

COUNTERCURRENT ELECTROPHORESIS ON PAPER  
IX. COMPARISON OF ELECTROPHORETIC, CHROMATOGRAPHIC AND  
STATIC DISTRIBUTION ISOTHERMS\*

KAREL PEJŠA, LUDMILA DOBÍHALOVÁ, ZDENĚK FIDLER AND JIRÍ DVOŘÁK

*Department of Physical Chemistry, Charles University,  
Prague (Czechoslovakia)*

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INTRODUCTION

The preceding parts of this series have shown the agreement of electrophoretic distribution isotherms with isotherms obtained by static measurements<sup>1</sup> and the suitability of the frontal method for the measurement of electrophoretic distribution isotherms<sup>2</sup>. The object of this paper is to show the agreement of all three kinds of distribution isotherms, *i.e.* electrophoretic, chromatographic and static and the influence of the swelling of the stationary phase on the results of the measurement of distribution isotherms. The frontal method<sup>2-4</sup> has been applied to the measurement of chromatographic distribution isotherms.

In order to calculate the values of the distribution isotherm  $q(c)$  (the amount adsorbed by 1 g of stationary phase) by our method the following quantities are measured:  $V$  = volume of the solvent passing through the column during the time  $t$ ,  $V_0$  = total volume of the column,  $x_0$  = weight of the stationary phase in the column,  $\rho$  = density of the stationary phase,  $c$  = concentration of the solution. Since the frontal method is involved, the derivation  $q'(c) = dq(c)/dc = q(c)/c$  and the DE VAULT<sup>5</sup> equation may be written in the form  $q(c) = [(\partial t/\partial x)_{c,w} - \alpha]c$ . Here  $\alpha$  is the pore space. Hence in the case of swelling material, 1 g of which takes up  $b$  ml of water we have the expression:

$$q(c) - bc = \left\{ \left[ \left( \frac{\partial t}{\partial V_0} \right)_c \frac{V}{t} - 1 \right] \frac{V_0}{x_0} + \frac{1}{\rho} \right\} c \quad (1)$$

For static measurement we already used<sup>1</sup> the equation:

$$q(c) - bc = \frac{V^*}{x} (c_0 - c) \quad (2)$$

where  $V^*$  is the volume of the solution,  $x$  the weight of the dry adsorbent,  $c_0$  the concentration of the original solution and  $c$  the concentration of the equilibrium solution.

Measurement of electrophoretic distribution isotherms for swelling material does not lead to values  $q(c)$ , but to values  $q(c) - bc$ . Thus the value of swelling water,

\* Part VIII: J. VACÍK AND M. JAKOUBKOVÁ, *J. Chromatog.*, 14 (1963) 456.

$b$ , can not be obtained by any of the methods for measuring the function  $q(c)$  nor by their combination. An independent method can be based on the comparison of the volume flow rate ( $dV/dt$ ,  $\text{cm}^3 \cdot \text{sec}^{-1}$ ) and the linear flow rate ( $dL/dt$ ,  $\text{cm} \cdot \text{sec}^{-1}$ ). The following equation then follows:

$$\frac{dV}{dt} = \rho S(\alpha - b) \frac{dL}{dt} \quad (3)$$

where  $S$  is the width of the paper strip,  $L$  the length in the direction of the flow, and  $\rho$  the weight of the paper in  $\text{g} \cdot \text{cm}^{-2}$ . The rate  $dL/dt$  can be measured by following a boundary on both sides of which the solutions possess the same adsorptive properties. The boundary of sodium phosphate solutions differing only in the radioactivity of phosphorus was used. The measurement mentioned leads to the definition of the value of swelling water as all the water that does not take part in the flow.

#### EXPERIMENTAL

The temperature was kept at  $20^\circ \pm 1^\circ$ , for all measurements. The structural parameters of the chromatographic papers used were taken from the preceding paper<sup>6</sup>. The concentrations are expressed in g/l.

