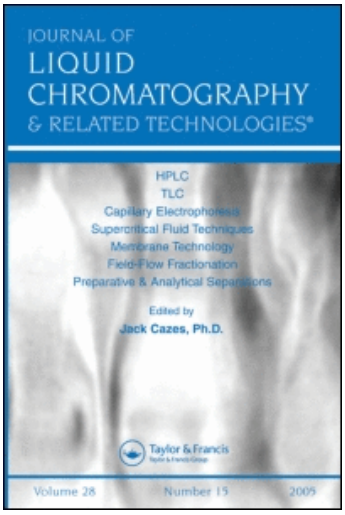

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SILANOPHILIC INTERACTION IN THE RETENTION MECHANISM OF PHARMACEUTICAL DRUGS ON A CYANOPROPYL BONDED SILICA COLUMN

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ABSTRACT

The mechanism of retention of several pharmaceutical drugs with basic, neutral and acidic properties have been examined on a cyano bonded silica stationary phases (Ultrasphere cyano). Clenbuterol, bromhexine, terphenadine, pirenzepine, astemizol, betamethasone, acetaminophen, indomethacin, diclofenac and nimesulide were chosen for this study. Methanol-phosphate buffer mixtures with low water levels were used as mobile phases. Different pH values and ionic strengths were considered. Under experimental conditions, the chromatographic behavior of basic drugs is more complex than the behavior of acidic and neutral ones. The predominant silanophilic interactions observed for drugs of basic characteristics may become quite useful in optimizing selectivity.

INTRODUCTION

The cyanopropil bonded silica column has been extensively used in the analysis of pharmaceutical drugs due to its great versatility (1-3).

Its reverse or normal phase behavior, according to the employed chromatographic system is responsible of its particular selectivity.

The retention mechanism in the reverse mode has been described in a previous work (4).

Some investigators have suggested that the retention behavior of a cyanopropil column is comparable with a short chain alkyl-bonded silica column (5).

The retention properties in normal phase mode have also been examined. It appears that residual silanols provide a behavior similar to silica phase, but as the polarity of the solvent increases, the masking effect on silanols makes possible the interaction on cyano sites (6).

It is established that basic drugs have specific interactions with residual surface silanols of the silica bonded phases. Silanophilic interactions can potentially supply a greater selectivity to a chromatographic system than hydrophobic ones When hydro-organic eluents leaned in water are used, it has been observed that interactions with silica may become the dominant effect and basic compounds show normal phase behavior (7-9). Moats and Leskinen (9) have studied the retention of some penicillins on polymeric, silica and cyano columns employing mobile phases with low water proportions.

We considered of interest to investigate the chromatographic behavior of several basic drugs frequently used in pharmaceutical

formulations on a cyano column. So, the influence of different parameters as pH, ionic strength and organic modifier proportions in the mobile phase, specially with low water levels, has been evaluated in the present study.

The experimental conditions have been proposed in order to elucidate the main interactional modes between solutes and stationary phases.

MATERIALS

Chemicals:

Acetaminophen, diclofenac, indomethacin, nimesulide, betamethasone, clenbuterol chlorhidrate, bromhexine chlorhidrate, pirenzepine chlorhidrate, terphenadine and astemizol were of pharmaceutical purity. Methanol and ammonium dihydrogen phosphate were HPLC grade. Sodium dihydrogen phosphate, phosphoric acid and ammonium hydroxide were analytical grade. Deionized, doubly distilled water was employed. The eluents were filtered through a 0.2 um membrane before using.

METHODS

Chromatographic Conditions:

A Beckman 332 liquid chromatograph with an Altex 322 model integrator was used. The fixed wavelenght detector was set at 254 nm. and the sensitivity was set at 0.04 aufs. A 150 x 4.6 mm I.D. 5 nm Ultrasphere Cyano column (Beckman Instruments U.S.A.) was employed. Twenty microliters samples were injected. The flow rate was 1 mL/min. All determinations were performed at room temperature (23ºC).

