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Stimuli-responsive bile acid-based metallogels forming in aqueous media

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ABSTRACT

The synthesis and gelation properties of a picolinic acid conjugated bile acid derivative in the presence of metal salts along with the stimuli-responsiveness of the systems are reported. The gels are formed in the presence of Cu^{2+} ions in the solvent systems composed of 30–50% of organic solvent (MeOH, acetonitrile, or acetone) in water. The gels respond to various stimuli: they can be formed upon sonication or shaking, and their gel–sol transformation can be triggered by a variety of chemical species. NMR, MS, and SEM techniques are exploited in order to gain a deeper insight on the self-assembled systems.

1. Introduction

The formation of highly ordered nanostructures from small molecules through non-covalent interactions, such as hydrogen bonding, π–π stacking, metal coordination, solvophobic forces, and van der Waals interactions, has widely been utilized as a bottom-up approach to design and prepare nanostructured materials with interesting properties [1,2]. Of these materials, supramolecular gels derived from low molecular weight gelators have attracted ever increasing attention because of the wide range of applications in which they may find use in our daily life. These applications range from *e.g.*, optoelectronics, light harvesting, and hybrid materials to tissue engineering, regenerative medicine, and drug delivery, as exhaustively reviewed several times [3–7].

The interest in the investigation of metal complexes as supramolecular metallogelators has been increasing only in the past decade. The availability and the diversity of metal ligand coordination that could readily induce or control the self-assembly process leading to the gel formation and thereby influence the properties of the formed gel has raised the interest towards metallogels. Especially the utilization of transition metal complexes as metallogelators has been found to lead to materials exhibiting interesting optical, catalytic, and magnetic properties [8]. In general, the incorporation of the metallic elements into the gel matrix can be achieved by either physical entrapment of noncoordinating metallic elements or by exploiting the metal–ligand interactions as the major driving force leading to the gel network formation. Metallogels have been shown to be responsive to external stimuli and exploit a range of spectroscopic, magnetic, catalytic, and redox properties that are inherent to the metals they contain.

Steroids are widespread natural products having a large and rigid steroidal nucleus combined with derivatizable functional groups leading to an adjustable polarity profile, which makes them attractive building blocks when designing novel low molecular weight gelators [9]. Bile acids are a group of steroidal compounds biosynthesized in the liver through several complementary pathways. Besides the rigid steroidal nucleus composed of four fused rings they have a short aliphatic side chain containing a carboxylic acid group in its end. In higher vertebrates the rings A and B of the steroidal nucleus are in *cis*-fusion, causing curvature to the steroidal nucleus, and thus making the molecules facially amphiphilic. The hydroxyl groups are located on the concave α-face, whereas the convex β-face possesses the three methyl groups [10]. Besides as low molecular weight gelators, bile acids and their derivatives have found use in pharmacology [11–13], asymmetric synthesis and chiral discrimination [14], or as receptors for molecular and ionic recognition [15,16], to name a few.

Herein, we report the synthesis and gelation properties of an aminoethyl amide derivative of deoxycholic acid conjugated *via* amide bond with picolinic acid (**4**, Scheme 1) in the presence of Cu^{2+} salts, along with the stimuli-responsiveness of the formed metallogels. Gelation properties of a series of aminoalkyl amides

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of different bile acids have been systematically studied by us lately [17]. *N*-(2-aminoethyl)-3α,12α-dihydroxy-5β-cholan-24-amide (**3**, Scheme 1) employed as a starting compound in the current study was not, however, shown to be capable of self-assembly leading to gelation in the conditions studied. Inspired by the free amino group of the compound we were prompted to synthesize a new conjugate with the desire of gaining better gelation capabilities. Because picolinic acid (**2**, Scheme 1) is known to act as a chelating agent in the human body, it was chosen as the compound to be conjugated with the deoxycholyl derivative. Indeed, compound **4** was shown to form gels in the presence of Cu^{2+} ions in the solvent systems composed of 30–50% of methanol, acetonitrile, or acetone in water. Moreover, the gels were shown to respond to various stimuli: they could be formed upon sonication or shaking, and their gel–sol transformation could be triggered by a variety of chemical species.

2. Experimental

2.1. Materials

Pyridine-2-carboxylic acid (**1**) (99%) was purchased from Aldrich. Other reagents used in the synthetic steps as well as the solvents used in chromatography and gelation studies were of analytical grade. Triethylamine and ethyl chloroformate were distilled prior to use. The mixed anhydride method used in the preparation of the target molecule and slightly modified by us has been reported previously [18–20].

2.2. Synthesis of compound 4

Compound **4** was synthesized by conjugating the freshly prepared pyridine-2-carboxylic acid anhydride (**2**) with *N*-(2-aminoethyl)-3α,12α-dihydroxy-5β-cholan-24-amide (**3**) prepared according to the previously reported synthetic protocol (Scheme 1) [17].

