

## PREPARATION AND IN-VITRO EVALUATION OF ALGINATE BEADS OF FLURBIPROFEN

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الغرض من هذه الدراسة هو تحضير وتقييم كريات من هيلام الألجينات كنظام بطى لمعدل انطلاق عقار الفلوربروفين عن طريق الفم. الفلوربروفين هو أحد العقاقير الغير كورتيكوستيرويدية التي تستخدم فى علاج الإنتهابات والألام الروماتيزمية والتي لها تأثيرات جانبية خطيرة على الجهاز الهضمى مثل التهيج والتقرح والنزف فى المعدة ولهذا تم تحضير هذه الكريات المحملة بالعقار بنسبة ١:٢ ، ١:٤ (عقار : بوليمر) لتلافى هذه الأعراض. ولقد اتضح من الدراسة أن معدل الطلاق العقار من الكريات الألجينية يتأثر بقوة بالأس الهيدروجينى لوسط الذوبان وأيضا نسبة العقار للبوليمر. فى كل الحالات كانت ميكانيكية معدل الإنطلاق تتبع نظام- الصفر. إن معدل انطلاق العقار من الوسط القاعدى أكبر بكثير من مثيله فى الوسط الحامضى. أيضا تم دراسة معدل انتفاخ كريات الألجينات الجافة ، ولقد وجد أن الكريات تبقى بدون تغيير فى الوسط الحامضى (الأس الهيدروجينى ١.٢ ، ٥.٨) ولكنها تنتفخ بسرعة فى الوسط القلوى عند ٧.٤ أس هيدروجينى إلى حجم أكبر من حجمه الأصى قبل التجفيف. ويمكن استخدام هذه الخاصية لتناول العقارات الحساسة للوسط الحامضى أو العقارات التي لها تأثير عكسى على الجهاز الهضمى بتحميلها على شكل كريات أالجينية. أيضا تم دراسة تأثير العقار والعقار المحمل على الكريات الألجينية على النشاط التقرحى لأمعدة الأرانب. ولقد وجد أن تحضير العقار فى صورة كريات أالجينية ليس لها أى نشاط تقرحى أو نزف لأمعدة الأرانب.

*The purpose of this study was to prepare and evaluate alginate gel beads as an oral controlled release system of flurbiprofen (FLUP). FLUP is one of the potent nonsteroidal antiinflammatory drugs (NSAIDs) which has deleterious side effects on the GIT such as irritation, ulceration and hemorrhage. The release of FLUP from alginate beads was strongly affected by pH of the dissolution medium and drug : polymer ratio. In all cases the drug release from alginate beads showed nearly zero-order mechanism. The release rate of drug was more rapidly in alkaline medium (pH 7.4) than that at pH 5.8. The swelling property of dried alginate beads was of interest. The beads remained unchanged in acidic medium (pH 1.2 and 5.8) but swelled rather rapidly in pH 7.4 phosphate buffer to a size greater than their original size before being dried. Such a pH-sensitive swelling properties could be advantageous for orally administered drug vehicles, especially when an acid-sensitive drug or drug that has adverse-effects on the GIT is incorporated in the beads. The effect of the intact drug as well as FLUP alginate beads on the ulcerogenic activity of the drug in the stomach of rabbit was carried out. It was observed that, the ulcerogenic activity of the intact drug disappeared and the mucosal surface didn't show hemorrhage or inflammation when the drug is loaded with alginate beads.*

### INTRODUCTION

Flurbiprofen has action similar to other non-steroidal anti-inflammatory drugs (NSAIDs) which have potent antiinflammatory effects in addition to the antipyretic and the analgesic activities.<sup>1</sup> So, it is used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and juvenile arthritis.<sup>2-5</sup> The molecule is slightly soluble in water, but it

is readily absorbed after oral administration, and approximately 95% of a dose is excreted in the urine within 24 hr. Chronic oral administration of drug may increase the chance of gastrointestinal damage including bleeding, ulceration and perforation.<sup>6</sup> In order to eliminate these adverse effects, enteric coated products and/or sustained release forms have been developed.<sup>7-10</sup>

In recent years, multiparticulate systems have received a considerable interest, where the alginate beads have a protective effect on the mucous membranes of the upper gastrointestinal tract (GIT).<sup>11,12</sup> Also, alginates suppressed food and acid reflux into the esophagus.<sup>13</sup>

Several reports illustrated that, alginate beads can be used for controlling drug release.<sup>14-18</sup>

The purpose of this study is to investigate a multiparticulate enteric preparation of the flurbiprofen, based on alginates. Flurbiprofen alginate beads were prepared and the effect of pH of the dissolution medium and drug to polymer ratio on the release profile of flurbiprofen were investigated. Moreover, the ulcerogenic activity of the intact drug and drug loaded beads were elaborated in rabbits stomach.

