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**Graphical Method of Determination of Resolution Range in TLC
Realised in the Systems Containing Binary and Ternary
Mobile Phases**

Graficzna metoda określania przedziału rozdzielczości w TLC
w układach chromatograficznych z dwu- i trójskładnikową fazą ruchomą

Графический метод определения интервала разложения в TLC
в хроматографических системах с дву и три составной подвижной фазой

INTRODUCTION

Adsorption thin-layer chromatography TLC as one among many method of separation of homogeneous mixtures is at present generally used analytical method. Owing to operations simplicity and relatively low-cost apparatus it can be used as a pilot technique for column liquid chromatography as well as for the theoretical considerations relating to chromatographic process. A greater possibility of choosing of optimal separation conditions creates an use of mixed mobile phases. By proper choosing of composition of mobile phase it can optimize a chromatographic process in the aspects of analysis time and resolution [1, 2].

In the past years a great interest arouse again the chromatographic systems containing ternary mobile phase. This interest tend towards more detailed quantitative description of the effects relating to energetical heterogeneity of adsorbent's surface, nonideality of mobile phase as well as towards improvement of resolution of different mixtures especially of mixtures containing the substances having similar k' values [3, 4].

In this paper we have attempted use a simple graphical method to evaluation and comparison of the resolution in the chromatographic systems containing binary and ternary mobile phases. Apart from many other methods the method described here may be helpful to evaluation of resolution of the mixtures containing the substances having similar chemical nature.

THEORETICAL

Partition coefficient for chosen pair of the substances is expressed by known equation [5]:

$$R_s = \frac{l_1 - l_2}{0,5(w_1 + w_2)} \quad (1)$$

where:

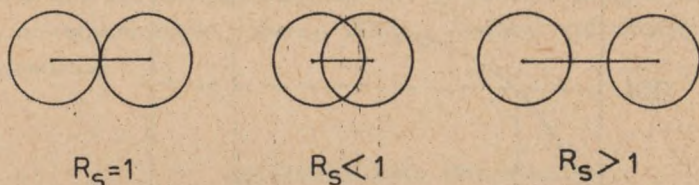
$$l_1 = \frac{R_{F1}}{1} \quad , \quad l_2 = \frac{R_{F2}}{1}$$

In above equation R_{F1} and R_{F2} denote R_F values of the substances chromatographed 1 and 2 respectively; 1 - distance between start line and solvent front; l_1 and l_2 - distances between start line and spots corresponding to the substances 1 and 2., l_1 and l_2 values are measured from spot centers, in the point of highest concentration; w_1 and w_2 width of the spots corresponding to the substances 1 and 2 respectively.

Measurements by adsorption thin-layer chromatography method were carried out in standard way using: manufactured chromatographic plates covered with adsorbent layer, calibrated micropipettes serving to spread of the chromatographed substances and special patterns constructed in Departement of Phisical Chemistry serving to spread

of the substances chromatographed and reading of R_M values. Such patterns were already used in our earlier investigations.

Basing on our previous investigations, we have assumed some simplifications permitting to rapid evaluation of resolution and usefulness of given mobile phase to chromatographic separation of given substances. Separation of given pair of the substances can be illustrated by the scheme presented in Fig. 1.



From the Fig. 1, results minimal R_S value is equal of 1-2. For higher R_S values, Chromatographic separation is satisfied indeed, but time of separation is too long, which is undesired in chromatographic separation process.

Thus it has assumed, that:

$$w_1 = w_2 = w = 0,7 \text{ cm}$$

$$l = 16 \text{ cm}$$

$$R_S \text{ min.} = 1,0$$

From above assumptions results that:

$$R_S = \frac{l_1 - l_2}{0,5(w_1 + w_2)} = \frac{l(l_1/l - l_2/l)}{w} = \frac{l(R_{F1} - R_{F2})}{w}$$

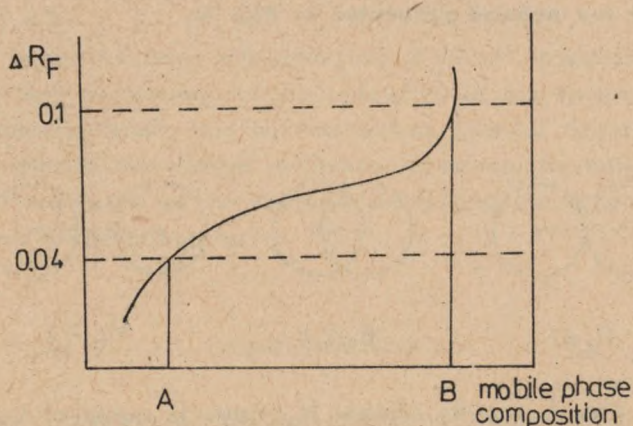
$$\Delta R_{F12} = \frac{R_S \cdot w}{l} \quad (2)$$

Substituting numerical data into the above equation we will obtain:

$$\Delta R_{F12 \text{ min}} = \frac{1 \cdot 0,7}{16} = 0,04$$

$$\Delta R_{F12 \text{ opt.}} = \frac{2 \cdot 0,7}{1,6} \approx 0,1$$

On the basis of presented relationships, we can define an optimal range of separation of given pair of substances in given mobile phase system Fig. 2.



