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Doxazosin–carrageenan interactions: A novel approach for studying drug–polymer interactions and relation to controlled drug release

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ABSTRACT

When a cationic drug like doxazosin mesylate (DM) is incorporated into matrix tablets made of anionic polyelectrolytes carrageenans (CARRs) of different types (κ-, ι-, λ-CARR), DM–CARR interactions have a strong impact on drug release. To investigate these interactions, special DM ion-selective membrane electrode was made and applied for construction of binding isotherms. Isotherms were treated by the Zimm–Bragg theory and cooperative binding model. It was demonstrated that binding of doxazosin cations, DH^+, to CARRs is cooperative. It starts at very low drug concentrations with strong electrostatic interactions followed by aggregation of DH^+ ions. Hydrophobic interactions between bound DH^+ substantially contribute to the extent of binding. The strength of interactions increases with increasing negative charge of CARRs. At saturation, the number of DM molecules bound per repeat unit depends on the charge and steric distribution of binding sites on CARRs. Drug release rates of DM from CARR matrices were in accordance with the cooperativity binding constants: the weakest binding resulted in the fastest release. However it was proven that prolonged drug release is possible only by several processes running simultaneously, i.e., by swelling and erosion of CARR matrices on one side and electrostatic interactions and cooperativity effects on the other.

1. Introduction

Charged polymers, polyelectrolytes, are often used as sustained release agents in pharmaceutical formulations (Bhardwaj et al., 2000; Coviello et al., 2007; Nanaki et al., 2010; Omidian and Park, 2008). Besides their swelling/water uptake and erosion properties, which are profitably used for sustained drug release, the release of oppositely charged drugs may be strongly affected by the occurrence of charge–charge and other interactions frequently leading to complex formation (Bonferoni et al., 2000; Graham et al., 1963; Lelham and Sundelöf, 1995). In majority of cases the result of polyelectrolyte–drug interactions is an additional prolongation of drug release.

The polyelectrolyte–drug complexes have been under focus of several researchers (Bonferoni et al., 2000; Graham et al., 1963; Lelham and Sundelöf, 1995; Persson et al., 2000). However, limited data is available concerning the understanding of the underlying mechanisms involved in the formation of drug–polyion complexes. Complexity of interactions depends on the nature of both, polyelectrolyte and drug, on the solvent medium and on temperature and has to be studied carefully. It is often still misinterpreted that only electrostatic interactions between ionic polymers and oppositely charged drugs are involved in complex formation.

In order to demonstrate the advantages that complexes between ionic polymers and drugs offer for "advanced" sustained drug release, only pre-made complexes have been investigated (Aguzzi et al., 2002; Bonferoni et al., 2000). One of the main goals was to find the optimal ratio between the drug and polymer, which would offer optimal properties for controlled drug release. For this purpose, drug–polymer complex formation has to be apprized from the view-point of the amount of drug bound by the polymer. In order to determine binding properties of a drug by an ionic polymer, the dialysis equilibrium technique, which requires the use of special dialysis cells, was usually used (Bonferoni et al., 2000; Lelham and Sundelöf, 1995). However, the main obstacle of the dialysis equilibrium technique approach lies in a large number of experiments needed for constructing the binding isotherm. In addition, experiments are time consuming, taking up to 2 days before the equilibrium is reached.

A different experimental approach was used to study the binding of small amphiphilic (surfactant) molecules by polyions. Special ionic surfactant selective membrane electrodes have been successfully applied to determine surfactant–polyelectrolyte binding isotherms (Benrraou et al., 1992; Hayakawa and Kwak, 1982;

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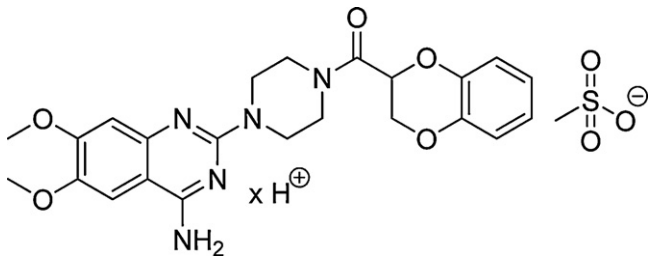

Fig. 1. Structural formula of doxazosin mesylate (DM: $C_{23}H_{24}N_5O_5 \times H^+ \times CH_3SO_3^-$). The protonated form of doxazosin, i.e., the doxazosin cation ($C_{23}H_{24}N_5O_5 \times H^+$), is abbreviated as DH^+ in the text.

Satake and Yang, 1976). The advantages of these specially designed membrane electrodes for binding studies were proven to include: (i) excellent sensitivity and reproducibility, normally far superior to results obtained from equilibrium dialysis experiments relying on spectrophotometric or volumetric concentration determinations, (ii) small sample volume, (iii) almost instantaneous binding determination, and (iv) electrode tolerance to large excess of inorganic electrolytes (Hayakawa and Kwak, 1982). Furthermore, binding isotherms were successfully analyzed and binding mechanism was explained by applying the theoretical treatment for cooperative binding with the nearest-neighbour interaction model, which is based on the Zimm–Bragg theory for the cooperative coil-to-helix transition (Satake and Yang, 1976). By this combined approach, it was demonstrated that the cooperative binding is a consequence of both electrostatic interactions between the polyion and oppositely charged surfactant ions and of the so-called hydrophobic interactions between bound surfactant ions, which are due to their amphiphilic nature. In the case of drug binding by ionic polymers, on the contrary, practically no theoretical treatment of the binding isotherms was performed that could shed more light on drug–polymer interactions.

Polyelectrolytes that have attracted much attention due to the possibility of the coil-to-helix transition and to the related ion specificity are carrageenans (CARRs). CARRs are linear, anionic, partially sulphated 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 sulphated galactans are classified according to the presence of the 3,6-anhydrogalactose on the 4-linked residue and the position and the number of sulphate groups (Reilly, 2005). The most important types of carrageenans are κ-, ι-, and λ-carrageenan, herein abbreviated as κ-, ι-, and λ-CARR, respectively.

In the ideal case, κ-CARR has only one sulphate group per disaccharide repeating unit, ι-CARR has two, whereas λ-CARR has three sulphate moieties per disaccharide unit. Thus, CARRs differ strongly in their linear charge density. Additional differences apply to their ability to form gels. κ- and ι-CARRs contain the 3,6-anhydrogalactose unit and are gelling polymers, whereas λ-CARR with only galactose residues is considered as a non gelling, water soluble polymer, which forms very viscous solutions (Rees, 1977; Rochas et al., 1986; Yuguchi et al., 2002). CARRs are often used in food products as thickeners and stabilizers; however, their usage is increasing also in pharmaceutical formulations. They are considered as 'generally recognized as safe' (GRAS) and are also of pharmaceutical grade. Polyelectrolyte nature of CARRs was shown to have a crucial influence on the drug release behaviour (Singh and Lelham, 1998).

Doxazosin (Fig. 1) is a cationic drug, a selective α_1-antagonist, used for the treatment of hypertension. It also blocks the α_1-receptors in the prostate gland and alleviates the symptoms of benign prostatic hyperplasia. Therapeutic dose is between 1 and 16 mg, available also as a once daily dosing in controlled release formulations, leading to increased patient compliance (Griffith, 2002). Despite its cationic properties, which give hydrophilicity to the molecule, doxazosin has a large hydrophobic character and can therefore be recognized as an amphiphile. It is most often used in the form of a mesylate. According to calculations of Kinsella et al. (2006), mesylate salt of doxazosin (doxazosin mesylate, DM) is most probably formed by protonation of one of the potentially protonisable nitrogens on the quinazoline ring, but the protonation site is not defined. In the following, the designation DH^+ will be used for the doxazosin cation.

