

Some problems of the optimization of the separation process in liquid chromatography*

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One of the optimization theory worked out by Ościk has been used for some physicochemical studies and optimization of chromatographic process. On the basis of the theoretical equations a retention in binary mobile phases can be predicted with good agreement for a lot of chromatographic systems. The influence of the different properties of chromatographic systems can be determined by use of the discussed optimization theory.

The possibilities of the use of the theory for some physicochemical studies as determination of the hydrophobicity have been demonstrated too.

1. INTRODUCTION

An extensive use of liquid chromatography for example in industrial, scientific and clinical laboratories has created a need to develop simple, reliable methods for at least an approximate prediction of separation conditions of mixtures. There are a lot of methods on the basis of which we can obtain retention value of a given substance in a given chromatographic system or differences of retention values of two adjacent solute spots on a chromatogram. Problem of optimization of separation conditions is as old as chromatography. It was born together with chromatography method. There are many methods on the basis on which we can obtain optimum separation conditions of a given solute

* Dedicated to Professor Emil Chibowski on the occasion of his 65th birthday.

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mixture. Generally, the optimization methods can be classified in two groups: theoretical and chemometric optimization methods [1-3].

Theoretical methods discuss retention mechanism, interactions in chromatographic system and their influence on solute retention. The fundamental equations in a greater part of optimization theories describe chromatographic system with binary mobile phases. For multi component mobile phases general theoretical equations are very complicated from the mathematical point of view or they need a lot of parameters, the determination of which is often a very difficult or time-consuming process. On the basis of chromatographic theory we can not only predict retention data, but by analyzed values of parameters of given theory we can determine which kind of interactions in given chromatographic system play a significant role in separation process. In liquid chromatography stationary phase can be closed in column (or capillary) – this kind of liquid chromatography is called column (or capillary) liquid chromatography. When a stationary phase forms thin layer on the inert support (glass, aluminum or polyethylene) – the kind of the liquid chromatography is called thin layer chromatography. In “*Handbook of TLC*” in the first chapter Sherma writes that “thin layer chromatography and paper chromatography comprise “planar chromatography”. TLC is the simplest of all widely used chromatographic methods to perform. A suitable closed vessel containing solvent and the coated plate are all that are required to carry out separations and qualitative and semi-quantitative analysis. With optimization of techniques and materials and the use of available commercial instruments, highly efficient separations and accurate and precise separations by employing specialized layers, apparatus and techniques” [1].

Due to these reasons thin layer chromatography can be a useful method to teste an optimization theories, because it is a cheap and non time-consuming chromatographic technique.

2. THERMODYNAMIC THEORY (OŚCIK'S EQUATION)

From a lot of optimization theories applied in our laboratory one of them are tested so called “*thermodynamic theory*” because it is based on the thermodynamics of conformal solutions [4-8].

The thermodynamic theory of adsorption from multicomponent solvent system, makes it possible to combines R_M values of a chromatographed solute with the composition of a mobile phase and the adsorption parameters of the given chromatographic system, resulting in the general equation:

$$R_M = \sum (x_i R_{Mi}) + \sum A_z (x_i - y_i) \quad (1)$$

In which: R_M is the value of solute obtained by using an i -component mobile phase, R_{Mi} is the R_M value of solute in pure solvent “ i ”, y_i is the volume fraction of more polar component in surface phase, x_i is the volume fraction of more polar component in bulk phase, $(y_i - x_i)$ is the adsorption excess of more polar component. This value can be obtained experimentally from isotherms of excessive adsorption or from the distribution function of the more polar component of mobile phase.

$A_z = \log k_{ij}$ represents intermolecular interactions between molecules of chromatographed substance and two components of mobile phase. The A_z value can be calculated by fitting the parameter to the basic equation or in some cases by experimental way.

In binary mobile phases the equation 1 can be described in the following form:

$$R_{M12} = x_1 \Delta R_{M12} + (y_1 - x_1) (\Delta R_{M12} + A_z) + R_{M2} \quad (2)$$

where “1” is the more polar component in binary mobile phase, $\Delta R_{M12} = R_{M1} - R_{M2}$ is the difference between R_M values of given solute in pure components of binary mobile phases.

$(y_1 - x_1)$ expresses the adsorption excess of more polar component “1” of the binary mobile phase and can be determined from adsorption isotherm [9-12] or y_1 value can be obtained [13-16] by using Kisielew and Ewerett equation:

$$y_1 = (K_1 x_1) / (x_2 + K_1 x_1) \quad (3)$$

K_1 is the distribution function of component “1” of the mobile phase. It may be assumed that for ideal or conformal regular mixtures K_1 can be determined from chromatographic data:

$$-\log K_1 = (\sum \Delta R_{M12s}) / n \quad (4)$$

where ΔR_{M12s} is the difference between R_M values in pure solvents for given chromatographed substance “ s ”, n -number of tested substance.

For obtaining of $R_{M1,2}$ value for given binary mobile phase, K_1 and A_z values ought to be determined.

The dependence of parameters K_1 and A_z of equation 2 on the structure of chromatographed substances was studied in systems with inactive solvents. The following substances of the group N, according to Pimental and McClellan classification [17] were chromatographed: naphthalene, anthracene, pyrene, chrysene, and 3,4-benzopyrene. Binary solvents N-N type such as: n-heptane – carbon tetrachloride, n-heptane – benzene, carbon tetrachloride – benzene and

carbon tetrachloride – toluene were used as mobile phase in all concentration range, Silica gel 60 (Merck) for TLC was used as adsorbent.

R_M values of the substances investigated in selected binary mobile phases were calculated from equation (2) (A_z values of the equation were calculated numerically – the best fit method). In all tested chromatographic system there was a good agreement of the calculated and experimental results. Thus in inactive chromatographic systems, in which do not exist specific intermolecular interactions the equation (2) allows to determine a theoretical function $R_{M12} = f(x_1)$. Attention should also be drawn to the simple relationship between R_M values and number of aromatic rings in the solute molecule. The smallest R_M values are obtained for substances of a relatively low number of aromatic rings.

K_1 parameters depend on the sitting area of the solute molecule – Figure 1. Stronger relationship between K_1 and sitting area of the solute molecule is observed for mobile phase for which differences between solvent strength parameter (S_T) of mobile phase components are the highest.

