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DEVELOPMENT OF A COMBINED PARTITION-CHROMATOGRAPHIC METHOD

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SUMMARY

The development of a partition-chromatographic method is discussed and new variants of the method that widen its range of application are proposed.

INTRODUCTION

The identification of the components of complex organic mixtures is one of the most important problems in analytical chemistry, particularly in gas chromatography, which is the most commonly used analytical method. The application of chromatographic methods alone gives valuable information, which, however, is sometimes insufficient for the reliable identification of chromatographic zones¹⁻³ owing to the limited accuracy of measurements of the retention data (especially those performed in different laboratories) and to the fact that these data are often very close for various compounds.

The problem of identification is considerably simplified when selective and super-selective stationary phases and adsorbents are used. However, the choice of selective phases (especially at elevated temperatures) is limited and the use of several columns with different sorbents is laborious.

Recently, considerable advances have been made as a result of the combination of gas chromatography with other physical and chemical methods^{4,5}. The following requirements are usually imposed upon a combined method: (1) high selectivity and informativeness; (2) simplicity of the experimental procedure; (3) high speed; (4) the possibility of carrying out trace analysis.

Therefore, the improvement of known combined methods and the development of new variants of them are very important. The combination of partition with chromatography, based on the equilibrium distribution of a substance between the heterogeneous phases and the chromatographic separation of the components of the mixture, satisfies all of the above requirements. As a rule, chromatography is also used for the determination of the distribution of the components between phases. As the data on chromatographic separation and on interphase distribution are important qualitative features of a substance, when considered together they considerably

simplify the qualitative identification of a substance and are of interest in quantitative analysis and some physico-chemical measurements.

The first work in which the partition-chromatographic method was used concerned methods of functional group analysis. In 1962, Suffis and Dean⁶ proposed to use the propylene glycol extraction of the components of a mixture dissolved in carbon tetrachloride for the selective removal of alcohols without chemical reaction. The characteristics of the distribution of a component between two phases was determined by analyzing samples of the carbon tetrachloride solution before and after extraction. The fraction of the substance in each phase was used to represent the distribution characteristics. Any component whose fraction in propylene glycol exceeded 50% was identified as an alcohol. As a preliminary step, acids and amines were removed from the mixture being analyzed by extraction with a dilute acid or alkali. Suffis and Dean⁶ reported that similar results can be obtained in different solvent systems by using, for example, cyclohexane, diethyl ether or carbon disulphide as a non-polar solvent and ethylene glycol, nitromethane or acetonitrile as a polar solvent. This method had advantages over the removal of alcohols by means of chemical reactions (using benzoyl chloride or 2,4-dinitrobenzoyl chloride) as no reagents are introduced into the mixture and the alcohols are not converted into other chemical compounds. This also makes it possible to separate and identify alcohols by other methods, for example spectral methods. Although the distribution characteristics were determined by Suffis and Dean⁶, they were not used for identification purposes, the use of the second phase (propylene glycol) serving only for the selective removal of alcohols.

Later, Lulova *et al.*⁷ developed a similar method for the identification of aromatic compounds in gasoline fractions by extracting them with selective immiscible solvents. The treatment of gasoline fractions with polar solvents reduced the content of aromatic compounds considerably.

Aromatic compounds in mixtures with aliphatic compounds in aqueous solutions were determined by an analogous method that involved a liquid-vapour system⁸. By repeated equilibrium between liquid and vapour phases with the removal of the latter and its replacement with the pure gas, only aromatic compounds, having relatively high distribution coefficients, remained in the solution.

The next step in developing the partition-chromatographic method was made by Beroza and Bowman⁹ in 1965. They used the distribution constant between two phases in combination with the chromatographic retention volume for the identification of unknown components. In order to identify trace amounts of pesticides, they used as the distribution characteristic the so-called extraction p value, which was determined by a simple procedure. A 5-ml volume of a polar phase was added to an equal amount of solution contained in a 10-ml calibrated centrifuge tube fitted with a ground-glass stopper. The tube was shaken for 1 min and then the upper (non-polar) phase was analyzed in the same way as previously. The p value was determined as the ratio of the amount of the analyzed substance in the non-polar phase after extraction (according to the data of the second analysis) to its initial amount (according to the data of the first analysis).

Later, Beroza and Bowman have published a number of papers¹⁰⁻¹⁴ describing improvements to their partition-chromatographic method. They were the first to propose the use of the characteristics of the chromatographic retention in combination

with those of the distribution of components between two immiscible liquid phases for quantitative analysis in gas chromatography.

It should be noted, however, that the principle of using the distribution value between two phases for component identification had been proposed earlier. Thus, Shriner and Fuson¹⁵ gave a procedure for the identification of organic acids by means of their distribution between two phases and the determination of the amount of acid in each of the phases (after their separation) by titration. The distribution coefficient for each of the acids in the given system of phases is constant and can be used for component identification. The identification of separated pure components can, of course, be performed by any physico-chemical method with the determination of the concentration of a substance in two phases, but chromatography has the greatest possibilities.

