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# Study of the retention behaviour of barbiturates by overpressured layer chromatography using silica gel bonded with tricaprylmethylammonium chloride

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ABSTRACT

The chromatographic behaviour of twelve barbituric acid derivatives was studied by overpressured layer chromatography using silica gel impregnated with tricaprylmethylammonium chloride (TCMA). The retention of the barbiturates decreased with increasing TCMA concentration on the layer. The $R_F$ values increased with increasing methanol content of the eluent. The pH and the inorganic salt concentration in the eluent had no effect on the retention. Similar results were obtained earlier for different types of compounds, *e.g.*, amino- and nitrosalicylic acids, pyrimidine derivatives, penicillins, cephalosporins and tetracyclines. Several similarities have been observed between chromatographic processes performed either on silica gel layers or on a dynamically modified silica high-performance liquid chromatographic column with eluents containing an ion-pairing reagent. It has been shown that no ion pairing occurs on silica gel layers impregnated with TCMA. Hydrophobic interactions play an important role in the retention of the compounds. The retention is controlled by the TCMA adsorbed on the silica gel layer. The results of the best separations are given.

## INTRODUCTION

The use of quaternary ammonium compounds in chromatographic practice is widespread. For ion-pairing chromatographic separations of acid-type compounds, cetrimide (cetyltrimethylammonium chloride or bromide) is frequently used. Several papers have been published by Hansen and co-workers [1–7] in which high-performance liquid chromatography (HPLC) on a dynamically modified silica column was used with eluents containing cetrimide. It was shown that cetrimide binds to the silanol groups of the silica gel; at the same time, a proton dissociates and the silica surface is hydrophobized. This process takes place during the preliminary equilibration or development.

Szepesi and co-workers [8,9] extended the model of the dynamically modified silica column to experiments involving overpressured layer chromatography (OPLC). In the OPLC technique developed by Tyihák and co-workers [10–12], the layer acts as a "spread-out" column. Instead of the equilibration usually performed in HPLC, preliminary impregnation seemed to be useful with thin layers. The retention of organic acids, studied under these conditions by Szepesi *et al.* [8], was highly dependent on the ion-pairing reagent concentration of the solution used for impregnation of the layers.

In an earlier study, we used tricaprylmethylammonium chloride (TCMA) for the separation of different amino- and nitrosalicylic acids [13]. TCMA is known to be an ion-pairing reagent and is used for the preparation of ion-selective membrane electrodes, *e.g.*, salicylate electrodes [14–17]. With eluents containing methanol and water but not TCMA, the retention of salicylic acid derivatives increased with increasing TCMA concentration of the impregnating solution. The pH had no effect on the retention in the range 2–12 [13]. We observed a similar retention behaviour for basic pyrimidine derivatives (minoxidil and its intermediates) [18], penicillins and cephalosporins [19] and tetracyclines

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(unpublished results), which have amphoteric characteristics.

Our findings suggest that no ion pairing occurs on silica gel layers impregnated with TCMA. The adsorption of TCMA on the silica gel (this may also be chemisorption) exhibits a Langmuir-type isotherm [18]. With increasing amount of adsorbed TCMA, the surface of the silica gel becomes hydrophobic. At 0.1–0.2 *M* TCMA concentration in the impregnating solution, the quaternary ammonium compound forms a monolayer on the silica surface and the retention is controlled by hydrophobic interactions.

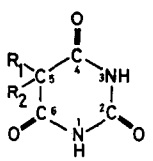
In this work, the retention behaviour of twelve barbituric acid derivatives (Fig. 1) was studied under similar conditions to those in the earlier work [13,18,19].

## EXPERIMENTAL

### *Chemicals and reagents*

Silica gel layers (Art. 5554) were obtained from E. Merck (Darmstadt, Germany). The layers were developed with methanolic TCMA solution overnight in an unsaturated normal chamber. Three sides of the plates were impregnated with Impres (Laboratory Instruments, Budapest, Hungary) as pretreatment for OPLC development.

