Characterization of micelles formed by sucrose 6-O-monoesters

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A B S T R A C T

In the present study we synthesized and purified several 6-O-sucrose monoesters by using classical synthetic methods and stereo-regioselective procedures. The compounds have different alkyl chain lengths, with the number of methylene units ranging between 7 and 17. For all the available compounds the physicochemical information concerning the formed micellar aggregates was evaluated by using fluorescent methodologies. Cmc for the series show a clear linear dependence with the length of alkyl chain. Structural properties of the aggregate, like cross-sectional area or aggregation numbers show clear dependence on the number of methylene units. For shorter esters, the cross-sectional area is constant, probably consequence of the size of sucrose head, whereas for the longer ones is dependent on the length of alkyl chain. Aggregation number is dependent of ester concentration for shorter compounds and independent for the longer ones. These results show for 6-O-sucrose esters with different length of alkyl chain that its properties depend on the balance of the size of alkyl chain and sucrose head.

Keywords: Sucrose ester Micelle Fluorescence

1. Introduction

Despite the significant number of publications on sugar fatty acid esters (SFAE) beginning in the mid-1950s, there is a renewed interest in these compounds with a noteworthy increase of studies in the last two decades. These studies have been motivated by their outstanding surface-active properties (surface tension-reducing capacity, penetrability into lipid bilayers, easiness of dispersion, and remarkable emulsifying power, among others) and their environmental advantages, when compared to surfactants derived from petrochemical industry [1,2]. SFAE are nontoxic and non-allergic surfactants, readily biodegradable in aqueous environments [2,3]. The raw materials involved in their synthesis are low-cost, simple, effortlessly accessible and renewable: fatty acids or their derivatives and sucrose [4–7]. SFAE have a large number of applications in many fields such as cosmetic and health care [8,9]. Like food additives [9], they have a very large variety of functions such as emulsifying, foaming, improving components mixing, improving water holding, preventing denaturation, and avoiding precipitation. Sucrose esters with short hydrocarbon chain are widely employed to solubilize membrane proteins without causing denaturation and to extract specific compounds (i.e. enzymes, receptors, and transport carriers), and can also be removed by simple dialysis [10,11]. These non-ionic carbohydrate-based surfactants contain usually glucose, xylose or sucrose [12] as hydrophilic head. The hydrophobic tail of these compounds corresponds to hydrocarbon chains (of different length with or without insaturations), as a substituent on one specific hydroxylic group of sucrose.

Mixtures of sucrose esters, usually commercial preparations, are used in most of the reported work on physicochemical properties (e.g. characteristic micellar parameters, and surface activity) and functional properties (e.g. emulsification capacity and rheological modifications) [13,14], and a few basic studies have been made with pure sucrose monoesters [15–20] and pure diesters [21]. Commercially available sucrose esters are typically mixtures of mono-, di- and minor quantities of highly polysubstituted isomers, being the proportion of each, responsible for their specific hydrophilic–lipophilic balance (HLB value), among other properties. The recent increase in the number of studies dealing with physicochemical properties of carbohydrate-based surfactants reflects recognition that the knowledge, regarding the influence of the hydrophilic and lipophilic counterparts on application-related properties, needs to be expanded and clarified for this kind of surfactants. The present study, involving fluorescence measurements (steady state and time resolved) and surface tension determinations, intends to obtain and systematize physicochemical information (critical micellar concentration, and properties of the micellar aggregate such as micropolarity, microfluidity, shape and aggregation number) of the micellar aggregates formed by six pure 6-O-sucrose esters, with hydrocarbon chains ranging between 7 and 17 methylene units to report a homogeneous set of properties for a complete series of compounds.

