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ION-PAIR REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY OF BASIC DRUGS USING SULPHONIC ACIDS

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SUMMARY

The use of sulphonic acid ion-pair reagents in the thin-layer chromatography of four basic drugs (all secondary amines) on C₁₈-bonded silica gel, paraffin coated silica gel and silica gel itself has been investigated. Effects of the ion-pair reagents were only obtained on C₁₈-bonded silica gel, and only then when the reagents were pre-coated onto the stationary phase. In general the largest reductions in the *R_F* values of the test compounds occurred when sodium dodecylsulphate was coated onto the plates.

INTRODUCTION

The use of ion-pair (IP) reagents to modify the chromatographic behaviour of ionisable compounds is an established technique in reversed-phase (RP) high-performance liquid chromatography (HPLC)¹. Although certain aspects of the mechanism, or mechanisms, by which these reagents exert their effects in HPLC still attract debate, the importance of parameters such as pH, reagent concentration, reagent structure etc., have been extensively investigated. In contrast, for thin-layer chromatography (TLC) much less is known. In previous studies²⁻⁵ we have investigated the use of IP reagents for the reversed- and normal-phase TLC of a number of organic acids on both silica gel and on a variety of bonded silicas, (mainly C₁₈). Here we describe further studies on the application of IP-RP-TLC of four basic drugs (Fig. 1) each of which contains a secondary amine in a "β-blocker"-type side chain (*i.e.*, a secondary amine connected, via a methylene bridge, to a secondary alcohol). Chromatography was performed using two sulphonic acid IP reagents (heptanesulphonic acid and sodium dodecylsulphate) on silica gel, three different types of C₁₈-bonded silica gel and paraffin coated silica gel.

EXPERIMENTAL

Chemicals and reagents

Heptanesulphonic acid (HSA) and sodium dodecylsulphate (SDS) were ob-

mg/ml, were spotted onto the origin of the variously treated TLC plates using 1- μ l glass capillaries. These plates were then developed in solvent systems composed of methanol-water containing 0, 20, 40, 60, 80 or 100% methanol. The effect of pH on chromatography was investigated by the addition of 0.1% trifluoroacetic acid to the solvent (to give a pH of *ca.* 2). The effect of adding 0.1 M IP reagent to the solvent (in the absence of IP reagent coated onto the plate) was also investigated.

Once developed plates were visualized by viewing under UV light at 254 nm.

RESULTS

On both silica gel and paraffin-coated silica gel all four test compounds gave unacceptable chromatograms, characterised by elongated streaks from origin to solvent front, irrespective of mobile phase composition. Coating either type of plate with SDS or HSA produced no improvement.

In contrast, on all three types of C_{18} -bonded TLC plate good chromatographic behaviour was observed for all four test compounds even when no IP reagent was present. Thus, as illustrated in Fig. 2, R_F values increased in a regular way as the elutotropic strength of the solvent was raised. Good results were observed over the

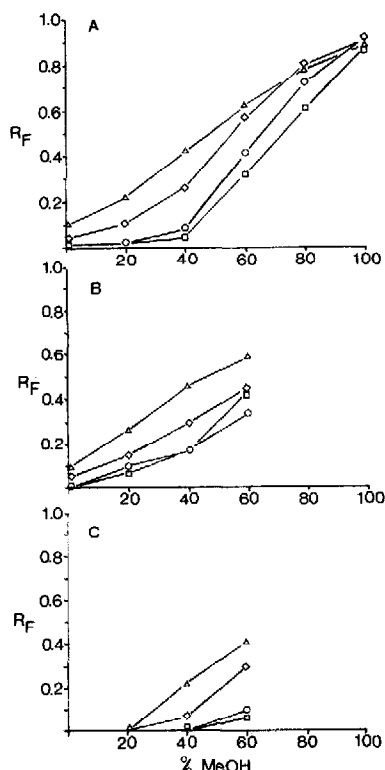


Fig. 2. Reversed-phase TLC of ICI 118 551 (○); ICI 141 292 (◇); ICI 45 520 (□); and ICI 50 172 (△) on C_{18} -bonded TLC plates supplied by (A) Merck, (B) Macherey-Nagel and (C) Whatman using methanol-water solvent systems. MeOH = Methanol.

whole range of solvent compositions on the Merck plates. However, neither Whatman or Macherey-Nagel plates gave acceptable chromatograms with solvent systems containing more than 60% of methanol (Fig. 2).