TCMA was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical-reagent grade. Barbituric acid derivatives were of pharmacopoeial quality and were kindly provided by Alkaloida and Chinoin Pharmaceutical Works (Tiszavasvári and Budapest, Hungary, respectively). Portions of 1 $\mu$l of 5 mg ml$^{-1}$ ethanolic solutions of barbiturates were applied.



barbituric acid

| derivatives | $R_1$ | $R_2$ |
|---|---|---|
| amobarbital | ethyl | isopenthyl |
| aprobarbital | allyl | isopropyl |
| barbital | ethyl | ethyl |
| butobarbital | ethyl | butyl |
| crotylbarbital | ethyl | crotyl |
| cyclobarbital | ethyl | cyclohexenyl |
| diallyl barbital | allyl | allyl |
| hexobarbital | cyclohexenyl | methyl , 3-methyl |
| pentobarbital | ethyl | 1-methylbutyl |
| phenobarbital | ethyl | phenyl |
| proxibarbal | allyl | $\beta$-hydroxypropyl |

Fig. 1. Structures of the studied barbituric acid derivatives.

### *Apparatus*

A Chrompres$^{25}$ OPLC instrument (Laboratory Instruments) was used for the development of the chromatograms at a membrane pressure of 20 bar and a flow-rate of 1 ml min$^{-1}$. The spots were detected under a UV lamp at 254 nm. For the determination of TCMA adsorbed on the layer, Fourier transform IR (FT-IR) spectrometry was used [19].

## RESULTS AND DISCUSSION

### *Effects of TCMA concentration on retention*

The effects of the TCMA concentration in the impregnating solution on the retentions of the barbiturates are shown in Fig. 2. The retentions increased with increasing TCMA concentration when eluents containing methanol and water were used. For pentobarbital, a significant increase in retention was observed at 0.01 *M* TCMA (Fig. 2A), whereas the retentions of the other compounds increased at 0.05 *M* or higher TCMA concentrations. The retention *vs.* TCMA concentration curves are very similar, and at 0.1–0.2 *M* TCMA concentrations only small differences exist in the retentions. In the studied TCMA concentration range, the smallest retention change was observed for proxibarbal (Fig. 2A).

These retention *vs.* TCMA concentration curves (Fig. 2) are very similar to those measured for barbiturates on a dynamically modified silica column with eluents containing cetrimide [20]. At a given cetrimide concentration, proxibarbal and barbital had the smallest and phenobarbital, pentobarbital and amobarbital the highest $k'$ values. This similarity indicates that the retention mechanism is sim-

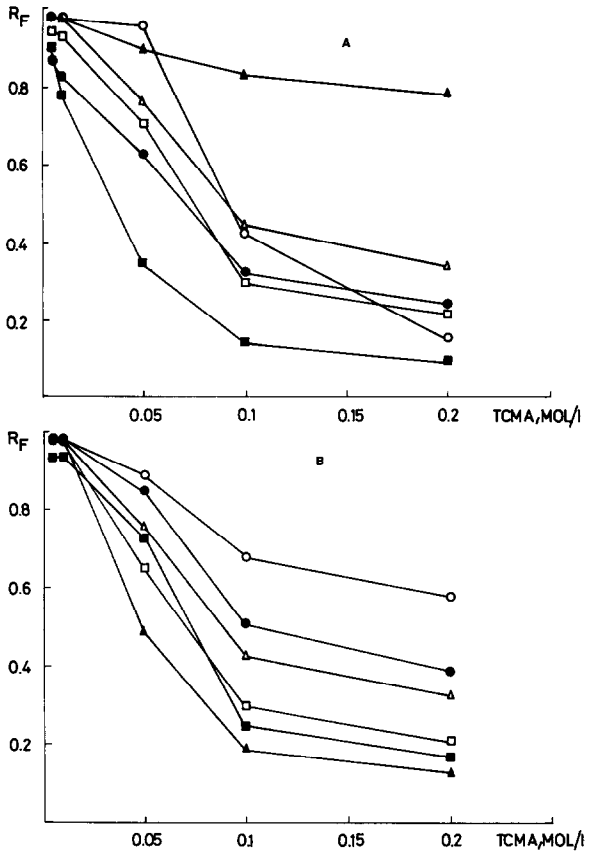



Fig. 2. Effect of TCMA concentration on retention. Eluent: methanol–water (30:70, v/v). (A) ○ = Barbituric acid; ● = hexobarbital; □ = cyclobarbital; ■ = pentobarbital; △ = crotylbarbital; ▲ = proxibarbal. (B) ○ = Barbital; ● = diallylbarbital; □ = butobarbital; ■ = phenobarbital; △ = aprobarbital; ▲ = amobarbital.

