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Synthesis and aqueous phase behavior of 1-glyceryl monooleyl ether

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Abstract

Synthesis of 1-glyceryl monooleyl ether (GME) has been accomplished yielding material of high purity (99.6%). The aqueous phase behavior of synthesized lipid has been investigated by using polarized microscopy and small angle X-ray diffraction. As a result, a partial temperature–composition phase diagram has been constructed. GME forms a reversed micellar solution and reversed hexagonal liquid crystalline phase at low and high hydration, respectively. The hexagonal phase coexists with excess water and is stable up to about 63 °C. These findings make GME an interesting alternative to glycerol monoesters in various fields of applications.

Keywords: 1-Glyceryl monooleyl ether; X-ray diffraction; Phase behavior; Phase diagram; Reversed hexagonal phase

1. Introduction

The polar lipid liquid crystalline phases have attracted both scientific and industrial attention in various fields of applications [1]. However, only a very limited number of lipids exhibit suitable phase behavior and particularly low toxicity to use in pharmaceutical applications, such as drug delivery [2]. In fact, the unsaturated monoglycerides belong to the very few systems showing these properties [3]. The pharmaceutical applications of the aqueous dispersions of the cubic and reversed hexagonal phases of glycerol monooleate (GMO) and GMO/triglycerides drug carriers have been recently considered [4]. It is noteworthy that lipid-based liquid crystalline vehicles can facilitate certain protection of incorporated drug molecules against proteolytic degradation in vivo [5]. On the other hand, GMO-based liquid crystalline phases and dispersions itself are sensitive to the ester bond hydrolysis [6] and action of hydrolytic enzymes, such as lipases [7,8]. Here we present the possible alternative for GMO-based liquid crystalline materials to further increase stability, bioavailability and protection of biomolecular drugs. The utilization includes the use of 1-glyceryl monooleyl ether (GME) whose advantage compared to the corresponding ester (GMO) is the lack of enzymatic degradation by lipases. Alternatively, the toxicity of GME is not known but considering the molecular structure is expected to be at least not higher when compared to GMO.

Non-polar ether lipids of the 1-*O*-alkyl-2,3-diacyl-*sn*-glycerol type are present at trace levels in most animal tissues, but they are the main constituents in the lever oils of various species of elasmobranch fish, such as dogfish and shark [9]. The highly valuable 1-*O*-alkyl-*sn*-glycerols are obtained on hydrolysis of lever oils, where the most abundant glyceryl ether is GME with $C_{18:1}$ alkyl moiety [10]. GME also corresponds to selachyl alcohol named after its source of original isolation from the lever oil of Selachoidei family fish.

Glyceryl ethers exhibit a variety of biological activities, such as antibacterial and antifungal action, immunological stimulation, antitumor properties [11–13]. Besides its biological effects, glyceryl ethers are amphiphilic and exhibit surface active properties. However, surprisingly few stud-

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ies of their physicochemical properties have been reported [14–16]. Therefore, in the present study, highly pure 1-glyceryl monooleyl ether was synthesized and its phase behavior in water was investigated by polarized microscopy and X-ray diffraction measurements. As a result, a partial temperature–composition phase diagram is constructed and discussed.

2. Experimental

2.1. Synthesis of oleyl p-toluene sulfonate

To an ice-cooled solution of oleyl alcohol (33.1 g, 0.105 mol) in dry pyridine (200 ml), small portions of *p*-toluene sulfonyl chloride (21.9 g, 0.115 mol) was added at −10 °C. The reaction mixture was stirred for 10 min maintaining the reaction temperature at −10 °C until the solution became clear, and then stirred for additional 2 h. The reaction mixture was kept in the refrigerator overnight at +4 °C, and then extracted with two portions of ether. The ethereal extracts were washed in course with acidified water (pH 4), two portions of cold water, and finally with solution of potassium hydrocarbonate. The solution was dried over anh. Na_2SO_4, and solvent evaporated to give 33 g of crude material. Purification over silica gel column (eluent benzene) afforded 26.9 g (60.9%) of pure compound. ^{1}H NMR and IR data are given in Table 1.

