Journal of Chromatography, 167 (1978) 291–305

CHROM. 11,087

ADJUSTMENT OF STATIONARY PHASE SELECTIVITY IN GAS–LIQUID CHROMATOGRAPHY BY USING MIXED LIQUID PHASES

J. F. K. HUBER and E. KENNDLER
Institute of Analytical Chemistry, University of Vienna, Waehringerstrasse 38, A-1090 Vienna (Austria)
and
H. MARKENS*
Laboratory of Analytical Chemistry, University of Amsterdam, Amsterdam (The Netherlands)

SUMMARY

Contrary to liquid–liquid chromatography, where binary, ternary and quaternary mixed solvents have been systematically investigated and extensively applied, very few systematic studies on mixed stationary liquid phases have been undertaken in gas–liquid chromatography (GLC). Except for mixtures of chemically analogous polymeric constituents resulting from production processes, little use is made of mixed solvents in GLC. In this work, "binary" mixed solvents were studied as stationary liquids in GLC.

Linear and non-linear relationships between the capacity factor and phase composition were found experimentally. In the non-linear case curves with minima were observed. It was confirmed experimentally that adsorption on the solid support is negligible. The shape of the relationship is discussed in terms of thermodynamic theory.

The exploitation of the selectivity of mixed solvents in GLC separations is demonstrated. It is shown that columns with composite solvents as stationary phases and coupled columns with single phases are equivalent if there is a linear relationship between the capacity factor and the stationary liquid phase composition. It is demonstrated that switching of columns can be successfully employed in achieving or optimizing a separation by GLC.

INTRODUCTION

The choice of the phase system is decisive for the success of a chromatographic separation as it determines the differential migration of the components of the sample. The ability of a phase system in chromatography to retard differently two compounds is characterized by the selectivity coefficient, which is defined as the ratio of the distribution coefficients of the two compounds in the phase system. The selectivity of a phase system for a given mixture of n components is characterized by the $n - 1$

* Present address: Institute for Physical Chemistry TNO, Zeist, The Netherlands.

selectivity coefficients of the components with consecutive values of the distribution coefficient.

In addition to the selectivity coefficients the absolute values of the distribution coefficients also have to be considered. They determine, together with the phase ratio, the capacity factors. The phase ratio can be varied in gas–liquid (GLC) and gas–solid chromatography (GSC) by about two orders of magnitude.

The chromatographic resolution depends on both the selectivity coefficient and the capacity factor, and both are adjusted by choosing an appropriate phase system. The effort necessary to achieve a given minimum resolution, R_{ji}, with a given phase system can be expressed by the number of theoretical plates, N_R, that is required, which is given by

$$N_R = R_{ji}^2 \left(\frac{1 + \kappa_n}{\kappa_n[r_{(n+1)n} - 1]}\right)^2 \tag{1}$$

with

$$r_{(n+1)n} = K_{(n+1)}/K_n$$

and

$$\kappa_n = K_n V_s / V_m$$

where $r_{(n+1)n}$ is the selectivity coefficient for the pair of consecutive components n and $n + 1$ that is most difficult to separate, κ_n is the capacity factor, K_n is the distribution coefficient of component n, $K_{(n+1)}$ is the distribution coefficient of component $n + 1$, and V_s and V_m are the volumes of the stationary and mobile phase, respectively, in the column.

By an appropriate choice of the phase system, the term $(1 + \kappa_n)/\kappa_n[r_{(n+1)n} - 1]$ can be adjusted to a minimum value. Often it is not easy or is even impossible to find a phase system that enables one to resolve all components. In GLC a suitable stationary liquid phase has to be found, and it would be expected that the search for this phase would be significantly facilitated if mixed liquid phases were also considered.

The use of mixed stationary liquid phases was first considered in the early days of GLC[1–29]. A crucial point is the question of whether there is a linear relationship between retention and stationary liquid composition. If there is such a linear relationship, the use of three equivalent approaches is possible: mixed phase columns, mixed bed columns with composite packings supporting different liquid phases, and coupled columns in series with different packings. In principle, a mixed bed column is not in equilibrium and should change slowly to a mixed phase column.

The discussion in the literature on the form of the dependence of the chromatographic retention data on the composition of the mixed stationary phase is controversial. A number of workers have reported linear[1,9,10,15,19,22,26–28] and others on curvilinear relationships[4,11,13,14,16,20]. It is obvious that the type of relationship depends on the solvent–solvent and solute–solvent interactions, and that different functions may be expected.

Although GLC with mixed stationary solvents is rarely applied in practice today, it is clear that the better exploitation of the selectivity potential of liquid phases promises a powerful approach to the solving of difficult separation problems.

THEORETICAL

In chromatography, the separation is caused by a difference in the distribution coefficients of the components in the stationary and mobile phases. The retention time, t_{Ri}, of a component i is given by

$$t_{Ri} = \frac{L}{u}(1 + K_{i0}V_s/V_m) \tag{2}$$

where L is the length of the chromatographic column, u is the velocity of the mobile phase and K_{i0} is the limiting value of the distribution coefficient of component i in the stationary and mobile phases at infinite dilution.

In gas–liquid systems, the distribution coefficient, K_{i0}, is determined at low pressures (up to a few bars) by the activity coefficient in the liquid phase and the vapour pressure of the pure component according to

$$K_{i0} = \frac{RT}{\gamma_{i0}p_i^0\bar{v}_s} \tag{3}$$

where γ_{i0} is the activity coefficient of component i in the liquid phase at infinite dilution, p_i^0 is the vapour pressure of the pure component i, $\bar{v}_s$ is the molar volume of the solvent, T is the temperature and R is the gas constant, assuming that the gas phase behaves like an ideal gas.

The activity coefficient, γ_{i0}, depends on the nature of the solvent. The nature of composite solvents is determined by their composition. With a simple model for the molecular interaction, the following expression for γ_{i0} can be derived for binary solvent mixtures[30,31]:

$$\ln \gamma_{i0}^{(1,2)} = \Phi_1 \ln \gamma_{i0}^{(1)} + \Phi_2 \ln \gamma_{i0}^{(2)} - \bar{v}_i\beta_{1,2}\Phi_1\Phi_2 \tag{4}$$

where $\gamma_{i0}^{(1)}$, $\gamma_{i0}^{(2)}$ and $\gamma_{i0}^{(1,2)}$ are the activity coefficients of component i at infite dilution in solvent 1, solvent 2 or their mixture 1, 2, respectively, $\Phi_1 = x_1\bar{v}_1/(x_1\bar{v}_1 + x_2\bar{v}_2)$ and $\Phi_2 = x_2\bar{v}_2/(x_1\bar{v}_1 + x_2\bar{v}_2)$ are the volume fractions of solvents 1 and 2, respectively, where x_1 and x_2 are the molar fractions and $\bar{v}_1$ and $\bar{v}_2$ the molar volumes of these solvents, $\bar{v}_i$ is the fictitious molar volume of the pure liquid solute i and $\beta_{1,2}$ is an empirical coefficient characteristic of the solvent mixture 1, 2.

