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Self-Assembly and Emulsification Properties of Hydrophobically Modified Inulin

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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.

47 **Introduction**

48

49 Inulin is a storage polysaccharide and can be found in many plants including chicory,
50 leek, onion, garlic, wheat, artichoke and banana but the main commercial source is
51 chicory[1]. It consists of linear chains of $\beta\,(2 \rightarrow 1)$ linked fructose units which are
52 terminated with a glucose residue. The fructose chains from chicory have a degree of
53 polymerisation, DP, typically of 2 - 60. Much effort has recently been devoted to the
54 chemical modification of inulin in order to develop industrial products with specific
55 characteristics. Several amphiphilic polymers obtained from inulin have been
56 prepared by esterification[2], etherification[3], and carbamoylation[4] using fatty acid
57 methyl esters (FAME), alkyl epoxides, and alkyl isocyanates, respectively. van
58 Kempen et al.[5] have also recently reported the modification of oligofructose dissolved
59 in dimethylsulphoxide with fatty acids of varying chain length using lipase as a
60 catalyst. It is evident from the literature that most of the reactions have been
61 undertaken in organic solvents which can dissolve both the inulin and the
62 hydrophobic reactant. Recently, Morros et al.[6] and Kokubun et al.[7, 8] reported the
63 modification of inulin in water using alkenyl succinic anhydrides. Kokubun et al.[7, 8]
64 prepared samples using octenyl and dodecenyl succinic anhydrides at varying degrees
65 of substitution. Surface tension and dye solubilisation measurements indicated that the
66 samples aggregated above a critical concentration referred to as the critical
67 aggregation concentration, CAC, which was found to decrease with increasing alkenyl
68 chain length and degree of substitution. It was also shown that these molecules are
69 effective emulsifiers and are able to stabilize oil-in-water emulsions[8]. The aim of the
70 present work was to extend the study of Kokubun et al.[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[9]. 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).

94

95 *Methods*

96

97 *Synthesis*

98

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

110

111

112 **Characterisation**

113

114 *NMR spectroscopy*

115

116 H^1 NMR spectra of the ASA-inulins were measured using a 500 MHz NMR
117 Spectrometer at 25 °C. 5 mg of sample were dissolved in 0.7g of DMSO- d_6 then
118 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[7]. 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 Millex-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

144 wavelength of 510nm using a Lambda 25 UV/VIS Spectrometer (Perkin Elmer). The
145 CAC was obtained from the point at which the absorbance first increased.
146
147 *Dynamic light scattering*
148
149 Dynamic light scattering measurements were performed using the Zetasizer Nano ZS
150 (Malvern Instruments Ltd, Malvern, UK) equipped with a 5 m_W He-Ne laser (λ_0
151 632.8nm) and a digital correlator at an angle of 175° to the incident beam. The
152 temperature was controlled to 25°C +/- 1°C. Samples were prepared by serial dilution
153 with filtered distilled water from a stock of 2.0g/dL to five sample concentrations in the
154 range of 0.004-1 g/dL. Samples were filtered using a Millex-GP 0.22 μm filter
155 (Millipore Ireland Ltd). Solutions were placed in disposable plastic cells with a cross
156 sectional area of $1cm^2$. 15 runs were performed for each sample and the collection time
157 was 180 seconds. Zetasizer Software 6.20 © 2002-2010 Malvern Instruments Ltd was
158 used for data analysis. The CAC was determined from the change in the slope of the
159 plot of the intensity of scattered light as a function of concentration. The hydrodynamic
160 diameter of the aggregates formed above the CAC was obtained from the
161 Stokes-Einstein relationship using the instrument software.
162
163 *Surface tension*
164
165 The surface tension of the ASA-inulin solutions at varying concentration was
166 determined using a Tensiometer K8 with a 4 cm circumference Du Nouy ring (RI 01
167 from Krüss GmbH). All measurements were repeated three times and the average taken.
168 The temperature was kept constant at 25°C +/- 1°C during all the measurements. The

169 CAC was determined from the change in slope of the plot of equilibrium surface
170 tension as a function of ASA-inulin concentration.
171
172 *Emulsification properties*
173 15w/w% oil-in-water emulsions were prepared by mixing 1.5g oil and 8g of a 2.5%
174 aqueous solution of the alkenyl succinylated inulin and 0.5g water to make 2.35%.
175 ASA-inulin emulsion samples contained in a 20mL glass tube using an IKA T25 Digital
176 ultra-Turrax homogenizer at 24000 rpm for 3 minutes. Emulsions were also prepared
177 using Tween 20 for comparison. The droplet size of the emulsions was measured by
178 laser diffraction immediately after preparation and after storing at room temperature for
179 a period of up to 21 days using the Mastersizer-2000 (Malvern Instruments, UK). Two
180 or three drops of the sample were introduced into the dispersion unit containing
181 distilled water. The dispersion unit pump speed was 2000 rpm and the obscuration was
182 between 10% and 30%. The refractive index of the dispersing medium and the
183 dispersed particles were 1.33 and 1.45 respectively. Measurements were carried out in
184 duplicate and the average value reported.
185
186
187 **Results and discussion**
188
189 *Characterisation*
190
191 Figures 1a and 1b show the ^{1}H NMR spectra of the native and OSA-inulin samples
192 respectively. The prominent peaks at 2.5 ppm and 3.35 ppm are from the solvent i.e.

