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Matrix tablets based on carrageenans with dual controlled release of doxazosin mesylate

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ABSTRACT

The use of polymeric polyelectrolytes as matrix-forming agents is far from optimally or fully understood. Polyelectrolyte carrageenan (CARR) matrices loaded with oppositely charged active substance doxazosin mesylate (DM) were investigated according to their water-uptake/erosion properties, *in situ* complexation ability of CARR with DM, and the possibility to achieve dual drug release control. Interactions between different CARR types (ι-, κ-, and λ-) and DM were confirmed by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and zeta potential measurements. Combination of water-uptake/erosion with *in situ* complexation prolonged DM release from CARR matrices for more than 24 h. The rate order of drug release was in accordance with the number of ester sulfate moieties per disaccharide unit of CARRs (κ (1) > ι (2) > λ (3)). The higher the charge on the CARR backbone, the higher the number of interactions with DM and the slower the drug release. Low pH, more vigorous hydrodynamics, and higher ionic strength resulted in faster drug release. Based on zeta potential measurements of DM and CARRs, proposed influence of counterion condensation and its effect on screening polyelectrolyte–drug interactions was confirmed to lower *in situ* DM–CARR complexation. Dual drug release control from polyelectrolyte matrices by water-uptake/erosion and *in situ* complexation offers many new approaches for designing controlled-release systems.

1. Introduction

Hydrophilic, swellable polymers are the main functional matrix excipients for the majority of modified-release tablet preparations (Omidian and Park, 2008). Cellulose ethers have been the most thoroughly investigated of all of the semi-synthetic polymers (Alderman, 1984; Baumgartner et al., 1998). Nevertheless, polymers of natural origin widely used in the food and cosmetics industry are now coming to the fore of pharmaceutical research (De Ruiter and Rudolph, 1997; Bhardwaj et al., 2000; Coviello et al., 2007).

For hydrophilic polymers, it is generally accepted that upon contact with water they hydrate and swell, forming a gel layer around the dry core that regulates the penetration of water into the matrix and the release of the active ingredient incorporated. Swelling and erosion of the functional polymers are therefore the two main mechanisms governing the release of the drug from the hydrophilic polymer matrices (Colombo et al., 2000; Baumgartner et al., 2002). However, the term "swelling" is often reserved for polymers that form a gel upon contact with water. Because some polymers such as λ-carrageenan do not form gels but rather very viscous solutions (Rees, 1977; Rochas et al., 1986; Yuguchi et al., 2002), "swelling" is denoted as a "water-uptake mechanism."

When ionic polymers (polyelectrolytes) are used as excipients in pharmaceutical formulations, the release of oppositely charged drugs may be strongly affected by the occurrence of charge–charge interactions or possible complex formation (Lelham and Sundelöf, 1995). In some cases, these events are considered negative and should be avoided because they represent drug–excipient incompatibility. On the other hand, polyelectrolyte–drug interactions (complexation) can also be exploited for controlled drug release (Bonferoni et al., 2000).

The polyelectrolytes that have attracted much attention due to their variations in ion specificity, charge density, and possibility of helix–coil transition, thus offering a large number of different applications, are carrageenans (CARRs).

CARRs are linear, anionic, partially sulfated galactans extracted from many species of red algae, the Rhodophyceae. They are composed of D-galactose residues linked alternately with α-(1→3) and β-(1→4) linkages. These sulfated galactans are classified according to the presence of 3.6-anhydrogalactose on the 4-linked residue and the position and number of sulfate groups (Rowe et al., 2005). The most important types of carrageenans are κ-, ι- and λ-carrageenan (Fig. 1). In theory and in the ideal case, κ-CARR has only

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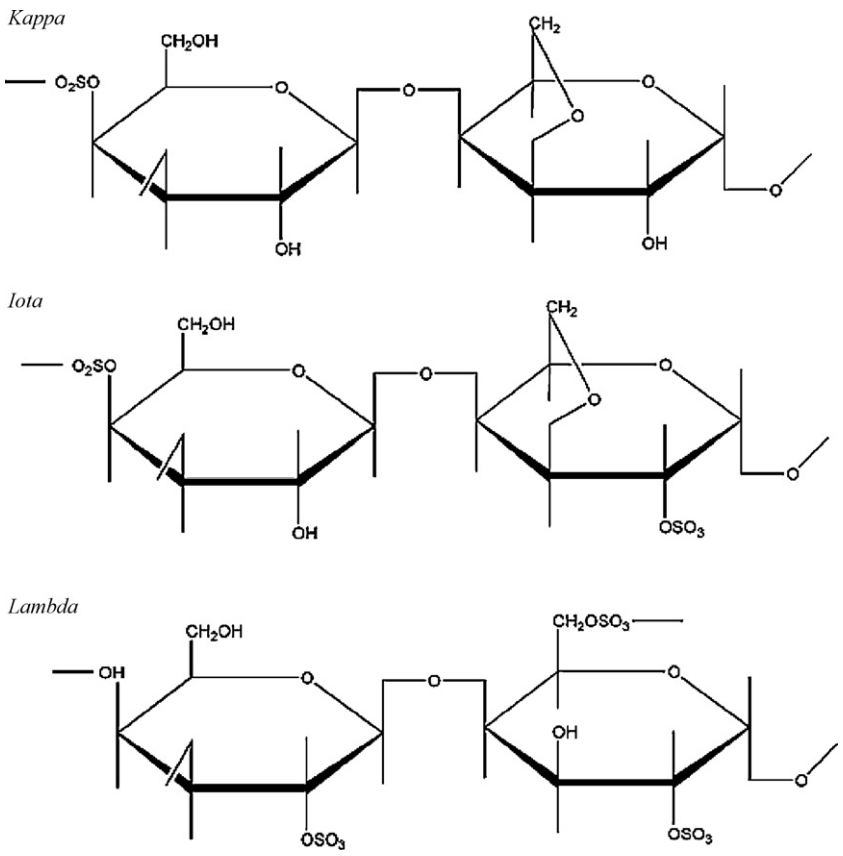

Fig. 1. The repeating unit structure of the carrageenan family (Rowe et al., 2005).

one sulfate moiety per disaccharide repeating unit, ι-CARR two, and λ-CARR can bear three sulfate moieties per disaccharide unit. κ- and ι-CARR contain the 3.6-anhydrogalactose unit and are gelling polymers, but λ-CARR has only galactose residues and is considered a non-gelling, water-soluble polymer, which forms very viscous solutions (Rees, 1977; Rochas et al., 1986; Yuguchi et al., 2002). CARRs are very often used in food products as thickeners and stabilizers; however, their usage is also increasing in pharmaceutical formulations. They are GRAS and are also of pharmaceutical grade. The polyanionic nature of CARRs was shown to have a possible crucial influence on drug release behaviour as well (Singh and Lelham, 1998).