ilar on silica gel layers impregnated with TCMA and on a dynamically modified silica column with eluents containing cetrimide, in spite of the fact that there is a difference between the alkyl moieties of the quaternary ammonium compounds. Cetrimide contains three methyl groups and one cetyl group and TCMA contains three capryl groups and one methyl group.

We also used eluents containing cetrimide at pH 10, where all the barbituric acid derivatives are fully dissociated. The chromatograms were developed on silica gel layers impregnated with 0.1 *M* TCMA. There was no difference between the retentions measured in the presence or the absence of cetrimide; it is possible that stronger interactions occur between the compounds and TCMA adsorbed on the silica surface than with cetrimide in the eluent.

*Effects of methanol content of the eluent*

With increasing methanol content of the eluent (from 10 to 50%), the retentions of the barbituric acid derivatives decreased (Fig. 3). On layers impregnated with 0.05 *M* TCMA, the retentions of some derivatives (barbituric acid, barbital and diallylbarbital) did not decrease with increasing methanol content, and the others showed only small differences compared with the results obtained on layers impregnated with 0.1 or 0.2 *M* TCMA. This can be explained by the fact that at 0.05 *M* or lower impregnating solution concentration not all the silanol groups of the silica gel are completely covered by TCMA, and the barbitals interact both with the silanol groups and with the capryl groups.

The same tendency was observed for amino- and nitrosalicylic acids [13], pyrimidine derivatives [18], penicillins and cephalosporins [19] and tetracyclines (unpublished results). Gazdag *et al.* [21] studied the retention characteristics of different organic acids on a dynamically modified silica column with eluents containing cetrimide. The $k'$ values decreased significantly at methanol concentrations higher than 40%. Their measurements in methanol–water showed that the adsorption of cetrimide on silica gel exhibited a maximum at about 50% methanol content. Gazdag *et al.* [21] suggested that the explanation for this is that cetrimide is adsorbed on the silica surface in two ways: by means of a silanophilic interaction and by ion exchange with the proton of the silanol group. The effect of the former process becomes stronger with increasing organic solvent content of the eluent, whereas that of the latter decreases.

On silica gel layers impregnated with TCMA, we could not perform direct experiments to study the correlation between the amount of adsorbed TCMA and the methanol content of the eluent. TCMA is an oily compound immiscible with water and methanol–water mixtures of low methanol content. Therefore, "indirect" investigations were made: the silica gel layers were impregnated with 0.1 *M* TCMA and the layers were then developed by means of OPLC, using eluents with different methanol contents (15–75%). The amount of adsorbed TCMA was measured by means of FT-IR spectrometry.