Eqn. 4 predicts a linear relationship between the logarithm of the activity coefficient $\gamma_{i0}^{(1,2)}$ and the composition of the solvent expressed in volume fractions if the two solvents form an ideal mixture ($\beta_{1,2} = 0$). If the solvents form a mixture with a negative $\beta_{1,2}$ value, a positive deviation from the linear relationship results for the logarithm of the activity coefficient $\gamma_{i0}^{(1,2)}$. Conversely, a negative deviation results if the solvents form a mixture with a positive $\beta_{1,2}$ value. A larger value of the activity coefficient means a smaller value of the distribution coefficient and *vice versa*.

From regular solution theory, the following expression for $\beta_{1,2}$ can be derived[31]:

$$\beta_{1,2} = \frac{(\delta_1 - \delta_2)^2}{RT} \tag{5}$$

where δ_1 and δ_2 are the solubility parameters of solvents 1 and 2, respectively. It can be seen that the regular solution theory predicts only positive values for $\beta_{1,2}$.

Substitution of γ_{i0} in eqn. 3 by means of eqn. 4, and considering that $\Phi_1 + \Phi_2 = 1$, leads to

$$K_{i0} = \frac{RT}{p_i^0 \gamma_{i0}} \frac{e^{\bar{v}_i \beta_{1,2}(\Phi_2 - \Phi_2^2)}}{\bar{v}_{1,2}(\gamma_{i0}^{(2)}/\gamma_{i0}^{(1)})\Phi_2} \quad (6)$$

where $\bar{v}_{1,2}$ is the molar volume of the solvent mixture. From eqn. 6, it can be seen that the relationship between the distribution coefficient, K_{i0}, and the volume fraction, Φ_2, becomes about linear if the exponent $\bar{v}_i \beta_{1,2}$ is positive and small, and the ratios of the activity coefficients and of the molar volumes of the two solvents, which determine the value of $\bar{v}_{1,2}$ are not too different from unity. In general, however, the relationship is non-linear. Even for ideal mixtures of the solvents ($\beta_{1,2} = 0$), a linear relationship is approached only for values near to each other for the ratio $\gamma_{i0}^{(2)}/\gamma_{i0}^{(1)}$ and the ratio of the molar volumes of the solvents. Experimentally has been observed that the distribution coefficient may go through a maximum or minimum[32]. In principle, eqn. 6 is too simple to describe the dependence of the distribution coefficient on the composition of the liquid phase in detail under all circumstances.

EXPERIMENTAL

Apparatus

For the measurements of retention data two dual-column gas chromatographs, both equipped with two flame-ionization detectors, a dual-channel amplifier and a temperature programmer were used (Siemens L402 for the work with phenolic compounds, Hewlett-Packard Model 5750B for the work with trimethylsilyl-3-methylhydantoins). One of the chromatographs was equipped with a column-switching device with external valves (Siemens).

Chromatographic columns were constructed from glass tubing of length 1 m and I.D. 2 mm, silanized in the usual way with dimethyldichlorosilane, (for phenolic compounds), and copper tubing of length 1 m and I.D. 4 mm (for trimethylsilyl-3-methylhydantoins). The detector signal was recorded by means of a potentiometric line recorder (Siemens Kompensograph III and Goerz Servogor R512).

Liquid sample solutions were injected into the injection ports of the gas chromatographs by means of a precision syringe of 1-μl capacity (Hamilton 7001N).

Small screw-capped reaction tubes (Sovirel) were used for silanization prior to injection.

Chemicals

Two types of solid supports were used: a synthetic low-surface-area silanized silica (Volaspher A 2; E. Merck, Darmstadt, G.F.R.) for the phenolic compounds and a silanized diatomite material (Chromosorb G HP; Johns-Manville, Denver, Colo., U.S.A.) for the trimethylsilyl-3-methylhydantoins, both with a particle size range of 100–125 μm.

The solvents used as stationary liquids were OV-101, OV-225 and Carbowax 20M (E. Merck, chromatography grade), OV-1 and OV-17 (Speciality Chemical,

Marietta, Ohio, U.S.A.) and ethylene glycol adipate (EGA; Applied Science Labs., State College, Pa., U.S.A.). The carrier gas was nitrogen of purity 99.995% (v/v) (Messer-Griesheim, Düsseldorf, G.F.R.).

The following test compounds for the chromatographic experiments and reagents and solvents for the synthesis of test compounds were used: phenols (from Fluka, Buchs, Switzerland, and E. Merck), amino acids (all from Ajinomoto, except sarcosine from J. T. Baker, Gross Gerau, G.F.R.), methyl isocyanate and dimethoxyethane (BDH, Poole, Great Britain), sodium hydroxide (E. Merck), hydrochloric acid, acetone and methanol (all AnalaR grade from BDH), and bis(trimethylsilyl)trifluoroacetamide (BSTFA; Serva, Heidelberg, G.F.R.).

Procedures

The constituents of the mixed liquid phases were first checked for their mutual miscibility by mixing them and heating the mixture under a microscope to the temperature of the chromatographic experiments. Complete miscibility was observed at all mixing proportions used. The solid support was coated with the stationary liquid in the usual way using solutions in chloroform. The column tubes were filled with the coated solid support and the packings were conditioned as usual. The hold-up time of the mobile phase was determined with methane as an inert tracer.

For the synthesis of 3-methylhydantoins, 5 mg of amino acid are dissolved in water and the pH of the solution is adjusted to 7–8 with 6 *N* hydrochloric acid or 6 *N* sodium hydroxide solution. In order to obtain a homogeneous solution, 5 ml of dimethoxyethane are added. Next, 5 ml of methyl isocyanate are added to the solution and the pH is readjusted to 7–8 by addition of 6 *N* sodium hydroxide solution. The addition of methyl isocyanate is repeated until the pH of the solution will not decrease further. An equal volume of 12 *N* hydrochloric acid is added to the solution, then it is boiled for 30 min. The major part of the solvent (dimethoxyethane, hydrochloric acid, water) is evaporated under reduced pressure, then the pH is adjusted to 2–3 by addition of 6 *N* sodium hydroxide solution and the remaining solvent is evaporated. In order to remove sodium chloride, hot water-free ethanol is added to the residue and the slurry is filtered. Next, the ethanol is evaporated from the filtrate and the 3-methylhydantoins are obtained. They are purified by recrystallization from water, acetone or ethanol.

The silanization of 3-methylhydantoins is carried out according to the following procedure[33,34]. A 2-mg amount of a given 3-methylhydantoin is placed in a reaction tube and 100 μl of methanol are added to dissolve the sample. A fraction of 10 μl is withdrawn by means of a syringe and placed in another reaction tube, then the solvent is evaporated and the tube is closed tightly using a cap with a septum. Next, 100 μl of BSTFA are added to the dry residue by means of a syringe and the solution is heated for 15 min at 140°. In this manner a solution of trimethylsilyl-3-methylhydantoin (TMS-3-MH) in BSTFA is obtained.

