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Research Paper

Effect of cations on the microstructure and in-vitro drug release of κ- and ι-carrageenan liquid and semi-solid aqueous dispersions

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Abstract

Objectives The main objective of this study was to determine the effect of potassium and calcium ions on the microstructure and release dynamics of kappa (κ) and iota (ι) carrageenan.
Methods The microstructure of the dispersions was imaged using a cryogenic scanning electron microscope. Franz-cell diffusion apparatus was used to determine the release kinetics of a model hydrophilic drug, sodium fluorescein, incorporated in selected polymer dispersions. Release profiles were analysed using Higuchi, Korsmeyer-Peppas and dual first-order models.
Key Findings Cryogenic scanning electron microscope images showed that κ-carrageenan forms hexagonal structures, whereas ι-carrageenan forms rectangular pores at low cation concentrations. In-vitro release studies showed sustained release profiles for all carrageenan systems; however the model drug, fluorescein, diffusion from ι-carrageenan with 0.06% w/v calcium was significantly higher than other ι-carrageenan systems. This may be attributed to improved tortuosity of this system. However further increase in cation concentration led to a reduction in fluorescein release from the matrices. The dual first-order release model illustrated two distinct release rates, an initial rapid release followed by a slow diffusion of fluorescein from the carrageenan matrices.
Conclusions The observed microstructural differences may account for the well known variation in mechanical properties of κ- and ι-carrageenan gels. The dual first order release model adds a new tool in the elucidation of release mechanisms from polymer matrices, where parallel processes contribute to drug release.
Keywords carrageenan; cation concentration; cryo-SEM; in-vitro release; iterative curve fitting

Introduction

The carrageenans are a family of water-soluble, anionic, linear polysaccharides extracted from marine red algae. These polymers are widely used in the food industry and, to some extent, in the pharmaceutical industry, applications ranging from formulation stabilisers and viscosity enhancers to drug release modifiers.[1,2] Carrageenans are also potential excipients in pharmaceutical applications as key components for mucoadhesive and ion-sensitive in-situ gelling formulations.[2,3] In-situ gelling formulations are systems that undergo sol to gel transition when introduced into the desired tissue, organ or body cavity. Such a system can be introduced into the target tissue in a minimally invasive manner and allows the incorporation of drugs by simple mixing. Moreover being simple aqueous dispersions these formulations are especially useful in the delivery of biomaterials such as peptides, proteins, oligonucleotides and DNA. With an increase in residence time, in-situ gelling formulations may increase the bioavailability and hence the efficacy of the therapeutic agent of interest.[4,5]

Chemically, carrageenan is a linear sulphated galactan, composed of alternating disaccharide repeating units of 3-linked β-D-galactopyranose (G units) and 4-linked α-D-galactopyranose (D units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA units). The three main commercially available carrageenans are iota (ι)-, kappa (κ)- and lambda (λ)-carrageenan, categorised according to the number and position of sulphate groups.[6,7] Due to high sulphate content, lambda carrageenan does not undergo sol–gel transition. Conversely, gellation of both κ- and ι-carrageenans may be promoted by the presence of cations owing

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to the neutralisation of coulomb repulsion force between carrageenan polymer units at the cross-link point. As ion concentration increases the neighbouring polymer chains become tightly aggregated and gel strength increases dramatically.[8]

The type and concentration of dispersed carrageenan and the nature and concentration of added cation dictates the resultant gel properties.[9–12] Tako and Nakamura[13,14] and Morris and Chilvers[15] have reported that calcium is a more efficient promoter of intermolecular association of ι-carrageenan, whereas potassium is most efficient in promoting cross linking of κ-carrageenan.[16] However, gels of ι-carrageenan are soft and elastic and those of κ-carrageenan are hard and brittle.[6,17] The concentration of cations required for inducing sol–gel transition in both κ- and ι-carrageenan dispersions is relatively low, making them ideal candidates for the rational design of in-situ gelling formulations. It follows that variation of carrageenan network characteristics in biological systems is inevitable.

To design drug delivery systems based on carrageenan it is essential to understand the rate and mechanism of transport of molecules incorporated in this polymeric system. The most important rate controlling mechanisms of polymeric systems are diffusion, swelling and erosion. Diffusion of drug molecules from these systems will be affected by the structural characteristics of the system as well as chemical interactions between the incorporated drug and the polymer matrix.[18–21] There are several well-established mathematical models that provide quantitative interpretation of drug release parameters from pharmaceutical dosage forms. These include first-order kinetics, Higuchi, Weibull, Korsmeyer–Peppas, Baker–Lonsdale models, etc.[22] However, the diffusion of drug within and release from the formulation often involves multiple steps or parallel processes. Such complexity reduces the efficiency of these models to interpret the release mechanism and no suitable method has previously been advanced.

