Journal of Chromatography, 323 (1985) 173–189
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 485

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF BASIC DRUGS ON SILICA COLUMNS USING NON-AQUEOUS IONIC ELUENTS

I. FACTORS INFLUENCING RETENTION, PEAK SHAPE AND DETECTOR RESPONSE

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(First received August 26th, 1984; revised manuscript received December 7th, 1984)

SUMMARY

The use of silica columns together with non-aqueous ionic eluents provides a stable yet flexible system for the high-performance liquid chromatographic analysis of basic drugs. At constant ionic strength, eluent pH influences retention via ionisation of surface silanols and protonation of basic analytes, pK_a values indicating the pH of maximum retention. At constant pH, retention is proportional to the reciprocal of the eluent ionic strength for fully protonated analytes and quaternary ammonium compounds. The addition of water up to 10% (v/v) has little effect on retention if the protonation of the analytes is unaffected. Thus, it is likely that retention is mediated primarily via cation exchange with surface silanols. However, additional factors must play a part with compounds such as morphine which give tailing peaks at acidic or neutral eluent pHs.

INTRODUCTION

It has been known for some time that efficient high-performance liquid chromatographic (HPLC) separations of basic analytes can be obtained using unmodified silica columns together with aqueous methanol or acetonitrile eluents containing a variety of additives[1–9]. Further studies have shown that silica columns used with non-aqueous, primarily methanolic, eluents modified by ionic compounds that are highly dissociated in organic media provide a stable yet flexible system for the analysis of basic drugs. Such systems possess a number of practical advantages, such as the

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0021-9673/85/$03.30

ability to analyse relatively large volume extracts directly and to use electrochemical oxidation for the detection of secondary and tertiary aliphatic amines[10]. The aim of the present paper is to summarise the evidence available as to the factors influencing retention, peak shape and detector response as an aid to the use of non-aqueous ionic eluent systems.

EXPERIMENTAL

Methanol (HPLC grade) was obtained from Rathburn (Walkerburn, U.K.) or from Fisons (Loughborough, U.K.), perchloric acid (60%), sodium hydroxide and sodium perchlorate monohydrate (all analytical reagent grade) from BDH (Poole, U.K.), and ammonium perchlorate from Aldrich (Gillingham, U.K.). The nomenclature of the drugs studied follows that of Martindale[11] and pK_a values were obtained from this same source unless otherwise stated.

Constant-flow reciprocating pumps (Applied Chromatography Systems, Model 750/04 or 400) were used with syringe-loading sample injection valves (Rheodyne, Model 7125, or Negretti and Zambra, Model M190). Column effluents were monitored by UV absorption (Applied Chromatography Systems, Model 750/11, or Laboratory Data Control, Spectromonitor III), or electrochemical oxidation using a V25 grade (carbonised at 2500°C) glassy carbon electrode (Le Carbone, Portslade, U.K.) in a wall-jet assembly with electronics similar to those described previously[12]. Stainless-steel columns (125 or 250 × 4.9 mm I.D.) containing Spherisorb S5W silica (Phase Separations, Queensferry, U.K.) obtained from Hichrom (Reading, U.K.) or packed from a methanol slurry were used unless otherwise stated.

The eluents were primarily methanolic solutions of perchloric acid or ammonium perchlorate of an appropriate pH and ionic strength. However, in experiments designed to investigate the influence of eluent pH on retention, sodium perchlorate was used to adjust the ionic strength. Acidic pHs were obtained by the addition of either perchloric acid (60%) or methanolic perchloric acid (0.1%, v/v) and methanolic sodium hydroxide (0.1 *M*) was used to obtain alkaline pHs. The precise volumes of methanolic sodium hydroxide added were not recorded but varied between *ca.* 0.5 and 40 ml/l over the range 7.5–11. Eluent pHs were measured using standard glass electrodes (Jenway, Dunmow, U.K., Model 6000 or Elkay, Basingstoke, U.K., Model OHP 1463000) calibrated against aqueous buffers, and no correction was applied[13]. The analyses were performed at ambient temperature (normally 22°C) and at a flow-rate of 2.0 ml/min. Analyte retention times were measured using Hewlett-Packard Model 3390A recording integrators. Mass distribution ratios (column capacity factors, k') were calculated using the formula $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the analyte and t_0 is the retention time of the non-retained peak (taken as first deviation of the baseline following the injection of 100 μl of acetone).

RESULTS AND DISCUSSION

Study of the retention mechanism(s) occurring when using bonded stationary phase materials with aqueous methanol or acetonitrile eluents containing inorganic salts, pairing ions and/or organic amines in the analysis of basic drugs has proved difficult[14]. However, unmodified silica used with a primarily methanolic eluent con-

taining an ionic modifier gives a simple and largely predictable system. The silica surface consists of siloxane (–Si–O–Si–) and silanol (–Si–OH) moieties. Most silanols are weakly acidic and thus only ionised at neutral or basic eluent pHs. However, some are strongly acidic and thus appreciably ionised even at low pH[15].

Factors affecting retention, peak shape and detector response

pH. Although alterations in eluent pH are useful in adjusting retention, not only the protonation of basic analytes but also the ionisation of the surface silanols may be influenced by such changes. However, with quaternary ammonium compounds only changes in silanol ionisation need be considered. The retention of the quaternary ammonium compound emepronium is plotted against eluent pH at constant ionic strength in Fig. 1. It is clear that the increase in the retention of emepronium with increasing pH is similar to the ionisation profile of the silica silanols[15], the increase in retention being greatest in the pH region 7–9. Sodium perchlorate was used to provide the eluent ionic strength since a modifier with no buffering capacity was required to facilitate pH changes without altering ionic strength unduly. How-