RESULTS AND DISCUSSION

The following problems were studied:

(1) is the distribution mechanism dominated by the gas–liquid solution, and can the adsorption be neglected?;

(2) what is the relationship between the capacity factor and the composition of a mixture of two solvents?;

(3) how can the selectivity of mixed phases be exploited in GLC?

Widely used stationary liquids, which are polymeric mixtures, were chosen for these investigations.

Test of the magnitude of adsorption

If gas–liquid solution phenomena have to be studied, adsorption effects must be excluded. Adsorption in gas–liquid–solid systems can occur on the gas–liquid and the liquid–solid interfaces, assuming that the solid is completely covered by the liquid. The total capacity factor in such a system is given by

$$\kappa_i = {}^s\kappa_i + {}^\sigma\kappa_i + {}^\lambda\kappa_i = \frac{{}^sK_{i0}V_s + {}^\sigma K_{i0}A_\sigma + {}^\lambda K_{i0}A_\lambda}{V_m} \tag{7}$$

where ${}^s\kappa_i$, ${}^\sigma\kappa_i$ and ${}^\lambda\kappa_i$ are the contributions to the capacity factor arising from solution in the stationary liquid s, adsorption on the liquid–solid interface σ and adsorption on the gas–liquid interface λ, respectively, ${}^sK_{i0}$, ${}^\sigma K_{i0}$ and ${}^\lambda K_{i0}$ are the corresponding distribution coefficients at infinite dilution, in which the concentrations are given per unit volume for the bulk phases and per unit interfacial area for the interfaces, A_σ is the surface area of the solid support and A_λ is the area of the liquid interface in the column[34].

In order to determine the contribution of the adsorption, the capacity factor is measured at different liquid loadings of the solid support. For sufficiently high liquid loadings, where the solid surface is completely covered with liquid, a linear relationship, $\kappa_i = a_0 + a_1(V_s/V_m)$ is predicted by eqn. 7, where a_0 represents the contribution due to adsorption. For a number of systems a_0 was determined by linear regression of the experimental data and the results are given in Table I. For experimental convenience, the mass of the solid support, m_σ, was used instead of V_m,

TABLE I

LINEAR REGRESSION, $\kappa_n = a_0 + a_1 \cdot V_s/m_\sigma$, OF THE CAPACITY FACTOR, κ_n, AND THE LIQUID LOADING, V_s, RELATED TO THE MASS, m_σ, OF THE SOLID SUPPORT

According to theory, $a_0 = {}^\sigma K_n A_\sigma/V_m + {}^\lambda K_n A_\lambda/V_m$ and $a_1 = {}^sK_n m_\sigma/V_m$.

Solid support, Chromosorb G HP; liquid loading, 6 different loadings in the range 0.05–1.2% (w/w); mobile phase, nitrogen. Sample: trimethylsilyl-3-methylhydantoins of different amino acids (Abbreviations given by capital letters: VALine, SERine, GLYcine, METhionine, SARcosine, THReonine).

Stationary liquid	*Temperature (°C)*	*Sample*	$a_1 \pm s_1$ *(g/mm³ × 10)*	$a_0 \pm s_0$	*Correlation coefficient,* r^2
EGA	170	TMS-3-MVALH	2.62 ± 0.03	0.14 ± 0.02	0.9996
		TMS-3-MSERH	3.99 ± 0.09	0.20 ± 0.04	0.9976
OV-17	170	TMS-3-MGLYH	1.64 ± 0.07	0.17 ± 0.04	0.9964
		TMS-3-MMETH	11.6 ± 0.25	0.56 ± 0.02	0.9990
OV-225	150	TMS-3-MGLYH	6.69 ± 0.25	0.23 ± 0.01	0.9996
		TMS-3-MMETH	46.2 ± 0.25	0.91 ± 0.02	1.0000
Carbowax 20M	150	TMS-3-MSARH	11.0 ± 0.01	0.50 ± 0.04	1.0000
		TMS-3-MTHRH	10.2 ± 0.25	1.36 ± 0.05	0.9988

which is allowable for a constant packing density and low liquid loadings, where V_m/m_G is virtually independent of V_s. The volume of the stationary liquid, V_s, was calculated by $V_s = m_s/\varrho_s$ from the mass, m_s, of the stationary liquid in the column and its density, ϱ_s, at the column temperature. The density was measured by means of pyknometry. It can be seen that in all instances a significant but small adsorption is observed, which is of the order of 2–10% at a liquid loading of 10 mm^3/g.

An example of a series of measurements, given in the form of regression data in Table I, is shown in Fig. 1, in which as usual the capacity factor is plotted as a function of the mass of liquid per unit mass of solid. It can be seen that the plot is linear in the measured range, which is confirmed by the data in Table I. This means, according to eqn. 7, that the contribution of adsorption is virtually constant. The contributions of solution (S) and adsorption (A) are indicated in Fig. 1.

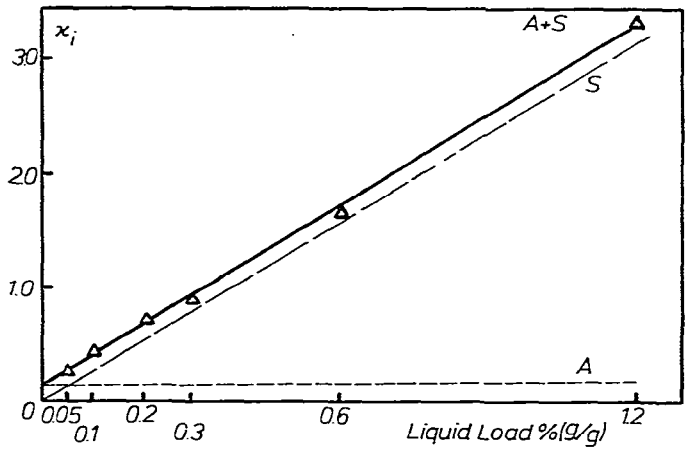

Fig. 1. Test plot for adsorption effects in GLC. A = adsorption; S = solution. Column: stationary liquid EGA coated on Chromosorb G HP, 100–125 μm; mobile phase, nitrogen, inlet pressure, 1.6 bar; temperature, 170°. Sample: 0.2 μl of a solution of TMS-3-MVALH in BSTFA, concentration 2 mg/cm^3.

Relationship between capacity factor and composition of mixed liquid phase

Mixed phases in chromatography can be replaced, in principle, by mixed beds or coupled columns if there is a linear relationship between the capacity factor and the composition (molar fraction, mass fraction, volume fraction) of the mixed phase. It is therefore important to elucidate the mathematical relationship between capacity factor and composition data. Recently it was strongly emphasized[26–28] that linear relationships are obtained if volume fractions are used as the composition data. From eqn. 6 we must conclude that this cannot be expected in general.