However, although the method has the advantages of simplicity, ease of performance in any laboratory, etc.; it has not yet found wide application. This may be due to various causes, such as (1) the inadequate accuracy of p value determinations and (2) the unsuitability of this value for large and small distribution coefficients, which limits the number of systems that are suitable for identification ($p = 0.1-0.9$).

We have developed several modifications to the partition-chromatographic method that widen the possibilities for its application, improve its precision and, consequently, improve the reliability of identifications. Thus, it was proposed to use for the identification purposes not a p value but a relative distribution coefficient determined as the ratio of the concentration of the analyzed component to that of the known component serving as a standard in two phases. It was also proposed to use, in addition to the already known liquid-liquid system, liquid-vapour, liquid-solid and ternary immiscible solvent systems¹⁶⁻¹⁸.

Taking account of the volumes of the co-existing phases and the sample size taken for chromatographic analysis, the p value can be expressed by the equation

$$p = \frac{A_n V_p W_s}{A_s V_s W_n - A_n W_s (V_n - V_p)} \quad (1)$$

where V_n , V_p and V_s are the volumes of the non-polar and polar phases and of the initial solution, respectively, before extraction; A_s and A_p are the areas or heights of the peaks of the analyzed component on the chromatogram of the initial solution and of the solution after extraction (distribution); and W_s and W_n are the volumes of samples of the initial solution and solution after extraction, respectively. When the volumes of co-existing phases and those of sample are equal, $p = A_n/A_s$.

The relative distribution coefficient, K_{rel} , can be calculated by the equation

$$K_{rel} = [(A_n^i V_n / W_n) / (A_n^{st} V_n / W_n)] / [(A_p^i V_p / W_p) / (A_p^{st} V_p / W_p)] \quad (2)$$

where i and st indicate the analyzed and standard components, respectively. As A^i and A^{st} are usually determined during one experimental run, eqn. 2 can be simplified to

$$K_{rel} = (A_n^i / A_n^{st}) / (A_p^i / A_p^{st}) \quad (3)$$

Thus, the relative distribution coefficient is a more stable value than the p value. It does not depend on the volumes of either co-existing phases or the sample

volume taken for analysis, or on other factors. This greatly improves the precision of measurements, simplifies the experimental procedure and broadens the analytical possibilities of the method.

In order to determine the optimum conditions for analyses performed by the partition-chromatographic method, we considered the dependence of the relative distribution coefficient on the experimental conditions.

RESULTS AND DISCUSSION

Liquid-liquid system

It has been established that equilibrium in this system is reached 2 min after the mixing and subsequent phase separation, which is a sufficiently short period.

The effective application of distribution coefficients for the qualitative description of a substance and also for quantitative analysis is possible because this coefficient remains constant under different experimental conditions and is independent of the concentrations of this and other analyzed substances. This is true only for dilute solutions when the activity coefficient is unity or is constant for both phases. It should be noted that the relative distribution coefficients are less sensitive than absolute distribution coefficients to variations in concentrations (if the standard has been chosen correctly). A change in K_{ubn} and K_{rel} with increasing concentration of substance is shown in Table I.

TABLE I

EFFECT OF CONCENTRATION ON THE DISTRIBUTION COEFFICIENT OF *n*-PROPANE (BENZENE AS STANDARD) IN THE SYSTEM ISOCTANE-N-METHYLPYRROLIDONE AT 20°

Distribution coefficient	Concentration (% v/v)									
	0.02	0.04	0.1	0.50	1.0	1.4	2.2	3.0	3.4	3.8
K_{rel}	0.29	0.29	0.29	0.29	0.28	0.28	0.28	0.27	0.27	0.27
K_{ubn}	0.097	0.100	0.105	0.104	0.104	0.105	0.112	0.113	0.114	0.109

Although the relative distribution coefficient changes only slightly with concentration, it is advisable to carry out the measurements under conditions such that the total concentration of the analyzed mixture does not exceed 0.5–2% (v/v), as at higher concentrations the "third" component begins to affect the values of K_{rel} .

A change in K_{ubn} varies greatly with temperature. However, when relative values are used, K_{rel} remains constant over a wide temperature range. This reduces the requirements for the precision of thermostatic control and makes it possible to operate at room temperature (Table II).

As was shown above, the values of K_{rel} are independent of the volumes of co-existing phases used for distribution and, consequently, these values determined for unsaturated (mutually non-equilibrated) solvents must coincide with the values for the system of saturated (mutually equilibrated) solvents. This was confirmed experimentally.

