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Interactions and hybrid complex formation of anionic algal polysaccharides with a cationic glycine betaine-derived surfactant

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ABSTRACT

The interaction between anionic algal polysaccharides ((κ)-, (ι)-, (λ)-carrageenans, alginate and ulvan) and a cationic glycine betaine (GB) amide surfactant possessing a $C_{18:1}$ alkyl chain has been studied using isothermal titration calorimetry (ITC), zeta-potential measurements, dynamic light scattering (DLS), transmission electron microscopy (TEM), atomic force microscopy (AFM), and surface tension measurements. It was observed that this cationic surfactant derived from renewable raw materials induced cooperative binding with the anionic polymers at critical aggregation concentration (CAC) and the CAC values are significantly lower than the corresponding critical micelle concentration (CMC) for the surfactant. The CMC of cationic GB surfactant was obtained at higher surfactant concentration in polysaccharide solution than in pure water. More interestingly, the presence of original polysaccharide/surfactant hybrid complexes formed above the CMC value was evidenced from (κ)-carrageenan by microscopy (TEM and AFM). Preliminary investigations of the structure of these complexes revealed the existence of surfactant nanoparticles surrounded with polysaccharide matrix, probably resulting from electrostatic attraction. In addition, ITC measurements clearly showed that the interactions of the κ-carrageenan was stronger than for other polysaccharides ((ι)-, (λ)-carrageenans, alginate and ulvan). These results may have important impact on the use of the GB amide surfactant in formulations based on algal polysaccharides for several applications such as in food, cosmetics, and detergency fields

1. Introduction

Algal polysaccharides represent a wide family of biopolymers that are derived from red seaweeds such as carrageenans, brown seaweeds such as alginates, and green seaweeds such as ulvans. These hydrocolloids have been widely used in industry (especially alginates and carrageenans) because of their rheological properties (gelling and thickening agents) and their biological activities (anticoagulant, antiviral, and immune-inflammatory) that are highly valuable in food, cosmetic and pharmaceutical applications (Kraan, 2012). Carrageenans are linear sulfated polysaccharides composed of repeating disaccharide units with alternating 3-linked β-D-galactopyranose and 4-linked α-galactopyranose or 3,6-anhydro-α-galactopyranose (Fig. 1a). There are three main varieties of carrageenan, which differ mainly in their degree of sulfation. Kappa (κ)-carrageenan is composed of alternating 3-linked β-D-galactose 4-sulfate and 4-linked 6-anhydro-α-galactopyranose with one elementary charge per disaccharide repeating unit. Iota (ι)-carrageenan has two sulfate groups per disaccharide repeating unit. Lambda (λ)-carrageenan has three sulfate groups per disaccharide unit, but they do not include any 3,6-anhydride bridge contrary to the first two carrageenans (Campo, Fabio, Braz da Silva, & Carvalho, 2009). (κ)-carrageenan and (ι)-carrageenan form gels, whereas no gel formation has been observed for the most highly charged (λ)-carrageenan. Alginates are anionic polysaccharides that contain linear blocks of covalently (1-4)-linked β-D-mannuronate (M) with the C5 epimer α-L-guluronate (G) (Benvegnu & Sassi, 2010). Within the structure of alginate, the M and G monomers are sequentially assembled in either homopolymeric repeating (MMM or GGG) blocks or heteropolymeric alternating (MGMG) blocks (Fig. 1b). It has been shown that the physical properties of alginates depend on the relative

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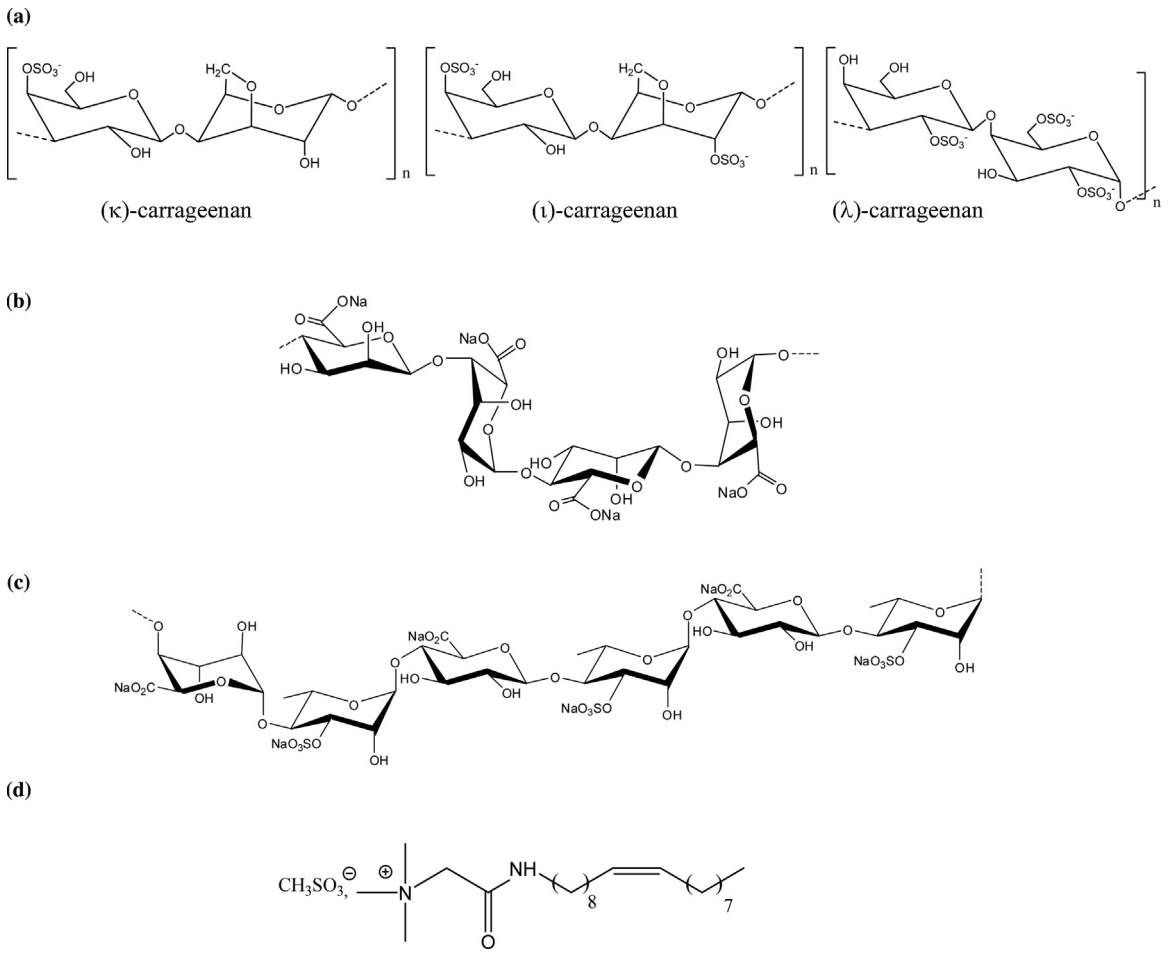

Fig. 1. Chemical structure of carrageenans (a); alginate (b); ulvan (c); and glycine betaine (GB) amide surfactant **1** (d).

proportion of these three types of blocks (Haug, Larsen, & Smidsrod, 1967; Smidsrod & Haug, 1972a; Smidsrod, Haug, & Whittington, 1972b). For example, the ability to form gels from alginate, by addition of calcium ions, involves the G blocks so the higher the proportion of these, the greater the gel strength (Draget & Taylor, 2011). Ulvans are complex polysaccharides composed of sulfate, rhamnose, xylose, iduronic and glucuronic acids as main constituents (Fig. 1c) (Lahaye & Robic, 2007). They possess structural properties close to glycosaminoglycans such as hyaluronan and chondroitin sulfate due to its content of glucuronic acid and sulfate constituents (Chiellini and Morelli, 2010; Dash et al., 2014). Ulvans form gels in the presence of boric acid, calcium ions and at a pH between 7.5 and 8.0 (Haug, 1976).

Amphiphilic versions of these polysaccharides could represent an attractive strategy to improve their properties and functions. They can be achieved either by chemical modification of the macromolecular backbone (Yang, Zhou, & He, 2013), or by interactions between the anionic biopolymer and surfactants of opposite charge. Many practical systems for industrial applications contain mixtures of polymers and surfactants, widely used in foods, cosmetics, detergents, personal-care products and mineral processing (Goddard & Ananthapadmanabhan, 1993; Ivanov, Kamenova, Georgieva, Kamenska, & Georgiev, 2006). The association of surfactants with polysaccharide chains can give rise in aqueous media to soluble or insoluble complexes/aggregates with various properties depending on several parameters, such as electrostatic, hydrophobic, dipolar, and hydrogen bonding interactions and steric hindrance effects. Changes in the structure of the polymer and/or the surfactant, the charge density of the polyanion and the relative ratio between the oppositely charged polymer–surfactant pair may also affect the structure and the physicochemical properties of these mixed systems. Polymer/molecule or polymer/macromolecule complexes resulting from electrostatic interactions were recently developed to exhibit attractive rheological properties or to form nanoparticle (NP) systems for drug delivery (Wu et al., 2007, 2014). In particular, new hybrid polymer/protein complex NPs were prepared by utilizing the electrostatic interaction between proteins and NPS made from diblock copolymer of poly(lactide-*co*-glycolic acid) (PLGA) and L-arginine-based polycation (PC). This study highlighted a novel strategy for the robust delivery of proteins and other relevant molecules.

Generally, when surfactants are introduced into a solution containing oppositely charged polysaccharide, electrostatic interactions occur until complete neutralization of the polyanionic biopolymer. Surfactants in the presence of oppositely charged polysaccharides start forming aggregates with the polysaccharides at the so-called critical association concentration, CAC, involving both strong electrostatic binding and aggregation of alkyl chains of the bound surfactant ions (Wang, Li, Li, & Niu, 2014; Holmberg, Jönson, Kronberg, & Lindman, 2003; Kogej & Kerjanc, 2001). CAC is usually far below the critical micelle concentration, CMC, of a particular surfactant.

