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OPTIMIZATION OF LIQUID PHASE MIXTURES*

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SUMMARY

The concept of window diagrams has been used to predict what lengths of dissimilar fused-silica capillaries should be serially coupled to achieve the optimum separation of two "real world" samples whose separation on a single column has not yet been reported. Complicating factors, including the role of the solute partition ratio and the velocity gradient of the carrier gas, are discussed. Separations of mixtures of (a) volatiles produced by yeast fermentation, and (b) solvents used in the preparation of food packaging films, were achieved in single passes on properly configured serially coupled columns composed of two precise lengths, one coated with polymethylsiloxane, and the other with polyethylene glycol.

INTRODUCTION

The optimization of gas chromatographic (GC) parameters such as separation and analysis time must logically be predicated on the use of open tubular capillary columns, because of their inherent superiority over packed columns with respect both to the above parameters[1] and to their increased inertness, greater convenience and lower overall cost[2]. The degree to which two given solutes are separated by a GC system at some given temperature is a function of (a) the breadth of the solute bands (which is influenced both by the width of the starting band or injection efficiency, and by band broadening or column efficiency), and (b) their relative retention, *i.e.*, liquid phase selectivity. While one well chosen liquid phase is best for separating two solutes, it does not always have the selectivity to adequately separate a mixture of three or more solutes. To predict what ratio of two liquid phases yields the best separation of three or more solutes, Maier and Karpathy[3] proposed a method based on the fact that the retention behavior of a solute in a liquid phase mixture could be predicted from a knowledge of its retention behavior in the pure liquid phases, and the relative amounts of those liquid phases in that mixture (all other things being constant). They suggested that the desired proportions of the two liquid phases could be

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realized by (1) series coupling predetermined lengths of columns packed with support coated (separately) with the two liquid phases, (2) packing the column with two differently coated solid supports that had been intimately mixed in the proper proportions and (3) packing the column with a solid support that had been coated with the appropriate mixture of the two liquid phases. They observed the best separation of a hydrocarbon mixture for case (3), but because it demanded ideal solution behavior, they removed it from further consideration. A very similar method was subsequently proposed by Laub and Purnell (*e.g.*, ref. 4); most of these examples employed packed columns whose efficiencies were sufficiently low that more selective liquid phases were required to affect separation. Most of the mixtures employed in those investigations can be completely resolved by a single liquid phase in a more efficient capillary system. Purnell *et al.*[5] recently employed a coupled capillary column and constructed a "window diagram" (see below) of the relative retentions of the solutes *vs.* the length fraction of one of the columns. The method of determining the length fraction was not specified, but apparently it involved a direct relationship between α and the length fraction. Although they were able to achieve a slightly shorter analysis time on the coupled capillary columns as contrasted to their packed column, resolution was not improved. They attributed this to their inexperience in the preparation of capillary columns. It should be noted that determining the appropriate length fraction is not as trivial as it might seem, as would surely have been demonstrated by a more challenging separation.

Two advantages are offered by the use of serially coupled columns to achieve liquid phase mixtures: it obviates the danger of non-ideal solution behavior that may occur with liquid phase mixtures (although this is rarely demonstrated), and because the preparation of highly efficient capillary columns is not a trivial matter, it is often preferable to be able to employ lengths of commercially manufactured columns.

On the other hand, the relationship between ϕ and the length fraction is rarely simple and direct. There are several complicating factors, of which the most serious is (usually) the velocity gradient in the column. Because of this, the second column in the series must always operate at higher linear gas velocities than does the first; the first column will contribute more (and the second column will contribute less) to the separation that would be implied by its length fraction alone. This complication will be discussed in more detail later.

