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Separation of minoxidil and its intermediates by overpressured layer chromatography using a stationary phase bonded with tricaprylmethylammonium chloride

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ABSTRACT

The retention behaviour of minoxidil (2,4-diamino-6-piperidinopyrimidine-3-oxide) and its intermediates (2,4-diamino-6-hydroxypirimidine, 2,4-diamino-6-chloropyrimidine and 2,4-diamino-3-N-oxo-6-chloropyrimidine) was studied by using silica gel layers impregnated with tricaprylmethylammonium chloride (TCMA). The retention of the compounds increases with increasing concentration of TCMA adsorbed on the silica gel. The pH and the ionic strength of the eluents do not affect the retention at all. The retention of the solutes decreases with increasing methanol content of the eluent, because of the TCMA-desorbing effect of methanol. On the basis of these and earlier findings, it was concluded that no ion-pairing occurs during the separation. A monolayer is formed on the silica surface at 0.1–0.2 *M* TCMA concentration in the impregnating solution, and hydrophobic interactions play an important role in the separation mechanism.

INTRODUCTION

The use of quaternary ammonium salts for the separation of different compounds in high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) is widespread. Tricaprylmethylammonium chloride (TCMA, Aliquat 336S), which has been applied as an active component of liquid membrane ion-selective electrodes, *e.g.*, salicylate-selective electrodes [1–4], proved very useful in the separation of different amino- and nitrosalicylic acids [5]. The chromatographic layers were impregnated with a methanolic solution of TCMA and the eluent did not contain any TCMA. Our further experience led us to study the retention characteristics of different basic compounds, *e.g.*, minoxidil (2,4-diamino-6-piperidinopyrimidine-3-oxide, Min) and its intermediates (2,4-diamino-6-hydroxypyrimidine, 6-OH; 2,4-diamino-6-chloropyrimidine, 6-Cl; and 2,4-diamino-3-N-oxo-6-chloropyrimidine, 3-N-oxo) (Fig. 1).

For development of the chromatograms, overpressured layer chromatography (OPLC), developed by Tyihák and co-workers [6–8], was used. Using OPLC one run takes only a few minutes, which is very important for in-process control, *e.g.*, in the production of minoxidil via its intermediates.

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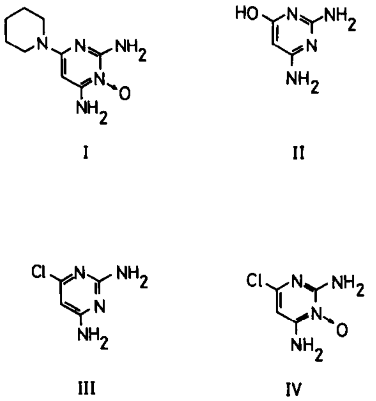

Fig. 1. Structures of minoxidil (I) and its intermediates: 2,4-diamino-6-hydroxypyrimidine (II), 2,4-diamino-6-chloropyrimidine (III) and 2,4-diamino-3-N-oxo-6-chloropyrimidine (IV).

This work was concerned with the separation of the above-mentioned compounds by using silica gel layers impregnated with TCMA; in addition, we attempted to elucidate the mechanism of the separation mode.

EXPERIMENTAL

Apparatus

For development of the chromatograms, a Chrompres[25] overpressured layer chromatograph (Labor Instruments, Budapest, Hungary) was used, at a membrane pressure of 20 bar and a flow-rate of 1 ml $\min^{-1}$. For TCMA adsorption studies, an OK 102/1 conductivity meter (Radelkis, Budapest, Hungary) was used. The spots were revealed under a UV lamp at 254 nm.

Chemicals and reagents

Silica gel plates were obtained from E. Merck (Darmstadt, Germany). Three sides of the plates were impregnated with Impres (Labor Instruments) for OPLC development and the layers were developed with methanolic TCMA overnight.

