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Research Paper

Synthesis, characterisation and physicochemical properties of hydrophobically modified inulin using long-chain fatty acyl chlorides

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ABSTRACT

A series of inulin derivatives were synthesized in aqueous solution using acyl chlorides with varying alkyl chain length (C10-C16). They were characterised using a number of techniques including MALDI TOF-MS, ^{1}H NMR and FTIR and their degree of substitution determined. The solution properties of the hydrophobically modified inulins were investigated using dye solubilisation and surface tension and it was 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 hydrophobe chain length. It was found that the C10, C12 and C14 derivatives formed stable oil-in-water emulsions and the emulsion droplet size decreased with increasing alkyl chain length. The C16 derivative was not able to produce stable oil-in-water emulsions; however, it was able to form stable water-in-oil emulsions. The fact that the derivatives are able to form micellar-like aggregates and stabilise emulsions makes them suitable candidates for the encapsulation and delivery of active compounds with potential application in food, cosmetic, personal care and pharmaceutical formulations.

1. Introduction

There is considerable interest nowadays in developing sugar-based surfactants derived from renewable resources for potential application in a broad range of industrial formulations where they may function as wetting agents, dispersants and emulsifiers. Typical examples are alkyl polyglycosides, sorbitan esters, and sucrose esters (Hill and LeHen-Freenbach, 2009) which find use, for example, in food, personal and home care products, cosmetics, pharmaceuticals agrochemicals, surface coatings etc. In recent years particular attention has been paid to the derivatisation of inulin (Exerowa et al., 2009; Han, Ratcliffe, & Williams, 2015; Kokubun, Ratcliffe, & Williams, 2013; Morros, Levecke, & Infante, 2010; Tadros and Vincent, 1983; Tadros, 1999; Tadros, Vandamme, Booten, Levecke, & Stevens, 2004). Inulin is an abundant reserve polysaccharide found in many plants including chicory, garlic, leek, artichoke and onion but the primary industrial source is chicory with over 350,000 t produced annually (Evans, Han, Ratcliffe, & Williams, 2016). It consists of β(2 → 1) linked fructose units which are terminated with a glucose residue andhas a degree of polymerisation of 2–60. It has been chemically modified by esterification, etherification, and carbamoylation in organic solvents using fatty acid methyl esters (FAME), alkyl epoxides, and alkyl isocyanates, respectively (Rogge & Stevens, 2004; Rogge et al., 2004; Stevens et al., 2001) to yield surfactant – like molecules. Van Kempen et al. (2013) recently reported the modification of oligofructose dissolved in dimethylsulphoxide with fatty acids of varying chain length using lipase as a catalyst. More recently a number of workers have produced inulin derivatives by reaction with alkenyl succinic anhydrides in water (Han et al., 2015; Kokubun et al., 2013; Morros et al., 2010) and it has been shown that these materials will form micellar aggregates in aqueous solution. Kokubun, Ratcliffe, and Williams (2014) and Han et al. (2015) have also shown that the succinylated inulins are able to produce stable oil-in-water emulsions and that their effectiveness is a function of the alkenyl chain length. Kokubun et al. (2015) reported that the dodecenyl derivatives produced emulsions with smaller droplet size than the octenyl derivatives and that droplet aggregation was inhibited by electrostatic repulsions which arise from the presence of the carboxylate group of the alkenyl succinate half ester formed during derivatisation.

In a previous paper, one of us, has reported on the esterification of starch in alkaline aqueous media using C6–C10 acyl chlorides (Fang, Fowler, Sayers, & Williams, 2004) and more recently Namazi, Fahi, and Dadkhah (2011). esterified starches under aqueous conditions using C8, C12 and C16 fatty acid chlorides. The aim of the present study was to use this approach to modify inulin to produce novel non-ionic esterified

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derivatives with varying alkyl chain length and to evaluate their solution and interfacial properties for potential commercial application as encapsulating and emulsifying agents.

2. Materials and methods

2.1. Materials

Inulin coded Fibruline® DS2 (Degree of Polymerisation, DP < 10) was supplied by Cosucra Chemicals. The inulin was dried at 70 °C for 24 h before use. Decanoyl chloride (C10), lauroyl chloride (C12), myristoyl chloride (C14) and palmitoyl chloride (C16) were obtained from Aldrich Chemical Co. 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 0.95 g/mL.

