ELSEVIER

International Journal of Pharmaceutics 115 (1995) 103–111

international journal of pharmaceutics

# Some aspects on characterization and properties of charged polysaccharides. An investigation of the system carrageenan/amitriptyline/water with relation to amphiphile adsorption and charge density

Ninus Caram-Lelham *, Lars-Olof Sundelöf

*Physical Pharmaceutical Chemistry, Uppsala University, BMC, Box 574, S-751 23 Uppsala, Sweden*

Received 13 July 1994; modified version received 18 August 1994; accepted 19 August 1994

**Abstract**

The importance of a precise characterization of the polymeric material for pharmaceutical applications is exemplified by an adsorption study of the system carrageenan/amitriptyline/water performed by means of dialysis equilibrium measurements. It is shown how differences in charge density and chemical composition lead to a wide variation in adsorption and phase separation properties. Simple physico-chemical methods are suggested that provide a both rapid and precise characterization of polyelectrolyte materials. In particular, charge density, structure of the monomeric unit and accurate determination of the Donnan equilibrium are discussed.

*Keywords:* Polysaccharide; Carrageenan; Charge density; Amitriptyline; Adsorption; Phase separation; Dialysis equilibrium

## 1. Introductory problem presentation

Water soluble polymers and macromolecules play an important role in biomedicine and pharmacy as well as in many technical applications. In this respect, the polysaccharides constitute a class of substances of particular interest due to their wide span of properties and their essential role in living matter. The polysaccharides are characterized by an almost unmatched variability which derives from the structural features of the basic building unit normally constituted by a mono- or disaccharide of specific structure (Wolfrom et al., 1969; Aspinall, 1982, 1983, 1985). Such a unit carries a number of hydroxyl groups that provide hydrophilic properties whereas the rest of the basic unit often must be regarded as fairly hydrophobic. This balance between hydrophobic and hydrophilic properties is basic to the action of the polysaccharides and it can be varied within large limits by substituting the hydroxyls either by longer or shorter hydrophobic chains or by charged groups. In the former case the molecule takes on predominantly hydrophobic properties still being water soluble at least in limited regions

* Corresponding author.


*SSDI* 0378-5173(94)00260-6

of concentration and temperature. In the latter case the hydrophilic properties will dominate.

Since the number of substituted groups can be varied as well as their distribution along the chain, it is evident that very complex molecules can be obtained. Some of the biologically most potent polysaccharides have basic structural units comprising several glucosic rings with very specific structures and charges (Brimacombe et al., 1964).

Obviously, such polymers are capable of interaction both with molecules of their own kind – which, for instance, is essential to the properties of the cartilage – as well as with other molecules, such as low molecular weight amphiphiles. Recently, a specific effect of the hyaluronan oligosaccharides on a high molecular weight hyaluronan has been observed (Heldin et al., 1993). An important feature also seems to be the complex conformational interaction exhibited, either in the form of helix-coil transitions or as networks when the concentration is sufficiently high. These helix-coil transitions are dependent on temperature and salt concentration and could be utilized to design control release systems.

It is clear from this short overview that in any study of the properties of systems containing such water soluble polymers it is essential for the interpretation of experimental results to have a very clear understanding of the degree of substitution and substitutent distribution, charge density, structural features in general, molecular size and size distribution, etc. This is by no means a simple task and it requires a wide arsenal of methods in order to succeed.

Charged amphiphiles (surfactants) and oppositely charged polymers (polyelectrolytes) normally show strong interaction primarily due to charge neutralization. In polysaccharides, for instance, this has the effect that the polar or hydrophilic properties of the polyelectrolyte become at least partially eliminated and the solution properties of the polymer will change drastically. Often the polymer becomes insoluble. After reaching a sufficient degree of neutralization, hydrophobic interaction can no longer be excluded as a more dominant element, possibly leading to reconformation of the polymer and clustering of amphiphile onto its backbone. As a consequence there could in exceptional cases even be a recharging of the polymer with the opposite charge due to formation of micellar clusters of amphiphile followed by an increase in solubility. It is to be expected that such an interaction, if present, will depend strongly on the polymer concentration and may give rise to large macroscopic effects.

