Carbohydrate Polymers 87 (2012) 1503–1506

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Hydrophobically modified alginate for emulsion of oil in water

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ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 4 September 2011
Accepted 12 September 2011
Available online 21 September 2011

Keywords:
Amphiphilic dodecanol alginate
Oil-in-water emulsion
Emulsifier
Stability

ABSTRACT

Dodecanol was covalently coupled to sodium alginate (NaAlg) via ester functions using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) as a coupling reagent to provide an amphiphilic dodecanol alginate (DA) for subsequent use in oil-in-water (O/W) emulsion application. The structure of DA was confirmed by FT-IR spectrometry. The stability of the emulsions prepared with different concentrations (0.3–1.2 wt%) of DA or 1.0 wt% NaAlg was evaluated by measuring droplet size, microstructure, viscosity and creaming. The results showed that the emulsions containing 1.0 wt% NaAlg, 0.3 and 0.5 wt% DA were unstable and the emulsions containing 0.8–1.2 wt% DA presented better stability during storage.

1. Introduction

Emulsions can be referred to as functional fluids in which one immiscible fluid is incorporated within another. Many food products can be categorized as oil-in-water (O/W) emulsions, which consist of small lipid droplets dispersed in an aqueous medium, e.g., milk, cream, ice-cream, beverages, dressings, dips, sauces, mayonnaise and desserts. Conventional emulsions are inherently thermodynamically unstable systems because the contact between oil and water molecules is unfavorable, and so they will always breakdown over time (Dickinson, 1992; Friberg, Larsson, & Sjoblom, 2004; McClements, 2005).

Food emulsions may become unstable due to a variety of different physicochemical mechanisms (Bengoechea, Romero, Aguilar, Cordobés, & Guerrero, 2010; McClements, 2007), including gravitational separation (creaming/sedimentation), flocculation, coalescence, partial coalescence, Ostwald ripening and phase inversion. Many natural and processed food products consist either partly or wholly in emulsions whose formation and stability are achieved by the use of emulsifiers. Emulsifiers are commonly used in food emulsion systems to increase their short- and long-term kinetic stability (Dickinson, 1992). A wide variety of different kinds of synthetic and natural emulsifiers are available for use in foods, including certain proteins, polysaccharides, phospholipids, small molecule surfactants and solid particles (Kaasgaard & Keller, 2010; Stauffer, 1999; Whitehurst, 2004). There is a trend within the food industry to replace synthetic emulsifiers with more natural ones. The most commonly used polysaccharides in food emulsions are gum arabic, modified starch, modified cellulose, galactomannans, and pectin (Gu, Decker, & McClements, 2004). However, these molecules are not particularly surface active and/or have to be used in relatively large quantities to make stable emulsions.

Alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks (Atkins, Nieduszynski, Mackie, Parker, & Smolko, 1973a, 1973b; Haug et al., 1967). Alginate has a number of free hydroxyl and carboxyl groups distributed along the backbone, therefore it is an ideal candidate for chemical functionalization. By forming alginate derivatives through functionalizing available hydroxyl and carboxyl groups, the properties such as solubility, hydrophobicity and physicochemical and biological characteristics may be modified (Yang, Xie, & He, 2011). Alginates are widely used as a gelling agent for thickening foods and cosmetics. Our strategy is based on hydrophobic modification of alginate, which is regarded as an emulsifier used in emulsions.

In this study, we intend to prepare amphiphilic alginate derivative by the addition of alkyl groups to the backbone of the native alginate. And the derivative will be used as an emulsifier to prepare an oil-in-water emulsion. The objective of this study is to investigate the influence of this emulsifier concentration on the stability of the emulsions by measuring particle size distribution, microstructure, viscosity, and creaming, so as to determine the range of experimental conditions where this amphiphilic alginate derivative can be used to improve emulsion stability by interfacial adsorption. This knowledge could be used to design and

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doi:10.1016/j.carbpol.2011.09.046

fabricate food and cosmetics emulsions with novel or improved properties.

