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# SYSTEMATIC DESIGN OF BINARY SOLVENT SYSTEMS FOR LIQUID–SOLID CHROMATOGRAPHY VIA RETENTION BEHAVIOUR OF MONO- AND DI-FUNCTIONAL STEROIDS ON SILICA GEL COLUMNS

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## SUMMARY

Based on the mechanism of adsorption chromatography, a classification of binary solvents and an adaptation of solvent systems for various solutes were investigated. The retention behaviour of 14 mono- and di-substituted steroid derivatives as a function of the composition of 21 binary solvents on a silica gel column was determined by high-performance liquid chromatography. The slopes and intercepts on the axes of the linear relationships between the logarithms of the capacity factors and the solvent concentrations were calculated and are discussed in relation to the solute and solvent characteristics. A simple procedure for providing optimal binary solvents for a given compound is demonstrated. The design of equi-eluotropic binary solvent systems was devised on the basis of the quantitative data for the retention–solvent ratio correlations.

## INTRODUCTION

Liquid–solid chromatography (LSC) has been widely used as a separation technique in various fields, especially in synthetic and natural product chemistry[1,2]. The selection of a suitable solvent system for given solutes in LSC has been recognized as the key to successful results. Although much data concerning this process are available, actual solvent systems have, in the past, often been selected empirically, with little consideration of the interactions between solute, mobile phase and stationary phase.

The investigation of the chromatographic behaviour of a group of compounds by employing various solvent systems that have been systematically prepared and the study of the quantitative relationship between retention and solvent compositions would seem to be an appropriate method to find criteria for practical solvent optimization. The retention characteristics of 14 steroid samples on a silica gel column as a function of the compositions of 21 binary solvents were therefore examined as a guide to the systematic design of LSC solvent systems, based upon recent experimental results and their mechanistic interpretations[3–5].

## EXPERIMENTAL

Chromatography was performed using a KP-9H reciprocating pump (Kusano Scientific Instruments, Tokyo, Japan), an RI 101 refractive index detector (Waters Assoc., Milford, Mass., U.S.A.) and a recorder (Ohkura Electric Co., Tokyo, Japan). Columns were constructed from a 200 mm × 4 mm I.D. glass tube provided with a PTFE conical inlet plug, as described recently by Hara[3]. The columns were filled by the mechanical tap–fill procedure. On-column injections were made with a 10-$\mu$l syringe (Kusano).

The silica gel packing was Wakogel LC-10H with a mean particle size of 10 $\mu$m, irregularly shaped, pore size 70 Å (Wako, Osaka, Japan). Technical-grade solvents (Wako) were used without further purification. Steroid samples were synthesized from cholesterol and androstenolone in our laboratory.

The flow-rate used was 1.0 ml/min with a pressure drop of 20–40 kg/cm$^2$. An amount of 1–5 mg of sample was dissolved in 1 ml of a mobile phase solvent. When the sample was not soluble in the mobile phase solvent, it was dissolved in dichloromethane. A volume of 1–5 $\mu$l of the sample solution was injected into the column. All of the results were obtained at a temperature of 15 $\pm$ 5°.

## RESULTS AND DISCUSSION

### *Correlation between retention and binary solvent composition*

Recent research on the mechanism of LSC using silica gel as adsorbent has shown that hydrogen bonding among the solute and solvent molecules and the active sites of the adsorbent contributes considerably to the adsorption–desorption equilibrium[4,5]. Hence a new classification of solvents with respect to their hydrogen-bonding activity can be usefully adapted to LSC mobile phases. The diluents (weak components, W) were divided into three classes, *viz.*, solvents unable to form hydrogen bonds with either the solute or with the adsorbent (class N in the classification proposed by Pimentel and McClellan, *e.g.*, aliphatic hydrocarbons), solvents that have $\pi$-electrons (*e.g.*, aromatic hydrocarbons) and solvents that have non-bonded electrons (*e.g.*, haloalkanes). These have been designated as "O-", "P-" and "N-type", respectively[3]. The polar solvents (strong components, S) were classified in two categories, *viz.*, electron-donor solvents (*e.g.*, ethers, esters and ketones) and electron-acceptor and -donor solvents with active protons (*e.g.*, alcohols). These have been designated as "B class" and "AB class", respectively[6].

Binary solvent systems that are widely used in LSC can be prepared in a systematic manner according to the following classifications:

(i) O + O, O + P, O + N, P + N;
(ii) O + B, P + B, N + B;
(iii) O + AB, P + AB, N + AB;
(iv) B + B, B + AB, AB + AB.

The arrangement of these solvent systems depends upon the eluotropic sequences and shows that the solvent strength of a class generally increases downwards and from left to right in these classifications.

Soczewiński *et al.*[5,7–10] investigated the correlation between the $R_M$ value [$R_M$ = log ([1/$R_F$] − 1) = logarithm of capacity factor, log $k'$] of a given solute and the con-

centration of the polar solvent in the binary system. A linear relationship between the $R_M$ value and the logarithm of strong solvent compositions was proposed by assuming solvation of the solute with the polar solvent in a mobile phase on the basis of the law of mass action. The following equation has been introduced and verified experimentally by employing certain phenols and amines as the solutes and *n*-pentane as a non-polar diluent by means of thin-layer chromatography (TLC)[7–9] and high-performance liquid chromatography (HPLC)[10]:

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

where $X_s$ is the concentration of the stronger component in the mobile phase and $c$ and $n$ are constants.

Scott introduced independently a similar relationship between $1/k'$ and the composition of the stronger solvent based on his own theoretical considerations[11–13]. The validity of the equation was also proved experimentally by using *n*-heptane as the diluent and mono-functional compounds as the solutes in HPLC.

For re-confirmation of Soczewiński's equation, Hara[3] examined the retention volumes of various steroids in HPLC. With *n*-hexane–ethyl acetate and *n*-hexane–2-propanol binary systems, the linear relationships given above were obtained in a medium range of S component concentrations ($X_s$).

These quantitative retention data agreed with the new concept of the mechanism of LSC, and should also be useful for the characterization of binary solvent systems in LSC. Therefore, the relationship between retention and polar solvent composition was investigated by using various solvents that were systematically prepared as described above, including not only O-type but also P- and N-type diluents.

The solvents selected in this work are commonly available volatile solvents that are especially useful for preparative work, such as *n*-hexane (O), benzene (P), methylene chloride, chloroform (N); diethyl ether, ethyl acetate, acetone (B); 2-propanol, ethanol and methanol (AB). The binary solvent types O + P, O + N and P + N were applied to solutes containing an acyloxy group in their molecules. Solvent types O + B, P + B, N + B, O + AB, P + AB and N + AB were provided for mono- and di-functional steroids containing acyloxy, keto and hydroxyl groups. The experimental results are illustrated in Figs. 1–5 and numerical data are shown in Table I. Sometimes, the tosylate and the acetate could not be detected in the chromatograms, and in the case of $N_1 + B_2$ and $N_2 + B_2$ systems linear relationships were not obtained for a few of the solute compounds, and in these instances no numerical data are reported.

