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DESIGN OF EQUI-ELUOTROPIC BINARY SOLVENT SYSTEMS FOR SILICA GEL LIQUID CHROMATOGRAPHY*

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SUMMARY

Based on the previously reported linear relationship between the logarithm of the capacity factor and the logarithm of the binary solvent composition, a procedure for finding concentrations of a stronger eluent or a diluent in the binary mobile phase with an approximately equal elution strength for general compounds was established using mono- and disubstituted steroid derivatives as solutes on a silica gel column. The design of an equi-eluotropic binary solvent system was compared with Snyder's and Neher's previously proposed calculations. This new procedure can be useful for the selection of an optimal solvent system that has maximal selectivity for a given sample mixture in liquid–solid chromatography.

INTRODUCTION

The optimization of the mobile phase in liquid chromatographic separations with a given solute mixture is of obvious importance. The solvent system is generally required to have a suitable elution strength and also a favourable selectivity for the particular sample compounds[1]. Because the interaction between the solvent and the solute is fairly specific, in liquid–solid chromatography, a number of solvent systems that have almost identical elution strengths are usually prepared. The most suitable solvent system with a desirable selectivity for the given solute can finally be found from these solvents. It is, therefore, necessary to provide a mobile phase that consists of different solvent components and having a similar elution strength. This procedure is defined as the design of an equi-eluotropic solvent system.

For controlling the retention of given solutes, binary solvent systems composed of a diluent or weak eluent (W) and a stronger eluent (S) are varied in their composition and are most commonly utilized in liquid chromatography. Snyder[1] has proposed a formula for obtaining the elution strength parameter of a binary mobile phase, ε^0_{AB}, on the basis of theoretical considerations by using the solvent strength parameters of the pure components, ε^0_A and ε^0_B, previously reported, and the molar

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fraction of the more strongly adsorbed component. An equi-eluotropic binary solvent system can be designed from Snyder's calculation[1,2]. Neher[3] also proposed a procedure for the design of an equi-eluotropic binary solvent system, which has been generally utilized because of its simplicity[1,2,4,5]. However, the observed R_F value in thin-layer chromatography used in his procedure is not consistent with the theoretical mobility or "real" R_F value obtained by column liquid chromatography[1,6]. Consequently, it is recommended that the latter data be used for the theoretical consideration[1]. In a previous paper[7], a correlationship between the retention behaviour of the mono- and disubstituted steroid derivatives on a silica gel column and a number of binary solvent compositions was determined quantitatively by using high-performance liquid chromatography. Based on these results, a new procedure for developing an equi-eluotropic binary solvent system is described in this paper.

EXPERIMENTAL

The chromatographic systems used and the conditions adopted were described in a previous paper[7].

RESULTS AND DISCUSSION

Binary mobile phases in liquid chromatography are composed of a diluent (W) and a stronger eluent (S). According to the concept of the adsorption–desorption equilibrium on a silica gel surface in liquid–solid chromatography, a linear relationship exists between the logarithm of the molar fraction of the stronger eluent and the logarithm of the capacity factor, as has been proposed by Soczewiński[8,9], Snyder[10] and Jandera and co-workers[11,12]:

$$\log k' = c - n \log X_s \tag{1}$$

where X_s is the molar concentration of the stronger eluent (S) and c and n are constants for a particular solute and binary solvent system. The constant c is related to the characteristics of the stationary phase whereas n is dependent on the solute structure, mainly its functional groups. The constants for monosubstituted cholestane and disubstituted androstane derivatives, with the more common non-ionic functional groups such as acyloxy, keto and hydroxy in their steroid nuclei, were determined. The packing was silica gel with a pore size of 70 Å, commonly used for thin-layer and column liquid chromatography. Pure solvents have been classified according to their degree of hydrogen bonding with a sorbent and a solute[7,13]. The solvents used were *n*-hexane (O), benzene (P), dichloromethane (N_1), chloroform (N_2), diethyl ether (B_1), ethyl acetate (B_2), acetone (B_3), 2-propanol (AB_1), ethanol (AB_2) and methanol (AB_3). Binary solvent systems were prepared[7].

If two solvent systems containing different stronger eluting solvents and the same diluent afford identical capacity factors for a particular solute compound, they are called equi-eluotropic and are related to each other according to eqn. 1 (ref. 7):

$$\log k'_{(1)} = \log k'_{(2)} = c_1 - n_1 \log X_{s(1)} = c_2 - n_2 \log X_{s(2)}$$

$$\log X_{s(1)} = \underbrace{\frac{c_1 - c_2}{n_1}}_{\text{(I)}} + \underbrace{\frac{n_2}{n_1} \log X_{s(2)}}_{\text{(II)}} \tag{2}$$

where $k'_{(1)}$ and $k'_{(2)}$, $X_{s(1)}$ and $X_{s(2)}$, c_1 and c_2 and n_1 and n_2 are the capacity factors, the molar ratio of stronger components and the two constants in eqn. 1 for a pair of equi-eluotropic solvent systems 1 and 2, respectively. According to eqn. 2, an equi-eluotropic mobile phase with a different component S can be readily determined. If the diluent is changed but the stronger eluent remains the same, an equi-eluotropic system with a different diluent can also be calculated.

