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ION-PAIR REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY OF ORGANIC ACIDS

RE-INVESTIGATION OF THE EFFECTS OF SOLVENT pH ON R_F VALUES

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SUMMARY

The effect of solvent pH on the ion-pair reversed-phase thin-layer chromatography of four organic acids (salicylic acid, gentisic acid, 2,5- and 2,6-dihydroxybenzoic acid) has been re-investigated. These acids were chromatographed on silica gel, paraffin-impregnated silica gel and on C_{18} -bonded silica gel with a total of four ion-pair reagents.

The R_F values of these compounds were unaffected by solvent pH when tetra-*n*-butylammonium iodide and tetraheptylammonium bromide were used as ion-pair reagents. However, when cetrimide and cetyltrimethylammonium bromide were used a significant dependence of R_F on the solvent pH was observed.

Some possible explanations for the observed differences between these ion-pair reagents are discussed, together with the potential usefulness of this phenomenon.

INTRODUCTION

Ion-pair (IP) chromatography has been extensively employed in the field of high-performance liquid chromatography (HPLC) in order to obtain the retention of highly polar ionic substances. However, despite the advantages of IP techniques for such compounds, there have been relatively few applications of ion pairing in thin-layer chromatography (TLC). Some examples which show the usefulness of IP-TLC are described in refs. 1–4. In a previous paper we reported the results of studies in which the reversed-phase (RP) IP-TLC of a range of organic acids was investigated using a number of IP reagents⁴. This work was performed with the aim of determining the importance of factors such as pH, solvent composition etc., on chromatography. Our results demonstrated that, using tetra-*n*-butyl ammonium iodide (TBA) as a model IP reagent, varying the pH of the solvent between 2 and 10 had no effect on the R_F values of the test acids.

We were therefore intrigued by the report of Szepesi *et al.*³, who found in their studies that “the effect of the pH of the eluent on the selectivity and efficiency of the

separation on KC-18 (a C₁₈-bonded plate manufactured by Whatman) was similar to that in HPLC using a chemically bonded phase". These workers found that a pH of 8 was optimal for bonded phases, whilst a pH of 5 gave the best results on silica gel.

A number of differences between the studies of Szepesi *et al.*³ and our own investigations⁴ are apparent. In particular, they used an over-pressurised TLC system, and cetrimide as the IP reagent. However, their results appeared so at variance with our experience that they prompted us to re-investigate the role of solvent pH in IP-RP-TLC using a wider range of IP reagents, including both TBA and cetrimide.

EXPERIMENTAL

Chemicals

The ion-pair reagents used in this study were purchased from either Fisons [cetrimide (bromide salt), cetyltrimethylammonium bromide (CTA), tetra-*n*-butylammonium iodide (TBA)] or Eastman-Kodak [tetraheptylammonium bromide (THA)]. Solvents were of analytical grade.

The test acids were obtained from the Aldrich (Gillingham, U.K.) and were used as received. All the TLC plates used in this study incorporated a fluorescent indicator (254 nm).

Preparation of TLC plates

Silica gel TLC plates (20 × 20 cm, plastic backed, Macherey-Nagel, Camlab, Cambridge, U.K.) were coated with the appropriate ion-pair by dipping them in a 0.07 *M* solution of reagent dissolved in a volatile solvent. In the case of TBA and THA the dipping solution was dichloromethane and ethanol (4:1, v/v). For cetrimide and CTA the dipping solution was dichloromethane.

Paraffin-coated silica gel TLC plates, were prepared as described in ref. 4, by dipping silica gel TLC plates (Macherey-Nagel, 20 × 20 cm, plastic backed) in a 0.07 *M* solution of the IP reagent containing 7½% (v/v) of heavy refined paraffin ("Nujol"). Apart from the inclusion of paraffin, the composition of the dipping solutions were the same as those used for coating the silica gel TLC plates with IP reagent alone.

