Preparation and Evaluation of Ketoprofen-loaded Calcium alginate beads

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Abstract: Ionotropic gelation method was used to entrap Ketoprofen (KP) into calcium alginate beads. KP is one of the non steroidal anti-inflammatory drugs (NSAIDs); it has a short half life (1.5-2 h) and deleterious side effects on GIT such as irritation and ulceration. Beads were investigated in-vitro for possible sustained drug release and in-vivo as a gastro-protective system for KP. The curing time of beads in CaCl\textsubscript{2} solution was determined and process variables, such as polymer concentration, polymer/drug ratio and co-entrapped polymers (Gelatin (G), Hydroxy propyl methyl cellulose acetate succinate (HPMCAS) and ethyl cellulose (EC)) were analyzed for their influence on bead properties. On the basis of differential scanning calorimetry (DSC) and IR-spectroscopy, alginate was found to be compatible with KP. Scanning electron microscopy (SEM) photographs showed that the prepared beads were spherical and small (about 1 mm diameter) with small cracks and fissures on the surface. KP encapsulation efficiencies were high (>90%), also results showed that, release profile in 0.1M HCl (pH 1) was slow. while in phosphate buffer (pH 7.4), complete drug release was exhibited for all formulations within 2 h. There was no improvement concerning the retarding of drug release with co-entrapped polymers. The mechanism of release was depending on swelling and erosion of beads. The swelling behavior was strongly dependent on pH of the medium; such a pH sensitive swelling could be advantageous for orally administered drug vehicles especially for acid sensitive drugs or drugs that have adverse effects on GIT. The Ulcerogenic effect of the free drug on the stomach was compared to that of drug encapsulated in alginate beads using rats. Results showed a significant mucoprotective effect of alginate beads compared to the free drug. In conclusion, alginate beads can be used as enteric coated formulations rather than ideal sustained release formulations.

Keywords: Ketoprofen, alginate beads, ionotropic gelation, anti-inflammatory.

1. Introduction

One approach for controlled release formulation of different therapeutic agents is the production of polymeric gel beads. The beads are discrete spherical microcapsules that serve as the solid substrate on which the drug is coated or encapsulated\textsuperscript{(1)}. Beads can provide sustained release properties and a more uniform distribution of drugs within the gastrointestinal tract\textsuperscript{(1,2)}.

Sodium alginate (SA) is water soluble salt of alginic acid, a naturally occurring non-toxic polysaccharide found in brown algae\textsuperscript{(3)}. It is a linear polymer containing two uronic acids, α-L- guluronic (G) and β-D-mannuronic (M) acids linked by (1-4) glycosidic linkages. It is also composed of homopolymeric blocks MM or GG and blocks with an alternating sequence (MG blocks)\textsuperscript{(4,5)}. 
Alginate has a unique gel-forming property in the presence of multivalent cations, such as calcium ions in an aqueous medium, which takes place mainly at junctions in the G-G sequence rich chain region, known as “egg box junctions”.

When divalent metal ions such as calcium, barium and stannous diffuse into an alginate solution, the rapid ion binding and formation of a polymeric network produces an inwardly moving gelling zone. In fact, alginate moves from the gel core towards this gelling zone, leading to the deletion of alginate within the core.

The gelling property of alginate with divalent metals has been used to prepare alginate beads for drug delivery system and this can be achieved by dropping the drug containing sodium alginate dispersion into calcium chloride bath.

As calcium alginate matrix formed is usually very permeable and little or no drug release can actually be controlled in the case of soluble drugs. Hence, a preferential use for alginate gel beads in the delivery of low solubility or macromolecular drugs has been suggested.

Ketoprofen (KP) is an anionic, non-selective non-steroidal anti-inflammatory drug (NSAID). It is a derivative of propionic acid and widely used for management and treatment of patients with rheumatic disease. The unwanted side effects of KP are due to inhibition of COX-1, while their therapeutic effects are due to inhibition of COX-2. So, the drug can cause gastric ulceration partly by directly irritating the gastric mucosa and partly by inhibiting the synthesis of cytoprotective prostaglandins. Although it is well absorbed (> 90%) after oral administration with peak plasma concentration occurring at 1-2 h, but the elimination of the drug is rapid with mean half life of only 1.5-2 h. So, KP is a good candidate for the preparation of controlled release formulations. Several trials have been undertaken to alleviate the drug’s side effects by modification of its dosage form, some of these trials include the use of other routes of administration as topical, rectal or transdermal systems have been developed.

