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## TERNARY SOLVENT SYSTEM DESIGN FOR LIQUID-SOLID CHROMATOGRAPHY

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### SUMMARY

The linear relationship between the logarithmic values of capacity ratio and the molar fraction of one or two stronger solvents ( $S$  or  $S_1 + S_2$ ) in ternary eluent systems containing two or one diluents, respectively ( $S + W_1 + W_2$  or  $S_1 + S_2 + W$ ), was determined by using steroid saponin as solutes in silica gel liquid-solid chromatography. The linear relationship was found to be reliable for concentrations of the  $S$  component as high as 100%. On the basis of this, an optimization procedure was established for a pair of model compounds in a ternary eluent by employing the retention-eluent composition correlation in two corresponding binary solvent systems.

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### INTRODUCTION

In the process of optimization in liquid-solid chromatography, binary eluents composed of a diluent and a stronger solvent have commonly been utilized. In ternary solvent systems made by adding one more component to a binary solvent system, the solvent strength and the selectivity for given samples can be more closely controlled and better resolution of the mixture can be achieved<sup>1-3</sup>. However, such systems are not commonly used, because the adaptation and design of the system is more troublesome than for corresponding binary systems.

Soczewiński and Jusiak<sup>4,5</sup> and Hara and co-workers<sup>6-13</sup> have systematically investigated the correlation between eluent composition and retention in binary solvent liquid-solid chromatography. They found in numerous instances a linear, theoretically expected relationship between the logarithm of the capacity ratio and the logarithm of the molar fraction of the stronger component for chromatographic systems of the silica-diluent + stronger solvent type. Such a relationship was found to be useful in the optimization of chromatographic systems. On the basis of these results, a rational method for the design of ternary solvent systems was established and is presented in this paper.

## EXPERIMENTAL

*Samples and columns*

Sapogenins and derivatives were prepared in Kunihiro's laboratory<sup>14,15</sup> and cholesterol was purchased from Wako (Osaka, Japan). Radialpack silica plastic cartridges (10 cm × 10 mm I.D.) (Waters Assoc., Milford, MA, U.S.A.) were used.

*Chromatography*

Model 7125 injector (Rheodyne, Berkeley, CA, U.S.A.) and a Model 45 pump (Waters Assoc.) were linked to a Model 401 differential refractometer (Waters Assoc.). Technical-grade solvents (Wako) were used. The flow-rates were 2.0 ml/min under a pressure drop of 500 p.s.i. An amount of 1 mg of sample was dissolved in 1 ml of eluent. A volume of 5  $\mu$ l of sample solution was injected into the column. The hold-up volume was measured by using *n*-hexane as a sample, and the capacity ratio,  $k'$ , was obtained with the equation  $k' = t_s/t_m$ , where  $t_s$  is adjusted retention time and  $t_m$  is dead time. The results were obtained at ambient temperature ( $23 \pm 2^\circ\text{C}$ ) and at a relative humidity of  $60 \pm 5\%$ .

## RESULTS AND DISCUSSION

A binary eluent in liquid-solid chromatography consists of a diluent (W) and a stronger solvent (S). When a ternary solvent system is prepared, a third component (W or S) is added to the binary system, W + S. As a result, the ternary eluents can be classified into two groups. The first contains two diluents and a stronger solvent, *i.e.*,  $W_1 + W_2 + S$  (solvent strength of the diluents defined as  $W_2 > W_1$ ) and the other contains a single diluent and two stronger solvents, *i.e.*,  $W + S_1 + S_2$  (similarly, the eluent strength of the stronger solvents is defined as  $S_2 > S_1$ ).

A linear relationship between the logarithm of the capacity ratio and the logarithm of the molar fraction of the stronger solvent in the binary system had been found for numerous systems<sup>4-13</sup>.

