

Spectrochimica Acta Part A 58 (2002) 1489-1498

SPECTROCHIMICA ACTA PART A

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### The interaction of Cu<sup>2+</sup> ions and NaDC micelles Y. Sun<sup>a</sup>, Z-L. Yang<sup>a</sup>, L. Zhang<sup>a</sup>, T-D. Hu<sup>b</sup>, R.D. Soloway<sup>c</sup>, S-F. Weng<sup>a</sup>, I-G. Wu<sup>a,\*</sup>

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Received 18 June 2001; accepted 30 July 2001

#### Abstract

By mixing an aqueous solution of  $CuCl_2$  with an NaDC aqueous solution of various concentration and initial molar ratio, seven coordinated samples with distinct appearances and characters were obtained. Their structures and components were investigated by FT-IR spectroscopy, EXAFS (the extended X-ray absorption fine structure), thermal analysis, X-ray diffraction, laser light scattering, TEM (transmission electron micrograph), element analysis and ICP (inductively coupled plasma) analysis. The following conclusions were given: (1) The complexes of  $Cu^2 + -NaDC$  with distinct appearances and properties were synthesized. (2) After  $Cu(DC)_2$  dissolved in NaDC aqueous solution, larger micelles (30–90 nm diameter) formed in the supernate, it is a mixed micelle with  $Cu(DC)_2$  and NaDC. So these micelles are a new kind of micelle containing two kinds of metal ions. This is a new result using metal ions as bridges to form micelle. (3) According to the different concentration of  $Cu^{2+}$  to NaDC, the complexes formed as gel or poly-crystals. Both the composition of gel complexes and the coordination structure of carboxyl groups with metal ions varied with the initial molar ratio of  $Cu^{2+}$  to Na<sup>+</sup>. The gel complexes exhibits the non-stoichiometric character. (4) These results are in agreement with physiological condition. All the different states such as gel, precipitate, micelles of various structures are present in bile of gallbladder. We can suggest an ideal model of the interaction between  $Cu^{2+}$  and bile salts in vivo. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Copper deoxycholate; Sodium deoxycholate; Steroid; Micelle; Gel; FT-IR spectra

#### 1. Introduction

Abbreviations:  $Cu2^+$ , copper ion; NaDC, sodium deoxycholate;  $Ca(DC)_2$ , calcium deoxycholate; NaC, sodium cholate;  $CuC_2$ , copper cholate.

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As the predominant ingredients of bile, bile salts is one of the most important bio-surfactants in vivo. In gallbladder, bile salts exist mainly as simple micelles or mixed micelles formed with phospholipid and cholesterol. Therefore, extensive studies have been made on the colloid chemical properties of bile salts. The aggregation behavior of bile salts, such as CMC, micelle size, aggregation number and model, and micelle formation, have been studied by the methods of crystal structure [1], conductance [2], viscosity [2], calorimetry [2,3], surface tension [4], fluorescence [5,6], NMR [5], time resolved light scattering and neutron scattering [6–9], small-angle X-ray scattering [10], micro-calorimetric titration [11], and mutual diffusion measurements [12]. In order to further understand the physiological function, the mixed micelle formation of bile salts with phospholipid, cholesterol and protein has been investigated [13– 15].

The addition of divalent metal ions can effect the size of simple and mixed micelles and the transition of mixed micelle to vesicle, in which the interaction of divalent metal ions with bile salts plays an important role [16-20]. Copper is a minor but important component of black-pigment gallstone, and it has been proposed that copper bilirubinate acts as a free-radical scavenger in bile, protecting phospholipids from peroxidation [17]. As one of main metal ions existing in bile, copper ion involves binding to bile salts or bile salts micelles [16,19]. So the nature of  $Cu^{2+}$  coordinating to bile salts or bile salts micelles may play an important role in the mechanism of black-pigment gallstone formation. Progress in understanding how bile salts interact with metal ions in solution [21–25] depends on knowledge of their coordination with metal ions. However, few crystal structure of divalent metal bile salts has been provided because it is difficult to obtain the single crystal of

