In vitro and in vivo studies of ibuprofen-loaded biodegradable alginate beads

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Abstract
The irritation effects of ibuprofen, a widely used non-steroidal anti-inflammatory drug (NSAID), were evaluated on mouse gastric and duodenal mucosa when suspended in 0.5% (w/v) sodiumcarboxymethylcellulose (NaCMC) solution and loaded in alginate beads. The ionotropic gelation method was used to prepare controlled release alginate beads of ibuprofen. The influence of various formulation factors on the encapsulation efficiency, as in vitro drug release and micromeritic properties, was investigated. Other variables included the alginate concentration, percentage drug loading and stirring speed during the microencapsulation process. Scanning electron micrographs of alginate beads loaded with ibuprofen showed rough surface morphology and particle sizes in the range of 1.15–3.15 μm. The yield of microspheres, as collected after drying, was generally 80–90%. Formulation code H showing t50% value of 3.5 h was chosen for in vivo trials because of the appropriate drug release properties. For in vivo trials, free ibuprofen (100 mg kg⁻¹), blank and ibuprofen (100 mg kg⁻¹) loaded alginate beads (formulation code H) were suspended in 0.5% (w/v) NaCMC solution and each group was given to six mice orally by gavage. NaCMC solution was used as a control in experimental studies. In vivo data showed that the administration of ibuprofen in alginate beads prevented the gastric lesions.

Keywords: Alginate beads, ibuprofen, microencapsulation, controlled release, gastric lesions

Introduction
Alginic acid, which is a natural acidic polysaccharide obtained from marine brown algal, has received much attention in pharmaceutical preparations, particularly as a vehicle for controlled drug delivery (Bodmeier and Wang 1993; Gürsoy and Çevik 2000; Çalış et al. 2002). Recently, alginate beads containing several substances have been prepared by the gelation of alginates with calcium cations (Acartürk and Takka 1999a) and the behaviour
of the release of these substances from alginate beads has been investigated (Hwang et al. 1995; Takka and Acartürk 1999b; Arica et al. 2002a).

Ibuprofen is a well-known hydrophobic oral non-steroidal anti-inflammatory drug (NSAID) (Wilson et al. 1989) and is the first phenylalkanoic acid approved by the FDA for general analgesic use. Similar to other drugs of this group, it has a wide spectrum of gastrointestinal side effects ranging from mild dyspepsia to gastric bleeding. The gastric irritation is mainly due to the free carboxylic acid group in the chemical formula (Goodman Gilman et al. 1985). Due to its short plasma half-life of 1–3 h following oral dosing and the gastric irritation, ibuprofen is an ideal candidate for preparing prolonged or controlled release drug products (Diamantis et al. 1980; Bodmeier and Chen 1989; Bakan et al. 1992; Leo et al. 2000; Sipahigil and Dortunç 2001; Arica et al. 2002b; Ladron et al. 2003; Saravanan et al. 2003). Alginate is known to be non-toxic when taken orally and to protect the mucous membrane of the upper gastrointestinal tract from the irritation of chemicals. Since the property of reswelling is susceptible to the environmental pH, the incorporation of acid-sensitive drugs into the beads protects them from the gastric juice (Segi et al. 1989). Therefore, drug-loaded alginate beads might provide these advantages for NSAIDs such as ibuprofen, which lead to gastric irritation. The formulation of ibuprofen as a controlled release dosage form of beads seems to be an important approach to overcome the potential problems in the gastrointestinal (GI) tract so as to achieve a reduction of the NSAID’s adverse effects (Lee et al. 1998; Çalış et al. 2003).

The aim of the present study was to develop ibuprofen-loaded alginate beads as a controlled release oral delivery system in vitro and to investigate the gastrointestinal side effects in vivo. The effects of the polymer concentration, stirring speed and drug loading on the particle size, encapsulation efficiency and the $t_{50\%}$ value were also investigated. The ionotropic gelation technique was selected to prepare controlled release ibuprofen alginate beads due to its simplicity, low cost and its success with poor aqueous solubility drugs and the production of beads. The effects of oral ibuprofen on gastric and duodenal mucosa, when administered free or in alginate beads, were compared.