At low values of the selectivity coefficient, a small change causes a large change in the resolution. An increase of 5% in the selectivity coefficient, *e.g.*, results in a two-fold increase in the resolution, as can be predicted from eqn. 1. Consequently, the relationship between capacity factor and phase composition must be described with corresponding accuracy. A rough estimate of this relationship is not sufficient for predicting retention data with the desired accuracy.

TABLE II

RETENTION DATA OF ALKYLPHENOLS ON MIXED STATIONARY LIQUIDS CONSISTING OF OV-101 AND OV-225

Columns, 1000 × 2 mm; stationary phase, mixture of OV-101 and OV-225 on Volaspher A2, total loading 5% (w/w); mobile phase, nitrogen, inlet pressure 1.6 bar; temperature, 140°.

n	*Compound*	*Ratio of OV-101 to OV-225 in stationary liquid (w/w)*									
		100:0	*75:25*	*50:50*	*25:75*	*100:0*	*100:0*	*75:25*	*50:50*	*25:75*	*0:100*
		Capacity factor, κ_n					*Selectivity coefficient, $r_{(n+1)n}$*				
1	Phenol	1.82	4.26	6.53	9.03	11.77					
2	*o*-Cresol	2.66	5.36	7.81	10.44	13.52	1.46	1.26	1.20	1.16	1.15
3	*m*-Cresol	2.97	6.58	9.94	13.37	17.77	1.12	1.23	1.27	1.28	1.31
4	*p*-Cresol	3.08	6.55	9.82	13.22	17.59	1.04	1.00	0.99	0.99	0.99
5	2,6-Dimethylphenol	3.62	6.04	8.10	10.20	13.01	1.18	0.92	0.82	0.77	0.74
6	2-Ethylphenol	4.11	7.70	11.02	14.54	18.95	1.14	1.27	1.36	1.43	1.46
7	2,4-Dimethylphenol	4.44	8.21	11.60	15.22	19.83	1.08	1.07	1.05	1.05	1.05
8	2,5-Dimethylphenol	4.46	8.20	11.57	15.19	19.75	1.00	1.00	1.00	1.00	1.00
9	3,5-Dimethylphenol	4.93	10.10	14.84	19.76	26.2	1.11	1.23	1.28	1.30	1.33
10	4-Ethylphenol	4.93	10.28	15.05	20.2	26.8	1.00	1.02	1.02	1.02	1.02
11	2,3-Dimethylphenol	5.18	10.07	14.53	19.15	25.3	1.05	0.98	0.97	0.95	0.95
12	3,4-Dimethylphenol	5.71	12.17	17.84	24.2	32.0	1.10	1.21	1.23	1.26	1.26
13	2,4,6-Trimethylphenol	6.38	9.51	12.18	15.02	18.52	1.12	0.78	0.68	0.62	0.58
14	2,3,6-Trimethylphenol	7.10	11.13	14.47	18.40	23.2	1.11	1.17	1.19	1.23	1.25
15	2-*tert.*-Butylphenol	8.03	13.66	18.32	23.5	30.4	1.13	1.23	1.27	1.28	1.31
16	2,3,5-Trimethylphenol	8.33	15.17	21.0	27.3	35.8	1.04	1.11	1.14	1.16	1.18
17	3-Methyl-6-isopropylphenol	8.90	14.90	19.97	25.3	33.0	1.07	0.98	0.95	0.92	0.92
18	2,3,5,6-Tetramethylphenol	12.97	20.0	25.5	31.6	40.5	1.46	1.34	1.28	1.25	1.23

TABLE III

RETENTION DATA OF CHLOROPHENOLS ON MIXED STATIONARY LIQUIDS CONSISTING OF OV-101 AND OV-225

Columns, 1000 × 2 mm; stationary phase, mixture of OV-101 and OV-225 on Volaspher A2, total loading 5% (w/w); mobile phase, nitrogen, inlet pressure 1.6 bar; temperature, 140°.

n	*Compound*	*Ratio of OV-101 to OV-225 in stationary liquid (w/w)*									
		100:0	*75:25*	*50:50*	*25:75*	*0:100*	*100:0*	*75:25*	*50:50*	*25:75*	*0:100*
		Capacity factor, κ_n					*Selectivity coefficient, $r_{(n+1)n}$*				
1	2-Chlorophenol	2.59	3.37	4.80	5.69	7.05					
2	Guaiacol	4.06	5.27	7.44	8.97	11.02	1.57	1.56	1.55	1.58	1.56
3	2-Chloro-5-methylphenol	4.40	5.49	7.60	8.92	10.74	1.08	1.04	1.02	0.99	0.97
4	2-Nitrophenol	5.11	6.44	8.89	10.43	12.70	1.16	1.17	1.17	1.17	1.18
5	2,4-Dichlorophenol	6.22	9.21	13.70	17.04	21.3	1.22	1.43	1.54	1.67	1.68
6	2,5-Dichlorophenol	6.24	9.25	13.86	17.21	21.7	1.00	1.00	1.01	1.01	1.02
7	3-Chlorophenol	6.38	15.87	27.7	37.3	49.2	1.02	1.72	2.00	2.17	2.27
8	4-Chlorophenol	6.47	16.18	28.3	38.1	50.3	1.01	1.02	1.02	1.02	1.02
9	2,3-Dichlorophenol	6.48	9.75	14.69	18.25	22.9	1.00	0.60	0.52	0.48	0.46
10	2,6-Dichlorophenol	7.21	10.02	14.64	17.90	22.2	1.11	1.03	1.00	0.98	0.97
11	4-Chloro-2-methylphenol	9.46	19.84	33.4	44.1	57.7	1.31	1.98	2.28	2.46	2.60
12	4-Chloro-3-methylphenol	10.33	23.1	39.8	53.2	69.8	1.09	1.16	1.19	1.21	1.21
13	2,3,5-Trichlorophenol	13.76	21.3	33.3	41.9	53.3	1.33	0.92	0.84	0.79	0.76
14	2,4,6-Trichlorophenol	14.78	20.8	31.1	38.0	47.6	1.07	0.98	0.93	0.91	0.89
15	2,4,5-Trichlorophenol	15.34	25.9	41.1	52.4	67.3	1.04	1.24	1.32	1.38	1.41
16	2,3,4-Trichlorophenol	16.04	27.0	43.3	55.1	70.8	1.05	1.04	1.05	1.05	1.05
17	2,3,6-Trichlorophenol	16.90	25.6	39.1	48.5	61.7	1.05	0.95	0.90	0.88	0.87

TABLE IV

CAPACITY FACTORS, κ_n, OF PHENOLIC COMPOUNDS ON MIXED STATIONARY LIQUIDS CONSISTING OF OV-17 AND CARBOWAX 20M

Column, 1000 × 2 mm; stationary phase, mixture of OV-17 and Carbowax 20M on Volaspher A2, total loading 5% (w/w); carrier gas, nitrogen, inlet pressure 1.6 bar; temperature, 200°.