Over the last decade, mixed systems composed of cationic surfactants and anionic polysaccharides were investigated increasingly. In particular, the interaction of lauric arginate (LAE), a food-grade cationic surfactant with a high antimicrobial activity, with various biopolymers was analyzed to evaluate the effect

of electrostatic attraction and complex/aggregate formation on physicochemical and antimicrobial properties (Asker, Weiss, & McClements, 2009; Bonnaud, Weiss, & McClements, 2010; Loeffler et al., 2014). In addition, Vinceković, Bujan, Smit, and Filipović-Vinceković (2005) investigated phase behavior in mixtures of *n*-dodecylammonium chloride, DDAC, and carrageenans. Structural characteristics of insoluble DDAC/carrageenan complexes revealed a lamellar ordering of surfactant and the formation of giant vesicles in these systems at low carrageenan concentrations.

In our laboratory, new cationic ester-type and amide-type surfactants based on natural glycine betaine (GB) and vegetable oils as renewable raw materials were recently developed on a kilogram scale using environmentally friendly starting materials and chemical processes. The aerobic ultimate biodegradability of these surfactants was evaluated applying the "CO_2 evolution" test. This test is included in the European Regulation on biodegradability of detergent surfactant (European regulation (EC) No. 648/2004). A surfactant is considered as readily biodegradable if the biodegradation level exceeds 60% within 28 days in this test. High biodegradation level (>70%) was reached with GB-derived surfactants that allowed these formulations to be classified as readily biodegradable (Goursaud et al., 2008). Some formulations containing these GB-derived surfactants were found to exhibit remarkable emulsifying properties for road making or other industrial applications (Goursaud et al., 2008). Unlike GB-esters, GB amide-type surfactants were found to exhibit weak pH sensitivity under neutral and basic conditions, thereby opening a larger range of application fields. Within this context, a GB amide surfactant **1** derived from oleyl amine as the lipophilic chain (betainylaminooctadec-9-ene methanesulfonate), was selected to evaluate the aqueous behavior of this original emulsifier in the presence of anionic polysaccharides.

In this study, we characterized the interactions between the cationic GB amide surfactant **1** (Fig. 1d) and various anionic algal polysaccharides (carrageenans, alginate, ulvan) in order to understand the formation of aggregates or complexes in aqueous solution. In the case of carrageenans, the three more commercially distributed forms (κ, ι and λ) were investigated and provided information on the influence of charge, position of sulfate groups and chemical structure in comparison with other biopolymers (alginate and ulvan). Furthermore, the effects of these interactions on the surface activity of surfactant in the presence of κ-carrageenan were evaluated, as an example. For that purpose, we used a variety of analytical methods, such as isothermal titration calorimetry, dynamic light scattering, surface tension measurements, transmission electron microscopy (TEM) and atomic force microscopy (AFM) to characterize these interactions as well as the structure of the mixed complexes.

2. Materials and methods

2.1. Chemicals

GB amide surfactant **1** (Fig. 1d) was prepared following a one-pot two step procedure as already reported (Goursaud et al., 2008). κ-Carrageenan was purchased from Gelimar (France) and furnished by the CEVA (Centre d'Etude et de Valorisation des Algues, Pleubian, France). The average molecular weight of the polysaccharide, 1484 000 g/mol, was determined from standard curve of pullulan (g/mol) and measured with size exclusion chromatography associated to a refraction index detector. In the case of ι-carrageenan and λ-carrageenan, they were also purchased from Gelimar (France) and the average molecular weight of polysaccharides were equal to 5152 715 g/mol and 2171 930 g/mol, respectively, using standard curve of pullulan (g/mol). Alginate was purchased from Cargill corporation and the average molecular weight of the polysaccharide is equal to 185 600 g/mol. Ulvan was extracted from *Ulva rotundata* and furnished by the CEVA. The average molecular weight of the polysaccharide is equal to 963 000 g/mol and was determined as before.

2.2. Solution preparation for analysis by isothermal titration calorimetry (ITC)

Cationic surfactant solution was prepared by dispersing 25 mg of GB amide surfactant **1** into 15 mL of Milli-Q water leading to a concentration of 3.6 mM. Polymer solutions (κ, ι, λ-carrageenans; alginate; ulvan) were prepared by dispersing 10 mg of polysaccharide into 100 mL of Milli-Q water to a final concentration of 0.1 g/L. Before titration, polymer and surfactant solutions were stirred thoroughly overnight to assure complete dissolution of both compounds.

2.3. Isothermal titration calorimetry (ITC)

An isothermal titration calorimeter (VP-ITC, LLC Microcal Inc., Northampton, MA, USA) was used to measure enthalpies of mixing at 30 °C. Fifty-eight 5 μL aliquots of cationic GB amide surfactant **1** solution (3.6 mM) were injected sequentially into a 1480 μL titration cell initially containing 0.1 g/L of polymer solutions. Each injection lasted 10 s with an interval of 240 s between successive injections. The solution in the titration cell was stirred at a constant speed of 307 rpm throughout the experiments. All solutions were degassed prior to the measurements being carried out. The resulting heat-flow time curves were integrated using the instrument's software to give interaction enthalpy-cationic surfactant concentration curves.

2.4. Surface tension measurement

Surface tension measurements were carried out using a Krüss apparatus with platinum ring method. The ring was thoroughly cleaned and flame-dried before each measurement. To measure the surface tension, the vertically hung ring was dipped into the liquid and then subsequently pulled out. The sample solution of GB derivative **1** was concentrated about 5 times the CMC and was progressively diluted with water containing or not κ-carrageenan at the concentration of 0.1 g/L. Three successive measurements were performed and the deviation standard deviation did not exceed ± 0.1 mN/m. The temperature was controlled to within ±0.1 °C by circulating thermostated water through the jacketed cell (conic vessel in PTFE), sample solution was continuously stirred using a magnetic stirrer before measurements. The CMC value was estimated from plots of surface tension as a function of surfactant concentration.

2.5. Size measurement by dynamic light scattering (DLS)

The average diameters of particles solutions formed after ionic interactions between a κ-carrageenan and various concentrations of GB amide surfactant **1** were measured using dynamic light scattering (Beckman Coulter instruments Delsa^{TM} Nano C particle analyser) at 25 °C. The Z-average diameter and the polydispersity index (PDI) of the samples were automatically provided by the instrument using cumulant analysis. The diameter obtained was an average value based on three consecutive measurements for each sample and calculated from the analysis of the auto-correlation function. The auto-correlation function of scattering data was analyzed via the CONTIN method to obtain the distribution of diffusion coefficients (D) of the solutes. The apparent hydrodynamic radius (R_h) was deduced from D by the Stokes–Einstein

equation $R_h = kT/6\pi\eta D$, where k is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity, respectively. The PDI provided by DLS was a reasonable way to appreciate if the sample size distribution could be considered as monomodal. All samples reported in that work had PDI values lower than 0.35, which could be considered as characteristic of a reasonable agreement of monomodal model with real droplet size distributions of the samples.

2.6. ξ-Potential measurement

The electrical charge of various suspensions of particles was assessed by ξ-potential measurements. The values of ξ-potential registered were performed with a Beckman Coulter instruments Delsa™ Nano C particle analyser at 25 °C. The ξ-potential was calculated from the measurement of the electrophoretic mobility of particles in an applied oscillating electric field using laser Doppler velocimetry. The different suspensions of particles (final volume of solution of 10 mL) were prepared by mixture of different concentrations of cationic surfactant **1** (ranged from 0.09–0.9 mmol/L, equivalent to 41.65–416.5 mg/L of GB amide surfactant **1**) with a κ-carrageenan solution at the concentration of 0.1 g/L.

2.7. Preparation of κ-carrageenan/cationic surfactant complex solutions for analysis by microscopy (TEM and AFM)

Cationic surfactant solution was prepared by dispersing 250 mg of GB amide surfactant **1** into 15 mL of Milli-Q water leading to a concentration of 16.67 g/L (36.02 mM). κ-Carrageenan solution was prepared by dispersing 100 mg of polysaccharide into 100 mL of Milli-Q water to a final concentration of 1 g/L (0.2494 mM). A first sample (**A**) containing a mixture of κ-carrageenan at a concentration of 0.095 g/L and cationic surfactant **1** at a concentration of 0.083 g/L was first prepared by mixing 9.5 mL of the κ-carrageenan solution and 0.5 mL of the cationic surfactant solution, and then 200 μL of the resulting solution was diluted by adding 1.8 mL of Milli-Q water. A second sample (**B**) containing a mixture of κ-carrageenan at a concentration of 0.0825 g/L and cationic surfactant **1** at a concentration of 0.29 g/L was then prepared by mixing 8.25 mL of the κ-carrageenan solution and 1.75 mL of the cationic surfactant solution of the cationic surfactant solution, and then 200 μL of the resulting solution was diluted by adding 1.8 mL of Milli-Q water.

2.8. Microscopy observation of the structure of κ-carrageenan/cationic surfactant complexes

2.8.1. TEM

A drop of 50 μL of each aqueous dispersion specimen was first placed on a carbon-coated TEM copper grid (Quantifoil, Germany) previously submitted to a glow-discharge to ensure hydrophilicity. The sample was then negatively stained with uranyl acetate (Merck, Germany). For that, the sample-coated TEM grid was successively placed on a drop of an aqueous solution of uranyl acetate (2% w/w) for negatively staining, and on a drop of distilled water for rincing. The grid was then air-dried before introducing them in the electron microscope. The samples were viewed using a JEOL JEM-1230 (Jeol, Japan) operated at 80 kV and equipped with a LaB_6 filament. All the micrographs were recorded on a Gatan 1.35 K × 1.04 K × 12 bit ES500W CCD camera. For each sample, the experiment – sample preparation and observation – was repeated to ensure repeatability, and to get enough images from different zones for further statistical analysis.

