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Responsiveness of κ-carrageenan microgels to cationic surfactants and neutral salts

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ABSTRACT

The objective of this study was to examine the responsiveness of chemically cross-linked κ-carrageenan microspheres to different types of neutral salt electrolytes as well as to surfactants of varying chain lengths. In the presence of increasing salt concentration microsphere size changed radically from D[4,3] values of 320 μm to approximately 160 μm. The level of salt concentration needed to bring about this change varied depending on electrolyte type. This common behaviour was attributed to the difference in free cationic counter-ions concentration between the inside and outside of the microsphere and can be explained due to the effect of the Donnan equilibrium. The rheological properties of these microgels in their swollen and collapsed states were also explored with results showing that the collapsed microspheres had a greater impact on the viscosity of the system probably as a result of some aggregation of the collapsed microgels at rest due to surface charge screening at these high salt concentrations. The effect of surfactant on microsphere size showed a dramatic drop in D[4,3] values from 320 μm to approximately 120 μm for BAC, DoTAB, MTAB and CTAB at specific critical concentrations. This critical aggregation concentration was found to increase linearly on a log–log scale with the critical micelle concentration of these surfactants in water, indicating that the alkyl chain length of the surfactants had an effect on the critical aggregation concentration.

1. Introduction

Covalently cross-linked gel networks or hydrogels swell when placed in water (Katchalsky, 1949) due to an osmotic effect which can be attributed to the presence of network counterions (Ricka & Tanaka, 1984). This swelling behaviour can be greatly reduced as a response to stimuli in the surrounding environment such as increase in ionic strength, change in pH (Tanaka et al., 1980), and also by applying an electrical field (Tanaka, Nishio, Sun, & Ueno-Nishio, 1982). These stimuli cause the swollen gel to go through a conformational transition change resulting in a collapsed polymer structure.

The effect of ionic solutes and non polar solvents on covalently cross-linked alginate beads (Moe, Skjak-Braek, Elgsaeter, & Smidsrod, 1993) and gellan gels (Annaka, Ogata, & Nakahira, 2000) has been previously explored. Results for both ionic polysaccharides showed a deswelling occurring with increases in salt concentration in the surrounding environment. The ionic contribution to swelling was thought to be the main factor in determining the degree of swelling of the gels. A marked hysteresis was also seen in the swelling curves of the alginate beads in the presence of Ca^{2+}, Sr^{2+} and Ba^{2+} indicating that the alginate gels still retains its ion binding properties after crosslinking. The swelling behaviour of partially hydrolyzed cross-linked carrageenan-graft-polymethacrylamide (H-κC-g-PMAM) in various salts solutions was investigated by Pourjauadi, Sadeghi, and Hosseinzadeh (2004). This is one of the few studies found in the literature in relation to the chemical cross-linking of κ-carrageenan. It was found that the swelling capacities of H- κC-g-PMAM in saline solutions were substantially reduced compared to distilled water. This effect was attributed to a charge screening that shielded the charge of the sulphate and carboxylate groups. The impact of monovalent, divalent and trivalent salts were also assessed, with trivalent salts forming cation bridges between the sulphate and anhydro-D-galactose residue resulting in lower swelling capacity in comparison to mono and divalent salts.

When considering the addition of these microspheres to a system it is also important to explore the rheological implications of this phase transition induced by salts. The rheological properties of a temperature sensitive microgel of poly (*N*-isopropylacrylamide) was investigated by Senff and Richtering (1999). Results showed that with increasing temperature, bead size and effective volume decreased causing zero shear relative viscosity to be greatly reduced at higher temperatures, but that at low volume fractions, the microgels exhibited hard sphere behaviour irrespective of swelling degree.