TCMA (Aliquat 336S) was purchased from Fluka (Buchs, Switzerland). Minoxidil and its intermediates were laboratory-made products of recrystallized quality; 1 μl of a 2 mg ml^{-1} methanolic solution was spotted. Methanol was of HPLC quality from E. Merck. All other chemicals were of analytical-reagent grade and were used as received.

RESULTS AND DISCUSSION

Retention on silica gel layers bonded with TCMA

The silica gel layers were impregnated with methanolic TCMA at concentrations from 0.005 to 0.2 *M* overnight. After evaporation of the methanol, the sample substances were spotted and the chromatograms were developed by OPLC as described under Experimental.

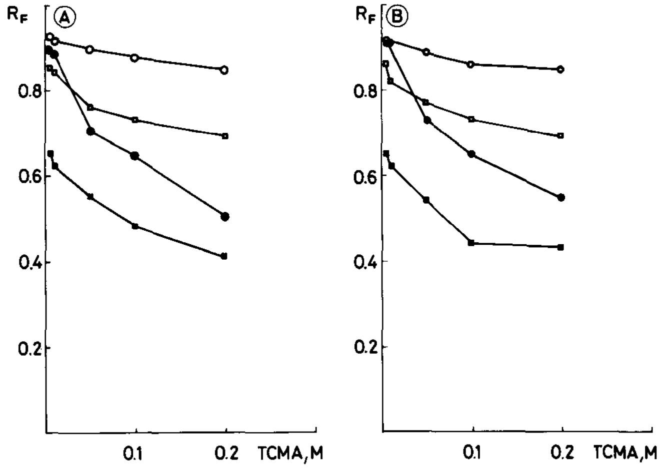

Fig. 2. Effect of TCMA concentration of impregnating solution on retention with methanol–aqueous Britton–Robinson buffer (50:50) eluent: (A) at pH 2.39; (B) at pH 9.40 (■) I; (○) II; (●) III; (□) IV (compound numbers as in Fig. 1).

Fig. 2 shows the effect of the TCMA concentration on the retention of minoxidil and its intermediates at pH 2.39 and 9.40. The eluent was aqueous Britton–Robinson buffer (0.05 *M*)–methanol (50:50). With increasing TCMA concentration, the retention increases for all the derivatives. Good separation can be achieved from 0.1 *M* TCMA. The efficiency of the separation (calculated in HETP values) is of the same order of magnitude for all the compounds as with 2.5% diethylamine on reversed-phase layers. It is notable that the HETP values are 2–3 times larger with 0.2 *M* than with 0.1 *M* TCMA. The pH of the eluent does not affect the retention.

Fig. 3A illustrates the effect of the methanol concentration on the retention.

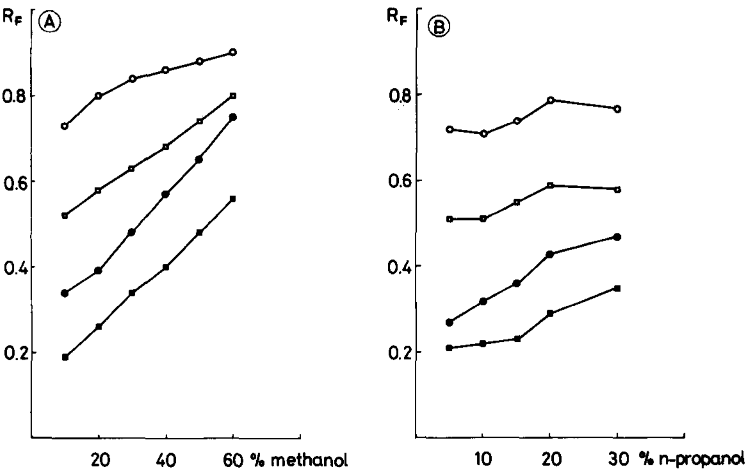

Fig. 3. Effects of (A) methanol and (B) *n*-propanol content of the eluent on retention on silica gel layers impregnated with 0.1 *M* TCMA. Symbols as in Fig. 2.

