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Gelation of charged bio-nanocompartments induced by associative and non-associative polysaccharides

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ABSTRACT

Vesicles composed of sodium oleate (NaO) and monoolein (MO) are adequate candidates for drug nanoencapsulation and controlled release due to their stability and perceived biocompatibility. The object of the present study is to design hydrogels based on those anionic vesicles and polymers of both non-associative and associative type. The selected macromolecules were *k*-carrageenan (KC), carboxymethyl cellulose (CMC) and hydrophobically modified carboxymethyl cellulose (HMCMC). While the polymer-vesicle association was probed by rheology, the influence of the polymer on the vesicle stability was monitored by cryo-TEM and calorimetric measurements.

The effects of the polymer on the rheological properties of surfactant aggregate solutions clearly depend on the polymer type: the storage moduli of the polymer–vesicle mixtures, compared to the vesicles alone, increases around 2 orders of magnitude if the polymer is non-associative and 4 orders of magnitude if the macromolecule is of associative type.

As the vesicles are added, the non-associative polymer networks tend to be disrupted, while the networks formed by associative polymer get more robust. These observations can be explained by fundamental changes in electrostatic/hydrophobic interactions: vesicles entrapped in KC networks convert the polysaccharide in a highly charged entity and favor high electrostatic repulsions between the chains; this encourages network collapse. The opposite picture is experienced in HMCMC systems, i.e., such network is stabilized by the presence of vesicles. This is ascribed to the enhanced hydrophobic association, compensating the electrostatic repulsions between vesicles and polymer chains.

1. Introduction

Encapsulation of both hydrophilic and hydrophobic drugs has been an important challenge for scientists. It can be achieved by entrapping the drug in a chemically crosslinked polymer matrix [1–4] or using surfactant aggregates or amphiphilic polymers for drug compartmentalization [5,6]. Physical networks have some advantages compared to those of chemical nature, mostly due to gel–liquid reversibility. Physical networks composed by vesicles and charged polysaccharides, with or without hydrophobic interactions are reported in literature [7–12]. These charged systems are considerably sensitive to salt, so that stability and collapsing of the network can be induced by electrolyte. Several reasons are behind such phenomenon: salting-out, reduction of electrostatic repulsion [13], and the different ability of salts to form ion binding to polyelectrolytes [14].

In this study we use anionic vesicles, with long kinetic stability and perceived biocompatibility, as nanocompartments. Although some work has been reported with biocompatible systems, most of the published studies concern noncompatible molecules, used for non-biological applications or for fundamental understandings. Monoolein (MO) is biocompatible [15,16] and sodium oleate (NaO) is known to improve the biocompatibility of some nanoparticles [17]. These 2 amphiphilic molecules form different structures in water. While the critical packing parameter (CPP) of MO exceeds one, NaO forms normal micelles [18,19]. In the dilute corner, spontaneously formed vesicles with long-term stability are found (for at least 1 year) [15]. Previous studies, concerning cubic liquid-crystalline phases of MO in water, show significant time effects due to MO hydrolysis [20]. It seems that MO is more stable when present in the mixed vesicles, since no time effects were seen within 1 year. The formation of such aggregates is not dependent on preparation procedures, and the vesicles can be also formed by adding NaOH to an aqueous dispersion of oleic acid and MO [15].

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Mixtures containing 1 wt% of NaO and 1 wt% of MO are near the molar stoichiometry and small unilamellar vesicles are in large excess with their size ranging from 10 to 70 nm, mostly below 25 nm. The situation is similar when MO content increases up to 2 wt%. The increase of the charge density in the system by incorporation of NaO enhances the vesicle polydispersity, diameters ranging from 10 to 200 nm, mostly below 40 nm [15].

Here we highlight on physical networks of MO–NaO vesicles and anionic polysaccharides. The vesicles were entrapped within the polymer network and the rheological response was reported. We selected three different polysaccharides: *k*-carrageenan (KC), carboxymethyl cellulose (CMC) and hydrophobically modified carboxymethyl cellulose (HMCMC).

2. Materials and methods

2.1. Materials

NaO or sodium 9-octadecenoate, is an anionic surfactant with a C_{18} hydrophobic chain and a carboxyl headgroup. The molecular weight is 304 Da and was purchased from Nu-Chek Prep, Inc. (Elysian, USA) with high purity (>99%).

MO, Rylo Mg 90 glycerol monooleate was kindly provided by Danisco Ingredients (Braband, Denmark).

KC is a polysaccharide, supplied by Fluka BioChemika (Buchs, Switzerland), with M_w of around 700 kDa. The basic structure of carrageenan is a linear polysaccharide made up of a repeating disaccharide sequence of β-D-galactopyranose linked through positions 1,3 (A residues) and α-D-galactopyranose residues linked through positions 1,4 (B residues). At room temperature, the observed gelation in carrageenan is caused by helix formation [21,22] and this can only occur in repeated structures where the B residue is in a1-C-4 conformation. All the gelling types of carrageenan, which include κ, β and ι, contain a 3,6-anhydro bridge on the B unit which forces the sugar to flip from a 4-C-1 conformation to a 1-C-4 conformation and can then form cross-link networks and gels. Formation of a helical structure is promoted by adding an appropriate electrolyte to a sufficient ionic strength and/or by lowering the temperature [23–28].

