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Characterization of a diltiazem-lambda carrageenan complex

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Abstract

In the present paper the interaction between lambda carrageenan, a natural sulphated polysaccharide, and diltiazem HCl, a Ca channel blocking agent, was studied. Dialysis equilibria were performed to quantify the binding capacity of lambda carrageenan for diltiazem. The relevance of the interaction to hydrophilic matrix systems was confirmed: a relationship was found between the binding capacity and the release profiles of matrix tablets containing a fixed amount of drug and different percentages of lambda carrageenan. Dialysis equilibria in buffered media showed that the interaction is quite insensitive to the pH of the medium (in the range 1.8–6.8), while it is reduced by increasing ionic strength; this behaviour is in line with the importance of ionic bonds in diltiazem-carrageenan interaction. On the basis of the calculated binding capacity, the complex was prepared, dried and milled. A preliminary characterization of the diltiazem-carrageenan complex in the solid state was effected by means of X-ray and DSC analysis. The amount of drug going into solution from the complex was not significantly affected by the pH of the medium (in the range 1.8–6.8), while it was increased by increasing ionic strength.

Keywords: Lambda carrageenan; Drug–polymer complex; Dialysis equilibria; Diltiazem HCl; Controlled release matrix tablets

1. Introduction

Whenever ionic polymers (polyelectrolytes) are used as excipients in pharmaceutical formulations, the release of oppositely charged drugs may be strongly affected by the occurrence of charge–charge interactions, which sometimes result in the formation of an insoluble product. In some cases, this occurrence is considered as a negative event, to be avoided as drug-excipient incompatibility. These polymer–drug interactions can also be exploited for controlled drug release; in this perspective, water insoluble complexes have been described between pectins and either nonsteroidal antiinflammatory drugs or phenothiazines (Takahashi et al., 1978, 1981). Earlier studies have compared the interaction capability of several hydrocolloids with some tranquilizers, hypotensive alcaloids and anthihistamines; in particular, sustained release of promazine from a complex with a carrageenan has been ascertained following intramuscular injection in rabbits (Graham et al., 1963a; Graham and Baker, 1963b). In the case of formulations aimed at oral controlled delivery, however, this approach strongly depends on the

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possibility to maintain a reliable control of the release in all the conditions of pH, ionic strength, etc. that the formulation faces along the gastrointestinal tract. For instance, in the case of polyelectrolytes carrying carboxylic groups, such as pectins, drug–polymer interaction is quite sensitive to low values of pH, probably due to the reduced dissociation of the polymers (Graham et al., 1963a). Consistent with these findings, the release rates of basic model drugs (namely salbutamol sulphate and chlorpheniramine maleate) from hydrophilic matrix systems based on Na carboxymethylcellulose and xanthan gum was higher at low pH values than at neutral pH. Instead, the release profiles of the same drugs (salbutamol sulphate and chlorpheniramine maleate) from matrices, based on lambda carrageenan, was controlled even at gastric pH values. This is conceivably attributed to the presence in the carrageenan molecule of strongly acidic sulphate groups that allow a certain degree of ionization to be mantained also at low pH (Graham et al., 1963a; Bonferoni et al., 1994). According to this hypothesis, matrix formulations containing HPMC and lambda carrageenan showed an almost pH independent release of very soluble basic drugs (Bonferoni et al., 1998a).

Interestingly the release control decreased on increasing drug load, which suggested that an optimal drug–polymer ratio was needed in order to keep drug release controlled. This point is of crucial importance in the case of high dosage freely basic drugs, like for example diltiazem HCl.

