Journal of Chromatography, 133 (1977) 355–358

CHROM. 9748

Note

Salt effects in reversed-phase thin-layer chromatography on silica gels

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(Received October 11th, 1976)

When evaluating a feasible method for extracting the isoxazolylpenicillins and their active metabolites after their quantitative separation by reversed-phase thin-layer chromatography (TLC) from the non-polar stationary phase (silica gel impregnated with silicone oil[1] and/or silanized silica gel plates), it appeared that neither aqueous buffer systems (pH *ca.* 7), nor organic solvents were useful for this purpose[2]. As the penicillins are relatively strong acids ($pK_a = 2.7$) and the apparent partition coefficient (octanol–water) of the isoxazolylpenicillins at pH 7 is *ca.* 0.01–0.04 (ref. 3), the failure to extract these substances from the thin layers by aqueous systems was unexpected. In order to obtain more insight into this behaviour, the adsorption of cloxacillin on a thin-layer (silica gel, S; silica gel impregnated with silicone oil, SO; silanized silica gel, SS) was investigated by adding 30 mg of the thin-layer material** to 1 ml of a cloxacillin solution (20 μg/ml) and shaking the suspension for 1 h at room temperature; after centrifugation, the remaining amount of cloxacillin in the supernatant was determined by bioassay[4].

Preliminary experiments showed that the adsorption on SO and SS increased with increasing salt content of the cloxacillin solution. S, on the contrary, showed virtually no adsorptive properties under the conditions used (Table I). These results suggest that the partition of cloxacillin between the lipophilic phase (either SO or SS) and the aqueous phase depends on the ionic strength.

The partition of cloxacillin between 15% silicone oil in *n*-hexane and water, or between octanol and water, however, showed no dependence of, for instance, the sodium chloride content ($\leqslant 1$ *M*) of the aqueous phase. Measurements of the pH of the suspensions showed that the thin-layer materials were "proton-active", *i.e.*, they changed the pH of the cloxacillin solutions. Table II summarizes the changes in the pH of buffered cloxacillin solutions (potassium phosphate buffer) following the addition of the thin-layer materials. From the results, it is clear that the different silica gels give a basic reaction in water and weakly buffered solutions. The same effect was observed for sodium chloride solutions. Increasing sodium chloride concentrations ($>10^{-1}$ *M*) increased the acidity of the suspension (pH < 6). The pH of acidic

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** The thin-layer material was obtained by scraping off the plates (pre-coated plates; Merck, Darmstadt, G.F.R.) and homogenizing the scrapings. A 30-mg amount of the material corresponds to about 2 cm^2 of the thin-layer.

TABLE I

ADSORPTION OF CLOXACILLIN ON THIN-LAYER MATERIAL

The results are the means of experiments in triplicate.

Solvent for cloxacillin (20 μg/ml)	*Fraction adsorbed*		
	*S**	*SO**	*SS**
Water	0.09	0.28	0.31
10^{-2} *M* potassium phosphate (pH 6.1)	0.04	0.54	0.63
10^{-2} *M* sodium chloride		0.50	0.65
10^{-1} *M* potassium phosphate (pH 6.1)	0.03	0.84	0.82
10^{-1} *M* sodium chloride	0.06	0.90	0.89

* The thin-layer material was added to the cloxacillin solutions in 30 mg/ml amounts.

systems (pH $\ll$ 6), on the contrary, was shifted to more basic values. For instance, 30 mg of SS suspended in 1 ml of Teorell buffer[5] of pH 3.9 shifted the pH to about 4.9.

The cation-exchanging property of silica gel, which apparently is responsible for this proton-activity, is well known[6]. This property, however, was not expected of silanized silica gel, as the reactive silanol groups of silica gel are considered to be replaced by non-polar dimethylsilyl groups. Silanized silica gel that is commercially available for column chromatography (Merck), on the contrary, did not show the above property. This material, at moderate pH, showed only weak adsorptive properties (Fig. 1). Consequently, it might be that the enhanced adsorption on SO and SS are pH effects. However, as can be seen from Fig. 1, salt effects are also involved. The influence of salt concentration on the adsorption is probably a thermodynamic phenomenon as a result of changes in the water structure or changes in the structure of the surface of the particular silica gel. With SS it is apparent that electrolytes exert some influence on its surface characteristics. For instance, electrolyte-free water, as much as 10 μl on a spot, causes swelling of the layer. Also, the sedimentation of SS in a salt solution is better than in a suspension of it in water.

The above characteristics of silica gel layers determine to a great extent the chromatographic behaviour of acids and/or bases when subjected to reversed-phase chromatography on SO and/or SS. Fig. 2a shows the dependence of the R_M values of some penicillins and of sulphathiazole and phthalylsulphathiazole on the sodium

TABLE II

pH CHANGES OF POTASSIUM PHOSPHATE SOLUTIONS DUE TO THE ADDITION OF THIN-LAYER MATERIALS

Values given are the pH of the solution after the addition of 30 μg/ml of the particular thin-layer material.

