Journal of Chromatography, 360 (1986) 236–240
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 615

Note

Reversed-phase ion-pair thin-layer chromatography of organic acids

Influence of ion-pair structure and solvent pH on R_F

J. A. TROKE and I. D. WILSON*
Department of Drug Metabolism, Hoechst Pharmaceutical Research Laboratories, Walton Manor, Walton, Milton Keynes, Buckinghamshire MK7 7AJ (U.K.)
(Received March 3rd, 1986)

In previous studies[1,2] we have investigated the influence of solvent pH on the R_F values of a number of aromatic acids on ion-pair reversed-phase thin-layer chromatography (TLC). These studies were conducted using a variety of types of TLC plate including silica gel, paraffin-coated silica gel and C_{18} bonded silica gel impregnated with a range of different ion-pair reagents.

One of the main results of these experiments was the observation that, with respect to the effects of solvent pH on the R_F value of the test compounds, the ion-pair reagents could be divided into two distinct classes (independent of the type of TLC plate used). Thus, one group, exemplified by tetra-*n*-butylammonium bromide (TBAI), were found to be insensitive to solvent pH, and the R_F values of the test acids did not vary between pH 2 and 11. However, compounds with a similar structure to cetrimide (tetradecyltrimethylammonium bromide) showed large changes in the R_F values of these compounds at different pH values.

As a working hypothesis we proposed that ion-pair reagents such as cetrimide, which possesses both a hydrophilic trimethylammonium group and a long hydrophobic, alkyl chain, were able to form a bilayer on the surface of the silica by interaction with acidic silanol groups[2]. The large changes in R_F observed on lowering the pH from 7 to 2 might then be explicable in terms of the breakdown of this bilayer as the ionisation of the silanol groups was suppressed.

In order to obtain more information on the structural requirements needed by an ion-pair reagent in order for the solvent pH to exert an effect on R_F we have now undertaken some further studies using ion-pair reagents coated onto paraffin-impregnated silica gel TLC plates. These investigations involved the synthesis of a number of ion-pair reagents containing a trimethylammonium group attached to alkyl chains varying in length from C_6 to C_{10}. In addition we have prepared a reagent possessing two trimethylammonium groups attached to either end of a C_{12} alkyl chain. The results of studies employing these reagents, where the effects of solvent pH and structure of the ion-pair reagent on R_F have been investigated, are detailed below.

EXPERIMENTAL

Chemicals

2,5-Dihydroxybenzoic acid, 2,6-dihydroxybenzoic acid, gentisic acid and salicylic acid were obtained from Aldrich (Gillingham, U.K.). Cetrimide was obtained from Fisons (Loughborough, U.K.). All chemicals and reagents were used as received.

Synthesis of ion-pair reagents

The appropriate bromoalkane (Aldrich), usually 0.05 mole, was mixed with a solution of trimethylamine in ethanol (45 ml, 33%, v/v) and left overnight at room temperature. The solution was then diluted with hexane (45 ml) and cooled to 0°C. This resulted in the crystallisation of the product which was filtered, washed with fresh ethanol–hexane (1:1, v/v) and then desiccated. Yields of between 5 and 10 g of each compound were obtained.

It should be noted that the lower-molecular-weight reagents (particularly hexyltrimethylammonium bromide) were very hygroscopic.

Preparation of TLC plates

Silica gel TLC plates, incorporating a fluorescent indicator, (20 × 20 cm, plastic backed, E. Merck; purchased from BDH, Poole, U.K.) were coated with the appropriate ion-pair reagent by dipping them in a 0.1 *M* solution of the reagent in dichloromethane. This solvent also contained 7.5% (v/v) of heavy refined paraffin ("Nujol") in order to simultaneously coat the silica gel with paraffin.

Chromatography

Ascending chromatography was performed in glass TLC tanks 20 × 20 × 5 cm using solvent systems composed of methanol and 0.01 *M* sodium phosphate buffer at either pH 2, 4, 7, or 9. For most of these experiments the solvent composition was methanol–water (1:1). All the plates coated with the different ion-pair reagents were developed simultaneously in the same TLC tank to minimise experimental variations.

