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# REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF ORGANIC ACIDS ON SILICA WITH CETRIMIDE AS CATIONIC ION-PAIR REAGENT

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## SUMMARY

Adsorption isotherms on bare non-bonded silica were determined with eluents containing methanol or acetonitrile as organic solvent in various concentrations, aqueous phosphate buffer (10 m*M*, pH 5.0) and cetrimide as ion-pair reagent. When methanol was used in the eluent, the ion-pair reagent concentration in the stationary phase was highly dependent on the methanol concentration, and at higher cetrimide concentrations in the mobile phase (50–100 m*M*) its change with methanol concentration is a curve with a maximum. The highest stationary phase coating can be achieved with 50% methanol, and in this range (40–60% methanol) the adsorption isotherms seem to follow the Langmuir equation. Using acetonitrile as organic solvent, the adsorbed cetrimide concentration rapidly decreases with increasing organic solvent concentration. The influence of the concentration of ion-pair reagent, both in the mobile and stationary phase, as well as that of the nature and concentration of organic solvent used in the eluent, on the retention of fifteen organic acids (sulphonic acids, aromatic carboxylic acids, phenols) were investigated in detail.

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## INTRODUCTION

Reversed-phase ion-pair chromatography on bare (non-bonded) silica is a technique as old as ion-pair chromatography on chemically bonded phases. Both systems were reported at the same time by Knox and Laird[1]. Because of the increasing popularity of the latter technique, the use of bare silica for ion-pair systems has been considered less effective, and the importance of ion-pair chromatography on silica columns has seemed to increase again only in the last few years. Since 1979, several papers have appeared dealing with reversed-phase ion-pair chromatography on silica, dynamically modified by different surfactants, dissolved in the eluent[2–12]. The work of Hansen and co-workers is quite significant in this field. They investigated the effects of the nature and concentration of different quaternary ammonium salts on retention[8,9], as well as that of pH, ionic strength and buffer concentration on the selectivity of the separation of different ionic and non-ionic compounds[11,12]. The modification of various silica packings with cetrimide has also been studied in detail[9,10].

Our recent papers[13,14] have introduced the ion-pair formation and separation

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of fifteen different organic acids on silica. The optimal conditions for high-performance liquid chromatographic (HPLC) experiments have previously been determined by over-pressurized thin-layer chromatography. In a continuation of our recent study, the main aim of the present work was to determine the adsorption isotherms on bare silica when methanol and acetonitrile are used as organic solvents and cetrimide as the ion-pair reagent in the eluent. The purpose of our investigations was also a study of the effects of the organic solvent concentration and pH of the eluent on the adsorption characteristics of cetrimide and on the retention data for organic acids.

## EXPERIMENTAL

A Liquochrom Model 2010 high-performance liquid chromatograph (Labor MIM, Esztergom-Budapest, Hungary), equipped with variable-wavelength UV (Labor MIM) and refractive index (RI) detectors (Waters Assoc.), loop-type injector and dual-channel recorder Type 185 (Kutesz, Budapest, Hungary) was used for the experiments.

The adsorption isotherms were determined from the breakthrough curves, according to the method of Bartha and Vigh[15]. Following each adsorption experiment the column was brought to the initial state by elution with methanol, containing 0.1 *M* phosphoric acid. The phosphoric acid was eliminated by washing the column with methanol, and prior to the next experiments the column was equilibrated with the eluent without ion-pair reagent.

The separations were performed on a LiChrosorb Si 60, 10-μm (250 × 4.6 mm I.D.) column (Chrompack, Middelburg, The Netherlands). The eluents were prepared from HPLC-grade methanol or acetonitrile and aqueous phosphate buffer solution (pH = 5.0), containing various amounts of cetrimide. The buffer solution was prepared from 0.1 *M* phosphate buffer (pH 5.0). The final buffer concentration of