The deswelling of charged polymer microgels in the presence of oppositely charged surfactants has received much attention (Göransson & Hansson, 2003; Nilsson & Hansson, 2005; Andersson, Rasmark, Elvingson, & Hansson, 2005). It has been established that the polymer counterions are replaced by the surfactant through ion exchange reactions (Hansson, 1998; Khokhlov, Kramarenko,

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Makhaeva, & Starodubtsev, 1992). This ion exchange causes the gels to go from a swollen state (seen in water) to a collapsed state, with the surfactant ions forming micelle-like aggregates in the gel (Khokhlov et al., 1992). The reduction in osmotic pressure when the counterions of the gel are removed causes the gel to collapse as a result of the elastic energy stored in the network (Andersson et al., 2005; Khokhlov et al., 1992). When surfactant levels exceed the critical aggregation concentration (cac), micelles form throughout the whole gel, causing it to become completely collapsed. Although the physical-chemistry behind such a collapse is well understood and has been shown on numerous synthetic polymers, to our knowledge, no work has studied this interaction on cross-linked natural polysaccharides like κ-carrageenan.

In our previous studies, the manufacture of κ-carrageenan microspheres for addition to food products was optimised (Ellis & Jacquier, in press). The consequent cross-linking of these microspheres (Keppeler S. & A., submitted for publication) resulted in highly swellable microsponges which could be used as a smart delivery system for the food industry. Therefore this study will be mainly concerned with the investigation into the behaviour of these microspheres in the presence of various monovalent and divalent salts and oppositely charged surfactants. The study of the evolution of the size of these microgels as a function of their ionic environment and the resulting flow behaviour of these dispersions should allow for a better understanding of the potential of these cross-linked k-carrageenan microspheres as smart release delivery systems.

2. Materials and methods

2.1. Materials

κ-Carrageenan (Gelcarin GP-91) from FMC Biopolymer, USA was used as is. Distilled water was used to prepare all microgel suspensions and rapeseed oil (Millbrook Farms, Co. Meath, Ireland) was used to form the emulsion continuous phase. Epichlorohydrin (99%, Sigma–Aldrich) and 2-Propanol (extra pure, Fluka) were used in the cross-linking reaction. All salts were supplied by Merck (p.a. grade). The surfactants MTAB (Myristyltrimethylammonium bromide), CTAB (Cetyltrimethylammonium bromide), DoTAB (Dodecyltrimethylammonium bromide), BAC (Benzalkonium Chloride), SDS (Sodium dodecyl sulphate) and Trition X-100 were all analytical grade from Sigma–Aldrich and used as received.

2.2. Methods

2.2.1. Preparation of cross-linked microspheres

Microspheres were prepared using a previously optimised method (Ellis & Jacquier, in press). Briefly, an emulsion of 4% w/w κ-carrageenan solution and rapeseed oil was formed using a Silverson L4RT rotor/stator mixer (Silverson Machines Ltd., UK) at 3000 rpm, followed by a fast tempering stage using a Heidolph mixer. The microspheres were then removed from the oil using a 100 mM KCl solution and centrifugation.

The chemical cross-linking of the microgels was based on a previously optimised method (Keppeler et al., submitted for publication). Briefly, the microspheres were mixed in an alcoholic alkaline solution (KOH 700 mM and 2-propanol 50% V/V) before adding the cross-linking agent, Epichlorohydrin, and left to react for 24 h with continuous stirring. The microspheres were then washed using distilled water.

2.2.2. Particle size analysis

Particle size analysis was carried out using a Malvern Mastersizer S (Malvern Instruments Ltd., Morchester, UK) fitted with a 300 RF range lens (0.05–900 μm) and a small volume sample dispersion unit. The Fraunhofer optical model was used as particle size was large enough for this model to give a good approximation of particle distribution (ISO13320, 1999). Deionised water was used as the dispersion media. Microspheres were added to the unit before injecting measured quantities of the selected salt or surfactant solutions into the unit. The De Brouckere diameter D[4,3] and Sauter Diameter D[3,2], were determined at each salt concentration. Reported values represent the average of at least three independent replicates for each sample.