## MATERIALS AND METHODS

### Materials

The following materials were used as received. Flurbiprofen (FLUP) [(±)-2-fluoro- $\alpha$ -methyl-4-biphenyl acetic acid] was purchased from Sigma Chemical Co., St. Louis, MO. Sodium alginate (Merck), calcium chloride (BDH). All other chemicals were of reagent grades.

### Methods

#### Preparation of alginate beads

Homogenous dispersion of flurbiprofen in aqueous solution of sodium alginate (6% w/v) was obtained. The bubble-free suspension was forced out of a 10-ml syringe nozzle ( $\approx$ 1 mm internal diameter) into gently stirred calcium chloride solution (0.1 M). The flow rate was kept constant of about 10 drops/min. The height of fall down to the surface of calcium chloride solution was 25 cm. Beads of alginate gel were allowed to remain in calcium chloride solution for 72 hr to ensure that, the beads have been cured, then they were separated by filtration, washed with distilled water and dried at room temperature for 24 hr, after that, they were transferred into vacuum desiccator over anhydrous calcium chloride at room temperature for another 24 hr. Alginate beads with two drug : polymer ratios (1:2 and 1:4) were prepared. In all cases the ratio of polymer to calcium chloride

solution volume was constant (1 g of alginate for 100 ml of CaCl<sub>2</sub> solution). The mentioned method was carried out at room temperature to minimize the variation in the viscosity of the alginate solution.<sup>19</sup>

### Drug loading

#### Indirect method

Aliquots from the filtered solution remaining after removal of the beads were assayed spectrophotometrically at 254 nm. The amount of FLUP entrapped in the beads was calculated from the difference between the total amount of FLUP added and the FLUP found in the filtered solution.

#### Direct method

20 mg of alginate beads were added to 40 ml of pH 7.4 phosphate buffer in 50 ml volumetric flask and dissolved completely and allowed to stand overnight. The solution was filtered and completed to a volume. The absorbance of FLUP was measured spectrophotometrically at  $\lambda_{\max}$  254 nm using UV spectrophotometer (Shimadzu, Kyoto, Japan) after appropriate dilution.<sup>19</sup>

#### Scanning electron microscope (SEM)

Scanning electron microscope model JSM-S400 LV. JEOL, Japan was used to characterize the shape and the surface of the beads after coating with carbon-gold layer under vacuum. The surface was screened and photographed.

#### Differential scanning calorimetry (DSC)

DSC analysis was used to characterize the thermal behaviour of the different beads components. This analytical method was carried out on isolated substances, their physical mixtures and empty and loaded beads.

The DSC patterns of alginate beads were carried out with a Shimadzu Model DSC-50 (Shimadzu, Kyoto, Japan). The measurements were done using the sample pan for liquid sample, at a scanning speed of 10°/min under N<sub>2</sub> gas stream, from 30° to 350°. Sample weight was about 5 mg.

#### Infra-red absorption spectroscopy (IR)

The IR spectra were obtained with a Shimadzu IR-470 infra-red spectrophotometer (Shimadzu, Kyoto, Japan) using the KBr disc

method. The discs were made under a pressure equal to 400 Kg/cm<sup>2</sup>.

#### Swelling rate of dried alginate beads

The resulting dried alginate beads were gently immersed in solutions of different pH (1.2, 5.8 and 7.4), to assimilate the pHs of GIT throughout the passage of the beads from stomach to intestine, at 37°. The diameter of each swelling beads, taken out of the solution, was measured by micrometer at three different position and the average of five particles was calculated at appropriate time intervals until the bead is burst. The magnitude of swelling was represented by the ratio of the diameter of a swelled bead to the corresponding diameter of the fully-cured bead before drying.