This proposed possible scenario rests on considerable general knowledge accumulated in our own and other laboratories over recent years. The main issue here is that it points to the necessity to consider effects of the nature indicated in many common formulations for drug administration. To this end the present paper will present some data on the model system, amitriptyline/carrageenan/water, selected to have implications for drug delivery formulation in general. The carrageenan family of polyelectrolytic polysaccharides has been chosen due to its variability both in struture and charge density as well as its common use. Some even recent investigations have treated the problem of drug-polymer interaction without many direct references to the investigation of polymeric structure (Heyd et al., 1975; Cadwallader et al., 1981; Zatz et al., 1987; Bonferoni et al., 1993). Amitriptyline was selected as the model amphiphile due to its charge as well as its merits as a model drug and good analytical detectability.

The experimental results have been collected over a wide composition interval of both amphiphile and polysaccharide from infinite dilution up to phase separation. In a wider perspective the intent will be to show how and to what extent certain properties change with polysaccharide structure and charge density in an attempt to bridge thermodynamic and conformational observations, as determined from dialysis equilibrium experiments and hydrodynamic measurements. For the moment only the effect of charge density and polysaccharide concentration on the interaction will be treated, also providing a theory for quantification of the experimental observations. Precise experimental methods are given for determination of charge density and for direct determination of the Donnan effect.

Different counterions are known to have spe-

cific effects on the system properties (Rees et al., 1969; Morris et al., 1980; Rochas et al., 1980). In the carrageenan system, for instance, substitution of sodium ions for potassium ions leads to gelation at much lower concentrations. Other ions introduce other effects. In the present paper only potassium ions are included in the system. The effect of the different ions on the carrageenan system is under investigation.

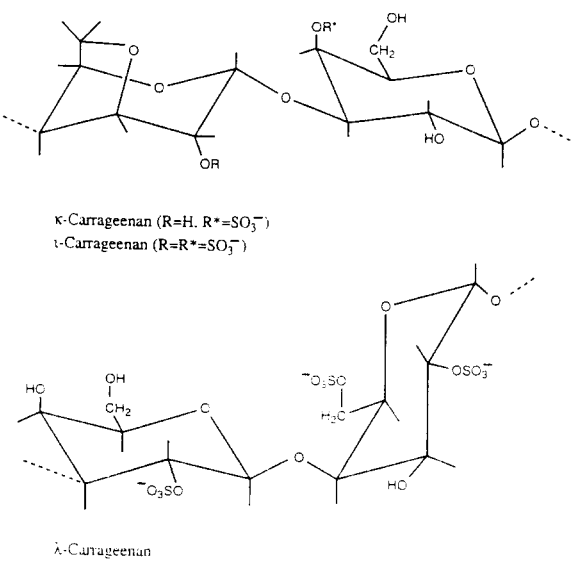
## 2. Materials and methods

### 2.1. Samples

Commercial samples of κ-, ι-, and λ-carrageenans were obtained from Sigma, the samples having designations (C-1263: lot no. 19F-06737), (C-4014: lot no. 27H-0373), and (C-3889: lot no. 21H-0322), respectively. Amitriptyline hydrochloride (drug grade purity) was a gift from Pharmacia AB. Anthrone and resorcinol were purchased from Sigma. All other chemicals were commercially available products of analytical grade.

The carrageenans constitute a polysaccharide family built up from subunits consisting of two galactose rings that carries an electric charge of up to three unit charges deriving from dissociated sulphate groups. The ideal structures indicate that the κ-, ι-, and λ-carrageenans should be characterized by one, two, and three charge units, respectively, per subgroup (see Scheme 1) (Aspinall, 1982, 1983, 1985). However, the structure of natural polymers and their derivatives seldom conform to simple and well defined structural schemes and the carrageenans are no exception to this. The characterization (see below) has revealed that although the three samples used show an increase in subunit charge in the order 'κ', 'ι', 'λ', the subunit charge does not conform to idealized models. This must mean that the samples show some unhomogeneity as to structure which must be taken into consideration when data are analysed.

Furthermore, the commercial samples do not have one single type of cation as counterion. Since different cations can give quite different sample properties (Rees et al., 1969; Morris et al., 1980; Rochas et al., 1980), especially at higher concentrations, the samples must be processed to have one single type of counterion. Finally, the commercial samples contain extra salt and even some other low molecular weight substances that also must be cleaned off.




Scheme 1. Idealised disaccharide repeating structure of the carrageenans.

### 2.2. Purification

The carrageenan sample was dissolved in double-distilled water at elevated temperature and stirred for 2 days. Undissolved carrageenan was removed by centrifugation at 7000 rpm for 30 min. 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 dialyzed aginst double-distilled water for 8 days.