In order to evaluate the contribution of a functional group to the retention, typical solutes having groups of medium polarity, *i.e.*, acyloxy, carbonyl and hydroxyl groups substituted at a sterically non-hindered position on a rigid lipophilic cholestane skeleton, were selected. The relationship between elution sequences and molecular structures of the mono-functional solutes was examined according to these data, and it was clearly observed that the retention of the solutes always increased in the following order for any solvent system: benzoate < acetate < ketone < $\alpha,\beta$-unsaturated ketone < alcohol. However, tosylate showed unusual behaviour. Although it was retained in larger amounts than acetates in *n*-hexane as diluent, the re-

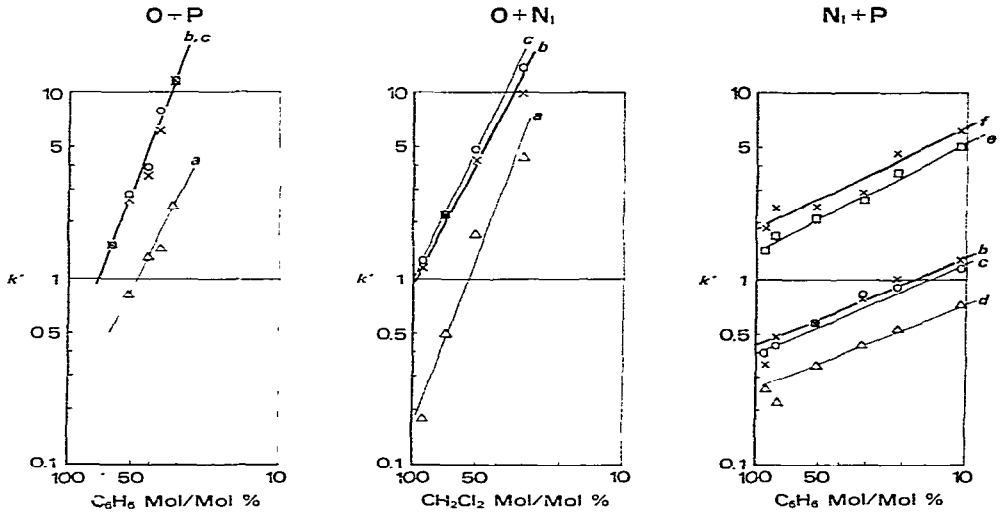



Fig. 1. Logarithm of capacity factor on silica gel as a function of logarithm of polar solvent composition in *n*-hexane–benzene–dichloromethane binary systems. Samples: a = 3$\beta$-benzoxy-5-cholestene; b = 3$\beta$-acetoxy-5$\alpha$-cholestane; c = 3$\beta$-acetoxy-5-cholestene; d = 3$\beta$-tosyloxy-5-cholestene; e = 5$\beta$-cholestan-3-one; f = 5$\alpha$-cholestan-3-one. Solvent systems: O + P = *n*-hexane–benzene; O + $N_1$ = *n*-hexane–dichloromethane; $N_1$ + P = dichloromethane–benzene.

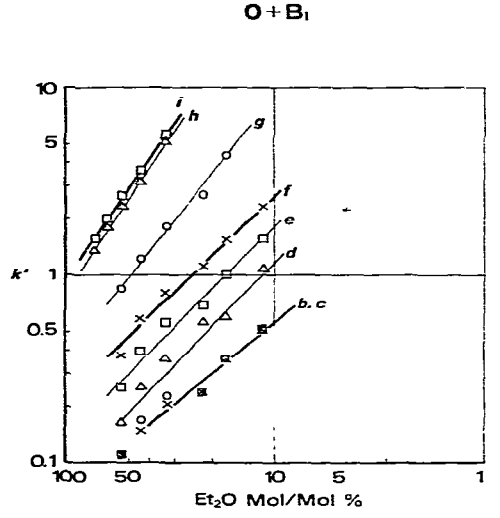
tention decreased considerably when the weak solvent was changed from *n*-hexane to benzene or dichloromethane.

With respect to a particular solute compound, the eluotropic sequence of various binary solvents was then considered. The weakest eluotropic system was the O + P type of solvent, which afforded the highest slope (*n*) and axis intercept (*c*). With benzoate and acetates as the solutes, the eluotropic character of a binary solvent increased in the following order: O + P < O + N < N + P.

In a comparison of the eluotropic sequences of various O + B and O + AB systems by using solutes such as acetates, ketones and alcohols, the eluotropic character was found to be in the increasing order $O + B_1 < O + B_2 < O + B_3 < O + AB_1$. Similarly, the sequences $P + B_1 < P + B_2 < P + B_3 < P + AB_1 < P + AB_2 < P + AB_3$ and $N + B_1 < N + B_2 < N + B_3 < N + AB_1$ were observed.

With a diluent of the O, P or N type, the following eluotropic sequences of the binary system were found: O + B < P + B < N + B and O + AB < P + AB < N + AB.

Snyder[4] and Soczewiński[13] have suggested that the slope (*n*) in the linear relationship of log *k' versus* log $X_s$ is equal to the ratio of the number of solute and polar solvent molecules which exchanged on the adsorbent surface. When a combination of a solute and a solvent that have the same active functional group in their molecules, *e.g.*,

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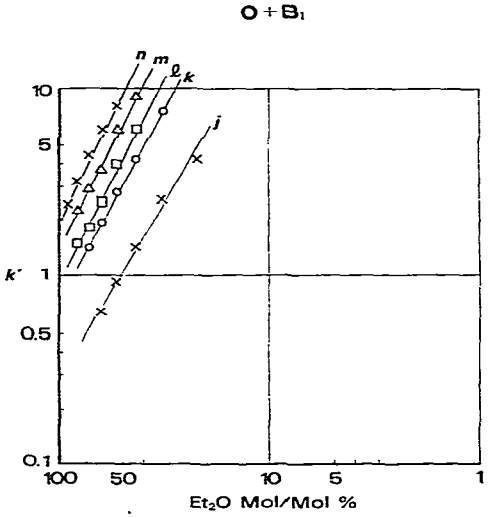


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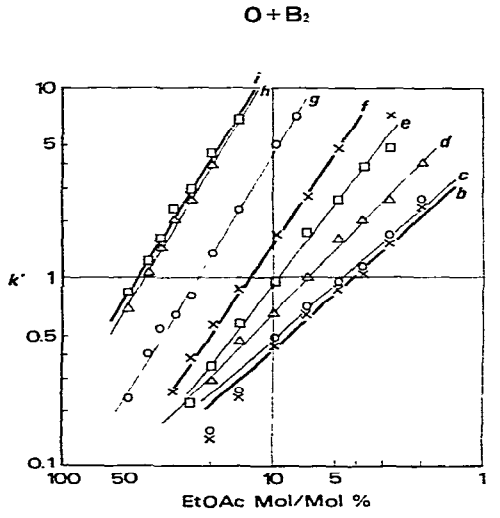


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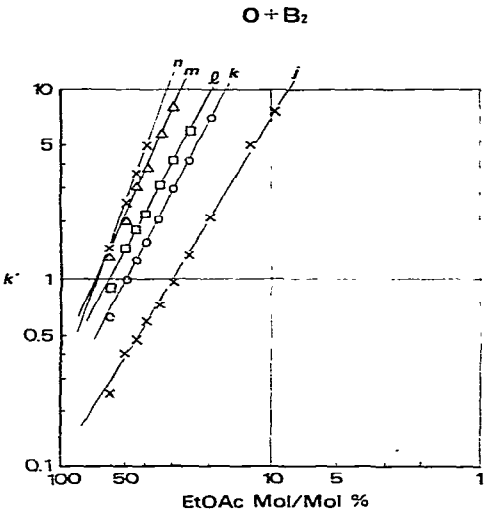


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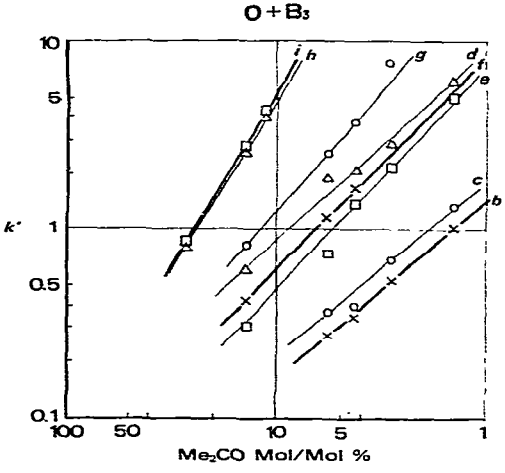


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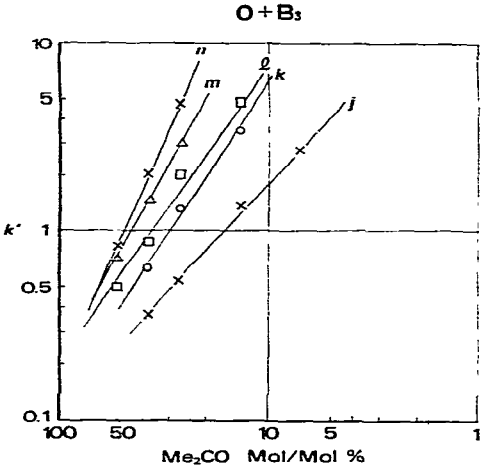


(B)

Fig. 2.

