STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, XLIX, 1, 2004

OPTIMIZATION OF THE TERNARY MOBILE PHASE USED IN THIN-LAYER CHROMATOGRAPHY BY THE "WINDOW DIAGRAMS" METHOD

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ABSTRACT. The most important problem of planar chromatography is the theoretical and experimental elaboration of methods for predicting mixture separation conditions that would eliminate the arduous choice of the optimum parameters of a chromatographic process.

In this paper "window diagrams" method is used for optimizing the solvent system used in separating of eight dyes by thin-layer chromatography. Kowalska retention model is used to determine the separation selectivity.

Among the interpretative methods for optimizing separation quality, those that employ window diagrams play a specific role, mostly because of the compulsory implementation of this approach with a reliable theoretical model of solute retention as an unavoidable precondition.

An advantage of this optimization procedure is that the optimum composition can be easily obtained even for multicomponent mobile phase.

INTRODUCTION

In analytical TLC, as in any other chromatographic method, the greatest effort is devoted to the purpose of obtaining the largest separation of the compounds of a mixture. Optimization of the quality of separation can be considered as one of the major practical tasks of the theory of chromatography.

Because separated mixtures usually contain compounds of very similar chemical and electronic structure, their separation is not simple. Interaction of such chromatographied substances with the surface of stationary phases and components of the mobile phase are similar and separation of mixtures with mono or binary mobile phases is practically impossible. Multi-component mobile phases are very often used in modern chromatographic analysis. Because of the many different compositions possible for ternary and quaternary mobile phases, determining the optimum composition of mobile phases with more than two components is rather complicated.

The several techniques used for optimization of separation selectivity can be grouped under three major strategies: *simultaneous methods*, *sequential methods* and *interpretative methods*.

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Among the interpretative methods for optimizing separation quality, those that employ window diagrams [1, 2] play a specific role.

Laub and Purnell [1, 2] in order to optimize the separation selectivity in gas chromatography first used the "window diagrams" method, which is an interpretative one. Ever since, the method has been widely used in gas chromatography (GC) and high performance liquid chromatography (HPLC).

In the case of direct phase liquid chromatography, is used the simplest variant of "window diagrams" method, where the efficiency of the chromatographic separation can be quantified by use of ΔR_f, which is responsible against the solvent composition. For any given pair of compounds, the value ΔR_f is given by relationship:

$$\Delta R_f = \frac{k_2 - k_1}{(1 + k_1)(1 + k_2)} \quad (1)$$

The k_1 and k_2 values can be calculated as a function of a binary solvent's composition:

$$\log k = a \log X_S \quad (2)$$

where X_S represents the molar fraction of the strongest solvent.

If all the pairs of solutes contained in a mixture are being considered, the maximum of ΔR_f values were arranged around a specific molar fraction of binary solvent system.

The advantage of this method resides in the fact that global optimum can be easily traced, either visually or by computer.

Same articles [3, 4] had compared optimization power of three different retention models suitable for liquid chromatography systems by aid of relationship proposed by Snyder [5], Schoenmakers [6] and Kowalska [7]. The direct optimization procedure was performed with aid of window diagrams method [3, 4]. The research studies concluded that Kowalska model is the most relevant model for obtaining the optimum results. According to this model, nonlinear relationship, which links the solute R_f coefficient with composition of binary mobile phase, is given below [8]:

$$R_f = A(X_1)^{1/2} + B(X_2)^{1/2} + C \quad (3)$$

where X_1, X_2 are the volume fractions of solvents and A, B, and C are, in the first instance, the constants of equation.

The separation selectivity α, offers the possibility of simple quantification of separation quality and for this practically reason the magnitude of α has very often been employed in procedures aimed at optimization of solute separation. The physico-chemical interpretation of α is given by the relationship:

$$\alpha = \frac{K_1}{K_2} = \frac{k_1}{k_2} \quad (4)$$

where K_1 and K_2 are the distribution coefficient of solutes 1 respectively 2 between the stationary and mobile phases, and k_1 and k_2 are the respectively capacity factors ($K_1 > K_2$ or $k_1 > k_2$).

