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DESIGN OF BINARY SOLVENT SYSTEMS FOR LIQUID-SOLID CHROMATOGRAPHY ON SILICA GEL COLUMNS USING MONO- AND DIFUNCTIONAL STEROIDS AS SOLUTES

SHOJI HARA* and AKIKO OHSAWA

Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03 (Japan)

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SUMMARY

In order to characterize binary solvents used in silica gel liquid-solid chromatography and to systematize their design, the capacity ratios in systems using various strong eluents such as tetrahydrofuran, dioxan and acetonitrile were determined by using mono- and disubstituted steroids as solutes. The strengths and selectivities of these solvent systems are discussed on the basis of retention indices. The design of equi-elutotropic binary systems containing these strong solvent components is presented.

INTRODUCTION

Binary solvent systems consisting of a diluent or weak component (W) and a stronger eluent (S) are commonly utilized to allow adjustment of the mobile phase in liquid-solid chromatography (LSC). In order to simplify the design of solvent systems, studies of the classification of solvents and of the correlation between the capacity ratios and binary solvent compositions for silica gel LSC were carried out. Retentions of fourteen mono- and disubstituted steroids in sixteen binary solvents were reported in previous papers^{1,2}. In the case of LSC, the solvent selectivity is the most valuable parameter for the resolution of a given mixture. An optimal system having good selectivity for a solute can be found by examining a variety of solvents. In continuation of the previous studies^{1,2}, we have investigated the characteristics of binary solvent systems containing tetrahydrofuran, dioxan or acetonitrile as the stronger eluting component.

These three common solvents have absorption peaks only in the shortwavelength UV region and are volatile, making them suitable for analytical and also preparative liquid chromatography. Although these solvents have often been employed in reversed-phase liquid chromatography, they have not yet been conventionally utilized in normal phase systems.

EXPERIMENTAL

The chromatographic systems used and the conditions adopted were described in a previous paper¹.

RESULTS AND DISCUSSION

It has been observed that hydrogen bonding is commonly involved in the adsorption-desorption equilibrium as a leading interaction between the solute and the solvent molecules at the active sites of the adsorbent surface. Based on these observations, solvent systems have been classified according to diluent type: O, P and N and stronger eluents: B (basic) and AB (acidic/basic)¹⁻⁵.

In previous studies^{1,2}, binary solvents were prepared using four diluents (W), *n*-hexane (O), benzene (P), dichloromethane (N₁) and chloroform (N₂), and six stronger eluting solvents (S), diethyl ether (B₁), ethyl acetate (B₂), acetone (B₃), 2-propanol (AB₁), ethanol (AB₂) and methanol (AB₃). To allow for an even wider spectrum of solvent systems, tetrahydrofuran (B₄), dioxan (B₅) and acetonitrile (B₆) were selected in this paper as additional S components. As examples of B₄, B₅ and B₆ solvents, the strength and the selectivity of the binary systems O + B₄, O + B₅, P + B₄, P + B₅, P + B₆, N₁ + B₄, N₁ + B₅, N₁ + B₆, N₂ + B₄, N₂ + B₅ and N₂ + B₆ were examined on silica gel. The O + B₆ system could not be tested because of its immiscibility.

The solutes were mono- and disubstituted steroids containing mediumly polar functions such as acyloxy, keto and hydroxyl groups. The same solutes were employed in previous work^{1,2}. The chemical structures of the solutes are shown in Fig. 1.

Correlation between retention and binary solvent composition

A linear relation between the logarithm of the capacity ratio and the mole fraction of the S component in binary solvents has been observed in silica gel LSC¹⁻⁸. The correlation has been shown to be

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

where *c* and *n* are constants, *k'* is the capacity ratio and *X_S* is the mole fraction of S in the binary system. Therefore, the retention data obtained by using B₄-W, B₅-W and B₆-W systems and the mole fraction of the S components were plotted using logarithmic scales. The experimental results are illustrated in Fig. 2. The two constants *c* and *n* were calculated by the least-squares procedure and are listed in Table I.

It was observed that the slope and the intercept of these plots increased in the order: esters (b, c) < 5β-ketone (e) < 5α-ketone (f) < α,β-unsaturated ketone (g) < alcohols (h, i). In Fig. 2, the retention data for three common types of solutes, esters (b), ketones (f) and alcohols (i), are indicated specifically by thick solid lines in order to clearly indicate relative retention behaviours. The elution order for the solutes mentioned above was found to be as same as that obtained previously¹ by using other B- and AB-W systems.

