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Alginate/chitosan particulate systems for sodium diclofenac release

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Abstract

Alginate/chitosan particles were prepared by ionic gelation (Ca^{2+} and Al^{3+}) for the sodium diclofenac release. The systems were characterized by electron microscopy and differential scanning calorimetry. The ability to release the active substance was examined as a function of some technological parameters and pH of dissolution medium. The release of sodium diclofenac is prevented at acidic pH, while is complete in a few minutes when pH is raised up to 6.4 and 7.2. The alginate/chitosan ratio and the nature of the gelifying cation allow a control of the release rate of the drug. The release mechanism was briefly discussed.

Keywords: Alginate/chitosan beads; Ca^{2+} and Al^{3+} ions; Sodium diclofenac; Alginate/chitosan ratio; Dissolution profiles

1. Introduction

The use of biodegradable polymeric carriers for the drug delivery systems has gained a wide interest, mainly for their biocompatibility and, among the microparticulate systems, microspheres show a special importance for providing local (Lorenzo-Lamosa et al., 1998; Hari et al., 1996) as well as temporal controlled release of the drug (Donbrow, 1992). Different types of polymers are encountered in the literature used to this purpose. In the case of the release of diclofenac are available: poly-3-caprolactone (Alonso et al., 2000), poly-/vinylalcohol (Chawla et al., 2000), poly-lactide-*co*-glycolide (Tuncay et al., 2000a,b). Also natural polymers found their application in this field: albumin (Tuncay et al., 2000a,b), alginate (Gursoy and Cevik, 2000; Acikgoz et al., 1995), carboxymethyl-cellulose (Arica et al., 1996), chitosan (Lim et al., 1997; McLaughlin et al., 1998). The preparation and characterization of the samples are quite similar in most cases.

The anti-inflammatory drug (as sodium salt) is dissolved in an aqueous solution containing the soluble polymer and, in the case of carboxylate-containing polymers (alginate, carboxymethyl cellulose), the formation of microspheres was

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obtained by the addition of Ca^{2+} or Al^{3+}: the hydrophilic colloids interact with metal ions to form crosslinked insoluble complexes, that precipitate incorporating the drug.

In this paper, we prepared microspheres of alginate containing sodium diclofenac and examined the different influence of Ca^{2+} or Al^{3+} ions on the microsphere morphology and the influence of different amounts of chitosan on the release of diclofenac. Among polyanionic polymers alginate has been widely studied and applied for its possibility to modulate the release, according to the properties of its carboxyl groups as well as its biodegradability and absence of toxicity. Also chitosan finds wide applications in pharmaceutical technology as tablet disintegrant, for the production of controlled release solid dosage forms (Miyazaky et al., 1994; Kas, 1997; Illum, 1998; Sezer and Akbuga, 1999) or for improvement of drug dissolution (Felt et al., 1998; Gupta and Ravi Kumar, 2000).

Diclofenac is a suitable candidate for incorporation into microspheres (Hosny et al., 1998; Gohel and Amin, 1999) to minimize its adverse effect after oral administration; in fact, alginate microspheres containing diclofenac start to release the drug after the pH of the environment increases above 7 (Sabnis et al., 1997), by-passing the gastric environment and avoid direct contact between the drug and the gastric mucosa.

2. Materials and methods

2.1. Materials

The following materials were obtained from the indicated suppliers and used as received: sodium alginate (low viscosity; viscosity of 2% solution 25 °C, $\approx$250 cps), chitosan (Practical grade from crab shell; Sigma, Barcelona, Spain). Calcium chloride hexahydrate, aluminum chloride hexahydrate (Sigma, Barcelona, Spain), di-sodium hydrogen phosphate anhydrous, potassium di-hydrogen phosphate, hydrochloride acid 35% and acetic acid glacial 100% (Merck, Barcelona, Spain), were of the highest purity level available. Sodium diclofenac was of pharmaceutical degree (Farchemia, Italy).