TABLE I

COMPOUNDS INVESTIGATED

| *No.* | *Name* |
|---|---|
| 0 | 2-Chloronicotinic acid |
| 1 | Nicotinic acid |
| 2 | Isonicotinic acid |
| 3 | Salicylic acid |
| 4 | Acetylsalicylic acid (*o*-acetoxybenzoic acid) |
| 5 | Niflumic acid |
| 6 | Veratric acid |
| 7 | Phenol |
| 8 | Cresol (mixture of *ortho*- and *para*-isomers) |
| 9 | Resorcinol |
| 10 | Pentachlorophenol |
| 11 | 5-Sulphosalicylic acid |
| 12 | *p*-Toluenesulphonic acid |
| 13 | Benzenesulphonic acid |
| 14 | 1,4-Naphthoquinone-4-sulphonic acid |

each eluent was constant, 0.01 $M$. The pH of the eluent was controlled before use by adjusting it, if necessary, to the required value with phosphoric acid or sodium hydroxide solution. The eluents were filtered and degassed prior to chromatography. The flow-rate was 1.0 ml/min. The compounds were detected at 260 nm.

All reagents were of analytical grade and were obtained from Reanal (Budapest, Hungary). The compounds investigated are listed in Table I.

## RESULTS AND DISCUSSION

### *Effect of methanol concentration on the adsorption isotherms of cetrimide*

The amount of the adsorbed cetrimide was determined for six different meth-

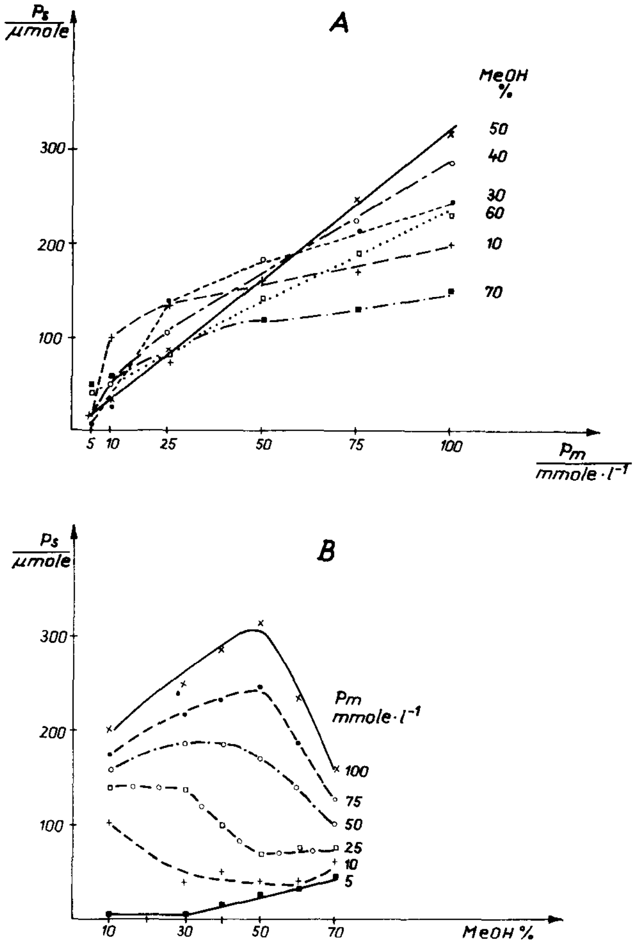

Fig. 1. Adsorption isotherms of cetrimide on silica from methanol (MeOH)-containing eluents (A) and stationary-phase concentration of cetrimide, $P_s$, as a function of the methanol concentration of the eluent (B) for various cetrimide concentrations ($P_m$). Column: LiChrosorb Si 60, 10 µm (250 × 4.6 mm I.D.). Eluent: methanol-aqueous phosphate buffer (0.02 $M$, pH = 5.0), flow-rate 1 ml/min. Detection: RI, temperature 25°C.

anol concentrations. The adsorption isotherms in various methanol–buffer eluents are shown in Fig. 1A. It may be seen that the amount of cetrimide adsorbed is highly dependent on the methanol concentration; the highest value can be achieved at 50% methanol. The isotherm data can be analyzed by keeping the mobile phase ion-pair concentration constant and plotting the stationary phase concentration against the methanol content of the eluent as shown in Fig. 1B. It is seen that when the eluent contains more than 50 m*M* cetrimide, the dependence, of the centrimide concentration in the stationary phase, $P_s$, on the methanol content of the eluent can be described by a curve with a maximum. When lower centrimide concentrations are used in the eluent, the dependence is more complicated, and at 5 m*M* the amount of adsorbed centrimide increases with increasing methanol concentration.