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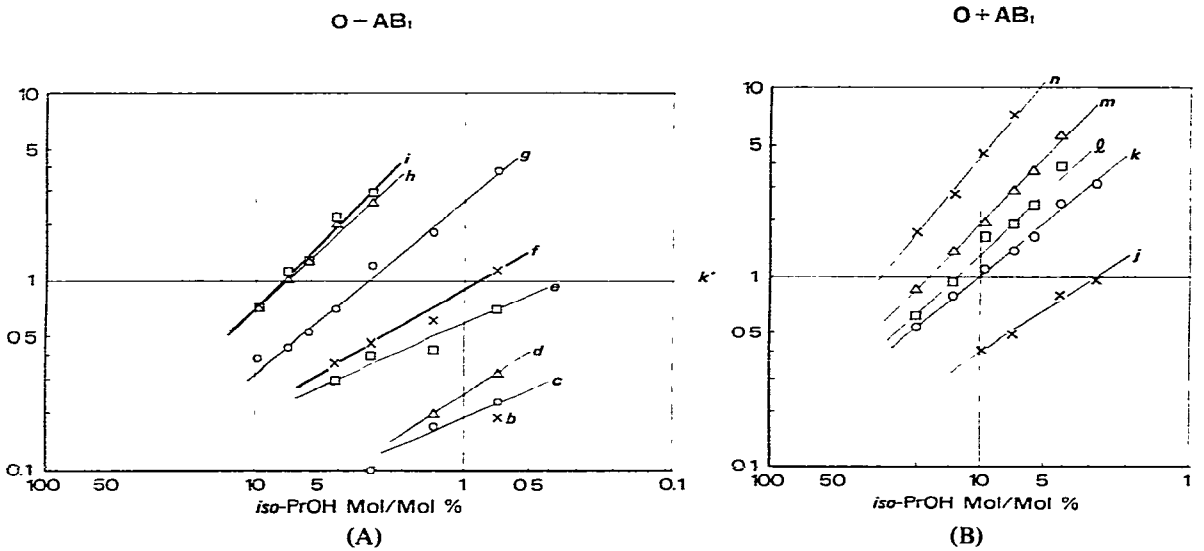
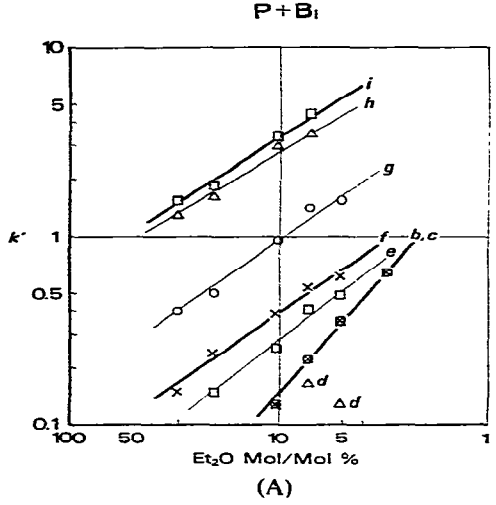


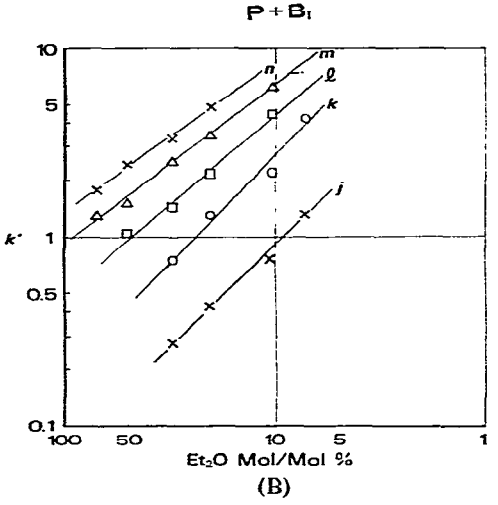

Fig. 2. Logarithm of capacity factor on silica gel as a function of logarithm of polar solvent composition in *n*-hexane–polar solvent binary systems. Samples: (A), mono-substituted cholestane derivatives: b = 3β-acetoxy-5α-cholestane; c = 3β-acetoxy-5-cholestene; d = 3β-tosyloxy-5-cholestene; e = 5β-cholestan-3-one; f = 5α-cholestan-3-one; g = 4-cholesten-3-one; h = 5-cholesten-3β-ol; i = 5α-cholestan-3β-ol. (B), di-substituted androstane derivatives: 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. 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.

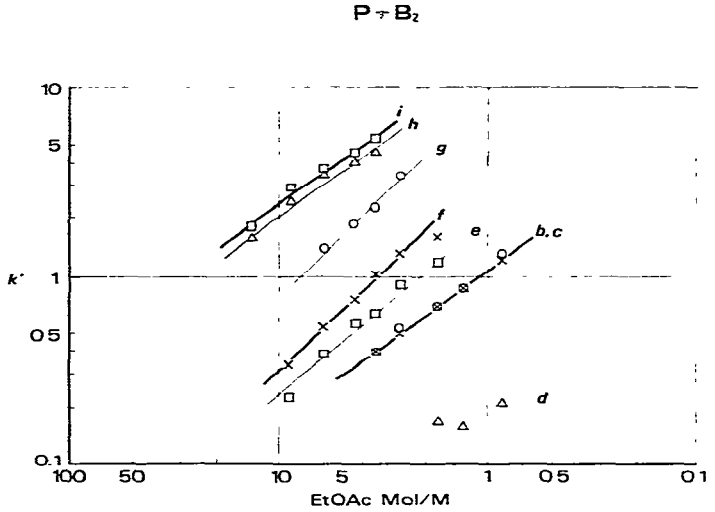
a combination of cholesterol and the 2-propanol–*n*-hexane system, is employed, the number of solute and polar solvent molecules exchanged on the adsorbent surface may be nearly identical, *i.e.*, $n \approx 1$. This assumption was consistent with the experimental results for O + B and O + AB systems, as shown in Table II.

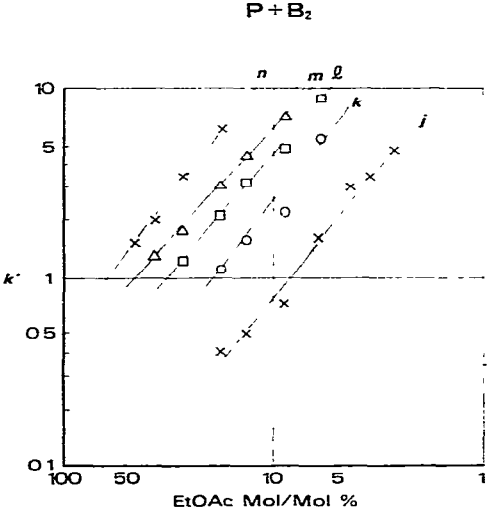
When the polarity of a diluent W increases, the exchange ratio of the stronger solvent and solute molecules may decrease because the participation of the diluent molecules is increased. In agreement with this conjecture, when the O component was changed to P or N, the systems gave smaller values of *n* than those corresponding to O + B or O + AB systems, with only a few exceptional cases, as shown in Table III.