Standard preparations:

Stock solutions containing 1 mg/mL of drugs were prepared in methanol.

Working solutions were obtained by appropiate dilution to get concentrations of: acetaminophen 10 ug/mL, indomethacin 10 ug/mL, diclofenac 20 ug/mL, nimesulide 20 ug/mL, betamethasone 10 ug/mL, terphenadine 150 ug/mL, bromhexine 20 ug/mL, pirenzepine 50 ug/mL, clenbuterol 20 ug/mL and astemizol 20 ug/mL.

Capacity Factors:

The capacity factors were calculated from the mean retention time of triplicate injections. A sample of 10% aqueous sodium nitrate was used to determine the column void volume.

RESULTS AND DISCUSSION

It has been reported (7-9) that on bonded-silica phases when minor water concentrations are used in the mobile phases, a demasking effect on the residual silanol groups is produced and as a consequence, a normal phase mode plays the predominant role in determining retention.

The experiments discussed here were carried out with organic aqueous eluents containing 70% (v/v) of methanol or more, considering pH values between 3 and 7.

Neutral drugs as betamethasone and acetaminophen were little retained (k' values minor to one) and no significant variations were observed when different pHs and organic modifier proportions were assayed (fig. 1 and 2). These observations suggest that solvophobic contribution to the overall retention appears to be minimal.

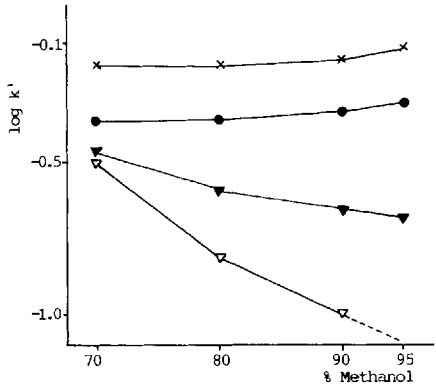

Figure 1 Plot of log k' versus volume percentage of methanol with phosphate buffer pH 7.0 (μ: 0.05). × betamethasone, ● acetaminophen, ▼ nimesulide, ▽ diclofenac and indomethacin.

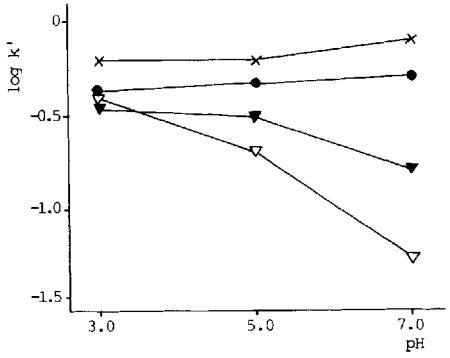

Figure 2 Plot of log k' versus phosphate buffer pH with the mobile phase methanol-phosphate buffer (95:5) (μ: 0.05). Drugs as in Fig. 1.

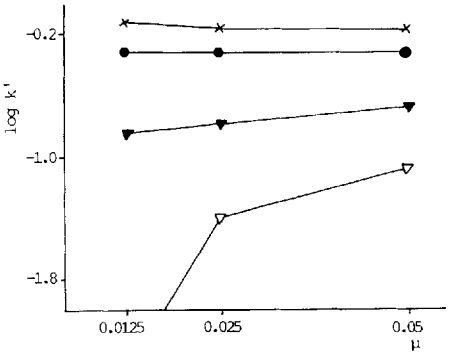

Figure 3 Plot of log k' versus ionic strength (μ) of the buffer pH 7.0 with the mobile phase methanol-phosphate buffer (90:10). Drugs as in Fig. 1.

Acidic drugs as diclofenac, indomethacin and nimesulide showed a marked decrease in the retention when pH increases (fig.2)

This effect is more pronounced yet with higher methanol proportions. Repulsion forces between negative charges of residual dissociated silanol groups and carboxilate ions of the drugs molecules are believed to be responsible of this behavior. At pH 7.0 or higher, when minor buffer concentrations are used, a decrease in the retention is produced (fig.3). Under these conditions the diminished masking effect of the buffer cations over the silanol groups probably produce a major repulsion. The above observations are in accordance with results previously reported (4).