Replotting the isotherms in a Langmuir representation (Fig. 2) shows that between 40 and 60% methanol an acceptable correlation can be obtained. A similar correlation is found with methanol-rich eluents at high centrimide concentration in the eluent and in methanol-lean eluents at low reagent concentration.

*Effect of acetonitrile concentration on the adsorption isotherms*

Fig. 3A shows the relationship between the amount of adsorbed cetrimide, $P_s$, and the cetrimide concentration in the eluent, $P_m$. It is seen that the amount of cetrimide adsorbed rapidly decreases with increasing acetonitrile concentration (Fig. 3B). Replotting the isotherms in a Langmuir representation reveals that the Langmuir isotherm equation is approximately valid only for low acetonitrile concentrations

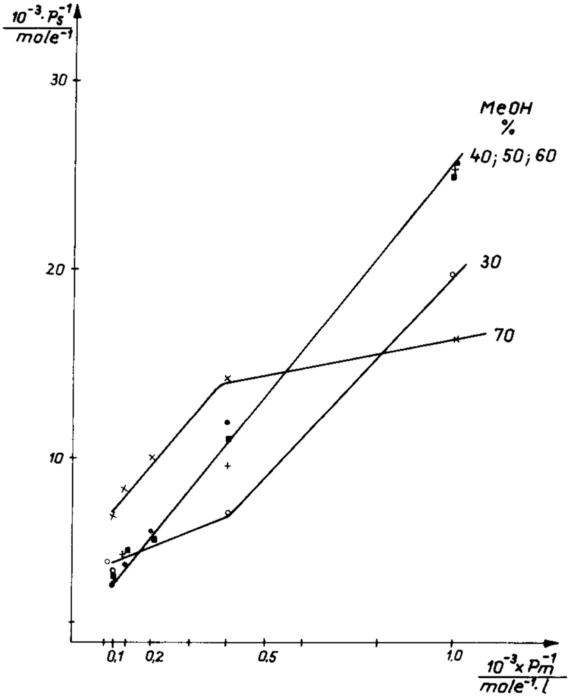

Fig. 2. Langmuir representation of adsorption isotherms of cetrimide in methanol-containing eluents (replotted from Fig. 1).

