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INFLUENCE OF TEMPERATURE ON SEPARATION PROCESSES IN ADSORPTION LIQUID CHROMATOGRAPHIC SYSTEMS

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SUMMARY

Temperature programming in liquid chromatography is not universally recommended in the literature. The present work was therefore undertaken to study this question using adsorptive systems. It was observed that in liquid phases that have the ability to solvate substance molecules, the values of the absorption partition coefficients, K°, may, contrary to expectations, either not change or increase with a temperature increase. These observations were made using di-*n*-butyl ether as the mobile phase. They were explained by analysing the influence of temperature on the stability of the solvated complex. In a cyclohexanone–*n*-heptane mobile phase, K° always showed a small but unmistakable decrease with increase in temperature, even though one of its components, cyclohexanone, has, like di-*n*-butyl ether, the ability to solvate the substances investigated. These cases were analysed taking the influence of solvation on adsorption into consideration, and cyclohexanone was found to have a considerably weaker solvating ability than di-*n*-butyl ether. In all remaining cases in which solvation did not occur as expected, K° decreased with increase in temperature. The practical importance of these observations is discussed, taking the influence of temperature change on the resolution factor, R_S, into consideration. Cases where temperature programming is advantageous are pointed out.

INTRODUCTION

The selection of satisfactory separation conditions is still a major problem in liquid chromatography. Although temperature is one of the parameters that influences the separation process, only a few examples have been published to illustrate the optimization of separation conditions in liquid chromatography through the use of optimal temperatures. Most reports were related to separations in ion-exchange systems[1–10]. Cases are known where the separation conditions in molecular sieve[11–15] and partition chromatography[16–18] have been improved in this manner. As concerns adsorption chromatography[19–27], very few examples have been reported, although it was in relation to adsorption systems that the importance of temperature was first pointed out[19]. There are different opinions about the advantage of using temperature programming to improve chromatographic separations. Kirkland[26] thought that such attempts were rarely successful, whereas Dybczyński[1], Chitumbo and Brown[14], Maggs[24] and Scott and Lawrence[23,25] found that separation conditions were improved in a number of instances following changes in temperature.

Hesse and Engelhard[22] and Snyder[27] also drew attention to the advantage of using temperature programming for solving the "general elution problem".

There are also differing opinions about the accuracy of temperature control during chromatographic investigations. According to Kirkland[26], there is no need to observe the temperature regime strictly, as the changes brought about in the retention parameters by a temperature change of 1°C do not exceed 1%. Maggs[24], however, states that, in order to achieve reproducibility of the retention parameters within 1%, it is necessary to keep the temperature constant to within ± 0.2°C.

Even more conflicting are opinions about the influence of temperature on retention parameters in adsorption systems[27]. Kirkland[26] and Snyder[27] consider that an increase in temperature in these systems is associated with a decrease in the adsorption partition coefficients for the components being separated. Lie Tien Chang[20], on the other hand, observed both absence of changes and an increase or a decrease in the retention parameters.

In view of this confused situation, we studied more closely the influence of temperature on separation processes in adsorption column systems.

INFLUENCE OF TEMPERATURE ON SELECTIVITY AND EFFICIENCY PARAMETERS IN ADSORPTION LIQUID CHROMATOGRAPHIC SYSTEMS

The dependence of selectivity parameters on temperature in adsorption systems, where the interactions between the substance and mobile phase are negligible, is expressed by Snyder's[27] equation:

$$\log K_T^0 = \log V_a + \left[1 - \frac{1 - 297/T}{1 - 297/a}\right] \alpha(S^0 - A_s \varepsilon^0) \qquad (1)$$

where K_T^0 is the linear adsorption partition coefficient at T°K; V_a is the surface volume of the adsorption layer; α is the surface activity of the adsorbent at 297°K; S^0 is the adsorption energy of the substance on the surface of an adsorbent having an activity of 1, with *n*-pentane as the mobile phase; A_s is the adsorbent surface area occupied by the substance molecule adsorbed; ε^0 is the elution strength of the mobile phase; and a is a constant when $\Delta H^0 = a\Delta S^0$, where ΔS^0 is the entropy and ΔH^0 is the enthalpy of adsorption.