#### Dissolution studies

The release studies of flurbiprofen were determined using USP XXI paddle apparatus. Flurbiprofen powder (33.33 and 20 mg) or its beads (equivalent to 33.33 and 20 mg drug for 1:2 and 1:4 drug to polymer ratio respectively) were introduced into 250 ml of the release medium of pH 5.8, and 7.4 using phosphate buffer (B.P.) at 37±1° and stirred at 50 rpm. Samples were withdrawn at specified time intervals and replaced with an equal volume of the dissolution medium. The drug concentration was determined spectrophotometrically at  $\lambda_{\max}$  254 nm. All the studies were carried out in triplicate.

#### Ulcerogenic Activity

The assessment of the ulcerogenic activity of flurbiprofen from the selected formulations was carried out in two groups of albino rabbits, three for each (1.5-2 Kg body weight). The rabbits were kept free of diet and water two hours before and after drug administration. An oral dose of the intact drug (10 mg) or an amount of beads equivalent to 10 mg of the drug (in colourless gelatin capsule) were administered twice daily, with 10 ml of water to each group for 7 days. At the end of experiment, the animals were sacrificed and the stomach was excised and opened along the greater curvature, washed with saline solution and examined for the presence of ulcers and then photographed.<sup>20</sup>

## RESULTS AND DISCUSSION

Homogeneous flurbiprofen alginate beads were obtained in a rapid production rate, especially, if the viscosity of the initial drug suspension is between 100 and 3000 cp. In this range, the sedimentation of the drug is minimized and reasonable flow rate is achieved.<sup>21</sup> The viscosity of flurbiprofen suspended in sodium alginate solution, in the present study, was ≈505 cp (Brookfield Viscometer, Massachusetts, USA).

#### Drug content and encapsulation efficiency

Total FLUP percent entrapment and the surface drug, obtained from the direct and the indirect methods, in alginate beads are shown in Table 1. Table 1 shows the physical characteristics of the alginate beads, which illustrated that less shrinkages was observed with the increase in the amount of drug loaded, viz. the diameter of dried beads of 1:2 > that of 1:4 drug to polymer ratio. The reproducibility of flurbiprofen content in the beads was found to be 30.25% and 17.65% for 1:2 and 1:4 drug : polymer ratio respectively. This may be attributed to the low solubility of flurbiprofen in calcium chloride solution or in water. Consequently, very small amount of the drug was lost during curing period or washing process. With the increase in loaded flurbiprofen, the encapsulation efficiency increased due to the decrease in the percent of drug lost during preparation process. This result is agreement with that reported by Østberg.<sup>21</sup>