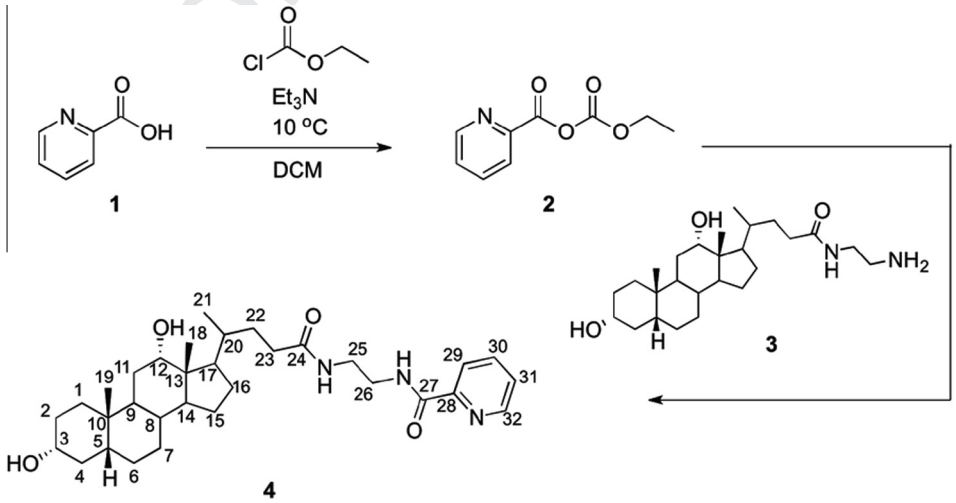
Synthesis of compound **4** was performed in N_2-atmosphere. In a round-bottomed three-necked 250 mL flask picolinic acid (pyridine-2-carboxylic acid; 5.3 mmol, 1 eq.) and dry dichloromethane (40 mL) were cooled on an ice-water bath to +10 °C, after which triethylamine (6.9 mmol, 1.3 eq.) was added to the solution from a dropping funnel, followed by a dropwise addition of ethyl chloroformate (6.9 mmol, 1.3 eq.) in dichloromethane (3 mL). The mixture was stirred at rt for 40 min, after which *N*-(2-aminoethyl)-3α,12α-dihydroxy-5β-cholan-24-amide (5.3 mmol, 1 eq.) in dichloromethane (30 mL) was added dropwise to the freshly prepared picolinic acid anhydride. The stirring was continued for 20 h on an oil bath (40 °C). The crude product obtained after the evaporation of the volatiles was dissolved in $CHCl_3$ (100 mL) and washed with water (2 × 75 mL), 0.1 M HCl solution (2 × 75 mL), water (75 mL), and finally with brine (2 × 75 mL). Then the yellowish organic layer was dried (Na_2SO_4), filtered, and the volatiles evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, CH_2Cl_2:MeOH 90:10) to yield the compound as a white solid.

2.3. Compound 4

Yield 84%. ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 8.56 (d, 1H, 32-CH), 8.38 (m, 1H, NH), 8.18 (d, 1H, 29-CH), 7.86 (m, 1H, 30-CH), 7.44 (m, 1H, 31-CH), 6.46 (m, 1H, NH), 3.94 (m, 1H, 12β-H), 3.61 (m, 3H, 3β-H + 26-CH_2), 3.49 (m, 2H, 25-CH_2), 2.24 (m, 1H, 23α/β-H), 2.09 (m, 1H, 23α/β-H), 1.890.96 (m, 26H), 0.95 (d, 3H, 21-CH_3), 0.89 (s, 3H, 19-CH_3), 0.63 (s, 3H, 18-CH_3). ^{13}C NMR ($CDCl_3$, 126 MHz, ppm): δ 174.3 (C-24), 165.6 (C-27), 149.5 (C-28), 148.1 (C-32), 137.5 (C-30), 126.4 (C-31), 122.4 (C-29), 73.1 (C-12), 71.8 (C-3), 48.3 (C-14), 47.1 (C-17), 46.5 (C-13), 42.1 (C-5), 40.4 (C-25), 39.4 (C-26), 36.5 (C-4), 36.0 (C-8), 35.3 (C-1), 35.2 (C-20), 34.1 (C-10), 33.7 (C-9), 33.4 (C-23), 31.6 (C-22), 30.5 (C-2), 28.7 (C-11), 27.4 (C6/7), 27.1 (C6/7), 26.2 (C-16), 23.6 (C-15), 23.1 (C-19), 17.4 (C-21), 12.7 (C-18). ESI TOF MS: $[M+Na]^+$ m/z = 562, $[M+K]^+$ m/z = 578. M.W. ($C_{32}H_{49}N_3O_4$) = 539.75. Elemental analysis: Found C, 70.73; H, 9.13; N, 7.67. Calc. for $C_{32}H_{49}N_3O_4$ $0.25H_2O$: C, 70.62; H, 9.17; N, 7.72.