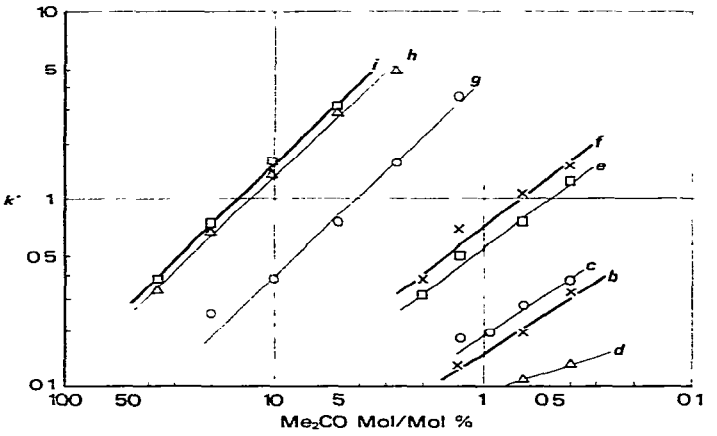
In order to compare the steepness of slope for various mono-substituted solutes, the *n* values for a group of compounds in *n*-hexane plus B or AB binary systems were assessed approximately relative to the values for alcohols as the standard compounds, and the following values were obtained: alcohol, 1.0; α,β-unsaturated ketone, 0.9; ketone, 0.6; and acetate, 0.5. Although the values of *n* for di-functional compounds were higher than those for mono-functional compounds, they were lower than the simple sums of the values for mono-functional compounds that contain corresponding substituents. These results can be interpreted as follows. Firstly, the di-functional solutes included a substituent at a sterically more hindered position than in the mono-functional solutes, for example at the 17-position rather than at the less hindered 3-position. Secondly, two-point adsorption of the di-functional compounds may not occur completely on the discontinuous active sites of the adsorbent surface[1]. For a quantitative interpretation of the behaviour of di-functional solutes, however, further experiments are required.



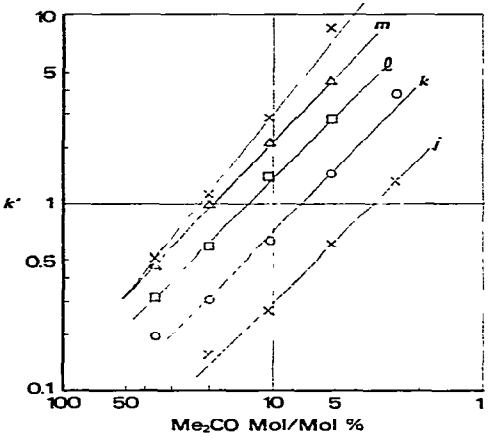




Fig. 3.

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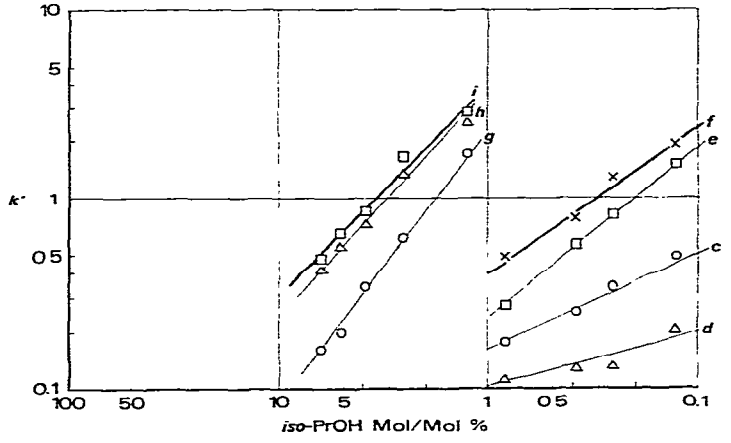
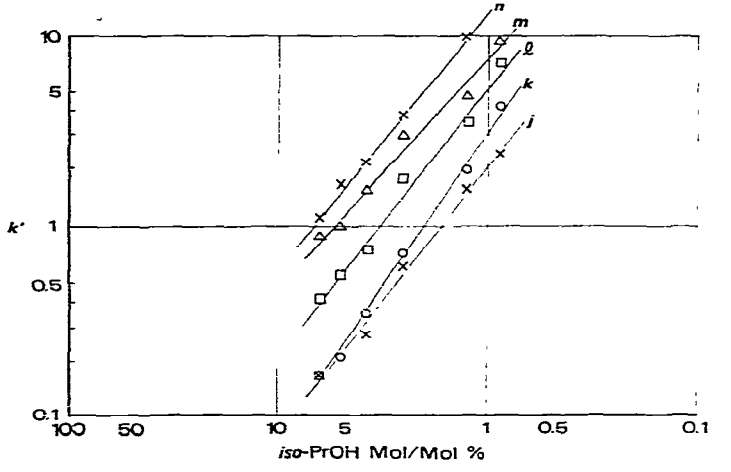
P + AB$_1$




(A)

P + AB$_1$




(B)

P + AB$_2$

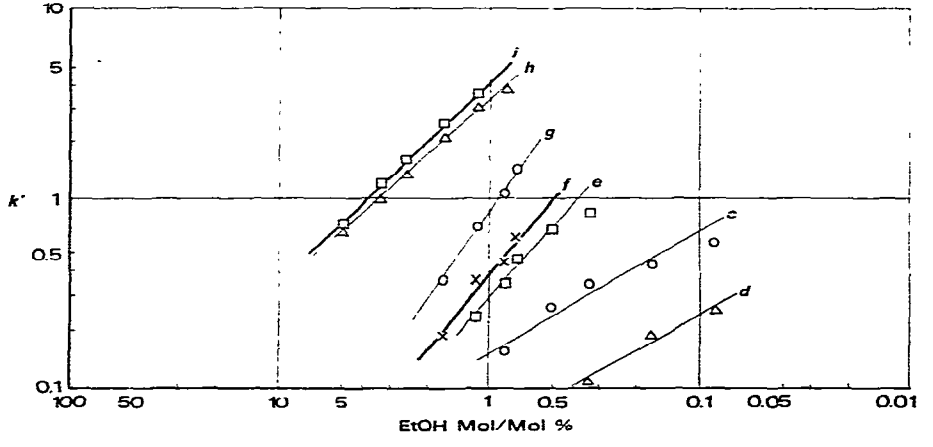
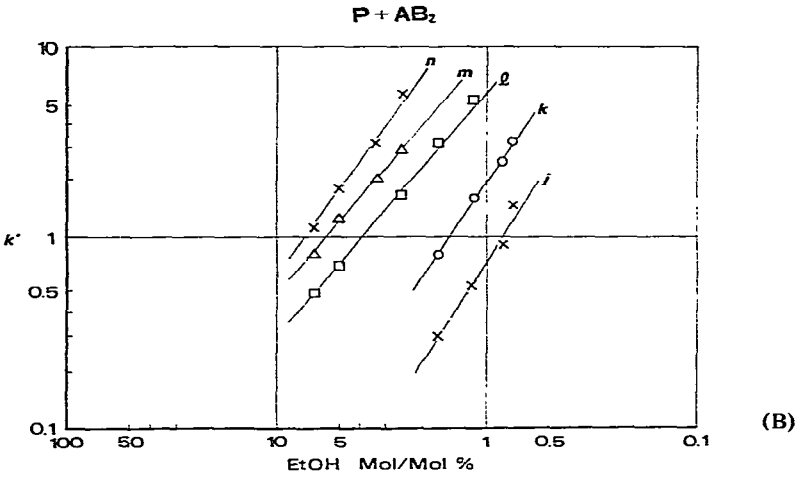



Fig. 3. (A)