Table 1 Reactants and resultants of deoxycholate complexes synthesis them. Vibrational spectroscopy is a sensitive probe to detect the interaction of metal ions with bile salts [26–28]. Therefore, in this work, a series of Cu deoxycholate complexes were prepared by varying the concentration of reactants. The coordination of Cu with carboxylic group of deoxycholate was investigated using EXAFS and FT-IR spectroscopic measurements. Furthermore, with the results of laser light scattering and TEM (transmission electronic micrograph), the possible mode of interaction of Cu<sup>2+</sup> ion with deoxycholate in aqueous solution was discussed.

#### 2. Experiments

#### 2.1. Materials

Sodium deoxycholate (NaDC) was obtained from Serve Company and dissolved in ethanol, filtered, and recrystallized. All other regents used were of analytical grade.

## 2.2. Preparation and characterization of Cu deoxycholate complexes

In our previous work, both of crystalline and gel state of complexes were obtained by reaction of  $Cu^{2+}$  ion with Na deoxycholate in aqueous solution (Table 1). When the concentration of NaDC was near or below CMC (0.01 M), or the concentration of NaDC was upon CMC (0.1 M) and at the same time the concentration of CuCl<sub>2</sub> was higher (1 M), the resultant precipitates exhib-

Synthesis	Final concentration of NaDC (mol $l^{-1}$ )	Final concentration of $CuCl_2$ (mol $1^{-1}$ )	Initial molar ratio (Cu <sup>2+</sup> :Na <sup>+</sup> )	Resultant state
Sample 1	0.1	0.5	1:2	Crystalline
Sample 2	0.01	0.1	1:2	Crystalline
Sample 3	0.1	0.1	5:1	Gel
Sample 4	0.1	0.1	2:1	Gel
Sample 5	0.1	0.1	1:1	Gel
Sample 6	0.1	0.1	1:2	Gel
Sample 7	0.1	0.1	1:3	Gel
Sample 8	0.1	0.1	1:4	Gel

ited crystalline-state complexes (Table 1). However, when the concentration of NaDC was upon CMC (0.1 M), and simultaneously the concentration of CuCl<sub>2</sub> was lower (0.1 M), the resultant precipitates exhibited gel-state complexes (Table 1). Moreover, the composition of crystalline-state complexes was regular, but that of gel-state complexes was varied with initial molar ratio of reactants. In this work, a series of complexes were prepared by the method of changing the concentration and initial molar ratio of CuCl<sub>2</sub> and Na deoxycholate in aqueous solution. CuCl<sub>2</sub> aqueous solution was added into Na deoxycholate aqueous solution with the concentration and initial molar ratios showed in Table 1. The precipitates were filtered off and washed with distilled water several times to dissolve the CuCl<sub>2</sub> and NaDC absorbed. Then they were stored over silicon-gel drier to be desiccated naturally.

All the complexes obtained above were identified by elemental analysis with an Elementar Vario EL instrument and an ICP-AES instrument of Leeman Company. The EXAFS was undertaken in Beijing Synchrotron Radiation Facility (BSRF) station. The spectra were recorded in transmission mode on X-ray beam line 4WIB with a ring energy of 2.2 Gev and ring currents between 40 and 70 mA. The radiation was monochromatized with a Si(III) double crystal. The mid FT-IR spectra were measured on a Nicolet Magna-IR 750 spectrometer using fluorulubemull technique with DTGS detector and 32 scans at 4 cm<sup>-1</sup> resolution. The X-ray powder diffraction diagrams were recorded on a Rigaku D/Max 2400 diffractometer with  $CuK_{\alpha}$  radiation ( $\lambda =$ 1.5418 nm) and a LiF monochromator, and the scanning velocity is  $4^{\circ}$  min<sup>-1</sup> and the scanning range is 5-40°. The differential thermal and thermogravimetric diagrams were recorded on a DT-40 thermal analyzer of Shimadzu Corporation with  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> reference substance and Pt simple cell. The calefactive pace is  $10^{\circ}$  min<sup>-1</sup>.