Constants for terms I and II were thus calculated for each solute compound. Table I shows the data which were obtained by exchanging two of the stronger components with each other, *i.e.*, $B_1 \leftrightarrow B_2$, $B_1 \leftrightarrow B_3$, $B_2 \leftrightarrow B_3$, $B_1 \leftrightarrow AB_1$, $B_2 \leftrightarrow AB_1$, $B_3 \leftrightarrow AB_1$, and using *n*-hexane (O) as diluent. For the examination of the effect of the structure of the solute molecule on equi-eluotropic correlations, average values of the constant terms given by mono- and disubstituted solutes and all samples, and also the standard deviations of the constants, were calculated by exchanging two of the stronger components with each other, *i.e.*, $B_1 \leftrightarrow B_2$, $B_1 \leftrightarrow B_3$, $B_2 \leftrightarrow B_3$, $B_1 \leftrightarrow AB$, $B_2 \leftrightarrow AB$ and $B_3 \leftrightarrow AB$, and using *n*-hexane (O), benzene (P), dichloromethane (N_1) and chloroform (N_2) as diluents, and also by exchanging the pair of diluents with each other, *i.e.*, $O \leftrightarrow P$, $O \leftrightarrow N$ and $P \leftrightarrow N$, using a stronger component [diethyl ether (B_1), ethyl acetate (B_2), acetone (B_3) or, 2-propanol (AB_1)] or another diluent [dichloromethane (N_1), benzene (P) or *n*-hexane (O)] (Table II).

TABLE I

CONSTANT TERMS I AND II IN EQN. 2 FOR A LINEAR RELATIONSHIP BETWEEN THE CONCENTRATIONS OF STRONGER COMPONENTS IN A PAIR OF BINARY SOLVENT SYSTEMS CONTAINING *n*-HEXANE AS A DILUENT

Stronger solvents: B_1 = diethyl ether; B_2 = ethyl acetate; B_3 = acetone; AB_1 = 2-propanol. 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	*Stronger component*											
	$B_1 \rightarrow B_2$		$B_1 \rightarrow B_3$		$B_2 \rightarrow B_3$		$B_1 \rightarrow AB_1$		$B_2 \rightarrow AB_1$		$B_3 \rightarrow AB_1$	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
b	0.0	1.1	0.6	1.0	0.5	0.9						
c	0.0	1.1	0.5	1.0	0.4	0.9	1.5	0.5	1.4	0.5	1.1	0.5
d	0.2	1.0	0.2	0.9	0.0	0.9	1.6	0.6	1.4	0.6	1.5	0.7
e	0.0	1.3	0.5	1.0	0.4	0.8	1.4	0.4	1.1	0.3	0.9	0.4
f	−0.3	1.5	0.6	1.0	0.6	0.7	1.4	0.6	1.2	0.4	0.9	0.6
g	0.0	1.2	0.7	0.9	0.6	0.7	1.4	0.6	1.1	0.5	0.8	0.7
h	0.2	1.1	0.5	1.1	0.2	1.0	1.4	0.6	1.2	0.5	0.9	0.5
i	0.2	1.1	0.4	1.1	0.2	1.0	1.4	0.7	1.1	0.6	0.9	0.6
j	0.3	1.0	0.9	0.6	0.7	0.7	1.5	0.4	1.3	0.4	1.0	0.6
k	0.2	1.0	0.7	0.8	0.5	0.8	1.5	0.4	1.3	0.4	0.9	0.5
l	0.3	0.9	0.9	0.7	0.6	0.8	1.5	0.5	1.2	0.5	0.8	0.7
m	−0.1	1.2	0.5	0.9	0.5	0.8	1.4	0.5	1.3	0.4	1.0	0.6
n	−0.3	1.3	0.2	1.1	0.4	0.9	1.3	0.6	1.2	0.4	0.9	0.5

TABLE II

MEAN VALUES OF CONSTANT TERMS I AND II IN EQN. 2 FOR A LINEAR RELATIONSHIP BETWEEN THE CONCENTRATIONS OF STRONGER COMPONENTS AND DILUENTS IN A PAIR OF BINARY SOLVENT SYSTEMS

Diluents: O = *n*-hexane; P = benzene; N_1 = dichloromethane; N_2 = chloroform. Stronger solvents: AB_2 = ethanol; AB_3 = methanol. Sample: a = 3β-benzoyloxy-5-cholestene. Other stronger solvents and samples as in Table I.

