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COMPARISON OF NON-HYDROPHOBIC C_{18} BONDED AND PARAFFIN-COATED SILICA GEL REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY PLATES

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SUMMARY

The chromatographic properties of C_{18} bonded reversed-phase thin-layer chromatography plates have been compared with paraffin-coated silica gel thin-layer chromatography plates for the reversed-phase chromatography of a range of organic compounds. These included non-steroidal anti-inflammatory drugs, ecdysteroids, aminophenols, hydroxybenzoic acids and antipyrine. Both types of plate can be used with mobile phases containing up to 100% water, allowing a wide choice of solvent systems. Although differences were found between bonded and coated plates in most applications they are interchangeable following minor adjustments to the mobile phase.

INTRODUCTION

Alkyl-bonded reversed-phase thin-layer chromatography (RP-TLC) plates are usually intensely hydrophobic[1,2], limiting the amount of water which may be added to the mobile phase. This in turn limits the range of solvent systems available, and may therefore considerably restrict the types of compound which can be chromatographed. The application of aqueous solutions, or biological fluids (plasma, bile, urine etc.) is also prevented. Paraffin-coated silica gel RP-TLC plates are not limited in this way, and may be used with entirely aqueous solvent systems and samples[3]. Recently a number of alkyl-bonded RP-TLC plates have become available which are compatible with high levels of water in the mobile phase or sample[4,5]. In an earlier report we described the use of a non-hydrophobic C_{12} bonded TLC plate, together with paraffin-coated silica gel for the RP-TLC of ecdysteroids (insect moulting hormones)[3]. We have also described the application of the C_{12} bonded plate in the determination of the stability of a novel β-blocking drug in biological fluids[6]. In this study we have compared C_{18} bonded non-hydrophobic RP-TLC plates with paraffin-coated silica gel for a wider range of compounds, including non-steroidal anti-inflammatory drugs (NSAIDs), aminophenols, ecdysteroids, antipyrine and some hydroxybenzoic acids.

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EXPERIMENTAL

Preparation of RP-TLC plates

Paraffin-coated plates were prepared as described previously[3]. Macherey-Nagel plastic-backed silica gel TLC plates, 20 × 20 cm were developed in a solvent system consisting of refined heavy paraffin oil (Nujol) in dichloromethane (7.5%, v/v). When the solvent reached the top of the plates, they were removed from the tank and allowed to dry in a fume cupboard.

Merck C_{18} bonded plates were washed in methanol and heated at 120°C for 15 min prior to use.

Chromatography

Ascending chromatography was performed in glass TLC tanks (20 × 20 × 5 cm), saturated with the solvent. Samples were applied as solutions in methanol using a 1-μl glass capillary. Plates were equilibrated over the solvent for 30 min prior to chromatography. Following chromatography, plates were visualised under UV light at 254 nm.

Bonded and coated plates were always chromatographed simultaneously in the same TLC tank.

RESULTS AND DISCUSSION

In order to compare paraffin-coated and C_{18} bonded plates, compounds covering a range of polarities, with a variety of functional groups, and in some cases the potential for ionisation, were chosen. In all cases the paraffin-coated and C_{18} bonded plates were chromatographed together in order to aid comparison. The results of these experiments for each of the classes of compound examined are described below.

RP-TLC of NSAIDs

The chromatography of four non-steroidal anti-inflammatory drugs, ibufenac, ibuprofen (a methyl analogue of ibufenac), isoxepac and a methyl analogue of isoxepac was investigated.

On the C_{18} bonded plates R_F values of 0.5 for all four compounds were obtained with mobile phases containing between 78 and 87% of methanol. A linear relationship between R_F and methanol concentrations over the range 60–80% was obtained for all four compounds (Fig. 1a). The best separation was seen with 60% methanol.

On paraffin-coated plates R_F values of 0.5 were obtained with *ca.* 55 and 60% methanol for isoxepac and its methyl analogue, and at *ca.* 74 and 78% for ibufenac and ibuprofen respectively. For isoxepac and its analogue a linear relationship between R_F and the methanol content of the mobile phase was observed over the range 40 to 60% methanol. A similar relationship was seen for ibufenac and ibuprofen for methanol concentrations of between 50 and 80%. Only small increases in R_F were observed when more than 80% methanol was included in the mobile phase (Fig. 1b). The best separation of these compounds was seen with 60% methanol.