The aim of this work was to design KP-loaded alginate beads as a controlled release oral delivery system. Ionotropic gelation technique was selected to prepare the alginate beads due to its simplicity, low cost and its high entrapment rates achieved with poorly water soluble drugs.

2. Material and methods

2.1. Materials

Ketoprofen (KP) was kindly provided by Amriya Pharm. & Chem. Ind. Co. (Cairo, Egypt). Sodium alginate, calcium chloride and gelatin were obtained from Chem. and pharma. Co. LTD (England). Ethyl cellulose (EC) was procured from Hercules, Wilmington, DE. (USA). Hydroxypropyl methylcellulose acetate succinate (HPMCAS) was procured from Shin Etsu, Tokyo (Japan). Potassium dihydrogen orthophosphate, sodium hydroxide, hydrochloric acid were purchased from El-Naser pharm. And chem. Co. (Egypt), and all other ingredients used were of pharmaceutical grade.

2.2. Determination of gelation rate of Ca-alginate beads:

The gelling rate was assessed during particle formation and was determined from weight changes of the beads in calcium chloride solution as described elsewhere.

The beads were prepared by forcing aqueous solution of sodium alginate through nozzles (0.65 mm i.d.) into gently stirred 0.1 M calcium chloride solution. Five beads were collected at appropriate time intervals and weighed after removal of surface moisture using filter paper.

The weight of five droplets was assumed to be the initial weight at t=0. The ratio of average bead weight after t to that of droplet was considered as weight fraction change of the curing beads in CaCl₂. Results were expressed as weight loss, corresponding indirectly to gelation due to water loss during matrix formation. The time at which no change in weight fraction was considered as the time at which the bead became fully cured. The gelling rate was determined for different alginate concentration (3-5% w/v) and each experiment was done in triplicate.
2.3. Preparation of KP-loaded Ca-alginate gel beads:

KP-loaded Ca-alginate beads were prepared using ionotropic gelation method \(^{(8, 13)}\). In this method KP was dispersed homogenously in alginate solution; this suspension was forced through needles into gently stirred CaCl\(_2\) solution (0.1M) at a constant rate of (10-12) drops/min. Ca-alginate beads were allowed to stand in CaCl\(_2\) solution until curing for 12 hrs, then filtered, washed three times with distilled water and dried at room temperature for 24 h and then placed in an oven at 45°C for another 24 h. Different formulations were prepared according to the following schedule:

a- Formulations prepared using different Na-alginate concentrations (3, 4, and 5% w/v) at constant polymer to drug ratio (1:1).

b- Formulations prepared using constant concentration of alginate (4% w/v) at different polymer to drug ratio (1:3, 1:1 and 3:1).

c- Formulations prepared using 2% w/v alginate, 2% of co-entrapped polymers (soluble polymer as Gelatin (G), enteric polymer as Hydroxypropyl methylcellulose acetate succinate (HPMCAS) and insoluble polymer as Ethyl cellulose (EC), and 4:1 polymer to drug ratio.

2.4. Drug polymer interaction study

2.4.1. Differential scanning calorimetric (DSC) studies:

The DSC patterns of the drug alone, excipients alone as well as physical mixtures of the drug with the investigated excipients (1:1 w/w) and the alginate beads at alginate concentration of 4% (1:1 w/w polymer to drug ratio) were examined. A Shimadzu model DSC-50 was used at a scanning rate of 10 °C/min from 30°C to 200 °C under nitrogen gas stream at a flow rate of 40 ml/min using 4-8 mg of sample.

2.4.2. Fourier transform infrared spectroscopy (FTIR) studies:

Samples (1-2mg) of the drug alone, alginate alone, physical mixture and alginate beads were mixed with potassium bromide (IR) grade, compressed into disks in the compressor under vacuum and scanned from 4000 to 800 cm\(^{-1}\) with an empty pellet holder as a reference.