Diluent	*Value*	*Stronger component*											
		$B_1 \rightarrow B_2$		$B_1 \rightarrow B_3$		$B_2 \rightarrow B_3$		$B_1 \rightarrow AB_1$		$B_2 \rightarrow AB_1$		$B_3 \rightarrow AB_1$	
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
O	Average value for monosubstituted cholestanes	0.0	1.2	0.5	1.0	0.4	0.9	1.5	0.6	1.2	0.5	1.0	0.6
	Average value for disubstituted androstanes	0.1	1.1	0.7	0.9	0.5	0.8	1.4	0.5	1.2	0.4	0.9	0.6
	Average value for all samples	0.1	1.1	0.6	1.0	0.4	0.8	1.5	0.5	1.2	0.5	1.0	0.6
	Standard deviation	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1
P	Average value for monosubstituted cholestanes	0.0	1.1	0.5	1.1	0.5	1.0	0.9	1.3	0.8	1.1	0.3	1.0
	Average value for disubstituted androstanes	−0.5	1.4	0.3	1.3	0.5	0.9	0.8	1.5	0.9	1.1	0.4	1.2
	Average value for all samples	−0.2	1.2	0.4	1.2	0.5	1.0	0.9	1.4	0.8	1.1	0.4	1.1
	Standard deviation	0.4	0.3	0.4	0.4	0.3	0.2	0.2	0.4	0.2	0.3	0.2	0.2
N_1	Average value for monosubstituted cholestanes	−0.3	1.7	0.2	1.2	0.3	0.7	1.1	1.5	0.8	0.9	0.8	1.3
	Average value for disubstituted androstanes	0.5	0.8	0.1	1.3	−0.7	1.9	1.0	1.5	0.6	2.1	0.7	1.1
	Average value for all samples	−0.1	1.5	0.1	1.2	0.0	1.0	1.0	1.5	0.7	1.2	0.7	1.2
	Standard deviation	0.4	0.5	0.1	0.2	0.5	0.6	0.1	0.3	0.1	0.6	0.2	0.4
N_2	Average value for monosubstituted cholestanes	0.0	1.5	−1.8	4.1	−1.3	2.9	1.1	3.7	0,9	2.5	0.8	1.1
	Average value for disubstituted androstanes			−1.8	4.2			−0.3	4.8			0.7	1.2
	Average value for all samples	0.0	1.5	−1.8	4.2	−1.3	2.9	−0.2	4.4	0.9	2.5	0.8	1.2
	Standard deviation	0.4	0.1	0.7	1.2	0.4	0.9	3.1	1.0	0.1	0.3	0.1	0.1

Stronger component	*Value*	*Diluent*											
		$O \rightarrow P$		$O \rightarrow N_1$		$O \rightarrow N_2$		$P \rightarrow N_1$		$P \rightarrow N_2$		$N_1 \rightarrow N_2$	
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
B_1	Average value for monosubstituted cholestanes	0.9	0.8	1.2	0.6	2.0	0.1	0.3	0.8	1.7	0.2	1.6	0.3
	Average value for disubstituted androstanes	1.3	0.5	1.5	0.4	2.0	0.1	0.4	0.9	1.6	0.2	1.3	0.2
	Average value for all samples	1.0	0.7	1.3	0.5	2.0	0.1	0.4	0.9	1.6	0.2	1.4	0.2
	Standard deviation	0.4	0.4	0.1	0.2	0.1	0.0	0.2	0.3	0.1	0.1	0.2	0.1
B_2	Average value for monosubstituted cholestanes	0.8	0.6	1.0	0.8	1.6	0.1	0.1	1.2	1.4	0.3	1.1	0.2
	Average value for disubstituted androstanes	0.9	0.6	1.5	0.2			1.0	0.4				
	Average value for all samples	0.8	0.6	1.1	0.6	1.6	0.1	0.4	1.0	1.4	0.3	1.1	0.2
	Standard deviation	0.1	0.1	0.3	0.3	0.1	0.0	0.4	0.4	0.2	0.1	0.3	0.1

$B_1 \rightarrow AB_2$		$B_1 \rightarrow AB_3$		$B_2 \rightarrow AB_2$		$B_2 \rightarrow AB_3$		$B_3 \rightarrow AB_2$		$B_3 \rightarrow AB_3$		$AB_1 \rightarrow AB_2$		$AB_1 \rightarrow AB_3$		$AB_2 \rightarrow AB_3$	
I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
1.0	1.5	0.8	1.5	0.9	1.3	0.8	1.4	0.5	1.3	0.3	1.5	0.2	1.4	0.1	1.6	−0.1	1.2
0.9	1.6	0.7	1.6	1.0	1.1	0.8	1.1	0.5	1.3	0.3	1.3	0.1	1.1	−0.1	1.1	−0.1	1.0
0.9	1.5	0.8	1.5	0.9	1.2	0.8	1.3	0.5	1.3	0.3	1.4	0.1	1.2	0.0	1.4	−0.1	1.1
0.2	0.4	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.3	0.1	0.3	0.3	0.4	0.3	0.6	0.1	0.2