193 DMSO and HDO respectively and the peaks from 3.4 ppm to 5.3 ppm are from the
194 inulin itself (Figure 1a)[7]. ^{1}H NMR signals at 0.85 ppm, 1.26 ppm and 1.94 ppm[11, 12]
195 correspond to the methyl and methylene groups of the octenylsuccinic anhydride
196 (Figure 1b). The amount of alkenyl chains incorporated into the modified samples was
197 calculated from the ratio of the area of the peak at 0.85 ppm to the area of the peaks
198 from 3.4 – 5.3 ppm and the results are shown in Table 1.
199
200 FTIR spectra of the native and OSA-inulin samples are presented in Figure 2. The
201 peaks for the native inulin at 3398cm^{-1}, 2930 cm^{-1} and 1028cm^{-1} indicate O-H
202 stretching, CH_2 stretching and C-O-C bending, respectively[7, 13]. The spectrum of
203 OSA-inulin shows two new peaks at 1576 cm^{-1} and 1734 cm^{-1} due to the formation of
204 the ester linkage. Similar findings were found for the other modified samples (data not
205 shown). The peaks are assigned to asymmetric COO- stretching and ester carbonyl
206 stretching respectively[13]. In studies on starch modification it has previously been
207 reported that the CH_2 stretching band at 2930cm^{-1} increased after modification
208 because of the contribution from the carbon chain associated with the alkenyl succinic
209 group[14]. However the CH_2 stretching band at 2930cm^{-1} for our modified inulins was
210 not comparably enhanced in agreement with the findings of Kokubun et al.[7].
211
212 *Critical aggregation concentration (CAC)*
213
214 The absorbance values obtained for the ASA-inulins in the presence of Sudan IV are
215 given in Figures 3a and 3b. It is noted that the values increase significantly above a
216 critical concentration which is attributed to the formation of micellar – like aggregates
217 and the dissolution of the dye in their hydrophobic core[7]. The CAC values for all the

218 ASA-inulins are summarised in Table 2. It is seen generally that the CAC decreases as
219 the alkenyl chain length increases as is expected and also as noted by Kokubun et al.[7]
220 and van Kempen et al.[5] for hydrophobically modified inulins. The actual CAC values
221 for the OSA- inulins are lower than those reported by Kokubun et al.[7] and this is
222 attributed mainly to the fact that the present samples have a significantly higher degree
223 of alkenylation. The values, however, are higher than those reported by van Kempen et
224 al.[5]. This is probably due to the fact that, in our study, modification was carried out
225 using alkenyl succinic anhydride, which leads to the incorporation of a free carboxylate
226 group present in the surfactant head group giving an anionic charge. This was not the
227 case for the samples produced by van Kempen et al. which were non-ionic[5]. It is well
228 known that ionic surfactants have a higher critical micelle concentration (CMC)
229 compared to non-ionic surfactants due to the fact that, for ionic surfactants, electrostatic
230 repulsions between the surfactant head groups in the micelle oppose the micellisation
231 process.
232
233 The scattering intensity of solutions of the ASA-inulin samples are plotted as a function
234 of concentration in Figure 4a and 4b. The sharp increase in the scattering intensity
235 above a certain concentration is attributed to the CAC and the formation of aggregates.
236 The CAC values are given in Table 2 and are of the same order of magnitude as the
237 values obtained by dye solubilisation.
238
239 The z-average hydrodynamic diameters of the ASA-inulin samples are given as a
240 function of concentration in Figure 5a and are plotted as a function of alkenyl chain
241 length in Figure 5b. It is noted that in general the size of the aggregates increases with
242 the alkenyl chain length as might be expected. The value for ODSA-inulin is a little

243 lower than might be expected from the trend shown and this is probably due to the fact
244 that it has a significantly lower degree of modification than the other samples. Kokubun
245 et al.[7] reported higher values of 13nm and 30nm compared to values of 4nm and 12nm
246 in this study for OSA- and DDSA- modified inulin samples respectively. The main
247 reason for the difference is attributed to the fact that the DP of the inulin used in the
248 present study was 2-8 while in the study of Kokubun et al.[7] the inulin had a DP of >10.
249 van Kempen et al.[5] also used inulin with a DP of 2-8 and reported values of between
250 4.3nm to 13.4nm for C8 – C18 modified inulins consistent to the values reported here
251 using inulin of a similar DP.
252
253 The surface tensions of the ASA-inulins at the air-water interface are shown as a
254 function of concentration in Figures 6a and 6b. The CAC value for each of the
255 samples was obtained from the inflexion in the curve and the results are reported in
256 Table 2. The values are in reasonable agreement with the values obtained using the
257 dye solubilisation and dynamic light scattering methods. The surface tension was
258 found to be between 31-34 mNm at the CAC for all of the samples apart from
259 ODSA-inulin which had a much higher value (43mNm). This discrepancy may be due
260 to its lower degree of modification. Kokubun et al.[7] reported values of~35-40mNm
261 for OSA-inulin and DDSA-inulin and van Kempen et al.[5] reported values of
262 ~34-40mNm for inulin samples modified with C8 – C16 alkyl chains.
263
264 The CAC values for the ASA- inulins obtained using the different techniques are
265 plotted as a function of the alkyl chain length in Figure 7 which clearly shows the
266 decrease in CAC with increasing alkyl chain length. The free energy decrease for the

267 transfer of a $-CH_2-$ unit from the bulk phase to the micelle (ΔG_{mic}) can be calculated by
268 equation (1) derived by Rosen[15, 16].
269
270 $$\log CMC = (\Delta G_{mic}/2.303RT)m + K_{mic} \quad (1)$$
271
272 Where m is the number of carbons in the alkyl chain and K_{mic} is a constant for the
273 same hydrophilic group. The calculated ΔG_{mic} per CH_2 is -0.79 kJ/mol at 25°C.
274 Zhang and Marchant[16] reported a ΔG_{mic} value for the *N*-alkylmaltoamide series of
275 -1.86kJ/mol at 25°C. van Kempen et al.[5] using the same procedure obtained a value of
276 -3.1 kJ/mol.
277
278 The surface excess, Γ , is related to surface tension by the Gibbs equation, which for a
279 1:1 ionic surfactant is given by equation (2) (5).
280
281 $$\Gamma = -\left(\frac{1}{2RT}\right)\left(\frac{d\gamma}{d\ln C}\right) \quad (2)$$
282
283 Surface excess values were determined from the slope of the lines for the plot of γ–lnC
284 just below the CAC[5, 17] and the results are shown in Table 3. The values obtained for the
285 surface excess were used to calculate the surface area occupied by each molecule, A,
286 using equation (3)
287
288
289 $$A = \frac{1}{\Gamma N_A} \quad (3)$$
290
291 where N_A is Avogadro's number.