n	*Compound*	*Ratio of OV-17 to Carbowax 20M in stationary phase (w/w)*				
		100:0	*75:25*	*50:50*	*25:75*	*0:100*
1	Guaiacol	1.83	2.41	3.42	3.91	4.71
2	2-Nitrophenol	2.42	3.06	3.97	4.33	5.26
3	2,3-Dichlorophenol	2.70	5.09	8.16	10.30	12.95
4	4-Chloro-3-methylphenol	3.97	10.24	18.61	24.7	31.6
5	2,3,5,6-Tetramethylphenol	4.89	6.68	9.32	10.79	12.58
6	N,N-Dimethyl-3-aminophenol	6.75	15.25	26.5	34.7	44.1
7	Vanillin	7.85	14.97	24.3	30.8	38.9
8	Veratral	9.87	12.82	17.21	19.77	23.0

The relationship between capacity factor and liquid phase composition was examined experimentally for "binary" mixtures of OV-101 and OV-225 and OV-17 and Carbowax 20M, with phenolic compounds, and OV-1 and OV-17 with TMS-3-MHs of amino acids as solutes. As the two solvents mixed are mixtures themselves, their mixture is not a true binary mixture of two components but a "binary" mixture

TABLE V

RETENTION DATA OF TRIMETHYLSILYL-3-METHYLHYDANTOIN DERIVATIVES OF AMINO ACIDS ON MIXED STATIONARY LIQUIDS CONSISTING OF OV-1 AND OV-17

Column, 1000 × 4 mm; stationary phase, mixture of OV-1 and OV-17 on Chromosorb G HP, 125–150 μm, total loading 4% (w/w); mobile phase, nitrogen, flow-rate 30 cm³/min at 25° and 1 bar; temperature, 170°. Sample: 0.2 μl of solution in BSTFA, concentration 2 mg/cm³.

n	*Amino acid (abbreviations by first three letters)*	*Ratio of OV-1 to OV-17 in stationary liquid (w/w)*									
		100:0	*90:10*	*60:40*	*30:70*	*0:100*	*100:0*	*90:10*	*60:40*	*30:70*	*0:100*
		Capacity factor, κ_n					*Selectivity coefficient, $r_{(n+1)n}$*				
1	SARcosine	2.17	1.72	2.59	5.54	11.50					
2	GLYcine	4.14	3.09	3.73	7.29	13.94	1.91	1.80	1.44	1.31	1.21
3	ALAnine	4.34	3.16	3.86	7.02	12.16	1.05	1.02	1.03	0.96	0.87
4	PROline	5.12	4.15	6.22	13.63	27.3	1.18	1.31	1.61	1.94	2.24
5	VALine	6.31	4.40	5.11	8.92	15.50	1.23	1.06	0.82	0.65	0.57
6	Iso-LEucine	9.07	6.47	7.26	13.05	22.0	1.44	1.47	1.42	1.46	1.42
7	LEUcine	9.24	6.58	7.40	13.50	23.2	1.02	1.02	1.02	1.03	1.05
8	SERine	11.70	8.58	9.68	16.98	28.6	1.27	1.30	1.31	1.26	1.23
9	HYP-1*	13.18	10.51	14.12	28.4	54.7	1.13	1.22	1.46	1.67	1.91
10	THReonine	14.44	10.53	11.44	19.64	34.3	1.10	1.00	0.81	0.69	0.63
11	HYP-2*	16.03	12.64	16.26	32.2	62.4	1.11	1.20	1.42	1.64	1.82
12	METhionine	23.0	18.40	25.2	49.8	96.7	1.43	1.46	1.55	1.55	1.55
13	ASPartic acid	24.1	18.23	21.6	41.1	77.3	1.05	0.99	0.86	0.83	0.80
14	PHEnylalanine	35.0	28.6	38.4	79.8	163	1.45	1.57	1.78	1.94	2.11
15	GLUtamic acid	35.4	28.1	33.8	63.1	120	1.01	0.98	0.88	0.79	0.74

* Hydroxyproline.

TABLE VI

LINEARITY TEST OF CAPACITY FACTOR (κ_n) AND COMPOSITION (%, w/w)

For experimental data, see Tables I, II and III. Regression according to $\kappa_n = a_0 + a_1 \cdot \%$ (w/w) for OV-225 and Carbowax 20M, respectively.

Compound	$a_0 \pm s_0$	$a_1 \pm s_1$	*Correlation coefficient*, r^2
Alkylphenols			
Phenol	1.75 ± 0.12	0.0991 ± 0.0019	0.9989
o-Cresol	2.60 ± 0.15	0.1072 ± 0.0025	0.9984
m-Cresol	2.85 ± 0.26	0.1456 ± 0.0043	0.9974
p-Cresol	2.91 ± 0.29	0.1428 ± 0.0047	0.9968
2,6-Dimethylphenol	3.61 ± 0.18	0.0918 ± 0.0030	0.9969
2-Ethylphenol	3.96 ± 0.27	0.1461 ± 0.0045	0.9972
2,4-Dimethylphenol	4.30 ± 0.30	0.1512 ± 0.0049	0.9969
2,5-Dimethylphenol	4.32 ± 0.29	0.1503 ± 0.0048	0.9970
3,5-Dimethylphenol	4.72 ± 0.44	0.209 ± 0.007	0.9965
4-Ethylphenol	4.72 ± 0.45	0.215 ± 0.007	0.9965
2,3-Dimethylphenol	4.98 ± 0.43	0.1975 ± 0.0071	0.9962
3,4-Dimethylphenol	5.47 ± 0.51	0.258 ± 0.008	0.9969
2,4,6-Trimethylphenol	6.36 ± 0.19	0.1192 ± 0.0031	0.9979
2,3,6-Trimethylphenol	6.96 ± 0.35	0.1581 ± 0.0057	0.9961
2-*tert.*-Butylphenol	7.87 ± 0.53	0.218 ± 0.009	0.9953
2,3,5-Trimethylphenol	8.09 ± 0.65	0.268 ± 0.011	0.9953
3-Methyl-6-isopropylphenol	8.70 ± 0.65	0.234 ± 0.011	0.9938
2,3,5,6-Tetramethylphenol	12.78 ± 0.80	0.267 ± 0.013	0.9929
Chlorophenols			
2-Chlorophenol	2.45 ± 0.14	0.0450 ± 0.0023	0.9922
2-Chloro-5-methylphenol	4.21 ± 0.20	0.0644 ± 0.0032	0.9925
2-Nitrophenol	4.88 ± 0.23	0.0767 ± 0.0038	0.9926
2,4-Dichlorophenol	5.90 ± 0.31	0.1519 ± 0.0050	0.9966
2,5-Dichlorophenol	5.93 ± 0.32	0.1535 ± 0.0052	0.9965
3-Chlorophenol	5.88 ± 0.57	0.428 ± 0.009	0.9986
4-Chlorophenol	5.97 ± 0.59	0.438 ± 0.009	0.9985
2,3-Dichlorophenol	6.14 ± 0.35	0.1656 ± 0.0057	0.9964
2,6-Dichlorophenol	6.83 ± 0.37	0.1511 ± 0.0060	0.9953
4-Chloro-2-methylphenol	8.76 ± 0.76	0.483 ± 0.012	0.9980
4-Chloro-3-methylphenol	9.40 ± 0.92	0.597 ± 0.015	0.9981
2,3,5-Trichlorophenol	12.77 ± 0.95	0.399 ± 0.016	0.9955
2,4,6-Trichlorophenol	13.89 ± 0.88	0.331 ± 0.014	0.9944
2,4,5-Trichlorophenol	14.34 ± 1.05	0.521 ± 0.017	0.9968
2,3,4-Trichlorophenol	14.91 ± 1.15	0.551 ± 0.019	0.9965
2,3,6-Trichlorophenol	15.83 ± 1.08	0.450 ± 0.018	0.9954
Various phenolic compounds			
Guaiacol	1.08 ± 0.10	0.0290 ± 0.0016	0.9911
2-Nitrophenol	2.42 ± 0.11	0.0278 ± 0.0018	0.9872
2,3-Dichlorophenol	2.70 ± 0.17	0.1028 ± 0.0028	0.9978
4-Chloro-3-methylphenol	3.88 ± 0.46	0.279 ± 0.007	0.9979
2,3,5,6-Tetramethylphenol	4.95 ± 0.25	0.0780 ± 0.0040	0.9921
N,N-Dimethyl-3-aminophenol	6.64 ± 0.60	0.376 ± 0.010	0.9980
Vanillin	7.79 ± 0.51	0.311 ± 0.008	0.9979
Veratral	9.90 ± 0.36	0.1326 ± 0.0059	0.9940