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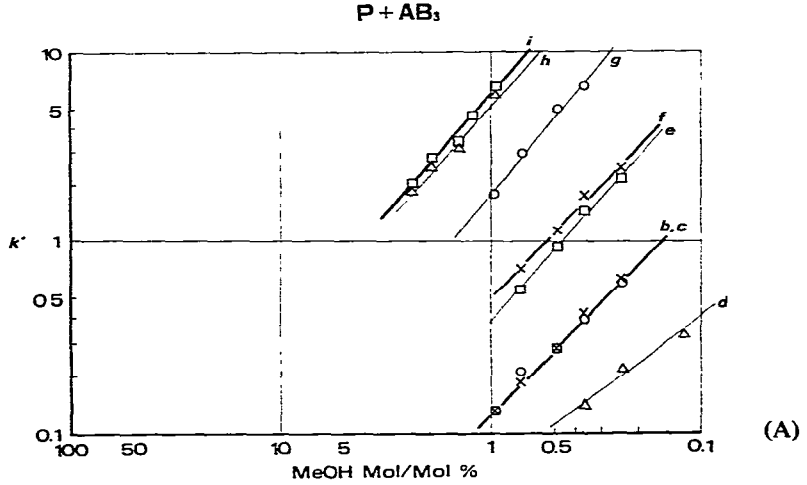
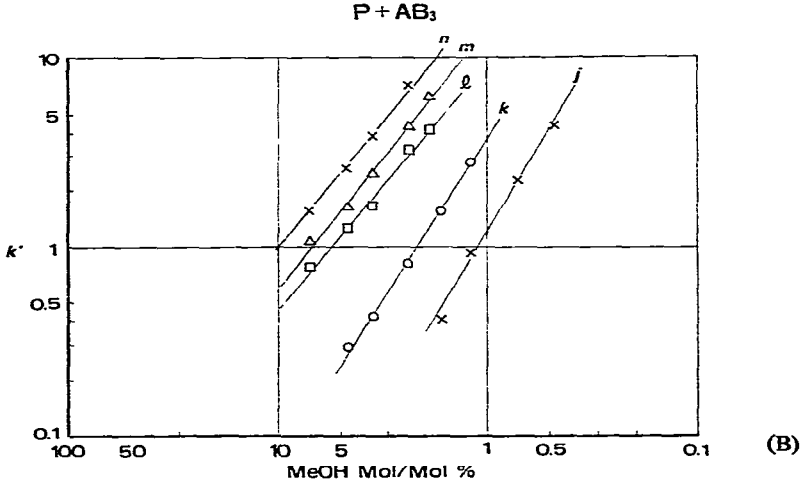
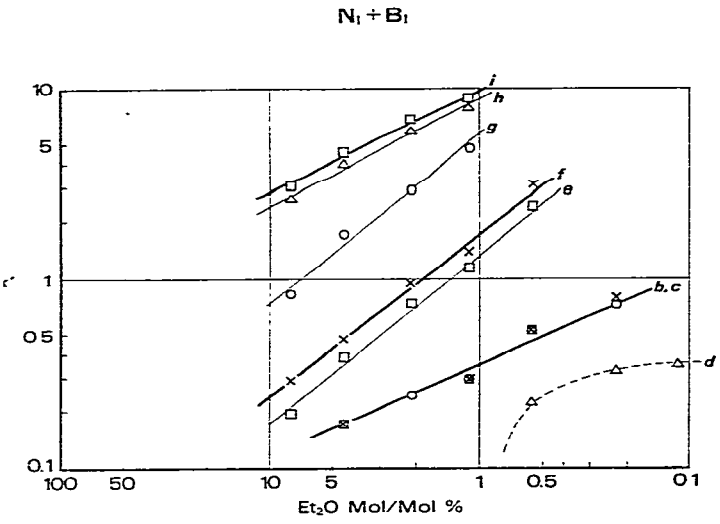



(A)




(B)

Fig. 3. Logarithm of capacity factor on silica gel as a function of logarithm of polar solvent composition in benzene–polar solvent binary systems. Samples as in Fig. 2. Solvent systems: P + $B_1$ = benzene–diethyl ether; P + $B_2$ = benzene–ethyl acetate; P + $B_3$ = benzene–acetone; P + $AB_1$ = benzene–2-propanol; P + $AB_2$ = benzene–ethanol; P + $AB_3$ = benzene–methanol.


N₁ + B₁
10
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100
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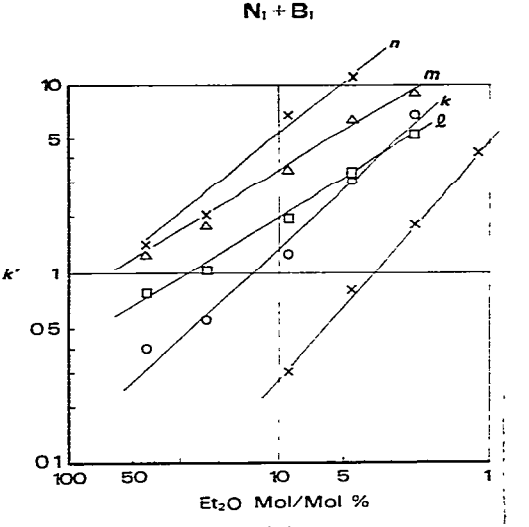
Et₂O Mol/Mol %

(A)


N₁ + B₁
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1
0.5
0.1
100
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10
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1
n
m
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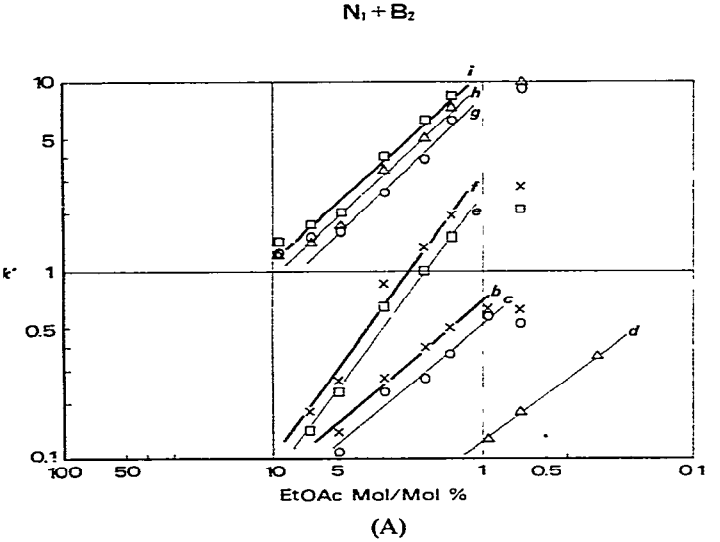
Et₂O Mol/Mol %

(B)


N₁ + B₂
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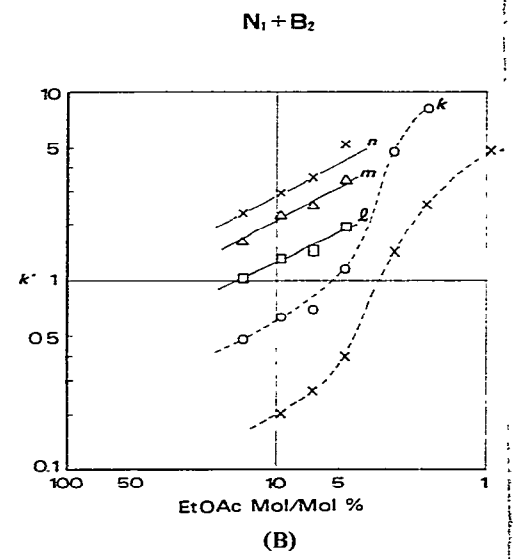
EtOAc Mol/Mol %

(A)


N₁ + B₂
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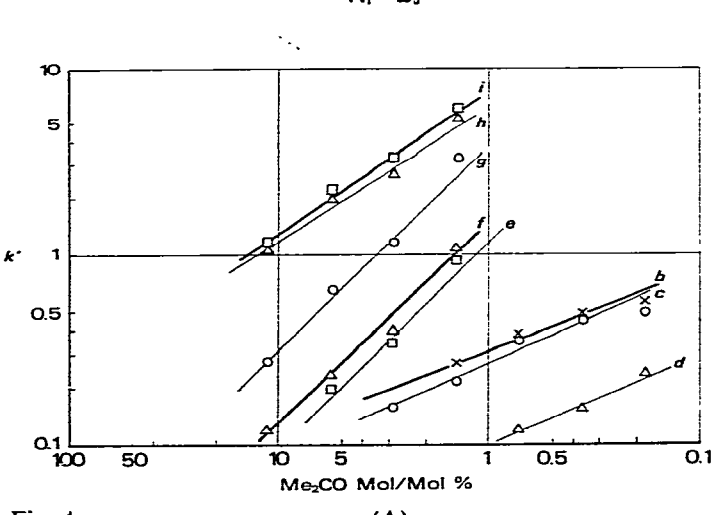
EtOAc Mol/Mol %