Our previous studies showed that simultaneous presence of CARRs and DM in a matrix formulation can be used for additional, more than 24 h sustained drug release (Pavli et al., 2010a,b). In the present paper we want to further investigate DM–CARR interactions, which caused this additional prolongation of DM release from CARR matrices. For this purpose comprehensive binding study of DM by various CARRs was performed. The aim of our study was three fold: (i) to construct special DM selective membrane electrode and to apply it to study the binding of DH^+ by CARRs under various experimental conditions, (ii) to provide theoretical treatment of binding isotherms based on the nearest-neighbour interaction model (Satake and Yang, 1976), and (iii) to explain the DM release mechanism from CARR matrix tablets based on the knowledge of DM–CARR interactions in solution. As far as we could ascertain, our report is the first on the use of such electrodes for the evaluation of the degree of drug binding by polymers in solution and presents a new approach in understanding the drug release mechanisms from advanced dosage form.

2. Materials and methods

2.1. Materials

Pharmaceutical grade carrageenans κ-CARR (Gelcarin GP 911 NF), ι-CARR (Gelcarin GP 379 NF), and λ-CARR (Viscarin GP 209 NF) were obtained from FMC Biopolymers (USA). Average molecular weight of κ- and ι-CARRs was in the range 400–600 kDa, whereas that of λ-CARR was 400–800 kDa. These CARRs were used without further purification, as received, and are in the following denoted as unpurified. Additionally purified, in our study denoted as purified, κ-, ι- and λ-CARR samples, were commercial reference products from Sigma Chemicals Co. (Bornem, Belgium), Types III (No. 127H-1222), V (No. 27F-0373) and IV (No. 58F-0604), respectively. They were thoroughly purified by dialysis and transformed into sodium salt forms by the procedures reported in the literature (Bongaerts et al., 1999, 2000; Denef et al., 1998). The solid salts were obtained by freeze drying and were stored in refrigerator.

Doxazosin mesylate (DM; $C_{23}H_{25}N_5O_5 \times CH_3SO_3H$; M = 547.58 g/mol), was supplied by Krka, d.d. (Slovenia). The solubility of DM in water at 25 °C is 2.6 mg/mL (=4.74×10^{-3} mol/L or shortly M) and at 37 °C it is 2.9 mg/mL (=5.3×10^{-3} M).

2.2. Potentiometry

2.2.1. Preparation of the membrane ionselective electrode (MIE)

The active part of the electrode is the membrane. A membrane selective to DH^+ was prepared from 23 wt.% poly vinyl chloride (PVC), 76 wt.% dioctyl phthalate (DOP) and 1.3 wt.% carrier complex (Hayakawa and Kwak, 1982; Satake and Yang, 1976). The carrier complex was prepared by dissolving equivalent amounts of DM and sodium dodecyl sulphate (SDS) in water. The resulting white precipitate, the insoluble DH^+–DS^- complex (where DS^- denotes the dodecyl sulphate anion), was washed repeatedly with water followed by drying in vacuum at 50–60 °C. The carrier complex was dissolved in 5 mL of tetrahydrofuran (THF) by heating.

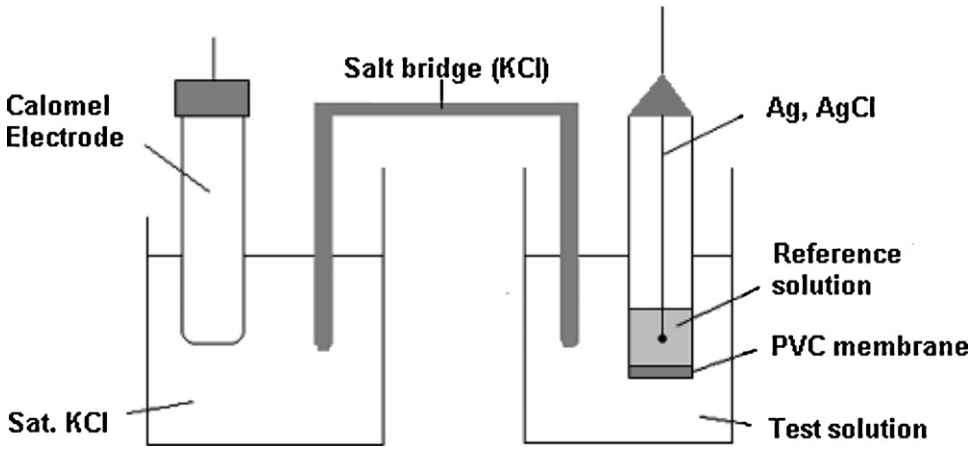

Fig. 2. A schematic representation of the galvanic cell with the membrane ion-selective electrode (MIE) sketched on the right side and the reference electrode (SCE) sketched on the left.

PVC was added into the heated mixture, followed by the addition of DOP. The clear viscous solution was cast into a petri dish of 5 cm diameter. THF was left to evaporate gradually, after which a thin solid membrane layer was obtained of approximately 1 mm thickness. A piece of the resulting PVC membrane was glued to the bottom of a hard PVC tube with a 1 cm diameter by using a THF solution of PVC as an adhesive. PVC tube was filled with the reference solution with a concentration 1×10^{-4} M DM in 0.01 M NaCl. The upper part of the tube was then closed with a covering containing a pinhole, enabling the contact between the applied Ag/AgCl electrode and the reference solution in the tube. As the reference electrode, the saturated calomel electrode (SCE) was used.

2.2.2. Galvanic cell

The potential difference, E, was measured for the following galvanic cell: SCE | sat. KCl || test solution | PVC membrane | reference DM solution | AgCl, Ag (where || denotes the salt bridge; Fig. 2). The potential difference was monitored with the pH-meter ISKRA (MA 5740) at 25 °C and 37 °C. The titration technique was used to determine the dependence of E on the total DM concentration, c^{t}_{DM}. First, E was determined as a function of c^{t}_{DM} in solution without added CARR; these data represented the calibration curve. 10 mL of triple distilled water was placed into the cell. Small amounts of DM solution with a concentration of 0.004 M were added into the cell with a microburette. After each addition of DM, the potential difference between the indicator and reference electrode was measured. It reached a constant value a few minutes after each addition. Calibration curves were determined at 25 °C and 37 °C. Afterwards, the dependence of E on DM concentration in the presence of CARR was measured. Instead of water, 10 mL of an aqueous CARR solution was placed into the cell. The concentration of CARR solution in the cell was 5×10^{-4} moles of polymer charges per volume, denoted as monomol/L. So-called 'monomolar' concentrations were calculated on the basis of the idealized CARR structures (Fig. 1). Small amounts of 0.004 M DM solution were then added to the CARR solution with a microburette. With each addition of DM solution, the same volume of the CARR solution with 2-times higher concentration compared to the CARR concentration in the cell was added. In this way, the concentration of CARR in the cell was kept constant during the whole experiment, i.e., DM additions did not cause dilution of CARR in the cell. After each DM addition, potential difference between the indicator and reference electrode was measured in the same way as previously.