#### Thermal analysis

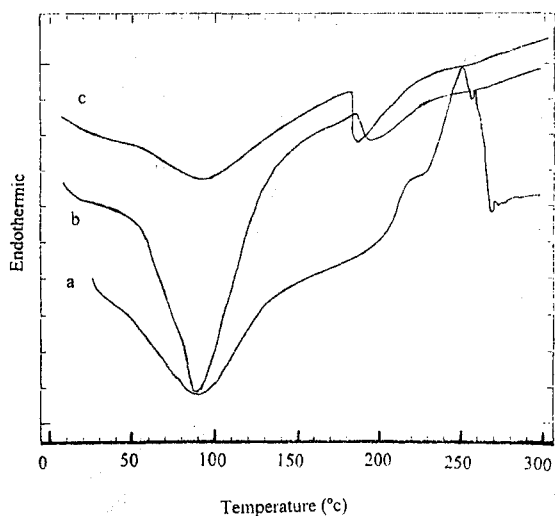
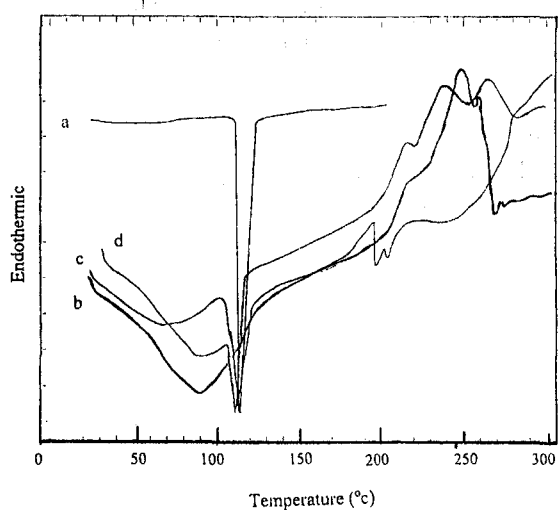
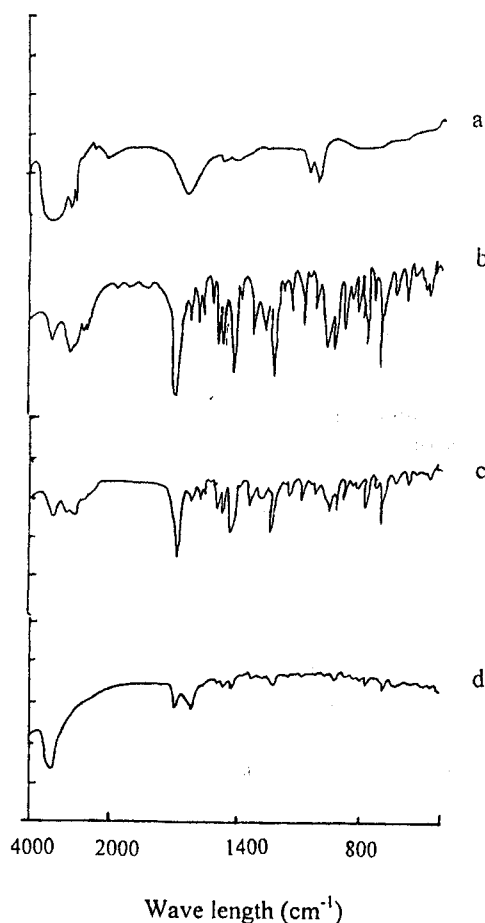
In the DSC studies several interaction can be identified: Alginate-Ca, the degradation exotherm of alginate at ≈250°, is absent and at 190°, an endotherm corresponding to alginate-Ca interaction is observed (Fig. 1). Also, the drug-alginate beads were analysed using DSC. The thermogram obtained was partially different from that obtained with the drug-alginate physical mixture (Fig. 2). This reveals that there were some interaction between FLUP and alginate.

#### IR spectroscopy

Fig. 3 shows infra-red (IR) spectra of FLUP crystals, sodium alginate, physical

**Table 1:** Physical characteristics of alginate beads loaded with flurbiprofen at 1:2 and 1:4 drug : polymer ratio.

D/P <sup>(1)</sup> ratio	Diameter (mm)		Shrink- age <sup>(2)</sup> %	Weight (mg/bead)	Drug content %	Drug surface (%) ±SD	Encapsulation efficiency <sup>(3)</sup> %
	Before drying	After drying					
1:2	4.97 ±0.20	2.22 ±0.03	223.87	4.03 ±0.15	30.25 ±0.85	3.10 ±0.011	90.76
1:4	4.11 ±0.10	1.48 ±0.02	277.7	3.15 ±0.025	17.65 ±0.35	2.80 ±0.015	88.25

<sup>(1)</sup> D/P = drug / polymer ratio<sup>(2)</sup> Shrinkage % =  $\frac{\text{diameter of beads before drying}}{\text{diameter of beads after drying}} \times 100$ <sup>(3)</sup> Encapsulation efficiency =  $\frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$ **Fig. 1:** Thermograms of the samples obtained from: (a) sodium alginate; (b) physical mixture of sodium alginate and calcium chloride; and (c) blank beads.**Fig. 2:** Thermograms of the samples obtained from: (a) FLUP; (b) sodium alginate; (c) physical mixture of FLUP and sodium alginate; and (d) FLUP-alginate beads.**Fig. 3:** IR spectra of (a) sodium alginate; (b) FLUP; (c) physical mixture of FLUP and sodium alginate; and (d) FLUP-alginate beads.

mixture of FLUP and alginate and FLUP-alginate beads. This study was done to investigate a possible interaction between FLUP and alginate. The IR spectrum of physical mixture as simply a superposition of each component, while that of the beads was obviously different from the physical mixture, especially in the carbonyl-stretching regions (1800-1650  $\text{cm}^{-1}$ ) and stretching vibration regions at 3800-3000  $\text{cm}^{-1}$ . This implies that a high ratio of drug was entrapped into the beads during their preparations.

