# The Effect of Hydrophobic Character of Drugs and Helix-coil Transition of κ-Carrageenan on the Polyelectrolyte-drug Interaction

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***Purpose.*** The extent of adsorption of different drug molecules to κ-carrageenan was investigated in order to evaluate the effect of drug hydrophobicity on the adsorption isotherm.

***Method.*** Dialysis experiments were used to determine the amount of drug adsorbed to the polyelectrolyte. The amount of drug on both sides of the membrane was determined spectrophotometrically after attaining equlibrium. CMC for the drugs were determined by the dye solubilisation method.

***Results.*** It is shown that the small differences in structure between the drug molecules used in this study still leads to considerable difference in adsorption properties, especially the onset of adsorption. It was also found that the slope of the adsorption isotherms among the drug molecules followed the same pattern as the CMC values for drugs. The extent of adsorption of drugs to the helix form of κ-carrageenan was much higher than to the coil form.

***Conclusions.*** These results suggest that the adsorption of charged drug molecules to an oppositely charged polymer is effected not only by the coulombic interactions, but also by the hydrophobicity of the drug. Furthermore, the adsorption of drug molecules to κ-carrageenan in the helix form is higher than for the coil form because of the shorter distance between the charges and the thereby enhanced hydrophobic interaction between bound drug molecules.

**KEY WORDS:** κ-carrageenan; drug adsorption; amphiphile-polyelectrolyte interaction; helix-coil transition; charge-to-charge interaction; hydrophobic interaction.

## INTRODUCTION

Polymers are high molecular weight substances that are used widely in pharmaceutical systems as adjuvants, suspending and emulsifying agents, etc, and increasingly as the basis of drug delivery systems. An overview of such systems is given by Florence and Attwood (1). The modern pharmaceutical literature indicates that in order to use polymeric substances in different drug formulations, there is a need for a deeper knowledge about the interaction between drug molecules and polymers. It is well known that small amphiphilic (surfactant) molecules—like many drug molecules—interact strongly with polymers, especially with polyelectrolytes. For an excellent review the reader is refereed to Goddard (2). The amphiphilic-polymer interactions can have different origins, the most common types being charge-charge and hydrophobic interactions. Several factors play a role in the adsorption of charged amphiphiles to oppositely charged polyelectrolytes. Most important is the overall chemical structure, especially the three dimensional arrangement of polar and hydrophobic segments, distance between charges and charge density. The surrounding medium (solvent) plays a decisive role since it largely regulates the degree of dissociation of ionisable groups ($pK_a$) and helps to determine the conformation. In previous papers (3,4) the effect of charge density, distance between the charged units and of the three dimensional structure of carrageenans on the adsorption of amitriptyline has been investigated. In this paper the effect of the hydrophobic character of a drug on the adsorption isotherm for the system κ-carrageenan/drug/salt/water is studied. Furthermore the isotherms for the adsorption of different drugs on κ-carrageenan in both the helix and the coil conformation are compared.

## MATERIALS

The κ-carrageenan sample was purchased from Sigma (St Louis, MO, USA), the sample having designation C-1263: lot no. 19F-06737. Amitriptyline hydrochloride (drug grade purity) was a gift from Pharmacia AB. The samples of Imipramine-HCl, Clomipramine-HCl, Chlorpromazine-HCl, Doxepin-HCl and anthrone were obtained by Sigma (St Louis, MO, USA). The dye Oil Orange SS (o-Toluenazo-β-Naphtol) was obtained from Tokyo Kasei Inc. All other chemicals were commercially available products of analytical grade.

The carrageenan sample was dissolved in hot double distilled water and stirred for two days. Undissolved carrageenan was removed by centrifugation at 7000 rpm for 30 minutes. In order to remove remaining salt and other low molecular weight substances the supernatant solution was placed in dialysis bags (molecular weight cut-off 12000–14000) and dialysed against double distilled water for eight days.

After these steps the dialysed solution contained a mixture of counterions and these were replaced by hydrogen ions by passing the solution through an ion exchange column (Amberlite IR 120H). To ensure complete conversion of the polysaccharide to acid form at least an eight fold excess of resin was used. The acid form could then be transformed into salt form with well defined counterions by neutralisation using an appropriate base. For the experiments described here 0.5 M KOH and 0.5 M NaOH were used, thus giving a polysaccharide salt in water solution with potassium and sodium counter ions respectively.