Adaptation of eq.3 for determination of separation selectivity α results in formula [9]:

$$\alpha = \frac{\left[1-\left(A_1\sqrt{x_1}+B_1\sqrt{x_2}+C_1\right)\right]\left(A_2\sqrt{x_1}+B_2\sqrt{x_2}+C_2\right)}{\left(A_1\sqrt{x_1}+B_1\sqrt{x_2}+C_1\right)\left[1-\left(A_2\sqrt{x_1}+B_2\sqrt{x_2}+C_2\right)\right]} \quad (5)$$

where A_i, B_i and C_i are the constants of eq. 3 for solute i.

This research is concerning to the optimization of separation quality of some dyes.

EXPERIMENTAL

All the solvents were of analytical grade.

The silicagel plates with normal phase $60F_{254}$ (Merck) were used for the chromatographic experiment.

The mixtures of methanol, petroleum ether and acetone were used as mobile phases. These solvents were selected according to Snyder classification.

The solutions of dyes (0.1%) were prepared in methanol and the compounds (0,2µl) were applied on plates as spots. The plates were developed on a 9 cm distance at room temperature. After developing, the plates were dried and detection was performed in 254nm UV light.

RESULTS AND DISCUSSION

In the present paper "window diagrams" method is used for the optimizing the composition of solvent system used in separation of eight dyes by thin-layer chromatography. The dyes were recently synthesized with intend to create a new set of dyes. The structural formulas of dyes are presented in Figure 1.

Until now, the "window diagrams" method has been used for the optimization of binary mobile phase only. For this reason, in the first step of optimization procedure there are used mixture of methanol (strong solvent $\varepsilon^0 = 5{,}1$) and petroleum ether (week solvent $\varepsilon^0 = 0$) as mobile phase. Three chromatographic experiments were performed with methanol-petroleum ether in various proportions (Table 1). The experimental values of R_f (Table 1) were put in the eq.3 in order to determine the coefficients A, B and C for each solute. The values of these coefficients are presented in Table 1.

The plots of α against X_1 (Figure 2) and the plots ΔR_f against X_1 (Figure 3) (X_1 represent the molar fraction of methanol) for each solute represent the "window diagram" showing relationship between α and X_1 respectively ΔR_f and X_1.

Table 1.

The R_f values and the coefficients A, B and C of eq.3 for each solute.

Nr. crt.	R_f			Coefficients		
	Mobile Phase (methanol - petroleum ether, v/v)			A	B	C
	40 : 60	80 : 20	50 : 50			
1	0.084	0.070	0.064	-1.821	-1.157	1.930
2	0.241	0.270	0.258	0.929	0.536	-0.669
3	0.325	0.353	0.333	0.110	0.002	0.254
4	0.482	0.482	0.441	4.524	-2.963	5.127
5	0.494	0.506	0.538	4.524	2.933	-4.127
6	0.506	0.482	0.516	1.765	1.216	-1.340
7	0.542	0.541	0.581	4.331	2.839	-3.900
8	0.807	0.812	0.871	0.144	0.286	0.626