2.5. Characterization of alginate beads

2.5.1. Size, weight, shrinkage % and water content of the beads:

The diameter of beads before and after drying was determined with slide caliper at three different positions for each bead. The mean diameter of 3 beads was calculated. In addition, the average weight of 10 beads before and after drying was determined and the mean of 3 determinations was considered as the bead weight. The shrinkage % and water content % of beads were calculated according to the following equations:

\[
\text{Shrinkage \%} = \left(\frac{\text{diameter of beads before drying}}{\text{diameter after drying}}\right) \times 100 \quad (1)
\]

\[
\text{Water content \%} = \left[\frac{\text{(weight of bead before drying} - \text{weight after drying})}{\text{weight before drying}}\right] \times 100 \\
\]

\[
\text{..............} (2)
\]

2.5.2. Drug content and entrapment efficiency determinations:

Weighed beads (10 mg) were immersed and dispersed in 100 ml of phosphate buffer at pH 7.4 for 12 h under magnetic stirring at room temperature. The solution was then filtered, and the KP content was assayed by UV spectrophotometer at wavelength of 260 nm. The percent drug loading and entrapment efficiency were calculated using the following equations \(^{(23, 24)}\). Results were based on triplicate determination.

\[
\text{Drug content \%} = \left(\frac{\text{amount of drug in beads}}{\text{weight of beads}}\right) \times 100 \quad (3)
\]

\[
\text{Encapsulation efficiency \%} = \\
\left(\frac{\text{actual drug content}}{\text{theoretical drug content}}\right) \times 100 \quad \text{...........} \quad (4)
\]

2.5.3. Morphology study of alginate beads:
The surface morphology and internal structure of KP Ca-alginate beads were observed using scanning electron microscopy (SEM). Samples of the dried beads were mounted onto stubs, sputter coated with gold in a vacuum evaporator, and photographed using scanning electron microscope.

2.5.4. Swelling rate of alginate beads:

Swelling rate of the beads was measured at different pH. An amount of beads (equivalent to 20 mg of drug) were placed in phosphate buffer (pH 7.4) or 0.1M HCl (pH 1) solutions (using dissolution apparatus Erweka DT-D6, Heusenstamm, Germany) at 37 °C. At different time intervals the beads were taken out and weighed after drying the excess water using filter papers. Their changes in weight (after correcting for drug loss) were measured during swelling. The swelling percent were determined by calculating the water uptake using the following equation:

\[ \text{Swelling index} \% = \left( \frac{w_t - w_0}{w_0} \right) \times 100 \] ........................ (5)

Where \( w_t \) is the weight of bead at each time and \( w_0 \) is the initial weight of bead before swelling (dry bead). Each determination was performed in triplicate.

Macroscopic and microscopic pictures during swelling in both media were taken using digital camera and microscope, respectively.

2.6. In vitro dissolution study:

The drug release was determined using USP 25 dissolution apparatus according to paddle method (II) at rotation speed of 50 rpm and temperature of 37 °C. KP powder (20 mg) or beads (equivalent to 20 mg drug) was placed into 500 ml of test solution (0.1 M HCl of pH 1, phosphate buffer of pH 7.4). At appropriate intervals, 5-ml samples were withdrawn. An equal volume of fresh medium was added to test solution to maintain constant volume. The content of KP was determined spectrophotometrically at \( \lambda_{\text{max}} 260 \) nm. Each experiment was performed in triplicate.

2.7. Ulcerogenic effect of KP entrapped in alginate beads:

The test was done according to the reported method(26). Briefly, the experiment was designed with nine adult male albino rats weighing (100-120 g) divided equally into 3 groups. The rats were fasted for 18 h prior to the experiment. A dose level of 50 mg/kg of the tested formula was orally administered with small amount of water on two successive days using oral lavage needle. Group 1 was the control (received only distilled water). Group 2 received KP powder. Group 3 received beads prepared using 4% alginate at polymer/drug ratio of 1:1. After 7 h of administration of the last dose, all rats were scarified. The rats' stomach were excised, opened along curvature and washed with saline. Stomachs were examined grossly under a binocular magnifier(27) and with SEM.

2.8. Statistical analysis

All results were expressed as mean values ± standard deviation (SD). The results were evaluated and analyzed statistically with the prism ver. 5. (Graph Pad Soft ware, USA) computer program. For statistical evaluation, data were analyzed by two way analysis of variance (ANOVA) using bonferroni post test. Differences between means were considered statistically non-significant (NS) if the P value was > 0.05. When 0.05 > P ≥ 0.01 the parameters taken as significantly (S) different and when 0.01 > P ≥ 0.001, the differences were regarded to be highly significant (HS).