2.2. Preparation of microspheres

Sodium diclofenac (0.25% w/v) was added to aqueous solutions of sodium alginate (1.2% w/v) and stirred up to complete dissolution. This solution was dropped using an hypodermic syringe into a second solution, containing Ca^{2+} (or Al^{3+}) ions (1.3% w/v) and chitosan, previously dissolved in acetic solution (0.5% v/v).

Microspheres formed immediately and were left into the original solution for 24 h to ensure internal gelification also. Then they were filtered, washed and dried at room temperature.

The whole preparation was carried out at room temperature. Table 1 lists the formulations prepared.

2.3. Loading and yield of the process

To evaluate the amount of the drug inside the microspheres, an indirect method was used (Fernández-Hervás et al., 1998). Aliquots from the filtered solutions remaining after removal of the beads were assayed spectrophotometrically at 276 nm. The amount of sodium diclofenac entrapped was calculated from the difference between the total amount of drug added and the sodium diclofenac found in the filtered solution.

2.4. Thermal analysis

Thermograms were obtained using differential scanning calorimeter (Mettler FP89HT), to evaluate the state of the active agent inside the microsphere of possible degradation.

All the samples were run at a scanning rate of 10 °C/min from 30 to 300 °C.

2.5. Scanning electron microscopy

Micrographs of the external surface were obtained by SEM (Philips XL 30), depositing on the sample a thin film of carbon. The shape and size of the beads were determined using an image analysis system (Scion Image) which was connected to the microscope.

The following parameters were selected to characterize the sodium diclofenac microspheres:

- area (A), it is the selected surface;
- perimeter/length (l), it is the length around the outside of the selection, or line length for line selections;
- diameter of the equivalent area circle (ECD), this is an equivalent diameter that is calculated as follows:

$$Dcirl = 2\sqrt{\frac{A}{\pi}}$$

where A is the area of the closed boundary of the particle.

- shape factor (s), this parameter is used to measure object complexity, namely contour complexity. The more the variation of the contour, the more the shape factor parameter is elevated. The value of s is determined as follows:

$$s = \frac{L^2}{4\pi A}$$

where L and A are the perimeter and the area of the closed boundary of the particle, respectively.

2.6. Release tests

Dissolution assays were carried out in triplicate for 6 h at 37 ± 0.5 °C. For the first 2 h, pH of the dissolution medium was buffered at pH 1.2 (HCl/NaCl, to mimic the gastric district). pH was then raised up to 6.6 (phosphate buffer) and maintained at this value for further 2 h. Finally, a little higher pH (7.4) was used up to the end of the experiment. The tests were performed into an apparatus as described in USP 23 (Turu Grau, mod. D-6).

At prefixed time (15 min), 3 ml of solution were withdrawn and spectrophotometrically assayed for the diclofenac content ($\lambda = 276$ nm) (Hitachi, mod. U-2000).

Dissolution tests were carried out taking into account the following parameters: type of gelifying ion and alginate/chitosan ratio.

3. Results and discussion

Side effects, mainly at the gastric level, are well known, following oral administration of an NSAID. Therefore the efforts of many researchers have been concerned to solve these problems, through a variety of techniques of protection of the gastric mucosa or alternatively to prevent the NSAID release in this district. In this paper we evaluate the potential utility of natural materials, such as alginate and chitosan in inhibiting sodium diclofenac release in the gastric environment. And since among the microparticulate systems, microspheres have a special interest as carriers for NSAID, mainly to extend the duration period of the dosage form, we aimed to investigate the

Table 1
Composition of the different formulations used for the preparation of sodium diclofenac microspheres

Batch	Cation	Chitosan (%)	Sodium alginate (%)	Alginate/chitosan
L1	Ca^{2+}	0.1	1.2	12
L2	Ca^{2+}	0.1	1.5	15
L3	Ca^{2+}	0.2	1.2	6
L4	Ca^{2+}	0.2	1.5	7.5
L5	Ca^{2+}	1.2	1.2	1
L6	Al^{3+}	0.1	1.2	12
L7	Al^{3+}	0.1	1.5	15
L8	Al^{3+}	0.2	1.2	6
L9	Al^{3+}	0.2	1.5	7.5
L10	Al^{3+}	1.2	1.2	1
L11	Al^{3+}	0.6	1.2	2
L12	Al^{3+}	0.4	1.2	3
L13	Al^{3+}	0	1.2	0