Solvent for cloxacillin (20 μg/ml)	*Thin-layer material added*			
	None	*S*	*SO*	*SS*
Water	5.2	7.73	7.50	7.30
$5 \cdot 10^{-3}$ *M* potassium phosphate	6.03	7.00	6.82	6.63
10^{-2} *M* potassium phosphate	6.11	6.72	6.56	6.48
$5 \cdot 10^{-2}$ *M* potassium phosphate	6.10	6.21	6.24	6.25
10^{-1} *M* potassium phosphate	6.06	6.20	6.20	6.20

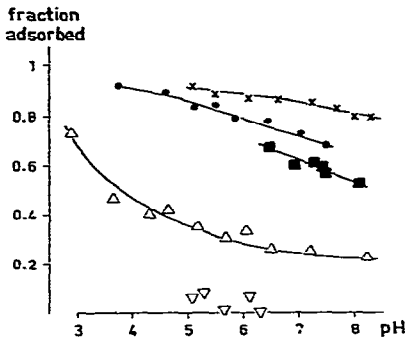

Fig. 1. Plot of the fraction of cloxacillin adsorbed *versus* the pH of the mixture of the cloxacillin solution and the adsorbent. Adsorbent (30 mg/ml of cloxacillin solution): ×, ●, ■ = silanized silica gel from thin-layer plates; △ = silanized silica gel for column chromatography; ▽ = silica gel from thin-layer plates. Cloxacillin (20 μg/ml) dissolved in: ●, △, ▽, Teorell buffer; × = Teorell buffer supplemented with 0.3 *M* NaCl; ■ = Teorell buffer diluted 1:1 with water. The results are the means of experiments in duplicate.

chloride concentration of the aqueous phase for the silanized silica gel system. The observed increase in the R_M values with increasing electrolyte concentration can be ascribed to (i) an increased adsorption owing to salt effects and (ii) an increased partition between the lipophilic layer and water because of the acidic reaction of the silica gel. The results were independent of whether the compounds were applied as the free acid or as the sodium salt. In water–acetone mixtures the compounds migrated with

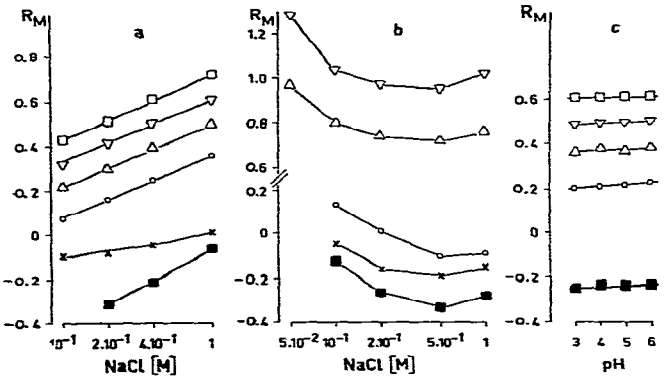

Fig. 2. (a) Plot of the R_M values of some acidic substances *versus* the NaCl concentration of the aqueous phase in the silanized silica gel reversed-phase TLC system. Developing solvent: NaCl solution–acetone (2:1). ■ = Phthalylsulphathiazole; × = suphathiazole; ○ = oxacillin; △ = cloxacillin; ▽ = flucloxacillin; □ = dicloxacillin. (b) Plot of the R_M values of basic substances *versus* the NaCl concentration of the aqueous phase. Developing solvent as in (a). ■ = Pseudoephedrine; × = amphetamine; ○ = *o*-phendimetrazine; △ = amitriptyline; ▽ = chloropromazine. (c) Plot of the R_M values of some penicillins *versus* the pH of the aqueous phase. Developing solvent: Teorell buffer-acetone (5:2). ■ = Methicillin; ○ = oxacillin; △ = cloxacillin; ▽ = flucloxacillin; □ = dicloxacillin. The stationary phase in all instances was the pre-coated silanized silica gel plate F_{254} (Merck). Each substance was spotted in duplicate on a plate in 2-μl amounts of a 2 mg/ml aqueous solution (the acids as the sodium salts; the bases as the hydrochloride salts). The results are the means of two chromatograms.

the solvent front, which is understandable on account of the basic reaction of the silica gels with water (Table II), thus making the acids more water soluble. Comparable results were obtained with the silicone oil–silica gel system.

As the physico-chemical interactions that determine the extent of adsorption on the silica gels may not be identical for different compounds, the shifts in the R_M values due to increasing salt concentrations, as can be seen for sulphathiazole and its phthalyl derivative (Fig. 2a), need not to be the same.

Fig. 2b shows the relationship between the R_M values of some basic substances and the sodium chloride concentration of the mobile phase. The initial decrease in the R_M value (*i.e.*, increase in the R_F value) of the compounds can be ascribed to the increase in acidity on the surface of the layer, thus favouring the solubility in the aqueous phase. At higher salt concentrations this increase in migration rate is counterbalanced by the increased adsorption (Fig. 2b).

Another aspect resulting from the cation-exchange property of the silica gels is that the R_M values of acidic and/or basic substances, when corrected for the degree of ionization at the pH of the eluting aqueous phase[7,8], do not obey the theory of partition chromatography, which demands constancy of the true R_M value[3,9,10]. In Fig. 2c, an example is given which demonstrates that under particular chromatographic conditions the R_M values of penicillins are almost independent of the pH of the eluting buffer. As the buffer used has a fixed cation concentration (68 mM Na^+), the pH range on the surface of the layer is probably much narrower than is suggested by the pH of the different aqueous phases.

Taken these facts together, it seems to be questionable whether reversed-phase TLC on systems with silica gels as the support for the lipophilic stationary phase can be used to determine the relative partition data for a heterogeneous series of compounds unless it is ascertained that, for instance, the shifts in R_M value due to changes in the electrolyte concentrations of the aqueous phase are identical for the different compounds. Most of the more lipophilic penicillins apparently fulfil the latter condition (Fig. 2a).

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