Stronger component	*Value*	*Diluent*											
		$O \rightarrow P$		$O \rightarrow N_1$		$O \rightarrow N_2$		$P \rightarrow N_1$		$P \rightarrow N_2$		$N_1 \rightarrow N_2$	
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
B_3	Average value for monosubstituted cholestanes	1.0	0.6	0.9	0.6	1.3	0.3	−0.1	0.9	0.9	0.5	0.7	0.7
	Average value for disubstituted androstanes	0.9	0.7	1.0	0.6	1.4	0.3	0.2	0.9	0.9	0.5	0.7	0.6
	Average value for all samples	0.9	0.7	1.0	0.6	1.4	0.3	0.0	0.9	0.9	0.5	0.7	0.6
	Standard deviation	0.3	0.1	0.3	0.2	0.1	0.1	0.3	0.2	0.1	0.1	0.2	0.2
AB_1	Average value for monosubstituted cholestanes	0.4	1.2	0.7	1.4	0.9	0.8	0.3	1.1	0.6	0.6	0.7	0.7
	Average value for disubstituted androstanes	0.4	1.5	0.8	1.3	1.1	0.7	0.3	0.9	0.5	0.5	0.2	0.6
	Average value for all samples	0.4	1.3	0.7	1.3	1.0	0.7	0.3	1.0	0.6	0.5	0.4	0.7
	Standard deviation	0.2	0.4	0.2	0.5	0.3	0.1	0.1	0.4	0.1	0.1	0.3	0.2

(Continued on p. 122)

TABLE II *(continued)*

Value	*Diluents 1 and 2*					
	N_1; $O \rightarrow P$		P; $O \rightarrow N_1$		O; $P \rightarrow N_1$	
	I	*II*	*I*	*II*	*I*	*II*
Average value	1.7	0.2	1.7	0.2	0.0	1.0
Standard deviation	0.0	0.0	0.0	0.0	0.5	0.3

Fig. 1 shows the correlation between the two concentrations of stronger components for a pair of equi-eluotropic solvent systems containing *n*-hexane (O) as a diluent, $X_{s(1)}$, $X_{s(2)}$, in terms of a particular solute compound. The data for mono- and disubstituted steroids and mean values for the two groups of samples are indicated by thin, dotted and thick lines, respectively. In order to compare our procedure with Snyder's and Neher's proposals for the design of an equi-eluotropic binary solvent system, the latter data were also plotted.

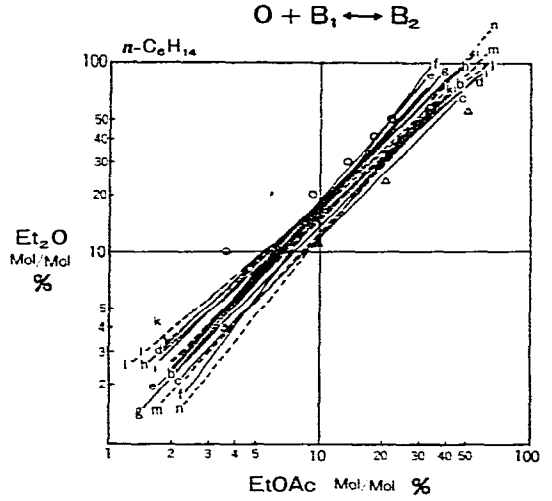
Fig. 1 shows that the correlation lines spread rather widely due to the particular solute compound. However, this deviation itself is a useful selectivity factor of the sample–solvent interaction, which makes possible the resolution of complex sample mixtures[1,10]. On the other hand, only a small difference between the average values for the mono- and disubstituted steroid derivatives is observed. Consequently, the mean values seem to be reliable for the general solute compounds. It is evident that Snyder's and Neher's data plotted in Fig. 1 are slightly different from our data.

For finding the effect of the diluent (W) on the molar percentages of the stronger eluents (S) in two equi-eluotropic solvent systems, the average values of the constant terms given by all of the samples as related to the weak components (O, P, N_1 and N_2) are plotted in Fig. 2. For finding the effect of the stronger solvent (S) or the diluent (W) on the molar percentages of the other weak eluents (W) in two equi-eluotropic solvent systems, the average values of the constant terms as related to the stronger component (B_1, B_2, B_3 and AB_1) and the weak component (O, P and N_1) are shown in Fig. 3. An equi-eluotropic concentration of a component in the given pair of binary solvent systems can be found readily by employing Figs. 2 and 3.