Ionic strength has little influence on the retention of neutral drugs.

Fig. 4 shows the influence of the variation of pH in the retention of basic drugs when a mobile phase with 70% (v/v) of methanol is used. The increasing retention with pH is mainly the

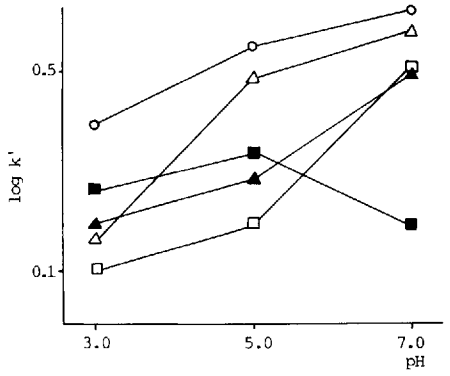

Figure 4 Plot of log k' versus phosphate buffer pH with the mobile phase methanol-phosphate buffer (70:30) (μ: 0.05). ○ astemizol, ■ bromhexine, ▲ terphenadine, △ pirenzepine, □ clenbuterol.

result of an ion-exchange interaction between dissociated surface silanols and protonated analites, at the range of pH values examined. However, bromhexine showed a different behavior which could be interpreted as a minor amine protonation grade at higher pH. The same observations were reported by Papp et al. (10) for aromatic amines in C18 alkyl silica bonded phases with methanol rich eluents.

When methanol proportion is set at 95% (fig. 5) basic drugs retentions decrease from pH 3 to pH 7. Under these conditions hydrogen-bonding interaction between amino groups of the analites and non dissociated silanol groups (11) would be expected to act as the principal mechanism of retention. These observations have been pointed out by other authors (12). As the pH increases this interaction diminishes. This is believed to be caused by the increasing of the silanol groups dissociation and the progressive

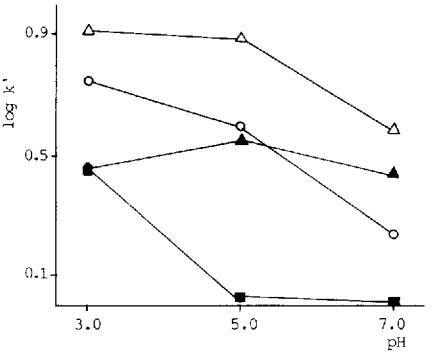

Figure 5 Plot of log k' versus phosphate buffer pH with the mobile phase methanol-phosphate buffer (95:5) (μ: 0.05). Drugs as Fig. 4.

minor protonation grade of the solutes, so a minor overall retention is observed. Under these conditions, the dielectric constant of the solvent diminishes progressively and thus, a minor silanols dissociation and a minor analites protonation are produced. Consequently the attractive electrostatic forces in the ion-exchange mechanism decrease.

However, at pH 7.0 and above 90% methanol concentration (fig.6) drugs retention increases. These observations could be interpreted as the result of a demasking effect over the silanol groups produced by the low water proportion in the mobile phases. This would suggest that an adsorption interaction becomes dominant.

Fig. 7 shows the influence of methanol concentration at pH 3.0. The pronounced increase in the retention of drugs between 80-95% (v/v) of methanol is probably due to non ionic interaction of the solutes with silanol groups. This has already been explained for pH 7.0 when methanol proportion above 90% was used. However at

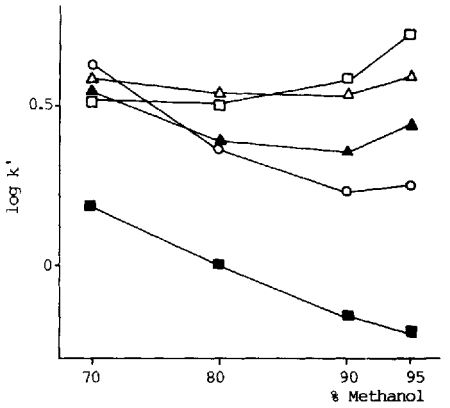

Figure 6 Plot of log k' versus volume percentage of methanol with phosphate buffer pH 7.0 (μ: 0.05). Drugs as in Fig. 4.