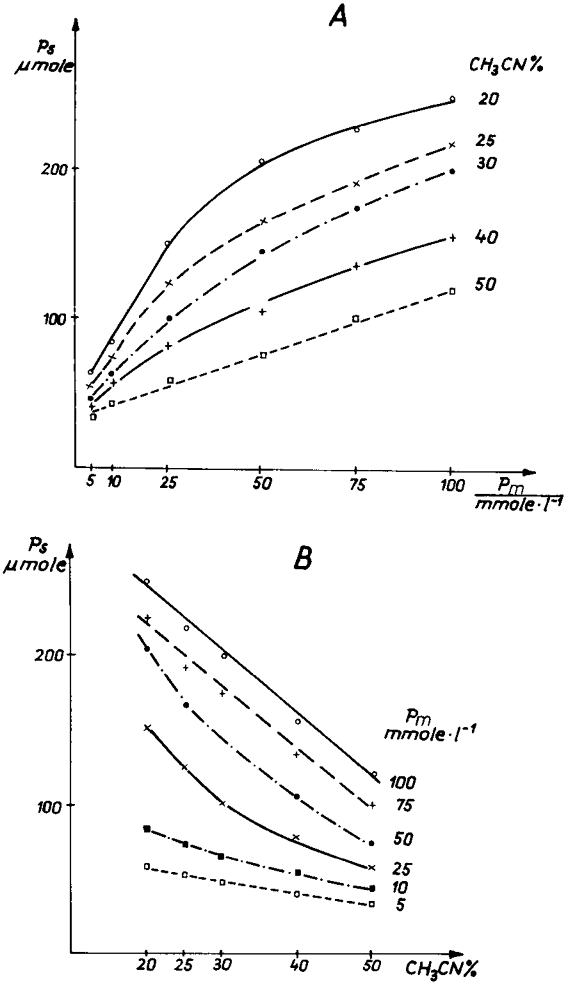

Fig. 3. Adsorption isotherms of cetrimide from acetonitrile-containing eluents (A) and stationary phase concentration of cetrimide as a function of the acetonitrile concentration of the eluent (B). Eluent: acetonitrile–aqueous phosphate buffer (0.01 *M*, pH = 5.0). For other conditions see Fig. 1.

(Fig. 4). The dependence of stationary phase ion-pair concentration on the modifier concentration in the eluent obtained for acetonitrile was different from that for methanol.

For these experiments (in eluents containing methanol and acetonitrile) a lower pH value of the eluent was used than recommended[8,12]. The lower pH results in increased column stability, and the necessary coating of cetrimide on the silica surface can also be achieved at pH 5.0. At this pH value the cation-exchange property of ionized silica can also be excluded.

The operation of a Langmuir equation in the given organic solvent concentration can be explained by the formation of no more than a monomolecular layer, while according to the literature[16], at higher pH, when more than 1 mmol $g^{-1}$ ce-

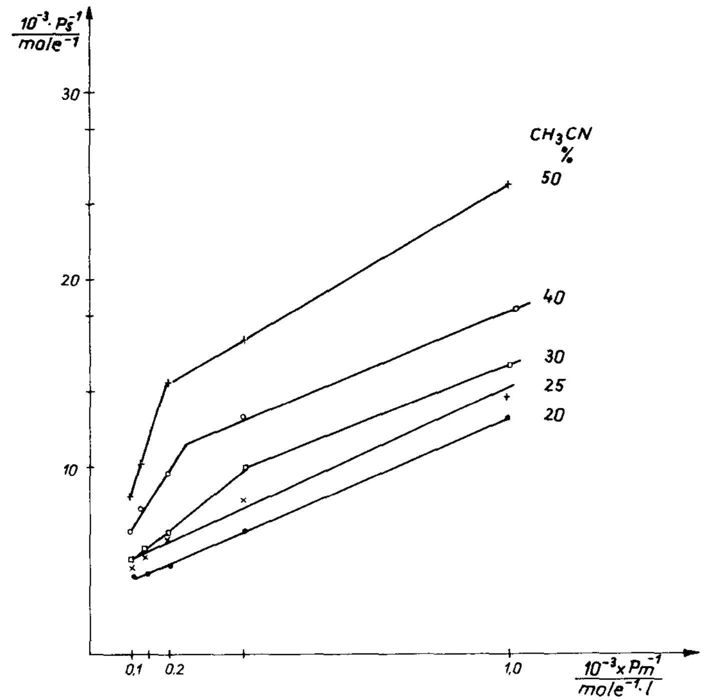

Fig. 4. Langmuir representation of adsorption isotherms of cetrimide in acetonitrile-containing eluents (replotted from Fig. 3).

trimide is adsorbed and an aqueous eluent is used, double and multiple layers are formed.

*Effect of eluent pH on the amount of adsorbed centrimide*

Constant buffer (0.01 *M*) and cetrimide ($5 \cdot 10^{-2}$ *M*) concentrations were used and the pH of the eluent was varied between 2 and 8. The dependence of the amount of adsorbed centrimide on the eluent pH is shown in Fig. 5. As is seen, the amount adsorbed rapidly increases when the pH is higher than 5.0.