2.8.2. Image analysis

The software Image J *(Research Services Branch NIMH & NINDS. Image J—Image processing and analysis in Java. Web site: http://rsb.info.nih.gov/ij/)* has been used for image analysis in order to compute a number of statistical sizes from which the size distribution, mean diameter and standard deviation were extracted. As the particle morphology was not rigorously spherical, the diameter was assimilated to the Feret number that corresponds to the longest distance between any two points along the selection boundary. The total number of particles computed was a minimum of 500 for each sample.

2.8.3. AFM

Samples were imaged over an area of 200 nm^2 to 20 μm^2 at 0.2 to 0.5 Hz in the SCANASYST mode operated in fluid on a BioScope Catalyst AFM (Bruker AXS, Santa Barbara, CA) using a small volume liquid cell and a set-point of around 1–2 nN. SCANASIST-Fluid AFM tips (Bruker AFM Probes, Camarillo, CA) with a nominal spring constant, resonant frequency and radius of 0.7 N/m, 150 kHz and 20 nm, respectively, were used for each experiments. AFM data were analyzed using the Nanoscope Analysis 1.5 software (Bruker AXS, Santa Barbara, CA).

2.9. Viscosity analysis

Viscosity measurements were performed using a RheoWin Mars III rheometer (Thermo Fisher Scientific) cone-plate model. The shear viscosity experiments were conducted over an extended shear rate range between 10 and 200 s^{-1}. The viscosity was measured for solutions corresponding to sample (**A**) containing a mixture of κ-carrageenan at a concentration of 0.095 g/L and cationic surfactant **1** at a concentration of 0.083 g/L, and sample (**B**) containing a mixture of κ-carrageenan at a concentration of 0.0825 g/L and cationic surfactant **1** at a concentration of 0.29 g/L. The same study was also investigated from solutions containing the same surfactant/polymer ratios but ten times more concentrated (sample (**A′**): 0.83 g/L of GB surfactant and 0.95 g/L of κ-carrageenan; sample (**B′**): 2.92 g/L of GB surfactant and 0.825 g/L of κ-carrageenan) as well as solutions containing either only GB surfactant at a 2.92 g/L concentration (sample (**C**)), or only κ-carrageenan at a concentration of 0.95 g/L (sample (**D**)). All measurements were twice realized at room temperature.

3. Results and discussion

3.1. Solution properties of cationic GB amide surfactant

The critical micelle concentration (CMC) of GB amide surfactant **1** was determined using ITC by measuring the enthalpy change resulting from titration into water at pH = 7. Heat flow versus time profiles resulting from sequential injections of 5 μL aliquots of surfactant solution (1.67 g/L) into 1438 μL titration cell initially containing water were measured. The surfactant concentrations in the injector were appreciably above the CMC, so that the injector contained mainly surfactant micelles with some surfactant monomers. Initially in the absence of anionic polysaccharide, a relatively large endothermic peak was observed when the surfactant solution was progressively injected into the reaction cell. These enthalpy changes are the result of micelle dissociation because the surfactant concentration in the cell was below the CMC (Fig. 2a). The endothermic nature of these peaks ($\Delta H > 0$) indicates that demicellization must lead to an increase in the overall entropy of the system at this temperature (30 °C), since micelle dissociation is thermodynamically favorable below the CMC ($\Delta G < 0$), and therefore $T\Delta S > \Delta H$. This entropy increase can be attributed to changes in

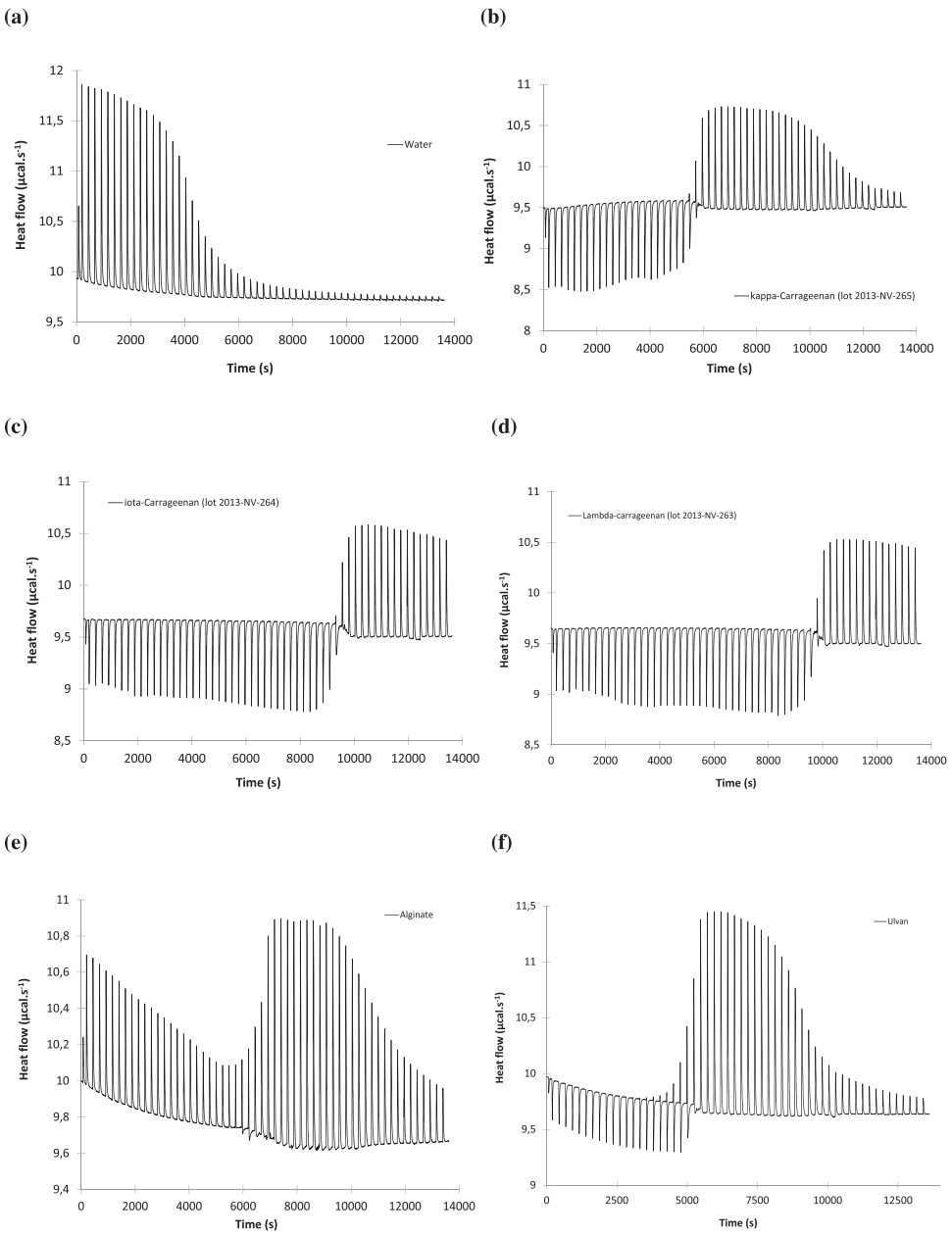

Fig. 2. Heat flow versus time profiles resulting from injection of 5 μL aliquots of 1.67 g/L (3.6 mM) of cationic GB amide surfactant into a 1438 μL titration cell containing different aqueous solutions at pH = 7 and 30.0 °C: (a) water; (b) 0.1 g/L of κ-carrageenan; (c) 0.1 g/L of ι-carrageenan; (d) 0.1 g/L of λ-carrageenan; (e) 0.1 g/L of alginate; (f) 0.1 g/L of ulvan.

the structural organization of water molecules around hydrophobic groups, to the release of surfactant monomers from micelles and of counterions associated with the surfactant head groups when micelles break down to monomers. After a certain number of injections, there was an appreciable decrease in the peak height because the surfactant concentration in the reaction cell exceeded the CMC and thus the micelles titrated into the reaction cell no longer dissociated. Above the CMC, the enthalpy change is therefore solely the result of micelle dilution effects. The dependence of the enthalpy change per injection on the surfactant concentration in the reaction cell was calculated by integration of the heat flow versus time profiles (Fig. 2a). The CMC of the surfactant was determined from the inflection point in the ΔH versus surfactant concentration curves which is equal to 0.23 ± 0.2 mM and corresponding to 106.43 mg/L of GB amide surfactant. A similar value of CMC (≈0.23 mM or 106.43 mg/L) was determined from tension surface measurement (Section 3.8).

