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Synthesis, characterization and emulsification properties of dodecenyl succinic anhydride derivatives of Gum Arabic

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Abstract

Gum Arabic, GA, has been chemically modified using dodecenyl succinic anhydride (DDSA) in aqueous solution and the products have been characterized and their solution and emulsification properties evaluated. FTIR was used to confirm that derivatisation had occurred and the hydrophobe incorporation and reaction efficiency was determined by a simple titration method. The derivatives, DGA5 and DGA10 were found to contain 5 and 10 %w/w DDSA respectively. Surface tension and dye solubilisation measurements demonstrated that the modified GA samples aggregated in solution at a specific concentration, referred to as the critical aggregation concentration, CAC. The value of the CAC was found to be a function of the degree of substitution and values of 0.1% and 0.04% were obtained for DGA5 and DGA10 respectively. The emulsification properties of the polymers were assessed by determination of the droplet size as a function of polymer concentration and time using laser diffraction. The droplet size decreased for all samples as the polymer concentration increased but the minimum droplet size obtained increased in the order DGA10 < DGA5 < GA indicating enhanced emulsification efficiency with increasing hydrophobe content. The effect of ageing on emulsion stability was assessed and the droplet size of emulsions prepared with GA and DGA5 increased with time but remained constant for samples prepared with DGA10. Emulsion creaming was followed using the Turbiscan and it was noted that DGA5 and DGA10 were much more effective than GA at reducing creaming. The results have demonstrated that the modified gum Arabic samples have potential application for microencapsulation.

Keywords:

Dodecenyl Succinic Anhydride derivatives, Gum Arabic, Emulsification properties, droplet size, creaming

Introduction

Gum Arabic, GA, is a complex polysaccharide obtained as an exudate from the trunks and branches of certain Acacia trees, namely, *Acacia senegal* and *Acacia seyal* which grow in various countries across the Sahelian belt of Africa. (Williams and Phillips, 2009; Kennedy, Phillips and Williams, 2012). It consists of galactose, arabinose, rhamnose and glucuronic acid together with a small amount of proteinaceous material which is present as an integral part of the structure. It is now generally recognised that the gum consists of three main fractions, commonly referred to as the arabinogalactan (AG), arabinogalactan-protein (AGP)and glycoprotein (GP) components, which differ mainly in molecular mass and protein content (Randall et al 1988, 1989; Osman et al 1994). The AG fraction represents about 90% of the gum and contains very little associated protein. It has a molecular mass of ~300kDa. Experiments using transmission electron microscopy, atomic force microscopy and small angle neutron scattering have shown that it consists of disk-like molecules with a diameter of ~20nm and thickness of ~ 2nm (Renard et al, 2006; Sanchez et al, 2008). The AGP fraction accounts for ~10% of the gum and has a molecular mass of ~1500kDa. It contains ~10% protein as an integral part of its structure and enzyme and alkali hydrolysis studies have shown that it has a 'wattle blossom' type structure, typical of AGPs generally. It is believed to consist of carbohydrate blocks of Mw ~4 x 10^4 attached to a polypeptide chain consisting of ~250 amino acids through O-serine and O-hydroxyproline residues together with short chains of arabinose linked to hydroxyproline (Mahendran et al, 2008). The carbohydrate blocks may be disk-like as is proposed by Sanchez et al (2008) for the AG fraction. The GP fraction represents about 1% of the total mass of the gum and contains ~20-50% protein. Little is known about its structure.

GA is widely used in the Beverage Industry as an emulsifier for the stabilisation of flavour oil-in-water emulsions and it is commonly accepted that its ability to emulsify is due to the presence of proteinaceous components within the AGP and GP fractions

(Randall et al 1988, 1989; Padala et al 2009). These provide amphiphilic characteristics which facilitate adsorption of the molecules onto the surface of the oil droplets. The AG fraction, which represents the bulk of the gum, is not involved in the emulsification process and it is for this reason that concentrations as high as 15% gum Arabic are required to produce a stable 20% oil-in-water emulsions (Randall et al 1988, 1989). There has been recent interest in chemically modifying the gum to enhance its emulsification efficiency and this has involved esterification using alkane- or alkene-substituted dicarboxylic acid anhydrides and in particular octenyl succinic anhydride (Ward, 2002a, 2002b). In addition Sarkar, et al (2011, 2013) recently produced an octenyl succinylated GA derivative for the encapsulation of mint oil and found that the modified gum had improved performance compared to the original GA.