3. Results and discussion:

3.1. The gelling rate of beads:

The process of gelation can be visually detected as the appearance of translucent circular boundary which gradually shrunk with progression of curing process and finally disappeared. As shown in Figure 1, formulations are arranged according to the decline in gelation rate in the following order: 3% alginate > 4% alginate > 5% alginate; In which the formula containing 3 % of alginate showed a higher rate of decline (\( w_t/w_0 = 50.83 \pm 1.99 \)) than that containing 4 % (\( w_t/w_0 = 59.503 \pm 3.91 \)) or 5% (\( w_t/w_0 = 67.85 \pm 7.09 \)) alginate after 72 hrs. This may be due to the difference properties of beads before drying as weight and water content.
It was noticed that for all formulations, gelation was completed at about 12 hrs and assumed to be fully cured.

3.2. Drug polymer interaction study:

The DSC thermograms (Figure 2) of the physical mixture and loaded beads had retained the characteristic endothermic peak of the drug at 95.38°C (onset = 90.16°C and ΔH of -150 J/g corresponding to its melting point indicating no significant interaction between alginate and drug. Figure 3 shows that the principal bands of KP (3000-2500, 1695 and 1655 cm⁻¹ corresponding to carboxylic OH, C=O stretching of acid and ketone, respectively) in both the physical mixture and loaded beads which confirms no significant interaction.

![Figure 1: Weight fraction changes of curing beads in 0.1 M CaCl₂ prepared using alginate (Alg.) with different concentrations.](image)

![Figure 2: Differential scanning colorimetry thermograms, (a) KP (b) Na-alginate (c) Physical mixture (d) Plain beads (e) KP-loaded beads.](image)

3.3. Characteristics of alginate beads:

Table 1 shows the physical characters of different alginate bead formulations. All beads are small in size with diameter about 1mm and of weight from 0.6-0.8 mg. There is no significant difference (P > 0.05) between the particle size of beads prepared with different alginate concentrations (3, 4 &5 % w/v). Statistical analysis revealed that the addition of some entrapped polymers (EC, HPMCAS and Gelatin) did not significantly (P > 0.05) affect the particle size of the bead after drying. The size of the beads was arranged according to shrinkage percentage. Less shrinkage (P > 0.001) was observed with alginate concentration 5% w/v (172%), with increase in amount of drug loaded (1:3) (152%) and after addition of EC (175.17%) or HPMCAS (168.3 %) which lead to bigger beads in size than pure alginate beads. It was concluded that an increase in percent of Na-alginate could lead to a great viscosity of the solution and hence a larger drop was dripped out of the nozzles as a result larger beads were formed and diffusivity was decreased (28). Also, EC and HPMCAS don't have swelling or shrinkage ability, so their addition may decrease the ability of beads to take water during curing. The water content in all formulations was between (90–93 %) and the encapsulation efficiencies of KP were high (>90%) for all formulations owing to the low solubility of KP in water and the minimum loss of the drug during preparation of the beads or the subsequent washing stages. This result is in agreement with the findings of Khazaeli et al. (28).

3.4. Morphology of beads

SEM micrographs, magnifications (x 35, x 75) showed that the dried beads were spherical with rough surface and low porosity Figure (4A, 4B). At high magnification (x1000), small cracks and fissures in the matrix sometimes appear irrespective of drying temperature and drug content. Small amount of drug crystals may appear on the surface which can be formed during drying (Figure 4C). Similar results were obtained by Fathy et al. (29) for alginate beads loaded with tiaramide.
Table 1. Characteristics of KP-loaded Ca-alginate beads of different composition.

<table>
<thead>
<tr>
<th>Bead property</th>
<th>Alginate conc. (% w/v)</th>
<th>Alginate (4 % w/v):Drug ratio</th>
<th>Polymer added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 %</td>
<td>4%</td>
<td>5%</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>Before drying</td>
<td>2.25 ± 0.14</td>
<td>2.39 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>After drying</td>
<td>1.16 ± 0.07</td>
<td>1.23 ± 0.05</td>
</tr>
<tr>
<td>Shrinkage (%)</td>
<td>Before drying</td>
<td>194.7 ± 0.61*</td>
<td>194.1 ± 3.03*</td>
</tr>
<tr>
<td></td>
<td>After drying</td>
<td>8.84 ± 0.39</td>
<td>8.38 ± 0.46</td>
</tr>
<tr>
<td>Weight (mg/bead)</td>
<td>Before drying</td>
<td>0.69 ± 0.13</td>
<td>0.83 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>After drying</td>
<td>92.16 ± 0.52</td>
<td>90.12 ± 0.65</td>
</tr>
<tr>
<td>Water Content (%)</td>
<td>Before drying</td>
<td>47.64 ± 0.10</td>
<td>47.95 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>After drying</td>
<td>95.29 ± 0.2</td>
<td>95.91 ± 0.09</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>Before drying</td>
<td>95.29 ± 0.2</td>
<td>95.91 ± 0.09</td>
</tr>
<tr>
<td>Encapsulation efficiency (%)</td>
<td>Before drying</td>
<td>95.29 ± 0.2</td>
<td>95.91 ± 0.09</td>
</tr>
</tbody>
</table>