When the content of polar component is higher than 5-10%, this relationship is represented by the equation

$$\log k' = c - n \log X_s \quad (1)$$

where  $k'$  is the capacity ratio,  $X_s$  is the molar fraction of the stronger solvent and  $c$  and  $n$  are constants ( $c = \log k'$  for a pure stronger solvent, *i.e.*,  $X_s = 1$ ). To examine the correlation between the retention of a solute and the composition of the ternary solvent system, we simplified the ternary system to a two-component system such as a binary (W + S) plus W or S. The correlation between the capacity ratio and the solvent composition of  $S/(W_1 + W_2)$  or  $(S_1 + S_2)/W$  was examined for various proportions of  $W_1/W_2$  or  $S_1/S_2$ , respectively.

*n*-Hexane (O) and benzene (P) were used as diluents and ethyl acetate ( $B_2$ ) and acetone ( $B_3$ ) were added as stronger solvents. The solvent codes used in this paper are those suggested by Hara *et al.*<sup>6,7</sup>.

The solvent strength of the ternary system must, in general, be greater than that of the corresponding binary system. Triterpenoid sapogenins obtained from ginseng were chosen as solutes, because they have three or more functions and exhibit

stronger adsorption activity than the mono- and disubstituted steroid derivatives that were used in earlier work on the systematic design of binary solvent systems<sup>6-10</sup>. Such functional groups contain an oxygen atom, together with acyloxy, keto carbonyl and hydroxy groups. Structural formulae of these substances are shown in Fig. 1.

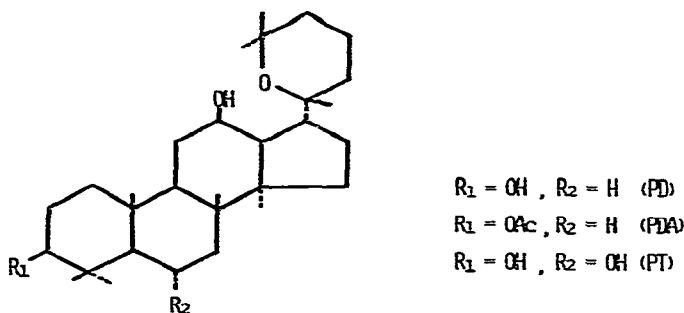


Fig. 1. Structural formulae of triterpenoid sapogenin and its derivatives.

### $W_1 + W_2 + S$ system

*n*-Hexane (O) and benzene (P) were used as diluents of  $W_1$  and  $W_2$ . The diluent molar ratios were 1:0, 2:1, 1:1, 1:2 and 0:1. Ethyl acetate ( $B_2$ ) was added to the diluent binary  $W_1 + W_2$ . The capacity ratio,  $k'$ , of panaxadiol (PD) and panaxatriol (PT) was determined for various compositions of the stronger component,  $X_{s/(w_1+w_2)}$ . The data obtained were plotted as a log-log graph. The results are illustrated in Fig. 2.

A linear relationship between the logarithm of  $k'$  and the logarithm of  $X_{s/(w_1+w_2)}$  was found, with a correlation coefficient of 0.9981–0.9998. All lines merged to a point on the vertical axis when the ratio of the stronger eluent (S) was increased to 100%.

These results indicate that the linear relationship discussed above was achieved at high concentrations of the S component. Therefore, it became possible to predict the capacity ratio of a solute in a pure stronger solvent by extrapolating the plots obtained for moderate concentrations of the S solvent. Based on these results, the retention in a ternary solvent system can be expressed as follows:

$$\log k'(W_1 + S) = c - n_1 \log X_s \quad (2a)$$

$$\log k'(W_2 + S) = c - n_2 \log X_s \quad (2b)$$

$$\log k'(W_1 + W_2 + S) = c - n_{12} \log X_{s/(w_1+w_2)} \quad (2c)$$

where  $c$  and  $n_1$ ,  $n_2$  and  $n_{12}$  are constants given by the systems:  $W_1 + S$ ,  $W_2 + S$  and  $W_1 + W_2 + S$ , respectively. It is assumed that the value of constant  $n_{12}$  is intermediate between  $n_1$  and  $n_2$ , because the constant  $n$  is considered to be an exchange ratio between solvent molecules and a solute on the active site of the adsorbent surface<sup>16</sup>. On the basis of such a concept, the constant  $n_{12}$  can be calculated approxi-