It is expected that the emulsification and encapsulation properties of the hydrophobically modified GA will be a function of both the concentration and length of the of alkyl chains although there are no reports on this in the literature. The aim of the present work, therefore, is to synthesise GA derivatives using different concentrations of dodecenyl succinic anhydride and to investigate their ability to stabilise oil-in-water emulsions.

Materials and methods

Materials

A kibbled GA sample (*Acacia senegal*) was obtained from the Gum Arabic Company, Sudan. (2-Dodecen-1-yl) succinic anhydride (DDSA), spectroscopic pure potassium bromide, petroleum ether, sodium hydroxide and hydrochloric acid were obtained from Fisher Chemicals UK. Sudan IV, dye content 80%, was obtained from Eastman Fine Chemicals. Medium-chain triglycerides, (MCT), were obtained from the Trec Nutrition Company and consisted of octanoic/decanoic acid triglycerides. Deionized water (Purite Select Fusion 40 Purite Pure Water System) was used throughout.

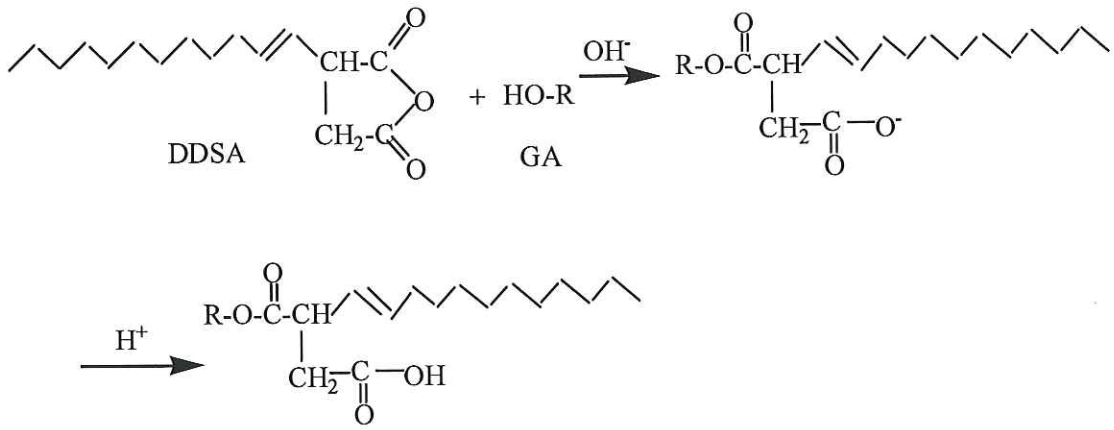
Methods

Molecular mass distribution of gum Arabic

The molecular mass distribution of the GA was determined by Gel permeation Chromatography (GPC) using 0.1M $NaNO_3$ as eluent. The system consisted of a Waters (Division of Millipore, USA) Solvent Delivery System Model 6000A connected to a Suprema column 3000A 10υm 8*300mm. A manual Rheodyne Model 7125 syringe loading sample injector equipped with 100μl sample loop was used. The column effluent was monitored using a DAWN DSP laser light scattering photometer equipped with a He–Ne laser with a wavelength of 633 nm (Wyatt Technology Corporation, USA), and a Wyatt Optilab DSP interferometric refractometer (Wyatt Technology Corporation, USA). A value of 0.141 ml/g was used for the refractive index increment, dn/dc.

Synthesis of dodecyl succinic anhydride (DDSA) gum Arabic derivatives

The DDSA derivatives of GA were prepared using dodecenyl succinic anhydride. The reaction proceeds according to the following scheme:



15g of GA was dispersed in 40ml of deionised water contained in a 250ml multi-necked flask using a stirrer (Heidolph Type: ST1 or RZR 50) with PTFE centrifugal shaft, 6mm diameter, 400mm. The pH was adjusted to between 8.30±0.1 using 1% NaOH solution using a peristaltic pump (P-1 Pharmacia Fine Chemicals). At the same time 30ml ethanol were added. Two reactions were performed using 1.5g and 3.2g DDSA