292

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

308

309 *Emulsion stability*

310

311 The droplet size distributions for the emulsions prepared using the various modified
312 inulin samples and Tween 20 were determined shortly after preparation and after 21
313 days and are presented in Figures 8a and 8b respectively. The corresponding $d_{3,2}$ and
314 $d_{4,3}$ values are given in Figures 9a and 9b. It was found that the modified inulin
315 samples with alkylene chain lengths C10-C18 produced emulsions with slightly
316 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.[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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406 OSA-inulin.

407

408 **Figure 2.** FT-IR spectra of unmodified inulin (Inutec H25P) and OSA-inulin.

409

410 **Figure 3a.** Absorbance at 510nm of OSA-, DSA-, and DDSA- inulin samples at

411 varying concentration in the presence of Sudan IV dye.

412

413 **Figure 3b.** Absorbance at 510nm of TDSA-, HDSA-, and ODSA- inulin samples at

414 varying concentration in the presence of Sudan IV dye.

415

416 **Figure 4a.** Scattering intensity as a function of concentration for OSA, DSA and

417 DDSA modified inulins.

418

419 **Figure 4b.** Scattering intensity as a function of concentrations for TDSA, HDSA,

420 ODSA for modified inulins.

421

422 **Figure 5a.** Z-average hydrodynamic diameter as a function of concentration for

423 modified inulins with different alkenyl chain lengths.

424

425 **Figure 5b.** Z- average hydrodynamic diameter as a function of alkenyl chain length for

426 ASA-inulins.

427

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429 concentration.
430
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432 concentration.
433
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435 alkenyl chain length.
436
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438 modified inulins with various length of alkenyl chain at room temperature on day 1.
439
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441 modified inulins with various length of alkenyl chain at 25 °C after storage at room
442 temperature for 21days.
443
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445 inulins at room temperature on day 1.
446
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448 inulins after storing at room temperature for 21days.
449

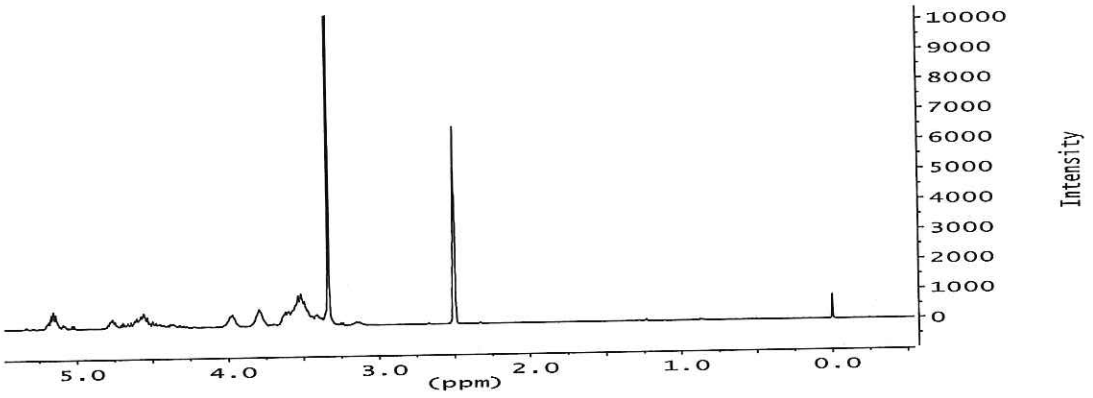
450 **List of Tables**

451

459

Figure 1

(a)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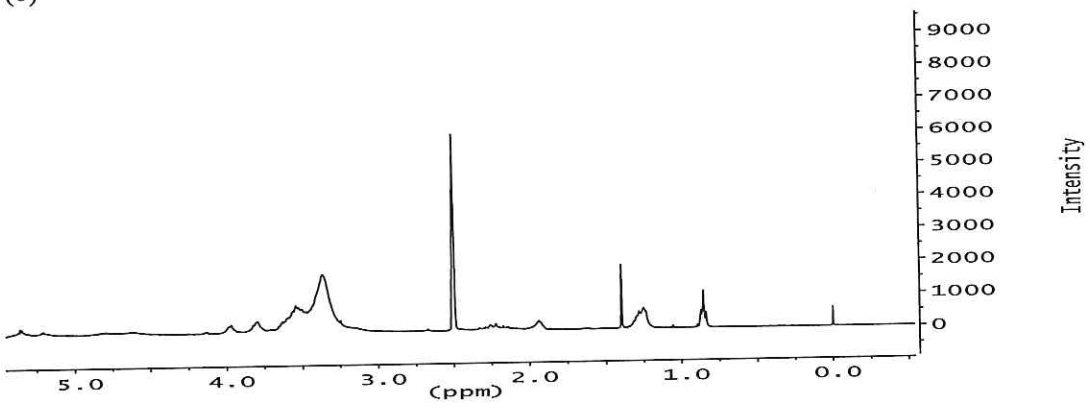
1.0
0.0

