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INFLUENCE OF ORGANIC BASES ON THE STABILITY AND SEPARATION PROPERTIES OF REVERSED-PHASE CHEMICALLY BONDED SILICA GELS

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SUMMARY

The stability of reversed-phase materials towards attack by various bases was tested. With strong bases, such as sodium hydroxide and quaternary ammonium hydroxides, the silicate structure of these materials is attacked rapidly, rendering columns useless within 1–3 days. With primary, secondary and tertiary alkylamines, however, and working with acetonitrile–water solutions at higher pH values (> 11), columns can be used for several weeks without appreciable degradation of the performance.

The separation characteristics of such basic systems were tested with model ergot alkaloids. In general, the use of a high pH permits the separation of compounds with minor structural differences by making use of their amphoteric properties.

It has been shown that the retention properties of the alkaloids tested are governed by the structural properties and concentration of the mobile phase bases, whereas the resolution of such systems is strongly influenced by the pH and the pK values of these amines. Triethylamine has been found to be a good choice if properties such as low aggressivity towards the silicate structure, ease of availability, handling and solubility are of importance, although other bases could be used with little decrease in performance.

INTRODUCTION

The use of chemically bonded phases based on a silica gel matrix in high-performance liquid chromatography (HPLC) is well known. They can be obtained commercially[1,2] as different types and with various particle sizes, in batch or as already pre-packed columns. The most widely used types are the reversed-phase chemically bonded materials, which are commercially available with carbon chain lengths of C_8 and C_{18} and with particle sizes of 5 and 10 μm. Their ready availability and fairly constant quality have contributed much to the great increase in the use of

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reversed-phase materials in HPLC. In fact, in the pharmaceutical HPLC work at Sandoz, more than 80% of all separations are carried out on such materials. An advantage of these materials based on silica gel is their high-pressure resistance up to several hundred bars, in comparison with purely organic polymeric materials, and the absence of swelling effects. A disadvantage, however, is their instability toward extreme pH values, particularly in the basic region.

Opinions on the stability of such materials differ. Vivilecchia *et al.*[3], for example, indicated the Si–C bond for C_{18} material to be stable between pH 1 and 9. For bonds of the type Si–O–SiR₃ a stability range of pH 3–10 has been reported[4]. The use of such phases in conjunction with salt-containing aqueous solvents may result in a further decrease in their long-term stability, even under less rigorous pH conditions[5,6]. Horvath *et al.*[6] claimed, in fact, that even at pH > 7 the life of the phases can be seriously reduced in the presence of aqueous salt solutions, and this effect would be enhanced when working at higher temperatures. They suggested that the small particles dissolve under the influence of hydroxyl and other small anions, which eventually causes the packing structure to collapse. The result is a drastically reduced efficiency and permeability of the column.

On the other hand, practical experience has shown that in many instances basic conditions are essential in order to achieve good separations, especially for acids, bases or zwitterions, on reversed-phase materials. In some extreme instances, *e.g.*, with some nonapeptides, separations were possible only above pH 9, independent of the choice of the other solvent parameters[7]. It seemed that it was absolutely necessary for the free amino groups in these peptides to be in a non-protonated state. Similar arguments hold for the separation of some isomers of ergot alkaloids, where in fact only a high pH leads to the described separation[8].

In an effort to improve the life of reversed-phase systems under such extreme conditions, mineral base systems were replaced successfully with organic bases, the lifetimes increasing from 2–3 days to several weeks.

In this paper we summarize experience gained with the operation of C_8 columns at high pH in systematic studies of the effect of organic bases on the stability of the reversed-phase material and on the separation characteristics of natural and 9,10-hydrogenated ergot alkaloids of the ergotoxine group[8–10].

EXPERIMENTAL

Stability tests

Apparatus. Measurements of rates of decomposition were carried out in batch experiments. During the exposure times, the erlenmeyer flasks were mounted on a mechanical shaking device (A. Kühner, Basle, Switzerland) with a linear movement. This apparatus is equipped for variable shaking speeds (50–150 rpm) and a variable vertical movement (20–120 mm). It can simultaneously accommodate two sets of 46 erlenmeyer flasks on three levels.

The silicate content was measured in the solutions by atomic-absorption spectrophotometry (AAS) with a Perkin-Elmer Model 403 instrument (Perkin-Elmer, Norwalk, Conn., U.S.A.). The instrument was equipped with a nitrous oxide gas burner and a silicon hollow-cathode lamp (Activion, Halstead, Great Britain). The combustion gas was a nitrous oxide–acetylene mixture.

