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### Some problems in the use of ternary mobile phases in thin-layer chromatography

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Single-component and multi-component solvents are used as mobile phases in high-pressure liquid chromatography, column chromatography and thin-layer chromatography (TLC). Single-component solvent systems constitute the least complex and the best known chromatographic systems, and separations of mixtures in this type of system are simple. The interpretation of chromatographic spectra in single-component systems is also easy.

More complex and more difficult to explain are two-component systems, because of additional interactions among the molecules of the solvent components. However, investigations in two-component solvent systems are now highly advanced.

In TLC, apart from some generally accepted rules, optimal conditions for the separation of mixtures are often established by means of successive tests. This procedure is easy in single-component and even in two-component systems, but is difficult in three-component systems. In three-component systems, molecular interactions are more complicated and the results are difficult to interpret or even to express in diagrammatic form, although some work has been carried out in this field<sup>1</sup>. Some relationships introduced into this kind of system are considered theoretically rather than applied in practice.

The  $R_F$  or  $R_M$  values as a function of the solvent composition are presented on spatial diagrams. This is very difficult for the average experimentalist in TLC. Therefore the results obtained for three-component solvent systems were interpreted by Gibbs' triangle.

## EXPERIMENTAL

The separation of the chosen mixtures of substances was carried out by TLC on aluminium oxide G (E. Merck, Darmstadt, G.F.R.) using three-component mobile phases.

The adsorbent was placed on a plate (0.3 mm thick layer) and activated by the method described previously<sup>2,3</sup>. The following model substances were used: acridine (class B according to the Pimental and McClellan<sup>4</sup> classification, based on the different abilities of substances to form hydrogen bonds), 3,4-benzacridine (class B), fluorenone (class N), anthracene (class N), phenanthrene (class N), fluoranthene

(class N) and chrysene (class N). The spots of the substances were observed in UV light or after spraying with  $\text{KMnO}_4$  reagent.

The following solvents were used as the three-component mobile phases: water (class AB\*), acetone (class B), chloroform (class A) and *n*-heptane (class N). The organic solvents were dried in order to improve the chromatographic process<sup>5,6</sup>.

The choice of the compositions of the three-component phases was based on binodal curves obtained by titration<sup>7,8</sup> or from the literature<sup>9</sup>. In each of the three-component solvents examined, the individual concentrations of all three components varied over the whole range of concentrations\*.

All measurements were made at a temperature of *ca.* 24°, which ensured an accuracy of the  $R_F$  values obtained of  $\pm 0.02 R_F$  unit.

## RESULTS AND DISCUSSION

In order to investigate the choice of the three-component mobile phase and the interpretation of the chosen chromatographic systems, a Gibbs triangle was used to construct a flat system of coordinates in which the  $R_F$  values of substances were plotted on the ordinate and the composition of the three-component mobile phase on the abscissa. The composition of the phase was obtained in the following way. On the binodal curve in the Gibbs triangle, some points representing different compositions of the three different solvents were chosen, and by joining these points, corresponding to the composition of a three-component mixture of the solvents, to the abscissa by perpendicular lines, a projection on this axis was obtained. In this way,  $R_F$  values of the separate substances were related to the projection of the composition of the solvents on the abscissa. Thus, instead of complex spatial curves for  $R_F = f(\text{composition of three-component solvent})$ , a flat two-dimensional diagram was obtained, similar to that generally used for systems with two-component mobile phases.

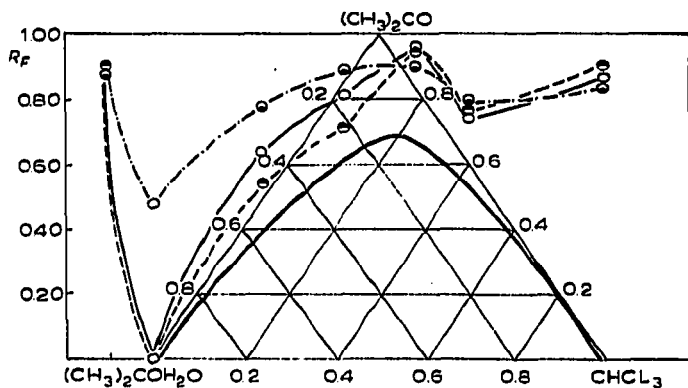


Fig. 1. Separation of acridine (●- - - ●), 3,4-benzacridine (● - - - ●) and fluorenone (○—○) on aluminium oxide using acetone-water-chloroform as mobile phase. The thick line represents the composition of the solvent system as a volume fraction.

\* Mixtures over the binodal curve as developing solvent systems constitute a separate problem and are being investigated at present. The results will be published in future papers.