After these steps the dialysed solution still contained different types 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 8-fold excess of resin was used. The acid form could then be transformed into salt form by neu-

tralization using an appropriate base. For the experiments described here 0.5 M KOH was used, thus giving a polysaccharide salt in water solution with potassium counterions.

The ion-exchange process excerts a fairly strong influence on the polymer and normally leads to some degradation. This should be taken into account in the experimental design. If experiments with different counterions should be compared one must always start from the same acid form and neutralise directly to the desired salt since repeated ion exchange would give a repeated degradation.

### 2.3. Solution preparation and concentration determination

After the purification steps and ion exchange the polysaccharide concentration is known only approximately. Part of it was therefore weighed, dried, and the dry weight determined. Drying was performed at 90°C overnight. Higher temperature caused discoloration. To ensure reliable concentrations the drying experiments were performed in triplicate. In all cases the solutions after the neutralization step had a weight-to-weight (g/g) concentration of approx. 0.002. These stock solutions were kept at 4°C. Immediately before an experiment a stock solution was brought to room temperature and diluted to appropriate concentrations.

In the quantitative treatment of experimental data it would be advantageous to know the molar concentration of polysaccharide subunits in solution. However, the discussion in section 2.1 indicates that this requires a precise knowledge of polysaccharide structure. Since this is only approximately known (cf. the discussion above concerning number of charges per subunit, i.e., charge density) only approximate figures will be available for the molar subunit concentration.

### 2.4. Characterization

For a very precise discussion it would be necessary to know the polysaccharide structure in detail. To perform such a characterization is a task which is both difficult and time consuming. Work is in progress in our laboratory on these matters. However, for the present purpose the main information needed is a fairly precise knowledge of the average amount of ionizable groups per subunit, the average charge density, $\beta$. This was determined for the solutions containing the acid form after ion exchange by titration to pH 7 with 0.1 M NaOH. It was assumed that the number of moles of NaOH consumed was identical to the number of sulphate groups in the sample. Combining these titration data with the assumption that all charges are carried by sulphate groups and that the ideal structure of the $\kappa$-, $\iota$-, and $\lambda$-carrageenans shown in Scheme 1 applies, it is possible to determine $\beta$. After passing the sample through the ion exchanger the average charge density was also estimated from flame photometry measurements. Both methods agree fairly well. The results of charge density determinations are collected in Table 1. The main significant differences within this polysaccharide family reside in the content of sulphate groups, which varies from none for agarose up to three for the $\lambda$-form.

It must be realized, however, that the calculation of $\beta$ from the titration experiments rests on

Table 1
Characteristics of $\kappa$-, $\iota$- and $\lambda$-carrageenans: a comparison between the ideal and the experimentally found structure

| Carrageenans | Ideal structure [a] | | | Experimental [a] | | |
|---|---|---|---|---|---|---|
| | $M_w$ | Charge density | Amount of anhydrogalactose | $M_w$ | Charge density | Amount of anhydrogalactose |
| Kappa | 424 | 1.0 | 1.0 | 428 | 1.03 | 0.82 |
| Iota | 543 | 2.0 | 1.0 | 482 | 1.49 | 0.59 |
| Lambda | 679 | 3.0 | 0.0 | 571 | 2.09 | 0.16 |

[a] All values are refered to the disaccharide unit with potassium ion as a counterion.

certain assumptions about structure (see Scheme 1). That the structures in our samples are not completely ideal is evident, at least for the λ-sample, since in this sample there is some presence of 3,6-anhydrogalactose. The content of 3,6-anhydrogalactose was determined by a resorcinol procedure outlined by Yaphe and Arsenault (1965). For the κ- and ι-samples, on the other hand, the amount of 3,6-anhydrogalactose was found to be lower than what should be the case for the ideal structure. These experimental findings indicate that the structure is at least not completely ideal. Hence the calculated numbers for β should not be taken to be accurate to more than say one decimal.

### 2.5. Dialysis

The dialysis experiments were performed in specially designed lucite cells consisting of two halves, each with a cavity volume of approx. 2 ml and a membrane area of some 3 $cm^2$. The membrane was clamped between two blocks which were held together by metal bolts. In some early cells there was no membrane support. The new cells were designed to allow appropriate membrane support and simple filling and sampling routines.