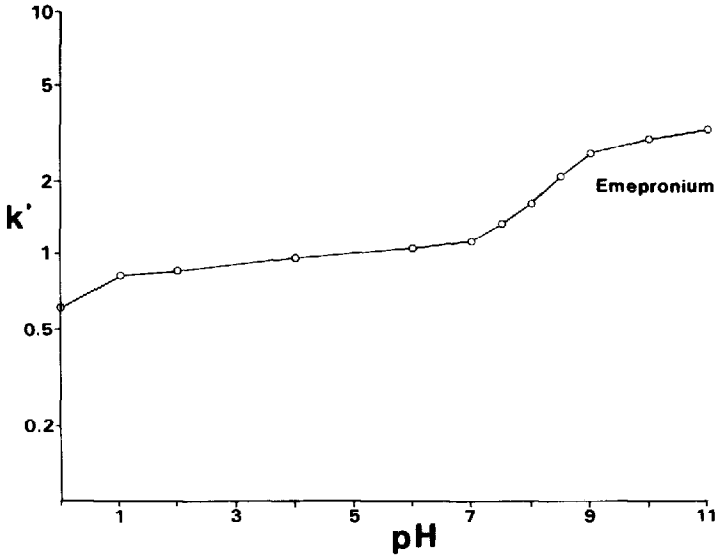

Fig. 1. Variation of retention with eluent pH at constant ionic strength for emepronium, a quaternary ammonium compound. Column: 125 mm Spherisorb S5W silica; eluent, sodium perchlorate (0.1 *M*) in methanol adjusted to an appropriate pH (see below); detection UV, 240 nm; injection, 10 µl of methanolic solution containing emepronium (10 mg/l). The eluent pH was adjusted by the addition of either perchloric acid or methanolic sodium hydroxide (0.1 *M*) as follows:

Eluent pH	*Addition*
0	200 µl/l perchloric acid (60%)
1	30 µl/l perchloric acid (60%)
2	3.8 ml/l 0.1% (v/v) methanolic perchloric acid
4	2.6 ml/l 0.1% (v/v) methanolic perchloric acid
6	1.8 ml/l 0.1% (v/v) methanolic perchloric acid
7	Nil
7.5 and above	(See text)

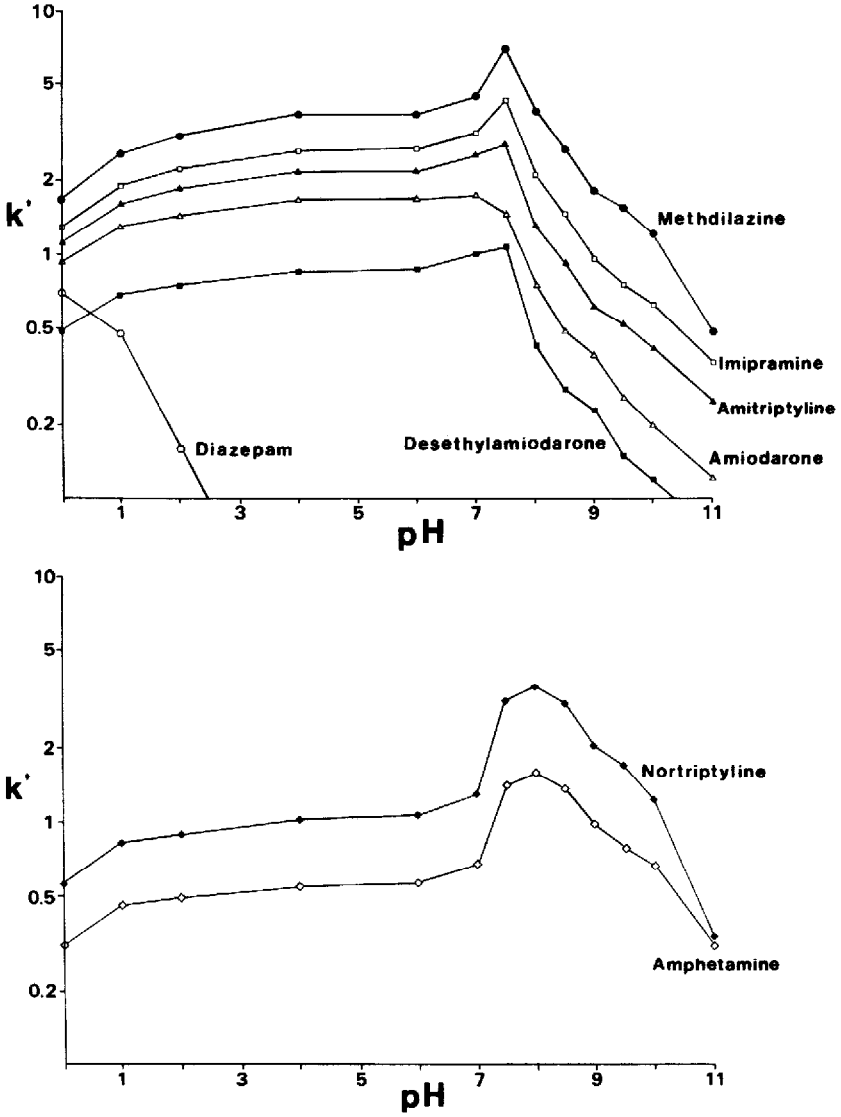

Fig. 2. Variation of retention with eluent pH at constant ionic strength for some test compounds. Column, 250 mm Spherisorb S5W silica; eluent, sodium perchlorate (10 m*M*) in methanol adjusted to an appropriate pH (see below); detection, UV, 254 nm; injection, 10 µl of solutions of each analyte (10 mg/l except amphetamine, 100 mg/l) in methanol. The eluent pH was adjusted by the addition of either perchloric acid or methanolic sodium hydroxide (0.1 *M*) as follows:

Eluent pH	*Addition*
0	100 µl/l perchloric acid (60%)
1	10 µl/l perchloric acid (60%)
2	2.6 ml/l 0.1% (v/v) methanolic perchloric acid
4	1.1 ml/l 0.1% (v/v) methanolic perchloric acid
6	0.85 ml/l 0.1% (v/v) methanolic perchloric acid
7	0.4 ml/l 0.1% (v/v) methanolic perchloric acid
7.5	0.3 ml/l 0.1% (v/v) methanolic perchloric acid
8 and above	(See text)

ever, the pH of eluents initially adjusted to between 8 and 10 slowly reverted to pH *ca.* 8. This was attributed to either absorption of atmospheric carbon dioxide or reaction with the glass solvent container, and the use of a polypropylene eluent reservoir combined with continuous helium sparging served to obviate this problem. Many column volumes (several hundred millilitres) were required to achieve constant effluent pH readings especially at intermediate pH values due to the buffering effect of silica.