C₁₈-bonded silica gel HPTLC plates

The C₁₈-bonded plates (manufactured by E. Merck, supplied by BDH, Poole, U.K.) were also coated with IP reagent by dipping. The coating solutions were the same as those employed for coating the silica gel TLC plates.

Chromatography

Ascending chromatography was performed in glass TLC tanks 20 × 20 × 5 cm using solvent systems composed of methanol and 0.01 *M* potassium phosphate buffer at pH 2, 4, 7, 9 and 11.

All three types of IP-impregnated TLC plates (silica gel, paraffin-coated silica gel and C₁₈-bonded silica gel) were developed simultaneously in the same tank to minimise experimental variations.

RESULTS

The effect of solvent pH on the R_F of four polar organic acids (gentisic acid, salicylic acid, 2,5-dihydroxybenzoic and 2,6-dihydroxybenzoic acid) was investigated over the pH range 2–11. TBA, THA, cetrimide and CTA were used as IP reagents in these studies, coated onto a variety of TLC adsorbents. The adsorbents used were silica gel, paraffin-coated silica gel, and C_{18} -bonded silica gel. In the case of the paraffin-coated plates the IP reagent was applied to the plate in the same solution as the paraffin, as described elsewhere⁴. Unless the reagents were coated onto the adsorbents IP formation was either inefficient or did not occur at all^{3,4}. The results obtained for each of the IP reagents are described below.

TBA

In the absence of TBA all four acids had R_F values of approximately 0.9, irrespective of adsorbent, eluotropic strength of the solvent or pH. A similar result was obtained when TBA was coated onto silica gel. However, when coated onto either C_{18} -bonded or paraffin-coated plates TBA gave excellent results, with any desired R_F between 0 and 0.9 possible using a solvent of appropriate eluotropic strength. For the same solvent composition the paraffin-coated plates gave a higher R_F than the C_{18} -bonded ones. These results compare well with those obtained in the previous study⁴. When the effect of solvent pH was re-examined using both C_{18} -bonded and paraffin-coated TLC plates the R_F value of the test compounds showed

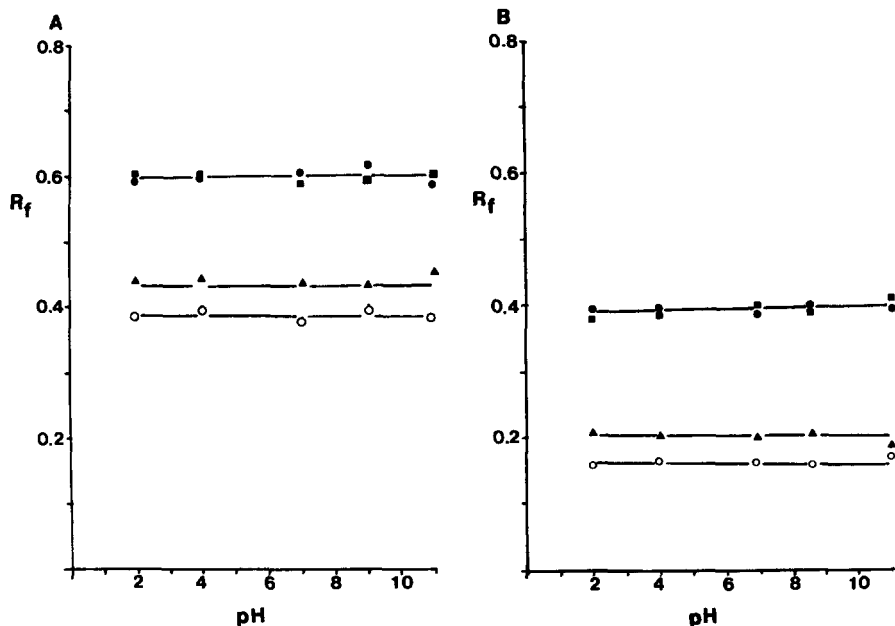


Fig. 1. Plots of R_F versus pH of the solvent [methanol-water (1:1)] showing the absence of any variation in the R_F values of the test compounds using TBA as IP reagent on paraffin-coated silica gel (A) and C_{18} -bonded RP-TLC plates (B). Key: (■) gentisic acid; (●) 2,5-dihydroxybenzoic acid; (○) 2,6-dihydroxybenzoic acid, (▲) salicylic acid.

