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Enzymatically prepared *n*-alkyl esters of glucuronic acid: The effect of freeze-drying conditions and hydrophobic chain length on thermal behavior

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Abstract

In this work, some of the physicochemical properties of enzymatically prepared *n*-alkyl esters of glucuronic acid are presented. Two questions are addressed. The first concerns the influence of post-purification freeze-drying conditions on octyl glucuronate thermotropic behavior. Depending on the amount of water added before freeze-drying, the α/β anomeric ratio determined by ^{1}H NMR is affected and differences are observed in DSC thermograms probably due to polymorphism. The second question concerns the effect of hydrophobic chain length on the thermal behavior. An increase of both transition temperature and transition enthalpy is observed by increasing the number of carbon atoms in the alkyl chain ($C8 < C10 < C12 < C14$). This kind of results can provide relevant information for the processing and the practical use of these nonionic surfactants.

Keywords: Nonionic surfactants; Glucuronic acid; Biosurfactants; Enzymatic synthesis; Lipase; DSC; X-ray diffraction; ^{1}H NMR

1. Introduction

Sugar-derived fatty esters constitute an interesting group of nonionic surfactants. They are biodegradable, non-toxic, non-skin-irritant, odorless and tasteless [1], which confers to them a particular interest in the food, pharmaceutical and cosmetic industry.

The classical chemical way of synthesis of these compounds shows some major drawbacks. This conventional approach requires high temperature which causes coloration, high energy consumption and the recovery of considerable amounts of side-products [1,2]. The use of biological catalysts under mild conditions can help to overcome these problems. The enzymatic synthesis has given new potentialities to sugar esters production [3].

In the last years, a lot of papers have been published on the lipase-catalyzed synthesis of sugar esters [4–7]. Those articles mainly describe the influence of various parameters (temperature, water activity, kind and amount of lipase, sugar/acyl donor molar ratio) on the conversion yields. However, few data on the physicochemical properties of the synthesized molecules are available. For this reason, new investigations on this novel class of surfactants are necessary for further development.

The particularity of the surfactants used within the present study is linked to the nature of the hydrophilic moiety: glucuronic acid. In previous papers, we reported on the synthesis and the surface properties of such compounds namely alkyl glucuronate (also known as alkyl glucopyranosiduronate) [4,8,9]. We now focus on their characterization by differential scanning

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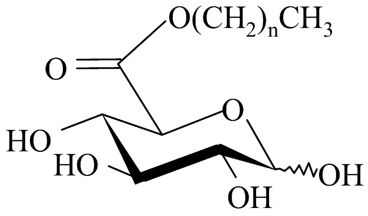

n= 7, 9, 11 or 13

Fig. 1. Ester of glucuronic acid and fatty alcohol.

calorimetry (DSC) in relation to X-ray diffraction and ^{1}H NMR. To our knowledge, no data on the thermal behavior of glucuronic acid esters are available in the literature. However, this kind of data can provide relevant information for the practical use of these surfactants in formulations.

2. Materials and methods

2.1. Ester synthesis and purification

Esters of glucuronic acid with different alkyl chain lengths (structure illustrated in Fig. 1) were prepared using immobilized *Candida antarctica* lipase B (Novozym SP 435, Novo Nordisk) in organic medium. Glucuronic acid (0.025 M) and fatty alcohol (0.025 M) were dissolved in 10 ml of *t*-butanol. After addition of 100 mg of lipase and 500 mg of activated molecular sieve (8–12 mesh, Sigma), enzymatic reaction was carried out in a shaking (150 rpm) water bath at 60 °C during 48 h. Lipase and molecular sieve were then removed by filtration and ester was extracted by dichloromethane (20 ml) and purified using silica gel chromatography (70/30 v/v chloroform/methanol as eluent). The purity of all synthesized products was proved to be more than 99% by HPLC-ELSD.

2.2. Ester powder preparation

Eluent from the purification step was evaporated using rotary vacuum evaporator (Rotovapor RE111, Büchi). Aliquots of 20 mg were then sampled and various amounts of water (0, 0.5, 5, 50 ml) were added to them. All samples were finally freeze-dried using a HETO DryWinner (condenser temperature: −40 °C, vacuum pressure: 50 hPa). So, esters were obtained in a white powder form. They were finally stored in the freezer over a desiccant.