According to eqn. 1, a temperature increase should always be associated with a decrease in K^0.

Eqn. 1 is used to describe adsorption in systems containing apolar and weakly polar solvents in which only negligible changes in α and V_a are brought about by a temperature increase caused by changes in the water content in the adsorbent. However, in systems containing polar mobile phases, where a temperature increase may change the water content in the adsorbent considerably, the direction and extent of changes in K^0 are difficult to predict with certainty.

It is even more difficult to estimate the influence of temperature on the formation of adsorption partition coefficients when the substance molecules undergo solvation by solvent molecules, because not only the equilibrium constants for the adsorption of the substance and the solvent, but also the solvation constant, have then to be considered.

The efficiency of typical adsorption chromatographic systems, for which $u \gg u_{opt}$ (the linear velocity of the mobile phase) is characterized by Gidding's equation[28]:

$$H = A + (C_K + C_m)u \tag{2}$$

where H is the height equivalent to a theoretical plate; $A = 2 \sum_{i=1} \lambda_i d_p$ represents eddy diffusion; $C_K = 2(1 - R)^2 k_a$ represents the kinetics of the sorption–desorption processes; $C_m = \omega_i d_p^2/D_m$ represents diffusion in the mobile phase deposited in the pores of the adsorbent particles; R is the retention coefficient; ω_i and λ_i are the structural parameters; d_p is the diameter of adsorbent particles; and D_m is the diffusion coefficient; k_a is the adsorption constant.

The influence of temperature on the theoretical plate height is mainly expressed by changes in C_m, the values of C_K usually being low[28]. The value of C_m decreases with increase in temperature owing to the increase in the diffusion coefficient in the expression describing that parameter.

EXPERIMENTAL

Apparatus and materials

Measurements were made using a DuPont Model 830 chromatograph with a detector of wavelength 253.7 nm. Steel columns of 50 × 0.2 cm I.D. filled with Type H 60 silica gel for thin-layer chromatography were used (E. Merck, Darmstadt, G.F.R.)*. The temperature of the columns was maintained constant to within ± 0.2°C.

As test substances we used 8-methylquinoline (8MQ), aniline (A), phenol (P), α-naphthylamine (α-NAM), α-naphthol (α-NOL), *p*-nitroaniline (PNA), *o*-nitroaniline (ONA), *p*-nitrophenol (PNP), *p*-hydroxyazobenzene (PHA), *p*-aminazobenzene (PAM), *p*-methyl-*o*-hydroxyazobenzene (OHA) and azobenzene (Az).

Benzene, ethylene chloride, di-*n*-butyl ether and cyclohexanone–*n*-heptane (3:7) were used as mobile phases.

Methods

The adsorbent for the column packing was prepared as follows. An aqueous suspension of silica gel was spread on glass plates, dried in the air for about 12 h, then activated for 2 h at 130°C. The plates with the activated layers were cooled in a desiccator. After having been removed from the plates, the adsorbent was used to fill the columns. The columns were dry-packed by the "bounce-and-tap" method. Measurements were carried out after washing the columns with the mobile phase to

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* We filled the columns with this silica gel because we intended to compare the results of this study with those of studies performed by thin-layer chromatography, which will be published in the near future.

equilibrate the system until constant values for the retention volume of the test substances were obtained.

The solvents were distilled, then filtered through the column packed with activated Type 60 silica gel of particle diameter 0.2–0.5 mm (Merck).