Procedure. Portions of 200 mg of the column packing material from the same batch were weighed into 500-ml erlenmeyer flasks to within $\pm$ 1%, and 20 ml each of the amine solutions with concentrations between 0.0125 and 0.5 *M* were added. The flasks were tightly stoppered and shaken on the machine at 80 rpm and with a vertical movement of 100 mm for various times.

For each data point, three flasks were simultaneously prepared and tested. The exposure times, time intervals and amine concentrations are given in Figs. 1–3. The tests were carried out at a thermostatically controlled temperature of 27 $\pm$ 2°.

At the pre-set times, the flasks were collected and the solutions filtered through a Solvinert filter, Type U.R. 1.5 (Millipore, Kloten, Switzerland). The residue was washed with 40 ml of acetonitrile–water (1:1, v/v). The silicon content was measured by AAS in the combined solutions. A sodium silicate reference solution in acetonitrile–water (1:1) served as the standard.

Reagents and materials. The following reagents were used for these tests: acetonitrile (ACN), for spectroscopy (E. Merck, Darmstadt, G.F.R.); water, doubly distilled; methylamine (MA), purum, 40% in water; dimethylamine (DMA), purum, 40% in water; trimethylamine (TMA), purum, 40% in water; tetramethylammonium hydroxide (TTMAH), purum, 10% in water; ethylamine (EA), purum, 70% in water; diethylamine (DEA), puriss, p.a.; triethylamine (TEA), puriss, p.a.; tetraethylammonium hydroxide (TTEAH), pract. > 20% in water; propylamine (PA), puriss.; dipropylamine (DPA), puriss.; tripropylamine (TPA), purum; tetrapropylammonium hydroxide (TTPAH), purum, 20% in water and tetrabutylammonium hydroxide (TTBAH), pract. 40% in water (all from Fluka, Buchs, Switzerland).

LiChrosorb RP-8, RP-18 and Si-100 (all 10 μm) were obtained from Merck.

Chromatography

Apparatus. The HPLC equipment consisted of a Waters Model 6000 pump (Waters Assoc., Milford, Mass., U.S.A.), a syringe-loading sample injector, Model 7105 (Rheodyne, Berkeley, Calif., U.S.A.), a Perkin-Elmer LC-55 variable-wavelength UV detector and a W+W recorder, Type 601 (W+W Electronics, München-stein, Switzerland). The retention data were measured on a Type 3352 B data system (Hewlett-Packard, Avondale, Pa., U.S.A.).

Procedure. The separations were carried out on 25 cm $\times$ 4.6 mm I.D. columns. The columns were packed with RP-8 by a dynamic slurry technique and were conditioned for 24 h with the corresponding mobile phase. All experiments were carried out on the same column and re-examined for each amine phase on a new column. Good agreement was observed between the two sets of data. The flow-rate was set at 3.5 ml/min $\pm$ 5%. Detection was carried out at 280 nm for the 9,10-dihydroergotoxine alkaloids and at 314 nm for the ergotoxine alkaloids. The separations were performed at room temperature (22 $\pm$ 1°). About 5 μl of a 0.1% (w/v) solution in the mobile phase were injected.

Reagents and materials. All of the reagents (see under *Stability tests)* were used without prior purification. The dihydroergotoxine and ergotoxine alkaloids were provided by Sandoz.

RESULTS AND DISCUSSION

Stability studies

   The influence of a strong organic base [0.1 M tetraethylammonium hydroxide solution in acetonitrile–water (1:1, v/v)] on non-bonded silica gel and RP-8 and RP-18 reversed-phase materials was tested (Fig. 1). From the results it can be seen that a protective function is exerted by the C_8 and C_{18} carbon chains bonded to the silica gel lattice. The non-bonded silica gel decomposes at a much faster rate than the reversed-phase materials. Of the two chemically bonded phases, the one with the C_{18} coverage is better protected than the C_8 type. It is also interesting that the kinetics of decomposition, are of first order, as expected. This was observed in all decomposition studies, a good lin/log representation being obtained.
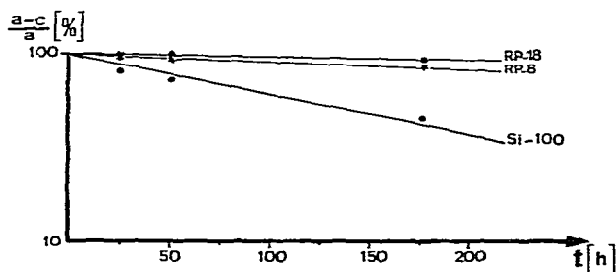

Fig. 1. Influence of a strong organic base (0.1 M tetraethylammonium hydroxide) solution in aceto-nitrile–water (1:1, v/v) on the dissolution rate of non-bonded LiChrosorb Si-100 and of reversed-phase LiChrosorb RP-8 and RP-18. a = starting amount (200 mg in 20 ml); c = amount of phase material dissolved.