2.2. Synthesis of 1-glyceryl monooleyl ether

Potassium (3.9 g, 0.1 mol) was heated in dry benzene (250 ml) until dispersion, and 1,2-isopropylidene glycerol (11.9 g, 0.09 mol) was added. The mixture was stirred and heated at 80–90 °C for 3 h. A solution of oleyl *p*-toluene sulfonate (26.9 g, 0.063 mol) in dry benzene (100 ml) was added dropwise slowly, and refluxed for additional 20 h. Reaction mixture was cooled, filtered with suction, washed with benzene and solvent evaporated. The residue was dissolved in ether, washed twice with water, dried over anh. Na_2SO_4, and ether was evaporated. The isopropylidene protecting group was removed by hydrolysis with boric acid. To a solution of 1,2-isopropylidene glyceryl monooleyl ether (24.5 g, 0.064 mol) in dioxane (100 ml), boric acid (8.0 g, 0.13 mol) was added and refluxed for 20 h. The organic phase was evaporated in vacuo and the residue dissolved in ether. The ethereal solution was washed with water and dried over anh. Na_2SO_4. Purification over silica gel column (eluent hexane–chloroform) afforded 10.1 g (28.1%) of the oily substance which crystallized in the refrigerator, mp 18–18.5 °C (17.6–18.5 °C according to Wood and Snyder [17]). ^{1}H NMR and IR data are given in Table 1. Mass spectrum, *m/z* 342 (M^+). Anal. Calc. for $C_{21}H_{42}O_3$, %: C, 73.63; H 12.36. Found: C, 73.78; H, 12.35.

2.3. Aqueous phase behavior

1-Glyceryl monooleyl ether/water mixtures were prepared by weighing appropriate amounts of lipid (ca. 200–300 mg) and Milipore water into glass vials (i.d. 9 mm). The vials were immediately sealed and allowed to equilibrate in the dark at 22 °C for at least 4 weeks before measurements. In order to obtain phase homogeneity, samples were centrifuged up-and-down at 1500 × *g* for 30 min. If needed, the centrifugation was repeated several times. The texture and homogeneity of the samples were examined by a polarizing microscope Zeiss Axioplan II (Carl Zeiss, Oberkochen, Germany).

2.4. X-ray diffraction

X-ray diffraction measurements were performed on a Kratky compact small-angle system equipped with an OED 50 M position-sensitive detector (Mbraun, Graz, Austria) containing 1024 channels of width 53.1 μm. Cu Kα nickel-filtered radiation of wavelength 1.542 Å was provided by a Seifert ID 3000 X-ray generator (Rich Seifert, Ahresburg, Germany) operating at 50 kV and 40 mA. A few milligrams of the samples were mounted between thin mica windows in a steel sample holder at the sample-to-detector distance of 277 mm. Temperature control within 0.1 °C was achieved by using a Peltier element. X-ray diffraction patterns were collected in the temperature range from 25 to 70 °C in increments of 3–6 °C in the heating direction. Because no significant difference was observed between different times, 10 min of pre-equilibration was typically used to collect the X-ray data. The recorded diffraction patterns were evaluated using 3D-View software (Mbraun, Graz, Austria) without any additional data treatment.

3. Results and discussion

Synthesis of 1-glyceryl monooleyl ether (GME; IUPAC name 3-[(9*E*)-9-octadecenyloxy]-1,2-propanediol) was accomplished via a reaction sequence involving the interaction of 1,2-isopropylidene glycerol potassium salt with oleyl