RESULTS AND DISCUSSION

In order to investigate the role of the alkyl chain length of the ion-pair reagent in the production of pH effects with the test acids; silica gel plates were prepared impregnated with both paraffin and ion-pair reagents with chain lengths of C_6, C_8, C_{10} and C_{14} (cetrimide). These plates were then spotted with methanolic solutions of four model compounds, namely 2,5-dihydroxybenzoic acid, 2,6-dihydroxybenzoic acid, gentisic acid and salicylic acid, and chromatographed using methanol–water (1:1) at solvent pH values of 2 and 4. These pH values were chosen on the basis of our previous study[2], which had shown that the largest changes in the R_F of the test compounds occurred in this range. The results of those experiments are shown in Fig. 1.

The C_{14} ion-pair reagent (cetrimide) gave the pH dependant changes in R_F values for the four acids anticipated on the basis of our previous work[2]. At the other

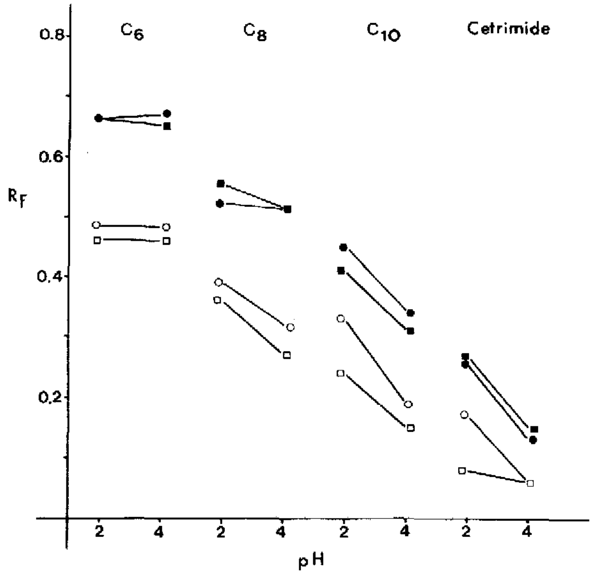


Fig. 1. Plots of R_F *versus* pH of the solvent (methanol–water, 1:1) at pH values 2 and 4 for 2,5-dihydroxybenzoic acid (■); 2,6-dihydroxybenzoic acid (□); gentisic acid (●); and salicylic acid (○) on paraffin-coated silica gel TLC plates simultaneously coated with either C_6, C_8, C_{10}, or cetrimide ion-pair reagents.

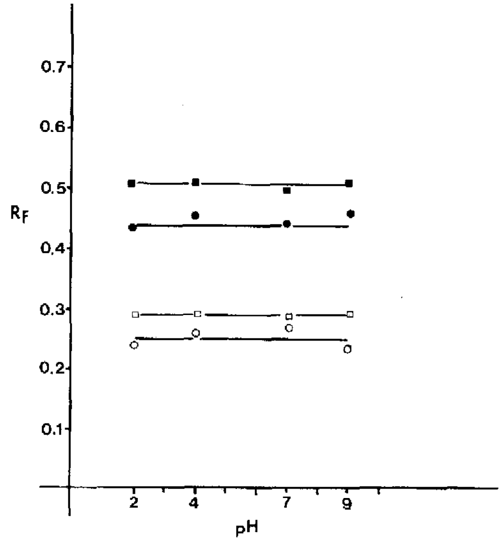
end of the range, the C_6 ion-pair reagent showed no change in the R_F values of any of the four test compounds on going from pH 4 to pH 2. However, on increasing the chain length to C_8 (or C_{10}) a higher R_F value was obtained for each of the four acids using a solvent pH of 2 compared to that attained using a similar solvent buffered to pH 4. Thus the C_6 ion-pair reagent behaved in a similar way to tetra-*n*-butylammonium bromide, whilst the longer-chain C_8 and C_{10} ion-pair reagents were similar in their properties to cetrimide.

As might be expected there was a clear trend to lower R_F values as the chain length of the ion-pair reagent increased (see Fig. 1).

Properties of the bifunctional bis-quaternary ammonium ion-pair reagent

The studies described above clearly demonstrated the need for a long alkyl chain to be present for pH effects to be observed. To determine whether both hydrophobic and hydrophilic groups must be present in order for these effects to be obtained the bifunctional ion-pair reagent 1,12-bis(trimethylammonium)dodecane dibromide was prepared. This compound is similar in size to cetrimide, but has hydrophilic trimethylammonium groups at each end of the alkyl chain; thus, unlike cetrimide, it does not possess a hydrophobic end. We anticipated therefore that, if a hydrophobic "tail" was necessary for the influence of solvent pH to be observed, this bifunctional ion-pair reagent would not show pH dependent changes in R_F.