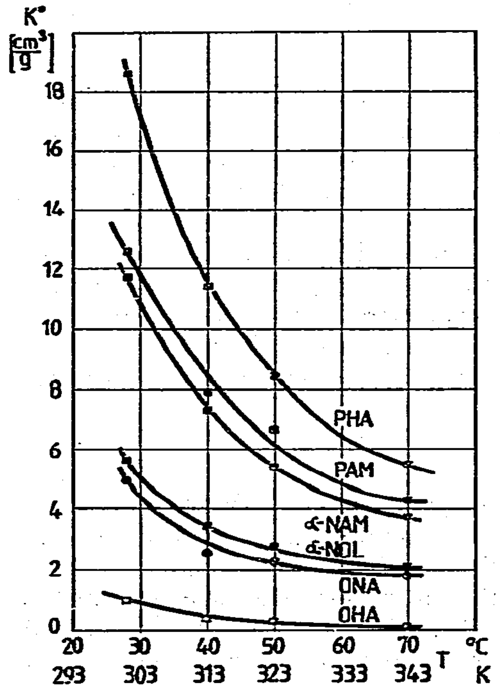
The test substances were injected into the column as 0.01 *M* solutions in volumes ranging from 1 to 4 μl, depending on the intensity of light adsorption. Separate measurements of the dependence of retention volume on amount of substance showed that the amounts of the substance injected were within the linear range of the run of the adsorption isotherm.

The retention parameters and the theoretical plate height were measured at least three times in independently packed columns.

RESULTS AND DISCUSSION

Figs. 1–4 show the courses of changes occurring in the adsorption partition coefficient with increase in temperature in systems where benzene, ethylene chloride, di-*n*-butyl ether and cyclohexanone–*n*-heptane (3:7) mixture were used as mobile phases.

It can be seen that in systems containing benzene, ethylene chloride or cyclohexanone–*n*-heptane as the mobile phase, the adsorption partition coefficients decreased with increase in temperature for all substances tested, as expected from



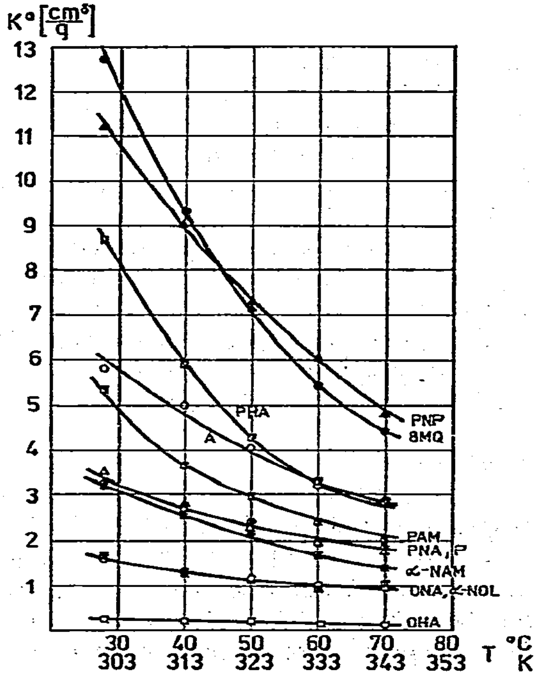
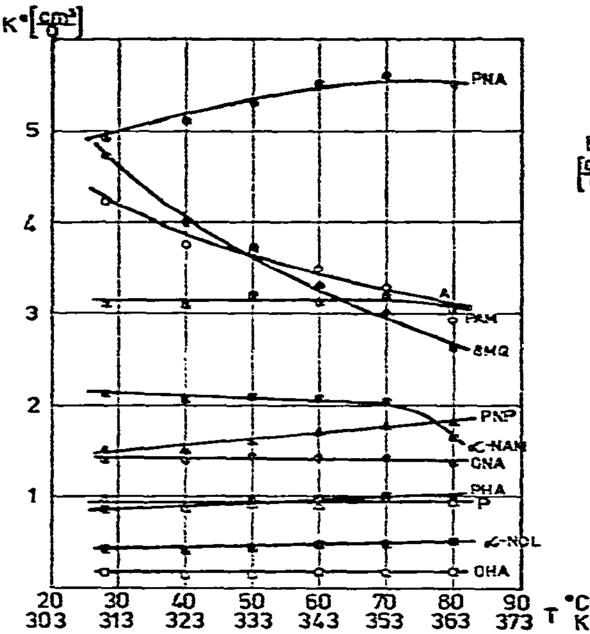


Fig. 1. Dependence of adsorption partition coefficient on temperature with benzene as mobile phase.

Fig. 2. Dependence of adsorption partition coefficient on temperature with ethylene chloride as mobile phase.