Table 2
Encapsulation efficiency (EE) and shape parameters of the different formulations; ECD, equivalent circular diameter

Batch	EE (%)	Area (mm^2)	Perimeter (mm)	Shape factor	ECD (mm)
L1	99.65 ± 5.28	11.35 ± 2.10	10.82 ± 3.03	0.825 ± 0.03	3.80 ± 0.52
L2	99.63 ± 7.99	11.98 ± 3.58	10.94 ± 2.85	0.798 ± 0.05	3.91 ± 0.99
L3	99.66 ± 7.21	9.30 ± 5.81	10.21 ± 4.11	0.897 ± 0.01	3.44 ± 1.01
L4	99.63 ± 10.02	10.51 ± 2.78	10.54 ± 4.08	0.847 ± 0.05	3.66 ± 0.21
L5	98.82 ± 5.21	8.70 ± 3.13	10.10 ± 3.66	0.938 ± 0.03	3.33 ± 0.09
L6	99.87 ± 4.11	14.37 ± 9.54	11.62 ± 3.85	0.752 ± 0.01	4.28 ± 0.24
L7	99.85 ± 10.32	14.41 ± 4.51	11.62 ± 2.10	0.749 ± 0.02	4.28 ± 0.54
L8	99.68 ± 7.41	12.99 ± 5.41	11.40 ± 5.92	0.801 ± 0.05	4.07 ± 0.65
L9	99.72 ± 3.33	13.14 ± 5.99	11.56 ± 5.21	0.813 ± 0.02	4.09 ± 0.54
L10	99.07 ± 6.52	10.01 ± 2.10	9.10 ± 2.45	0.662 ± 0.01	3.57 ± 0.81
L11	99.3 ± 11.45	10.22 ± 5.47	9.09 ± 2.66	0.648 ± 0.03	3.61 ± 0.77
L12	99.49 ± 7.41	10.30 ± 2.33	9.32 ± 1.48	0.675 ± 0.05	3.62 ± 0.63
L13	99.76 ± 5.23	10.04 ± 4.10	9.20 ± 3.21	0.675 ± 0.04	3.58 ± 0.41

possible applicability of chitosan treated alginate beads as a controlled release system for soluble salts. We prepared microspheres containing sodium diclofenac starting from natural polysaccharides by ionotropic gelation method and examined the effects of various factors (alginate/chitosan ratio, electrolyte concentration and nature of bead).

3.1. Microsphere characterization

3.1.1. Morphology

Alginate/chitosan microspheres containing sodium diclofenac were evaluated for particle size, yield and encapsulation efficacy and surface morphology. The ionic gelation method gave beads with a high diameter ranging from 2 to 4 mm (Table 2). The drug encapsulation yield was more than 98% in all the cases, and the efficacy was neither affected by the alginate amount nor the crosslinking ion used. So, this method is useful to encapsulate ionic drugs with a high water solubility.

Although the encapsulation of the drug was approximately 100% in all the formulations, the use of different ions, such as calcium or aluminum, determines the mechanical properties of alginate gels and the egg-box structure of the beads, which gives it the spherical morphology. Fig. 1 shows a microsphere prepared adding Ca^{2+}, exhibiting acceptable sphericity and a notable surface porosity, with a shape factor greater than 0.80 in all the cases. This morphology was found independent of the starting composition, provided that Ca^{2+} ions were the gelifying agent. Due to the adhesive properties of chitosan, microspheres tend to agglomerate (Lim et al., 1997; Ganza-González et al., 1999; Murata et al., 1999).

The aspect and morphology of the particulates prepared with Al^{3+} ions is different: no formulation enabled the formation of a spherical morphology; on the contrary, the particles are flattened, disk-shaped with a collapsed center. The surface appears smooth and little porous (Fig. 2), with a shape factor less than 0.80. The trivalent ions cause more points of aggregation between two contiguous alginate chains, binding them so strictly and quickly that, as a consequence, there is no time to get spherical forms, during their formation.