Several groups have described the factors that influence the rate of cationic drug release from κ-carrageenan gel matrices.[8,23] Mangione *et al*.[23] suggested that the release of ketoprofen incorporated in κ-carrageenan gels was dependent on K^+/Na^+ ratio, whereas Naim *et al*.[8] reported either an increasing or decreasing release rate for metformin incorporated in κ-carrageenan gels depending upon KCl concentration. Nevertheless, assessment of drug release characteristics from carrageenan systems at low polymer and cation concentrations (<10 mM) is scarce. Moreover, there are neither comparative studies between drug release properties of ι- and κ-carrageenan gels nor investigations of anionic drug release from either.

The aim of this study is to determine the effect of monovalent cations (potassium) and the divalent cation (calcium) on the microstructure and diffusion of a model anionic drug from κ- and ι-carrageenan formulations. Potassium and calcium were selected due to their abundance in biological fluids[24] and because carrageenans have been shown to respond to these cations rapidly. Furthermore, this study will evaluate the application of iterative curve fitting methods to solve non-linear hypothetical models of release, and compare these results to those obtained by conventional models.

Materials and Methods

Materials

Carrageenan, Genuvisco type × 930-03 (κ) and type × 931-03 (ι) were kind gifts from CP Kelco (Lille Skensved, Denmark) and were used as received. The κ-carrageenan sample consisted of 5.1% (w/w) potassium, 1.6% (w/w) calcium and 0.87% (w/w) of sodium ions whereas the ι-carrageenan sample consisted of 5% (w/w) potassium, 0.031% (w/w) calcium and 4.8% (w/w) sodium ions (ICP-OES). Molecular weight of κ-carrageenan sample is 22 9666 (±49 088) g/mol and that of ι-carrageenan is 25 7666 (±29 652) g/mol (SEC-MALLS). Potassium chloride (KCl) and calcium chloride dihydrate ($CaCl_2.2H_2O$) were of analytical grade and purchased from Scharlau Chemie (Barcelona, Spain). Sodium fluorescein (MW 376.27) was purchased from Sigma-Aldrich (St Louis, USA). Water purified by ion exchange (Millipore, Bedford, USA) to a resistance of 18.2 MΩ cm at 25°C was used in the preparation of formulations and ion solutions.

Preparation of carrageenan/salt/water dispersions

Carrageenan dispersions were prepared by mixing the powder in an appropriate volume of water with continuous magnetic stirring at 80°C. Formulations were prepared by diluting the stock polymer solution in an appropriate volume of water and then adding the required amount of either 2% w/v KCl or $CaCl_2.2H_2O$ solution. For the in-vitro release studies, the appropriate volume of 5 μg/ml sodium fluorescein solution was added to the formulation (to make 0.5 μg/ml) and mixed thoroughly using a vortex before the addition of the required amount of salt solution. All formulations were stored for 24 h at ambient temperature (*ca.* 25°C) before analysis.

Microstructure imaging using cryogenic scanning electron microscopy

The microstructure of 0.4% (w/v) κ- and ι- carrageenan in the absence or presence of either KCl or $CaCl_2.2H_2O$ was examined using a cryogenic scanning electron microscope (cryo-SEM; Philips XL30S FEG, Netherlands). Samples were cured for 12 h then loaded onto rivets and plunge frozen in liquid nitrogen slush (−200°C). These were then loaded into the cryo chamber (Gattan Alto 2500, UK), fractured with a razor blade and sublimed at −80°C under vacuum for 30 min. Samples were then sputter-coated with gold (Polaron SC 7640 sputter coater, Quorum Technologies, UK) and viewed at an accelerating voltage of 5 kV while being maintained at −180°C.

In-vitro release of sodium fluorescein from carrageenan aqueous dispersions

The release of sodium fluorescein (hereinafter fluorescein) from either κ- and ι-carrageenan systems (0.4% (w/v)) in the absence of added cations and in the presence of KCl (0.06% w/v and 0.12% w/v) or $CaCl_2$ (0.06% w/v and 0.12% w/v) was investigated using a Franz cell diffusion apparatus (Logan instruments Corp., Somerset, USA). The standard Franz cell (receptor chamber volume of 12 ml) with an orifice size of 15 mm providing a diffusion area of 1.77 cm^2 was mounted on to a FDC-6 transdermal diffusion cell drive console (Logan

Instruments Corp., Somerset, USA). Temperature was maintained at 37 ± 1°C by a water jacket (VTC-220 heat circulator, Logan Instruments Corp., Somerset, USA). Receptor chambers were filled with balanced salt solution (BSS, pH 7.4) and were separated from the donor chambers by a cellulose membrane with a 12400 Dalton cut-off (Sigma-Aldrich, St Louis, USA). A magnetic stirrer was placed in each receptor chamber to enable adequate mixing.