   In a study of the influence of different organic bases on the reversed-phase material, acetonitrile–water (1:1, v/v) was used with RP-8. The results are shown in Fig. 2.
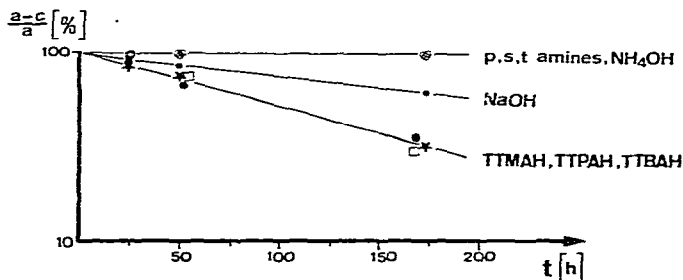

Fig. 2. Influence of primary (p), secondary (s) and tertiary (t) amines, tetramethyl- (TTMAH), tetra-propyl- (TTPAH) and tetrabutylammonium hydroxide (TTBAH), ammonium hydroxide and sodium hydroxide in acetonitrile–water (1:1, v/v) on the dissolution rate of LiChrosorb RP-8.

   The rate of decomposition over *ca.* 200 h can be seen to be negligible (or at least small) for ammonia and primary, secondary and tertiary amines. For ammonia this result would be expected as it has a considerably lower pK value than those of the amines.

A considerable increase in decomposition can be observed with strong bases such as sodium hydroxide and alkylammonium hydroxide solutions. Tetramethylammonium, tetrapropylammonium and tetrabutylammonium hydroxide show an even greater tendency than sodium hydroxide to attack the silicate structure. The behaviour of such strong bases can also be seen in Table I, which gives elemental analytical data for RP-8 material treated for 2 weeks with 0.1 M solutions of the bases. The results of C, H and N analyses are identical, within experimental error, for untreated materials and those treated with primary, secondary or tertiary amines. For quaternary ammonium bases, the proportion of organic material (C and H microanalyses) increases owing to loss of silicate material as a result of destruction of the silicate structure. It can be assumed that the organic layer (C_8 or C_{18}) is cleaved from the silicate surface (as has also been suggested by other workers[6]) and that the support structure is destroyed.

TABLE I

C, H AND N ANALYSES OF LICHROSORB RP-8 TREATED FOR 2 WEEKS WITH A 0.1 M SOLUTION OF BASES IN ACETONITRILE–WATER (1:1, V/V)

Treatment in acetonitrile–water (1:1, v:v) with	C (%)	H (%)	N (%)
Acetonitrile–water only	12.1	2.5	0.6
Propylamine (0.1 M)	12.0	2.5	0.2
Dipropylamine (0.1 M)	12.1	2.4	0.1
Tripropylamine (0.1 M)	11.8	2.4	0.1
Tetramethylammonium hydroxide (0.1 M)	17.8	3.9	1.2
Tetraethylammonium hydroxide (0.1 M)	16.0	3.0	0.6
Tetrapropylammonium hydroxide (0.1 M)	37.1	7.3	0.6
Tetrabutylammonium hydroxide (0.1 M)	38.6	7.9	0.3

The microphotographs in Fig. 3 seem to confirm the above postulation. A breakdown and a reduction in particle size can be seen to have resulted on the treatment with strong bases such as sodium hydroxide and tetrapropylammonium hydroxide. This would also explain the decrease in column permeability as the destruction proceeds. In contrast, treatment with weaker organic bases such as propylamine at pH* 13 did not result in significant alterations to the particle distribution of untreated material, where pH* = pH_s — [($E-E_s$)/0.05906]; E = electromotive force measured in a 0.05 M aqueous monopotassium phthalate solution at 25°; E_s = electromotive force measured in the experimental solution at 25°; and pH_s = pH of the 0.05 M monopotassium phthalate solution (= 4.005 at 25°)[11,12].