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2. Materials and methods

2.1. Chemicals

6-Dodecanoyl-2-dimethylaminonaphthalene, Laurdan, from Molecular Probes and dibutylaniline, DBA, from Sigma were used as received. Pyrene, from Merck, was recrystallized twice from ethanol. Solvents from Merck were HPLC quality. Water was purified with Milli-Q equipment from Waters. Surcose monoester β-D-fructofuranosyl-6-O-capryl-α-D-glucopyranoside (MCS), purchased from Dojindo Molecular Technologies, was used as received. The sucrose monoesters β-D-fructofuranosyl-6-O-lauryl-α-D-glucopyranoside (MLS), β-D-fructofuranosyl-6-O-miristyl-α-D-glucopyranoside (MMS), β-D-fructofuranosyl-6-O-palmitoyl-α-D-glucopyranoside (MPS) and β-D-fructofuranosyl-6-O-stearyl-α-D-glucopyranoside (MSS) were synthesized by a modification of the Osipow–Snell method [22,23], that yields a complex mixture of mono- (mainly 6-O and presumably 1-O), di- and tri-esters. The sucrose monoester β-D-fructofuranosyl-6-O-octyl-α-D-glucopyranoside (MOS) was synthesized by the Lindhart procedure [4]. Both methods yield a relatively complex mixture of mono (mainly 6-O and presumably 1-O) and polyesters. Chromatography on silica column was employed to isolate pure monoesters of all synthesized compounds, briefly, the reaction mixture was solubilized in chloroform and eluted from a semipreparative silica gel column by using chloroform:methanol:water (20:5:0.7) as mobile phase. Thin layer chromatography (using the same mobile phase and staining with a butanolic solution of urea–orthophosphoric acid) showed mainly one compound in the purified sample. The NMR spectra, obtained in a Bruker ADX 300 spectrometer, with DMSO-δ6 containing 5% of CH3OD to avoid micellization, were in good agreement with previously reported spectra for several monoesters [4,24]. The chemical structures of the studied compounds are summarized in Fig. 1.

2.2. Fluorescence spectroscopy

Steady state fluorescence measurements of Laurdan and pyrene emission were accomplished in a Fluorolog 2.2. Fluorescence spectroscopy. The values of generalized polarization of Laurdan, GP (LAU 3 μM, λex = 364 nm, λem = 440 nm and 490 nm), not corrected against DMSO value, were calculated by using the expression proposed by Parasassi et al., Eq. (1) [25,26]

\[ GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}} \]  

(1) [25,26]

Pyrene measurements involve the use of the ratio of its first and third emission bands (Py ratio or \( \frac{I_1}{I_2} \), \( \lambda_1 = 372 \text{ nm} \), and \( \lambda_3 = 384 \text{ nm} \), with excitation at \( \lambda_{ex} = 337 \text{ nm} \)) [27].

Pyrene time resolved fluorescence measurements were performed by using a Hamamatsu R928 photomultiplier mounted in a homemade housing and fitted to a PTI 01-001 monochromator exit slit port. Photomultiplier signals were monitored with a 500-MHz and 1 GSa/s Hewlett-Packard digital oscilloscope, model 54540A. All emission measurements were performed at 25.0 ± 0.1 °C, controlling temperature with a Haake thermoregulated bath.

2.3. Measurements of sucrose ester critical micellar concentration

The values of the critical micellar concentration, cmc, were determined with fluorescent techniques by using both, pyrene or Laurdan emission as probes. Surface tension measurements performed with a Du Noüy tensiometer (K8 Krüss, measurement range 5–90 mN/m with Pt–Ir ring of 20 mm) were used also to determine cmc values. From plots of the change in emission intensity or surface tension values against surfactant concentration, cmc was determined from the point of slope change. All described measurements were performed at 25.0 ± 0.1 °C.

2.4. Measurement of sucrose ester aggregation number

Aggregation number, \( n_{agg} \), were determined by employing time resolved fluorescence quenching experiments. Recorded fluorescence decay curves (Fig. 5) in presence of quencher were fitted to the Infeita–Tachiya equation [28,29], Eq. (2), by using GraphPad Prism Version 4.00 for Windows, GraphPad Software, San Diego CA, USA:

\[ I = I_0 \exp(-A_2t - A_3 [1 - \exp(-A_4t)]) \]  

(2)

where \( A_2, A_3 \) and \( A_4 \) are simple fitting parameters. The micelle aggregation number, \( n_{agg} \), may be determined from [30]

\[ n_{agg} = A_3 \left\{ \frac{C - \text{cmc}}{[Q]} \right\} \left[ 1 + \varepsilon \right]^2 \]  

(3)

being \( \varepsilon \) a correction factor equal to:

\[ \varepsilon = \frac{A_2 - \tau^{-1}}{A_2 A_4} \]  

(4)

3. Results and discussion

3.1. Cmc determination

Cmc values determined by using surface tension measurements and fluorescence procedures are included in Table 1. The values are on the order of those reported for the same compounds enzymatically and non-enzymatically synthesized [1,4,12,13,19]. Cmc values

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>I/III (Py)</th>
<th>GP (Laurdan)</th>
<th>Surface tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOS</td>
<td>3.2600</td>
<td>nd</td>
<td>2.3400</td>
</tr>
<tr>
<td>MCS</td>
<td>1.4500</td>
<td>1.8000</td>
<td>0.4300</td>
</tr>
<tr>
<td>MLS</td>
<td>0.3427</td>
<td>0.3505</td>
<td>0.2726</td>
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<tr>
<td>MMS</td>
<td>0.0484</td>
<td>0.0238</td>
<td>0.0191</td>
</tr>
<tr>
<td>MPS</td>
<td>0.0150</td>
<td>0.0080</td>
<td>0.0055</td>
</tr>
<tr>
<td>MSS</td>
<td>0.0122</td>
<td>0.0100</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1