2. Experimental

2.1. Materials

Sodium alginate (NaAlg, $\bar{M}_n$~430 kDa, M/G = 0.18), dodecanol, 4-(N,N-dimethylamino) pyridine (DMAP), toluenesulfonic acid (TSA), formamide (FA), dimethyl formamide(DMF), absolute ethanol were bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) was purchased from Sangon Biotech Co., Ltd (Shanghai, China). Sunflower oil was obtained from a local supermarket. Distilled water was used for the preparation of all solutions.

2.2. Synthesis and purification of the amphiphilic dodecanol alginate (DA)

DA was prepared according to the literatures (Vallée et al., 2009; Yang, Zhang, & Wen, 2007) with some modifications. NaAlg (2.0 g) was partially protonated in 70 mL of FA/DMF (10/9, v/v) containing TSA (1.0 g) by stirring at 55 °C for 30 min. Thereafter, the reaction between the carboxylic acid groups of protonated sodium alginate and the hydroxyl of dodecanol was carried out at 45 °C for 30 h, after adding of EDC-HCl (0.76 g), DMAP (0.95 g) and dodecanol (9.4 g). The reaction mixture was poured into 4 volumes of ethanol. The precipitated product was separated by centrifugation, thoroughly washed with absolute ethanol, then dissolved in the distilled water and neutralized by adding 1.5 wt% Na_2CO_3 solution. The solution was dialyzed against the distilled water for 4 days and lyophilized to get the pure product.

2.3. Preparation of emulsions

Emulsions contained sunflower oil (10 wt%). The aqueous phase contained various concentrations of DA (0.3–1.2 wt%). Oil in water emulsions were prepared by first mixing the oil into the aqueous phase using a superfine homogenizer FA25 (Fluko Equipment Shanghai Co., China) at 10,000 rpm for 3 min, followed by an ultrasonic processor VCX 750 (Sonics & Materials Inc., USA) with the energy output of the probe setting to 300 W for 3 min.

2.4. Fluorescence microscopy

Images of the emulsion microstructure were acquired using a DMRXA fluorescence microscope system (Leica, Germany). Approximately 10 mL of emulsion was placed in a test tube, and moderate rhodamine 6G (Rh6G) aqueous solution (1 mg/mL) was added and mixed for 30 min. The mixture was then dropped on a microscope slide and covered with a coverslip before observed.

2.5. Droplet sizing and creaming measurements

The droplet size distribution in the emulsions was measured using a BT-9300H laser particle size analyzer (Bettersize Co., China), and the data were analyzed using an optical model for a fluid with real parts of the complex refractive index set to 1.333 and 1.449 for the continuous and dispersed phases, respectively.

Creaming experiments were performed in 12 mm × 140 mm capped test tubes. Ten grams of emulsion was transferred into a test tube, and then stored at room temperature. The cream layer was determined visually and measured. The extent of creaming can then be simply characterized by a creaming index ($CI = 100 \times H_S/H_T$) of the emulsion at each observation time t, where H_T is the total height of the emulsion and H_S is the height of the serum layer.

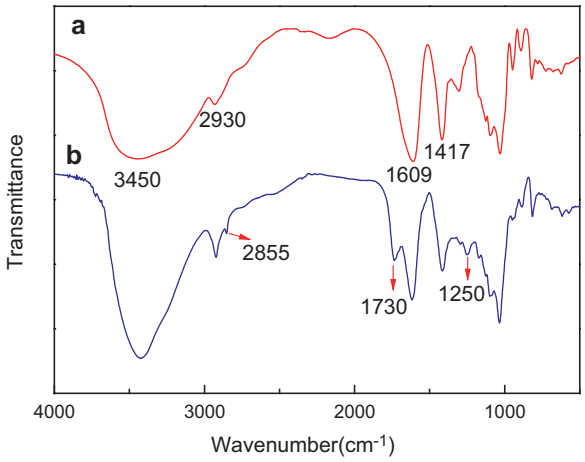

Fig. 1. FT-IR spectra of (a) NaAlg and (b) DA.

2.6. Steady shear measurements

Steady shear measurements were conducted in a rheometer (Haake RS600, Thermo Electron Co., USA) using a cone-and-plate geometry, with a cone angle of 1° and a diameter of 60 mm. The samples were introduced onto the plate for 5 min to eliminate residual shear history, and then carry out experiments immediately. The measuring device was equipped with a temperature unit that gave good temperature control (25 ± 0.05 °C) over an extended time in this work.