If the effect of the velocity gradient is ignored and both columns have the same phase ratios, the length of column containing liquid phase A (L_A) is related to the length of column containing liquid phase B (L_B) by the equation

$$L_A = \phi_A(L_A + L_B) = \phi_A L \tag{1}$$

where the total length, L, is equal to L_A plus L_B. If the phase ratios of the two columns differ, then

$$\frac{L_A}{\beta_A} = \frac{\phi_A L}{\beta} \tag{2}$$

where β_A and β_B are the phase ratios of columns A and B, respectively, and β is the average phase ratio of the coupled column, given by the relationship:

$$\beta = (L_A\beta_A + L_B\beta_B)/L \tag{3}$$

Substituting eqn. 3 into eqn. 2, the length of either segment can be calculated:

$$\frac{L_A}{\beta_B} = \frac{\phi_A L^2}{L_A\beta_A} + (L - L_A)\beta_B \tag{4}$$

Rearranging yields an equation that is second order with respect to L_A and L:

$$(\beta_A - \beta_B)\, L_A^2 + \beta_B L L_A - (\phi_A\beta_B)L^2 = 0 \tag{5}$$

Application of the quadratic equation yields

$$L_A = \frac{-L\beta_B \pm [L^2\beta_B^2 + 4(\beta_A - \beta_B)\phi_A L^2\beta_A]^{1/2}}{2(\beta_A - \beta_B)} \tag{6}$$

or

$$L = \frac{-\beta_B L_A \pm [L_A^2\beta_B^2 + 4(\beta_A - \beta_B)\phi_A\beta_A]^{1/2}}{2(\phi_A\beta_A)} \tag{7}$$

Either equation can be employed, depending on whether a certain total column length is desired (eqn. 6), or whether one wishes to use an existing column of length L_A (eqn. 7).

The concept of window diagrams, proposed by Laub and Purnell and their co-workers (*e.g.*, refs. 4,5) is based on the fact that

$$K_{D(A+B)} = K_{D(A)}\phi_A + K_{D(B)}\phi_B \tag{8}$$

where the subscripts A and B denote the liquid phase for that distribution constant in question, and $\phi_{A,B}$ represents the volume fraction of the specified liquid phase. Inasmuch as ϕ_A plus ϕ_B equals 1:

$$K_{D(A+B)} = K_{D(B)} + \phi_A(K_{D(A)} - K_{D(B)}) \tag{9}$$

From this it is apparent that a plot of $K_{D(A,B)}$ *vs.* ϕ_A must be linear; *i.e.*, the distribution constant of a solute in that mixture is a linear function of the amount of either of the liquid phases comprising that mixture.

The present study, an offshoot of our recycling work[6], was directed toward using these principles in a more efficient system to achieve the separation of two "real world" mixtures that have plagued a number of investigators.

EXPERIMENTAL

Test mixtures

Test mixture I was composed of solutes produced by alcoholic fermentation, whose separation is of considerable industrial interest. At least two separate columns

are traditionally employed to separate these components; their separation in a single chromatographic column has not yet been reported. Solutes, listed in Table I, were present in roughly equal amounts, with the exception of ethanol, which amounted to 30% of the mixture. The industrial importance of this separation was drawn to our attention by Liddell[7]. Test mixture II was composed of solvents (Table II) used in a variety of food packaging films. Determination of residual solvent in these films and in the packaged food is of obvious interest, both to regulatory agencies and to segments of the food processing industry. The mixture was suggested to us by Kolb[8], who had previously worked with this mixture[9].

Gas chromatography

GC separations employed a Varian 3700 gas chromatograph equipped with an inlet splitter and make up gas adaptor, both from J & W Scientific. Fused-silica capillary columns were obtained from the same source. The DB-1 polymethylsiloxane bonded phase (equivalent to SE-30) was 14 m × 0.32 mm I.D. with a liquid phase film thickness of 1.0 μm. Two polyethylene glycol (Carbowax) columns were employed, differing only in length (31 m and 21 m). Both were 0.25 mm I.D. with liquid phase film thicknesses of 0.25 μm. All columns were operated at 60°C and at average linear carrier gas velocities of 8–50 cm/sec with helium carrier gas.

The window diagrams were generated with BASIC computer programs on an Apple II plus computer, using a Paper Tiger 460G graphics printer (Integral Data Systems, Milford, NH, U.S.A.). The graphics printer routines were from Computer Stations (Granite City, IL, U.S.A.). These will be published separately.

For the purposes of testing the window diagrams using capillary columns, 15–20 cm of thin walled heat shrink PTFE tubing (0.025-in. expanded I.D., 0.010-in. recovered I.D., 0.004-in. wall; Zeus Industrial Products) was used to connect columns. Shrinking was accomplished with a heat gun at a maximum temperature of about 350°C.