Results represent mean ± SD of 3 observations. *: HS (P > 0.001)
3.5. Swelling rate of beads

The swelling behavior of dried KP alginate beads in different pH (1 & 7.4) is illustrated in Figure 5. As shown in this figure the beads exhibited high swelling rate in pH 7.4, while less swelling rate was noticed in pH 1. The swelling of beads at pH 7.4 was caused by the exchange of the cross-linking calcium ions with non-gelling Na or K ions (components in buffer)\(^{30}\). So the repulsion force between the negatively charged carboxylate group (i.e. \(\text{–COO}^-\) groups) of alginate increased and the degree of cross-linking decreased due to loss of Ca\(^{2+}\) ions. This ultimately results in a rather loose structure and hence the beads take up more water until bursting of the beads take place and the beads start to disintegrate\(^{31}\). Maximum swelling for the beads was reached at 1.5 h in phosphate buffer (pH 7.4) after which erosion and break down of beads took place; these results are in agreement with the findings for Al-Kassas et al.\(^{32}\).

The same beads tend to shrink when exposed to acidic environment (pH 1). Ouwerx et al.\(^{33}\) have shown that at low pH values (< 4), the carboxylate groups of alginate are protonized and hence the electrostatic repulsion among these groups decreased and shrinkage is favored so the interior water is rejected outside the bead and a decrease in its weight takes place. Such a pH-sensitive swelling property could be advantageous for orally administered drug vehicles, especially for acid sensitive drugs or drugs that have adverse effects on the GIT.

Figures (6a and 6b) show the stages of swelling of beads in both media (pH 1 and pH 7.4). The beads keep their intact form for up to 4 h in acidic pH; no change in sphericity and no erosion were observed (Figure 6a). While in pH 7.4 the beads remained intact only for 1.5 h (Figure 6b).
Figure 5: Swelling behavior of KP-loaded Ca-alginate beads (4% w/v) in 0.1M HCl (pH 1) and phosphate buffer (pH 7.4).

Figure 6: Swelling stages microphotographs (X 40) of Ca-alginate beads (4%w/v) in: (a) 0.1M HCl (pH 1), (b) phosphate buffer (pH 7.4).

3.6. In vitro dissolution study

3.6.1. Release of KP in 0.1 M HCl (pH1):

Figure 7a shows the release profiles of KP in 0.1 M HCl (pH1) from different alginate beads formed using different alginate concentrations (3, 4, &5% w/v). The release rate of KP from all formulations was significantly (p < 0.001) slow and sustained. Only 10-15% of the drug was released after 4h and no deformation in the shape of beads was observed as confirmed previously in the swelling study. While almost 63% of the drug was released from drug alone at the same time. This may be due to the stability and non swelling property of alginate in acidic pH (34) and the conversion of Ca-alginate to insoluble alginic acid (35).

Also there was no significant (p > 0.05) effect of changing drug loading on its release from the beads as shown in Figure 7b, but the release was still more sustained (p < 0.001) than that of the drug alone. So, it is expected that the release of KP from alginate beads would be minimized in the stomach, leading to less potential local adverse effects on gastric mucosa (36).
3.6.2. Release of KP in phosphate buffer (pH 7.4):

Figure 8a shows the release of KP in phosphate buffer (pH 7.4) from powder or Ca-alginate beads at different alginate concentrations (3, 4 & 5% w/v). The release of drug from its powder was very fast and completed within 15 min due to the higher solubility of KP in this medium (8.147 mg/ml). All Ca-alginate beads (3, 4 & 5% w/v) showed significant ($p < 0.001$) decrease in the rate of release than that of the drug alone, but all formulations released the drug completely after 3 h. Also the drug release rate significantly ($0.05 > P \geq 0.01$) decreased with increasing alginate concentration from 3 to 5% w/v during the first hour (from 0.5 to 1 h) until the erosion of beads occurred then all formulations became superimposed.