(b)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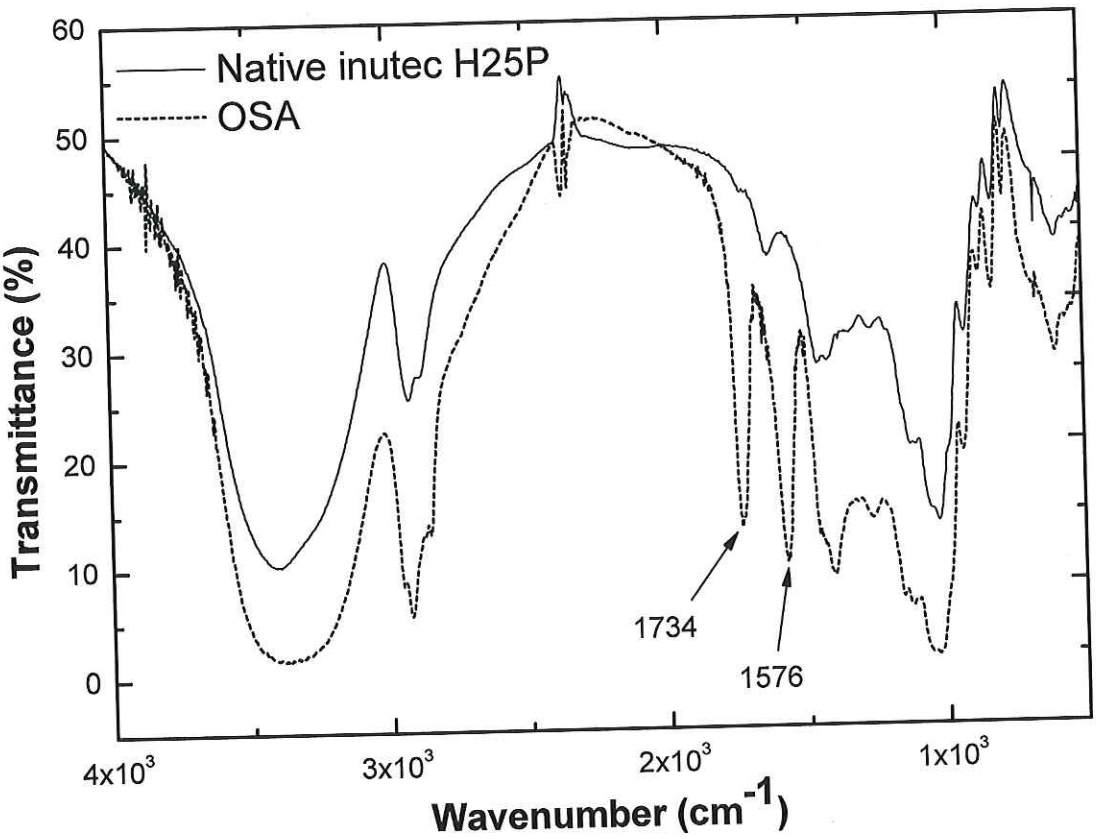
(ppm)
2.0
1.0
0.0

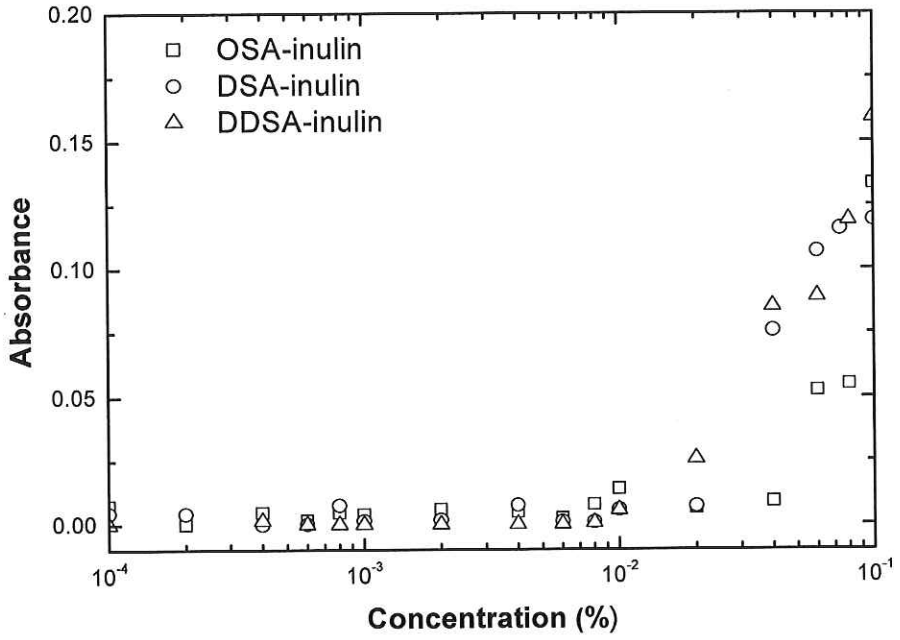
482 Figure 2.



483
484

485

486 Figure 3a



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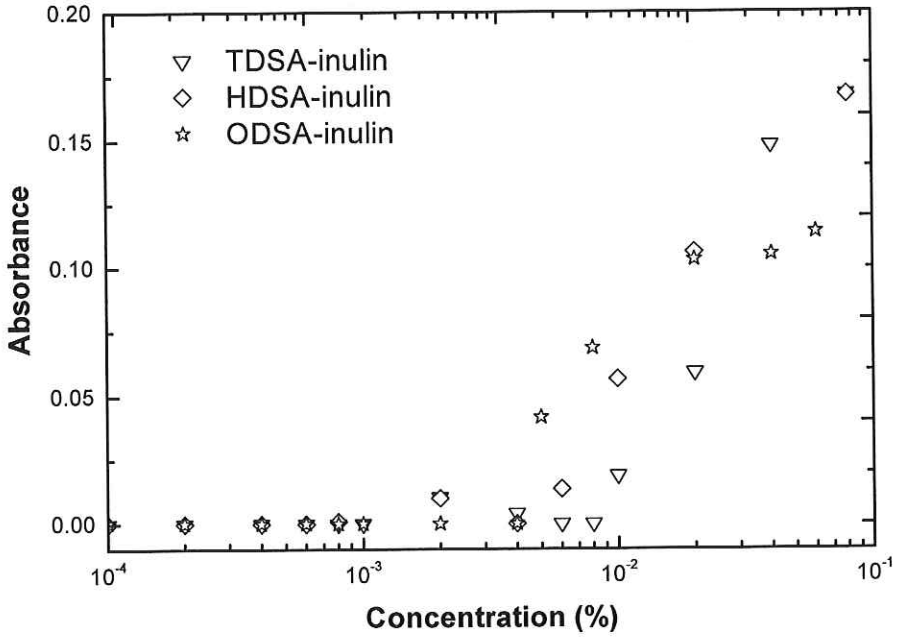
489 Figure 3b.