Formulations (1 g) were loaded onto the donor compartments and 200 μl samples were removed from the receptor compartments at predetermined intervals. Withdrawn samples were diluted with 1 M sodium hydroxide (NaOH) and agitated with a vortex mixer before analysis. Measurements were performed in quadruplicate for each formulation and samples were analysed using a fluorescence spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, USA) employing excitation and emission wavelengths of 490 nm and 510 nm, respectively.

The proportion of fluorescein released from samples over 8 h was plotted against time and the area under the curve (AUC) of each of the resultant release profiles was calculated according to the trapezoidal method. Data was then fitted to three kinetic equations, namely, Higuchi (equation 1), Korsmeyer–Peppas (equation 2) and a dual first-order release (equation 3) model, in an attempt to elucidate the release mechanism from the formulations under investigation. The dual first-order release model was optimised through Origin Pro 8 software (OriginLab Corporation, Northampton, USA) and the models were iterated using the Levenberg-Marquardt method. Kinetic parameters obtained as well as AUC data were then analysed using a one-way analysis of variance followed by Tukey's pairwise comparison at 99% confidence interval using SPSS 16.0 software (SPSS Inc, Illinois, USA).

$$Q_t = k_H t^{0.5} \quad (1)$$

Where Q_t is the amount of drug release in time (t) per unit area and k is the release constant.

$$Q_t = A\left(t - t_{lag}\right)^n \quad (2)$$

Where A is a constant characteristic of the dosage form, t_{lag} is the lag time and n is the release exponent indicative of the release mechanism.

$$Q_t = A_1\left(1 - e^{-k_1\left(t - t_{lag}\right)}\right) + A_2\left(1 - e^{-k_2\left(t - t_{lag}\right)}\right) \quad (3)$$

Results

Microstructure of κ- and ι-carrageenan systems

The electron micrographs of 0.4% (w/v) ι-carrageenan in the absence and presence of cations reveal substantial variations in polymer arrangement (Figure 1a–e). In the absence of added cations, the polymer strands demonstrated no visible order. Addition of 0.06% (w/v) or 0.12% (w/v) $CaCl_2$ or 0.12% (w/v) KCl induced a dramatic change in polymer arrangement, with the polymer strands forming rectangular pores (Figure 1b–e) due to intrahelical cross-linking. However, the rectangular pores observed in the presence of potassium ions were much narrower compared with the pores formed in the presence of calcium ions. Further increases in calcium ion concentration to 0.4% (w/v) had a negative impact on the ι-carrageenan gel structure (Figure 1d); where the fine filamentous network observed was disrupted with aggregation of polymer strands.

In contrast κ-carrageenan formed hexagonal networks in the presence of KCl (Figure 1g and h). This hexagonal structure was only partially visible in the κ-carrageenan gels with added $CaCl_2$ (Figure 1j). Moreover, a fine, woven arrangement was observed within these pores. As the added KCl concentration was increased to 0.4% (w/v) the fine network arrangement of the κ-carrageenan systems was also disrupted (Figure 1i).

In-vitro release of sodium fluorescein from carrageenan aqueous dispersions

Figures 2 and 3 show the release profiles of fluorescein from κ-carrageenan and ι-carrageenan systems, respectively. Statistical analysis of the area under the curve of carrageenan aqueous dispersions demonstrated the amount of fluorescein released from these was significantly ($P < 0.01$) lower than that from control solution, fluorescein (0.5 μg/ml) dissolved in BSS. Moreover, the release of fluorescein from ι-carrageenan with 0.12% (w/v) $CaCl_2$ and κ-carrageenan with 0.12% (w/v) KCl was significantly lower than that from other carrageenan formulations ($P < 0.01$) (Table 1).

Release data obtained fitted the Higuchi model reasonably well ($R^2 > 0.99$) and statistical analysis of the data showed that release rate of fluorescein from carrageenan dispersions was significantly ($P < 0.01$) lower than that of control solution (Table 2).