RESULTS

Chromatograms of test mixture I run on pure DB-1 and pure Carbowax are shown in Figs. 1 and 2. An analysis temperature of 60°C was chosen because of the high volatility of the solutes and the lower operating temperature limit of Carbowax. Fig. 1 shows that the DB-1 column failed to separate methanol and acetaldehyde, while in Fig. 2 it can be seen that the Carbowax column failed to separate 2-methylbutanol from 3-methylbutanol. The method of Laub and Purnell[4] was used to estimate the optimum mixture of these two liquid phases. The distribution constant of each solute was determined at 60°C on columns of known phase ratio, coated with pure polyethylene glycol or DB-1 (Table I). The two points (for each solute) were then connected by a straight line, producing a plot of the volume fraction of DB-1, *vs.* K_D (Fig. 3). This plot also reveals the elution order of those solutes as functions of the volume ratios of those two liquid phases at that temperature. The relative retention (α = larger K_D/smaller K_D) of every possible solute pair as a function of the liquid phase volume ratio is calculated; larger values (which would not be separation-limiting) are rejected by the computer, and those that are on-scale are plotted as in Fig. 4. The highest "window" in such a plot indicates what volume ratio of those liquid phases

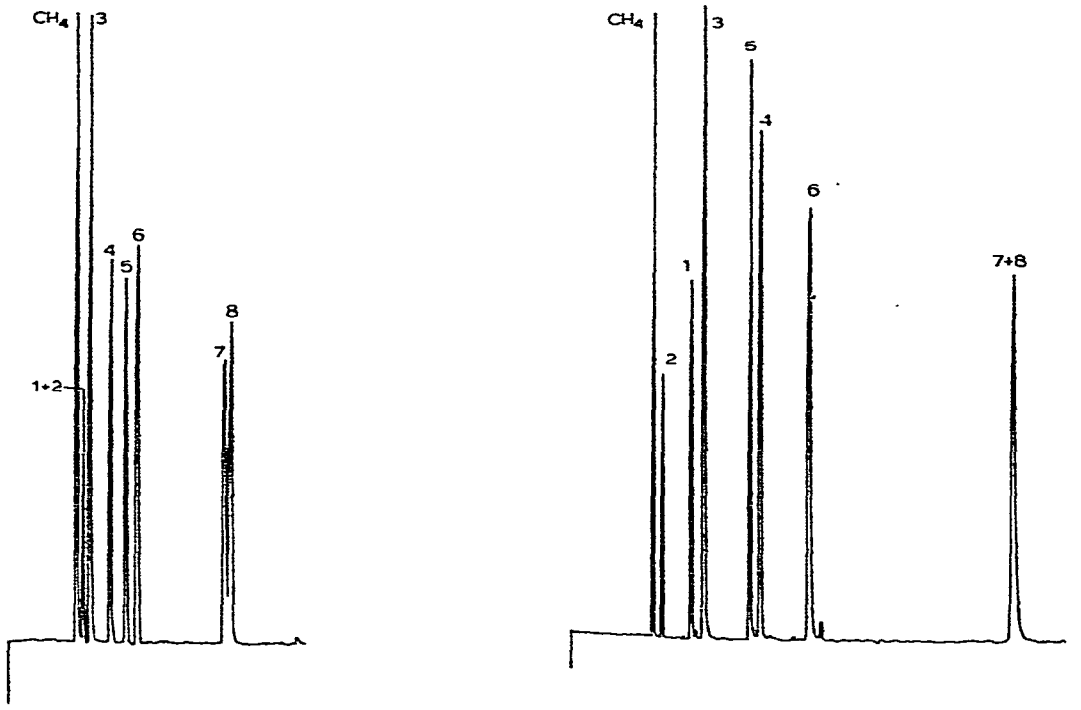


Fig. 1. Chromatogram of test mixture I on an 11 m × 0.32 mm fused-silica capillary, coated with 1.0-μm bonded film of polymethylsiloxane (DB-1); split injection (*ca.* 1:100), 60°C isothermal, u = 26 cm/sec. See Table I for compound identification.

Fig. 2. Chromatogram of test mixture I on a 30 m × 0.25 mm fused-silica capillary, coated with a 0.25-μm film of polyethylene glycol (Carbowax); 60°C, u = 30 cm/sec. See Table I for compound identification.