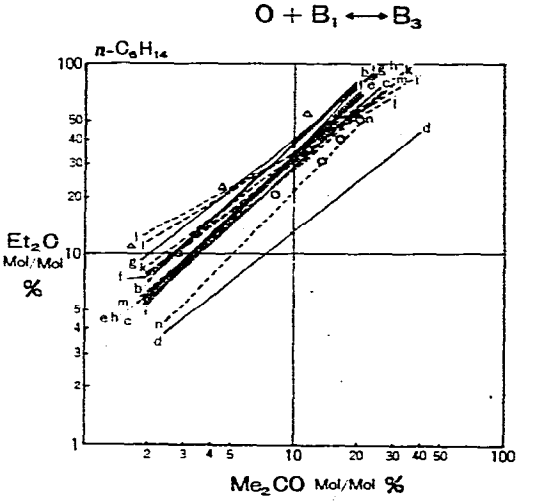
In order to optimize a binary solvent system for a given solute mixture in silica gel liquid–solid chromatography, a solvent system with a suitable elution

Fig. 1. Correlation between the concentrations of the two stronger components for a pair of equi-eluotropic binary solvent systems containing *n*-hexane as a diluent in terms of a particular solute compound. Thin solid lines, monosubstituted cholestane derivatives; thin broken lines, disubstituted androstane derivatives; thick solid lines, average value for monosubstituted cholestanes; thick broken lines, average value for disubstituted androstanes. △, Neher's data obtained with cyclohexane–binary solvent systems (ref. 3, p. 249); ○, Snyder's data obtained with *n*-pentane–binary solvent systems. Methanol was used instead of 2-propanol (ref. 1, p. 379). Solvent systems: O + B_1 = *n*-hexane–diethyl ether; O + B_2 = *n*-hexane–ethyl acetate; O + B_3 = *n*-hexane–acetone; O + AB_1 = *n*-hexane–2-propanol. 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.