**Experimental**

**Materials**

Ibuprofen was obtained from Eczacıbaşı (Istanbul, Turkey). Sodium alginate was kindly contributed by Pronova Biopolymers (Pronatal (60/10; Oslo, Norway). Calcium chloride was purchased from Merck (Darmstadt, Germany). All the solvent and chemicals (Merck, Darmstadt, Germany) were of analytical grade and were used as obtained from the manufacturers.

**Methods**

*Preparation of ibuprofen-loaded alginate beads.* Sodium alginate was dissolved in distilled water at a concentration of 2–4% (w/w). Ibuprofen was added to 20 mL of alginate solution and dispersed thoroughly by stirring. This solution was dropped into 50 mL of 0.1 M CaCl$_2$ solution by stirring via a magnetic stirrer through a syringe (needle) at a dropping rate of 2 mL min$^{-1}$. The formed spherical calcium-alginate gel beads were cured in 0.1 M CaCl$_2$ solution at room temperature for 1 h with gentle stirring. Beads were collected by filtration and washed twice in 50 mL of distilled water and dried under vacuum at room temperature.
The effects of different concentrations of alginate, stirring speed, drug loading and also the in vitro release rate of ibuprofen were evaluated. The investigated parameters likely to affect the microencapsulation of ibuprofen are given in Table I.

Characterization of ibuprofen-loaded alginate beads: surface morphology. The beads were mounted on metal stubs with conductive silver paint and sputtered with a 150 Å thick layer of gold in a Bio-Rad (UK) sputter apparatus. The morphological characteristics of the beads were then examined by Jeol Scanning Electron Microscope (SEM ASID-10, Japan) at an acceleration voltage of 80 KV.

Determination of encapsulation efficiency of alginate beads. Fifty milligrams of the beads were accurately weighed, diluted with distilled water to 25 mL each and placed in an ultrasonic water bath for 30 min. The samples were left to equilibrate for 1 day. Aliquot samples of 10 mL were removed through a filter and assayed spectrophotometrically (Shimadzu UV-160 A, Japan) at 265 nm. The determination was carried out in triplicate and the mean drug encapsulation efficiency was calculated.

Determination of the mean particle size. Particle size distribution of the beads was determined by sieve analysis (Endocott Ltd., London, UK).

In vitro release studies

The release of ibuprofen from alginate beads was performed using a bath-shaker. The weighed amounts of ibuprofen-loaded samples were put into a glass vessel. The dissolution mediums (30 mL) were 0.1 N HCl solution (pH 1.2) and phosphate buffer solution (pH 7.4). The glass vessel was then immersed into the water bath. Temperature of the dissolution vessel was maintained at 37 ± 0.5°C. At scheduled time intervals, 1 mL of sample was taken from the vessel and the amount of ibuprofen was determined by spectrophotometer (Shimadzu UV-160 A, Japan) at 265 nm.

Percentage yield value. The percentage yield value is defined as the quantity of beads produced as a function of loaded drug and polymer.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Concentration of alginate (%)</th>
<th>Stirring speed (rpm)</th>
<th>Drug loading (%)</th>
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In vivo studies

Pharmacology. Locally bred female albino mice weighing 22±2 g were used during in vivo studies. The animals were housed in groups of six and acclimatized to laboratory conditions for at least 2 days before the experiments, with food and water ad libitum. Twenty-four hours before the experiment, the food was withdrawn, but free access to water was allowed.

Free ibuprofen (100 mg kg⁻¹), blank alginate beads and ibuprofen (100 mg kg⁻¹) loaded alginate beads were each suspended in 0.5% (w/v) NaCMC solution. Each group was given orally by gavage to six mice. Blank 0.5% (w/v) NaCMC, used as a control, was also administered orally by gavage to six mice.

The animals were sacrificed 7 h after the oral administration and their stomachs and duodenums were dissected. The existing haemorrhagic lesions were inspected under microscope with ocular micrometer. The specimens were then put into 10% formalin solution for microscopic examinations. All procedures used in this in vivo study were approved by Hacettepe University's Ethics Committee (2001/78-4).

Histology. After removal of the stomachs and duodenums of mice, the tissues were placed in 10% buffered formaldehyde solution and fixed for 72 h. Afterwards, tissues were processed according to routine light microscope technique. First, they were dehydrated in ascending degrees of ethyl alcohol (70, 80, 90, 96, 99%) and then cleared in xylene and embedded in paraffin. Five micrometre paraffin sections were cut and stained with hematoxylin-eosin, examined and photographed with an Olympus BH-2 light microscope.