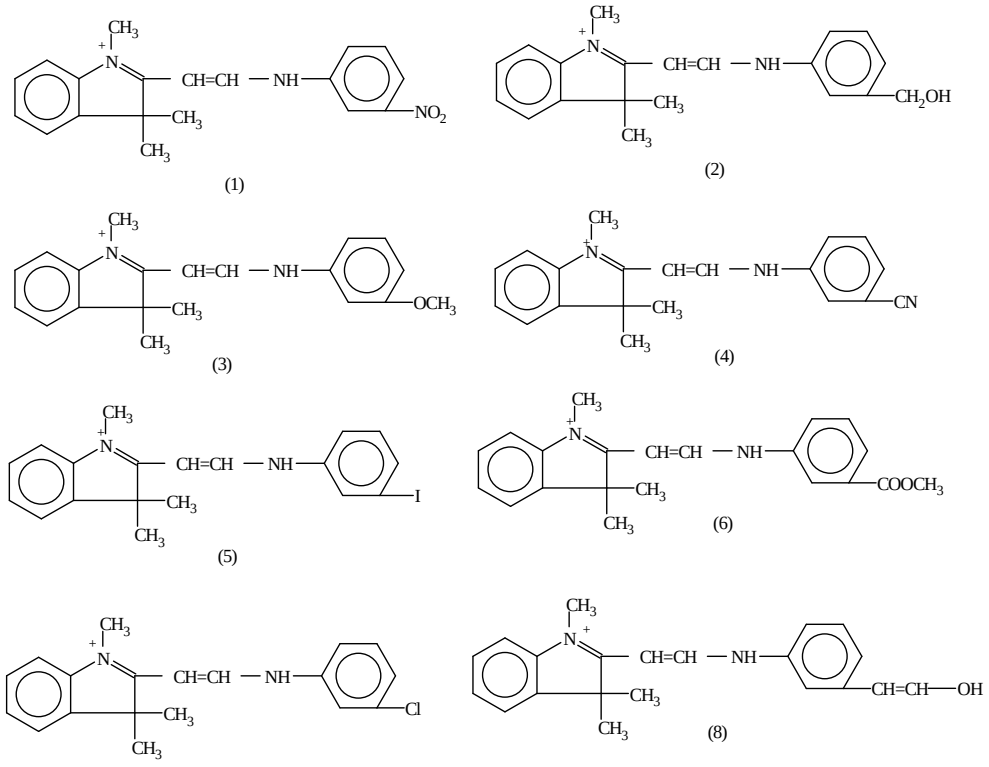

Fig. 1. *The chemical structures of dyes.*

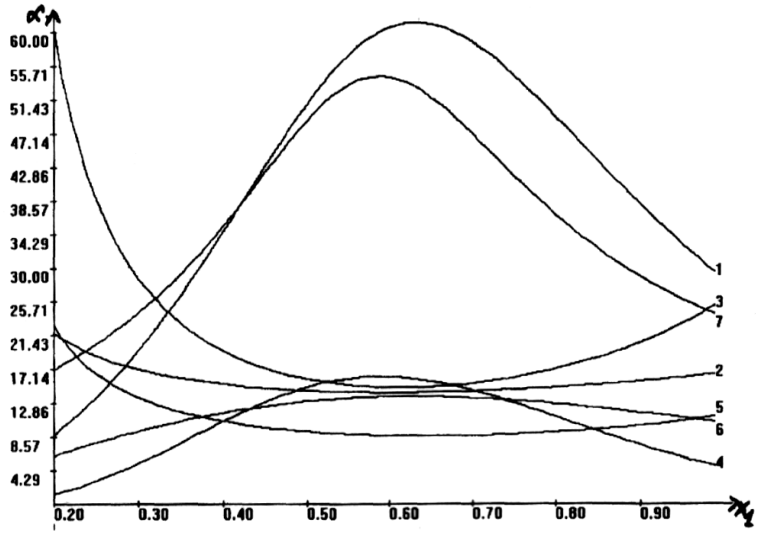

Fig. 2. *Window diagrams showing relationship between α and X_1 obtained by using methanol - petroleum ether mixture as mobile phase.*

From Figure 2, it can be observed that the greatest difference as a sum of differences between the values lies around the methanol fraction of 0.9 ± 0.02. This fact can also be proved by watching the plots ΔR_f of against X_1 (Figure 3).

