# LIQUID ADSORPTION CHROMATOGRAPHY WITH MIXED MOBILE PHASES

## III. INFLUENCE OF MOLECULAR AREAS OF SOLVENTS AND CHROMATOGRAPHED SUBSTANCES ON THE CAPACITY RATIO

M. JARONIEC, J. K. RÓŻYŁO and B. OŚCIK-MENDYK

*Institute of Chemistry, M. Curie-Skłodowska University, 20031 Lublin (Poland)*

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## SUMMARY

A new equation for the capacity ratio in liquid adsorption chromatography with a two-component mobile phase, involving the differences in molecular areas of solvents and chromatographed substances, has been derived. The special case of this equation is the logarithmic relationship of Snyder and Soczewiński, which is frequently used for describing the dependence of the capacity ratio on the composition of the mobile phase. According to our treatment the relationship of log (capacity ratio) to log(ratio of mole fractions of the more efficient solvent in the stationary and mobile phases) is linear for liquid mixtures by unit activity coefficients. This dependence has been examined by using thin-layer chromatographic data and excess adsorption isotherm for the binary solvent mixture. The excess adsorption data were used to calculate the mole fractions of the more efficient solvent in the stationary phase for different compositions of the mobile phase.

## INTRODUCTION

In two preceding papers of this series[1,2] the effects of non-ideality of the mobile phase and energetic heterogeneity of the adsorption system have been discussed. The theoretical considerations were presented by assuming identical molecular areas for the solvents and chromatographed substances.

In liquid adsorption chromatography the following expression for the capacity ratio[3-6]

$$\log k'_{(AB)S} = \log k'_{(B)S} - n\log x_B \tag{1}$$

is frequently used[7-9] to describe the experimental data $k'_{(AB)S}$ vs. $x_B$. In eqn. 1 $k'_{(AB)S}$ and $k'_{(B)S}$ are the capacity ratios of the chromatographed substance(S) in the binary mobile phase A–B and pure solvent B, respectively; $x_B$ is the mole fraction

of the more efficient eluent in the binary mobile phase A–B; and $n$ is the ratio of adjusted molecular areas of substance S and solvent B.

A discussion of the derivation of eqn. 1 was presented in ref. 10. In this paper it has been stated that the treatment of Jandera and Churáček[4] gives the full mathematical justification for eqn. 1 with $n = 1$. However, Soczewiński's approach to liquid adsorption chromatography leads to eqn. 1 with $n \neq 1$. Eqn. 1 predicts the correct value for $x_B = 1$; however, for low values of $x_B$ it is not valid.

In this paper a more general expression for the capacity ratio is derived. This expression involves the differences in molecular areas of solvents and chromatographed substances. The theoretical discussion is presented for homogeneous adsorbent surfaces and binary mobile phases.

## THEORETICAL

The chromatographic process for the substance S in the binary mobile phase A–B relates to the adsorption from a three-component liquid mixture A–B–S, when the concentration of S is infinitely low. The adsorption from A–B–S may be represented by the following reactions:

$$r\,B_m + A_s \rightleftarrows r\,B_s + A_m \tag{2}$$

$$p\,S_m + A_s \rightleftarrows p\,S_s + A_m \tag{3}$$

$$p\,S_m + r\,B_s \rightleftarrows p\,S_s + r\,B_m \tag{4}$$

where $r$ and $p$ are the ratios of the molecular area of A to those of B and S, respectively; the subscripts m and s refer to the mobile and surface phases. The equilibrium constants $K_{BA}$ and $K_{SA}$, characterizing reactions 2 and 3, are expressed as follows:

$$K_{BA} = x_A y_B^r / y_A x_B^r \tag{5}$$

and

$$K_{SA} = x_A y_S^p / y_A x_S^p \tag{6}$$

where $x_i$ and $y_i$ ($i = $ A,B,S) are the mole fractions of the $i$-th component in the mobile and surface phases, respectively. Combination of eqns. 5 and 6 gives the following expression for the equilibrium constant $K_{SB}$:

$$K_{SB} = K_{SA}/K_{BA} = (y_S/x_S)^p \cdot (x_B/y_B)^r \tag{7}$$

which characterizes reaction 4.