2.2.3. Potentiometric method for the determination of the free DH^+ concentration

The potential difference E of the galvanic cell in Fig. 3 is defined as the difference between the indicator MIE (E_{ind}) and reference SCE (E_{ref}) electrode potentials. By taking into account that E_{ref} is constant and writing the Nernst equation for the potential of the MIE electrode, one obtains the following expression for E:

$$E = E_{ind} - E_{ref} = E^{0}_{ind} + \left(\frac{RT}{F}\right) \ln(c_{DH^+}) - E_{ref}$$

$$= E' + \left(\frac{2.303RT}{F}\right) \log(c_{DH^+}) \quad (1)$$

Here, E^{0}_{ind} is the standard electrode potential of MIE, R is the gas constant (8.314 J K^{-1} mol^{-1}), T is the absolute temperature, F is the Faraday constant (96 485 As mol^{-1}), c_{DH^+} is the concentration of monovalent DM^+ ions in mol/L, and $E'(= E^{0}_{ind} - E_{ref})$ includes the constant potential terms. In the last part of Eq. (1), the natural logarithm was replaced by the decade one. According to Eq. (1), E is a linear function of $\log(c_{DH^+})$. The numerical value of the constants $2.303RT/F$, which determines the theoretical slope of the E vs. $\log(c_{DH^+})$ plot, is equal to 0.0592 and 0.0615 V at 25 and 37 °C,

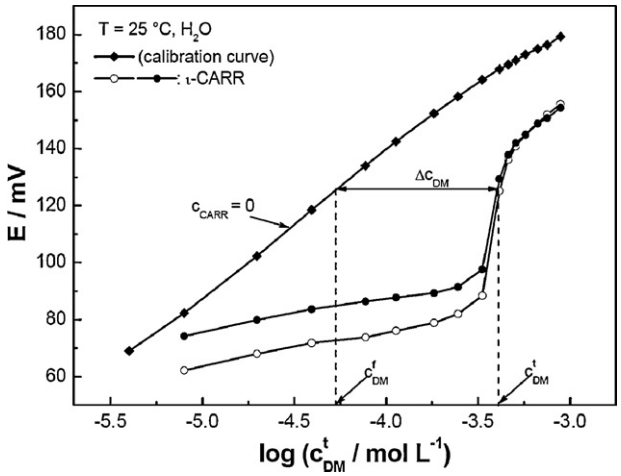

Fig. 3. The potential difference, E, vs. the logarithm of the total DM concentration, $\log(c^{t}_{DM})$; calibration curve (♦): $c_{CARR} = 0$; curves in the presence of CARR with $c_{CARR} = 5 \times 10^{-4}$ monomol/L: purified (empty circles) and unpurified (filled circles) ι-CARR at 25 °C. The evaluation of the amount of bound DM, Δc_{DM}, and the corresponding concentration of free DM, c^{f}_{DM}, are indicated.

respectively. Eq. (1) is the basis for the determination of the free DH^+ concentration in solution from the measured value of E. Note that c_{DH^+} is equal to c_{DM}.

2.3. Drug release from CARR matrix tablets

From homogeneously mixed powders of CARR of each type and doxazosin (8 mg per tablet), matrix tablets (300 ± 10 mg) were compressed using a tableting machine (Killian SP 300, Germany). The hardness of all tablet formulations was adjusted to 80–120 N (VanKel VK 200, USA; hardness tester; $n = 6$); tablets were flat-faced with a diameter of 10 mm. Dissolution studies were performed on a fully calibrated dissolution apparatus using the basket method (USP Apparatus I, VanKel Dissolution Apparatus, model VK 7000, USA). Paddle speed was kept at 150 rpm, the volume of the dissolution medium (0.0075 M phosphate buffer, pH 7.0) was 900 mL and T was 37.0 ± 0.5 °C. At predetermined time intervals, 10 mL samples (not replaced) were withdrawn, filtered through 0.45 μm membrane filters, and analyzed by UV spectrophotometry at 249 nm (HP diode array UV spectrophotometer, 8453, Germany). All dissolution studies were performed at least six times.

3. Results and discussion

3.1. Performance of the electrode, binding isotherms and the model of cooperative binding

The potential of MIE depends on the concentration of free (unbound or un-associated) DM in solution (c^f_{DM}) and is not affected by either aggregated/associated or polymer-bound DM (Benrraou et al., 1992; Hayakawa and Kwak, 1982, 1983; Kogej, 2007; Kogej and Škerjanc, 2001; Satake and Yang, 1976). Consequently, the electrode may be used for the determination of the amount of DM bound by carrageenans. In DM solutions in the absence of polyelectrolyte ($c_p = 0$; c_p is the concentration of CARR), the response of the electrode follows Eq. (1). The E vs. log(c_{DM}) plot is linear in a broad concentration range as presented by the calibration curve (Fig. 3).

In the presence of a polyelectrolyte like CARR, the observed potentiometric curve deviates from the linear calibration line due to the adsorption/binding of DM by CARR. The difference between the curves measured in the presence of CARR and the calibration curve gives information on the amount of DM that is bound by CARR. This quantity is expressed as Δc_{DM}: $\Delta c_{DM} = c^t_{DM} - c^f_{DM}$, where c^t_{DM} is the total DM concentration in solution. The data are presented in the form of binding isotherms, where degree of binding represented by β (=$\Delta c_{DM}/c_p$) is plotted vs. log(c^f_{DM}) (Fig. 4). Binding isotherms determined by this approach are superior in comparison with the equilibrium dialysis data (Fig. S1). Although the same trend of β values is observed the low quality of the data obtained from equilibrium dialysis prevents reliable theoretical treatment similar to the one presented in this paper.

The shape of binding isotherms for the DM binding by ι-CARRs (Fig. 4) is similar to those reported in the literature (Hayakawa and Kwak, 1982, 1983; Shirahama, 1998; Goddard, 1993). The degree of binding is very small at c^f_{DM} below $\sim 0.7 \times 10^{-5}$ M for both the purified and unpurified CARR samples. Above a well defined concentration, known as the critical association concentration (CAC) (Linse et al., 1998), a very steep increase is observed, after which β levels off. Such sigmoid shape of binding isotherms is well-known in the field of surfactant binding by polyelectrolytes (Kogej and Škerjanc, 2001) and indicates the cooperative nature of DM binding by CARR polyions, resembling the polyelectrolyte–surfactant case. The cooperative process involves strong electrostatic interactions between DH^+ and oppositely charged CARRs, which depend

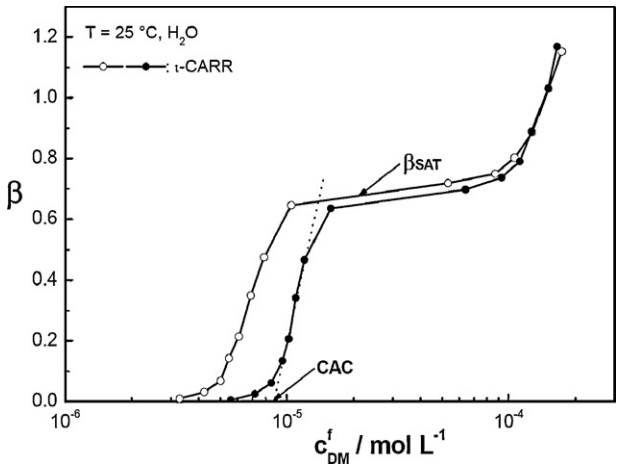

Fig. 4. Examples of binding isotherms for the DM binding by purified (empty circles) and unpurified (filled circles) ι-CARR at 25 °C: CAC denotes the critical association concentration and β_{SAT} the saturation degree of binding (see text).

significantly on the polyion charge density, and the so-called hydrophobic interactions between the bound DH^+ cations, arising from the amphiphilic nature of DM. Due to strong accumulation of DH^+ cations in the electrostatic field of the polyions the distance between DH^+ ions is sufficiently small for the hydrophobic interactions between the hydrophobic parts of DM to take place. Similarly to the surfactant–polyion case, where surfactant is bound in the form of polyion-induced micelles, one can propose that DH^+ is bound by CARR polyions in the form of aggregates and not as single ions. This is a characteristic property of cooperative binding described in the model below.

