

PREPARATION, CHARACTERIZATION, AND *IN VITRO* RELEASE OF KETOPROFEN LOADED ALGINATE MICROSPHERES

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ABSTRACT

Objective: Ketoprofen is a nonsteroidal anti-inflammatory drug which could benefit if fabricated in a sustained delivery system. This work aims to develop the sustained release delivery system for ketoprofen using natural polymers.

Methods: Alginate microspheres were prepared employing crosslinking method employing calcium chloride. The formation and compatibility of microspheres was confirmed by FT-IR. The formed microspheres were evaluated for percentage yield, drug content, drug loading, and encapsulation efficiency. *In vitro* release studies were carried out in phosphate buffer (pH 7.4).

Results: FT-IR spectra exhibited the successful compatible encapsulation of ketoprofen in alginate microspheres. The formed microspheres exhibited good drug loading and encapsulation efficiency. The drug release was sustained over a period of 8 hours. There was a better release profile in phosphate buffer in formulations containing 3:1 polymer to drug ratio.

Conclusion: It was feasible to prepare alginate-based microspheres capable of sustaining ketoprofen release over an extended period of time.

Keywords: Microspheres, Sustained release, Ketoprofen, Alginate.

INTRODUCTION

Microspheres are one of the multiparticulate drug delivery systems that are employed for prolonged or controlled drug delivery. Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000 μm , containing dispersed drug¹. They could be administered orally or injected into the body due to their small size. Biocompatibility of the polymer forming the microspheres and its degradation products is essential for any material that will be in contact with living tissues². Additionally, biodegradable polymers are preferred for drug delivery applications, since the need for removal of the depleted device is eliminated. Although the number of biodegradable polymers is large, only a limited number of polymers are suitable for drug delivery applications. Suitable candidates must not only be biodegradable but also fit the high prerequisites of biocompatibility, processability, and storage stability if it is to be useful for biomedical applications³.

Alginates are naturally occurring substances found in brown algae which have received considerable attention as a vehicle for controlled drug delivery. Alginates are block polymers which mainly consist of mannuronic acid (M), guluronic acid (G) and mannuronic-guluronic (MG) blocks. Alginate is known to be nontoxic when taken orally and has a protective effect on the mucous membranes of the upper gastrointestinal tract⁴. Oral nonsteroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal disturbances at a high incidence, which is an obstacle in increasing doses to obtain sufficient therapeutic effect. Ketoprofen is a propionic acid derivative NSAID and is used in an anti-inflammatory and analgesic drug for the treatments of rheumatoid arthritis and osteoarthritis⁵. Ketoprofen exhibits poor tolerability profile. Some common adverse effects are gastrointestinal tract complaints such as nausea, vomiting and epigastric discomfort⁶.

The aim of this work is to formulate and characterize alginate microspheres for the sustained delivery of a model drug, ketoprofen.

MATERIALS AND METHODS

Materials

Ketoprofen was purchased from Modern Pharma (Sana'a, Yemen). Sodium alginate was purchased from B. M. S (Italy). All chemicals used were of analytical grade and were used as received.

Preparation of microspheres

Sodium alginate microspheres containing ketoprofen were prepared by cross-linking technique followed by Fattah et al⁷ and Bert et al⁸ with minor modifications. Sodium alginate was dissolved gradually in distilled water at different concentrations. Drug polymer solution was prepared by dissolving the drug slowly into previously prepared alginate solution with continuous and uniform stirring for 30 minutes. A gelation medium was prepared separately by dissolving 5g of calcium chloride in 100 ml distilled water. Bubble free dispersion medium was extruded by dropping through glass syringe (20G) into the gently agitated CaCl_2 solution. The agitation was carried out by the propeller at 200rpm.

After 1 hour, 2 ml of isopropyl alcohol were added drop wise to harden the formed microspheres⁹. After 10 minutes, the microspheres were removed by filtration from the solution and washed with de-ionized water. The microspheres were dried at 45°C until they attained constant weight.

Characterization of microspheres

FT-IR

The IR spectrum of the drug, polymer and prepared microspheres were obtained to confirm the encapsulation of the drug in the microsphere as well as the stability of the formed microspheres. Measurements were performed using Perkin Elmer Spectrum 65 (Wisconsin, MI, USA). The particles were finely grounded with KBr to prepare the pellets under a hydraulic pressure of 600 dynes/cm² and spectra were scanned between 4000 to 400 cm⁻¹.

Percentage yield

The dried microspheres were weighed and the percentage yield of the prepared microspheres was calculated by using the following formula.