#### Characterization of alginate beads

Fig. 4 shows air-dried calcium alginate matrices with and without the model drug. During air drying, the gel beads lose their initial sphericity and become small, dense matrices in which FLUP crystals are embedded. Cracks and fissures in the matrix surface are observed irrespective of drying temperature and drug content. Crystals are also seen on the surface. These can be formed during drying, by crystallization of dissolved drug that migrates along with water to the bead surface.

#### Swelling rate of dried alginate beads

The swelling behaviour of dried FLUP alginate beads in different buffer solutions at pHs 1.2, 5.8 and 7.4 was illustrated in Fig. 5. The magnitude of swelling was represented by the ratio of the diameter of the swelling beads to the corresponding diameter of the fully-cured beads before drying.<sup>22</sup> The value 100% indicates that the swelling particles reached the original size of the hydrated beads before being dried. It should be noted that, no swollen was observed at pH 1.2, while a slight swelled (~22-31%) was observed at pH 5.8 by the end of the experimental time. In pH 7.4 the dried beads swelled to its original size in about 1.5 hours followed by further swelling beyond its original size, then it gradually burst and dispersed over several hours. These results depict that, the dried beads keep their intact form in the stomach and when transferred to the intestine, the particles are likely to swell and function as matrices for controlled-release of incorporated drug in the intestine.

#### In-vitro release studies

The drug release profiles from beads are shown in Fig. 6. Under conditions mimicking those in the duodenum, pH 5.8, only a small amount of drug was released during the test (6h). This corresponded to the drug deposited on the surface of the beads. Once this drug was removed, the insoluble nature of the pH-dependent polymer and the controlled release characteristics of the Ca-alginate interpolymeric complex presented the release of the FLUP.

Alginate is known not to swell in acidic medium<sup>23</sup> and both types of beads remained intact during the 6 hr test and no change in shape were noted.

At low pH, the non-swelling should reduce the matrix permeability and limit the drug diffusion.

The release of FLUP from beads in phosphate buffer of pH 7.4 (Fig. 6) shows that ~45% of the entrapped FLUP is released in 6 hr in 1:2 beads and 35% in 1:4 beads. The release behaviour could be explained on the basis of swelling results (Fig. 5). Beads at pH 5.8 showed slight swelling rate while at pH 7.4 showed rapid rate with large swelling percent. The swelling created a porous structure that brought more liquid inside the beads.

It was reported that, water-soluble drugs are released both by diffusion through the polymer gel and by gel erosion while water insoluble drugs, as FLUP, are released primarily by erosion mechanism.<sup>24</sup> Additionally, the higher solubility of FLUP at pH 7.4 could be considered as an important factor in increasing the release rate. The effect of FLUP : sodium alginate ratio on the release of the drug in phosphate buffer at pH 5.8 and 7.4 was studied. It was observed that, the greater the content of the drug, the larger the amount of drug released especially at pH 7.4 (Fig. 6). The alginate beads constituting a matrix of cross linking insoluble gels. As the polymer content increases the gel network is progressively increased. On the other hand, FLUP is water insoluble substance and its release is strongly influenced by the swelling and erosion of alginate gel matrix with the result that, the release of the drug has a significant dependency on the FLUP alginate ratio. Similar findings were reported by Kierstan *et al.*<sup>25</sup> and Kim and Lea.<sup>19</sup>