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493 Figure 4a.

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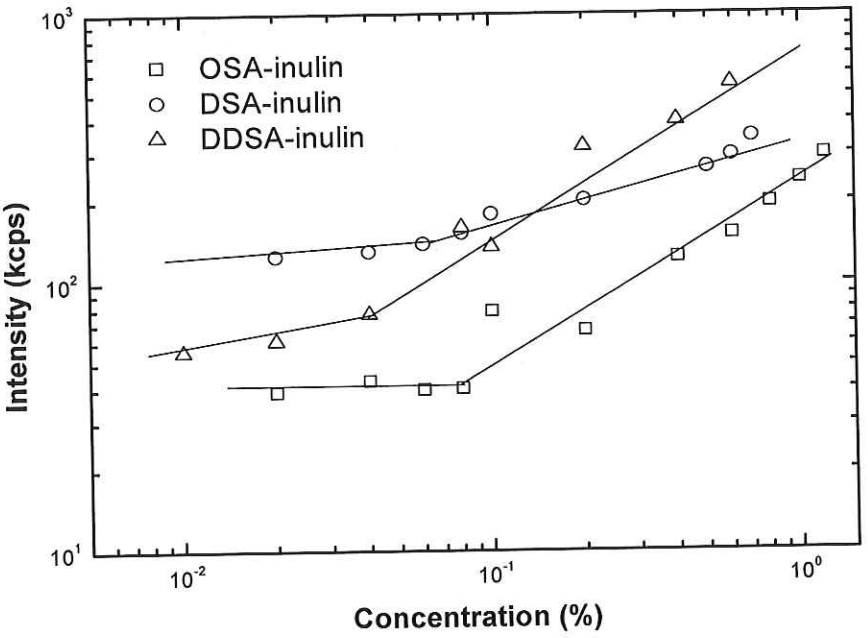

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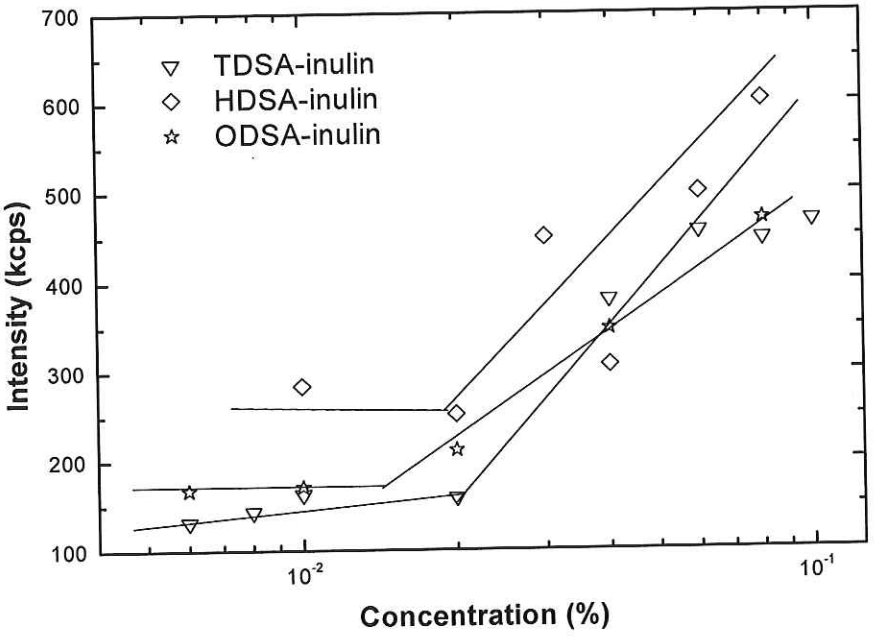

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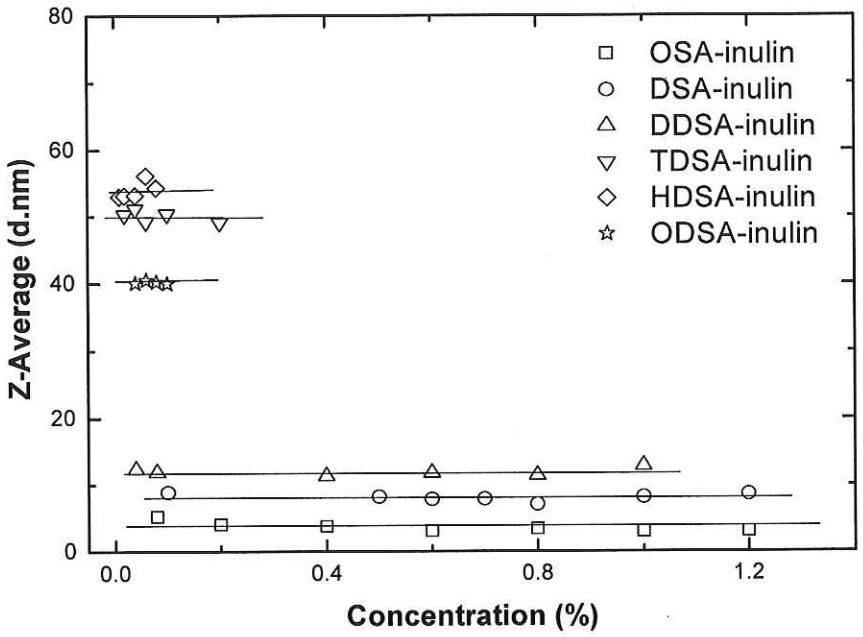