2.2. Methods

Synthesis. A series of acylated inulins was synthesized according to the method of Namaziet al. (2011). Inulin (Fibruline® DS2), 10 g was added to 50 ml NaOH solution (20%w/v) at room temperature and gently stirred until dissolved. A predetermined amount of fatty acid acyl chloride was added dropwise to the reaction vessel with stirring at room temperature. The reaction was allowed to continue until the modified products precipitated in water. Precipitation was completed over 1.5 h. The solid product obtained was recovered by freeze drying and washed by Soxhlet extraction with cyclohexane for 12 h to completely remove unreacted fatty acid chlorides as tested by FTIR. The product was then dried in a vacuum oven.

2.3. Characterisation

2.3.1. MALDI TOF mass spectrometry

The degree of polymerization for the inulin sample was obtained using an Applied Biosystems Voyager DE-PRO MALDI-TOF mass spectrometer. 2,5-dihydroxy benzoic acid (DHB) was used at 10 mg/mL in 50% acetonitrile-deionised water solution as the matrix solution. Sample preparation involved the dissolution of 10 mg of DS2 inulin with 1 ml of matrix solution. Following mixing this was then further diluted 1:9, sample to matrix solution. Samples were introduced into a stainless steel 100-well plate via the dried drop method. Measurements were completed in linear positive mode with a nitrogen laser. Ion acceleration was set at 15 KV, grid voltage 93% and guide width 0.05% with a 100 nanosecond extraction delay time. Laser intensity and number of shots fired were varied and repeated multiple times in order to obtain clear spectra. Analysis of the spectra was undertaken with Applied Biosystems Data Explorer Version 4. 0. 0. 0, an automated advanced baseline correction and Gaussian peak smoothing was used on the spectra during processing.

2.3.2. NMR spectroscopy

H^1 NMR spectra of the esterified inulins were measured using a 500 MHz NMR Spectrometer at 25 °C. 5 mg of sample were dissolved in 0.7 g of D_2O then added into a 5 mm thin wall NMR tube and dissolved at 25 °C. The spectra recorded using the Pulse Program ZG30 with a 30° pulse and a delay of 1 s together with Mnova 7.0 software.

2.3.3. Fourier-transform infrared spectroscopy (FTIR)

The esterified inulins samples were dried in an oven at 70 °C overnight. 1 mg of sample was milled with 100 mg 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 13 mm pellet die (Max 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.

2.4. Critical aggregation concentration (CAC)

2.4.1. Dye solubilisation

Stock solutions of 1% esterified inulins were prepared and diluted to give solutions of various concentrations. 10 mg of the dye was added to 10 ml of inulin solution and left stirring 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). Experiments were also carried out by adding 1 ml of a solution containing 5 mg Sudan IV in 10 ml cyclohexane rather than adding the solid dye. The absorbance of the solutions was determined at a wavelength of 510 nm using a Lambda 25 UV/VIS Spectrometer (PerkinElmer). The CAC was obtained from the point at which the absorbance first increased.

2.4.2. Surface tension

The static surface tension of the esterified inulin solutions at varying concentration was determined by the Du Nouy ring method using a Tensiometer K8 and a 4 cm circumference platinum ring RI 01 from Krüss GmbH. All measurements were repeated three times. 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 concentration.

2.5. Emulsification properties

15% w/w oil-in-water emulsions were prepared by adding 8.5 g of 1.5% acylated inulin solution (C10, C12 and C14) to 1.5 g of MCT contained in a 20 ml tube. Emulsification was achieved using an IKA T25 Digital Ultra-Turraxhomogeniser at 24000 rpm for 3 min. 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.

15% water-in-oil emulsions were prepared by adding 3 ml of the C16 modified inulin solution at varying concentration (0.5–1.5%) to 17 ml MCT. These emulsion samples were contained in a 30 ml glass tube by using an IKA T25 Digital Ultra-Turrax homogenizer at 24000 rpm for 3 min. The droplet size was monitored using a BT-1600 image particle size analyser (Dandong Bettersize instrument Ltd., China). This consisted of an optical microscope (Nikon YS100) and a CCD camera (HV2001UC). A small drop of emulsion was placed onto a microscopic slide with a well and a cover slip placed on top and photomicrographs (20× magnification) were taken.