An analogous approach was used in the study of the effect of pH on the retention of some basic drugs possessing different pK_a values (Fig. 2). Thus, for diazepam (pK_a 3.3) retention was greatest at pH 0. The slight increase in the ionisation of the surface silanols obtained on going from pH 0 to 2, as reflected in the increased retention of stronger bases, did not compensate for the greatly decreased protonation of diazepam. The retention of the other compounds increased with increasing eluent pH in a manner similar to that observed for emepronium (Fig. 1) until pH 7. At pHs greater than 7 the retention of amiodarone (pK_a 6.6, information from Labaz, Brussels, Belgium) decreased rapidly, while the retention of the remaining compounds increased between pH 7.0 and 7.5, and the retention of amphetamine (pK_a 9.9) and nortriptyline (pK_a 9.7) also increased slightly between pH 7.5 and 8.0, before decreasing at higher pH values. (N.B. It was only feasible to obtain retention data at intervals of 0.5 pH units in the range 7–10, where silanol ionisation and analyte protonation are changing rapidly, and thus the pH giving maximum retention for an individual analyte may not be precisely as shown). Clearly, changes in eluent pH can exert a profound effect on retention and can be used to adjust the elution sequence for analytes with different pK_a values. In addition, the pK_a of an individual compound may often be used to predict retention in general terms by analogy with the results presented in Fig. 2, despite the fact that pK_a values measured in water may not be strictly applicable to methanolic solution.

In addition to influencing retention, the eluent pH can affect the peak shapes given by certain analytes at constant retention. This does not arise from extraneous

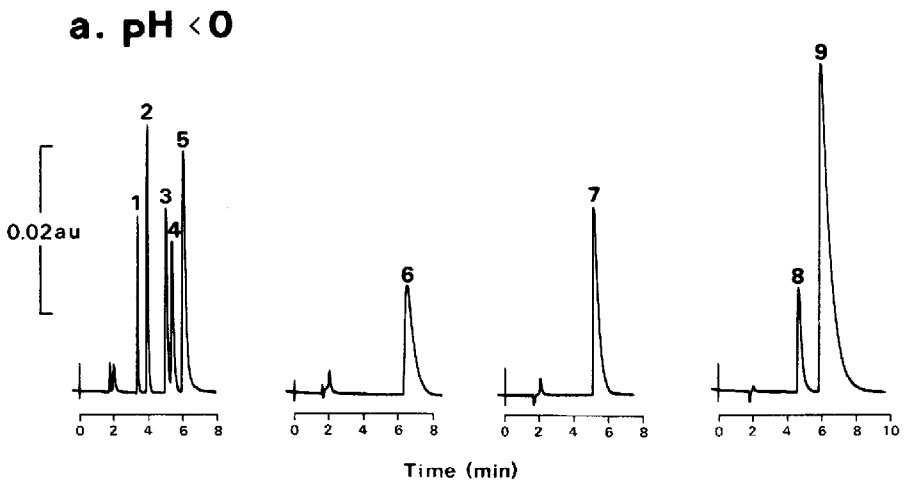

Fig. 3.

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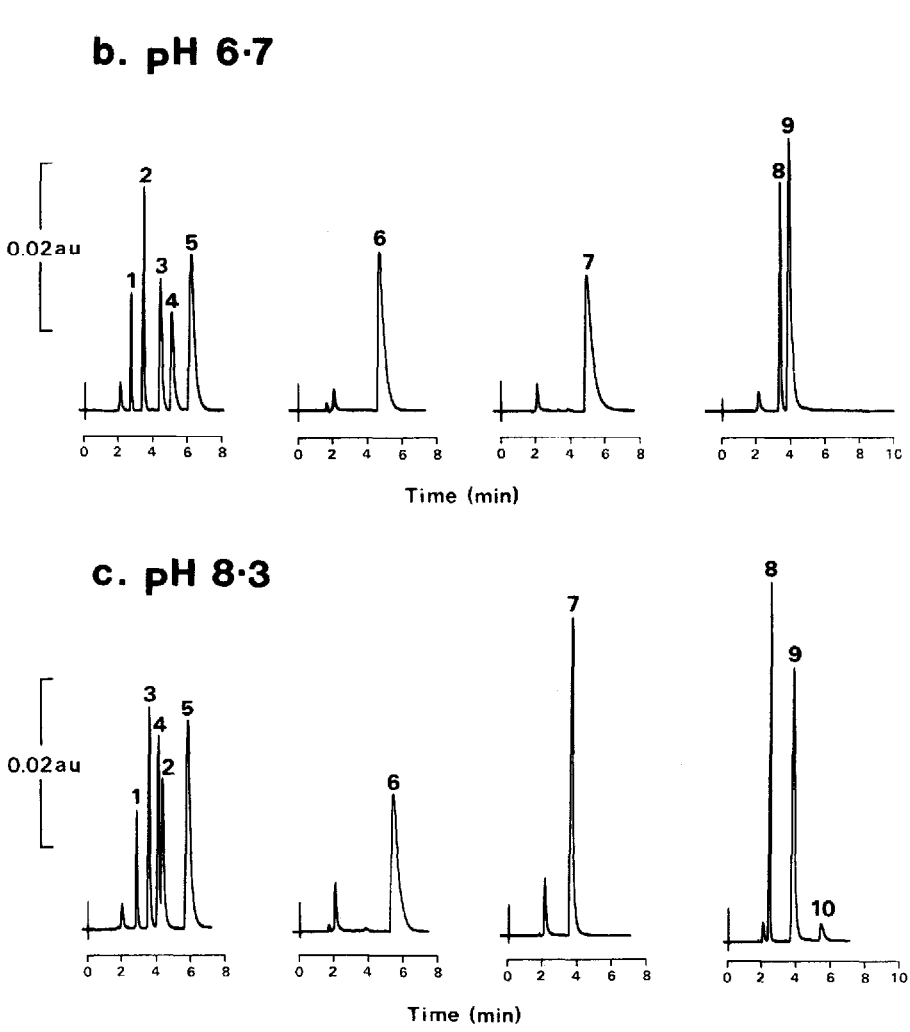

Fig. 3. Effect of eluent pH on the peak shapes of certain analytes. Column, 250 mm Spherisorb S5W silica; detection, UV, 254 nm; injection, 20 μl of methanolic solutions (10 mg/l) containing amphetamine (1) (100 mg/l), nortriptyline (2), amitriptyline (3), imipramine (4), methdilazine (5), emepronium (6) (50 μl, 250 mg/l), morphine (7) (50 μl, 50 mg/l), flurazepam (8), quinine (9) (50 mg/l) and dihydroquinine (10) (impurity in quinine solution). Eluent: methanol containing (a) pH <0, perchloric acid (60%) 0.05% (v/v) except flurazepam/quinine 0.10% (v/v); (b) pH 6.7, ammonium perchlorate (20 m*M*) plus 2 ml/l methanolic sodium hydroxide (0.1 *M*), (c) pH 8.3, ammonium perchlorate (20 m*M*) plus 60 ml/l methanolic sodium hydroxide (0.1 *M*).