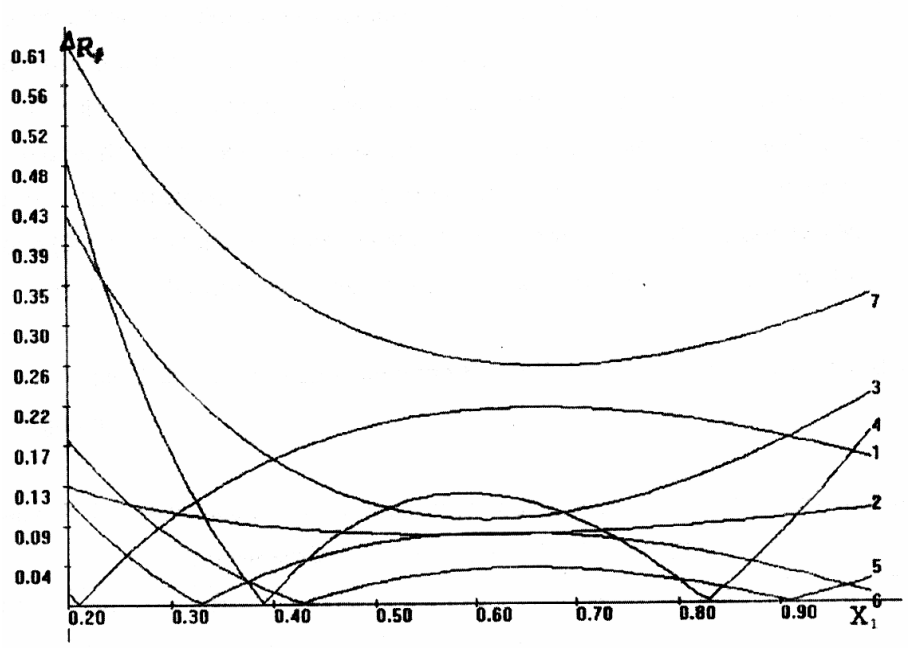

Fig. 3. *Window diagrams showing relationship between ΔR_f and X_1 obtained by using methanol - petroleum ether mixture as mobile phase*.

In accord with these models, the optimum composition is methanol - petroleum ether 9 : 1 (v/v).

With a mixture of more than two solutes it is again necessary to use the "window diagrams" approach in order to select the most suitable separation conditions. Therefore, in the second step the composition of ternary mobile phase was optimized in order to obtain a good separation of compounds. The procedure are similar with that used in first step but the strong solvent (I) is acetone (ε^0 = 5,1) and the mixture methanol-petroleum ether (9 : 1 v/v) represents the week solvent (II) (ε^0 = 4,6). The results are given in Table 2.

From Table 2, it can be observe that the R_f values increase with increasing of solvent I molar fraction.

In order to find out which proportion of methanol - petroleum ether - acetone mixture provides the optimum results, there are plotted again α and ΔR_f against X_1 (X_1 is molar fraction of acetone) for each solute.

Table 2.

The R_f values and the coefficients A, B and C (from eq.3) of each solute.

Nr. crt.	R_f			Coefficients		
	Mobile Phase (I – II, v/v)			A	B	C
	50 : 50	30 : 30	70 : 30			
1	0.077	0.055	0.072	0.903	0.610	-0.867
2	0.344	0.244	0.333	3.344	2.276	-3.092
3	0.411	0.333	0.389	3.344	2.276	-3.092
4	0.566	0.511	0.544	2.575	1.776	-2.144
5	0.589	0.522	0.567	2.977	2.037	-2.534
6	0.600	0.533	0.589	2.609	1.744	-2.117
7	0.622	0.578	0.628	1.280	0.793	-0.673
8	0.844	0.850	0.818	0.669	0.563	0.089

The plots of ΔR_f against X_1 (Figure 5) show that the greatest difference, as a sum of differences between the ΔR_f values, lies around the acetone fraction of 0.35. Same thing can be ascertained by watching the plots of α against X_1 (Figure 4). Thus, it was established that the optimum composition of solvents mixture I - II is 35 : 65.