The polysaccharide concentration was determined by the anthrone method as described previously (5).

## EXPERIMENTS

The dialysis experiments were performed in specially designed cells, previously described (4). The dialysis membrane was of a regenerated cellulose type, Spectra/Por 2, purchased from Spectrum and with a molecular weight cut-off in the interval 12000 to 14000. The membranes were rinsed in water for several days before use. It was tested experimentally to ensure that the absorption of the drugs used to this membrane was negligible. The dialysis cells were filled in one compartment with a solution containing only κ-carrageenan and in the other compartment with a solution containing only drug. The cells were placed in an air thermostat regulated to 25 ± 0.2°C. Each experiment lasted some 24 hours which was found to be sufficient to attain dialysis equilibrium.

After that equilibrium was attained the solutions on both sides of the membrane were analysed with respect to the content

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of drug. The drug concentrations were measured spectrophotometrically at well defined wavelengths.

The CMC values for the different drugs were determined by the dye solubilisation method. Salt solutions (0.03 M) with different drug concentrations were prepared and an excess amount of dye (Oil Orange SS) was added. The solutions were equilibrated over night at room temperature on a rotating table. The excess of dye was then separated off by centrifugation and the absorbance of supernatant was determined spectrophotometrically at 495 nm. Representative data for a few drugs are given in Figure 2.

## RESULTS AND DISCUSSIONS

Figures 3 and 4 show the extent of adsorption of the positively charged drugs to the negatively charged polyelectrolyte. The degree of binding is conveniently expressed as the number of moles of drug adsorbed per polymer charge, and denoted $\xi$. This number is defined by the relation