Due to the described cooperative process, very extensive DM binding by CARRs is detected above the CAC. The stronger is the interaction between DM and CARR the lower is the CAC (Kogej and Škerjanc, 2001). In practice, CAC can be determined from the steep part of the β vs. log c^f_{DM} curve by extrapolating the data points in the cooperative region to $\beta = 0$ (Fig. 4). The steep cooperative part of the isotherm is followed by a plateau region of almost constant β values and increasing c^f_{DM}, which indicates saturation of the polyion with DM. The value of β in the saturation region is herein denoted as β_{SAT} (Fig. 4). After the plateau region, the slope of the isotherm increases again, which is most probably due to some different type of DM aggregation/association in solution close to the solubility limit of the drug in the aqueous medium.

In studies dealing with surfactant binding by polymers (Benrraou et al., 1992; Lelham and Sundelöf, 1996; Satake and Yang, 1976), such binding isotherms were successfully analyzed using the theoretical treatment of cooperative binding based on the nearest-neighbour interaction model (Lelham and Sundelöf, 1996; Schwarz, 1970). The above described characteristics of the DM–CARR binding isotherms encouraged us to use the same treatment also in the present study.

According to the Zimm–Bragg model, the binding of a ligand, L^+, by a polymer is represented by two equilibria:

$$(00) + L^+ \overset{K}{\longleftrightarrow} (01) \quad (2)$$

$$(01) + L^+ \overset{Ku}{\longleftrightarrow} (11) \quad (3)$$

where (00) represents two unoccupied polyion binding sites, (01) an unoccupied binding site next to an occupied one, and (11) two adjacent occupied sites. In our case ligand L^+ is DH^+ and K is the binding constant for the DH^+ binding to an isolated binding site on the CARR polyion. The quantity u is the cooperativity parameter, which is determined by hydrophobic interactions between the bound DH^+ ions. The product Ku is the cooperative binding

constant that applies to the binding of DH^+ to a site adjacent to an already occupied site and thus parameter u reflects the contribution of hydrophobic interactions to such binding; these interactions clearly affect (increase) the binding constant. Binding is regarded as non-cooperative if u equals 1 (i.e., $Ku = K$), as cooperative if u is larger than 1 ($Ku > K$) and as anti-cooperative if u is lower than 1 ($Ku < K$). According to the model, only one ligand can bind per one binding site on the polyion, which means that the maximum possible value of β is 1. The term "site" is used in the framework of the model merely to indicate that one ligand can occupy (block) only one charged group on the polyion. In reality, DM binds to the electrostatic atmosphere of the highly charged polyions.

Schwarz (1970) and Satake and Yang (1976) derived the following expression for the degree of binding, β, which can be used to analyze the binding isotherm:

$$\beta = \frac{1}{2}\left[1 + \left[\frac{Kuc^{f}_{DM} - 1}{((1 - Kuc^{f}_{DM})^2 + 4Kc^{f}_{DM})^{1/2}}\right]\right] \quad (4)$$

The slope of the isotherm at the half-bound point (at $\beta = 0.5$) is related to parameter u by the relationship:

$$\left(\frac{d\beta}{d \ln c^{f}_{DM}}\right)_{\beta=0.5} = \frac{u^{1/2}}{4} \quad (5)$$

and the product Ku is determined from

$$Ku(c^{f}_{DM})_{\beta=0.5} = 1 \quad (6)$$

Eqs. (4)–(6) were used to analyze the experimental binding isotherms in DM–CARR mixed solutions and yielded values of K, Ku and u.

Eq. (4) was derived by different approaches (Schwarz, 1970; Satake and Yang, 1976; Shirahama et al., 1981) and its limitations have been critically reviewed in the literature (Goddard, 1993; Shirahama, 1998; Shirahama et al., 1981). It has been pointed out that the model applies to the region of the isotherm (usually at $\beta < 0.5$) where ligand binding does not seriously alter the electrostatic potential on the polyion. Consequently, larger deviations between calculated and experimental isotherms are usually observed for β values approaching 1. The determination of the binding constant Ku (related to the position of the isotherms on the x-axis, cf. Eq. (6)) is quite accurate, whereas parameter u (related to the slope of the isotherm at $\beta = 0.5$, cf. Eq. (5)) is less accurately determined. Still, the model is very appropriate when the purpose of calculating parameters Ku and u is to compare structurally very similar systems, as is the case with carrageenans used in our study. This is also the way how the model was applied in our investigation.

3.2. Binding of DM by CARRs at 25 °C

In Fig. 5, plots of E vs. log c^{t}_{DM} are shown for all systems at 25 °C. The response of the MIE in the absence of CARR is linear from around 7×10^{-6} to around 3×10^{-4} M DM with a slope 51.2 mV/decade, which is close to the theoretical value of 59.2 mV/decade (cf. Eq. (1)). The deviations from linearity observed at higher concentrations may be attributed to the proximity of the solubility limit of DM. Curves in the presence of CARRs are different for each CARR type and differ also for purified and unpurified CARRs.

Binding isotherms constructed from the data in Fig. 5 are shown in Fig. 6. In order to be able to apply the model of cooperative binding to treat the isotherms, the degree of binding is calculated per one binding site on the polyion, i.e., per one charged/sulphate group. Therefore, c_p in equation for β is expressed in moles of sulphate groups on CARR chains per volume. Note that in ι- and λ-CARRs case this concentration is not equal to the concentration of repeat units. Thus, the thin lines in Fig. 6 are drawn through

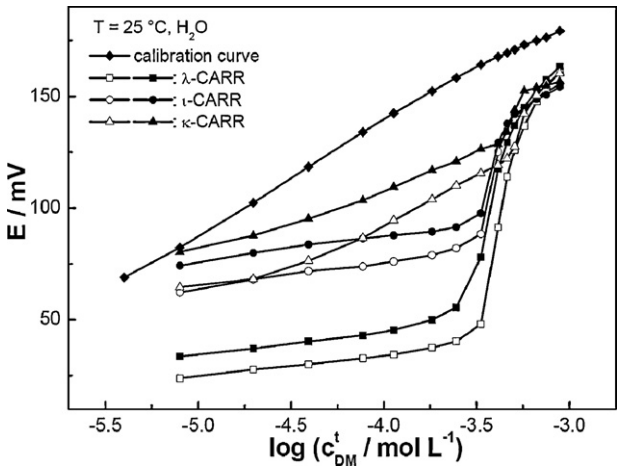

Fig. 5. The dependence of E on the logarithm of the total DM concentration, log(c^{t}_{DM}), at 25 °C: calibration curve (♦) and curves in the presence of purified (empty circles) and unpurified (filled circles) CARRs: λ- (□/■), ι- (○/●) and κ-CARR (Δ/▲).

experimental points to lead the eye and the thick lines represent the two-parameter fit of binding isotherms according to Eq. (4). It can be seen that Eq. (4) gives a satisfactory fit of the data points for β values below approximately 0.5–0.7, in agreement with the above discussion on model limitations. One of the reasons for deviations of the calculated isotherms from the measured data points at increasing beta values could also be the fact that the electrolyte ($NaCH_3SO_3$) concentration in solution increases upon DM binding by CARRs due to the release of Na^+ (originating from CARRs) and $CH_3SO_3^-$ ions (originating from DM) into the solution. At a 1:1 molar ratio between DM ions and charged groups on CARRs and for complete binding of DM by CARRs this would amount to a $NaCH_3SO_3$ concentration of 5×10^{-4} M. The electrolyte screens attractive interactions between oppositely charged CARRs and DM and thus affects the degree of binding. However, it can be deduced from the data in the literature (Goddard, 1993; Hayakawa and Kwak, 1982, 1983; Kogej and Škerjanc, 2001) that the effect of such low electrolyte concentrations (5×10^{-4} M) on binding isotherms is negligible.

