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Relation between structural and release properties in a polysaccharide gel system

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Abstract

The potential utility of κ-carrageenan gels for preparing drug release devices is here shown. Structural properties of κ-carrageenan gels prepared with different salt composition and containing Ketoprofen sodium salt, as model drug, have been evaluated with static light scattering and rheological measurements. These properties have been correlated with release profiles *in vitro* at pH 5.5. Release properties from gelled matrices have been compared with those obtained by two commercial products containing the same drug. Results show that: i) in this system it is possible to easily control the gel texture by using different cationic concentration; ii) the kinetics of drug release by κ-carrageenan gels are dependent on the structural properties of matrices; iii) in the typical interval time used in classical local applications, all gel samples release the loaded drug almost completely, at difference with the commercial products. All these findings can provide useful suggestions for the realization of classical topical release systems.

Keywords: K-carrageenan; Ketoprofen; Gel structural properties; Drug release; Franz's cells

1. Introduction

In the last years, a number of natural polysaccharides forming gels were studied to prepare drug-release devices. The drug release rate is strongly dependent on the characteristics of the gel structure, which, in some cases, can be easily modified [1–3]. Under this respect matrices based on κ-carrageenan deserve great interest. κ-carrageenan a linear water soluble polysaccharide is extracted from different species of red algae with a primary structure based on alternating disaccharide units of α-(1-3)-D-galactose-4-sulphate and β-(1-4)-D-galactose. In aqueous solutions and in presence of various cations, κ-carrageenan forms thermoreversible gels on cooling. It is extensively used as thickening, gelling agent, texture enhancer or stabilizer in the food, pharmaceutical and cosmetic industries [4–6]. More recently drug delivery systems based on κ-carrageenan gels were tested *in vitro* [7,8]. In this study the structural properties of κ-carrageenan gels have been correlated to its capability of release an active agent.

Previous studies on κ-carrageenan gels have shown that the internal structure of the gel can be easily and opportunely modulated using different proportions of K^+ and Na^+ ions [9,10]. In fact, these cations play a different and specific role in the κ-carrageenan gelation pathway: the K^+ induces a coil→double helix transition preceding and promoting the formation of an ordered gel, whereas the Na^+ ion does not promote the coil→double helix transition, but takes part in the aggregation process probably through a phase separation mechanism. In this case the resulting structure is more disordered with respect to that one obtained in the presence of K^+ [10]. Both different gelation mechanisms (i.e. the formation and lengthening of the helices induced by K^+ ions, or coil aggregation induced through phase separation by Na^+ ions) can compete when the two cations are both present in solution depending on the K^+/Na^+ ratio and temperature [10]. The gelation pathway can be modulated to produce gels with different texture.

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The aim of the present study is to evaluate i) the possibility of modulating the release rate of a model drug loaded in the κ-carrageenan gels by controlling the gel texture through the use of different cationic concentration, ii) the relationship between salt composition and release profiles iii) the potential advantages offered by κ-carrageenan matrices as drug delivery system in comparison with other commercial pharmaceutical products.

In order to perform this study three κ-carrageenan gels prepared with different salt composition have been loaded with Ketoprofen sodium salt, a known non steroidal anti-inflammatory drug (NSAIDs); the obtained Ketoprofen release profiles have been compared with those obtained by Artrosilene Gel® and Fastum Gel®, two commercial products containing the same active agent.

2. Materials and methods

2.1. Materials

The κ-Carrageenan (type X-6913, Lot 63-80270) was from Copenhagen Pectin A/S, Denmark. The κ-Carrageenan powder was dissolved in Millipore deionised water at 70 °C (in the presence of 200 ppm sodium azide as bacteriostatic agent) and stirred at the same temperature for 2 h. [9,11]. The pH of the solution was 8.7 to prevent hydrolysis during preparation [11,12]. Then, the solution was dialysed against Millipore water to eliminate excess salt. Hot Millipore water, containing the appropriate amount of KCl and/or NaCl, was added to set the final ionic composition. The solution was finally filtered at high temperature through a 0.22 μm Corning filter.

Ketoprofen sodium salt was obtained from salification of the acidic form (as supplied by Sigma) using NaOH 0.1 M in equimolar amount and freeze — drying the final solution.

The sodium salt form was chosen because of the very low solubility of the acidic form. The use of the sodium salt form at 2.5% w/w determines a contribution of approximately 100 mM of Na^+ ions which is sufficient to appreciably affect the aggregation process [10].

