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STUDIES IN REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

I. ADSORPTION ISOTHERMS OF TETRAALKYLAMMONIUM ION-PAIR REAGENTS ON LICHROSORB RP-18 IN METHANOL-WATER ELUENTS*

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SUMMARY

The adsorption isotherms of symmetrical tetraalkylammonium ion-pair reagents (tetramethyl, tetraethyl, tetrapropyl and tetrabutyl) on LiChrosorb RP-18 in 25 mM H_3PO_4 - NaH_2PO_4 aqueous buffer were determined from breakthrough curves at a constant Br^- counter ion concentration of 200 mM, maintained by the addition of sodium bromide. The Langmuir and Freundlich isotherms were tested with the data and a poor fit was observed at higher mobile phase concentrations.

The adsorption isotherms of tetrabutylammonium bromide were measured in the same system, in 0-70% (v/v) methanol-aqueous buffer eluents, at seven eluent compositions. Stationary phase ion-pair reagent concentration *versus* methanol concentration (at constant mobile phase pairing ion concentrations), and stationary phase *versus* mobile phase pairing ion concentration (at constant methanol concentrations) plots were obtained. With these plots, pre-selected stationary phase ion-pair reagent concentrations can be set (either held constant or varied according to a programme) under widely varying chromatographic conditions.

INTRODUCTION

Reversed-phase ion-pair chromatography is firmly established as the method of choice for the separation of ionic organic compounds, and recently even of non-ionic compounds. The main variable used to control the separations is the mobile phase concentration of the pairing ion.

Recent results¹⁻³ indicate that retention in ion-pair chromatography depends, primarily, on the surface concentration of the ion-pair reagent adsorbed on the stationary phase. Therefore, both an improved understanding of ion-pair separation processes and the meaningful design of ion-pair separations require a knowledge of the adsorption isotherms of various ion-pair reagents under widely varying chromatographic conditions.

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There are several publications dealing with the adsorption isotherms of ion-pair reagents on reversed-phase packings. Deelder *et al.*⁴ determined the adsorption isotherms of sodium alkylsulphonate pairing reagents on various reversed-phase packings. The effects of temperature, propanol concentration and counter ion concentrations were studied. Knox and Hartwick¹ measured the adsorption isotherms of sodium alkyl sulphates on ODS-Hypersil from methanol-aqueous phosphate buffer (1:4) of pH 6 at constant ionic strength.

Knox and Laird⁵ measured the adsorption isotherm of cetrimide on SAS silica. Terweij-Groen *et al.*⁶ studied the influence of various chromatographic parameters on the adsorption of cetrimide on to LiChrosorb RP-8. Van de Venne *et al.*⁷ measured the adsorption isotherm of octylamine from aqueous phosphate buffer, at constant ionic strength, on LiChrosorb RP-18. Jansson *et al.*⁸ determined the adsorption isotherm of N,N-dimethyloctylamine on LiChrosorb RP-8 from pH 2.2 phosphate buffers with various *n*-pentanol concentrations.

Schill and co-workers^{9,10} determined the adsorption isotherm of tetrabutylammonium ion on LiChrosorb RP-8 and μ Bondapak C₁₈ and used phosphate buffers of various pH and acetonitrile concentrations. Taylor and Hung^{11,12} studied the adsorption of several ion-pair reagents, both positively and negatively charged, on ODS-Hypersil in pure water. Sodium lauryl sulphate and cetrimide were also examined using 0, 30 and 60% (v/v) acetonitrile-water eluents.

We would not find, however, any reports of the determination of the adsorption isotherms of ion-pair reagents at constant ionic strength and temperature as a function of the methanol concentration of the eluent over wide, but chromatographically meaningful, mobile phase pairing ion and methanol concentrations. Therefore, this work was initiated in order to obtain a comprehensive database relating mobile phase and stationary phase ion-pair reagent concentrations to the retention data of similarly and oppositely charged and neutral solutes. The adsorption isotherm data were to be tested against the major isotherm equations, as reversed-phase ion-pairing models often postulate Freundlich- or Langmuir-type behaviour. Also, more information was to be gained about the effect of mobile phase methanol concentration on both the adsorption isotherms and the retention data. This paper deals with the adsorption isotherms, and subsequent papers will detail the retention data.