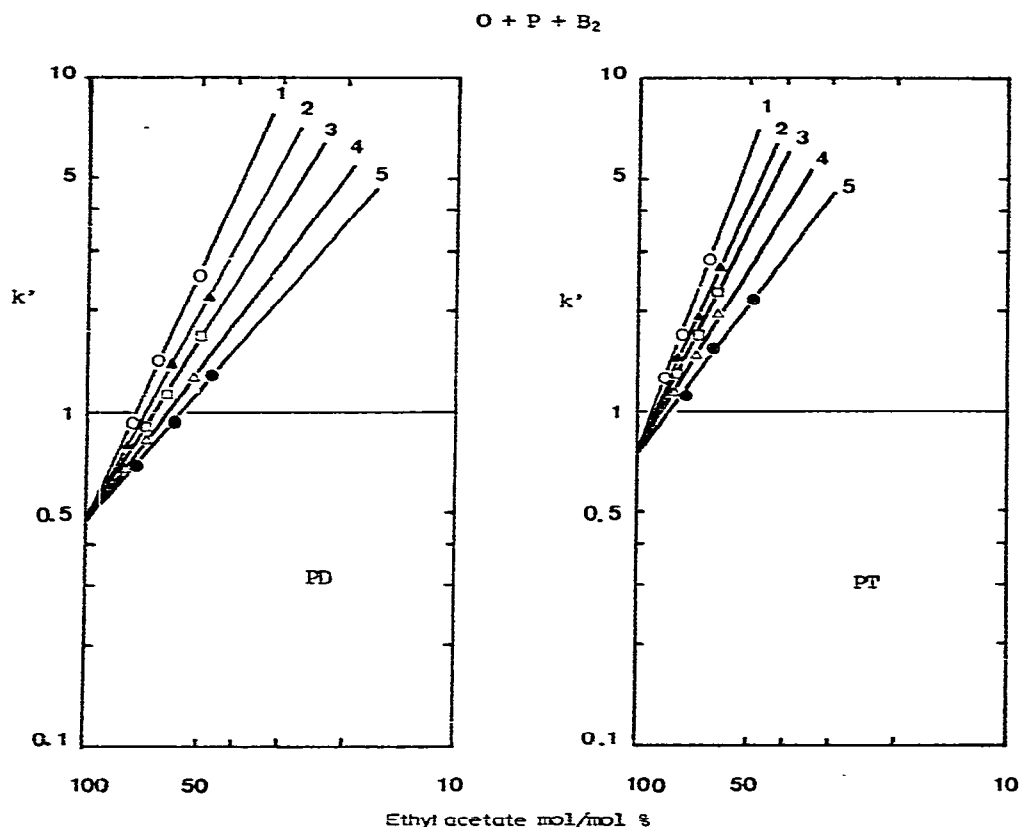


Fig. 2. Correlation between the logarithm of the capacity ratio ( $k'$ ) and the logarithm of the stronger solvent composition  $X_S/(w_1 + w_2)$  in a ternary eluent system. Samples: PD = panaxadiol; PT = panaxatriol. Solvents: O = *n*-hexane; P = benzene; B<sub>2</sub> = ethyl acetate. Eluent systems: 1 = O + B<sub>2</sub>; 2 = O + P + B<sub>2</sub> (O/P = 2); 3 = O + P + B<sub>2</sub> (O/P = 1); 4 = O + P + B<sub>2</sub> (O/P = 1/2); 5 = P + B<sub>2</sub>.