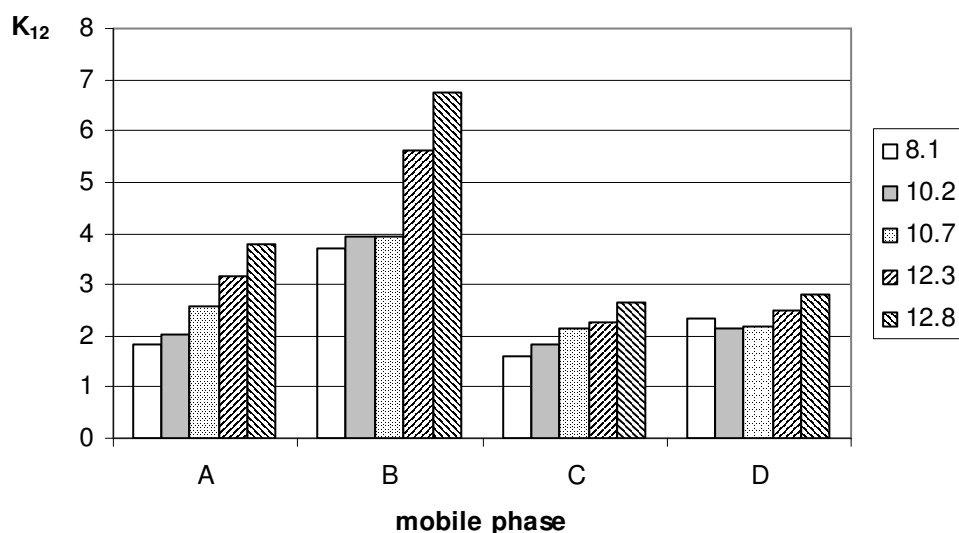


Fig. 1. K_{12} values of polyaromatic hydrocarbons with different sitting area (A_s) [\AA^2] in given mobile phases: A: $C_7H_{16}-CCl_4$, $C_7H_{16}-C_6H_6$, $CCl_4-C_6H_6$, $CCl_4-C_6H_5CH_3$. Chromatographed PAH (in order of increasing A_s values): naphthalene, anthracene, pyrene, chrysene, 3,4-benzopyrene.

In Figure 2. A_z values of tested aromatic hydrocarbons are presented. Positive A_z values mean that solute stronger interacts with more polar component of mobile phase, negative – that solute stronger interact with less polar component of binary mobile phase.

From the theoretical point of view K_1 ought to be characteristic for chromatographic system adsorbent – mobile phase (adsorption equilibrium constant). In the same mobile phase some dichlorophenol derivatives have been studied. K_1 values obtained on the basis of the equation 4 generally are similar in given mobile phase.

This is reflected by the values of function $R_{M12} = f(x_1)$ of the substances studied, calculated not only from individual K_1 values but also from averaged K_1 values obtained as arithmetical average of K_1 values calculated from chromatographic data (equation 4). In Figure 2 the K_{12} values obtained as average values of ΔR_{M12} values of PAH, and phenol derivatives and as average values of two group of the investigated solutes are compared. As it can be seen from the Figure 2 for mobile phase systems with the solvent at different elution strength the K_{12} parameters are different from the two groups of solutes. It means that in such systems a specific interactions existing between solutes and components of the mobile phases.

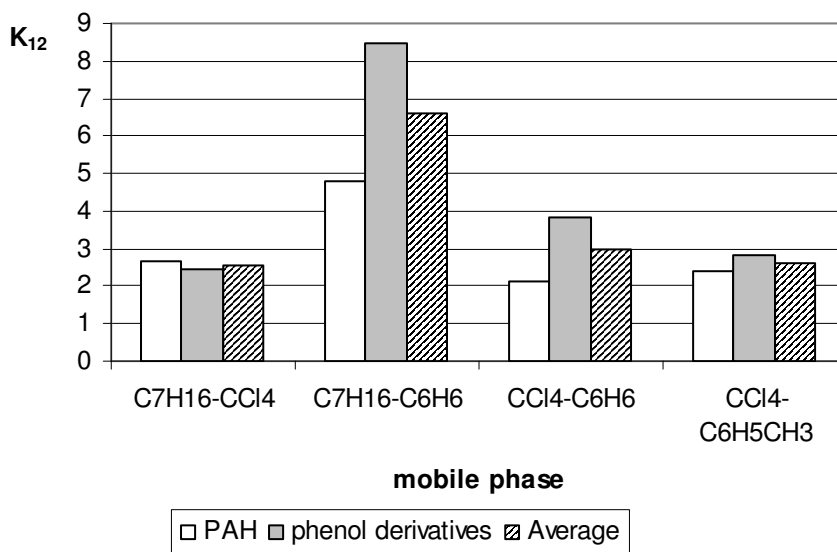


Fig. 2. K_{12} obtained as an average K_{12} values of PAH, phenol derivatives and as an average of K_{12} of all chromatographed solutes.

The comparison of the R_M values obtained experimentally and from equation (2) by use of K_{12} values calculated as average for PAH and all (PAH and phenol derivatives) solutes are presented in Figure 3. Relatively small differences between these values suggested that it was possible to use average K_1

values in system of binary inactive solvents (N-N) for a lot of chromatographed solutes, independently from the class of the solutes.