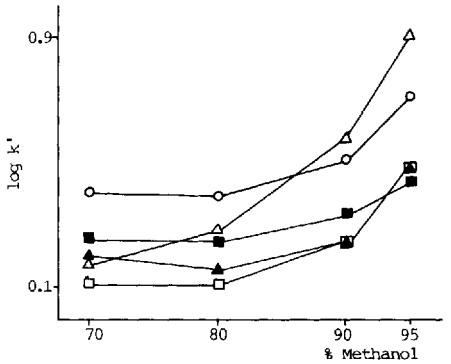

Figure 7 Plot of log k' versus volume percentage of methanol with phosphate buffer pH 3.0 (μ: 0.05). Drugs as in Fig. 4.

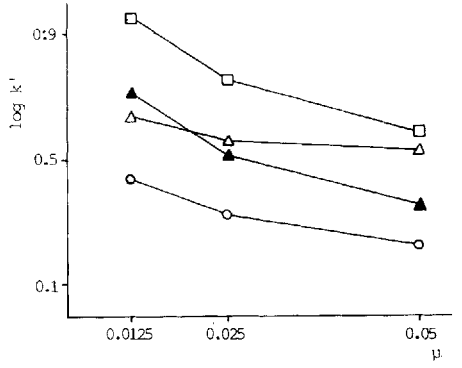

Figure 8 Plot of log k' versus ionic strength (μ) of the buffer pH 7.0 with the mobile phase methanol-phosphate buffer (90:10). Drugs as in Fig. 4.

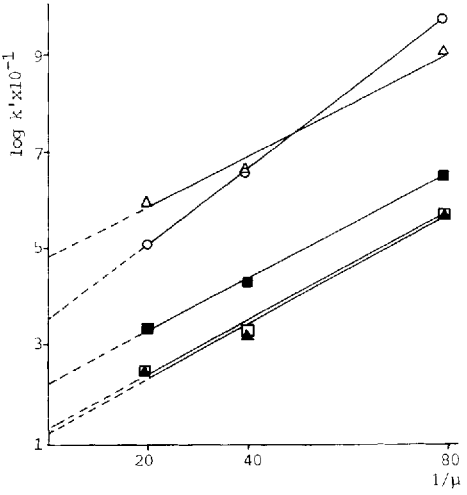

Figure 9 Plot of log k' versus 1/ionic strength of the buffer pH 3.0 with the mobile phase methanol-phosphate buffer (90:10). Drugs as in Fig. 4.

pH 3.0 the electrostatic interaction contribution to the retention is less pronounced, so no preliminar decrease in the plot is observed and different curve profiles are obtained respect to pH 7.0 (fig.6)

Fig. 8 represents the influence of the ionic strength of the buffer on the retention of the basic drugs studied. For all the pH values examined the retention of the solutes decreases with the increment of the ionic concentration because it is assumed that inorganic cations compete with protonated organic molecules for the active charged silanol sites.

The plot of log k' vs. the inverse of ionic strength of the buffer employed (fig.9), shows a typical lineal decrease of an ion exchange mechanism. But the positive intercept (at infinite buffer concentration) reveals that there is an additional non ionic retention mechanism though the ion exchange interaction has been eliminated. This behavior has also been observed in naked silica phases (12).

In sumary, the investigations on the retention modes for basic drugs on a cyano column, when high proportions of methanol are used, suggest that the silanophilic interactions play the main role and their effects would provide the particular characteristics on the chromatographic selectivity.

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