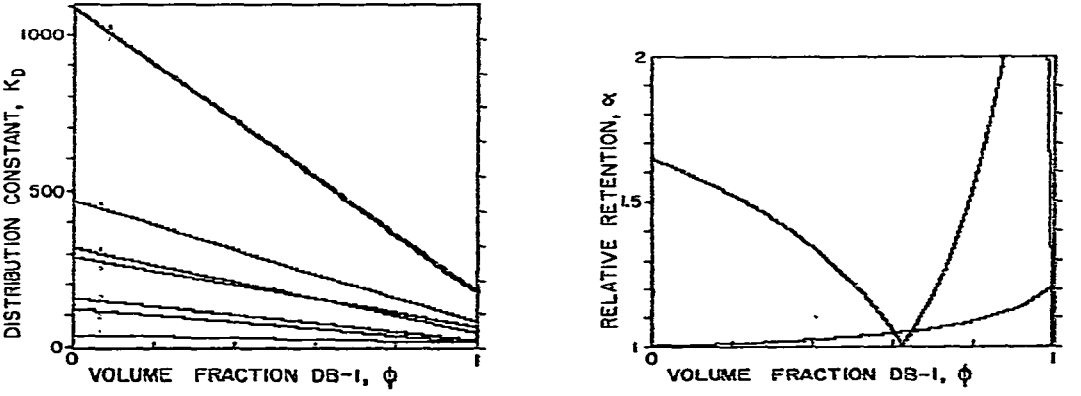


Fig. 3. Computer plot of the distribution constants (K_D) of the individual solutes *versus* the volume fraction of DB-1 (ϕ) for test mixture I. See Table I for compound identification.

Fig. 4. Computer plot of the relative retentions of separation-limiting solute pairs *versus* the volume fraction of DB-1 for test mixture I.

TABLE I

K_D VALUES (60°C) FOR SOLUTES OF TEST MIXTURE I

Solute	*Peak No.**	*DB-1*	*Carbowax*
Methanol	1	8	117
Acetaldehyde	2	8	30
Ethanol	3	16	153
n-Propanol	4	39	324
sec.-Butanol	5	57	294
Isobutanol	6	71	475
3-Methylbutanol	7	172	1099
2-Methylbutanol	8	178	1099

* As used in Figs. 1, 2 and 6.

produces the highest relative retentions for those limiting solutes at that temperature. In this particular case, the relative retention of acetaldehyde and methanol rapidly approaches unity as the last traces of Carbowax are removed from the system, and the optimum window would appear to be at *ca.* 99.5% DB-1 and 0.5% Carbowax.

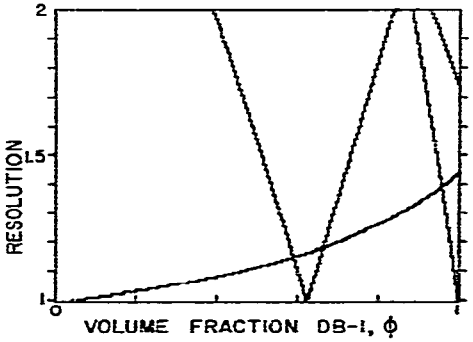


Fig. 5. Computer plot of resolution (*Rs*) *versus* volume fraction of DB-1 (ϕ) for test mixture I. See text for details.

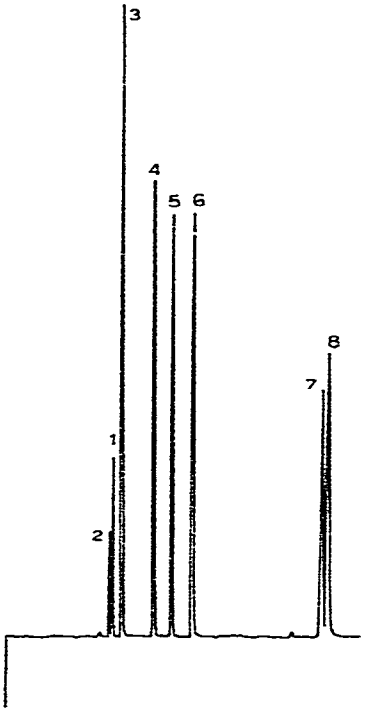


Fig. 6. Chromatogram of test mixture I on a serially coupled column composed of a 3.7 m × 0.25 mm section coated with a 0.25-μm film of polyethylene glycol (Carbowax), followed by a 28.5 m × 0.32 mm section coated with a 1.0-μm bonded film of polymethylsiloxane (DB-1); split injection, 60°C, u = 28 cm/sec. See Table I for compound identification.