The drug was incorporated under stirring into the filtered colloidal dispersion at 60 °C, to avoid destabilization of the Ketoprofen. The dispersion was then put in the cuvette for static light scattering measurements or in the rheometer plate for viscoelastic measurements at 20 °C and kept at this temperature for 24 h to obtain stable gels.

Commercial products, used in release experiments with Franz's cells, were Fastum Gel ®, containing 2.5% (w/w) Ketoprofen acid form and Artrosilene Gel ®, containing Ketoprofen lysine salt 5% (w/w).

2.2. Static light scattering measurements

Static light scattering measurements were done using a Brookhaven Instrument BI-9000 digital correlator and an ILT 550 Argon laser tuned at 514.5 nm. Samples, prepared as described above, were put into a thermostatic cell compartment of Brookhaven Instrument BI200-SM goniometer system at 20 °C. The sample was kept at this temperature for 24 h and heated at 37 °C before starting the measurement. The intensity of light scattered by gelled samples was measured at different scattering vector $q = 4\pi n \sin(\theta/2)/\lambda_0$, where n is the refraction index of solution, λ_0 is the wavelength of the incident light and θ is the scattering angle. Due to the non-ergodic character of the gel, the intensity was measured over different regions of the specimen, using a motor-driven cell holder.

2.3. Rheological measurements

Viscoelastic spectra under low amplitude oscillatory shear were measured on a controlled stress AR-1000 rheometer (TA Instruments, UK) using a titanium cone-plate geometry (angle 0.0174 rad, radius 20 mm, gap 26 μm). All experiments were done in the 0.02–30 Hz frequency range at 4×10^{-3} strain. The hot solution was loaded on the rheometer plate, set at 65 °C. Then, the temperature was quickly lowered to 20 °C. The sample was kept at this temperature for 24 h and heated at 37 °C before starting the measurement. The temperature was controlled by the built-in Peltier system. The thin sample-air interface was coated with silicone oil to avoid solvent evaporation.

2.4. Drug release studies

Release studies, performed at pH = 5.5, were done using Franz's cells to simulate the topical release [13]. Franz's cells with a receiving compartment volume of 4.2 ml and an effective diffusion area of 0.95 cm^2 were used in this study. The receiving phase was a buffer solution at pH 5.5 (K_2HPO_4, KH_2PO_4), 70 mM. The buffer, degassed before using, was continuously stirred (600 rpm) and maintained at 37 °C during all the experiment. A cellulose acetate membrane with a pore size of 0.45 μm and a thickness of 115 μm was used. 500 mg of each gel was placed on the membrane surface previously moistened with the receiving phase. Aliquots (100 μl) of the receiving phase were withdrawn at proper time intervals and replaced with an equal volume of fresh buffer solution. Sink conditions were maintained during the experiments. The amount of drug released was determined by measuring the absorbance at 260 nm of the removed aliquots. UV measurements were done using a Jasco V-530 UV–VIS spectrophotometer.

3. Results and discussions

The sol–gel transition of many polysaccharide systems strongly depends on the concentration and type of ions in solution [14,15]. In the case of κ-carrageenan, K^+ and Na^+ ions play a fundamental role in establishing the gelation pathway [9,10]. Previous studies, performed on gels without drug [10], have shown that it is possible, by using an appropriate $[K^+]/[Na^+]$ ratio, to obtain gels with very different structural properties, spamming from an ordered matrix formed by double helix aggregation, up to a much more disordered structure, formed by coil condensation.

Since the aim of this study is to correlate the structural properties of different gels with the release profiles of a loaded drug, it is necessary to evaluate how the drug addiction may change the

structural properties of the κ-carrageenan matrices. In order to investigate this aspect, static light scattering (SLS) measurements were performed on three different gels of 0.4% (w/w) κ-carrageenan at different K^+ and Na^+ concentrations and containing 2.5% (w/w) Ketoprofen sodium salt. Results were compared with those obtained on the same samples without drug.

Table 1 reports the cationic concentrations used for preparing the three 0.4% (w/w) κ-carrageenan gels with or without the drug.