2.4. NMR spectroscopy

^{1}H, ^{13}C, ^{13}C DEPT-135, and 2D PFG ^{1}H,^{13}C HMQC and HMBC NMR spectra used for characterization of the prepared compound was recorded with a Bruker Avance DRX 500 MHz spectrometer equipped with a 5 mm diameter broad band inverse detection probehead operating at 500.13 MHz in ^{1}H and 125.77 MHz in ^{13}C experiments, respectively. The ^{1}H NMR chemical shifts are referenced to the signal of residual $CHCl_3$ (7.26 ppm from internal TMS). The ^{13}C NMR chemical shifts are referenced to the centre peak of the solvent $CDCl_3$ (77.0 ppm from internal TMS). A composite pulse decoupling, Waltz-16, has been used to remove proton couplings from ^{13}C NMR spectra. Assignment of the ^{13}C NMR



Scheme 1. Synthesis route leading to compound **4**.

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chemical shifts has been made with the help of 2D NMR spectra and literature [21].

2.5. Gelation studies

Gelation tests for compound **4** in the presence of metal salts were performed as follows: 5 mg of compound **4** was weighed in a test tube and 150–250 μL of MeOH/MeCN/acetone was added. After that, depending on the solvent composition, 50–225 μL of deionized water was added. Finally, depending on the desired ligand:metal molar ratio (2:1, 1:1, or 1:2), 125 μL or 250 μL of aqueous metal salt solution was added, resulting in the total solvent volume of 500 μL. The obtained suspension or solution was then subjected to ultrasound for ca. 1 min and heated with a heat gun until a clear mixture was obtained. The solution was allowed to cool, after which observations with regard to gelation were made. The test tube was inverted, and if the solid mass did not move, it was defined as a gel. If the gel was formed already while sonicating, no heating was performed.

2.6. Microscopy

Scanning electron micrographs were taken with Bruker Quantax400 EDS microscope equipped with a digital camera. Samples of the xerogels were prepared either by placing a hot, clear solution of the gelator and metal salt in an appropriate solvent mixture or a scooped sample of the preformed gel on carbon tape placed over the sample stub, and after drying in air for two days, coated with gold in a JEOL Fine Coat Ion Sputter JFC-1100.

2.7. Mass spectrometry

The mass spectrometric experiments were performed with a QSTAR Elite ESI-Q-TOF mass spectrometer equipped with an API 200 TurboIonSpray ESI source from AB Sciex (former MDS Sciex) in Concord, Ontario (Canada). The gel samples were prepared by utilizing ligand:Cu^{2+} molar ratio of 3:1 and 1:1 and 30% of MeOH as the co-solvent. In order to investigate the effect of the counter anion both $CuSO_4$ $5H_2O$ and $Cu(NO_3)_2$ $3H_2O$ were exploited as the copper source. For the measurements, the gel-phase samples were dissolved and diluted in MeCN to obtain 9.25 μM samples for measurements.

3. Results and discussion

Compound **4** was synthesized with an overall yield of 84% by modifying the well-known synthesis method reported in the literature (Scheme 1). Conventional analysis methods were utilized in order to prove the identity, and the full data are provided in the experimental section. Compound **4** itself did not form gels in any of the common solvents tested; in general, it was shown to be rather poorly soluble in aromatic solvents, but then apparently too soluble in more polar solvents.

Table 1
Q2 Gelation properties of varying **4**:Cu^{2+} molar ratios in aqueous solvents.

	Solvent composition	Ligand:Cu^{2+} molar ratio		
		2:1	1:1	1:2
$CuSO_4 \cdot 5H_2O$	MeOH 50%	Gs	S	S
	MeOH 40%	Gs	Gs	Gs
	MeOH 30%	Gs	Gs	Gs
	MeCN 50%	S	S	S
	MeCN 40%	S	S	S
	MeCN 30%	G	G	G^t
	Acetone 50%	Gs → C	PG → C	PG → C
	Acetone 40%	Gs	Gs	Gs → C
	Acetone 30%	Gs	Gs	Gs
$Cu(NO_3)_2 \cdot 3H_2O$	MeOH 50%	S	S	S
	MeOH 40%	S	S → PG	S → PG
	MeOH 30%	PG	Gs	Gs
	MeCN 50%	S	S	S
	MeCN 40%	S	S	S
	MeCN 30%	S	S	S
	Acetone 50%	S	S	S
	Acetone 40%	S	S	S
	Acetone 30%	S	G	Gs

G = gel formed after heating and cooling; Gs = gel formed after sonication; G^t = transparent gel formed after heating and cooling; PG = partial gel; PG = partial weak gel; S = clear blue solution; C = crystallizes.

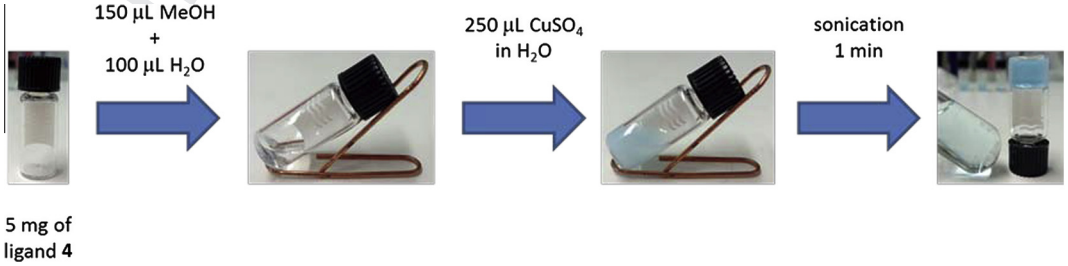

Fig. 1. Sonication-induced gel formation of compound **4** with $CuSO_4$ (1:1) in aqueous MeOH (30%).