However, the relative retention for a pair of 5β (e) and 5α (f) ketones in B₄, B₅ and B₆ systems was found to be smaller than that in other stronger solvent systems such as B₁, B₂, B₃, AB₁, AB₂ and AB₃. Details of this comparison were given previously¹. These results show that the former solvent systems have lower selectivity for the

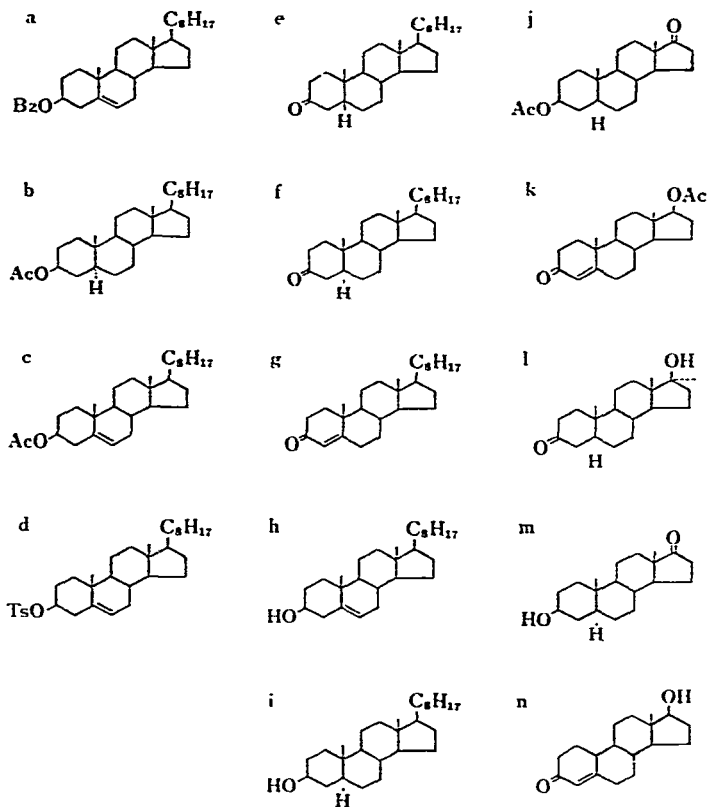


Fig. 1. Structural formulae of the steroids investigated. For identification, see Table I.

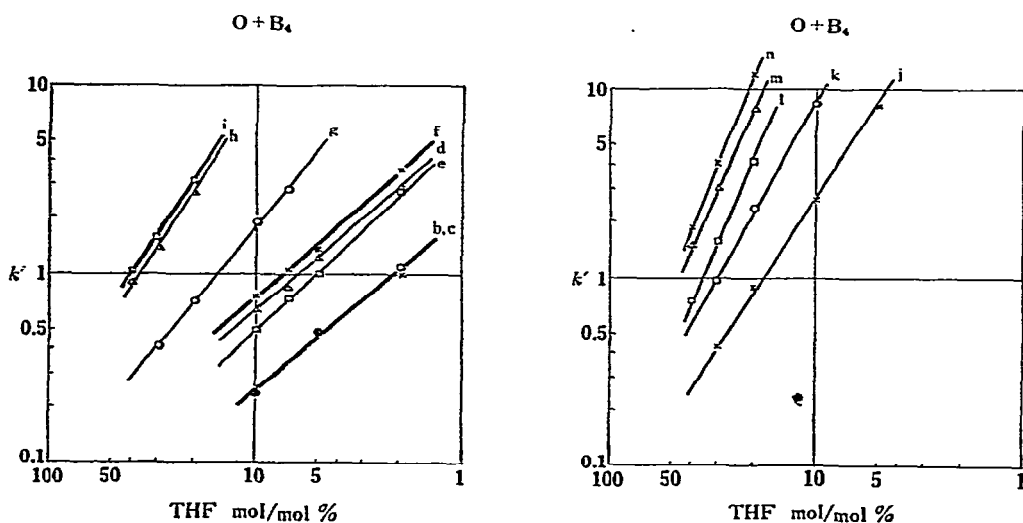


Fig. 2.

