Thermochimica Acta 643 (2016) 1–12

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Lithocholic acid derivative in the presence of dimethyl sulfoxide: Morphology and phase transitions

M. Ordon[a,b], Yu. Gorshkova[b], M.D. Ossowska-Chruściel[a,*]

[a] Siedlce University of Natural Sciences and Humanities, Institute of Chemistry, 3-go Maja 54, 08-110 Siedlce, Poland
[b] Joint Institute for Nuclear Research, Frank Laboratory of Neutron Physics, Joliot-Curie 6, 141980 Dubna, Russian Federation, Russian Federation

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form
10 September 2016
Accepted 12 September 2016
Available online 14 September 2016

Keywords:
Derivative of lithocholic acid
Gel–Sol–Gel
DSC
SANS
2D-FTIR

ABSTRACT

We report on the properties of a new organogelator, which is an ether derivative of lithocholic acid (7OPhOLCA). The correctness of the chemical structure and purity of 7OPhOLCA was confirmed by thin layer chromatography, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, elemental analysis (EA) and infrared spectroscopy (IR). Phase transition temperatures and enthalpies of the gel were obtained by differential scanning calorimetry (DSC) and polarizing optical microscopy (POM). Changes in the vibrational spectra depending on the temperature modifications were studied using the technique of FTIR Spectroscopy with 2D correlation analysis. The small angle neutron scattering method (SANS) was used to determine the morphology and internal structure of the investigated system. It was found that the substance forms a non-transparent stable gel with a spherulite organization at the macro-scale level. The morphology of the self-assemblies and internal structure at the nano-scale level are quite different with variation of the temperature in the Gel phase. A further increase in temperature leads to the formation of the sol phase again. It turns out that the temperature of the gel-sol transition changes significantly with the concentration of 7OPhOLCA. The results of the DSC and SANS measurements indicated the reversible behavior of the Gel-SolGel transition with hysteresis on the temperature during heating and cooling.

1. Introduction

Over recent years an interest in supramolecular gels based on LMOG's (low molecular mass organic gelators) has been increasing, as these substances can constitute an alternative biomaterial to polymer gels. Since 1990 the number of publications on gels has increased from near five to at least five hundred papers per year [1]. The potential applications of LMOG's arise from the fact that they are sensitive to physical stimuli, such as temperature, light, ultrasound or chemical stimuli: metal ions or anions [2]. LMOG's are used in drug delivery, three-dimensional cell culture [3], tissue engineering and regenerative medicine or photoelectronics [4].

Both natural and synthetic substances may be used as organogelators. They may be applied as three-dimensional (3D) networks formed from derivatives of peptides [5,6], saccharides [7], urea's [8], or as natural nucleobases hydrogels [9–11].

Sterols – natural solid alcohols – are also commonly used for the synthesis of hydro- and organogelators. Their chemical structure is based on a saturated tetracyclic hydrocarbon perhydrocyclopentanophenanthrene system, usually known as a steroid or sterane nucleus [12]. The steroid system has an elongated shape and is sufficiently stiff. Conformational changes within the ring under the influence of a solvent are not possible. LMOG's based on sterols are most often formed as long, thread-like structures that can easily immobilized solvent molecules. Additionally, thanks to the presence of one or more hydroxyl groups, they can readily form hydrogen bonds with water or organic proton acceptor solvents.

A series of azo- and polyhydroxy derivatives forming the gels has been characterized in the literature. Some of them can even consist of the di-sterane system [13]. Steroids are substances that are prevalent in nature and they contain easy-to-access hydroxyl groups. They can easily form a hydrogen bond with the proton acceptor molecules.

Latest papers report on self-assembly LMOG's with 2,3-dihydroxycholestane steroids [14]. As it was described in these papers, the presence of two hydroxyl groups in the molecule should facilitate the formation of hydrogen bond connections. This makes them ideal as organogelators.