EXPERIMENTAL

Materials

Experiments were carried out with tetramethylammonium chloride (TMACl), tetraethylammonium chloride (TEACl), tetrapropylammonium bromide (TPrABr) and tetrabutylammonium bromide (TBABr) (Fluka, Buchs, Switzerland). Orthophosphoric acid, sodium dihydrogen orthophosphate, sodium bromide and methanol were reagent-grade materials from Reanal (Budapest, Hungary).

Chromatographic system

The chromatographic system, which served a dual purpose, is shown in Fig. 1. It consisted of eluent reservoirs A and B, pumps P1 and P2 (Model 6000 A; Waters Assoc., Milford, MA, U.S.A.), six-port injection valves 1 and 2 (Model 7010; Rheodyne, Cotati, CA, U.S.A.), thermostatted columns C1 and C2 (250 × 4.6 mm I.D.;

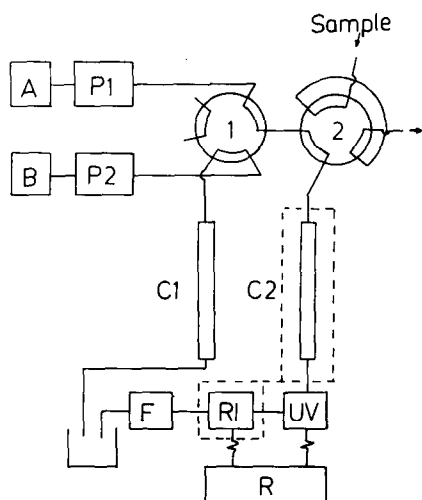


Fig. 1. The chromatographic system.

Chrompack, Middelburg, The Netherlands), UV (254 nm) and refractive index (RI) detectors (LC 4010, 4020; Varian Aerograph, Walnut Creek, CA, U.S.A.) and a dual-channel recorder (M 4220; Knauer, West Berlin, G.F.R.). The columns were packed with 10 μm LiChrosorb RP-18 (Merck, Darmstadt, G.F.R.), with a nitrogen BET surface area of 170 m^2/g and a carbon content of 17% (w/w). Adsorption isotherms were recorded by switching in a step-function manner with valve 1 between eluents A and B, which contained the ion-pair reagent in different concentrations. Column C1 was used to eliminate pressure surges on switching from eluent A to B.

Method

Eluents were prepared by weighing to 0.01 g into a 1-l volumetric flask the calculated amount of methanol yielding the pre-selected percentage composition (density 0.796 g/ml). Then, H_3PO_4 and NaH_2PO_4 yielding 25 mM in the final eluent were weighed in from 1 M stock solutions, followed by the solid tetraalkylammonium compound (1–150 mM). Finally, sodium bromide was added to give a total Br^- concentration of to 200 mM. Distilled water was slowly added while keeping the temperature at 25.0°C. After careful equilibration, the last few microlitres of water were added, and the weight of the full flask was determined. Thus, eluent concentrations in % v/v, w/w, w/v, etc., could be calculated and eluent preparation became very reproducible.

The pH of the filtered (through GF/A and GF/D glass-fibre filters (Whatman, Clifton, NJ, U.S.A.) and degassed eluent was measured by a combined glass electrode and digital pH meter (OP-208; Radelkis, Budapest, Hungary), calibrated with aqueous buffers. Measured pH values were corrected according to Bates¹³.

Isotherms were measured by switching valve 1 and recording the breakthrough curve of the new eluent¹. Both step-by-step and single-step breakthrough curves yielded identical stationary phase concentrations ($\pm 5\%$ relative). Once the isotherm at a given methanol concentration was recorded, the column was desorbed by pumping 500 ml of ethanol–50 mM phosphate buffer of pH 3 (1:1). The dead volume was

determined by injecting eluents enriched in potassium bromide. In the absence of an ion-pairing reagent this gave values identical with potassium iodide as recommended in ref. 14, but in the presence of tetraalkylammonium compounds potassium iodide was retained compared with potassium bromide.