TABLE II

EFFECT OF TEMPERATURE ON RELATIVE DISTRIBUTION COEFFICIENTS IN THE SYSTEM ISOCTANE-N-METHYLPYRROLIDONE

Temperature (°C)	Analyzed mixture I				Analyzed mixture II					
	Ethanol	<i>n</i> -Propanol	<i>n</i> -Butanol	<i>n</i> -Pentanol	Benzene (standard) K_{abs}	<i>n</i> -Pentane	<i>n</i> -Hexane	<i>n</i> -Octane	<i>n</i> -Nonane	<i>n</i> -Decane (standard) K_{abs}
20	0.21	0.28	0.33	0.36	0.36	0.41	0.49	0.74	0.82	10.45
30	0.21	0.28	0.33	0.36	0.38	0.41	0.50	0.72	0.83	9.19
40	0.20	0.26	0.32	0.35	0.40	0.42	0.51	0.72	0.81	8.6
50	0.19	0.23	0.31	0.33	0.43	0.40	0.49	0.72	0.79	5.0

Distribution in the liquid-vapour system

For the qualitative analysis of relatively volatile compounds with boiling points less than 150–180°, it has been found extremely convenient to use distribution in liquid-vapour systems, in which there is no need to seek a second phase that is immiscible with the first. The experimental and analytical procedures are simplified considerably.

In order to improve the accuracy and reproducibility of the determination of distribution characteristics in liquid-vapour systems, as well as in liquid-liquid systems, it is advisable to find relative values of the distribution coefficient.

In contrast to liquid-liquid systems, the distribution coefficient in liquid-vapour systems depends on the experimental conditions to a great extent. The time needed for equilibrium to be established is much greater than that in liquid-liquid systems (1.0–1.5 h), but in half an hour the relative distribution coefficient is almost equal to the equilibrium value.

The distribution constant is constant only for dilute solvents, *i.e.*, in the region where Henry's law is valid. However, this region is limited, but the use of relative distribution coefficients makes it possible to widen this region (Table III).

TABLE III

EFFECT OF CONCENTRATION ON THE DISTRIBUTION COEFFICIENT OF ETHANOL (ACETONE AS STANDARD) IN THE SYSTEM WATER-STEAM AT 50°

Distribution coefficient	Concentration (% v/v)						
	0.012	0.132	0.331	2.13	2.73	3.33	3.93
K_{rel}	5.9	5.91	6.03	6.07	6.11	6.09	6.10
K_{abs}	1093	1099	1098	1100	1131	1279	1415

It follows from Table III that the linear region (Henry's law) is observed at concentrations less than 0.3%. These results agree with published data.

It is known that the logarithm of the distribution coefficient depends linearly on the reciprocal of the temperature. As the slopes of the lines that characterize the heat of dissolution of a substance are different for different substances, changes in

the distribution coefficient with temperature cannot be neglected even when relative distribution coefficients are used, and the temperature must be maintained constant within $\pm 0.5^\circ$.

One of the main advantages of the partition-chromatographic method is the possibility of identifying organic compounds with the use of only one highly effective chromatographic column. The column has to be chosen so as to provide maximum information on the qualitative composition of a mixture, *i.e.*, the number of separated peaks should be a maximum. Evidently, long capillary and packed capillary columns possess such properties.

The solvents used in the partition-chromatographic method should meet the following requirements: selectivity, high purity, stability, passivity towards substances being analyzed, availability, and ease of standardization. In liquid-liquid systems, upon mixing they must form a binary system that is readily separated.

Retention of the solvents on a chromatographic column must prevent overlapping with the elution zones of the analyzed components, and the solvents must not contain admixtures with retention times that are close to those of the analyzed components. It is advisable to choose solvents that are eluted much earlier or later (the latter is preferable) than the analyzed substances. It should be borne in mind that because of the mutual solubility of the phases (the liquid-liquid system), the analysis of any of them may reveal the chromatographic zone of the other phase.

Selective detectors simplify the choice of a solvent that is not recorded by the detector. For example, an electron capture detector is insensitive towards hydrocarbons; a flame ionization detector gives only a slight response when solvents such as water, formamide, carbon disulphide or fluorinated hydrocarbons are used. The choice of the solvents for a liquid-vapour system is simpler because liquid immiscibility is not required.

For quantitative analysis, it is convenient (and sometimes necessary) to choose the analyzed substance itself as one of the phases.

A proper choice of a standard substance is of great importance. A substance introduced into the mixture or present in the initial mixture and characterized by a single peak on the chromatogram may serve as a standard. The concentration of the standard substance must be of the same order as that of the analyzed components.

Experiments on the determination of the distribution coefficient in a liquid-liquid system are carried out in a 10-ml test-tube fitted with a ground-glass stopper. A 5-ml volume of each of the solvents forming the binary system of immiscible liquids is placed in the tube (Fig. 1). There is no need to saturate the solvents with each other in a preliminary step when determining K_{rel} . Next, the analyzed mixture is introduced into the tube (not more than 200 μ l) and the mixture is shaken for 2 min. After mixing, the contents of the tube are subjected to phase separation. Thermostatic control is necessary only for the determination of absolute values of the distribution coefficient.