2.6. Rheology

The steady shear viscosity and storage and loss moduli of the emulsion samples were determined using an AR2000 rheometer (TA instrument, USA) fitted with a plategeometry (4 cm diameter flat plate sin 940215). All measurements were carried out at 25° C. In the steady shear measurements a constant and increasing shear rate was applied on the sample and the stress was measured simultaneously. The shear rate was increased from 0.01 to 1000 s^{-1} and then decreased from 1000 to 0.01 s^{-1}. In the oscillatory measurements, initially a frequency

sweep was performed to determine the linear viscoelastic region, whereby the amplitude was kept constant and the frequency is decreased from 1 to 0.01 Hz.

3. Results and discussion

3.1. Characterization

The MALDI-TOF MS spectrum for the unmodified inulin is presented in Supplementary data Fig. 1. The spacing between the peaks corresponds to a molar mass of 162 Da which is the mass of a fructose unit. It is evident that inulin chains with DP 2 to DP 18 are present. The results are consistent with those reported by Borromei et al. (2009) and Evans. Han et al. (2016) and Evans, Gallagher, Ratcliffe, and Williams (2016) for other commercial inulin samples. The average DP was determined to be 8 as calculated using Eq. (1)

$$DP = (\Sigma n_i DP_i)/(\Sigma n_i) \quad (1)$$

The degree of substitution was determined by ^{1}H NMR and the spectra obtained for the C10 modified material are given for example in Supplementary data Fig. 2. The prominent peak at 4.70 ppm is from the solvent (Barclay, Ginic-Markovic, Johnston, Cooper, & Petrovsky, 2012). The peaks from 3.30 ppm to 4.23 ppm and the peak at 5.35 ppm are from the inulin itself (Kulminskaya et al., 2003). After comparing with the ^{1}H NMR spectra of native inulin in the same solvent D_2O with spectra obtained by Kulminskaya et al. (2003), it is evident that acetylation had occurred. The ^{1}H NMR signals at 0.8 ppm (peak e in Supplementary data Fig. 2), as a triplet, showed three protons of the terminal methyl group of the acyl chain. Peak a at 2.0–2.25 ppm, peak b at 1.45 ppm, and peak d at 1.2 ppm are related to the methylene group and is in agreement with the results of Namazi et al. (2011). The peaks for the methylene groups (at 2.2 and 1.45 ppm) were close to the ester bond which indicated successful esterification. Similar results were found for the other modified inulins. The amount of alkyl chains incorporated into the modified samples was calculated from the ratio of the area of the peak at 0.8 ppm to the area of the peaks from 3.35 to 4.30 ppm and 5.35 ppm and the results are shown in Supplementary data Table 1. The degree of substitution (DS) was found to decrease with increasing length of the acyl chain. This is consistent to the work reported by Namazi et al. who synthesized acyl derivatives of starch and attributed this decrease to steric hindrance (Namazi et al., 2011).

FTIR spectra of the unmodified inulin and esterified inulin DS2C10 samples are presented in Supplementary data Fig. 3. The peaks for the native inulin at 3375 cm^{-1}, 2935 cm^{-1} and 1062 cm^{-1} indicate O—H stretching, CH_2 stretching and C—O—C bending, respectively (Fares, Salem, & Khanfar, 2011; Kokubun et al., 2013). The spectrum of DS2C10 shows two new peaks at 1563 cm^{-1} and 1437 cm^{-1} which are attributed to the carbonyl ester group (Namazi et al., 2011). Similar results were found for the other esterified inulin samples. The peaks are assigned to asymmetric C═O stretching and ester carbonyl stretching respectively (Fares et al., 2011; Namazi et al., 2011). The C—H stretching band absorbance at 2935 cm^{-1} for our esterified inulins was increased in intensity upon grafting in agreement with the findings of Namazi et al. (2011).

3.2. Critical aggregation concentration (CAC)

The absorbance values obtained for the esterified inulins in the presence of solid Sudan IV are given in Fig. 1a and b. 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 (Kokubun et al., 2013). The absorbance values obtained for the same samples in the presence of Sudan IV which is dissolved in cyclohexane are given in Fig. 2a and b and the results are very similar to those obtained by addition of the solid dye. The values of the CAC are shown in Supplementary data Table 2.

