

PREPARATION AND EVALUATION OF SULINDAC ALGINATE BEADS

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السوندك هو أحد العقاقير الغير كورتيكوستيرويدية التي تستخدم في علاج الالتهابات والآلام الروماتيزمية ولها تأثيرات جانبية خطيرة على الجهاز الهضمي مثل التهيج والتقرح والنزف في المعدة ولتلافي هذه الأعراض تم تحضير كريات من الجينات الكالسيوم محملة بالعقار بنسب ١:٢ ، ١:٤ (بوليمر:عقار). وقد تم دراسة تأثير نسبة البوليمر الي العقار والأس الهيدروجيني للأوساط (٧,٤ ، ٥,٥) على معدل إطلاق العقار من الكريات الألجينية المحضرة . وقد وجد أن معدل انطلاق العقار كان قليلا جدا في الأوساط الحامضة ومعقولا عند ٧,٤ أس هيدروجيني وفي كل الحالات كانت ميكانيكية معدل الانطلاق تتبع نظام الانتشار (نموزج هيجوشى). كما أوضحت الدراسة العملية أن معدل انطلاق العقار يقل بزيادة تركيز البوليمر . وقد تم دراسة تأثير العقار المحمل على الكريات الألجينية على النشاط التقرحي لأمعدة الأرانب وذلك بمقارنته بالعقار الغير محمل (العقار الحر) وقد وجد أن تحضير العقار في صورة كريات الالجينية ليس له أى نشاط تقرحي أو نزف لأمعدة الأرانب.

Sulindac (SUL) is one of the potent non steroidal anti-inflammatory drugs (NSAIDS) which has deleterious side effect on the gastro intestinal tract (GIT) such as irritation, ulceration and hemorrhage. Calcium alginate beads loaded with sulindac were prepared at 2/1 and 4/1 polymer to drug ratio. The effect of polymer to drug ratio and the pH of the dissolution medium (5.5 and 7.4) on the release profile of sulindac was investigated. In all cases the release rate mechanism followed Higuchi model with sustained release of sulindac from alginate beads. The Higuchi model mechanism was confirmed by the linearization of the data when spherical matrix model was adopted. The study investigated that as the polymer to drug ratio increased the amount of drug released progressively decreased. Also the release rate was significantly slower in the acidic side (pH 5.5) than that of pH 7.4. The effect of the intact drug as well as sulindac 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 was disappeared and the mucosal surface showed free of hemorrhage and inflammation when the drug is loaded with alginate beads.

INTRODUCTION

Algenic acid/Na alginate has a broad range of applications ranging from pharmaceutical and food adjuvant to an immobilization matrix for cells and enzymes due to its cation-induced gelation.^{1,2}

Because of the reswelling properties of alginate beads which are susceptible to pH medium, the beads have the following advantages:(1) acid-sensitive drugs are protected from gastric juice, (2) the reswelling process of

beads in the intestine offers controlled-release drug delivery system, (3) appropriately-sized particles of beads avoid local build-up of released drugs, (4) alginate is known to be nontoxic when taken orally.³

It was reported that alginate beads have a protective effect on the mucous membranes of the upper gastrointestinal tract (GIT).^{4,5} Also, alginates suppressed a food and acid reflux into the oesophagus.⁶

Several reports illustrated that alginate beads can be used for controlling drug release,

e.g: theophylline,⁷ propranolol,⁸ herbicides,⁹ metribuzin,¹⁰ and ibuprofen.¹¹

However, porosity gives alginate beads not only a fast release pattern of incorporated drugs but also a very low efficiency of incorporation of low molecular weight drugs,⁹ except for sparingly soluble drugs or drugs bound to macromolecules through covalent or noncovalent bonds.¹²

It is well documented that non steroidal anti-inflammatory drugs (NSAIDs) can cause GIT deleterious disorders such as irritation, ulceration and hemorrhage.^{13,14} Several trials have been carried out to eliminate and/or suppress these side effects. The formulations of these drugs, in the form of sustained or controlled release dosage forms, reduce or eliminate GIT irritation and bleeding with maintaining constant drug blood level over an extended period.

