Journal of Chromatography, 509 (1990) 157–169
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 390

Liquid chromatography of liquid substances characterized by limited solubility in the eluent

E. B. GUGLYA*
A. V. Topchiev Institute of Petrochemical Synthesis, U.S.S.R. Academy of Sciences, Leninski Pr. 29, 117912 Moscow (U.S.S.R.)
and
A. A. ZHUKHOVITSKII
Moscow Institute of Steel and Alloys, Moscow (U.S.S.R.)

ABSTRACT

A new version of liquid chromatography using component distribution between immiscible phases of a liquid sample and a liquid eluent has been developed. The method, called chromaextraction, is performed on a low-sorbing column packing under high loading conditions, similarly to the gas chromatographic method of chromadistillation. They both differ from traditional chromatographic techniques in the lack of a sorbent, as the separated mixture itself may be regarded as the stationary liquid phase.

When one component possessing limited solubility in the eluent is eluted, the breakthrough curve forms a rectangular zone corresponding to a saturated solution of this component. Ordinary isocratic elution of a mixture leads to frontal-type partial separation. The first zone contains all components and the number of components decreases by one in subsequent next zone, the last zone containing only the least soluble single solute.

To obtain a complete separation in adjacent zones of components, a restriction mode of operation may be applied. This is achieved by preliminary injection of an additional component, possessing the highest (but still limited) solubility in the eluent. Extraction of solutes arising on the restrictor rear boundary and further zone boundaries results in the separation of single solute zones, in order of decreasing solubility.

It was found, that the chromatographic process under overloading conditions with limited solubilities of substances is similar on different supports, owing to the occurrence of sample–eluent demixing. The application of a restrictor allows the separation of large samples to be improved and dilute solutions to be enriched up to saturation during separation on a sorbent.

Experiments were performed using PTFE microcolumns, packed with nonporous stainless-steel powder or with octadecyl silica. Two- and three-component mixtures of nitroalkanes were eluted with water and nitromethane was used as the restrictor. Separations of benzene, toluene and halobenzenes, eluted with water–

methanol, are other examples of the successful application of the restrictor mode of operation

INTRODUCTION

The wide application of chromatography to the separation and analysis of mixtures is due to the diversity of chromatographic modes using different types of separation systems and modes of operation. Zhukhovitskii and co-workers[1,2] proposed an original gas chromatographic method with extreme column overloading. The method, called chromadistillation, is based on the selective distribution of components between a quasi-stationary liquid phase of a separated mixture and a mobile phase consisting of its saturated vapour. A mixture is injected onto an inert column packing and moves through the column by means of a carrier gas. Application of a negative temperature gradient or injection of an additional component (called a "restrictor"), posessing maximum volatility leads to complete separation in adjacent zones of pure substances, in order of decreasing volatility. Chromadistillation is achieved by injection of much larger samples than is possible in traditional elution chromatography. Hence this method appears to be particularly suited to preparative-scale separations and to the enrichment of impurities.

When a column packed with sorbent rather than an inert support used under overloading conditions, the amount of liquid sample is sufficiently high for distillation to play a significant role in separation. Such a mode of operation may be regarded as intermediate between the usual gas chromatography and chromadistillation[3].

It seemed to be expedient to apply the same principle in liquid chromatography[4,5]. The idea is to use a mixture itself as a quasi-stationary liquid phase. It is clear that limited miscibility of two phases must exist when using a mobile liquid phase. To satisfy this demand, a large amount of a mixture consisting of slightly soluble compounds should be injected onto the column without preliminary dissolution in the eluent or in another solvent. This differs from the usual practice in liquid chromatography and places certain restrictions on the application of standard instruments.

It should be mentioned that the problem of limited solubility of substances in the eluent has not been sufficiently studied, and it is often regarded as only a negative phenomenon. However, two chromatographic techniques are known that use limited solubility in a positive sense. The first is precipitation chromatography, proposed by Baker and Williams[16] for the separation of polymer molecules. The method is based on equilibrium between a polymer gel in the stationary phase and a saturated solution in the mobile phase. Recurrence of precipitation is effected by combination of a solvent gradient (to higher solubility) and a temperature gradient along the column (to lower solubility). In modern high-speed versions of liquid chromatography[7], a temperature gradient is not applied and precipitation is achieved by the exclusion effect.

Schwarz[8] developed a chromatographic method for the measurement of solubility in liquid–liquid systems. Initially the column is filled with a liquid substance, then a solvent is fed into the column. When the excess of the first liquid has been replaced, the remainder is held on a support as a stationary film, which is dissolved by

the mobile phase. Measurement of the stationary liquid boundary velocity permits the determination of solubility.

We have demonstrated the possibility of effecting separations under such conditions and examined the features of the elution of one-, two- and three-component mixtures from two types of packing (a low-sorbing support and octadecylsilica). The method of separation on a low-sorbing packing, in which components of the sample are extracted by a liquid eluent under chromatographic conditions, is named chromaextraction.

EXPERIMENTAL

Experiments were performed on a JASCO Familic-100-N instrument (Japan) with a Uvidec-100-III variable-wavelength UV detector. The columns were PTFE tubes (15 cm × 0.5 mm I.D.) packed with non-porous particles of stainless-steel powder (40-μm fraction) or with Finepack Sil C_{18} (10-μm fraction) with the use of a high-pressure syringe.