Percentage yield = $\left\{ \frac{\text{The weight of microspheres}}{\text{The weight of polymer + drug}} \right\} * 100$

Drug content

The various batches of the microspheres were subjected for drug content analysis. Accurately weighed microsphere samples were mechanically powdered. Powdered microspheres were dissolved in

adequate quantity of phosphate buffer PH 7.4 then filtered. The UV absorbance of the filtrate was measured using a UV spectrometer at 262 nm[10].

Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency were determined for all batches using the following formulas. Values are expressed as percentage.

Drug loading = {Weight of drug in microspheres / Microspheres sample weight} * 100

Encapsulation efficiency = {Actual weight of drug in sample/ Theoretical weight of drug} * 100

In vitro drug release

The study of the release of ketoprofen was carried using 50 ml of phosphate buffer pH 7.4 as a dissolution medium. 20 mg equivalent ketoprofen containing alginate microspheres was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Five millilitres of sample were withdrawn at specific time interval for 8 hours. The sample volume was replaced by an equal volume of fresh medium. The concentration was determined spectrophotometrically at 262 nm.

Table 1: Composition of alginate microspheres

Formulation	Alginate content (g)	Drug content (g)
F1	4.5	3
F2	4.5	1.5
F3	3	3
F4	3	1.5

RESULTS AND DISCUSSION

Four different formulations were prepared varying in polymer and drug concentrations (Table 1). The drug to polymer ratio of the different formulations was 2:3, 1:3, 1:1, and 1:2.

The entrapment and stability of ketoprofen in the alginate microspheres were investigated by infrared spectroscopy study (IR). Fig. 1 represents the IR spectra of ketoprofen, alginate, and formed microspheres. Ketoprofen spectrum shows absorption bands for C=O stretching vibration of acid, C=O stretching vibration of ketone, O-H band and C=C stretching vibration of the aromatic ring appeared at 1697, 1655, 3380, and 3100 cm^{-1} , respectively.

IR spectrum of alginate showed intense absorption bands at 1796 cm^{-1} and 1874 cm^{-1} due to C=O vibrations of an acid group. Multiple bands in the range of 3400-3600 cm^{-1} can be attributed to O-H alcohol and acid. The IR spectrum of the alginate microspheres encapsulating ketoprofen showed the broad bands in the 3658, 3620, 3413 cm^{-1} range, which are attributed to the stretching vibration of O-H group of ketoprofen and alginate compounds.

The distinctive peak which appeared at 3100 cm^{-1} was assigned for the C=C aromatic group of ketoprofen. Also a strong intensity band was observed at 1658 cm^{-1} due to C=O ketone of ketoprofen. Similarly, the C=O acids of ketoprofen and alginate were observed at 1795 cm^{-1} and 1886 cm^{-1} .

Thus, the IR study indicates the stable nature of ketoprofen in the prepared alginate microspheres. IR spectra of the microspheres indicate that there is no strong interaction between the drug and the polymers. Hence, drug excipients compatibility was established which indicates the stable nature of the drug during the entrapment process.

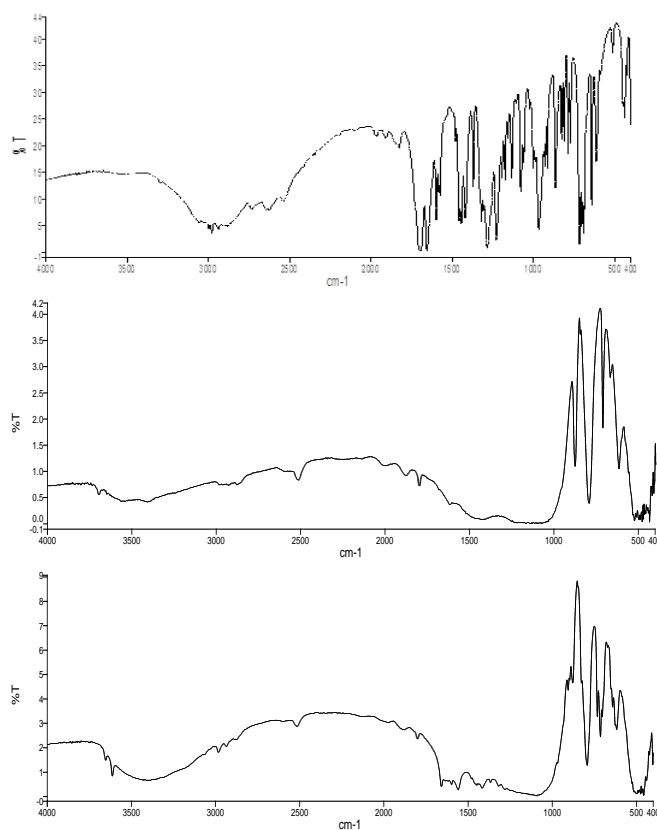


Fig. 1: IR spectra of ketoprofen (upper), alginate (middle), and ketoprofen-loaded microspheres (lower)