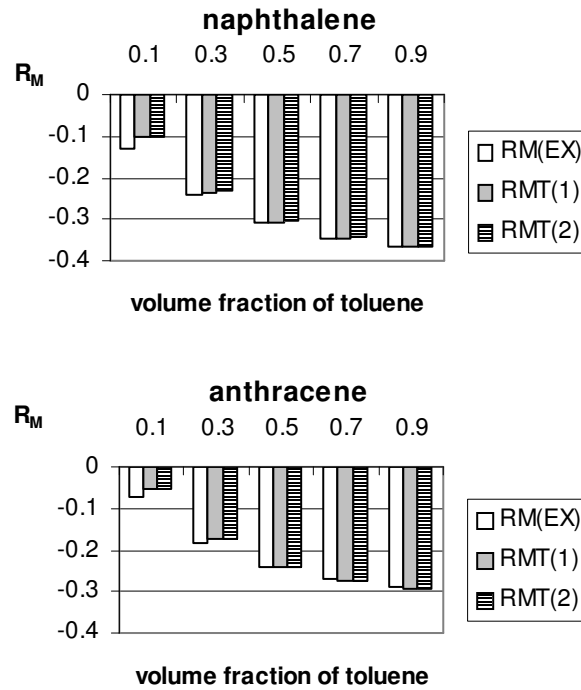


Fig. 3. Comparison of R_M values obtained experientially ($R_{M(EX)}$) and on the basis of the equation 2 by use K_{12} values obtained as an average of K_{12} values of PAH ($R_{M(1)}$) and as an average of K_{12} of PAH and phenol derivatives ($R_{M(2)}$). Mobile phase: $\text{CCl}_4\text{-C}_6\text{H}_5\text{CH}_3$.

The equation 2 can be used to predict separation of two substances as well. It is known that the separation of n-component mixture consists of the separation of adjacent spots on chromatographic plate. So the problem of separation of n-component mixture is limited to the separation of (n-1) pairs. The equation 2 can be used for prediction of separation results of two substances x and y .

For substances x the equation 2 assumes the form:

$$R_{M12}^x = x_1 \Delta R_{M12}^x + (y_1 - x_1) (\Delta R_{M12}^x + A_z^x) + R_{M2}^x \quad (2a)$$

For substance y the form

$$R_{M12}^y = x_1 \Delta R_{M12}^y + (y_1 - x_1) (\Delta R_{M12}^y + A_z^y) + R_{M2}^y \quad (2b)$$

Subtracting equation 2b from equation 2a we obtain:

$$\Delta R_{M12}^{xy} = x_1 \Delta(\Delta R_{M12})^{xy} + (y_1 - x_1) (\Delta(\Delta R_{M12})^{xy} + \Delta A_z^{xy}) + \Delta R_{M2}^{xy} \quad (5)$$

According to assumptions of the presented theory (for substances of similar structure $\Delta R_{M12}^x = \Delta R_{M12}^y$) equation 5 is reduced to a simpler form:

$$\Delta R_{M12}^{xy} = (y_1 - x_1) \Delta A_z^{xy} + \Delta R_{M2}^{xy} \quad (5a)$$

Where: $\Delta R_{M12}^{xy} = R_{M12}^x - R_{M12}^y$, $\Delta A_z^{xy} = A_z^x - A_z^y$, $\Delta R_{M2}^{xy} = R_{M2}^x - R_{M2}^y$.

As it can be seen from the equation 5 the ΔR_{M12}^{xy} values depend on:

- The properties of a mobile phase and adsorbent - $(y_1 - x_1)$ and K_1 values
- Structure of the substance and differences between interaction of substance x and y with component of mobile phases (ΔA_z^{xy} value).
- Interactions between the less active solvent in binary mobile phase and separated solutes (ΔR_{M2}^{xy})

The $(y_1 - x_1)$ values characterised a given (adsorbent–binary mobile phase) system via K_1 parameter. For a given chromatographic system ΔR_{M12}^{xy} values are a function of two variables: ΔA_z^{xy} and ΔR_{M2}^{xy} (Figures 4a,b,5).

Figures 4 a,b demonstrate the relationship between ΔR_{M12}^{xy} value and composition of mobile phase for different K_1 values.

When K_1 value increases, the separation of the pair of substances for the same A_z value is better- it means that the solutes can be easier separated in the mobile phases in which solvents have a different elution strength. With mobile phases in which K_{12} values is higher and chromatographed solutes have the same retention in the pure solvents, better separation of the chromatographed solutes in the mobile phase with less volume fraction of the more polar component is obtained. The second parameter which determined the separation of the solute pairs is ΔR_{M2}^{xy} -if ΔR_{M2}^{xy} value is higher, then separation of given solutes is simpler, it means that solutes which migrated with different velocity in less active component of mobile phase, can be easier separated. With increasing ΔR_{M2}^{xy} value ΔR_{M12}^{xy} is higher (Figure 5). Figure 6 shows separation of acridine and 8-aminoquinoline on magnesium silicate, and comparison of ΔR_{M12}^{xy} values obtained by experiment and on the basis of the equation 5a. From the analysis of the equation (5a) it follows, that for a good separation of the pair of solutes mobile phase ought to contain the solvent with a different elution strength and

second (less active solvent) can be so chosen, then interactions between the solvent molecules and separated solutes are different.

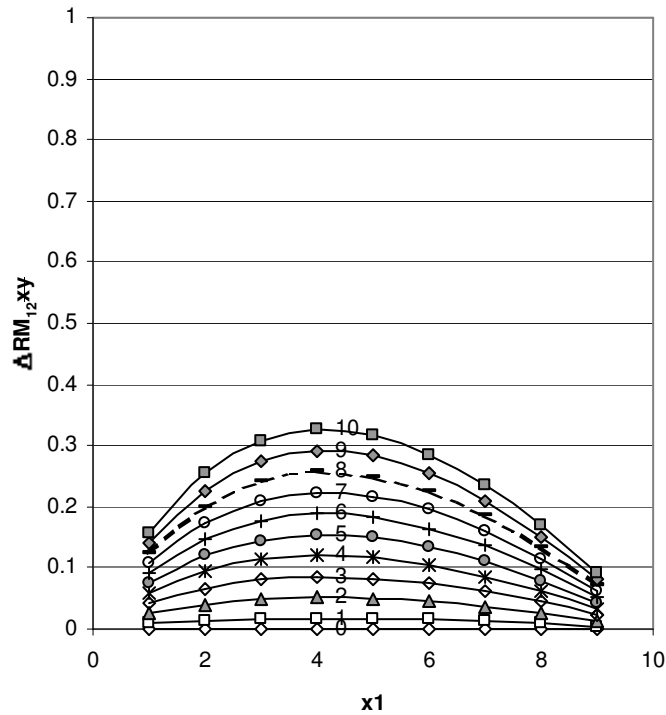


Fig. 4a. The relationship between ΔR_{M12}^{xy} values and composition of binary mobile phases calculated on the basis of eq. 5a for $K_1 = 2$, $\Delta R_{M2}^{xy} = 0$ and different ΔA_z^{xy} : 0 - $\Delta A_z^{xy} = 0$; 1 - $\Delta A_z^{xy} = 0.1$; 2 - $\Delta A_z^{xy} = 0.3$; 3 - $\Delta A_z^{xy} = 0.5$; 4 - $\Delta A_z^{xy} = 0.7$; 5 - $\Delta A_z^{xy} = 0.9$; 6 - $\Delta A_z^{xy} = 1.1$; 7 - $\Delta A_z^{xy} = 1.3$; 8 - $\Delta A_z^{xy} = 1.5$; 9 - $\Delta A_z^{xy} = 1.7$; 10 - $\Delta A_z^{xy} = 1.9$.