# 2.3. $Cu(DC)_2$ was dissolved in NaDC aqueous solution and characterization of the supernate

 $Cu(DC)_2$  crystalline complex was added into 0.05 M NaDC aqueous solution, and the mixture

was stirred using an electromagnetic stirrer for 48 h, with some precipitate present in the mixture. Then the precipitate was removed from the solution by centrifugation at 12 000 rpm for 30 min. The supernate obtained was identified with an ICP-AES instrument of Leeman Company, and was examined by laser light scattering at 25 °C, with the testing angle 90°. The medium viscosity was 0.8904 cp and the medium refraction index was 1.33287. The TEM was observed under a HITACHI H-800 electron microscope after negative staining (uranyl acetate, pH 6.0), with Magnification  $\times$  33 000.

#### 3. Results

## 3.1. Binding of $Cu^{2+}$ to deoxycholate (DC) in crystalline complex

The element analysis results indicated that the component of crystalline Cu deoxycholate complexes (Sample 1 and Sample 2) were  $Cu(DC)_2 \cdot 5H_2O$ . Fig. 1 showed the results of differential thermal and thermogravimetric analysis, indicating that the loss of weight was 5.59% corresponding to the loss of three H<sub>2</sub>O molecules at



Fig. 1. The results of differential thermal and thermogravimetric analysis of crystalline complex.



Fig. 2. The high-temperature FT-IR spectra of crystalline complex. (a) Crystalline complex at 25 °C; (b) crystalline complex at 100 °C; (c) crystalline complex at 210 °C; (d) crystalline complex at 220 °C; (e) HDC.

lower than 130 °C. In addition, at 210 and 220 °C the complex absorbed heat but did not lose weight.

In order to gain further information about changing of the complex structure with temperature, the variable temperature FT-IR spectra showed in Fig. 2 were measured. The results were listed in Table 2. The FT-IR spectrum of complex at 130 °C was similar to that at 25 °C. Especially, the bands corresponding to the asymmetric and symmetric stretching vibrations of the anion COO<sup>-</sup> groups did not change, indicating that three H<sub>2</sub>O molecules lost at 130 °C did not coordinate to Cu<sup>2+</sup>. As the temperature increases to 210 °C, the distinct variation was observed by comparison with that at 25 °C. Two new bands of COOH and OH located at 1724 and 3533 cm<sup>-1</sup> were observed. The COO<sup>-</sup> asymmetry

stretching vibration bands ( $v_{as}$ ) shifted from 1593 to 1607 cm<sup>-1</sup>, and the COO<sup>-</sup> symmetry stretching vibration bands ( $v_s$ ) of it shifted from 1418 to 1408 cm<sup>-1</sup>. It provided the evidence that the complex hydrolyzed one H<sub>2</sub>O molecule at 210 °C to produce deoxycholic acid and alkaline copper deoxycholate. When the temperature further increased to 220 °C, the COO<sup>-</sup> band disappeared, and the intensity of COOH and OH bands increased. It indicates that the other H<sub>2</sub>O molecule hydrolyzed at 220 °C. These results suggested that two H<sub>2</sub>O molecules coordinated to Cu<sup>2+</sup> and involved in H-bonding to two COO<sup>-</sup> groups. The change of Cu(DC)<sub>2</sub> · 5H<sub>2</sub>O complex with enhancement of temperature occurred as follows:

 $Cu(DC)_{2} \cdot 5H_{2}O \xrightarrow{130^{\circ}C} Cu(DC)_{2} \cdot 2H_{2}O \xrightarrow{210^{\circ}C} Cu(OH)$  $(DC) \cdot H_{2}O + HDC \xrightarrow{220^{\circ}C} Cu(OH)_{2} + 2HDC$ 

Occurrence of hydrolysis or dehydration of a metal carboxylate complex under high temperature related to intensity of the binding of  $H_2O$  molecule to metal ion and H-bonding of  $H_2O$  molecule to COO<sup>-</sup>. Therefore, the results proposed that only two of five  $H_2O$  molecules coordinated with  $Cu^{2+}$  and simultaneously involved in H-bonding with COO<sup>-</sup> of deoxycholate.