Results and discussion

Morphology, yield and particle size distribution

Scanning electron micrographs of blank and ibuprofen-loaded alginate beads and their surface morphology are shown in Figures 1(a) and (b), respectively. Figures 1(a) and (b) show beads having rough surface morphology and visible large wrinkles. A similar type of morphology was found previously for alginate types when solid drug was microencapsulated (Lee et al. 1998). The yield of microspheres, as collected after drying, was generally 80–90%. Yields were slightly lower (70–75%) when the concentration of the polymer solution for preparation was 2% (w/w).

The mean particle sizes of the obtained alginate beads are shown in Table II. Ibuprofen-containing alginate beads were in the size range of 1.15 ± 0.4–3.15 ± 0.6 mm. Both ibuprofen-loading amount (5–10% w/w) and stirring speed (50–100 rpm) seemed to affect the values of particle size. Thus, the particle size increased significantly by increasing ibuprofen-loading. Furthermore, it was also observed that the particle size was inversely proportional to the percentage of the polymer in the range of 2–4% (w/w) when two different stirring speeds were used, as beads prepared in 2% (w/w) alginate concentration had smaller ranges of distribution than those prepared with 4% (w/w) alginate.

The size of the spherical matrix could easily be controlled by varying the stirring speed of the system and the concentration of alginate added to the aqueous medium. At a stirring speed of 50 rpm, the mean particle diameter and the size distribution of the beads increased significantly. The tendency of the droplets to coalesce and aggregate at the slower stirring speed (50 rpm) appeared to be correspondingly high, resulting in larger mean bead diameters. This low stirring speed might have decreased the uniformity of the mixing force throughout the emulsion mixture, hence resulting in a wider size distribution of the final beads. At a stirring speed of 100 rpm, the particle size changed to a lesser extent.
At this higher stirring speed, a vigorous, uniform, increased mechanical shear might have resulted. This suggests that the size of the droplets formed during microencapsulation might, therefore, be closely related to the size of the final beads, which increased significantly by decreasing the stirring speed. Other researchers have previously reported a similar inverse relationship between the stirring speed and the mean size (Kawashima et al. 1989; Adeyeye and Price 1991).

Figure 1. Scanning electron micrographs: (a) surface morphology of blank alginate beads, (b) surface morphology of ibuprofen loaded alginate beads (H coded formulation).
Furthermore, the viscosity of the polymer solution significantly affected the bead size distribution. The smallest beads were produced when alginate was used at a low concentration (2%). At low drug-loading (5%), smaller particles (1.15 ± 0.4 mm) were produced than with high drug loading (10%), indicating increased coalescence of coacervate droplets. Finally, the mean particle size was increased from 1.15 ± 0.4 to 3.15 ± 0.6 mm when the polymer concentration was increased from 2% to 4% (w/w). Low polymer concentration resulted in decreased inner phase viscosity, which might efficiently promote the break-up of coacervate droplets and prevent coalescence.

**Encapsulation efficiency**

The encapsulation efficiencies were found to be in the range of 6.5 ± 1.5–14.8 ± 2.1% for ibuprofen containing alginate beads (Table II). Loading efficiency was increased from 6.5 ± 1.5 to 14.8 ± 2.1% for 1.15 ± 0.4 and 3.15 ± 0.6 mm beads, respectively. The small particle size of beads (1.15 ± 0.4 mm, formulation code A) showed a lower encapsulation efficiency of 6.5 ± 1.5% when compared to 14.8 ± 2.1% with the big particle size of beads (3.15 ± 0.6 mm, formulation code H) \((p < 0.05)\). The entrapment efficiency was related to the particle size of the ibuprofen-loaded beads. On the other hand, the encapsulation efficiencies were determined to increase as their mean particle sizes increased.

At high polymer concentration (4%), the percentage of the encapsulation efficiency was determined to be increased. Beads with a lower polymer concentration (2%), however, entrapped drug in the range of 6.5 ± 1.5–8.9 ± 1.1%. Evaluation of the variation of the polymer concentration showed that ibuprofen was highly entrapped when the polymer concentration was high. Also, increasing polymer amounts in solution seem to stabilize the droplets against merging and improved ibuprofen entrapment (Ça˘ılı¸s et al. 2001; Arica et al. 2002b).