Drug release from CARR matrices was prolonged up to 12 h (Picker, 1999a,b; Naim et al., 2004). CARRs were also combined with other well-established excipients for controlled release, such as cellulose ethers, to additionally prolong drug release (Nerurkar et al., 2005). Combinations of different CARRs themselves (Gupta et al., 2001) or with inert excipients (Picker, 1999a) were also studied. These investigations have shown that CARRs may interact with some basic drugs (Hugerth, 2001), which can lead to additional decreases in the drug release rate (Hariharan et al., 1997). A special method for preparation of a diltiazem hydrochloride–λ-CARR complex was proposed (Aguzzi et al., 2002) and almost all further CARR studies with a basic drug focused on the preparation of the complex before tablet preparation. To the best of our knowledge, no comparison of characteristics of prepared complexes with all three CARR types and a basic drug has been done to date, and no *in situ* complexation (instead of special complex preparation) has been evaluated. Detailed explanation of processes controlling drug release from matrices achieved by a combination of complexation and water-uptake/erosion mechanisms and the environmental impacts on these processes is still lacking.

Doxazosin mesylate (DM) is a cationic drug and selective α_1-antagonist used to treat hypertension. It also blocks α_1-receptors in the prostate gland and alleviates the symptoms of benign prostatic hyperplasia. The therapeutic dose is between 1 and 16 mg, also available as once-daily dosing in controlled-release formulations, which lead to increased patient compliance (Williams and Lemke, 2002).

The aim of our study was to focus on achieving dual drug release control from CARR matrices based on water-uptake and erosion on the one hand, and drug–polyelectrolyte complexation on the other. To accomplish this, various polyelectrolyte behaviours of all three major CARR types were exploited to achieve *in situ* complexation with the cationic drug DM. Complexes were characterized by DSC, SEM, and zeta potential measurements. Water-uptake and erosion mechanisms of CARR matrices were also studied. The magnitude of polymer–drug interactions can completely change the release profile and offers fine-tuning of the release profile in a desirable manner. An understanding of these interactions together with the impact of ionic strength and hydrodynamics is valuable in elucidating the effects of drug release *in vivo*.

2. Experimental

2.1. Materials

Carrageenan (CARR) ι (Gelcarin GP 379 NF), κ (Gelcarin GP 911 NF), and λ (Viscarin GP 209 NF) were obtained from FMC Biopolymers (USA). The average molecular weight (MW) for ι- and κ-CARR was in the range of 400–600 kDa and for λ-CARR it was 400–800 kDa. The active substance doxazosin mesylate (DM) with a MW of 547.58 g/mol was supplied by Krka (Slovenia). The saturated solubility of DM was determined experimentally in the following

media, to verify the pH dependency of solubility: pH 1.2 hydrochloric acid: 0.0603 mg/ml, pH 4.5 phosphate buffer: 2.79 mg/ml, pH 7.0 phosphate buffer: 1.84 mg/ml and water: 3.64 mg/ml.

2.2. Preparation of physical mixtures and pastes

Physical mixtures of DM with each selected CARR were prepared in 30:70, 50:50, and 70:30 w/w ratios by simple blending of the components in a mortar for 10 min at room temperature. To study drug–excipient interactions, a paste was prepared according to the procedure described by Bonferoni et al. (2000). Physical mixtures with different proportions of DM and CARR were made and small amounts of water were added dropwise until paste properties were achieved. The paste was then dried in a drying chamber at 45 °C for 24 h.

2.3. Preparation of matrix tablets

The drug dosage was chosen to be 8 mg per tablet. Each CARR type and DM was homogeneously mixed using a laboratory-model drum blender. Predetermined amounts of these tablet mixtures were compressed directly using a 10 mm diameter flat-faced punch with bevelled edges on a single-punch tabletting machine (Killian SP 300, Germany). The hardness of all the tablet formulations was adjusted to 80–120 N (VanKel VK 200, USA; hardness tester; $n = 6$). The weight of each tablet was 300 ± 10 mg. For water-uptake and erosion studies, pure CARRs without DM were compressed.

2.4. DSC measurements

DSC experiments were carried out on a Mettler TA 1 Star software apparatus (Mettler Toledo, Switzerland) equipped with a DSC 25 cell. Samples of about 5–10 mg were weighed (Mettler M3 microbalance) in pierced aluminium pans and scanned under a nitrogen atmosphere over a temperature range of 0–300 °C at a heating rate of 40 °C/min.

2.5. Scanning electron microscopy (SEM)

Pure substances (DM and ι-, κ-, and λ-CARR), physical mixtures, and pastes were carefully pressed onto double-sided adhesive carbon tape (SPI Supplies, USA) and imaged with a field emission scanning electron microscope (SEM, Supra 35 VP, Carl Zeiss, Germany) operated at 1 keV.