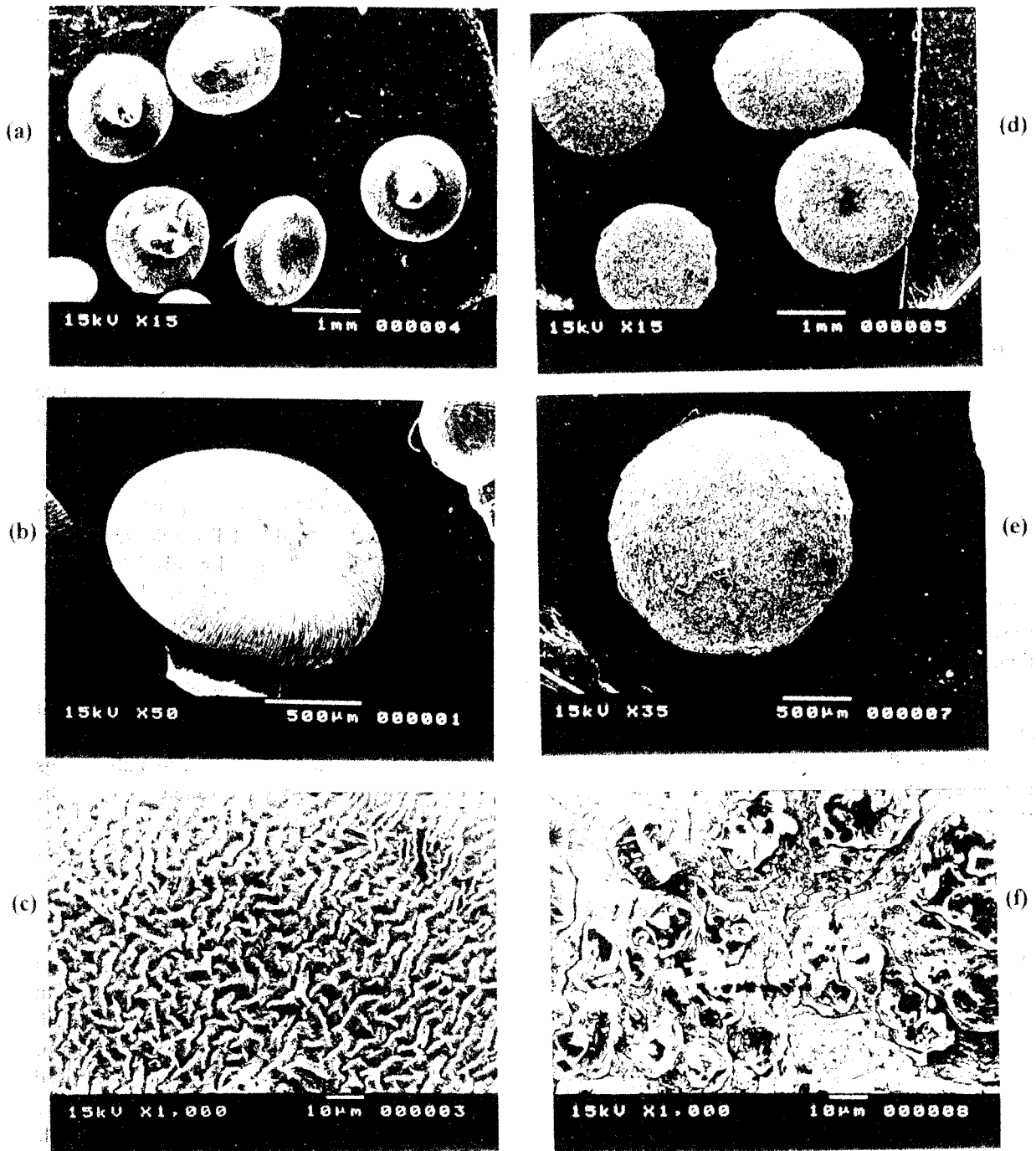


Fig. 4: Scanning electron micrographs of (a,b) shape and (c) surface of dried matrix beads and (d,e) shape and (f) surface of dried FLUP-alginate beads.