It is to be noted here that these microgels are quite monodispersed in size with average values in water for D[4,3] of 318 ± 3 μm and D[3,2] of 244 ± 9 μm giving a polydispersity index of 1.3 (determined on 11 independent replicates).

The final microgel concentration in the dispersion unit was estimated to be in the order of 0.2 mM expressed in carrageenan sulphate units.

2.2.3. Rheological analysis

Steady Stress Sweep tests were carried out on the microsphere suspensions using a stress controlled rheometer (model SR-2000, Rheometric Scientific, Piscataway, NJ, USA) with couette geometry (bob diameter 29.5 mm and internal diameter of cup 32 mm) at 25 °C.

3. Results and discussion

3.1. Effect of various salts

The responsiveness of the cross-linked microspheres to a saline environment was studied by measuring the evolution of the microgels diameter as a function of electrolyte concentration and type. In Fig. 1 the effect of NaCl (1:1 electrolyte), $MgCl_2$ (1:2 electrolyte) and Na_2SO_4 (2:1 electrolyte) on microspheres D[4,3] values is presented. Initially (below 0.05 M) there was minimal response to increased electrolyte concentration in the surrounding solution. But as salt concentration increased to 1 M for the three electrolyte types, D[4,3] values showed a marked decrease from 320 μm to approximately 160 μm. The effect of NaCl results in a gradual reduction in D[4,3] values, occurring between 0.1 and 1 M. A similar behaviour is observed for Na_2SO_4 but at slightly lower salt concentrations. In the case of $MgCl_2$, a much sharper decrease in microsphere size is seen, with complete microsphere collapse occurring between 0.09 and 0.12 M. A similar decrease in D[4,3] was seen for the for the other 1:2 electrolytes studied $ZnCl_2$ and $CaCl_2$, (results not shown).

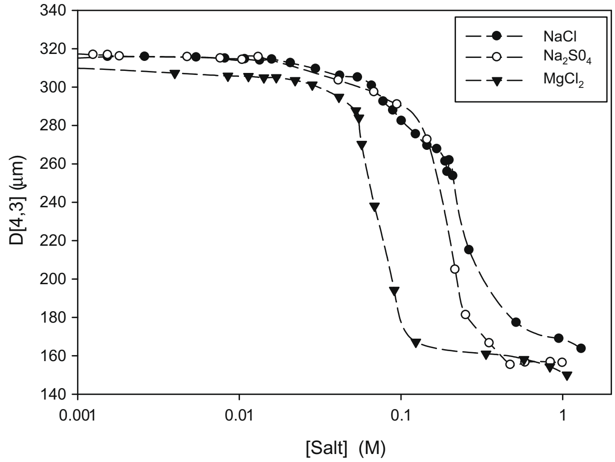

Fig. 1. Evolution of D[4,3] diameter of cross-linked κ-carrageenan microspheres as a function of neutral salts concentration. Effect of electrolyte type.

The marked decrease in microsphere size when salt in the external environment is increased is in accordance with the theory of the Donnan equilibrium. It predicts that the difference in ionic concentration between the outside and inside of the gel or microsphere should be reduced when salt in the surrounding medium is increased. The microsphere therefore acts a semi-permeable membrane, with a high number of fixed negative polymer charges inside and a corresponding high concentration of small cationic counterions to balance electroneutrality. However as salt concentration in the surrounding solution is increased, this imbalance in free ion concentration between the inside and outside of the membrane will decrease, causing the microspheres to shrink. This type of swelling behaviour in cross-linked polymers has also been exhibited with cross-linked alginate beads (Moe et al., 1993) and gellan beads (Annaka et al., 2000). The varying cations will therefore have an effect on the concentration at which this gel collapse happens as seen in Fig. 1.