no significant variation over the whole range, as illustrated in Fig. 1. These results confirmed our original observation and reinforced our view that solvent pH was not an important factor in the control of R_F in IP-RP-TLC using TBA. This result did not however, preclude the possibility that TBA might be an atypical IP reagent. For this reason we continued our investigations with THA, cetrimide and CTA.

THA

Chromatography on THA-coated silica gel was more successful than on TBA-coated plates. Thus, not only did the test compounds not chromatograph at the solvent front, but it was also possible to vary their R_F by changing the solvent composition. However, the chromatographic characteristics of THA-coated silica gel were not particularly good, with irregular spot shape and excessive tailing. Despite these limitations the results obtained when the solvent pH was varied showed that the R_F value did not change appreciably between pH 2 and 11. Similarly the R_F values of the test compounds was unaffected by solvent pH when the same experiments were performed using C_{18} -bonded and paraffin-coated RP-TLC plates. As was observed with TBA, for the same solvent composition paraffin-coated RP-TLC plates gave a higher R_F value than their C_{18} -bonded equivalents. The TBA-coated silica gel gave the highest R_F values of all.

Cetrimide

In marked contrast to TBA and THA cetrimide-coated silica gel gave excellent

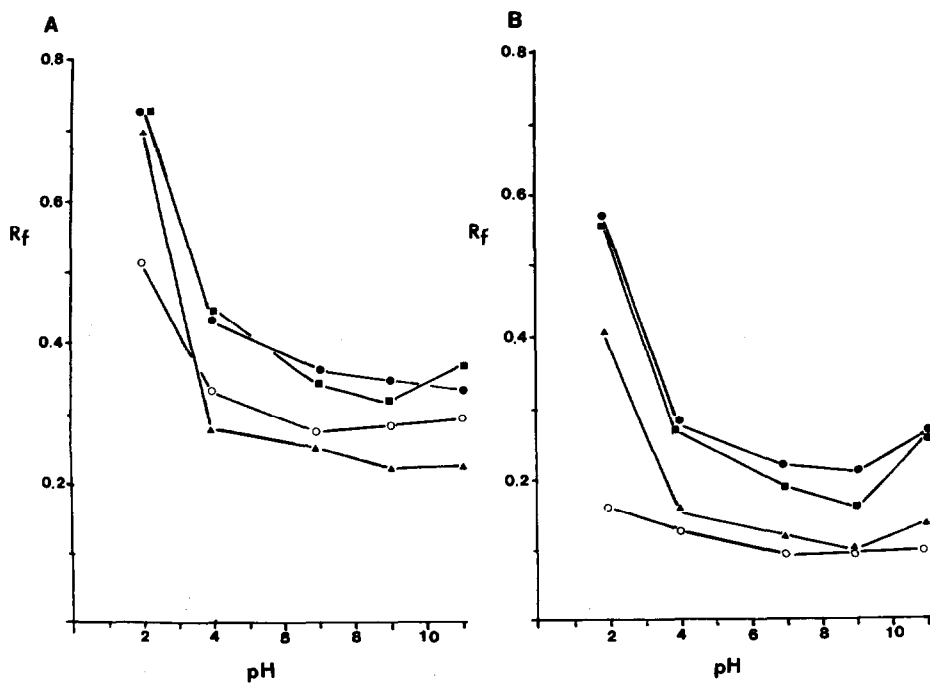


Fig. 2. Plots of R_F values of the test acids versus solvent pH on silica gel coated with cetrimide (A) or paraffin-coated silica gel also coated with cetrimide (B). Symbols as for Fig. 1. The solvent was methanol-water (1:1).