This rapid release can be explained on the basis of swelling and erosion. In phosphate buffer the rapid swelling created porous structure and brought more liquid inside the beads. Moreover, ion exchange with phosphate buffer and formation of the solute alginate led to erosion of the beads. Other studies have reached similar results, when using Ca-alginate beads.

Using alginate concentration of 4% w/v, different alginate/drug ratios (1:3, 1:1 and 3:1) were used to study the effect of drug loading on the in vitro release of drug in phosphate buffer. As shown in Figure 8b, slight differences between different ratios were obtained and the results revealed that the release rate was slightly faster with increase in alginate content from (1:3 to 3:1) alginate /KP ratio. This difference can be explained according to Tateshita et al. who found that the release of nifedipine from Ca-alginate beads increased with decrease in nifedipine content in solution. They suggested that the decrease in drug content may lead to increase in surface area of particles by means of good dispersion of drug in alginate gel.

3.6.3. The effect of co-entrapped polymers on release of KP in phosphate buffer (pH 7.4):

Both soluble (HPMCAS, gelatin) and insoluble (EC) polymers were incorporated in the formulation in the hope that their presence would lead to changes in the structure and barrier function of beads and give more controlled release properties in phosphate buffer.

Figure 9 shows the effect of incorporating polymers on release of KP from Ca-alginate formulations and revealed that the addition of these polymers resulted in no improvement concerning the retarding of drug release in phosphate buffer pH 7.4 and in some cases; drug release was even faster than that of pure Ca-alginate beads as shown when adding gelatin.

Polymers used in our work have been used by other authors in order to modify the release properties and similar results were obtained.
3.6.4. Effect of swelling of Ca-alginate beads on the release rate:

The swelling percent of the beads prepared using different alginate concentrations (3, 4 & 5 % w/v) was plotted against KP release at the same time to examine the effect of swelling on KP release rate. Figure 10 illustrates good direct linear correlations between beads swelling and percent of the release in phosphate buffer (pH 7.4). These correlations in phosphate buffer are good confirmation for release mechanism where significant swelling of the beads was found.

3.7. Ulcerogenic activity

Observation of the gastrointestinal mucosa of rats for the presence of lesions following oral administration of selected formulations as well as reference drug had been taken as an indication for the ulcerogenic effects.

Gross observation of rat stomach indicated wide spread erosions, ulceration and several hemorrhagic steaks in the rats treated with drug powder (2nd group) due to gastric mucosal injury (Figure 11b).

The membranes of rats' stomach treated with KP-loaded alginate beads (3rd group) (Figure 11c) revealed no abnormal naked eye changes in all tested rats and were similar to that of control (1st group) (Figure 11a).
Scanning electron microscopy of the control (1st group) stomach of rat revealed normal lining simple columnar epithelium without goblet cells in the fundic region. Normally, the stomach lining is protected by a layer of mucus (Figure 12a).

The 2nd group of rats treated with ketoprofen powder showed marked signs of ulceration as abrasion and sloughing of the epithelium (cracked clay appearance). Luminal surface and pit cells are exfoliated and the damage extends from the luminal surface into the glands (Figure 12b). Several mechanisms have been proposed to account for the development of gastric damage (41), among these explanations, the direct physical damage by the drug particles, loss of the protective mucous layer and acidic influence of the drugs (42).

**Figure 10**: Correlation between KP release and swelling of beads in phosphate buffer (pH 7.4).

**Figure 11**: Representative gross appearance photos for gastric mucosa of rats: (A) Control (1st group), (B) KP powder (2nd group) showing marked hemorrhagic streaks and (C) alginate beads.

**Figure 12**: Representative micrographs using scanning electron microscope for gastric mucosa of: (A) healthy rat with no dug treatment (1st group, Control), (B) rat treated with KP powder (2nd group) showing sloughing of the epithelium and marked abrasions, (C) rat treated with alginate beads (3rd group).
Significant reduction in the gastric mucosal injury and valuable protection of submucosal cells of rats' stomach treated with KP-loaded alginate beads (3rd group). The stomach lining covered by a layer of mucus and photomicrographs revealed normal appearance compared to control group (Figure 12c). This is may be due to the protective effect of alginate beads on the stomach by keeping their intact form with the absence of swelling and erosion that prevent KP release in the stomach resulting in a decrease in the contact between the drug and stomach. This is in agreement with the finding previously reported by Fathy M. (43) and El-Gindy G. A. (37).

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