The internal structure of these gels was characterized by determining their fractal dimension, d_f, defined by the relationship $M \propto R^{-d_f}$, where M is the mass of the fractal object within a sphere of radius R [16,17]. The value of this parameter in a gelled system allows determining the unravelling of the polymer chain in the space, i.e. how the polymeric network fills the space. The fractal dimension value can be determined by measuring, over a large q range, the light scattered intensity, $I(q)$, and applying the relationship:

$$I(q) = S(q) \cdot P(q) \propto q^{-d_f}$$

where $S(q)$ is the structure function, q is the scattering vector and $P(q)$ is the form factor which, for this system, can be considered equal to 1 due to the small size of single polymer chains with respect to the light wavelength.

Fig. 1 show log–log plots of $S(q)$, for all samples containing Ketoprofen (straight lines are the data best fit). The d_f values of samples with and without drug are reported in Table 1. In all cases, the drug presence does not modify the fractal dimension. For sample A, a value $d_f \approx 1.3$ was found, which is consistent with an overall loose packing of ramified double-helices [10]. The gel prepared with 3.5 mM KCl and 300 mM NaCl (sample B) has the highest d_f value (2.4). This result has been explained by considering that the small amount of K^+ is not sufficient to promote a significant conformational ordering (double helix), so that the aggregation process follows from a liquid–liquid phase transition (demixing process) leading to a disordered more compact structure [10]. Finally, sample C presents an intermediate d_f value. In this case the K^+ concentration is sufficient to promote the double helix formation, followed by a disordered aggregation as a consequence of the presence of Na^+ [10].

To obtain more information on the internal structure of gels containing Ketoprofen sodium salt, the visco-elastic properties were investigated by mechanical spectra measurements. The results, reported in Fig. 2, show that: (i) for each sample the elastic modulus, (G′), and the viscous modulus, (G″), are almost flat with G′ larger than G″, as expected for solid-like materials, (ii) the values of visco-elastic modules are very different for the three samples and (iii) they are dependent on the ionic composition but independent on the ionic strength. Mechanical spectra of the commercial products Artrosilene gel® and Fastum gel® were performed in the same conditions for comparison.

Table 1
Ionic composition and fractal dimension for κ-carrageenan 0.4% (w/w) gels with and without 2.5% (w/w) Ketoprofen sodium salt

Sample	$[K^+]$ mM	$[Na^+]$ mM	d_f — drug	d_f — no drug
A	150	0	1.3±0.1	1.1±0.1
B	3.5	300	2.4±0.1	2.3±0.1
C	20	300	1.7±0.1	1.8±0.1

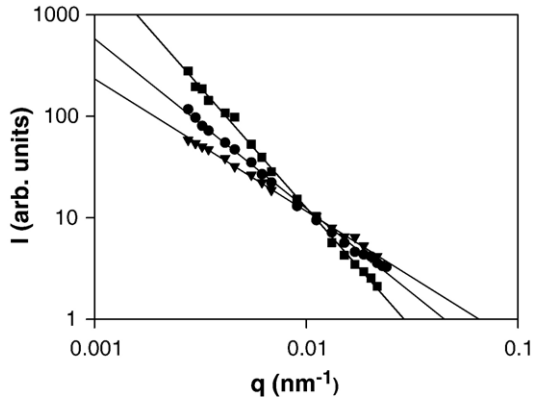

Fig. 1. Log–log plot of the light scattered, $I(q)$, vs. q of κ-carrageenan samples containing drug: sample A triangles; sample B squares; sample C circles. Samples composition is reported in Table 1.

Rheological results for κ-carrageenan gels lead to conclude that it is possible to modulate the visco-elastic properties of gels by opportunely varying the K^+ and Na^+ concentrations. In particular, for the gel prepared with 3.5 mM KCl and 300 mM NaCl, the Na^+ ions have a dominant effect in the aggregation process: in this case the gel network will contain a great number of