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 12000–14000. The membranes were rinsed in water for several days before use. It was tested experimentally that the absorption of amitriptyline by this membrane was negligible.

The dialysis experiments were performed at a constant temperature of 22.0 ± 0.2°C. Each experiment lasted some 24 h which was shown to be sufficient to attain dialysis equilibrium.

### 2.6. Analysis

After attaining equilibrium the solutions on both sides of the membrane were analysed with respect to content of amitriptyline, potassium ions, and polysaccharide. The amitriptyline concentration was determined spectrophotometrically at 240 nm in a Beckman DU 68 spectrophotometer. In separate experiments it was shown that the presence of polysaccharide did not affect the spectral properties of amitriptyline. The potassium ion content was determined by flame photometry using LiCl as internal standard. It was shown that neither amitriptyline nor carrageenan did have an influence on these determinations. The carrageenan concentration, finally, was determined colorimetrically with a modified anthrone method (Caram-Lelham et al., 1994).

## 3. Theory

In order to treat the experimental dialysis data quantitatively the following simplified theoretical approach was developed. We let II denote the dialysis cell compartment containing polymer and I represents the other compartment. Initially, the polymer only contained its own negatively charged sulphate groups together with a matching quantity of counterions solely consisting of $K^+$ ions. The amitriptyline sample, in its dissociated form, contained only positively charged amitriptyline ions, denoted $A^+$, and $Cl^-$ ions. This initial equality between number of sulphate groups and potassium ions on the one hand and between amitriptyline ions and chloride ions on the other considerably simplifies the stoichiometry. From the chemical point of view, however, it can be anticipated that the absence of any extra electrolytes may emphasize the polyelectrolytic behaviour of the carrageenans.

It is convenient to introduce the following definitions. Let $C_1$ denote the monomer molarity of polymer and $C_p^*$ the molarity of charges carried by the polymer if completely dissociated. Let β denote the (average) number of charges per monomer and $M_1$ the monomer molecular weight. As discussed in section 2, β is determined in independent experiments. If $c_p$ is the mass concentration of polymer (mass per volume unit, i.e., litre) we then have the relations:

$$C_1 = c_p/M_1 \quad (1)$$

$$C_p^* = C_1 \cdot \beta \quad (2)$$

At dialysis equilibrium and if osmotic effects due to polymer are small enough to be omitted and

also assuming complete dissociation of the amphiphile, it holds that:

$$[K^+]_I \cdot [Cl^-]_I = [K^+]_{II} \cdot [Cl^-]_{II} \quad (3)$$

$$[A^+]_I \cdot [Cl^-]_I = [A^+]_{II} \cdot [Cl^-]_{II} \quad (4)$$

Electroneutrality requires the equalities:

$$C_p^* + [Cl^-]_{II} = [K^+]_{II} + [A^+]_{II} \quad (5)$$

$$[K^+]_I + [A^+]_I = [Cl^-]_I \quad (6)$$

Eq. 3 and 4 give the following identity for the ratio, $r$, between the ionic concentrations on the two side of the membrane. Essentially, $r$ is a direct measure of the Donnan effect.

$$r = [K^+]_{II}/[K^+]_I = [Cl^-]_I/[Cl^-]_{II} = [A^+]_{II}/[A^+]_I \quad (7)$$

Since $[K^+]_I$ and $[K^+]_{II}$ are measured directly, $r$ can be determined experimentally.

Suppose now that the following chemical equilibrium governs the adsorption process, where $P_I^-$ denotes the active (charged) site on the monomer unit in the polymer and $P_IA$ the 'adsorption complex':

$$A^+ + P_I^- \rightarrow P_IA \quad (8)$$

and

$$C_I = [P_IA] + [P_I] \quad (9)$$

We can then define:

$$[A^+]_{tot,II} = [P_IA] + [A^+]_{II} \quad (10)$$

Here we have assumed that the only interaction taking place is the neutralization of polymer charge by the additive ion $A^+$. We then introduce the quantity $\xi$ defined as the fraction of the initial (total) charge of the polymer that has been neutralized by 'adsorption' in accordance with Eq. 8.

$$\xi = \{[A^+]_{tot,II} - [A^+]_{II}\}/C_p^* = \{[A^+]_{tot,II} - r \cdot [A^+]_I\}/C_p^* \quad (11)$$

Obviously, the unambiguous determination of the dialysis equilibrium, of the Donnan effect, and of the charge density in separate experiments provides a basis for considerable insight into the interaction process. It has been found that the maximum value of $\xi$ that can be attained, i.e., maximum adsorption before phase separation, is strongly dependent on the charge density of the polymer chain. In this paper, however, the quantitative analysis will not be carried further.