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Compound **4** bearing a pyridine group, a classic metal-coordination site, along with the aminoethyl amide linker between the deoxycholic acid and picolinic acid was anticipated to be able to interact with metal cations. Intrigued by the possibility of obtaining metallogels Cu^{2+}, Ag^{+}, and Zn^{2+} cations were tested. Cu^{2+} was shown to give the most promising results in the preliminary tests, and was thus chosen to be the metal cation concentrated on. Three different Cu^{2+} salts, namely $CuSO_4{\cdot}5H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, and $CuCl_2{\cdot}2H_2O$ were employed. The gels were shown to be formed in water with 50–30% of methanol, acetonitrile, or acetone as the co-solvent according to the Table 1. Since hydrogels are better compatible with bodily tissues than organogels, the water content of the solvent mixture was increased to as high a level as possible. If it exceeded 70% precipitation instead of gel formation occurred.

The counter anion of the copper salt was shown to play a role in the gel formation. The different properties of the copper salts, for example in terms of complex formation in aqueous solutions, may have affected on the behavior of the systems. In the studied conditions, $CuSO_4{\cdot}5H_2O$ was shown to promote gel formation more often than $Cu(NO_3)_2{\cdot}3H_2O$. Additionally, in the case of $Cu(NO_3)_2$-

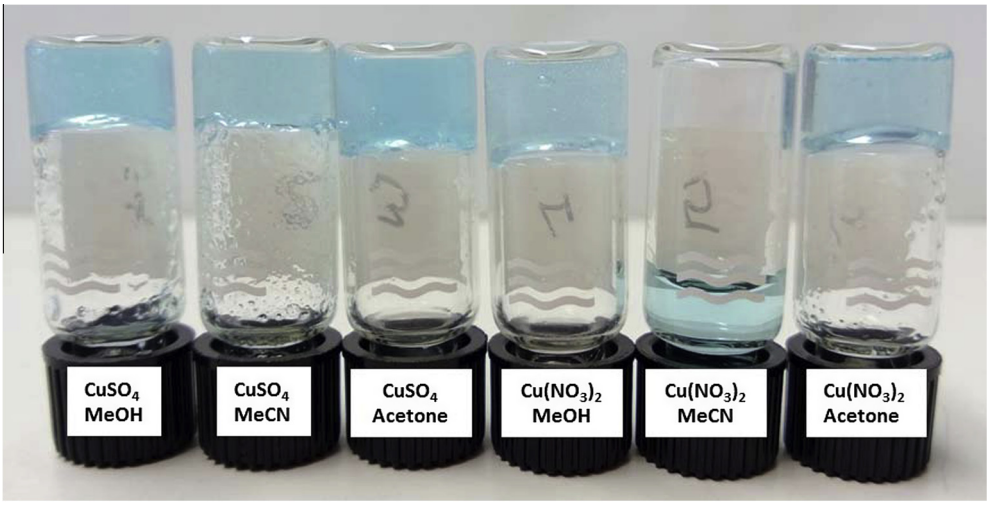

Fig. 2. Appearance of the samples with ligand:Cu^{2+} molar ratio of 1:1 in aqueous solvents (30% of organic solvent).