(B)


N₁ + B₃
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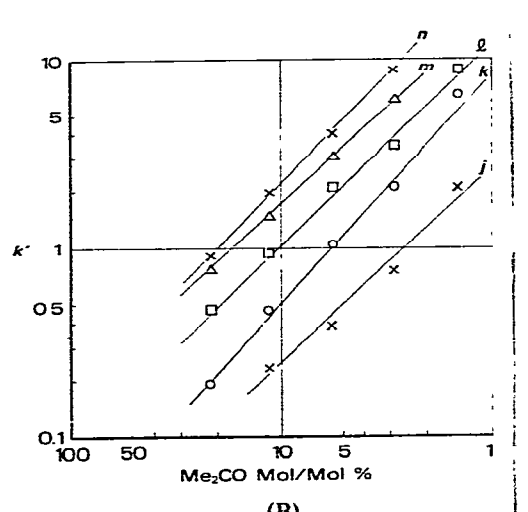
Me₂CO Mol/Mol %


N₁ + B₃
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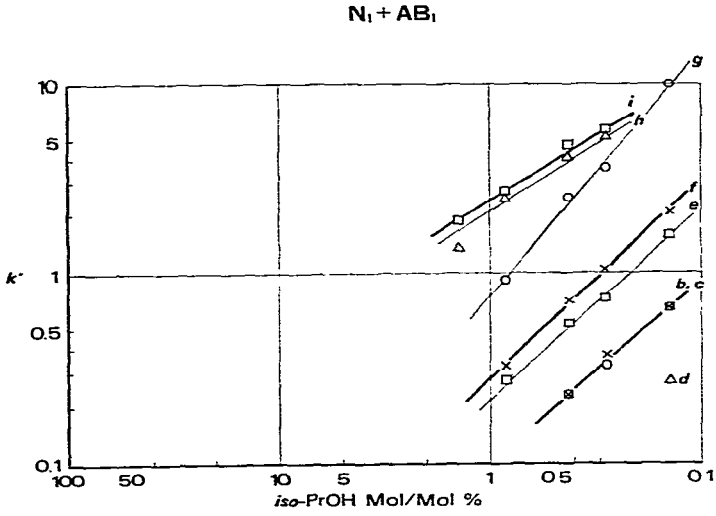
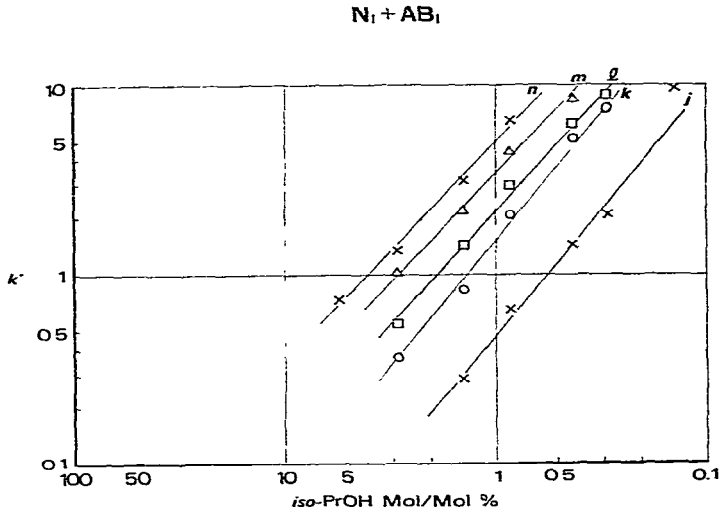
Me₂CO Mol/Mol %

(B)




(A)




(B)

Fig. 4. Logarithm of capacity factor on silica gel as a function of logarithm of polar solvent composition in dichloromethane–polar solvent binary systems. Samples as in Fig. 2. Solvent systems: $N_1 + B_1$ = dichloromethane–diethyl ether; $N_1 + B_2$ = dichloromethane–ethyl acetate; $N_1 + B_3$ = dichloromethane–acetone; $N_1 + AB_1$ = dichloromethane–2-propanol.

On the other hand, the axis intercepts ($c$) have been considered to be constants that vary depending upon the phase ratio (ratio of solvent volume to adsorbent weight) for a given column, the surface area of an adsorbent, etc.[13]. Experimental results revealed that the constant $c$ increases in parallel with the constant $n$ for a given solvent system.

By utilizing the above data, the quantitative relationship of log $k'$ *versus* log

TABLE I

CONSTANTS OF THE LINEAR RELATIONSHIP BETWEEN RETENTION AND SOLVENT COMPOSITION

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: a = 3β-benzoxy-5-cholestene; 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.

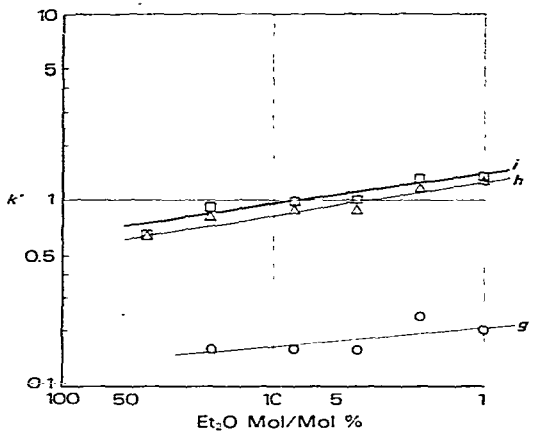
| *Sample* | *O + P* | | *O + N₁* | | *N₁ + P* | |
|---|---|---|---|---|---|---|
| | *c* | *n* | *c* | *n* | *c* | *n* |
| a | 3.77 | 2.26 | 5.22 | 3.01 | | |
| b | 5.40 | 2.91 | 4.30 | 2.17 | 0.58 | 0.47 |
| c | 5.40 | 2.91 | 4.59 | 2.30 | 0.57 | 0.49 |
| d | | | | | 0.30 | 0.44 |
| e | | | | | 1.30 | 0.58 |
| f | | | | | 1.35 | 0.54 |

| *Sample* | *O + B₁* | | *O + B₂* | | *O + B₃* | | *O + AB₁* | | *P + B₁* | | *P + B₂* | | *P + B₃* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* |
| b | 0.64 | 0.89 | 0.63 | 1.00 | 0.14 | 0.93 | | | 0.45 | 1.27 | 0.04 | 0.81 | −0.80 | 0.70 |
| c | 0.64 | 0.89 | 0.65 | 0.97 | 0.24 | 0.89 | −0.72 | 0.46 | 0.45 | 1.27 | 0.04 | 0.81 | −0.71 | 0.73 |
| d | 1.22 | 1.15 | 0.94 | 1.13 | 0.94 | 1.01 | −0.59 | 0.70 | | | | | −1.02 | 0.36 |
| e | 1.39 | 1.13 | 1.39 | 1.43 | 0.85 | 1.17 | −0.23 | 0.48 | 0.31 | 0.86 | 0.31 | 0.93 | −0.26 | 0.83 |
| f | 1.51 | 1.09 | 1.80 | 1.61 | 0.91 | 1.12 | −0.05 | 0.65 | 0.36 | 0.77 | 0.59 | 1.08 | −0.14 | 0.85 |
| g | 2.40 | 1.43 | 2.41 | 1.76 | 1.35 | 1.26 | 0.41 | 0.91 | 0.76 | 0.78 | 0.96 | 1.07 | 0.65 | 1.05 |
| h | 3.30 | 1.71 | 2.94 | 1.82 | 2.50 | 1.82 | 0.85 | 0.99 | 1.07 | 0.62 | 1.09 | 0.78 | 1.17 | 1.06 |
| i | 3.22 | 1.63 | 2.93 | 1.78 | 2.56 | 1.84 | 0.96 | 1.10 | 1.22 | 0.69 | 1.16 | 0.78 | 1.25 | 1.08 |
| j | 3.23 | 1.89 | 2.67 | 1.82 | 1.46 | 1.20 | 0.32 | 0.73 | 1.05 | 1.09 | 1.27 | 1.37 | 0.56 | 1.10 |
| k | 3.97 | 2.05 | 3.53 | 2.09 | 2.51 | 1.71 | 0.90 | 0.90 | 1.58 | 1.15 | 1.80 | 1.40 | 1.00 | 1.15 |
| l | 4.32 | 2.18 | 3.60 | 2.03 | 2.45 | 1.57 | 1.13 | 1.02 | 1.58 | 0.94 | 1.94 | 1.29 | 1.27 | 1.14 |
| m | 4.49 | 2.17 | 4.65 | 2.55 | 3.33 | 2.01 | 1.40 | 1.12 | 1.67 | 0.86 | 2.00 | 1.21 | 1.47 | 1.15 |
| n | 4.77 | 2.24 | 5.41 | 2.95 | 4.33 | 2.56 | 1.93 | 1.31 | 1.71 | 0.78 | 2.58 | 1.45 | 1.84 | 1.37 |