AB — optimal separation range

EXPERIMENTAL

Experimental investigations illustrating the theoretical considerations, were realised by adsorption thin-layer chromatographic method. Measurements were carried out at the temperature of $20^{\circ} \pm 1^{\circ}\text{C}$. Silica gel 60 G Merck was used as adsorbent. Following solvents were used as the mobile phases: carbon tetrachloride, benzene, chloroform, acetone, ethyl-methyl ketone, dioxane, cyclohexane, toluene, methanol and their binary and ternary mixtures. Chromatographic measurements in the systems containing binary mobile phases were carried out for two pure solvents - less and more polar and for their mixtures on the following molar fraction values: 0,1; 0,3; 0,5; 0,7 and 0,9. Ternary mobile phases were prepared in such way, that two less polar solvents were mixed in the proportion of 1:1 and then, the third, more polar solvent was added in suitable proportions. The solvents were chosen on the basis of Pimentel and Mc Clellan classification, according to ability of solvents to hydrogen bond formation [6].

In order to evaluation of resolution in the chromatographic systems investigated, we have chosen different pairs of polycyclic aromatic hydrocarbons belonged to N group of Pimental and Mc Clellan classification. On the basis of the eq.1, we have calculated ΔR_{F12} values, which could be also rapidly and directly related from the chromatogram. In our investigation we have used nonactive substances because such substances are difficult to separation and their chemical structure excludes additional interactions e.g. interactions of adsorbent - chromatographed substance. As it has stated earlier, all the chromatograms were developed at the distance of 16 cm. and the results obtained are presented graphically in the form of relationships between ΔR_{F12} values and mobile phase composition.

RESULTS AND DISCUSSION

On the basis of our investigations we have stated that determination of optimal separation range for chosen pairs of the substances separated in binary systems eg. in carbon tetrachloride - benzene or carbon tetrachloride - chloroform systems are not possible. The separation is either poor or differences in ΔR_{F12} values are too great, which requires a long analysis time. Fig. 3.

Addition of third, more active component of mobile phase has caused a significant separation of such concentration ranges in which separation was good. This optimal concentration range is 0,3 - 0,5 molar fraction of mobile phases containing dioxane and ethyl-methyl ketone and 0,3 - 0,7 molar fraction for the mobile phases containing chloroform, acetone and dioxane. The best chromatographic separations of the pairs of nonactive aromatic hydrocarbons were obtained on silica gel using ternary mobile phases containing acetone and ethyl-methyl ketone. The fact of increased interaction of active component of mobile phase with adsorbent caused by localization of this component, has been once more confirmed. In these cases when polar solvents are used, formation of hydrogen bonds between solvent and the chromatographed substance as well as formation of associates inside of mobile phase, so called secondary solvation effect, can take place [7]. Moreover, in such cases, a specific configuration of polar molecules of solvent and chromatographic substance can exist.

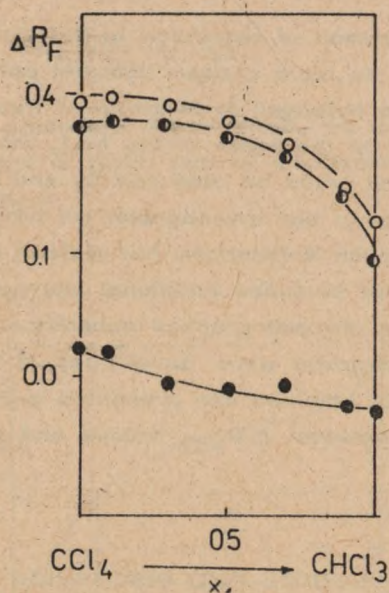


Fig. 3. Dependence of ΔR_{F12} values on the composition of mobile phase for following pairs of the substances chromatographed: ● anthracene - pirene, ○ anthracene - fluorenone, ◐ pirene - fluorenone, on silica gel 60 G Merck. Mobile phase: carbon tetrachloride - chloroform.