3.2. Characterization of interactions of cationic GB amide surfactant with different families of carrageenans: Kappa (κ), iota (ι) and lambda (λ)

In this part of work, the enthalpy changes associated with interactions between increasing amounts of cationic surfactant **1** solution at the concentration of 3.6 mM and different types of carrageenans (κ, ι and λ) at the concentration of 0.1 g/L were measured by ITC at 30 °C. According to the structure (positions of sulfate groups) and the number of charges along the polysaccharide chain, and the molecular weight, polysaccharides associated by ionic interactions with cationic surfactant could exhibit particular behaviors such as the formation of particles (in suspension) or aggregates (precipitate form). The energy of such interactions can be quantified by ITC tool that permitted to plot heat flow versus times profiles, as illustrated in Fig. 2b–d. We observed that each injection of 5 μL aliquots of cationic surfactant in the reaction cell

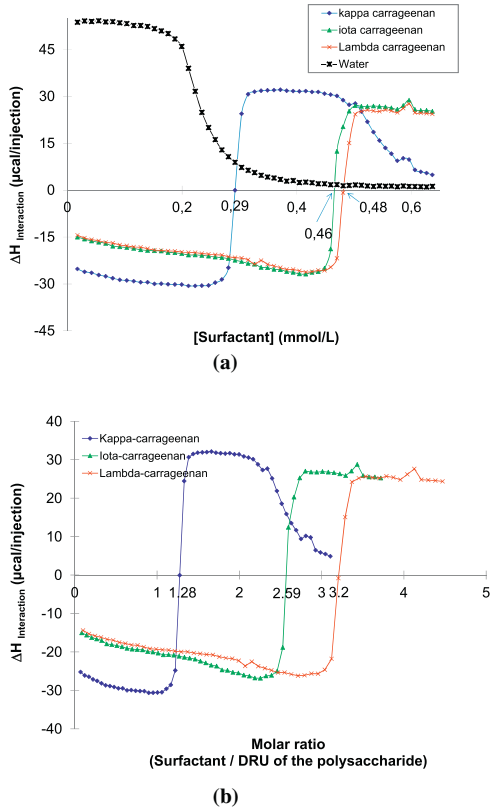

Fig. 3. (a) Interaction enthalpy change ($\Delta H_{interaction}$) versus GB surfactant concentration (mM) when 1.67 g/L of GB surfactant solution (pH = 7) was injected into a reaction cell containing 0.1 g/L of (κ)-, (ι)-, (λ)-carrageenans at pH = 7, 30 °C. Profiles of interaction enthalpy change ($\Delta H_{interaction}$) were obtained by subtracting the ΔH profile of GB surfactant solution (i.e. $\Delta H_{interaction} = \Delta H_{polysaccharide} - \Delta H_{surfactant}$); (b) Interaction enthalpy change ($\Delta H_{interaction}$) versus molar ratio of GB surfactant to disaccharide repeating unit (DRU) of (κ)-, (ι)-, (λ)-carrageenans (pH = 7, 30 °C).

containing 0.1 g/L of polysaccharide was marked by an appreciable change in the enthalpy titration curves (enthalpy value became more negative) compared to the control solution without anionic polysaccharide (Fig. 2a). It is convenient to divide the enthalpy versus surfactant concentration profiles into a number of different regions for the cationic surfactant/carrageenan systems depending on the proposed interaction that takes place (Fig. 3a).

In the case of κ-carrageenan three regions were observed. Indeed at relatively low cationic surfactant concentrations [0 < *c* < 0.29 mM], the interaction enthalpy was highly exothermic (heat release resulting from the formation of the complexes) and gradually decreased with increasing surfactant concentration, indicating strong surfactant binding until saturation is reached. It is likely that the positive head polar group of GB amide surfactant bound to sulfate groups on the κ-carrageenan through electrostatic attractions, as schematized in Fig. 14. In other word, we supposed that surfactant micelles titrated in the reaction cell broke down into monomers, which then bound to κ-carrageenan. Afterward at intermediate cationic surfactant concentrations [0.30 < *c* < 0.48 mM], the interaction enthalpy became highly endothermic, giving evidence of dissociation of non-bound micelles as they were titrated into the reaction cell. However in this region, the interaction enthalpy was less endothermic than in the absence of κ-carrageenan which showed that there was still an exothermic interaction between cationic surfactant likely under micelle-like or particle-like form and sulfate groups onto polysaccharide backbone. Furthermore, we can assume that the polar head group of GB surfactant bound to sulfate groups of the κ-carrageenan, interacts with surfactants under a monomer form

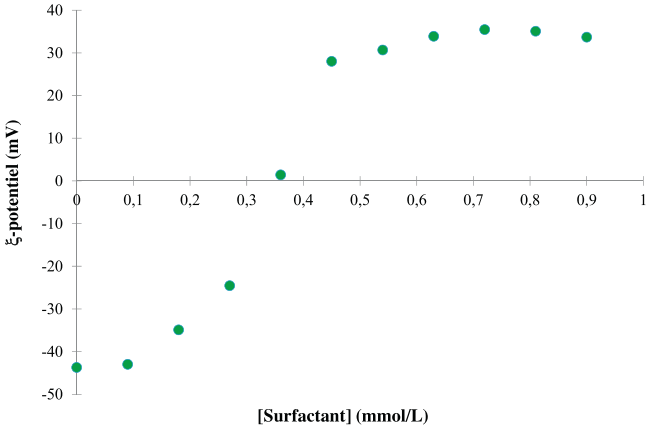

Fig. 4. Dependence of the ζ-potential of GB surfactant/κ-carrageenan solutions on the cationic GB surfactant concentration in the reaction cell. A cationic GB surfactant solution (1.67 g/L at pH = 7) was titrated into aqueous κ-carrageenan solution (0.1 g/L at pH = 7).

in the reaction cell, as illustrated in Fig. 14 that gives an overview in this domain of concentrations investigated. The simple mechanism of interactions schematized in Fig. 14 may be an ideal case in a dilute solution (below CAC) considering that a polymer chain does not interact with other polymer chains. After the polar head groups of cationic surfactant are bound to the totality of polymer sulfate groups, it is probable that associations occurred between surfactants bound to the polymer within the complex formed. It is also possible that associations between the surfactant/polymer complex structures operated in the solution through a reorganization of the complexes, as recently reported in the literature (Wang et al., 2014; Yin et al., 2014). Thus, in the case of dodecyltrimethylammonium bromide (DTAB)/(ι)-carrageenan systems, Yin et al. proposed a mechanism based on the rearrangement of the DTAB/polymer complex structure from lamellar-type structure to spherical-type structure prompted by the hydrophobic interactions.

At relatively high cationic surfactant concentrations [0.49 < *c* < 0.65 mM], the interaction enthalpy became less endothermic indicating that the surfactant concentrations in the aqueous phase exceed the CMC and additionally was close to zero suggesting that little further interactions occurred (i.e. micelle dissociation no longer occurred). Consequently, additional micelles appeared in the solution, as illustrated in Fig. 14. The CMC value was equal to 0.523 mM which represented 242 mg/L of cationic surfactant.

In the case of ι- and λ-carrageenans only two regions were distinguished (Fig. 3) unlike κ-carrageenan. In the manner of κ-carrageenan, at relatively low cationic surfactant concentrations [0 < *c* < 0.46 mM] for ι-carrageenans and [0 < *c* < 0.48 mM] for λ-carrageenans, the interaction enthalpies were highly exothermic and gradually decreased with increasing surfactant concentration, indicating strong surfactant binding until saturation is reached. Nevertheless, the interaction enthalpy changes of ι- and λ-carrageenans presented less magnitude for exothermic and endothermic curves than the one of κ-carrageenan, showing that the nature of interactions of cationic surfactant with various types of carrageenans was different. In addition, we can notice that higher cationic surfactant concentrations (0.46 mM for ι-carrageenans 0.48 mM for λ-carrageenans) were required to reach neutral interaction enthalpy (close to zero) while 0.29 mM of surfactant was required for κ-carrageenan. This result can be explained by the difference of carrageenan chemical structures, particularly the number of sulfate groups along the polysaccharide backbone. For these two ι- or λ-carrageenans, we did not determine CMC values due to the limited volume of the reaction cell of ITC instrument. In addition, we observed that ι- or λ-carrageenan/cationic surfactant

complexes formed large aggregated structures which precipitated instantaneously.

3.3. Influence of sulfated groups of carrageenans onto interaction enthalpy

The effect of charge of various types of carrageenans was featured plotting interaction enthalpy as a function of molar ratio of cationic surfactant to disaccharide repeating units of different types of carrageenans (Fig. 3b). We observed that the molar ratios of cationic surfactant to polysaccharide were about 1.28, 2.59, 3.20 for κ-carrageenan, ι-carrageenan, λ-carrageenan, respectively. These values correspond to neutral interaction enthalpy and they increase with the number of sulfate groups contained in the macromolecular chain. Furthermore, these values are higher than the molar ratio corresponding to the amount of cationic surfactant required to neutralize the charges of the disaccharide repeating units. Two assumptions can be put forward: the first one suggests that surfactants introduced in excess interact under a micelle-type or particle-type form with the polymer/surfactant complexes after neutralization of the polysaccharide charges, and the second one results from the difference of average molecular weights of the various carrageenans. These measurements clearly indicate that there is a distinct behavior of these carrageenans towards GB amide surfactant. These anionic polysaccharides increase the effective CMC by surfactant binding through attractive electrostatic interactions. In particular, the CMC value of the GB amide surfactant **1** in the κ-carrageenan solution was observed for a molar ratio of 2.51 which is lower than with ι- and λ-carrageenans. For the latter, the limited volume of reaction cell of ITC did not permit to perform CMC value measurements.

One of the limitations of ITC is that it only measures the overall enthalpy change of a system and it is not possible to directly isolate the contributions of different physico-chemical mechanisms such as micelle dissociation, monomer binding, micelle/particle binding, biopolymer conformational changes, and biopolymer aggregation. ITC should therefore be used in conjunction with other analytical techniques that can provide complementary information about the system. For this reason, we used microelectrophoresis measurements, TEM and AFM in the case of κ-carrageenan to provide further insights into aggregation and binding interactions. Indeed, very few studies concerning the formation of complexes between κ-carrageenan (Lopez-Pena & McClements, 2014) and cationic surfactants were reported in the literature as it is the case for ι-carrageenan (Bonnaud et al., 2010; Gawel et al., 2013; Yin et al., 2014). Thus, microelectrophoresis measurements were not carried out for the two other carrageenans (i.e. iota and lambda). However, these studies could be achieved in the near future.

