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IN-VITRO DEVELOPMENT AND EVALUATION OF METFORMIN BEADS AIMED FOR ORAL THERAPY OF TYPE II DIABETES

Annotation: *In this study, prepare entrapped metformin HCL beads using sodium alginate as the polymer for oral administration and to evaluate the sustain release of the drug (in vitro release studies).*

Keywords: *Metformin Hydrochloride, ionotropic gelation, Sustained release, encapsulation efficiency, Diabetes.*

IN-VITRO РАЗРАБОТКА И ОЦЕНКА МЕТФОРМИНОВЫХ ШАРИК, НАЗНАЧЕННЫХ ДЛЯ ОРАЛЬНОЙ ТЕРАПИИ ДИАБЕТА ТИПА II

Аннотация: *Цель исследования приготовить захваченные шарики метформина HCL с использованием альгината натрия в качестве полимера для перорального введения и оценить длительное высвобождение лекарственного средства (исследования высвобождения in vitro).*

Ключевые слова: *in-vitro разработка и оценка метформиновых шарик, назначенных для оральной терапии диабета типа ii.*

1. Introduction:

Metformin hydrochloride is an oral hypoglycemic agent, it widely used for the treatment of type 2 diabetes and to reduce the risk of diabetes- related end-points such as myocardial infarction, stroke, amputation, renal failure, blindness, and death[1, c.3709][2, c.114]. The joint guidelines issued by the American Diabetes Association and the European Association for the Study of Diabetes strongly and repeatedly suggest that metformin should be used as the first-line agent of choice alongside lifestyle modification at diagnosis of type 2 diabetes[3, c.17][4, c.1022].

Metformin is the only biguanide available, it is water soluble but the poor bioavailability (~ 52%) and short half-life (~ 1.5 – 4.7 h) of this drug make the development of extended-release formulations desirable, in order to improve patient compliance and reduce the dosing frequency, resulting in better glycemic control and less side effects such as diarrhoea, nausea, anorexia, vomiting and weight loss are increased[5, c.820][6, c.1227][7, c.403].

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[8, c.25]. Beads can provide sustained release properties and a more uniform distribution of drugs within the gastrointestinal tract[8, c.25] [9, c.293].

Sodium alginate, a salt of alginic acid, is a natural, polyanionic, non-toxic water-soluble copolymer of α -L-gluronic acid and β -Dmannuronic acid residues, commonly utilized in the design of sustained drug delivery systems[7, c.403] [10, c.1]. It has the ability to form a gel network in the presence of divalent cations (cross-linking agent) such as calcium in aqueous media. Thus, alginate beads are successfully prepared by ionotropic gelation technique [11, c.1497]. Sodium carboxymethyl cellulose (Na CMC) is a Swellable polymer generally used with Sodium alginate for prolonged released and sustained release action or activity [12, c.189].

The main objective of this study was to develop micro-particulate bead system of metformin HCL by ionotropic gelation technique employing sodium alginate and

sodium carboxymethyl cellulose as rate controlling polymers for sustained-release delivery.

2. MATERIALS AND METHODS:

2.1. Materials: Metformin HCl (Sigma-Aldrich/UK), Sodium Alginate (BDH Chemicals Limited/ UK), calcium chloride (Sigma-Aldrich/UK), HCL 37% (Sigma/Germany), monopotassium phosphate and dipotassium phosphate (Fluka Steinheim/Germany), Sodium carboxymethyl cellulose (Sigma-Aldrich/UK), distilled water.

2.2. PREPARATION OF STANDARD GRAPH

Pure drug sample of Metformin HCl was taken in volumetric flask of 10 ml capacity and the volume was made up with Phosphate buffer at pH 7.4 Stock solution prepared by using 10 mg/10ml equivalent to 1000 µg/ml. Concentrations of 3 µg/ml, 5 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml and 15 µg/ml were prepared and absorbance was taken at 232 nm [13,c.56] [14,c.34].

