the thermodynamic constants of adsorption equilibrium. Adsorption isotherms can be used to find the thermodynamic parameters of the molecular interactions on adsorbents with various surface chemistries and to predict the retention of organic compounds in chromatographic columns.

There are various chromatographic methods for the measurement of excess adsorption isotherms. In some instances coincidence of adsorption isotherms determined by static and dynamic methods has been found[1–5]. However, the conditions of the dynamic chromatographic process are distinct from those of static isotherm measurements. Therefore, the question of the thermodynamic equilibrium of chromatographic processes remains open. A comparison of adsorption isotherms obtained by different chromatographic methods may be used to evaluate the possibility of describing the adsorption equilibrium.

In this paper, we briefly describe the theories of frontal chromatographic and elution disturbance methods, applied in the calculation of excess adsorption isotherms, and compare various chromatographic methods for the determination of adsorption isotherms.

THEORY

General

The main influence on the retention and separation of components of mixtures

in liquid adsorption chromatography (LAC) is the adsorption interactions of mole-
cules of test compounds with the surface of the stationary phase. If we use binary or
ternary eluents, the separation of the components of mixtures must be described by
the theory of multi-component adsorption. In isocratic chromatography the station-
ary phase is in adsorption equilibrium with the mobile phase. The equilibrium surface
concentrations of eluent components are determined by the corresponding adsorp-
tion isotherms. The adsorption interactions of molecules of sample compounds in-
jected into a column add a correction due to the excess adsorption of one eluent
component from another.

Therefore, if we wish to describe the retention behaviour in LAC correctly, we
must take into account the adsorption effects of all components of the chromato-
graphic system. The thermodynamic description of this system must take the dynamic
character of adsorption into account.

De Vault's equation combined u, the linear velocity of moving the concentrated
band along the column, with Γ, the excess adsorption of the compound being inves-
tigated[1]:

$$u = \frac{w}{V_0' + S' \cdot \dfrac{d\Gamma}{dc}} \tag{1}$$

where w is the flow-rate of the eluent, V_0' the dead volume per unit column length,
S' the effective surface area of the adsorbent per unit column length and $d\Gamma/dc$ the
derivative of the excess adsorption isotherm of the investigated compound at the
stated equilibrium concentration c. Eqn. 1 combines the retention parameter of the
chromatographic column with the slope of the adsorption isotherm at every concen-
tration band, and in principle allows to describe the adsorption equilibrium in a
chromatographic system. Below we consider the methods for the determination of
adsorption isotherms from chromatographic data.

Minor disturbance method

The minor disturbance method has been used for the determination of ad-
sorption isotherms in solution and of dead volumes[2–4]. However, the interpretations
of this method in the papers cited only obscure the clear adsorption picture of the
chromatographic process. If we transform eqn. 1, with $LS' = S$, the total surface
area of the adsorbent in the column, and $LV_0' = V_0$, then we obtain:

$$S \cdot \frac{d\Gamma}{dc} = V_r - V_0 \tag{2}$$

where V_r is the retention volume, equal to Lw/u, and L is the column length.

Obviously, the minor disturbance method must give V_r, the retention volume
described by eqn. 2. In practice, this method involves equilibration of the column
with a solution of the investigated compound at equilibrium concentration followed
by injection of the sample of the same solution approximating the equilibrium con-
centration. It can be seen from eqn. 2 that the accurate determination of the dead
volume (V_0) is important.

   The problem of the determination of the dead volume of the mobile phase in the chromatographic system has been extensively discussed[4–12]. Knox and Kaliszan[4] contributed an interesting review of various chromatographic methods for the determination of dead volumes and showed the applicability of the minor disturbance method for these purposes. However, we believe that Knox and Kaliszan's interpretation of the principles of this method is not quite correct. The dead volume of the mobile phase in the chromatographic system must be determined as the whole volume of the liquid phase from the injector to the detector (with a correction for the lag of the signal from the detector).