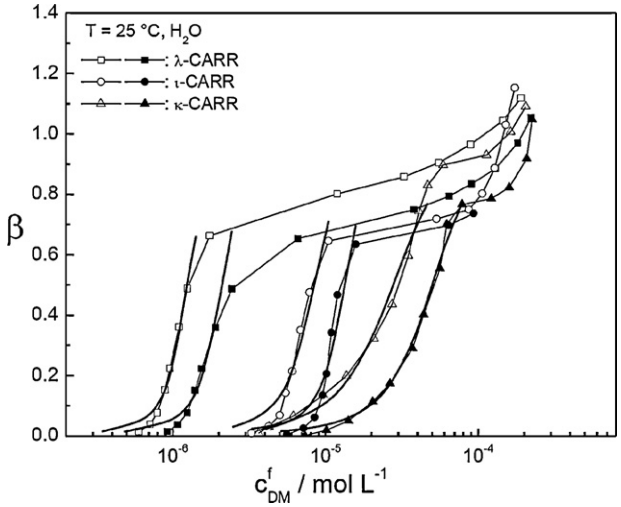

Fig. 6. Binding isotherms for DM binding by purified (empty circles) and unpurified (filled circles) CARRs at 25 °C: λ- (□/■), ι- (○/●) and κ- (Δ/▲) CARR. The thick lines represent the two-parameter fit of binding isotherms (Eq. (4)) and the thin lines are drawn through experimental points to lead the eye.

Table 1
Critical aggregation concentration, CAC, values for DM binding by CARRs at 25 and 37 °C.

	T (°C)	$10^6 \times$ CAC (mol/L)	
		Unpurified	Purified
λ-CARR	25	1.1	0.8
	37	1.2	1.0
ι-CARR	25	9	4.8
	37	16	14
κ-CARR	25	39	17
	37	39	34

The positions of the isotherms along the log c_{DM}^{f} axis (i.e., CAC values) indicate that DM is most strongly bound by λ-CARR (CAC is the lowest), followed by ι- and κ-CARRs. The sequence λ > ι > κ is in accordance with the number of charged ester sulphate groups per disaccharide unit and thus with the polyion charge density. Binding isotherms for unpurified CARRs are shifted to the right, i.e., to higher CAC values, indicating a weaker interaction in comparison with the purified forms (Table 1). However, the sequence λ > ι > κ is retained. The shift to higher CAC can be explained by the screening effect of simple salts, which are certainly present in the unpurified CARR samples. In purified CARRs, the simple salts and any other impurities were removed by exhaustive dialysis against triple distilled water. In unpurified CARRs, however, the dialysis and ion-exchange procedures were not performed. This means that some monovalent and possibly also divalent salts and other low molecular substances may be present in these samples. The composition and the amount of these impurities depend on the process of preparation and treatment of CARRs. Although no analysis of eventual low molecular weight salts in our pharmaceutical grade CARRs was performed, it is very likely that the ionic strength of solutions in the case of unpurified CARR samples is higher compared to the purified ones. Higher ionic strength contributes to stronger screening of electrostatic interactions between the sulphate groups on CARRs and DH^+ ions, which results in a weaker attraction and consequently in higher CAC values. Additionally, some cations that are present as impurities can predominantly bind to certain CARR types, for instance Ca^{2+} to ι-CARR, K^+ to κ-CARR (Zhang et al., 1992). These specific cations are usually more strongly bound than others, which results in an even more expressed screening effect. All binding parameters obtained by the model treatment are reported in Table 2.

The cooperativity constants *Ku*, which are equal to the reciprocal of the free DM concentration at $\beta = 0.5$ (Eq. (6)), clearly show that DM binding is the strongest by λ-CARR, followed by ι- and κ-CARRs. This is in accordance with the charge density of the polyions and with the previously observed decreasing trend in CAC. Similar conclusion is suggested by *K* values.

Table 2
The calculated *K*, *Ku*, and *u* values for DM binding by λ-, ι-, and κ-CARRs at 25 °C and 37 °C.

	25 °C			37 °C		
	$K/10^3$	$Ku/10^5$	u	$K/10^3$	$Ku/10^5$	u
λ-CARR						
Purified	23	8.1	36	10.3	5.4	52
Unpurified	18	5.1	29	19.2	4.0	21
ι-CARR						
Purified	6.7	1.2	18	2.0	0.52	26
Unpurified	2.9	0.75	26	1.3	0.46	35
κ-CARR						
Purified	6.0	0.36	6	1.8	0.18	10
Unpurified	2.5	0.2	8	1.2	0.16	13

Differences in *Ku* values are rather high: *Ku* for the interaction of DM with the purified λ-CARR is approximately 7 and 23 times higher than that for purified ι-CARR and κ-CARR, respectively. In the case of unpurified CARRs, *Ku* values are smaller in comparison with the purified polysaccharides for all three CARRs. However, the relative differences between *Ku* values are maintained. Lower *Ku* values for unpurified CARRs can be attributed to higher ionic strength of solutions and to the resulting electrostatic screening effect.

The nature of the binding process of DM by CARRs can be further explained by considering the values of the cooperativity parameter *u*. Our results (Table 2) show that *u* is between 6 and 52 in all cases, indicating that binding is always cooperative. The higher are the *u* values the more pronounced are hydrophobic interactions between bound DH^+ ions. An alternative interpretation of *u*, which will assist in the following interpretation of drug release mechanism, is in terms of aggregation numbers of DM clusters that are formed in the polyion domain: a larger cooperativity parameter indicates larger clusters or aggregates (Satake and Yang, 1976). Literature data on *u* values in different surfactant–polyion pairs range from 2 to a few 1000 (Benrraou et al., 1992; Hayakawa and Kwak, 1983; Satake and Yang, 1976), which classifies our DM–CARR system on the lower limit. Among others, the cooperativity depends strongly on structural features of the ligand, for example on the effectiveness of packing of single ligand molecules into clusters that eventually bind to the polyion. DM structure (Fig. 1) suggests that packing of a larger number of DM molecules into aggregates with a hydrophobic interior and a hydrophilic surface may be difficult due to its rather rigid structure. It is therefore reasonable to expect that the aggregation numbers of DM aggregates that form at CARR polyions are small.

The sequence of *u* values in the case of both purified and unpurified CARRs is as follows: λ > ι > κ, and parallels the *Ku* sequence. Higher charge density of CARR results in higher *u* values due to stronger electrostatic attraction forces between DM cations and CARR polyanions that drive DM molecules close together and facilitate hydrophobic interactions between the hydrophobic parts of the DM molecule.