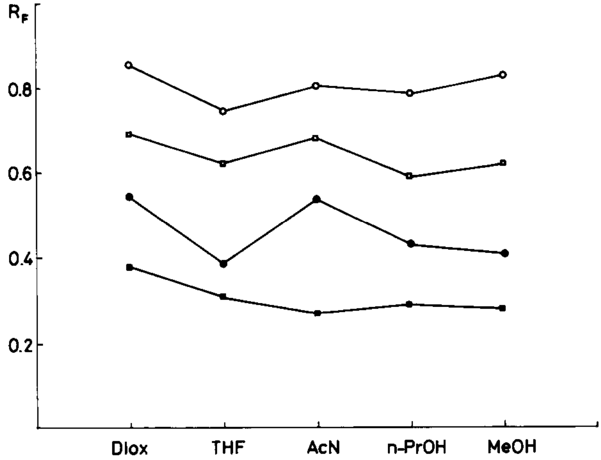

Fig. 4. Effects of different organic solvents on retention on silica gel layers impregnated with 0.1 *M* TCMA in eluents containing 80% water. Symbols as in Fig. 2. Diox = Dioxane; THF = tetrahydrofuran; ACN = acetonitrile; n-PrOH = *n*-propanol; MeOH = methanol.

The silica gel was impregnated with 0.1 *M* TCMA and the pH of the eluent was 2.39. From 10 to 60% methanol, the retention decreases almost linearly with increasing methanol content. With *n*-propanol instead of methanol, the retention of all the compounds decreases only slightly from 5 to 30% *n*-propanol content (Fig. 3B). At 30% and higher *n*-propanol concentrations, the shape of the spots becomes irregular. With other solvents instead of methanol, in a ratio of 8:2 with aqueous buffer solution at pH 2.39, the retention changes according to Fig. 4. No pronounced effect was observed in any solvent studied.

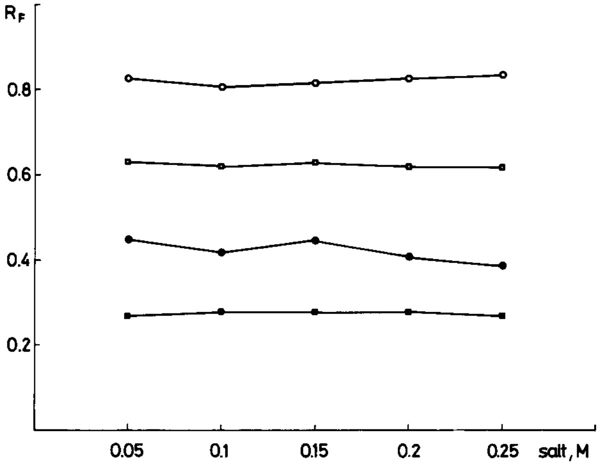

Fig. 5. Effects of ionic strength on retention on silica gel layers impregnated with 0.1 *M* TCMA in methanol–water (20:80) eluent. Symbols as in Fig. 2.

The ionic strength of the eluent, which was adjusted with Britton–Robinson buffer (0.05 *M* constant concentration) plus sodium chloride in various concentrations, does not affect the retention (Fig. 5).

For practical use, the best conditions are a silica gel layer impregnated with 0.1 *M* methanolic TCMA with eluents containing 10–50% of methanol and 90–50% of water. The R_s values are as follows with an eluent containing 10% of methanol: Min/6-OH = 7.94, Min/6-Cl = 2.50, Min/3-N-oxo = 4.83, 6-OH/6-Cl = 7.36, 6-OH/3-N-oxo = 3.50 and 6-Cl/3-N-oxo = 3.36; and with an eluent containing 50% of methanol: Min/6-OH = 5.67, Min/6-Cl = 2.77, Min/3-N-oxo = 3.76, 6-OH/6-Cl = 3.58, 6-OH/3-N-oxo = 2.11 and 6-Cl/3-N-oxo = 1.35. The spots are compact and the presence of TCMA does not affect the UV absorption characteristics of minoxidil and its intermediates.