   The dead volume of the mobile phase is determined strictly by the use of eqn. 2. It is known that the excess adsorption of the "pure" (without eluent) individual component being investigated is zero. Integrating eqn. 2 over the whole concentration range of x molar fractions, we obtain

$$V_0 = \int_0^1 V(x)\, dx \tag{3}$$

Hence the dead volume can be determined by integration of the retention volumes of minor disturbance peaks, measured over the whole range of equilibrium concentrations of the components of the mobile phase. Knox and Kaliszan[4] used the ex-
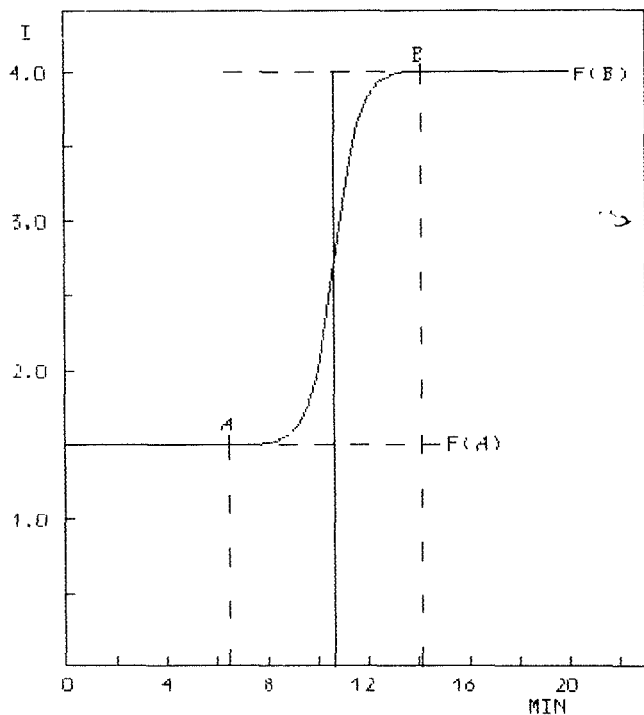

Fig. 1. Experimental frontal chromatogram.

perimental data published in ref. 5. In principle, the determination of V_0 values from these data leads to a straight line, parallel to the abscissa and crossing the experimental curve, giving two portions of equal area (Fig. 2B).

As was seen from eqn. 2, the calculation of the excess adsorption of one component from solutions was based on the minor disturbance method. The adsorption isotherms of acetonitrile from water calculated from our own data and from results published in ref. 5 are shown in Fig. 2A. As can seen, the comparison gives good agreement.

Frontal methods

The excess adsorption isotherms can be calculated from the chromatographic front by using eqn. 1. However, the application of frontal chromatography to the
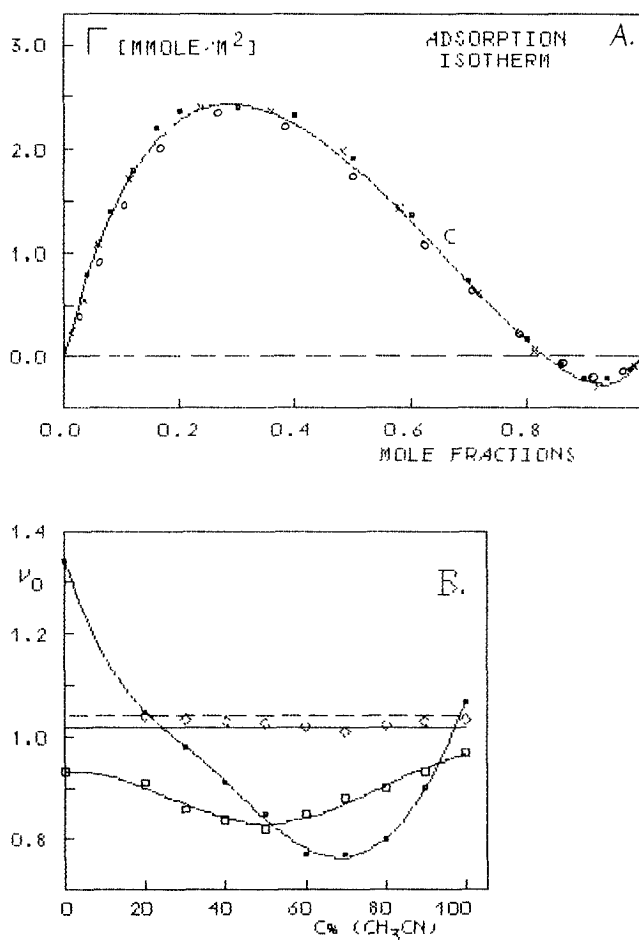

Fig. 2. (A) Isotherm of the adsorption of acetonitrile from water on Separon-C_{18}, measured by various methods· ■ = total frontal; × = differential; ○ = minor disturbance method (B) Retention volumes for the minor disturbance method.

investigation of adsorption isotherms in refs. 13 and 14 requires a strict correction for the chromatographic band broadening. This correction sometimes utilizes the data obtained by the static method. In the investigation of the excess adsorption from solutions on hydrophobic adsorbents (for example, during the adsorption of acetonitrile from aqueous solutions on LiChrosorb RP-18 this cannot be done, because these solutions do not wet the surface of such adsorbents).