2.3. Differential scanning calorimetry

Ester powders were analyzed by differential scanning calorimetry with a DSC 2920CE (TA Instruments). DSC curves were recorded during heating from −40 to 200 °C at a scan rate of 5 °C/min. Indium and dodecane were used to calibrate the temperature and enthalpy reading. All these DSC experiments were made using unhermetic aluminum pans of matched mass, i.e., the empty sample and reference pans were of equal mass to within ±0.10 mg. The analyzed sample mass was generally of 1.50 ± 0.25 mg.

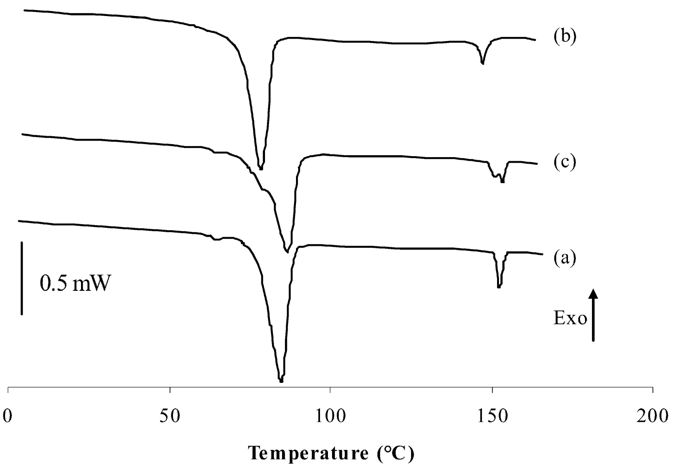

Fig. 2. DSC curves of octyl glucuronate: (a) freeze-dried without addition of water, (b) freeze-dried after dispersion in water at ratio 1/2500 w/w, (c) freeze-dried after dispersion in water at ratio 1/250 w/w.

2.4. NMR spectra

^{1}H NMR spectra were recorded on a Bruker AMX-300 (300 MHz) as d_6-DMSO solutions. The anomeric ratios were estimated from these spectra using OH-1, H-1, OH-2 and OH-3.

2.5. X-ray diffraction

X-ray diffraction patterns at room temperature were registered on a Phillips PW3710 diffractometer using a Ni-filtered CuK_α radiation, generated by an anode device operating at 40 kV and 30 mA, in conjunction with a proportional detector. The patterns were first recorded in a broad range of $4 \leqslant 2\theta \leqslant 50°$, and then in a reduced range of $15 \leqslant 2\theta \leqslant 27°$ at $0.02\theta\ \mathrm{s}^{-1}$.

3. Results

In a preliminary step, we observed that different thermograms could be obtained for a same compound, depending on postpurification treatments. So, a more systematic study was led in order to determine the influence of some freeze-drying conditions on the thermal behavior of glucuronic acid esters. We first focused on octyl glucuronate which presents interesting surface properties [8].

3.1. DSC thermograms of octyl glucuronate

Fig. 2 presents DSC curves for enzymatically prepared octyl glucuronate, purified by silica gel chromatography, and freeze-dried after addition of various amounts of water. The curve for octyl glucuronate freeze-dried without addition of water, just after the evaporation of the eluent from the purification step, shows two main endotherms. The first (onset at 77.4 °C, maximum at 84.7 °C) corresponds to 24.9 kJ mol^{-1}, while the second (onset at 151.7 °C, maximum at 153.2 °C) to 2.0 kJ mol^{-1}.