2.6. Zeta potential measurements

Photon correlation spectroscopy (Zetasizer Nano ZS; Malvern Instruments, UK) was used to determine the zeta potential of charged DM and CARR particles by observing their electrophoretic mobility in an electrical field. Samples were prepared by dispersing each substance in water, 0.1 M HCl, pH 4.5 phosphate buffer and pH 7.0 phosphate buffer. The concentration of DM was always higher than it's saturation solubility in each media, since zeta potential could only be determined on solid sub-micron particles. Ultrasonification was used to get sub-micron particles of DM. In case of CARRs experimental concentration of 0.3 g/L resulted in a formation of colloidal dispersion, which allowed the measurements. The temperature of the samples was controlled at 25 °C. All measurements were performed at least six times.

2.7. Water-uptake and erosion studies

Water-uptake and erosion studies were performed using a dissolution apparatus equipped with paddles (USP Apparatus II, VanKel Dissolution Apparatus, model VK 7000, USA). In addition to this standard dissolution apparatus, arrangements were made to prevent the matrix from sticking to the glass walls. These involved the inclusion of a stainless steel mesh device that fit precisely under the paddle into the lower portion of the standard dissolution vessel. The paddle speed was maintained at 75 rpm and the medium used was 900 ml of pH 7.0 phosphate buffer without or with 40 mM NaCl. Experiments were performed six times. After 0.5, 1, 2, 4, and 8 h, the mesh assemblies supporting the partially hydrated matrices were carefully removed and the tablets were lightly dried with tissue paper to remove excess surface water prior to being weighed. Water-uptake was calculated according to Eq. (1):

$$\text{water-uptake}\ \ (\%) = \frac{m_t - m_r}{m_r} \times 100 \quad (1)$$

m_t: mass of hydrated tablet after the determined time of water-uptake (g); m_r: mass of remaining dry tablet after water-uptake (g).

After the hydrated tablets' weight was determined, they were dried at 70 °C in a vacuum oven for 2 days before being reweighed to determine the remaining dry weight. The percent of erosion was calculated according to Eq. (2):

$$\text{erosion}\ \ (\%) = \frac{m_0 - m_r}{m_0} \times 100 \quad (2)$$

m_0: mass of dry tablet before water-uptake (g).

2.8. Drug release measurements

Dissolution studies were performed on a fully calibrated dissolution apparatus using the basket method (USP Apparatus I, VanKel Dissolution Apparatus, model VK 7000, USA). Basket speed was kept at 150 rpm. The dissolution medium was 900 ml of either 0.0075 M phosphate buffer pH 7.0 or phosphate buffer with 0.04 M NaCl. Because ionic strength in gastrointestinal fluids ranges from 0.010 to 0.166 (Baumgartner et al., 2008), an ionic strength of 0.04 was chosen for this study. We used NaCl as an electrolyte that is physiologically present in gastrointestinal fluids. The temperature was maintained at 37 ± 0.5 °C. At predetermined time intervals, 10 ml samples (not replaced) were withdrawn, filtered through a 0.45 μm membrane filter, and analyzed UV-spectrophotometrically at 249 nm (HP diode array UV spectrophotometer, 8453, Germany). All dissolution studies were performed at least six times.

To simulate *in vivo* conditions, drug release studies were performed on a fully calibrated USP III dissolution apparatus (BioDis, VanKel, USA). Each vessel was filled with 250 ml of 0.1 M HCl (1st and 2nd rows: 2 h), 0.1 M phosphate buffer pH 4.5 (3rd row: 0.5 h), and 0.0075 M phosphate buffer pH 7.0 (4th, 5th, and 6th rows: 21.5 h). The same procedure, with the addition of 0.04 M NaCl, was used to study the influence of ionic strength.

The other experimental conditions were as follows: a reciprocating cylinder was used as a sample holder, reciprocation speed was 10 dips/min, polypropylene screens (405 μm) were used, and the temperature was maintained at 37 ± 0.5 °C. At each predetermined time interval 5 ml samples were withdrawn, filtered through a 0.45 μm membrane filter, and analyzed UV-spectrophotometrically at 249 nm. All of the dissolution studies were performed at least six times.

3. Results and discussion

3.1. Complex characterization

Ionic interactions of CARRs with DM were confirmed and explained by use of differential scanning calorimetry (DSC) (Pavli et al., 2010). The thermal properties of pure DM and ι-CARR are