$$\xi = \frac{[D^+]_{tot,II} - [D^+]_I}{C_p^*}$$

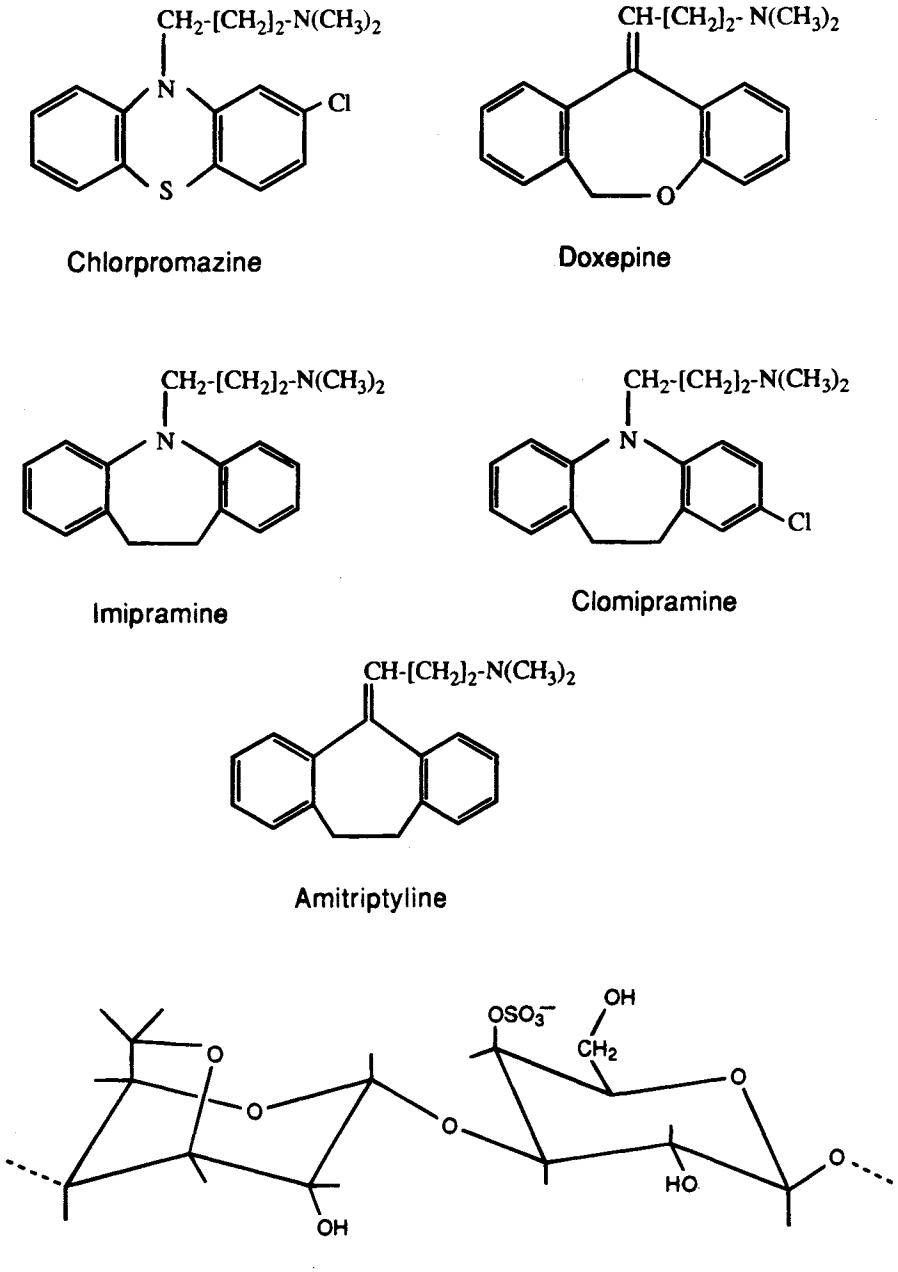


**Fig. 1.** Primary structure of κ-carrageenan and structure formulae of drugs.

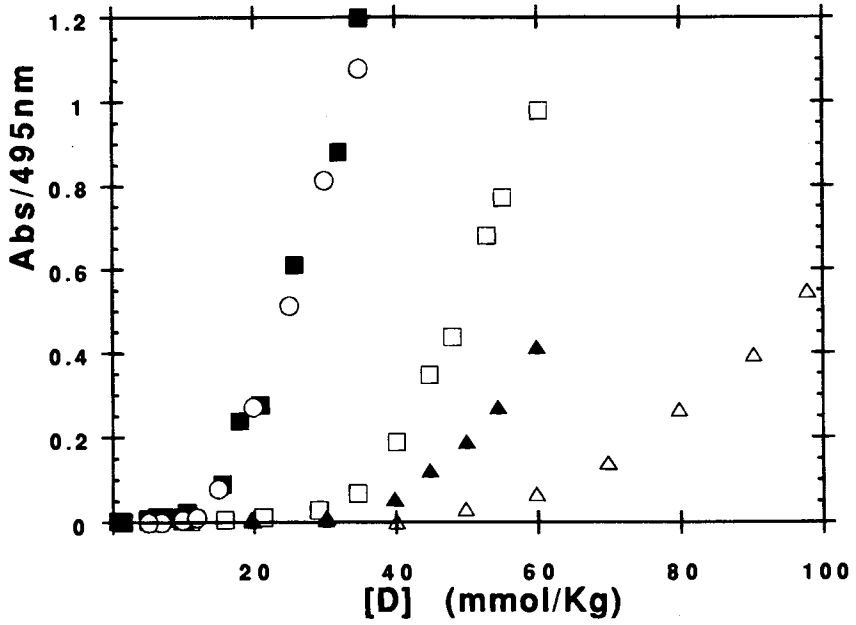


**Fig. 2.** Absorbance at 495 nm of solubilised Oil Orange as a function of total drug concentration ([D]) in 0.03 M NaCl at 25°C for, △ doxepin, ▲ imipramine, □ amitiptyline, ■ chlorpromazine and ○ clomipramine.

where $[D^+]_{tot,II}$ is the total concentration of the drug at equilibrium in the cell compartment containing polysaccharide and $[D^+]_I$ the corresponding drug concentration in the compartment without polysaccharide. $C_p$*, the molar concentration of the ionic polymer sites, can be calculated from the charge density of the polysaccharide and the polymer concentration in the cell. The charge density of the κ-carrageenan; sample used in this study was 1.03, as determined previously (4). The charge density is defined as the number of unit charges per disaccharide unit. The polymer concentration was kept at 0.5 mg/ml throughout this study since it was shown in a previous investigation that this concentration is low enough to avoid any marked polymer-polymer interaction (4).