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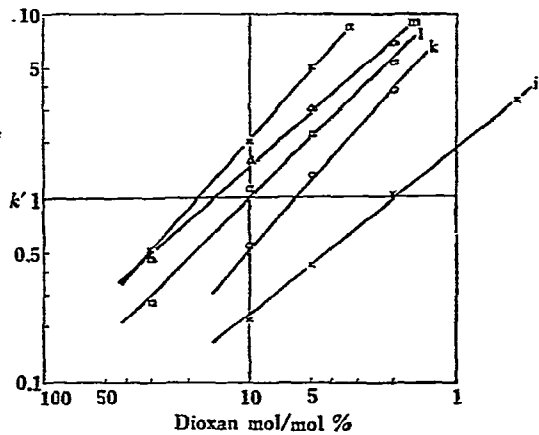
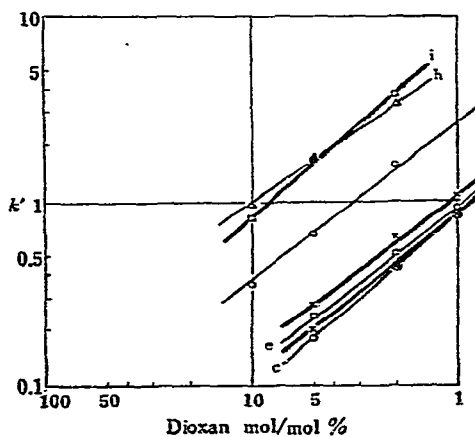
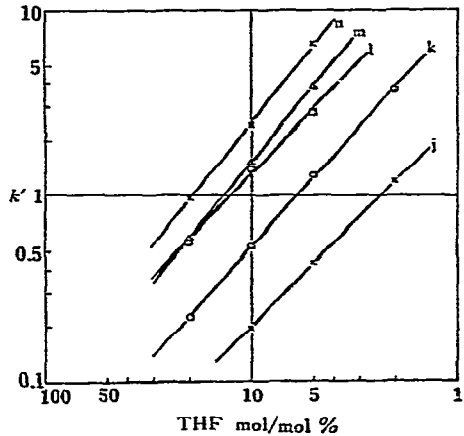
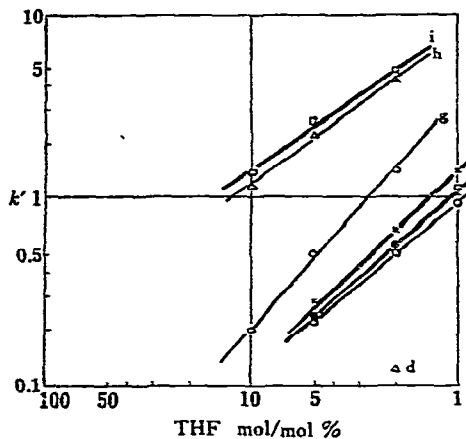
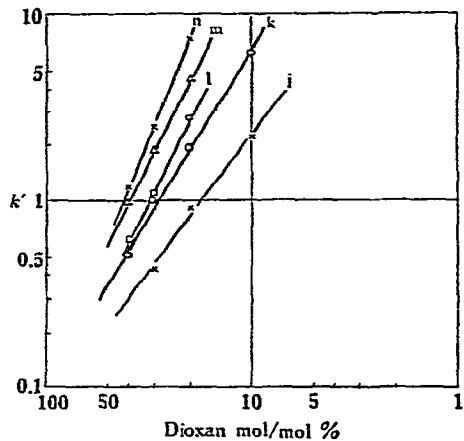
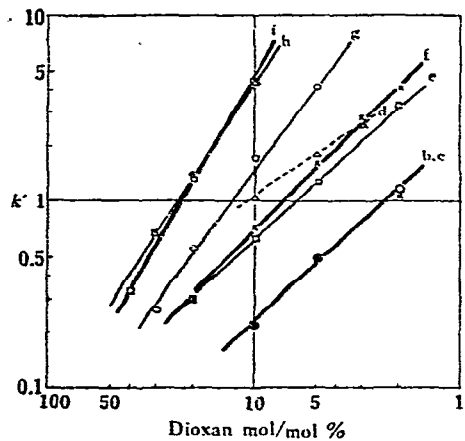


Fig. 2.