In order to determine the distribution coefficient, an aliquot of the solution (0.4–2 μ l) is taken with a microsyringe and analyzed chromatographically.

Similar experiments in a liquid-vapour system are carried out in a 50–100-ml glass bottle with a self-closing rubber stopper and a plastic screw-stopper that has a hole for the syringe needle (Fig. 2). A 20–50-ml volume of a solvent containing not more than 500–1000 μ l (according to the volume of the bottle) of the analyzed mixture or of the substance is poured into the bottle, which is then placed in a thermostatically

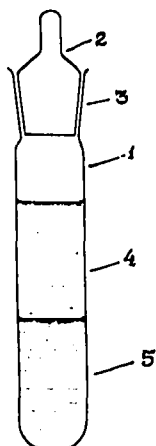


Fig. 1. Tube for the determination of distribution coefficients in liquid-liquid systems. 1 = Test-tube; 2 = stopper; 3 = ground end; 4 = upper phase; 5 = lower phase.

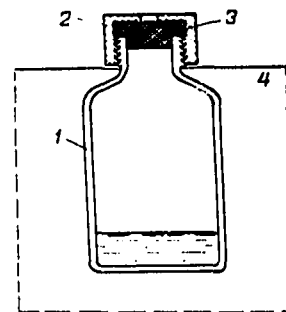


Fig. 2. Vessel for the determination of the distribution coefficients of volatile compounds in liquid-vapour systems. 1 = Glass bottle; 2 = plastic stopper; 3 = seal; 4 = thermostat.

controlled bath for 1.5 h. The phase analysis is performed after equilibrium has been established. The vapour phase (0.1–2 ml) is sampled by means of a syringe pre-heated to the bath temperature, and the liquid phase (0.2–1.0 μ l) is sampled with a micro-syringe.

For some phase systems and components and also for certain types of detectors (for instance, an electron capture detector), during an experimental run it is advisable to check whether the distribution coefficient is constant and the detector response is linear. These requirements may be satisfied when the experiments are conducted with different volume ratios.

The reproducibility of measurements of the relative distribution coefficient is satisfactory (the coefficient of variation is not more than 3.5%), and will improve with increasing standardization of the experimental conditions.

The results obtained by the partition-chromatographic method can be presented as the relationship between the distribution value and the number of carbon atoms in a homologous series of compounds. Such relationships for some classes of compounds in a number of solvent systems (liquid-liquid and liquid-vapour) are shown in Fig. 3. It can be seen that the partition-chromatographic method opens wide opportunities for structural determinations.

The reliability of identification increases (as in chromatography) with the number of systems in which the distribution values are determined. It is more convenient to present the experimental results in logarithmic form, and Fig. 4 shows the logarithmic dependences of the relative distribution coefficients in one solvent system on those in another system. It can be seen that the proper choice of the solvents makes it possible to identify both the group and the individual composition of the analyzed mixture.

The partition-chromatographic method allows one to use not only the data on distribution but also purely chromatographic values (retention volumes or reten-