Sulindac [5-fluoro-2-methyl-1-(4-methyl sulphanylbenzylidene) indane-3-yl] acetic acid is one of the potent (NSAIDs). It is a prodrug and its sulfide metabolites is an inhibitor of cyclooxygenase. It is used in musculoskeletal and joints disorders such as osteoarthritis and rheumatoid arthritis.¹⁵ Sulindac suppresses colon carcinogenesis in man and experimental animals,¹⁶ due to its specific cyclooxygenase-2 inhibitor.¹⁷

Reports on sulindac beads are scarce and its ulcerogenic activity has not been justified. For these reasons sulindac alginate beads were prepared and the effect of pH of the dissolution medium and polymer to drug ratio on the release profile of sulindac were investigated in these study. Moreover the ulcerogenic activity of the intact drug and drug loaded beads were elaborated in rabbits stomach.

EXPERIMENTAL

Materials

- * Sulindac (SUL) confirmed to the USP standard and has particle size < 100 μm .
- * Sodium alginate (merck)
- * Calcium chloride (B D H)
- * All other chemicals were of reagent grades.

Methods

Preparation of alginate beads

Sulindac was dispersed homogeneously in aqueous solution of sodium alginate (6% w/v). The bubble-free suspension was forced out of a 10 ml syringe nozzles (~1mm i.d) into gently stirred calcium chloride solution (0.1 M). The flow rate was kept constant of about 10-12 drops /min. The height of fall down to the surface of CaCl_2 solution was 25 cm. Alginate gel beads were allowed to stand in CaCl_2 solution for 72 h. to be fully cured, then the beads were separated by filtration, washed with dist. water and dried at room temperature for 24 h. followed by placement in vacuum at room temperature for additional 24 h. Alginate beads with two polymer/drug ratios (2/1 and 4/1) were prepared. In all cases the ratio of polymer to CaCl_2 solution volume was constant (1 g of alginate for 100 ml. of CaCl_2 solution). The procedure were carried out at room temperature to minimize the variation in the viscosity of the alginate solution.¹²

Determination of drug content

Alginate beads (20 mg.) 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 sulindac was measured spectrophotometrically (Shimadzu, Kyoto, JAPAN) at 285 nm after appropriate dilution.¹²

Scanning electron microscope

Scanning electron microscope (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.

Swelling rate of dried gel beads

The resulting dried beads were gently incubated in solutions of different pH (1.2, 5.5 and 7.4), to assimilate the pHs of GIT through out the passage of the beads from stomach to intestine, at 37°C. The diameter of each swelling beads, taken out of the solution, was

measured with micrometer at three different position and the average of five particles was calculated at 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.

Release studies

The release of sulindac was determined using USPXX1 paddle apparatus. Sulindac powder (33.33 and 20 mg) or its beads (equivalent to 33.33 and 20 mg drug for 2/1 and 4/1 polymer to drug ratio respectively) were introduced into 200 ml of the release medium of pH 5.5, and 7.4 using phosphate buffer B.P at 37°C. The drug concentration (of the withdrawn samples at time intervals) was determined spectrophotometrically at $\lambda 285$ nm. The mean of three determinations were reported.

Ulcerogenic activity

The assessment of the ulcerogenic activity of sulindac from the selected release formulations was studied in two groups of albino rabbits, (three for each 1.5-2 Kg). The rabbits were kept free of diet and water two hours before and after drug administration. An oral dose of the intact drug (8 mg) or an amount of beads equivalent to 8 mg of the drug (in gelatin capsule) were administered twice daily, with 10 ml 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 ulcer and then photographed.¹⁸

RESULTS AND DISCUSSION

A rapid production of homogeneous sulindac gel beads is obtained if the viscosity of the initial drug suspension is between 90 and 3000 cP. In this range, drug sedimentation is minimized and reasonable flow rate is achieved.¹⁹ The viscosity of sulindac suspended in sodium alginate solution, in the present study, was ~490 cP (Brookfield Viscometer, Massachusetts U.S.A.).