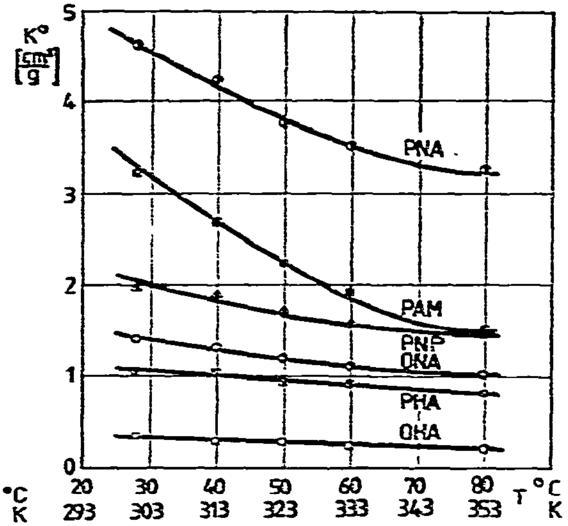


Fig. 3. Dependence of adsorption partition coefficient on temperature with di-*n*-butyl ether as mobile phase.

Fig. 4. Dependence of adsorption partition coefficient on temperature with cyclohexanone–*n*-heptane (3:7) as mobile phase.

eqn. 1. In systems containing di-*n*-butyl ether as the mobile phase, the adsorption partition coefficient decreased with increase in temperature only for 8-methylquinoline, aniline and α-naphthylamine. For the remaining substances, the $K^0 = f(T)$ changes differed from the theoretically predicted course, as a temperature increase either increased K^0 or left it unchanged.

In analysing the different courses of the $K^0 = f(T)$ changes for the various substances in systems containing di-*n*-butyl ether, the possibility was taken into consideration that the abnormalities observed in their retention parameters might be caused by the following factors: (a) increased surface activity of the adsorbent owing to its decreased water content at increased temperature, and (b) solvating influences in the mobile phase.

First we measured the changes in the water content of the adsorbent with increase in temperature, by measuring the increased content of water collected in the mobile phase during equilibration inside the column at 70°C and comparing it with the water content in the same phase at 28°C. The water content was measured by the Karl Fischer method with an accuracy of ± 5 ppm using a Beckman Model KF 4B apparatus. At 70°C, the water content in the silica gel was found to have decreased by 0.6%. The changes in V_a and parameters for silica gel, which corresponded to the decrease in water content, were then measured and compared with the values determined for these parameters at 28°C. The values of V_a and parameters for silica gel are given in Table I.

TABLE I

THE VALUES OF V_a AND PARAMETERS FOR TYPE 60 SILICA GEL AT 28 AND 70°C

These values were determined by the following methods: (a) by weight with an accuracy of *ca.* 0.2% by drying the adsorbent for 6 h at 200°C; (b) experimentally by Snyder's method[27] from measurements of the adsorption partition coefficient for naphthalene and pyrene, using *n*-hexane as mobile phase; (c) assuming $V_a = 0.00035$ (specific surface area)— 0.01 % H_2O (ref. 27), and by interpolating the data for the silica gel applied in this study to the data for the silica gels characterized by Snyder[27]

Temperature (°C)	*Water content in the gel (%)*	V_a *(cm³/g)*	
28	4.2*	0.128**	0.74**
70	3.6	0.139***	0.78***

* Method (a).
** Method (b).
*** Method (c).

Estimation of the changes occurring in the surface properties of the adsorbent showed that a 0.6% decrease in water content at 70°C did not influence the retention parameters of the substances used in the system studied. It seems, therefore, that the abnormal course of changes occurring in the retention parameters for systems containing di-*n*-butyl ether as the mobile phase should be ascribed to intermolecular interactions between the mobile phase and the substances studied.

Electron-donating solvents, such as di-*n*-butyl ether, are known to cause solvation of OH and NH_2 groups[27,29,30]. In systems in which solvation takes place, adsorption may be described by the dependence given by Soczewiński[31]:

$$K^0 = \frac{K_{AZ}X_{AS}}{K_{AS}X_S(1 + K_{ZS}X_S)} \cdot \frac{W}{V_m} \qquad (3)$$

where K_{AZ} is the adsorption equilibrium constant; K_{AS} is the adsorption equilibrium constant for the solvents; K_{ZS} is the stability constant for the solvate; X_{AS} is the surface concentration for the functional adsorbent groups occupied by solvent molecules; X_S is the solvent concentration; W is the adsorbent weight; and V_m is the liquid phase volume.