mately from the additivity principle

$$n_{12} = X_{w_1}n_1 + X_{w_2}n_2 \quad (2d)$$

It was found that the calculated value of  $n_{12}$  was very close to the experimentally obtained value of  $n_{12}$

#### *W + S<sub>1</sub> + S<sub>2</sub> system*

Ethyl acetate (B<sub>2</sub>) and acetone (B<sub>3</sub>), which were selected as two stronger solvents, S<sub>1</sub> and S<sub>2</sub> were added to the diluent *n*-hexane (O). Stronger solvent binary systems, S<sub>1</sub> + S<sub>2</sub>, were composed using molar ratios of 1:0, 2:1, 1:1, 1:2 or 0:1. The capacity ratios of PD and PT in various compositions of the stronger solvents,  $X(S_1 + S_2)/w$  were determined. The retention of the solutes and the composition of the ternary solvent were plotted on a log-log scale and the results are illustrated in Fig. 3. A linear relationship was obtained with a correlation coefficient of 0.9930–0.9996. The experimental results can be expressed as follows:

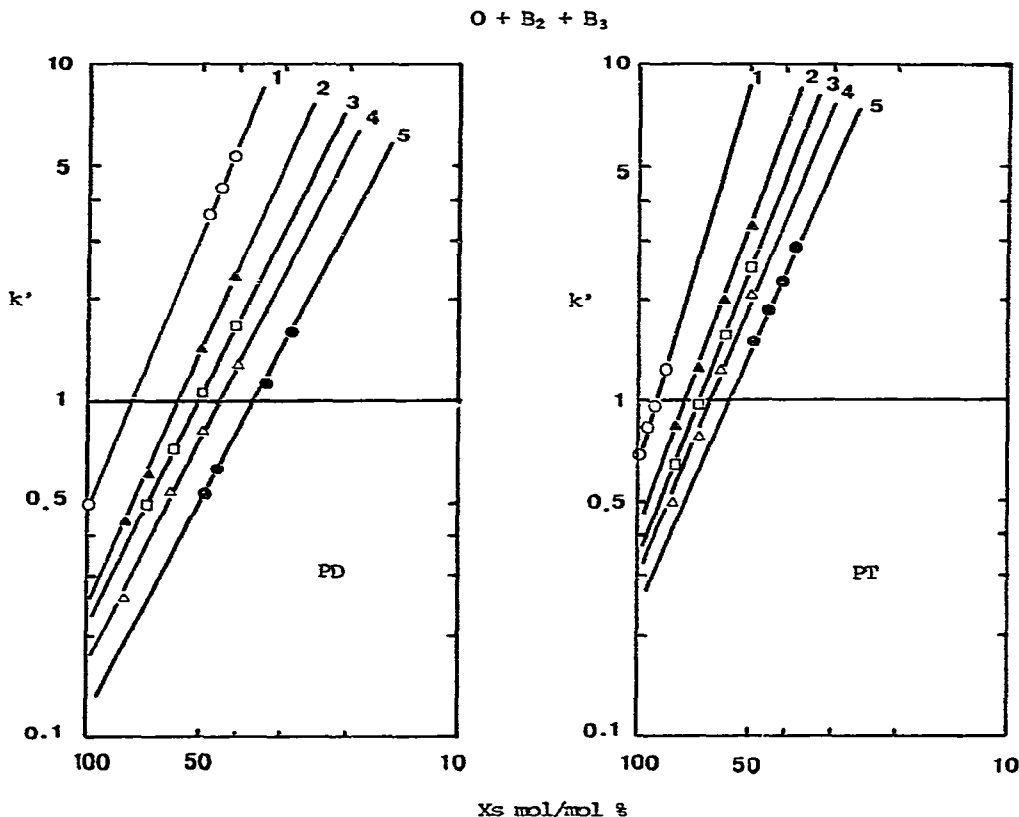


Fig. 3. Correlation between the logarithm of the capacity ratio ( $k'$ ) and the logarithm of the stronger solvent composition  $X (s_1 + s_2)/w$  in a ternary eluent system. Samples as in Fig. 2. Solvents: O = *n*-hexane; B<sub>2</sub> = ethyl acetate; B<sub>3</sub> = acetone. Eluent systems: 1 = O + B<sub>2</sub>; 2 = O + B<sub>2</sub> + B<sub>3</sub> (B<sub>2</sub>/B<sub>3</sub> = 2); 3 = O + B<sub>2</sub> + B<sub>3</sub> (B<sub>2</sub>/B<sub>3</sub> = 1); 4 = O + B<sub>2</sub> + B<sub>3</sub> (B<sub>2</sub>/B<sub>3</sub> = 1/2); 5 = O + B<sub>3</sub>.

$$\log k'(W + S_1) = c_1 - n_1 \log X_{s_1} \tag{3a}$$

$$\log k'(W + S_2) = c_2 - n_2 \log X_{s_2} \tag{3b}$$

$$\log k'(W + S_1 + S_2) = c_{12} - n_{12} \log X(s_1 + s_2)/w \tag{3c}$$