3.4. Electrical characteristics of cationic GB amide surfactant/κ-carrageenan complexes

The electrical characteristics of cationic GB amide surfactant/κ-carrageenan complexes were measured by microelectrophoresis to obtain additional information about the nature of the interactions involved (Fig. 4). Indeed, the interactions (hydrogen bonding, hydrophobic interactions and van der Waals forces) will depend strongly on the nature of the biopolymers (namely their neutral or charged chemical structure) and as well as the nature of the cationic agent. Titration experiments of the κ-carrageenan were carried out so as to mimic the ITC experiments: the ξ-potential (electrical charge on the surface) of the complexes was measured for aqueous solutions containing the cationic surfactant at different concentrations and κ-carrageenan at a 0.1 g/L concentration. The ξ-potential of κ-carrageenan solution was highly negative ($\xi = -44$ mV) in the absence of cationic GB derivative (Fig. 4), which can be attributed to the presence of sulfate groups along the carrageenan chain at pH = 7. When increasing amounts of cationic surfactant were injected into polysaccharide solutions, the ξ-potential was increasingly less negative and then it became increasingly positive, which suggests that cationic surfactants formed complexes with κ-carrageenan through electrostatic interactions. Charge neutralization ($\xi = 0$ mV) for κ-carrageenan occurred when cationic GB surfactant concentration reached 0.34 mM which is equivalent to 157.33 mg/L. This concentration corresponded in ITC experiment (Fig. 3a) to the point where micelles dissociate in the reaction cell and where the polysaccharide with cationic surfactant is under a neutral complex form. This observation put forward the interactions of micelles/particles with the cationic surfactant/κ-carrageenan complexes because the interaction enthalpy observed was less endothermic than the one containing exclusively cationic surfactants. Furthermore, for values of ξ-potential from −25 mV to +28 mV and especially for a value of ξ-potential around 0 mV, we observed the formation of large visible aggregates. As a result, we characterized the size of these complexes by DLS for all solutions prepared in the experimental conditions used for ITC and ξ-potential experiments.

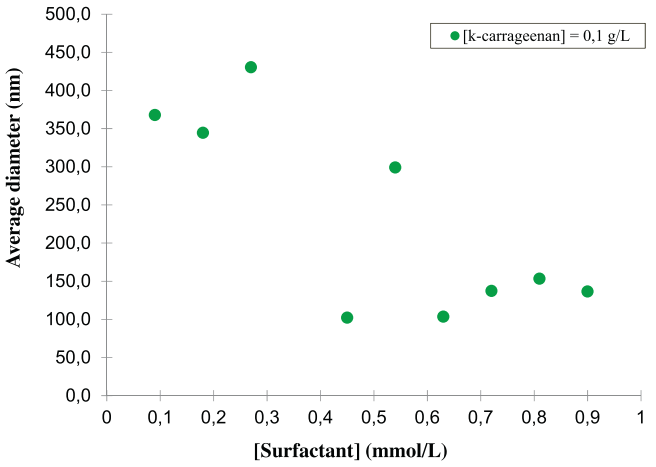

Fig. 5. Size measurements by DLS at 25 °C of aggregated particles (ionic interactions between polysaccharide and surfactant) as a function of the concentration of cationic GB surfactant.

3.5. Influence of cationic GB amide surfactant concentrations on the size of complexes formed with κ-carrageenan by electrostatic interactions

The size of aggregated particles formed by electrostatic interactions between κ-carrageenan and cationic GB derivative in aqueous solutions were measured by dynamic light scattering at 25 °C (Fig. 5). We observed that particles size strongly depended on cationic surfactant concentrations in aqueous solutions. For concentrations of cationic GB surfactant ranging from 0.09 mmol/L to 0.27 mmol/L (corresponding to 41.65 mg/L to 125 mg/L), the average diameter of particles were comprised between 340 nm and 430 nm for values of ξ-potential inferior to zero mV (Fig. 4). Critical aggregation concentration (CAC) corresponding to the neutralization of charges ($\xi \approx 0$ mV) of the κ-carrageenan was determined around 0.34 mM which is equal to 157 mg/L of cationic surfactant. At this CAC, complexes were presented under a precipitate form in the aqueous solution. Therefore, the size measurement of the system was not possible to carry out by DLS indicating that large aggregates were formed. In contrast, for higher concentrations of cationic GB surfactant ranging from 0.45 mmol/L to 0.9 mmol/L (corresponding to 208 mg/L to 416.5 mg/L), the neutralized polysaccharide/surfactant

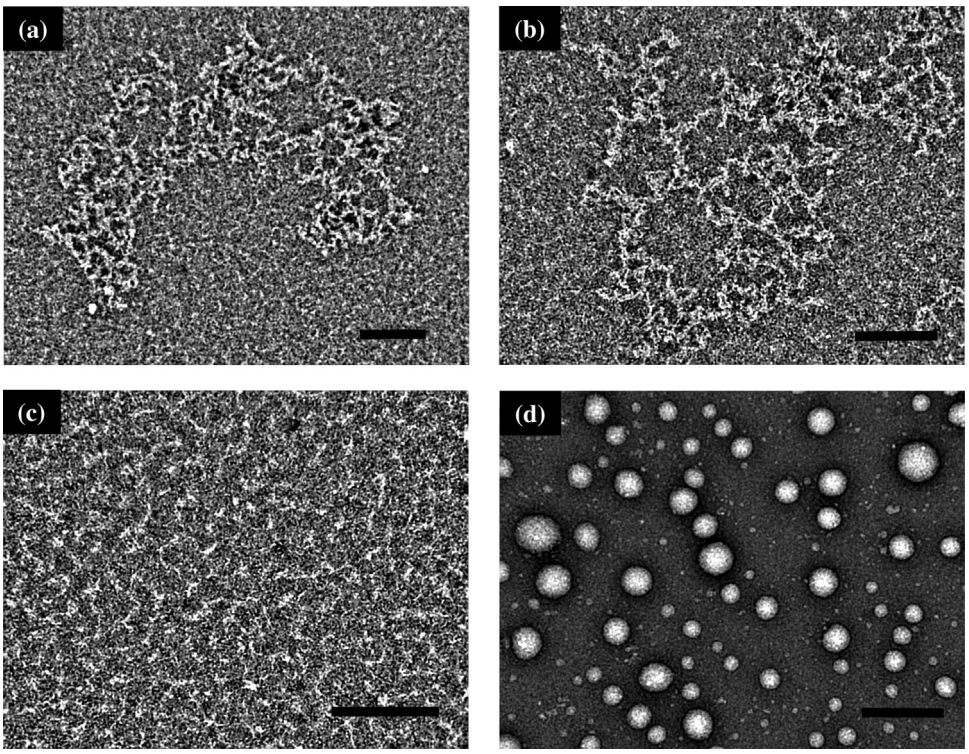

Fig. 6. Negatively stained TEM of aqueous solution (sample (**A**)) containing κ-carrageenan at a concentration of 0.095 g/L and GB amide surfactant **1** at a concentration of 0.083 g/L (0.179 mmol/L). (a)–(b): Aggregates resulting from rolled up κ-carrageenans; (c) mixture of κ-carrageenans with either rolled up or rigid chains; (d) spherical particles. Scalebar: 0.5 μm for (a–d).

aggregates become positively charged. Because of the charge reversal, the polysaccharide/surfactant aggregates resolubilize. Smaller assemblies (with sizes inferior to 160 nm) were obtained that could represent promising materials for engineering nanosystems suitable for drug delivery applications. Moreover, the ξ-potential values of these assemblies in solution were positive ($\xi > +28$ mV), as shown in Fig. 4. In the case of ι- and λ-carrageenans, important aggregation phenomena characterized by the formation

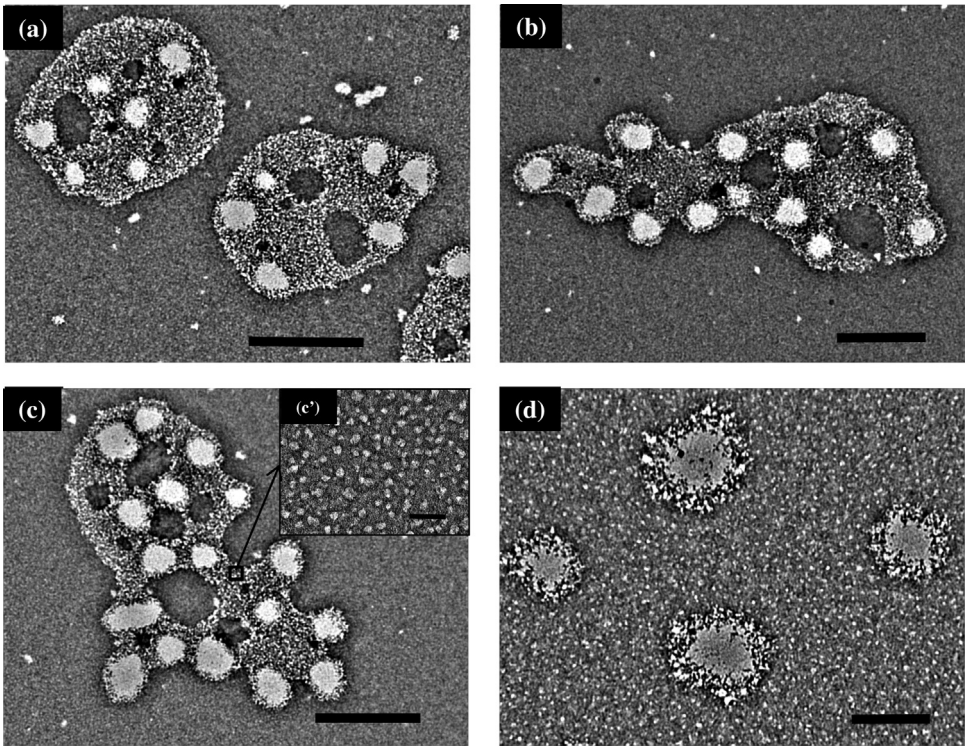

Fig. 7. Negatively stained TEM of aqueous solution (sample (**B**)) containing κ-carrageenan at a concentration of 0.0825 g/L and GB amide surfactant **1** at a concentration of 0.29 g/L (0.627 mmol/L). (a)–(c): Polymer surfactant multiparticle complexes; (d) isolated surfactant nanoparticles surrounded with polymer. Scalebar: 1 μm for ((a)–(c)), 200 nm for (d), and 50 nm for the inset (c′).