**Ibuprofen release from alginate beads**

The release profiles obtained after the release of ibuprofen from alginate beads are shown in Figure 2. The small particle size of beads (formulation code A) showed a \(t_{50\%}\) value of 1.1 h compared to 3.5 h with the big particle size of beads (formulation code H). Beads with smaller diameters (1.15 ± 0.4 mm, formulation code A) showed higher percentages of release than bigger particles (3.15 ± 0.6 mm, formulation code H) \((p < 0.05)\). The higher
and faster drug release displayed by the beads with smaller diameter could be attributed to
the decrease of alginate matrix density related to the increase in porosity. The smallest
sized beads presented a very fast release rate. In fact, 50% of the loaded drug was released
in 1.1 h from the beads owing to the highest surface area. Beads having biggest particle
size showed the slowest release rate. Fifty per cent of the entire loaded drug was released
in 3.5 h.

The differences between the release profiles of alginate beads with increasing polymer
concentrations are shown in Figure 2. As the alginate concentration of the prepared
beads increased, the release rate was decreased. The release of 50% of the drug was realized
in 1.1 h (formulation code A) and 2.6 h (formulation code E) for the alginate concentrations
of 2% (w/w) and 4% (w/w), respectively. Concomitantly, the \( t_{50}\% \) value increased from 1.1
to 2.6 h (Table II). Figure 2 clearly illustrates that the drug release from the beads depended
on the polymer concentration of the prepared formulation. An inverse relationship
was observed between polymer concentration and drug release from the prepared
beads. Beads containing 2% (w/w) alginate released the drug more rapidly, while
those with 4% alginate exhibited a relatively slower drug release profile. At low polymer
concentration (2%), the \( t_{50}\% \) value was decreased. Beads with a higher polymer
concentration, however, released nearly all of the encapsulated drug within a 7 h period.
From 0–7 h, the release from the beads with 4% (w/w) polymer concentration was
significantly slower than the release from the beads prepared in 2% (w/w) polymer concen-
tration, except for formulation code D. All formulations were capable of retarding
drug release below 10% for 2 h in 0.1 N HCl solution (pH 1.2) (data not shown). The
release rate of all ibuprofen-loaded alginate beads was relatively slow in acidic pH
media within the initial 2 h, while their release rate increased rapidly when the medium
pH changed from acid to 7.4. This suggested that ibuprofen was thoroughly encapsulated
in alginate beads.

Figure 2. The release profiles of ibuprofen loaded alginate bead formulations in PBS at pH 7.4 and
37 ± 0.5°C. Each point represents the mean value ± SEM (n = 6).
The release of ibuprofen was considered to occur mostly by diffusion but could be accelerated by the weight loss of the alginate polymers. After 1 h, 15–50% of the drug was released; however, more than 50% of the initial drug load remained in the beads. This suggested that during the first hours there was not a considerable release of ibuprofen. The bead structure changed significantly over time, indicating that there was substantial hydration and swelling of the polymeric matrix.

It is important to note that the alginate gel might have acted as a barrier to the penetration of the medium, thereby suppressing the diffusion of ibuprofen from the swollen alginate matrix. Similarly, Gallardo et al. (1998) reported that the release of ibuprofen was modulated by the diffusion of the drug through the swollen matrix.

The batch (formulation code H) containing 4% (w/w) alginate polymer demonstrated a satisfactory drug release property from among the beads and was chosen for in vivo trials. The profile was characterized by 15% and 72% ibuprofen release at the end of the 1st and 7th h of dissolution, respectively.

In vivo studies

In vivo studies were carried out with free ibuprofen (100 mg kg\(^{-1}\)), blank alginate beads, ibuprofen (100 mg kg\(^{-1}\)) loaded alginate beads (formulation code H) and as a control 0.5% (w/v) NaCMC solution.