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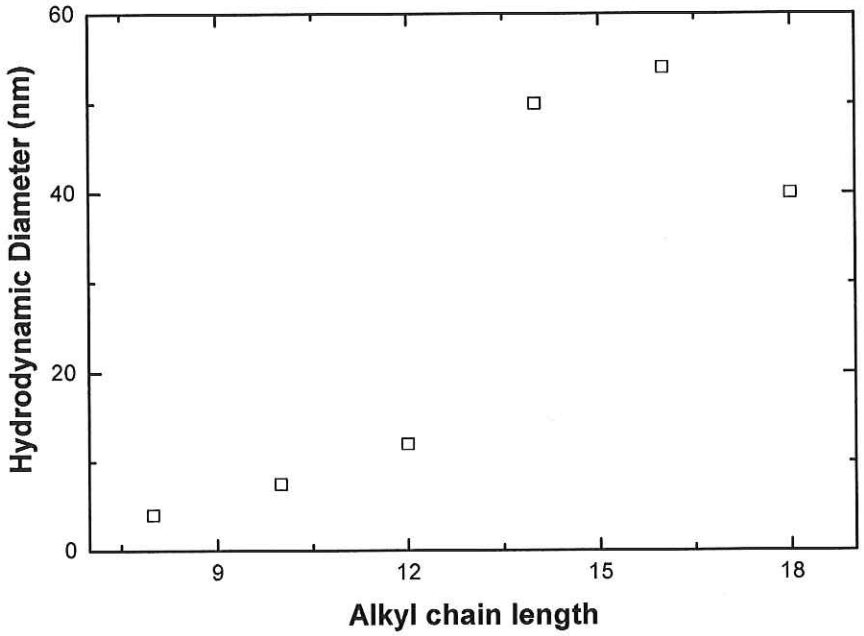

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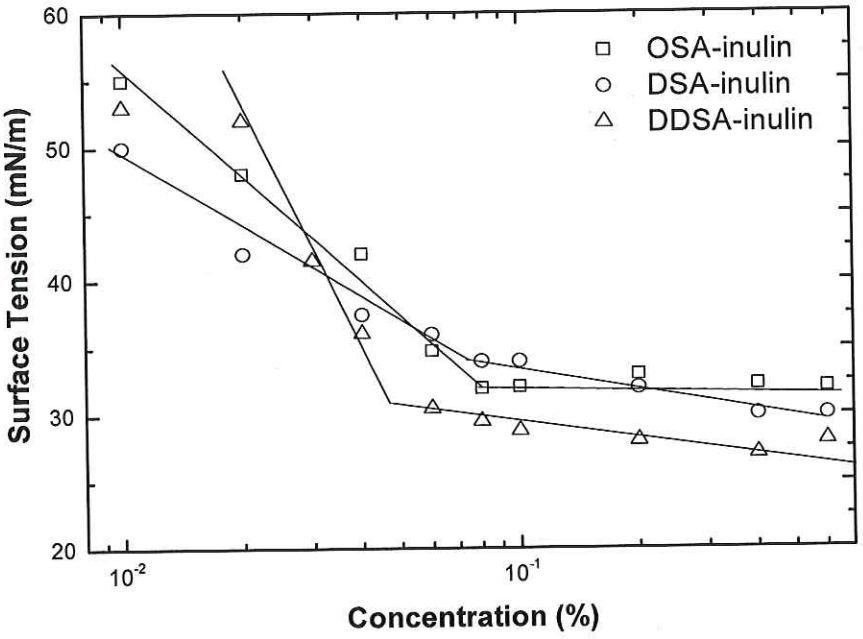

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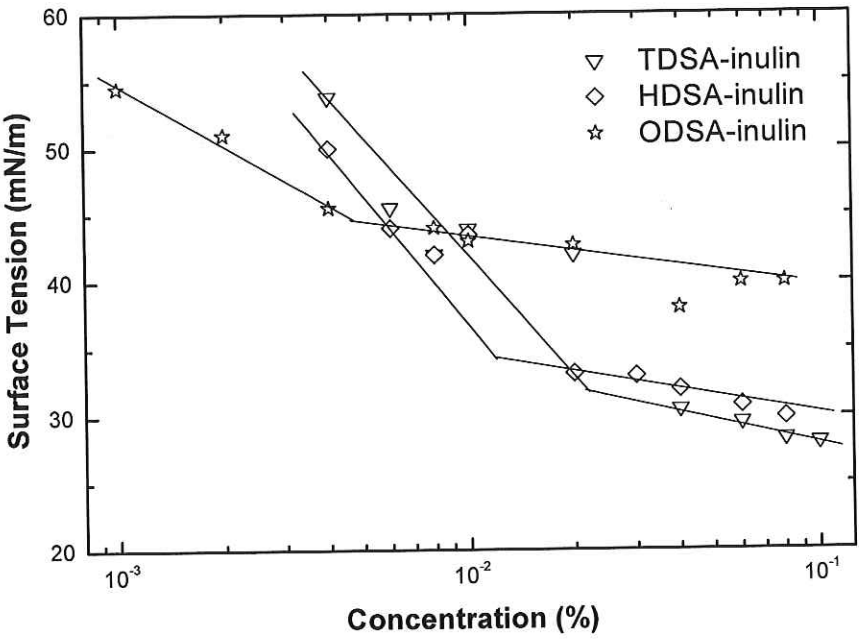