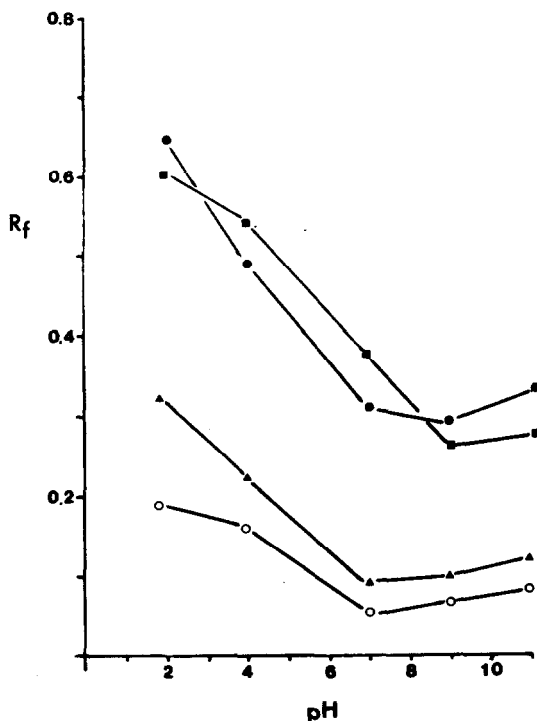


Fig. 3. Plot of R_F versus pH on C_{18} -bonded RP-TLC plates with cetrimide as IP reagent. The solvent was methanol-water (6:4). Symbols as for Fig. 1.

results when the chromatography of the test compounds was examined. Similarly paraffin-impregnated and C_{18} -bonded RP-TLC plates coated with cetrimide also gave good results for the four acids tested. For the same solvent composition the highest R_F values were observed on silica gel, with paraffin-coated plates giving higher R_F values than C_{18} -bonded phases. This result is therefore similar to that obtained with THA. However, when the effects of solvent pH on R_F were examined we were surprised to note a marked pH dependence in R_F for a constant solvent composition. So, for example, at pH 2 compounds such as gentisic acid, and 2,5-dihydroxybenzoic acid had R_F values (on silica gel coated with cetrimide) of approximately 0.76. These R_F values decreased dramatically at pH 4 to approximately 0.45, with a further, less marked, decrease when the pH was increased towards pH 11 (Fig. 2A). At pH 11 a slight increase in R_F was observed compared to the value obtained at pH 9. Essentially similar results were seen using paraffin-coated RP-TLC plates (Fig. 2B).

When the same experiments were performed on C_{18} -bonded RP-TLC plates a decrease in R_F with increasing solvent pH was also observed. However, unlike the silica gel and paraffin-coated plates there was no sudden reduction in R_F between pH 2 and 4, but instead a more regular decrease was obtained (Fig. 3).

CTA

In many respects the chromatographic properties of CTA-coated plates were similar to those impregnated with cetrimide. Thus excellent results were obtained

when this reagent was coated onto either silica gel, paraffin-coated silica gel, or C_{18} -bonded RP-TLC plates. Like cetrimide this IP reagent showed a clear dependence of R_F of the test compounds on pH, with the highest values obtained for acidic solvent systems. On silica gel and paraffin-coated silica gel RP-TLC plates the large decrease in R_F between pH 2 and 4, originally observed with cetrimide, was repeated with CTA. On the C_{18} -bonded plates a more gradual decrease in R_F was noted.