The effect of pH on R_F was briefly investigated as on high-performance liquid chromatography (HPLC) a large increase in retention time is obtained with this type

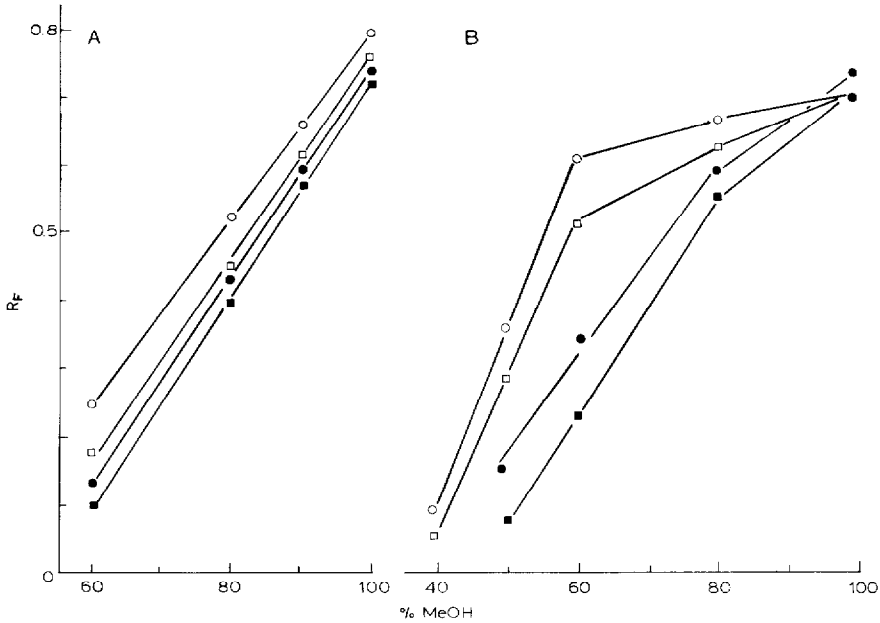

Fig. 1. (A) effect of mobile phase composition on the R_F of isoxepac (○), methyl isoxepac (□), ibufenac (●) and ibuprofen (■) on C_{18} bonded RP-TLC plates. (B) effect of mobile phase composition on the R_F of isoxepac (○), methyl isoxepac (□), ibufenac (●) and ibuprofen (■) on paraffin coated plates.

of compound when the mobile phase is acidified. A similar effect on RP-TLC could prove useful for optimising separations, particularly between acidic and non-acidic compounds. However, addition of formic acid (up to 1%) produced only a small decrease in R_F, on either type of plate.

On C_{18} bonded plates the chromatographic behaviour of all four NSAIDs was very similar. This was not the case on paraffin-coated plates where two distinct types of chromatography were seen. Thus isoxepac and its methyl analogue were similar to each other in chromatographic properties, as were ibufenac and ibuprofen. However, the two pairs of compounds exhibit quite different chromatography when compared to each other. The reasons for this are not clear, but it may be that on bonded plates the properties of the carboxyl group predominate, whilst on coated plates the structure of the drug assumes greater importance in determining chromatographic properties. Good spot characteristics were obtained on paraffin-coated and C_{18} bonded RP-TLC plates, and both appear suitable for the chromatography of these compounds.

RP-TLC of hydroxybenzoic acids

The chromatography of a range of hydroxybenzoic acids including 4-hydroxy-, 2,3-dihydroxy-, 2,4-dihydroxy-, 2,5-dihydroxy-, 2,6-dihydroxy- and 3,4-dihydroxybenzoic acids was examined. Neither bonded nor paraffin coated plates were suitable for the chromatography of these compounds. With both bonded and coated plates only poorly defined streaks were obtained. The addition of acid (1% formic or 1% phosphoric acid) to suppress ionisation failed to improve the chromatography.

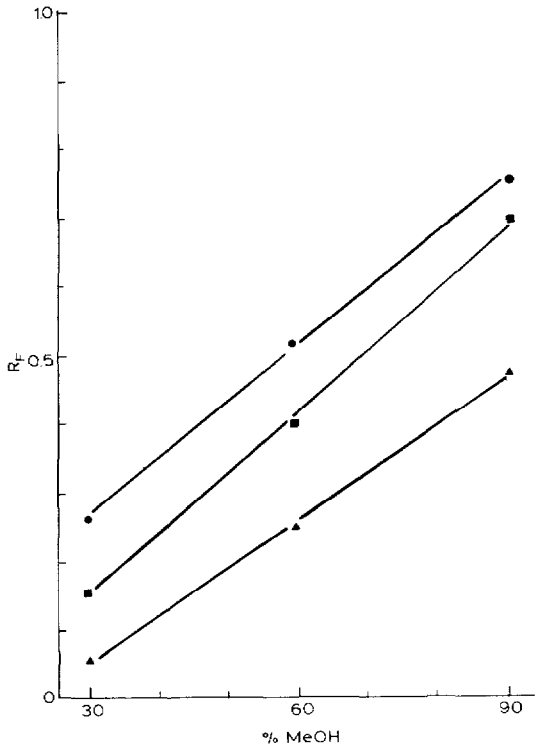

Fig. 2. Effect of mobile phase composition on the R_F of *p*-aminophenol (▲), *m*-aminiophenol (●) and *o*-aminophenol (■) on C_{18} bonded RP-TLC plates.