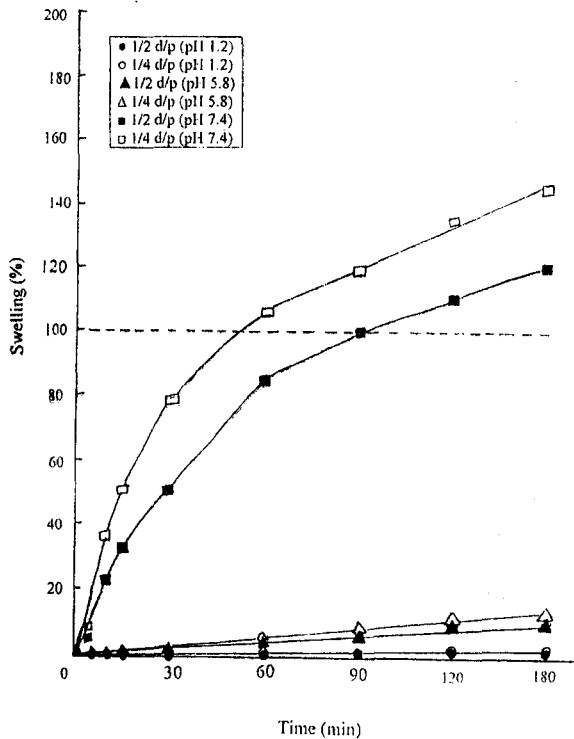


Fig. 5: Effect of dissolution medium pH on the swelling rate of dried FLUP-alginate beads prepared at 1:2 and 1:4 drug to polymer ratios.

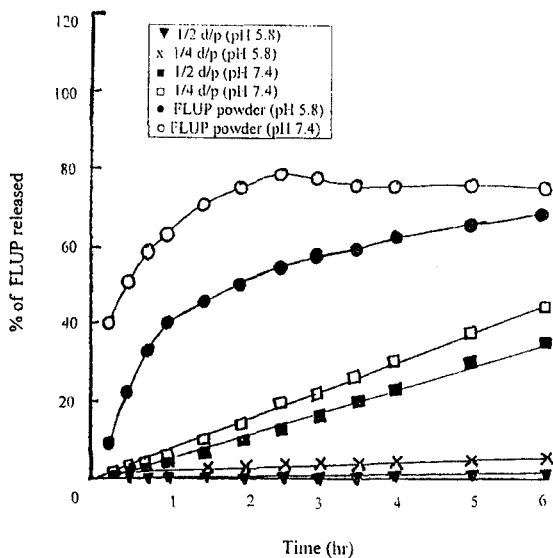


Fig. 6: Effect of dissolution medium pH on the release of FLUP from alginate beads prepared at 1:2 and 1:4 drug to polymer ratios.

### Kinetic of drug release

Analysis of the release data of FLUP from different beads were carried out using zero and first order kinetics as well as Higuchi Model, using the following equation:

$$q/A = 2 C_0 (Dt/\pi)^{0.5} \dots\dots\dots (1)$$

where  $q$  = amount of drug released (mg),  $D$  = diffusion coefficient ( $\text{cm}^2/\text{min}$ ),  $C_0$  = initial concentration of the drug in the beads,  $A$  = the surface area of the diffusion layer (bead), and  $\pi$  = constant. If  $K = 2C_0A (D/\pi)^{0.5}$ , equation (1) can be simplified to equation (2).

$$q = K (t)^{0.5} \dots\dots\dots (2)$$

Preference of some mechanisms was based on the correlation coefficient ( $r$ ) for the parameters involved. Further confirmation for the mechanism has been provided by plotting the percentage of drug release versus time, which was found to be linear (Fig. 6). The mathematical treatment of the release data, table 2, is in favor of zero-order mechanism.

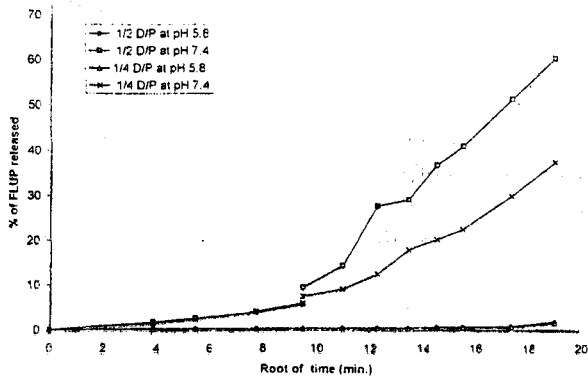
Swelling and erosion of alginate beads may have occurred during dissolution, so the kinetic of drug release was analyzed by adopting spherical matrix model.<sup>26</sup> In this equation of drug fraction released is related to the time according to the following equation:

$$3/2 [1 - (1-F)^{2/3}] - F = K.t \dots\dots\dots (3)$$

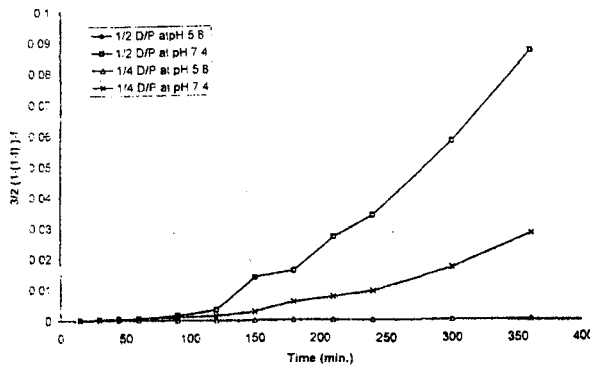
where  $F$  is the fraction of drug released and  $K$  is the release rate constant. Figures 7 and 8, and the correlation coefficient values (Table 2) proved that neither Higuchi model nor spherical matrix model were not applied since the two figures do not show a linear plot.

### Ulcerogenic activity

The results obtained throughout the study of the swelling of the beads at pH 1.2, 5.8 and 7.4 and the very slow release rates in acidic pH led to study of the effect of ulcerogenic activity of the free and loaded FLUP, in alginate gel beads, on rabbits stomach. The results obtained were prosperous and encouraging. The gastric mucosa of the animals administered free drug showed marked ulceration and hemorrhage with complete disappearance of mucosal surface in the ulcer region of the fundus and pylorus (Fig. 9) with numerous pin point ulcers on the lesser and greater curvature of the stomach. Loading of FLUP in alginate beads, induced no



**Fig. 7:** Effect of pH of the dissolution medium on the release of FLUP from alginate beads according to Higuchi's model.



**Fig. 8:** Plot of the release according to the spherical matrix model showing the effect of pH of the dissolution medium on the release of FLUP from alginate beads.

**Table 2:** Kinetic parameters for release of FLUP alginate beads prepared with 1:2 and 1:4 drug : polymer ratio at different pHs.

D/P ratio	pH of dissolution medium	Release order				Higuchi Diffusion model			Spherical matrix model	
		Zero		First		q vs t <sup>0.5</sup>		D (cm <sup>2</sup> /min)	r	slop (μg/ml.min) (Kx10 <sup>-5</sup> )
		r	K (μg/ml.min)	r	Kx10 <sup>-5</sup> (min <sup>-1</sup> )	r	K (μg/t <sup>0.5</sup> )			
1:2	5.8	0.862	0.003	0.861	3.85	0.793	0.07186	9.54x10 <sup>-4</sup>	0.726	0.0014
1:2	7.4	0.993	0.178	0.987	250.0	0.941	3.467	2.22	0.943	23.5
1:4	5.8	0.883	0.003	0.882	3.20	0.832	0.0630	1.02x10 <sup>-2</sup>	0.745	0.00098
1:4	7.4	0.995	0.101	0.988	3.29	0.941	1.970	10.05	0.928	7.16

r = Correlation coefficient  
 K = Specific release rate constant  
 D = Diffusion coefficient  
 q = Amount of drug release (mg)





Fig. 9: Stomach of rabbit given the pure drug (gross appearance).



Fig. 10: Stomach of rabbit given the drug loaded in alginate beads (gross appearance).

ulceration on the rabbits stomach with free appearance of hemorrhage and inflammation (Figure 10). This is due to the protective effect of alginate beads on the stomach by keeping their intact form with the absence of swelling and erosion mechanism. This led to a decrease in the points of contact between the drug and stomach, accordingly less or no ulcerogenic activity would happen.

### CONCLUSION

In conclusion, FLUP alginate beads were prepared using sodium alginate as gelling agent. The behaviour of the beads at low pH and the high release at high pH are of great interest for the delivery of drugs such as NSAIDs into the small intestine. The swelling property of the dried gel particles and the very slow release rate

in the simulating gastric fluid, led to study of the effect of FLUP beads on the ulcerogenic activity of the drug in the rabbits stomach. The results were encouraging which revealed the absence of ulcers and hemorrhage when drug is loaded as alginate beads.

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