Self-Assembly and Emulsification Properties of Hydrophobically Modified Inulin

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Self assembly and emulsification properties of hydrophobically-modified inulin

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Key words:
inulin, succinylation, critical aggregation concentration, dye solubilisation, surface tension, dynamic light scattering, oil-in-water emulsions
Abstract

A series of alkenylated inulin samples were synthesized in aqueous solution using alkenyl succinic anhydrides with varying alkenyl chain lengths (C8-C18). The inulin derivatives (ASA-inulins) were characterized using NMR and FTIR and their degree of substitution determined. The solution properties of the ASA-inulins were investigated using dye solubilisation, surface tension and dynamic light scattering and all three techniques confirmed that the molecules aggregated in solution above a critical concentration (critical aggregation concentration, CAC). The value of the CAC was found to be reasonably consistent between the different techniques and was shown to decrease with increasing alkenyl chain length from 0.08% for the octenyl succinylated sample to 0.005% for the octadecenyl succinylated sample. The hydrodynamic diameter of the ASA-inulins above the CAC was determined from dynamic light scattering studies and was shown to increase with alkenyl chain length from 4 nm for the octenyl derivative to 55 nm for the hexadecenyl derivative. All of the ASA-inulins were shown to be able to produce oil-in-water emulsions with a droplet size similar to emulsions prepared using Tween 20 on storing for 21 days. The fact that the derivatives are able to form micellar-like aggregates and stabilize emulsions makes them suitable candidates for the encapsulation and delivery of water insoluble active compounds with potential application in food, cosmetic, personal care and pharmaceutical formulations.
Introduction

Inulin is a storage polysaccharide and can be found in many plants including chicory, leek, onion, garlic, wheat, artichoke and banana but the main commercial source is chicory\textsuperscript{1}. It consists of linear chains of $\beta (2 \rightarrow 1)$ linked fructose units which are terminated with a glucose residue. The fructose chains from chicory have a degree of polymerisation, DP, typically of 2 - 60. Much effort has recently been devoted to the chemical modification of inulin in order to develop industrial products with specific characteristics. Several amphiphilic polymers obtained from inulin have been prepared by esterification\textsuperscript{2}, etherification\textsuperscript{3}, and carbamoylation\textsuperscript{4} using fatty acid methyl esters (FAME), alkyl epoxides, and alkyl isocyanates, respectively. van Kempen et al.\textsuperscript{5} have also recently reported the modification of oligofructose dissolved in dimethylsulphoxide with fatty acids of varying chain length using lipase as a catalyst. It is evident from the literature that most of the reactions have been undertaken in organic solvents which can dissolve both the inulin and the hydrophobic reactant. Recently, Morros et al.\textsuperscript{6} and Kokubun et al.\textsuperscript{7, 8} reported the modification of inulin in water using alkenyl succinic anhydrides. Kokubun et al.\textsuperscript{7, 8} prepared samples using octenyl and dodecenyl succinic anhydrides at varying degrees of substitution. Surface tension and dye solubilisation measurements indicated that the samples aggregated above a critical concentration referred to as the critical aggregation concentration, CAC, which was found to decrease with increasing alkenyl chain length and degree of substitution. It was also shown that these molecules are effective emulsifiers and are able to stabilize oil-in-water emulsions\textsuperscript{8}. The aim of the present work was to extend the study of Kokubun et al.\textsuperscript{8} and prepare a series of
alkenyl succinylated inulin derivatives with varying alkenyl chain length (C8 to C18) and to investigate their solution and interfacial behavior.

Materials and methods

Materials

Inulin coded INUTEC® H25P was supplied by Beneo Biobased Chemicals. This material has previously been characterised using MALDI-TOF and it was found that it contained molecules with a DP between 2 and 8 consistent with the data supplied by the suppliers. The inulin was dried at 70 °C for 24 hours before use. Octenyl succinic anhydride (OSA) and dodecenyl succinic anhydride (DDSA), were obtained from Aldrich Chemical Co.. Decenyl succinic anhydride (DSA), tetradecenyl succinic anhydride (TDSA), hexadecenyl succinic anhydride (HDSA), and octadecenyl succinic anhydride (ODSA) were obtained from Tokyo Chemical Industry UK Ltd, Oxford and were used as received. Deuterated dimethyl sulphoxide (DMSO-\(d_6\); 99.9 atom % D) and potassium bromide were obtained from Sigma-Aldrich Chemie GmbH. Sudan IV, a water insoluble diazo dye, was obtained from Eastman Kodak Company.