Table 1
NMR and IR data

Compound	^{1}H NMR ($(CD_3)_2CO$) δ, J (Hz)	IR (cm^{-1})
Oleyl *p*-toluene sulfonate	1.0–1.9 (m, 29H), 2.2 (m, 2H, CH_2), 2.6 (s, 3H, CH_3), 4.15 (t, 2H, CH_2), 5.5 (t, 2H, CH=CH), 7.6 and 7.95 (two d, 4H, aromatic protons)	1654, 1598, 1364, 1177 and 1098
1-*g*lyceryl monooleyl ether	1.0–1.7 (m, 31H), 2.2 (m, 2H, OCH_2), 3.5–3.8 (m, 7H, CH_2OCH_2 and CH), 5.45 (t, 2H, CH=CH)	3368, 1650, 1466 and 1122

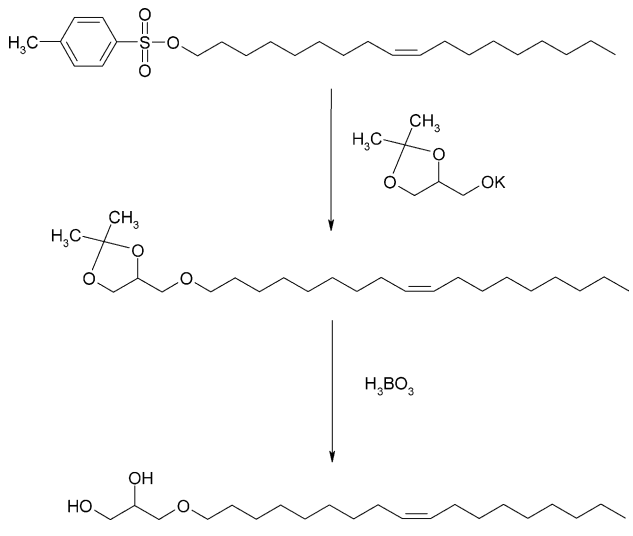

Fig. 1. Synthesis scheme of the 1-glyceryl monooleyl ether.

p-toluene sulfonate according to Fig. 1 [17]. The progress of reaction was monitored by thin-layer chromatography, for which specific conditions have been estimated. The isopropylidene protecting group was removed by hydrolysis with boric acid in the last step of the synthesis. To obtain the material of high purity, column chromatography was used though the general yield of product was moderate (about 28%). GC–MS analysis confirmed 99.6% purity of the synthesized material.

The aqueous phase behavior of GME was investigated in the water content and temperature ranges of 10–40% (w/w) and 25–70 °C, respectively. The phases were identified by X-ray diffraction and their location in the temperature–composition diagram of the binary GME/water system is shown in Fig. 2a. The phase boundaries and coexisting region, drawn to conform to both experimental data and the Gibbs phase rule, are presented in Fig. 2b. In addition, Fig. 3 shows representative X-ray diffractograms as a function of temperature for the sample containing 19.88% (w/w) water.

At room temperature and up to about 12% (w/w) water, a fluid isotropic reversed micellar solution (L_2 phase) is formed. This phase does not show more than a diffuse diffraction, and the X-ray data seen in Fig. 3 indicate a broadening of the first reflection of the hexagonal phase, similar to those found for other L_2 phases [18]. The absence of a Bragg peak indicates a disordered structure without long-range order. The L_2 phase occupies the high temperature part of the diagram at all hydration levels.

At higher water content, the L_2 phase transforms into a reversed hexagonal liquid crystalline phase (H_{II}), which consists of the reversed type cylindrical aggregates of amphiphilic molecules arranged in a hexagonal packing [19]. This phase can be easily distinguished from the L_2 phase by its optical anisotropy and characteristic texture seen in polarizing microscope (Fig. 4). The X-ray diffraction measurements of the H_{II} phase indicate three strong Bragg peaks with relative positions in ratios $1:\sqrt{3}:\sqrt{4}$, which can be in-