of two composite solvents. The results are given in Tables II–V. In addition to the capacity factors, κ_n, the selectivity coefficients, $r_{(n+1)n}$, with respect to two consecutive components n and $n + 1$, which are the crucial parameters in the selection of a phase system according to eqn. 1, are also given.

The experimental data were correlated by means of linear regression and the results for the phenolic compounds are given in Table VI. Plots for a number of these compounds are shown in Fig. 2a. As it is cumbersome to give the composition of mixtures of polymeric liquid phases in terms of volume fractions according to eqn. 6, the mass percentage was used in a first approach. As can be seen from the precision of the linear regression, the accuracy of the prediction of retention data in mixed phases from the values in the unmixed solvents ($a_0 = \kappa_{n,\text{OV-101}}$, $a_0 + 100a_1 = \kappa_{n,\text{OV-225}}$) is better than the required 5%, on average. Considering these results in the light of eqn. 6, where volume fractions are used, one must bear in mind that mass fractions approach volume fractions if the densities of the unmixed solvents are nearly the same, which is the case with OV-101 and OV-225.

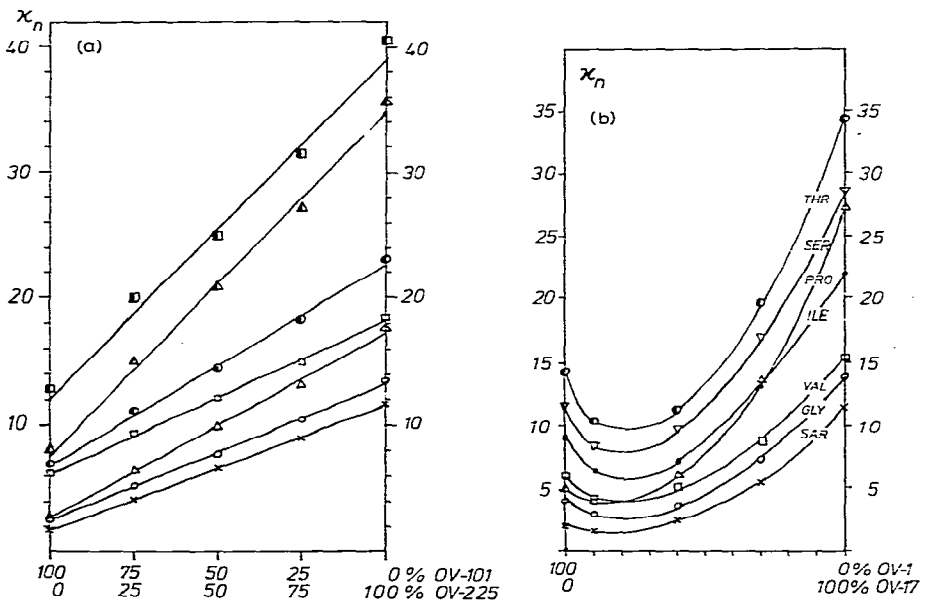

Fig. 2. Linear and non-linear relationships between capacity factor and stationary phase composition. (a) Column: see Table I. Sample: ×, phenol; ○, *o*-cresol; △, *m*-cresol; □, 2,4,6-trimethylphenol; ◐, 2,3,6-trimethylphenol; ◭, 2,3,5-trimethylphenol; ◧, 2,3,5,6-tetramethylphenol; solution in ethyl acetate. (b) For experimental conditions and abbreviations, see Table V.

For the TMS-3-MH derivatives of amino acids, curvilinear relationships with minima are found, as can be seen from Fig. 2b. In the literature[1,10,23,26–28], deviations from linearity are attributed to solvent–solvent interactions. Such types of interactions cannot occur in mixtures of dimethylsilicones (OV-1) and phenylmethylsilicones (OV-17) and the non-linear behaviour in this instance must have another reason. In this work experimental evidence was found for the existence of non-linear