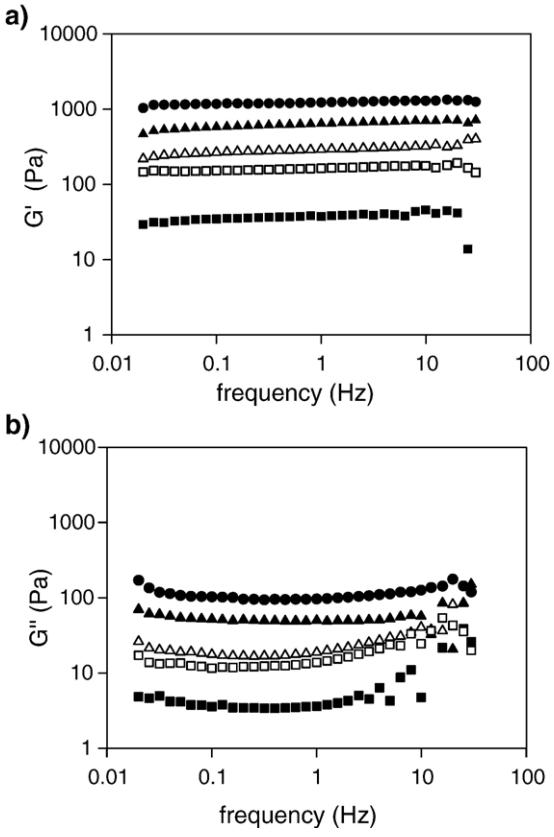

Fig. 2. Mechanical spectra of κ-Carrageenan gels: sample A full triangles, sample B full squares, sample C full circles, Artrosilene gel open squares, and Fastum gel open triangles. Panel a) elastic modulus, panel b) viscous modulus.

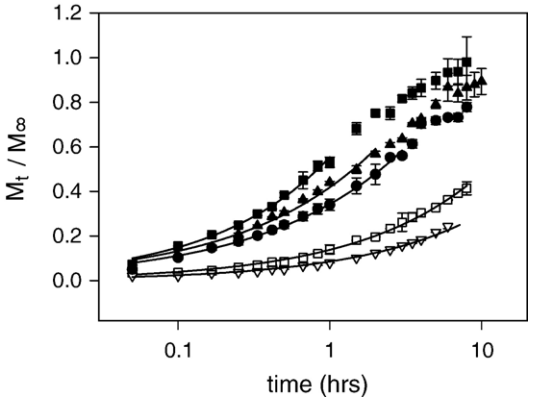

Fig. 3. Release profiles at pH 5.5 of Ketoprofen from κ-carrageenan gels and two commercial products. Sample A full triangles; sample B full squares; sample C full circles; Artrosilene gel open squares and Fastum gel open triangles. Solid lines are data fit to a power law (Eq. (1) in the text).

very weak bonds, since the flexible coils can be arranged in a huge number of non-specific mutual contacts. This justifies the formation of a very disordered gel with a low G′ value and high d_f value. On the opposite, in presence of 150 mM of KCl the gelation mechanism is governed by the formation of rigid double helices, which aggregate into super-strands. This regular structure presents a low d_f value and high G′ value. Finally, the gel prepared with 20 mM KCl and 300 mM NaCl is still able to form strands and helices, but the presence of Na^+ introduces in these structures a number of defects enhancing the flexibility and bond number. In this case, in fact, interconnected regions of partially formed double helices or more flexible and shorter super-strands are obtained, as suggested in the literature [18].

All these findings agree with previous results obtained in absence of Ketoprofen, supporting the hypothesis that the drug does not interfere significantly in the aggregation process.

To evaluate the release profile of Ketoprofen from each gel and correlate the release with the different structural properties of each system, drug release experiments were done using the Franz's cells. Results, reported in Fig. 3, were compared with those obtained using two commercial pharmaceutical products: Fastum Gel™ and Artrosilene Gel®, containing Ketoprofen acid at 2.5% (w/w) and its lysine salt at 5% (w/w), respectively.

Fig. 3 shows that: i) the profile of drug release is affected by the salt composition of the gels; ii) κ-carrageenan gels release a very high amount of drug; iii) the release from commercial pharmaceutical products is incomplete and it is very slow in comparison with the release from κ-carrageenan gels.

Release profiles are quite interesting as they suggest that the release can be controlled by simply choosing the appropriate $[K^+]/[Na^+]$ ratio. Moreover, the release from sample B is almost complete in 8 hrs. In the same time interval samples A and C release a high amount of drug. At difference, commercial products release, in the same interval, a much smaller drug amount (<30%). It is important to emphasize that the time window chosen in this study corresponds to the characteristic time elapsing between two subsequent topical applications; consequently it is very important that a consistent amount of drug is released in this interval time.

To obtain more information about the kinetics of release, data are fitted to the simple power law:

$$\frac{M_t}{M_\infty} = (Kt)^n \tag{1}$$

where M_t is the amount of drug released at time t, M_∞ is the amount of loaded drug in the gel, K is the rate constant and n is an exponent characterizing the release mechanism.