A syringe pump allowed the mobile phase flow-rate to be varied from 1 to 30 μl/min. A tube-type injector was used in the stop-flow mode of operation, the sample size being from 0.2 to 1.9 μl. If it was necessary to inject larger volumes of substances immiscible with the eluent, repeated injections were made.

Some experiments were performed on a Bruker LC31-B instrument equipped with a UV detector, a loop-type injector and a stainless-steel column (5 × 0.46 cm I.D.) packed with LiChrosorb RP-18 (10-μm fraction). This type of column was less suitable for our experiments owing to an undesirable increase in the column inlet pressure when substances of low solubilities were injected.

When a column packed with stainless-steel powder was used, distilled water served as the eluent and nitroalkanes were the substances to be separated. Water–methanol was used as the eluent for the reversed-phase separation of benzene, toluene and halobenzenes.

RESULTS AND DISCUSSION

Columns packed with a low-sorbing non-porous support were used to ensure that the following conditions were satisfied:

(1) The surface of the support is wettable better by the sample phase than by the eluent. When a liquid sample characterized by limited miscibility with the eluent is injected onto the column, it spreads on the column packing and a quasi-stationary liquid film is created. The thickness of the stationary liquid film is constant, as has been demonstrated by others[6].

(2) Conditions for mass transfer between the mobile and stationary phases are satisfactory. When a flow passes through the column the both phases are equilibrated to saturated concentrations.

(3) The amount of sorbate per unit layer volume is negligible at any solute concentration because the surface is inert or the surface area is very small. Only at solute concentrations close to saturation does the sorption increase abruptly. This means that one of the two liquid phases of the demixing solution precipitates onto the solid surface.

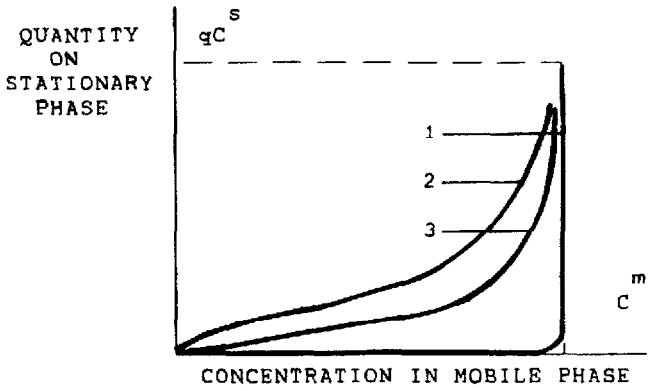

Fig. 1. Different types of single-solute adsorption isotherms from demixing solution of liquids. 1 = Extremely concave (non-sorbing wettable surface); 2 = σ-shaped; 3 = concave.

The sorption isotherm of a single component from a demixing solution on an inert wettable surface is an extremely concave curve (Fig. 1). It consists of two segments; the horizontal segment goes from zero to solubility point c_0^m and the vertical segment passes through this point and rises to qc_0^s. When a mobile phase flows through a sorbent layer, c_0^m and c_0^s correspond to the equilibrium concentrations in the mobile and stationary phases, respectively, q is the largest stationary portion of liquid and qc_0^s is the maximum amount of the component in the stationary phase.

When a dose Q of a liquid substance is injected into a packed column, which is then flushed with an eluent, it spreads over the surface of the particles and occupies a length of column that is proportional to Q (Fig. 2), the mobile and stationary phases being then equilibrated to saturation. The front boundary of the solution zone moves through the column at an eluent velocity α, if sorption from the mobile phase does not occur. The front boundary retention volume is equal to the void volume of the column. At the rear boundary of the solution zone permanent dissolution of the stationary liquid by the eluent takes place. The velocity U of the boundary can be determined from the mass balance equation:

$$\alpha c^m s^m = U(c^s s^s + c^m s^m) \tag{1}$$

where c^m and c^s are the concentrations of the mobile and stationary solutions, respectively (here and subsequently the symbol "0" is omitted) and s^m and s^s are the column sections occupied by mobile and stationary phases, respectively or

$$U = \alpha/[1 + qc^s/(1-q)c^m] \tag{2}$$

Eqn. 2 is also well known in the theory of non-linear ideal chromatography and describes the desorption boundary movement for the case of a concave isotherm. When dissolution is finished, the rear boundary velocity becomes equal to the front boundary velocity α. Hence the resulting volume V of the zone on a breakthrough curve is proportional to the sample size:

$$V = 100\, q/c^m \tag{3}$$

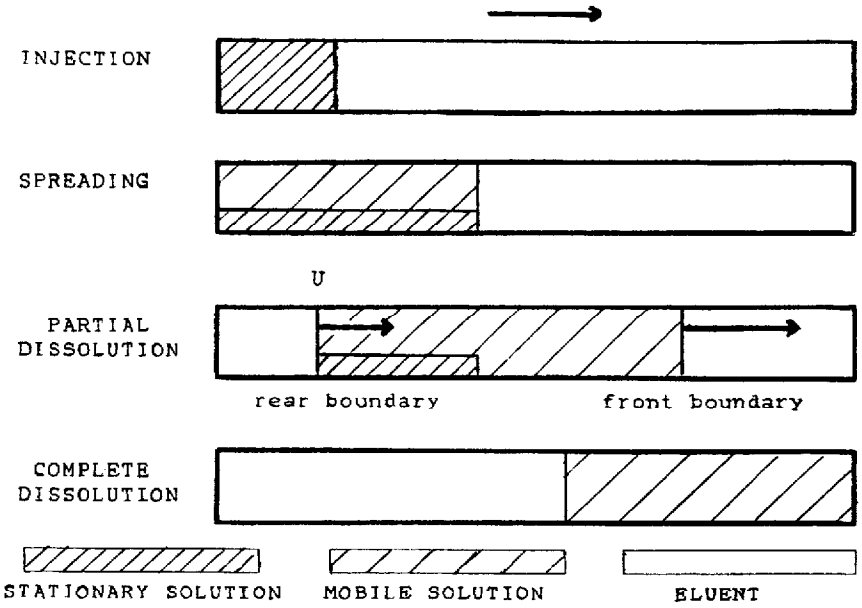

Fig. 2. Zone diagram of elution of one component from an inert packing.

where the solubility c^m is expressed in volume percent. As the concentration of the eluate is constant, the elution chromatogram has the form of a rectangular step, and the height of the step does not depend on sample size.

For experimental testing of this conclusion, some individual substances were eluted with water. In Fig. 3A the elution curves for different doses of nitroethane are depicted. Rectangular chromatograms are observed, the zone heights being identical and lengths increasing with increase in dose. Similar results were obtained when nitromethane and 2-nitropropane were eluted. If the sample size Q is known, the solute concentration generated at the column outlet can be calculated according to eqn. 3 from the length of the zone measured on the chromatogram at half of the zone height. By comparison with literature data it was found that the solute concentrations are approximately equal to the solubilities of eluted substances (9.5, 4.6 and 1.7 vol.-% for nitromethane, nitroethane and 2-nitropropane, respectively).

If the eluate concentration is known, it is possible to determine the maximum amount Q_{max} of quasi-stationary phase retained on the column packing. First the column is completely filled with a liquid substance, then another liquid (solvent) is fed into the column. When the excess of the first liquid has been removed, there is a liquid stationary film along the whole column length. Measurement of the solution zone volume on the chromatogram permits Q_{max} to be evaluated. When nitroalkanes were eluted with water this was about 25% and 15% of the void volume of the column when the flow-rate was *ca.* 0.2 and 1 mm/s, respectively.

When a column packed with octadecylsilica is used, the third condition for chromaextraction performance is not satisfied when the adsorption is high for all solute concentrations. Experiments carried out earlier[9] showed that the adsorption

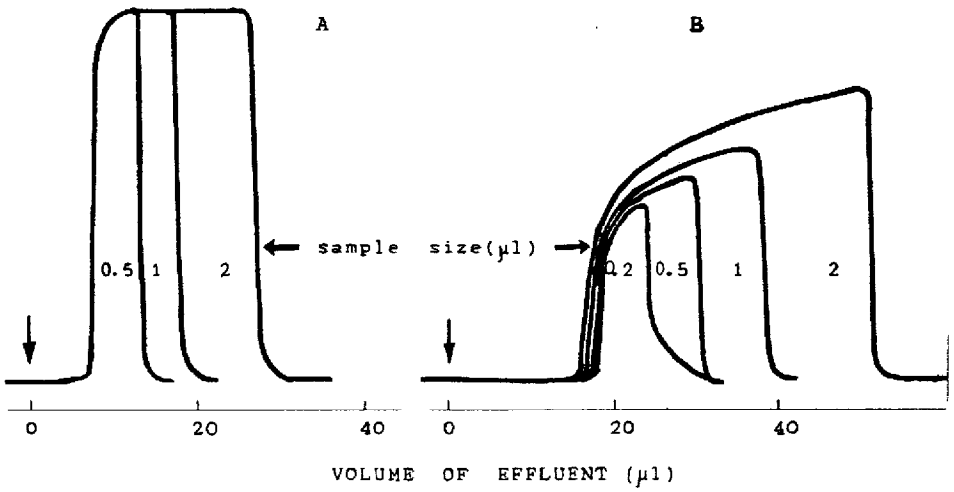

Fig. 3. Dependence of elution curve profiles on nitroethane sample size. Columns, 150 × 0.5 mm I.D.: (A) stainless-steel powder, 40 μm; (B) Finepack Sil C_{18}, 10 μm. Eluent, water at 2 μl/min; UV detection at 245 nm.

isotherms of nitroalkanes from aqueous solution on octadecylsilica are σ-shaped curves (Fig. 1, type 2). Adsorption of other substances dissolved in water–methanol was also examined, the solubilities of the sorbates being several percent. It was found that the isotherms of benzene, toluene and other aromatics are concave curves over almost the whole of the concentration interval (Fig. 1, type 3).

In accordance with the expected types of isotherms, the elution curves of nitroalkanes have a diffuse rear part when small doses are eluted (convex section of the isotherm). Further increases in the sample size result in sharpening of the peak "tail" and in dispersion of the peak front (Fig. 3B). In this way the peak shape approaches a step, with the step length and height approximating the values on chromatograms obtained using a low-sorbing packing. Hence in the case of limited solubilities in the elutent and at high loadings the solution zone concentration approaches saturation on a sorbing support.