Figure 3. Section from stomach of ibuprofen-loaded alginate beads (H coded formulation) group. Gastric mucosa with continuous and intact surface epithelium was observed. Gastric glands were structurally normal in appearance. Haematoxylin-eosinX200.
Figures 3–6 show photographs of mouse stomach and duodenal mucosa after administration of blank alginate beads, free ibuprofen (100 mg kg\(^{-1}\)) and ibuprofen-loaded alginate beads containing an equivalent amount of drug. The mucosa of the stomach and duodenum are composed of surface epithelium, lamina propria and muscularis mucosae. The lamina propria of the stomach is occupied by closely packed gastric glands. In the control NaCMC solution, blank and ibuprofen-loaded alginate bead groups, neither ulcer nor haemorrhage was observed macroscopically. Microscopically the structures of the gastric mucosa were normal. Surface epithelium of the stomach lined by columnar cells was intact and continuous. Neither erosion nor haemorrhage was observed in the gastric mucosa. Therefore, the lesion incidence was 0/6 for each of these three groups (number of lesion incidence/total number of animals in the group; \(n = 6\) for each group). Gastric glands in the lamina propria were also intact; they were neither dilated nor distorted (Figure 3). The duodenal mucosa, like the gastric mucosa, was also normal both macroscopically and microscopically. The structures of the villi extending towards the lumen,
the Lieberkühn crypts (intestinal glands) and the other layers of the duodenal mucosa were all normal histologically (Figure 4).

In the macroscopical examination of the stomach of the group receiving free ibuprofen in NaCMC solution, small erosive areas ≤1 mm in diameter were observed in some regions. Histologically there were a few eroded areas in the gastric mucosa. The size of these lesions was $0.31 \pm 0.05$ mm (mean ± SEM) in diameter and the incidence was 6/6 (number of lesion incidence/total number of animals in the group; $n=6$). In these eroded areas, only the surface epithelium was found to be exfoliated, whereas the gastric glands in these regions were not dilated or distorted. No haemorrhage was observed in these areas (Figure 5). In the same group, the macroscopical examination of the duodenum revealed no ulceration. The duodenal mucosa was found to be normal in histological examination as in the other groups (Figure 6).

Due to the effects of some drugs, chemicals, bacteria or of various circulatory problems, mucosal defects (erosions) accompanied by congestion of blood vessels and haemorrhage or deep defect of the mucosa extending into the submucosa and even into the muscular layers of the stomach and duodenum can be observed.

In this study, only the free ibuprofen in NaCMC solution caused a few eroded areas in the gastric mucosa, whereas no erosion was observed in the duodenal mucosa in this group. In the gastric mucosa only the epithelium was exfoliated; there was no congestion. Erosions were limited to the superficial mucosa and in the eroded areas’ necrotic tissue; distorted areas were not observed. There was no ulcer formation, but superficial mild mucosal erosion in the gastric mucosa. Both the gastric mucosa and duodenal mucosa

Figure 5. Stomach from the free ibuprofen in NaCMC solution group. One of the eroded areas was observed. The surface epithelium was exfoliated, whereas gastric glands were intact. Haematoxylin-eosinX200.
were structurally normal in other groups. According to these findings, it was concluded that ibuprofen used in this dosage may lead to superficial mild mucosal erosion in the gastric mucosa, but not in the duodenal mucosa. The erosive effect of ibuprofen disappeared when it was entrapped into alginate beads and alginate beads themselves did not lead to any damage in gastric and duodenal mucosa. Several trials have been similarly undertaken to decrease the ulcerogenic activity of NSAIDs. Chitosan microspheres of diclofenac sodium have been reported to reduce gastric mucosal injury and enhance its anti-inflammatory activity when compared to plain drug (Açıkgoz et al. 1995).

Conclusions

In conclusion, the present study reveals the characteristics of ibuprofen-loaded alginate bead formulations both in vitro and in vivo. The ionotropic gelation method was successful in producing ibuprofen-loaded beads. The formulation variables, drug-loading, polymer percentage and the stirring speed used influenced the encapsulation efficiency, micromeric and in vitro drug release characteristics of the prepared beads. Ibuprofen-loaded alginate beads in particular demonstrated a satisfactory sustained-release profile, suggesting that alginate is an effective natural polymer to control drug release from beads. The gastro protective effect of ibuprofen-loaded alginate beads was the main achievement of this study since none of the gastric side effects of free ibuprofen was observed using these formulations. Therefore, one can assume that the beads are promising pharmaceutical forms by providing controlled-release drug delivery systems and by covering the gastric lesions that NSAIDs may cause.
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References