In order to interpret the diagram,  $R_F$  values for the pure solvents were represented by points corresponding to the apexes of the Gibbs triangle, and  $R_F$  values corresponding to the pure components on the apexes of the triangle, opposite the abscissa, were inserted on the left of the triangle.

These diagrams permitted, on the one hand, an easy choice of a particular three-component mobile phase, and, on the other, the simple diagrammatic presentation of the relationship  $R_F = f(\text{composition of a three-component mobile phase})$ . Hence there is no need for a complex choice of the phase by using the method of successive tests.

The compositions obtained for the three-component mixture enabled appropriate developing solvents to be chosen by means of which mixtures of substances were chromatographed. Fig. 1 presents the separation of acridine, 3,4-benzacridine and fluorenone on aluminium oxide using a mixture of acetone, water and chloroform as the mobile phase. Fig. 1 shows that the best separation of the selected model substances (the highest  $\Delta R_F$  values) was obtained by using a mixture of chloroform-acetone-water in volume fractions of 0.02:0.38:0.60 and 0.12:0.62:0.36. With other volume fractions of these three components, no separation of the model substances was obtained.

In order to determine the effect of the type of mobile phase on the efficiency of separation of mixtures of substances, the same components were separated by using a mobile phase consisting of *n*-heptane, acetone and water. The results (Fig. 2) show that the highest  $\Delta R_F$  values were obtained by using two different compositions of the mobile phase (two values of  $\Delta R_F$  on the curve of  $R_F$  versus solvent composition, *i.e.*, *n*-heptane-acetone-water in the volume fractions 0.02:0.42:0.56 and 0.21:0.70:0.03). No separation of the model mixtures of substances was obtained with other compositions of the mobile phase.

An examination was then made of the separation of substances of very similar structure by standard chromatographic methods. The model substances used were anthracene, phenanthrene, fluoranthene and chrysene. The results of the separation (Fig. 3) show that the highest  $\Delta R_F$  values were obtained when the ratio of the compo-

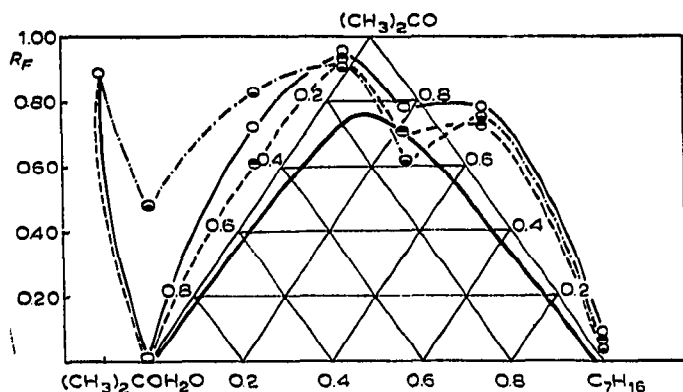


Fig. 2. Separation of acridine, 3,4-benzacridine and fluorenone on aluminium oxide using *n*-heptane-acetone-water as the mobile phase. Symbols as in Fig. 1.

nents of the mobile phase (chloroform–acetone–water) was 0.034:0.34:0.626. As a result, a distinct separation of anthracene and chrysenes from phenanthrene and fluoranthene was obtained. With other compositions, the separation of these components was not achieved.

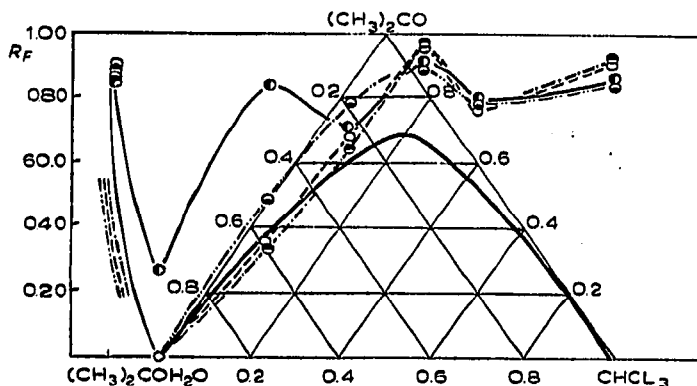


Fig. 3. Separation of anthracene (○—○), phenanthrene (●—●), fluoranthene (●—●—●—●) and chrysenes (●—●—●—●) on aluminium oxide using chloroform–acetone–water as the mobile phase. Thick line as in Figs. 1 and 2.

Hence optimal conditions for the separation of substances by using a three-component mobile phase were established without the use of the method of successive tests. The method described is time saving and requires a small number of reagents, which is very important in TLC, in which multicomponent mobile phases are used.

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