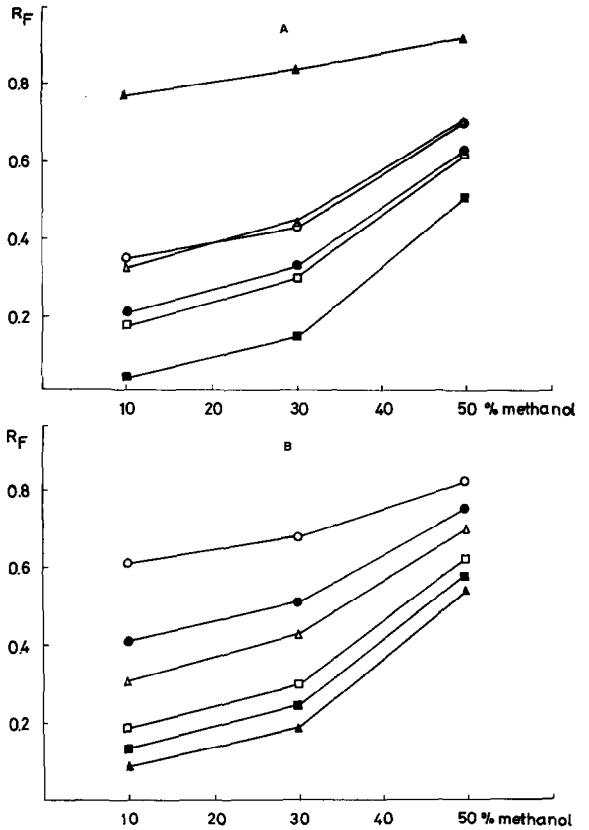



Fig. 3. Effect of methanol content of the eluent on retention. Silica gel impregnated with 0.1 *M* TCMA. Symbols in (A) and (B) as in Fig. 2.

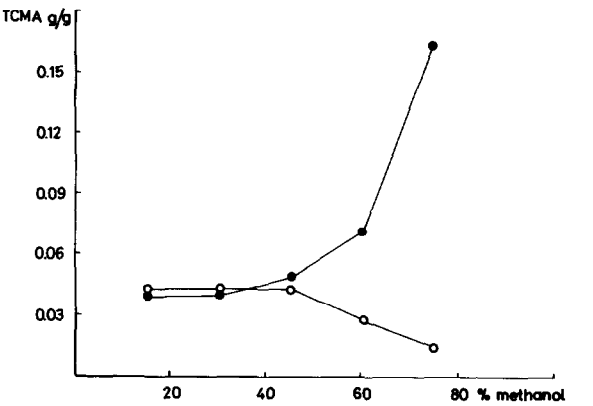



Fig. 4. Effect of methanol content of eluent on TCMA adsorbed on silica gel impregnated with 0.1 *M* TCMA. ○ = Near the start; ● = near the front.

In our opinion, this "indirect" method is better in OPLC or thin-layer chromatographic (TLC) developments, because it reflects the true situation existing during the chromatographic process. The results of the experiments can be seen in Fig. 4. With increasing methanol content of the eluent the amount of adsorbed TCMA, removed from near the start, decreases whereas that near to the front increases. With methanol–water mixtures of high methanol content, most of silanol groups became free again, and the hydrophobic interactions existing between the capryl groups and barbiturates diminished.

*Effects of pH and inorganic salt concentration in the eluent on retention*

The effect of the pH of the eluent on the retention of barbituric acid derivatives was investigated at pH 2 and 10, to study the chromatographic behaviour of undissociated species (for barbiturates, $pK_a \approx 3$–7) and dissociated species. It was found that the pH of the eluent had no effect on the retention; it makes no difference to the retention of the dissociated or undissociated form of a given compound. The same finding was obtained with amino- and nitrosalicylic acids [13], pyrimidine derivatives [18], penicillins and cephalosporins [19] and tetracyclines (unpublished results). During their experiments on reversed-phase ($C_2$-bonded) high-performance TLC layers impregnated with cetrimide and other quaternary ammonium compounds (tetrabutylammonium chloride, tetramethylammonium chloride), Tomkinson *et al.* [22] also observed that the solvent pH was not an important factor in the determination of the final retentions of dihydroxybenzoic acids.

It was found by Hansen and co-workers [2,4,7] that the selectivity of the chromatographic techniques based on a dynamically modified silica column was independent of the pH of the silica surface. In their opinion, the presence of cetrimide and buffer in the eluent can control the surface characteristics. Gazdag *et al.* [21] measured the adsorption of cetrimide on silica, and observed that cetrimide did not adsorb on the silica surface at low pH. Above pH 4, the extent of adsorption suddenly increased. This is in accordance with the dissociation

TABLE I

$R_s$ VALUES OF BARBITURATES ON SILICA GEL LAYERS IMPREGNATED WITH 0.05 *M* TCMA

Eluent: methanol–water (30:70) containing 0.25 *M* sodium chloride. Abbreviations: bacid = barbituric acid; barb = barbital; diallyl = diallylbarbital; buto = butobarbital; pheno = phenobarbital; hexo = hexobarbital; cyclo = cyclobarbital; pento = pentobarbital; crotyl = crotylbarbital; apro = aprobarbital; amo = amobarbital; proxi = proxibarbal.