Fig. 3 recalls the feature of a drop touching a water surface, suggesting that instantaneous gelification blocks the situation of the very first contact between the two solutions, the one containing alginate and the other one the inorganic ions.

3.1.2. Differential scanning calorimetry

Thermograms of both types of microspheres do not offer clear peaks of identification (Figs. 4 and 5) (Ford and Timmins, 1989).

Calcium chloride shows two endotherm peaks in the temperature range 180–220 °C; while

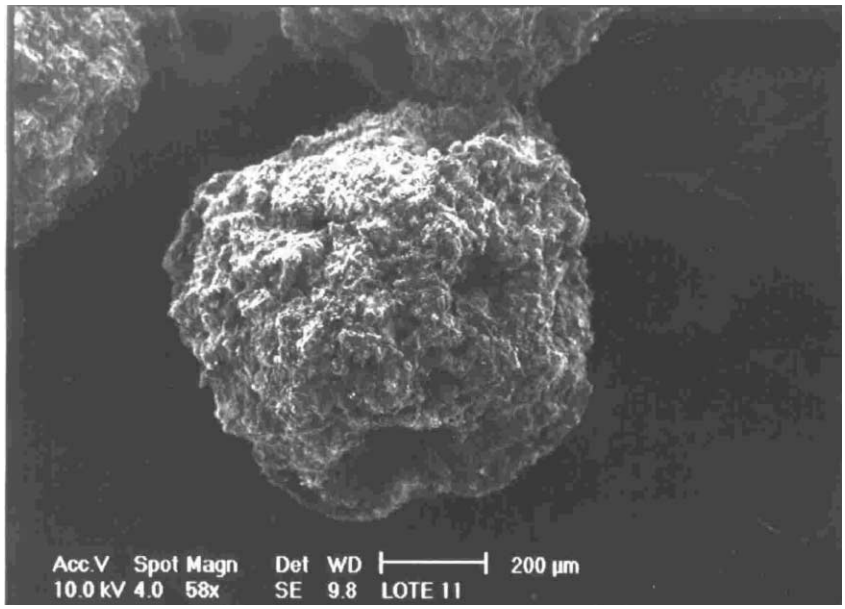

Fig. 1. Scanning electron micrographs of sodium diclofenac microspheres formulated with calcium chloride.

sodium alginate decomposes at about 240 °C with a broad exotherm. Peaks of the single components are not visible when combined into a microsphere, whose thermogram shows only a broad and small endotherm, probably related to dehydration, present at a temperature about 120 °C. The same applies to Al-microspheres: in this case the endotherm peak present in the thermogram of $AlCl_3 \cdot 6H_2O$ is also absent. This can be interpreted as follows: that a chemical reaction occurred in solution, modifying the starting reagents and that a strong interaction is present among all the compounds inside the formulation; this fact additionally acts as a stabilizer, since degradation endotherms both for alginate and sodium diclofenac are absent at the highest temperatures of the thermogram.

3.1.3. Effect of the ion on the release process

For the preparation of chitosan treated alginate beads containing sodium diclofenac, we dissolved sodium diclofenac in the aqueous solution of sodium alginate. The addition of the divalent (or trivalent) ions produced (Kondo, 1979) a partial neutralization of carboxylate groups present on the alginate chain, forming an insoluble (but permeable) transitory thin gelatinous film:

$$2n\text{NaAlginate} + n\text{Ca}^{2+} \rightarrow n\text{Ca(Alginate)}_2 + 2n\text{Na}^{+}$$

Ionic gelation is the result of the formation of an 'egg box' between facing units of two different chains and depends on the inorganic ion/alginate ratio. When the solution has a polycationic chain (e.g. protonated chitosan) this acts as a crosslinking thus improving the microsphere hardness.