RESULTS

Adsorption isotherms of tetraalkylammonium salts in aqueous phosphate buffer

The adsorption isotherms of the various tetraalkylammonium salts on Li-Chrosorb RP-18 measured in aqueous phosphate buffer are shown in Fig. 2. Except for TMACl, which is only slightly adsorbed, the isotherms correspond to the L-type ones in Giles *et al.*'s classification¹⁵.

The adsorption isotherms of TEACl, TPrABr and TBABr are plotted in the Langmuir representation in Figs. 3 and 4. It can be seen that for TPrABr and TBABr the data fail to satisfy the equation even at mobile phase concentrations as low as 5 mM.

It is also apparent from Fig. 2 that the isotherms fail to satisfy the Freundlich equation.

Thus, in agreement with other workers^{8,11}, and contrary to assumptions often used in reversed-phase ion-pair chromatography, it can be concluded that the adsorption isotherms of the higher tetraalkylammonium ion-pair reagents in aqueous eluents cannot be described with sufficient precision with either the Freundlich or the Langmuir isotherms.

Jansson *et al.*⁸ suggested that in aqueous eluents protonated amines and quaternary ammonium ions are adsorbed by two different sites on the surface of reversed-phase packings, presumably by the alkyl chains and the residual hydroxy groups. As these two mechanisms lead to isotherms with different natures, it is not surprising that the measured composite isotherm cannot be described by any of the single isotherm equations. Composite adsorption is suggested by our failure to correlate the

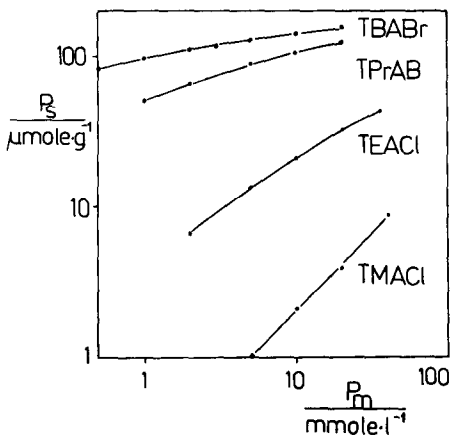


Fig. 2. Adsorption isotherms of tetramethylammonium chloride, tetraethylammonium chloride, tetrapropylammonium bromide and tetrabutylammonium bromide on LiChrosorb RP-18 from 25 mM H_3PO_4 -25 mM NaH_2PO_4 (pH 2.1) buffer at constant counter ion concentration ($[Br^-] = 200$ mM) at 25°C.

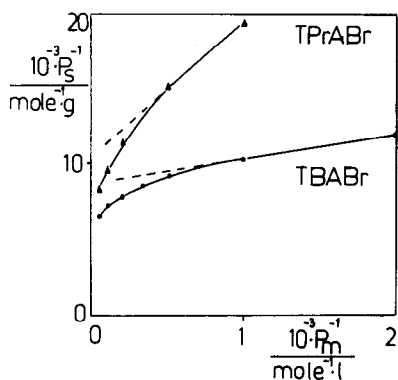


Fig. 3. Langmuir representation of the adsorption isotherms of tetrapropylammonium and tetrabutylammonium bromide (replotted from Fig. 1).

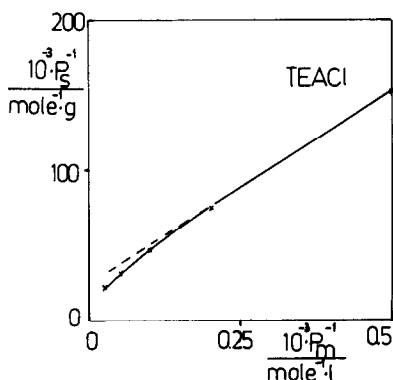


Fig. 4. Langmuir representation of the adsorption isotherm of tetraethylammonium chloride (replotted from Fig. 1).

stationary phase concentrations of the pairing ion and its hydrophobicity (chain length), although obviously the extent of adsorption increases with increasing chain length.