To further understand the mechanism of fluorescein release from the systems, data were fitted to the Korsmeyer–Peppas model and dual first-order release models. A significant difference between the release exponent of the control solution and the polymer formulations was observed with the Korsmeyer–Peppas model ($P < 0.01$) (Table 3). Moreover, the release exponent for all formulations was above 0.5, illustrating an involvement of more than one diffusion process in the release of fluorescein from the polymer systems. The lag time (presumably required for fluorescein to establish a uniform concentration gradient) for the release process obtained through this model, however, appeared to be an overestimation of the expected values.

To test the hypothesis that at least two mechanisms affect the release of fluorescein from these systems (anomalous transport), a dual first-order release model was constructed (Table 4). Statistical analysis of the initial rate of release (k_1) of fluorescein from the formulations showed no significant difference compared with the control solution. However this model illustrates a significantly lower second rate constant (k_2) for all carrageenan formulations, compared with the control solution.

Statistical analysis of the t_{lag} values obtained from the dual first-order model shows no significant difference between the carrageenan systems and the control solution ($P > 0.05$). The average t_{lag} value obtained from the first-order model was

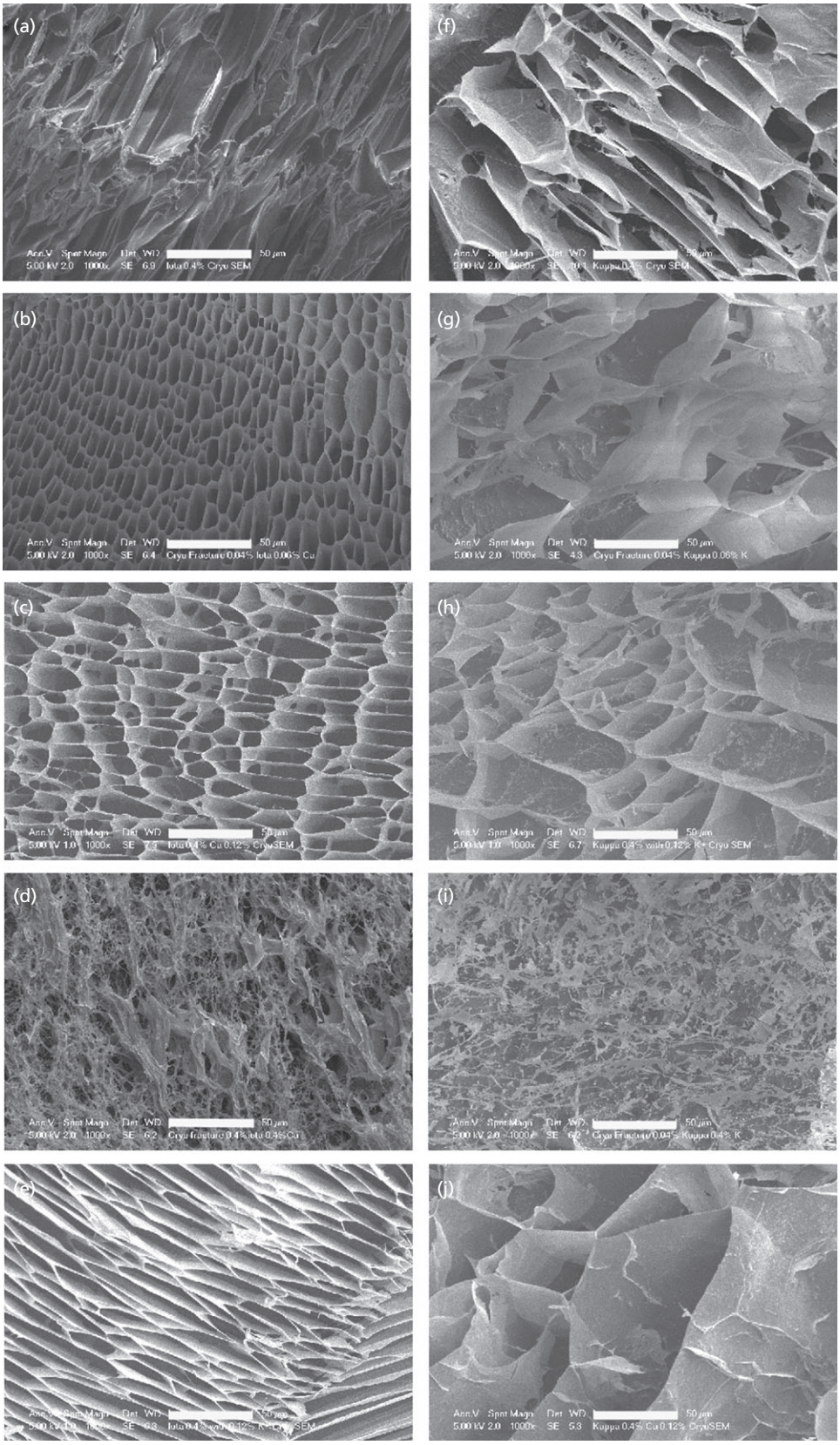

Figure 1 Microstructure imaging using cryogenic scanning electron microscopy. Images of ι-carrageenan in the absence of added cations (a), in the presence of 0.06% w/v (b) 0.12% w/v (c) or 0.4% w/v $CaCl_2$ (d) or 0.12% w/v KCl (e) and of κ-carrageenan in the absence of added cations (f), in the presence of 0.06% w/v (g), 0.12% (h) or 0.4% w/v KCl (i) or 0.12% w/v $CaCl_2$ (j) (scale bars = 50 μm).