The influence of temperature on K^0 will be expressed by changes in the constants in eqn. 3, mainly in K_{AZ} and K_{ZS} (changes in K_{AS} will be negligible in comparison with the changes in the other constants). As a temperature increase will decrease K_{AZ} and K_{ZS} to various extents, depending on their ratio, K^0 for the substances will either decrease, increase or remain constant.

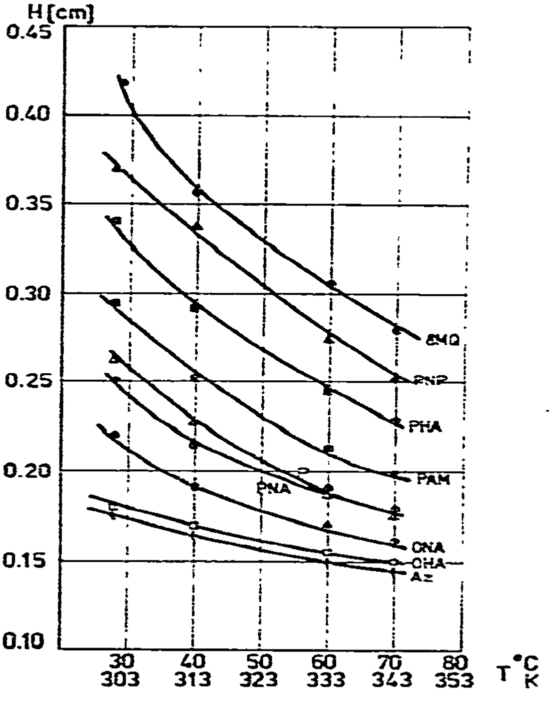
Such situations are illustrated in Fig. 3. The curves show that the reactions of 8-methylquinoline with di-*n*-butyl ether are weak, unspecific reactions (no hydrogen bond is formed) which make the solvation balance approximately zero for the molecule adsorbed upon the surface of the adsorbent and that of the aqueous molecule. In such instances K^0 depends on reactions between the substance and the adsorbent, which are characterized by the adsorption equilibrium constant, K_{AZ}, with the solvation constant $K_{ZS} = 0$. With increasing temperature K_{AZ} decreases, leading to a decrease in K^0, as can be seen in Fig. 3. The molecules of substances containing OH or NH_2 functional groups form solvation complexes with di-*n*-butyl ether by hydrogen-bond formation between the OH or NH_2 groups and the solvent molecule.

Solvation is stronger, the more strongly hydrogen is protonated in the molecule of the proton donor substance. Owing to the mesomeric effect due to the presence of the NO_2 group in its molecule, *p*-nitrophenol has stronger acidic properties than phenol (nitrophenol, $K_A = 7.1 \cdot 10^{-8}$; phenol, $K_A = 1 \cdot 10^{-10}$) and undergoes stronger solvation. It may therefore be expected that the stability constant K_{ZS} for the solvate will, for *p*-nitrophenol, have a significantly higher value than K_{ZS} for phenol. The increase in K^0 for nitrophenol with increase in temperature (see Fig. 3) seems to indicate that K_{ZS} undergoes a greater decrease with increasing temperature than does the adsorption equilibrium constant K_{AZ}.

In a similar manner it is possible to explain the significant increase in K^0 for *p*-nitroaniline which, owing to the mesomeric effect related to the presence of the NO_2 group in the molecule, shows a considerable decrease in alkalinity compared with aniline (*p*-nitroaniline, $K_B = 1.3 \cdot 10^{-12}$; aniline, $K_B = 3.5 \cdot 10^{-10}$) and may undergo strong solvation.

The lack of significant changes in K^0 with increasing temperatures for phenol, *α*-naphthol and *p*-aminobenzene may, in light of the above explanations, be an effect of changes in the adsorption constants K_{AZ} and the solvation constants K_{ZS}.