The Donnan effect is considered to be almost negligible because of the very low polysaccharide concentration as compared to the salt concentration used in the dialysis experiments. Furthermore, the Donnan effect will be of the same magnitude for both the coil and the helix systems and therefore it can be

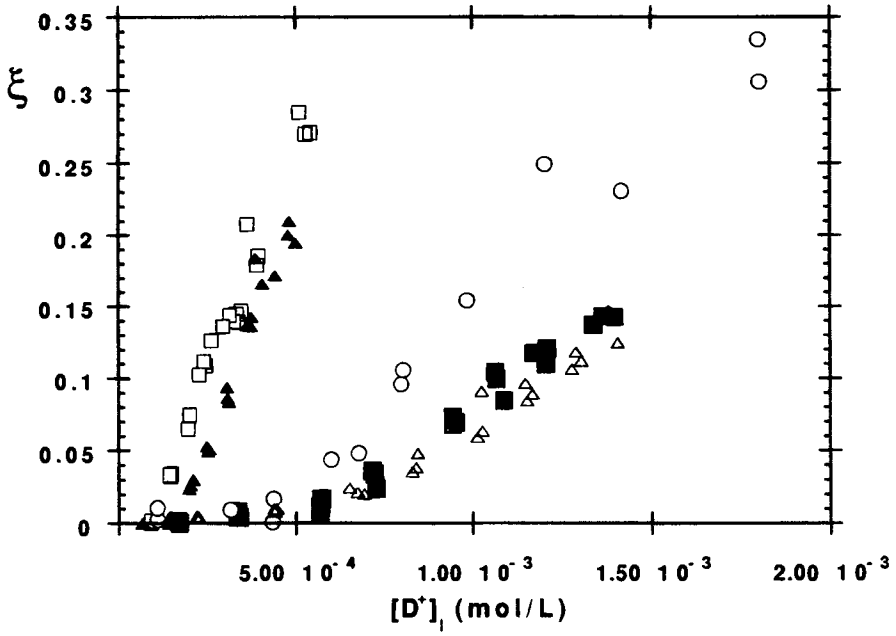


**Fig. 3.** Binding isotherms in terms of degree of binding, ξ, vs. $[D+]_I$ for κ-carrageenan in 0.03M NaCl at 25°C, △ doxepin, ■ imipramine, ○ amitiptyline, ▲ clomipramine, □ chlorpromazine.

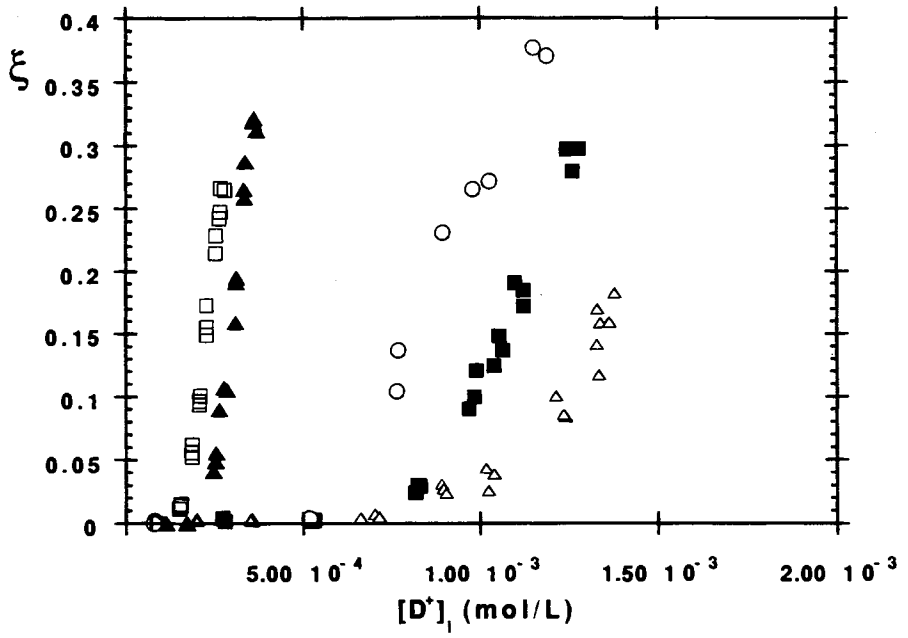


**Fig. 4.** Binding isotherms in terms of degree of binding, ξ, vs. $[D+]_f$ for κ-carrageenan in 0.03M KCl at 25°C, △ doxepin, ■ imipramine, ○ amitiptyline, ▲ clomipramine, □ chlorpromazine.

supposed not to appreciably change the adsorption differences between them.

