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Note

Impregnation of silica gel with tetraalkylammonium salts in adsorption chromatography of neutral aromatic compounds

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Several groups of compounds which are normally difficult to separate have been resolved by use of systems which take advantage of differences in the properties of a common functional group of the solute in relation to a suitable reagent coated on the stationary phase. For example, carboxylic acids have been separated on paper impregnated with a phosphate buffer, polyhydric phenols on stationary phases modified with borate buffers or boric acid and olefins on stationary phases containing silver salts.

The present paper describes the impregnation of silica gel with tetraalkylammonium salts in order to obtain a modified phase, suitable for use in certain separations, *e.g.*, in thin-layer chromatography (TLC) of several types of aromatic pesticides and related compounds, and of some moderately polar acetanilides. A somewhat similar use of tetraalkylammonium salts is to be found in chromatography on Bentones, where the original sodium or potassium cations of the montmorillonite clays are partially exchanged for highly lipophilic tetraalkylammonium ions[1,2]. The effects observed may be connected with the basis for the selective extractions of tetraalkylammonium salts utilized in modern ion-pair techniques[3,4].

EXPERIMENTAL

Chemicals

Biphenyl (Kebo, Stockholm, Sweden), *p*-terphenyl (Merck, Darmstadt, G. F.R.), diphenyl ether (Kebo) and dibenzofurane (Kebo) were used. Dibenzo-*p*-dioxin was kindly supplied by Dr. E. Pettersson, Royal Institute of Technology, Stockholm, Sweden. Clophen A 50 (Bayer, Leverkusen, G.F.R.) was used as a representative of technical polychlorinated biphenyls (PCBs). Pure samples of 2,2′,3′,4,5-pentachlorobiphenyl[5] and 2,2′,3,4,5′-pentachlorobiphenyl[5] were supplied by Dr. G. Sundström of this laboratory. 2,2-Bis(4-chlorophenyl)acetonitrile (*p,p*′-DDCN) was synthesized according to Grummit and Marsh[6]. DDT and some of its metabolites were obtained from Analytical Standards, Unilab Research Corporation, Berkeley, Calif., U.S.A.; 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (*p,p*′-DDT); 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1,1-trichloroethane (*o,p*′-DDT); 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p*′-DDE); 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene (*o,p'*-DDE); 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (*p,p'*-DDD); 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethane (*o,p'*-DDD); 2,2-bis(4-chlorophenyl)-1-chloroethylene (*p,p'*-DDMU) and 4,4'-dichlorobenzophenone (*p,p'*-DBP).

Tetraalkylammonium salts, not absorbing UV light (254 nm), were represented by tetramethylammonium hydroxide (Eastman-Kodak, Rochester, N.Y., U.S.A.; 25% solution in water), tetraethylammonium hydroxide (E. Merck, 20% solution in water), tetrabutylammonium hydroxide (Fluka, Buchs, Switzerland; 40% solution in water) and hexadecyltrimethylammonium bromide (Fluka). The corresponding fluorides, chlorides, bromides, nitrates and sulphates were prepared by neutralization with the appropriate acid. Neutralization of tetrabutylammonium hydroxide with phosphoric acid to pH 7.0 gives a mixed phosphate, which for simplicity is here designated as tetrabutylammonium hydrogen phosphate.

n-Hexane (Kebo, redistilled) and diethyl ether (Mallinckrodt, St. Louis, Mo., U.S.A.; Analytical Reagent) were used as mobile phases.

Chromatography

Thin-layer chromatography (TLC) was performed at room temperature on pre-coated silica plates with a fluorescence indicator (Merck; DC-Fertigplatten, Kieselgel 60 F_{254}, 20 × 20 × 0.025 cm). The plates were impregnated by dipping for *ca.* 10 sec into 0.1–0.8 *M* solutions of the tetraalkylammonium salts in ethanol–water (3:2), drying by means of blotting paper and activation at 110° overnight. The compounds (5–10 μg) in hexane or diethyl ether were applied to the layer by means of a capillary pipette.