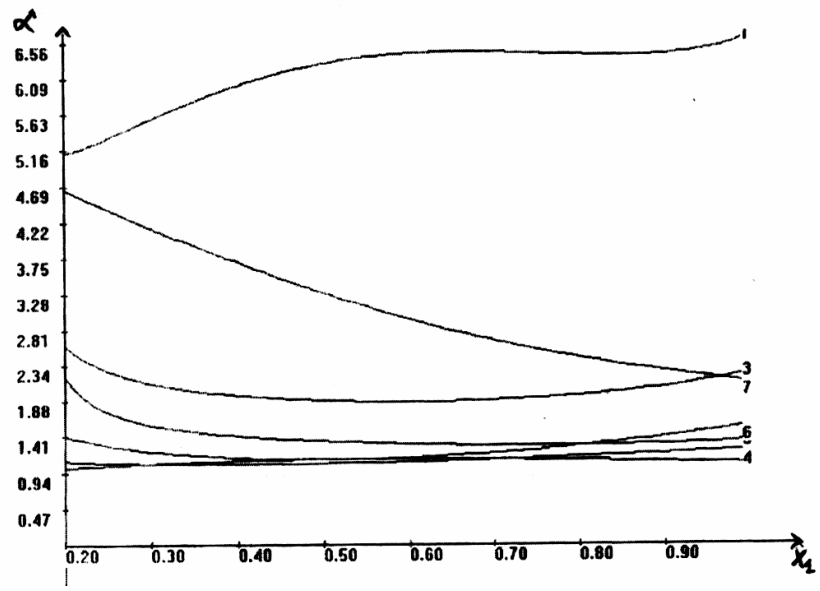

Fig. 4. *Window diagram showing the relationship between α and X_1 in case of ternary mobile phase (methanol - petroleum ether - acetone).*

Therefore, considering the fact that the optimum composition of solvent II is 9:1, it can conclude that in the case of the ternary mobile phase, the optimum composition acetone - methanol - petroleum ether is 35 : 58.5 : 6.5.

The mixture was further used to separate the eight dyes. As a result of chromatographic experiment, it was establish that this mobile phase mixture, acetone : methanol : petroleum ether in a volumetric proportion of 35 : 58.5 : 6.5 provides the best separation of the studied compounds.

In conclusion, for the first time the composition of ternary mobile phase was optimized using "window diagrams" method and retention model. The advantage of this method is that that optimum composition of mobile phase can be easily obtained.

Introduction of a physico-chemical logical to the optimization procedure is an ultimate goal of chromatography theory, and particularly of all efforts that aim to model solute retention.

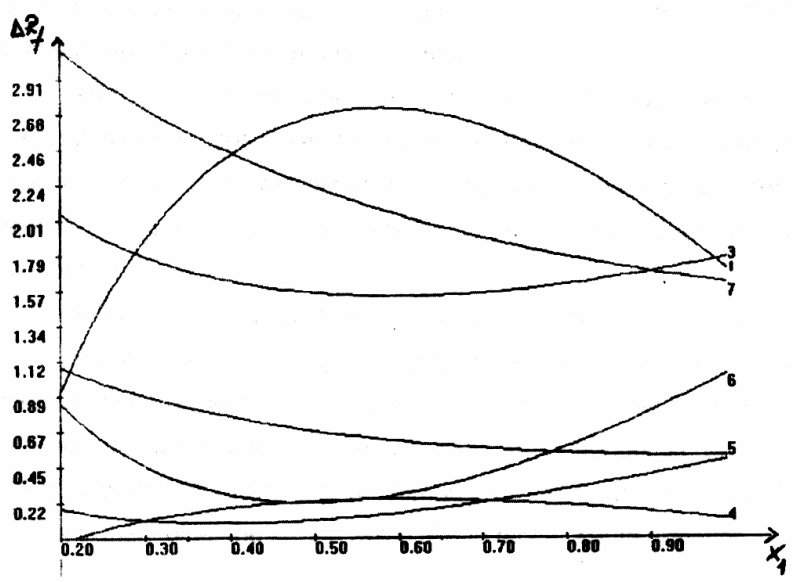

Fig. 5. *Window diagram showing the relationship between ΔR_f and X_1 in case of ternary mobile phases (methanol - petroleum ether - acetone).*

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