It is interesting to compare A_z values obtained from measurement of partition coefficient and obtained by chromatographic data. The value A_z can be calculated by fitting this parameter to the two parameter equation. According to its physical interpretation A_z parameter can be calculated from measurement of partition coefficient k_{12}^∞ . Because the components of the binary phase are completely miscible, the k_{12}^∞ coefficient, and hence the A_z value, can be obtained indirectly by measuring the partition coefficient of a given substance between component "1" of the mobile phase and water ($k_{1,w}^\infty$) and than between component "2" and water ($k_{2,w}^\infty$). k_{12}^∞ values can be calculated as follows:

$$k_{12}^\infty = k_{1,w}^\infty / k_{2,w}^\infty \quad (6)$$

The A_z values obtained as best fit parameter from the eq. 2 and A_z values obtained experimentally are presented in Figure 7.

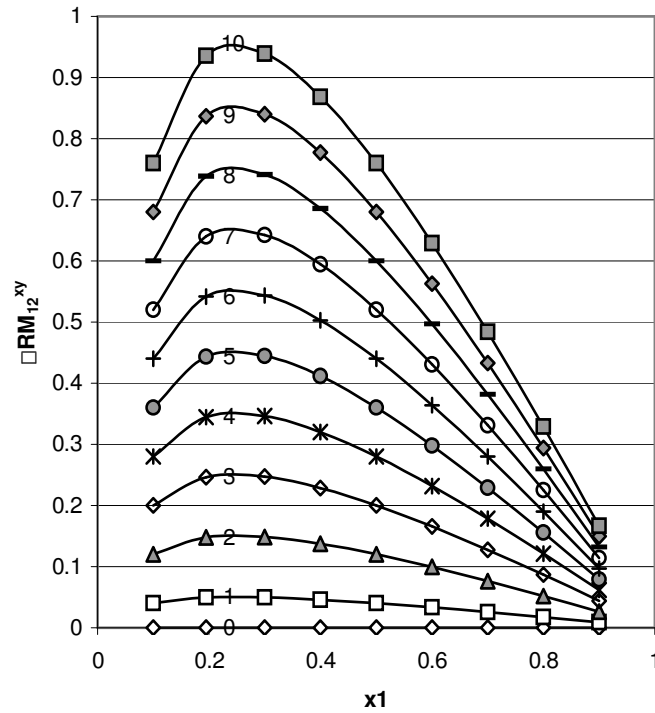


Fig. 4b. The relationship between ΔR_{M12}^{xy} values and composition of binary mobile phases calculated on the basis of eq. 5a for $K_1 = 7$, $\Delta R_{M2}^{xy} = 0$ and different ΔA_z^{xy} : 0 - $\Delta A_z^{xy} = 0$; 1 - $\Delta A_z^{xy} = 0.1$; 2 - $\Delta A_z^{xy} = 0.3$; 3 - $\Delta A_z^{xy} = 0.5$; 4 - $\Delta A_z^{xy} = 0.7$; 5 - $\Delta A_z^{xy} = 0.9$; 6 - $\Delta A_z^{xy} = 1.1$; 7 - $\Delta A_z^{xy} = 1.3$; 8 - $\Delta A_z^{xy} = 1.5$; 9 - $\Delta A_z^{xy} = 1.7$; 10 - $\Delta A_z^{xy} = 1.9$.

Values of A_z parameter calculated theoretically by fitting this parameter to the equation 2 ($A_{(ZC)}$) are different from A_z values obtained on the basis of the equation 6 (partition data $A_{(ZP)}$). The A_z values calculated from the partition coefficients for all tested solutes are higher than obtained as the best fit parameter. The R_M values calculated on the basis of parameter $A_{(ZC)}$ and $A_{(ZP)}$ are similar. It means that A_z value does not influence strongly R_M values. Experimental determination of the A_z parameter is a very long and time-consuming process, and via experiment only A_z values for binary system, in which components are immiscible in water can be determined. The data presented in the Figures 8 a,b demonstrated that A_z parameter can be obtained

via chromatographic measurement, which is a simpler process than partition experiment.

The differences between A_z values obtained from chromatographic and partition data suggest that the stationary phase influences the values of the parameters.

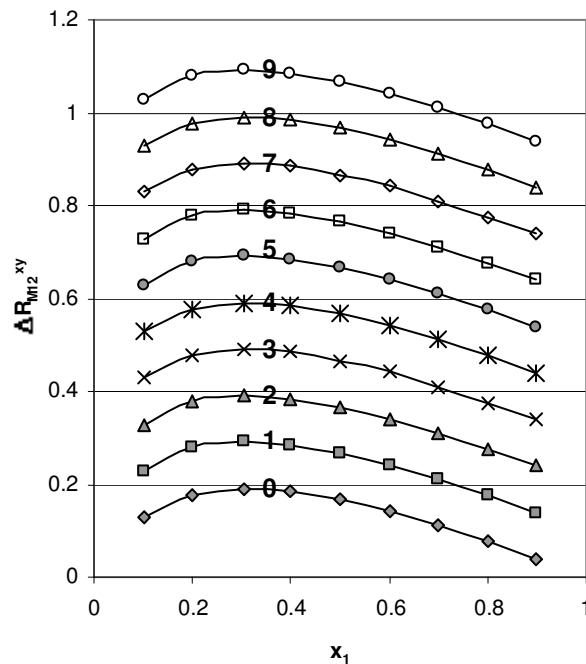


Fig. 5. The relationship between ΔR_{M12}^{xy} values and composition of binary mobile phases calculated on the basis of eq. 5a for $K_1 = 5$, $\Delta A_z^{xy} = 0.5$: and different ΔR_{M2}^{xy} : 0 - $\Delta R_z^{xy} = 0$; 1 - $\Delta R_z^{xy} = 0.1$; 2 - $\Delta R_{M2}^{xy} = 0.2$; 3 - $\Delta R_{M2}^{xy} = 0.3$; 4 - $\Delta R_{M2}^{xy} = 0.4$; 5 - $\Delta R_{M2}^{xy} = 0.5$; 6 - $\Delta R_{M2}^{xy} = 0.6$; 7 - $\Delta R_{M2}^{xy} = 0.7$; 8 - $\Delta R_{M2}^{xy} = 0.8$, 9 - $\Delta R_{M2}^{xy} = 0.9$.