The regularities of the elution of two-component and more complex mixtures are more complex than that of a single component. They are based on the distribution of the components in a demixing multi-component system. A binary mixture and the eluent comprise a three-component system, which may be described by equilibrium diagrams of different types. Here we do not list all possible types but only the two simplest types with limited mutual solubility of the components. In Fig. 4A neither component 1 nor 2 (mixture) is completely soluble in eluent 3. Two demixing liquid phases may exist at any ratio of the first two components. If component 1 only possesses a limited solubility, the area of demixing corresponds to a high content of this component (Fig. 4B).

When a mixture is injected into a column two phases are generated, the first being a solution of the mixture in the mobile phase and the second the mixture itself with the eluent dissolved in it (composition M_{II}, S_{II}, in Fig. 4). According to the phase diagram, the mobile phase should be enriched compared with the stationary phase by

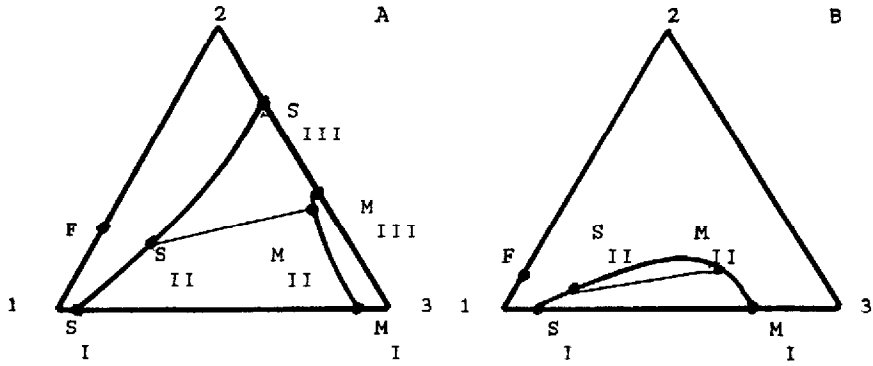

Fig. 4. Three-component solubility diagrams.

the more soluble component 2, hence an excess of component 1 remains in stationary phase. As a consequence, such an extraction process under chromatographic conditions leads to separation on zone II (mixture) and zone I (component 1) and a boundary between the zones appears (Fig. 5). The boundary velocity U_{II} and also U_I can be determined from the mass balance equation for component 1 by analogy with eqns. 1 and 2. Further, it is possible to demonstrate that $U_I < U_{II}$, but the proof is cumbersome. An elucidation of the problem with some additional assumptions may be found in the theory of chromadistillation[3].

The ordinary elution of a binary mixture by a slowly dissolving eluent from a non-sorbing packing results in separation into two zones. Three boundaries, possessing different velocities α, U_{II} and U_I are formed. The process continues until complete dissolution of the mixture occurs, then all the boundaries move with the same velocity α. At the column outlet zone II is registered first and zone I follows immediately after. In this version of partial separation the maximum sample size may be drifted over the whole column length without deterioration of separation.

Elution of binary mixtures of nitroalkanes by water was performed. The chromatograms in Fig. 6A consist of two extended zones. The height of the first depends on the composition of the initial mixture, and it may be approximated by the expression $h_1\chi_1 + h_2\chi_2$, were h_1 and h_2 are the heights of single-component zones and χ_1 and χ_2 are the molar fractions of substances in the initial mixture. The discrepancy between the measured and assumed values is not more than 10%. The heights of the

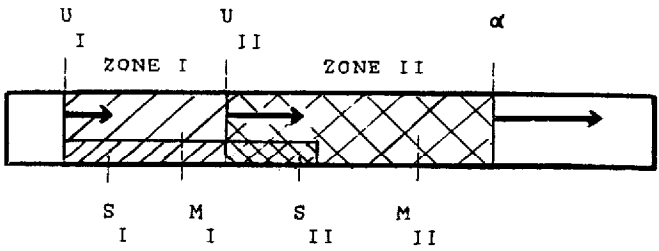

Fig. 5. Zone diagram of elution of a two-component mixture.