2.3. PREPARATION OF METFORMIN BEADS

After preliminary studies, Na alginate beads containing metformin HCL in various ratios with Na CMC were prepared (as shown in table. 1). Sodium alginate solutions of 1% concentrations was prepared by dissolving 1 gr of alginate in 100 ml of purified water, the solution was put in mechanical stirrer for 30 min till formation of clear solution and 1 gr of Metformin HCL was dispersed in this solution under constant stirring for uniform mixing.

The dispersion was sonicated for 30 minutes to remove air bubbles. The resultant dispersion was dropped through a syringe needle into 100 ml of 10 % (w/v) calcium chloride solution at room temperature.

Beads formed were allowed to stand for 30 min for the curing reaction, then the beads were filtered, washed with distilled water and subsequently oven-dried at room temperature for 24 h [12,c.189] [13,c.56] [15,c.1].

Table 1: Composition of Metformin hydrochloride beads formulation

Formulation	Drug (w/v %)	Na alginate (w/v %)	Na CMC (w/v %)	cross-linking agent (CaCl ₂ ; w/v%)
F1	1	1	-	10%
F2	1	1	2	10%
F3	1	2	-	10%
F4	1	2	2	10%
F5	1	3	-	10%
F6	1	3	2	10%

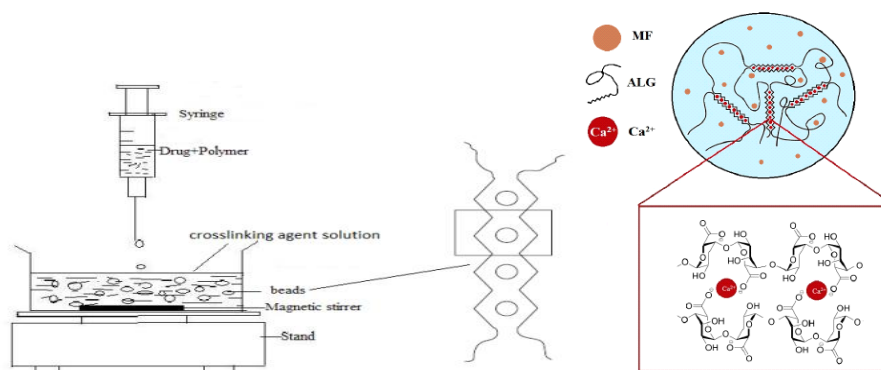


Fig. 1: Structure of CaCl₂ cross-linked ALG beads containing MF.

2.4. EVALUATION OF BEADS:

A) SWELLING INDEX:

Weighted beads from each formulation were soaked in 7.4 Phosphate Buffer at 37 c°. After 24 h, the beads were removed and their weights are measured again. Swelling index was calculated. Each experiment was performed in triplicate[16, c.627].

$$\text{swelling index} = \frac{\text{weight of wet beads} - \text{weight of dry beads}}{\text{weight of dry beads}} \times 100$$

B) DRUG CONTENT AND ENTRAPMENT EFFICIENCY DETERMINATIONS

100 mg alginate beads from each formulation crushed and dissolved in 100 ml of phosphate buffer pH 7.4 under magnetic stirring at room temperature for 4 h, then the solution was filtered, and the metformin HCL content was assayed by UV spectrophotometer at wavelength of 232 nm. Each experiment was performed in triplicate[16,c.627] [17,c.433].

$$\text{Drug content \%} = \frac{\text{amount of drug in beads}}{\text{weight of beads}} \times 100$$

Drug encapsulation efficiency (EE) was computed from the formula:

$$\text{Encapsulation efficiency \%} = \frac{\text{actual drug content}}{\text{theoretical drug content}} \times 100$$

C) IN- VITRO DISSOLUTION STUDY

The in-vitro release beads were performed using USP type II dissolution test apparatus in 900 ml of medium (0.1N HCL) for the first 2 h. and then in phosphate buffer pH 7.4 at temperature $37 \pm 0.5^{\circ}\text{C}$ and stirring rate of 50 rpm for the rest 6 h. A 5 ml sample of the solution was removed from the apparatus after different time intervals. The volume of each sample was replaced with the same volume of Phosphate buffer (pH 7.4) to maintain the sink conditions. The released amount of Metformin alginate beads was analyzed using UV- spectrophotometer at 232 nm. Each experiment was performed in triplicate^{16,17}.