We have developed[15] a variant of the frontal method for the determination of adsorption isotherms. The theory of this method has been considered in detail in ref. 13. Our variant makes it possible to correct the kinetic and diffusion effects in chromatographic systems.

For the determination of adsorption isotherms by frontal chromatographic methods, a series of frontal chromatograms for several concentrations of the investigated compounds are prepared. For each chromatogram the point corresponding to the retention time is determined. This point characterizes only the value of the adsorption from solution at a given concentration and does not depend on the band broadening[15,16]. The selection of this point is based on the application of the material balance equation. In ideal chromatography, in the absence of longitudinal diffusion, the front of a sample substance will be a vertical line that moves along the column at constant speed. The retention time will determine the adsorption value, Γ, in this instance:

$$mS\Gamma^{(v)} = (wt_R - V_0)c_0 \tag{4}$$

where m is the mass of adsorbent in the column, S is the specific surface area of the adsorbent, $\Gamma^{(v)}$ is the excess volume adsorption of the investigated substance and c_0 is the molar concentration of the investigated substance. The concentration front of the investigated compound broadens on moving through the column. The amount substance that moves in front of the ideal concentration front must be equal to that behind the ideal front. The retention time of the ideal front may be calculated from the chromatogram of the real front:

$$\int_0^{t_R} [c(t) - c_e] \, dt = \int_{t_R} [c_e - c(t)] \, dt \tag{5}$$

where $c(t)$ is the concentration of the investigated compound at the front and c_e is the initial equilibrium concentration. This equation is illustrated in Fig. 1. Eqn. 5 was derived on the basis of material balance conditions and it can therefore, be applied to the description of a front of any form.

It must be noted that the complete frontal method posesses an additional possibility for the simple determination of the dead volume. As the excess adsorption of a pure component is zero, we can show that the retention time of the average mass point of the displacement front of one pure component by another will be strictly equal to the dead volume of the chromatographic system.

The second variant of frontal chromatography for the measurement of the excess adsorption isotherm is the differential (step-by-step) method. In this method,

the retention time of the concentration front is measured relative to the previous equilibrium concentration. The correct transformation of eqn. 2 to eqn. 4 has been described in ref. 13. In this instance the retention volume is described by

$$V = V_0 + mS \cdot \frac{\Delta \Gamma}{\Delta c} \tag{6}$$

where Δ is the difference of the corresponding values for $N - 1$ and N equilibrium concentrations in the chromatogram. This method has been used for the calculation of the adsorption values at the equilibrium concentrations using the equation

$$\Gamma = \sum_{i=2}^{n} \left[\frac{(V_i - V_0) - (V_{i-1} - V_0)}{mS} (c_i - c_{i-1}) \right] \tag{7}$$

or, if the concentration decreases significantly:

$$\Gamma = \int_{0}^{c} \frac{V(c) - V_0}{mS} \cdot dc \tag{8}$$

Eqn. 8 corresponds in practice to a transition to the minor disturbance method.

It is necessary to make some remarks concerning the effective column volume and equilibrium of the chromatographic process. The dead volume of a column must depend on the sizes of the investigated molecules and on the pore structure of the adsorbent. The presence of micropores in the adsorbent will lead to exclusion effects relative to the molecules of different sizes. This effect will influence the equilibrium of the chromatographic process. As has been shown in ref. 15, the chromatographic process is close to thermodynamic equilibrium if the elution velocity is less than 0.1 cm/s and only for adsorbents with average pore diameters > 10 nm. These data are in good agreement with the calculations described in ref. 17. Therefore, if we want to obtain reproducible results from chromatographic analysis, it is necessary to use macroporous adsorbents with pore diameters > 10 nm. In this instance, the methods described here for the determination of the void volume will be applicable.

EXPERIMENTAL

The automatic chromatographic system for measuring adsorption isotherms by total frontal and differential frontal methods is shown in Fig. 3. It allows us to measure the adsorption values by both methods, using the same chromatographic system, by transforming one to the other by carrying out a simple change in the computer program.