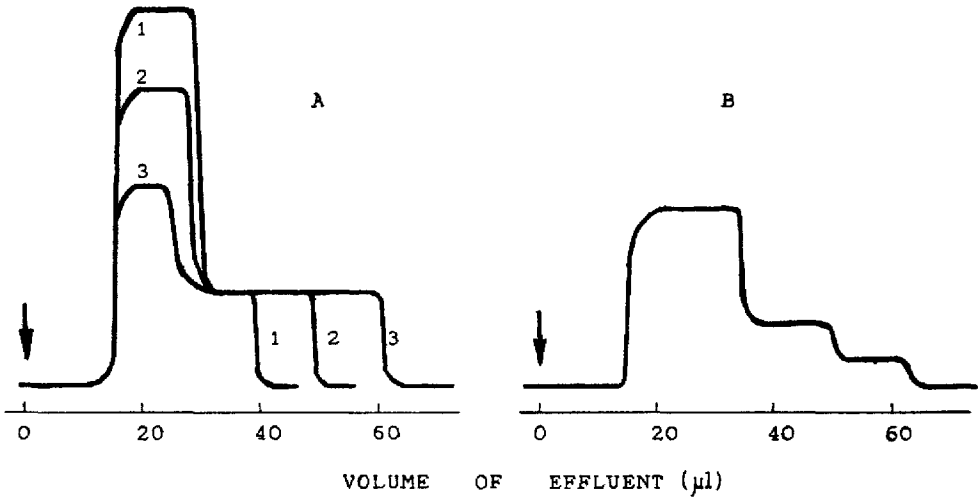

Fig. 6. Partial separation of (A) two-component and (B) three-component mixtures. Mixtures: (A) nitromethane–2-nitropropane, (1) 4:1, (2) 1:1, (3) 1:4; (B) nitroethane–2-nitropropane–2-nitrobutane, 12:4:1; sample size, 1 μl. Other conditions as in Fig. 3A.

second zone in Fig. 6A are identical and equal to the height h_1 of the less soluble component zone. In Fig. 6B a three-step chromatogram of a three-component mixture is shown. The preceding discussion confirms that the first zone on the chromatogram contains all components and the number of components decreases by one in each following zone, the last zone being a saturated solution of the least soluble compound. This type of partial separation seems to be the most similar to frontal-desorption separation.

In Fig. 7 other examples of partial separations of substances with different solubilities are given. In the first instance the binary mixture contains several percent of the less soluble component and the second is the reverse. As follows from Fig. 7A, the less soluble non-basic component forms a zone of saturated solution at the end of the chromatogram, even being at low concentration in the initial mixture. The last non-trivial result allows such a mode of operation to be recommended for the enrichment of impurites having a lower solubility than the basic component.

When an impurity component is completely soluble it forms a peak at the beginning of the chromatogram (Fig. 7B). The reason is that the concentration of this component in the mixed zone is sufficiently high for the extent of its boundary region. When a 1-butanol–acetone mixture is eluted with water, the peak height of the completely soluble acetone is proportional to its content in the initial mixture. 1-Butanol is not detectable, and therefore a negative step is registered immediately after the peak. When the concentration of acetone rises up to about 10% the separation deteriorates, and eventually complete miscibility of the sample and eluent occurs.

Some separations of compounds possessing limited solubility were executed on a sorbent packing under overloading conditions. The chromatograms in Fig. 7C show the separation of toluene and benzene (solid line) and the elution curve of toluene only (dashed line), the latter being given for comparison. A clear feature is

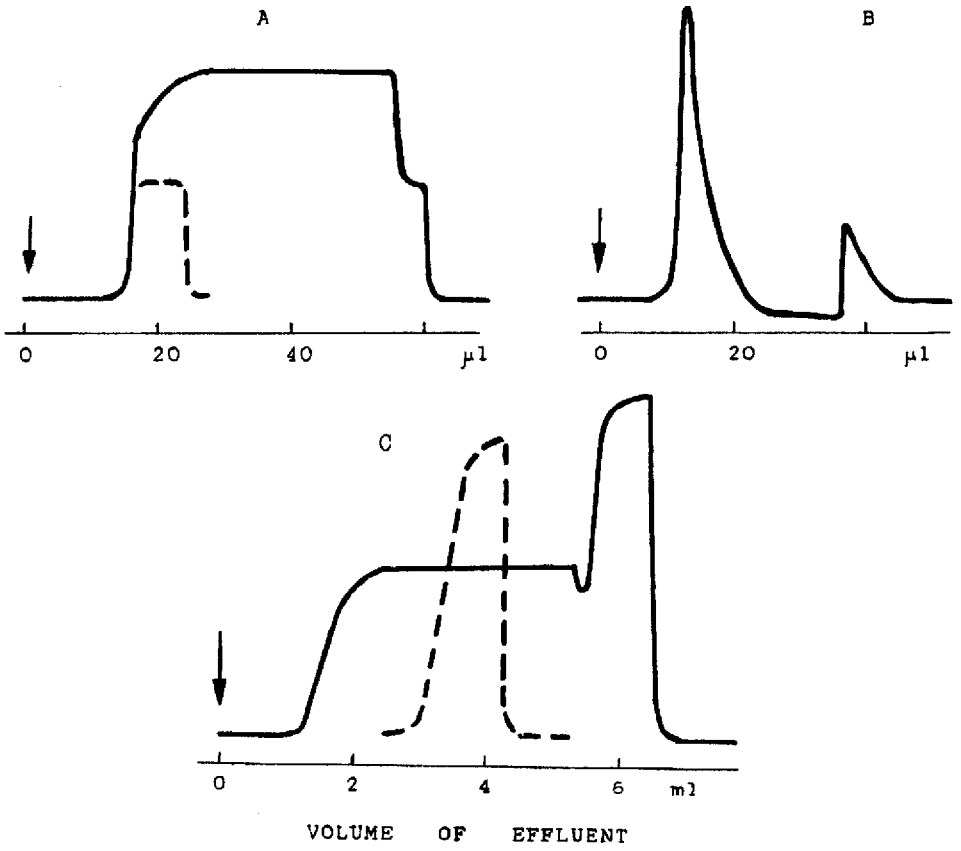

Fig. 7. Partial separation of two-component mixtures containing (A,C) a less soluble and (B) a more soluble non-basic component. Columns: (A,B) 150 × 0.5 mm I.D., stainless-steel powder, 40 µm; (C) 50 × 4.6 mm I.D., LiChrosorb RP-18, 10 µm. Eluent, (A,B) water at 2-µl/min; (C) water–methanol (30:70) at 0.5 ml/min; UV detection at (A) 245, (B) 220 nm and (C) 260 nm. Mixtures: (A) nitroethane–2-nitropropane (95:5), 1 µl; (B) acetone–1-butanol (2:98), 1 µl; (C) benzene–toluene (95:5), 100 µl.