137 respectively which were dissolved in 15 ml of ethanol and added at 25°C. The reaction
138 was left to continue for 7h when the pH became stable. Once the reaction was finished,
139 the resultant product was neutralized with 5% HCl solution to a pH of 6.0. The product
140 was transferred to a 500ml round-bottom flask and put on a rotary evaporator to
141 remove water and it was then freeze dried yielding a white powder. The powder was
142 purified by Soxhlet extraction for 6h using petroleum ether as solvent. Finally, the
143 sample was dried in an oven at 50°C overnight. The products are referred to as DGA5
144 and DGA10 corresponding to 5 and 10 w % hydrophobe incorporation respectively.
145
146 *Hydrophobe incorporation determined by titration*
147 1g DGA5 and DGA10 was accurately weighed using electrical balance with 0.1mg
148 accuracy and dissolved in 15ml deionized water by stirring for 10 min at room
149 temperature. The acid groups of modified GA were completely ionized by the addition
150 of 0.05M NaOH solution until a pH of 9.00. Then, all the basic groups of GA-DDSA
151 were titrated with 0.05M HCl solution, using a pH-electrode. The procedure was
152 undertaken on an unmodified GA sample as a blank. Titrations were performed in
153 duplicate and the average value taken. The % (w/w) hydrophobe incorporation was
154 calculated as follows:
155 $$HI = \frac{266 \times C\Delta V}{\mathrm{Wsample} - C\Delta V * 266} \times 100\% \ (\%\mathrm{w/w})$$
156 where △V (l) = titre for 1g sample – titre for 1g blank, the volume used during the
157 titration; C the molarity of HCl solution; 266, molecular weight of DDSA; W_{sample}, the
158 dried sample weight.
159
160 *FTIR spectroscopy*
161 Samples were ground using a mortar and pestle then mixed with dry potassium
162 bromide (1:100) and KBr disks produced. FTIR spectra were obtained using a Perkin
163 Elmer FT-IR Spectrometer Spectrum RXI.
164

Critical aggregation concentration (CAC)

The CAC was determined using the surface tension and dye solubilisation techniques.

Surface tension measurements

The du Noüy ring method was used to determine the surface tension of solutions of GA and its derivatives at different concentrations using Kruss K8 surface tensiometer (Kruss GMBH Germany). Measurements were carried out in triplicate and the average value recorded. The CAC was determined from the inflection in the plot of the surface tension versus concentration.

Dye solubilization

10mg of Sudan IV dye was added to 10ml of the GA samples at varying concentration in water. The samples were mixed at room temperature overnight and filtered using a Millex-GP 0.22um filter (Millipore Ireland Ltd) into disposable UV grade 10mm path length cuvettes (CXA-110-0053, Fisher Scientific Ltd). The absorbance of the solutions was measured at a wavelength of 510nm using a Perkin Elmer Lambda 25 UV/VIS spectrometer. Measurements were performed in duplicate and the average value taken. The CAC was determined as the point at which the absorbance increased. GA was used as blank in this measure.

Emulsification properties

Oil-in-water emulsions (10% v/v) were prepared by adding 2ml MCT to 18ml gum solution at various concentrations and mixing using an IKA Ultraturrax T25 mixer (T25, IKA, Germany) set at 24000 min^{-1} for 4 min. The droplet size was measured over a period of time by laser diffraction using the Mastersizer 2000.The emulsion was added dropwise using a plastic pipette to the water in the dispersion unit of the instrument until the obscuration was about 12%. The measurements were made at room temperature and were performed in triplicate and the average value determined. The refractive indices of water and MCTs were taken as 1.33 and 1.45, respectively.

Emulsion creaming was assessed using the Turbiscan MA 2000. The emulsions (6%w/w GA and its derivatives) were decanted into a glass cylindrical cell and then