Given these premises, the aim of the present work was twofold:

1. To study the interaction between lambda carrageenan and diltiazem HCl, in order to understand the possible relevance to the formulation of oral hydrophilic matrix systems. Diltiazem HCl is a Ca channel blocking agent good candidate to prolonged release formulations. Data have recently been presented in the literature on the use of equilibrium dialysis experiments to study the interaction of carrageenans with some basic drugs (Caram Lelham and Sundelof, 1995, 1996). Accordingly to this method, dialysis equilibria were performed to quantify the binding capacity of lambda carrageenan for diltiazem. This binding capacity was related to the release profiles of matrix tablets containing a fixed amount of drug and different percentages of lambda carrageenan. The sensitivity of the interaction to pH and ionic strength was also studied through dialysis equilibria in buffered media.
2. To possibly isolate and characterise the complex between lambda carrageenan and diltiazem. On the basis of the calculated binding capacity, the complex was prepared, dried and milled; the effect of the medium pH and ionic strength on its solubility was moreover investigated. A preliminary characterization of the diltiazem-carrageenan complex in the solid state was also performed, by means of X-ray and DSC analysis.

2. Materials and methods

2.1. Materials

Lambda carrageenan Viscarin GP 209 (Prodotti Gianni, Milan, I). Diltiazem HCl (Profarmaco, Milan, Italy). Hydroxypropylmethylcellulose Methocel K4M (Colorcon, Orpington, UK). Lactose USP XXIII.

2.2. Interaction isotherms

The interaction isotherms were obtained by using dialysis tubes 12 000–14 000 cut off (Visking, Emanuele Mires, Milan, Italy), previously boiled for 15 min in distilled water and carefully washed. Each dialysis bag was filled with 10 ml of 0.5% w/v carrageenan solution, it was closed and put in 90 ml of a diltiazem HCl solution, where it was maintained under agitation at 37°C until equilibrium was reached (24 h). Dialysis tubes did not allow the polymer to get out, but allowed the drug to diffuse into, and eventually to interact with the polymer. Both carrageenan and drug solutions were prepared in distilled water; initial drug concentration outside the dialysis bag was ranging between 0.3 and 10 mM. After the equilibrium was attained, final drug concentration outside the dialysis bags was assayed spectropho-

tometrically at 238 nm wavelength (Spectracomp, Advanced Products, Milan, Italy). From the difference between the initial and the final amount of drug outside the dialysis bag, the amount of drug bound to the polymer could be calculated.

The data obtained have been interpreted according to the following equation (Klotz et al., 1946):

$$r = n*x/(K_d + x) \quad (1)$$

where:
r, amount of drug bound (μmol/mg of polymer) at the equilibrium
x, concentration of drug unbound (mM) at the equilibrium (as measured outside the dialysis bags)
K_d, dissociation constant (mM)
n, maximum binding capacity for the drug (μmoles/mg of polymer)
Eq. (1) can be linearized as follows:

$$1/r = 1/n + K_d/n*1/x$$

From a $1/r$ versus $1/x$ plot, a preliminary estimate of the n and K_d parameters has been obtained. This estimate has been employed to start a non linear fitting programme (MINSQ, Micromath Scientific Software, Salt Lake City, UT), which also provide us with the uncertainty expressed as standard deviation (SD) of the parameter estimate.

The effect of the pH on diltiazem-carrageenan interaction was assessed by means of dialysis equilibrium studies. Diltiazem HCl solutions (90 ml) 1 and 10 mM in 0.05 M HCl–NaCl and NaOH–NaCl buffers whose initial pH ranged between 1.0 and 7.0, were equilibrated as previously described with carrageenan solutions (10 ml 0.5% w/v) in the same buffers: the final pH in the dialysis bags was always recorded.

In the same way also the effect of ionic strength was assessed. Diltiazem HCl solutions (90 ml) 1 and 10 mM in pH 3.0 HCl–NaCl buffers ranging in concentration between 0.001 and 0.5 M were equilibrated with carrageenan solutions (10 ml 0.5% w/v) in the same buffers.