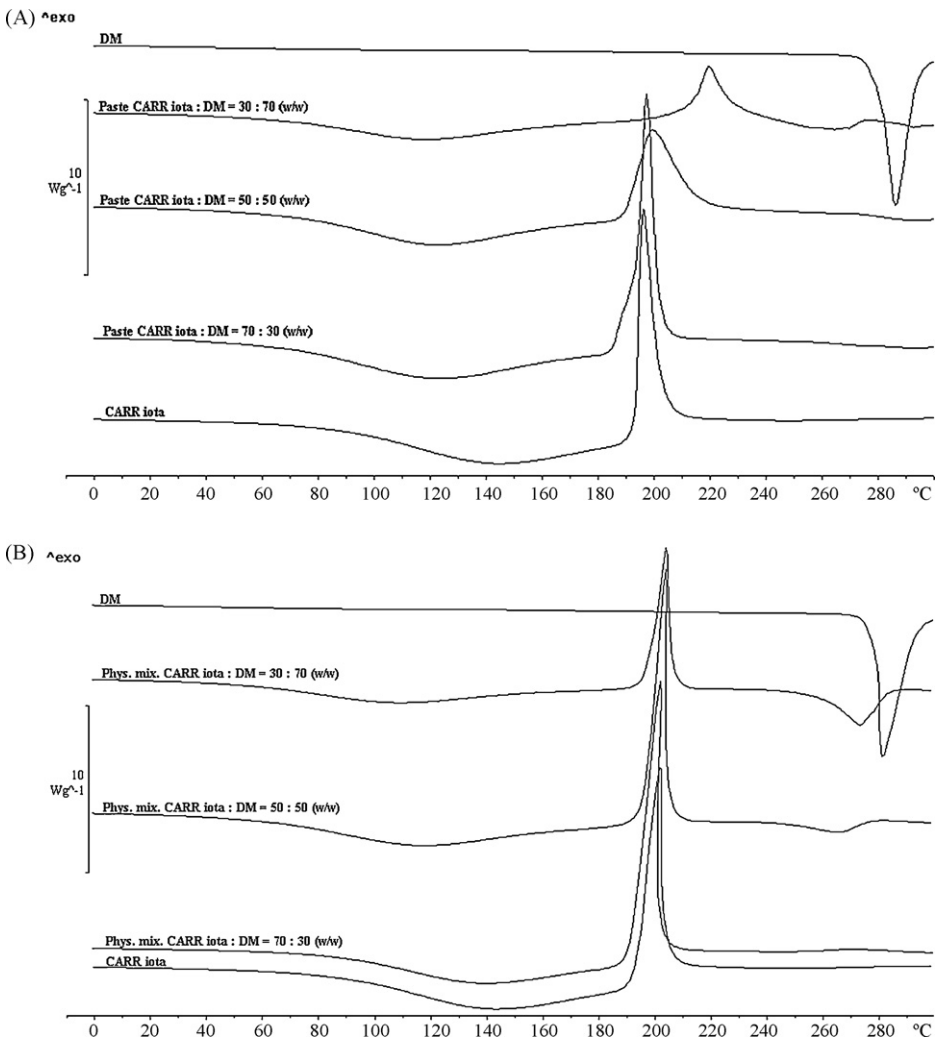

Fig. 2. DSC curves for doxazosin mesylate (DM), ι-CARR, and ι-CARR:DM pastes (A) and physical mixtures (B) in various ratios: 30:70 (w/w); 50:50 (w/w), and 70:30 (w/w). Samples of about 5–10 mg were weighed in pierced aluminium pans and scanned under a nitrogen atmosphere at a heating rate of 40 °C/min.

presented in Fig. 2. DM has one endothermic peak at 280 °C, which represents melting of the pure drug crystals. In case of ι-CARR as well as for other CARRs, a strong exothermal effect between 190 and 220 °C can be observed, which most probably represents the breakdown of the ester sulfate moieties on polysaccharide chains.

Due to the interactions between CARR and DM, obvious peak shifts and shape changes of the CARR desulfation peak in accordance with the disappearance of the DM melting peak are obtained in DSC curves of pastes prepared from ι-CARR:DM as an example (Fig. 2A). The range of thermal effects depends on the ratio of DM to CARRs. In general, the higher the DM:CARR ratio, the higher the number of interactions and the more pronounced the thermal effects. Additionally, thermal effects were more expressed in pastes than they were in physical mixtures of DM and CARRs (Fig. 2B).

According to the chemical nature of both substances, the presence of ionic interaction between the cationic DM and anionic CARRs was predicted. The DM molecule has five nitrogen atoms with varying proton affinities (from 880 to 1072 kJ/mol; Kinsella et al., 2006), and nitrogen with the highest proton affinity (i.e., proton acceptor ability) is assumed to be involved in the interaction with the anionic ester sulfate moieties of the CARRs (Fig. 3).

Ionic interactions between DM and CARRs are believed to inhibit desulfation of CARRs at temperatures observed for pure substances, which can be seen as the desulfation peak shift towards higher temperatures. In addition, the DM melting peak cannot be seen due to ionic interactions.

Interactions between DM and CARRs were also confirmed by the SEM characterization of pastes prepared from pure CARR and physical mixtures of CARR and DM at different mass proportions. Fig. 4 shows SEM photographs of pure DM, a paste made from pure ι-CARR, a physical mixture of ι-CARR:DM in a 70:30 ratio, and the same ratio of substances prepared as a paste (physical mixture wetted with water and dried).

Pure DM exists as long, cubic-shaped crystals (Fig. 4). In the physical mixtures some adherence of CARR particles to the drug

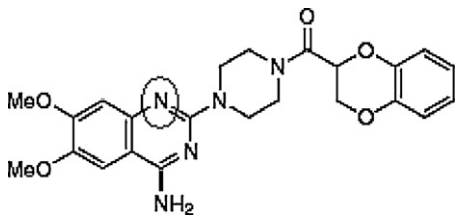

Fig. 3. Doxazosin molecule; calculations by Kinsella et al. (2006) suggest that the circled nitrogen is involved in the interaction with the anionic ester sulfate moieties of the CARRs.