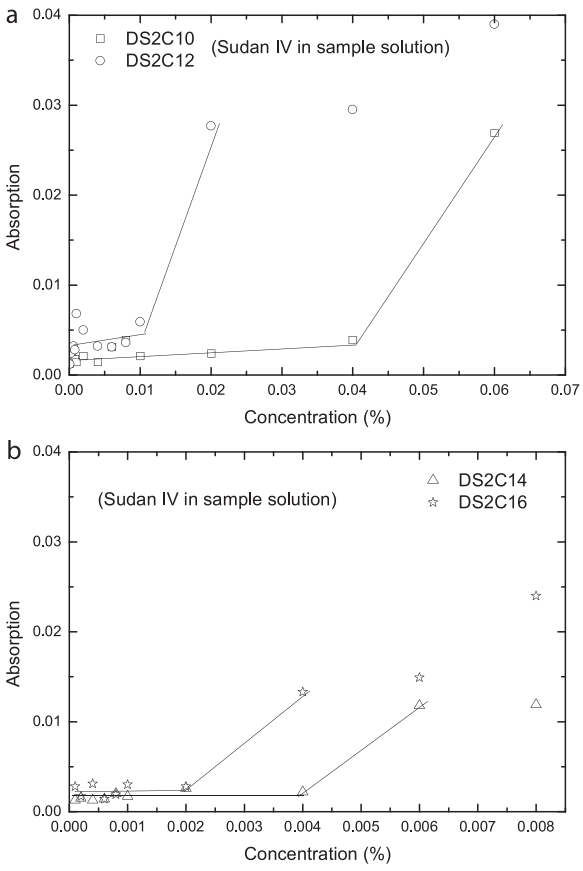

Fig. 1. a. Absorbance at 510 nm of DS2C10 and DS2C12 at varying concentration in the presence of Sudan IV dye. b. Absorbance at 510 nm of DS2C14 and DS2C16 at varying concentration in the presence of Sudan IV dye.

It is seen generally that the CAC decreases as the alkyl chain length increases as expected and also as noted by Kokubun et al. (2013) and Kempen, Schols, Van der Linden, and Sagis (2014) for hydrophobically modified inulins (Kokubun et al., 2013; van Kempen et al., 2014). The actual CAC value for the DS2C10 is a little lower than the value of 0.06% for the DSA-inulin sample prepared by Kokubun et al. (2013) and this is attributed mainly to the fact that DSA inulin was prepared using decenyl succinic anhydride and has a carboxylated charged group in the head group, while the acylated ones do not (Kokubun et al., 2013).

The surface tensions of the esterified inulins at the air-water interface were determined by the Du Nouy ring method and are shown as a function of concentration in Fig. 3a and b. The CAC value for each of the samples was obtained from the intercept in the plot and the results are reported in Supplementary data Table 2. The values are in reasonable agreement with the values obtained by dye solubilisation.

The minimum surface tension was found to be 45 mN/m for DS2C10, 60.5 mN/m for DS2C12, 62 mN/m for DS2C14 and 54 mN/m for DS2C16 *.i.e.* C10 > C16 > C12 > C14. The fact that the minimum surface tension does not follow the trend of increasing alkyl chain length may be due to differences in the degree of substitution and the position of attachment. Kokubun et al. (2013) reported values of ~35–40 mN/m for OSA-inulin and DDSA-inulin and van Kempen et al. (2014) reported values of ~34–40 mN/m for inulin samples modified with C8–C16 alkyl chains (Kokubun et al., 2013; van Kempen et al., 2014).

The CAC values for the esterified inulins obtained using the different techniques are plotted as a function of the alkyl chain length in Fig. 4 which clearly shows the decrease in CAC with increasing alkyl chain

