Highly Ordered Structures Self-assembled by NaDC under Metal-ion-mediation

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Sodium deoxycholate self-assembled into fan-like structural aggregates with uniform size and shape. Additional metal ions led the aggregates to transfer from fan-like to fractal aggregates. The fractal aggregates might provide an insight into understanding the formation of a gallstone because fractal structures were found in human being gallstones.

Because of its specific structure that consists of a hydrophobic steroid ring and a hydrophilic portion comprising the hydroxy groups and the ions with a concave hydrophilic surface on one side and a convex hydrophobic surface on the other side of the steroid moiety, the aggregation behavior of bile salts in both solution and solid/crystal is so distinguish and quite complex from the conventional surfactants and has attracted significant attention for their unique physical and chemical properties.[1] Naturally, bile salts are synthesized in the liver and stored in the gallbladder, so aggregation of bile salts and its relationship with metal ions may increase our knowledge to understand more clearly about solubilization of cholesterol and gallstone disease in human being.[2] The cross section of many gallstones exhibits a concentric ring structure composed of various small particles with a fractal character. A nonlinear scientific concept suggested to be considered in understanding gallstone formation.[3] Here, we used a simple and visual method to observe the aggregation morphology of sodium deoxycholate (NaDC) by optical micrograph. The well-ordered self-assembly aggregates of NaDC and the fractal structures formed upon addition of the metal ions were observed on the glass slide. The growth mechanism of the aggregation was also discussed from the experiments.

The organized precipitates of NaDC were obtained by dropping a droplet about 60 µL of NaDC solution of a certain concentration on the glass slide. The growth of fan-like precipitates proceeded around the rim of the droplets while water is drying up under the ambient condition about 25–30 °C. The fan-like structural aggregates prevailed in the sample of about 5×10^{-4} to 4×10^{-3} mol/L NaDC, which were observed clearly under an optical microscope, as shown in Figures 1a and 1b. The essential units of the fan-like precipitates were needle-like crystals with the length of about 0.1–0.3 mm. A great number of needle-like crystals assembled to form a fan-like structure or the circular or semicircular aggregates consisting of several fan-like structures. The higher the concentration of NaDC solution is, the more dense the aggregates is.

Furthermore, the effects of metal ions Na^+, Ca^{2+}, Co^{2+}, and Cu^{2+} on NaDC aggregated precipitates have been evaluated, respectively. The shape and size of the fan-like precipitates varied apparently with metal ions added to NaDC micellar solution, as shown in Figure 2. Sodium ions, which are the predominant cations in bile, were assumed to be the cations primarily involved in the formation of bile salt micellar aggregates.[4] The influence of NaCl on NaDC micellar solution can be visually observed by the shape and size of the precipitates of the mixed solution. The size of the NaDC aggregated precipitates with the radius of about 0.2–0.4 mm increased in a different way upon increasing the NaCl concentration, as shown in Figure 2a. It is worth noting that with increasing the concentration of the NaCl to about 1×10^{-2} mol/L, a conversion states with many ramified tiny aggregates formed, as shown in Figure 2b. After that, the aggregates became lager and changed to tree-like fractal structure shown in Figure 2c. The relevant solution was gel and further changed to chaotic white cloudy suspension with further increasing the concentration of NaCl. The carboxylate group has a higher affinity for Ca^{2+} than other anionic groups.[5] It was suggested that the addition of Ca^{2+} ions to NaDC micellar solution could give rise initially to the preferential binding of the Ca^{2+} ions to free anions and small oligomers and subsequently, to the binding to micellar aggregates.[6] In our experiment, the densely downy spherical aggregates with smaller size of the bile salts were obtained when $CaCl_2$ was added to the micellar solution of NaDC, as shown in Figure 2d. With the concentration of the Ca^{2+} increased, neighboring aggregates attracted to accumulate each other, as shown in Figure 2e. Then, the larger aggregates formed, and the white macroscopic fractal structures in aqueous solution can be seen. Dropping the solution on the glass slide we can see the scene shown in Figure 2f. The divalent Co^{2+} could also interact with NaDC micelle.[7] In the presence of 1×10^{-3} mol/L $CoCl_2$, the shape and size of the NaDC aggregated precipitates were not changed obviously, as shown in Figure 2g. With the concentration of Co^{2+} increased to about 1×10^{-2} mol/L, the size of the aggregated precipitates changed to form the numerous smaller fan-like structures thickly dotted in Figure 2h. Further each fan-like aggregate attracted and accumulated together to form the pink macroscopic fractal structures in aqueous solution. Dropping the solution on the glass slide we can see the scene shown in Figure 2i. Cu^{2+} was suggested to act as bridges to form micelle.[8] The effect of $CuCl_2$ on the shape and

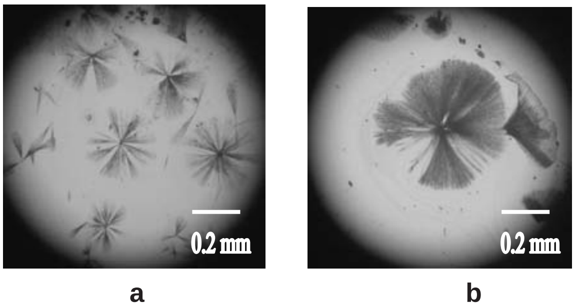

Figure 1. Optical microscopy images of aggregated NaDC precipitates with 160 magnification (a) NaDC = 5×10^{-4} mol/L; (b) NaDC = 4×10^{-3} mol/L.