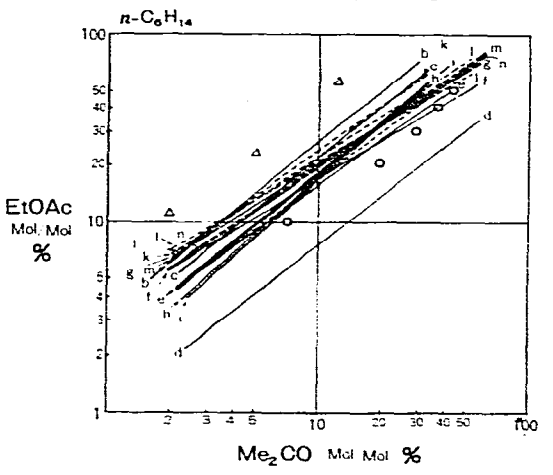
O + B_1 ⟷ B_2



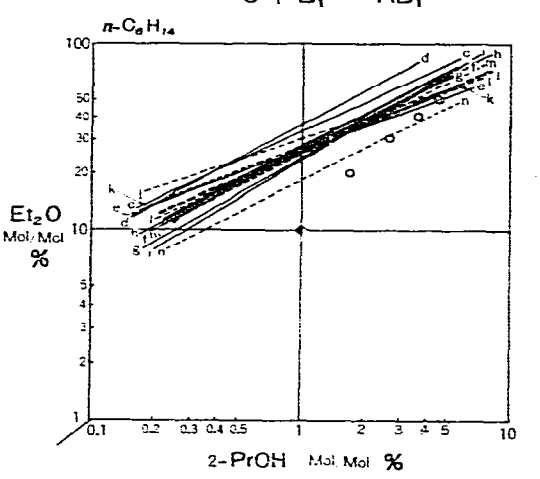
O + B_1 ⟷ B_3



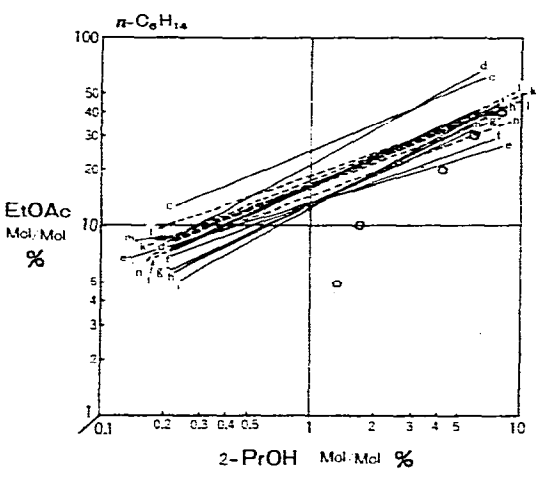
O + B_2 ⟷ B_3



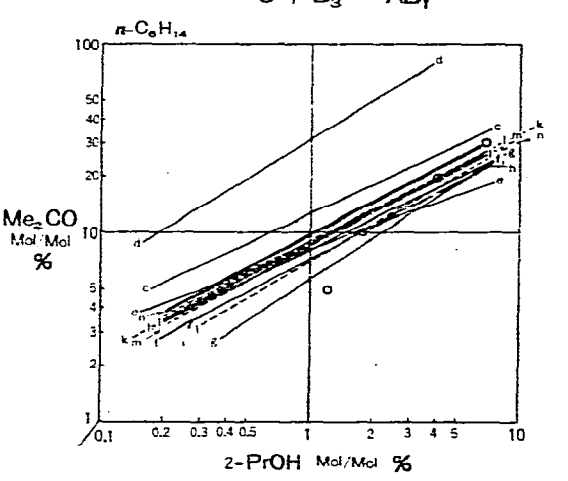
O + B_1 ⟷ AB_1



O + B_2 ⟷ AB_1



O + B_3 ⟷ AB_1



strength is first prepared. If the molecular structure of a given sample or the functional groups included in the solute are known, a preferable solvent combination and a suitable binary solvent composition can be assumed by consulting the correlation between the retention behaviour of the solute and the binary solvent composition in terms of the chemical structure of the sample compound, as described previously[7]. For the selection of an optimal mobile phase with a favourable selectivity for a given sample mixture, equi-eluotropic binary solvent systems are prepared successively. The stronger eluents are usually exchanged in turn by consulting the correlation between the concentrations of a pair of stronger components, $X_{s(1)}$ and $X_{s(2)}$, in a pair of binary solvent systems. The ratio of $X_{s(1)}$ to $X_{s(2)}$ in a pair of solvent systems containing the same diluent can be found directly by using Fig. 2 as described here. The diluents can also be exchanged by using Fig. 3 in a similar manner.

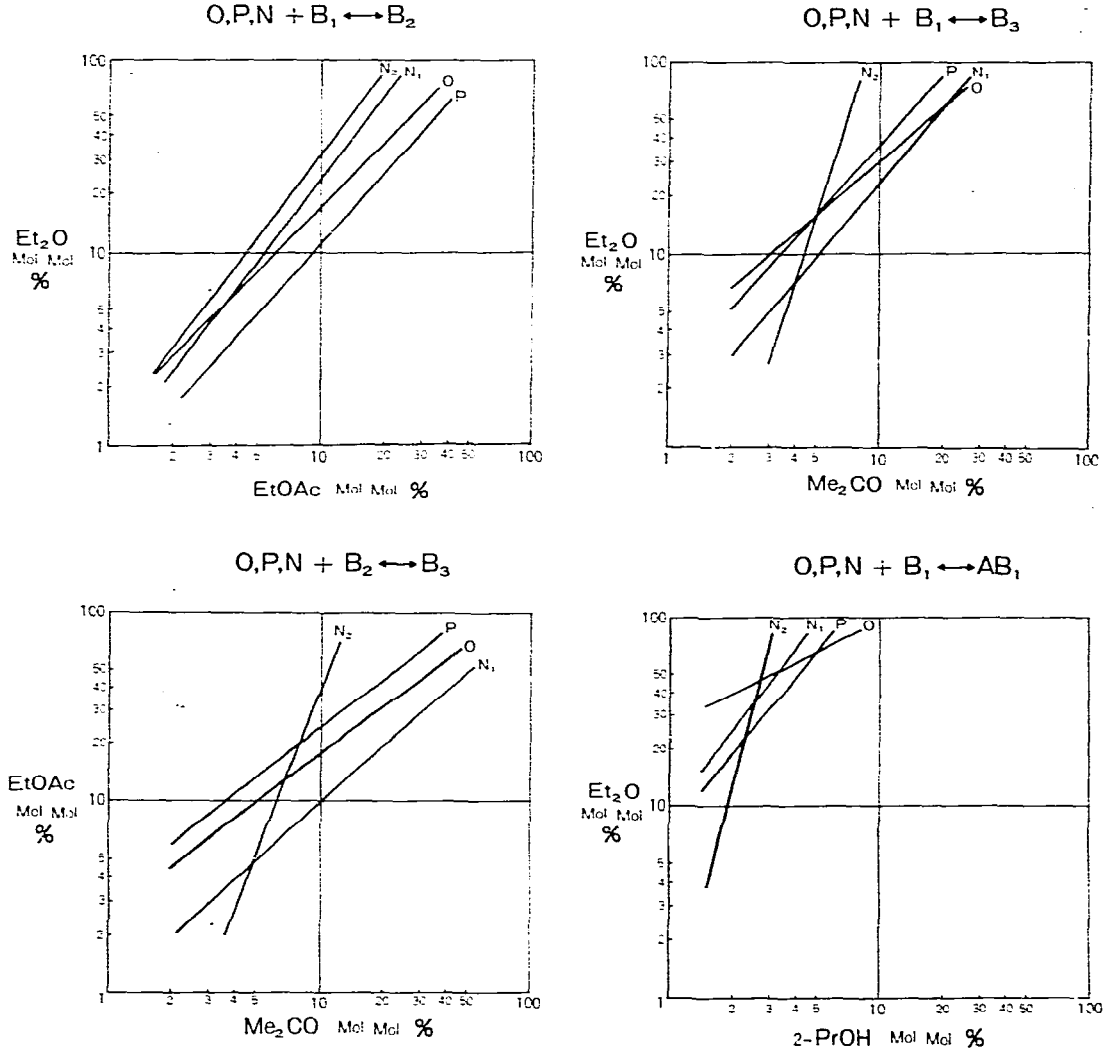


Fig. 2.