## 4. Results and discussion

The main results are presented in Table 1. If experimental data are compared with corresponding values for the ideal structure, it is obvious that none of the samples conform to the ideal behaviour. In the case of the $\kappa$-carrageenan sample the charge density is identical to the ideal one but the amount of anhydrogalactose is 18% lower than expected. The $\iota$-carrageenan sample has a charge density of only 1.49 instead of 2 and the amount of anhydrogalactose is as much as 41% lower than according to the ideal structure. The $\lambda$-carrageenan sample, finally, has a charge density only slightly above 2 instead of 3 for the ideal model. It contains also as much as 16% anhydrogalactose instead of none in the ideal structure. These observations show clearly that sample designations must be followed up by careful testing. The variations in charge densities and the irregularities in structure that have been found experimentally are due to many factors, some of which are related to the cultivation and harvesting of red algae as well as to the further processing of the carrageenan substance (Therkelsen, 1982).

In Scheme 1 the ideal basic structures for the three types of carrageenans are shown. The important structural effects deriving from varying degrees of charge densities and presence or absence of anhydrogalactose are obvious. Furthermore, the distribution of the 'defects', as revealed by Table 1, may not be evenly distributed along the chain which introduces still another parameter to be considered in a more complete characterization.

To indicate the high precision of the charge density determinations some titration curves are shown in Fig. 1. Such titrations are fairly easy to perform and give a precise value of the charge density (within a few per cent). Since the charge density seems to play a central role for the degree of interaction between amphiphilic charged drugs

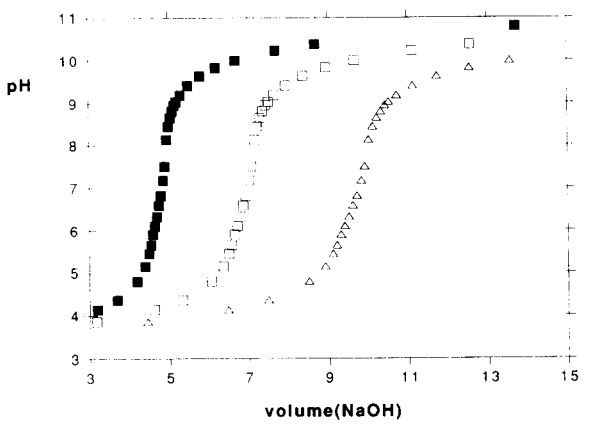



Fig. 1. Representative curves for titration by NaOH of a constant amount of the acid form of κ-carrageenan (■), ι-carrageenan (□) and λ-carrageenan (△); volume in ml.

and a polyelectrolyte such charge density determinations are strongly recommended.

A clear indication of the large variation with structure for the interaction between a drug and the carrageenan main chain is illustrated in Fig. 2. The parameter $\xi$ is by its definition (see Eq. 11) a direct measure of the relative adsorption of amphiphile to a charge site. Thus, the results presented in Fig. 2 indicate that for a given drug concentration the adsorption equilibrium varies considerably between the three carrageenan samples used. It is strongest for the λ-sample. The ι-sample seems to follow the same trend as the λ-sample when $\xi$ is plotted vs free drug concentration. However, the experimental data for the ι-sample seem to show some displacement along the $[A^+]_I$-axis. This displacement has not been possible to explain. It is experimentally reproducible. The κ-sample gives a much different behaviour with a more flat variation of $\xi$ with free drug concentration. The data have been obtained up to values of $[A^+]_I$ where phase separation begins. After this point no experiments have been performed. It is seen from the results that at phase separation the system is far from saturation but the adsorption limit increases with increasing charge density (see Fig. 3). Although this cannot be directly ascertained by the experiments it is likely that adsorption to some extent is also regulated by the disaccharide structure.