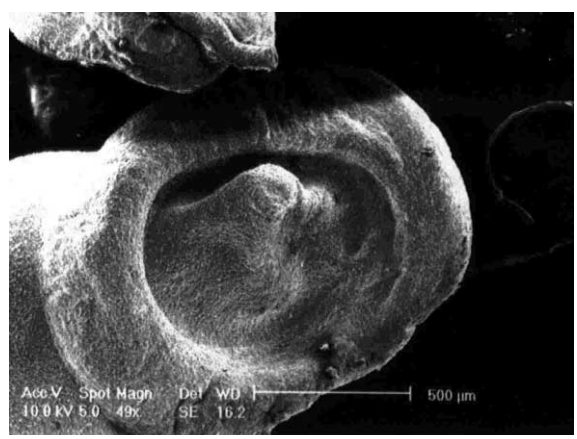

Fig. 2. Scanning electron micrographs of sodium diclofenac microspheres formulated with aluminum chloride.

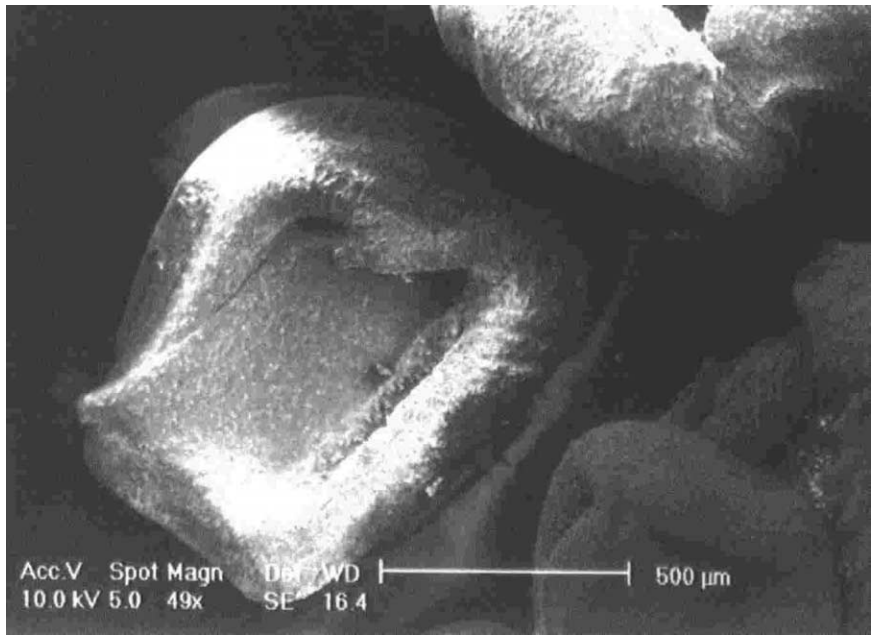

Fig. 3. Scanning electron micrographs of the structure formed from a drop touching a water surface, during the microsphere fabrication process. These sodium diclofenac microspheres were formulated with aluminum chloride.

On the addition of inorganic cations into the solution containing anions (alginate and diclofenac) two different reactions can start: the gelation of alginate chain and the (possible) precipitation of an insoluble diclofenac salt. However, the precipitation of diclofenac as an insoluble salt should make its recovery difficult during the dissolution test, even at varying pH. On the contrary, when the experimental conditions are favorable, diclofenac is released from the microspheres and appears in solution. Therefore it can be assumed that diclofenac is still present in a soluble form inside the microsphere, as a sodium salt or as a complex with the polycation chitosan readily available. In fact, since inorganic ions were added dropwise, they were always present in the solution in highly deficit concentrations with respect to the alginate anion units. At the composition used, alginate carboxylate groups strongly exceed those present in diclofenac; furthermore, the polyanion rapidly reacts with each cation blocking it into a thermodynamically very stable 'egg box'. This capture of inorganic ions prevents competition towards diclofenac anion, which could not react with Ca^{2+} or Al^{3+} and form insoluble products. The mutual neutralization between oppositely charged alginate and chitosan decreases the solubility of the whole system; the same could occur in the formation of a complex between chitosan cations and diclofenac anions. Therefore the driving force for the microsphere formation is a decrease of solubility. This mechanism explains the high efficiency of the microsphere formation in depleting diclofenac from the solution, irrespective of the different experimental conditions examined. The precipitate inside a microsphere has a complex composition and structure.