The high aggressiveness of the quaternary ammonium bases is not surprising as they are very strong bases and OH^- solvation at the surface of the support material seems to be more important than steric factors related to the cation.

The results of a detailed decomposition study with the weak bases of primary, secondary and tertiary amines are presented in Fig. 4. The results were collected over a period of 200 h and equimolar concentrations of all of the amine bases were used. It can be seen that within each group of amines (methyl-, ethyl- and propylamines)
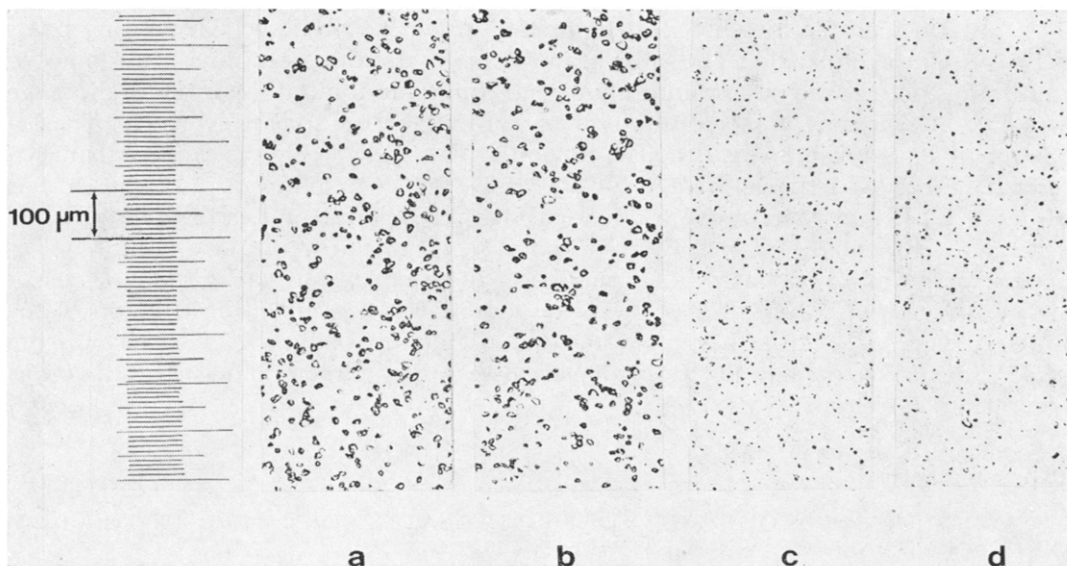
Fig. 3. Photographs of (a) untreated material, and materials treated with (b) 0.1 M propylamine, (c) sodium hydroxide and (d) tetrapropylammonium hydroxide.

the extent of attack on the silica gel lattice seems to decrease from the mono- and the di- to the triamines; at any rate the triamines definitely show a lower tendency for attack.

A plot of reaction rate constants (k) (as determined from Fig. 4) against pK values taken from the literature[13] is shown in Fig. 5. As expected for the first-order kinetic reactions, a reasonably linear relationship is observed between dissolution constant, k and pK values. Another interesting trend is the overall decrease in attacking tendency towards the silica matrix in the order methyl > ethyl > propyl bases. It
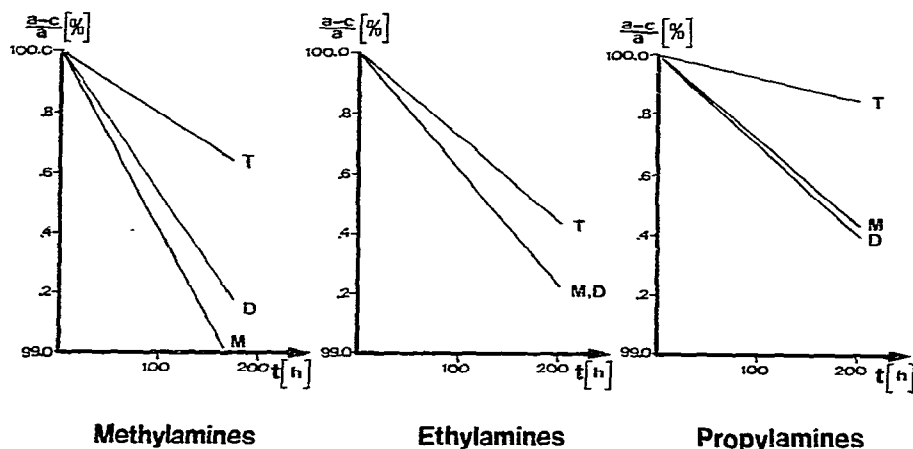