that the less soluble non-basic component is "driven back" when the more soluble basic component is present.

This result may be explained by means of the previous discussion. We can compare separations obtained on a non-sorbing support and on a sorbent. (Fig. 7a and c) when the solubilities of the components and their ratio in the initial mixture are not very different. On both chromatograms the first elongated zone contains the most soluble basic component almost at saturation, the concentration of the other solute being low. Only behind the mixed zone does that of the less soluble component appear. The solute concentration in the last zone is close to saturation irrespective of the initial composition of the mixture. Hence it may be affirmed that if the amount of liquid sample is very large, the distribution of the components is determined to a considerable extent by liquid–liquid equilibrium and not by adsorption on the surface of the solid support.

Another interpretation of this phenomenon is that the changes in the band retention and concentration of substances are influenced by multi-component adsorption at high concentrations. The band concentration and retention increase is known as the restriction effect[3]. It is the reverse of the displacement effect[10], which leads to a decrease in band retention. Both are concerned with non-linear chromatography where the sorption isotherms are concave (restriction) or convex (displacement). Just as the displacement effect allows the displacement mode of operation to be performed, so the restricton effect may be used for execution of the restricton mode, as was done earlier in chromadistillation. In our method the least sorbed component, called the "restrictor", must possess the highest (but still limited) solubility and be injected before a mixture.

The restricton mode of operaton is applied first for the separation of a two-component mixture on a low-sorbing packing, the most soluble compound 2 being used as a restrictor. Initially a large amount of the restrictor is placed on the column as a stationary phase (Fig. 8). The eluent dissolves the stationary film and the restrictor rear boundary moves. Then a mixture of 1 + 2 is placed on the vacant part at the inlet of the column. A boundary is created between the restrictor and the mixture, and extraction of components at this boundary occurs. The velocity U_{III} of the boundary can be found from the mass balance equation of component 1:

$$U_{III} = \alpha/[1 + c^{s}_{II,1} q_{II}/(1 - q_{II}) c^{m}_{II,1}] \tag{4}$$

where q_{II} is a portion of stationary liquid in zone II (Fig. 8) and $c^{s}_{II,1}$ and $c^{m}_{II,1}$ are the concentrations of component 1 in the same zone in stationary and mobile phase, respectively.

At the inlet part of the column a boundary between the mixture and the less soluble component 1 appears just as in ordinary elution and its velocity is

$$U_{II} = \alpha/[1 + c^{s}_{II,2} q_{II}/(1 - q_{II}) c^{m}_{II,2}] \tag{5}$$

where $c^{m}_{II,2}$ and $c^{s}_{II,2}$ are the concentrations of component 2 in zone II in the mobile and stationary phase, respectively.

If the distribution factor of the less soluble component is the larger, *i.e.*, $c^{s}_{II,1}/c^{m}_{II,1} > c^{s}_{II,2}/c^{m}_{II,2}$, from eqns. 4 and 5 it follows that $U_{II} > U_{III}$ and after a period of time the boundaries coincide, so the mixed zone disappears. Hence the possibility of separation in such a mode of operation depends on several factors: column length,

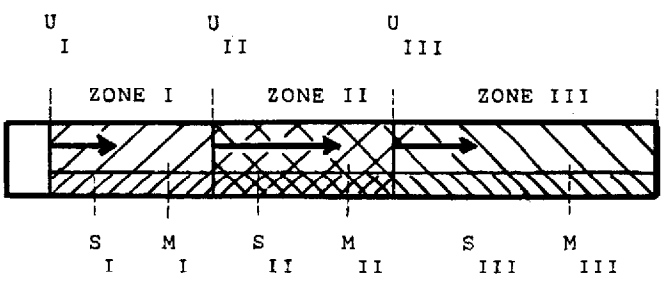

Fig. 8. Zone diagram of restriction mode of a two-component separation.

distribution factors of the components (boundary velocities), amounts of mixture and restrictor and time interval between injections of mixture and restrictor.

Earlier we assumed that the amount of mixture (sample) is suitable for occupying the part of the column vacated by the restrictor. If this is not so and the amount of mixture is greater, it is pushed forwards and a mixed zone of variable composition forms. If the amount of mixture is smaller, it is dissolved by the eluent and carried to the restrictor rear boundary. Further extraction generates a new mixture zone. Therefore, the last two situations may be converted into the former one.