195 periodically scanned by the detection head of the device with a pulsed near infrared
196 light source (wavelength 850 nm) from 0 h to 24 h at intervals of 30 min at 30℃. The
197 backscattered profiles along the height of the cell were collected as raw data.
198
199 **3. Results and discussion**
200 *Molecular mass distribution of GA*
201 The refractive index and molecular mass GPC elution profiles of GA is presented in
202 Figure 1 and the weight average, Mw, and number average, Mn, molecular mass
203 values were found to be 620kDa and 300kDa respectively. These values are within the
204 range reported by others for gum Arabic (Idris et al 1998; Al-Assaf et al 2005;
205 Mahendran et al 2008). The main peak, corresponding to the AG fraction is centered at
206 an elution volume of 9.5mL. The small shoulder on this peak, centered at around
207 8.5mL corresponds to the AGP fraction (Mahendran et al 2008).
208
209 *Characterization of dodecyl succinic anhydride (DDSA) modified GA*
210
211 *% hydrohobe incorporation*
212 The amount of hydrophobe incorporated into the GA was determined by titration and
213 found to be 5.8 %w/w and 10.3 %w/w respectively for DGA5 and DGA10. This
214 corresponds to reaction efficiencies of 57.6% and 48.3%.
215
216 *FTIR spectroscopy*
217 Figures 2a and 2b show the FTIR spectra of GA, DGA5 and DGA10 and the FTIR peak
218 assignment is shown in Table 1. It can be seen from the figures that at 1720 - 1740cm^{-1}
219 for DGA5 and DGA10, there is a peak attributable to the ester bond (-COOR) which is
220 not present for GA itself. For DGA10, the peak is more obvious than for DGA5 due to
221 the higher degree of substitution.
222
223 *Determination of the CAC*
224 Surface tension measurements

225 The surface tensions of the gum solutions are shown in Figure 3 as a function of
226 concentration. For the GA sample the surface tension deceases with concentration as
227 expected. It is assumed that the decrease is due to the adsorption of the gum Arabic
228 molecules at the air-water interface through the protein moieties present. For the
229 modified samples the surface tension also decreases with concentration but there is a
230 distinct inflection in the curve which we attribute to the formation of micellar-type
231 aggregates brought about by hydrophobic association of the molecules through the
232 alkyl chains. The CAC are 0.1 % and 0. 04% for the DGA5 and DGA10 samples
233 respectively.
234
235 Dye solubilization
236 The absorbance values of solutions of gum Arabic and its derivatives in the presence
237 of Sudan IV dye are presented in Fig. 4. The absorbance shows a gradual increase
238 with concentration for GA indicating that the dye is able to bind to specific regions
239 within the GA molecules. Fang et al (2010) have recently shown that fatty acids also
240 able to bind to gum Arabic although the actual binding site is not known. For the DGA5
241 and DGA10 samples there is an abrupt increase in the absorbance at concentrations
242 of 0.1 % and 0. 04 % respectively. This increase corresponds to the CAC and the
243 values are in close agreement with the CACs determined by surface tension.
244
245 *Emulsification properties*
246 The droplet size of emulsions prepared using the gum samples are shown in Figures
247 5a and 5b as a function of concentration. It is noted that the droplet size deceases with
248 increasing gum concentration for all of the samples but the final droplet size is smaller
249 in the order DGA10 < DGA5 < GA indicating that the emulsification effect increases
250 with increasing hydrophobe content. Presumably increasing the hydrophobe content
251 facilitates more rapid adsorption onto the droplets as they are created under shear thus
252 reducing droplet aggregation and coalescence.
253 The droplet sizes of the emulsions were determined as a function of time and the
254 results are presented in Fig. 6a and 6b. It is noted that the droplet size increased

slightly with time for the GA and DGA5 stabilised emulsions but remained almost constant for the emulsions prepared using DGA10. It is evident that the DGA10, therefore, provides an improved electrosteric barrier preventing droplet flocculation.

The creaming behavior of the emulsions was followed using the Turbiscan and the backscattering profiles (BS), taken at 30mins intervals over a 24h period are reported in Figures 7a, b, and c for DGA10, DGA5 and GA respectively. The results show that the BS profiles for DGA5 and DGA10 remain constant over a 24h period while for GA it changes significantly confirming that creaming occurs much more rapidly. This result infer that modified GA like DGA5 and DGA10 can stable oil-in-water emulsions, even the oil volume contents reach 10% v/v.

Conclusions

This paper demonstrates that hydrophobically modified GA can be readily synthesized in aqueous solutions using dodecenyl succinic anhydride, and the products DGA5 and DGA10 have been characterized and their solution and emulsification properties have been evaluated by means of FTIR, surface tension, dye solubilisation and so on. The GA derivatives have been shown to aggregate in solution at a critical concentration which depends on the amount of hydrophobe incorporated and hence could be used to dissolve active compounds for application, for example, in functional foods and nutraceuticals. The derivatives have been shown to have superior emulsification properties to GA and hence have potential application in microencapsulation.

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