DISCUSSION

The mechanism(s) of IP chromatography are still a matter for considerable debate⁵. For IP-HPLC three models have been proposed, the IP model, the dynamic ion-exchange model, and the ion-interaction model. In the IP model reagent and solute combine in the solvent to form a neutral species which is then able to partition into the stationary phase. The ion-exchange model assumes that the solute interacts with IP reagent adsorbed on the stationary phase. In the case of the ion-interaction model a somewhat more complex situation is proposed requiring the formation of an electrical double layer at the surface of the stationary phase. The primary layer is composed of IP reagent whilst an oppositely charged secondary layer of ions forms above this. The sample solute competes for access to the secondary layer, from which it is electrostatically attracted into the primary layer, where it may also be subject to lipophilic attraction to the non-polar stationary phase. Absorption of a solute ion into the primary layer results in an extra charge being present and a further molecule of IP reagent then enters to neutralise it. Thus a pair of ions is now present in the primary layer, although not necessarily as an ion pair. This partition, or adsorption, of ions onto the stationary phase is a dynamic process, and chromatographic separation is therefore possible.

Whether the models proposed for IP-HPLC apply equally well to IP-TLC is not yet clear. However, whatever mechanisms are responsible for IP-RP-TLC the IP reagents which we have examined fall into two distinct classes, those which show pH-dependent effects on R_F and those which do not. Clearly the explanation for this must reside in some physicochemical difference between the two classes of IP reagent. If only TBA and cetrimide are considered, the former showing no pH dependency whilst the latter giving a marked dependence of R_F on solvent pH, then the difference cannot simply be molecular weight as they differ by only a single methylene group. In shape however TBA and cetrimide are very different (see Fig. 4). Thus TBA is a symmetrical and (by comparison with cetrimide) compact molecule. Cetrimide in contrast is unsymmetrical, and has its quaternary nitrogen placed at one end of a long, lipophilic, alkyl chain. It should also be noted that as a consequence of these differences in lipophilicity the water solubility of TBA is much greater than that of cetrimide. It does not therefore seem unreasonable to suppose that such different IP reagents will give rise to quite different layers when coated onto TLC plates.

The type of layer formed seems also to depend in part on the adsorbent. Thus whilst silica gel and paraffin-coated silica gel gave rather similar results for cetrimide the C_{18} -bonded plates showed a different type of pH-dependent behaviour. This may be explicable in terms of the acidic silanol groups present on the silica gel. In the case of silica gel and the paraffin-coated silica gel all of the silanol groups are present and available to interact with the basic quaternary ammonium IP reagent. It can be

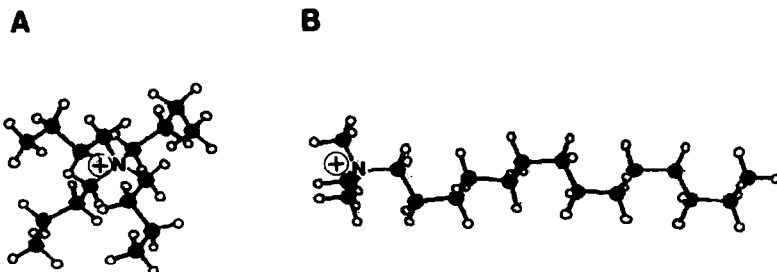


Fig. 4. Structures of TBA (A) and cetrimide (B) produced by a Chemical Graphics program (Chemical Design, Oxford, U.K.) on a Vax 11/750 computer.

postulated that, for an IP reagent like cetrimide on silica gel, this would lead to the production of a lipophilic layer on the surface of the silica gel in which the "tails" of further cetrimide molecules might sit, with their hydrophilic ion-pairing ends protruding into the aqueous mobile phase. This would enable cetrimide to act with an ion-exchange (or perhaps ion-interaction) type of mechanism when acidic compounds, such as the test acids, were chromatographed upon such plates. This type of layer is illustrated in Fig. 5A. As the pH of the mobile phase was lowered the ionisation of the silanols would be suppressed abolishing (or greatly reducing) the extent of the interaction between them and the IP reagent. This might lead to the situation shown in Fig. 5B, where a simple IP mechanism might apply.

In the case of C_{18} -bonded plates many of the silanol groups are removed when the silica gel is reacted with the silylating reagents used to prepare the bonded phase. Thus, the number available for interaction with cetrimide is limited.