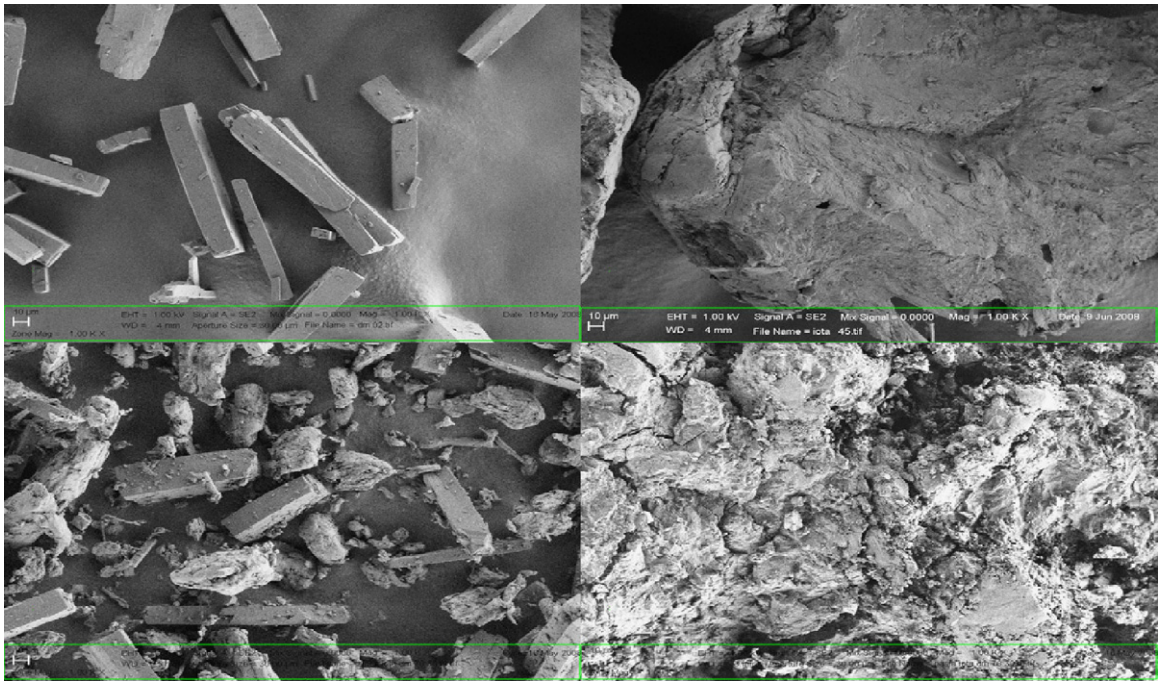

Fig. 4. SEM photos (1000× magnification) of DM (upper left), a pure ι-CARR paste (upper right), a physical mixture of ι-CARR:DM in a 70:30 ratio (lower left), and an ι-CARR:DM paste in a 70:30 ratio (lower right).

crystals can easily be observed. The aqueous paste prepared from pure ι-CARR has a homogeneous appearance, which results from the formation of a very dense polymer structure. However, the structure of the aqueous paste made from a mixture of DM and ι-CARR, is different from the latter, less cohesive (Fig. 4), because the drug was dissolved in the vehicle and interacted with the ester sulfate moieties on the CARR chains. This assumption was confirmed by the disappearance of the DM melting peak on the DSC curves of dried paste with the same composition. On the other hand, different paste structures could also result from the formation of DM amorphous particles, which could precipitate from the aqueous solution upon drying. However, this assumption was rejected because the DSC measurements detected no Tg or cold crystallization peak followed by a DM melting peak. The amorphous form of DM was studied in detail by Grčman et al. (2002).

3.1.1. Evaluation of ionic interactions between CARRs and DM in different media by zeta potential measurements

Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle (Delgado et al., 2005). Using the known zeta potential of the species, it is possible to predict the magnitude of attraction or repulsion forces between charged particles. Pure anionic substances have a negative zeta potential whereas pure cationic ones have a positive zeta potential. The net difference of zeta potentials between oppositely charged species shows how favourable the interaction is. The results of λ-CARR and DM zeta potential measurements are presented in Table 1.

Table 1
Zeta potential of DM and λ-CARR in different media.

Zeta potential (Z)[a]	Water (mV)	pH 4.5 medium (mV)	pH 7.0 medium (mV)
DM	70.2	5.49	19.6
λ-CARR	−105.0	−39.9	−57.9

[a] Zeta potential measurements made in 0.1 M HCl were unrepeatable.

λ-CARR is most highly charged in purified water (−105.0 mV), followed by pH 7.0 phosphate buffer (−57.9 mV) and pH 4.5 phosphate buffer (−39.9 mV). The same order of zeta potential was measured for DM: purified water (70.2 mV) > pH 7.0 phosphate buffer (19.6 mV) > pH 4.5 phosphate buffer (5.49 mV).

The zeta potential results indicate that strong attractive interaction persists between DM and CARRs and therefore *in situ* complex formation during drug release can be achieved. The results also indicate that the zeta potential is medium dependent, so it is obvious that this will have strong influence on the magnitude of interactions and thus also on *in situ* complexation. According to our results (Table 1), the highest zeta potential is obtained in water, which can be expected, since no other electrolytes that could influence the zeta potential of the particles, are available in media. In case of phosphate buffer the rate order of zeta potential can be explained in the same way, since ionic strength of pH 7.0 phosphate buffer and thus the concentration of electrolytes is much lower compared to pH 4.5 phosphate buffer.

3.2. Characterization of CARR matrices

CARR matrices were designed to control the release of DM not only by polymer water-uptake and erosion, but also by *in situ* complexation between drug and polymers. Therefore, this combination of drug release mechanisms was evaluated more specifically.

In order to distinguish these processes and to gain the basic knowledge of the mechanisms involved in drug release from CARR matrices, erosion and water-uptake studies were performed on pure polymer matrices without DM. In addition, the influence of increased ionic strength of media was also evaluated.

3.2.1. Erosion studies of ι-, κ-, and λ-CARR matrices

Results of erosion studies (Fig. 5) indicate that erosion was the fastest for κ-CARR, reaching 100% in 8 h, followed by both ι- and λ-CARR with 83% erosion. From the results presented, it is obvious that erosion is very rapid, exhibiting an almost linear profile with no lag time. Because all CARR matrices were completely eroded

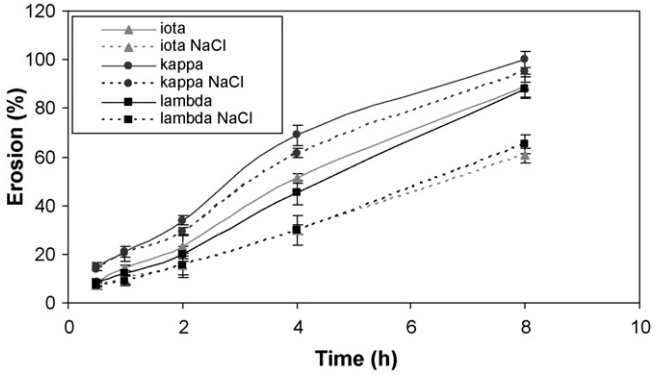

Fig. 5. Erosion of pure CARR matrices in pH 7.0 phosphate buffer (solid lines) and pH 7.0 phosphate buffer with increased ionic strength (dotted lines). The results represent the mean ± SD of six measurements.