Critical micellar concentrations (in mM units) of sucrose esters determined by fluorescence measurements and surface tension determinations, at 25 °C.
determined at 25 °C show noticeable differences when photo-
physical and physical measurements are compared, but the trends
observed for the cmc dependence on the number of methylene
units are clearly comparable, especially when the cmc values for
compounds with longer chains are omitted (Fig. 2). The cmc values
determined from both fluorescence and surface tension measure-
ments show an inverse dependence with the number of methylene
units (n). Longer alkyl chains, lower water solubility, hence aggrega-
tion occurs at lower concentration. If values for MSS are excluded,
the dependence of cmc on the hydrocarbon chain length can be
fitted according to the empirical equation of Stauff–Klevens (first
employed and reported on polyoxyethylenic surfactants series) as
can be seen in Fig. 2 [31]. This behavior, as expected, is consequence
of the enhancement of chain hydrophobicity with the increase in
the number of methylene groups.

For MSS, experimental difficulties (perhaps adsorption on Pt–Ir
ring), do not allow the determination of cmc from surface tension
measurements. For MOS, the value obtained is in accordance with
the value reported by Garofalakis et al. [12], but is several times
lower than the one reported by Molinier et al. [19].

3.2. Micelles micropolarity and microviscosity

The Py l1/l3 ratio and Laurdan GP are useful dimension-
less parameters to determine cmc values, and also contain
physical–chemical information of the microaggregate, not reported
previously for these compounds. Py scale informs about polarity
near the probe localization, meanwhile Laurdan generalized
polarization is a measure of microviscosity (fluidity) and/or water
accessibility. Both properties were determined when micelliza-
tion begins and at higher sucrose ester concentrations (when the
property reaches a plateau). The values determined for the
I/III ratio at concentrations equal to the cmc and at higher con-
centrations are shown in Table 2. For short esters, the values for
I/III ratio indicate a polar surroundings for the probe. For
longer chain derivatives, I/III ratio values do not show a clear
dependence on alkyl chain length, suggesting that probe senses
environments of the same polarity for all cases. These results are
indicative of two possible situations: the sensed micro-aggregates
have indeed similar polarities independent on the alkyl chain,
for n ≥ 12, indicating a similar but not identical hydrocarbon
packing for all systems or the probe migrates towards regions
of similar characteristics. According to the Py scale the sur-

Table 2

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>I/III</th>
<th>Plateau</th>
<th>cmc</th>
<th>GP</th>
<th>Plateau</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOS</td>
<td>1.40</td>
<td>1.20</td>
<td>0.097</td>
<td>0.095</td>
<td></td>
</tr>
<tr>
<td>MCS</td>
<td>1.25</td>
<td>1.20</td>
<td>−0.019</td>
<td>−0.080</td>
<td></td>
</tr>
<tr>
<td>MLS</td>
<td>1.10</td>
<td>0.92</td>
<td>−0.325</td>
<td>−0.341</td>
<td></td>
</tr>
<tr>
<td>MMS</td>
<td>1.00</td>
<td>0.98</td>
<td>−0.326</td>
<td>−0.343</td>
<td></td>
</tr>
<tr>
<td>MPS</td>
<td>1.08</td>
<td>0.93</td>
<td>−0.371</td>
<td>−0.367</td>
<td></td>
</tr>
<tr>
<td>MSS</td>
<td>1.18</td>
<td>0.90</td>
<td>−0.216</td>
<td>−0.215</td>
<td></td>
</tr>
</tbody>
</table>

Experiments performed at 25 °C.

For all studied systems there is a slight reduction of I/III ratio
when micelle concentration increases, result compatible with an
increase in the aggregation number at higher surfactant concen-
tration.

The profiles of Laurdan generalized polarization (GP) values
when plotted against sucrose ester concentration show clear break-
points for shorter chain compounds (MOS to MMS), reaching a
plateau. For all the measured compounds GP values are indepen-
dent of the surfactant concentration, after micellization. The GP
value for Laurdan incorporated to micelles, shows a reverse depen-
dence with the increase of the alkyl chain length. This result can
be understood in terms of the presence of bulky and highly substi-
tuted surfactant heads limiting water accessibility. Then the effect
of the alkyl chain length is of secondary relevance over certain chain
length. Octyl and lauryl esters, the shorter ones, show higher val-
ues of GP, indicating a more closed surface, with reduced water
penetration, but higher polarity, according I/III ratio.