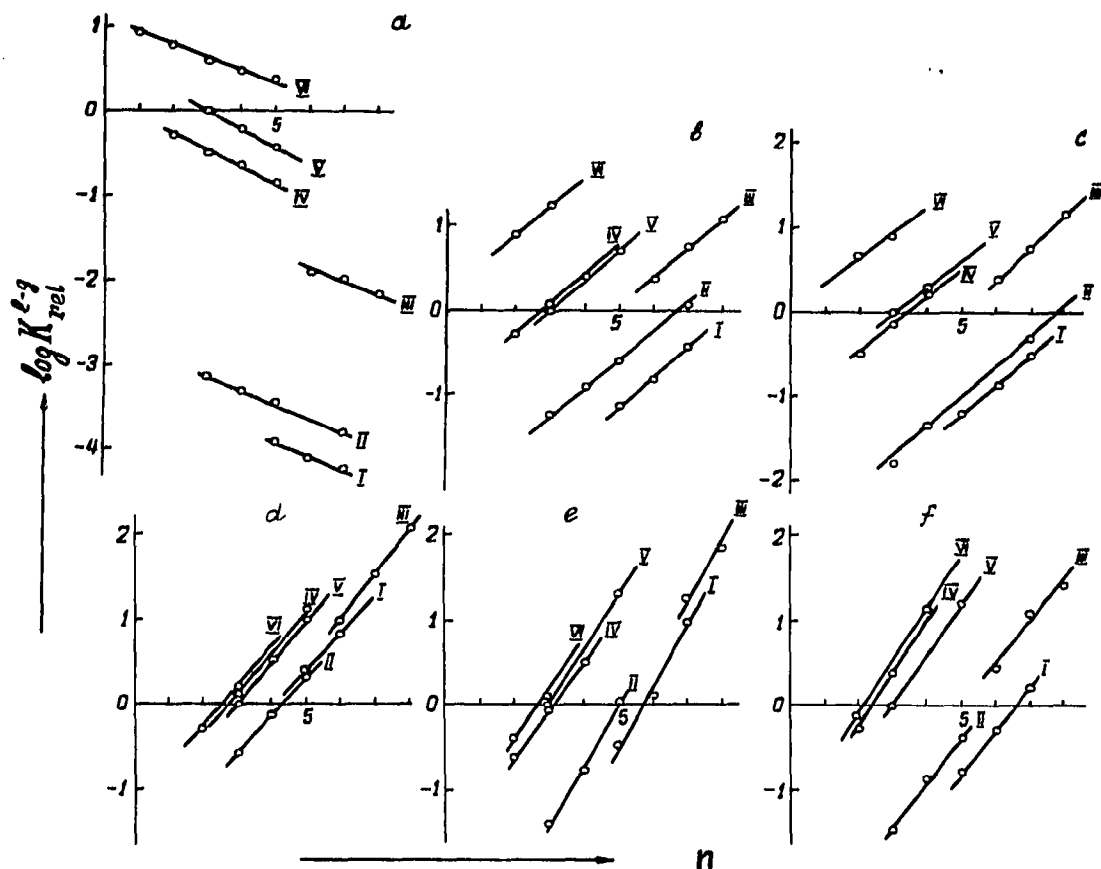


Fig. 3. $\log K_{rel}^{l-g}$ as a function of the number of carbon atoms, n , for some homologous series in liquid-vapour systems at 50°. Solvents: a = water; b = dimethylformamide; c = N-methylpyrrolidone; d = isooctane; e = benzene; f = ethyl acetate. Homologous series: I = *n*-alkanes; II = α -alkenes; III = aromatic hydrocarbons; IV = *n*-aldehydes; V = methyl ketones; VI = *n*-alcohols.

tion times). Therefore, combined dependences (distribution-chromatography) can also be used, such as the dependence of the logarithm of the relative distribution coefficient in both liquid-vapour and liquid-liquid systems on the logarithm of the relative retention volume for the stationary liquid phase in the column. This is very convenient for the identification of components in practice (Fig. 5).

The results obtained for several solvent systems (more than two) can be presented graphically as proposed by Berezkin and Walraven¹⁹. The differences in the logarithms of K_{rel} are plotted in Cartesian coordinates. As can be seen from Fig. 6, the classes of compounds fall into certain characteristic regions, thus making group identification of the components possible. Sometimes these regions overlap but this effect can be eliminated by the use of a more selective system. The system of three immiscible liquids is of interest (see Fig. 7).

For a number of problems, it is expedient to choose systems that contain a solid component, especially such selective adsorbents as molecular sieves, graphitized