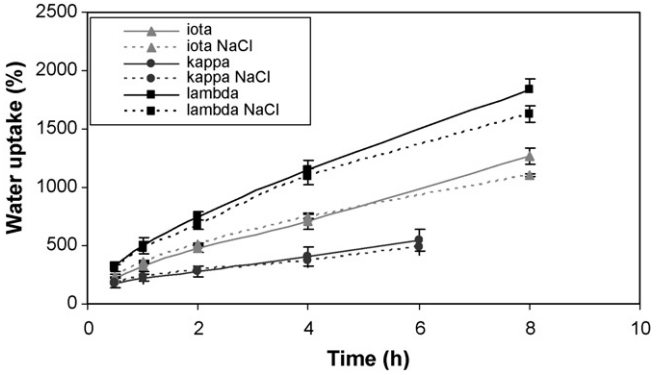

Fig. 6. Water-uptake of pure CARR matrices in pH 7.0 phosphate buffer (solid lines) and pH 7.0 phosphate buffer with increased ionic strength (dotted lines). The results represent the mean ± SD of six measurements.

between 8 and 9 h, we were able to determine the time period during which drug release from CARR matrices will be controlled only by water-uptake and erosion mechanisms.

The results of erosion of CARR matrices (κ > ι = λ) are a bit surprising because it was expected that the degree of polymer erosion would correlate with the number of sulfate moieties on disaccharide units of CARRs (λ (3) > ι (2) > κ (1)). Namely, κ-CARR with the lowest number of ester sulfate moieties per disaccharide unit could thus form stronger and more cohesive gels due to the smaller repulsion forces between polymer chains, and erosion should be the slowest. On the other hand, higher charge densities on the λ type of polymer should result in higher repulsions and therefore faster erosion. However, according to our erosion results κ-CARR erosion was the fastest, followed by a similar erosion rate of λ- and ι-CARR matrices. Based on these results it can be concluded that the erosion process of CARR matrices is not only dependent on polymer charge densities, but is also influenced by the molecular weight of polymer chains, hydrophobic effect, media composition, and conformational changes. This is especially important because CARRs are of natural origin and sequence differentiations from the ideal structure and conformations can be present (Van de Velde et al., 2002).

Because CARRs are anionic polymers, the presence of counterions is expected to affect the polymer structure. Erosion studies were thus performed under increased ionic strength. The results (Fig. 5) clearly show that increased ionic strength suppressed the erosion process. The reason is the charge screening effect of counterions (Rivas and Moreno-Villoslada, 1998; Baumgartner et al., 2008) neutralizing the charge on the CARR polymer backbones. Consequently, this led to lower repulsion between charged CARR chains and resulted in more cohesive polymer networks through more expressed Van der Waals forces and hydrophobic effect (Lelham and Sundelöf, 1995).

The presence of NaCl does not change the rank order of erosion of different CARRs compared to media without NaCl (κ > ι = λ). However, the impact of increased ionic strength is more evident in case of λ- and ι-CARR, which is probably due to their higher charge densities compared to κ-CARR, in which the effect of NaCl is insignificant. The higher charge densities of ι and λ result in more pronounced counterion condensation on their ester sulfate moieties.

Results of erosion studies of CARR matrices with incorporated DM were very similar to that of pure CARR matrices (data not shown). However, after precise visual inspection very small particles, especially in the case of loaded ι- and λ-CARR matrices, were found in media after complete matrix erosion. Because these were not observed in the case of pure CARR matrices, it led us to the assumption that these particles represent complexes between DM and CARRs.

From the erosion results it can be concluded that drug release from ι- and λ-CARR matrices, governed by erosion, will be much more sustained when matrices are exposed to media with dissolved ions such as NaCl.

3.2.2. *Water-uptake studies of ι-, κ-, and λ-CARR matrices*

Results of water-uptake studies (Fig. 6) indicate that water-uptake is the highest in case of λ-CARR at 1800% after 6 h, followed by ι-CARR at 1250% and κ-CARR at around 550%. The water-uptake process is rapid for all CARRs, exhibiting an almost linear time profile with no lag time. The degree of water-uptake correlates with the number of ester sulfate moieties on CARRs (λ (3) > ι (2) > κ (1)).

The presence of electrolytes in the medium has only a limited effect on water-uptake behaviour. NaCl in the medium suppresses water-uptake, but the effect was not as pronounced as in the erosion studies. Ester sulfate moieties have a strong tendency to interact with water, and counterion (sodium) condensation should shield these interactions. However, sodium ions themselves attract water with solvent drag and therefore the amount of water absorbed remained practically unchanged.

At first glance it seems that our water-uptake results are not in accordance with the study published by Picker (1999b), who found that water-uptake of κ-CARR was faster than ι-CARR. However, it has to be stressed out that their group used a different method for water-uptake studies with no hydrodynamic influence involved.

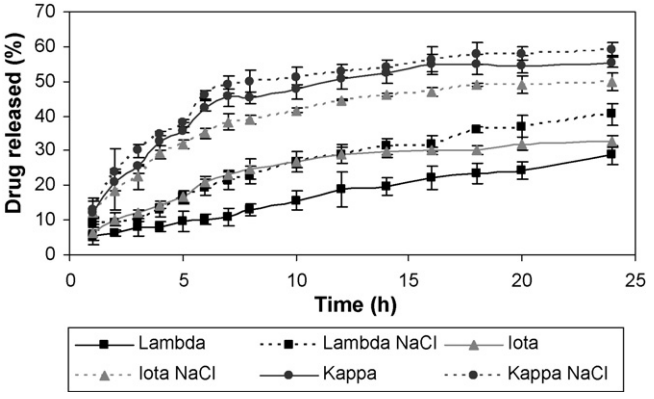

Fig. 7. DM release from different CARR matrices. Solid lines represent drug release in a pH 7.0 phosphate buffer, and dotted lines show drug release in a phosphate buffer with added NaCl. The results present the mean ± SD of six measurements.

3.2.3. Drug release from CARR matrices: dual drug release control

Our results show that DM release from CARR matrices was sustained for more than 24 h (Fig. 7). The slowest release was observed from λ-CARR, in which only 29% of DM was released in 24 h, followed by ι-CARR with 32% of drug released and κ-CARR with 55% of DM released.