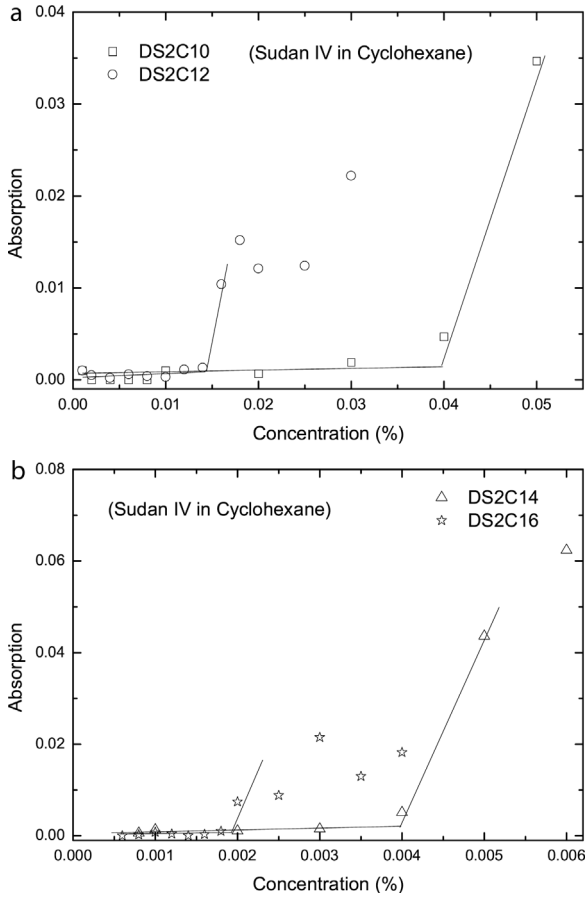

Fig. 2. a. Absorbance at 510 nm of DS2C10 and DS2C12 at varying concentration in the presence of Sudan IV dye in cyclohexane. b. Absorbance at 510 nm of DS2C14 and DS2C16 at varying concentration in the presence of Sudan IV dye in cyclohexane.

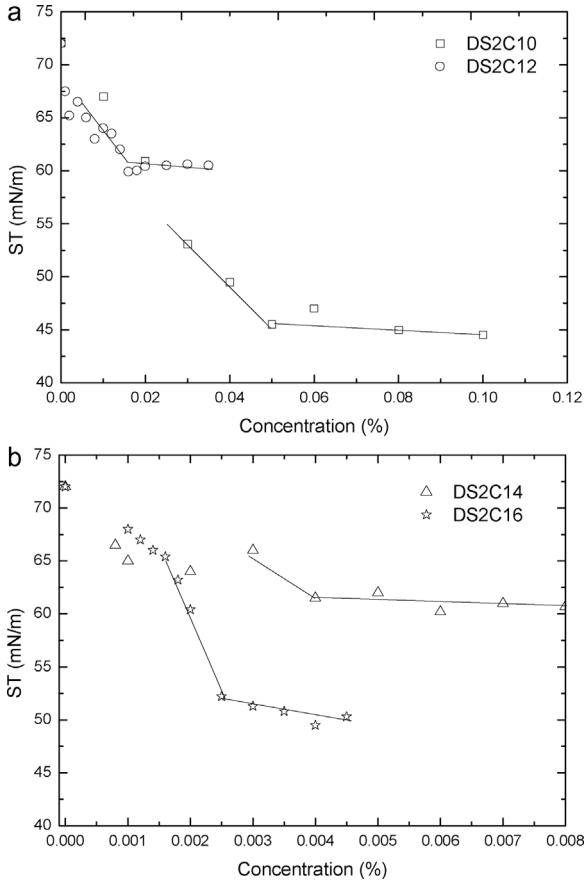

Fig. 3. a. Surface tension of DS2C10 and DS2C12 as a function of concentration. b Surface tension of DS2C14 and DS2C16 as a function of concentration.

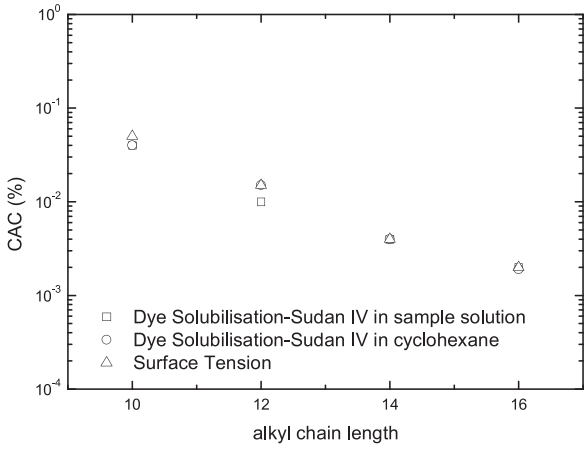

Fig. 4. Critical aggregation concentrations of the esterified inulins as a function of alkyl chain length.

length as expected from the work of other researchers (Molina-Bolivar & Ruiz, 2009). This is despite the fact that the DS2C10 and DS2C12 have a much higher DS than the DS2C14 and DS2C16 samples.