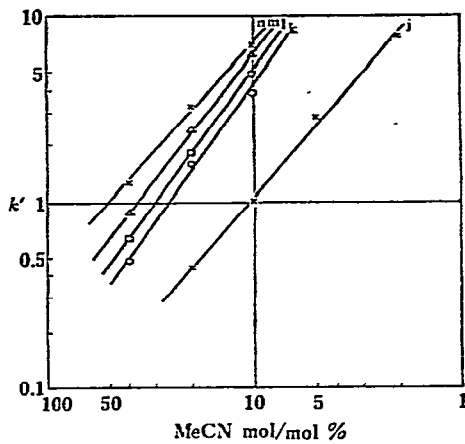
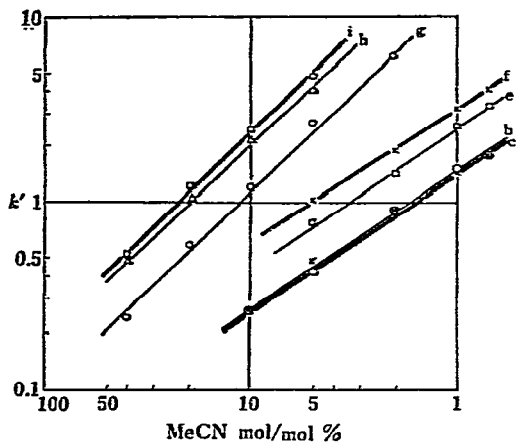
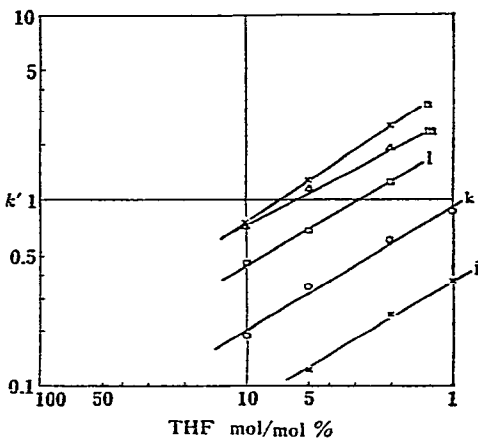
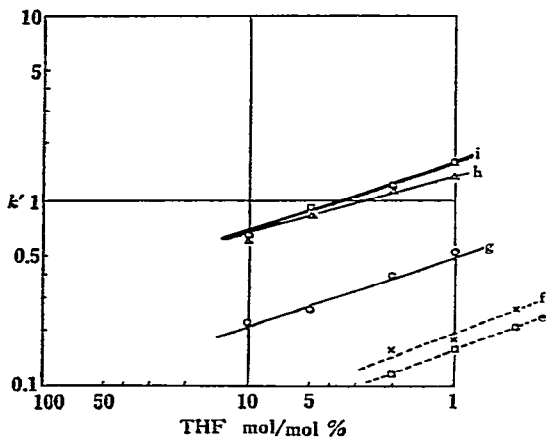
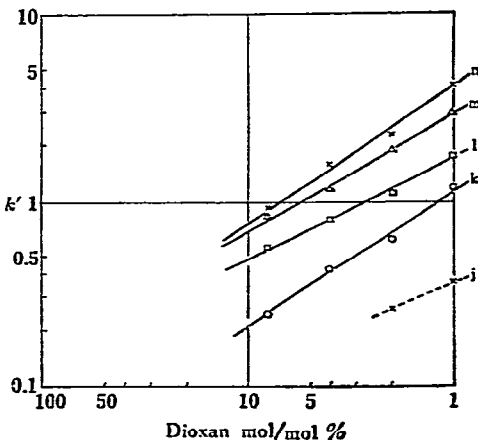
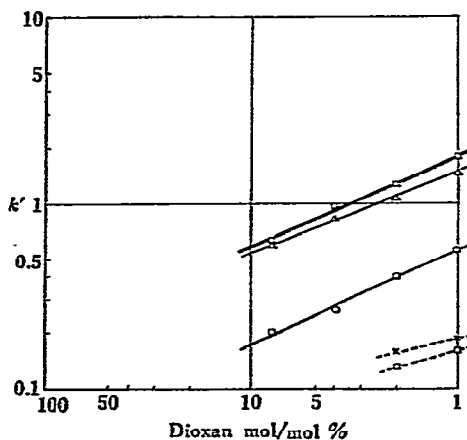
N₁+B₄N₁+B₄N₁+B₅N₁+B₅

Fig. 2.