During our studies it was found that only one isomer with trans-diaxial hydroxy groups was able to create a hydrogel with the proposed solvents. Moreover, the presence of the gel was observed only in chloride solvents or benzene or DMSO. The phenomenon

* Corresponding author.
E-mail address: dch@uph.edu.pl (M.D. Ossowska-Chruściel).

http://dx.doi.org/10.1016/j.tca.2016.09.013
0040-6031/

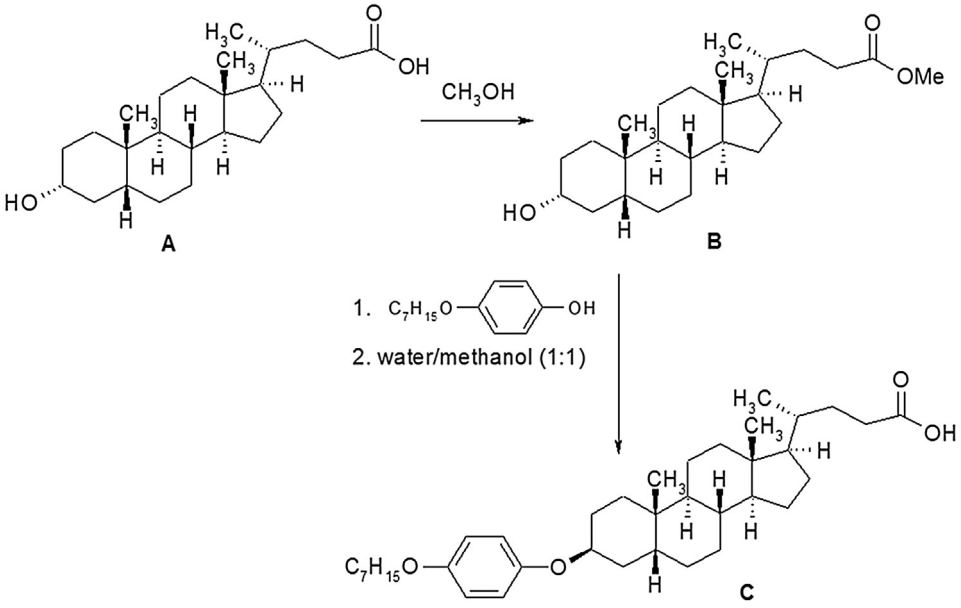

Fig. 1. Scheme of synthesis of 4-heptyloxyphenylo-lithocholic acid ether – 7OPhOLCA.

of self-assembly after dissolving this derivative alkyl alcohol and higher alkanes did not occur. This indicates that the type of a solvent and the conformation of the asymmetric center at which the hydroxyl group is substituted have a significant influence on the gelation process.

In recent years a new group of ionic and neutral sterols derivatives containing basic amino groups has been described [15]. An ionic derivative forms the Gel phase only with chloride benzene. Dietoxyamino-derivatives are able to self-assembly in chloride benzene or in a solvent with an addition of water, but not in pure water.

A special group of sterols are bile acids, which are characterized by the presence of one or more hydroxyl groups in the sterane ring. The presence of the carboxyl group further enhances the ability of these substances to form intermolecular hydrogen bonds. The main representative of this homologous series is lithocholic acid (LCA) [16].

It is known that a molecule of LCA can easily form hydrogen bonds with proton donor-acceptor solvents. This feature makes the dimethyl sulfoxide (DMSO) is an ideal solvent. It has the ability to form hydrogen bonds thanks to the presence of the carbonyl oxygen and acidic protons in the methyl group. Moreover, in small concentrations it is non-toxic to cells [17].