factors such as deterioration of the column bed since good peak shapes are still obtained for other analytes. The influence of eluent pH on the peak shapes of analytes at similar retention times and on the same chromatographic system is illustrated in Fig. 3. Some drugs such as amphetamine, nortriptyline, amitriptyline, imipramine and methdilazine give, if anything, better peak shapes under strongly acidic conditions than at pH 6.7, while flurazepam and quinine are strongly retained and give

badly tailing peaks with a perchloric acid-modified eluent but reasonable peak shapes at pH 6.7 and, especially in the case of quinine, at pH 8.3. On the other hand, morphine gives a slightly worse peak shape on going from strongly acidic conditions to pH 6.7, followed by dramatic improvement at pH 8.3. It is possible that these differences in peak shape are due to differences in the solvation of basic analytes depending on their degree of protonation. Evidence for this view is that the peak shape given by the quaternary ammonium compound emepronium was similar at each pH. The presence of more than one protonatable or ionised group on the molecule may be important in this respect although, for example, imipramine and methdilazine both contain two such moieties yet still show acceptable peak shapes under the conditions studied.

A further consideration in the choice of eluent pH is detector response whence, conversely, information as to the protonation of an analyte under different pH conditions can be obtained. For example, aniline shows strong absorption at 283 nm at pH 5 and above in methanolic solution but not at pH 0.3 owing to protonation of the chromophore. Thus, at pH 0.3 the analyte is retained but there is no absorption at 283 nm, whereas at pH 9.2 there is strong absorption at 283 nm but the analyte is not retained (Fig. 4). The analysis of amphetamine (primary amine), nortriptyline

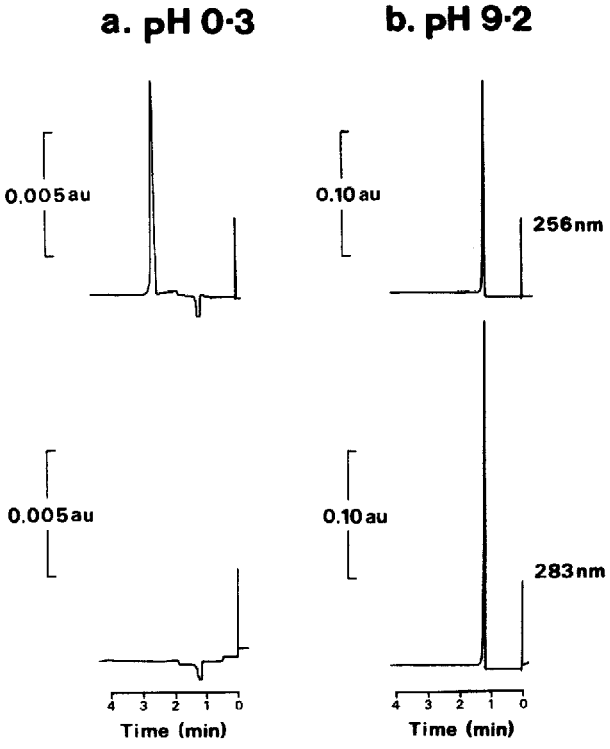

Fig. 4. Effect of eluent pH on the retention and UV absorption at 283 nm of aniline. Column, 150 mm Spherisorb S5W silica; eluent, methanol containing 0.02% (v/v) perchloric acid (*ca.* 2 m*M*) adjusted to an appropriate pH with methanolic ammonium hydroxide (0.1 *M*); detection, UV, 256 and 283 nm; injection, 10 μl of a solution of aniline (100 mg/l) in methanol.

(secondary amine), amitriptyline (tertiary amine), imipramine (tertiary amine and imidazoyl nitrogen) and methdilazine (phenothiazine sulphur/nitrogen and alicyclic tertiary amine) using UV (254 nm) and electrochemical (+1.2 V) detection is illustrated in Fig. 5. At pH 6.7 an electrochemical response was obtained for all of the above except amphetamine. This provides evidence for the presence of both protonated (retention on the column) and non-protonated (response at the detector) basic moieties since aliphatic amines such as amitriptyline are only oxidisable at this potential when present in the non-protonated form. This is emphasized by the results obtained at pH 0.6 where only imipramine and methdilazine give an electrochemical response.

Thus three factors, *i.e.* retention, peak shape and detector response, especially the electrochemical response of secondary and tertiary amines, have to be considered in the choice of eluent pH. Obviously a strongly acidic eluent is indicated in the

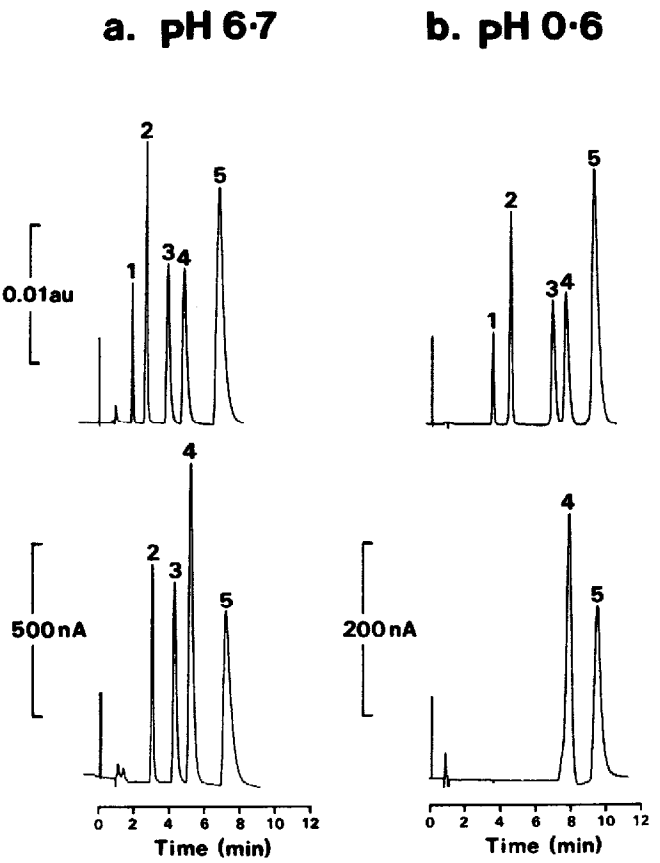

Fig. 5. Influence of eluent pH on the electrochemical detection of secondary and tertiary amines. Column, 125 mm Spherisorb S5W silica; detection, UV (254 nm) and electrochemical oxidation (+1.2 V applied); injection, 20 μl of a solution containing amphetamine (1) (100 mg/l), nortriptyline (2), amitriptyline (3), imipramine (4) and methdilazine (5) (all 10 mg/l) in methanol. Eluent: methanol containing (a) pH 6.7, ammonium perchlorate (10 m*M*) plus 1 ml/l methanolic sodium hydroxide (0.1 *M*); (b) pH 0.6, perchloric acid (60%) 0.01% (v/v) (*ca.* 1 m*M*).

analysis of very weak bases such as most benzodiazepines, and such an eluent can also be used in the analysis of many stronger bases. However, an eluent pH of 6.7 provides a compromise between retention, peak shape and electrochemical response for many analytes although a higher pH is advantageous in the analysis of compounds such as morphine and quinine. Quaternary ammonium compounds may be analysed most selectively at a relatively high pH since the retention of basic drugs is minimised (Fig. 2).