| *Sample* | *N₂ + B₁* | | *N₂ + B₂* | | *N₂ + B₃* | | *N₂ + AB₁* | |
|---|---|---|---|---|---|---|---|---|
| | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* |
| g | −0.70 | 0.08 | −0.74 | 0.11 | −0.53 | 0.45 | | |
| h | 0.09 | 0.18 | 0.18 | 0.29 | 0.49 | 0.68 | −0.10 | 0.64 |
| i | 0.15 | 0.17 | 0.13 | 0.23 | 0.34 | 0.51 | −0.05 | 0.66 |
| j | −0.71 | 0.09 | | | | | −0.06 | 0.59 |
| k | −0.43 | 0.12 | | | −0.04 | 0.74 | | |
| l | 0.11 | 0.18 | | | 0.30 | 0.56 | −0.14 | 0.73 |
| m | 0.32 | 0.17 | | | 0.52 | 0.59 | 0.11 | 0.68 |
| n | 0.47 | 0.17 | | | 0.75 | 0.70 | 0.27 | 0.78 |

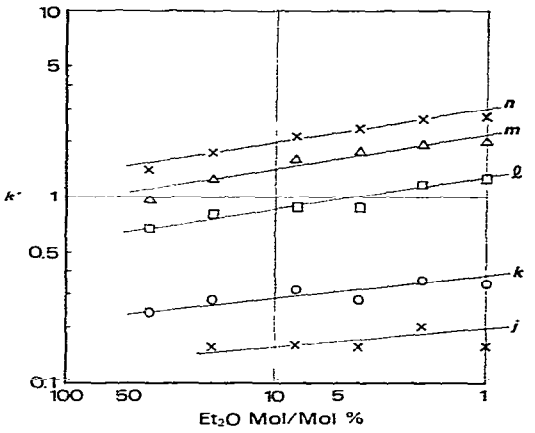
| $P + AB_1$ | | $P + AB_2$ | | $P + AB_3$ | | $N_1 + B_1$ | | $N_1 + B_2$ | | $N_1 + B_3$ | | $N_1 + AB_1$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | *n* |
| | | | | −0.90 | 1.10 | −0.46 | 0.48 | −0.15 | 0.91 | −0.50 | 0.41 | −0.98 | 0.92 |
| −0.77 | 0.49 | −0.80 | 0.63 | −0.90 | 1.10 | −0.46 | 0.48 | −0.28 | 0.91 | −0.56 | 0.49 | −0.98 | 0.92 |
| −0.97 | 0.28 | −1.23 | 0.62 | −1.19 | 0.80 | | | −0.90 | 0.81 | −1.00 | 0.48 | | |
| −0.62 | 0.89 | −0.52 | 1.27 | −0.42 | 1.23 | 0.12 | 0.86 | 0.40 | 1.48 | 0.08 | 1.08 | −0.67 | 0.98 |
| −0.40 | 0.76 | −0.41 | 1.34 | −0.28 | 1.13 | 0.23 | 0.84 | 0.53 | 1.47 | 0.14 | 1.03 | −0.57 | 1.03 |
| 0.37 | 1.48 | −0.07 | 1.60 | 0.25 | 1.32 | 0.76 | 0.89 | 0.91 | 1.04 | 0.57 | 1.06 | −0.12 | 1.26 |
| 0.59 | 1.22 | 0.53 | 0.99 | 0.71 | 1.22 | 0.94 | 0.55 | 0.99 | 1.01 | 0.78 | 0.70 | 0.32 | 0.69 |
| 0.61 | 1.12 | 0.62 | 1.05 | 0.78 | 1.27 | 0.98 | 0.51 | 1.06 | 0.97 | 0.85 | 0.74 | 0.38 | 0.67 |
| 0.32 | 1.39 | −0.14 | 1.69 | 0.10 | 1.86 | 0.65 | 1.22 | | | 0.41 | 1.02 | −0.34 | 1.29 |
| 0.51 | 1.63 | 0.28 | 1.61 | 0.59 | 1.71 | 1.13 | 1.00 | | | 0.87 | 1.17 | 0.18 | 1.33 |
| 0.73 | 1.42 | 0.76 | 1.31 | 0.98 | 1.31 | 0.96 | 0.67 | 0.61 | 0.51 | 1.05 | 1.05 | 0.32 | 1.20 |
| 0.89 | 1.23 | 0.98 | 1.30 | 1.18 | 1.37 | 1.19 | 0.65 | 0.91 | 0.59 | 1.22 | 0.99 | 0.53 | 1.15 |
| 1.13 | 1.38 | 1.30 | 1.53 | 1.33 | 1.33 | 1.58 | 0.85 | 1.01 | 0.56 | 1.47 | 1.13 | 0.70 | 1.14 |

$N_2 + B_1$



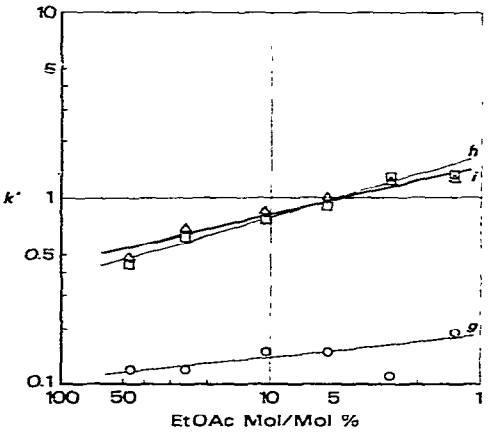

(A)

$N_2 + B_1$



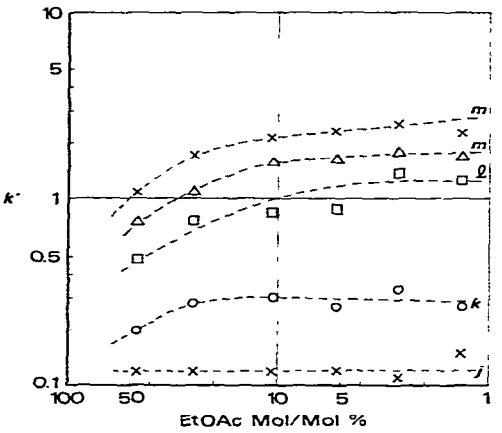

(B)

$N_2 + B_2$



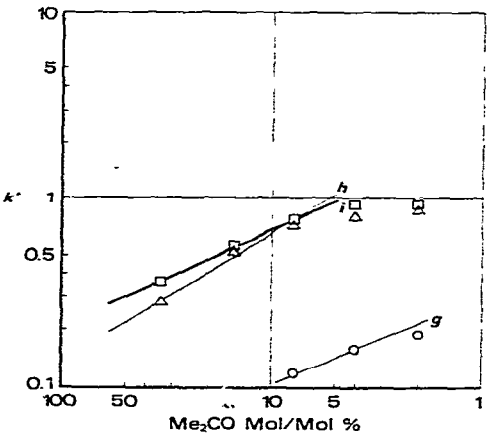

(A)

$N_2 + B_2$



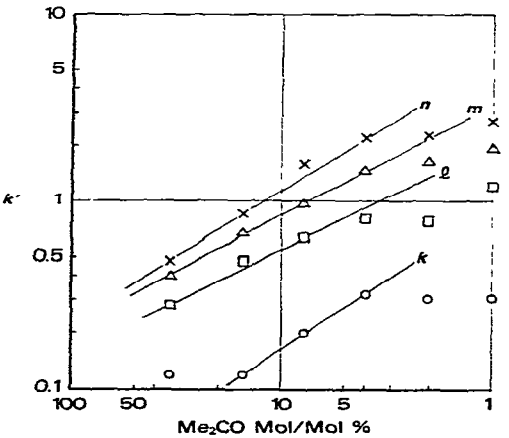

(B)

$N_2 + B_3$




(A)

$N_2 + B_3$




(B)

Fig. 5.