In order to explain that problem the chromatograms they were developed on silica gel layers with different pore diameter (60, 100, 200 and 500 Å) and specific surface area.

Microporous structure of adsorbents is one of important parameters influencing the optimization of chromatographic process. The role of the microporous structure of the adsorbent in TLC has been discussed in detail by Snyder [18] and Geiss [19]. Practical possibilities of controlling the process of thin layer chromatography have also been discussed by Różyło [20]. It has been observed that R_M values (migration of the chromatographed solutes) depends on

the pore diameter (and specific surface area of the adsorbent) of silica gel (Figure 9 a,b) [21-23].

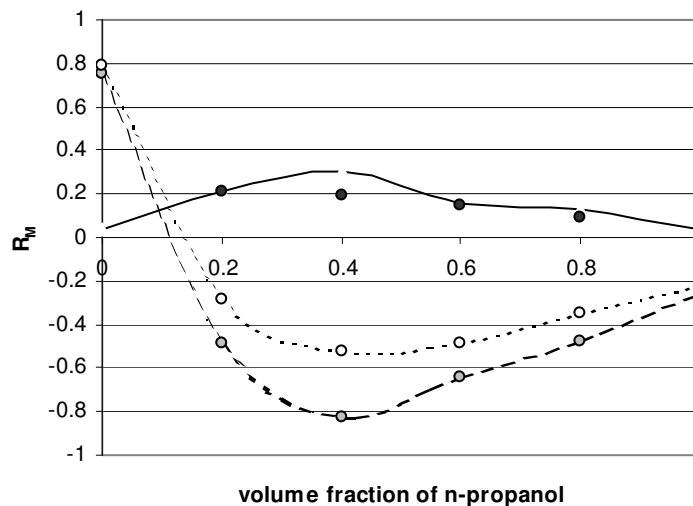


Fig. 6. The comparison of separation of acridine(▲) and 8 aminoquinoline (■) ΔR_{M12} obtained on the basis of experimental (line) and theoretical data obtained by eq. 5a (●). Adsorbent – silica gel, mobile phase: benzene – n-propanol.

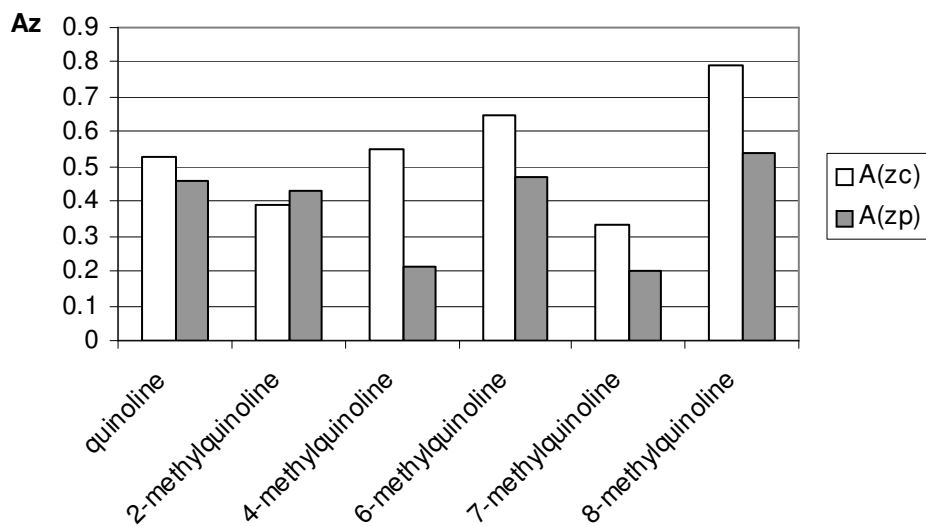


Fig. 7. The comparison of the A_z parameter obtained on the basis of the chromatographic ($A_{(zC)}$) and partition ($A_{(zP)}$) data.

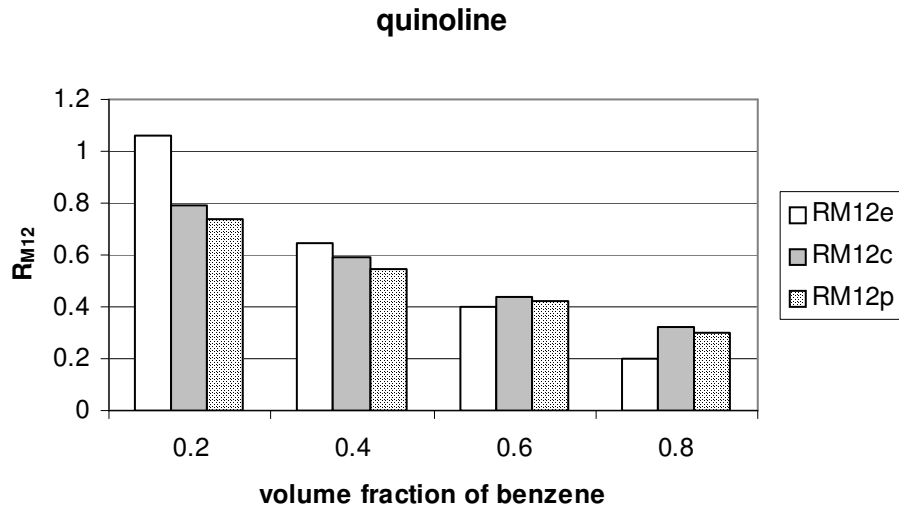


Fig. 8a. Comparison of R_M values of quinoline obtained experimentally (R_{Me}) and calculated for A_z values obtained from chromatographic data (R_{Mc}) and from partition data (R_{Mp}).