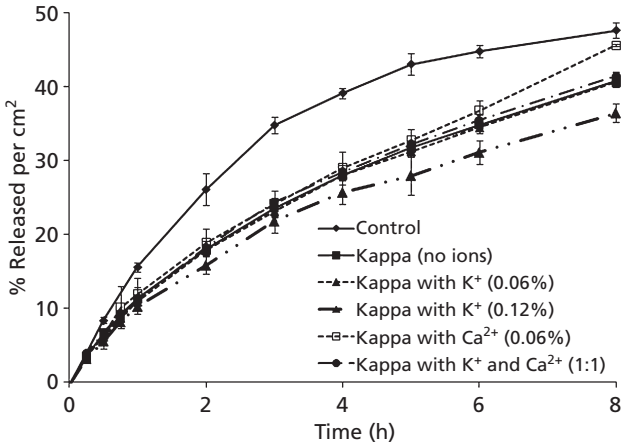

Figure 2 Percentage of fluorescein released per cm^2 from κ-carrageenan systems over 8 h. Data points represent mean ± SD, $n = 4$.

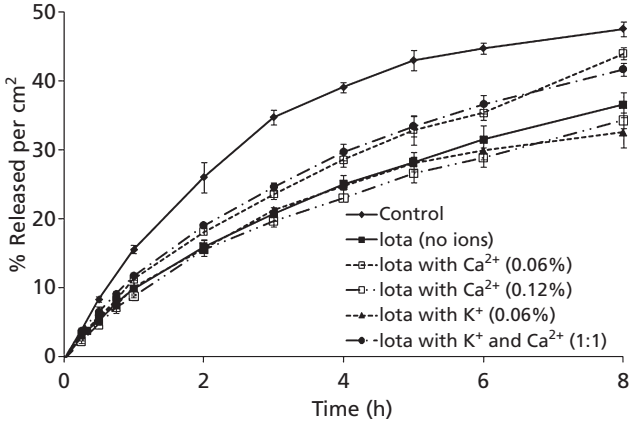

Figure 3 Percentage of fluorescein released per cm^2 from ι-carrageenan systems over 8 h. Data points represent mean ± SD, $n = 4$.

found to be 1.2 min; however these values varied considerably between formulations.

Discussion

Microstructure of κ- and ι-carrageenan systems

Due to the high water content of these polymeric systems, cryo-SEM was used to minimize the artefacts that can be introduced by conventional sample preparation. Rapid freezing with liquid nitrogen reduces the ice crystal formation, preserves the spatial structure of the gels and prevents the distortion of the sample due to dehydration.[25–27]

Significant variations in spatial structure of the systems were observed in the absence and presence of the selected cations. In the absence of added cation, both systems had no large porous network characteristic. However, some areas of irregular hexagonal porous arrangement were observed in κ-carrageenan systems in the absence of added cations. This may be due to the presence of considerably large concentrations of potassium ions in unpurified carrageenan samples.

Table 1 Area under the percentage drug released vs time curve (AUC) of carrageenan dispersions

Formulation	AUC ± SD (% h/cm²)
BSS (control)	483.4 ± 11.3
κ-Carrageenan	363.7 ± 5.5
κ-Carrageenan with K^+ (0.06% w/v)	359.8 ± 7.0
κ-Carrageenan with K^+ (0.12% w/v)	322.9 ± 12.0
κ-Carrageenan with Ca^{2+} (0.06% w/v)	384.6 ± 20.5
κ-Carrageenan with $K^+ : Ca^{2+}$ (1 : 1)	370.2 ± 8.6
ι-Carrageenan	324.7 ± 17.2
ι-Carrageenan with K^+ (0.06% w/v)	313.9 ± 11.8
ι-Carrageenan with Ca^{2+} (0.06% w/v)	366.8 ± 12.8
ι-Carrageenan with Ca^{2+} (0.12% w/v)	303.6 ± 8.7
ι-Carrageenan with $K^+ : Ca^{2+}$ (1 : 1)	381.0 ± 10.5

Data are mean ± SD, $n = 4$.