The alginate/chitosan microsphere represent an efficient system for controlling the release of diclofenac (Yotsuyanagi et al., 1991). At acidic pH, the release is low for 2 h: the amount of the released diclofenac did not exceed 6–7%, independent of the microsphere size. At acidic pH, alginate is protonated into the insoluble form of the alginic acid: this displays properties of swelling that explains the low aliquot of the release. In this case the release is hindered by chitosan: its positively charged groups strongly interact with algi-

nate and diclofenac ions, both reducing swelling and release and, possibly, the protonation of their carboxylated groups. At pH 6.4 a rapid increase of the release rate was observed up to 100%. The deprotonation of the alginic acid causes the disintegration of the microsphere systems and the complete release of diclofenac as soluble ions.

At increasing pH, the increasing deprotonation of chitosan weakens the extent of the interactions inside the microsphere; moreover, diclofenac is soluble as anions and can be brought into the solution. The release is complete in a few minutes. The role of the phosphate anions present in the buffer of the dissolution medium in sequestering the Ca^{2+} ions and taking away a factor for the insolubility of alginate chains cannot be excluded. This aspect should, however, play differently in the presence of Ca^{2+} or Al^{3+} ions, even though the release profiles overlap perfectly in both cases. All the formulations examined show a dissolution profile as that reported in Fig. 6.

3.1.4. Effect of alginate/chitosan ratio on the release process

The case of the alginate/chitosan ratio is different. The presence of chitosan increases the control of the release from the microsphere, since, at increasing concentration, it can form a network of bondings between the two polymer chains.

This was expected, since at increasing chitosan amount into the formulations, interactions between the two polymers should have been in-