Methylamines   **Ethylamines**   **Propylamines**

Fig. 4. Dissolution rate of LiChrosorb RP-8 in 0.1 M mono- (M), di- (D) and tri- (T) -methyl-, -ethyl- and -propylamine solutions in acetonitrile–water (1:1, v/v).

seems that for the rather similar dissociation conditions of these weak mono-, di- and tri-substituted bases, steric effects have a noticeable influence on the dissolution rate of the silica gel lattice. Further, it seems that for strong bases such as the tetraamines this effect is counteracted by the strong dissociation.
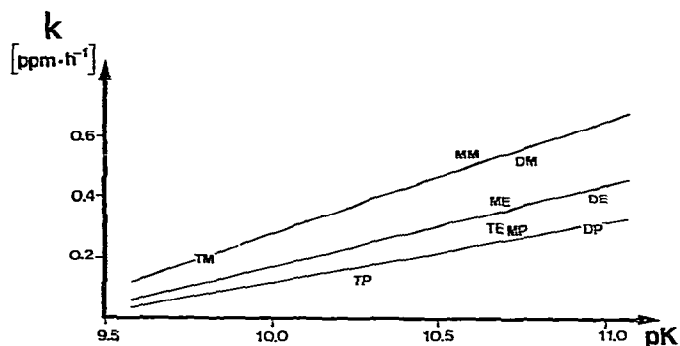

Fig. 5. Dissolution constants (k) *versus* pK values of the amines listed in Fig. 4. The symbols correspond to those in the reagent list.

In view of the above tendency for attack and its solubility in aqueous solutions, triethylamine seems particularly suitable for practical use. We therefore tested the dependence of the rate of decomposition on the amine concentration by use of triethylamine as a model system (Fig. 6). A linear dependence of k on pH* is observed.
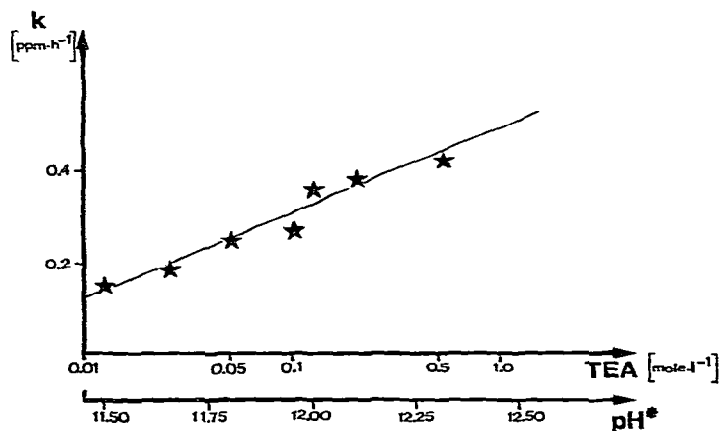

Fig. 6. Dependence of rate of decomposition of LiChrosorb RP-8 on concentration of triethylamine solution.

Another dependence that we wished to examine was the influence of acetonitrile content on the decomposition in 0.1 M triethylamine solutions. A difference in decomposition rate between 1:4 and 4:1 (v/v) acetonitrile–water mixture could be determined (for RP-8 and TEA-solutions we found that the amount of silicon dissolved in parts per million was approximately equivalent to the percentage of water in the eluent). The wetting agent seems to play a role.

From the results in Figs. 1–6, it can be concluded that the rate of attack of bases on the silica lattice seems to be governed by the strength of the base (pK values), concentration of the base and structural effects to varying degrees.