Table 1
Composition (mg) of the matrix tablets

	C0	C10	C25*	C50	C100
Diltiazem HCl	40	40	40	40	40
Methocel K4M	100	90	75	50	0
Carrageenan	0	10	25	50	100
Lactose	60	60	60	60	60

* Drug/carrageenan ratio (w/w) corresponding to the calculated maximum binding capacity

2.3. Matrix preparation and testing

Diltiazem HCl, lambda carrageenan, hydroxypropylmethylcellulose and lactose were mixed 15 min in turbula mixer (W. Bachofen, Basel, Switzerland); 200 mg of the mixture were directly compressed at 3 tons for 60 s, in a hydraulic press for KBr discs (Perkin Elmer) equipped with flat punches 10 mm diameter. The compositions of the matrix tablets are given in Table 1. The thickness of the tablets was in all cases between 1.8 and 1.9 mm; the crushing strengths decreased with the increase of carrageenan amount in the tablet, ranging between 16.1 kg (formulation C0) and 3.8 kg (formulation C100).

The dissolution test was performed in USP apparatus 1 at 100 rpm in 900 ml distilled water, at 37°C.

According to the power law model (Peppas, 1985), the parameters K (apparent release rate constant) and n (diffusional exponent) were obtained from the early portion of the curve (up to 60% drug release) by means of a non-linear regression programme (MINSQ).

2.4. Preparation of the complex

The complex was prepared by means of two different procedures.

The first procedure was in accordance with the literature (Nath et al., 1984; Bubnis et al., 1998): solutions of carrageenan and drug in distilled water were mixed, taking into account the drug–polymer ratio. The precipitate was collected by centrifugation (Sorvall RC 5B, Ing. Terzano and C, Milan, Italy), washed and freeze dried.

A second procedure was moreover used (Bonferoni and Caramella, 1998b). Diltiazem HCl and carrageenan powders in the ratio 1.6:1 (w/w),

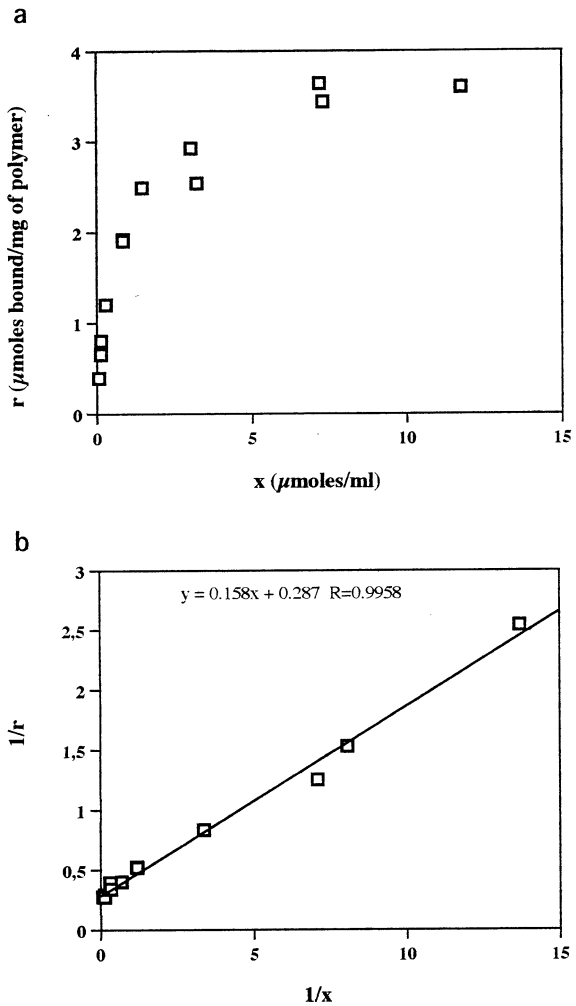

Fig. 1. Interaction isotherm of diltiazem and lambda carrageenan in distilled water; non-linearized (a) and linearized (b) form.

corresponding to the maximum binding capacity as calculated from the interaction isotherm, were blended in a turbula mixer. The minimum amount of distilled water necessary to obtain a paste was added and kneading was effected at 37°C for about 20 min. To wash away eventually unreacted species (either drug or polymer) the precipitate was washed a few times with distilled water, it was dried in oven at 45°C overnight, milled (RMO Retcsh GMbH miller, Haan, Germany) and sieved < 105 μm. The content in diltiazem was assayed spectrophotometrically (wavelength, 238 nm), after dissolution in HCl 0.1 M.