Ionic strength. The second major influence on retention is the eluent ionic strength, increases in ionic strength producing decreases in retention at constant pH. A linear relationship between log k' and log (ionic strength) was observed for strong bases and a quaternary ammonium compound under strongly acidic conditions and the plots given by each compound had similar slopes (Fig. 6). The addition of perchloric acid provided a simple means of adjusting the eluent ionic strength in this experiment. It is likely that the change in pH did not affect the results because any change in silanol ionisation is small over this limited pH range (Figs. 1 and 2). However, weak bases such as benzodiazepines behave differently, reduced protonation of

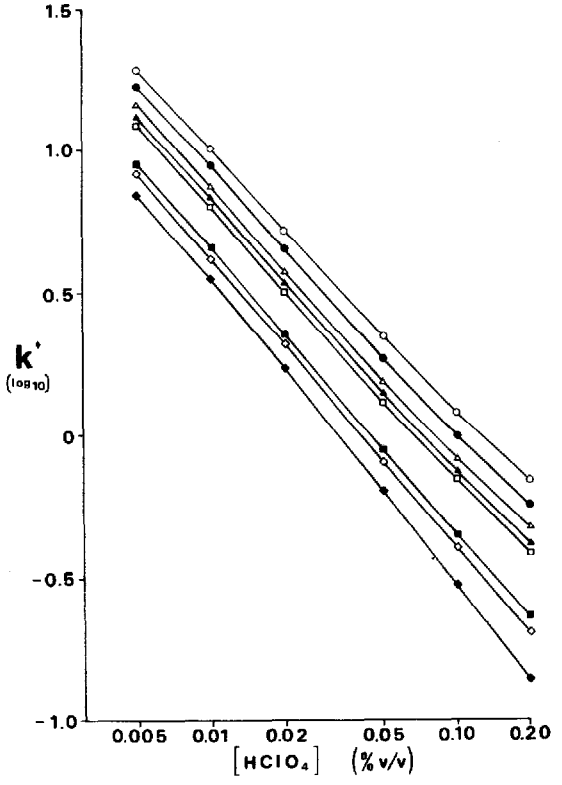

Fig. 6. Effect of changes in eluent perchloric acid concentration on the retention of some test compounds. Column, 250 mm Spherisorb S5W silica; eluent, methanol containing perchloric acid (60%) detection, UV, 254 nm; injection, 100 μl of solutions (10 mg/l except amphetamine, 100 mg/l) of each analyte in methanol (emepronium 10 μl of 1 g/l solution). Key: ◆ = amphetamine; ◇ = desethylamiodarone; ■ = nortriptyline; □ = amiodarone; ▲ = amitriptyline; △ = imipramine; ● = methdilazine; ○ = emepronium.

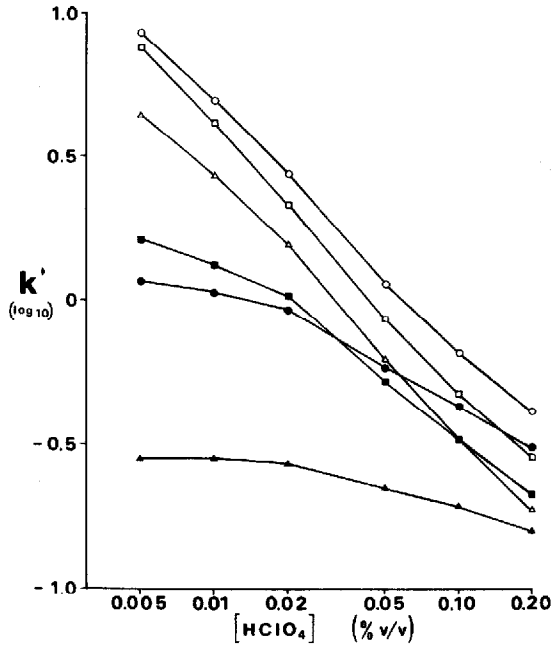

Fig. 7. Effect of changes in eluent perchloric acid concentration on the retention of some benzodiazepines. Injection, 100 µl of methanolic solutions of each analyte (10 mg/l). See legend to Fig. 6 for chromatographic conditions. Key: ▲ = lorazepam; △ = nitrazepam; ■ = oxazepam; □ = nordiazepam; ● = temazepam; ○ = diazepam.

very weak bases such as lorazepam (pK_a 1.3), temazepam (pK_a 1.6) and oxazepam (pK_a 1.7) at lower acid concentrations probably accounting for the changes in the slope of the log/log plots given by these compounds (Fig. 7).

Results similar to those presented in Fig. 6 were obtained at pH 6.7 using ammonium perchlorate as eluent modifier, although the slopes given by the log/log plots of the basic drugs were less than that given by the quaternary ammonium compound emepronium (Fig. 8). This difference may be due to the presence of a proportion of each base in the non-protonated form —evidence from the electrochemical response of secondary and tertiary amines shows that even with relatively strong bases such as nortriptyline a proportion of each analyte is present as the free base at pH 6.7 (Fig. 5). At pH 8.3 the difference between the slopes of the log/log plots given by the quaternary ammonium compound and the basic drugs was more pronounced (Fig. 9), the relatively strong bases amphetamine and nortriptyline giving plots intermediate in slope between that of emepronium and those of weaker bases. Clearly, this effect could be used to adjust the elution sequence of certain analytes, although this may be of practical value only in the analysis of compounds such as morphine which give tailing peaks at acidic or neutral eluent pHs (Fig. 3) and analysis times will also be affected at constant flow-rate.