3. RESULTS AND DISCUSSION

Standard calibration Curve of metformin HCL was shown in fig. 2

Table 2: Standard graph of Metformin HCl (n = 3)

c (µg/ml)	abs ± SD
3	0.275 ± 0.219
5	0.382 ± 0.042
8	0.621 ± 0.016
10	0.728 ± 0.015
12	0.862 ± 0.104
15	0.988 ± 0.009

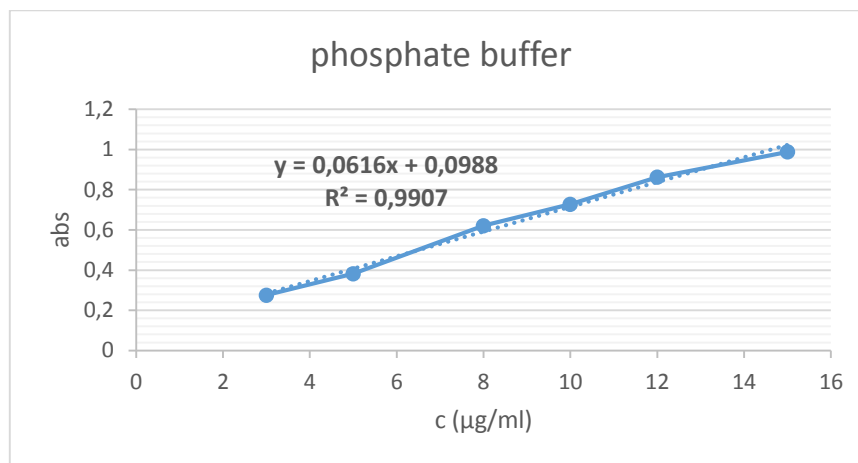


Fig. 2: Standard graph of Metformin HCl

The prepared metformin HCL-loaded alginate beads were spherical in the wet and dry state (as shown in fig. 3 and 4). Physicochemical properties of metformin HCl-loaded alginate beads were shown in table 3.

Table 3: physicochemical properties of metformin HCl-loaded alginate beads

Formulation	%Drug content ± SD (n = 3)	Encapsulation efficiency (%)	Swelling index (pH = 7.4)
F1	60.75 ± 0.105	73.27	84
F2	55.16 ± 0.042	70.81	89
F3	67.49 ± 0.012	76.34	86
F4	62.51 ± 0.241	72.25	88
F5	74.65 ± 0.095	92.98	90
F6	73.23 ± 0.527	91.12	92

The results obtained in this study showed that swelling ability of hydrogel beads not only depends on pH of medium used but also on the concentration of matrix forming polymers and the strength of cross linking agent used. Low concentration of calcium chloride leads probably to a loose gel, so using calcium chloride in the concentration of 10% gives more structured gel and the drug is more retained inside the beads and this led to an increase in the drug encapsulation efficiency[18, c.35] ,

since the existence of physical entanglements of cross-linked alginate-calcium chloride complex of lower dimensions controlling the drug diffusion flow within the beads[19, c.156] [20, c.642].



Fig. 3: Metformin HCL- loaded alginate beads in wet and dry state (F6)

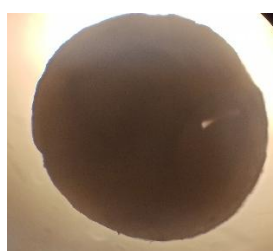


Fig. 4: Optical microscope images of metformin HCL-loaded alginate beads (F6)

Alginate swelling and dissolution are pH dependent. At acidic pH, as a result of reduction of the electrical repulsion between the negatively charged alginate molecules and positively charged ions in the medium, polymer is protonated and creates insoluble form of alginic acid, but it swells in neutral or basic pH [15, c.1]. [21, c.1100].

The swelling of the microcapsules increased with an increasing amount of polymers[18, c.35]. The increase in the swelling (%) that can be explained by the fact that with rising pH of the swelling medium, the ratio COO^-/COOH on CMC also increases because of increasing ionization of carboxylic groups and this results in a greater repulsion among the $-\text{COO}^-$ bearing CMC chains[22, c.1].