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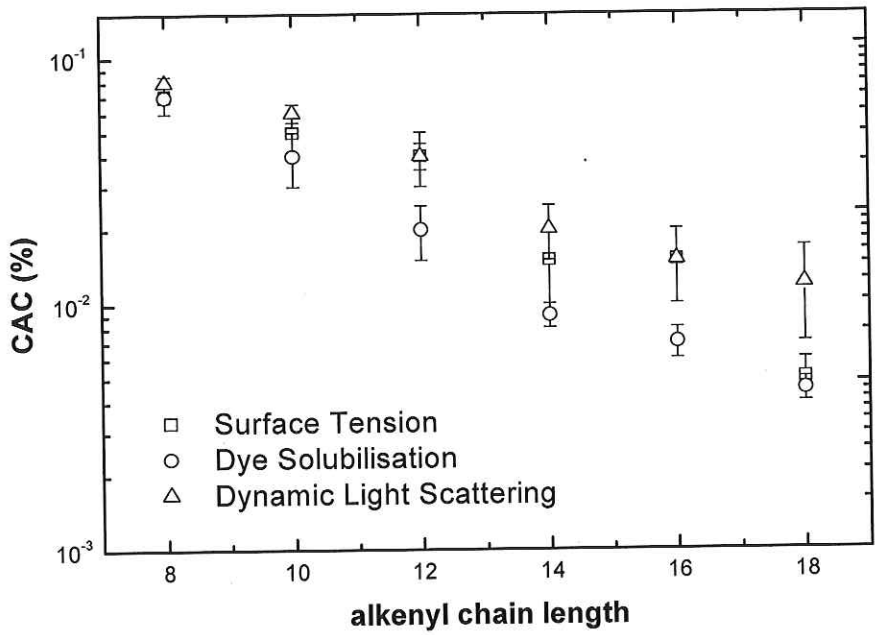
515 Figure 7.



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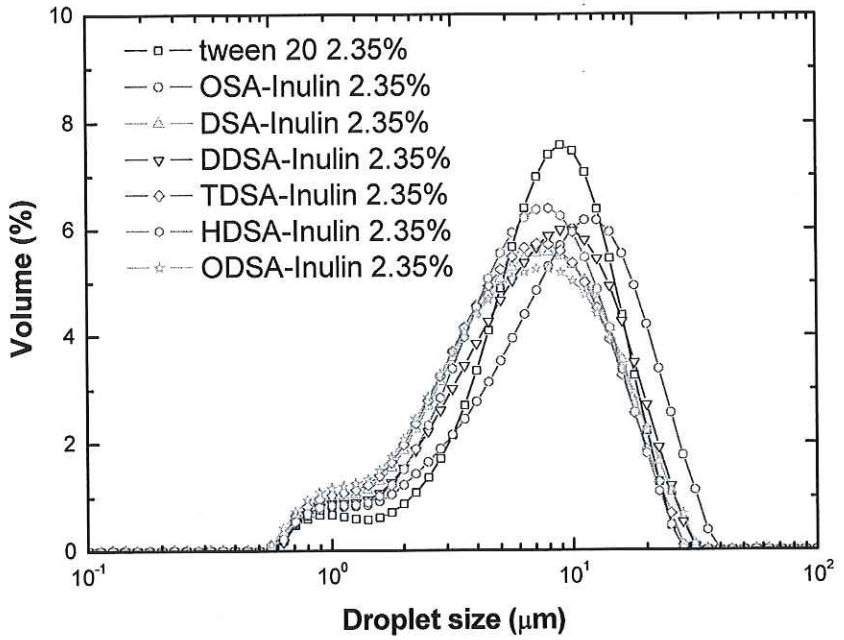

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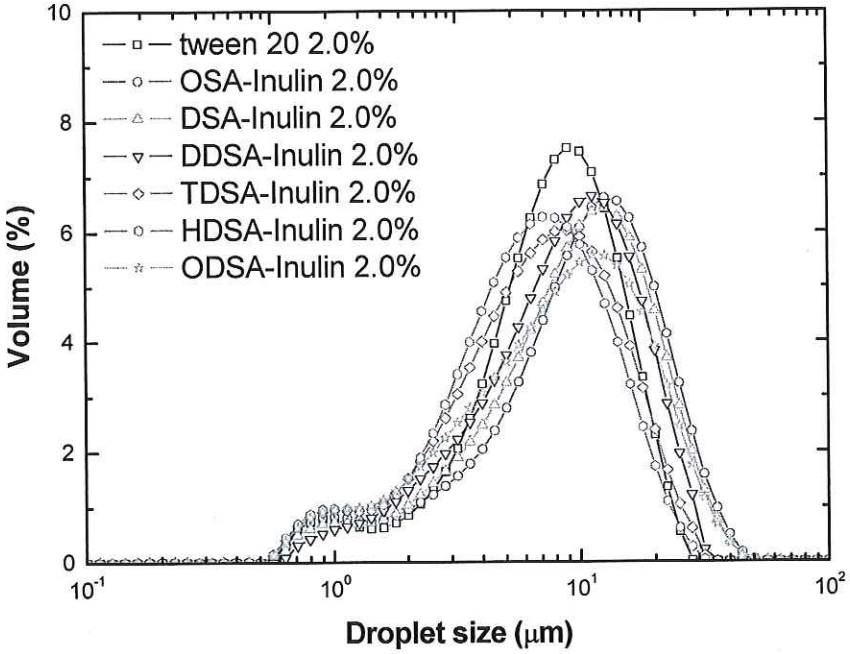