2.5. Solid-state characterisation of the complex

2.5.1. X-ray diffraction

X ray powder diffraction was performed with a Philips PW 1800/10 diffractometer, equipped with a Digital Microvax 2000, with a specific software APD 1700. Wavelengths: CuKα1 = 1.54060 Å, CuKα2 = 1.54439 Å. Scan range was 2–50° 2θ with a scan speed 0.02° 2θ/s. A graphite crystal monocromator was used.

2.5.2. DSC analysis

DSC and TGA analysis were performed with a Mettler TA4000 apparatus equipped with DSC and TG 50 cells. Samples were scanned from ambient temperature to 350°C at 10 K/min either under static or nitrogen atmosphere.

2.5.3. Solubility

The solubility of the complex at 37°C was assessed by measuring the drug concentration in equilibrium with the solid, in distilled water and in buffers differing for either pH or ionic strength. To assess the effect of the pH, 0.05 M HCl–NaCl and NaOH–NaCl buffers at the following values of initial pH were used: 1.8; 3.0 and 7.0. The final pH was always recorded.

To assess the effect of ionic strength, pH 3.0 HCl–NaCl buffers ranging in concentration between 0.001 and 0.5 M were used. After 16 h under agitation, the samples were filtered (0.45 μm Millipore filters) and the concentration of diltiazem HCl was spectrophotometrically read.

3. Results and discussion

3.1. Dialysis equilibria

Fig. 1a and b show the interaction isotherm of diltiazem HCl and lambda carrageenan in distilled water. In Fig. 1a, the amount of drug bound (μmol/mg of polymer) versus the drug concentration determined at the equilibrium (after 24 h) is given. In Fig. 1b, the same data transformed according to the linearized form of the interaction

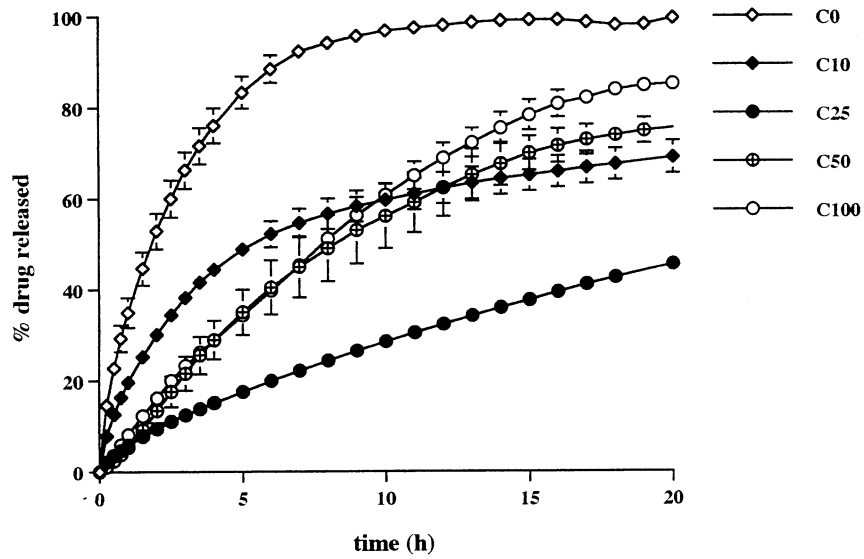

Fig. 2. Diltiazem release profiles in distilled water from the matrix tablets containing different drug-carrageenan ratios (mean ± SD of three tablets).

isotherm are given. From the slope and intercept of this linearized form, the interaction parameters n (maximum binding capacity) and K_d (dissociation constant) are obtained. This estimate of the interaction parameters was used in a non linear fitting procedure that resulted in a dissociation constant of 0.74 (±0.11 SD) mM, and in a maximum binding capacity of 3.71 (±0.14 SD) μmol/mg of polymer. The maximum binding capacity corresponds to 63% w/w drug loading in the complex.