Retention studies

Influence of acetonitrile. The capacity factors [$k' = (t_R - t_0)/t_0$, where t_R = retention time and t_0 = dead volume time] and selectivity coefficients ($\alpha = k'_2/k'_1$) for ergotoxine alkaloids are shown in Figs. 7a and 7b, respectively. The four components show typical reversed-phase behaviour. The k' values decrease, as expected, with increasing acetonitrile content in the aqueous mobile phase, while the selectivity remains virtually unchanged. The same behaviour was observed with 9,10-dihydroergotoxine alkaloids.
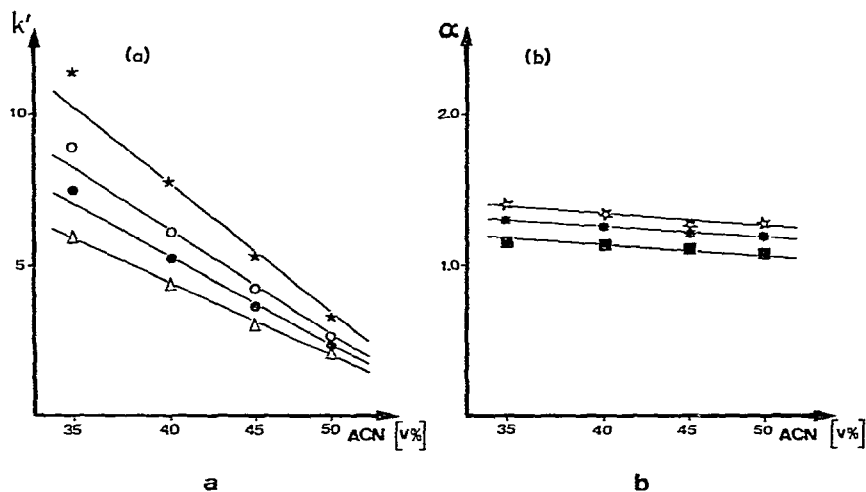

Fig. 7. Influence of acetonitrile content on the capacity factors (k') and selectivity coefficients (α) of the four ergotoxine alkaloids. ★, β-Ergocryptine; ○, ergocristine; ●, α-ergocryptine; △, ergocornine; *, β-ergocryptine–ergocristine; ■, ergocristine–α-ergocryptine; ☆, α-ergocryptine–ergocornine, in 0.05 M triethylamine solution.

Methanol can be used instead of acetonitrile, but its elution power is lower. One also observes a change in the order of elution of ergocristine and α-ergocryptine on changing from acetonitrile to methanol.

In general, it can be said that acetonitrile is a more suitable organic eluent for both ergotoxine and 9,10-dihydroergotoxine components, as its use results in better selectivity, higher plate numbers and a lower pressure drop. Further studies on the influence of various organic bases on retention behaviour were therefore carried out in acetonitrile–water media.

Influence of amines. The ergotoxine and the 9,10-dihydroergotoxine alkaloids were again chosen as model systems in studies of the influence of the base systems on this type of amphoteric compound. In a first simplified approach, the behaviour of the four ergotoxine compounds was determined as a function of the concentration of triethylamine, which has already been used extensively in reversed-phase separations[7,8].

The plots of k' *versus* log (triethylamine concentration) in Fig. 8 show that there is a linear decrease in k', corresponding to an increase in elution power, until the lines converge as expected in a single point at $k' =$ zero. Abnormal behaviour is exhibited by ergocristine, with a steeper descending slope that converges earlier with the $k' = 0$ line.
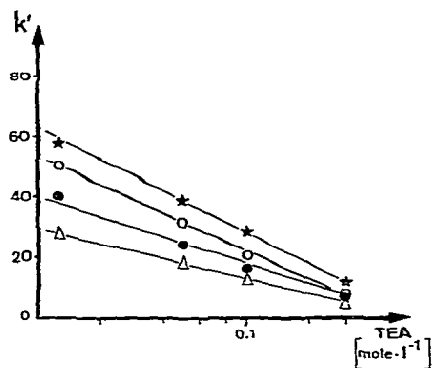

Fig. 8. Influence of triethylamine concentration on the capacity factors (k') of the ergotoxine alkaloids. Symbols as in Fig. 7a.

The same results, with the same abnormality for ergocristine, were observed for the k' patterns on all of the primary, secondary and tertiary amine bases of interest. On superimposing the plots for the mono-, di- and trisubstituted bases of the methyl-, ethyl- and propylamines and shifting them along the concentration axis until all lines converge at the $k' = 0$ position, almost complete overlap was obtained (Fig. 9). By plotting, *e.g.*, the lowest concentration (0.0125 M) for each amine on the abscissa, as has been done in Fig. 9, an indication of the relative elution powers of the tested bases is obtained. This elution power (elution strength) has been designated as ε^*. Fig. 9
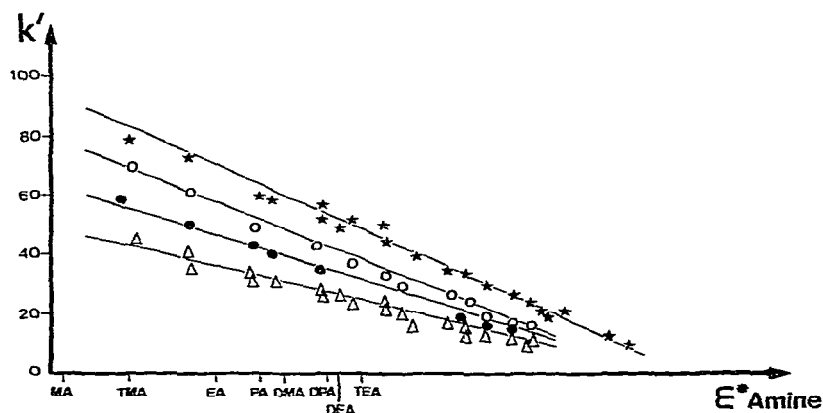