##### *Material*

Ground glass (Simax), kieselguhr (Kaznějov),  $\text{Al}_2\text{O}_3$  (for chromatography, Lachema), polyamide, cellulose (Whatman) and Whatman Nos. 1, 2 and 4 chromatographic papers were used as the stationary phase. Loose materials (except cellulose) were purified by washing and sieve screening and their densities were determined by pycnometry. 0.1 and 1.0 *N* acetic acid (analytical reagent purity) was used as the mobile phase. The measurement of distribution isotherms was carried out for different dyes and the dye Kashmir Blue T.G. Extra (Bayer, Elberfeld, Germany) was used for reference. The isotope  $^{32}\text{P}$  added to non-active  $\text{Na}_3\text{PO}_4$  was used for measuring the swelling water.

##### *Chromatographic measurements*

A burette (10 ml) was filled with the stationary phase. A constant level above the stationary phase was automatically maintained by adding solution from the storage flask. The movement of the front was measured by reading the volume on the burette. The flow of the solvent was determined by measuring the volume of the liquid which passed through the column. The flow rate was kept constant throughout.

Paper chromatography was performed by the descending method in a vapour-saturated space. The movement of the front was read on the scale in units of length. Velocity was then expressed as the volume rate. The flow of solvent was determined by measuring the volume of liquid necessary to keep the level in the upper trough constant.

##### *Static measurements*

A paper strip and/or cellulose powder were dried ( $115^\circ$ , 4 h). To the weighed amount ( $x$ ) of paper or cellulose a volume ( $V^*$ ) of solution was added corresponding to the

ratio  $V^*/\lambda$  chosen. The paper was kept with the solution in closed glass vessels for 120 h. The original and final concentrations were determined by spectrophotometry at a suitable wave length.

#### Measurement of swelling water

This was performed in a wet chamber by means of a Geiger-Müller counting tube GM 30/50B, Tesla. The concentration of  $\text{Na}_3\text{PO}_4$  was 0.001 M.

### RESULTS AND DISCUSSION

An example of the direct results of frontal measurement is given in Fig. 1 and an example of a distribution isotherm for swelling material is given in Fig. 2. The values of  $q(c)$  were calculated using  $b = 0.66$ . In all cases of non-swelling materials, smooth curves were obtained whereas swelling materials resulted in curves with a maximum.

The curves  $c_0 - c$  obtained from static measurements deviate from the line  $c_0 = c$  under the influence of the transition of the dye into the cellulose phase and under the influence of swelling. In all cases the influence of the transition of the dye dominated and  $c < c_0$ . An example is given in Fig. 3.

By performing both the measurements described for the dye Kashmir Blue on Whatman No. 4 chromatographic paper these results can be compared with the values obtained electrophoretically in the preceding paper<sup>2</sup> (see Fig. 4). The course of the curves is seen to be identical and the absolute values show a satisfactory agreement.

The swelling water was found to be 0.54, 0.66 and 0.65 in 0.0, 0.1 and 1.0 N acetic acid, respectively (water vapour saturated atmosphere, 20°, Whatman No. 4 paper).

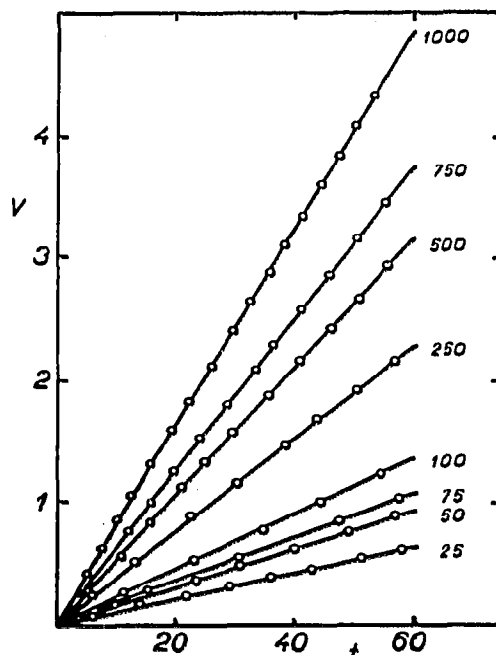


Fig. 1. Movement of the Methylene Blue front (0.1 N acetic acid, Whatman No. 4 paper) at different dye concentrations (indicated on the curves in p.p.m.).