When these experiments were repeated with Merck C_{18} -bonded TLC plates which had been coated with either SDS or HSA at 0.1 M a significant decrease in R_F values compared to the control was observed, together with an improvement in spot shape. These results are illustrated in Fig. 3A (HSA) and 4A (SDS). As can be seen, compared to the control, both reagents cause the curves produced by plotting the R_F values of the test compounds *versus* the methanol content of the mobile phase to be shifted to the right. The largest effect was seen with SDS. Coating the plates with either reagent using concentrations of less than 0.1 M was not particularly effective at lowering R_F . A similar lack of effect was observed when the reagents were present in the solvent but had not been coated onto the plate.

As was observed with the Merck C_{18} -bonded plates the chromatography of all four test compounds could be accomplished on the Macherey-Nagel C_{18} -bonded

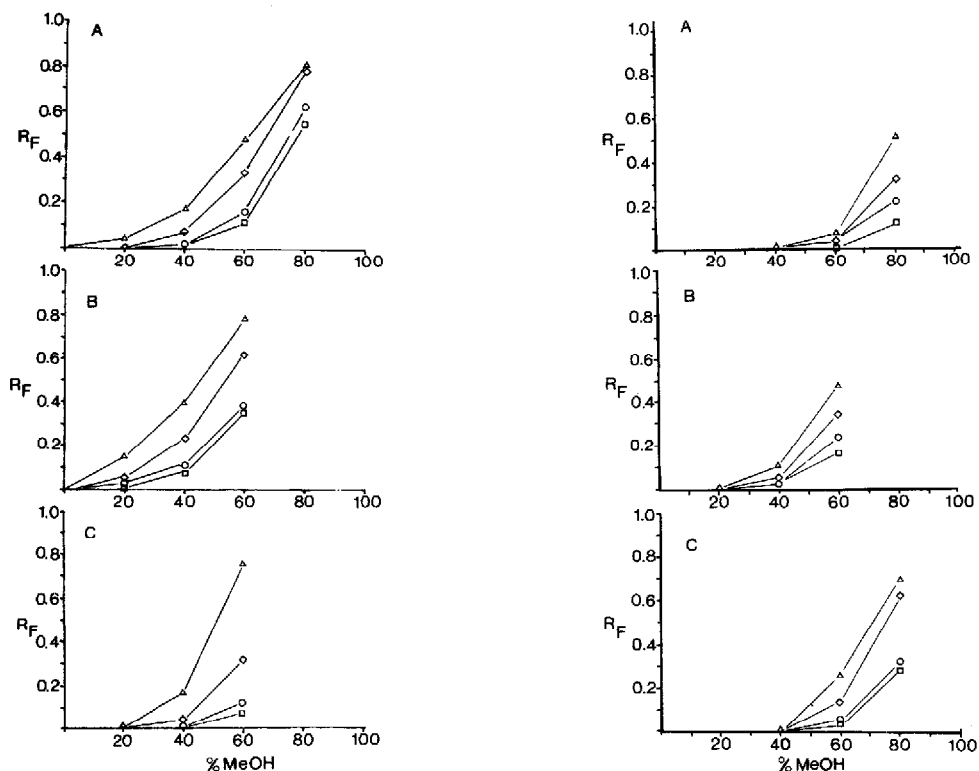


Fig. 3. IP-RP-TLC of ICI 118 551; ICI 141 292; ICI 45 520; and ICI 50 172 on (A) Merck, (B) Macherey-Nagel and (C) Whatman C_{18} -bonded TLC plates coated with 0.1 M heptanesulphonic acid using methanol-water solvent systems. Key as for Fig. 2.

Fig. 4. Reversed-phase ion-pair TLC of ICI 118 551; ICI 141 292; ICI 45 520; and ICI 50 172 on (A) Merck, (B) Macherey-Nagel and (C) Whatman C_{18} -bonded TLC plates coated with 0.1 M sodium dodecylsulphate using methanol-water solvent systems. Key as for Fig. 2.