| Compounds | $R_s$ | Compounds | $R_s$ | Compounds | $R_s$ | Compounds | $R_s$ | Compounds | $R_s$ |
|---|---|---|---|---|---|---|---|---|---|
| bacid/barb | 2.5 | barb/pheno | 10.9 | diallyl/crotyl | 1.2 | pheno/cyclo | 0.6 | cyclo/apro | 3.0 |
| bacid/diallyl | 5.1 | barb/hexo | 9.4 | diallyl/apro | 1.8 | pheno/pento | 2.3 | cyclo/amo | 2.6 |
| bacid/buto | 8.5 | barb/cyclo | 9.2 | diallyl/amo | 8.1 | pheno/crotyl | 4.5 | cyclo/proxi | 11.8 |
| bacid/pheno | 8.3 | barb/pento | 10.4 | diallyl/proxi | 8.6 | pheno/apro | 3.9 | pento/crotyl | 5.7 |
| bacid/hexo | 7.5 | barb/crotyl | 6.2 | buto/pheno | 0.3 | pheno/amo | 2.2 | pento/apro | 5.3 |
| bacid/cyclo | 7.5 | barb/apro | 6.8 | buto/hexo | 1.4 | pheno/proxi | 13.5 | pento/amo | 0.7 |
| bacid/pento | 8.4 | barb/amo | 13.5 | buto/cyclo | 0.3 | hexo/cyclo | 0.8 | pento/proxi | 12.5 |
| bacid/crotyl | 5.8 | barb/proxi | 4.7 | buto/pento | 2.7 | hexo/pento | 3.5 | crotyl/apro | 0.6 |
| bacid/apro | 6.1 | diallyl/buto | 5.9 | buto/crotyl | 4.6 | hexo/apro | 2.4 | crotyl/amo | 6.9 |
| bacid/amo | 9.7 | diallyl/pheno | 5.7 | buto/apro | 4.0 | hexo/amo | 3.9 | crotyl/proxi | 9.7 |
| bacid/proxi | 0.4 | diallyl/hexo | 4.2 | buto/amo | 2.7 | hexo/proxi | 12.3 | apro/amo | 6.3 |
| barb/diallyl | 4.9 | diallyl/cyclo | 4.6 | buto/proxi | 14.3 | cyclo/pento | 2.6 | apro/proxi | 10.2 |
| barb/buto | 11.6 | diallyl/pento | 6.6 | pheno/hexo | 1.6 | cyclo/crotyl | 3.5 | amo/proxi | 15.8 |

of the silanol groups. The anionic sites readily bind the cationic cetrimide, whereas at acidic pH the binding of proton is favourable.

Our findings with different types of compounds [13,18,19] suggest that the desorption of TCMA on silica gel is almost independent of the pH [19], so the retention seems to be unchanged.

With eluents containing 0.05–0.25 *M* of sodium chloride and 30% of methanol, the retentions of the barbiturates did not change on silica gel layers impregnated with 0.1 *M* TCMA. There was no difference between the retentions measured as a function of the salt concentration at pH 2 and 10. The diameter of the spots decreased an in parallel the efficiency of the chromatographic process became better with increasing salt concentration in the eluent.

*Separation of barbituric acid derivatives*

On the basis of the above results, it was found that on silica gel layers impregnated with TCMA the retention is controlled by the surface concentration of TCMA adsorbed on the silica gel. It is controlled directly via the concentration of the impregnating methanolic TCMA solution (adsorption), and indirectly via the methanol content of the eluent (desorption).

The results of the best separations on silica gel impregnated with 0.05 *M* TCMA, with an eluent containing 30% of methanol and 0.25 *M* of NaCl, are given in Table I. The $R_s$ values (resolution) are $<1$ in several instances, but barbituric acid can be separated from proxibarbal ($R_s = 2.1$) and hexobarbital from cyclobarbital ($R_s = 1.6$) with an eluent containing 10% of methanol and 0.25 *M* of NaCl on silica gel impregnated with 0.05 *M* TCMA. The other derivatives which have $R_s$ values $<1$ in Table I cannot be separated.

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