Thus, from eqn. 7 we have:

$$(y_S/x_S)^p = K_{SB}\,(y_B/x_B)^r \tag{8}$$

The capacity ratio, $k'$, is defined as follows:

$$k'_{(AB)S} = \beta(y_S/x_S) \tag{9}$$

where $\beta$ is the constant, in a first approximation characteristic for a given adsorbent and independent of the solvents in the mixture (cf. ref. 1).

Eqns. 8 and 9 give:

$$k'_{(AB)S} = \beta \, K_{SB}{}^{1/p} \, (y_B/x_B)^n \tag{10}$$

and

$$R_{M(AB)S} = \log k'_{(AB)S} = C + n \log(y_B/x_B) \tag{11}$$

where

$$n = r/p \text{ and } C = \log(\beta \, K_{SB}{}^{1/p}) = \log k'_{(B)S} = R_{M(B)S} \tag{12}$$

In eqns. 11 and 12 the symbols $R_{M(AB)S}$ and $R_{M(B)S}$ denote $R_M$ values, used in thin-layer chromatography (TLC), for the A–B solvent mixture and the pure solvent B, respectively.

At higher values of $x_B$ the mole fraction $y_B$ tends to unity, and then eqn. 11 reduces to eqn. 1, which was discussed by Snyder[3], Soczewiński[5], Jandera and Churáček[4]. However, for $r = p$ (identical molecular areas of all components), eqn. 11 becomes:

$$\log k'_{(AB)S} = C + \log(y_B/x_B) \tag{13}$$

According to the theory of liquid adsorption the mole fraction of the component B may be expressed as follows[11]:

$$y_B = \frac{x_B/k'_{(B)S}}{x_A/k'_{(A)S} + x_B/k'_{(B)S}} \tag{14}$$

Combining eqns. 13 and 14 and taking into account the definition of $C$ (cf. eqn. 12), we have:

$$\frac{1}{k'_{(AB)S}} = \frac{x_A}{k'_{(A)S}} + \frac{x_B}{k'_{(B)S}} \tag{15}$$

Eqn. 15 was discussed in refs. 10 and 11; it results from Snyder's theory of liquid adsorption chromatography[3], modified recently by Jaroniec et al.[11] It follows from eqn. 15 that the dependence $1/k'_{(AB)S}$ vs. $x_B$ should be linear. Such a dependence for $k'_{(AB)S}$ was discussed by Scott and Kucera[12] and Soczewiński[5] via different theoretical approaches.

For practical purposes another form of eqn. 11 may be more useful. In adsorption from solutions the excess adsorption data are directly measured. Therefore, the mole fraction $y_B$ may be replaced by the excess of the component B in the surface phase, i.e.,

$$y_B = n_B^e/n^s + x_B \tag{16}$$

where $n^s$ is the total number of moles of all components in the surface phase.

Eqns. 11 and 16 give the following relationship:

$$R_{M(AB)S} = \log k'_{(AB)S} = C + n \log[n_B^e/(n^s x_B) + 1] \tag{17}$$

This relationship gives the chance to compare experimental data obtained by two independent methods: the dependences $k'_{(AB)S}$ vs. $x_B$ and $R_{M(AB)S}$ vs. $x_B$ may be obtained by high-performance liquid chromatography and TLC, respectively, whereas the excess adsorption data $n_B^e$ vs. $x_B$ may be measured by a classical method[13]. The excess adsorption isotherm is characteristic for a given binary mobile phase A–B, because the chromatographed substance S appears in the liquid mixture at infinitely low concentration and its influence on $n_B^e$ values may be neglected.