RESULTS AND DISCUSSION

The chromatographic properties of the silica gel were markedly changed on

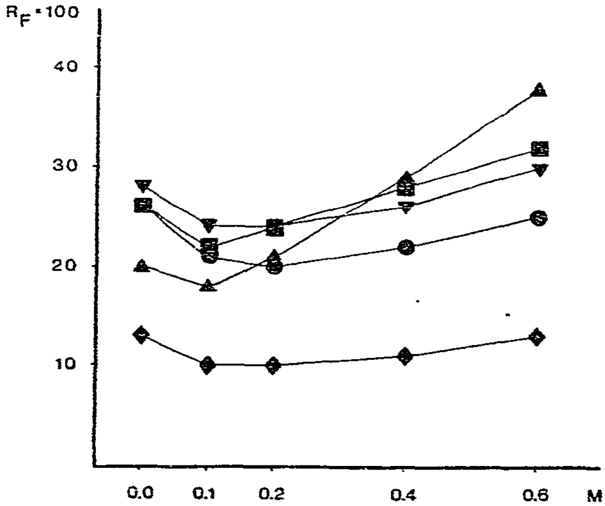
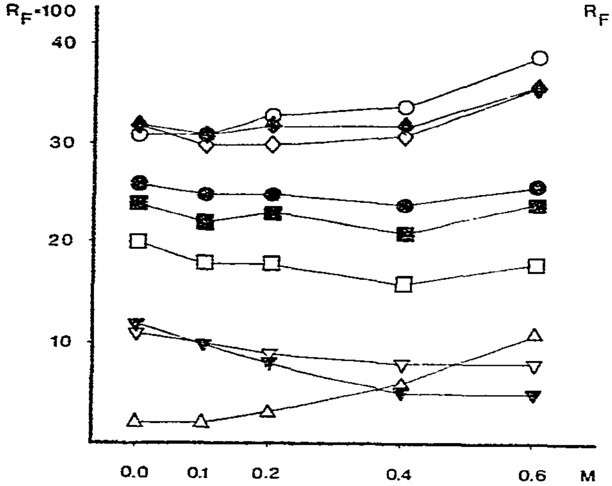


Fig. 1. Mobilities of some aromatic compounds on silica gel, and on silica gel impregnated with solutions of hexadecyltrimethylammonium bromide of increasing concentration. Mobile phase, hexane. Compounds in elution order on silica gel: ▼ = biphenyl; ■ = dibenzo-*p*-dioxin; ● = dibenzofurane; ▲ = diphenyl ether; ◆ = *p*-terphenyl.

impregnation of the gel with any of the tetraalkylammonium salts listed, as exemplified by the behaviour of some representative aromatic compounds on silica gel treated with hexadecyltrimethylammonium bromide (Fig. 1). Alterations in the impregnation conditions such as changes in the concentration of the solutions used for impregnation (Figs. 1 and 2) and changes in the type of cation (Fig. 3) or anion (Fig. 4) often caused pronounced changes in the relative mobilities of the test compounds, sometimes even an interchange in the order of elution.



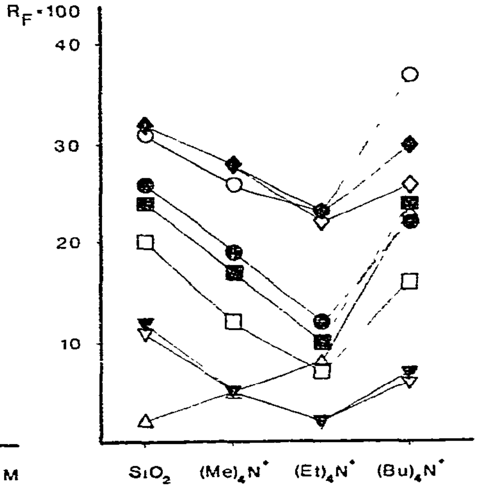
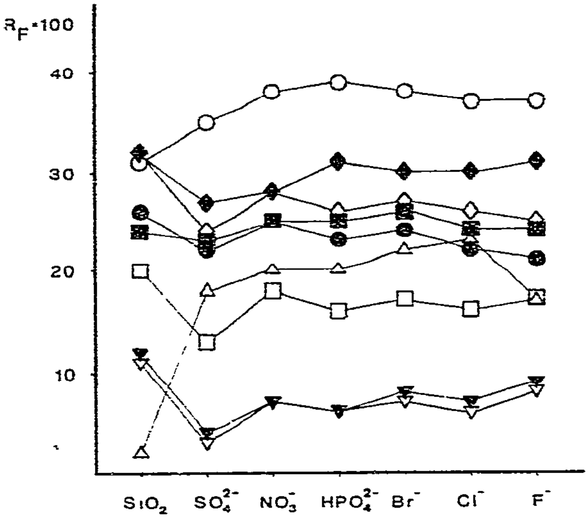