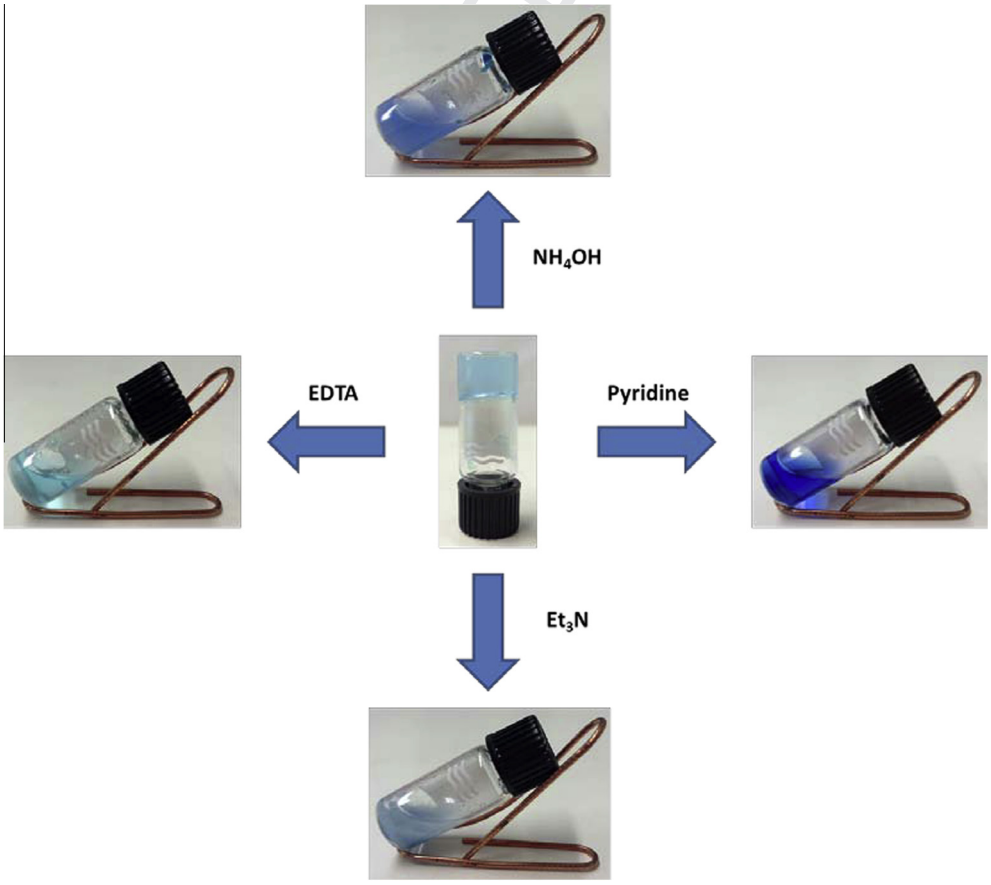

Fig. 3. Illustration showing the gel–sol transformation of Cu^{2+}-gels of compound **4** by the addition of external chemical stimuli.

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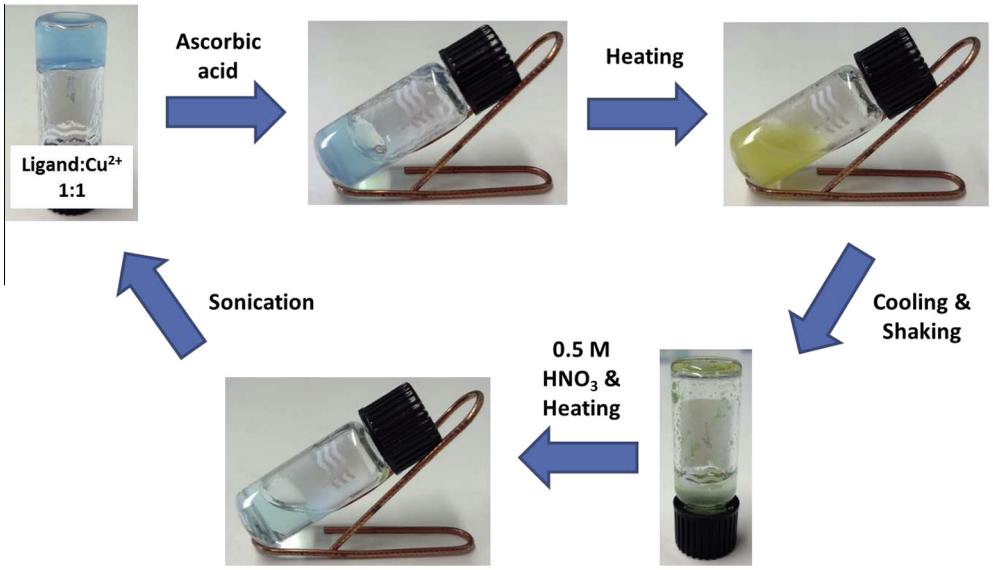

Fig. 4. Illustration of the reversible gel–sol transformation induced by the reduction of Cu^{2+} to Cu^{+} by ascorbic acid and subsequent oxidation back to Cu^{2+} by HNO_3.