Only one  $v_{as COO^-}$  was observed at 1593 cm<sup>-1</sup> in FT-IR spectrum of Cu deoxycholate at room temperature, indicating that two COO<sup>-</sup> groups in Cu deoxycholate molecule bound to Cu<sup>2+</sup> with the same mode. The  $v_{s COO^-}$  was at 1418 cm<sup>-1</sup>. The  $\Delta v$  was 175 cm<sup>-1</sup>.  $\Delta v$  of asymmetric and symmetric stretching vibration ( $v_{as} - v_s$ ) usually depends on the coordinating mode of COO<sup>-</sup> group with metal ion [29].  $\Delta v$  of monodent mode is the highest (> 200 cm<sup>-1</sup>),  $\Delta v$  of chelating mode is the lowest (< 100 cm<sup>-1</sup>), and  $\Delta v$  of the

Table 2

The data of high-temperature FT-IR spectra of copper deoxycholate  $(cm^{-1})$ 

Assignment	СООН	$\rm COO^{as}$	CH2	COO <sub>s</sub> <sup>-</sup>
Copper deoxycholate at 25 °C	_	1593	1446	1418
Copper deoxycholate at 130 °C	_	1593	1446	1417
Copper deoxycholate at 210 °C	1724	1607	1447	1408
Copper deoxycholate at 220 °C	1728	_	1449	_
Deoxycholic acid	1715, 1699	-	1446	_



Fig. 3. (a) The coordination of Na with deoxycholate in NaC complex; ([30]). (b) The possible coordination of Cu with deoxycholate in crystalline complex.

bridging one is media ( $100-200 \text{ cm}^{-1}$ ). Comparison with NaC complex ( $\Delta v = 175 \text{ cm}^{-1}$ , and COO<sup>-</sup> groups coordinate to Na<sup>+</sup> with monodent mode. Another O atom in COO<sup>-</sup> group forms hydrogen band with a H<sub>2</sub>O molecule, and the O atom in this H<sub>2</sub>O molecule coordinates with Na<sup>+</sup>, these two COO<sup>-</sup> groups coordinated with Cu<sup>2+</sup> with a mode similar to COO<sup>-</sup> group in NaC complex.

From the results of EXAFS, it was known that in the crystalline complex the coordinating number was 6 and the coordinating atoms were all oxygen atoms. The shift of OH bands in 3700- $3000 \text{ cm}^{-1}$  region (Fig. 2) suggested that both the two hydroxyl groups of each deoxycholate anion bound to Cu<sup>2+</sup>. However, the two hydroxyl groups coordinated with the same Cu<sup>2+</sup> maybe come from two different deoxycholate anions.

Based on above results, the possible coordination of  $Cu^{2+}$  ion with deoxycholate in the crystalline complex was suggested as follows (Fig. 3).

# 3.2. Binding of $Cu^{2+}$ with the deoxycholate (DC) in gel complexes

We found that the gel complexes usually obtained by reacting  $Cu^{2+}$  ion with NaDC in

aqueous solution when the concentration of  $Cu^{2+}$  ion was lower and that of NaDC was higher than its CMC.

Fig. 4 showed a series FT-IR spectra of gel precipitates (Sample 3–7), and NaDC in solid and in  $D_2O$ . Table 3 gave the characteristic frequency data and their assignments. From Fig. 4, the FT-IR spectra of gel precipitates varied with the initial molar ratio of CuCl<sub>2</sub> and NaDC, and were evidently different from that of the crystalline one showed in Fig. 2.