Medium-chain triglyceride (MCT) was obtained from Nisshin Oillio Group, Ltd, Tokyo, Japan. It had been prepared from triglycerides and was a mixture of C8 and C10 fatty acids at the mixing ratio of 75:25. The density of oil was 0.95g/mL. Tween 20, 97% (Aldrich, Gillingham, UK).
Methods

Synthesis

Inulin, 25g was dissolved in deionised water, 75g, at 25°C and a predetermined amount of ASA dissolved in ethanol and added to the vessel with stirring through a dropping funnel. As the solubility of higher ASAs was poor even in ethanol, it was required to increase the amount of ethanol and warm the solutions to prevent solidification in the dropping funnel. Throughout the reaction the pH was maintained at pH 8.3-8.5 through addition of aqueous sodium hydroxide, (1, 3 or 10% w/w). The reaction was continued until no further pH change was observed, typically this was about 6 hours. The reaction mixture was then brought to pH 6.0 by addition of aqueous hydrochloric acid, 5%. The solid product was recovered by freeze drying and washed by Soxhlet extraction with cyclohexane for 6 hours, prior to drying in a vacuum oven.

Characterisation

NMR spectroscopy

$^1$H NMR spectra of the ASA-inulins were measured using a 500 MHz NMR Spectrometer at 25 °C. 5 mg of sample were dissolved in 0.7g of DMSO- $d_6$ then added into a 5mm thin wall NMR tube and dissolved at 25°C. The spectra were
recorded using the Pulse Program ZG30 with a 30 degree pulse and a delay of 1s
together with Mnova 7.0 software.

Fourier-transform infrared spectroscopy (FTIR)

The ASA-inulin samples were dried in an oven at 70°C overnight. 1mg of sample was
milled with 100mg of dried KBr using an agate mortar and pestle for several minutes
to obtain a fine powder. A thin pellet was produced using a 15 ton manual press and a
P/N 03000 13mm pellet die (maximum load 10.0 tons) from Specac Limited. The
FTIR spectra were recorded in the range 4000-400 cm⁻¹ using a Perkin-Elmer FTIR
spectrometer RX 1 taking 16 scans at a resolution of 4 cm⁻¹. Spectral analysis and
display were performed using the interactive Perkin-Elmer Read-IR3 version 3.0
software.

Critical aggregation concentration (CAC)

Dye solubilisation

The CAC was determined using the dye solubilisation technique as described
previously. Stock solutions of 1% ASA-inulins were prepared and diluted to give
solutions of various concentrations. 10mg of the dye was added to 10ml of inulin
solution and the samples were left agitating at 40°C overnight. The solution was then
filtered to remove insoluble dye particles using a Milllex-GP 0.22 μm filter (Millipore
Ireland Ltd) into disposable UV grade 10 mm path length cuvettes (CXA-110-0053
from Fisher Scientific Ltd). The absorbance of the solution was determined at a
wavelength of 510nm using a Lambda 25 UV/VIS Spectrometer (Perkin Elmer). The CAC was obtained from the point at which the absorbance first increased.

Dynamic light scattering

Dynamic light scattering measurements were performed using the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) equipped with a 5 mW He-Ne laser (λ₀ 632.8nm) and a digital correlator at an angle of 175° to the incident beam. The temperature was controlled to 25°C +/- 1°C. Samples were prepared by serial dilution with filtered distilled water from a stock of 2.0g/dL to five sample concentrations in the range of 0.004-1 g/dL. Samples were filtered using a Milllex-GP 0.22 μm filter (Millipore Ireland Ltd). Solutions were placed in disposable plastic cells with a cross sectional area of 1cm². 15 runs were performed for each sample and the collection time was 180 seconds. Zetasizer Software 6.20 © 2002-2010 Malvern Instruments Ltd was used for data analysis. The CAC was determined from the change in the slope of the plot of the intensity of scattered light as a function of concentration. The hydrodynamic diameter of the aggregates formed above the CAC was obtained from the Stokes-Einstein relationship using the instrument software.