A note seems appropriate at this point on the observed cooperativity of the binding process of DM by CARRs on one hand and on the absence of well-defined cooperative effects in solutions of pure DM on the other. The self-association tendency of DM in solutions without added polyelectrolyte is considerably weaker in comparison with conventional surfactants. This is seen from the calibration line (Fig. 3 or 5). In the case of ionic surfactant like cetylpyridinium chloride (CPC) the *E* vs. log c_{DM}^{t} plot shows a distinctive change in slope at the critical micelle concentration (CMC), which is attributed to the highly cooperative process of micelle formation (Kogej and Škerjanc, 2001; Kogej, 2007). Above the CMC, *E* is virtually constant because the concentration of free surfactant is constant. In the DM case, however, the *E* vs. log c_{DM}^{t} curve only gradually deviates from linearity when the solubility limit of DM is approached, excluding any highly cooperative association process in DM solutions. Probably clusters or aggregates of DM form in a step-wise manner, proceeding from dimers, to trimers, etc. The cooperativity emerges only in the presence of CARR polyions owing to the strong accumulation of DH^+ cations in their vicinity. It has been calculated (Kogej and Škerjanc, 2001; Kogej, 2007) that the local concentration of monovalent ions close to the oppositely charged polyion may exceed their average concentration in solution by a factor of more than 1000 (up to almost 10,000 times in the case of most highly charged λ-CARR). Consequently, the solubility limit of DM in the proximity of the polyion is greatly exceeded. Such high DM concentrations could never be achieved in an aqueous medium in the absence of the polyion. Strong accumulation of hydrophobic parts of DM then drives the formation of

polyion induced DM clusters, which are held together by the CARR chain.

In comparison with purified samples, u values (Table 2) are larger for unpurified κ- and ι-CARRs but smaller for unpurified λ-CARR, although differences are small and some, as for example for κ-CARR, in the range of the uncertainty of the method. Similar to κ- and ι-CARRs case, it was found that the addition divalent salts leads to a marked increase in u for the binding of cationic surfactants by dextrane sulphate (Hayakawa and Kwak, 1983), which was attributed to changes in polymer chain conformation and to larger surfactant aggregates. The same is likely to be the cause of the increased cooperativity also in κ- and ι-CARRs case. It is well known that the presence of specific cations, e.g., K^+ or Ca^{2+}, induce a coil-to-helix transformation of the κ- and ι-CARR chains (De Ruiter and Rudolph, 1997; Yuguchi et al., 2003). The formation of a helical state was shown to result in a smaller inter-charge distances on CARR backbones, higher charge density (Hugerth and Sundelöf, 2001). A higher cooperativity was found also for amitriptiline binding by predominantly helical state of ι- and κ-CARRs (Hugerth and Sundelöf, 2001). The spatial distribution of binding sites may be more favourable in the helical conformation, leading to more efficient DM–DM hydrophobic interactions. This finally results in somewhat higher u values, as is observed experimentally (Table 2).

All isotherms reach a saturation region where β vs. log c_S^f curves level off, i.e., they reach a more or less well-pronounced plateau defining β_{SAT}. As seen from Fig. 6, β_{SAT} values are similar for DM binding by λ- and ι-CARRs, between 0.8 and 0.7, almost irrespective of their purity. These values imply that between 0.7 and 0.8 DH^+ ions are bound per one sulphate group. In the case of κ-CARR, the plateau region is narrower compared to λ- and ι-CARRs and β_{SAT} values are higher: $\beta_{SAT} > 0.8$ for the purified and $\beta_{SAT} > 0.9$ for the unpurified κ-CARR, pointing to an almost quantitative association between DH^+ and κ-CARR. The finding that β_{SAT} values are lower for λ- and ι-CARRs than for κ-CARR is surprising, since these two carrageenans both have higher charge density than κ-CARR. However, lower β_{SAT} values are perhaps a consequence of a less favourable spatial distribution of ester sulphate groups in this case, which causes that charges are less accessible for DM binding due to steric hindrance. This clearly becomes very important in the saturation region when the number of DM molecules close to the polyion is the largest. DM is a rather bulky and rigid molecule that requires considerable amount of space when it binds to charged groups on the CARR chain. If the distance between polyion charges is too small or if their position is unfavourable, this may result in a lower degree of binding per binding site. It has to be stressed that lower β_{SAT} values do not imply that the overall amount of DM bound to λ- or ι-CARR is smaller compared to κ-CARR. The degree of binding was namely calculated per one sulphate group and not per one repeat disaccharide unit (see above). Since λ-CARR contains three sulphate groups per repeat disaccharide unit and ι-CARR two, β_{SAT} values calculated per one repeat unit increase by a factor of 3 and 2, respectively. Thus the ratio of β_{SAT} values is actually λ:ι:κ = 2.4:1.4:1.

The width of the plateau region after the levelling off of β (Fig. 6) decreases with the decreasing number of charged groups per disaccharide unit on the CARR backbones. However, a steep increase of β to values larger than 1 is observed again for all CARR samples for c_{DM}^f above 1×10^{-4} M. Such increase is observed also in systems containing conventional surfactants and is therein due to free micelle formation, ensuring saturation. However, the possibility of self-association of DM after all the binding sites on the polyion have been occupied and free DM appears in solution was ruled out in the above detailed discussion of the E vs. log c_{DM}^t curve. Therefore, additional binding of DM to the DM–CARR complex is more likely to explain this observation. This may lead to a specific type of ordering of CARR chains in conjunction with amphiphilic DM in the form of bilayers, which is a frequently observed structure

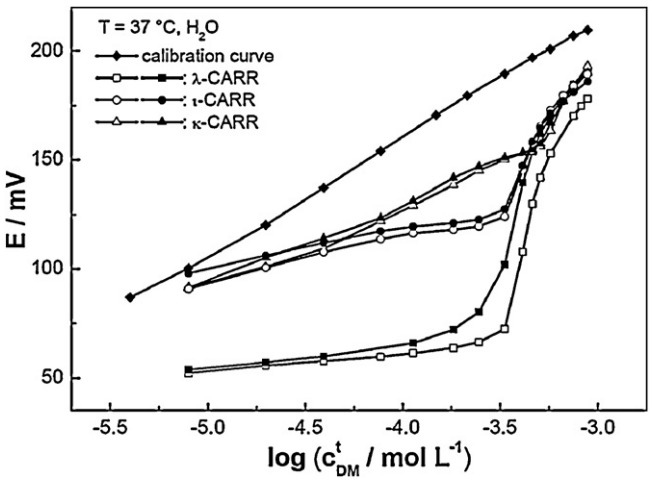

Fig. 7. The dependence of E on the total DM concentration at 37 °C: symbols are the same as in Fig. 5.

in the case of surfactant mixtures with rigid carrageenan polyions (Kogej et al., 2001) and results in formation of a three dimensional network.

3.3. Binding of DM by CARRs at 37 °C

The binding of DM by CARRs was investigated also at the body temperature 37 °C. A linear response of MIE to DM concentration in a wide concentration range was observed, from around 4×10^{-6} M to around 9×10^{-4} M (Fig. 7). The slope of the calibration curve at 37 °C was equal to 54.4 mV/decade and agrees favourably with the theoretical value 61.5 mV/decade.

The results obtained for the electrode response in the presence of CARRs show similar behaviour as those obtained at 25 °C They were used for the construction of binding isotherms (Fig. 8) and were treated according to previously described procedures. All binding parameters are reported in Table 2.