Using lengths calculated according to eqn. 7, a column of these proportions was assembled; separation differed from that obtained on pure DB-1 only in that a slight shoulder was noticeable on the first peak (acetaldehyde plus methanol). This poor separation was attributable to the fact that these two solutes exhibit very small partition ratios, *ca.* 0.2, at that window. Although these proportions of the two liquid phases produced the highest relative retentions for the overall mixture, separation was less than adequate, in that resolution is also affected by the partition ratio, k.

A plot of resolution *vs.* ϕ confirmed this (Fig. 5); the best resolution window occurs at 95% DB-1 and 5% Carbowax. Utilizing eqn. 7, a column was constructed of 28.5 m DB-1 (thick film, wide bore) plus 3.7 m Carbowax. With the Carbowax segment connected to the inlet, methanol and acetaldehyde exhibited a resolution of 1.72 (Fig. 6); when the DB-1 segment was connected to the inlet, the resolution of these two solutes was 1.09. On the other hand, the resolution of the two methylbutanol isomers was 1.45 in the first configuration, and 1.59 in the second.

Fig. 7 shows test mixture II run on a column coated with pure DB-1, on which acetone and isopropanol, and methyl acetate and dichloromethane coeluted, and Fig. 8 shows the mixture on a column coated with pure Carbowax, where 2-butanone,

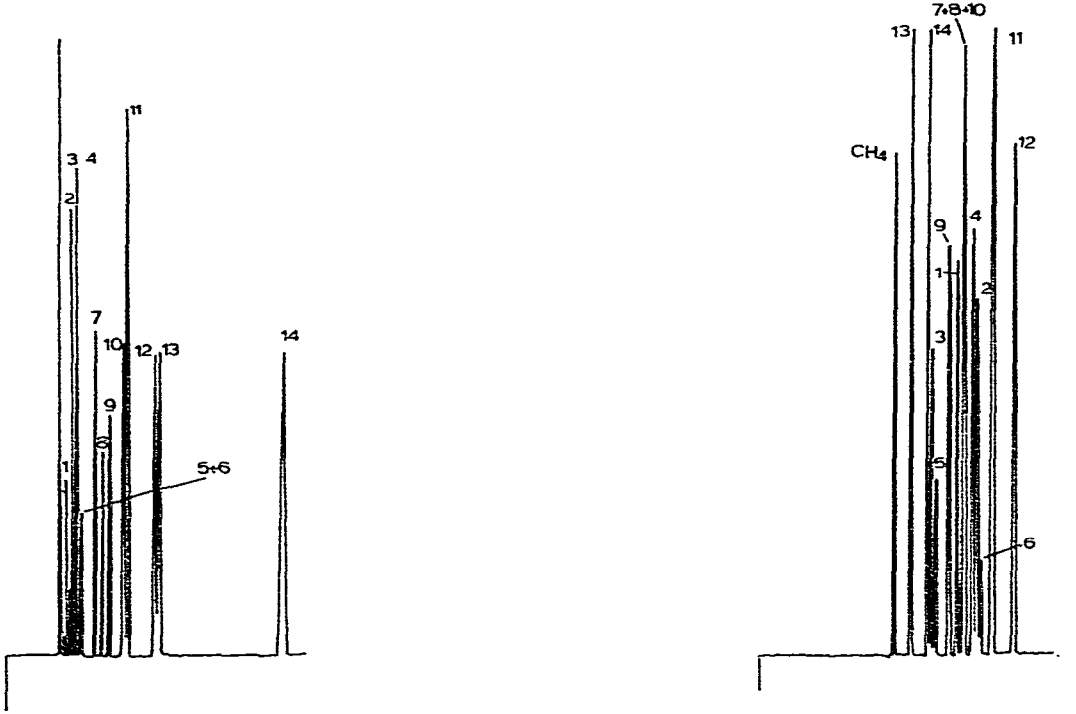
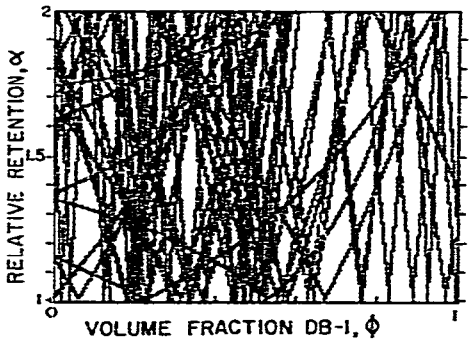