In Fig. 2 the evolution of the microspheres diameter as a function of NaCl and KCl (1:1 electrolytes) concentration is shown. The effect of these two 1:1 electrolytes is broadly similar, but the decrease in size of the microspheres is observed at much lower potassium concentrations with complete microsphere collapse occurring between 0.015 and 0.1 M. The difference in the point at which microsphere shrinking occurs may be due to nature of κ-carrageenan. Potassium is a specific monovalent cation (Mangione et al., 2005) known to bind to κ-carrageenan. This may bring about a coil to helix transition in the carrageenan polymer within the microgel, akin to gelation of the free polymer, with direct binding (condensation) of the K^+ onto the gel. As sodium is a non specific monovalent cation in relation to κ-carrageenan, this could explain the much larger concentrations of NaCl needed to bring about collapse. It is also worth noting here that due to the saline conditions rich in potassium during the cross-linking reaction, the carrageenan in the microgels is essentially in potassium form. The specific binding of these potassium ions to carrageenan could also result in a specific lag phase before collapse induced by the presence of non-binding cations in the environment.

3.2. Rheological properties

The salt induced deswelling of the microgels from 320 to 160 μm corresponds to an 8-fold increase in carrageenan concentration inside the microgel and this dramatic change in polymer concentration could potentially have an impact on the flow behaviour of these particles in water (Ellis & Jacquier, 2009). The flow curves of the swollen microspheres dispersed in distilled water were therefore compared to the collapsed microspheres in a 1 M solution of NaCl (Fig. 3). The swollen microspheres up to 48% (v/v) show predominately Newtonian behaviour. In comparison the collapsed microspheres at the same volume fraction exhibit a complex flow behaviour, with an apparent yield stress followed by a shear thinning region, that yield to a Newtonian region at high shear stress characterised by viscosity values close to the ones exhibited by the swollen microspheres.

At a higher phase volume ratio of 54% (v/v), the swollen microspheres show evidence of an apparent yield stress followed by strong shearing thinning at higher stress, while the collapsed microspheres also show evidence of an apparent yield stress and shear thinning region, before reaching a minimum viscosity plateau at high stress.

In the case of collapsed microgels, the presence of apparent yield stress at low phase volume ratios (results not shown) and a somewhat stronger yield stress at high phase volume ratios than for the swollen microgels could be an indication of some particle aggregation at rest, which maybe due to surface charge screening as a result of high salt concentrations. Also, the prolonged shear thinning region exhibited by the swollen microgels could be due to the high deformability of these particles because of their relatively low carrageenan content resulting in some possible deformation in the direction of flow.

3.3. Effect of surfactants

The responsiveness of cross-linked carrageenan microspheres to the surrounding concentration of a range of surfactants was also assessed (Fig. 4). At the beginning of each experiment, the microspheres, when placed in distilled water, exhibited an average volume diameter D[4,3] of 320 μm. As surfactant concentration increased to approximately 0.17 mM, there was no significant effect to microsphere size. As surfactant concentrations increased further, a sharp decrease in size or collapsing of the microspheres occurred at a specific concentration range for each surfactant. CTAB caused a decrease in microsphere size at 0.18 mM, with D[4,3] values reducing from 320 to 120 μm over a small concentration range (between 0.18 and 0.35 mM). In the case of MTAB, the gel collapse started at a slightly higher surfactant concentration of 0.22 mM, with complete collapse by 0.36 mM. Increasing concentrations of BAC showed microsphere size beginning to decline at a surfactant concentration of 0.28 mM, with a final D[4,3] value of 120 μm at