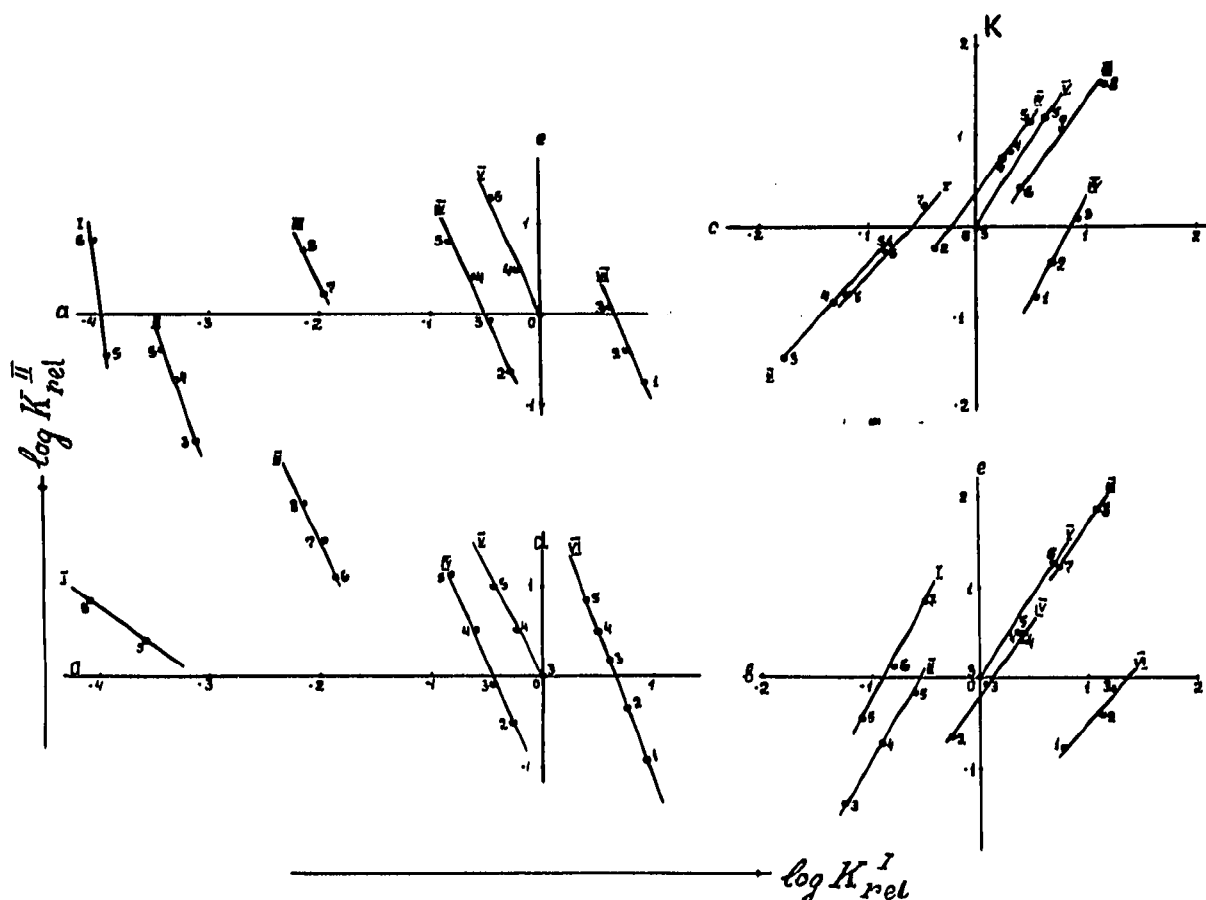


Fig. 4. Logarithmic dependence of relative distribution coefficients in liquid-vapour systems at 50° for two solvents. Solvents and classes of compounds as in Fig. 3. Figures near the experimental points denote the number of carbon atoms in the molecule.

carbon black and polymeric solvents. It should be noted, however, that the distribution coefficient in solid-vapour and solid-liquid systems depends to a greater extent on the concentration of the component to be determined, and therefore the method can be used only in a limited concentration range.

The partition-chromatographic method permits the application of other selective systems, including chemical reactions and complexing. The method is also promising for determining the absolute and relative concentrations of the components of admixtures whose analyses are assuming increasing importance in science and technology. In this case, the major component can be used as one of the phases.

The requirements for increased sensitivity of analytical methods are constantly growing. The methods of concentration and separation of major components [distillation, extraction (distribution), sublimation, etc.] may considerably increase the sensitivity. Distribution (extraction) has the advantage of higher selectivity and the possibility of using the data on distribution as the qualitative characteristics of the components.