Fig. 5: swelling of metformin HCl- loaded alginate beads in phosphate buffer.

Among the cellulose derivatives, carboxymethyl cellulose can form insoluble salts when the polymer chains share polyvalent metal ions by a crosslinking technique. The entrapment efficiency of the beads formed with Na CMC and Na alginate was found to be decreasing. The reason for decreased entrapment could be due to the difficulty in the formation of microspheres due to the high concentration of hydrophilic polymer[23, c.301].

Drug release profile of metformin HCL from Na alginate beads for all formulations was shown in fig. 6 and 7. All formulations released amounts of the drug (<30%) in the HCl medium, due to the poor solubility of alginate in a pH less than 3. Adding Na CMC showed better sustained release (F2, F4 and F6), which is similar to the results reported by DHANARAJU MD et. al. [23, c.301]. This may be due to the fact that the higher porosity of alginate beads results in faster release.

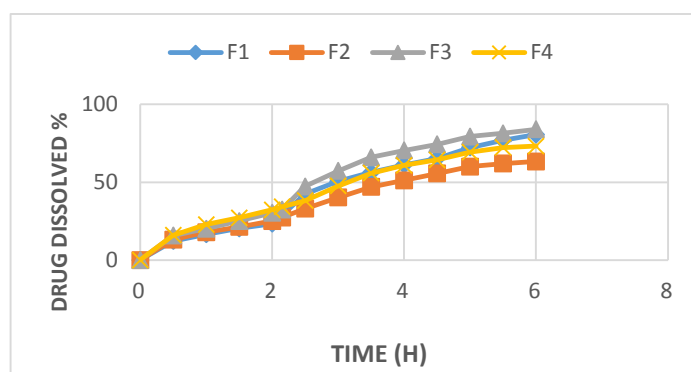


Fig. 6: Drug release profile of metformin HCL from Na alginate beads for F1-F4

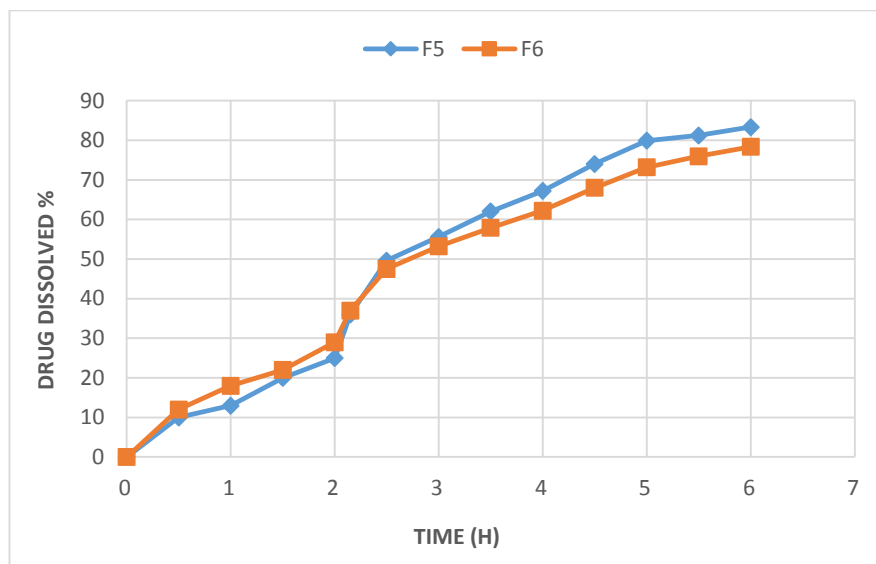


Fig. 7: Drug release profile of metformin HCL from Na alginate beads for F5, F6

The cross linking agent form three dimensional bonding structure with the Sodium CMC inside the microcapsules This three dimensional bonding results in extended crosslinking through the whole microcapsule producing hard microcapsules with lower water uptake and thus leading to slow release of drug in the phosphate buffer[23,c.301]. [24,c.203].

CONCLUSIONS:

This study shows that metformin HCl-loaded alginate bead formulations may be useful in prolonging the hypoglycemic effect of orally administered metformin. This is capable of increasing patient compliance with the medication and decrease the degree of side effect of metformin.

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