In Fig. 9A the separation of binary mixtures of the same composition as in Fig. 6A is shown, with the difference that the more soluble component is used as a restrictor. All the chromatograms consist of two zones with identical heights (but different lengths), equal to the heights on the chromatograms of the individual substances, *i.e.*, the compositions of the zones c_i^m correspond to single-solute saturated solution. The absolute amount of each component i, calculated as $q_i = V_i c_i^m/100$, where V_i is the volume of the zone in a mixed chromatogram, does not differ from the known initial value by more than 10%. The restrictor need not be a component of the mixture to be separated; the only essential requirement is limited solubility of this component in eluent, but higher than that of the other components. In Fig. 9B the chromatogram of ternary mixture of nitroalkanes is shown, the restrictor not being a component of the initial mixture. The chromatogram contains four steps, each representing a zone of saturated solution of a single compound, in order of decreasing solubility. The step heights on chromatogram are equal to those of the individual substances, so the completeness of separation is confirmed.

It is possible to carry out the restriction mode of operation on a sorbent packing. This is especially useful for large sample separations. In this instance extraction of the solutes on the restrictor rear boundary is an additional factor for improving the separation process. Two- and three-component separations under overloading condi-

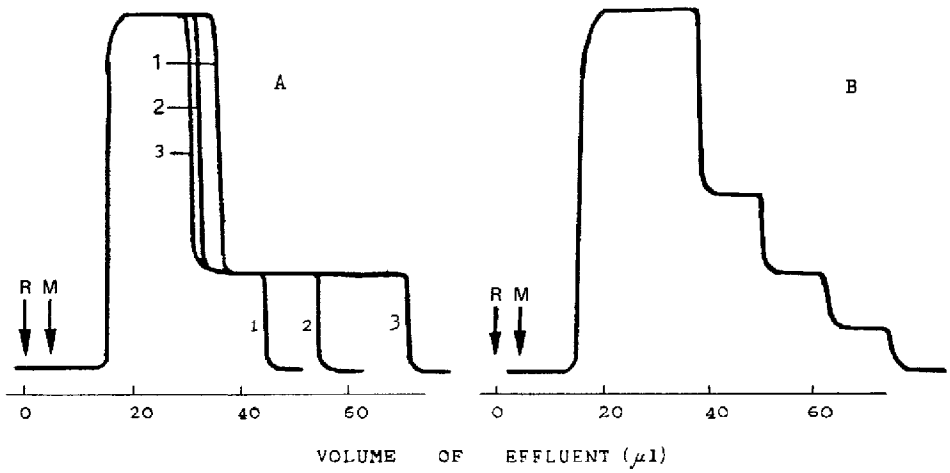

Fig. 9. Restriction mode of separation of (A) two-components and (B) three-components mixtures. Restrictor: nitromethane, (A) 2 μl, (B) 3 μl. Mixture and restrictor injections are indicated by M and R, respectively. Other conditions as in Fig. 6.