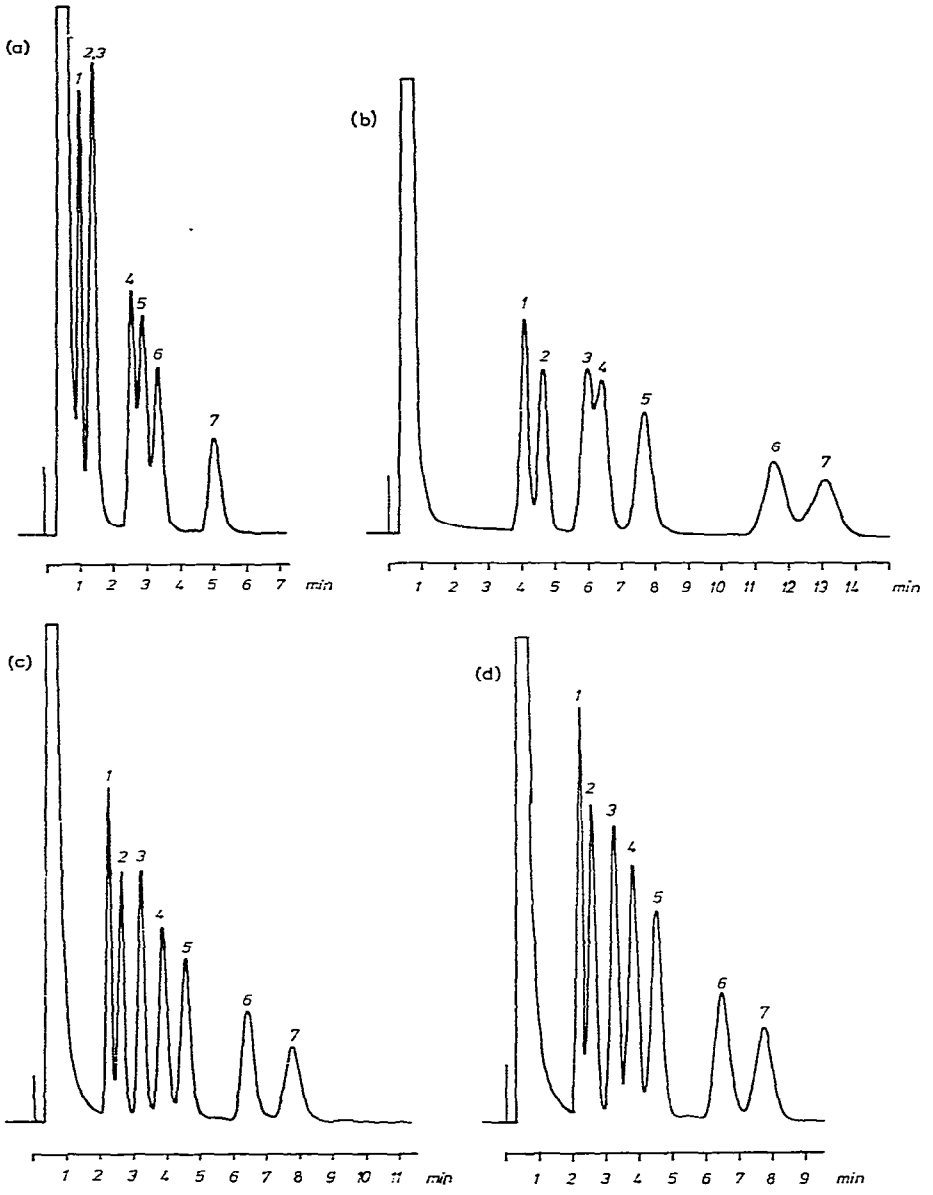

Fig. 3. Separation of alkylphenols on mixed OV-101–OV-225 stationary phases. Column, 1000 × 2 mm; stationary phase on Volaspher A2, 100–125 μm, total loading 5% (w/w); mobile phase, nitrogen, flow-rate, 8.3 cm^3/min at 20° and 1 bar, inlet pressure, 1.6 bar; temperature, 140°. Sample: (1) phenol; (2) *o*-cresol; (3) *m*-cresol; (4) 2,4,6-trimethylphenol; (5) 2,3,6-trimethylphenol; (6) 2,3, 5-trimethylphenol; (7) 2,3,5,6-tetramethylphenol; solution in ethyl acetate. (a) Chromatogram on stationary phase OV-101; (b) chromatogram on stationary phase OV-225; (c) chromatogram on stationary phase OV-101 + OV-225 (1:1, w/w), homogeneously mixed (d) chromatogram on two columns coupled in series, 500 × 2 mm each, stationary phase OV-101 and OV-225, respectively.