The HLB values of the esterified samples was determined from Eq. (2) (Hu, Chen, & Huang, 2011).

$$\mathrm{HLB} = 20 \times \left(1 - \frac{M_O}{M}\right) \tag{2}$$

Where M_0 is the molecular weight of hydrophobic groups; and M is the molecular weight of the sample. The HLB values from calculation are shown in Supplementary data Table 2. The DS of DS2C14 and DS2C16 were very low (0.0415 and 0.0424) and it was not meaningful to calculate a value for them.

The free energy decrease for the transfer of a $-CH_2-$ unit from the bulk phase to the micelle (ΔG_{mic}) can be calculated by Eq. (3) derived by Rosen (Rosen, 1976; Zhang & Marchant, 1996).

$$\log \mathrm{CMC} = (\Delta G_{mic}/2.303RT)m + K_{mic} \tag{3}$$

Where m is the number of carbons in the alkyl chain and K_{mic} is a constant for the same hydrophilic group. The calculated ΔG_{mic} for per CH_2 of esterified inulins (DS2C10-DS2C16) is -1.48 kJ/mol compared with the value for ASA-inulins of -0.79 kJ/mol at 25 ° Zhang and Marchant (1996) reported a ΔG_{mic} value for the *N*-alkylmaltoamide series of -1.86 kJ/mol at 25 °C (Zhang & Marchant, 1996). van Kempen et al. (2014) using the same procedure obtained a value of -3.1 kJ/mol (van Kempen et al., 2014).

The surface activity mainly depends on the chain length and the polarity of the head group of the surfactant molecule. The extent of surfactant adsorption at a liquid surface is expressed in terms of its surface excess concentration, Γ, and is related to surface tension by the Gibbs equation, which for a non-ionic surfactant is given by Eq. (4) (Becerra, Toro, Zanocco, Lemp, & Gunther, 2008; van Kempen et al., 2014)

$$\Gamma = -\left(\frac{1}{RT}\right)\left(\frac{d\gamma}{d\ln C}\right) \tag{4}$$

Surface excess values were determined from the slope of the lines for the plot of $\gamma - \ln C$ just below the CAC (Becerra et al., 2008; Garofalakis, Murray, & Sarney, 2000; van Kempen et al., 2014). It should be pointed out that there is some uncertainty in the values which

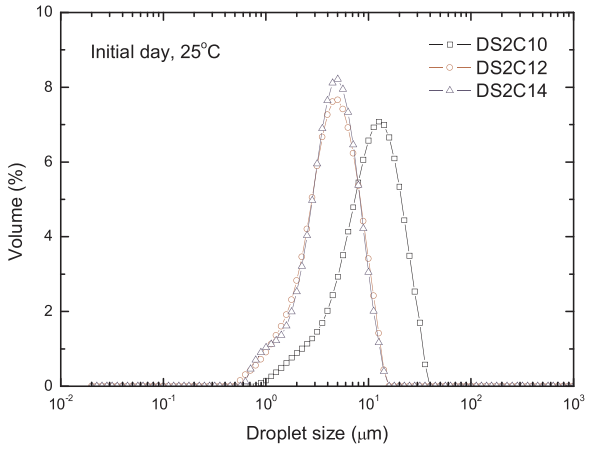

Fig. 5. Droplet size distributions of oil in water emulsions prepared with 1.5% DS2C10, DS2C12, DS2C14 solutions respectively on the initial day at 25 °C.

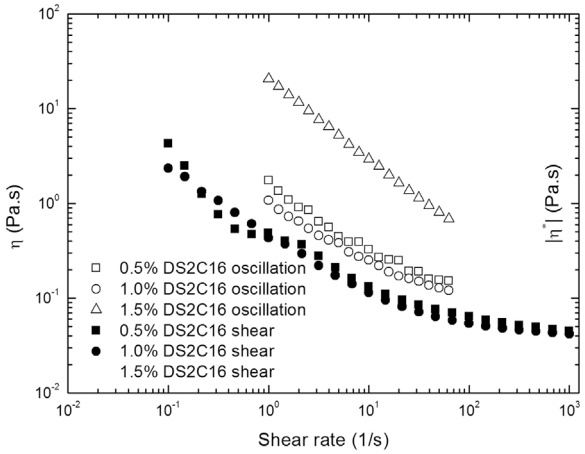

Fig. 6. The steady shear and dynamic viscosity as a function of shear rate for 0.5%, 1.0%, and 1.5% DS2C16 water in oil emulsion solution at 25 °C.