As was the case with silica gel and paraffin-coated silica gel when the pH is lowered that portion of the cetrimide interacting with the silanols is reduced. However, whereas with the silica gel and paraffin-coated plates the reduced interaction of the IP reagent with the silanol groups might lead to the breakdown of the postulated layer (Fig. 5B), the C_{18} layer would still provide a lipophilic zone in which the lipophilic portion of cetrimide might sit. This possibly explains in part why the dra-

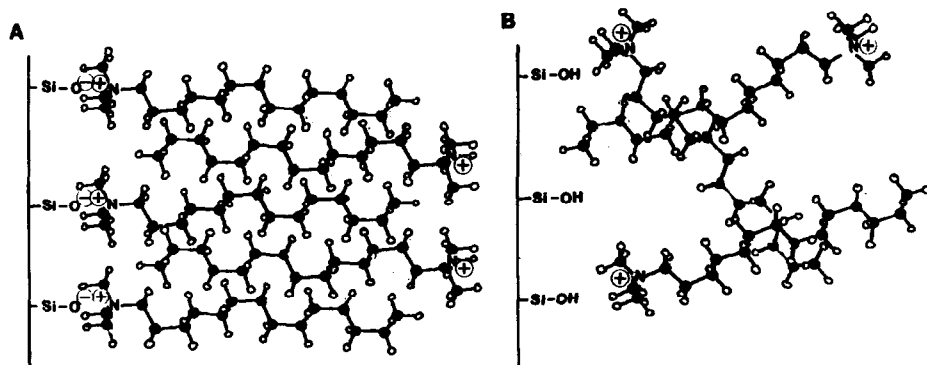


Fig. 5 (A) Hypothetical bilayer resulting from the interaction of cetrimide with ionised silanol groups on silica gel. (B) The breakdown of the bilayer illustrated in A because of the suppression of ionisation of the silanol groups at a solvent pH of 2.

matic increase in R_F noted with cetrimide on silica gel and paraffin-coated plates on going from pH 4 to 2 is not seen on C_{18} -bonded plates. An ion-interaction or ion-exchange mechanism rather than ion-pairing seems likely on these C_{18} -bonded plates.

With IP reagents such as TBA the construction of the hypothetical layer postulated for cetrimide (and similar compounds) looks less likely. For, although a proportion of the available TBA may indeed interact strongly with surface silanols on the silica gel they will not produce a non-polar layer as, unlike cetrimide, the quaternary ammonium ion is in the centre of the molecule and not at one end. Furthermore, TBA (and THA) are hydrophilic, water soluble, compounds. Thus, it is very likely that even were a layer formed it would rapidly breakdown as the reagent dissolved in the advancing solvent. Layers of cetrimide (and CTA) on the other hand, being much less water soluble, would probably be more stable. Thus this difference in water solubility may provide a partial explanation for the lack of ion-pairing effect with TBA coated onto silica gel compared with cetrimide.

It must be emphasised that, in the absence of further experimental evidence, much of the above discussion is highly speculative.

CONCLUSIONS

Whatever their cause the differences observed between the two types of IP reagent in this study probably explains why some workers have encountered pH-dependent effects in IP-RP-TLC whilst we did not. These results are both interesting and useful, for, whilst the underlying reasons for the different types of behaviour requires further study, there are immediate practical benefits to the chromatographer. Thus systems may be chosen where pH is an important factor in the separation or alternatively pH effects may be excluded completely. This, therefore provides another way in which the selectivity of an IP-RP-TLC system might be manipulated. For example, it should be possible using a pH "sensitive" IP reagent to change the R_F values of acidic compounds whilst leaving non-acids unaffected, thus enabling a separation to be optimised.

Further work is clearly required in this area in order to provide explanations for the behaviour of the different IP reagents, and to explain differences between the bonded and paraffin-coated TLC plates. Studies designed to provide further insights into these problems are in progress.

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