where  $c_1, n_1; c_2, n_2$  and  $c_{12}, n_{12}$  are the intercepts and slopes corresponding to the solvent systems:  $W + S_1; W + S_2$  and  $W + S_1 + S_2$ , respectively.

Constants  $c_{12}$  and  $n_{12}$  were found to have intermediate values between  $c_1, c_2$  and  $n_1, n_2$ , respectively; the  $n_{12}$  value could be calculated from eqn. 2d and the  $c_{12}$  value from eqn. 3d:

$$c_{12} = X_{s_1} c_1 + X_{s_2} c_2 \tag{3d}$$

Although a slight difference was observed between the calculated values and the experimental values; it seems possible to predict the retention for a given ternary solvent

system on the basis of the retention behaviour for a pair of corresponding binary systems.

In previous papers<sup>7-9</sup>, commonly available volatile solvents that are used for the preparative research work in liquid-solid chromatography were classified in several types and the strength of the solvents in the binary systems was evaluated. By considering such a classification and a relative strength, a solvent for the ternary system can be defined as one of the components in the system;  $W_1 + W_2 + S$  or  $W + S_1 + S_2$  due to the sequence of the solvent strength ( $W_1 < W_2 < S$  or  $W < S_1 < S_2$ ). Additionally, the solvent composition of the ternary system can readily be optimized by adapting the given sample mixture according to the linear relationships expressed by eqns. 2a-c and 3a-c. The procedure described in this report seems to be especially useful for controlling the retention of the weak polar compounds such as steroid and terpenoid mixtures by employing weak polar solvent systems.

#### *An example of controlling the resolution of a pair of solutes*

Based on the correlation between the retention and the composition of the ternary solvent system discussed above, optimization of the resolution of a pair of solutes was attempted.