Fig. 9. Influence of the sum of the tested bases on the capacity factors (k') of the ergotoxine alkaloids. Symbols as in Fig. 7a.

shows that monomethylamine has the lowest and triethylamine* the highest elution power. A check with the pK values of these bases revealed no correlation between pK and elution strength.

The general conclusion to be drawn from these results is therefore that the elutropic series for these bases is governed by hydrophobic rather than base strength parameters. The general order of elution powers is lowest for the methylamines, intermediate for the ethylamines and highest for the propylamines. A possible explanation for this phenomenon could be the formation of ion pairs, which would alter the polarity of the alkaloids.

As it is the separation of the four components of the ergotoxine alkaloids that poses particular problems and which is of great interest, the relationship between resolution and concentration of the individual bases was studied. The resolution was plotted as normalized resolution, R_n**, equivalent to the log (concentration) scale. A simplified plot of the results obtained with triethylamine is shown in Fig. 10. As in Figs. 8 and 9, abnormal behaviour of ergocristine is noticeable in the R_n curve for the pair β-ergocryptine–ergocristine. A study of resolution with all of the other primary, secondary and tertiary amines revealed the same pattern. A superimposition of the R_n plots obtained for the eight amines tested, similar to that in Fig. 9 for k' values, is shown in Fig. 11. The spread of results is indicated by the hatched zones. Contrary to the retention (k') behaviour shown in Fig. 9, the log (concentration) scale can be converted into a pH scale, *i.e.*, the resolution is dependent on pH* and hence on the base strength (pK values) rather than on structural phenomena as observed for the k' dependence.
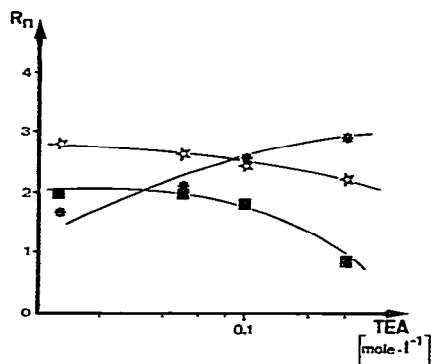

Fig. 10. Influence of triethylamine concentration on the normalized resolution (R_n) of the ergotoxine alkaloids. Symbols as in Fig. 7b.

The optimal separation conditions can be chosen from Fig. 11 and would be in the area of convergence of the plots somewhere between pH* 12 and 12.5, resulting in an equidistant separation of the four components, which also permits the best time optimization. Such a separation is demonstrated in Fig. 12b for triethylamine. The

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* Tripropylamine, which in this respect should be the most powerful base, has not been tested owing to solubility difficulties.

** $R_n = 10 \left(\dfrac{\alpha - 1}{\alpha} \right) \left(\dfrac{k'}{1 + k} \right), \quad N = 1600.$
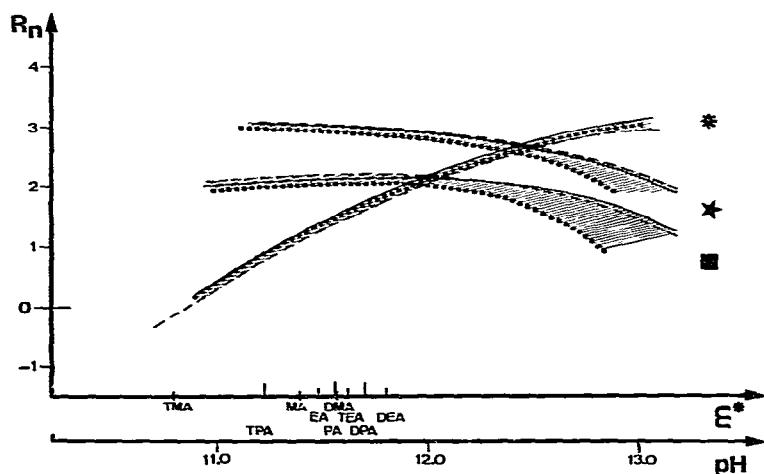
Fig. 11. Influence of the sum of the tested bases on the normalized resolution (R_n) of the ergotoxine alkaloids. Symbols as in Fig. 7b; ———, methylamines; – – –, ethylamines; · · · · · ·, propylamines.