Solvent composition. The effect of eluent water content upon the retention of a number of compounds at pH 6.7 and constant ionic strength is shown in Fig. 10. Following the addition of 10% (v/v) water the k' values decreased and peak shapes

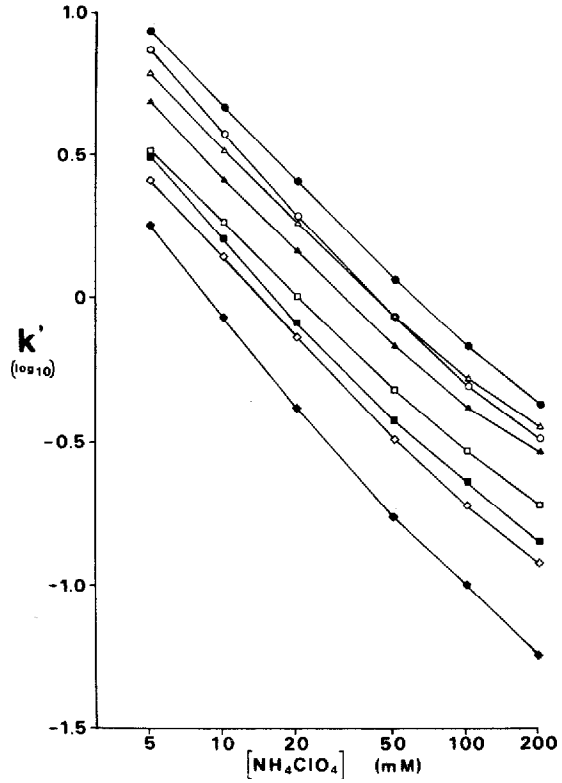

Fig. 8. Effect of changes in eluent ionic strength on the retention of some test compounds at pH 6.7. Column, 250 mm Spherisorb S5W silica; initial eluent, methanolic ammonium perchlorate (0.2 *M*) plus 20 ml/l methanolic sodium hydroxide (0.1 *M*); detection, UV, 254 nm; injection, 10 μl of methanolic solutions of each analyte (10 mg/l except amphetamine, 100 mg/l) (emepronium 100 μl of 10 mg/l solution). For key see legend to Fig. 6.

improved slightly. The retention of some longer retained compounds again decreased at a water content of 20% (v/v), but the retention of all of the compounds studied then increased with increasing water content up to 60% (v/v) and peak shapes deteriorated. The elution sequence was unchanged with the exception that desethylamiodarone and amiodarone showed marked increases in retention at higher water contents, which may be attributed to the lipophilic nature of these compounds. Water contents above 60% (v/v) were not studied because very long retained, broad peaks were obtained. The addition of water up to 10% (v/v) to a perchloric acid-modified eluent had little overall effect on the retention or peak shape of these same compounds. In contrast, with benzodiazepines the addition of 0.5% (v/v) water caused a decrease in the retention of temazepam and oxazepam and the addition of up to 10% (v/v) water gave rise to further decreases in retention and a change in elution sequence (Fig. 11). Since benzodiazepines are only partially protonated under non-aqueous conditions at low pH, it is possible that these changes in retention are attributable to changes in protonation. Peak shapes were unaffected with the exception that flurazepam showed an improved peak shape at higher eluent water contents.

We have not studied systematically the effect of alterations in solvent com-

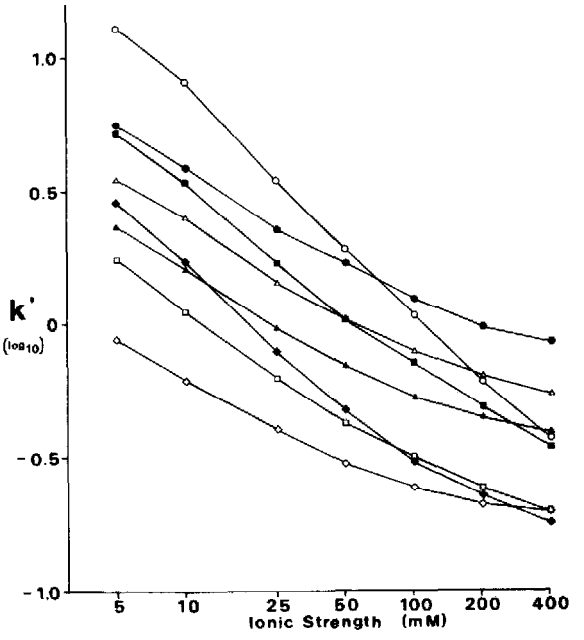

Fig. 9. Effect of changes in eluent ionic strength on the retention of some test compounds at pH 8.3. Column, 250 mm Spherisorb S5W silica; initial eluent, methanolic ammonium perchlorate (0.4 *M*) plus 300 ml/l methanolic sodium hydroxide (0.4 *M*); detection, UV, 254 nm; injection, 10 µl of methanolic solutions of each analyte (10 mg/l except amphetamine, 100 mg/l) (emepronium 100 µl of 10 mg/l solution). For key see legend to Fig. 6.

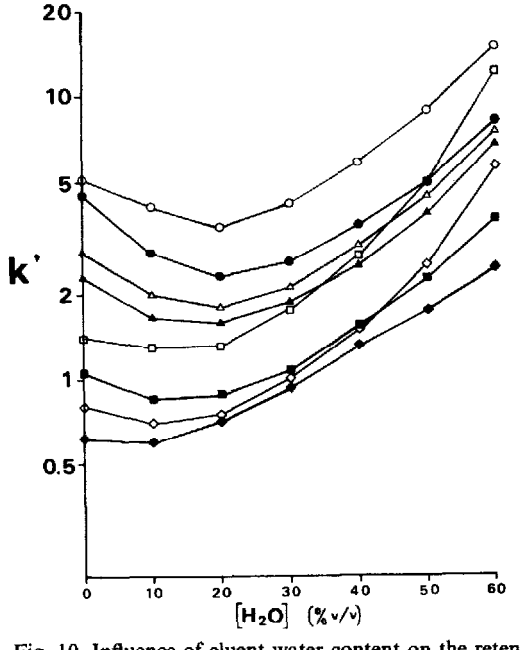

Fig. 10. Influence of eluent water content on the retention of some test compounds at pH 6.7. Column, 100 × 4.6 mm I.D. Spherisorb S5W silica; eluent, ammonium perchlorate (10 m*M*) plus 1 ml/l methanolic sodium hydroxide (0.1 *M*) in methanol or methanol–glass-distilled water; detection, UV, 254 nm; injection, 10 µl of solutions (10 mg/l except amphetamine, 100 mg/l) of each analyte in methanol. For key see legend to Fig. 6.