Fig. 6 shows the dependence of the capacity ratios, $k'$, of some model compounds, listed in Table I, on the eluent pH when constant methanol and ion-pair reagent concentrations are used in the eluent. The retentions of sulphonic acids and phenols change significantly with increasing eluent pH. This can be explained in case of the sulphonic acids by a higher cetrimide load on the silica surface, and the increased retention of phenols relates to their more complete ionization and reaction with ion-pair reagent both in the mobile and stationary phases.

*Effects of the mobile- and stationary-phase concentration of the ion-pair reagent on the selectivity of the separation of organic acids*

In Fig. 7 the dependence of log $k'$ values obtained for the compounds listed in Table I on the mobile and stationary phase concentrations of cetrimide, $P_m$ and $P_s$, is illustrated when methanol is the organic solvent in the eluent. Similarly, log $k'$

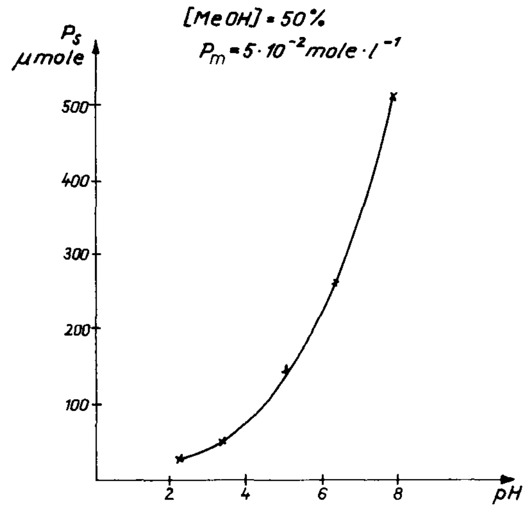

Fig. 5. Influence of eluent pH on the amount of adsorbed centrimide. Eluent: methanol–aqueous phosphate buffer (0.01 *M*) (1:1) containing 50 m*M* cetrimide. Other conditions as in Fig. 1.

plots when acetonitrile is the organic solvent are shown in Fig. 8. Both figures show the influence of the organic solvent concentration on the retention of the compounds (Figs. 7C and 8C).

From a comparison of Figs. 7 and 8 it can be concluded that in methanolic eluents the log *k'* *vs.* $P_m$ or $P_s$ curves do not significantly differ from each other, the dependences of log *k'* values on the methanol concentration being similar. Quite different log *k'* *vs.* $P_m$ or $P_s$ curves were obtained for eluents containing acetonitrile. The selectivity of the separation depends on the reagent concentration in the mobile

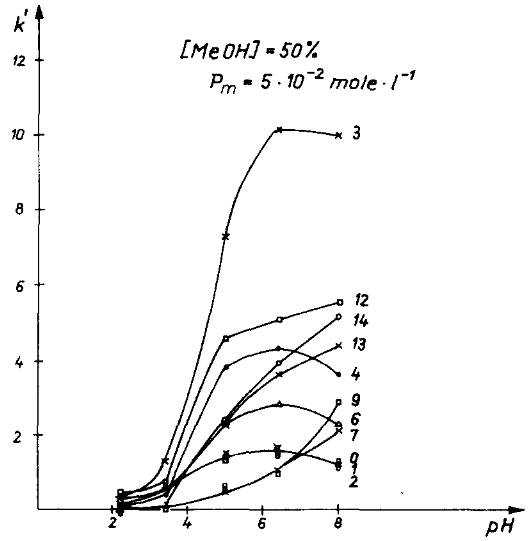

Fig. 6. Dependence of capacity ratios, *k'*, on the eluent pH. Conditions as in Fig. 5; compounds as in Table I.