Fig. 7. Chromatogram of test mixture II on an 11 mm × 0.32 mm fused-silica capillary, coated with 1.0-μm bonded film of polymethylsiloxane (DB-1); split injection, 60°C isothermal, u = 26 cm/sec. See Table II for compound identification.

Fig. 8. Chromatogram of test mixture II on a 30 m × 0.25 mm fused-silica capillary, coated with a 0.25-μm film of polyethylene glycol (Carbowax); 60°C, u = 28 cm/sec. See Table II for compound identification.



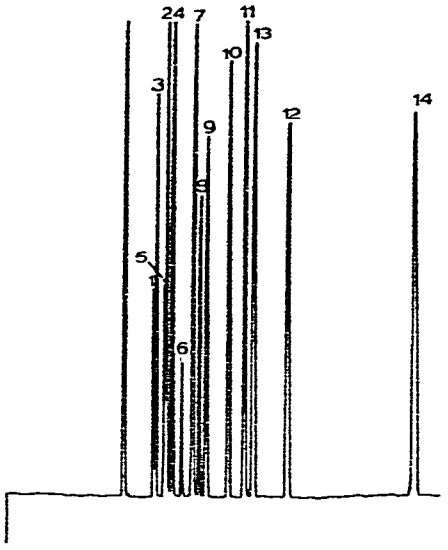


Fig. 9. Computer plot of relative retentions (α) of individual pairs of solutes in test mixture II, *versus* the volume fraction of DB-1 (ϕ). See text for details.

Fig. 10. Chromatogram of test mixture II on a serially coupled column composed of a 14.0 m × 0.32 mm section coated with a 1.0-μm bonded film of polymethylsiloxane (DB-1), followed by a 23.1 m × 0.25 mm section coated with a 0.25-μm film of polyethylene glycol (Carbowax); split injection, 60°C, u = 30 cm/sec. See Table II for compound identification.

ethyl acetate and isopropyl acetate eluted as a single peak. The distribution constants are listed in Table II.

A window diagram of α *vs.* ϕ is shown in Fig. 9, which indicates two approximately equal windows, one at 80% DB-1, and the other at 86% DB-1. Using

TABLE II

K_D VALUES (60°C) FOR SOLUTES IN TEST MIXTURE II

Compound	*Peak No.**	*DB-1*	*Carbowax*
Methanol	1	8	118
Ethanol	2	16	153
Acetone	3	21	70
Isopropanol	4	23	145
Methyl acetate	5	32	78
Dichloromethane	6	31	162
2-Butanone	7	53	130
Ethyl acetate	8	63	128
Tetrahydrofuran	9	75	102
Isopropyl acetate	10	96	130
Benzene	11	102	183
n-Propyl acetate	12	144	224
n-Heptane	13	149	30
n-Octane	14	336	63

* As used in Figs. 7, 8, 10, 11 and 13.

eqn. 7, 14 m of the DB-1 column were joined to 23.1 m of Carbowax. The resultant chromatogram (with the DB-1 segment connected to the inlet) is shown in Fig. 10, where it can be seen that the most difficult solutes to resolve are methanol–acetone, and methyl acetate–ethanol. Since the calculated column lengths can only approximate the desired ratios of the two liquid phases (largely because of the carrier gas velocity gradient discussed earlier), short lengths of column were removed by trial and error until the optimum resolution of all solutes was achieved. This resulted in a column composed of 13.35 m of DB-1 and 23.1 m Carbowax. The chromatogram obtained with this column is shown in Fig. 11, and resulted in a resolution of 1.12 for methanol and acetone, and 1.19 for methyl acetate and ethanol.