Adding water to octyl glucuronate before freeze-drying modifies the DSC curve. So, a shift of the endothermic peaks

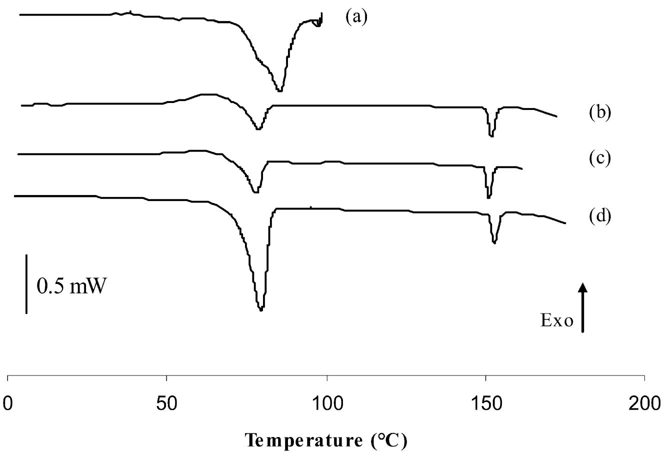

Fig. 3. DSC curves of octyl glucuronate freeze-dried without addition of water for a sequence of heating, cooling and reheating scan: (a) first heating to 100 °C, (b) second heating after cooling at 20 °C for 30 min, (c) second heating after cooling at 20 °C for 2 h, (d) second heating after cooling at 20 °C for 24 h.

to lower temperatures is observed when dispersing octyl glucuronate in a large amount of water (ratio 1/2500 w/w). In this case, the first endotherm appears at an onset temperature of 72.6 °C (maximum at 78.6 °C) and the second at 145.1 °C (maximum at 147.3 °C). The associated enthalpies remain close to those determined for the sample freeze-dried without addition of water, respectively 24.8 and 1.9 kJ mol^{-1}.

When dispersing the sample in a 10 times smaller amount of water (ratio 1/250 w/w) before freeze-drying, an overlapping is observed at each endothermic peak. The DSC profile seems to be intermediate compared to those presented for the sample without water and dispersed in an excess of water before freeze-drying.

Fig. 3 shows DSC thermograms of octyl glucuronate for a sequence of heating, cooling and reheating scan. The sample freeze-dried without addition of water was first heated at 100 °C, i.e., above the first endothermic peak. It was then cooled at 20 °C and kept at this temperature for different time and finally re-heated to 200 °C. When the sample was kept at 20 °C for 30 min or 2 h, an exotherm is observed before the first endotherm. This exotherm completely disappears when the sample is kept at 20 °C for 24 h. In comparison to the first scan, the second one shows a shift of the first endotherm to a lower temperature (onset at 73.0 °C, maximum at 78.6 °C).

It is also worthy to note that after the compound has been heated to 160 °C and cooled down to 20 °C, no peak is observed upon reheating DSC run.

3.2. NMR analysis of octyl glucuronate

As the glucuronic acid moiety of octyl glucuronate presents an anomeric carbon, the anomeric ratio (α/β) of the samples freeze-dried in different hydration conditions was determined from ^{1}H NMR data (Table 1). It can be deduced that the addition of water to octyl glucuronate before freeze-drying modifies its anomeric ratio. Indeed, octyl glucuronate obtained directly after the evaporation of the eluent from the purification step gives an anomeric ratio (α/β) of 71/29, while this compound dispersed in a large amount of water (200 mg octyl glucuronate in 500 ml water) and freeze-dried gives 60/40. The anomeric ratio of octyl glucuronate dispersed in a 10 times smaller amount of water is 70/30.

Table 1
^{1}H NMR data (d_6-DMSO solution) of octyl glucuronate: (a) freeze-dried without addition of water, (b) freeze-dried after dispersion in water at ratio 1/2500 w/w, (c) freeze-dried after dispersion in water at ratio 1/250 w/w

H	ppm	(a) (%)	(b) (%)	(c) (%)
OH 1β	6.78	27	40	27
OH 1α	6.53	73	60	73
H 1α	4.92	70	59	68
H 1β	4.33	30	41	32
OH 2β + OH 3β	4.99–4.97	29	40	31.6
OH 2α + OH 3α	4.80; 4.61	71	60	68.4