These considerations may easily be generalized for non-ideal liquid mixtures. Let $g_i y_i$ and $f_i x_i$ denote the activities of the $i$-th component in the surface and mobile phases, respectively; then, $g_i$ and $f_i$ are the activity coefficients. Eqn. 8 may be rewritten as follows:

$$(y_S/x_S)^p = K_{SB}(y_B/x_B)^r(g_B/f_B)^r(f_S/g_S)^p \tag{18}$$

The activity coefficients $g_B$, $g_S$, $f_B$ and $f_S$ refer to a three-component solution A–B–S, and they may be approximated by expressions known from the literature[13,14]. Eqns. 11 and 17 describe the adsorption model, when both surface and mobile phases are ideal. Eqn. 18 generates equations analogous to eqns. 11 and 17 but for non-ideal phases; they are:

$$\log k'_{(AB)S} = C + n \log(y_B/x_B) + n \log(g_B/f_B) + \log(f_S/g_S) \tag{19}$$

and

$$\log k'_{(AB)S} = C + n \log\left(\frac{n_B^e}{n^s x_B} + 1\right) + \log[(g_B/f_B)^n(f_S/g_S)] \tag{20}$$

In the adsorption from solutions the ideality of the surface phase and non-ideality of the mobile phase are frequently assumed[15]. For such an adsorption model eqns. 19 and 20 are reduced to the following expressions:

$$\log k'_{(AB)S} = C + n \log(y_B/x_B) + \log(f_S/f_B^n) \tag{21}$$

and

$$\log k'_{(AB)S} = C + n \log\left(\frac{n_B^e}{n^s x_B} + 1\right) + \log(f_S/f_B^n) \tag{22}$$

For higher values of $x_B$ the mole fraction $y_B$ is close to unity and eqns. 19 and 21 become:

$$\log k'_{(AB)S} = C - n \log x_B + \log[(g_B/f_B)^n(f_S/g_S)] \tag{23}$$

and

$$\log k'_{(AB)S} = C - n \log(x_B f_B) + \log f_S \tag{24}$$

Eqn. 24 is identical with that obtained by Slaats *et al.*[16] in terms of Snyder's theory of liquid adsorption chromatography.

The most general equation is eqn. 19; this equation may be written in a slightly different form involving the excess adsorption data, $n_B^e$ (see eqn. 20). Eqns. 1, 11, 13, 15, 17, 21, 22, 23 and 24 are special cases of eqn. 19. Applying the analytical expressions for the activity coefficients, appearing in eqns. 19–24, we obtain a new form of these equations. The simple forms of eqns. 19–24 may be obtained, when for the activity coefficients the expressions resulting from the theory of regular solutions will be used[1]. In this paper eqn. 17 has been examined by using TLC data and excess adsorption isotherm, and compared with eqn. 1.

## EXPERIMENTAL

Experiments were carried out to test eqn. 17 for the following chromatographic system: solute (S)–cyclohexane (A)–benzene (B) on silica gel H. TLC measurements were performed for *o*-nitrophenol and four dichlorophenol isomers.

*Chemicals*

Benzene, cyclohexane and *o*-nitrophenol (puriss. grade) were obtained from Polskie Odczynniki Chemiczne (P.O.Ch., Gliwice, Poland). Four dichlorophenol isomers (for Lab. use only) were obtained from L. Light (Colnbrook, Great Britain). The silica gel H (type 60) was from Merck (Darmstadt, G.F.R.).

*TLC measurements*

The TLC measurements were made under thermostatted conditions at 20°. Silica gel H was the adsorbent, and cyclohexane-benzene was the mobile phase. The adsorbent layers were 0.3 mm thick and were activated for 2 h at 135°. The chromatograms were developed by the ascending technique to a distance of 16 cm. The spots were visualized using the universal reagent[17]. The $R_F$ values were converted into $R_M$ values using the equation of Bate-Smith and Westall[18]. Figs. 1 and 2 show the relationship of the $R_M$ values to the composition of the mobile phase for the investigated substances.