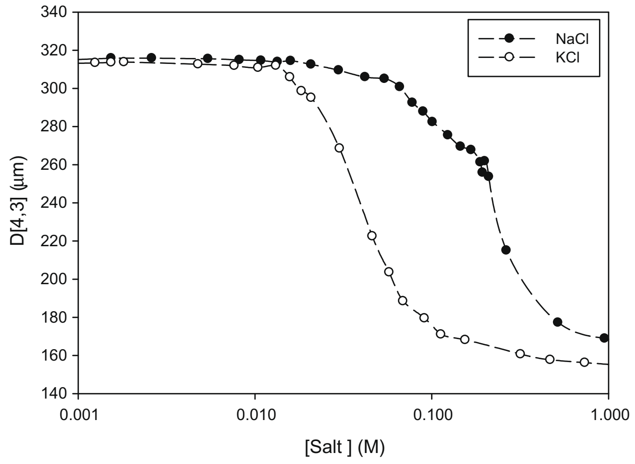

Fig. 2. Evolution of D[4,3] diameter of cross-linked κ-carrageenan microspheres as a function of neutral salts concentration. Effect of cation type.

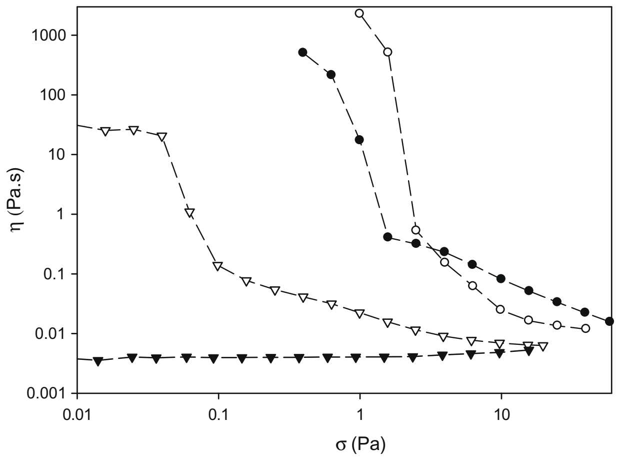

Fig. 3. Flow behaviour of swollen (closed symbols) and collapsed (open symbols) microspheres dispersions at various phase volume ratios (triangles ϕ = 48%; circles ϕ = 54%).

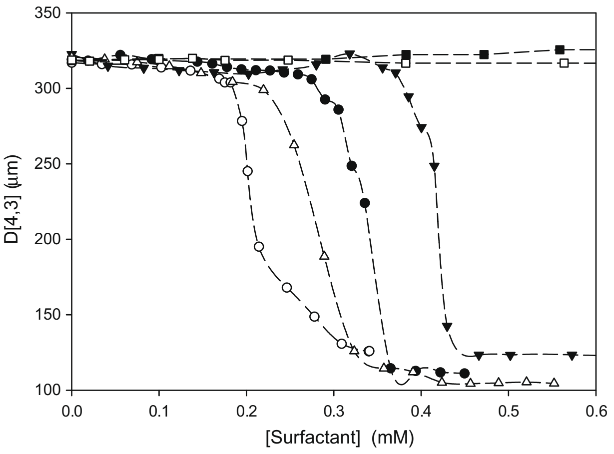

Fig. 4. Effect of surfactant concentration on D[4,3] diameter of the microspheres. (●) BAC; (○) CTAB; (△) MTAB; (▼) DoTAB; (■) SDS; (□) Triton X-100.

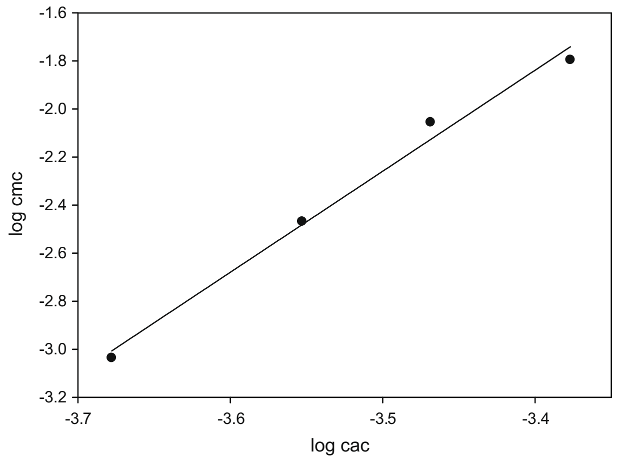

Fig. 5. Double logarithmic plot of the critical aggregation concentration versus critical micelle concentration.