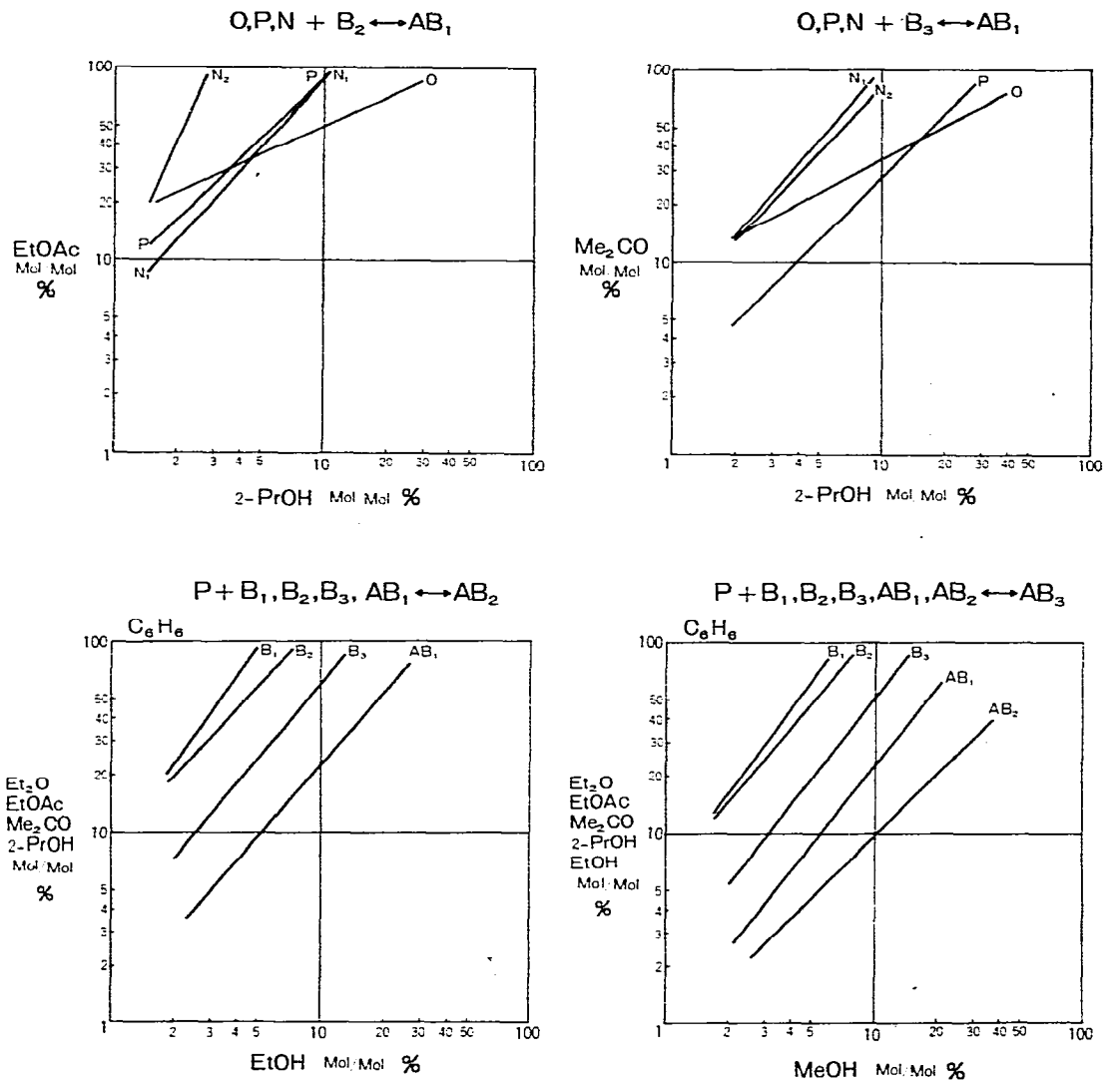


Fig. 2. Correlation between the concentrations of the two stronger components for a pair of equi-eluotropic binary solvent systems with various diluents. Interchange for S components. Solvents: O = *n*-hexane; P = benzene; N_1 = dichloromethane; N_2 = chloroform; B_1 = diethyl ether; B_2 = ethyl acetate; B_3 = acetone; AB_1 = 2-propanol; AB_2 = ethanol; AB_3 = methanol. Samples as in Fig. 1.

The procedure for the design of equi-eluotropic solvent systems described here is simple and convenient for the systematic preparation and determination of the optimal mobile phase in silica gel liquid chromatography. It can be useful not only for column liquid chromatography but also for thin-layer chromatography. Although it may appear to be a rough approximation, it has been used successfully in our laboratory as a very practical technique, especially in preparative separations in synthetic and natural product chemistry research.