3.3. Surface activity

Surface tension measurements allow the determination of sev-
eral important parameters. Accepting that Gibbs adsorption model
describes the behavior of micellization for sucrose esters, from
the plot of surface tension values against the natural logarithm of
concentration, before micellization, is possible to determine the
surface excess and the molecular area, according Eqs. (5) and (6):

\[ \Gamma = -\left(\frac{1}{RT}\right) \left( \frac{\partial \gamma}{\partial \ln C} \right)_{T,P} \]  
\[ A = \frac{1}{N_{A}T} \]  

Fig. 3 shows profiles obtained for surface tension variation upon
MMS addition, equivalent plots are obtained for all other SFAEs.
None of the compounds studied gave cloudy solutions at work
temperatures, as reported for other pure sugar esters [12].

Surface excess values were determined where a linear
dependence between surface tension and concentration natu-
ral logarithm was observed. The surface excess values depend
inversely on the hydrocarbon chain length, the area occupied by
each molecule of surfactant increases with the increase of the
length of alkyl chain. The values determined for A (cross-sectional
area per molecule) are different for each ester, see Table 3. So, A
not only depends on the bulky hydrophilic head group dimensions
(sucrose plus solvation sphere) but also on packing and stereo-
chemistry of whole structure [12]. Values calculated for A are on
the order of the reported ones for pure enzymatically synthesized
6-O-monoesters, for 6-O-laurylsucrose an 6-O-palmitylsucrose Fer-
rer et al. [1] report values of 44 Å² and 91 Å², respectively (at
25 °C), and Garofalakis et al. [12] 53 Å² and 54 Å² (at 32 °C). Garo-
falakis also reports values of cross-sectional areas for samples corresponding to mixtures of several monoesters, results that are somehow lower than ours, and always show independence on the alkyl chain length. Our results for cross-sectional areas show a constant value around 40–50 Å² for alkyl chains of 7–11 methylene units, but an important increase can be observed for longer alkyl chains, being the lauryl derivative (11 units) the breakpoint of this behavior. These results are shown in Fig. 4. Summarizing, the effect of the hydrophobic chain on the micelle properties, is only observed when the alkyl chain overpasses certain length (in this case the corresponding to a lauryl chain). According to the GP values reported in Table 2, this increase of the cross-sectional area seems not to be accompanied by a higher water penetration into the micelle, being the sucrose head groups an important barrier.

Table 3 also includes the values of surface tension at concentrations higher than cmc, γ°lim, which is a measure of the surfactant ability of each compound. The γ°lim values determined for the studied compounds are somehow higher than the reported ones [12].

The critical packing parameter, Cpp, defined only from interactions between amphiphilic molecules within the aggregate and geometrical considerations [32], is a useful criterion to predict the aggregate geometry [12,33].

\[
\text{Cpp} = \frac{v}{(a_0 l_c)}
\]

where \(v\) corresponds to the hydrocarbon chain volume, assuming an incompressible fluid; \(a_0\) is the optimal headgroup area and \(l_c\) is the maximum effective length that the hydrophobic chains can assume, which corresponds to a semiempirical parameter known as the critical chain length. According to Tanford [34], for saturated hydrocarbon chain with \(n\) carbon atoms these parameters can be

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>(I) ((10^{-10} \text{ mol cm}^{-2}))</th>
<th>(A) ((\text{Å}^2 \text{ molecule}^{-1}))</th>
<th>Cpp</th>
<th>(\gamma°\text{lim}) ((\text{mN m}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOS</td>
<td>4.08</td>
<td>40.71</td>
<td>0.50</td>
<td>–</td>
</tr>
<tr>
<td>MCS</td>
<td>3.31</td>
<td>50.20</td>
<td>0.42</td>
<td>–</td>
</tr>
<tr>
<td>MLS</td>
<td>3.64</td>
<td>45.60</td>
<td>0.46</td>
<td>43.14</td>
</tr>
<tr>
<td>MMS</td>
<td>2.51</td>
<td>66.20</td>
<td>0.32</td>
<td>48.68</td>
</tr>
<tr>
<td>MPS</td>
<td>1.57</td>
<td>105.80</td>
<td>0.20</td>
<td>47.06</td>
</tr>
</tbody>
</table>

Fig. 3. Cmc determination of MMS employing I/III, GP and surface tension measurements.