Fig. 2. Mobilities of some chlorinated aromatic pesticides on silica gel, and on silica gel impregnated with solutions of hexadecyltrimethylammonium bromide of increasing concentrations. Mobile phase, hexane. Compounds in elution order on silica gel: ◆, 2,2′,3,4,5′-pentachlorobiphenyl; ◇ = 2,2′,3′,4,5-pentachlorobiphenyl; ○ = *p,p′*-DDE; ● = *o,p′*-DDE; ■ = *o,p′*-DDT; □ = *p,p′*-DDT; ▼ = *o,p′*-DDD; ▽ = *p,p′*-DDD and △ = *p,p′*-DBP.

Fig. 3. Mobilities of some chlorinated aromatic pesticides on silica gel, and on silica gel impregnated with 0.4 *M* solutions of tetramethyl-, tetraethyl- and tetrabutyl-ammonium chlorides, respectively. For other details see Fig. 2.

By a suitable choice of the impregnation conditions it was possible to achieve an improved resolution in the chromatography of some otherwise poorly separated mixtures. A commercial mixture of PCBs, Clophen A 50, which on chromatography in the system silica gel–hexane gives three closely spaced bands (R_F 0.32–0.40), was resolved into *ca.* ten bands (R_F 0.16–0.60) on a silica layer treated with a neutral solution of tetrabutylammonium phosphate (0.8 *M*). Similarily, a change in the mobilities of polychlorinated biphenyls relative to certain DDT metabolites could be achieved, as exemplified by the separation between *p,p′*-DDE, 2,2′,3′,4,5-pentachlorobiphenyl and 2,2′,3,4,5′-pentachlorobiphenyl (Fig. 4). These two biphenyls are known to cause strong interference in pesticide analysis[7].

Improvements in the separation between *p,p′*-DDT, *o,p′*-DDT and their main metabolites could also be obtained through a suitable choice of the impregnation conditions and mobile phase (Fig. 5.) The impregnation technique proposed excludes the use of mobile phases such as chlorinated hydrocarbons and of more polar solvents which dissolve the salts used. However, improved separation of moderately polar



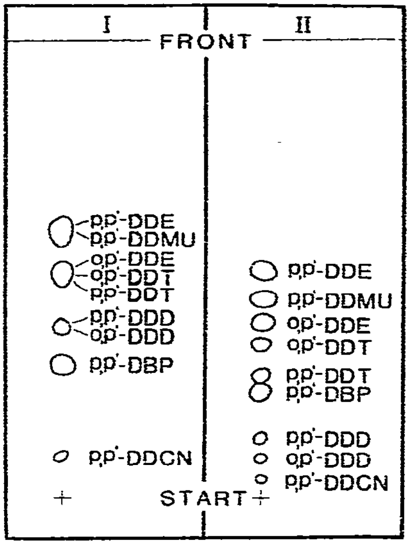


Fig. 4. Mobilities of some chlorinated aromatic pesticides on silica gel, and on silica gel impregnated with 0.4 *M* solutions of tetrabutylammonium sulphate, nitrate, hydrogen phosphate, bromide, chloride and fluoride, respectively. For other details see Fig. 2.

Fig. 5. Chromatography of *p,p'*-DDT and *o,p'*-DDT and their main metabolites on silica gel (I) and on silica gel impregnated with a 0.4 *M* solution of hexadecyltrimethylammonium bromide (II). Mobile phase, diethyl ether–hexane (1:19).

compounds could also readily be obtained by the use of diethyl ether as a mobile phase as exemplified by the chromatography of acetanilides (Fig. 6).