The R_F values for the test acids, on TLC plates impregnated with both the reagent and paraffin, using solvents with pH value of 2, 4, 7 and 9 are shown in Fig. 2. Poor spot shape precluded the use of solvents of pH 11. Clearly, over the range of solvent pH values for which it was possible to obtain results, the R_F values of the



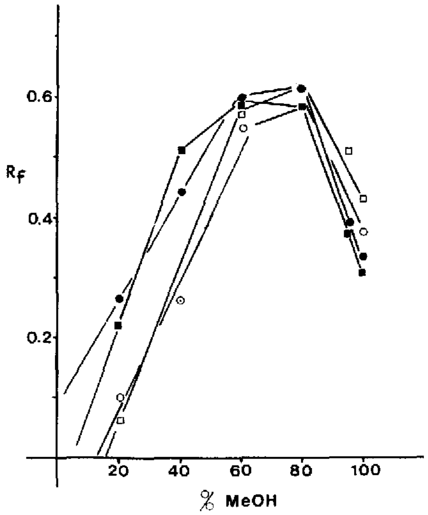


Fig. 2. Plots of R_F *versus* pH of the solvent (methanol–water, 2:3) showing the absence of variations in the R_F of the test compounds over the pH range 2 to 9 with 1,12-bis(trimethylammonium)dodecane bromide as ion-pair reagent on paraffin coated silica gel. Symbols as for Fig. 1.

Fig. 3. Plots of the variation of R_F of the four test acids (symbols as for Fig. 1) with solvent composition (methanol–water 0 to 100%) on paraffin coated plates simultaneously impregnated with 1,12-bis(trimethylammonium)dodecane bromide.

test compounds do not vary appreciably. This result confirms the need for both hydrophilic and hydrophobic regions on the reagent in order to obtain pH dependent changes in R_F.

Because of the unusual structure of this novel reagent we investigated its chromatographic properties further by examining the variation in R_F of the four test acids with solvent composition (from 0 to 100% methanol). These results are illustrated in Fig. 3 and show some unusual features. Thus, with up to 60% methanol in the solvent there is a regular increase in the R_F values of the test compound in line with the increasing eluotropic strength of the mobile phase. Compared with the other reagents examined in this study the bifunctional ion-pair reagent most closely resembles the C_8 reagent in terms of the R_F values for the test compound for methanol–water (1:1).

However, by 80% of methanol in the solvent the R_F values of all the test compounds appear to have reached a maximum. For higher concentrations of methanol, most unusually, a *decrease* in R_F values was observed. This was also accompanied by a reversal of the order of migration previously noted. This in turn suggests a change in the mode of chromatography from reversed to normal phase. This type of chromatographic behaviour was not observed with the C_8 ion-pair reagent; in addition it should be noted that in the absence of the ion-pair reagents all the test compounds chromatographed at, or near, the solvent front, irrespective of the solvent composition.

As yet we are unable to offer a plausible explanation for these unusual effects observed with high concentrations of methanol.

CONCLUSIONS

These studies have confirmed our original observations that ion-pair reagents such as cetrimide, which possess a hydrophilic trimethylammonium group attached to a long lipophilic alkyl chain, differ from ion-pair reagents such as TBAI in showing effects on R_F which are dependent on the pH of the solvent. Such effects are only observed when the length of the alkyl chain is increased from C_6 to C_8 and C_{10}. The long-chain (C_{12}) ion-pair reagent possessing hydrophilic trimethylammonium groups attached to each end did not show pH effects. This confirms the need for both hydrophilic and hydrophobic portions in order to obtain such effects, and demonstrates that it is not enough merely to have a long chain.

The mechanisms by which these pH effects are caused still require further work in order to understand them. We are currently performing further studies in order to shed more light on this phenomenon. In addition, the intriguing properties of the bifunctional 1,12-bis(trimethylammonium)dodecane bromide are being further investigated.

REFERENCES

1 S. Lewis and I. D. Wilson, *J. Chromatogr.*, 312 (1984) 133.
2 I. D. Wilson, *J. Chromatogr.*, 354 (1986) 99.