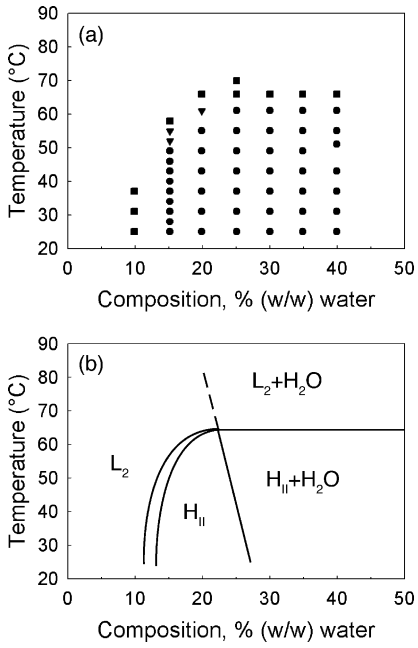

Fig. 2. (a) Identity and location of the phases in the 1-glyceryl monooleyl ether/water system as determined by X-ray diffraction in the heating direction over the 25–70 °C temperature and 0–40% (w/w) water composition ranges. The phases are labeled as follows: squares, L_2; circles, H_{II}; reversed triangles, $L_2 + H_{II}$. (b) Temperature–composition phase diagram of the 1-glyceryl monooleyl ether/water system based on both the data in (a) and the Gibbs rule.

dexed as $hk = 10$, 11 and 20 reflections (Fig. 3). Such relationship proves the two-dimensional hexagonal structure, whereas the reversed morphology can be confirmed by the phase position in the diagram and variation of the calculated lattice parameter, $a = (2d_{hk}/\sqrt{3})(h^2 + k^2 + hk)^{1/2}$ (d_{hk} are the interlayer spacings obtained from Bragg peak positions), with the water content.

The calculated a increases from 48 to close to 58 Å in the water content range of 13–27% (w/w) and a temperature of 25 °C (Table 2). The a value remains unchanged upon the further addition of water. This fact indicates that the H_{II} phase

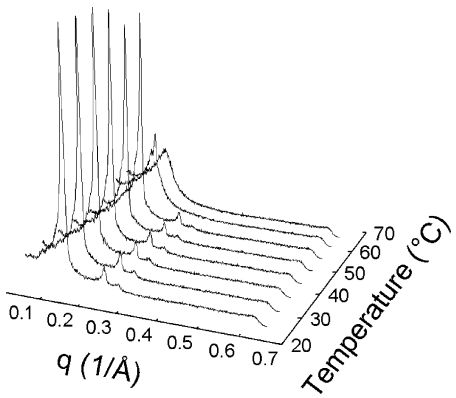

Fig. 3. Representative temperature dependent X-ray diffractograms of the 1-glyceryl monooleyl ether/water sample of 80.1/19.9% (w/w) composition showing the $H_{II} \rightarrow L_2$ phase transition.

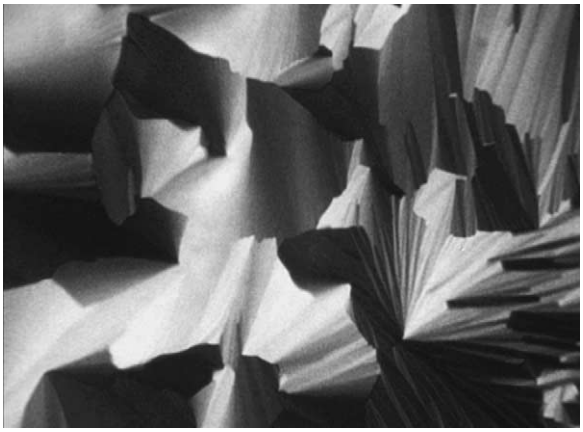

Fig. 4. Polarizing microscopy image of the H_{II} phase of 1-glyceryl monooleyl ether/water sample of 30/70% (w/w) composition at 25 °C.

is in equilibrium with excess water and proves the reversed nature of the hexagonal arrangement [20]. As the temperature is increased, the H_{II}/H_{II} + water boundary moves to lower hydration. The measured lattice parameter also decreases with temperature. The change in a as a function of temperature is determined as about 0.06 and 0.2 Å/°C at lower and higher hydration, respectively. The upper limit for the H_{II} phase is approximately 63 °C. At higher temperatures, GME forms only L_2 phase.