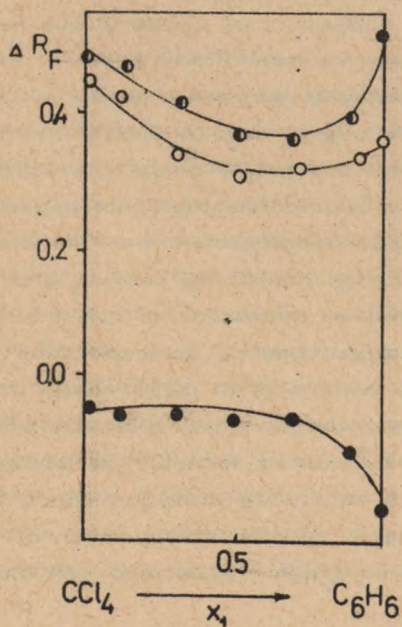


Fig. 4. Dependence of ΔR_{F12} values on mobile phase composition for following pairs of the substances chromatographed: ● anthracene - pirene, ○ anthracene - fluorenone, ◐ pirene - fluorenone on silica gel 60 G Merck. Mobile phase: carbon tetrachloride - benzene.

If given substance not posses functional groups in molecules, this substance is unlocalized in the adsorption phase and cannot assume any specific configuration. Fig. 5, 6, 7.

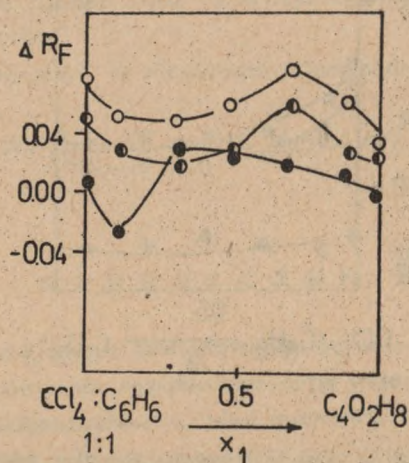


Fig. 5. Dependence of ΔR_{F12} values on the composition of mobile phase for following pairs of the substances chromatographed: ● naphthalene - anthracene, O anthracene - chrysene, ◐ fluorantene - pirene on silica gel 60 G Merck. Mobile phase: carbon tetrachloride - benzene - dioxane.

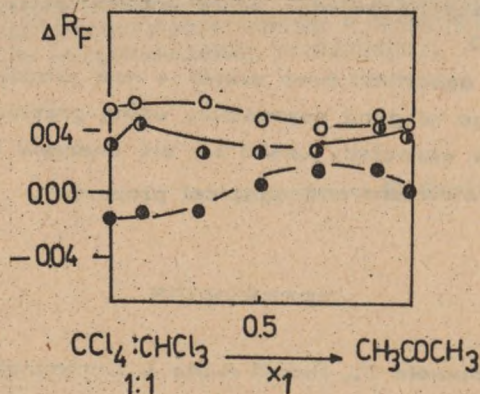


Fig. 6. Dependence of ΔR_{F12} values on the composition of mobile phase for following pairs of the substances chromatographed: ● anthracene - pirene, O anthracene - fluorenone, ◐ pirene - fluorenone on silica gel 60 G Merck. Mobile phase: carbon tetrachloride - chloroform - acetone.

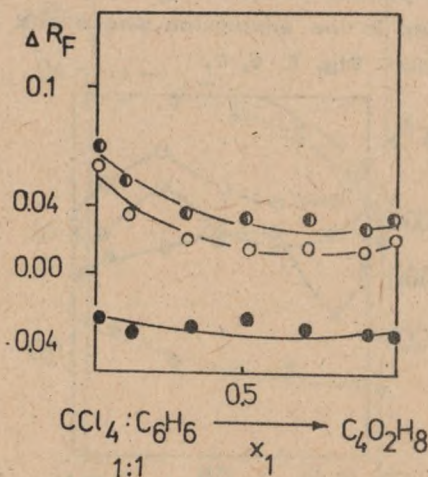


Fig. 7. Dependence of ΔR_{F12} values on the composition of mobile phase for following pairs of the substance chromatographed: ● anthracene - pirene, ○ anthracene - fluorenone, ⊙ pirene - fluorenone on silica gel 60 G Merck. Mobile phase: carbon tetrachloride - benzene - dioxane.

In the case on this, the adsorption parameters are determined mainly by the interactions between adsorbent and polar component of mobile phase [8].

Investigations described here create a new possibility of determination of the range of good separability using graphical method. This method can be especially useful for the analysis of the substances having similar structure and chemical properties.

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S T R E S Z C Z E N I E

W pracy podjęto próbę zastosowania prostej metody graficznej do określania i porównania rozdzielczości w układach chromatograficznych z dwu- i trójskładnikową fazą ruchomą. Problem przedstawiono teoretycznie oraz eksperymentalnie, rozdzielając wybrane pary węglowodorów wielopierścieniowych.

Р Е З Ю М Е

В данной работе сделано попытку применения простого графического метода для определения и сопоставления разложения в хроматографических системах с дву и три составной подвижной фазой. Проблему представлено теоретически и экспериментально, разделяя избранные пары макрокольцевых углеводов.

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