To determine the optimum average carrier gas velocity for this particular separation, a Van Deemter plot was generated, which indicated that the optimum was 30–40 cm/sec under these conditions. At a velocity of 35 cm/sec however, methyl acetate and ethanol co-eluted. Plots of resolution of these two solvent pairs *vs.* the average linear carrier gas velocity are shown in Fig. 12. Although all of these solutes exhibited optimum velocities of *ca.* 35 cm/sec, resolution between methanol and ethyl acetate was maximum somewhere below 7 cm/sec, while that between acetone and methanol is best at a velocity of *ca.* 40 cm/sec. It seems probable that this is due to the carrier gas velocity gradient operating in the column.

To test this hypothesis, the column was reversed and the Carbowax end connected to the inlet. By comparing the elution order of the solutes to those indicated by

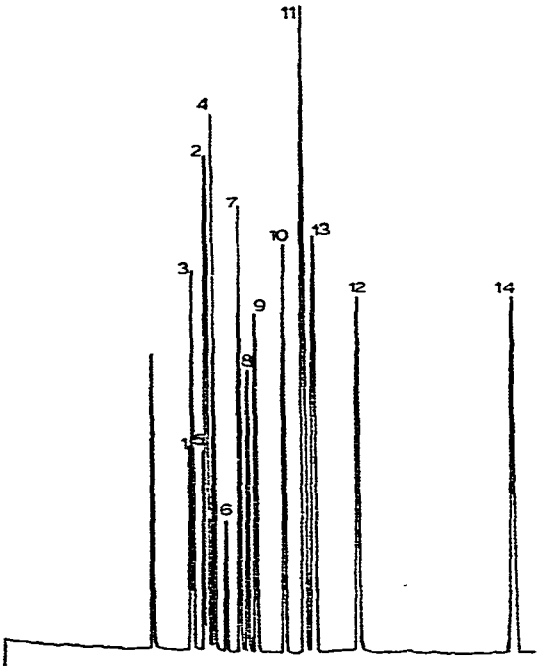


Fig. 11. Chromatogram of test mixture II on a serially coupled column composed of a 13.35 m × 0.32 mm section coated with a 1.0-μm bonded film of polymethylsiloxane (DB-1), followed by a 23.1 m × 0.25 mm section coated with a 0.25-μm film of polyethylene glycol (Carbowax); split injection, 60°C, u = 30 cm/sec. See Table II for compound identification.

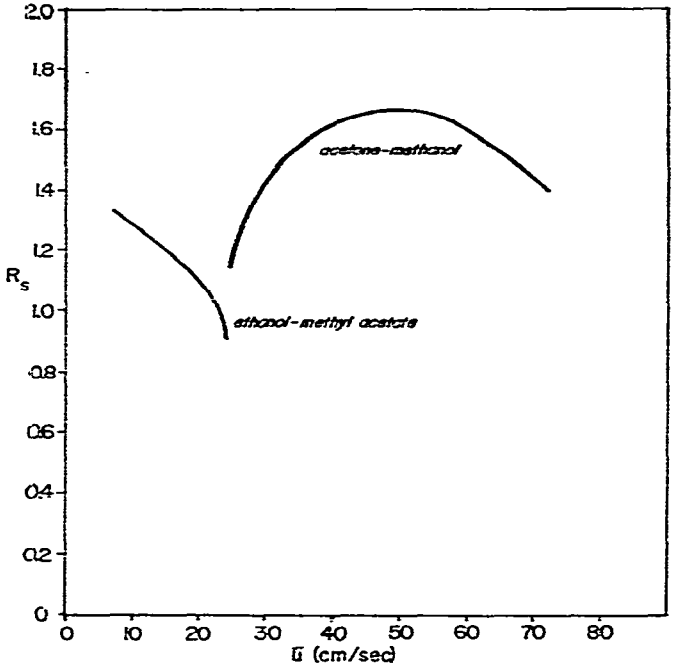


Fig. 12. Plot of resolution (R_s) *vs.* u for ethanol–methyl acetate, and acetone–methanol on a serially coupled column composed of a 13.35 m × 0.32 mm section coated with a 1.0-μm bonded film of polymethylsiloxane (DB-1), followed by a 23.1 m × 0.25 mm section coated with a 0.25-μm film of poiyethylene glycol (Carbowax); split injection, 60°C.

plotting the values in Table II (as was done in Fig. 3 for the values in Table I), it was determined that because the gas velocity at the inlet end is lower than the average and that at the outlet end is higher than the average, the "apparent" liquid phase mixture in this configuration (Carbowax preceding DB-1) approximated 66% DB-1 instead of the 80% desired. Small segments of the Carbowax column were removed by trial and error until the correct elution order was observed. This resulted in a column composed of 13.35 m of DB-1 and 13.1 m of Carbowax. A chromatogram run on this column is shown in Fig. 13.