Fig. 4. Dependence of cross-sectional area with alkyl chain length.
calculated using the following equation:

$$
\begin{align*}
L_c & \leq L_{\text{max}} \approx (0.154 + 0.1265n) \text{nm} \\
v & \approx (27.4 + 26.9n) \times 10^{-11} \text{nm}^3
\end{align*}
$$

where \( n \) is the number of carbon atoms in the sucrose ester, any hydrophobic chain should only consider the methylene units. The values calculated for the critical packing parameter of the different sucrose esters are included in Table 3. For short alkyl chain sucrose esters (MOS, MCS, and MOS), the determined Cpp values indicate that the most favored structure corresponds to cylindrical micelles [35]. The value of Cpp for MMS is in the limit between cylindrical and spherical micelles and MPS should form neatly spherical aggregates (Cpp < 0.33) [35]. Previous studies performed on series of sucrose esters did not report this behavior, showing Cpp values almost constant and independent of the alkyl chain length. Our data indicate that alkyl chains can move (inside the micelle) with less restriction in spherical micelles (when sucrose esters have more than 12 methylene units), than in cylindrical ones. In addition, it is observed that the random movements (less fluid environment of the probe) correlate with head group cross-sectional areas in these type micro-aggregates.

### 3.4. Micelle aggregation number determination

The method based on the fluorescence quenching to determine micelle sizes (number of molecules per micelle) relies on the following assumptions: the micelles must be monodisperse; probe and quencher must be hydrophobic and have to be located inside the micelles, with residence times much longer than the unquenched fluorescence life-time of the probe; the observed fluorescence is emitted from probes located in micelles only when there is no quencher, i.e. static quenching occurs; and the random association of probe and quencher with micelles is described by Poisson distribution.

The aggregation numbers determined for micelles of sucrose esters are given in Table 4, and can be fairly compared (are smaller) with the values reported by Kawaguchi et al. [15] The quenching of Pyrene emission by DBA for all studied sucrose ester micelles occurs in the manner predicted by Eqs. (2) and (3) as can be seen in Fig. 5. The aggregation numbers obtained from steady-state measurements, \( N_s \), (not reported) despite being incorrect, allow us to explain the behaviour of each sucrose ester as a function of surfactant concentration. For the octyl sucrose ester (MOS, shortest hydrophobic alkyl chain studied) the aggregation number shows a positive dependence with concentration or square root of concentration, micelles increase in size systematically, taking values of 50 near to the cmc and reaching a value of 250 monomers at 40 mM. This behavior has been proposed to be a consequence of the transition from micelles to a lamellar phase and has been observed and reported for several Tritons [36]. Furthermore, Cpp values obtained for octyl ester predict micelles of cylindrical shape, so concentration dependence of aggregation number should be expected [35]. The capryly derivative, MCS, in contrast with the response observed for other non-ionic surfactants [37,38], shows an initial reduction of the aggregation number reaching a plateau, \( N_s = 79 \), for concentrations higher than 12.5 mM. The remaining sucrose esters, with longer alkyl chains, show \( N_s \) values independent on its concentration. The aggregation numbers determined from TRFQ data, \( N_D \), for longer alkyl chains were found to be concentration independent in the range studied. The values determined for \( N \) are smaller than the previously determined using X-ray scattering [15]. Despite the data available for the self-diffusion coefficient of micelles correspond not only to pure regioisomers [19], also a different behavior between short and long chain substituted sucrose esters is reported.

The existence of an important effect of the sucrose head and its solvation core on the micelle properties is postulated, compatible with the work of Molinier et al. [20], related with the regiochemistry of the ester and the formation of intramolecular hydrogen bonds. According to our results, the properties of the formed micelles are ruled by sucrose moiety for esters with shorter alkyl chains, being only balanced for relatively long alkyl chains. Even more, longer alkyl chains directly affect the cross-sectional area of sucrose in the surface of microaggregate.

Summarizing, the physical chemical properties of the micellar aggregates of a whole series of pure 6-o-sucrose esters were determined and reported. Cmc values of all studied compounds are comparable with previously reported ones showing the expected linearity against number of methylene units. Aggregation numbers, most of them not previously available, show different dependence on concentration depending on the size of substituents. Cross-sectional areas were calculated from surface activity measurements, allowing us to predict shapes by using the critical packing parameter. These parameters show a breakpoint when they are analyzed as function of alkyl chain length. Additionally, properties as micropolarity and microfluidity of micellar aggregates are reported. So, new and systematic information of the micelles formed by pure surfactants with sucrose heads substituted at position 6-0, was collected.

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