

## Light scattering studies on micelle formation by some penicillins in aqueous solution

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The aggregation of a number of penicillins, both in water and in electrolyte solution, has been examined by total intensity light scattering methods. Micellar association was noted for carfecillin, flucloxacillin, cloxacillin, phenethicillin and penicillin V and critical micelle concentrations and micellar aggregation numbers were determined. Association of penicillin G in water and electrolyte was limited to dimer and trimer formation.

Early studies of the physico chemical properties in solution of penicillin G by Hauser et al (1947, 1949, 1950) reported a high surface activity and the presence of large colloidal aggregates. These results were in contrast to those of Kumler & Alpen (1948) who observed only a slight surface activity. Careful purification removed the highly surface active impurities present in some samples giving batches with little surface activity (Few & Schulman 1953). The reported presence of large aggregates was similarly disputed. Conductimetric measurements (Woodbury & Rosenblum 1947; Goyan 1949) and determinations of osmotic coefficients (Lund & Pederson-Bjergaard 1949) indicated a lack of any significant association. McBain et al (1949), working over a much higher concentration range, noted colloidal behaviour at concentrations in excess of  $0.25 \text{ mol dm}^{-3}$  from cryoscopic and dye-solubilization studies. Light scattering measurements by Hocking (1951) indicated the presence of small aggregates (molecular weight 'not greater than 3000') in the presence of  $0.5 \text{ mol dm}^{-3}$  potassium bromide. More recent studies by Thakkar & Wilham (1971) of the concentration dependence of  $^1\text{H}$  nmr spectra suggest aggregation of penicillin G in  $\text{D}_2\text{O}$  through hydrophobic interactions of the benzyl side chains. A critical micelle concentration (cmc) of approximately the same magnitude as that reported by McBain et al (1949) was interpolated from the nmr data.

### Methods

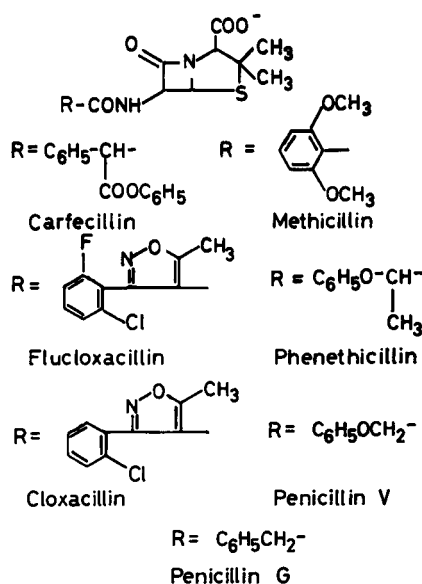
In the present study we have studied the association characteristics of a number of penicillins by light scattering techniques, both in water and in the presence of added electrolyte. Measurements were made at 303 K with a Fica 42000 photogoniometer using a wavelength of 546 nm. The solutions were freshly

prepared and clarified by ultrafiltration through  $0.1 \mu\text{m}$  Millipore filters. The refractive index increments were measured at 546 nm by differential refractometry. The following penicillins were used as received: carfecillin sodium, cloxacillin sodium, flucloxacillin sodium, methicillin sodium, phenethicillin potassium (Beecham Pharmaceuticals), penicillin V potassium (Eli Lilly & Co Ltd), penicillin G potassium (Sigma).

### Results and discussion

Figs 1 and 2 show the light scattering intensity at an angle of  $90^\circ$ .  $S_{90}$ , plotted as a function of the molal concentration of the penicillin. Any deviation of the scattering curves from those calculated for unassociated monomers occurred at clear inflection points indicative of a micellar mode of association. Cmc values are given in Table 1. Plots of  $(m_2/\Delta S_{90})$  against the molality of the micellar species  $m_2$  (where  $\Delta S_{90} = S_{90} - S_{90 \text{ cmc}}$ ) were linear with positive slopes. Treatment of the data using the theory of Anacker & Westwell (1964) yielded the values of aggregation number,  $N$  and degree of dissociation  $\alpha$  as given in Table 1.

The two phenyl rings of carfecillin confer a greater hydrophobicity to this compound and this is reflected in its lower cmc and higher aggregation number. The micellar properties of the other penicillins under examination are unusual in that added electrolyte, although



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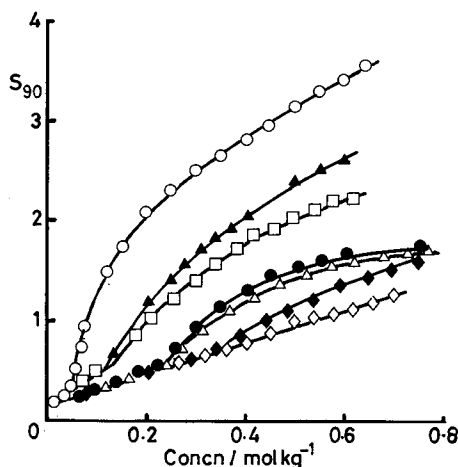


Fig. 1. Variation of the scattering ratio,  $S_{90}$ , with concentration for aqueous solutions of  $\circ$  carfocillin;  $\blacktriangle$  flucloxacillin;  $\square$  cloxacillin;  $\bullet$  phenethicillin;  $\diamond$  methicillin;  $\triangle$  penicillin V;  $\blacklozenge$  penicillin G at 303 K.

decreasing the cmc has surprisingly little effect on micelle size. The  $\alpha$  values are unusually high implying that most of monomers constituting the micelle are not in close association with their counterions.

