CHROM. 4868

The influence of pH in buffered reversed-phase thin-layer chromatography of penicillins and cephalosporins

The R_M values of penicillins and cephalosporins, as determined by a previously described reversed-phase TLC method[1,2], were shown to correlate well with the antibacterial activity[3]. This was in agreement with findings that the lipid-solubility of unionized molecules is one of the important factors in determining the passage of drugs through biological membranes[4,5]. A decrease in the permeability of a membrane to weak acids or bases can result from a change in the pH of the medium, which ionizes

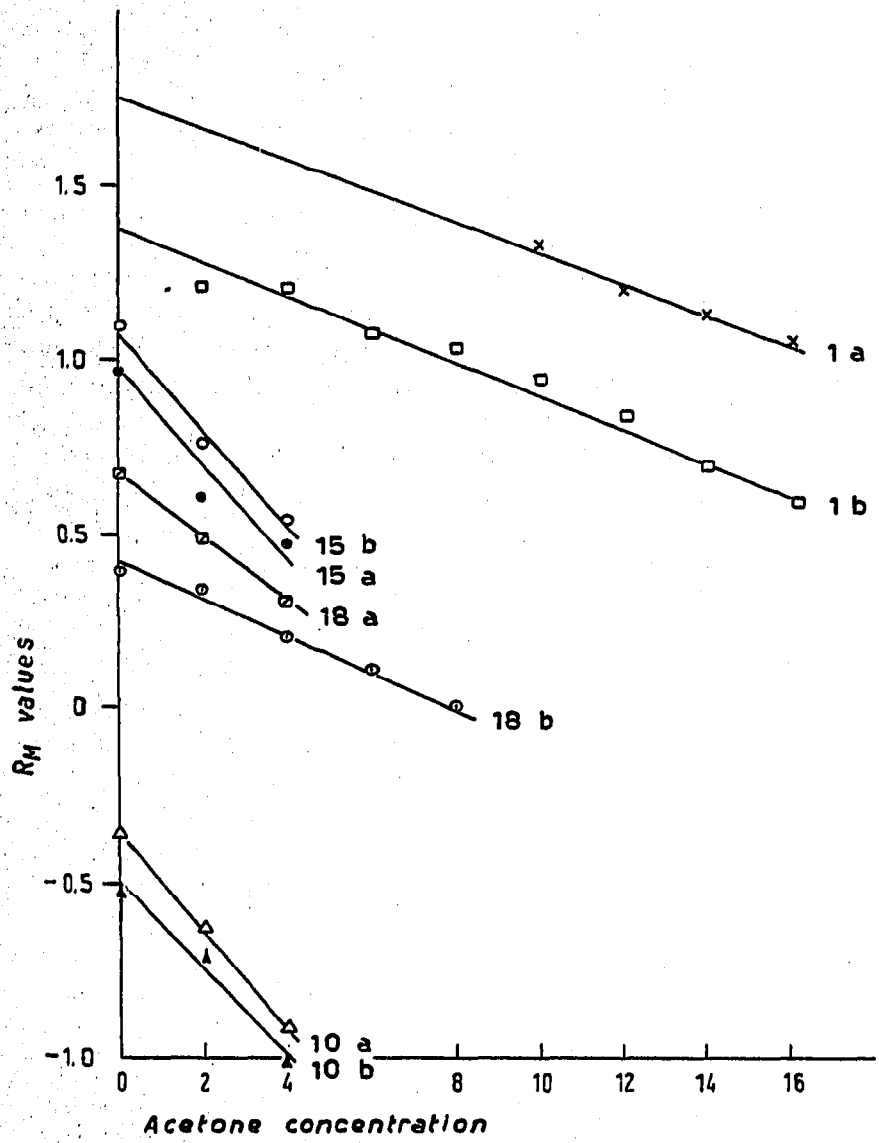
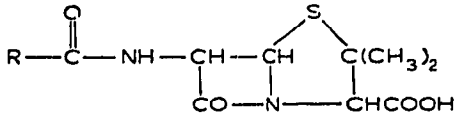


Fig. 1. The R_M values of some compounds plotted against the composition of the mobile phase. The straight lines were calculated from the R_M values in the range of linearity. Each point represents the mean of 8 determinations. The compounds are numbered as in Tables I and II. The pH 2.6 and 9.4 of the mobile phase correspond respectively to (a) and (b).

TABLE I

LIST OF THE PENICILLINS ACCORDING TO THE DECREASING LIPOPHILIC CHARACTER OF THEIR MOLECULES AS EXPRESSED BY THEIR EXTRAPOLATED R_M VALUES BOTH AT pH 2.6 AND 9.4
Carbenecillin was previously indicated as carboxybenzylpenicillin[1].