The percentage yield of formulations was found out to be 71.40%, 87.51%, 77.24%, and 75.58% for formulations F1-F4 respectively (Table 2). Higher drug content (and hence, percentage yield) was

observed in formulations containing 3g alginate polymer and 3g ketoprofen (formulation containing 1:1 drug to polymer ratio). A study reported that various factors such as drug to albumin ratio,

concentration of surfactant, stirring rate of the emulsion and average size of microspheres could affect drug loading[11]. They found that drug loading could be increased by increasing drug to albumin ratio, decreasing surfactant concentration and increasing the stirring rate. The results of the variation in drug loading and encapsulation efficiency with polymer: ketoprofen ratio are shown in table 3. Higher percentage of loading was obtained by increasing the amount of the drug with respect to alginate. Formulations

exhibited good encapsulation efficiencies between the range of 70.88% to 96.12% depending on the composition of the different microspheres. The high percentage yields obtained in our study indicates that this method was very useful for adoption in the formulation of ketoprofen microspheres. Higher percentage yield was observed for formulations containing higher drug to polymer ratio. These findings comply well with results reported in other studies [12].

Table 2: Drug content and percentage yield of microspheres.

Formulation	Percentage yield (%)	Drug content (G)
F1	71.40	1.774
F2	87.51	1.005
F3	77.24	2.68
F4	75.58	1.017

Table 3: Drug loading and encapsulation efficiency of microspheres

Formulation	Drug loading (%)	Encapsulation efficiency (%)
F1	33.12	74.31
F2	19.11	70.88
F3	59.98	96.12
F4	32.55	85.32

In vitro drug release studies were carried out employing phosphate buffer (pH 7.4). Fig. 2 and 3 show the cumulative release of ketoprofen from alginate microspheres in phosphate buffer. The drug release was prolonged for up to 8 hours in all formulations. Formulation F2 with 3:1 polymer to drug ratio showed lowest burst release.

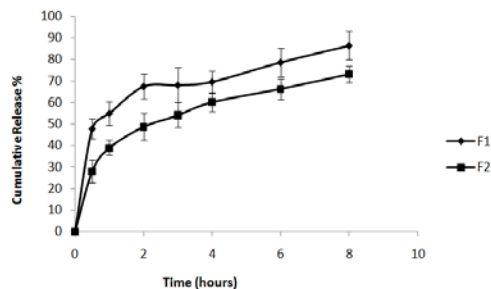


Fig. 2: *In vitro* release of ketoprofen in phosphate buffer from formulations F1 and F2. (n=4)

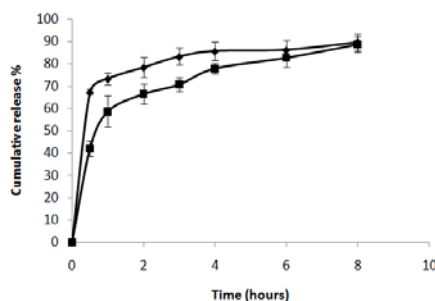


Fig. 3: *In vitro* release of ketoprofen in phosphate buffer from formulations F3 and F4. (n=4)

The release of ketoprofen from the alginate microspheres could be attributed to diffusion and/or erosion. The drug could diffuse out of the alginate microspheres. Secondly, the drug can be released from the alginate microspheres through the erosion of the matrix. The erosion is due to the removal of the cross-linker bivalent cation,

calcium, from the alginate microspheres [13]. Even if the microspheres did not burst, large swelling occurred and thus increased matrix porosity. These findings comply well with the higher drug to polymer ratio used in formulation F1 [14].

The disruption of the alginate matrix occurred fast in a phosphate buffer because of the chelating action of phosphate ions and at pH 7.4, the affinity to phosphate was higher than that to alginate, and consequently, the drug was released from alginate microspheres through the continuous erosion of the microspheres. Both of our formulations exhibited a sustained release of risperidone over a period of 8 hours. A slower release pattern was observed for formulation containing higher amounts of the polymer. Kilicarsan and Baykara investigated the effect of the drug/polymer ratio on properties of the verapamil loaded microspheres [15].