Although the association of penicillin G in water and electrolyte is limited to dimer and trimer formation, it does however appear to commence at a reasonably well-defined critical concentration. The cmc in the absence of electrolyte is of the same order as the values of  $0.25 \text{ mol dm}^{-3}$  determined by McBain et al (1949) and

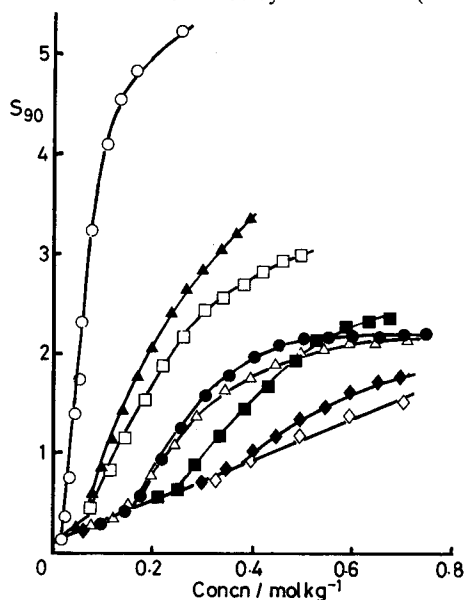


Fig. 2. Variation of the scattering ratio,  $S_{90}$ , with concentration for  $\circ$  carfocillin;  $\blacktriangle$  flucloxacillin;  $\square$  cloxacillin; and  $\diamond$  methicillin in  $0.15 \text{ M NaCl}$ ;  $\bullet$  phenethicillin;  $\triangle$  penicillin V and  $\blacklozenge$  penicillin G in  $0.15 \text{ M KCl}$  and  $\blacksquare$  penicillin G in  $0.5 \text{ M KCl}$  at 303 K.

Table 1. Micellar properties of penicillins at 303 K.

Compound	Solvent	cmc ( $\text{mol kg}^{-1}$ )	Aggregation	
			No.	$\alpha$
Carfocillin sodium	$\text{H}_2\text{O}$	0.048	8	0.3
	$0.15 \text{ M NaCl}$	0.020	21	0.4
Flucloxacillin sodium	$\text{H}_2\text{O}$	0.112	5	0.4
	$0.15 \text{ M NaCl}$	0.064	6	0.5
Cloxacillin sodium	$\text{H}_2\text{O}$	0.135	5	0.6
	$0.15 \text{ M NaCl}$	0.075	5	0.5
Phenethicillin potassium	$\text{H}_2\text{O}$	0.270	7	0.7
	$0.15 \text{ M KCl}$	0.160	9	0.8
Penicillin V potassium	$\text{H}_2\text{O}$	0.240	5	0.6
	$0.15 \text{ M KCl}$	0.172	6	0.8
Penicillin G potassium	$\text{H}_2\text{O}$	0.32	2-3	0.7
	$0.15 \text{ M KCl}$	0.30	2-3	0.7
	$0.50 \text{ M KCl}$	0.25	3	0.7
Methicillin sodium	$\text{H}_2\text{O}$ $0.15 \text{ M NaCl}$		No aggregation	

$0.25$ – $0.275 \text{ mol dm}^{-3}$  from the nmr measurements of Thakkar & Wilham (1971). The light scattering measurements of Hocking (1951) were over a lower concentration range than the critical concentration indicated in this study for association in  $0.5 \text{ mol dm}^{-3}$  electrolyte.

Ong & Kostenbauder (1975) have shown that penicillin G in the micellar form is 2.5 times more stable than a solution below the cmc under conditions of constant pH and ionic strength. The results presented here may thus be of consequence in a consideration of the stability of solutions of these penicillins at high concentrations, such as may be encountered in hospital usage.

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#### REFERENCES

- Anacker, E. W., Westwell, A. E. (1964) *J. Phys. Chem.* Ithaca 68: 81–93
- Few, A. V., Schulman, J. H. (1953) *Biochim. Biophys. Acta* 10: 302–310
- Goyan, F. M. (1949) *J. Am. Pharm. Assoc. (Sci. Ed.)* 38: 161–164
- Hauser, E. A., Phillips, R. G., Phillips, J. W. (1947) *Science* 106: 616
- Hauser, E. A., Phillips, R. G., Phillips, J. W., Vavruch, I. (1949) *J. Phys. Coll. Chem.* 53: 287–294
- Hauser, E. A., Marlow, G. J. (1950) *J. Phys. Coll. Chem.* 54: 1077–1087
- Hocking, C. S. (1951) *Nature* 168: 423–424
- Kumler, W. D., Alpen, E. L., (1948) *Science* 107: 567
- Lund, C. G., Pedersen-Bjergaard, K. (1949) *Ibid.* 109: 149–151
- McBain, J. W., Huff, H., Brady, A. P. (1949) *J. Am. Chem. Soc.*, 71: 373–374
- Ong, J. T. H., Kostenbauder, H. B. (1975) *J. Pharm. Sci.*, 64: 1378–1380
- Thakkar, A. L., Wilham, W. L., (1971) *Chem. Comm.* 320–322
- Woodbury, D. T., Rosenblum, C. (1947) *J. Biol. Chem.*, 171: 447–453