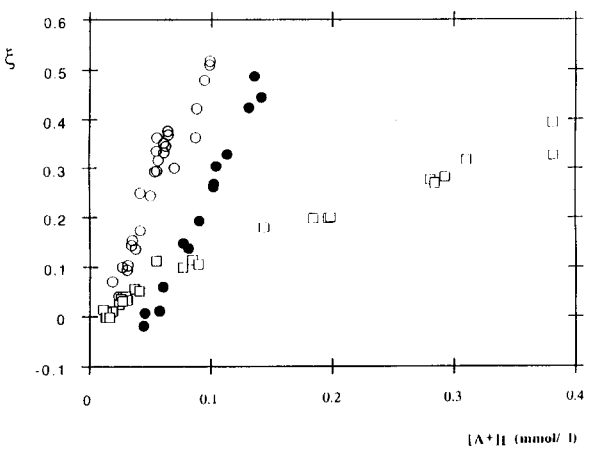



Fig. 2. $\xi$ as a function of amitriptyline concentration at equilibrium for the κ-carrageenan (□), ι-carrageenan (●) and λ-carrageenan (○) samples.

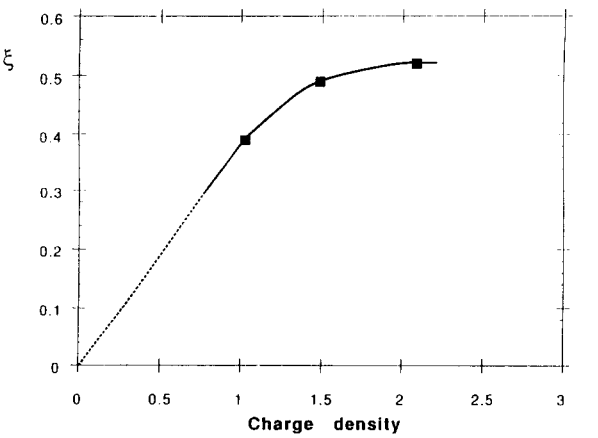



Fig. 3. $\xi$ as a function of charge density.

Fig. 4 gives the variation of $\xi$ with $[A^+]_I$ for the κ-sample at three different concentrations. There is seen to be a profound decrease in adsorption as polymer concentration increases. Doubling the carrageenan concentration from 1 to 2 mg/ml leads to a decrease in adsorption by more than a factor of two. In other words, an increase in polymer concentration leads to a decreased tendency to adsorb the drug.

At the same time for the higher κ-carrageenan concentrations, the phase separation is obtained at lower $\xi$-values. This means that at high poly-

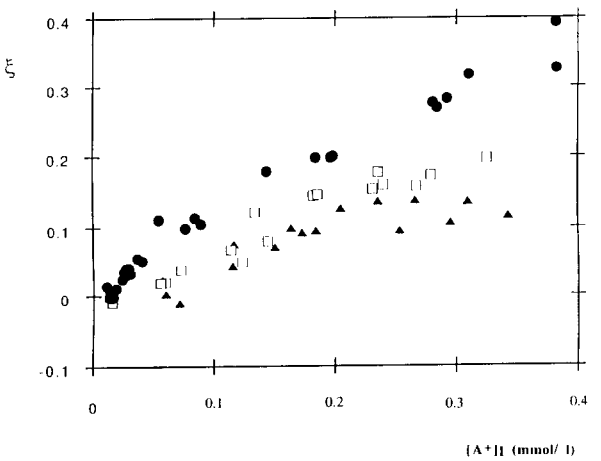



Fig. 4. $\xi$ as a function of amitriptyline concentration at equilibrium for the $\kappa$-carrageenan sample with polymer concentrations 1.0 mg/ml (●), 1.5 mg/ml (□) and 2.0 mg/ml (▲).

mer concentrations a lower degree of charge neutralization is required in order to reach phase separation. This is not surprising because the phase separation is caused by interaction between polymer chains which is favoured by high polymer concentration and charge neutralization (in this case by amitriptyline) which increase the hydrophobicity of the polymer chains.

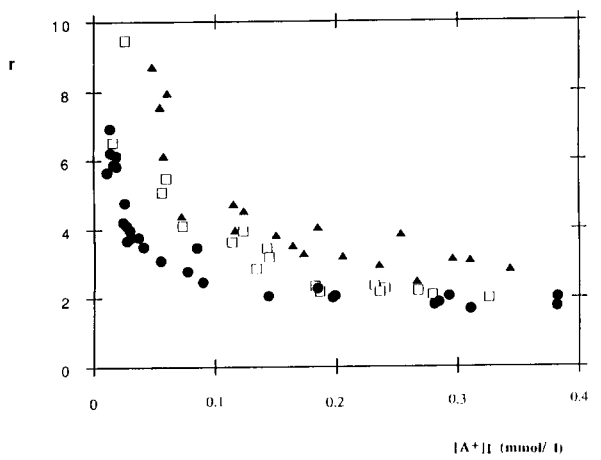



Fig. 5. The 'Donnan ratio' $r$ as a function of amitriptyline concentration at equilibrium for the $\kappa$-carrageenan sample with polymer concentrations 1.0 mg/ml (●), 1.5 mg/ml (□) and 2.0 mg/ml (▲).