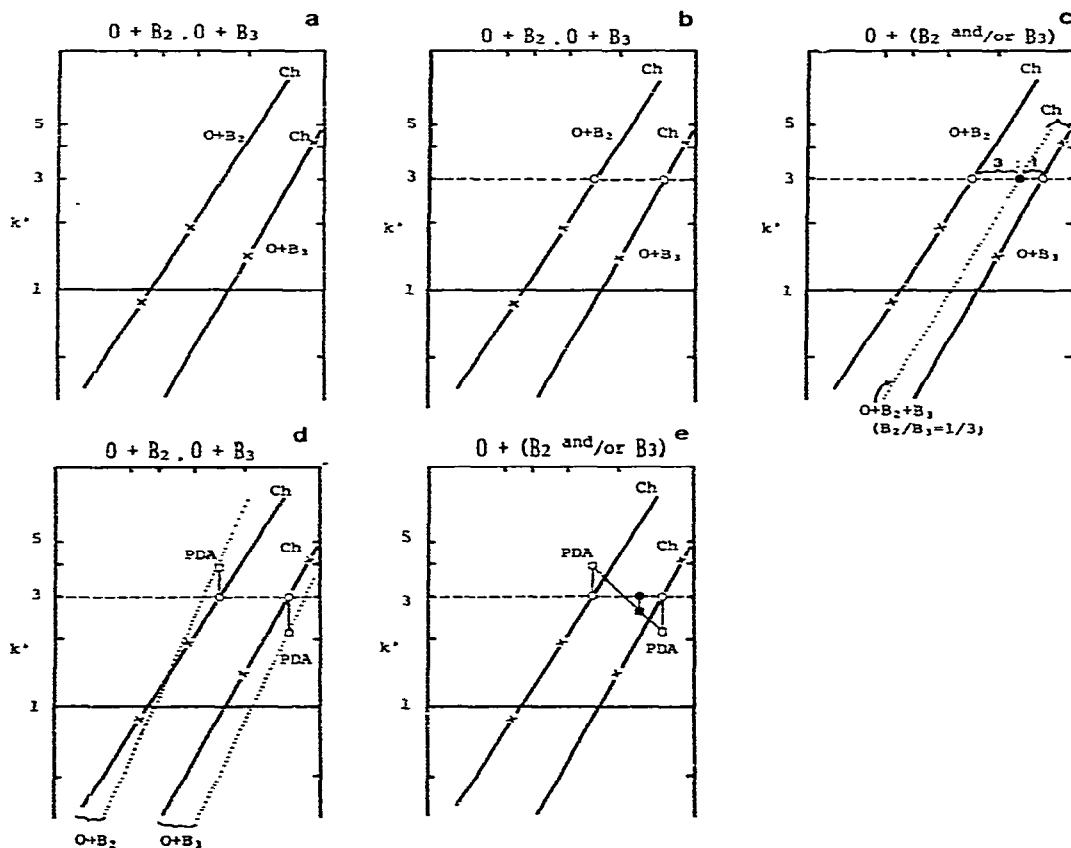


Fig. 4. Example of optimization procedure for the resolution of a pair of solutes. Model compounds: Ch = cholesterol; PDA = panaxadiol 3-acetate. Solvent components: *n*-hexane (O), ethyl acetate ( $B_2$ ), acetone ( $B_3$ ). For details see text.

Cholesterol (Ch) and panaxadiol acetate (PDA) were selected as a pair of model compounds and a ternary mixture consisting of *n*-hexane (O), ethyl acetate ( $B_2$ ) and acetone ( $B_3$ ) was adapted. First, the retention behaviour of Ch as a standard solute was determined by two binary eluents containing the same diluent. A pair of capacity ratios obtained by applying binaries of the type O +  $B_2$  and O +  $B_3$  is plotted in Fig. 4c (crosses). On the basis of eqn. 1, the correlation between the retention and the composition of the binary solvents can be quantitatively expressed as two straight lines. The proper composition of the binary systems O +  $B_2$  and O +  $B_3$  can be predicted by determining the points where these lines intersect with a horizontal line drawn to represent the capacity ratio ( $k' = 3$ , see broken line and open circles in Fig. 4b).

According to a model experiment described in the section on the W +  $S_1$  +  $S_2$  system, it is possible to control the retention directly by adjusting the solvent composition of the ternary solvent system. When a stronger eluent binary  $B_2$  +  $B_3$  is prepared with a composition ratio of 1:3, the molar ratio of the ternary system O +  $B_2$  +  $B_3$  with a capacity ratio of 3 for Ch can be found at the intersection with the horizontal broken line in Fig. 4c. This point was determined by proportional division of a segment of the dotted line according to the ratio of  $S_1$  versus  $S_2$ . This is represented by the closed circles.