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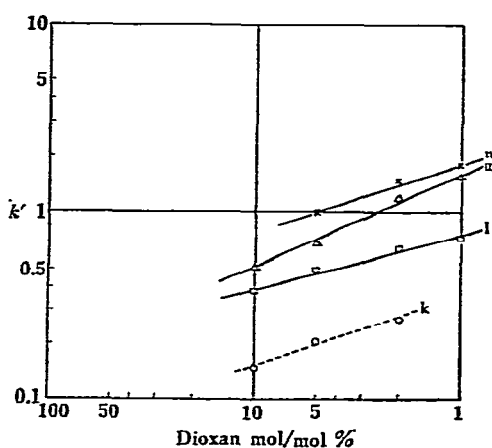
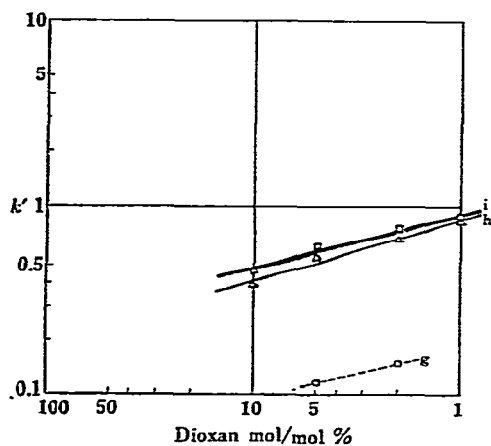
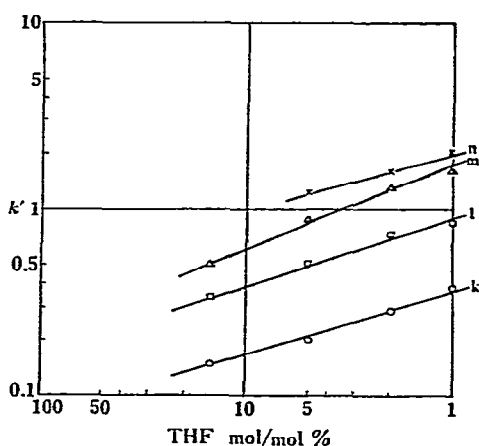
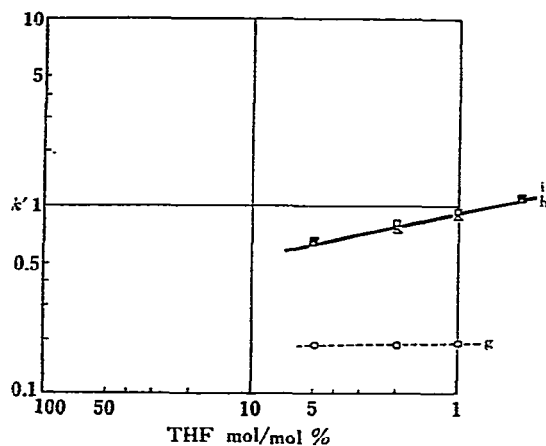
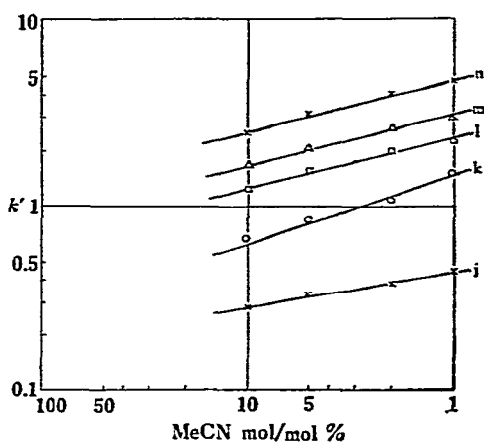
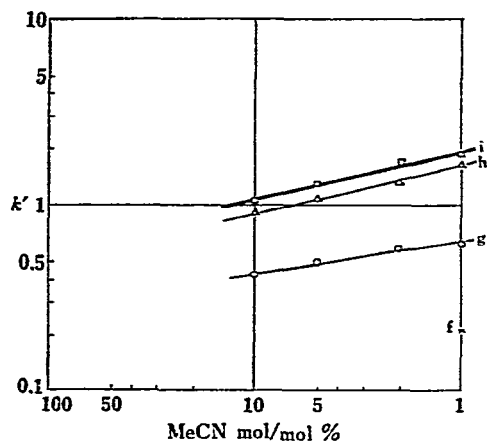


Fig. 2.