Figure 3 clearly shows that below a certain—and in our case low—concentration of drug, no appreciable binding to κ-carrageenan is observed, while above this concentration the drug ions bind to the polyion roughly in proportion to the drug concentration. However, it is seen that the concentration for the onset of adsorption and the shape and slope of the adsorption isotherms are different for the drugs studied. In order to explain these differences, it is necessary to take into account the nature of the interaction between the drug molecules and the polyelectrolyte.

As mentioned before, the most important type of interaction between a small charged amphiphile and an oppositely charged polyelectrolyte is the charge-charge interaction. The importance of the such coulombic interaction has been shown by Dubin and his coworkers by investigating the adsorption of mixed micelles (charged and uncharged amphiphiles) to the polyion (6). In addition to the charge-charge interaction, it has been shown that the amphiphile ions bind cooperatively to polyelectrolyte not only because of electrostatic interactions between amphiphile ion and polyion, but also through hydrophobic interactions between the bound amphiphile ions.[7–9]

Furthermore, it is well-known that the magnitude of such interactions is influenced by the properties of the polyelectrolyte, amphiphile and the solvent (7–9).

The systems presented in figure 3 have the same composition, the only differences being the small variation in structure of the drug molecules. All the drugs chosen for this study have the same hydrophilic part (tertiary amine, see Fig. 1). This similarity is reflected in the very small differences in their $pK_a$ values (Table 1) (10). The structural differences between these drugs reside in the hydrophobic parts. These variations in hydrophobicity lead to moderate differences in the CMC values (Table I) (11,12). The lower the CMC value, the higher is the hydrophobicity.

We are fully aware of the fact that CMC itself cannot be used as an absolute measure of hydrophobicity, however, since the hydrophilic parts of the drug molecules studied here are the same, CMC can be used as some sort of a relative measure of the hydrophobicity of the drug molecules. In addition, logP values for these drugs show the same tendency as the CMC values presented in this paper (Table I) (10).

By comparing the hydrophobicity of the drugs (CMC-data in Table I) and data presented in Fig. 3, it is clear that the hydrophobic interactions play a considerable role in determining the adsorption pattern of drug molecules to κ-carrageenan. It should be noted that an increase in hydrophobicity in this case not only shifts the drug concentration at which the binding starts toward a lower concentration but changes also the general appearance of the binding isotherm. The slopes of these isotherms are presented in Table II. A drug molecule with high hydrophobicity gives a steeper slope.

It should be borne in mind that the electrostatic and hydrophobic interactions may be effected differently when the system is changed. For example, the electrostatic interactions

**Table I.** CMC, logP, and $pK_a$ Values for Drug Molecules

| Compound | CMC (mmol/Kg) | | | | $pK_a$[e] | Log P[e] |
|---|---|---|---|---|---|---|
| Doxepin-HCl | 62[a] | 50[b] | 68[c] | 57[d] | 9.0 | 2.4 |
| Imipramine-HCl | 50[a] | 42[b] | 48[c] | 38[d] | 9.5 | 2.5 |
| Amitriptyline-HCl | 30[a] | 35[b] | 44[c] | 34[d] | 9.4 | 3.0 |
| Clomipramine-HCl | 20[a] | 24[b] | 23[c] | 14[d] | — | 3.3 |
| Chlorpromazine-HCl | 22[a] | — | — | 14[d] | 9.3 | 3.4 |

[a] Determined by light scattering in water at 30°C[11,12].
[b] Determined by pH in water at 30°C[11].
[c] Determined by conductivity in water at 30°C[11].
[d] Determined by dye solubilization in 0.03 M NaCl at 25°C.
[e] Reference nr 10.

**Table II.** The Slope Values for Adsorption Isotherms of Drug to Both Helix and Coil Form of κ-carrageenan

| | Slope of adsorption isotherms (L/mol) | |
|---|---|---|
| | 0.03 M NaCl (coil) | 0.03 M KCl (helix) |
| Doxepin-HCl | 159 ± 10 | 372 ± 42 |
| Imipramine-HCl | 167 ± 6 | 594 ± 29 |
| Amitriptyline-HCl | 231 ± 19 | 577 ± 32 |
| Clomipramine-HCl | 648 ± 32 | 2343 ± 119 |
| Chlorpromazine-HCl | 615 ± 26 | 2423 ± 82 |

are affected by introduction of an external salt which results in an extensive shift of the amphiphile concentration at which the binding starts toward a higher concentration. This is due to a competitive effect and also to electrostatic shielding of the oppositely charged ions of the polyelectrolyte and amphiphile (7,8). Similar results published previously (4) where the adsorption of amitriptyline to κ-carrageenan in the absence of the external electrolyte was investigated, support these conclusions. An external salt may also effect the extent of hydrophobic interaction through the changes in the three dimensional structure of the polyelectrolyte. This will be further discussed below.