Drug content and encapsulation efficiency

Calcium alginate beads were prepared by dripping suspension of sulindac in sodium alginate solution into gently stirred calcium chloride solution.

The physical characteristics of the produced beads were illustrated in Table 1, which depicts that less shrinkage was observed with the increase in the amount of loaded drug, viz. the diameter of dried beads of 2/1 > that of 4/1 polymer to drug ratio. The reproducibility of sulindac content in the beads (30.75% and 17.81% for 2/1 and 4/1 polymer/drug ratio respectively) was also observed, this may attributed to the low solubility of sulindac in calcium chloride solution or in water (<0.003 mg/ml).²⁰ Consequently very small amount of the drug was lost during curing period or washing process. With the increase in drug loaded, the encapsulation efficiency increased due to the decrease in the percent of drug lost during preparation process. This is valid with that reported by T. Østberg.¹⁹

Shape and surface characteristics

Scanning electron microscope micrographs, Fig. 1 showed that the surface of the dried beads is uneven rough with some loss in their sphericity and became small dense matrices in which sulindac crystals are embedded. Cracks and fissures in the matrix are observed, few crystals are also seen on the surface. These can be formed during drying, by crystallization of dissolved drug that migrates along with the water to the bead surface.



Fig. 1: Scanning electron micrograph of dried sulindac alginate beads.

Swelling rate of dried gel beads

Figure 2 shows the swelling behavior of dried sulindac beads in different buffer solution at pH 1.2, 5.5 and 7.4. 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.³ 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 swelling was observed in pH 1.2, while in pH 5.5, very slight swelling (~12-18%) was observed at the end of the experimental time. In pH 7.4 the dried beads swelled to its original size in about 1.5 hour 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.

Release study

Release profile of sulindac from powder (<100 μm) or its beads prepared at 2/1 and 4/1 polymer/drug ratio into phosphate buffer of pH 5.5 and 7.4 was illustrated in figure 3. The release of sulindac from its powder was completed in about 15 min at pH 7.4 and 30 min at pH 5.5, this is due to the higher solubility of sulindac in solution of pH 7.4 than that of pH 5.5 (3.4 and 0.35 mg/ml respectively).²⁰

The release of sulindac from its beads (2/1 or 4/1 polymer to drug ratio) in pH 5.5 is very slow when compared to that of pH 7.4, the latter is seemed to be reasonable. This behavior could be explained on the basis of swelling results (Fig. 2). Beads at pH 5.5 showed slight swelling rate while at pH 7.4 showed rapid rate with large swelling percent. The swelling created porous structure and 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 sulindac) are released

primarily by erosion mechanism.²¹ Additionally, the higher solubility of sulindac in pH 7.4 could be considered as an important factor in increasing the release rate. The effect of sulindac/sodium alginate ratio on the release of the drug into phosphate buffer at pH 5.5 and 7.4 was studied. It was observed that the greater the content of alginate, the slower was the rate of release of the drug (Fig. 3). The alginate beads constituting a matrix of cross linking insoluble gel. As the polymer content increases the gel network is progressively increase. On the other hand, sulindac 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 sulindac alginate ratio. Similar findings were reported by Kierstan *et al.*²² and C. K. Kim and E. J. Lea.¹²

Kinetic of drug release

The data of sulindac release were fitted to first-order and Higuchi's equations. Zero order was not applied since the plot of the amount of drug released versus time don't show a linear plot (Fig. 3). Table 2 summarized the correlation coefficient (r) and the release rate constant, corresponding to different release mechanisms, for sulindac release from 2/1 and 4/1 (polymer / drug ratio). The (r) values obtained depict that the release mechanism is diffusion controlled, as plots of the percent of drug released versus square root of time were found to be linear (Fig. 4).