To control the separation of a pair of solutes, the retention behaviour of PDA as a second compound was then determined. Two capacity ratios for PDA were obtained by using two binaries, O +  $B_2$  and O +  $B_3$ , which provide a capacity ratio of 3 for Ch. These points are indicated by open squares in Fig. 4d. In this instance, elution sequences of a pair of solutes in the O +  $B_2$  and O +  $B_3$  systems were reversed. The chromatograms obtained in this experiment are illustrated in Fig. 5a and c.

The distance between two points indicated by the open squares and open

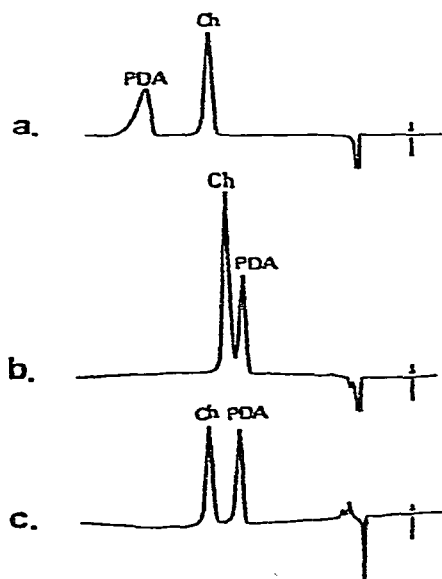


Fig. 5. Chromatograms showing the controlling process for the resolution of a pair of model compounds. Solvent components as in Fig. 4. (a) O +  $B_2$  ( $B_2$ : 24.5%); (b) O +  $B_2$  +  $B_3$  ( $X_3$ : 15%,  $B_2$ : $B_3$  = 1:3); (c) O +  $B_3$  ( $B_3$ : 12.6%).

circles in Fig. 4d is directly related to the logarithm of the separation factor. The resolution of a mixture can be controlled by adjusting the solvent system and the solvent composition.

The retention of PDA and the resolution of a mixture of Ch and PDA for the ternary solvent O + B<sub>2</sub> + B<sub>3</sub> which corresponded to a  $k'$  value of 3 for Ch were directly predicted by determining the point indicated by the closed square in Fig. 4e. Such an assumption was confirmed experimentally and the chromatogram obtained is shown in Fig. 5b.

As shown above, the optimization process for the ternary solvent system was elaborated systematically. First, a standard solute was selected and the retention behaviour of the solute was quantitatively determined by using a pair of binary solvent systems. Capacity ratios of the second solute are determined by using the same binaries. From these experimental data, ternary solvent systems, for which the separation factor is an average of the values obtained for the two corresponding binary systems, can be directly prepared. The systematic design of ternary solvent systems described in this paper can be applied effectively to control the retention and to optimize the separation of complex mixtures. The limitations of the method are due to the assumptions relating to the additivity of the slopes ( $n$ ) of  $\log k'$  vs.  $\log X_S$  plots as well as of the intercepts  $c$  (Eqns. 2d and 3d). The assumptions are realistic for limited differences in the solvent strengths of the components of the binary diluent  $W_1 + W_2$  or of the binary polar solvent  $S_1 + S_2$ . For larger differences in  $\epsilon^0$  values the  $\log k'$  vs.  $X_S$  plot becomes concave (*cf.*, corresponding plots for eluent strength of binary eluents<sup>17</sup>). As stated earlier, the linear  $\log k'$  vs.  $\log X_S$  relationship which follows from the Snyder–Soczewiński displacement model, does not apply to dilute solutions of polar solvents ( $X_S < 0.05$ ). The approach is thus based on semi-empirical equations and although simpler, is less general than that proposed recently by Glajch and Snyder<sup>3</sup> for multi-component eluents.

For larger differences in  $\epsilon^0$  values the linear equations 2d and 3d could probably be substituted by quadratic equations.

A microcomputer program for control of the resolution and the separation described in this report has been developed in Hara's laboratory. The program was found to be useful and reliable in analytical and synthetic chemistry research.

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