The increase in temperature causes a shift of the isotherms to higher c_{DM}^f and to higher CAC values (Table 1), i.e., lower Ku values. On the other hand, β_{SAT} values are more or less unaffected by the increase in temperature: they are around 0.8, 0.7, and 0.9 for

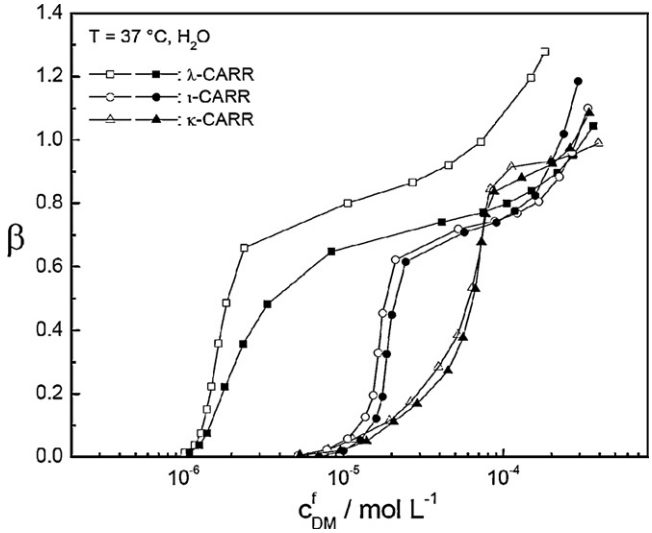

Fig. 8. Binding isotherms for DM binding by purified (empty circles) and unpurified (filled circles) CARRs at 37 °C: symbols are the same as in Fig. 6.

both purified and unpurified λ-, ι- and κ-CARRs, respectively (for an easier comparison of the isotherms obtained at 25 and 37 °C see Fig. S2). In most cases the slope of the isotherms in the cooperative region slightly increases, which results in higher *u* values; however, the decrease in *Ku* is more pronounced than the increase in *u*, in particular for the purified forms. Similarly to the cooperativity binding constant *Ku*, the constant *K* is also lower at 37 °C. This is expected, since *K* is influenced primarily by electrostatic attraction between the DH^+ cations and CARR polyanions, and these are influenced by the thermal energy of molecules. Thus, lower *K* indicates weaker interaction between DH^+ and CARRs in the initial stage of binding and is a consequence of higher kinetic energy of molecules at higher temperature.

In the discussion of the temperature dependence of *Ku* one has to keep in mind that the combined cooperative binding constant, the product *Ku*, includes effects of both, electrostatic binding and cooperativity, i.e., of hydrophobic interactions. Santerre et al. (1985) have presented temperature dependence of binding of a conventional cationic surfactant to an anionic polyelectrolyte. In the temperature range between 5 and 50 °C, the change of *Ku* with temperature was remarkably small which is in agreement with our results. Furthermore a clear maximum in *Ku* values and simultaneously in *u* values was reached at around 30 °C. The authors have compared this finding with the temperature dependence of CMC. There are numerous reports of temperature minima in CMC of conventional surfactants at around 25–30 °C, which are parallel to the maximum in *Ku*. The maximum in *Ku* of course indicates a sign reversal in the enthalpy of binding. A positive slope of the ln *Ku* vs. $1/T$ plot points to a negative (exothermic) enthalpy of binding, and this is observed also in the case of DM binding by CARRs. Somewhat surprising is the effect of temperature on *u* in DM–CARR solutions, which is just the opposite of its effect on *Ku*: *u* is higher at 37 °C than at 25 °C. The reason for a higher cooperativity parameter at 37 °C is probably different flexibility of CARR chains at different temperatures. Increased chain flexibility at higher *T* may facilitate the formation of larger DM aggregates. It should be stressed, however, that our data apply to two temperatures only and that the studied temperature range is rather narrow. A more thorough investigation of temperature dependence would be needed to make more reliable conclusions on the effect of temperature on DM binding by CARRs.

3.4. Relation between binding results and drug release from matrix tablets

The release profiles of DM from CARR matrix tablets are shown in Fig. 9. The low ionic strength buffer (0.0075 mM phosphate buffer) was used because binding studies were conducted in conditions of low ionic strength as well. It can be seen that the release of DM is prolonged for considerably more than 24 h, although the tablets are fully disintegrated after approximately 9 h, irrespective of the CARR type. This limit is indicated by the dotted line in Fig. 9 and was identified visually. After 9 h, only very small particles remain in the release medium, which are slowly eroding with time. In contrast to tablet disintegration, the drug release rate within 9 h depends strongly on the CARR type: it is the fastest in the case of κ-CARR, followed by ι- and λ-CARRs.

The obtained drug release behaviour is not very common for hydrophilic polymers, especially CARRs, since they can typically prolong the drug release up to 12 h (Naim et al., 2004; Picker, 1999). This is usually achieved by common drug release mechanisms from matrix tablets, i.e., by hydration, swelling and erosion of the matrix, which results in the formation of a gel layer (in the case of ι- and κ-CARRs) or in a very viscous layer (in the case of λ-CARR) around the unhydrated matrix core (Rees, 1977; Rochas et al., 1986; Yuguchi et al., 2002). This gelly like or very viscous layer around the matrix tablet coupled with dissolution and

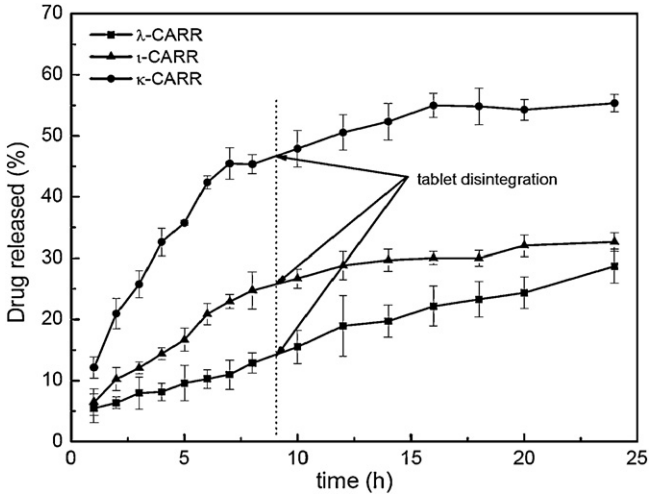

Fig. 9. DM release from different CARR matrices at 37 °C in a pH 7.0 phosphate buffer. The results present the mean ± SD of six measurements.

diffusion of the drug governs the drug release rate. However, it is obvious from the release profiles that other drug release mechanisms are involved in the case of DM–CARR tablets that additionally reduce the drug release rate. These mechanisms are clearly indicated in the present paper through fundamental binding studies and include strong electrostatic binding of DM by CARRs reinforced by cooperative self-association of amphiphilic DM molecules in the electrostatic field of the polyions. By combining binding and drug release studies we can now explain the drug release mechanism in detail.

Drug release order during the first 9 h (κ > ι > λ) agrees with the trend of DM binding by various CARRs as demonstrated by *Ku* values (λ > ι > κ): the stronger the binding of DM, the slower its release rate. λ-CARR with the highest linear charge density most strongly attracts DM molecules and consequently the release rate is slowest in this case. However, the electrostatic interactions alone cannot explain the very large differences between DM releases from CARR tablets. An important contribution comes from self-association of DM molecules in the vicinity of the carrageenan polyions. The arising DM aggregates with a considerably higher charge compared to individual DM molecules bind to the polyion as multivalent counterions and this leads to further substantial decrease in the overall release rate. The cooperativity parameter *u* (Table 2) suggests that the largest aggregates form at the λ-CARR and the smallest ones at the κ-CARR chain. Furthermore, these aggregates start to form only above a threshold concentration of DM molecules in the media, i.e., above the CAC, which again depends strongly on the CARR type: the sequence of CAC values (Table 1) is κ > ι > λ, meaning that DM aggregates first start to form in λ-CARR solutions and last in κ-CARR ones. All the exposed features fuse into a strong synergistic effect, which results in the most strongly sustained DM release in λ-CARR solutions.