3. Results and discussion

3.1. Characterization of DA

NaAlg was hydrophobically modified by use of the coupling agent EDC-HCl to form ester linkages between dodecanol molecules and the carboxylate moieties on the alginate polymer backbone. The structures of samples were confirmed by FT-IR (Tensor 27, Bruker) as shown in Fig. 1.

From the spectrum of NaAlg (Fig. 1a), it was being observed that a broad peak at 3450 cm^{-1} was due to the stretching vibrations of O–H, and a small peak at 2930 cm^{-1} was attributed to the C–H stretching vibrations of methyne groups. The bands at 1090 and 1030 cm^{-1} were assigned to C–O–C stretching vibrations of the saccharide structure. It was further noted that two strong peaks at 1609 and 1417 cm^{-1} were assigned to asymmetric and symmetric stretching vibrations of carboxyl groups. Comparing that of NaAlg (Fig. 1a), the spectrum of DA (Fig. 1b) contained the characteristic hydroxyl and carboxyl bands, but also featured additional peaks. The peak of 2855 cm^{-1} was assigned to methylene groups of dodecyl, and the peaks of 1730 and 1250 cm^{-1} were attributed to the C=O and C–O component of an ester bond respectively. The appearance of the peaks suggested that dodecyl groups successfully grafted onto alginate.

Substitution ratio ($N_{dodecyl}/N_{hexuronic}$) was determined by gas chromatography (Agilent GC 6890N, column SE 30 Chromosorb W-HP, length 2 m; injection temperature 280 °C, column temperature 230 °C; nitrogen flow 25 mL/min) on aliquots (100 mg) first subjected to alkaline hydrolysis, followed by toluene extraction of the resulting dodecyl alcohol. It was proved that substitution ratio value of the DA was 8.47%.

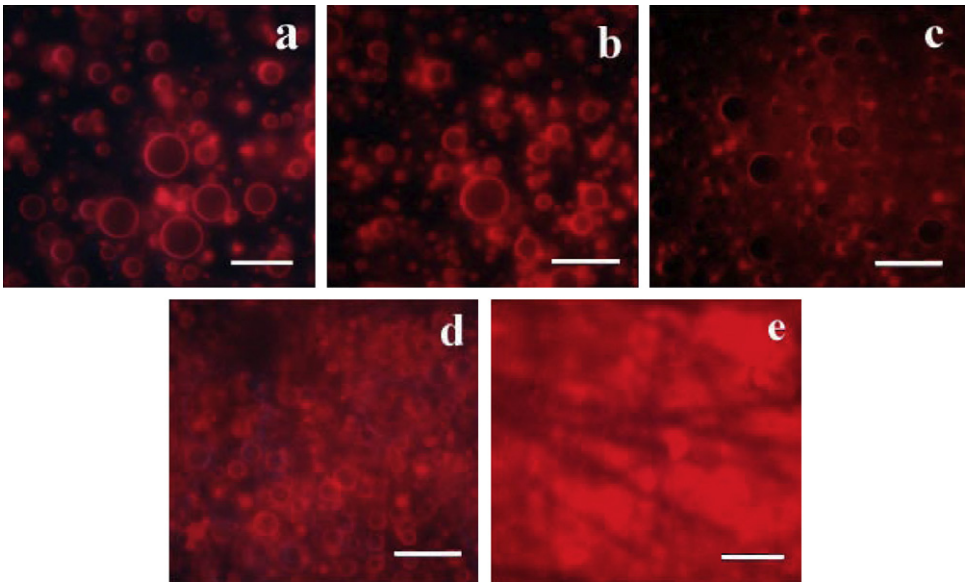

Fig. 2. The fluorescence photomicrograph of the emulsions prepared with DA (a) 0.5 wt%, (b) 0.8 wt%, (c) 1.0 wt%, (d) 1.2 wt%, and (e) NaAlg 1.0 wt%.