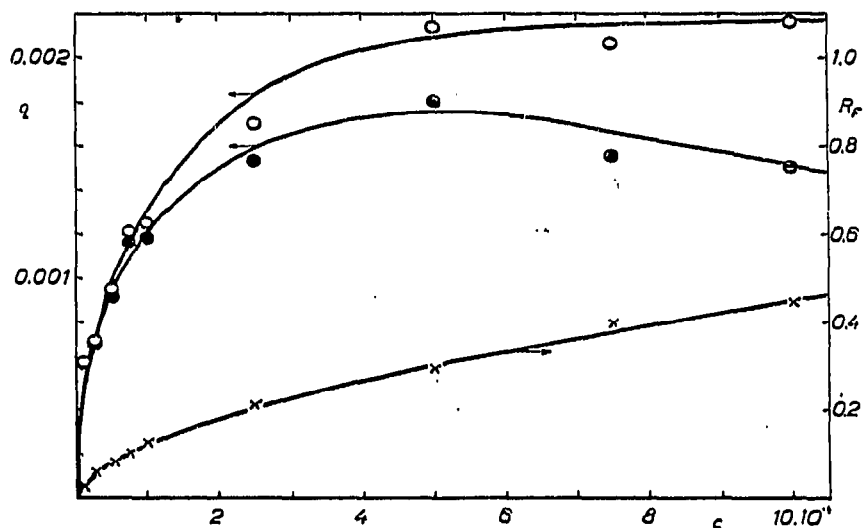


Fig. 2. Chromatographic isotherms  $q(c)$  (O),  $q(c) - bc$  (●), and the concentration dependence of  $R_F$  (x) for Methylene Blue and cellulose powder in 0.1 N acetic acid.

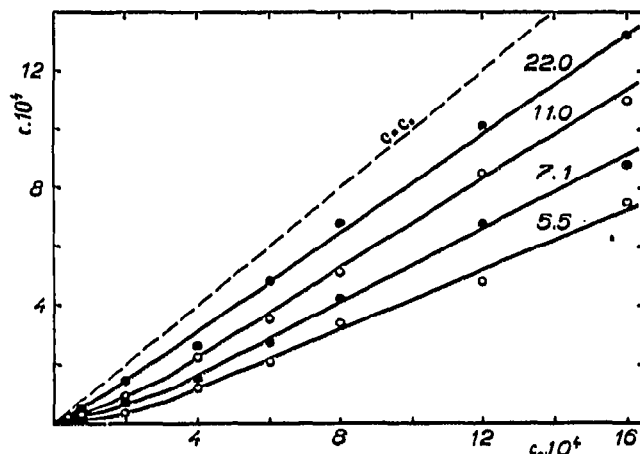


Fig. 3. Static relationship between equilibrium concentration  $c$  and original concentration  $c_0$  for Naphthol Black and Whatman No. 4 paper in 0.1 N acetic acid. Ratio  $V^*/x$  indicated at the curves.

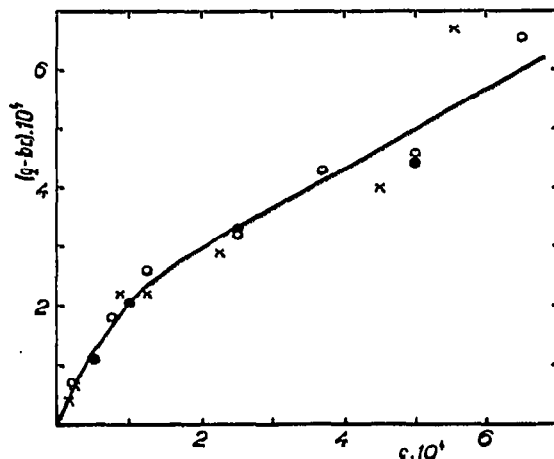


Fig. 4. Comparison of electrophoretic (O), chromatographic (●) and static (x) distribution isotherms for Kashmir Blue and Whatman No. 4 paper in 0.1 N acetic acid.

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## SUMMARY

It has been shown that swelling of the stationary phase influences the static and chromatographic measurements of distribution isotherms. If the swelling of paper is disregarded when calculating the adsorbed amount from the results of both static and chromatographic measurements the values obtained are diminished. The distribution isotherm can even show a maximum. The value of swelling water was measured for the conditions used. For measurement of chromatographic distribution isotherms the frontal method was applied. The identity of the electrophoretic, chromatographic and static distribution isotherms was proved using Kashmir Blue in 1.0 *N* acetic acid on Whatman No. 4 chromatographic paper as an example.

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