The magnitude of the Donnan effect as a function of $[A^+]_i$ for three different concentrations of the $\kappa$-sample is seen in Fig. 5. Obviously, the Donnan effect is quite important at the lower drug concentrations. It is also strongly dependent on polymer concentration.

Apart from the adsorption analysis just described, no attempt has been made in this paper to analyze the experimental data in terms of more sofisticated theories, since the main aim has been to point out the magnitude of the adsorption effects and their variation with structure and charge density. Work is in progress to widen these studies and to try to deepen the theoretical approach.

## Acknowledgements

Financial support from the Swedish Natural Science Research Council, the Swedish Council for Engineering Sciences, and Pharmacia AB is gratefully acknowledged. We also thank Mr Andreas Hugerth for technical assistance.

## References

Aspinall, O.G., *The Polysaccharides,* Academic Press, New York, Vol. 1, 1982.

Aspinall, O.G., *The Polysaccharides,* Academic Press, New York, Vol. 2, 1983.

Aspinall, O.G., *The Polysaccharides,* Academic Press, New York, Vol. 3, 1985.

Bonferoni, M.C., Rossi, S., Tamayo, M., Pedras, J.L., Dominguez-Gil, A. and Caramella, C., On the employment of $\lambda$-carrageenan in a matrix system: I. Sensitivity to dissolution medium and comparison with Na carboxymethylcellulose and xanthan gum. *J. Controlled Release,* 26 (1993) 119–127.

Brimacombe, J.S. and Webber, J.M., *Mucopolysaccharides,* Elsvier, Amsterdam, Vol. 6, 1964.

Cadwallader, D.E. and Madan, D.K., Effect of macromolecules on aqueous solubility of cholestrol and hormone drugs. *J. Pharm. Sci.,* 70 (1981) 442–446.

Caram-Lelham, N., Cleland, R.L. and Sundelöf, L.-O., Temperature and salt optimization of $\kappa$-carrageenan fractionation by DEAE-cellulose. *Int. J. Biol. Macromol.,* 16 (1994) 71–75.

Heldin, P. and Pertoft, H., Synthesis and assembly of the hyaluronan-containing coats around normal human mesothelial cells. *Exp. Cell Res.,* 208 (1993) 422–429.

Heyd, A. and Dhabhar, D.J., Enhancing fluidity of concentrated antacid suspensions. *J. Pharm. Sci.*, 64 (1975) 1697–1699.

Morris, E.R., Rees, D.A. and Robinson, G.J., Cation-specific aggregation of carrageenan helices: Domain model of polymer gel structure. *J. Mol. Biol.*, 138 (1980) 349–362.

Rees, D.A., Steele, I.W. and Williamson, F.B. Conformational analysis of polysaccharides: III. The relation between stereochemistry and properties of some natural polysaccharide sulfates. *J. Polym. Sci. C*, 28 (1969) 261–276.

Rochas, C. and Rinaudo, M., Activity coefficients of counterions and conformation in kappa-carrageenan systems. *Biopolymers*, 19 (1980) 1675–1687.

Therkelsen, G.H., In Whistler, R.L. and BeMiller, J.N. (Eds), *Industrial Gums*, Academic Press, New York, 1982, pp. 145–180.

Wolfrom, M.L. and Tipsin, R.S., *Advances in Carbohydrate Chemistry and Biochemistry*, Academic Press, New York, Vol. 24, 1969.

Yaphe, W. and Arsenault, G.P., Improved resorcinol reagent for the determination of fructose, and of 3,6-anhydrogalactose in polysaccharides. *Anal. Biochem.*, 13 (1965) 143–148.

Zatz, J.L. and Woodford, D.W., Prolonged release of theophylline from aqueous suspensions. *Drug Dev. Ind. Pharm.*, 13 (1987) 2159–2178.