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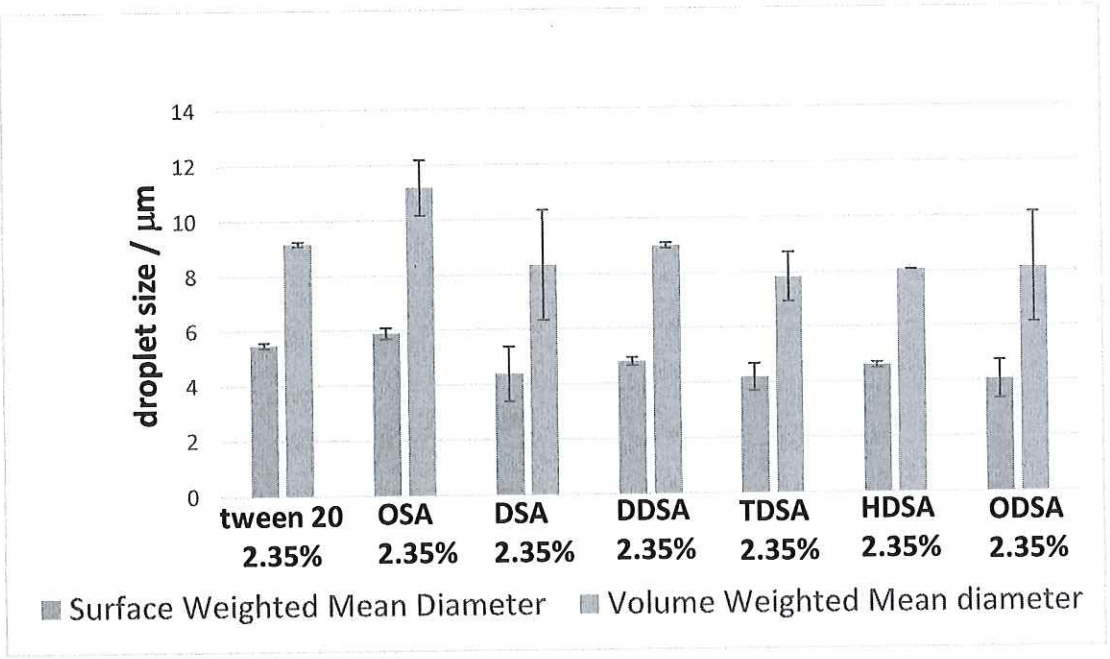

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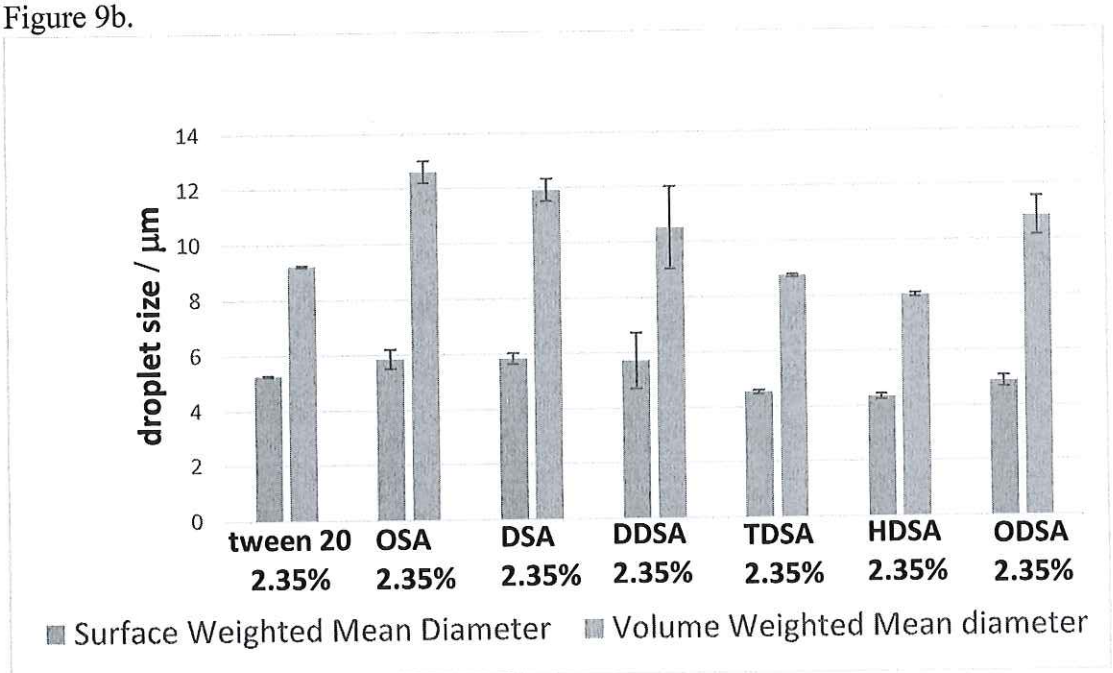

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528

529 Table 1. Degree of substitution of the modified inulins.

Sample	% substitution / moles
OSA	29.6
DSA	28.7
DDSA	22.7
TDSA	26.2
HDSA	39.8
ODSA	12.9

530

531

532 Table 2. Critical Aggregation Concentrations of the modified inulin samples using
533 various techniques.

CAC % / sample	Dynamic Light Scattering (Intensity)	Dye Solubilisation	Surface tension
OSA	0.08±0.005	0.07±0.01	0.07±0.01
DSA	0.06±0.005	0.04±0.01	0.05±0.01
DDSA	0.04±0.01	0.02±0.005	0.04±0.01
TDSA	0.02±0.005	0.009±0.001	0.015±0.005
HDSA	0.015±0.005	0.007±0.001	0.015±0.005
ODSA	0.012±0.005	0.0045±0.0005	0.005±0.001

534

535

536 Table 3. Surface excess (Γ) and molecular cross-sectional area (A) determined at 25°C.

Surfactant	Maximum Surface excess $(\Gamma) \times 10^{-6}\ (mol/m^2)$	Molecular area (nm^2)
OSA	2.24	0.74
DSA	1.58	1.06
DDSA	2.52	0.66
TDSA	1.66	1.00
HDSA	1.94	0.86
ODSA	0.93	1.78

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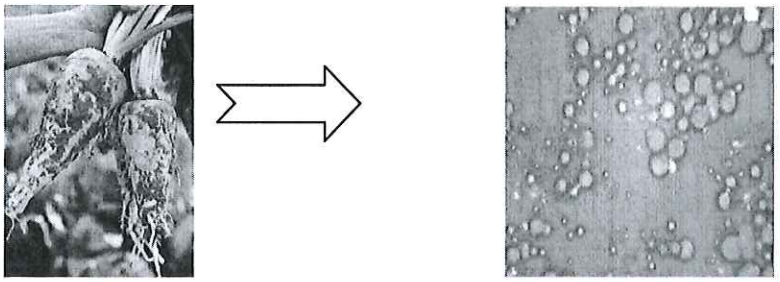
540 **Table of Contents graphic**

541

542 Inulin Biosurfactant



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