Surface tension

The surface tension of the ASA-inulin solutions at varying concentration was determined using a Tensiometer K8 with a 4 cm circumference Du Nouy ring (RI 01 from Krüss GmbH). All measurements were repeated three times and the average taken. The temperature was kept constant at 25°C +/- 1°C during all the measurements. The
CAC was determined from the change in slope of the plot of equilibrium surface tension as a function of ASA-inulin concentration.

**Emulsification properties**

15w/w% oil-in-water emulsions were prepared by mixing 1.5g oil and 8g of a 2.5% aqueous solution of the alkenyl succinylated inulin and 0.5g water to make 2.35%. ASA-inulin emulsion samples contained in a 20mL glass tube using an IKA T25 Digital ultra-Turrax homogenizer at 24000 rpm for 3 minutes. Emulsions were also prepared using Tween 20 for comparison. The droplet size of the emulsions was measured by laser diffraction immediately after preparation and after storing at room temperature for a period of up to 21 days using the Mastersizer-2000 (Malvern Instruments, UK). Two or three drops of the sample were introduced into the dispersion unit containing distilled water. The dispersion unit pump speed was 2000 rpm and the obscuration was between 10% and 30%. The refractive index of the dispersing medium and the dispersed particles were 1.33 and 1.45 respectively. Measurements were carried out in duplicate and the average value reported.

**Results and discussion**

**Characterisation**

Figures 1a and 1b show the $^1$H NMR spectra of the native and OSA-inulin samples respectively. The prominent peaks at 2.5 ppm and 3.35 ppm are from the solvent i.e.
DMSO and HDO respectively and the peaks from 3.4 ppm to 5.3 ppm are from the
inulin itself (Figure 1a).\textsuperscript{7} \textsuperscript{1}H NMR signals at 0.85 ppm, 1.26 ppm and 1.94 ppm\textsuperscript{11, 12}
correspond to the methyl and methylene groups of the octenylsuccinic anhydride
(Figure 1b). The amount of alkenyl chains incorporated into the modified samples was
calculated from the ratio of the area of the peak at 0.85 ppm to the area of the peaks
from 3.4 – 5.3 ppm and the results are shown in Table 1.

FTIR spectra of the native and OSA-inulin samples are presented in Figure 2. The
peaks for the native inulin at 3398 cm\(^{-1}\), 2930 cm\(^{-1}\) and 1028 cm\(^{-1}\) indicate O-H
stretching, CH\(_2\) stretching and C-O-C bending, respectively\textsuperscript{7, 13}. The spectrum of
OSA-inulin shows two new peaks at 1576 cm\(^{-1}\) and 1734 cm\(^{-1}\) due to the formation of
the ester linkage. Similar findings were found for the other modified samples (data not
shown). The peaks are assigned to asymmetric COO- stretching and ester carbonyl
stretching respectively\textsuperscript{13}. In studies on starch modification it has previously been
reported that the CH\(_2\) stretching band at 2930 cm\(^{-1}\) increased after modification
because of the contribution from the carbon chain associated with the alkenyl succinic
group\textsuperscript{14}. However the CH\(_2\) stretching band at 2930 cm\(^{-1}\) for our modified inulins was
not comparably enhanced in agreement with the findings of Kokubun et al.\textsuperscript{7}.

Critical aggregation concentration (CAC)