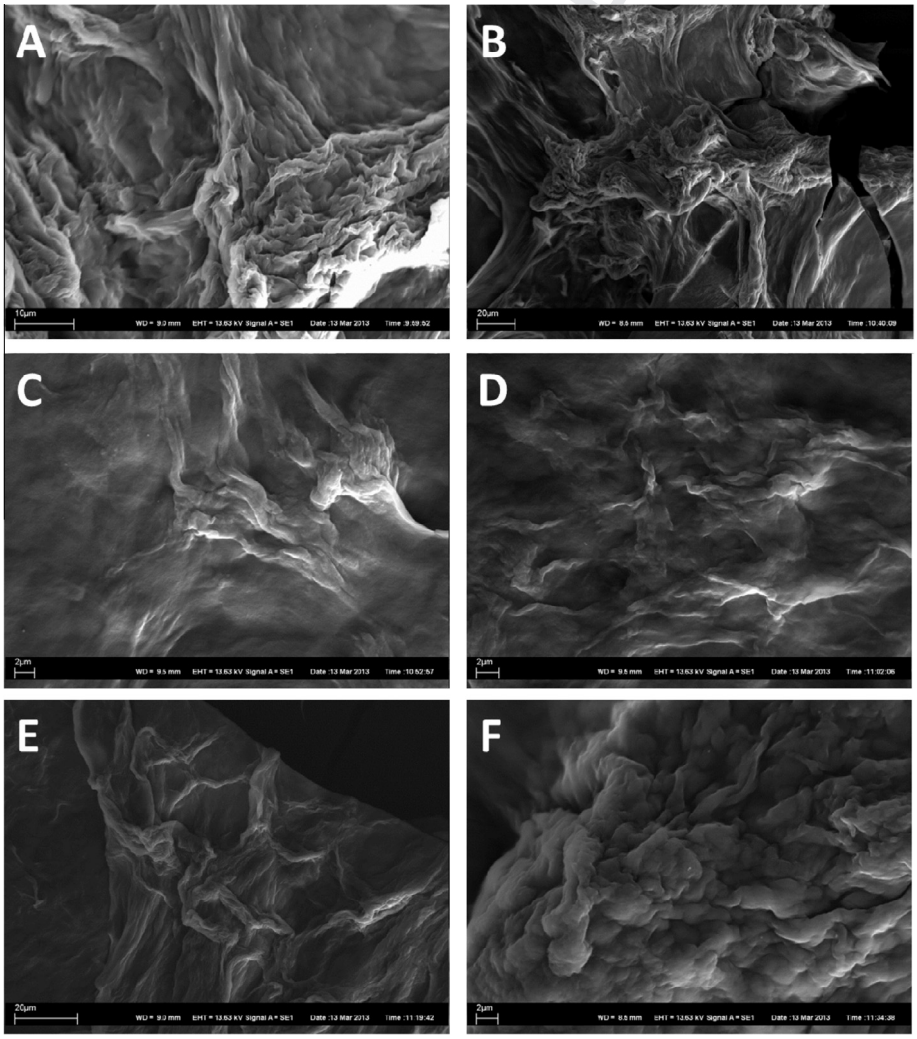

Fig. 5. SEM images of the xerogels of compound 4 formed in the presence of Cu^{2+}. Samples prepared by scooping a portion of the preformed gel on a sample stub. (A) Ligand:Cu^{2+} 2:1 gel in MeOH, (B) Ligand:Cu^{2+} 2:1 gel in MeCN, (C) Ligand:Cu^{2+} 2:1 gel in acetone, (D) Ligand:Cu^{2+} 1:1 gel in MeOH, (E) Ligand:Cu^{2+} 1:1 gel in MeCN, (F) Ligand:Cu^{2+} 1:1 gel in acetone. Scale bars: (A) 10 μm, (B) 20 μm, (C) 2 μm, (D) 2 μm, (E) 20 μm, (F) 2 μm.

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·3H_2O, in most of the cases ligand:Cu^{2+} molar ratio of 1:1 or 1:2 was required for the gel formation, and the gels were only formed in rather high water concentrations. However, the gels formed with $Cu(NO_3)_2·3H_2O$ were shown to be rather stable, whereas the gels formed with $CuSO_4·5H_2O$ were shown to crystallize/precipitate moderately quickly. $CuCl_2·2H_2O$, however, was only able to give clear blue solutions; no gels were detected. Interestingly, very recently Džolić and Cametti with their co-workers reported the metallogel formation of pyridyloxalamide in alcohols in the presence of only $CuCl_2$ of several other Cu^{2+} salts tested [22].

Gelation was shown to take place by solely sonication (denoted Gs in Table 1, Fig. 1) in several cases. If sonication was not sufficient enough in order to promote gelation, the traditional heating and cooling-cycle was performed. Interestingly, in some cases the gelation of the clear blue solution obtained after the heating and cooling/settling period of one day could be induced by vigorous shaking. Similar phenomenon of mechanically triggered gel formation has been recently reported by us [23] and others [24]. In most of the cases rather translucent light blue gels were obtained (Fig. 2).

The gel systems obtained were shown to act as responsive soft materials responding to a variety of chemical stimuli (Figs. 3 and 4). Three drops of pyridine, triethylamine, or aqueous ammonia or small amount of solid disodium salt of EDTA (ethylenediamine tetra-acetic acid) were added onto the surface of a preformed gel. The upper layer of the gel was immediately shown to transform into a solution, and after a gentle agitation, the gel–sol transformation was completed. In the case of pyridine an intensively blue-colored clear solution was obtained (Fig. 3).

The gels also showed reversible redox-responsiveness (Fig. 4): the Cu^{2+} ions could be reduced to Cu^{+} by adding ascorbic acid (1 eq. in 20 μL of water) onto the preformed gel. The upper layer of the gel was immediately shown to transform into solution. In order to speed up the process the sample was heated until a clear yellow solution was obtained. When cooled down, the clear yellow solution first became opaque and finally after some shaking a light green solution with some green precipitate was obtained. In order to oxidize Cu^{+} again to Cu^{2+} 20 μL of 0.5 M nitric acid was added to the solution, which was then heated until a clear light blue solution was obtained. This solution was finally sonicated in order to revive the gel.

In order to gain some visual insight into the microscopic morphology of the dried self-assembled materials the scanning electron microscopy (SEM) was utilized. The xerogel samples for the SEM were prepared by following two different protocols: in the first one, the gel was formed by the heating–cooling cycle and allowed to stabilize, after which the preformed gel was scooped onto a sample stub covered with the carbon tape (Fig. 5). In the second experiment, the clear hot solution containing the ligand, copper salt, and the aqueous solvent was pipetted onto the sample stub and allowed to dry (Fig. 6). As expected, and observed by us previously [23], as well, differences in the sample appearance between these two sample preparation protocols were observed. The sample appearance, however, was shown to be independent of the ligand:Cu^{2+} molar ratio, or the solvent system used.