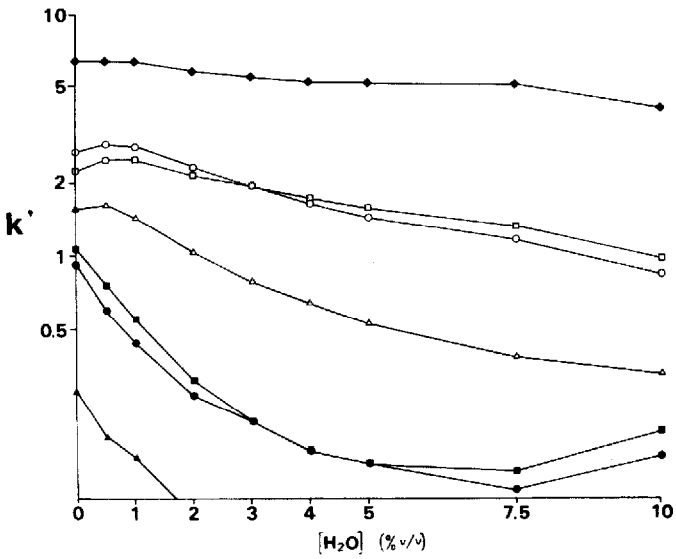

Fig. 11. Influence of eluent water content on the retention of some benzodiazepines under strongly acidic conditions. Column, 250 mm Spherisorb S5W silica; eluent, perchloric acid (60%) 0.02% (v/v) in methanol or methanol–glass-distilled water; detection, UV, 254 nm; injection, 20 μl of solutions (10 mg/l, flurazepam 50 mg/l) of each analyte in methanol. See legend to Fig. 7 for key (◆ = flurazepam).

position on retention or peak shape other than those discussed above. However, we have not observed any major changes following the addition of up to 20% (v/v) of *n*-hexane, methyl *tert.*-butyl ether or diethyl ether to a methanolic eluent at acidic or neutral (6.7) pH. At higher pHs an increasing proportion of basic analytes will be present in the non-protonated form, and this may be a factor in changes in retention achieved by altering the solvent such as the resolution of codeine and morphine achieved at pH 9.2 and constant ionic strength by using methanol–chloroform (40:60) rather than methanol[10]. This may be due to alterations in the solvation of the non-protonated species and thus the use of solvents such as acetonitrile which possess different solvating properties to water–methanol may prove useful.

Nature of the ionic modifier. We have not studied systematically the effect of variations in the nature of the cation used on retention, although preliminary results have shown that different competing ions can influence retention without altering the elution sequence. Thus, substituted amines possess stronger eluting power than sodium, potassium or ammonium ions. However, ammonium salts are useful modifiers because, in addition to acting as a competing ion, the ammonium ion is partially dissociated at neutral and basic pHs thus buffering the eluent pH and giving stable retention times. Ammonium salts have the disadvantage that at neutral and basic pHs ammonia is oxidisable by the electrochemical detector. The alternative modifiers, however, have greater disadvantages: sodium and potassium salts of strong acids have no buffering capacity while substituted amines are more easily oxidised than ammonia.

The anion used does not have any major influence on retention. Methanolic solutions of hydrochloric and nitric acids give rise to longer retention than perchloric

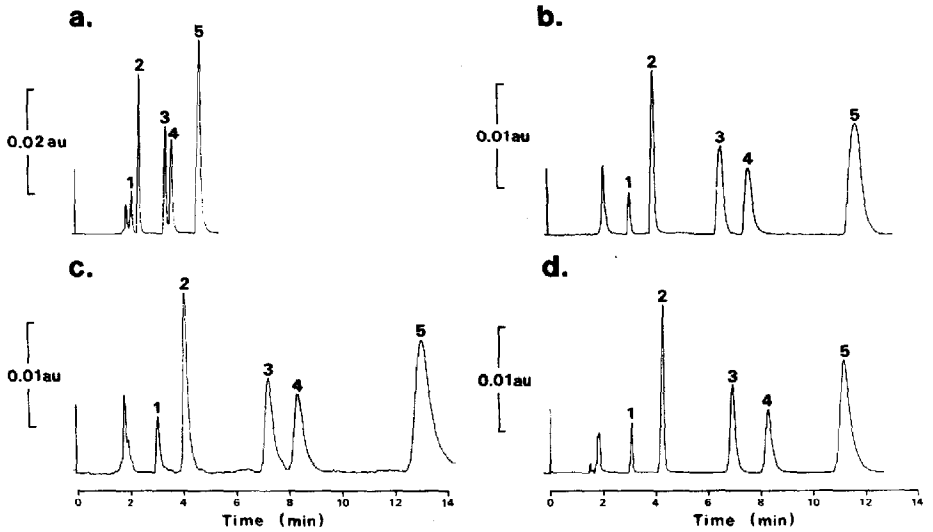

Fig. 12. Influence of the origin of the packing material on the retention of some test compounds. Eluent, ammonium perchlorate (10 m*M*) plus 1 ml/l methanolic sodium hydroxide (0.1 *M*) in methanol; detection, UV, 254 nm; injection, 20 µl of a solution containing amphetamine (1) (100 mg/l), nortriptyline (2), amitriptyline (3), imipramine (4) and methdilazine (5) (all 10 mg/l) in methanol. Column: 250 mm packed with (a) Partisil 5 (Whatman); (b) Hypersil (Shandon Southern); (c) Syloid 74 (W. R. Grace, London, U.K., fractionated by aqueous sedimentation to give a nominal particle size of 7 µm), and (d) Spherisorb S5W silica.

acid at an equivalent concentration when used in the analysis of relatively strong bases, presumably because these compounds are not fully dissociated in methanol. In addition, hydrochloric or nitric acid-modified eluents cannot be used in the analysis of very weak bases such as benzodiazepines. However, at neutral or basic eluent pHs nitrate, chloride or perchlorate salts give virtually identical retention for a range of basic drugs. Halides such as chloride or bromide are oxidisable by the electrochemical detector, and bromide and nitrate have relatively high UV cutoffs. Perchlorates are used routinely since they are compatible with the detection systems and are adequately soluble in methanol. The dilute solutions used for analytical purposes are safe but evaporation to yield potentially explosive residues should be avoided. Camphorsulphonic acid provides a useful alternative especially when strongly acidic, high ionic strength eluents are required.

Nature of the packing material. The silica used as an HPLC column packing material exhibits variations in physical properties such as pore size, surface area and surface silanol concentration depending on its method of production[16]. Differences in retention were observed between Partisil 5 and the other packings tested using an ammonium perchlorate-modified eluent (Fig. 12), although good efficiencies and peak shapes were obtained in each case and the elution sequence was identical.