Swelling and erosion of alginate beads may have occurred during dissolution, so the kinetic of drug release was analyzed by adopting spherical matrix model.²³ In this equation the drug fraction released is related to the time according to the following equation: $3/2[1-(1-F)^{2/3}]-F=K.t$, where F is the fraction of drug released and K is the release rate constant. Figure 5 and the correlation coefficient values obtained (Table 2) proved the linearization of the data. This confirming that the release mainly followed diffusion mechanism.

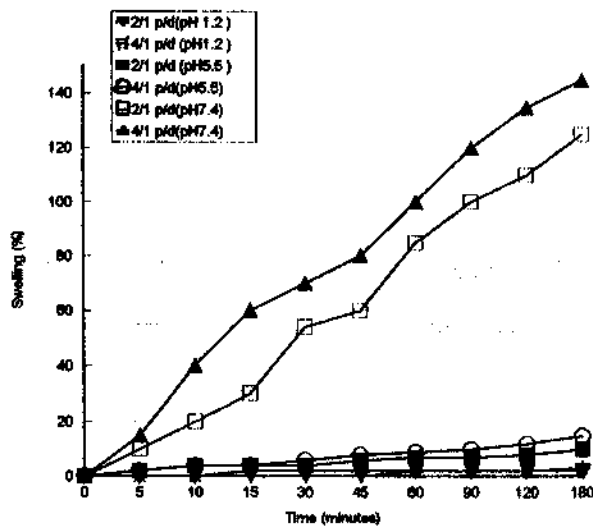


Fig. 2: Effect of dissolution medium pH on the swelling rate of dried sulindac alginate beads prepared at 2/1 and 4/1 polymer to drug ratio.

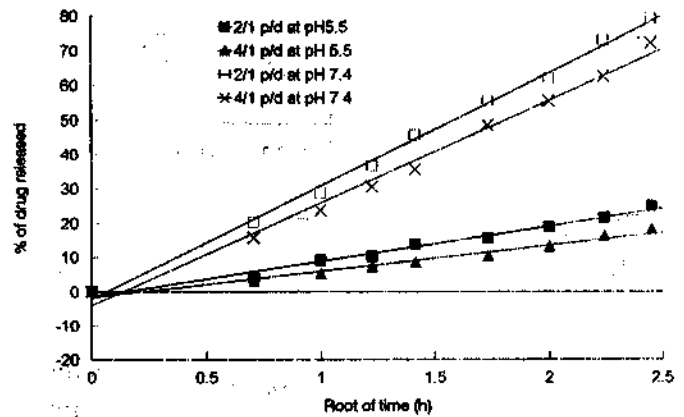


Fig. 4: Higuchi plot showing the effect of pH of the dissolution medium (5.5 and 7.4) on the release of sulindac from alginate beads prepared at 2/1 and 4/1 polymer to drug (p/d) ratio.

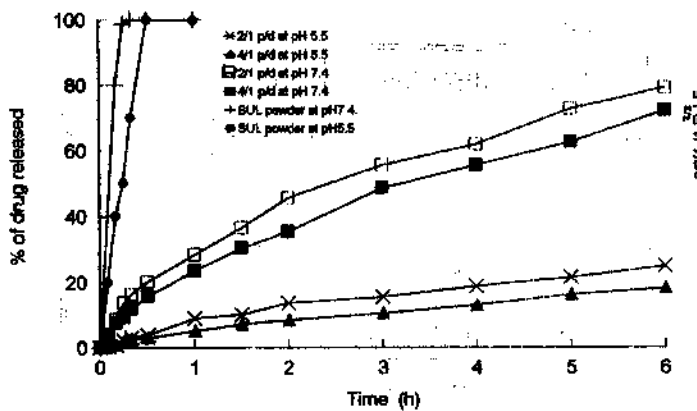


Fig. 3: The effect of pH of dissolution medium on the release of sulindac from alginate beads prepared at 2/1 and 4/1 polymer to drug ratio into phosphate buffer (pH 5.5 and 7.4) at 37°C (mean of three experiments).