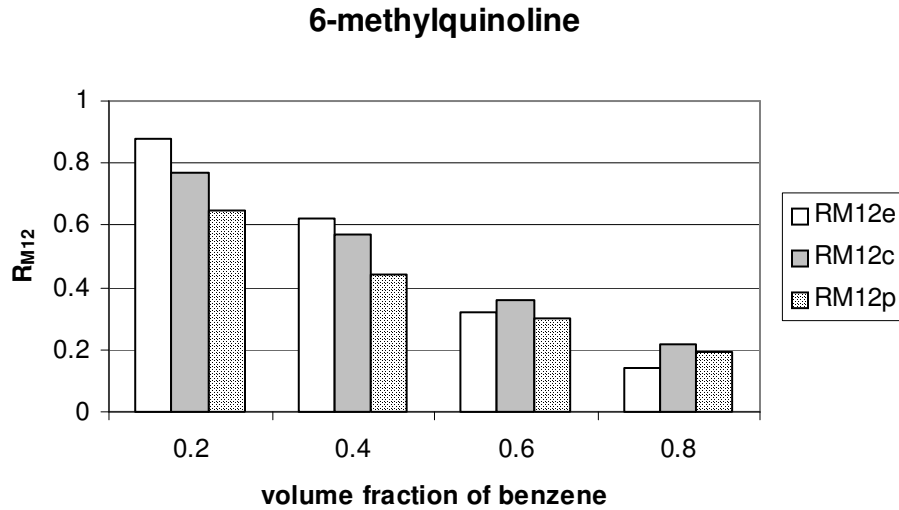


Fig. 8b. Comparison of R_M values of 6-methylquinoline obtained experimentally (R_{Me}) and calculated for A_z values obtained from chromatographic data (R_{Mc}) and from partition data (R_{Mp}).

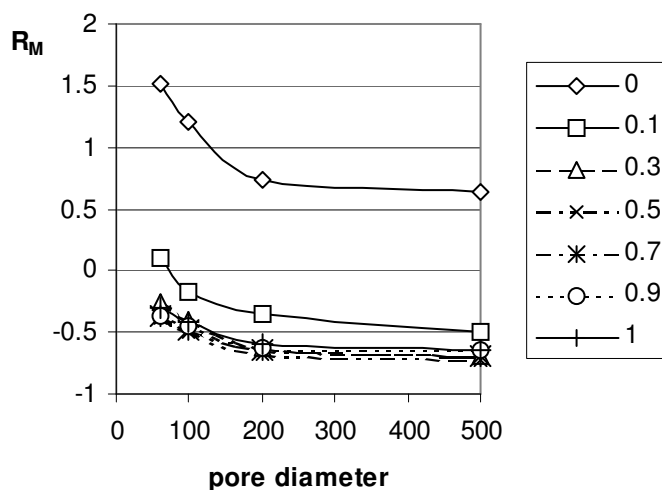


Fig. 9a. R_M values of chrysene obtained in mobile phase n-hexane-benzene on silica gel layer with different pore diameter.

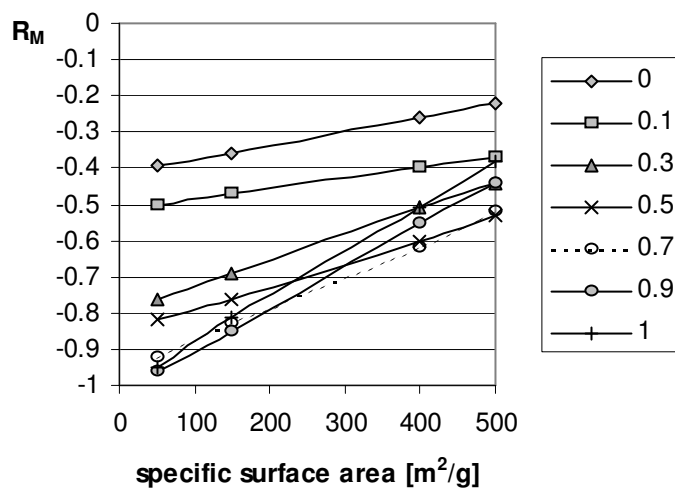


Fig. 9b. R_M values of chrysene obtained in mobile phase n-hexane-benzene on silica gel layer with different specific surface area.

On the basis of the experimental data it was concluded that in the case of substance showing neither electron donor nor electron acceptor properties the R_M change in a regular manner and a linear relationship exists between the R_M

values and specific surface area of the adsorbent independently of the nature of the mobile phases (Figure 9b). This relationship can be expressed as the following equation:

$$R_M = as + b \quad (7)$$

where a and b are the parameters of the straight line, s is the specific surface area of silica adsorbent [m^2/g]. “ a ” values depend on the kind of chromatographed substance and mobile phase composition. The smallest dependences between retention and specific surface area are observed for benzene the biggest – for n-hexane (Figure 10).

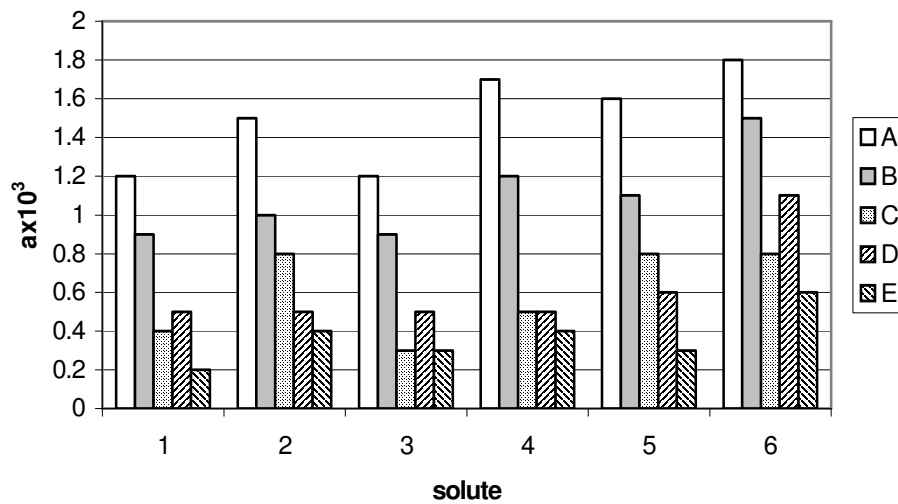


Fig. 10. Values of “ a ” parameter from eq. 7 obtained for different mobile phase for investigated PAH. 1-naphthalene, 2-2,6 dimethylnaphthalene, 3-fluoranthene, 4-anthracene, 5-chrysene, 6-pyrene. Mobile phase: A: n-hexane, B-methylcyclohexane, C-n-hexane-benzene (1:1 v/v), D- cyclohexane-benzene (1:1 v/v), E-benzene.