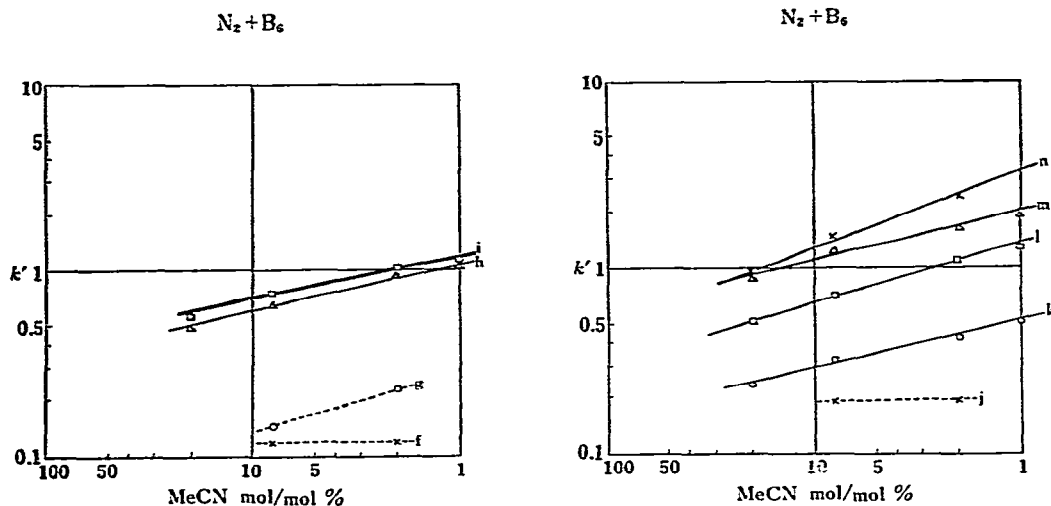


Fig. 2. Logarithm of capacity ratios on silica gel as a function of the logarithm of the concentration of the stronger solvent. In the examples shown here, *n*-hexane, benzene, dichloromethane and chloroform were used as the diluent. Samples (small letters) as in Table I.

separation of such a pair of diastereomeric isomers. It should be noted that the relative retentions for a pair of diastereomeric ketones (e, f) in P-binary systems such as $P + B_4$ and $P + B_5$ were much smaller than that in the other binary systems. When using B_4 , B_5 and B_6 solvent systems, one solute (3β -benzoyloxy-5-cholestene) was eluted so fast that its capacity ratio could not be determined. The elution behaviour of tosylate (d) varied greatly depending on the solvent system applied; it was slightly retained in binary solvents containing benzene (P) as the diluent.

The two constants for difunctional compounds were larger than those for monofunctional compounds. Whereas the slopes were smaller than the simple sums of the values for monofunctional compounds containing corresponding substituents, the intercepts were slightly larger than the sums of the values for the corresponding monosubstituted solutes.

The eluotropic strength of the W and S components in the binary systems was examined for each solute compound. When the slopes and the intercepts for the various diluents with the same stronger eluent were compared using the same solute, it was clear that both constants decreased in the order: $O > P > N_1 > N_2$. In terms of the eluotropic strength of the S component in binary systems containing the same W component, it was found that whereas the strengths of B_4 and B_5 are very close, B_6 is weaker than these in binary systems containing the same W component.

Design of equi-eluotropic binary solvent systems

To determine the relative strengths of the basic and basic/acidic eluents, data given previously¹ were incorporated and compared with the results obtained here. It was concluded that the strengths of B_4 and B_5 are comparable with those of acetone (B_3) in O- and P-binary systems. The strength of B_6 is almost the same as that of ethyl

TABLE I

CONSTANTS IN THE LINEAR RELATIONSHIP BETWEEN RETENTION AND SOLVENT COMPOSITION

Solvents: O = *n*-hexane; P = benzene; N₁ = dichloromethane; N₂ = chloroform; B₄ = tetrahydrofuran; B₅ = dioxan; B₆ = acetonitrile. Samples: b = 3β-acetoxy-5α-cholestane; c = 3β-acetoxy-5-cholestene; d = 3β-tosyloxy-5-cholestene; e = 5β-cholestan-3-one; f = 5α-cholestan-3-one; g = 4-cholesten-3-one; h = 5-cholesten-3β-ol; i = 5α-cholestan-3β-ol; j = 3β-acetoxy-5α-androstan-17-one; k = 17β-acetoxy-4-androsten-3-one; l = 17β-hydroxy-17α-methyl-5α-androstan-3-one; m = 3β-hydroxy-5α-androstan-17-one; n = 17β-hydroxy-19-nor-4-androsten-3-one.