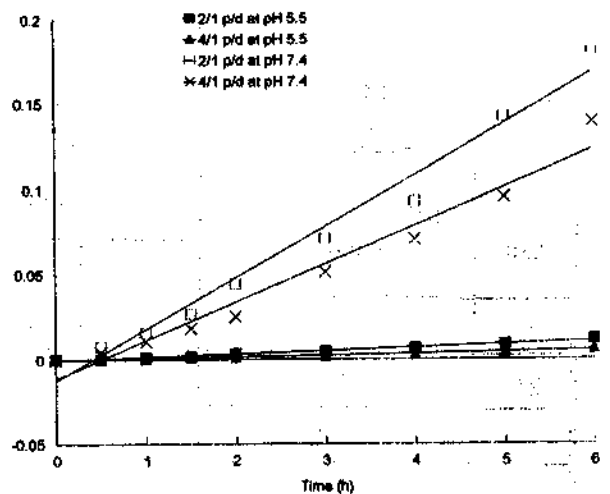


Fig. 5: Plot of the release according to the spherical matrix model showing the effect of pH of the dissolution medium on sulindac release from alginate beads prepared at 2/1 and 4/1 polymer to drug ratio.

Table 1: Characteristics of Ca alginate beads loaded with sulindac at 2:1 and 4:1 polymer : drug ratio.

P/D ratio	diameter (m.m)		Shrinkage	Weight	Drug	Encapsulation
	Before drying	After drying	%	(mg/bead)	content %	Efficiency %
2/1	4.58 ± 0.15	1.85 ± 0.02	246.77	3.28 ± 0.02	30.75 ± 0.67	92.25
4/1	3.98 ± 0.07	1.32 ± 0.01	298.47	2.82 ± 0.03	17.82 ± 0.2	89.07

Mean ± S.D

P/D: polymer ratio / drug

$$\text{Shrinkage \%} = \frac{\text{diameter before drying}}{\text{diameter after drying}} \times 100$$

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Table 2: Effect of the dissolution medium pH on the release kinetics of sulindac alginate beads prepared at 2/1 and 4/1 polymer to drug ratio.

P/D ratio	pH of dissolution medium	Frist Order		Higuchi diffusion model		Spherical matrix model	
		r	K (h ⁻¹)	r	D (cm ² . hr)	r	K
2/1	5.5	0.979	0.019080	0.992	10.26840	0.992	0.00189
2/1	7.4	0.996	0.106893	0.998	32.86890	0.990	0.03011
4/1	5.5	0.990	0.013488	0.986	07.55930	0.984	0.01101
4/1	7.4	0.995	0.086000	0.995	29.80110	0.984	0.02224

- r : correlation coefficient.
 K : specific release rate constant.
 D : diffusion coefficient.

Ulcerogenic Activity

The results obtained throughout the study of the swelling behaviour of the beads (in pH 1.2, 5.5 and 7.4) and the very slow release rates in acidic pH led to study the effect of ulcerogenic activity of the free and loaded sulindac, in alginate 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 (plate I) with numerous pin point ulcers on the lesser and greater curvature of the stomach. Loading of sulindac in alginate beads, induced no ulceration on the rabbits stomach with free appearance of hemorrhage and inflammation (plate II) 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 decrease in the points of contact between the drug and stomach,

accordingly less or no ulcerogenic activity may happen.

Conclusion

Sulindac alginate beads were prepared using sodium alginate as gelling agent. The ratios of polymer to drug were 2/1 and 4/1. The shape characteristics and the release profile were studied. In all cases the release kinetics in favor of diffusion mechanism. The effect of the dissolution medium pH and polymer/drug ratio on the release rate was also studied. At acidic pH the release rates were very slow when compared to that of pH 7.4. The swelling behavior of the beads and the very slow release rate in the simulating gastric fluid, led to study the effect of sulindac beads on the ulcerogenic activity of the drug in the rabbits stomach. The results were encouraging which investigated the absence of ulcers and hemorrhage when drug is loaded as alginate bead.



Plate I: Stomach of rabbit given the intact drug (gross appearance).



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

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