Parameter “ b ” from the equation 7 is obtained for specific adsorbent surface equals 0. For this reason, this parameter represents intermolecular interaction in chromatographic system with hypothetical adsorbent surface 0. Such adsorbent does not influence the interaction between solute and components of the mobile phase. Another relationship can be observed in the case of solutes, which contain active substituents in their molecules (Figures 11 a,b). In this case the relationships between R_M values and specific surface area are parabolic (Figure 11b). Because of specific properties of the investigated substances (phenol, acridine, quinoline and its methyl derivatives) only N-AB systems containing active solvents, according to the classification of Pimentel and

McCellan can be used, because in N-N systems the R_M values are very high in all concentration range (the solutes practically stay on the start line). From the mathematical point of view the relationships can be presented in the following form:

$$R_M = ls^2 + as + b \quad (8)$$

where “ l ” denotes the parameter describing the specific intermolecular interactions of the substance in the chromatographic system, “ a ” represents the parameter describing the non-specific intermolecular interactions between the chromatographed solute and other components of the chromatographic system, “ b ” – is the parameter describing the intermolecular interactions between the chromatographed substance and the mobile phase components.

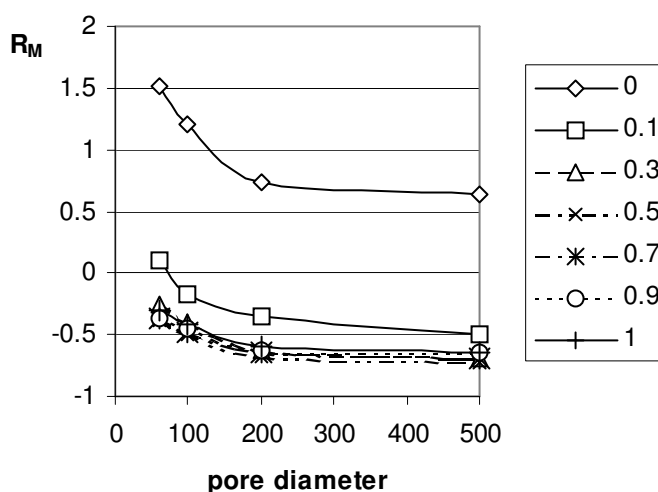


Fig. 11 a. R_M values of quinoline obtained in mobile phase benzene-ethanol on silica gel layer with different pore diameter.

If specific intermolecular interactions do not take place, than $l=0$ and the equation 8 is simplified to the equation 7. Similarly as in the case of the equation 7, the parameter of the equation 8 can be determined and tabularized [21-23]. The obtained results seem to suggest that it is possible to tabulate values of parameters of the equations 7 and 8 in order to calculate the dependence of $R_M = f(s)$. The relationship described here can be used for the calculation of the R_M values measured on silica having any specific surface area, as well as for the determination of specific surface area of given silica adsorbent on the basis of the chromatographic data. On the basis of the equation 7 and 8 the R_M values of

a given substance on the adsorbent can be predicted with a good agreement. It permits to eliminate a series of complicated and time consuming measurements.

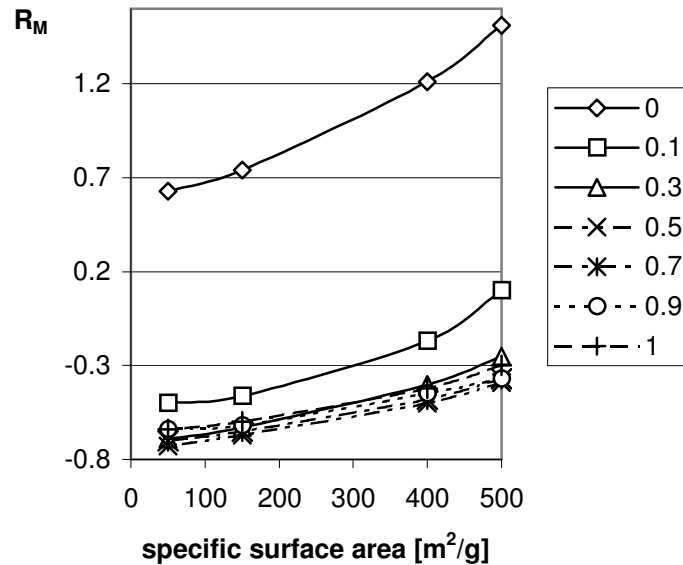


Fig. 11 b. R_M values of 6-methylquinoline obtained in mobile phase benzene-ethanol on silica gel layer with different specific surface area.

The connection of the values of parameters describing theoretical relationship with physico-chemical quantities being characteristic of chromatographic system will allow us to avoid a series of tiring experimental measurements and to obtain greater universality of chromatographic data.

The use of the thermodynamic optimization theory in physicochemical investigations – the determination of the hydrophobicity of the active biological compounds.

The equation 2 can be presented in linear form [24, 25].

$$G(x_1) = \frac{x_1(1-x_1)}{R_{M12} - x_1R_{M1} - (1-x_1)R_{M2}} = ax_1 - b \quad (9a)$$

The equation was tested from in many chromatographic systems [24,25].

R_{M12} , R_{M1} and R_{M2} have been obtained from experiment. On the basis of the equation 2 the K_{12} and A_z parameter can be calculated from linear relationship.