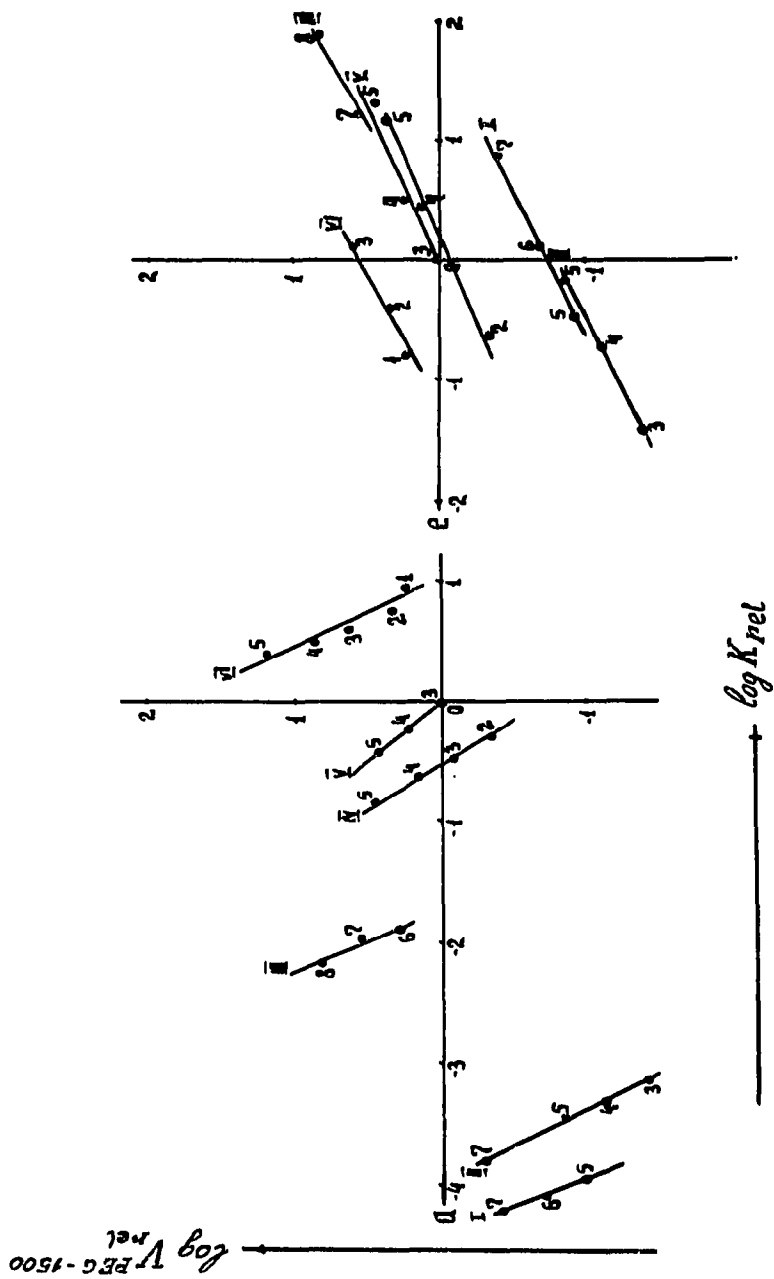


Fig. 5. Logarithm of relative retention volume for polyethylene glycol 1500 at 80° ($\log V_{rel}^{PEG-1500}$) as a function of the relative distribution coefficient in liquid-vapour systems at 50°. Solvents and classes of compounds as in Fig. 3.

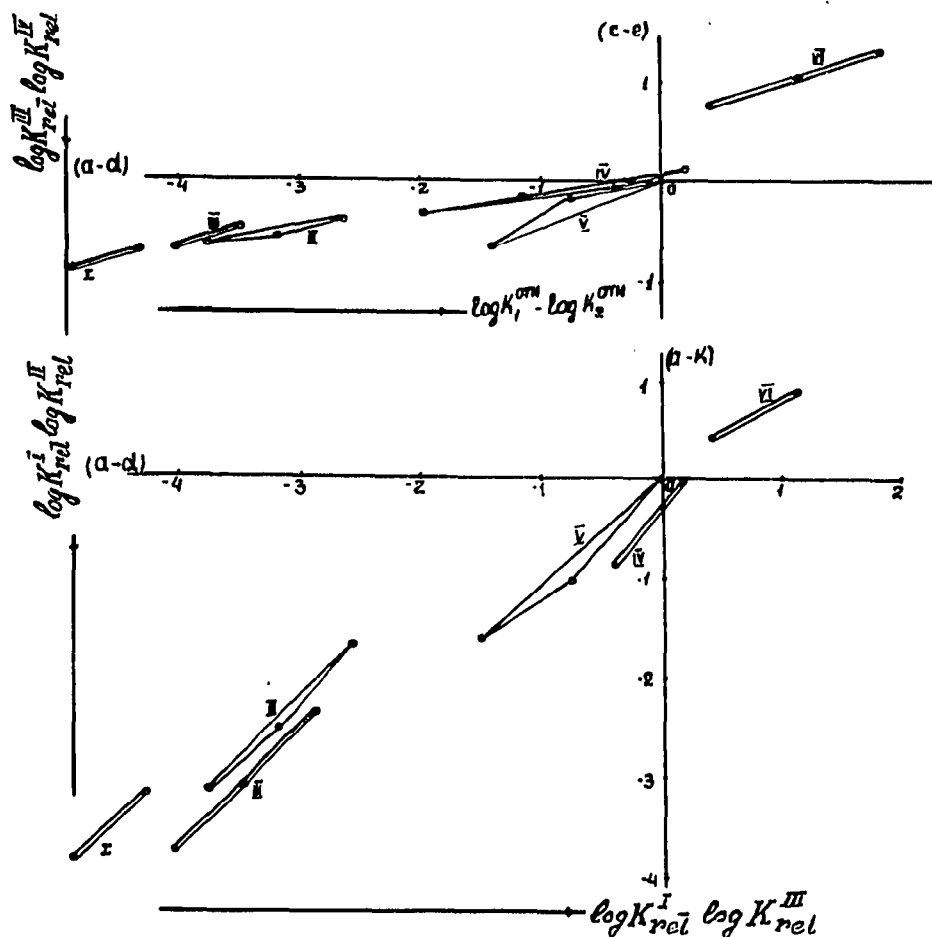


Fig. 6. Logarithmic relationship between differences in relative distribution coefficients for three and four liquid-vapour systems at 50°. Solvents and classes of compounds as in Fig. 3.

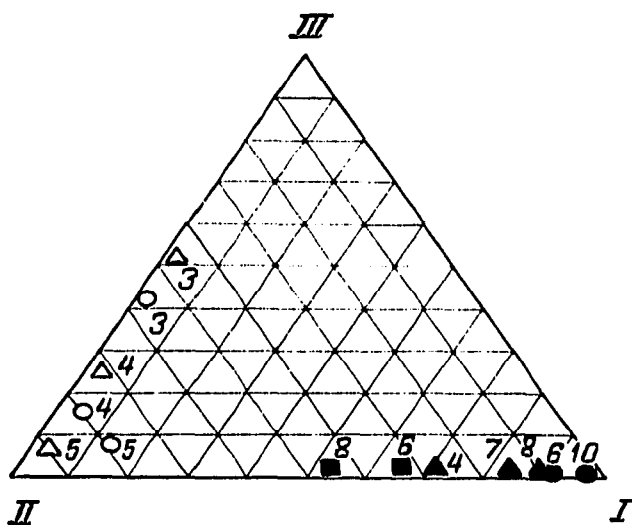


Fig. 7. Component distribution in the ternary system isooctane (I)-aniline (II)-water (III) at 20°. Compounds: ●, *n*-alkanes; ▲, α -alkenes; ■, aromatics; ○, methyl ketones; △, *n*-alcohols. Figures denote the number of carbon atoms in the molecule.