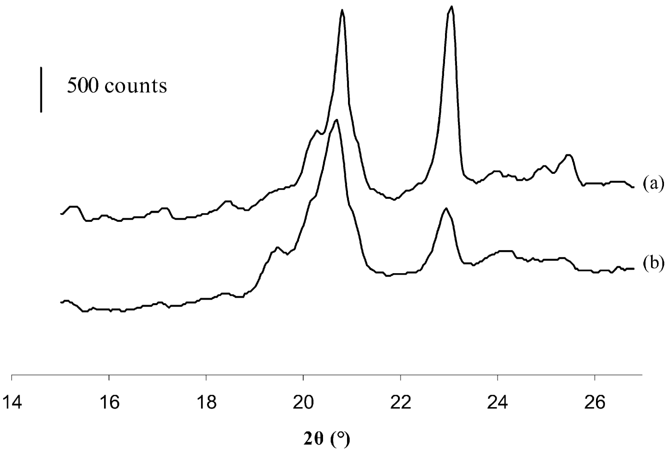

Fig. 4. X-ray diffraction pattern of octyl glucuronate at 20 °C: (a) freeze-dried without addition of water, (b) freeze-dried after dispersion in water at ratio 1/2500 w/w.

3.3. X-ray diffraction patterns of octyl glucuronate

Fig. 4 compares X-ray diffraction patterns of octyl glucuronate freeze-dried without addition of water and freeze-dried after addition of a large amount of water. Two main characteristic diffraction peaks (centered at $2\theta = 20.8°$ and $2\theta = 23.0°$) are clearly observed for the powder obtained by freeze-drying without addition of water. A smaller peak also appears at $2\theta = 20.4°$. In comparison, a single peak at $2\theta = 20.6°$ is observed for the powder obtained by freeze-drying after addition of water. The peak at $2\theta = 23.0°$ is also observed but its intensity is lower than the one determined with octyl glucuronate freeze-dried without addition. Other differences between the patterns of both samples can be noted, in particular at $2\theta \sim 19.5°$ and in the $24 < 2\theta < 26°$ range.

Fig. 5 shows that a flat diffractogram is present when measurement is performed at 100 °C on octyl glucuronate freeze-dried without addition of water. A return of the same sample to 20 °C for 24 h leads to a new pattern, different from the initial one, but quite similar to that obtained for octyl glucuronate freeze-dried after addition of a large amount of water.

Table 2
Peak temperatures and enthalpies of glucuronic acid esters (C8–C14, all samples freeze-dried without addition of water) extracted from DSC curves

Surfactant	Peak 1		Peak 2	
	$T_{\max}$ (°C)	ΔH (kJ mol^{-1})	$T_{\max}$ (°C)	ΔH (kJ mol^{-1})
Octyl glucuronate	84.7	24.9	153.6	2.0
Decyl glucuronate	91.1	29.1	163.5	1.4
Dodecyl glucuronate	94.3	35.1	163.6	1.0
Tetradecyl glucuronate	98.8	41.2	158.1	0.4

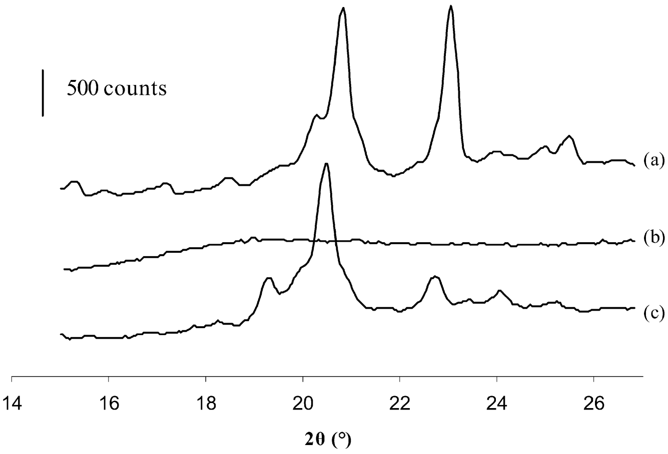

Fig. 5. X-ray diffraction pattern of octyl glucuronate freeze-dried without addition of water: (a) at 20 °C, (b) at 100 °C, (c) at 20 °C after heating at 100 °C and then cooling at 20 °C for 24 h.

3.4. *Effect of hydrophobic chain length on the thermal behavior*

As octyl glucuronate, other esters of glucuronic acid (C10–C14) exhibit two endothermic peaks during heating. Both enthalpy and temperature of the first peak are increased when the chain length is augmented whereas enthalpy of the second peak decreases (Table 2).

