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Short Communications

Small Angle Scattering of X-Rays in Aqueous Solutions of Bile Acid Salts

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Freezing point determinations, studies on the solubilization of water-insoluble lipophilic dyes, hydrocarbons etc. and electrical conductance measurements have provided conclusive evidence that an association to micelles takes place in aqueous bile acid salt solutions [1-7]. Up to now, however, it has generally been considered that in contrast to the solutions of association colloids of the paraffin chain salt type these solutions do not give long spacing X-ray interferences. The lack of interferences has been taken as evidence that the order in the micelles of the bile salts is of much lesser degree than in the micelles of compounds of the paraffin chain type [8,9].

McBain and Hoffman [8] X-rayed 20 % aqueous solutions of sodium desoxycholate for 12—15 hours with a film-to-sample distance of 200 mm. They reported that there was no suggestion of "a long spacing line, and therefore nothing corresponding to a McBain micelle, a Hess micelle, or an interparticle spacing. Evidently in these solutions the colloid consists of particles too irregular in shape and size and distance from each other to give either a lattice spacing from within the micelles or an interparticle spacing between them".

We have, however, been able to obtain X-ray patterns with aqueous solutions of bile acid salts.

Experimental. The X-ray unit, a Philips Norelco X-ray unit with Cu target, operated at 45 kV, 15 mA; Ni-filtered radiation was employed. Three cameras were used: 1) A small angle vacuum camera with collimator slits, principally of the same design as described by Harkins [10]. Collimator width 0.2 mm, distance between collimators 110 mm, sample-to-film distance 229 mm. 2) A small angle vacuum camera with pinhole collimators of the design described by Kiessig [11]. Sample-to-film distance 212 mm. 3) Pinhole powder camera, diameter 114.6 mm.

Desoxycholic acid (Hoffman La Roche, Basle) was purified by the ether-choleic acid method, dissolved in absolute ethanol and recrystallized twice; m.p. 174—175° C. The acid was dissolved in absolute ethanol, and neutralized with ethanolic sodium ethylate. The crystallized sodium salt was filtered and dried in vacuum over P_2O_5 at 78 °C. All solutions were made by weighing, redistilled water ($\varkappa < 10^{-6}$ mho) being used. The solutions were X-rayed at 25 °C in glass-capillaries, diameter 0.7 mm, wall thickness 0.01 mm. Times of exposure: 1) camera with collimator slits 20 h, 2) camera with pinhole collimators 60 h, 3) powder camera 3 h. The exposed films were measured with a Zeiss nonrecording photometer. Three parallel runs were made on every film, readings being taken at every 0.1 mm of the film.

Results. The diffraction pattern for sodium desoxycholate solutions obtained with the small angle vacuum cameras resembles those for solutions of association colloids of paraffin chain type. We have, however, observed only one intensity maximum in the former patterns; this maximum is not so distinct as the so-called I-band of the last mentioned colloids.

Fig. 1 shows the shape of the photometric curves of the halo obtained with different desoxycholate concentrations. For solutions below the 0.05 M concentration the curves are identical with those for pure water, but already in the 0.06 M solution there occurs a very small difference. With increasing concentration the peak becomes more and more pronounced.

In spite of the long exposure time the patterns in all cases are rather weak. It is difficult to determine the exact position of the intensity maximum. If, however, an attempt is made to determine the maxi-

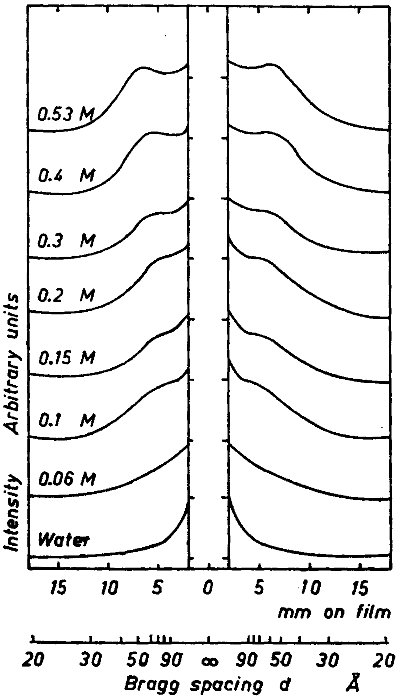

Fig. 1. Photometer traces of the small angle scattering from aqueous solutions of sodium desoxycholate.

mum, and if it is supposed that Bragg's law is valid in this case (compare Guinier's and Fournet's criticism of earlier methods of treating the experimental results [12]), the spacings given in Fig. 2 are obtained. It is seen that the spacing value undergoes a variation with increasing concentration. With the powder camera a short spacing of 6.0 – 6.2 Å was obtained in addition to the water halo of about 2.7 – 3.3 Å. This short spacing is not altered by changes in the concentration. We have obtained a pattern of similar character in solutions of sodium cholate.