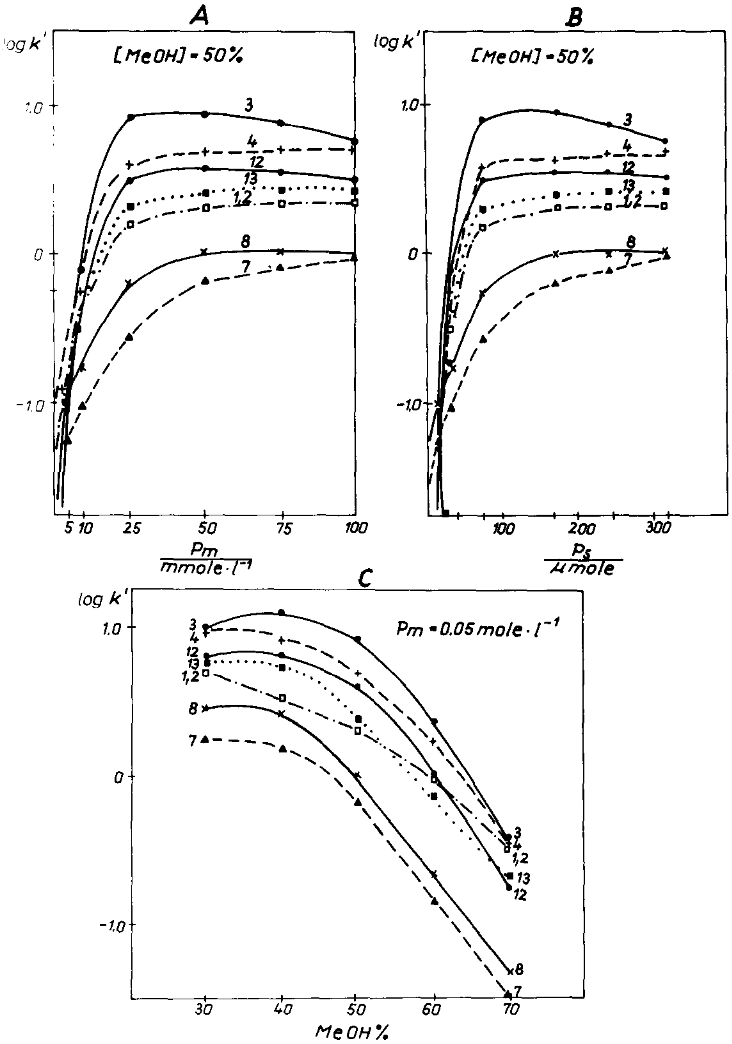

Fig. 7. Dependence of retention of the compounds investigated on centrimide concentration in the mobile (A) and stationary (B) phases and on the methanol concentration in the eluent (C). Conditions as in Fig. 1; compounds as in Table I.

and stationary phases as well as on the acetonitrile concentration in the eluent. This is shown in Fig. 8C. Plotting the log $k'$ values against the acetonitrile concentration resulted in nearly linear correlations for the compounds investigated.

A more complicated dependence of the retention of particular acids (such as pentachlorophenol, niflumic acid and sulphosalicylic acid) on $P_m$ or $P_s$ as well as on organic solvent concentration has been found. This is illustrated in Fig. 9. It shows that log $k'$ *vs.* $P_s$ curves have maxima both in methanol- and acetonitrile containing eluents. This can be explained by micellar formation of cetrimide in the mobile phase at high centrimide concentrations[17,18]. Fig. 9 also shows the dependence of the log