From the gelation experiments it was clear that Cu^{2+} was essential for gel formation. The role of the cation in the process was further investigated by utilizing NMR spectroscopy. First, the ^1H NMR spectra were measured in absence of the cation for samples of 0.17%, 0.5%, and 1% in concentration (**4** in MeOD:D_2O mixture). The spectra appeared well-resolved. Increasing of the concentration did not have any particular effect on the values of the chemical shifts. From the spectra it was obvious that there was no gel formed. When the cation was added to the sample solutions, the overall resolution and S/N in all spectra dramatically decreased. The most probable explanation is, naturally, the paramagnetic nature of Cu^{2+}.

The spectra were measured for ligand:Cu^{2+} 1:1 samples of 0.17%, 0.5% (gel), and 1% (gel) in concentration (**4**:Cu^{2+} in MeOD:D_2O mixture). Increasing of the concentration resulted in

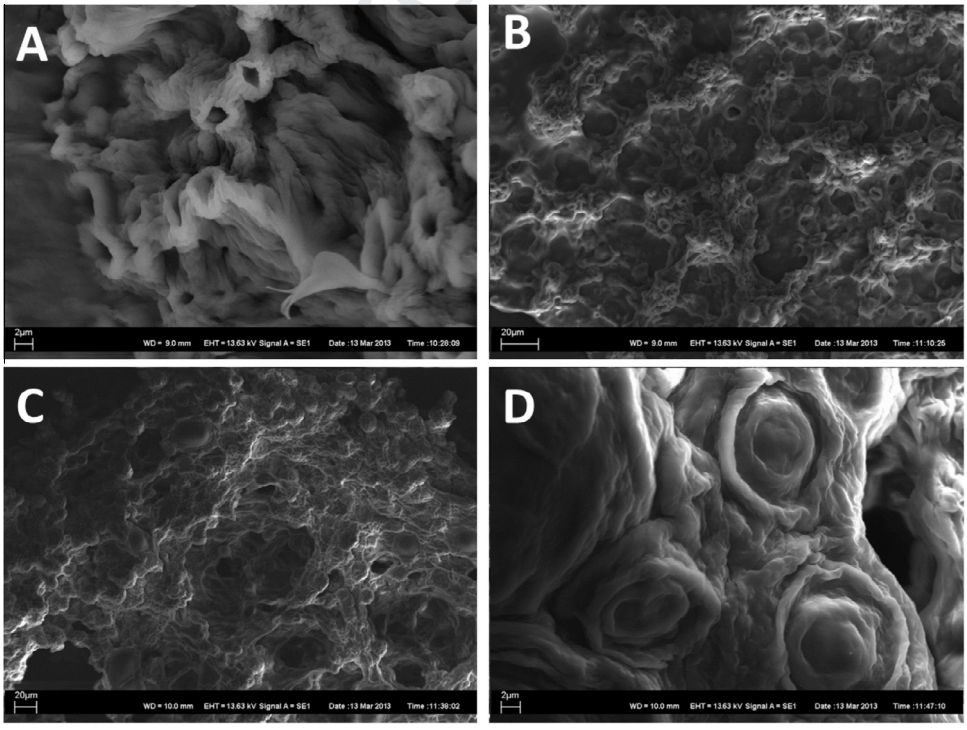

Fig. 6. SEM images of the xerogels of compound **4** formed in the presence of Cu^{2+}. Samples prepared from a hot clear solution. (A) Ligand:Cu^{2+} 2:1 in MeOH, (B) Ligand:Cu^{2+} 1:1 in MeOH, (C and D) Ligand:Cu^{2+} 1:1 in acetone. Scale bars: (A) 2 μm, (B) 20 μm, (C) 20 μm, (D) 2 μm.