The very slow release of DM cannot be explained simply by the water-uptake and erosion results because both processes take place only up to 8 h, depending on the CARR type (Figs. 5 and 6). Additional drug release prolongation and the slower release rate result from *in situ* complexation between negatively charged CARRs and positively charged DM, which was confirmed by DSC and SEM measurements and discussed above.

The rate order of drug release from CARRs (κ > ι > λ) is again in accordance with the number of sulfate moieties (κ (1) > ι (2) > λ (3)). The higher the charge on the CARR backbones, the higher the interaction with DM and the slower the drug release.

Despite the total erosion of CARR matrices in less than 8 h, the incorporated drug is only partially released. This supports our previous finding from erosion studies that the complex particles of DM and CARRs still persist in the dissolution medium after full matrix degradation. These particles thus represent the reservoir for drug release, further enabling dual drug release control. There are no literature data indicating that complexation of CARR and a basic drug can result in interactions with such a magnitude that drug release could be controlled for more than 24 h.

From the drug release results (Fig. 7) it can be seen that drug release slowly reaches a plateau for κ- and ι-CARR; for κ-CARR this is in 16 h and for ι-CARR 12 h, but no plateau is observed in the release profile of DM from λ-CARR matrices. It seems that after 8 h, when the tablets are completely eroded, some undissolved drug remained entrapped in these complex particles and was slowly released by particle erosion until only DM bound to CARR was left. In the case of λ-CARR, complex particles together with undissolved drug were more tightly bound due to the higher number of interactions, and so erosion of these particles was slower and did not reach a plateau in 24 h.

Increasing ionic strength of media resulted in faster DM release from CARR matrices (Fig. 7). Drug release from λ-CARR increased to 35% in 24 h and from ι- and κ-CARR drug release in 24 h reached 48% and 59%, respectively. Again, continuous drug release was observed after the matrices completely eroded, until a plateau was reached. The reason is the same as in media without added NaCl.

Higher ionic strength was expected to result in faster drug release because sodium ions compete with DM molecules for interaction points on the CARR backbones. This competition resulted in a lower degree of interactions between DM and CARRs, leading to faster drug release.

However, the effect of NaCl was not expressed to the same extent for all CARR types. The most dramatic difference can be seen in the much faster release rate of DM from the ι-CARR matrix. This phenomenon can be explained by the various counterion specificities of CARRs and connected structural changes. It is known that CARRs have the ability to undergo coil-to-helix transition in solution depending on the temperature, type, and concentration of ions (Nilsson and Piculell, 1991). According to a study by Janaswamy and Chandrasekaran (2001), ι-CARR in the presence of sodium cations changes its coil-to-helix conformation, which can be followed by the formation of double helix. This double-helix conformation can significantly affect drug release from the ι-CARR matrix. When a double helix is formed, fewer interactions are possible between ι-CARR and DM, resulting in significantly faster release than expected. On the other hand, only single chain conformation of κ-CARR exists in the presence of monovalent ions like sodium, indicating no reduced interaction sites on κ-CARR chains (Yuguchi et al., 2003). The same was confirmed by the study of Mangione et al. (2005), where in the presence of sodium cations κ-CARR was found in the coil state at room temperature. According to these authors, only at high sodium concentration and low temperature conformational transition from coil to helix could be induced, however, this still remains a matter of the debate.

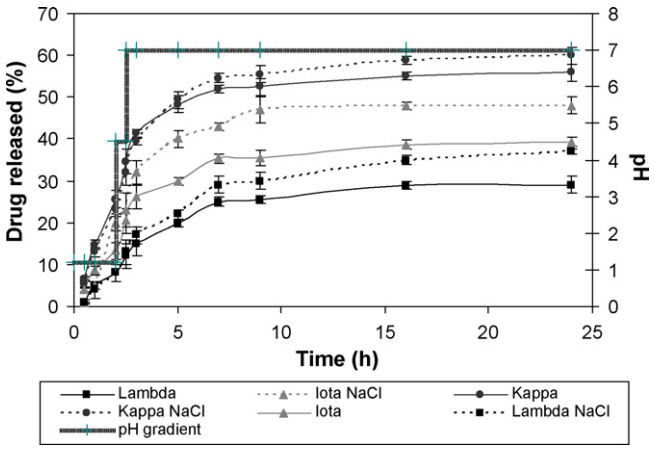

Fig. 8. DM release from different CARR matrices. Solid lines present drug release in media with a pH gradient from 1.2 to 7.0, and dotted lines present drug release in the same media with added NaCl. The pH gradient was maintained as follows: 2 h pH 1.2, 30 min pH 4.5, and 21.5 h pH 7.0. The results represent the mean ± SD of six measurements.

Drug release from λ-CARR matrix is also not supposed to be affected to the extent that ι-CARR is, due to its specific structure. As mentioned before, λ-CARR does not form gels, a fact that is associated with its lack of ability to form double helices.

3.2.4. The influence of pH variation and increased hydrodynamic conditions on drug release from CARR matrices

Results of DM release from CARR matrices obtained in pH 7.0 phosphate buffer on a USP 1 apparatus showed the potential of dual drug release control: by the water-uptake/erosion process and *in situ* complexation. In addition, we wanted to determine how CARR matrices loaded with DM would perform under conditions more similar to *in vivo* conditions. Therefore we performed drug release studies on a BioDis system that allows the concurrent simulation and variation of pH, ionic strength, and hydrodynamics in the gastrointestinal tract (Esbelin et al., 2006).

In our experiments, pH was varied from 1.2 to 7.0. The results obtained (Fig. 8) indicate that simulated *in vivo* conditions influenced the release profiles from CARR matrices.

DM release was the slowest in λ-CARR, followed by ι- and κ-CARR. The comparison of these results with those obtained on the USP 1 apparatus (Fig. 9) shows significant differences in the rate of drug release over 24 h, in which faster drug release was observed on the BioDis apparatus.