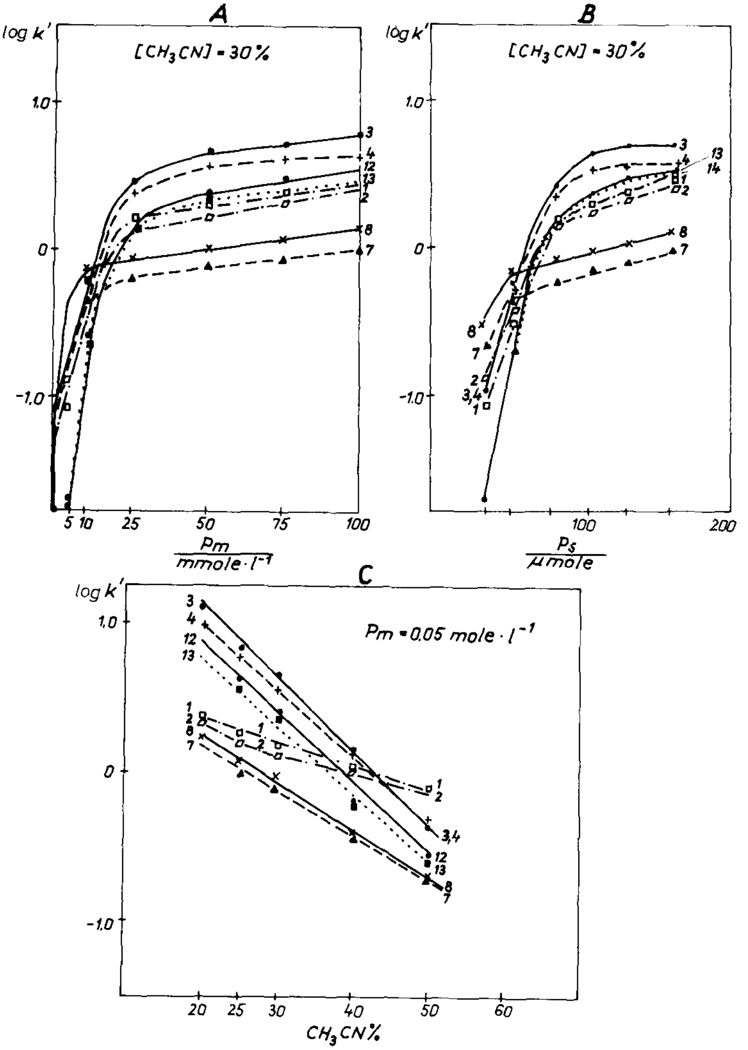

Fig. 8. Dependence of retention of the compounds investigated on cetrimide concentration in the mobile (A) and stationary (B) phases and on the acetonitrile concentration in the eluent (C). Conditions as in Fig. 3; compounds as in Table I.

$k'$ values of these three acids on the organic modifier concentration. When methanol is used as solvent, the elution order depends on the methanol concentration.

## CONCLUSIONS

The adsorption isotherms of cetrimide were determined on bare (non-bonded) silica with methanol and acetonitrile as organic solvents in the eluent (pH 5.0). Although a significant difference in selectivity was found between the methanol- and acetonitrile-containing eluents, the capacity ratios obtained with both eluents were