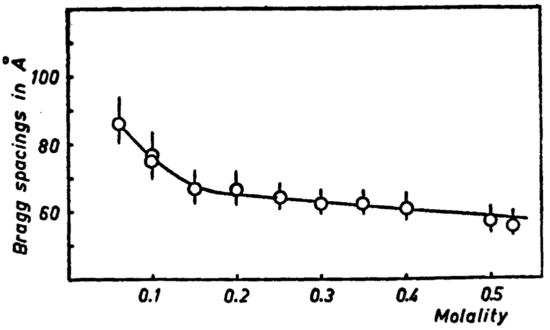

Fig. 2. Graph of the variation of the "band spacing" with sodium desoxycholate concentration.

Previously [5-7] it has been shown that micelle formation begins in sodium desoxycholate solutions at the 0.005 M concentration and becomes fully developed from 0.01 M upwards; a third concentration limit where many of the properties of the desoxycholate solutions are altered lies at 0.045 M. The small angle X-ray patterns thus have been observed in desoxycholate solutions down to the third concentration limit. The spacings in Fig. 2 vary with the colloid concentration in a similar way as the I-band spacing in solutions of association colloids of the paraffin chain type.

The micelles in bile salt solutions are thus regular enough in shape and size to give a small angle X-ray pattern similar in type to those given by micelles in solutions of paraffin chain association colloids. This does not exclude the possibility that the micelles of these two compound groups possess different structures.

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On the Solvent Dependence of the Spectra of Complex Ions

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In recent studies of the absorption spectra of dissolved complexes of transition group elements the so-called crystal field bands are treated as due to electronic transitions between levels caused by the perturbation of the electric fields of the ligands in the first co-ordination sphere. In order to examine the correctness of the assumption of the non-influence of the second sphere we have measured the spectra of some robust complexes of iron(II), chromium(III) and cobalt(III) in other solvents than water. Acetone, nitromethane, chloroform, ethanol, and especially methanol were used as solvents, and in the cases where the complexes were sufficiently soluble the spectra of the solutions were measured at a Cary recording spectrophotometer.

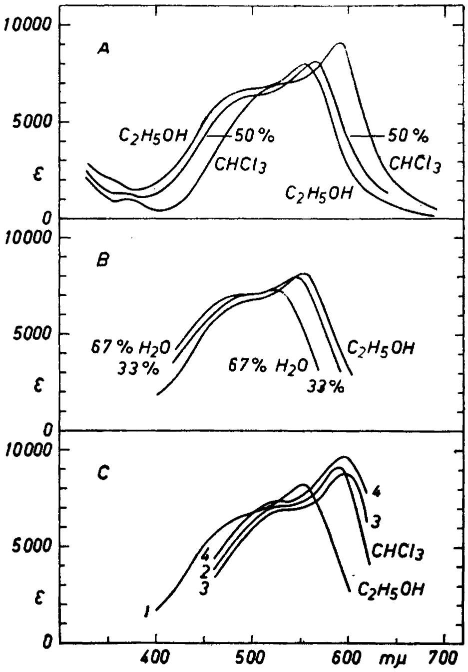

Fig. 1. Extinction curves for $[Fe(o\text{-}phen)_2(CN)_2]$ in different solvents.

A. In $CHCl_3$, 50 % $CHCl_3$+50 % C_2H_5OH and C_2H_5OH
B. In C_2H_5OH, 67 % C_2H_5OH+33 % H_2O, 33 % C_2H_5OH+67 % H_2O
C. In C_2H_5OH (curve 1), $CHCl_3$ (curve 2), 50 % $CHCl_3$ + 50 % petrolether (curve 3), 50 % $CHCl_3$ + 50 % CCl_4 (curve 4).

Of the complexes examined dicyano-bis(*o*-phenanthroline)iron(II) was prepared according to Barbieri[1] and recrystallized from chloroform until the spectrum was unchanged by further recrystallization. (Found: Fe 10.81; N 16.32; C 60.90. Calc. for $[Fe(o\text{-}phen)_2(CN)_2],3H_2O$: Fe 10.70; N 16.22; C 60.25). Tris(*o*-phenanthroline)-iron(II) bromide was prepared according to Blau[2] and recrystallized once. The Reinecke's salt $K[Cr(NH_3)_2(SCN)_4]$ was prepared after Nordenskjöld[3]. An analyzed preparation of $(NH_4)_3[Cr(SCN)_6]$, $4H_2O$ was kindly made to us and measured in water, methanol and acetone by C. E. Schäffer. Pure samples of Nos. 4—10 in Table 1 were kindly put at our disposal by Professor Fred Basolo, who prepared[4] the compounds during his stay at this laboratory.

The data for 10 complex salts are summarized in Table 1. For each absorption band are given the value of λ_{max} (wavelengths of maximum in mμ) and ε_{max} (molar extinction coefficient) in water, and for the organic solvents the changes in λ_{max} and ε_{max} as % of values in water. The examples given in the table show that λ_{max} usually is practically independent of the solvent. A significant change of λ_{max} is only found in one case, *viz.* $[Co(en)_2(SCN)_2](SCN)$. This compound has a crystal field band (ε_{max} =

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