3.2. Influence of the interaction on drug release in water

Fig. 2 shows the release profiles of diltiazem from the matrices containing different drug/carrageenan ratios. To make the comparison easier, in Table 2 the parameters of the power equation K and n describing the release curves are given (Peppas, 1985). The release test has been performed in distilled water so that the results can be interpreted by taking into account the interaction parameters obtained in the dialysis equilibrium studies. In the formulation C0, which is based on HPMC only, the main controlling mechanism is given by the gelation of the polymer, and the release is typically diffusive, as indicated also by the value of n, close to 0.5. In all the other formulations, the presence of carrageenan results in lower initial release rates, conceivably due to drug–polymer interaction. Clearly different be-

Table 2
Power law model parameters: apparent release rate constant K and diffusional exponent n (±SD of the estimate)

	C0	C10	C25*	C50	C100
K (%/h^n)	34.69 (±0.178)	19.47 (±0.087)	5.87 (±0.044)	8.75 (±0.614)	8.88 (±0.219)
n	0.596 (±0.006)	0.620 (±0.005)	0.684 (±0.003)	0.811 (±0.033)	0.839 (±0.013)

* Drug/carrageenan ratio (w/w) corresponding to the calculated maximum binding capacity

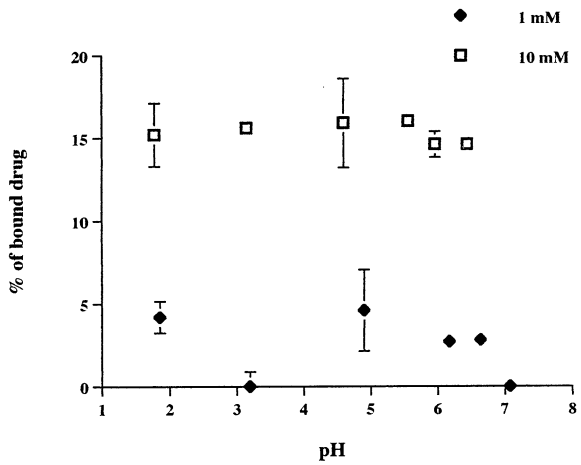

Fig. 3. Effect of pH on dialysis equilibria between carrageenan and diltiazem 1 and 10 mM solutions (mean ± SD of three replicates).

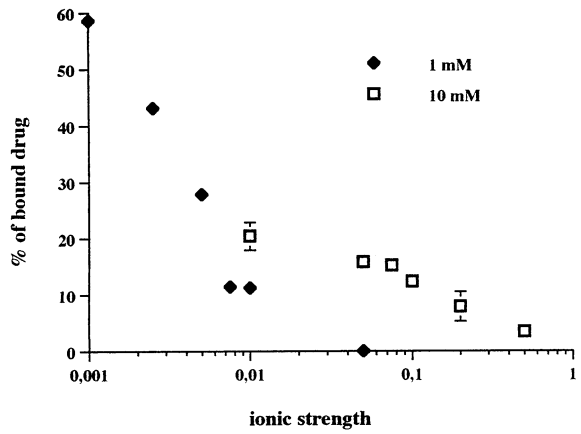

Fig. 4. Effect of ionic strength on dialysis equilibria between carrageenan and diltiazem 1 and 10 mM solutions (mean ± SD of three replicates).

haviour can be seen between the the formulation C10, in which drug/carrageenan ratio was higher than the calculated maximum binding capacity, and the three formulations C100, C50 and C25, in which drug/carrageenan ratio was below or equal (in the C25) the calculated maximum binding capacity. Accordingly to the results of the dialysis equilibrium studies, in the C10 formulation the amount of carrageenan (10 mg) is not enough to bind the whole drug dose but only about 40% of it (16 mg). This accounts for the presence of an initial burst of some not interacting drug. It must be remembered that the diltiazem-carrageenan complex has very low solubility in water (from which it was obtained by precipitation); moreover its high molecular weight is likely to impair diffusion from a matrix tablet containing HPMC. While salts can diffuse into the matrix and displace the drug from the complex in buffered media, this cannot happen in distilled water; it is therefore conceivable that in distilled water the release of the drug from the carrageenan complex can only follow matrix erosion. Previous studies showed that tablets based on carrageenan were subject to faster erosion with respect to those based on HPMC (Bonferoni et al., 1993, 1995). In C10, the relatively high content of HPMC with respect to the lambda carrageenan makes the erosion of the matrix quite slow. Probably for this reason the release curve tends to level off; this occurs when about 60% of the dose has been released. It can be noticed that at this point of the release curve, the ratio between the amount of the drug that is retained in the matrix (about 40%) and the total amount of the carrageenan in the matrix, corresponds to the calculated maximum binding capacity.