On impregnation of the silica gel layers with 0.1 *M* cetrimide (cetyltrimethylammonium bromide) instead of 0.1 *M* TCMA, the R_F values of the compounds are similar, or slightly lower on the cetrimide-bonded layers with Min and the 6-OH derivative (Fig. 6). The compactness of the spots and the efficiency are the same with either TCMA or cetrimide.

Several experiments were performed on RP-18 reversed-phase layers. Using eluents containing 60–95% of methanol, Min can be separated from its intermediates, but the intermediates cannot be separated from each other. In acetonitrile–water eluents a 10–30% water content is suitable for the separation of all the compounds. The efficiency is greatest with a 30% water content. At lower water contents the spot diameters are large, indicating interactions between the solutes and the residual silanol groups. The presence of diethylamine or triethylamine in the eluent affects the efficidency only with Min, especially above 1.5%. The large elongated spots of the intermediates are not suitable for quantitative measurements.

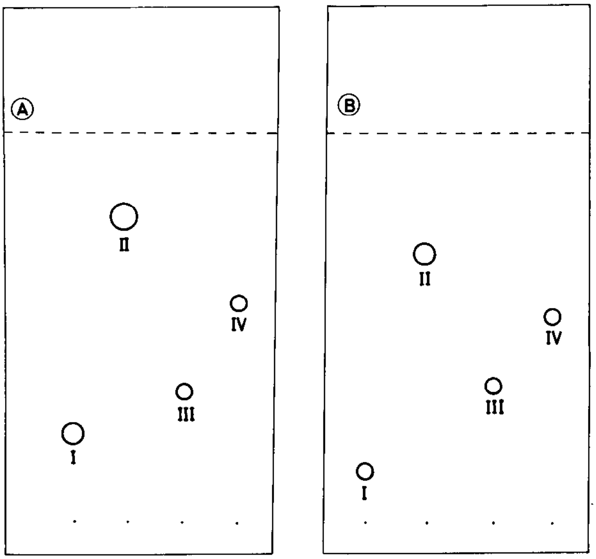

Fig. 6. Retention of minoxidil and its intermediates on silica gel layers impregnated with (A) 0.1 *M* TCMA and (B) 0.1 *M* cetrimide in methanol–water (20:80) eluent. Compound numbers as in Fig. 1.

Adsorption and desorption characteristics of TCMA on silica gel layers

Szepesi and co-workers [9–11] discussed in detail the adsorption behaviour of cetrimide on different layers. They concluded that the relative retention of different acidic compounds was highly dependent on the reagent concentration of the solution used for the immersion of the layers.

Tomkinson *et al.* [12] studied the retention behaviour of several dihydroxybenzoic acids on reversed-phase (C_2-bonded) HPTLC plates impregnated with tetra-*n*-butylammonium bromide (TBA), tetramethylammonium bromide (TMA) or cetrimide. Coating the plate with TBA or cetrimide gave the best chromatographic results, but these were largely ineffective in the eluent. TMA, in contrast, provided satisfactory results only when it was present in the eluent.

Our earlier investigations [5] with different amino- and nitrosalicylic acids on silica gel layers impregnated with TCMA gave the same results, *i.e.*, the retention was dependent on the TCMA concentration in the impregnating solution. In our case the eluent did not contain any TCMA. The pH of the eluent had no effect on the retention. Tomkinson *et al.* [12] also observed that the solvent pH is not an important factor in determining the final R_F of dihydroxybenzoic acids. This result was surprising in comparison with ion-pairing HPLC separation, where the effective concentrations of the ion-pairing reagent used are much lower and the pH plays a dominant role in the control of the retention.

On the basis of these findings, it seems that no ion pairing occurs during the separation. The separation mechanism is closely connected with the adsorption–desorption characteristics of the quaternary ammonium compound on the layer; further, there are interactions between the adsorbed quaternary ammonium compound and the solute.