4. Discussion

All octyl glucuronate samples, enzymatically prepared, purified and lyophilized from aqueous solutions at different concentrations, are white solid. At room temperature, they all exhibit birefringent texture under the polarization microscope, indicating the presence of crystalline structure. However, the amount of added water before freeze-drying affects the thermal behavior of the compound. Indeed, if all DSC thermograms of octyl glucuronate present two endotherms, the temperature at the peaks position depends on the initial concentration of ester.

Carbohydrate-derived surfactants are expected to exhibit thermotropic liquid-crystalline properties. So, the appearance of two endothermic peaks can be explained in the following way. Similarly to other sugar-based surfactants such as glucose [10–13], lactose and lactitol derivatives [14], the first endotherm might indicate a transition to an anisotropic liquid-crystalline phase. The second peak corresponds to the melting of the liquid-crystalline phase to an isotropic solution. It can be noted, in particular, that DSC curve of octyl glucuronate has exactly the same features as the thermogram of octyl α-glucoside and that the enthalpies of the second peak for these two compounds are nearly the same [13].

The self-organizing phenomenon is frequent for soft condensed matter. Amphiphiles molecules (surfactants, phospholipids, etc.) can associate themselves to form micellar, hexagonal, lamellar or cubic phases. Measurements of X-ray diffraction profiles can provide information about the underlying phase structure of octyl glucuronate, but a more extensive interpretation could only be possible with a small-angle X-ray (SAXS) analysis (measurements not performed in this study). SAXS is recorded at very low angles (typically 0.1–10°), providing information about the interlayer spacings of partially ordered materials. Therefore, analyzing our wide-angle X-ray diffraction data, the presence of two main diffraction peaks observed for octyl glucuronate freeze-dried without addition of water and freeze-dried after addition of a large amount of water may suggests a characteristic lamellar arrangement [15,16].

Difference in thermotropic transition temperature as a function of freeze-drying conditions is probably due to the polymorphism of octyl glucuronate. This is supported by the difference appearing in X-ray diffraction patterns which can be attributed to a loss of order in structural arrangement after changes in lyophilization (water addition). Addition of a large amount of water before lyophilization leads to a less stable form with a lower transition temperature (~78 °C). NMR analysis performed on the same samples indicates that the addition of water reduces the α/β anomeric ratio. So, it is observed that a higher α-anomer content, resulting from an equilibrium in water instead of chloroform/methanol (purification medium), affects polymorphic forms of octyl glucuronate. Other authors have previously reported on the influence of isomer ratio on the thermal behavior of glucose-based surfactants. For C_8G_1 alkylglucosides, Nilsson et al. [10] demonstrate that the α-anomer has a more stable crystal structure that the β-anomer. Boyd et al. [11] present the dependence of the thermotropic transition temperature on the diastereoisomer ratio for glucose-derived surfactants. They attribute the effect of the diastereoisomer ratio on the thermotropic behavior to the significant difference in molecular shape resulting from very subtle structural difference between the surfactants.

In our case, DSC measurements also imply that the less stable form can be obtained by heating of the more stable form and cooling down to room temperature.

Regarding to the effect of hydrophobic chain length, an increase of both transition temperature and transition enthalpy is observed by increasing the number of carbon atoms in the alkyl chain. The increase in transition or melting temperature with the molecular mass is a well-known phenomenon in homologous series of lipids or surfactants [14,15], and is due to the decrease of chain ends (and therefore defects) inside a crystal domain as the molar mass is increased [17], in our case as the number of carbon atoms of the alkyl chain is increased. The transition enthalpy increase with increasing chain length (C8 $<$ C10 $<$ C12 $<$ C14) is expected because the van der Waals (London) interactions are stronger when the number of aliphatic carbons increases [18].

5. Conclusion

This study provides new physicochemical data on unusual nonionic surfactants produced by enzymatic synthesis. These results serve as an indication to surfactant product investigators that the conditions of post-synthesis treatments must be taken into account when studying the thermal behavior of carbohydrate-based esters, since these conditions can have significant effects on the isomer ratio and crystalline organization.

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