In OH stretching vibration region  $(3000-3700 \text{ cm}^{-1})$ , the crystalline complex had a series bands at 3444, 3360, 3338, 3318 cm<sup>-1</sup>. However, each of Sample 3–7 had only a broad band at ~ 3398 cm<sup>-1</sup>. This suggested that OH group coordinated with Cu<sup>2+</sup> varied.

In C=O stretching vibration region (1400–1800 cm<sup>-1</sup>), crystalline complex has one sharp  $v_{as COO^-}$  at 1592 cm<sup>-1</sup>, and a sharp  $v_{s COO^-}$  at ~1415 cm<sup>-1</sup>. However, in Sample 3–7 two  $v_{as COO^-}$  bands at ~1614 and ~1554 cm<sup>-1</sup> are observed,



Fig. 4. A series FT-IR spectra of crystalline complex (Sample 1–2), gel precipitates (Sample 3–7), undissolved substance and NaDC in solid state. (a) Sample 1; (b) Sample 2; (c) Sample 3; (d) Sample 4; (e) Sample 5; (f) Sample 6; (g) Sample 7; (h) undissolved substance; (i) NaDC in  $D_2O$ .

Table 3

	ОН	СН	СООН	COO <sub>as</sub>	COO <sub>s</sub> <sup>-</sup>
Sample 1	3444, 3360, 3378, 3318	2951, 2923, 2855	_	1593	1418
Sample 2	3444, 3360, 3378, 3319	2951, 2923, 2856	_	1593	1418
Sample 3	3407	2935, 2863	1708	1613, 1553	1415
Sample 4	3406	2936, 2864	1708	1614, 1553	1416
Sample 5	3396	2935, 2863	1708	1614, 1554	1414
Sample 6	3388	2935, 2863	1707	1614, 1554	1414
Sample 7	3388	2934, 2863	1707	1613, 1556	1413
NaDC (reagent, s)	3263	2931, 2861	_	1563	1404
NaDC (in D <sub>2</sub> O solution)	3411	2940, 2868	-	1552	1406

The characteristic frequency data of FT-IR spectra for gel-like complex and their assignments

and the  $v_{s COO^-}$  at ~1415 cm<sup>-1</sup> became much broader compared with crystalline complex. From the curve-fitting results of  $v_{as COO^-}$  bands showed in Fig. 5 and Table 4, each of them is composed of two peaks: one at ~1610 cm<sup>-1</sup>, the other at ~1556 cm<sup>-1</sup>.

The ICP analysis results of gel complexes given in Table 5 demonstrated that there was not only copper but also sodium in gel complex, and with the increase of CuCl<sub>2</sub> to NaDC initial molecular ratio, the content of copper increased, and that of sodium decreased. In Fig. 6, the relative intensity of the peak at ~ 1610 cm<sup>-1</sup> increased with the increase of the content of copper in gel complex and that of the peak at ~ 1556 cm<sup>-1</sup> linear increased with the increase of the content of sodium. It is suggested that the peak at 1610 cm<sup>-1</sup> was corresponded with the  $v_{as}$  band of COO<sup>-</sup> mainly coordinating with Cu<sup>2+</sup>, and the

Table 4	
The curve-fitting results of vac COC	) <sup>-</sup> of gel complexes

	Initial	Relative	Relative
	molar ratio $(Cu^{2+}\cdot Na^{+})$	intensity of $1610 \text{ cm}^{-1}$	intensity of $1556 \text{ cm}^{-1}$
	(Cu .iva )	band	band
Sample 3	2:1	0.49	0.51
Sample 4	1:1	0.46	0.54
Sample 5	1:2	0.33	0.67
Sample 6	1:3	0.25	0.75
Sample 7	1:4	0.23	0.77



Fig. 5. The curve-fitting results of  $v_{as}$  COO<sup>-</sup> of gel complexes. (a) Sample 3; (b) Sample 4; (c) Sample 5; (d) Sample 6; (e) Sample 7.