In order to fully appreciate all these effects, binding isotherms are redrawn by plotting the degree of DM binding calculated per one repeat unit of the CARR polyions, instead of per one charged group. Such plots are shown in Fig. 10 for the case of un-purified CARR samples at 37 °C, which were used also in drug release experiments reported in Fig. 9.

It can be seen that the saturation degree of DM binding presented in this way is by far the greatest in the λ-CARR case. At saturation, one repeat unit of λ-CARR binds around 2.2 molecules of DM, whereas that of κ-CARR binds only around 0.9. Besides, the plateau region corresponding to saturation extends to considerably lower free DM concentrations in λ-CARR solution. When DM–CARR

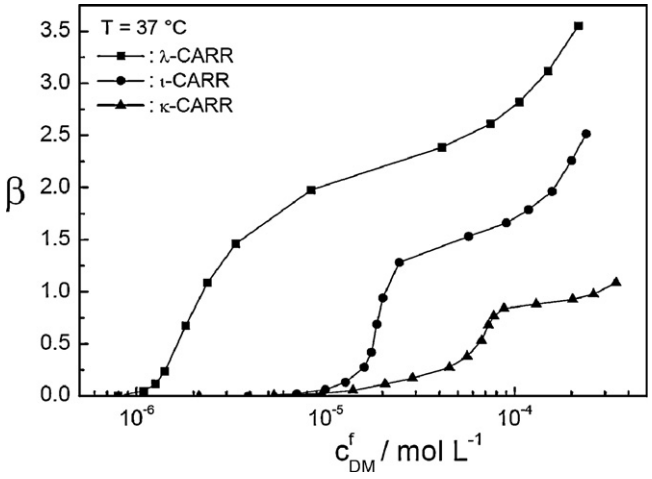

Fig. 10. Binding isotherms for DM binding by unpurified carrageenans at 37 °C: the degree of binding β is calculated per one repeat unit of the carrageenan chain (symbols are the same as in Fig. 6).

complex is being released from the tablet formulation, the drug is gradually (and continuously) dissolved from the complex into the medium, as long as its concentration in the medium is maintained at a sufficiently low level. Continuous drug release moves us along the binding isotherm to the left, i.e., from the plateau region to the cooperative part and finally to the part where β drops virtually to zero. Evidently, free DM concentration in the presence of λ-CARR (this is what is measured in drug release experiments) is the lowest, meaning that we are virtually all the time above the CAC, i.e., in the cooperative region of binding, where the binding is reinforced as described above. On the other hand, with κ-CARR, the free drug concentration in equilibrium with the complex from the matrix tablet is by at least one order of magnitude higher than in the λ-CARR case. Diffusion of DM from the tablet matrix is thus most strongly hindered by λ-CARR. Within the 24 h period the concentration of DM released from the λ-CARR formulation increases with a constant slope and the amount of drug released after 24 h is only around 25%. With κ-CARR, the release rate within the first 7 h is high, leading to a 45% of DM release within 7 h. Afterwards, the release rate drops substantially and thus the amount of the released drug increases to only around 55% after 24 h. The initial rather fast release rate in this case points to the weakest interaction between DM and κ-CARR. A similar release profile with a distinct decrease in slope after around 8 h and with approximately 25% release up to this point (and only around 30% release within 24 h) is registered with ι-CARR formulation.

A possible explanation for the changed DM release rate in κ- and ι-CARRs tablet formulation after the initial 7–8 h could be the altered binding regime. It has been found that surfactants arrange into three-dimensional bilayer-like structures in conjunction with the rigid carrageenan chains (Kogej et al., 2001). It can be visualized that the amphiphilic DM molecules likewise form such structures. Since the interaction of DM with carrageenans is weaker in comparison with conventional surfactants, such ordering presumably occurs only above a certain critical degree of binding to carrageenans. Thus, increasing DM concentration leads to the formation of large ordered structures, which can strongly hinder drug release. With λ-CARR chains the critical DM concentration for such bilayer-like ordering is presumably not reached within the investigated time period of 24 h and the release profile is governed solely by the mechanisms described above.

It is important to note that all the described mechanisms are running simultaneously. When DM dissolves from the solid matrix it immediately binds to CARRs, whereas the excessive/unbound DM molecules diffuse out of the matrix. Since a dynamic DM concentration gradient between the hydrated layer around the matrix core and the outside medium is maintained during drug release, the exact binding process and thus also drug release through the hydrated layer is best described by both, the binding isotherm plots and the release profiles. In the microenvironment of the matrix tablet where concentration of DM is very high, the right part of the binding curve (above β_{SAT}) approximately describes the binding process and thus the drug release mechanism. The opposite is true for the regions, where DM concentration is low, i.e., outside the matrix in the surrounding solution where some dissolved CARR molecules are present as well. Here, binding is best described by the left, i.e., the cooperative, part of the binding isotherm around or above the CAC. The actual release profile depends on the sum of DM binding to CARRs in different regions, i.e., in the hydrated layer around the matrix core and in solution.

Finally, one can argue about the practical relevance of the studied systems since a rather low amount of the drug (55% or less) is released from the investigated matrixes within a 24 h period. However, it must be pointed out that the studied conditions are not equal to *in vivo* ones, where other amphiphilic molecules can be present as well. It was found for example (data in preparation for publication) that the presence of anionic surfactants such as sodium dodecylsulphate, SDS, in the release medium can significantly affect the interactions between DM and CARRs and even lead to a complete DM release in less than 24 h. This indicates that such systems can be used *in vivo*; however, it is essential that they are fully characterized.

4. Conclusions

On the basis of binding isotherms, determined by a special doxazosin mesylate, DM, selective membrane electrode, interactions between DM and anionic polysaccharides carrageenans, CARRs, in solution were evaluated and explained in detail. The most important result is that binding of DM to CARRs is cooperative in nature. The cooperativeness is a result of the initial strong electrostatic attraction between DM and CARRs and of the subsequent self-association of DM molecules in the vicinity of CARR chains. This self-association tendency is a consequence of DM amphiphilicity and gives rise to DM binding in the form of highly charged aggregate-like structures. These structures are formed of several tens of DM molecules and in the final stage of binding block between 70 and 90% of ester sulphate groups on the polyion. DM binding to CARRs is strongly influenced by the charge of CARRs but to a lesser extent by temperature.

Strong ionic interactions are responsible also for sustained release of DM from solid CARR matrix tablets. Different mechanisms have appeared in this process. First, swelling and erosion of tablets, accompanied by drug diffusion from solid matrices, takes place; this step determines the initial release rate. At the same time strong electrostatic interactions between DM and CARRs in solution, which are reinforced by additional hydrophobic interactions between bound DM molecules (self-association), contribute to further prolongation of DM release from CARR tablets. It has to be stressed that these additional hydrophobic interactions can substantially alter the drug release rate; however they are often neglected when studying drug release mechanisms based on drug–polymer interactions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.09.019.

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