Table 2 Release rate constants for the carrageenan dispersions obtained from the Higuchi model

Formulation	K_H	Adjusted R^2
Control solution	35.7 ± 0.8	0.983
κ-Carrageenan	28.8 ± 0.2	0.999
κ-Carrageenan with K^+ (0.06%)	28.7 ± 0.4	0.999
κ-Carrageenan with K^+ (0.12%)	24.1 ± 1.1	0.991
κ-Carrageenan with Ca^{2+} (0.06%)	31.2 ± 0.2	0.997
κ-Carrageenan with $K^+ : Ca^{2+}$ (1 : 1)	29.2 ± 0.2	0.999
ι-Carrageenan	26.3 ± 1.3	0.999
ι-Carrageenan with K^+ (0.06%)	24.1 ± 0.8	0.993
ι-Carrageenan with Ca^{2+} (0.06%)	30.9 ± 0.2	0.998
ι-Carrageenan with Ca^{2+} (0.12%)	23.1 ± 0.6	0.993
ι-Carrageenan with $K^+ : Ca^{2+}$ (1 : 1)	29.9 ± 0.9	0.998

Data are mean ± SD, $n = 4$.

The formation of a regular porous structure in these systems with the addition of cations indicates the cross-link formation. The hexagonal honeycomb structure of κ-carrageenan systems is consistent with the known high tensile properties of these systems.[16]

The negative impact of high concentrations of cation on the microstructure of carrageenan networks (Figure 1d and i) is consistent with the physical appearance of the systems (formation of gel clumps), where excess amounts of cation is responsible for polymer aggregation and phase separation. This may be due to the saturation of cross-link points, where polymer helices interact, followed by rapid aggregation of polymer chains in close proximity. This may prevent long-range cross-linking of the polymer chains that form a cohesive three-dimensional network. This phenomenon has been employed to formulate carrageenan beads for drug delivery.[28–31]

MacArtain *et al*.[11] described a similar trend for κ-carrageenan in the presence of calcium ions. They studied the effect of 1 : 1 and 4 : 1 calcium : κ-carrageenan, and reported the formation of coarser strands with a more open network at higher counter ion concentrations. Moreover other natural polymer dispersions sensitive to ions (such as Gellan gum and psyllium polysaccharides) have been shown to

Table 3 Kinetic parameters for the carrageenan dispersions obtained from Korsmeyer–Peppas model

Formulation	A	t_{lag} (h)	*n*	Reduced Chi square	Adjusted R^2
Control solution	24.9 ± 3.6	0.6 ± 0.3	0.3 ± 0.07	11.2 ± 4.3	0.959 ± 0.015
κ-Carrageenan	12.8 ± 0.6	0.2 ± 0.02	0.6 ± 0.02	0.3 ± 0.2	0.998 ± 0.001
κ-Carrageenan with K^+ (0.06%)	12.8 ± 1.1	0.2 ± 0.08	0.6 ± 0.04	0.7 ± 0.9	0.996 ± 0.005
κ-Carrageenan with K^+ (0.12%)	11.5 ± 0.7	0.2 ± 0.03	0.6 ± 0.04	1.6 ± 0.4	0.988 ± 0.004
κ-Carrageenan with Ca^{2+} (0.06%)	12.6 ± 2.6	0.1 ± 0.07	0.6 ± 0.09	0.6 ± 0.5	0.996 ± 0.002
κ-Carrageenan with K^+ : Ca^{2+} (1 : 1)	12.9 ± 0.8	0.2 ± 0.01	0.6 ± 0.03	0.5 ± 0.3	0.997 ± 0.002
ι-Carrageenan	11.5 ± 0.7	0.2 ± 0.003	0.6 ± 0.02	0.3 ± 0.1	0.998 ± 0.001
ι-Carrageenan with K^+ (0.06%)	11.7 ± 0.9	0.2 ± 0.01	0.5 ± 0.04	1.2 ± 0.3	0.989 ± 0.004
ι-Carrageenan with Ca^{2+} (0.06%)	12.4 ± 0.8	0.2 ± 0.02	0.6 ± 0.03	0.5 ± 0.07	0.997 ± 0.001
ι-Carrageenan with Ca^{2+} (0.12%)	10.6 ± 0.5	0.2 ± 0.02	0.6 ± 0.02	0.6 ± 0.4	0.994 ± 0.003
ι-Carrageenan with K^+ : Ca^{2+} (1 : 1)	13.3 ± 0.4	0.2 ± 0.008	0.6 ± 0.02	0.8 ± 0.4	0.995 ± 0.002

Data are mean ± SD, $n = 4$.