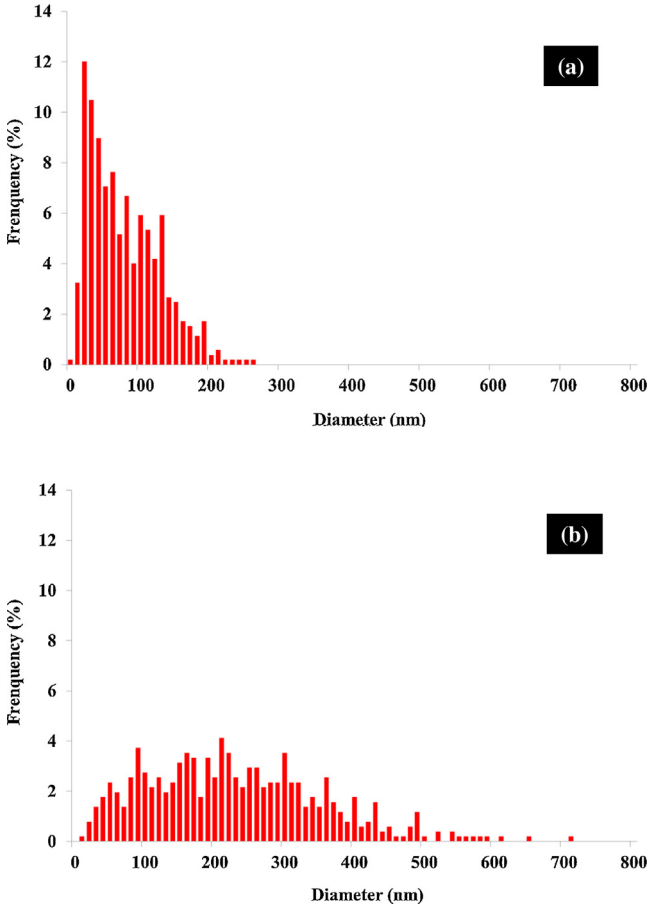

Fig. 8. Particle size distribution of (a) sample (**A**) and (b) sample (**B**) established from the TEM analysis. (a) Size distribution of the spherical-shaped nanoparticles of sample (**A**). (b) Size distribution of the surfactant dense nanoparticles of sample (**B**), with no taking into account the surrounding polymer network. Mean diameter is 81.8 nm and 234.1 nm, and standard deviation is 50.4 nm and 125.7 nm, for sample (**A**) and sample (**B**), respectively.

of very large aggregates occurred that did not make possible size measurements by DLS.

3.6. Microscopy of cationic GB amide surfactant/κ-carrageenan complexes

The effect of surfactant/polysaccharide molar ratio on the structure of the complexes was evaluated using negatively stained TEM and AFM in liquid imaging from two samples (**A**) and (**B**) containing κ-carrageenan at a close concentration (0.095 g/L and 0.0825 g/L, respectively) and cationic surfactant **1** at two different concentrations (0.083 g/L and 0.29 g/L, respectively). The negatively stained TEM technique appeared suitable to investigate the particles structures of samples (**A**) and (**B**). However, TEM using a negatively staining has to be applied carefully as typical artefacts can sometimes arise from the drying step of the sample. Then, AFM experiments in liquid environment were conducted in parallel to TEM from samples (**A**) and (**B**) to complete the structural characterization and verify the effect of the aqueous environment/drying step on the structures. Figs. 6 and 7 present typical TEM images after negatively staining for samples (**A**) and (**B**), respectively, and Figs. 9 and 10 show typical AFM images acquired from samples (**A**) and (**B**), respectively, always kept in their aqueous environment.

At 0.083 g/L (0.179 mmol/L, sample (**A**)), the cationic surfactant concentration is below the complete neutralization of the polymer ($(\xi < 0)$. Investigating sample (**A**), TEM revealed the presence of several aggregates resulting from the rolling-up of the polysaccharide chains (Fig. 6a and b) in addition to very well-defined spherical particles (Fig. 6d). The particle size statistical analysis established from the corresponding TEM images is shown in Fig. 8a. The mean diameter deduced from the distribution is 82 nm with diameters ranging from 10 to 200 nm. Mixtures of κ-carrageenans with either rolled up or rigid chains were also visualized as shown in Fig. 6c. At 0.29 g/L (0.627 mmol/L), the cationic surfactant concentration is above the CMC of pure surfactant **1** (0.11 g/L, 0.23 mmol/L). Interestingly, singular polymer/surfactant hybrid structures were observed from sample (**B**), characterized by the presence of dense surfactant nanoparticles surrounded with a polymers layer (Fig. 7). The particle size statistical analysis of the dense surfactant nanoparticles is shown in Fig. 8b. The deduced mean diameter is 234 nm with a broader distribution with diameters ranging from 20 to 500 nm. These original systems include both multi-particles embedded within the polysaccharide matrix (Fig. 7a–c) and isolated surfactant nanoparticles linked to polymers at their periphery, giving a typical core-shell hybrid morphology (Fig. 7d). These results clearly showed the existence of interactions between micelle-like or particle-like aggregates made with cationic GB surfactant **1** and polysaccharide chains. The samples (**A**) and (**B**) were also investigated by AFM operated in liquid environment. The AFM images corresponding to sample (**A**) also indicated the presence of aggregates as a network of polysaccharide chains (Fig. 9a–c) along with spherical-liked particles appearing with a relatively broad distribution (Fig. 9d). Such structures confirmed the results previously obtained with TEM with the difference that the polysaccharide-based aggregates were more interconnected under the aqueous environment. Investigating the sample (**B**) by AFM, polymer/surfactant hybrid structures were found with morphologies very close to the ones previously described by TEM (Fig. 10a–c). These structures were also explained as dense surfactant nanoparticles surrounded with a polymers layer except that the nanoparticles were swelled in comparison with their dehydrated form seen by TEM. Higher magnification observation of isolated nanoparticles (Fig. 10d) confirmed the global size range of the dense surfactant cores. However, the peripheral polymer layer was no clearly seen, which was explained by the dropping of the AFM resolution when scanning in liquid.

Further investigations particularly based on laser scanning confocal fluorescence microscopy are in progress to give additional information about the structure of these complexes. Especially, the effect of dilution to explain the mechanism of formation of these systems will be soon described.

3.7. Viscosity measurement of surfactant/κ-carrageenan complexes at two different surfactant/polymer ratios and concentrations

The viscosity level of GB surfactant/κ-carrageenan complex solutions corresponding to sample (**A**) (0.083 g/L of GB surfactant and 0.095 g/L of κ-carrageenan) and sample (**B**) (0.29 g/L of GB surfactant and 0.0825 g/L of κ-carrageenan) was found to be close to the one of water (~1 mPa s). Conversely, samples (**A′**) and (**B′**) characterized by the presence of surfactants and polymers at the same ratios but at ten times superior concentrations, exhibited different viscosity values (Fig. 11). At low GB surfactant concentration (0.83 g/L; sample (**A′**)), the viscosity of complex solution was shear rate dependent, it decreased when the shear rate increased from 10 to 200 s^{-1} exhibiting typical shear-thinning or pseudoplasticity behavior. The presence of more GB surfactant (2.92 g/L; sample (**B′**)) dramatically changed the viscosity profile of the GB surfactant/κ-carrageenan complex solution. The viscosity values became comparable to those obtained with water or sample (**C**) containing exclusive surfactant (2.92 g/L), showing a Newtonian behavior. Finally, the presence of only κ-carrageenan