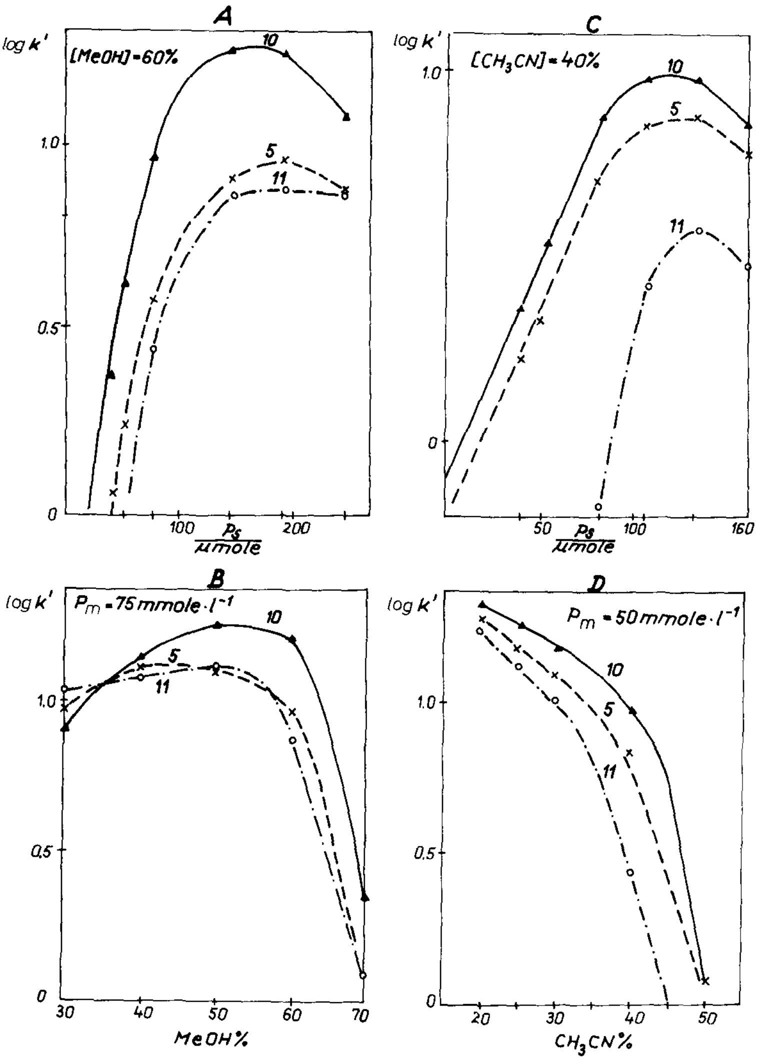

Fig. 9. The retention of pentachlorophenol, niflumic acid and sulphosalicylic acid as a function of the stationary phase concentration of cetrimide in 60% methanol in phosphate buffer (A), in 40% acetonitrile in phosphate buffer (C) and of the methanol concentration (B) and acetonitrile concentration (D) in the eluent at constant concentration of cetrimide in the mobile phase.

compared when the cetrimide concentrations both in the mobile and stationary phases, the pH of the eluents and the buffer concentrations were identical. This is shown in Fig. 10. As is seen, a good correlation was obtained when the *k*' values are compared at two different concentrations of cetrimide in the stationary phase.

When the adsorption isotherms were determined in methanol–aqueous phosphate buffer and acetonitrile–aqueous phosphate buffer, the measured values within a given solvent concentration range could be described by the Langmuir isotherm equation.

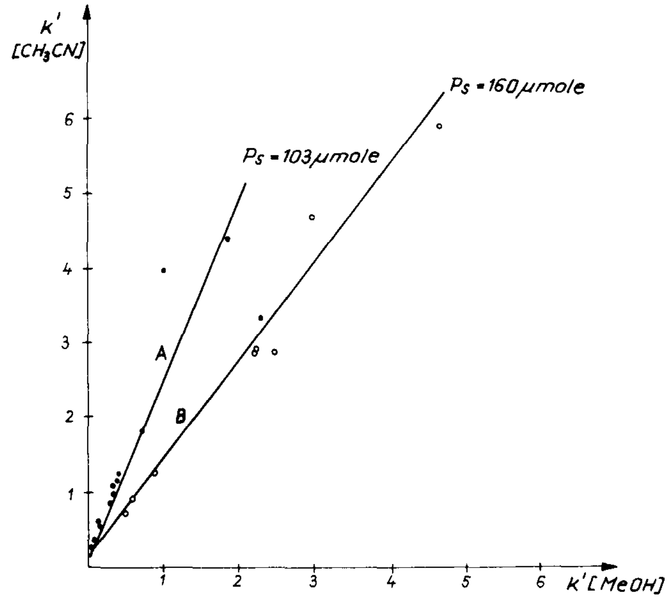

Fig. 10. Correlation between the retentions obtained in methanol- and acetonitrile-containing eluents at a constant concentration of cetrimide in the stationary and mobile phases. A, Methanol concentration, 70% (v/v); acetonitrile concentration 40% (v/v); $P_m$ = 50 m*M*; $P_s$ = 103 µmol; buffer concentration 0.01 *M*, pH 5.0. B, Methanol concentration 50% (v/v); acetonitrile concentration 25% (v/v); $P_m$ = 50 m*M*; $P_s$ = 160 µmol; buffer concentration 0.01 *M*, pH 5.0.

The influence of the nature and concentration of organic solvents in eluents containing cetrimide has been described by Hansen and Helboe[19]. Their experiments and observations differ from ours due to different experimental conditions. They used cetrimide in a low concentration range, and demonstrated the necessary coating of ion-pair reagent by using a high eluent pH.

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