base concentration was chosen to give pH > 12.00. In contrast, a pH* of 11 or 13 will result in a poor resolution of the α-ergocryptine–ergocristine and ergocristine-β-ergocryptine pairs, as can be seen in Figs. 12a and 12c.

   Similar studies carried out with 9,10-dihydroergotoxine alkaloids yielded the same type of results and curves. The irregular behaviour of ergocristine in the dihydro form was again observable; a wider spread of data was observed in the superimposition of R_n versus pH* curves which made the optimal area (pH 12–13) more difficult to locate. An explanation of this behaviour of ergocristine is not yet possible.
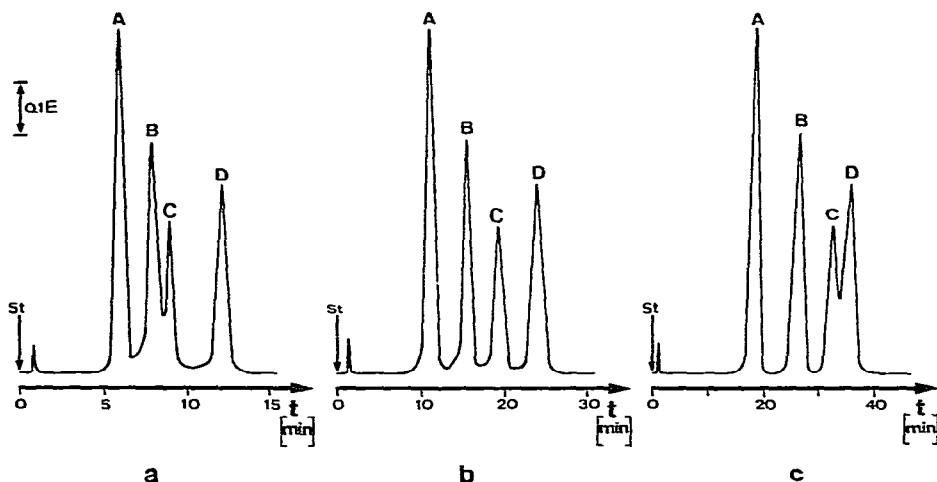

Fig. 12. Separation of artificial test mixture of ergotoxine alkaloids (A = ergocornine, B = α-ergocryptine, C = ergocristine, D = β-ergocryptine): (a) at pH* 13.0 (0.1 M dipropylamine solution), (b) at pH* 12.5 (0.05 M triethylamine solution) and (c) at pH* 11.0 (0.05 M trimethylamine solution) [acetonitrile–water (1:1, v/v)].

CONCLUSIONS

The stability studies revealed that it is possible to use organic bases of the primary, secondary and tertiary alkylamine type to achieve a higher pH without rapidly destroying the reversed-phase material. Sodium hydroxide and the strongly basic quaternary ammonium hydroxides, on the other hand, attack the silicate structure and, although the organic C_8 or C_{18} layers protect the silicate lattice to some extent, the materials will usually break down within 1–3 days. Cleavage of the organic layer from the silicate particles and collapse of the silicate structure occur, as suggested by the microscopic studies and the elemental analysis. This supports the postulation made by Horvath *et al.*[6] of such a base attack.

The other amines tested exhibit, from both the stability and the separation points of view, very similar properties and a definite choice of the optimal base is virtually impossible. It seems, however, that triethylamine, which has been used extensively for such separations in the past, comes close to an optimal choice if all of the parameters, such as minimal silicate attack, retention and separation properties, availability, handling and good solubility are considered.

From the separation studies, a general conclusion can be drawn as to the usefulness of working at higher pH for substances with very similar structural properties but possessing different acidic, basic or amphoteric behaviour. The possibility, therefore, of being able to carry out chromatographic work at high pH values will greatly enhance the potential of HPLC.

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