3.2. Microstructure of emulsions

Fig. 2 shows the fluorescence microscopy images of various O/W emulsions, which were prepared with DA (0.5–1.2 wt%) or NaAlg (1.0 wt%). Because of opposite charges attracted, the fluorescent dye of Rh6G with positive charges bound to the DA or NaAlg with the negative charges, so that the region of macromolecules appeared red. Oil droplets, however, appeared nonfluoresced black spherical area because there was no interaction between Rh6G and the molecules of oily phase. In DA systems (Fig. 2a–d), we could clearly observe a red ring encompassing a dark spot. The bright layer outside oil droplet showed that the macromolecular membrane formed due to the DA molecular adsorption at the oil/water interface. But in NaAlg system (Fig. 2e), a large area appeared bright red. We know that NaAlg, without hydrophobic groups, is a kind of water-soluble macromolecules, so distributes homogeneously in continuous phase of the emulsion.

3.3. Emulsion stability

The emulsion stability was evaluated by droplet size measurements and visual inspection of the emulsions after different storage times at room temperature. The obtained emulsions, in this study, presented polydisperse systems and the diameter of the droplets fell somewhere in the range of 0.1–10 μm. The measurements of droplet size and creaming index of the emulsions are shown in Table 1. In the emulsions containing DA, varying the concentrations of DA from 0.3 to 1.2 wt% caused slightly a decrease in median diameter of droplets from 2.93 to 1.93 μm, which apparently did not change significantly during the storage period. However, the droplet median diameter of the emulsion containing NaAlg increased distinctly from 1.98 to 7.33 μm over the 30-day storage period. Moreover, for the 1.0 wt% NaAlg, 0.3 and 0.5 wt% DA emulsions, CI increased from 0 to 80.5, 65.5 and 60.9, respectively, and for the emulsions containing higher DA concentrations (0.8–1.2 wt%), CI unchanged at 0 over the 30-day storage period. The instability of the emulsions is also clearly visible in the photograph shown in Fig. 3. In the emulsions of 1.0 wt% NaAlg, 0.3 and 0.5 wt% DA it was obvious to visually discern two layers, thus indicating that creaming had occurred. Remarkably, in the NaAlg emulsion a lower serum layer was transparent because it contained no droplets that scatter light. During storage process the droplets in the emulsion of NaAlg tend to aggregate with one another and form larger ones. Since they have a lower density than the surrounding liquid, the droplets move upwards rapidly and a droplet-depleted serum layer is formed at the bottom of the container. But in the emulsions of 0.3 and 0.5 wt% DA a turbid serum layer appeared at the bottom, where the smaller droplets may remain dispersion. It is most likely related to the fact that DA adsorb to the surface of droplets to form a protective coating that prevent the droplets from aggregating with one another. At higher DA concentrations (0.8–1.2 wt%) the emulsions did not appear creaming, and presented better stability during storage.

3.4. Viscosity of emulsion

The shear viscosity for the emulsions is shown in Fig. 4. The viscosity of emulsion containing 1.0 wt% NaAlg was about an order

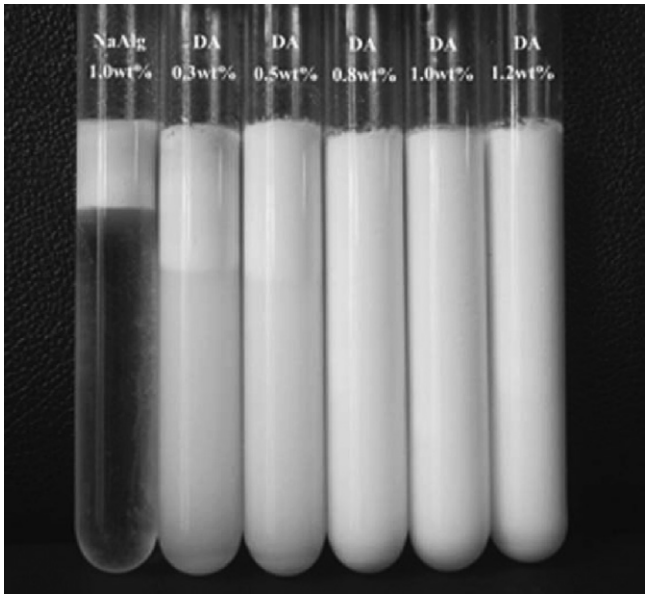

Fig. 3. Photograph of the emulsions over 30-day storage period at room temperature.