CMC, was supplied by BDH Chemicals in the sodium salt form and with low M_w. CMC is fully ionized at pH 7 and is almost nonionic at pH 2 [29–31].

HMCMC is a semiflexible polyanion [29], in the sodium salt form; it was received as a gift by Akzo Nobel Surface Chemistry. The CMC backbone is modified by tetradecyl groups with a degree of hydrophobic substitution of 1.2% (1.2 C14 chains per 100 sugar units). The charge density (percentage of carboxymethyl groups) is 80% at pH 8 (80 negative charges per 100 sugar units). The weight average molecular weight is around 879 kDa. The overlap concentration is around 0.06 wt% [29,30].

The chemicals were used without any further purification and double distilled and deionised water was used. pH of the samples was around 9, thus the carboxyl groups were fully ionized.

2.2. Methods

Vesicle stock solutions were prepared by weighting the desired amount of surfactant and water in glass ampoules containing a small magnetic stirring bar. The polymer was added afterwards to the vesicle solution. The samples were sealed, mixed in a vortex, and homogenized at 80 °C. The mixtures were stored for 1 week before measurements. To analyze their textures, samples were observed in a polarized microscope.

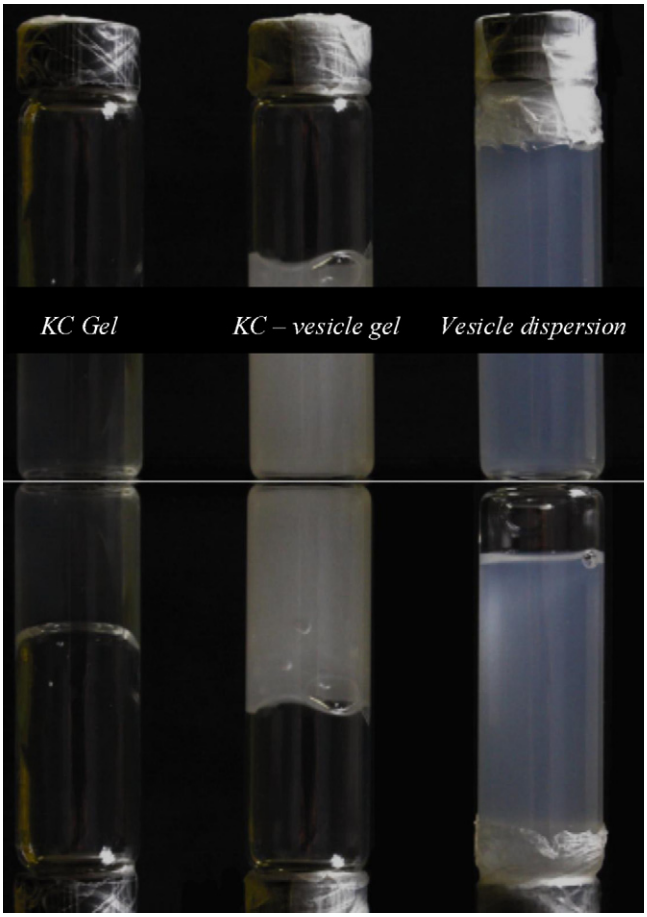

Fig. 1. Pictures of selected samples; from left to right: 0:0:2KC, 1:1:2KC and 1:1:0.

The prepared mixtures were labeled following the nomenclature "*a*:*b*:*cP*", being *a* the concentration of NaO (wt%), *b* the concentration of MO (wt%), *c* the concentration of polysaccharide (wt%), and *P* the polysaccharide name.

The experiments were performed far from the melting transition of the surfactants alkyl chains to prevent the network properties to be influenced by the different states [32].

While the sample containing only NaO is transparent, which results from the micellar phase reported in the literature [18,19], the remaining samples exhibit bluish color, which is usually related to the existence of vesicles.

Polymer-free solutions are liquid-like but when polymer is added, a gel-like behavior is observed (if polymer solution is intrinsically a gel). Fig. 1 shows selected images of the samples.

Rheological measurements were conducted using a shear strain controlled rheometer RFS III (Rheometrics, USA) equipped with a couette and a microcouette, cylinder geometry (gap 1.06 mm, inner radius 17 and 7 mm, respectively), according to the viscosity of the samples. The temperature was controlled by a water circulator apparatus (±0.5 °C). To prevent errors due to evaporation, the measuring geometries were surrounded by a solvent trap containing water. Two different kinds of experiments were carried out: (a) steady flow experiments were performed in a shear rate range 0.02–1700 s^{-1}. Sometimes it was not possible to cover all shear rate range due to the instrumental limits imposed by the viscosity of the samples. The minimum and the maximum torque that the instrument can measure are 0.04 and 1000 g cm, respectively. To be sure that the samples were in steady flow, the flow equilibrium time was measured by transient experiments (step-rate test) and