Table 5The ICP analysis results of gel complexes

	Initial molar ratio (Cu <sup>2+</sup> :Na <sup>+</sup> )	Cu%	Na%
Sample 1	1:2	7.40	_
Sample 2	1:2	7.40	_
Sample 3	2:1	4.12	1.58
Sample 4	1:1	3.97	1.70
Sample 5	1:2	3.57	2.03
Sample 6	1:3	3.35	2.27
Sample 7	1:4	3.24	2.39

peak at 1556 cm<sup>-1</sup> was related to the  $v_{as}$  band of COO<sup>-</sup> mainly combining with Na<sup>+</sup>. This was improved further by the fact that in crystalline Cu(DC)<sub>2</sub> complex COO<sup>-</sup>  $v_{as}$  is at 1592 cm<sup>-1</sup> and in NaDC (in D<sub>2</sub>O) COO<sup>-</sup>  $v_{as}$  is at 1552 cm<sup>-1</sup>.

In C–H stretching vibration region (2800–3000 cm<sup>-1</sup>), the peak shape and position of gel complexes at ~ 2935 and 2863 cm<sup>-1</sup> were much more similar to that of NaDC in D<sub>2</sub>O (NaDC exhibits micelle state in aqueous solution) at 2931 and 2961 cm<sup>-1</sup>, but more different from that of crystalline complexes at 2923 and 2855 cm<sup>-1</sup>. It was suggested that the deoxycholate in the gel complexes exhibits NaDC micelle structure.

The results of X-ray diffraction analysis showed in Fig. 7 demonstrated that on comparing with the crystalline complex, the crystallinity of gel complexes was much lower and the structure of them varied. The X-ray powder diffraction diagrams of gel complexes were different from both that of NaDC and that of  $Cu(DC)_2$  crystalline complex. Comparing NaDC and  $Cu(DC)_2$  crystalline complex, they were a new kind of complexes with distinct structure.

### 3.3. Analysis of supernate of $Cu(DC)_2$ crystalline complex dissolved in NaDC aqueous solution

The ICP analysis results of the supernate demonstrated that the concentration of copper and sodium were 0.0025 and 0.033 mol  $1^{-1}$ , respectively. These results gave evidence that  $Cu^{2+}$  could react with simple NaDC micelles to form mixed micelles of  $Cu(DC)_2$  and NaDC in aqueous solution. This hypothesis can be further testified by the laser light scattering results which showed that the diameter of the micelles of the NaDC +  $Cu^{2+}$  was 61.3 nm, while that of NaDC micelle was only 1.2–1.5 nm [6,7]. The addition of  $Cu^{2+}$  strikingly increased the size of simple NaDC micelles.  $Cu^{2+}$  are attracted to bile salt micelles and change micelle size through competition and displacement of Na<sup>+</sup>.

This can be further testified by the TEM. In the micrograph of NaDC +  $Cu^{2+}$  supernate (Fig. 8), numerous typical spherical micelles with the diameter of 30–90 nm can be seen. However, in NaDC aqueous solution, only dot-like micelle with the size of several nm can be seen. This suggested that  $Cu^{2+}$  can change micelle size by interacting with NaDC micelle.

ICP analysis results indicated that there was sodium (Na = 1.51%) in the undissolved substance below the supernate. FT-IR spectra of this dissolved substance was similar to that of gel complexes (Fig. 2), and it was demonstrated that  $Cu(DC)_2$ -NaDC mixed-micelle settled down to produce gel complexes.

Based on above results, it was suggested that  $Cu^{2+}$  reacted with the COO<sup>-</sup> groups of NaDC micelles. The DC<sup>-</sup> micelles connected each other by ways of  $Cu^{2+}$  bridges to form the gel complexes with macromolecular structure. All of our experiment results gave supports for the model.