| Sample | O + B ₄ | | O + B ₅ | | P + B ₄ | | P + B ₅ | | P + B ₆ | |
|--------|--------------------|------|--------------------|------|--------------------|------|--------------------|------|--------------------|------|
| | c | n | c | n | c | n | c | n | c | n |
| b | 0.32 | 0.92 | 0.39 | 1.01 | 0.04 | 0.95 | -0.05 | 0.90 | 0.16 | 0.75 |
| c | 0.32 | 0.92 | 0.39 | 1.01 | -0.01 | 0.91 | -0.08 | 1.02 | 0.14 | 0.75 |
| d | 0.76 | 0.95 | | | | | | | | |
| e | 0.74 | 1.00 | 0.82 | 1.04 | 0.03 | 0.95 | -0.02 | 0.88 | 0.34 | 0.72 |
| f | 0.85 | 0.94 | 0.97 | 1.08 | 0.14 | 1.05 | 0.02 | 0.85 | 0.50 | 0.69 |
| g | 1.65 | 1.39 | 1.54 | 1.36 | 0.52 | 1.21 | 0.42 | 0.84 | 1.11 | 1.04 |
| h | 2.79 | 1.76 | 2.28 | 1.65 | 0.87 | 0.81 | 0.74 | 0.75 | 1.28 | 0.95 |
| i | 2.86 | 1.75 | 2.43 | 1.76 | 0.94 | 0.80 | 0.86 | 0.94 | 1.46 | 1.07 |
| j | 2.03 | 1.60 | 1.78 | 1.44 | 0.44 | 1.11 | 0.26 | 0.91 | 1.26 | 1.24 |
| k | 2.82 | 1.90 | 2.57 | 1.78 | 0.94 | 1.22 | 0.98 | 1.26 | 2.08 | 1.49 |
| l | 4.11 | 2.64 | 3.23 | 2.19 | 1.26 | 1.16 | 1.03 | 1.04 | 2.13 | 1.44 |
| m | 4.15 | 2.49 | 3.52 | 2.20 | 1.49 | 1.31 | 1.16 | 1.01 | 2.15 | 1.37 |
| n | 4.74 | 2.80 | 4.36 | 2.67 | 1.78 | 1.38 | 1.61 | 1.30 | 2.13 | 1.26 |

| | N ₁ + B ₄ | | N ₁ + B ₅ | | N ₁ + B ₆ | | N ₂ + B ₄ | | N ₂ + B ₅ | | N ₂ + B ₆ | |
|---|---------------------------------|------|---------------------------------|------|---------------------------------|------|---------------------------------|------|---------------------------------|------|---------------------------------|------|
| | c | n | c | n | c | n | c | n | c | n | c | n |
| g | -0.30 | 0.38 | -0.26 | 0.50 | -0.20 | 0.17 | | | | | | |
| h | 0.13 | 0.29 | 0.18 | 0.42 | 0.21 | 0.25 | -0.03 | 0.25 | -0.07 | 0.32 | 0.03 | 0.24 |
| i | 0.20 | 0.35 | 0.22 | 0.41 | 0.28 | 0.23 | -0.03 | 0.25 | -0.04 | 0.27 | 0.05 | 0.20 |
| j | -0.44 | 0.66 | | | -0.36 | 0.18 | | | | | | |
| k | -0.05 | 0.66 | 0.04 | 0.76 | 0.15 | 0.35 | -0.43 | 0.34 | | | -0.26 | 0.28 |
| l | 0.28 | 0.64 | 0.26 | 0.58 | 0.34 | 0.24 | -0.04 | 0.37 | -0.12 | 0.28 | 0.14 | 0.33 |
| m | 0.44 | 0.57 | 0.47 | 0.60 | 0.48 | 0.24 | 0.23 | 0.43 | 0.18 | 0.48 | 0.32 | 0.28 |
| n | 0.61 | 0.72 | 0.61 | 0.68 | 0.67 | 0.25 | 0.33 | 0.29 | 0.26 | 0.36 | 0.51 | 0.39 |

acetate (B₂) in a P-binary system. The strengths of B₄, B₅ and B₆ systems containing an N component are greater than the corresponding binary systems containing O or P components.

A general procedure for finding the concentrations of S and W components in binary mobile phases with approximately equal elution strengths was established previously², and the design of an equi-elutotropic binary solvent system based on the linear relationship in eqn. 1 became feasible². This expression is

$$\log X_{S(1)} = \frac{c_1 - c_2}{n_1} + \frac{n_2}{n_1} \log X_{S(2)} \quad (2)$$