The formulations C25, C50 and C100 show particularly low and quite similar initial release rates. Release profiles of C50 and C100 clearly suggest an erosive instead of diffusive release mechanism: as previously observed (Bonferoni et al., 1998a), higher carrageenan percentages correspond to higher erosion sensitivity in carrageenan-HPMC matrix systems.

3.3. Influence of pH and ionic strength on drug–polymer interaction

The effect of pH and ionic strength on carrageenan-diltiazem interaction equilibria is illustrated in Fig. 3 and Fig. 4. In Fig. 3 the percentage of drug bound to the polymer versus the pH measured at the equilibrium in the dialysis bag is given. For both 1 and 10 mM diltiazem HCl solutions, no influence of pH could be detected in the range from 1.7 to 7.0: this is in accordance with the strong acidic character of the

sulphated polymer. Instead, the increase in ionic strength resulted in a decrease in the percentage of drug bound to the polymer (Fig. 4). The highest sensitivity to ionic strength was observed for the lowest drug concentration: this again confirms the competitive effect of the ions of the medium.

3.4. Isolation of the complex

A first attempt was performed to obtain the complex by preparing solutions in distilled water of lambda carrageenan and diltiazem HCl, and by mixing them according to the maximum binding capacity determined by means of dialysis equilibria. A precipitate was obtained, that resulted however quite difficult to recover by filtration for filter clogging. Centrifugation was made difficult by the viscosity of the suspension, so that the total recovery was quite low (about 10%).

An alternative procedure to obtain the complex was also followed (Bonferoni and Caramella, 1998b): the drug and the polymer were mixed as dry powders and therefore wetted with small volumes of distilled water and kneaded. This procedure resulted in higher recoveries: the precipitate formed was much less fine, allowing easier centrifugation and washing.

3.5. Solid-state characterisation of the complex

The complexes obtained with the two procedures have been compared for drug content and by means of DSC analysis. The drug content of the complex obtained from mixing solutions resulted 1.42 μmol/mg of complex, in good accordance with the theoretical value. Different batches of complex prepared according to kneading procedure had a mean ($\pm$SD) drug content of 1.41 ($\pm$0.0406) μmoles of DTZ/mg of complex ($n=7$), meaning a drug loading of 63.6% ($\pm$1.85%). Also in this case a good correspondence was obtained with the drug content as expected on the basis of the isotherm interaction studies, whose results were in this way confirmed. No rigorous stability studies were performed on the complex, although the drug content was checked to remain constant during the study. The complexes prepared according to the two procedures have been compared also by means of the DSC profiles, which resulted superimposable.

The preliminary DSC characterization showed that while the thermal effects of both individual components were present in the physical mixture (Fig. 5b), in the complex the melting endotherm of the drug was absent, and the exothermal effect due to thermal decomposition of the free polymer (about 190°C) was less pronounced. This indicates