Table 4 Kinetic parameters for the carrageenan dispersions obtained from dual first-order release model

Formulation	A_1	K_1	A_2	K_2	t_{lag} (H)	Reduced Chi square	Adjusted R^2
Control solution	29.7 ± 2.1	0.4 ± 0.05	20.3 ± 4.5	0.4 ± 0.04	0.04 ± 0.03	0.9 ± 0.9	0.996 ± 0.003
κ-Carrageenan	8.9 ± 4.3	1.5 ± 0.9	60.8 ± 16.4	0.1 ± 0.04	0.03 ± 0.05	0.2 ± 0.2	0.998 ± 0.001
κ-Carrageenan with K^+ (0.06%)	12.1 ± 2.4	0.8 ± 0.09	69.4 ± 12.9	0.1 ± 0.01	0	0.1 ± 0.1	0.999 ± 0.001
κ-Carrageenan with K^+ (0.12%)	14.6 ± 1.6	0.7 ± 0.1	87.6 ± 10.7	0.04 ± 0.01	0.03 ± 0.03	1.8 ± 0.2	0.987 ± 0.002
κ-Carrageenan with Ca^{2+} (0.06%)	8.8 ± 4.1	1.5 ± 0.3	100 ± 0	0.1 ± 0.007	0.06 ± 0.04	0.5 ± 0.2	0.997 ± 0.001
κ-Carrageenan with K^+ : Ca^{2+} (1 : 1)	12.5 ± 5.1	1.3 ± 1.2	78.2 ± 24.8	0.1 ± 0.05	0.02 ± 0.03	0.3 ± 0.2	0.998 ± 0.001
ι-Carrageenan	6.7 ± 3.3	1.4 ± 0.6	56.7 ± 15.7	0.1 ± 0.06	0.04 ± 0.04	0.2 ± 0.1	0.999 ± 0.001
ι-Carrageenan with K^+ (0.06%)	14.5 ± 2.1	0.4 ± 0.1	27.3 ± 9.9	0.2 ± 0.1	0.01 ± 0.02	0.8 ± 0.6	0.993 ± 0.006
ι-Carrageenan with Ca^{2+} (0.06%)	7.2 ± 1.7	1.3 ± 0.3	84.9 ± 26.1	0.1 ± 0.05	0.04 ± 0.02	1.0 ± 0.4	0.995 ± 0.002
ι-Carrageenan with Ca^{2+} (0.12%)	10.8 ± 2.0	0.9 ± 0.2	94.8 ± 10.4	0.04 ± 0.004	0.08 ± 0.04	0.3 ± 0.3	0.997 ± 0.003
ι-Carrageenan with K^+ : Ca^{2+} (1 : 1)	11.8 ± 5.9	0.7 ± 0.2	66.1 ± 29.5	0.1 ± 0.08	0	0.4 ± 0.3	0.998 ± 0.002

Data are mean ± SD, $n = 4$.

demonstrate a similar trend; the microstructure of the polymer systems is disrupted by an increase in ion concentration beyond a given threshold.[32,33]

In-vitro release of sodium fluorescein from carrageenan aqueous dispersions

Complying with the Donnan's exclusion theory, it was noted that the average loading concentration of the model hydrophilic molecule in carrageenan formulations was 5–10% less than that for the control solution.[34] Nevertheless, the comparatively low release rates of fluorescein from carrageenan formulations can be attributed to the inherent characteristics of the formulations, such as net charge, amount of imbibed water, average pore size, pore size distribution and pore interconnections.[34–36] Release profiles demonstrated that after 8 h none of the carrageenan formulations had proceeded to exhaustion. The effect of incorporation of both potassium and calcium at a ratio of 1 : 1 in the carrageenan systems was also investigated, but the release data demonstrated no synergistic effect between the two cations. The control solution demonstrated slow release, suggestive of rate-limiting behaviour in the semi-permeable cellulose membrane that was used to separate the donor and receptor compartments.

The widely used Higuchi model describes drug release as a diffusion process (Fick's Law), which is square root time dependent and can be applied to analyse the release profiles of water-soluble drugs from semi-solid or solid matrixes.[22] Analysis of the release profiles using this empirical model shows that diffusion dictates the release of fluorescein from the carrageenan polymer matrix.