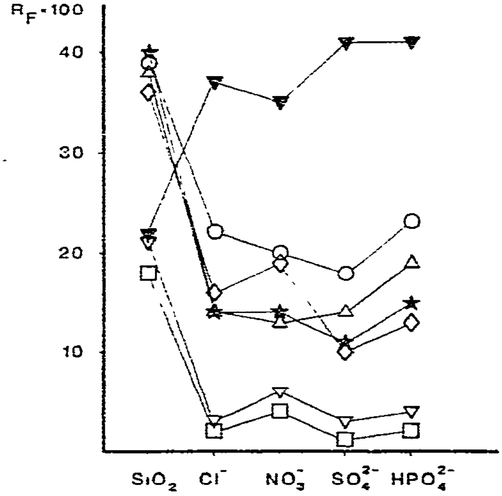


Fig. 6. Mobilities of several acetanilides on silica gel, and on silica gel impregnated with 0.4 *M* solutions of tetrabutylammonium chloride, nitrate, sulphate and hydrogen phosphate, respectively. Mobile phase, diethyl ether. Compounds in elution order on silica gel: ★ = 2,4,6-trichloroacetanilide; ○ = 2-chloroacetanilide; △ = 2,6-dichloroacetanilide; ◇ = 2,4-dichloroacetanilide; ▼ = N-methylacetanilide; ▽ = acetanilide and □ = 4-chloroacetanilide.

The mechanism of the observed effects is obviously complex, but is dependent on the concentrations used for the impregnations (Figs. 1 and 2). This dependence was valid over the range of concentrations giving hardly any observable effects (*ca.* 0.025 *M*) to concentrations giving deteriorations of the chromatographic properties (*ca.* 1–2 *M*, depending on the salt used), possibly being related to the completion of monomolecular layers. This interpretation is supported by rough calculations using estimated values of the areas occupied by the salts[8] and the specific area of the silica gel[9].

In the range of concentrations generally used in this work the surface of the adsorptive layer should be highly heterogeneous, and the resulting chromatographic properties should depend not only on the reduced free area of the silica gel but also on the individual contributions from each ion pair adsorbed. This description permits certain tentative rationalizations of the more drastic effects. The ion pairs are probably adsorbed on the layer via strong hydrogen bonds between the "naked" anions and the silica acid framework, whereas the tetraalkylammonium ions are directed towards the mobile phase. This mode of adsorption should result in a decreased accessibility of the protons of the stationary phase to hydrogen bonding. The electrostatic field generated by the ion pairs should simultaneously influence a permanent or induced dipole in a solute molecule and direct its positive end towards the layer; this is inconsistent with efficient hydrogen bonding of the pertinent solute molecules through the protons of the silica layer. This should mean that the field consistent with the adsorption of the ion pairs on the layer and the change in the accessibility of the protons are concurrent factors, responsible for the increased R_F values of certain compounds observed on the impregnated layers, *e.g.*, 4,4'-dichlorobenzophenone (Figs. 1, 3 and 4). On the other hand, when the solute contains sufficient numbers of acidic hydrogen atoms in pertinent dipolar groups for efficient binding to the free electron pairs of the silica gel, the directing effects of the field, mentioned above, obviously will facilitate the binding to the layer. This is believed to be the main reason for the marked decrease in R_F values of the acetanilides on the impregnated layers (Fig. 6). However, a reduction in the extent of adsorption of the mobile phase (diethyl ether) on the layer due to reduced hydrogen bonding, and a resulting decrease in the ability to replace the solute, might have some contribution to the effects. The adsorption of N-methylacetanilide, on the contrary, should depend strongly on the protons of the stationary phase, and as expected marked increases in the mobility of this compound were observed on the impregnated phases (Fig. 6).

Compounds having a less pronounced tendency to participate in hydrogen bonding, such as the chlorinated aromatic hydrocarbons and ethers, gave rather unpredictable mobilities on the different stationary phases studied, and several factors may be involved, including the attraction of induced dipoles to the ion pairs and steric effects; the latter are presumably responsible for the marked dependence on the size of the tetraalkylammonium ions (Fig. 3).

The present description stresses the surface active properties of the salts, an idea supported by the absence of similar effects on the impregnation of silica gel with hydrophilic salts like ammonium chloride (0.4 and 5 *M* solutions), while anionic detergents such as potassium oleate gave observable alterations. The presented material is evidently too small to allow definite conclusions about the mechanisms, but it may serve as an indication of the utility of the technique in different fields of preparative and analytical chemistry, *e.g.*, in connection with high-pressure liquid chromatography.

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