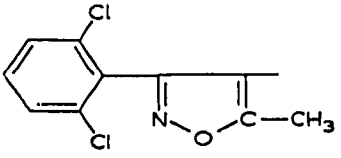
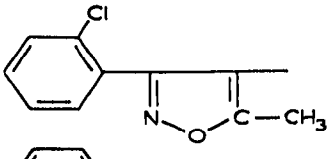
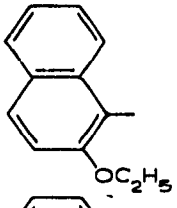
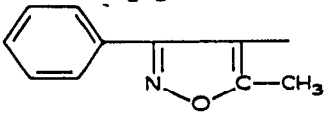
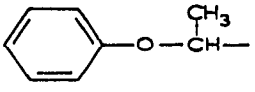
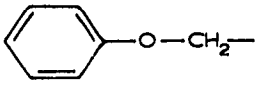
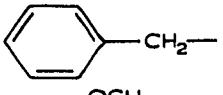
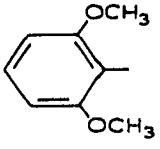
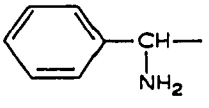
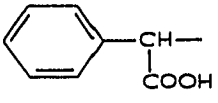
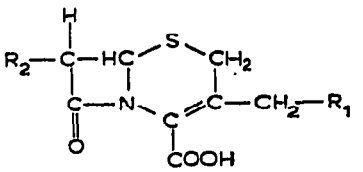
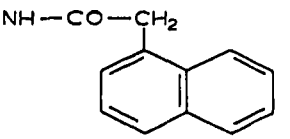
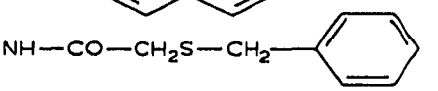
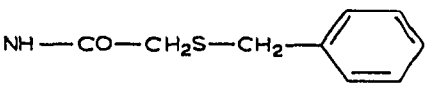
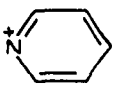
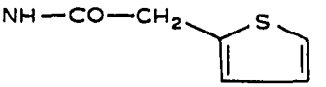
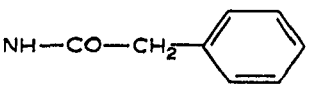
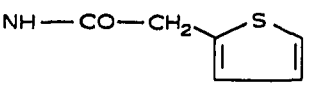
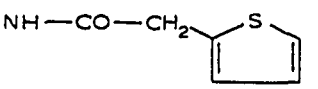
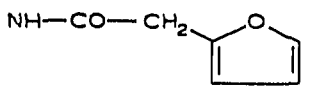
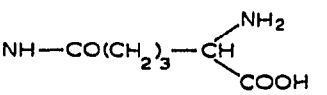
No.	*Compound*	*R*	*R_M value*	
			pH 2.6	*pH 9.4*
1	Dicloxacillin		1.76	1.43
2	Cloxacillin		1.67	1.21
3	Nafcillin		1.43	1.20
4	Oxacillin		1.39	0.96
5	Phenethicillin		1.35	0.91
6	Phenoxymethylpenicillin		1.17	0.89
7	Benzylpenicillin		0.84	0.45
8	Methicillin		0.78	0.41
9	Ampicillin		0.11	0.05
10	Carbenecillin		−0.37	−0.50

TABLE II

LIST OF THE CEPHALOSPORINS ACCORDING TO THE DECREASING LIPOPHILIC CHARACTER OF THEIR MOLECULES AS EXPRESSED BY THEIR EXTRAPOLATED R_M VALUES BOTH AT pH 2.6 AND 9.4
Cephaloridine was not tested in the previous work on cephalosporins[2].



No.	*Compounds*	R_1	R_2	R_M *value*	
				pH 2.6	*pH 9.4*
11	(Acid)	$O-CO-CH_3$	$NH-CO-(CH_2)_6-CH_3$	1.94	1.72
12	(Acid)	$O-CO-CH_2$		1.59	1.40
13	(Sodium salt)	$O-CO-CH_3$		1.48	1.20
14	(Sodium salt)	N_3		1.37	1.17
15	(Cephaloridine)			0.99	1.08
16	(Sodium Cephaloram)	$O-CO-CH_3$		0.86	0.63
17	(Sodium Cephalotin)	$O-CO-CH_3$		0.69	0.43
18	(Acid)	N_3		0.67	0.42
19	(Acid)	$O-CO-CH_3$		0.38	0.22
20	(7-Amino-cephalosporanic acid)	$O-CO-CH_3$	NH_3^+	−0.17	−0.26
21	(Potassium cephalosporin C)	$O-CO-CH_3$		−0.49	−0.57

the molecules[4]. The present work shows that such a reversed-phase TLC method can demonstrate the influence of the pH of the mobile phase on the partitioning of penicillins and cephalosporins between the polar mobile and the non-polar stationary phases.