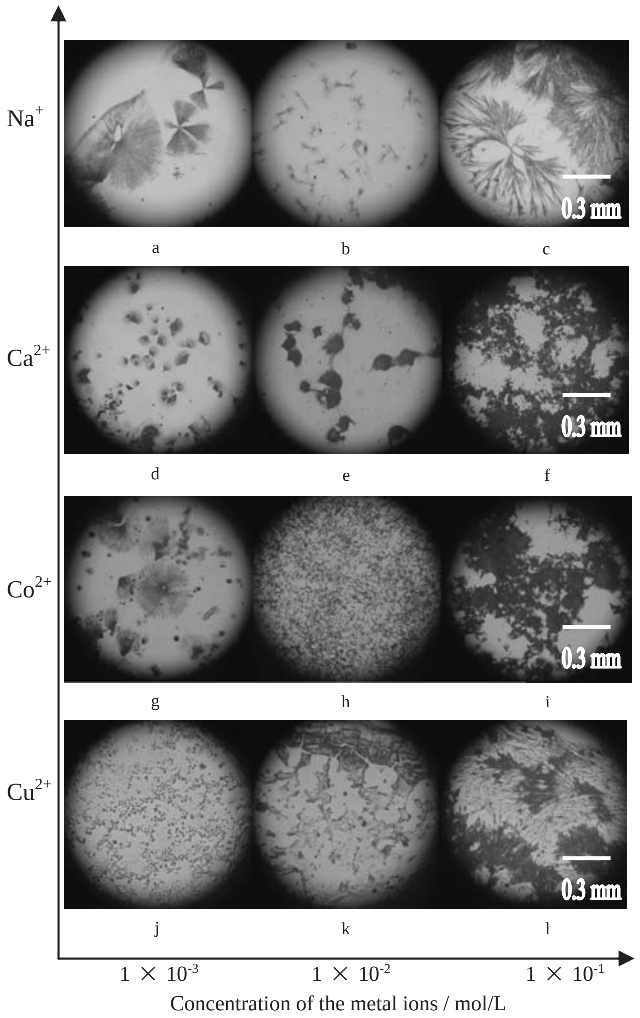

Figure 2. Optical microscopic images with 160 magnification of NaDC in the presence of metal ions Na$^+$, Ca^{2+}, Co^{2+}, and Cu^{2+}. NaDC = 4×10^{-3} mol/L; Concentration of the metal ion was 1×10^{-3}, 1×10^{-2}, 1×10^{-1} mol/L, respectively.

size of NaDC aggregates was obviously different from the former three. We cannot find the fan-like structures in presence of Cu^{2+}, as shown in Figures 2j and 2k. When the concentration of the Cu^{2+} was increased to 1×10^{-1} mol/L, the tree-like fractal structures can be found on the glass slide, as shown in Figure 2l. Subsequently, these tree-like structured aggregates accumulated together to form the large blue fractal precipitates in the aqueous solution.

From the fact we can infer that the aggregation process is progressive, leading to larger structures as the concentration of bile salt monomers or the ionic strength are raised. The morphology of the precipitates largely depended on the species and concentration of the metal ions. Unique coordination features of NaDC with metal ions dominated the geometries of the particles/aggregates. Effects of metal ions played an important role in the transition of NaDC's aggregates to fractal structure. Fractal character was observed in many nonlinear nonequilibrium dynamical systems,[9] and the fractal structure of bile salts was thought to have more relationship with gallstone formation.[3] It indicated that nonequilibrium growth existed when the NaDC crystals formed in the presence of metal ions. At the same time, minor changes in the chemical composition and/or the microenvironment may lead to very different precipitate patterns with a variety of shapes, color appearances, and structures. It demonstrated that the macroscopic structure of the aggregated precipitates can be controlled by adjusting the microenvironment of the solution system.

In summary, we have successfully observed the fan-like self-assembly of bile salts. Metal-mediated assembly of nonequilibrium growth characterized by the fractal precipitates was also demonstrated. The phenomena were well reproducible. The presented results may provide an insight into fractal growth of bile salts and be helpful to explain the formation of a gallstone. Also, owing to its biochemical activities, the bile salt aggregates with specific structures are expected to be useful in the application of biochemical field. Further characterization of the specific structures is undertaking to clarify the assembly process and the formation mechanism.

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