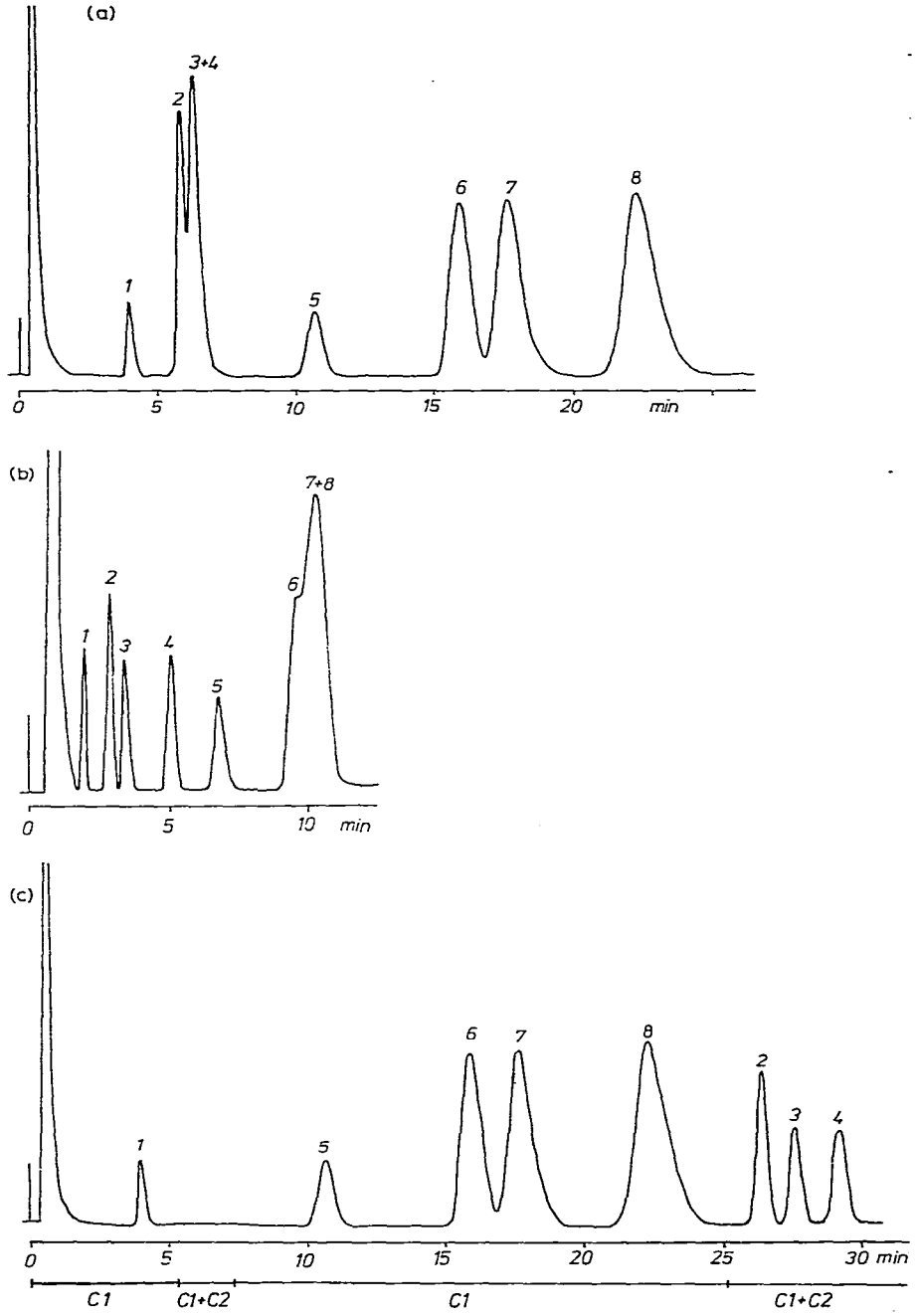

Fig. 4. Separation of phenolic compounds by column switching. Sample: (1) phenol; (2) *p*-cresol; (3) 2,5-dimethylphenol; (4) 2,4,6-trimethylphenol; (5) 3-methyl-6-isopropylphenol; (6) 2,4,6-trichlorophenol; (7) 2,3,5-trichlorophenol; (8) 2,4,5-trichlorophenol; solution in ethyl acetate. Column, total loading 5% (w/w) on Volaspher A2, 100–125 μm; mobile phase, nitrogen, flow-rate, 8.3 cm^3/min at 20° and 1 bar; temperature, 140°. (a) Column C1, 1000 × 2 mm; stationary phase OV-225; (b) column C2, 2000 × 2 mm; stationary phase OV-101; (c) columns C1 and C2 coupled by means of a switching device.

relationships in simple systems. Future work is intended to find an explanation for this phenomenon.

Chromatographic exploitation of the selectivity of mixed phases

For the choice of a phase system for a chromatographic separation on the basis of eqn. 1, tables with selectivity coefficients and capacity factors are required. It can be seen from Table V that for the separation of TMS-3-MHs with OV-1/OV-17 stationary liquids, unmixed OV-17 is the best choice. For the separation of phenolic compounds on OV-101/OV-225 stationary liquids, the situation is more complicated. As can be seen from Tables II–IV, there is no single phase that can resolve all species. Some pairs of compounds are resolved over the whole composition range, others only within limited ranges, and others cannot be resolved at all.

For groups of compounds, it is possible to demonstrate the need for mixed phases. They are not sufficiently resolved on the unmixed solvents, but can be separated with satisfactory resolution on a mixed liquid phase. An example is shown in Fig. 3, which also shows the equivalence of mixed phases and coupled columns, if the assumption of a linear dependence of the capacity factor on the phase composition is fulfilled.

Even if it is not possible to separate a group of compounds on a single mixed phase, a satisfactory resolution can be achieved by column switching with the unmixed solvents. An example is shown in Fig. 4.

We feel that at present the potential selectivity of mixed liquid phases in GLC is not being sufficiently exploited in practice. Especially in combination with the gradual adjustment of the phase selectivity by column switching, the solution of unsolved separation problems may be expected in future. The exploitation of non-linear effects of the solvent composition needs further investigation.

REFERENCES

1 W. H. McFadden, *Anal. Chem.*, 30 (1958) 479.
2 J. A. Barnard and H. W. D. Hughes, *Nature (London)*, 183 (1959) 250.
3 E. O. A. Haathi, W. J. A. VandenHeuvel and E. C. Horning, *Anal. Biochem.*, 2 (1961) 344.
4 A. Waksmundzki, E. Socjewinski and Z. Suprynowicz, *Collect. Czech. Chem. Commun.*, 27 (1962) 2001.
5 A. A. Zhukhowitskii, M. S. Selenkina and N. M. Turkeltaub, *Russ. J. Phys. Chem. (Engl. Transl.)*, 36 (1962) 519.
6 T. Matsuda and H. Yatsugi, *Bunseki Kagaku (Jap. Anal.)*, 11 (1962) 1116.
7 A. H. Duvall and W. F. Tully, *J. Chromatogr.*, 11 (1963) 38.
8 M. Mitooka, *Nippon Kagaku Zasshi*, 84 (1963) 923.
9 G. P. Hildebrand and C. N. Reilley, *Anal. Chem.*, 36 (1964) 47.
10 R. A. Keller and G. H. Stewart, *Anal. Chem.*, 36 (1964) 1186.
11 A. Waksmundzki and Z. Syprynowicz, *J. Chromatogr.*, 18 (1965) 232.
12 H. Miyake, M. Mitooka and T. Matsumoto, *Bull. Chem. Soc. Jap.*, 38 (1965) 1062.
13 A. W. Littlewood and T. Wilmott, *Anal. Chem.*, 38 (1966) 1031.
14 J. H. Purnell, S. P. Wasik and R. J. Juvet, *Acta Chim. Acad. Sci. Hung.*, 50 (1966) 201.
15 J. C. Touchstone, C. H. Wu, A. Nikolski and T. Murawec, *J. Chromatogr.*, 29 (1967) 235.
16 S. A. Reznikov, *Russ. J. Phys. Chem. (Engl. Transl.)*, 42 (1968) 906.
17 A. Islam and A. Darbre, *J. Chromatogr.*, 43 (1969) 11.
18 M. I. Molera, J. A. G. Dominguez and J. F. Biarge, *J. Chromatogr. Sci.*, 7 (1969) 305; 11 (1973) 538; 14 (1976) 299.
19 C. L. Young, *J. Chromatogr. Sci.*, 8 (1970) 103.

20 J. C. Touchstone, T. Murawec and A. Nikolski, *J. Chromatogr. Sci.*, 8 (1970) 221.
21 M. Mitooka, *Bunseki Kagaku (Jap. Anal.)*, 20 (1971) 1542; 21 (1972) 189, 197, 614, 717 and 729.
22 Y. N. Boglovsky, V. M. Sakharov and I. M. Shevchuk, *J. Chromatogr.*, 69 (1972) 17.
23 G. W. Pilgrim and R. A. Keller, *J. Chromatogr. Sci.*, 11 (1973) 206.
24 A. S. Mel'nikov and B. A. Azaivov, *Zh. Anal. Khim.*, 28 (1973) 791; 29 (1974) 365.
25 S. V. Levy and T. Schwartz, *Clin. Chim. Acta*, 54 (1974) 19.
26 J. H. Purnell and J. M. Vargas de Andrade, *J. Amer. Chem. Soc.*, 97 (1975) 3585 and 3590.
27 R. J. Laub and J. H. Purnell, *J. Amer. Chem. Soc.*, 98 (1976) 30 and 35.
28 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
29 K. van de Casteele, H. de Pooter and G. F. van Sumere, *J. Chromatogr.*, 121 (1976) 49.
30 J. P. O'Connel and J. M. Prausnitz, *Ind. Eng. Chem., Fundam.*, 3 (1964) 347.
31 J. H. Hildebrand, J. M. Prausnitz and R. L. Scott, *Regular and Related Solutions*, Van Nostrand Reinhold, New York, 1970, pp. 130–134.
32 I. L. Leites and F. P. Ivanovskii, *Khim. Prom.*, (1962) 653.
33 P. Hagel, *Thesis*, University of Amsterdam, 1970.
34 H. Markens, *Thesis*, University of Amsterdam, 1974.
35 J. F. K. Huber and R. G. Gerritse, *J. Chromatogr.*, 80 (1973) 25.