GENERAL DISCUSSION

The results presented here suggest that retention on silica column/non-aqueous ionic eluent systems is mediated primarily via cation exchange with surface silanols.

Only positively charged species are retained, and the degree of retention is influenced by the ionisation of the silanols and the protonation of basic analytes (Figs. 1 and 2). In addition, the effect of eluent ionic strength on retention (Figs. 6–9) is that expected for a weakly acidic cation-exchanger. The reduction in the slope of the log/log plots for basic drugs at higher pH values (Figs. 8 and 9) is in agreement with that predicted for partially protonated bases on a conventional cation-exchange column[17]. These conclusions are supported by the observation that large volume sample injections can be performed using a zero ionic strength ("non-eluting") solvent with no loss of column efficiency[10]. On the other hand, non-protonated bases are not retained and this suggests that interaction of the relatively non-polar free bases with surface siloxanes does not contribute to retention (Fig. 2). Similarly, there is no evidence that interaction between un-ionised silanols and protonated bases is a major influence since retention is also at a minimum under strongly acidic conditions except for benzodiazepines which are only partially protonated even at very low pH values (Figs. 1 and 2). Finally, the differences in retention between the different packings tested (Fig. 12) are those expected if the major difference between these materials lies in the number of surface silanols.

Three factors remain which are difficult to reconcile with the postulated simple ion-exchange mechanism. Firstly, the elution sequence of a series of fully protonated amines and a quaternary ammonium compound, *i.e.* $1° < 2° < 3° < 4°$, means that the affinity of the ions for the packing material increases with increasing ionic size, which is the opposite of that predicted from an ion-exchange model. One possible explanation lies in greater solvation of the less substituted analytes to give them larger effective ionic radii and hence shorter retention in an analogous manner to that observed for inorganic cations on a conventional ion-exchange column[18]. Secondly, a number of drugs such as morphine show asymmetric peaks at acidic or neutral eluent pH values. This poor performance cannot be explained satisfactorily on the basis of a simple ion-exchange mechanism and additional factors such as changes in solvation of protonated species must play a part. Finally, the changes in retention caused by alterations in solvent composition at constant pH and ionic strength other than those attributable to changes in protonation (Fig. 11) may also be due to changes in solvation/solubility.

Many workers have used eluents consisting of methanol or acetonitrile and/or a chlorinated solvent modified by the addition of ammonium hydroxide in the HPLC of basic drugs on silica. Since it is likely that ammonium hydroxide is at least partially dissociated in such solvents, it is possible that the mechanism of retention in such systems is similar to that under discussion here. The relationship between retention and eluent ionic strength has been studied for a number of analytes using silica columns and methanolic solutions of the sodium salts of strong acids, *i.e.* sodium bromide and perchlorate, as eluents[19]. Differences in retention between eluents containing equivalent concentrations of these salts were observed although the elution sequence was similar. These differences in retention were attributed to differences in ion-pair formation, perchlorate forming stronger ion-pairs and thus giving decreased retention. However, no account was taken of eluent pH (*cf.* Fig. 2). Differences in the slopes given by log/log plots of k' against eluent ionic strength were also observed, the slopes given by quaternary ammonium compounds and secondary amine tricyclic antidepressants being greater than those of tertiary amine tricyclics and phenothi-

azines. These results are similar to those obtained in the present study at pH 8.3 (Fig. 9) and thus could possibly be explained on the same basis.

Sugden *et al.*[2] reported decreases in retention for basic drugs on silica using methanol–water (70:30) containing ammonium nitrate as eluent on going from mildly basic to acidic conditions. This was attributed to changes in ion-pair formation rather than silanol ionisation and Crommen[3], using acidic aqueous eluents of an appropriate pH and ionic strength, also concluded that ion-pair formation was the major influence on the retention of the compounds studied. In contrast, Wheals[4], Hansen[5] and Svendsen and Greibrokk[8] concluded that a number of retention mechanisms including ion-exchange were operating depending on the pH, ionic strength and the nature of the solvent and of the analyte. In particular, Hansen[5] reported that the retention of opiates using eluents containing 50–90% (v/v) water could be attributed to ion-exchange but at eluent water contents down to 1% (v/v) several (unstated) mechanisms were thought to apply. Further work from this group[6,7] has concentrated on the addition of a "reversed-phase-forming" agent (long-chain quaternary ammonium compound) to an aqueous methanol eluent although it was concluded that the retention of quaternary ammonium analytes was still mediated by cation-exchange with surface silanols.

Bidlingmeyer *et al.*[9] using acetonitrile–water (60:40) reported similar results to those presented here as to the effect of pH and ionic strength on the retention of basic drugs. However, a linear relationship was observed between log k' and eluent water content in the range 30–70% (v/v) water at pH 7.8 and constant ionic strength and the elution sequence was unchanged (*cf.* Fig. 10). It was thought that surface siloxanes might contribute to increased retention at higher eluent water contents via an "ion-interaction effect" similar to that proposed for reversed-phase ion-pair liquid chromatography[14]. (N.B. water contents below 30% (v/v) were not studied because the chosen modifier was not adequately soluble). Finally, Bidlingmeyer *et al.*[9] also used methanol–water (80:20, v/v) containing butylamine as eluent in the analysis of some secondary and tertiary amine tricyclic antidepressants. Changes in eluent pH gave rise to changes in elution sequence analogous to those shown in Fig. 2 but were interpreted as being due to modification of the silica surface by adsorbed butylamine. Similarly, the observation that substituted amines possessed stronger eluting power than ammonia was also attributed to adsorption of the amine to the silica surface.

CONCLUSIONS

It is evident that there are a number of potential mechanisms whereby basic drugs may be retained upon unmodified silica. When using methanol alone as eluent solvent, an ion-exchange model can provide practically useful information as to the effect of eluent pH and ionic strength on retention, pK_a values indicating the pH of maximum retention. However, this simple model cannot predict the effect of changes in eluent pH or solvent composition on peak shape or those of changes in solvent composition on retention other than by analogy with the results presented here. The study of the factors influencing retention of basic drugs on unmodified silica, and also on bonded stationary phase materials where residual silanols may contribute significantly to retention[2,4,9], using aqueous methanol or acetonitrile eluents may benefit from the application of a simplified model.

ACKNOWLEDGEMENT

We thank B. B. Wheals and our colleagues at the Metropolitan Police Forensic Science Laboratory and at the Poisons Unit for help and encouragement.

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