RP-TLC of aminophenols

On the C_{18} bonded plates the order of separation of the aminophenols was *meta* (*m*) > *ortho* (*o*) > *para* (*p*). A linear relationship between R_F and the methanol content of the mobile phase was seen between 30 and 90%, with good resolution over the whole range (Fig. 2). For 60% methanol, R_F values of 0.4, 0.52 and 0.25 were obtained for *o*-, *m*- and *p*-aminophenol, respectively. The aminophenols were not well retained on paraffin-coated plates, the only suitable solvent system proving to be 100% water. In this system R_F values of 0.56 (*o*), 0.68 (*m*) and 0.86 (*p*) were obtained. Whilst both separation and spot shape were good, there is clearly less opportunity for adjusting the separation by altering the mobile phase composition on the coated compared to the bonded plates. It is interesting to note that the coated plates exhibited a different selectivity compared to the bonded plates. On coated plates, the order of separation of the aminophenols was *p*, *m*, *o*, whilst on the bonded plates the order was *m*, *o*, *p*. It is not clear why this should be so; however the anomalous behaviour of these compounds on RP-TLC has been noted by others[7]. Modification of the pH of the mobile phase with aqueous ammonia solution (specific gravity 0.88), had no effect on the R_F values of the aminophenols on the coated plates [at either 1, or 10% (v/v) aqueous ammonia]. When the same experiments were performed on C_{18} bonded plates it was observed that as little as 1% ammonia in the solvent caused the adsorbent to wrinkle and become detached from the glass backing.

This was not an effect of pH since 0.1 *M* sodium hydroxide solution did not produce the same result. Solvent systems containing ammonia have been used by us with C_2, C_8 and C_{18} bonded hydrophobic RP-TLC plates from the same manufacturer, and with C_{12} bonded non-hydrophobic RP-TLC plate[3] without difficulty. It would therefore appear that this problem is restricted to this particular type of plate.

RP-TLC of ecdysteroids

On coated plates R_F values of 0.5 were obtained for ecdysone and 20-hydroxyecdysone with *ca.* 50 and *ca.* 40% methanol in the mobile phase. For an R_F value of 0.5 on C_{18} bonded plates concentrations of 70 and 60% methanol were required for ecdysone and 20-hydroxyecdysone respectively (Fig. 3). Linear relationships between methanol content and R_F for ecdysone were seen over the range 30–70% for coated and 40–90% for bonded plates. The linear range for 20-hydroxyecdysone was somewhat narrower, extending between 40–50% on coated, and 50–80% on C_{18} bonded plates. Best separations between ecdysone and 20-hydroxyecdysone were obtained with solvent compositions of between 40 and 50% methanol on coated plates. For bonded plates, little difference in the degree of separation was seen with between 50 and 80% methanol in the mobile phase. The largest separation of *ca.* 0.2 R_F units was obtained on paraffin-coated plates, compared with *ca.* 0.125 R_F units on C_{18}

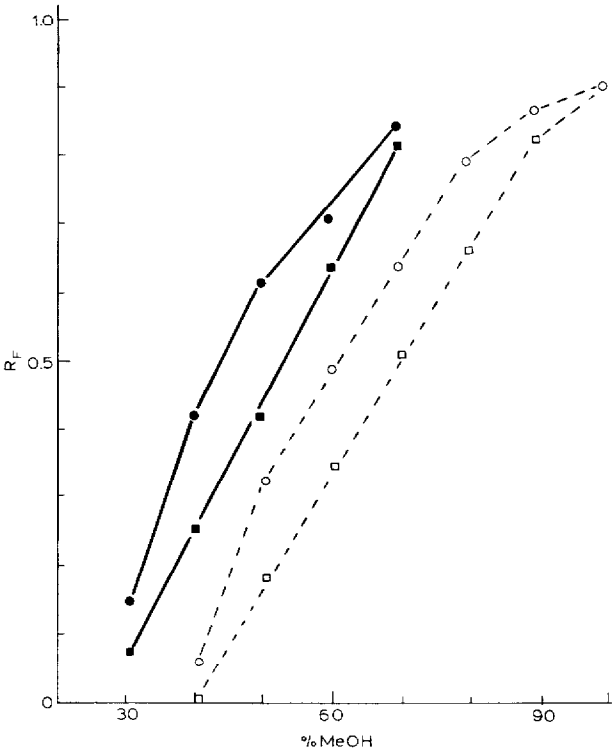

Fig. 3. Effect of mobile phase composition on the R_F of ecdysone (■, □), and 20-hydroxyecdysone (●, ○), on paraffin-coated (——) and C_{18} bonded (- - -) RP-TLC plates.