0.37 mM. The microsphere collapse in the presence of DoTAB however was slightly different. First, it would seem that at approximately 0.32 mM, the microspheres swelled slightly, before a sharp drop in size at 0.42 mM. Both anionic SDS and neutral Triton X-100 were also assessed in these experiments, however neither had an effect on the microsphere diameter up to 10 mM concentration.

It is obvious that the profound effect these surfactants have on the microspheres is not linked to the Donnan equilibrium as in the case of neutral salts presented previously, where the shrinking of the microgels was due to a reduction in the counterion concentration difference. In this concentration range (up to 1 mM) no change in size was observed for any salt previously studied. This dramatic gel collapse being only seen in the case of cationic surfactants would suggest some specific electrostatic interaction with the negatively charged carrageenan polymer. This interaction is reminiscent of polymer precipitation by oppositely charged surfactants. In the present case, where polymer freedom is somewhat limited by the chemical crosslinks, no precipitation occurs but a dramatic decrease in gel size is seen.

The absorption of these oppositely charged surfactants seems to take place in two phases. The initial stage at low concentrations, where absorption has no effect with microsphere size remaining steady is known as the lag region which has been seen by many other studies (Göransson & Hansson, 2003; Nilsson & Hansson, 2005). The point at which this stage ends varies between surfactant types, with the surfactants of longer chain length (see Table 1) such as CTAB, causing collapse of the microspheres at the lowest surfactant concentration. It can be said that the point of collapse is quite surfactant specific, causing collapse of the microspheres within a narrow concentration range. This critical concentration, reminiscent of a critical aggregation concentration (cac) is shown for each surfactant in Table 1, along with the critical micelle concentrations (cmc) (Rosen, 2004). The double logarithmic plot of these two critical concentrations (Fig. 5) shows a linear increase of the log of cac values with the corresponding log of surfactant cmc in water ($r^2 > 0.995$). It can therefore be assumed that the collapse of the carrageenan microgels is due to the specific aggregation of the surfactant molecules inside the gel matrix as pseudo-micelles, with the electrostatic binding of the positively charged ammonium surfactants to the carrageenan sulphate groups and the surfactants hydrophobic alkyl chain forcing the polymer strands close together, resulting in a remarkable water expulsion from the gels.

Table 1
Responsiveness of κ-carrageenan microgels to cationic surfactants.

Surfactant	n_c	cac (mM)	cmc (mM)*	Final D[4,3] (μm)	Final D[3,2] (μm)
DTAB	12	0.42	16	118 ± 6	59 ± 1
MTAB	14	0.28	3.4	111 ± 8	45 ± 5
CTAB	16	0.21	0.92	129 ± 5	55 ± 7
BAC	–	0.34	8.8	131 ± 6	51 ± 6

* Rosen (2004).

4. Conclusion

Cross-linked κ-carrageenan microspheres have shown the ability to swell dramatically in distilled water and undergo a shrinking or collapse in the presence of both neutral salt and cationic surfactant in the surrounding solution. The shrinking brought about by neutral electrolytes was attributed to the Donnan equilibrium, with specific binding of K^+ causing microsphere shrinking at a lower concentration and within a narrower concentration range than the other monovalent salt, NaCl. The impact of neutral salt induced shrinkage on the flow behaviour of the resulting dispersions was also examined, where it was found that collapsed microspheres show signs of aggregation at rest, probably due to a decrease in microgel surface charge, and that swollen microspheres show signs of deformation at high shear, due to the low polymer concentration in the gels. The microgel size collapse induced by oppositely charged surfactants was found to occur at very specific critical concentrations linked to the surfactant cmc.

Overall, these microspheres have shown response to a number of triggers such as salts and surfactants, which is essential in the development of targeted delivery systems.

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