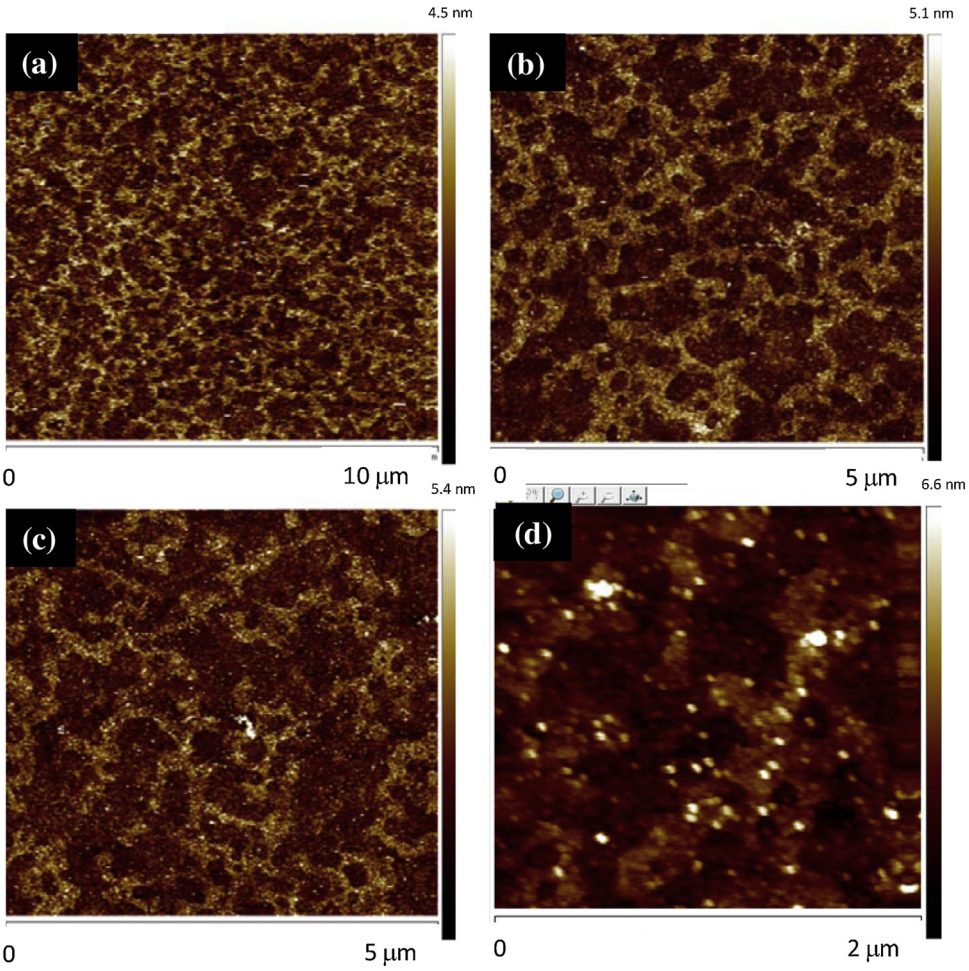

Fig. 9. Height AFM in liquid of aqueous solution (sample (**A**)) containing κ-carrageenan at a concentration of 0.095 g/L and GB amide surfactant **1** at a concentration of 0.083 g/L (0.179 mmol/L). (a)–(b): Aggregates resulting from rolled up κ-carrageenans; (c) mixture of κ-carrageenans with either rolled up or rigid chains (see black arrows); (d) spherical particles. Scanning surfaces are: (a) 10 μm × 10 μm; (b)–(c) 5 μm × 5 μm; (d) 2 μm × 2 μm.

at a concentration of 0.95 g/L (sample (**D**)) led to constant viscosity values but at a higher level than samples (**B**′) and (**C**). These preliminary results clearly demonstrated the importance of the GB surfactant addition into κ-carrageenan solution to modulate the rheological properties. Further investigation could be envisaged to correlate these viscosity data with the nature of GB surfactant/κ-carrageenan complexes at high concentrations.

3.8. *Effect of interactions between κ-carrageenan and cationic surfactant on the CMC value*

The variation of surface tension of Milli-Q water in the presence or absence of κ-carrageenan was measured as a function of cationic GB surfactant concentration, as shown in Fig. 12. Thus, we studied the effect of interactions between the anionic polysaccharide and GB surfactant on the critical micelle concentration (CMC). The CMC of cationic GB surfactant in water can change in the presence of κ-carrageenan. Indeed, we observe in the absence of anionic polysaccharide that the lower value of surface tension of 36 mN/m was reached at 0.23 mM of cationic surfactant (equivalent to 106.43 mg/L) that is in agreement with the value determined from the ITC measurements (Fig. 2a). On the contrary, the presence of κ-carrageenan changes considerably the amount required to obtain low value of surface tension. The CMC value was then reached at 0.63 mM of cationic surfactant (equivalent to 291.5 mg/L) in comparison with the previous case where lower concentrations of cationic surfactant were efficient. This value is slightly different from the one that was obtained from ITC measurements (0.523 mM). This difference can be probably explained by the less accurate capability of the tensiometer to measure interaction phenomena when complexes (anionic polymer/cationic surfactant) are formed in solution. Furthermore, we can notice for each concentration of GB derivative added into polymer solution at 0.1 g/L that the surface tension values of water are clearly higher than those determined without anionic polysaccharide as it was reported in the literature for other anionic polysaccharides (Gawel et al., 2013; Desbrieres, 2010). The plateau of 36 mN/m is reached when the polymer chain is saturated with the cationic surfactant.

3.9. *Influence of the anionic polysaccharide type*

In this study, we compared the interactions of cationic GB surfactant **1** with various anionic polysaccharides typically used in animal, plant or human foods: κ-carrageenan, alginate and ulvan. These polysaccharides have different molecular structures, molecular weights and charge characteristics as outlined in the introduction. Titration experiments in the presence of 0.1 g/L of anionic polysaccharide (κ-carrageenan, alginate or ulvan) in the reaction cell caused a significant modification of the enthalpy curves (Fig. 2b, e and f, respectively) compared to the control aqueous solutions containing only cationic GB amide surfactant (Fig. 2a). The enthalpy change versus cationic surfactant

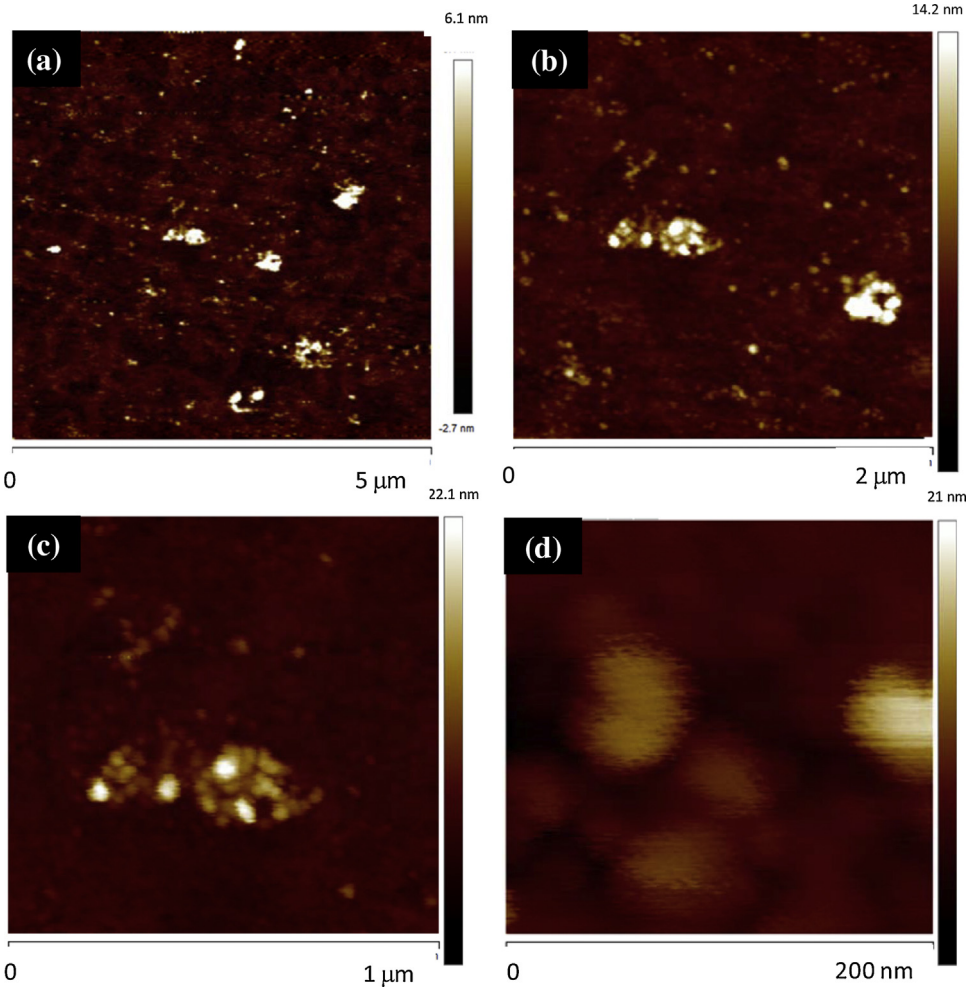

Fig. 10. Height AFM in liquid of aqueous solution (sample (**B**)) containing κ-carrageenan at a concentration of 0.0875 g/L and GB amide surfactant **1** at a concentration of 0.29 g/L (0.627 mmol/L). (a)–(c): Polymer surfactant multiparticle complexes and isolated surfactant nanoparticles; (d) high magnification view of surfactant nanoparticles. Scanning surfaces are: (a) 5 μm × 5 μm;(b) 2 μm × 2 μm; (c) 1 μm × 1 μm; (d) 200 nm × 200 nm.

concentration profiles for the different anionic polysaccharides are shown in Fig. 13. The enthalpy change in the presence of κ-carrageenan versus cationic surfactant concentration was already detailed in Sections 3.2 and 3.3. The presence 0.1 g/L of alginate in the reaction cell caused a slight modification in the titration enthalpy curves (Figs. 2e and 13). We observed that the interaction enthalpy was endothermic and the intensity of peaks was lower than interaction enthalpy of the control sample, indicating that interactions between alginate and cationic surfactant s were weaker than that measured for κ-carrageenan and ulvan. Neutral interaction enthalpy was reached at 0.32 mM (equal to 148.1 mg/L of cationic surfactant) which was higher than the amount required for obtaining neutral interaction enthalpy for κ-carrageenan and ulvan. This unexpected result may be explained by the conformation adopted by the alginate chain that could inhibit the surfactant approach close to the anionic groups and also by the presence of carboxylate groups on the mannuronate and guluronate units instead of sulfate moieties that could be less favorable to strong interactions with the cationic surfactants. Moreover, the presence of alginate in aqueous media leads to

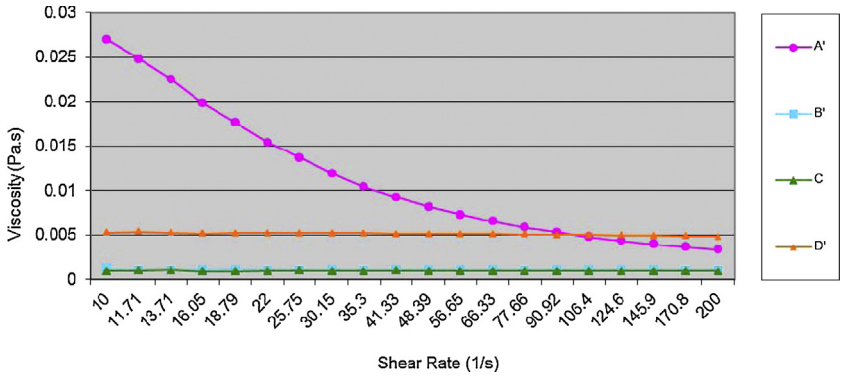

Fig. 11. Effect of shear rate on dynamic viscosity of sample (**A**′): 0.83 g/L of GB surfactant and 0.95 g/L of κ-carrageenan; sample (**B**′): 2.92 g/L of GB surfactant and 0.825 g/L of κ-carrageenan); sample (**C**) containing only GB surfactant at a 2.92 g/L concentration; sample (**D**) containing only κ-carrageenan at a concentration of 0.95 g/L.