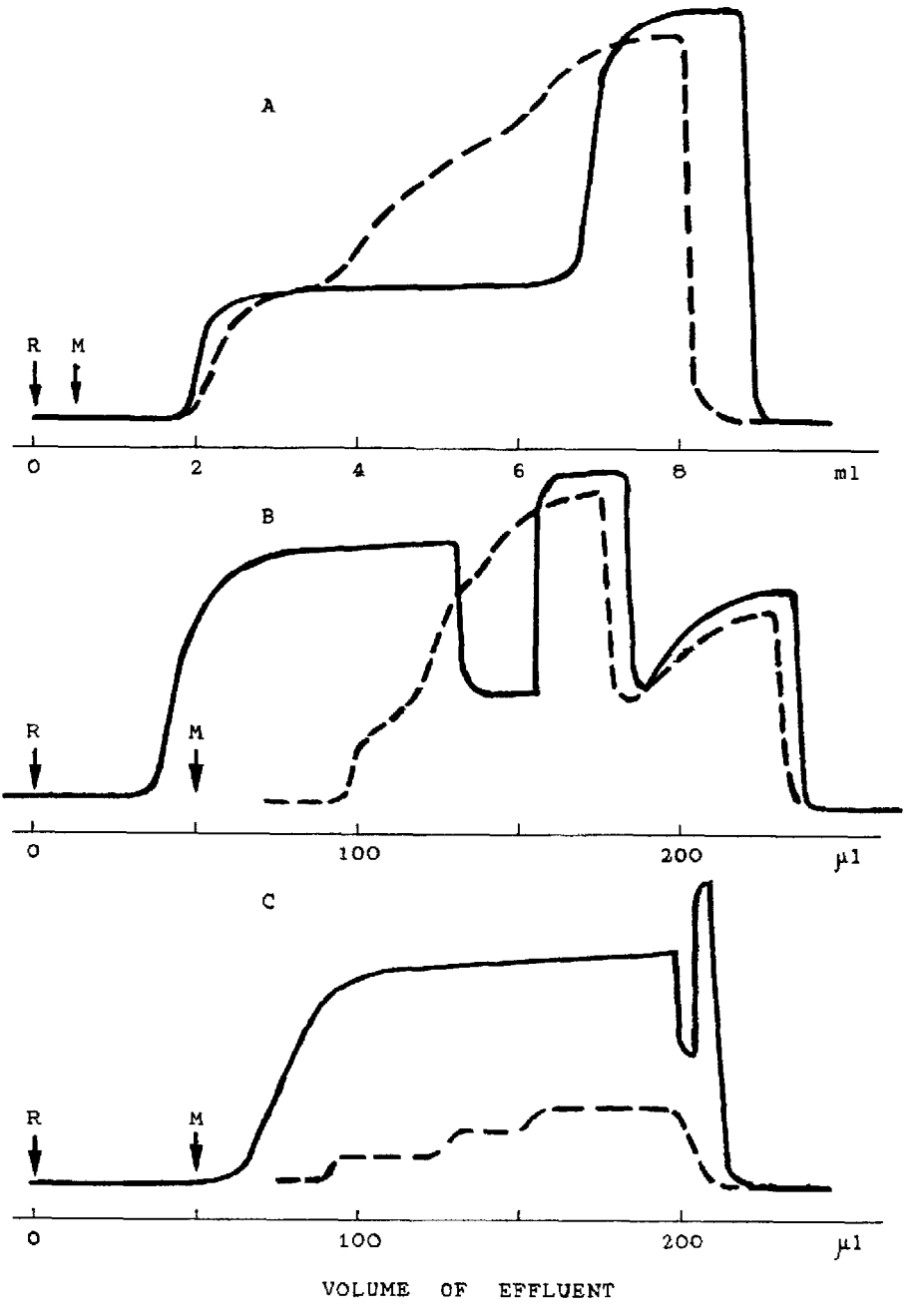

Fig. 10. Restriction (solid lines) and elution (dashed lines) modes of separation on sorbent packing. Columns: (A) 50 × 4.6 I.D., LiChrosorb RP-18, 10 µm; (B,C) 150 × 0.5 mm I.D., Finepack Sil C_{18}, 10 µm. Eluent: water–methanol, (A,B) 3:7, (C) 4:6; UV detection at 260 nm. Restrictor: benzene, (A) 50 µl, (B,C) 4 µl. Mixtures: (A) benzene–toluene, (7:3), 100 µl; (B) chlorobenzene–iodobenzene–*m*-dibromobenzene (10:5:3), 0.7 µl; (C) chlorobenzene–iodobenzene (2:1), saturated solutions in eluent diluted 5-fold, 80 µl.

tions are depicted in Fig. 10. The dashed lines correspond to the ordinary elution mode of separation, which is not satisfactory. When the restrictor is applied (solid lines) the separation is improved, the profile of the curves approximating a rectangular shape. As at non-sorbing support, the restrictor may be either a component of the mixture (Fig. 10A) or not (Fig. 10B).

It is also possible to increase the concentration of a dilute solution up to saturation by means of a restrictor by analogy with displacement enrichment. An example of the simultaneous separation and increase in band concentration of a feed solution diluted in 5-fold compared with the saturated composition is shown in Fig. 10C.

However, there are some differences in the features of the restriction effect on a low-sorbing layer and on a traditional sorbent, especially for strongly sorbed, poorly soluble substances. The latter interact much more weakly with all other components, including the restrictor, than with the solid support. Hence the last zone in Fig. 10B does not change on either chromatogram (the first being obtained with the restrictor and the second without it) whereas the others are altered.

CONCLUSIONS

To perform the chromaextraction method, it is necessary to use a liquid eluent that forms a demixing system with a liquid sample and a non-sorbing column packing that is more wettable by the sample phase than by the eluent phase.

Ordinary isocratic elution of a mixture of substances, characterized by different solubilities in the eluent, allows a partial frontal-type separation to be obtained, so the least soluble component may be obtained at a saturated concentration in the eluent irrespective of the composition of the initial mixture.

To carry out a complete separation an additional component, a restrictor, must be placed on the column previously. As a result, adjacent zones of saturated solutions of individual substances elute in order of decreasing component solubilites. Application of a restrictor also improves separations on sorbent packings when high loadings and limited solubility of the substances occur.

REFERENCES

1 A. A. Zhukhovitskii, S. M. Yanovskii, V. P. Shwarzman and I. A. Revelskii, *Zh. Fiz. Khim.*, 49 (1975) 2954.
2 A. A. Zhukhovitskii, S. M. Yanovskii and V. P. Shwarzman, *J. Chromatogr.*, 119 (1976) 59.
3 S. M. Yanovskii, *Usp. Khim.*, 55 (1986) 1163.
4 E. B. Guglya, A. A. Zhukhovitskii, S. M. Revelskii and S. M. Yanovskii, *Zh. Fiz. Khim.*, 58 (1984) 669.
5 E. B. Guglya and S. M. Yanovskii, *Zh. Fiz. Khim.*, 61 (1987) 108.
6 C. A. Baker and R. J. P. Williams, *J. Chem. Soc.*, (1956), 2352.
7 D. W. Armstrong and R. E. Boehm, *J. Chromatogr. Sci.*, 22 (1984) 378.
8 F. P. Schwarz, *Anal Chem.*, 52 (1980) 10.
9 E. B. Guglya and S. M. Yanovskii, *Zh. Fiz. Khim.*, 60 (1986) 3069.
10 Cs. Horváth, A. Nahum and J. H. Frenz, *J. Chromatogr.*, 181 (1981) 365.