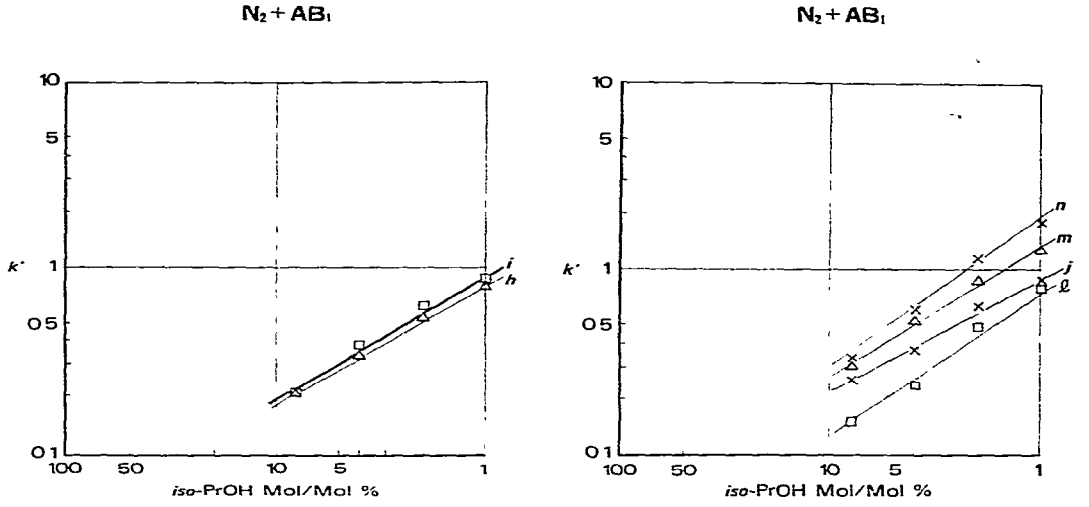



Fig. 5. Logarithm of capacity factor on silica gel as a function of logarithm of polar solvent composition in chloroform–polar solvent binary systems. Samples as in Fig. 2. Solvent systems: $N_2 + B_1$ = chloroform–diethyl ether; $N_2 + B_2$ = chloroform–ethyl acetate; $N_2 + B_3$ = chloroform–acetone; $N_2 + AB_1$ = chloroform–2-propanol.

TABLE II

SLOPES ($n$) FOR O + B AND O + AB SYSTEMS

| *Solute* | *Solvent system* | *n (sample in parentheses)* |
|---|---|---|
| Acetate | O + $B_2$ | 1.00 (b); 0.97 (c) |
| Ketone | O + $B_3$ | 1.17 (e); 1.12 (f) |
| Alcohol | O + $AB_1$ | 0.99 (h); 1.10 (i) |

TABLE III

SLOPES ($n$) FOR DIFFERENT SOLVENT SYSTEMS

| *Solute* | *Solvent system* | *n (sample in parentheses)* | *Solvent system* | *n (sample in parentheses)* |
|---|---|---|---|---|
| Acetate | P + $B_2$ | 0.81 (b); 0.81 (c) | $N_1 + B_2$ | 0.91 (b); 0.91 (c) |
| Ketone | P + $B_3$ | 0.83 (e); 0.85 (f) | $N_1 + B_3$ | 1.08 (e); 1.03 (f) |
| Alcohol | P + $AB_1$ | 1.22 (h); 1.12 (i) | $N_1 + AB_1$ | 0.69 (h); 0.67 (i) |
| | | | $N_2 + AB_1$ | 0.64 (h); 0.66 (i) |

$X_s$ can be predicted for a particular solute compound if the hydrogen-bonding activity of its functional group with the polar solvent and with the silanol groups on the silica surface can be estimated. If two points obtained by a pilot TLC or analytical HPLC experiment are plotted on the graph to show the correlation between retention and polar solvent composition, the constants $c$ and $n$ can be obtained more precisely. Thus the practical optimization of binary solvent systems for HPLC can be readily accomplished.

*Design of equi-eluotropic binary solvent systems*

It is well known that the eluotropic sequence of a solvent system varies depending upon the particular solute used in LSC. Snyder[1,4] defined the secondary solvent effect or solvent selectivity on the polar adsorbent surface, in addition to the primary solvent effect or solvent strength. In LSC, a significant change in the relative solute retention is observed whenever proton-donor or -acceptor solutes and solvents are involved[4].

Although the solvent selectivity can be interpreted in terms of hydrogen bonding, quantitative prediction of the solvent selectivity for given solute pairs is not generally possible. The following process is required in order to optimize the solvent system. Various solvents with approximately equal eluting powers (equi-eluotropic solvents) are first prepared, then a particular solvent with the most favourable selectivity for a given mixture is chosen by trial-and-error. The latter step is usually carried out by TLC, and the data can be extrapolated to the HPLC system as described earlier[3,14,15].

According to the solvent strength parameter ($\varepsilon^\circ$) defined by Snyder[1], binary solvents having identical primary effects can be adequately prepared[1]. However, in practice, the application of this theory requires a set of tedious calculations, although a simplification of this procedure has been attempted[16]. On the other hand, Neher[17] arranged single and binary solvents in an equi-eluotropic series on the basis of average TLC $R_F$ values for 20 steroids[17]. The graphical form of Neher's calculation has been widely accepted because of its simplicity[16]. However, it is desirable that these data be re-confirmed by means of carefully controlled HPLC studies, because column chromatography offers more accurate data than TLC, as Snyder pointed out earlier[4]. Therefore, another design of the equi-eluotropic solvent system by employing HPLC data with a theoretical basis has been developed.

As a linear relationship between $\log k'$ and $\log X_s$ was obtained for each solute, two equi-eluotropic binary solvents containing a different stronger component (S) in the same diluent (W) can be correlated as follows:

$$\log k'_1 = \log k'_2 = c_1 - n_1 \log X_{s(1)} = c_2 - n_2 \log X_{s(2)} \tag{2}$$

where the two solvent systems are indicated by the subscripts 1 and 2; $k'_1$ 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, stronger solvent concentrations, axis intercepts and slopes, respectively.

In eqn. 2, concentrations of the stronger components in solvents 1 and 2 were linearly related. On the above premises, the following expression can be derived for an equi-eluotropic solvent composition as a function of another polar solvent composition:

$$\log X_{s(2)} = \frac{c_2 - c_1}{n_2} + \frac{n_1}{n_2} \cdot \log X_{s(1)} \tag{3}$$

According to eqn. 3, a binary solvent W + $S_2$ whose eluotropic power is identical with that of another binary solvent W + $S_1$ can be prepared readily, and

*vice versa*, provided that two constant terms are known. Similarly, a binary solvent $W_2 + S$ whose eluotropic power is identical with that of another binary solvent $W_1 + S$ can also be prepared, and *vice versa*. The two constant terms in eqn. 3 were calculated with respect to a particular solute by employing the axis intercept ($c$) and slope ($n$) from eqn. 1.

The concept of the equi-eluotropic solvent system can be applied to solute compounds in general, and the average values of the two constant terms obtained can be utilized for the design of equi-eluotropic solvent systems.

The constants obtained for the systematization of binary solvent systems were calculated on the basis of a limited number of steroid derivatives. Hopefully, similar data for other groups of compounds will be obtained and the applicability of this procedure will be improved further.

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