DISCUSSION

Window analysis seems well suited to predicting binary phase mixtures that can achieve the separation of complex mixtures that resist separation even on high resolution columns coated with a single liquid phase. Both test mixtures investigated fall into this category, and both were resolved using coupled capillary columns to achieve the desired ratio of the two liquid phases.

Calculation of the requisite column lengths is complicated by the carrier gas velocity gradient operating in the column. While the velocity gradient itself is well understood (*e.g.*, refs. 10–13), the matter is further complicated by the fact that the velocity gradient and the column length are interdependent. Neither is it readily apparent which column parameter is most closely related to ϕ.

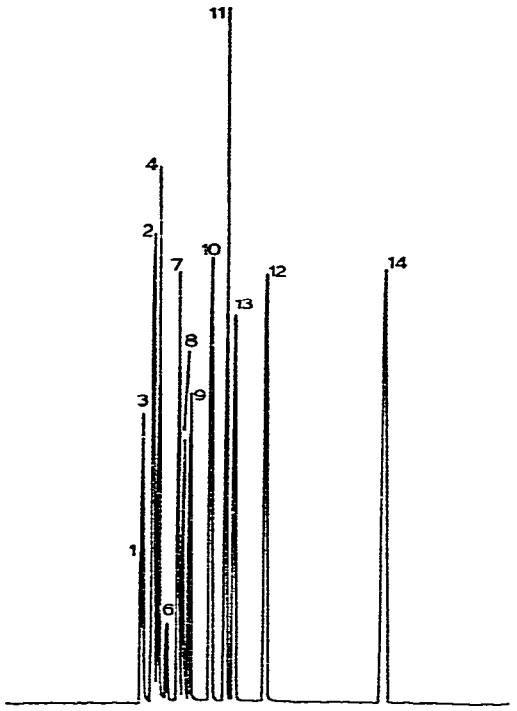


Fig. 13. Chromatogram of test mixture II on a serially coupled column composed of a 13.1 m × 0.25 mm section coated with a 0.25-μm film of polyethylene glycol (Carbowax), followed by a 13.35 m × 0.32 mm section coated with a 1.0-μm bonded film of polymethylsiloxane (DB-1); split injection, 60°C, u = 40 cm/sec. See Table II for compound identification.

In practice, however, excellent results can be achieved by using the window diagram concept to approximate the proper liquid phase mixture; the required lengths of column are then calculated using eqn. 6 or 7, as appropriate. The calculated lengths usually require some modification, because the section which is first in sequence (*i.e.*, the inlet end) will need to be shorter than that calculated, and/or the second segment (that connected to the detector) will need to be longer than that calculated. Which of these choices is best will depend on the lengths of column available, and their inner diameters. For example, the DB-1 column employed with test mixture II was a wide-bore column (0.32 mm I.D.). When this was first, only a small adjustment (−64 cm) was required; when the Carbowax segment (0.25 mm I.D.) was first, a larger adjustment (−900 cm) was necessary to achieve the same results.

Columns coated with premixed liquid phase mixtures offer another possibility. There have been some notable successes in the coating of fused-silica capillaries with premixed liquid phases[14,15]. It is probable that column suppliers will soon offer commercial columns coated with liquid phases premixed to the analyst's specifications and designed for the optimal separation of specific mixtures.

An alternate approach to minimizing the velocity gradient would be to place a restriction at the detector outlet and operate at higher average column pressures[16]. Neither of these possibilities was investigated in the present study.

The observed partition ratio exhibited by any solute is the sum of the times spent in the two liquid phases, quantity divided by the time spent in the gas phase. Hence in series coupled columns coated with dissimilar liquid phases, the apparent partition ratio is affected by the carrier gas velocity, a situation that is not encountered with columns coated with a pure (or homogeneously mixed) liquid phase. It should be possible to set the length of the second column segment so that the linear velocity is optimized with respect to resolution.

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