*Measurements of excess adsorption isotherm*

The excess adsorption isotherm for cyclohexane–benzene on silica gel H was measured at 20°. The silica gel used was the same as in the TLC measurements. A binary solution of known composition was prepared for each experimental point. The amounts of liquid and adsorbent were measured gravimetrically. Equilibrium was obtained by shaking the flask with the adsorbent and liquid mixture in a water bath for 24 h. Compositions of binary liquid mixtures were measured with a gas chromatograph, type Chromatron GCHF 18.3, with a thermal conductivity detector. Hydrogen, purified by using a filter containing molecular sieve 5A pellets, was used as the carrier gas at a flow-rate of 50 cm³/min. A column, 100 cm × 4 mm I.D., containing the partition packing (10% silicone oil on Polysorb) was used. The chromatographic measurements were performed at 80° ± 0.1°.

The excess isotherm was calculated using the procedure described in ref. 13.

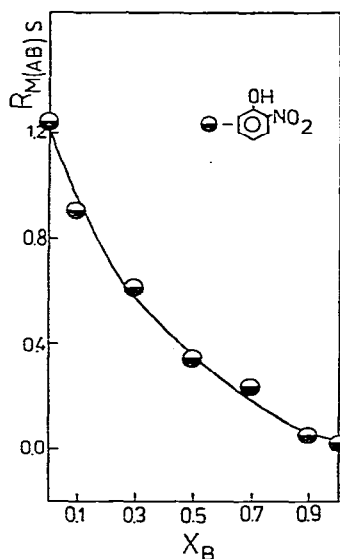The excess isotherm for benzene–cyclohexane on silica gel is shown in

Fig. 1. $R_{M(AB)S}$ *vs.* $x_B$ dependence for *o*-nitrophenol in benzene (B)–cyclohexane (A) solvent mixture on silica gel H.
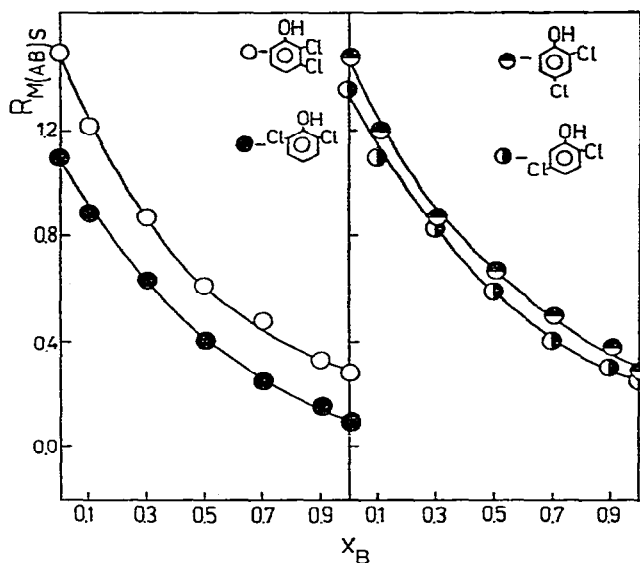
Fig. 2. $R_{M(AB)S}$ *vs.* $x_S$ dependences for dichlorophenols in benzene (B)– cyclohexane (A) solvent mixture on silica gel H. ○, 2,3-Dichlorophenol; ◐, 2,4-dichlorophenol; ◑, 2,5-dichlorophenol; ●, 2,6-dichlorophenol.

Fig. 3. The surface phase capacity $n^s$ is 2.05 mmole/g for benzene–cyclohexane adsorption. The value of $n^s$ was evaluated by the graphical method of Schay and Nagy[19].
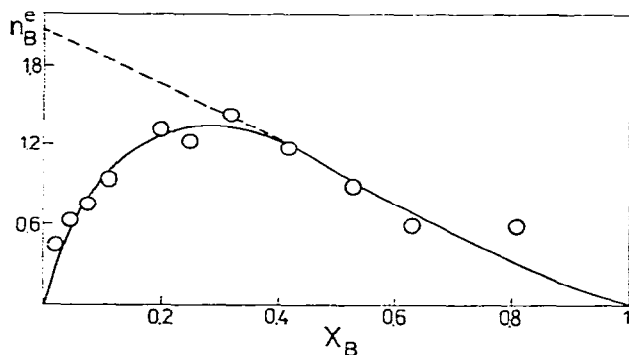


Fig. 3. Excess adsorption isotherm for benzene from cyclohexane on silica gel at 20°.