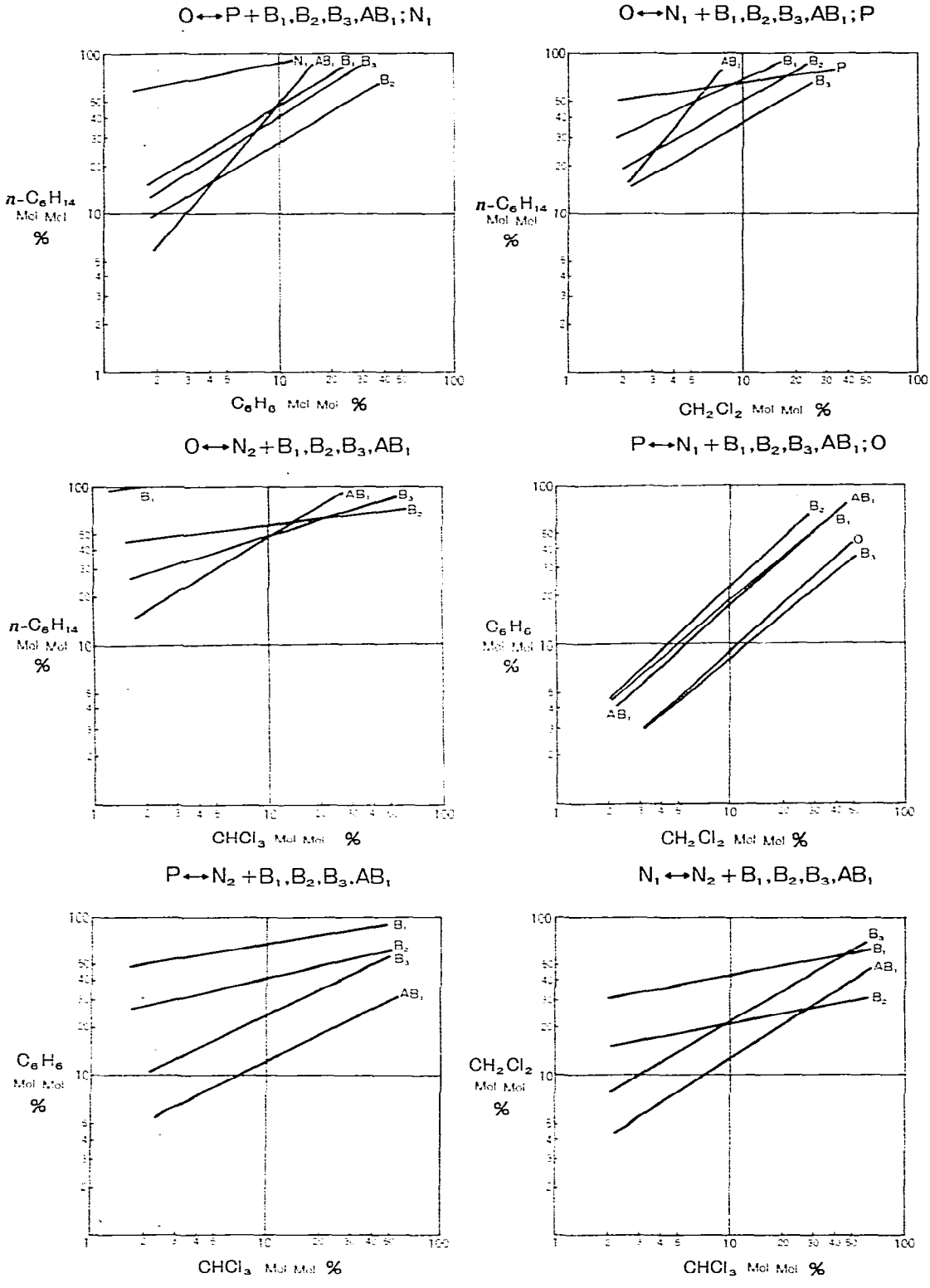


Fig. 3. Correlation between the concentrations of stronger eluents or a diluent for a pair of equi-eluotropic binary solvent systems with various diluents. Interchange for W components. Solvents as in Fig. 2. Samples: a = 3β-benzoyloxy-5-cholestene; others as in Fig. 1.

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REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
2 D. L. Saunders, *Anal. Chem.*, 46 (1974) 470.
3 R. Neher, *Steroid Chromatography*, Elsevier, Amsterdam, 2nd ed., 1964.
4 Z. Deyl, K. Macek and J. Janák, *Liquid Column Chromatography*, Elsevier, Amsterdam, 1975.
5 D. L. Saunders, *J. Chromatogr. Sci.*, 15 (1977) 372.
6 F. Geiss, *Die Parameter der Dünschicht-Chromatographie*, Vieweg, Braunschweig, 1972.
7 S. Hara, Y. Fujii, M. Hirasawa and S. Miyamoto, *J. Chromatogr.*, 149 (1978) 143.
8 E. Soczewiński, *Anal. Chem.*, 41 (1969) 179.
9 E. Soczewiński, *J. Chromatogr.*, 130 (1977) 23.
10 L. R. Snyder, *Anal. Chem.*, 46 (1974) 1384.
11 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
12 P. Jandera, M. Janderová and J. Churáček, *J. Chromatogr.*, 148 (1978) 79.
13 S. Hara, *J. Chromatogr.*, 137 (1977) 41.