TLC plates without difficulty (Fig. 2B). With the Macherey-Nagel plates, using HSA as the reagent, there was reduction in the R_F value of the test compounds compared to their control values up to 40% of methanol in the solvent. However, at 60% methanol there was a tendency for the R_F values obtained by using HSA to be higher than for the uncoated control plates. This was particularly noticeable for ICI 50 172 and ICI 141 292 (compounds 1 and 4, respectively; Fig. 1). Above 60% methanol all four compounds ran at the solvent front (in the absence of IP reagent these compounds gave elongated streaks above 60% methanol). Using SDS as ion-pair reagent on the Macherey-Nagel plates resulted in decreased R_F values compared to the control until methanol contents of above 60% were reached when the test compounds ran at the solvent front. The results for the Macherey-Nagel plates with HSA and SDS are summarised in Fig. 3B and 4B respectively.

Successful chromatography of the test compounds was also achieved on the Whatman C_{18} -bonded TLC plates in the absence of IP reagents (Fig. 2C). Coating these plates with HSA resulted in a small decrease in the R_F values of ICI 50 172 and ICI 141 292 in the solvent system methanol-water (40:60, v/v). However, with 60% methanol in the solvent the R_F values of 3 of the compounds (ICI 118 551, ICI 141 292 and ICI 45 520) were very close to their control values, whilst that of ICI 50 172 was very much higher (0.76 against a control value of 0.41). With solvents containing 80%, or more, methanol all the test compounds chromatographed at, or near, the solvent front. Coating the Whatman plates with SDS resulted in a large reduction in R_F values for all solvent compositions tested and, in addition, extended the range of usable solvent compositions up to 80% methanol. The results for the Whatman plates coated with HSA and SDS are illustrated in Figs. 3C and 4C, respectively.

In previous studies with quaternary ammonium ion-pair reagents (e.g., tetramethylammonium) we observed that, on the whole, the effect of solvent pH in IP-RP-TLC was much less critical than in HPLC. Limited studies were therefore performed on the Merck plates in order to investigate the effects of acidifying the solvent (to ensure that the amino groups on the test compounds were fully ionised) and of buffering the C_{18} -bonded TLC plates by dipping them into buffer solutions, as well as ion-pair reagent, prior to chromatography. No effect on the R_F values of the test compounds was seen as a result of any of these modifications (on either control or ion-pair coated plates). It should be noted however, that spot shape was slightly worse on plates dipped in pH 4.4 buffer.

DISCUSSION

The poor chromatography of the test compounds on both bare silica gel and silica gel coated with paraffin was expected, but not the lack of any discernible improvement when the IP reagents were present. Indeed this result contrasts markedly with our previous experiences using such systems with quaternary ammonium IP reagents²⁻⁵. However, similar results were reported by Szepesi *et al.*⁶ for 10-camphorsulphonic acid on silica gel in studies on ion-pair overpressurised TLC. Interestingly, by first coating the silica with the quaternary ammonium ion-pair reagent centrimide, followed by 10-camphorsulphonic acid, they were able to obtain acceptable results.

In these studies we obtained good chromatography for all four test compounds on the various C₁₈-bonded TLC plates tested, even in the absence of added IP reagents. The fact that we were able to obtain acceptable chromatography despite the lack of an IP reagent is probably, at least in part, due to the presence of residual silanols on the surface of the silica gel. It is well established that such groups can act as weak ion exchangers which are capable of interacting with basic compounds (often to the detriment of the chromatography, particularly in HPLC). The lack of any significant effect on chromatography following the inclusion of either of the reagents in the mobile phase is entirely consistent with our previous results on the quaternary ammonium IP reagents and acidic test compounds. It does, however, contrast with the previous results of Jost and Hauck⁷ who successfully used acidic IP reagents in the solvents. To some extent this may reflect our choice of test compounds rather than any fundamental difference, and indeed one of the test compounds employed (coedine) by these authors was seemingly unaffected by the presence of 0.1 M reagent in the solvent. Clearly this is an area worth careful investigation if an IP system is being considered for a particular analyte. The lack of effect of pH on R_F is also consistent with our previous results with certain types of quaternary ammonium IP reagent²⁻⁵. It is however, still somewhat surprising given the importance of this parameter in HPLC.

All three types of C₁₈-bonded plate showed similar selectivities, and these were not modified greatly either by the use of an ion-pair reagent or the type of reagent used. As might have been predicted the more lipophilic SDS gave lower R_F values than the shorter chain SDS.

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