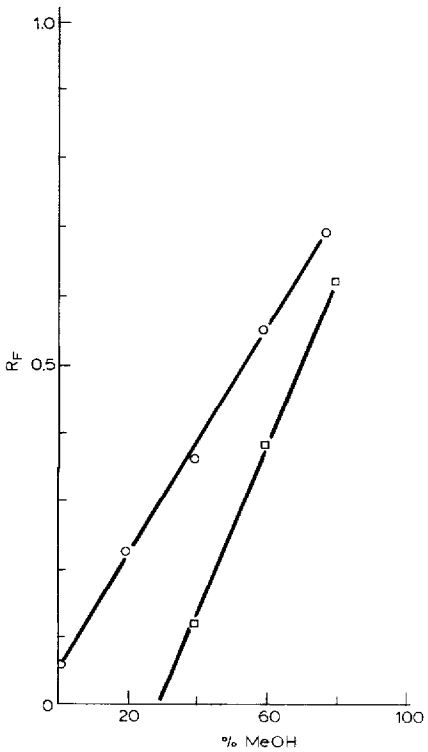

Fig. 4. Effect of mobile phase composition on the R_F of antipyrine on paraffin-coated (O) and C_{18} bonded RP-TLC plates (□).

bonded plates. Both types of plate appear suitable for the separation of the ecdysteroids.

RP-TLC of antipyrine

Antipyrine on paraffin-coated plates had an R_F value of 0.5 using *ca.* 45% methanol in the mobile phase. To achieve a similar R_F value on bonded plates required *ca.* 70% methanol. With no organic modifier in the mobile phase antipyrine had an R_F value of 0.08 on coated plates, whilst on the bonded plates up to 20% methanol may be present without migration. With both types of plates good spot shapes were obtained on chromatography. There was a wide range over which a linear relationship between R_F values and methanol content was obtained (0–50% for coated and *ca.* 40–80% for bonded), as shown in Fig. 4. Both types of plate appear suitable for the chromatography of antipyrine.

CONCLUSIONS

Both paraffin-coated and C_{18} bonded types of plate were suitable for the chromatography of all the compounds tested except the hydroxybenzoic acids. Usually a linear relationship between methanol content and R_F was observed, extending over

a wide range of solvent compositions. This property should be especially useful in metabolic studies where metabolites are more polar than the parent drug. In such cases the solvent strength could easily be modified to take this into account. Relatively large changes in solvent composition are required to cause appreciable changes in R_F in contrast to normal-phase TLC, where small changes in solvent composition may cause large changes in R_F. This means that in RP-TLC, R_F is relatively insensitive to small changes in the composition of the mobile phase such as those which might result from day to day variation in the preparation solvent. Both types of plate were suitable for solvents containing up to 100% water with relatively short development times. For bonded plates run in 40 and 60% methanol, development times were *ca.* 0.23 cm min^{-1}, with similar values also being obtained for paraffin-coated plates (for a 4.5-cm run). Invariably the C_{18} bonded plates required a higher percentage of methanol in the mobile phase to achieve the same R_F as the coated plates. The greatest differences in chromatography between bonded and coated plates were observed with the aminophenols, both in terms of solvent strength and selectivity. Differences in selectivity were also seen for the NSAIDs. The lack of effect of acidification of the mobile phase on the R_F of the NSAIDs, which are all organic acids, is surprising especially when compared to HPLC. In part this may be explained by the acidic nature of the uncapped silanols on the surface of the silica. Because these are acidic they may already suppress the ionisation of these weakly acidic drugs to such an extent that the addition of further acid is without effect.

We found both types of RP-TLC plates easy to use, and suitable for most applications. With appropriate modification of mobile phase composition, bonded and coated plates appear interchangeable.

The ability to apply entirely aqueous samples (such as urine, bile, or deproteinised plasma) onto the plates greatly increases the scope and usefulness of RP-TLC for biomedical applications.

Given the excellent chromatographic properties of both types of plate, the choice of which system to use then becomes one of economy or convenience. The paraffin-coated plates are cheap in comparison to the bonded plates. However the latter require no preparation before use and the consequent saving in time might be important in some circumstances.

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