Jaroniec proved that the equation 1 and 2 can be used not only in adsorption but in reversed phase systems as well. The use of the linear form of the equation in reversed phase systems is used in QSAR studies for determination of hydrophobicity of the solutes.

Hydrophobicity is one of the important parameters influencing the biological activity of the compounds. Hydrophobicity has been measured or calculated in many different ways:

- From octanol – water solubility differences – as logarithm of partition coefficient in the n-octanol/water systems
- From side chain polarities as calculated by quantum chemical techniques
- From the relative distributions at the surface and in the core of proteins
- From the atomic constitution of the side chains

Determination of the capacity ratio of the solute from water and n– octanol for many solutes is very difficult and solute-consuming process. In many cases the determination of the P by conventional extraction method is impossible from different reasons. There is a linear correlation between $\log k_w$ and $\log P$ known as Collander-type equation [26]:

$$\log P = a \log k_w + b \quad (10)$$

where $\log P$ is partition coefficient of the substance between n-octanol and water, k_w – retention coefficient obtained in pure water in reversed phase chromatography.

However in RP- chromatography direct measurement of the k_w in pure water is for a lot of the solutes impossible from two reasons:

- stationary phases with chemically bonded aliphatic hydrocarbons are not wetted by pure water and rich water mobile phases
- hydrophobic solutes practically don't migrated in pure water or in rich-water mobile phases.

So determination of the hydrophobicity is not a simple process.

For determination of $\log k_w$ parameters a few methods were proposed. One of them is linear extrapolation of the relationship between $\log k$ and composition of the aqueous mobile phase – volume fraction of the organic modifier in aqueous mobile phase ($\log k = f(x_1)$). This method is not precise, because the linear relationship between $\log k$ values and volume fraction of the organic modifier exist only in limited range (for small concentration of organic modifier the relationships do not exist) [27, 28]. Janicka and Kwietniewski show that the

Ościk's equation can be used for determination $\log k_w$ values successfully [27-31]. Because in literature in QSAR studies $\log k_w$ are used, the equation 9a can be described as follows:

$$G(x_1) = \frac{x_1(1-x_1)}{\log k - x_1 \log k_1 - (1-x_1) \log k_w} = ax_1 - b \quad (9b)$$

in the equation a composition of binary aqueous mobile phase are known (x_1 values is known), k and k_1 can be obtained experimentally but k_w value cannot be obtained experimentally, but it can be determined numerically.

$\log k_w$ values can be obtained from chromatographic data by extrapolation, when the values were used in equation 9b linear relationship has been obtained (Figure 12a). By decreasing of $\log k_w$ value more linear relationship can be obtained, in the end above w given $\log k_w$ values a linear relationship (with a high R^2 values) are observed.

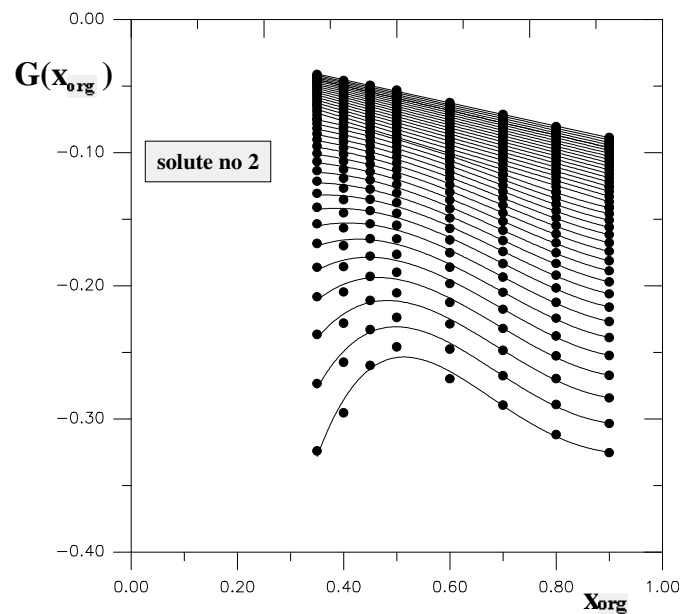


Fig. 12 a. $G(x_1)$ vs. x_1 relationships obtained for different $\log k_w$ values applied in linear form of Ościk's equation.

The $\log k_w$ value is defined as a value which was evaluated by linear extrapolation of the linear part of the relationship $dR^2/d \log k_w$ – Figure 12b.

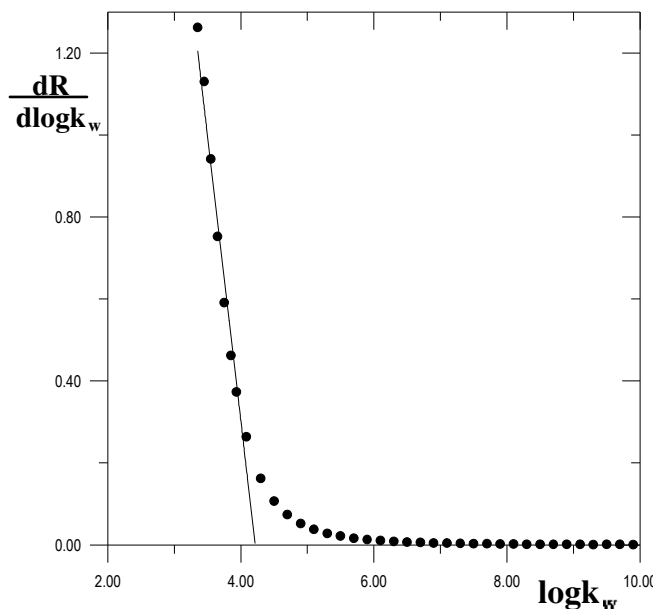


Fig. 12.b. Determination of $\log k_w$ by linear extrapolation of $dr/d(\log k_w)$ vs. $\log k_w$ relationship towards $dr/d(\log k_w) = 0$.

On the basis of the equation 9b and described procedure, $\log k_w$ values were determined for many groups of the solutes and compared with $\log P$ values. There is a good linear relationship between $\log k_w$ obtained by eq. 9b and $\log P$ – better than between $\log k_w$ obtained by extrapolation method.

The presented theory can be used for optimization procedures and for physico-chemical investigations as well.

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- Studies on new supports in planar chromatography
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