When the adsorption isotherm is measured by the differential method, pump 1 pumps the eluent and pumps 2 and 3 pump the solutions of the investigated compound by increasing (or decreasing) the concentration in the loop of the corresponding valves 4 and 5. When a solution of a certain concentration is displaced from the loop of valve 4, the loop of valve 5 simultaneously fills with the solution of the next
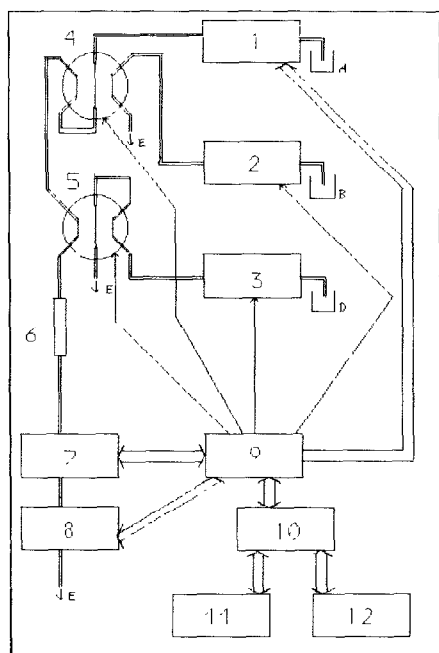
Fig. 3. Schematic diagram of the experimental system designed for frontal chromatography: 1, 2, 3 = pumps; 4, 5 = valves; 6 = column; 7 = detector; 8 = flow meter; 9 = interface; 10 = computer; 11 = plotter; 12 = display.

concentration from pump 3. When the equilibrium concentration appears in the detector, the computer turns valves 4 and 5 simultaneously and stops pump 3. The computer also turns on pump 2 to fill the loop of valve 4 with the next concentration of the solution of the investigated compound. During this time, pump 1 displaces the solution from loop 5 into the column. This system allows the total isotherm to be measured automatically. Measurement is carried out by either increasing or decreasing the concentration. This allows additional checking of the equilibrium and the entire desorption (the retention time of middle-mass points must coincide when passing through the isotherm forwards and backwards).

It is also possible to use only two pumps and one valve in the same chromatographic system for the determination of the adsorption isotherm by the total frontal method. In this instance, pump 1 pumps the pure eluent through the column and pump 2 fills the loop of valve 4 consecutively with solutions of various concentrations, while valve 5 is always in the "probe injection" position and this pump 3 is always shut off.

For measurement of the adsorption isotherm by the elution method a Model 600 liquid chromatograph (Laboratorní Přístroje, Prague, Czechoslovakia) was used with a Model RD-601 refractive index detector. The adsorption isotherm was determined by calculating the retention times of the minor disturbance peaks of acetonitrile–water solutions. The system dead volume was determined by the above-described method. The adsorption isotherm was calculated from eqn. 6.

The adsorption isotherm of acetonitrile from water on silica gel Separon-C_{18} measured by all the methods described is shown in Fig. 2. As can be seen, all methods give comparable results.

CONCLUSION

The direct investigation of adsorption interactions of compounds with stationary phases has been carried out by chromatographic methods. Suggested variants of the frontal method allow the equilibrium of the chromatographic process to be verified, corrected for the kinetic and diffusion effects of the broadening chromatographic zones.

The methods described allow these investigations to be made directly on commercial chromatographic equipment and to define the dead volume on a thermodynamic basis both in normal-phase and in reversed-phase chromatography, which is only a variant of LAC.

The importance of applying the thermodynamic approach to the dynamic adsorption process is noteworthy. In many instances it makes it possible to determine the applicability of suggested theoretical ideas. For example, Knox and Kaliszan[4] applied the concentration approach to the minor disturbance method and obtained virtually the same results as those presented here. However, when they calculated the adsorption isotherm of acetonitrile from water on a reversed-phase adsorbent, they considered the positive adsorption over the full concentration range. Although, as can be seen from Fig. 2B, at high concentrations of acetonitrile in water the difference $V_R - V_0$ is positive and from eqn. 4 the value of the excess adsorption in this region must be negative.

It can be seen that many questions can be answered with a strict thermodynamic approach to the chromatographic separation of complicated mixtures with multi-component eluents, accounting for the adsorption effects on each component.

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