Effects of methanol concentration on the adsorption isotherms of tetrabutylammonium bromide

The limited solubility of sodium bromide in methanol precluded the determination of adsorption isotherms above 70% (v/v) methanol. Again, the overall Br^- and phosphate buffer concentrations of the final eluent were kept constant at 200 mM and at 25 mM H_3PO_4 and 25 mM NaH_2PO_4 , respectively. As the pK of H_2PO_4^- increases with increasing methanol content¹³, the pH of the eluents increased in the range 2.1–3.4.

The adsorption isotherms of TBABr in various methanol–buffer eluents are shown in Fig. 5 and Table I.

It can be seen that the amount of TBABr adsorbed decreases rapidly with increasing methanol concentration, but the shape of the isotherms does not change. It can be also seen that the isotherms fail to follow the Freundlich equation.

Replotting the isotherms in a Langmuir representation (*cf.*, Fig. 6) reveals that they do not follow the Langmuir equation in methanol–buffer eluents, and the deviation is especially large in methanol-lean eluents (0–25%, v/v).

The isotherm data can also be analysed by keeping the mobile phase pairing ion concentration constant and plotting the stationary phase pairing ion concentration against the eluent methanol content, as shown in Fig. 7. It can be seen that the stationary phase concentration of the pairing ion changes rapidly and non-linearly with increasing methanol concentration of the eluent.

Fig. 7 can also be used for another purpose. With its help, one can relate those mobile phase pairing ion concentrations and methanol concentrations which establish identical stationary phase concentrations of the pairing ion (*cf.*, Fig. 8). As earlier observations hinted at the primary role of the stationary phase ion-pair reagent concentration, it is reasonable to expect that the relationship between k' and methanol

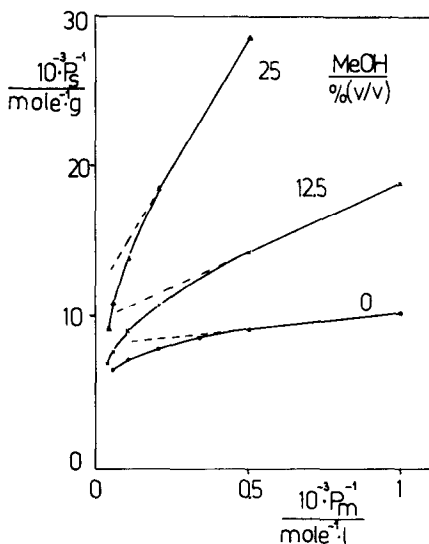
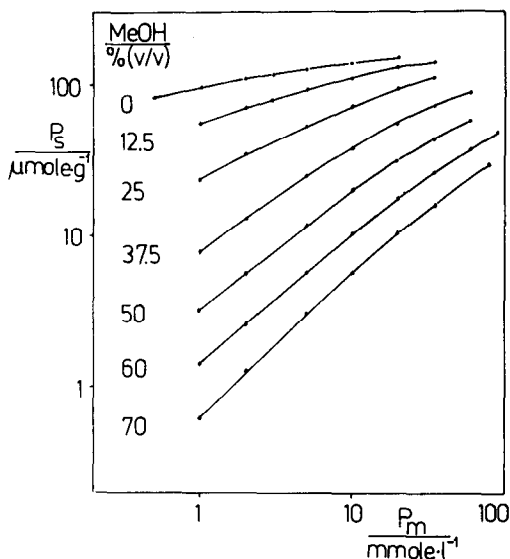


Fig. 5. Adsorption isotherms of tetrabutylammonium bromide from 0, 12.5, 25, 37.5, 50, 60 and 70% (v/v) methanol (MeOH)-aqueous phosphate buffer eluents (25 mM H₃PO₄-25 mM NaH₂PO₄; pH 2.1-3.4, [Br⁻] = 200 mM; 25°C).

Fig. 6. Langmuir representation of the adsorption isotherms of TBABr from 0, 12.5 and 25% (v/v) methanol-aqueous phosphate buffer eluents. Conditions as in Fig. 4.