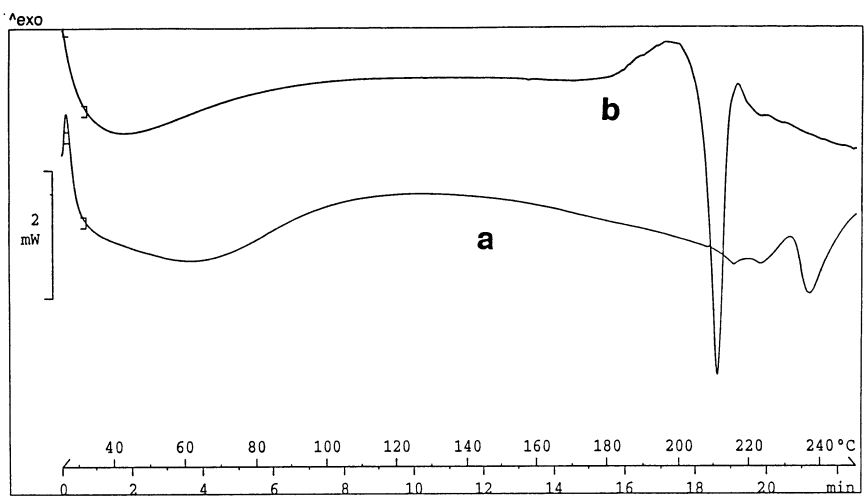

Fig. 5. DSC curves of the complex (a) and of the physical mixture having the same composition as the complex (b).