Studies of the relationship $K^0 = f(T)$ in a system containing the two-component mobile phase cyclohexanone–*n*-heptane were performed for a cyclohexanone concentration of $X_S = 0.377$, for which the value of K^0 for the substances investigated



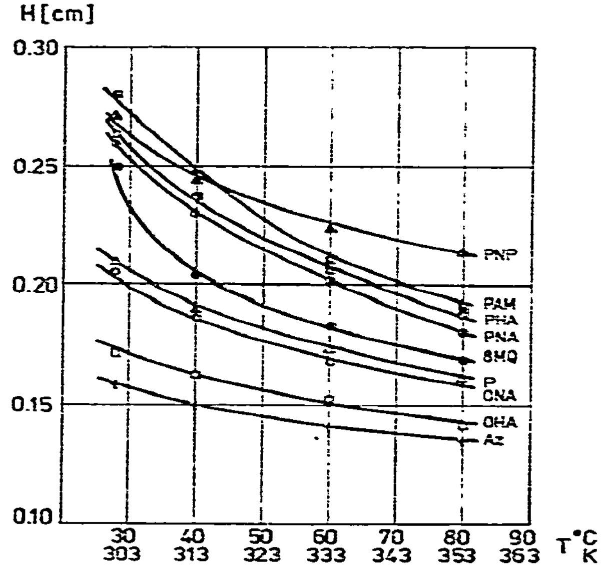


Fig. 5. Dependence of HETP temperature with ethylene chloride as mobile phase. $u = 0.4$ cm/sec.

Fig. 6. Dependence of HETP on temperature with di-*n*-butyl ether as mobile phase. $u = 0.4$ cm/sec.

approximated that for di-*n*-butyl ether. The course of changes in $K^0 = f(T)$ for the above mobile phase is illustrated by the curves in Fig. 4.

It can be seen that, the K^0 decreases, although the mobile phase contained an electron-donating solvent which, in a similar manner to di-*n*-butyl ether, causes solvation of molecules in substances with OH and NH_2 groups. It should be taken into consideration, however, that ketones, being electron-donating solvents, cause weaker solvation than do ethers. This can account for the evident, although not very great, decrease in K^0 with increase in temperature in the system discussed. Previous studies on intermolecular interactions have shown that solvation of the OH and NH_2 groups in electron-donating solvents decreases in the order ethers > ketones > esters[29].

Figs. 5 and 6 illustrate the course of changes in the theoretical plate height with increasing temperature in systems containing ethylene chloride and di-*n*-butyl ether, respectively, as mobile phase. The plate height decreased in both systems with increase in temperature, in agreement with eqn. 2.

The findings from these studies lead to the following conclusions:

(a) It can be assumed that, with increase of temperature, the distance between the zone centres for the substances being separated will either decrease, increase or remain unchanged. A temperature increase will, as demonstrated, contribute at the same time to decreasing the width of the substance zones;

(b) It can be assumed that the degree of separation of two components, expressed by[26]

$$R_S = \frac{K_B^0 - K_A^0}{1/2(W_A + W_B)} \qquad (4)$$

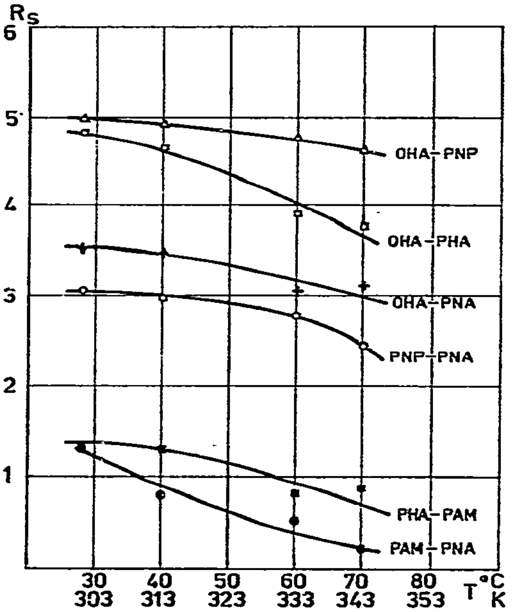


Fig. 7. Dependence of resolution factor on temperature with ethylene chloride as mobile phase.