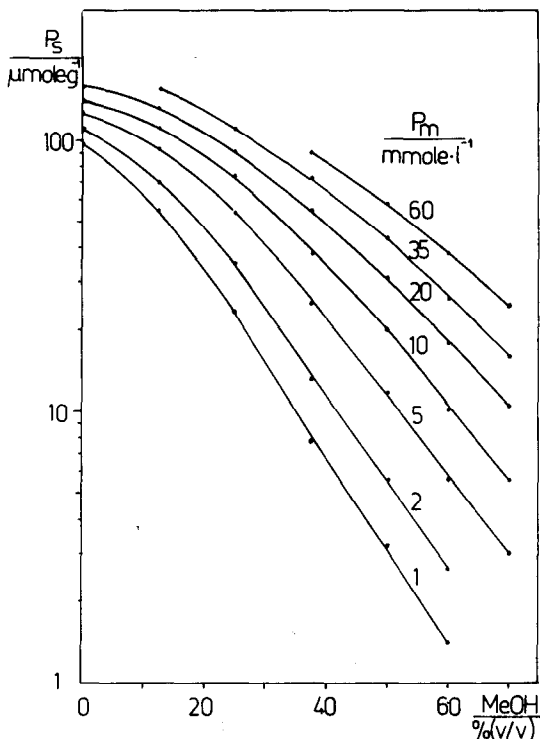


Fig. 7. Stationary phase concentration of TBABr (at constant TBABr mobile phase concentration) as a function of the methanol concentration of the eluent.

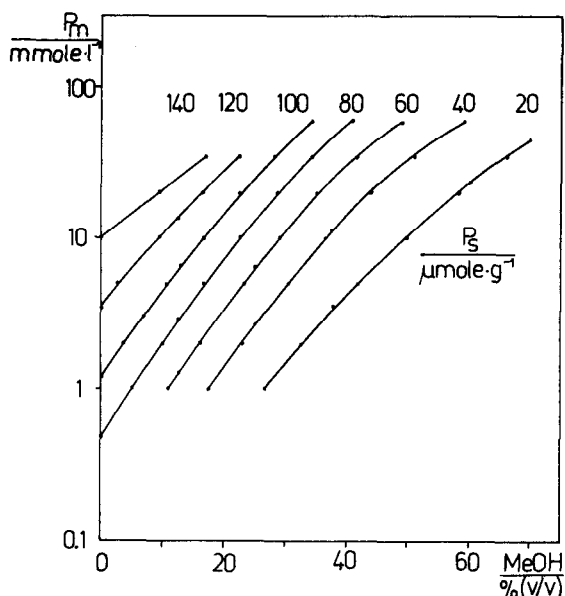


Fig. 8. Mobile phase TBABr concentration (at constant stationary phase TBABr concentration) as a function of the eluent methanol concentration.

and pairing ion concentrations can be treated in a more direct way. It will be shown in Part II¹⁶ that this is indeed the case.

It can also be seen from Fig. 8 that in methanol-rich eluents (60–70%, v/v) the surface concentrations of the pairing ion are very low, even at very high mobile phase pairing ion concentrations. On the other hand, in aqueous eluents even very low pairing ion concentrations (1–2 mM) establish very high surface concentrations (60–70% of the maximum observed values).

A practical consequence of this observation is that if low retention is experienced in methanol-rich eluents with moderate mobile phase pairing ion concentrations, then it is better to select another, more hydrophobic pairing ion, rather than try to increase the mobile phase concentration of the first one.

CONCLUSION

The adsorption isotherms of symmetric tetraalkylammonium ion-pair reagents (methyl to butyl) were determined on LiChrosorb RP-18, from an aqueous phosphate buffer (25 mM H_3PO_4 –25 mM NaH_2PO_4) that also contained 200 mM bromide. The measured values could not be described by either the Freundlich or the Langmuir isotherm equations.

The adsorption isotherms of tetrabutylammonium bromide were determined in 0–70% (v/v) methanol–aqueous buffer eluents. These isotherms also failed to satisfy the Langmuir and Freundlich equations. The stationary phase concentration of TBABr (at constant mobile phase concentration) changed sensitively, and non-linearly, with the methanol concentration of the eluent. These plots allow for the

selection of pre-determined ion-pairing conditions under widely varying chromatographic conditions.

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