The significantly lower release rate of fluorescein from ι-carrageenan with a K^+ ion system could be related to the negative charge of ι-carrageenan, which is not significantly lowered by the addition of monovalent cations. Accordingly the electric repulsion between the negatively charged polymer and the fluorescein may have led to the reduced permeation of the solute from the system.[36] In addition, the tortuosity (the product of pore size and pore interconnections) of ι-carrageenan formulations may have contributed to the reduced release rates (Figure 1e). This can be elaborated from the increased fluorescein release from ι-carrageenan systems with 0.06% (w/v) $CaCl_2$, where the addition of calcium ions has reduced the net charge repulsion between the solute and the polymer as well increased the tortuosity of the system (Figure 1b). The release rate constants of κ-carrageenan systems in the absence of added cations and in the presence of 0.06% (w/v) of KCl or $CaCl_2$ are also consistent with the

observed high tortuosity of these systems. This is in agreement with the findings of Walther *et al*.[37] and Loren *et al*.[38] Both studies reported an increase in diffusion of molecules incorporated in κ-carrageenan gels with an increase in void or pore size.

Conversely, ι-carrageenan with 0.12% (w/v) $CaCl_2$ and κ-carrageenan with 0.12% (w/v) KCl systems exhibited lower fluorescein release rate constants, despite the observed highly porous (large void) network. Hence, the known high viscoelastic properties of these systems are likely to have contributed to the reduced release rate.[39] As described by the Stokes–Einstein equation, the diffusion coefficient of a molecule is inversely proportional to the viscosity of the medium.[40] Thus, beyond a specific cation concentration, the viscosity of the carrageenan systems may dictate the release rate of molecules incorporated.

The exponent of the Korsmeyer–Peppas model is accepted to be suggestive of the diffusional release mechanisms from the polymeric matrix systems.[22] As described by Peppas,[41] for a slab, an *n*-value of 0.5 describes Fickian diffusion, whereas an *n*-value of 0.5–1 can be explained by anomalous transport where more than one release process occurs. Nonlinear curve fitting of the experimental data demonstrates the involvement of more than one diffusional process in the release of fluorescein from the carrageenan systems. It is therefore logical to employ a model that can accommodate two processes. However, where one or both processes follow first-order kinetics no simple deterministic method is available. At present, iterative curve-fitting techniques allow the parameters of such models to be determined.

With the above knowledge a mathematical model was then developed to incorporate two release processes. The dual first-order release model thus provides two release constants for a given formulation, with a considerably lower second release constant. This decrease in diffusivity of fluorescein from the polymer matrix is due to the increase in diffusion front, which necessitates further passage of molecules through the polymer network.

Calculated lag values from the dual first-order model varied considerably between formulations, demonstrating the lack of significance of this parameter for this polymeric system. The model was iterated without the t_{lag} parameter, but the values obtained for all other parameters were similar to the values reported in this paper. This demonstrates the dual first-order equation to be an adequate empirical model in systematically investigating the parameters involved in release of molecules from polymer matrices.

Conclusions

Cryo-SEM images of the κ- and ι-carrageenan systems at various ionic conditions confirm the structural variations of these polymeric systems. Interestingly the introduction of a small amount (<10 mM) of selected counter ion is able to induce significant variation in polymer cross-linking. Nevertheless, an excessive amount of incorporated cation has a negative impact on the polymer cross linking. These micrographs may also provide a correlation between the microstructure and the release profiles of a model drug from the polymer-based matrix systems. ι-Carrageenan in the absence of added cations and ι-carrageenan with added KCl are the systems with lowest cross links and they exhibited lower release rates than ι-carrageenan with $CaCl_2$ (0.06% w/v), which formed regular intra-helical cross links. Viscosity also plays a significant role in drug release from these polymer matrices as illustrated by the reduced release rate of κ- and ι-carrageenan at 0.12% (w/v) KCl and $CaCl_2$ concentration, respectively.

The parallel processes that may simultaneously contribute to the release of a drug from hydrated polymer systems render standard deterministic methods (i.e. Higuchi and Korsmeyer–Peppas) of limited value. We have demonstrated that nonlinear derivatives that allow scope for the simultaneous contribution of more than one process are more consistent with release data. These methods therefore add a new tool in the elucidation of release mechanisms.

Importantly this study also shows that, ι-carrageenan gels induced by calcium ions are as effective as κ-carrageenan gels in controlling the release of incorporated hydrophilic molecules. Considering the physical and rheological properties, ι-carrageenan appears to be a promising candidate for in-situ gelling formulations.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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