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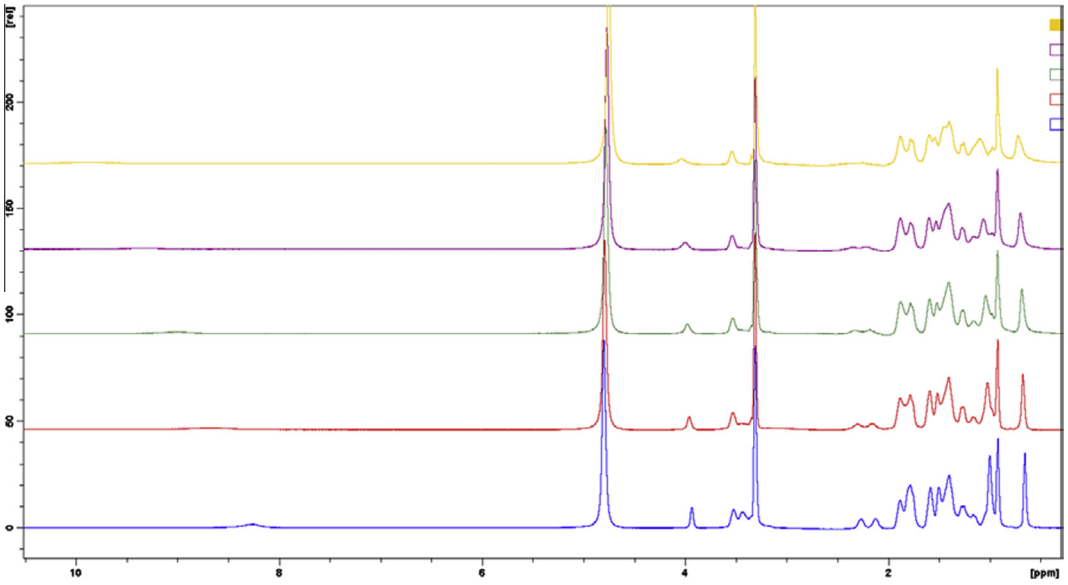

Fig. 7. Samples with 1% of **4** in MeOD with varied ligand:Cu^{2+} molar ratios: 10:1 (undermost) → 10:2 (5:1) → 10:3 → 10:4 (5:2) → 10:5 (2:1) uppermost.

gel formation, which was evident from the ever broadening signals of ^{1}H NMR spectra due to restriction of molecular tumbling as a result of gel formation. The ligand:Cu^{2+} molar ratio was further increased to 1:2, and the measurements repeated for the same set of concentrations. The appearances of the spectra were close to identical, when compared with the ligand:Cu^{2+} molar ratio of 1:1. When the temperature was increased from 30 °C to 60 °C in 5 °C temperature steps for the 1% sample of ligand:Cu^{2+} in a molar ratio of 1:2, the breakdown of the gel network could be deduced from the ever improving resolution of the ^{1}H NMR spectra. The pyridine protons became visible and were observed to move towards the upper field region as the gel was melting (*i.e.*, the temperature was increased). This indicates pyridine playing an important role in the process leading to gel formation, most probably by chelating Cu^{2+} ions. When the gel-forming network breaks down the Cu^{2+} cation does not interact with pyridine as intensively, and consequently withdrawal of the pyridine electrons by the positively charged cation diminishes. This results in decreased deshielding, which can be seen as movement of the chemical shift to the upper field.

Next, the molar ratio of copper to ligand was increased from 1:10 to 1:2. Due to the simultaneous effects of unfavorable NMR properties of copper and restricted molecular tumbling due to gel formation, not much but the decrease in resolution as a consequence of the increasing amount of copper could be observed. The measurements were further repeated in pure MeOD, a solvent which did not induce gel formation. Paramagnetic properties of Cu^{2+} affected on the appearance of the spectrum, but when the amount of Cu^{2+} was increased, the pyridine protons broadened and moved to downfield direction (Fig. 7). This further confirms the role of pyridine chelating copper as the key process in the gel formation. A tendency of the 12 β proton on the steroidal skeleton to move to downfield direction was observed as well.

In addition to the role we were also interested in the coordination degree of copper in the current gel-forming system. Encouraged by the work of Bunzen and coworkers [25], the complex responsible for gelation was tried to be determined by using mass spectrometry. The samples were prepared by using ligand:Cu^{2+} molar ratios of 3:1, 2:1, and 1:1 and 30% of MeOH as the co-solvent. In order to investigate the effect of the counter anion both sulfate and nitrate were exploited as the copper source. For the measurements, the gel samples were further dissolved and diluted in MeCN to obtain 9.25 μM samples for measurements.

Unfortunately, for compound **4** the results were not straightforward. For each sample, a collection of different ligand:metal adducts were observed in the spectrum. While copper undoubtedly is essential to gel formation, based on the mass spectrometric experiments a certain ligand:copper complex inducing the gel formation is not likely – rather several different adducts synergetically end in gelation.

4. Conclusions

In this work we report the synthesis and structural analysis of an aminoethyl amide derivative of deoxycholic acid conjugated *via* amide bond with picolinic acid. Furthermore, the compound is shown to be able to form gels in the presence of Cu^{2+} ions in the solvent systems composed of 30–50% of methanol, acetonitrile, or acetone in water. The formed gels are shown to respond to various stimuli: they can be formed upon sonication or shaking, and their gel–sol transformation can be triggered by a variety of chemical species. The gels also show reversible redox-responsiveness: the Cu^{2+} ions can be reduced to Cu^{+} followed by gel–sol transition. Oxidation of the Cu^{+} ions again to Cu^{2+} revives the gel. The essential role of the pyridine moiety chelating the Cu^{2+} ions in the process leading to gel formation was proved by NMR experiments. Mass spectrometry and SEM techniques were used in further characterization the systems.

The important physiological roles of bile acids and picolinic acid combined with the formation of stimuli-responsive gels in aqueous media responding to several stimuli may in the future lead to fascinating biomedical applications, especially due to the neuroprotective, immunological, and anti-proliferative activities of picolinic acid.

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