To clarify this question, we determined the adsorbed TCMA concentrations on silica gel (and other) layers. For this purpose, a calibration graph was constructed on the basis of the conductivity of the methanolic TCMA solutions. The graph is linear from 0 to 0.01 *M* TCMA. The layers were impregnated with methanolic TCMA overnight. After drying, the silica gel was scraped off the plates along the horizontal axis in 1-cm strips. One strip was taken from near the start-line, another from the middle of the layer and the last from near the solvent front. The strips were then treated with 30–40 ml of methanol under reflux on a water-bath for 2 h. After cooling, the volume of the suspension in a volumetric flask was diluted to 50 ml with methanol and the conductivity was measured. Four TCMA concentrations (from 0.05 to 0.3 *M*) were used for the experiments. There was no difference between the conductivities of the strips taken from different sites of the layer. This means that no TCMA concentration gradient exists along the layer after the impregnation; a uniform bonding is formed on the layer.

The relationship between the concentration of the impregnating solution and the amount of TCMA adsorbed calculated in grams per gram of sorbent is illustrated in Fig. 7. The adsorbed TCMA concentration increases almost linearly at impregnating solution concentrations up to 0.1 *M*. Above 0.1 *M*, a slight increase can be observed. This is in accordance with the TCMA concentration (in the impregnating solution) *vs.* R_F values (Fig. 2). The quaternary ammonium compound is bonded to the silanol groups, and it is very likely that at a certain impregnating solution concentration a TCMA monolayer is formed on the silica gel surface. This concentration

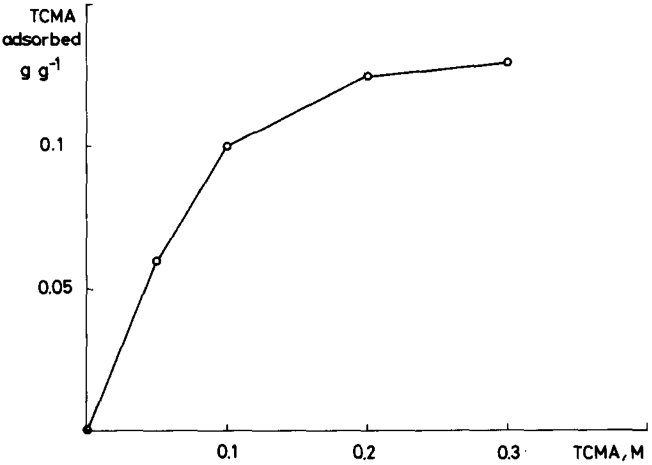

Fig. 7. Amount of TCMA adsorbed on silica gel layer (g g^{-1}) *vs*. TCMA concentration of impregnating solution.

seems to be between 0.1 and 0.2 *M*. As a consequence, the polarity of the surface decreases and the retention of the solutes increases. A hydrophobic interaction can be taken into account between the capryl groups and the solutes. This is why the pH of the eluent has no effect on the retention.

Another question to be clarified is the retention-decreasing effect of methanol or *n*-propanol (Fig. 3). When the TCMA impregnating layers are developed with methanol–water eluents of different compositions and the TCMA concentration is measured on the layers, a concentration gradient can be observed. At the start-line, strong TCMA desorption occurs and the amount of TCMA remaining on the layer is dependent on the eluent composition. As expected, the concentration of the retained TCMA is the lowest at methanol–water (75:25), and it is about twice as high if the eluent contains 25% of methanol. This explains the characteristic R_F *vs*. methanol content curves (Fig. 3).

Near the front-line, TCMA accumulates on the layer, which is why a linear relationship does not exist for the R_F *vs*. methanol concentration curves for the 6-OH derivative. Furthermore, band broadening can be observed at high methanol concentrations (60% or more). This means that a silanophilic interaction is preferred with increasing methanol content in the eluent. The desorption of the quaternary ammonium compound makes the silanol groups free. The same processes can be taken into account on layers impregnated with other quaternary ammonium salts in comparable concentrations. The retention difference observed between cetrimide and TCMA-impregnated layers with Min and the 6-OH derivative is due to the special individual interaction between the solute and the cetyltrimethyl (cetrimide) and tricaprylmethyl (TCMA) groups. For a given quaternary ammonium compound, this individual interaction depends on the polarity of the solute.

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