The absorbance values obtained for the ASA-inulins in the presence of Sudan IV are
given in Figures 3a and 3b. It is noted that the values increase significantly above a
critical concentration which is attributed to the formation of micellar – like aggregates
and the dissolution of the dye in their hydrophobic core\textsuperscript{7}. The CAC values for all the
ASA-inulins are summarised in Table 2. It is seen generally that the CAC decreases as the alkenyl chain length increases as is expected and also as noted by Kokubun et al.\textsuperscript{7}\footnotemark[7] and van Kempen et al.\textsuperscript{5} for hydrophobically modified inulins. The actual CAC values for the OSA-inulins are lower than those reported by Kokubun et al.\textsuperscript{7} and this is attributed mainly to the fact that the present samples have a significantly higher degree of alkenylation. The values, however, are higher than those reported by van Kempen et al.\textsuperscript{5}. This is probably due to the fact that, in our study, modification was carried out using alkenyl succinic anhydride, which leads to the incorporation of a free carboxylate group present in the surfactant head group giving an anionic charge. This was not the case for the samples produced by van Kempen et al. which were non-ionic\textsuperscript{5}. It is well known that ionic surfactants have a higher critical micelle concentration (CMC) compared to non-ionic surfactants due to the fact that, for ionic surfactants, electrostatic repulsions between the surfactant head groups in the micelle oppose the micellisation process.

The scattering intensity of solutions of the ASA-inulin samples are plotted as a function of concentration in Figure 4a and 4b. The sharp increase in the scattering intensity above a certain concentration is attributed to the CAC and the formation of aggregates. The CAC values are given in Table 2 and are of the same order of magnitude as the values obtained by dye solubilisation.

The z-average hydrodynamic diameters of the ASA-inulin samples are given as a function of concentration in Figure 5a and are plotted as a function of alkenyl chain length in Figure 5b. It is noted that in general the size of the aggregates increases with the alkenyl chain length as might be expected. The value for ODSA-inulin is a little
lower than might be expected from the trend shown and this is probably due to the fact
that it has a significantly lower degree of modification than the other samples. Kokubun
et al.\textsuperscript{7} reported higher values of 13nm and 30nm compared to values of 4nm and 12nm
in this study for OSA- and DDSA- modified inulin samples respectively. The main
reason for the difference is attributed to the fact that the DP of the inulin used in the
present study was 2-8 while in the study of Kokubun et al.\textsuperscript{7} the inulin had a DP of $>10$.
van Kempen et al.\textsuperscript{5} also used inulin with a DP of 2-8 and reported values of between
4.3nm to 13.4nm for C\textsubscript{8} – C\textsubscript{18} modified inulins consistent to the values reported here
using inulin of a similar DP.

The surface tensions of the ASA-inulins at the air-water interface are shown as a
function of concentration in Figures 6a and 6b. The CAC value for each of the
samples was obtained from the inflexion in the curve and the results are reported in
Table 2. The values are in reasonable agreement with the values obtained using the
dye solubilisation and dynamic light scattering methods. The surface tension was
found to be between 31-34 mNm at the CAC for all of the samples apart from
ODSA-inulin which had a much higher value (43mNm). This discrepancy may be due
to its lower degree of modification. Kokubun et al.\textsuperscript{7} reported values of $\sim$35-40mNm
for OSA-inulin and DDSA-inulin and van Kempen et al.\textsuperscript{5} reported values of
$\sim$34-40mNm for inulin samples modified with C\textsubscript{8} – C\textsubscript{16} alkyl chains.

The CAC values for the ASA- inulins obtained using the different techniques are
plotted as a function of the alkyl chain length in Figure 7 which clearly shows the
decrease in CAC with increasing alkyl chain length. The free energy decrease for the
transfer of a –CH$_2$– unit from the bulk phase to the micelle (Δ$G_{mic}$) can be calculated by

equation (1) derived by Rosen$^{15,16}$.

\[
\log\text{CMC} = \left(\frac{\Delta G_{mic}}{2.303RT}\right)m + K_{mic} \quad (1)
\]

Where m is the number of carbons in the alkyl chain and $K_{mic}$ is a constant for the same hydrophilic group. The calculated Δ$G_{mic}$ per CH$_2$ is -0.79 kJ/mol at 25°C. Zhang and Marchant$^{16}$ reported a Δ$G_{mic}$ value for the N-alkylmaltoamide series of -1.86kJ/mol at 25°C. van Kempen et al.$^5$ using the same procedure obtained a value of -3.1 kJ/mol.