## RESULTS AND DISCUSSION

In this paper the TLC data for the mobile phase benzene–cyclohexane are analysed. This mobile phase shows only slight deviations from ideal behaviour. Therefore, these experimental data may be described by means of an equation assuming the ideality of the mobile phase. For this purpose we shall examine eqns. 17 and 1; the first is the exact equation for LC with a two-component ideal

mobile phase; the second is an approximate one. Up to now eqn. 1 has frequently been used to characterize the liquid chromatographic (LC) data. In this paper the chromatographic parameters obtained from both equations are compared. Such a comparison gives information about the applicability of eqns. 17 and 1 to experimental chromatographic systems.

From TLC measurements the experimental dependence $R_{M(AB)S}$ vs. $x_B$ has been obtained. However, the adsorption measurements gave the dependence $n_B^e$ vs. $x_B$. Using these experimental functions the dependence $R_{M(AB)S}$ vs. $\log [n_B^e/(n^s x_B) + 1]$ may be plotted according to eqn. 17. The ordinate of this linear dependence determines the $R_{M(B)S}$ value, and the slope gives the ratio of adjusted molecular areas of solute and solvent. In Fig. 4 the straight lines, plotted according to eqn. 17, are shown for substances chromatographed in benzene–cyclohexane as mobile phase. It follows that experimental points plotted according to eqn. 17 fall on straight lines. In Table I the parameters $C$ and $n$ of the straight lines of Fig. 4 are summarized. In this table the $R_M$ values for pure solvent B are also given and compared with the $C$ values, because for $n_B^e = O$, i.e. $x_B = y_B = 1$, the parameter $C$ is equal to $\log k'_{(B)S} = R_{M(B)S}$. It can be seen from Table I that the values of $C$ are very close to the $R_{M(B)S}$ values.
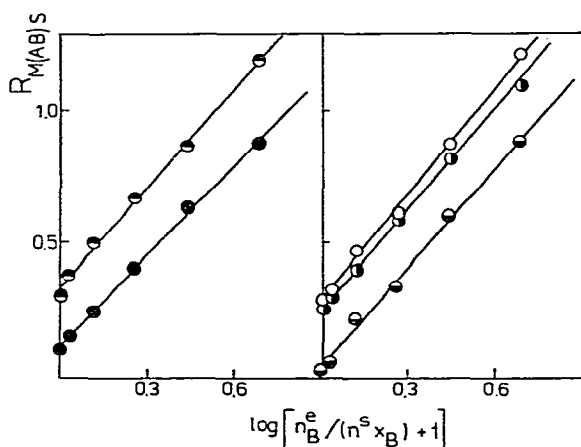


Fig. 4. Linear dependences $R_{M(AB)S}$ vs. $\log[n_B^e/(n^s x_B) + 1]$ for substances investigated plotted according to eqn. 17. The labelling of substances is as in Fig. 2 and Fig. 1.

TABLE I

PARAMETERS OF THE STRAIGHT LINES PRESENTED IN FIGS. 4 AND 5 FOR o-NITRO-PHENOL AND DICHLOROPHENOL ISOMERS IN BENZENE (B)–CYCLOHEXANE (A) ON SILICA GEL H

| Substance S | $R_{M(A)S}$ | $R_{M(B)S}$ | Eqn. 17 | | Eqn. 1 | |
|---|---|---|---|---|---|---|
| | | | $C$ | $n$ | $C$ | $n$ |
| o-Nitrophenol | 1.24 | 0.02 | 0.04 | 1.24 | 0.04 | 1.14 |
| 2,3-Dichlorophenol | 1.50 | 0.28 | 0.29 | 1.30 | 0.28 | 1.16 |
| 2,4-Dichlorophenol | 1.48 | 0.29 | 0.34 | 1.22 | 0.30 | 1.20 |
| 2,5-Dichlorophenol | 1.36 | 0.25 | 0.25 | 1.28 | 0.25 | 1.10 |
| 2,6-Dichlorophenol | 1.10 | 0.09 | 0.12 | 1.14 | 0.09 | 1.04 |