Using the partition-chromatographic method, we have identified traces of various components when solving such practically important problems as the determination of traces of admixtures in monomers in the atmosphere around industrial sites, etc.²⁰⁻²².

REFERENCES

- 1 V. G. Berezkin, in K. V. Chmutov and K. I. Sakodinskii (Editors), *Uspekhi Khromatografii*, Nauka, Moscow, 1972, p. 215.
- 2 V. G. Berezkin, *J. Chromatogr.*, 98 (1974) 477.
- 3 D. A. Leathard and B. C. Shurlock, *Identification Techniques in Gas Chromatography*, Wiley-Interscience, New York, 1970.
- 4 L. S. Ettre and W. H. McFadden (Editors), *Ancillary Techniques of Gas Chromatography*, Wiley-Interscience, New York, 1969.
- 5 V. G. Berezkin, *Analytical Reaction Gas Chromatography*, Plenum Press, New York, 1968.
- 6 R. Suffis and D. E. Dean, *Anal. Chem.*, 34 (1962) 480.
- 7 N. I. Lulova, S. A. Leontyeva, V. M. Sakharov and V. S. Voskov, *U.S.S.R. Author's Certificate*, 284,417, Appl. March 28, 1968; Publ. October 14, 1970; *Bull. Izobr.*, No. 32 (1970) 121.
- 8 C. McAuliffe, *U.S. Pat.* 3759086, Appl. May 24, 1971; Publ. September 18, 1973; *Ref. Zh. Khim.*, 15 (1974) 1295.
- 9 M. Beroza and M. C. Bowman, *Anal. Chem.*, 37 (1965) 291.
- 10 M. C. Bowman and M. Beroza, *Anal. Chem.*, 38 (1966) 1544.
- 11 M. C. Bowman and M. Beroza, *Anal. Chem.*, 38 (1966) 1427.
- 12 M. C. Bowman and M. Beroza, *Anal. Chem.*, 38 (1966) 837.
- 13 M. Beroza and M. C. Bowman, *J. Ass. Offic. Agr. Chem.*, 48 (1965) 358.
- 14 M. C. Bowman and M. Beroza, *J. Ass. Offic. Agr. Chem.*, 48 (1965) 943.
- 15 R. L. Shriner and R. C. Fuson, *The Systematic Identification of Organic Compounds*, Chapman & Hall, New York, 4th ed., 1956.
- 16 V. G. Berezkin, V. D. Loshchilova and A. G. Pankov, *Zh. Anal. Khim.*, 28 (1973) 2432.
- 17 V. G. Berezkin, V. D. Loshchilova and A. G. Pankov, in K. I. Sakodinskii, U. N. Bogoslovsky, K. V. Alekseeva and N. I. Lulova (Editors), *Sostoyanie i Perspektivi Razvitiya Gazovoi Khromatografii*, Summaries of Proceedings of VI U.S.S.R. Scientific and Technical Conference, Central Board of Vavilov Scientific and Technical Society of Instrument Making Engineering, Moscow, 1973, p. 21.
- 18 V. G. Berezkin, V. D. Loshchilova and A. G. Pankov, *U.S.S.R. Author's Certificate*, 392,405, Appl. June 18, 1970, Publ. July 17, 1973; *Bull. Izobr.*, No. 32 (1973) 112.
- 19 V. G. Berezkin and J. J. Walraven, *Zh. Anal. Khim.*, 26 (1971) 1442.
- 20 A. G. Pankov, V. V. Shugaev, A. V. Bitkina, V. V. Afanasyeva and V. D. Loshchilova, in V. N. Salyaev (Editor), *Toksikologiya i Gigiena Produktov Neftekhimii i Neftekhimicheskikh Proizvodstv*, II U.S.S.R. Conference, Yaroslavl', 1973, p. 163.
- 21 V. D. Loshchilova, A. G. Pankov and V. G. Berezkin, in G. A. Stepanov (Editor), *Issledovanie i Razrabotka Tekhnologii Protsesov Polucheniya Monomerov dlya SK*, Yaroslavl', 1973, p. 183.
- 22 A. G. Pankov, V. D. Loshchilova, N. A. Novikov and V. G. Berezkin, *Issledovanie i Razrabotka Tekhnologii Protsesov Polucheniya Monomerov dlya SK*, Yaroslavl', 1973, p. 188.