The R_M values of the penicillins and cephalosporins are reported in Tables I and II. The non-polar stationary phase consisted of silicone oil. The aqueous mobile phase was sodium acetate veronal buffer at pH 2.6 or 9.4, alone or in various proportions with acetone. The pH of the mobile phase was measured after each chromatographic run and found to be practically unchanged. The details of the TLC method have already been published[1].

Results and discussion

As previously pointed out[1,2], there is a range of linear relationship between R_M values and acetone concentration in the mobile phase (Fig. 1). The plots in Fig. 1 also show that a lipophilic compound such as dicloxacillin at pH 2.6 did not move from the starting line until a 10 % acetone concentration was used as mobile phase. On the other hand, at pH 9.4 a 2 % acetone concentration was sufficient to move the compound. More hydrophilic compounds such as compound No. 18 and carbenecillin migrated with a 0 % acetone concentration in the mobile phase both at pH 2.6 and 9.4. However the R_M values obtained at pH 2.6 were higher than those at pH 9.4. The only exception was cephaloridine, the R_M values of which were higher at pH 9.4 than at pH 2.6 (Fig. 1). By means of the equations of the straight lines it was possible to calculate an R_M value for each compound at 0 % acetone in the mobile phase at pH 2.6 and 9.4 (Tables I and II). In practice, the compounds migrated as round spots both at acid and basic pH's.

Higher R_M values indicate a more lipophilic nature; this means that at an acid pH more molecules are unionized and therefore are more lipophilic than at a basic pH. This is in agreement with the acidic character of penicillins and cephalosporins[6]. The behavior of cephaloridine could be considered a consequence of the basic character of the pyridine ring in its molecule which is ionized at an acid pH.

The influence of pH on the chromatographic behavior of acids and bases has also been pointed out by several other investigators[7–10]. HOWE[8] in particular, showed that, for several series of organic acids, an acid or basic mobile phase, where the acids were unionized or ionized, respectively, caused different migrations of each compound. BUSH[10] suggested the use of a formula derived by SOCZEWIŃSKI[11] for the calculation of the pK of an acid or base from paper chromatographic data. The classification of the compounds is practically the same as that previously found with a mobile phase of pH 7.4[1,2].

$$R_M \text{ (acid)} = 0.190 + 1.096\, R_M \text{ (basic)}$$

In Fig. 2, the R_M values for each compound at pH 2.6 are plotted against those at pH 9.4. It can be seen that there is a linear relationship between the two series of data, expressed by the equation:

This means that all the compounds tested experimentally show the same increase in their R_M value when one changes the pH of the mobile phase from 2.6 to 9.4. Cephaloridine, which was not used in calculating the equation of the straight line, shows the greatest deviation from the regression line. In fact, its R_M values

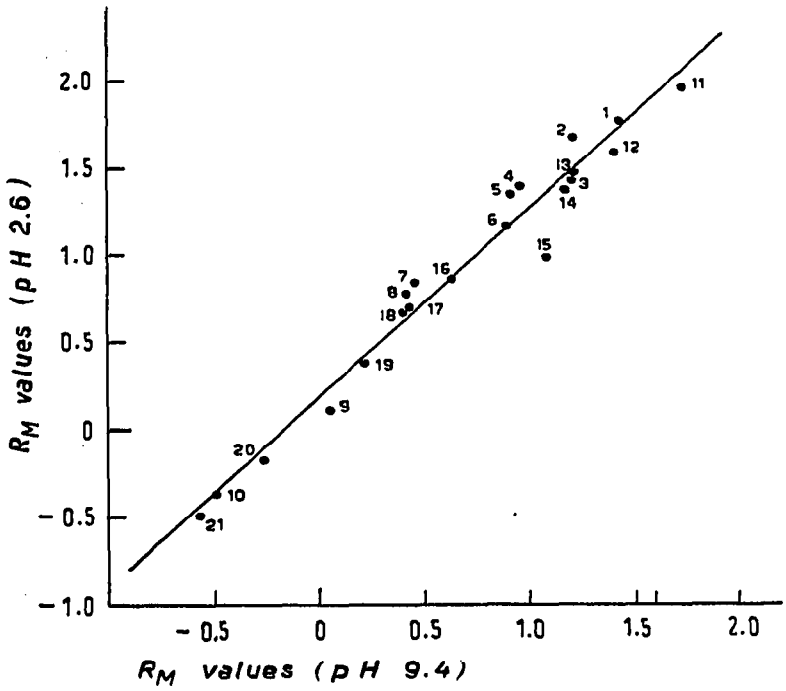


Fig. 2. The R_M values at pH 2.6 are plotted against those at pH 9.4. The compounds are numbered as in Tables I and II.

decrease by changing from a basic to an acidic pH in the mobile phase. The present data seem to suggest that buffered reversed-phase TLC can be used as a system, which permits one to study changes in the lipophilic character of a compound according to variations in the pH of the medium. The TLC technique described here could possibly provide a model system for studying the penetration of molecules through biological membranes.

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