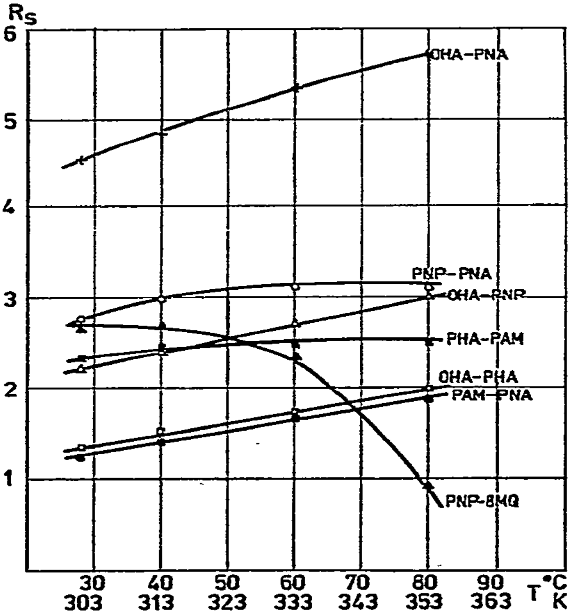


Fig. 8. Dependence of resolution factor on temperature with di-*n*-butyl ether as mobile phase.

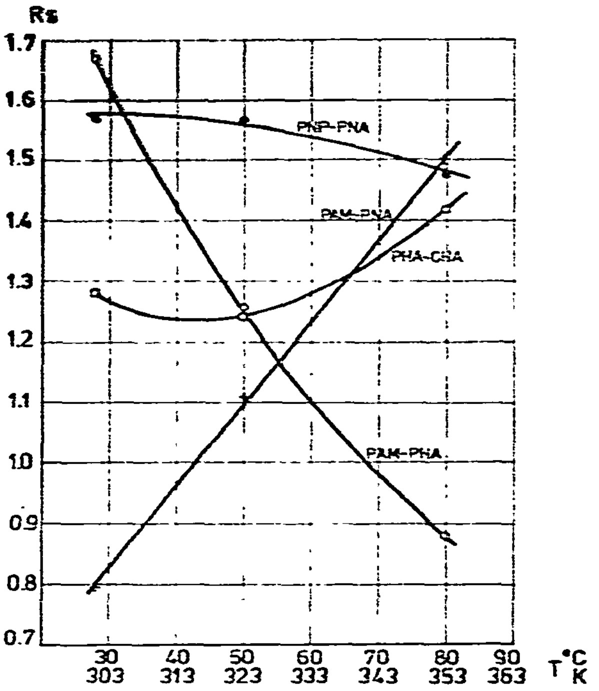


Fig. 9. Dependence of resolution factor on temperature with cyclohexanone–*n*-heptane (3:7) as mobile phase.

where W_A, W_B are the zone widths of the two substances, will, with a temperature increase, either decrease, increase or remain unchanged, depending on the kind of components being separated and the kind of mobile phase. The above conclusion is confirmed by the curves obtained in this study and presented in Figs. 7–9.

The trend of changes in R_S with increase in temperature is defined, above all, by the course of changes occurring in the retention parameters of the components being separated, because, as shown in the graphs of $H = f(T)$, the widths of substance zones always decrease with increasing temperature. A decrease in R_S was obtained for substances for which K^0 decreased with temperature, as shown in Figs. 2–4. An increase or a lack of change in R_S were obtained for substances for which K^0 increased or did not change significantly with increase in temperature.

CONCLUSIONS

The use of temperature programming in adsorption systems may improve the separation of substances if it is conducted in systems where the molecules of the substances are solvated by the molecules of the solvent, and where the decrease in the solvation constant with increase in temperature is greater than, or similar to, the decrease in the equilibrium constant, in accordance with eqn. 3.

In systems with considerable differences in the elution times of the components, the use of increased temperatures helps to shorten the time of analysis without affecting the degree of separation.

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