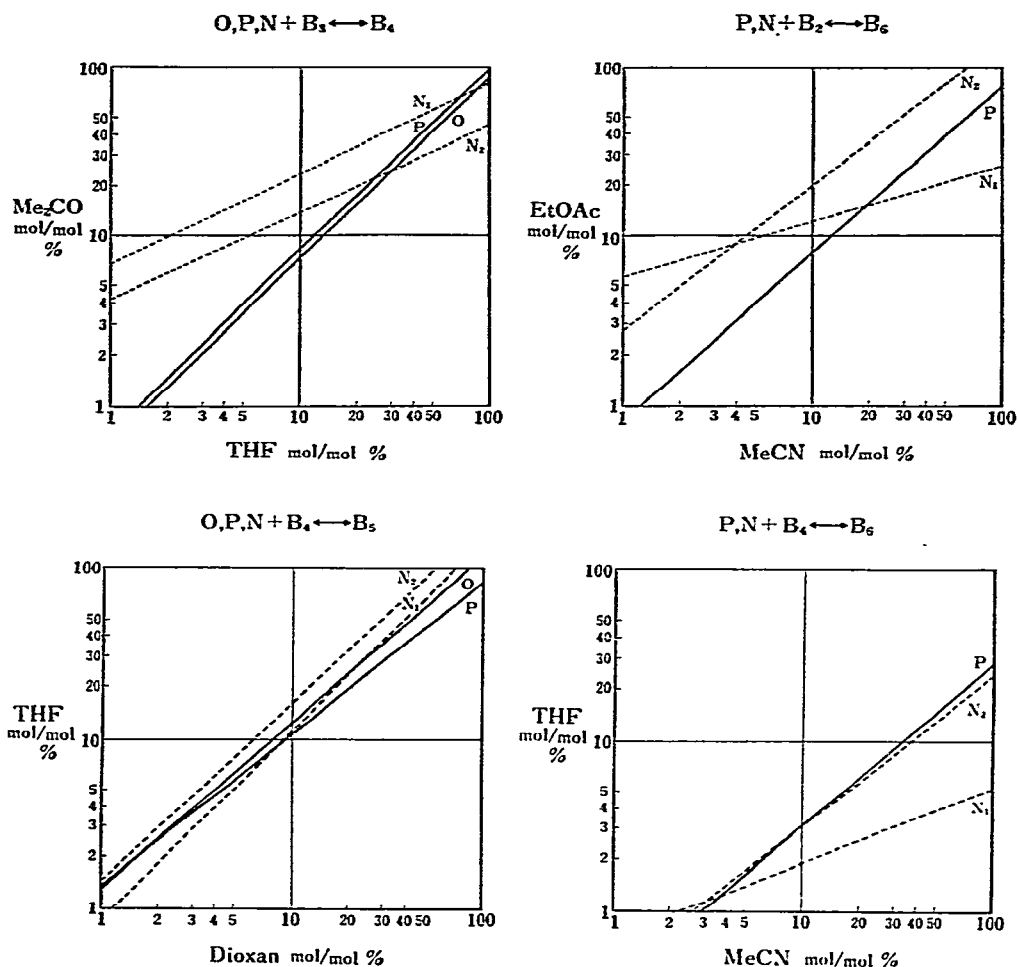


Fig. 3. Correlation between the concentrations of the stronger components for a pair of equi-elutotropic binary solvent systems with various diluents. Interchange for S components. Solvents: O = *n*-hexane; P = benzene; N₁ = dichloromethane; N₂ = chloroform; B₂ = ethyl acetate; B₃ = acetone; B₄ = tetrahydrofuran; B₅ = dioxan; B₆ = acetonitrile. Samples as in Table I.

where $X_{S(1)}$ and $X_{S(2)}$, c_1 and c_2 and n_1 and n_2 are the molar ratios of S and the two constants in eqn. 1 for a pair of equi-elutotropic solvent systems 1 and 2, respectively.

Constants for terms I and II were thus calculated for each solute compound. In order to determine the effect of the diluent on the molar percentages of the stronger eluent in two equi-elutotropic solvent systems, the average values of the constant terms given by all of the samples as related to the weak components (O, P, N₁ and N₂) were calculated. Examples comparing S components having similar strengths are illustrated in Fig. 3, where the correlations between the strengths of binary systems are indicated.

The present experimental results suggest that B₄-, B₅- and B₆-W systems are adjustable for a variety of solutes having medium polarity. A few of the B₄-binary solvents were applied to the optimization of the mobile phase in the separation of

several natural products. These results have already been reported^{4,5}. It is evident that the procedure for designing B₄, B₅ and B₆ binary solvent systems broadens the spectrum of mobile phases available for LSC, and that this procedure can be useful in the systematic design of chromatographic systems having optimal eluting strengths and selectivities.

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