Faster drug release on the BioDis is a result of combined effect of pH, medium composition, and stressful hydrodynamics. DM solubility in different media (Section 2.1) also has some influence on drug release; however, this influence is less expressed due to "sink" conditions used during dissolution studies. Moreover, drug release from CARR matrices in the presence of NaCl is faster compared to media without NaCl (Fig. 8), even if solubility of DM is lower. Experimentally determined solubility of DM in pH 7.0 phosphate buffer was 1.84 mg/ml, while in pH 7.0 phosphate buffer with NaCl solubility of 0.040 mg/ml was determined.

More stressful hydrodynamics results in a faster matrix erosion compared to hydrodynamic conditions in USP 1, as observed in our study, in which all CARR matrices disintegrated at about the same time, in 7 h. The effect of pH and media composition is

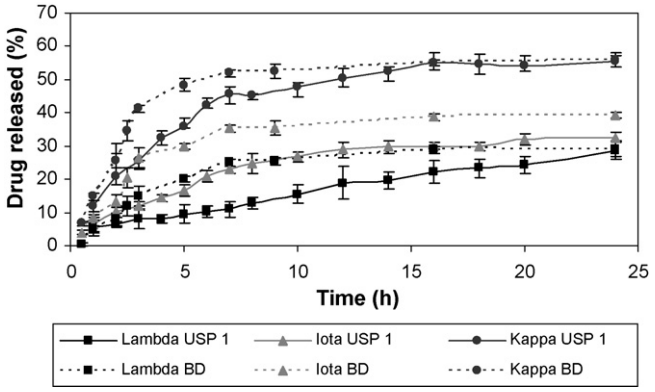

Fig. 9. DM release from different CARR matrices on BioDis and USP 1 apparatuses. Solid lines present drug release on the USP 1, and dotted lines present drug release on the BioDis. The pH gradient on the BioDis was maintained as follows: 2 h pH 1.2, 30 min pH 4.5, and 21.5 h pH 7.0. The results represent the mean ± SD of six measurements.

more complex. Low pH (1.2) should affect interactions between the basic drug and CARRs, as was observed by Bonferoni et al. (1993). Cationic–anionic interactions between two species at low pH should be lower because CARRs become less ionized. It seems that this is also the case for the release during the first 2 h on the BioDis, resulting in a faster drug release rate. However, this effect was less pronounced than expected, maybe also due to lower DM solubility at this pH (Section 2.1). Low solubility of DM in 0.1 M HCl medium is a bit surprising. However, it is known also from the literature (Herbig et al., 1995) that solubility of doxazosin could be higher at low pH, but only in the absence of chloride ions.

In media with pH 4.5 and pH 7.0 the situation is different. At these conditions both CARRs and DM are ionized, drug solubility is in the same order rank, and the pH effect is not dominant, but ionic strength influences drug release.

The highest ionic strength was in the pH 4.5 medium ($\gamma = 0.1$), followed by pH 7.0 ($\gamma = 0.02$). Based on water-uptake and erosion studies, higher ionic strength decreased the erosion process whereas water-uptake remains more or less unaffected. If water-uptake/erosion were the only mechanisms governing the drug release, then drug release would be the slowest in the pH 4.5 medium (between the 2nd and 3rd hour of drug release, Figs. 8 and 9) and the fastest in the pH 7.0 medium (from the 3rd hour to the end of the drug release study). However, the results show the opposite because sodium ions compete with DM molecules for ester sulfate moieties on CARRs. The higher the concentration of sodium counterions, the lower the complexation of DM with CARRs and the faster the release. The same parallel could be made for DM release in media with added NaCl (Fig. 7).

To confirm the hypothesis that sodium counterions supersede DM molecules and to explain the difference in drug release in different media on the BioDis, zeta potential measurements are of great value (Table 1). These results indicate that sodium and phosphate ions in the media lowered the potential of both species by adhering to their oppositely charged surfaces, resulting in lower attraction. The net zeta potential difference between λ-CARR and DM is much higher in pH 7.0 phosphate buffer (77.5 mV) compared to pH 4.5 phosphate buffer (45.48 mV), which explains the higher attraction and consequently slower drug release in media with pH 7.0. Zeta potential measurements clearly confirmed the postulated hypothesis of correlation between sodium ions concentration and DM release rate from CARR matrices.

Because 24 h drug release is far from complete on both apparatuses used (Figs. 8 and 9), the insoluble complex particles of DM with CARRs offer the potential of drug depot. However, the question remains whether the complexed DM is really available for absorption *in vivo* or whether it will be eliminated unabsorbed through the faeces as a complex with CARRs.

Our further studies concerning the usage of drug depot benefit revealed that the incorporation of an ionic surface active agent can at least partially block the complexation. The addition of ionic surfactant in concentrations that provide surface tension close to *in vivo* values, 30–45 mN/m (Kalantzi et al., 2006), has a profound effect on raising the drug release rate. Therefore it can be expected that naturally occurring surfactants such as lecithin and taurocholate would cause similar effects. Nevertheless, at least steric factors and critical micelle formation must also be considered when comparing different types of surfactant. This investigation is still in progress and will be published upon completion of the study.

4. Conclusions

We have demonstrated that dual controlled drug release from CARR matrices can be achieved. In addition to water-uptake and erosion processes, *in situ* complexation between DM and CARRs took place during drug release. The combination of both mechanisms resulted in release prolongation of more than 24 h, which is more than two times longer compared to traditional CARR matrices. Interactions between negatively charged CARRs and positively charged DM, confirmed by DSC, SEM, and zeta potential measurements, can be tuned to achieve the desired release rate, also offering drug depot for continuous slow drug release. The type of carrageenan (ι, κ and λ) and its structural characteristics have a significant effect on drug release. It was shown that the higher the number of ester sulfate moieties on CARR, the slower the release rate. Hydrodynamics and ionic strength, especially counterion condensation, can also alter drug release due to structural characteristics of CARRs. However, the question remains what the behaviour of such a dual controlled drug release form is *in vivo*. Anionic surfactant was found to have a major impact on DM–CARR interactions and can offer additional tuning of drug release from these systems. The study of the influence of naturally occurring surfactants and the usage of the drug depot is still in progress.

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