The power law can be used for fitting the release data at short time intervals with $M_t/M_\infty \leq 0.6$, as shown in Fig. 3. The calculated exponent, n, gives an indication of the release mechanism. Indeed, $n=0.5$ when the release is controlled by diffusion and $n=1$ for a time-dependent release (Case-II transport). For $0.5<n<1$ the release is called "anomalous" and both swelling and diffusion play an important role [19,20].

Table 2 shows that the drug release depends on the gel structures. Release from samples A and C is governed by pure diffusion, whereas for sample B, like as for commercial products, the release appears to be anomalous.

The amount of Ketoprofen released has been also plotted as a function of the square root of time as shown in Fig. 4. A linear region can be observed for each κ-carrageenan sample until $t^{1/2}=1.44$. Data have been compared with those obtained from Artrosilene gel and Fastum gel in the same interval time. The linear behaviour indicates that the release is well

Table 2
n and D values, calculated by data fit to Eqs. (1) and (2) in the text, for κ-carrageenan gels and commercial products

Sample	N	D (cm^2/h)
A	0.498	0.1523
B	0.569	0.2508
C	0.490	0.1035
Artrosilene gel	0.540	0.0159
Fastum gel	0.616	5.7255e−3

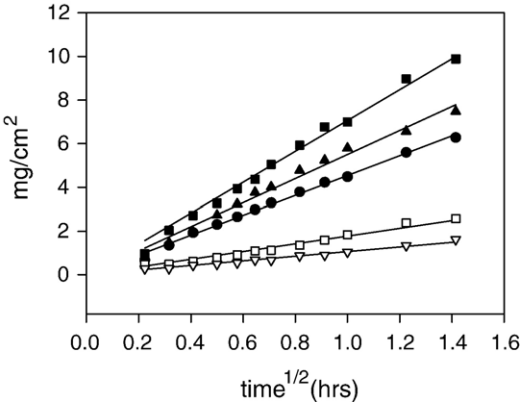

Fig. 4. Ketoprofen amount released from κ-carrageenan gels and commercial products as function of square root of time. Sample A full triangles, sample B full squares, sample C full circles, Artrosilene gel open squares, Fastum gel open triangles. Solid lines are data fit to the Eq. (2) in the text.

described by the mathematical model postulated by Higuchi for the release of a drug from semisolids [21,22]:

$$q = 2C_0(Dt/\pi)^{1/2} \quad (2)$$

where q is the amount release per unit area, C_0 is the initial drug concentration in the gel, D is the diffusion coefficient of the drug and t is the time. D values, calculated from the slope in figure, for κ-carrageenan gels and for commercial products are reported in Table 2.

The resulting n and D values are in good agreement with the structural characteristics of κ-carrageenan samples. Indeed, D decreases on increasing the visco-elasticity. Moreover, in samples A and C, which are more elastic (having G′ larger) and ordered (having d_f smaller) than sample B, the release is controlled by diffusion ($n = 0.5$), and D value are lower than that of sample B, which is less elastic and ordered.

4. Conclusions

The study of κ-carrageenan gels containing a drug and prepared with different salt compositions shows the versatility of this polymer for the realization of gel matrices apt to drug release for external uses. Under the chosen experimental conditions, the incorporation of a drug in sodium salt form does not seem to change the gelation mechanism discussed in details in previous works [9,10]. The structural and visco-elastic characteristics of gels can be controlled by the K^+/Na^+ ions composition.

Drug release studies, using Franz's cells, allowed evaluating the release properties of κ-carrageenan gels. Data have been compared with those obtained from two commercial products. These results suggest that the drug release kinetics in κ-carrageenan gels is affected by the salt composition, and is related to visco-elastic and structural properties. The release by all κ-carrageenan samples is almost completed in 8 hrs, at difference with commercial products. This result seems very promising for external drug formulations, considering that a time interval of several hours between two subsequent applications is generally required.

Overall, this work suggests that structural characteristics of κ-carrageenan gels can be easily modulated by choosing the appropriate salt solution ($[K^+]$ or $[Na^+]$ or mixture). This, in turn, determines the release kinetic without significantly interfere with the mechanism of drug release. This aspect can provide useful advantages for the pharmaceutical development of external gels.

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