They found that the drug release profile could be sustained by increasing polymer amount. Similarly, insulin and diaminopyridinemicroparticles were prepared by solvent evaporation method and drug/polymer ratio was shown to affect microspheres characteristics and drug release [16,17]. The initial burst release could be related to the surface drug as well as small size microspheres which might be due to the fact that smaller particles offered more surface area to release the drug. A similar release pattern has been reported elsewhere in which about 50% of the loaded drug released within 1 h, which was attributed to the amount of drug adsorbed on the surface of microspheres[18].

CONCLUSION

Alginate-based microspheres of ketoprofen were successfully prepared and separated by cross-linking technique. The formed microspheres showed good percentage yield and encapsulation efficiency. *In vitro* drug release showed prolonged release of ketoprofen employing phosphate buffer as a release media. Slower drug release was observed with increasing the polymer concentration. It was feasible to prepare alginate-based microspheres capable of sustaining drug release over an extended period of time. Such a system has several advantages for drug delivery. In the case of ketoprofen, this system could reduce the GIT-related side effects inherent to the drug. The drug delivery system could serve to deliver many drugs that could benefit from such sustained delivery.

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REFERENCES

- Vyaas SP, Khur RK. Targeted and controlled drug delivery-Novel carrier systems. Delhi: CBS publishers and distributors; 2006.
- Kronenthal RL. Biodegradable polymers in medicine and surgery. In: Kronenthal RL, Oser Z, Martin E, editors. Polymers in medicine and surgery. New York: Plenum Press; 1975.
- Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
- Kim CK, Lee EJ. The controlled release of blue dextran from alginate beads. Int J Pharm 1992;79:11-9.
- Fossgreen J, Kirchheiner B, Petersen FO, Tophoj E, Zachariae E. Clinical evaluation of ketoprofen in rheumatoid arthritis-double blind cross over comparison with indomethacin. Scand J Rheumatol Suppl 1976;14:93.
- Hilgendorf C, Spahn LH, Regardh CG, Lipka E. Caco-2 versus caco-2/HT29-MTX cocultured cell lines: permeabilities via diffusion, inside and outside-directed carrier-mediated transport. J Pharm Sci 2000;89:63.
- Fattah DJ, Grant KE, Gabar Meshali MM. Physical characteristics and Release behaviour of salbutamol sulfate Beads prepared with different Ionic polysaccharides. Drug Dev Ind Pharm 1998;24:541-7.
- Bert O, Haglund, Satyanarayana M, Steven HN, Michel A. Preparation and evaluation of sustained release agarose beads containing ibuprofen and indomethacin. Control Drug Develop Ind Pharm 1994;20:947-57.
- Tafaghodi M, Sajadi Tabasi SA, Jaafari MR. Formulation and characterization and release studies of alginate microsphere encapsulated with tetanus toxoid. J Biomat Sci Polymer Ed 2006;17:909-24.
- Venkatesan P, Manavalan R, Valliappan K. Preparation and evaluation of sustained release loxoprofen loaded microspheres. J Basic Clin Pharm 2011;2:159.
- Chou, WH, Tsai TR, Hsu SH, Cham TM. Preparation and *in-vitro* evaluation of nifedipin-loaded albumin microspheres cross-linked by different glutaraldehyde concentrations. Int J Pharm 1996;144:241-5.
- Sudhamani T, Noveenkumarreddy K, Ravi Kumar VR, Revathi R, Ganesan V. Preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery. Int J Pharm Res Dev 2010;1:119-25.
- Sanghvi SP, Nairn JG. Effect of viscosity and interfacial tension on particle size of cellulose acetate trimellitate microspheres. J Microencapsulation 1992;9:215-27.
- Perumal D. Microencapsulation of ibuprofen & Eudragit RS 100 by the emulsion solvent diffusion technique. Int J Pharm 2001;218:1-11.
- Kilicarslan M, Baykara T. The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. Int J Pharm 2003;252:99-109.
- Gibaud S, Bonneville A, Astier A. Preparation of 3, 4-diaminopyridine microparticles by solvent-evaporation methods. Int J Pharm 2002;242:197-201.
- Venkatesan P, Muralidharan C, Manavalan R, Valliappan K. Selection of better method for the preparation of microspheres by applying analytic hierarchy process. J Pharm Sci Res 2009;1:64-78.
- Sahin S, Selek H, Ponchel G, Ercan MT, Sargon M, Hincal AA, Kas HS. Preparation, characterization and *in-vivo* distribution of terbutaline sulfate loaded albumin microspheres. J Cont Rel 2002;82:345-58.