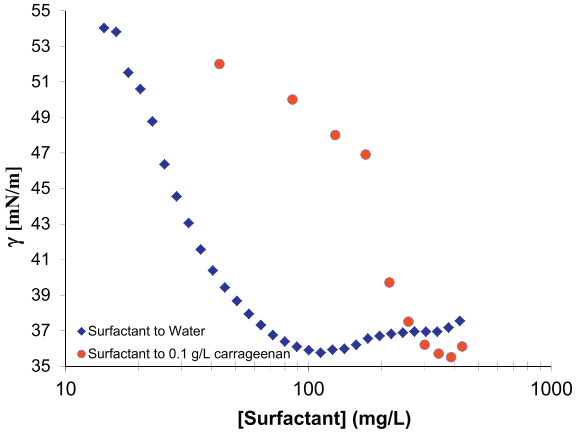

Fig. 12. Effects of surfactant addition on the surface tension of water (◆) and aqueous solution (●) containing 0.1 g/L of κ-carrageenan.

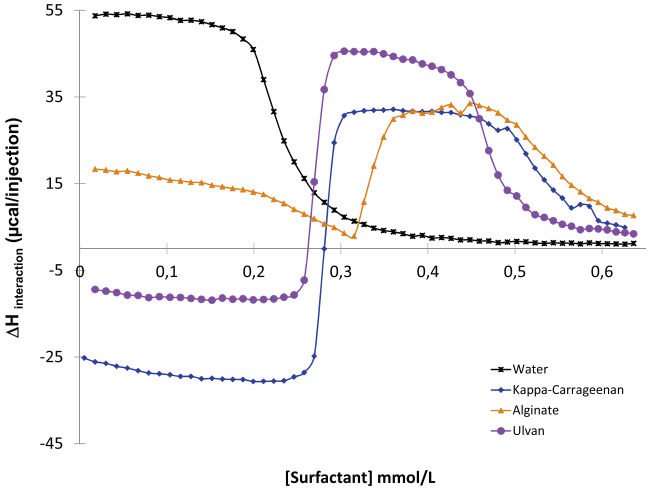

Fig. 13. Interaction enthalpy change ($\Delta H_{interaction}$) versus GB surfactant concentration (mM) when 1.67 g/L of GB surfactant solution (pH = 7) was injected into a reaction cell containing 0.1 g/L of various types of anionic polysaccharides (κ-carrageenan; alginate; ulvan) at pH = 7, 30 °C. Profiles of interaction enthalpy change ($\Delta H_{interaction}$) were obtained by subtracting the ΔH profile of GB surfactant solution (i.e. $\Delta Hi_{interaction} = \Delta H_{polysaccharide} - \Delta H_{surfactant}$).

a higher CMC, determined from the inflection point at around 0.53 mM and equal to 245 mg/L of cationic surfactant. Each injection of cationic surfactant solution in the reaction cell containing 0.1 g/L of alginate was marked by the formation of large complexes which were clearly visible.

In the case of ulvan, we observed an appreciable change in the enthalpy titration curves (Figs. 2f and 13) compared to the control sample without polysaccharide addition. However, the interaction enthalpy curve for the ulvan/cationic surfactant complexes became less exothermic than the one of the κ-carrageenan/cationic surfactant complexes. That suggested that cationic GB surfactant bound less strongly along the ulvan chain than for κ-carrageenan chain. On the other hand, cationic surfactant bound more importantly along the ulvan chain than with alginate chain. Neutral interaction enthalpy in the case of ulvan was reached at 0.27 mM, equal to 125 mg/mL of cationic surfactant. This value is lower than the one obtained for other polysaccharides studied hereby. This result may be explained by the difference of chemical structures of ulvan possessing both sulfate and carboxylate groups of complex sugars in comparison with carrageenan and alginate. The presence of ulvan also caused an significant decrease in the effective CMC of the cationic surfactant which was reached at 0.469 mM, equivalent to 217 mg/L of cationic surfactant. However, this CMC value was higher compared to the control and it was reached at lower concentration compared to carrageenan and alginate solutions. During each injection of cationic surfactant solution in the reaction cell containing 0.1 g/L of ulvan, the formation of large complexes was observed.

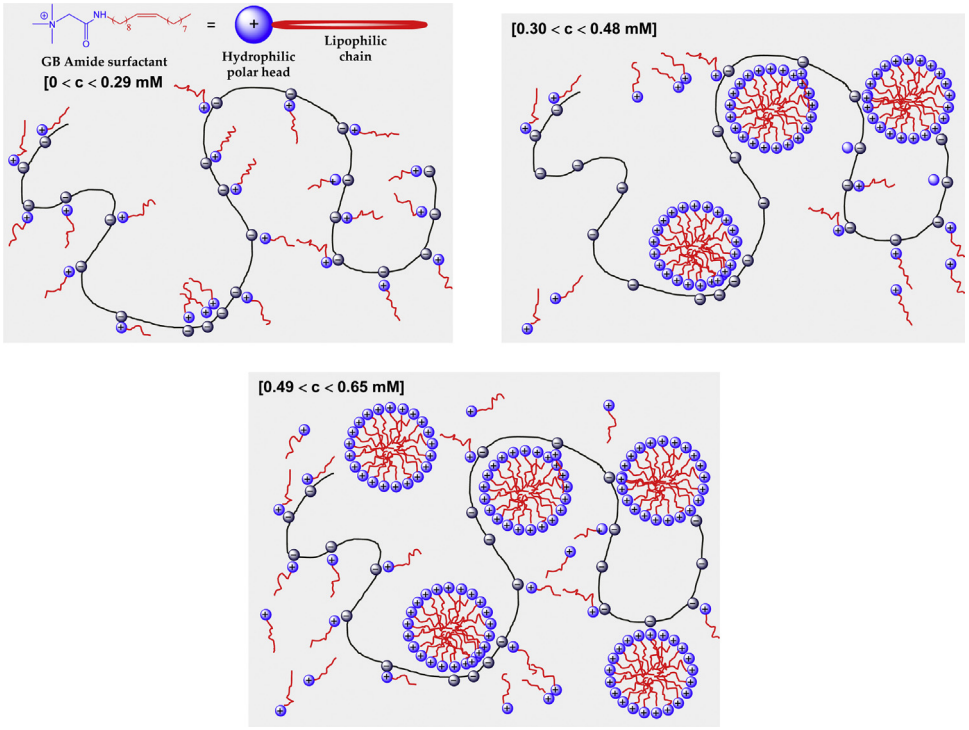

Fig. 14. Schematic representation of the mechanism of interactions between GB surfactant and anionic polymers (carrageenans, alginate or ulvan). As an example, the interval of GB surfactant concentrations corresponds to the amounts interacting with κ-carrageenan and forming complexes.

4. Conclusion

In summary, we have studied the interactions between cationic glycine betaine amide surfactant **1** and anionic algal polysaccharides which were *kappa*, *iota* and *lambda* carrageenans, alginate and ulvan using the Isothermal Titration Calorimetry technique. Indeed, ITC was capable to detect an appreciable enthalpy changes associated with the dissociation of micelles (from GB amide surfactant) in the biopolymer-free solutions and with the binding of surfactant to anionic algal polysaccharides in solution. Therefore, the ITC technique can provide valuable information about the formation of complexes or aggregates in solution. The enthalpy versus surfactant profiles highlighted that different physicochemical phenomena occurred in the surfactant/algal polysaccharide solutions depending on surfactant and biopolymer concentrations, such as micelle dissociation, monomer binding and micelle/particle binding. The interaction of GB amide surfactant with polysaccharides may have important implications for some applications: for example, complex formation may impact the ability of the GB amide surfactant to act as antimicrobial agents or they may impact their foaming or emulsifying properties. We have shown that in all surfactant concentration regions, the monomeric surfactant absorbed on the anionic polysaccharides chains through the electrostatic attraction. In addition, we observed that the interaction (associative behavior) of the κ-carrageenan was stronger than for other polysaccharides (*iota* and *lambda* carrageenans, alginate and ulvan). We highlighted the existence of critical aggregation concentration CAC in the case of κ-carrageenan (corresponding to the saturation of the binding sites of the polysaccharide at the critical saturation concentration). Original polymer/surfactant hybrid structures were observed by microscopy, both TEM and liquid AFM, above the CMC that gives evidence of the ability of charged polysaccharides to interact with micelles-like or nanoparticle-like systems composed of cationic surfactants. Additional studies based on TEM, AFM and laser scanning confocal fluorescence microscopy are in progress to provide more detailed information about the structure of these complexes. For other polysaccharides, further investigations would be needed to determine the CAC. The interactions of GB amide surfactant with alginate seemed less strong than for carrageenans and ulvan that can be explained by the difference of chemical structure of algal polysaccharides. Furthermore, the presence of algal polysaccharides in solution led to an increase of the CMC values of GB amide surfactant in comparison with the control surfactant solution. After saturation of the binding sites of the κ-carrageenan, the surface tension decreased further on increasing surfactant concentration until it reached the CMC of the surfactant. We have also shown for κ-carrageenan that the stability of the complexes and the electrical charge depend of the amount of GB amide surfactant used. These new polymer–surfactant complexes could find applications in cosmetics (for example, topical applications), food, detergency, paint, petroleum recovering, and polysaccharide extraction.

Acknowledgments

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