#### 4. Discussion

The present studies have demonstrated the reaction between  $Cu^{2+}$  ions and NaDC that varied with the concentrations of  $Cu^{2+}$  and NaDC.

We synthesized the complexes of  $Cu^{2+}$ -NaDC with distinct appearances and properties. According to the different concentration of  $Cu^{2+}$  to NaDC, the complexes formed as gel or polycrystals [1]. When the concentration of NaDC is below CMC, because NaDC exists as mono-molecular,  $Cu^{2+}$  react with the mono-molecules of NaDC directly to produce crystalline precipitate. The component of it is  $Cu(DC)_2 \cdot 5H_2O$  [2]. When the concentration of NaDC is upon CMC and the concentration of  $Cu^{2+}$  is higher (at 0.5 or 1 M), the micelles of NaDC are destroyed completely by high ionic strength of aqueous solution.



Fig. 6. (a) The relation of relative intensity of the  $v_{as}$  COO<sup>-</sup> at 1610 cm<sup>-1</sup> with the content of copper; (b) the relation of relative intensity of the  $v_{as}$  COO<sup>-</sup> at 1556 cm<sup>-1</sup> with the content of sodium.



Fig. 7. The results of X-ray diffraction analysis. (a) Sodium deoxycholate; (b) crystalline complex (Sample 1); (c) gel complex (Sample 6).

Therefore,  $Cu^{2+}$  react with NaDC molecule to produce crystalline precipitate, which is similar to that when the concentration of NaDC is below



Fig. 8. Transmission electron micrograph of the structure of NaDC-Cu<sup>2+</sup> system after negative staining (uranyl acetate, pH 6.8). Numerous typical mixed-micelles are seen. Magnification  $\times$  33 000.

CMC. The component of it is also  $Cu(DC)_2$ . 5H<sub>2</sub>O. In the two reaction condition above, only simple  $Cu(DC)_2 \cdot 5H_2O$  complex was formed [3]. When the concentration of NaDC is upon CMC and the concentration of  $Cu^{2+}$  is lower (at 0.04 M or more lower), the micelles structure was still retained. Cu<sup>2+</sup> associate with NaDC micelles to produce gel precipitate. NaDC micelles connect with each other through the  $Cu^{2+}$  bridges to form macromolecular structure. Therefore, in this reaction condition, Cu(DC)<sub>2</sub>-NaDC mixed complexes formed. In these mixed complexes, the coordination structure of Na<sup>+</sup> varied from that in NaDC micelle, and the coordination structure of  $Cu^{2+}$ was different from that in Cu(DC)<sub>2</sub> crystalline complex. Furthermore, with the increase of molar ratio of CuCl<sub>2</sub> and NaDC, the copper content increases and the sodium content decreases in resultants. So it was a kind of complex with non-stoichiometric character.

After  $Cu(DC)_2$  dissolved in NaDC solution, large micelles (30–90 nm diameter) formed in the supernate. These micelles are a new kind of micelle containing two kinds of metal ions ( $Cu^{2+}$  and Na<sup>+</sup>). This is a new result using metal ions as bridges to form micelles.

The concentration of  $Cu^{2+}$  is usually lower than 0.05 M and that of bile salts is upon CMC in human bile, so the condition in [3] is very similar to that in human bile. The results in [3] are in agreement with physiological condition. All the different states such as gel, precipitate, micelles of various structures are present in bile of gallbladder. We can presume that when the conjugated bile salts interact with  $Cu^{2+}$ , the micelles of them can also connect with each other through metal ion bridges to produce mixed micelles with much bigger size. This can be suggested as an ideal model of the interaction between divalent metal ions and bile salts in vivo.

#### Acknowledgements

This work was supported by State Key Project of Basic Research (G1998061307) and Key Project (39730160 and 20023005) of National Natural Science Foundation of China.

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