Table 1
The measurements of droplet size and creaming index of the emulsions after different storage times at room temperature.

Batches	1 h		24 h		1 week		1 month	
	MD[a] (μm)	CI[b]	MD (μm)	CI	MD (μm)	CI	MD (μm)	CI
1.0 wt% NaAlg	1.98	0	2.91	1.3	6.13	52.6	7.33	80.5
0.3 wt% DA	2.92	0	2.93	0	3.04	36.2[c]	3.10	65.5[c]
0.5 wt% DA	2.72	0	2.70	0	2.93	28.5[c]	3.05	60.9[c]
0.8 wt% DA	2.23	0	2.25	0	2.24	0	2.25	0
1.0 wt% DA	2.06	0	2.07	0	2.07	0	2.10	0
1.2 wt% DA	1.94	0	1.93	0	1.97	0	1.96	0

[a] Median diameter.
[b] Creaming index.
[c] A turbid serum layer at the bottom of the emulsion.

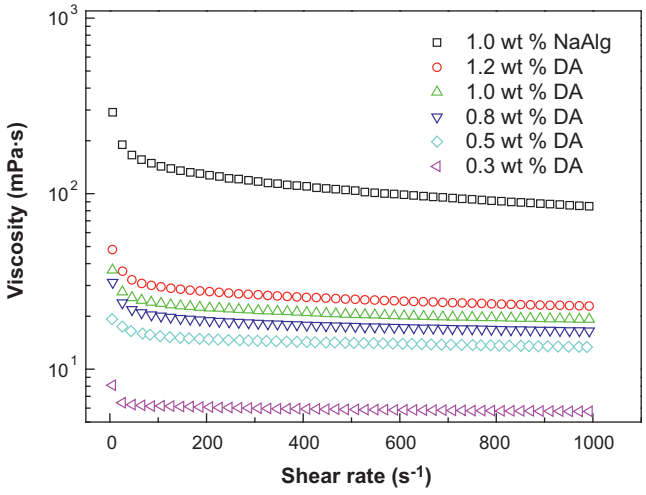

Fig. 4. Shear rate dependence of the viscosity for the emulsions at 25 °C.

of magnitude higher than that of the same concentration DA. The result clearly demonstrates that the DA molecules are mainly adsorbed at the surface of droplets, and the NaAlg molecules are mainly distributed in continuous phase of the emulsion, because the viscosity of an emulsion is proportional to the viscosity of the continuous phase (Allouche, Tyrode, Sadtler, Choplin, & Salager, 2004; Tyrode, Allouche, Choplin, & Salager, 2005). This result consists with microstructure analysis of the emulsions. Moreover, as can be seen from Fig. 4, the viscosity of emulsion increases with DA concentration. At low DA concentrations (0.3–0.5 wt%) the viscosity of emulsions was too small to slow down the upward movement of bigger droplets, so led to creaming as indicated by similar visual appearances in Fig. 3. Higher DA concentrations (0.8–1.2 wt%) increased the emulsion stability. This is due mainly to increase in both viscosity of emulsion and strength of protective layer of droplet with DA concentration increasing.

4. Conclusions

In this research, we synthesized an amphiphilic macromolecule DA and then, prepared oil in water emulsions with DA or NaAlg. The DA, as emulsifier, can be adsorbed on the oil/water interface of droplets in emulsion to prevent the droplets from aggregating with one another. In the emulsions containing DA, varying the concentrations of DA from 0.3 to 1.2 wt% cause a decrease in droplet size and an increase in viscosity, so increase the emulsion stability. However, in the emulsion containing 1.0 wt% NaAlg, there is not a protective coating on surface of droplets to prevent the aggregation of droplets, and creaming had occurred during storage, though the viscosity was about an order of magnitude higher than that of the same concentration DA.

Acknowledgements

We gratefully acknowledge the financial support from the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (08KJB530007) and the Open Project Program of the State Key Laboratory of Food Science and Technology (SKLF-KF-200906).

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