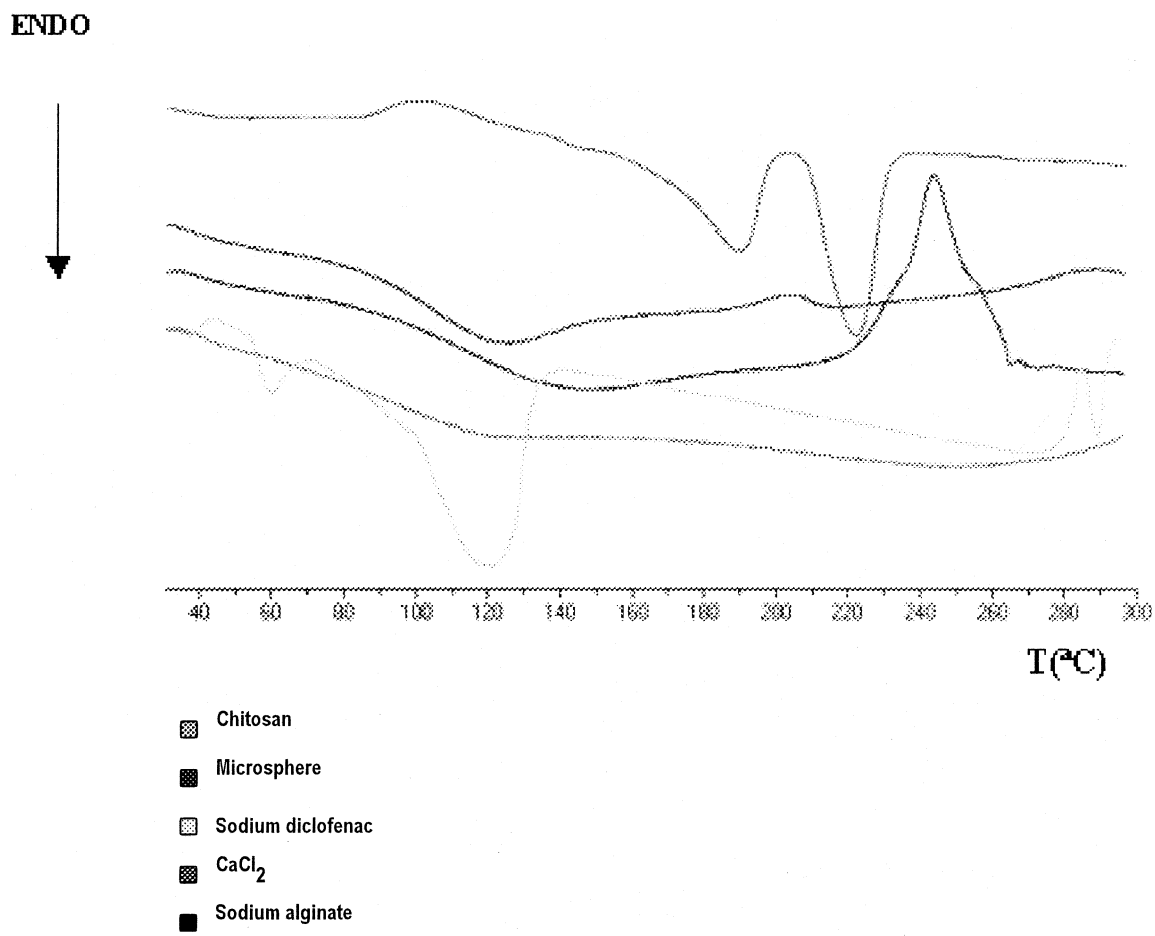

Fig. 4. Thermograms corresponding to the sodium diclofenac microspheres with calcium chloride.