It has been shown that the well known eqn. 1 is a special case of eqn. 17. Fig. 5 presents the linear dependences plotted according to eqn. 1. According to the theoretical prediction the experimental points show deviations from the straight line at low concentrations of $x_B$, whereas eqn. 17 works in the whole region of concentrations of the more efficient eluent. The parameters $n$ and $C$, evaluated according to eqn. 1, are summarized in Table I and compared with those calculated using eqn. 17. It follows from this comparison that the approximate eqn. 1 may be used to determine the parameter $n$ for ideal adsorption systems, because both values of $n$, obtained from eqns. 1 and 17, are similar.
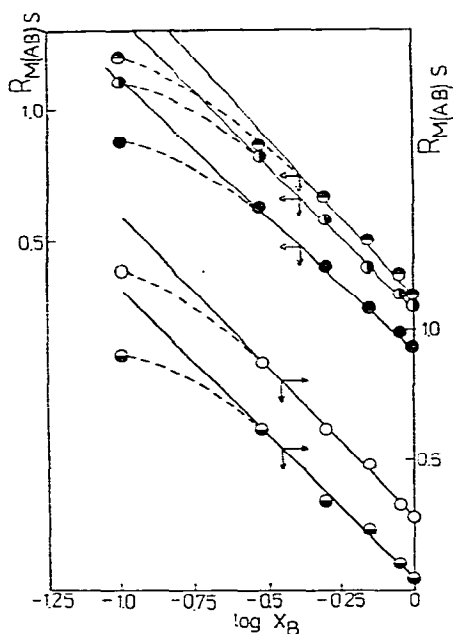


Fig. 5. Linear dependences $R_{M(AB)S}$ vs. $x_B$ for substances investigated plotted according to eqn. 1. The labelling of substances is as in Fig. 2 and Fig. 1.

Interesting information obtained from analysis of the parameter $n$, is the ratio of the molecular areas of substance and solvent. The numerical values of $n$ may be useful in discussion of the orientation of molecules on the adsorbent surface. The molecular areas of the substances chromatographed in benzene–cyclohexane mobile phase are greater than those for the benzene admolecule. Since molecules of the chromatographed substances are larger than those of benzene, the fact that $n > 1$ may be due to a similar orientation of molecules of benzene and dichlorophenol isomers on the surface. Another conclusion may be drawn on the basis of comparison of $n$ values for all dichlorophenol isomers investigated since these values are similar, it means that the mechanisms of adsorption are also similar.

It seems that experimental dependences plotted according to eqn. 17 confirm the applicability of this equation for analysing TLC data. It is very interesting that this good agreement of theory with experimental points was observed for data

obtained by two separate techniques, namely, the correlation between $R_M$ and $n_B^e$ values was investigated. More detailed analysis of the parameter $n$ will be made in the future on the basis of a great number of chromatographic systems.

CONCLUSIONS

It has been shown that eqn. 17 describes well the experimental dependence $R_{M(AB)S}$ vs. $\log[n_B^e/(n^s x_B) + 1]$ in the whole region of concentrations of $x_B$. For LC with two-component ideal mobile phases this equation exactly predicts the chromatographic parameters $n$ and $R_{M(B)S} = C$, the physical meaning of which has been explained in the preceding section. Table I shows that values of the parameters $n$ and $C$ obtained by means of eqns. 17 and 1 are very similar. These results show that eqn. 1, used by many authors to characterize chromatographic systems, predicts good values of $n$ and $C$.

A further conclusion concerns the theoretical description of LC with two-component mobile phases. According to this formulation we can derive all the equations which were used to describe LC with two-component ideal and non-ideal mobile phases. Moreover, this formulation creates new possibilities for derivation of new expressions describing LC with non-ideal mobile phases.

ACKNOWLEDGEMENTS

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