In this work we present a new derivative of LCA, 4-heptyloxyphenyloxylithocholic acid ether (7OPhOLCA) as an organogelator in deuterated dimethyl sulfoxide (DMSO-d_6). Five calibration solutions with different 7OPhOLCA concentrations in DMSO-*d*6 were prepared for checking the gelation degree. The resulting gels were characterized by differential scanning calorimetry (DSC), transmitted light intensity (TLI), polarized optical microscopy (POM), small angle neutron scattering (SANS) and the technique of two-dimensional Fourier transform infrared correlation spectroscopy (2D-FTIR).

2. Material and methods

2.1. Synthesis

The synthesis of the organogelator – 4-heptyloxyphenyl-olithocholic acid ether (7OPhOLCA) – was carried out starting with lithocholic acid (acronym–LCA) (99.8% purity), which was purchased from Sigma–Aldrich Company. Phase behavior of this compound was described in the literature [16]. The first step of the synthesis was the esterification reaction of lithocholic acid using dried methanol (Fig. 1). LCA (Fig. 1A) had been previously dried over phosphorus oxide (P_2O_5). The methanol was a reaction medium and a solvent. The methanol of purity 99,8% was purchased from Sigma–Aldrich Company. Methanol was further dried by addition of magnesium turnings and distillation of the protection from moisture. 3 g LCA was dissolved in 45 ml anhydrous methanol. The reaction was stirred for about 15 h at a temperature of 35–40 °C and was monitored by thin layer chromatography (TLC). The eluate was a mixture of methylene chloride: acetone in a ratio of 9:1. After the reaction, the methanol was removed by rotary evaporation. The methyl ester of lithocholic acid (Fig. 1B) was isolated using column chromatography.

The next step was etherification of the hydroxyl group of the ester (B) using 4-heptyloxy-4′- hydroxybenzene ($OHC_6H_4OC_7H_{15}$) in Mitsunobu reaction [18]. The product was purified using column chromatography (eluate dichloromethane). The resulting ether was hydrolyzed in a mixture of water-ethanol (1:1) at 40–45 °C for 10 h. The hydrolysis was monitored by thin layer chromatography. The crude 4-heptyloxyphenylolithocholic acid 7OPhOLCA (Fig. 1C) was purified using column chromatography (eluate dichloromethane–methanol 95:5) and by crystallization from ethyl acetate (three times) to constant melting point, T_m = 128.7 °C.

The purity of 7OPhOLCA was denoted by TLC (R_f = 0.738, CH_2Cl_2:CH_3OH 9.5:0.5) and spectroscopy method ^{1}H NMR, ^{13}C NMR (Varian 400 MR), Fig. 2.

The correctness of the structure of 7OPhOLCA was verified by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis: ^{1}**H NMR** (400 MHz, $CDCl_3$ δH 7.26 ppm, 25 °C, TMS): δ 6.82–6.84 (d, 2H, —Ph orto to —O—Ph), δ 6.81–6.79 (d, 2H, —Ph orto to —O-steroid), δ 4.46 (s, 1H, O—CH—, ring A), δ 3.90–3.87 (t, 2H, —CH_2- α to COOH), δ 2.44–2.36 (m, 1H, steroid), δ 2.29–2.22 (m, 1H, —steroid), δ 2.17–1.99 (m, 2H, —CH_2-, β to —OPh), δ 1.98–1.71 (m, 9H, steroid), δ 1.58 —1.25 (m, 19H, aliphatic and steroid), δ 1.15–1.05 (m, 6H, —CH_2- aliphatic), δ 0.98 (s, 3H, —CH_3 steroid), δ 0.93–0.87 (m, 6H, —CH_3, steroid), δ 0.66 (s, 3H, —CH_3, aliphatic). ^{13}**C NMR** (400 MHz, $CDCl_3$ δC 77.00 ppm, 25 °C): δ 120.04 (COO), 153.23 (ArC—O-steroid), 151.53 (ArC—O—R), 117.62 (Ar2C,orto to O-steroid), 115.26 (Ar2C, orto to O—R), 73.27 (C—O, ring A steroid), 68.53 (aliph.-C-O-R), 56.62 (steroid,