The electrostatic interactions between the polyions and the drug molecules are also highly dependent on the pH-value of the solution since the $pK_a$-values of the polyelectrolyte ions and the drug ions in relation to pH in the solution will define the extent of ionisation of charged substituents as well as of the drug. In the present investigation, the solutions were not buffered and the pH values varied between 4 and 7. However, both drug molecules (Table I) and sulphate groups on the κ-carrageenan are fully charged in this pH range.

The influence of the charge density on the adsorption isotherm becomes obvious when different polyelectrolytes are compared. This was shown in a previous paper (4) where different carrageenans with different charge densities were compared regarding adsorption of amitriptyline. The observed "order" of the adsorption isotherms for a given drug was λ-carrageenan > ι-carrageenan > κ-carrageenan which correlates with the charge density for these polymers. Even the differences in chemical structure should contribute to some degree. This clearly demonstrates the dominant effect of the electrostatic interaction and is an important reminder for the applications since it was also shown[4] that different batches of the same type of carrageenan may have different charge densities. This emphasises the necessity of charge density determinations for the product specification.

Since the cooperative effect in the binding of drug molecules to a polyelectrolyte is to a large degree governed by hydrophobic interaction between bound drug molecules as well as with hydrophobic parts of the polyelectrolyte, we may expect this effect to be sensitive to the distance between the charges on the polymer and to the detailed chemical structure of the polymer backbone.

Comparing Figs. 3 and 4, it is clearly seen that the adsorption isotherms are much steeper in the case of KCl than in the case of NaCl. This significant increase in cooperativity in the case of KCl is due to a lowering of the distance between the neighbouring sulphate groups (charges) on the κ-carrageenan caused by the formation of a helical structure (3). It is a well-known fact (13,14) that at 25°C the κ-carrageenan molecules exist in the coil form in the presence of 0,03 M NaCl and in the helix form in the presence of 0,03 M KCl. Furthermore, it has been shown that the charge-charge distance for κ-carrageenan is much shorter when the molecules are in the helix form than in the coil form (15). A shorter distance between neighbouring charges leads up to a limit to higher hydrophobic interactions between the bound drug molecules which will increase the cooperativity and thereby make the adsorption isotherm steeper. This helix-coil transition is proposed to explain the differences in adsorptions that are seen in Figs. 3 and 4.

The results presented in Table 2 show the differences in the slope of the adsorption isotherms for the drugs investigated in both 0,03 M NaCl and 0,03 M KCl. The slope is referred to the linear part of the adsorption isotherm. The values for the helix form (0,03 M KCl) are clearly higher than the corresponding values for the coil form (0,03 M NaCl) which is in accord with our previous findings (3). In the presence of NaCl, the ratio between the slope values follow the same pattern as the CMC values, i.e., the highest slope value corresponds to the lowest CMC value (highest hydrophobicity). In the case of KCl, the same comparison becomes less distinct because the adsorption isotherms are much steeper which makes the values more uncertain. This is supported by high standard deviation values for helix form comparing to the coil form.

Table I shows that the presence of an external salt lowers the CMC values, but the order among the drugs is not changed. It should also be observed that the drug concentrations used in this investigation are far below the CMC values even in the presence of 0.03 M salt. This means that there are no free micelles in the systems studied. At the same time, the bound drug molecules may form some type of aggregates along the polymer chain.

From the results presented it can be concluded that even if the electrostatic interaction is the dominant one, the hydrophobic interactions between bound drug molecules play an important role for the degree of adsorption of drugs to the polyelectrolytes. In addition, the distance between the charges on the polyions governs the magnitude of the hydrophobic interaction and thereby the cooperativity. It is also shown that by changing the three dimensional structure of the polyelectrolyte, in this case κ-carrageenan, one may change the charge-charge distance. This latter observation may have application for controlled drug delivery. Since the helix-coil transition of κ-carrageenan can be controlled not only by changing the salt type and salt concentration, but also by changing the temperature, it is possible to influence the adsorption and the desorption of drug molecules.

## ACKNOWLEDGMENTS

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