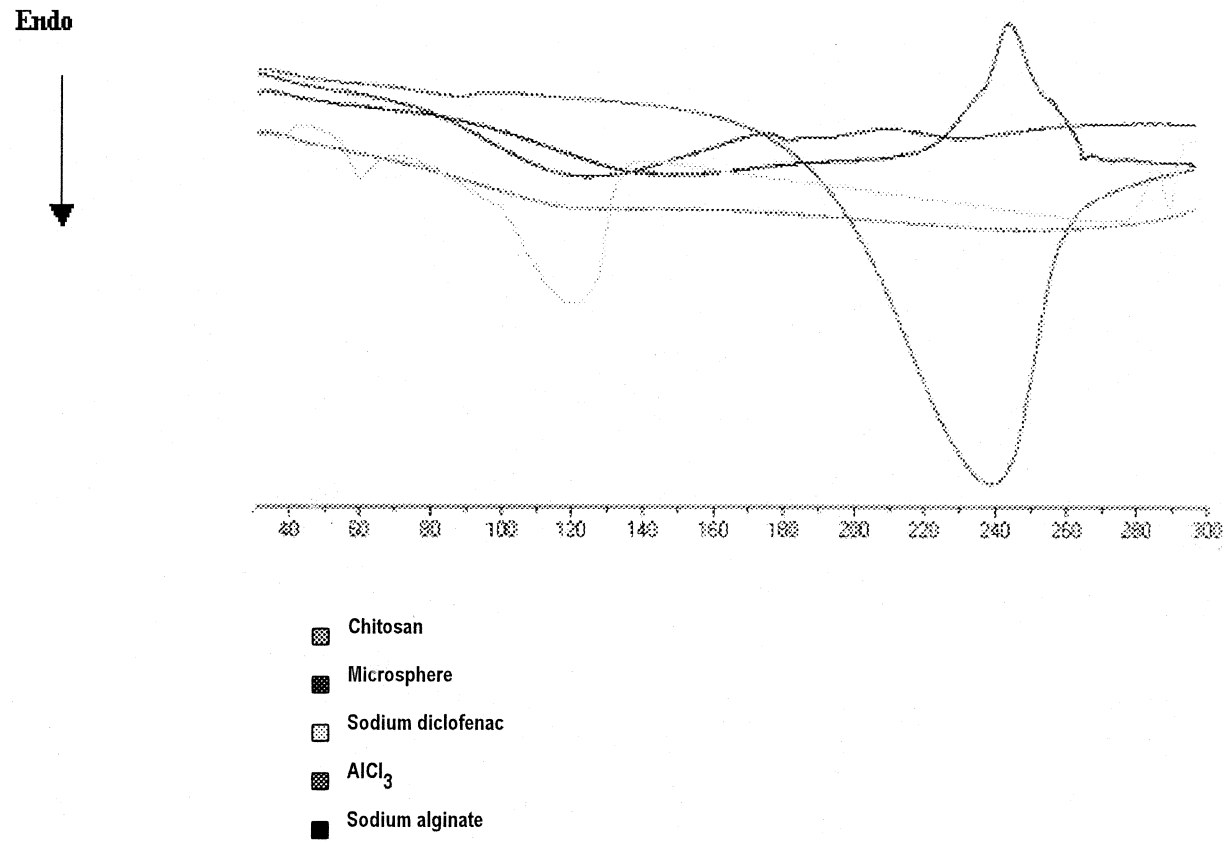

Fig. 5. Thermograms corresponding to the sodium diclofenac microspheres with aluminum chloride.

creased, forming a closer network, which should decrease the diffusion of the drug outwards of the bead. Therefore, to deeper examine this parameter the alginate/chitosan ratio was changed to value 1, 2 and 3 w/w. In these cases, differences were more evident. At the ratio 1:1, while Ca^{2+} offered a dissolution profile as the previous ones, in the case of Al^{3+} microspheres, the release is lowered below 50%. This result confirms what was reported in the literature that increasing chitosan concentrations decrease the release percentage (Fig. 7). At 2:1 and 3:1 ratios the starting solutions were viscous and the final microsphere did not have a suitable morphology for technological application. At alginate/chitosan 1:1 (w/w) ratio the use of Al^{3+} in gelation reduces the release below 50% with respect to usual conditions, where

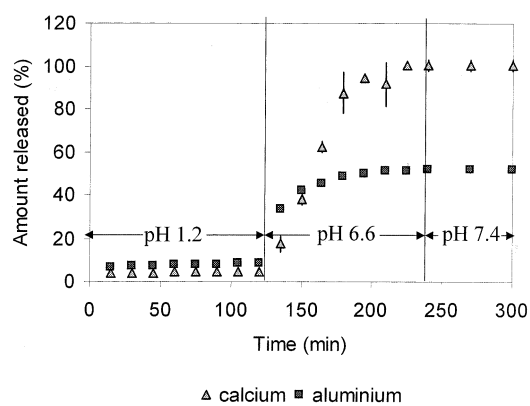

Fig. 6. Effect of the ionic substance (Ca^{2+} or Al^{3+}) on the sodium diclofenac release process.

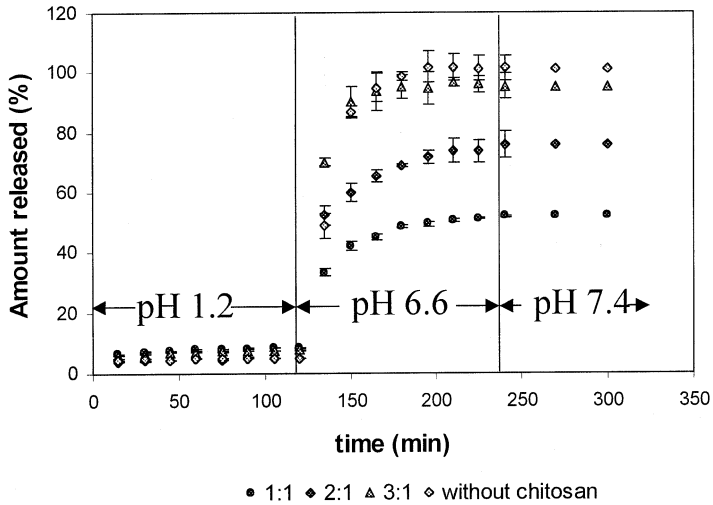

Fig. 7. Effect of the alginate/chitosan ratio on the sodium diclofenac release process.

the ratio was kept at the 3:1 value. This fact introduces an interesting variable in preparing microspheres, that together with the prevention of the release at the gastric level, an extension of the drug release over longer periods of time can be introduced.

Acknowledgements

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