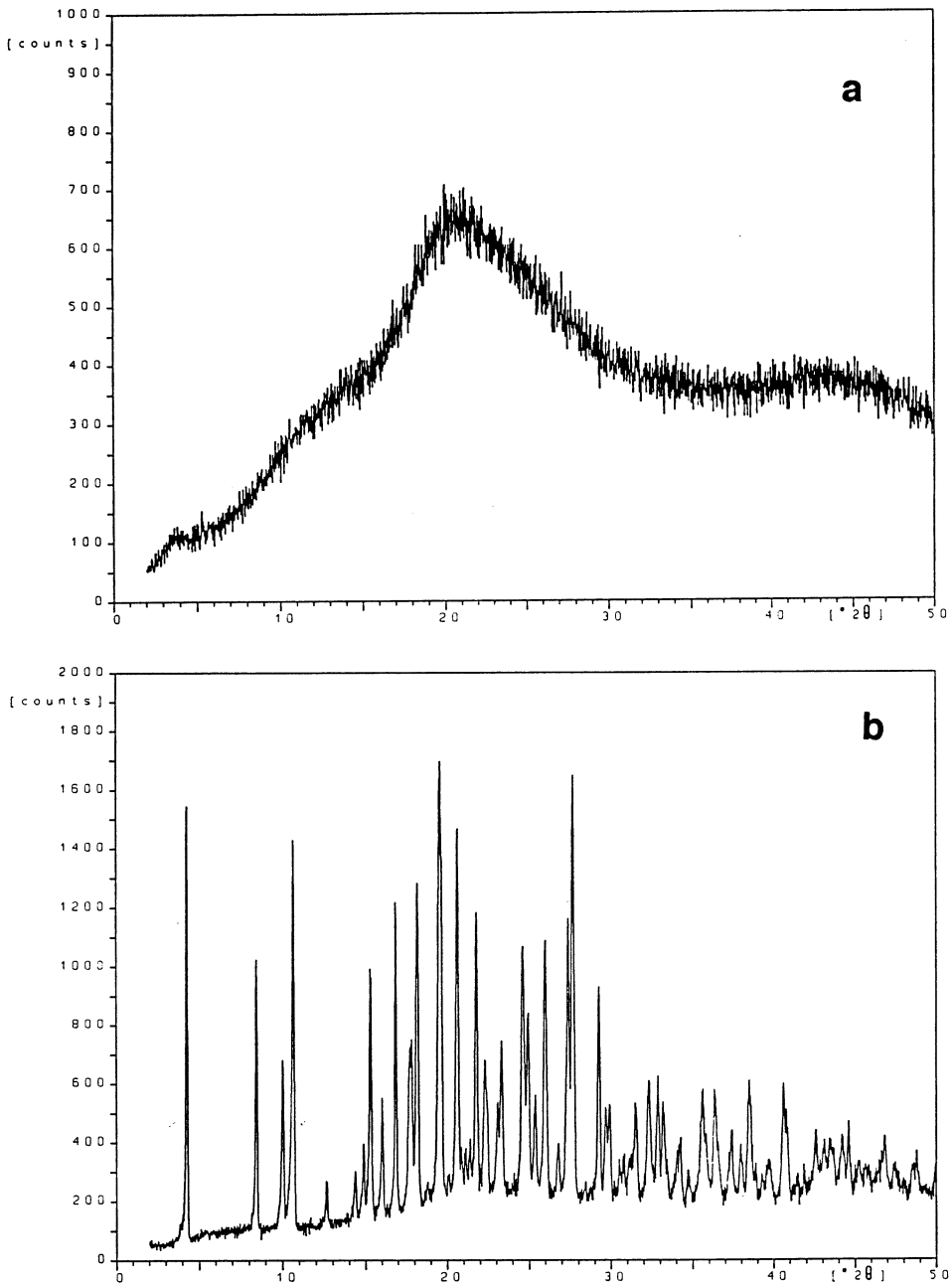

Fig. 6. Powder X-ray diffraction patterns of the complex (a) and the physical mixture having the same composition as the complex (b).

that the drug bound to the polymer is amorphous, and the thermal stability of carrageenan is increased as a result of this interaction (Fig. 5a).

This result has been confirmed by means of X ray analysis: the crystalline structure of diltiazem could be still recognised in the diffraction pattern of the drug–polymer physical mixture (Fig. 6b),

while the complex was found to be amorphous (Fig. 6a).

The effect of ionic strength on the solubility of the drug from the complex is illustrated in Fig. 7. The solubility increases with the increase of the ionic strength; this is in accordance with the result obtained in the dialysis equilibrium studies and confirms once more the displacement of the drug by means of the competing ions. No significant effect of the pH on DTZ release was detected according to an Anova test between pH 1.8, 3.0 and 6.8 (values measured at the equilibrium).

4. Conclusions

The results obtained confirm the importance of ionic bonds in diltiazem-carrageenan interaction. In accordance with the strong acidic nature of carrageenan, the dialysis equilibrium studies indicated that the interaction is quite insensitive to the pH of the medium (in the range 1.8–6.8), while it is reduced by increasing ionic strength; it must be however remembered that the ionic strength was varied in this study well beyond the physiological range.

The maximum binding capacity of the polymer calculated from dialysis equilibrium studies was successfully related to the release profiles of the drug from hydrophilic matrices containing different carrageenan-drug ratios. This confirms the interaction occurring in hydrophilic matrices in which a drug and a polymer of opposite charge are involved: following matrix hydration, a product is formed whose solubility depends on the competing action of medium ions. Water penetration rate, interaction constants of the drug–polymer complex and eventual presence of additional gelifying polymers (like HPMC) play a role in controlling drug release rate and profile.

This hypothesis was confirmed by the isolation and identification of the complex, which could be univocally characterised by means of X-ray diffraction and DSC.

The amount of drug going into solution from the complex was not significantly affected by the pH of the medium (in the range 1.8–6.8): this can be useful in oral controlled release dosage forms that must face different pH values along the intestinal tract. The positive effect of ionic strength can be also useful to complete the release and assure higher bioavailability. More systematic studies of the effect of the medium parameters are in progress.

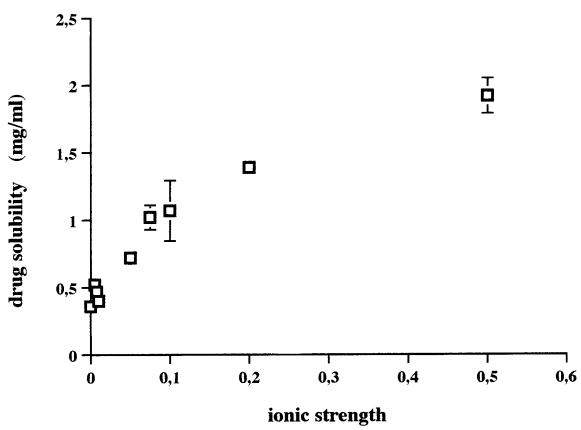

Fig. 7. Effect of ionic strength on the solubility of diltiazem from the complex (mean ± SD of three replicates).

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