The surface excess, $\Gamma$, is related to surface tension by the Gibbs equation, which for a 1:1 ionic surfactant is given by equation (2) ($^5$).

\[
\Gamma = -\left(\frac{1}{2RT}\right)\left(\frac{dy}{d\ln C}\right) \quad (2)
\]

Surface excess values were determined from the slope of the lines for the plot of $\gamma$–lnC just below the CAC$^{5,17}$ and the results are shown in Table 3. The values obtained for the surface excess were used to calculate the surface area occupied by each molecule, A, using equation (3)

\[
A = \frac{1}{\Gamma N_A} \quad (3)
\]

where $N_A$ is Avogadro’s number.
The area occupied per molecule was found to vary between 0.66 and 1.06 nm$^2$ for the inulins modified using C8-C16 alkenyl chains and are of a similar order to values reported by van Kempen et al.$^5$ of 0.46 to 0.91 nm$^2$ and Stevens et al.$^4$ who reported an area per molecule of 0.9 nm$^2$ for a hydrophobically modified inulin sample. The high value obtained for the ODSA inulin (1.78 nm$^2$) may be a consequence of its low solubility which is likely to result in molecular aggregation. The values for A are larger than those reported for simple sugar-based surfactants with one or two sugar residues. For example, Soutani et al.$^{18}$ reported values of 0.05 – 0.2 nm$^2$ for hydrophobically modified fructose and sucrose surfactants while Garofalakis et al.$^{19}$ reported values of 0.29 – 0.68 nm$^2$ for surfactants based on xylose, galactose, sucrose and lactose. For the modified inulins the area occupied will ultimately depend on the number and position of the hydrophobic groups attached to the backbone. If there are several hydrophobic groups randomly distributed along the backbone, the carbohydrate moieties would be expected to lie flat at the interface and occupy a much larger surface area than inulin molecules with the hydrophobic groups attached at one end.

**Emulsion stability**

The droplet size distributions for the emulsions prepared using the various modified inulin samples and Tween 20 were determined shortly after preparation and after 21 days and are presented in Figures 8a and 8b respectively. The corresponding $d_{3,2}$ and $d_{4,3}$ values are given in Figures 9a and 9b. It was found that the modified inulin samples with alkylene chain lengths C10-C18 produced emulsions with slightly smaller droplet sizes than the C8 inulin and the Tween 20. There was little change
observed in the droplet size on storing for 21 days. This is consistent with the study
by Kokubun et al.\textsuperscript{8} which showed that DDSA-inulin yielded emulsions with a droplet
size smaller than OSA-inulins (both with DS 12 mole%).

The work described in this paper has demonstrated that inulin can be successfully
modified in aqueous solution by alkylene succinic anhydrides with alkenyl chain
lengths varying from C8-C18 and the derivatives have been shown to form
micellar-like aggregates in solution. The CAC values have been found to decrease
with increasing alkenyl chain length while the hydrodynamic size of the aggregates
has been shown to increase with increasing alkenyl chain length. It has also been
shown that the inulins are able to produce stable oil-in-water emulsions with a droplet
size similar to emulsions formed with Tween 20. The dye solubilisation experiments
have demonstrated that the ASA-inulins are able to form micellar-like aggregates and
dissolve hydrophobic compounds within their hydrophobic core. This coupled to the
fact that they can stabilize oil-in-water emulsions makes them suitable candidates for
the encapsulation and delivery of water insoluble active compounds with potential
application in food, cosmetic, personal care and pharmaceutical formulations.

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Figure 1

(a)

(b)
Figure 3a

![Graph](image1)

Figure 3b.

![Graph](image2)
Figure 4a.

Figure 4b.
Figure 6a.

Figure 6b.
Figure 7.
Figure 8a.

Figure 8b.
Table 1. Degree of substitution of the modified inulins.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% substitution / moles</th>
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<tr>
<td>ODSA</td>
<td>12.9</td>
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Table 2. Critical Aggregation Concentrations of the modified inulin samples using various techniques.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CAC (%)</th>
<th>Dynamic Light Scattering (Intensity)</th>
<th>Dye Solubilisation</th>
<th>Surface Tension</th>
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<td>Surfactant</td>
<td>Maximum Surface excess $(\Gamma) \times 10^{-6}$ (mol/m²)</td>
<td>Molecular area (nm²)</td>
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<td>--------------------------------------------------------</td>
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Inulin → Biosurfactant