The obtained X-ray diffraction data were further applied to estimate the structural characteristics of the GME-based H_{II} phase. Using simple geometric considerations, the calculated a allows to approximate the lipid length (l) and the radius of the water cylinders (R_W) [21,22]. The value of l is simply calculated as $l = (a - 2R_W)/2$, where $R_W = a[\sqrt{3}(1-\phi)/2\pi]^{1/2}$ and ϕ is the volume fraction of lipid. To simplify calculations, we assumed equal partial specific volumes of water and GME. In this case, ϕ stands for the lipid weight fraction.

The calculated structural parameters for the GME/water H_{II} phase are summarized in Table 2. The results give an indication that the size of water channels in the H_{II} phase increases with increasing the water content, but slightly decreases with increasing temperature. Although the monolayer thickness becomes smaller at higher temperatures, it is practically constant at different hydration levels.

Table 2
Structural parameters of the H_{II} phase formed in the GME/water system

Water content (%, w/w)	Temperature (°C)	a (Å)	R_W (Å)	l (Å)
15.15	25	48.05	9.82	14.21
	28	47.78	9.76	14.13
	31	47.64	9.73	14.08
	34	47.41	9.69	14.02
	37	47.29	9.66	13.98
	40	47.08	9.62	13.92
	43	46.93	9.59	13.88
19.88	25	53.36	12.49	14.19
	31	52.88	12.38	14.06
	37	52.58	12.31	13.98
	43	52.27	12.24	13.90
	49	51.62	12.08	13.73
25.08	25	57.39	15.09	13.61
	31	56.66	14.90	13.43
	37	55.00	14.46	13.04

Earlier studies of the GME/water system have indicated formation of two liquid crystalline phases, the H_{II} and neat (lamellar liquid crystal) phase at low and high hydration, respectively [14]. The neat phase has been found to coexist with excess solution phase at hydrations higher than 30 wt.% (w/w). In our study, we did not observe any indications of the lamellar liquid crystalline phase formation even by cooling of some of the samples to 0 °C. The large difference observed in phase diagrams may be attributed to the fact that only 92% pure GME has been used in that study, whereas our preparation consists of 99.6% of GME. More complicated phase behavior could arise from the impurities containing saturated alkyl chains since the transition temperatures for both H_{II} and neat phase to L_2 phase have been also found to be higher.

The observed features of the aqueous phase behavior of GME are dramatically different from that of an ester analog, GMO (commonly known as monoolein), although the molecular structures are very similar. It is well known that, at room temperature and with increasing water content, GMO forms three liquid crystalline phases, lamellar and two reversed bicontinuous cubic phases [23,24]. At higher temperatures, the GMO lamellar and cubic phases transform to the L_2 and H_{II} phases, and the phase behavior becomes similar to that of GME at lower temperatures. The differences as well as some similarities in phase behavior and types of the liquid crystalline phases formed can be attributed to the greater hydrophobicity of the GME compared to GMO. Another reason is that due to the acyl migration all GMO preparations contain some (up to 12% [25]) of the 2-isomer which is expected to have larger cross-sectional area of the polar head groups at the bilayer surface. The expectation then is that the 2-isomer will tend to flatten out the lipid/water interface with the favourable formation of phases with less negative curvatures. In contrast, the spontaneous isomerization to 2-isomer is not present in GME preparation. Therefore, GME consisting of only 1-isomers will favour liquid crystalline phases with more negative curvatures such H_{II} phase.

4. Conclusions

A partial temperature–composition phase diagram of the synthetic 1-glyceryl monooleyl ether (GME) in water is constructed. With increasing water content, GME forms reversed micellar solution and reversed hexagonal liquid crystalline phase. The latter phase coexists with excess water. The features of the phase behavior and increased chemical stability make GME a possible alternative to a well-known and widely used glycerol monooleate in various kinds of food, cosmetic and pharmaceutical applications.

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