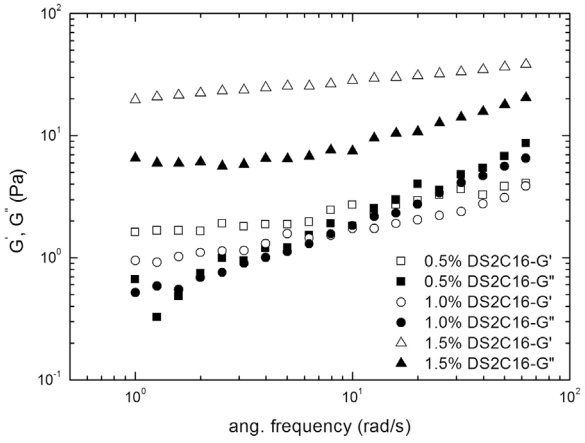

Fig. 7. The storage moduli and loss moduli for 0.5%, 1.0%, and 1.5% DS2C16 at 25 °C as a function of frequency.

are quoted in the Supplementary data Table 3. The values obtained for the surface excess were used to calculate the surface area occupied by each molecule, A, using Eq. (5)

$$A = \frac{1}{\Gamma N_A} \tag{5}$$

Where N_A is Avogadro's number.

The area occupied per molecule was found to vary between 0.166 and 1.45 nm^2 for the inulins modified using acyl chlorides (C10-C16), compared to the values for ASA-inulins of 0.66–1.06 nm (Han et al., 2015; Namazi et al., 2011). The reason for the low value for DS2C16 may be due to the fact that it is not very soluble. The value of 1.05 nm^2 for DS2C12 is similar to the results which were reported by van Kempen et al. (0.91 nm^2) and Stevens et al. (0.9 nm^2) for hydrophobically modified inulin samples (Stevens et al., 2001; van Kempen et al., 2014). For the modified inulins the area occupied will ultimately depend on the number and position of the hydrophobic groups attached to the backbone.

3.3. Emulsion stability

Fig. 5 and Supplementary data Fig. 4 show the droplet size distribution and $d_{3,2}$, $d_{4,3}$ values of 15% MCT oil-in-water emulsions prepared using 1.5% DS2C10, DS2C12 and DS2C14, respectively, on the initial day at 25 °C. It is seen that the droplet size decreases with the alkyl length increase. The droplet sizes of emulsions after storing for varying times at 25 °C and 50 °C are presented in Supplementary data Table 4. It is seen that although DS2C14 produced emulsions with the smallest droplet size the emulsions were not stable over time. Besides, the droplet size was measured also at 50 °C and some emulsions were unstable. DS2C16 was unable to form oil-in-water emulsions, however, it was found that it could form water-in-oil emulsions. Photomicrographs obtained for the droplets of water-in-oil emulsions prepared using varying concentrations of DS2C16 were obtained using a BT-1600 image particle size analyser and are presented in Supplementary data Fig. 5. It is evident that the droplet size decreased significantly from approximately ~8 μm to ~1 μm as the DS2C16 concentration increased from 0.5% − 1.5%.

3.4. Emulsion rheology

The shear and dynamic viscosities of water-in-oil emulsions prepared using various concentrations of DSCC16 (0.5%–1.5%) are plotted as a function of shear rate in Fig. 6. The plot shows that the samples have typical shear thinning behavior and that the dynamic viscosity is greater than shear viscosity. The fact that the systems do not obey the Cox-Merz rule is indicative of the presence of a weak network structure. Fig. 7 shows the G' and G" values for emulsions prepared using varying concentrations of DS2C16. For emulsions prepared using 0.5%, 1.0% DS2C16 the G' and G" values cross over at an angular frequency of ~10 rad/s indicating they are fairly fluid. For the sample prepared using 1.5% DS2C16 G' is greater than G" over the frequency range studied and both are independent of frequency. This is typical of a weak gel.

4. Conclusions

It has been shown that inulin can be successfully modified in aqueous solution using fatty acyl chlorides to yield amphiphilic molecules which associate in solution above a critical concentration to form micellar-like aggregates. It was found that the C10, C12 and C14 derivatives were able to form oil-in-water emulsions while the C16 derivative produced water-in-oil emulsions. It is evident that these materials have potential application as encapsulating and emulsification agents in, for example, the formulation of cosmetics, personal care, surface coatings and pharmaceuticals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2017.09.008.

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