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Research Article

Development of Microcapsules of Glimepiride using Fenugreek Seed Extract

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Abstract

Glimepiride microcapsules were prepared by ionic gelation method as a sustained release formulation. Fenugreek seed extract and sodium alginate were used as coating polymers in different ratios 1:1, 1:2, 1:3 and 1:4 to obtain microcapsules. The formulations were characterized for sieve analysis, encapsulation efficiency, drug loading, swelling index and scanning electron microscopy. The microcapsules were discrete, large, almost spherical and free flowing with maximum encapsulation efficiency about 97%, swelling index as 168% and particle size 879 to 998 µm. Glimepiride release from these microcapsules extended over longer period of time depending on the concentration of fenugreek seed extract and sodium alginate coat. Kinetic study showed drug release was diffusion controlled and followed zero order kinetics. *In vivo* study showed that the blood glucose level after challenged to rats, in Glimepiride microcapsules was 198 mg/dl and in microcapsules containing Glimepiride and fenugreek seed extract was 173 mg/dl, showed synergistic effect.

1. INTRODUCTION

Microencapsulation is a process in which very thin coatings of polymeric materials are deposited around the particles of solids or droplets of liquids.[1] Microcapsules were prepared by ionic gelation method in which the ionic polymer interact with oppositely charged ion to initiate cross linking.[2] Microcapsules usually have a particle size range between 1-2000 µm.[3] Glimepiride is a oral hypoglycaemic drug, used for diabetes treatment and it acts on pancreatic beta cell membrane causes depolarization by reducing conductance of ATP sensitive K^+ channels. This enhances Ca^{2+} influx so the rate of insulin secretion at any glucose concentration is increased. Glimepiride has half life of 5 h and the dose about 8 mg, were used as the core in microencapsulation.[4] Fenugreek seeds are aromatic, bitter, carminative, galactogouge, antibacterial and may be eaten raw or cooked. Fenugreek seeds contain gel fiber, it reduces the rate of glucose absorption and may also delay gastric emptying and thereby preventing the rise in blood sugar levels following a meal. In fenugreek seeds, the gum (gel fiber) fraction consists of galactomannan which is made up of galactose and mannose units. The gum also resembles guar gum in structure and is very viscous (15 centipoise) when dissolved in water.[5] The Glipizide microcapsules using gum karaya[6] and Gliclazide microcapsules using xanthan gum by ionic gelation method were prepared but until now microcapsules coated with fenugreek seed extract has not been developed.

The objective of the present study were formulation of microcapsules containing combination of fenugreek seed mucilage and hypoglycaemic drug like Glimepiride, they showed the synergistic effect.

2. MATERIALS AND METHODS

2.1 Materials

Glimepiride was obtained as gift sample from Zim Laboratories Pvt. Ltd. (Nagpur, India). Fenugreek seeds were purchased from local suppliers of Nagpur. Sodium alginate and calcium chloride were purchased from Loba Chemical (Mumbai, India).

2.2 Extraction of Fenugreek Seed

The plant materials (seeds) were collected, washed with water to remove dirt, dried, crushed and powdered. The powder was soaked in water for 5-6 h, boiled for 30 min, and allowed to stand for 1 h so that all the mucilage was released into the water. The materials were then squeezed from muslin bag to remove the marc from the solution. Then three volumes of acetone were added to the filtrate to precipitate the mucilage. The mucilage was separated, dried in an oven at a temperature less than 50°C, and the dried powder was passed through a no. 80 sieve and retained on sieve no. 100 and stored in a desiccators.[7]

2.3 Preparation of Microcapsules

Sodium alginate (1g) and Fenugreek seed mucilage (1g) were dissolved in purified water (32 ml) to form a homogeneous polymer solution. The active substance, Glimepiride (2g), was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (15% w/v) solution (40 ml) through a syringe with a needle (No. 23). The added droplets were retained in the calcium chloride solution for 15 min to complete the curing reaction and to produce spherical rigid microcapsules having coat: core ratio (1:1).[6] Similarly, microcapsules with different coat: core ratio were also prepared. The microcapsules were collected by filtration, washed with water and dried over night at room temperature. The compositions of the microcapsules formulations are listed in Table 1.

Table 1: Formulation of various batches of microcapsules

Batches	Ratio Drug: SM and SA	Drug (D) (g)	Seed Mucilage (g)	Sodium alginate (g)	Calcium Chloride (g)
F1	2: 2	2	1	1	6
F2	2: 3	2	2	1	6
F3	2: 4	2	3	1	6
F4	2: 5	2	4	1	6
F5	2: 3	2	1	2	6
F6	2: 4	2	1	3	6
F7	2: 5	2	1	4	6

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2.4 Evaluation of Microcapsules

Percentage yield[8], particle size analysis[9], drug content[10], encapsulation efficiency[11] and Swelling index[12] were calculated as per reported procedures.

2.4.1 Morphology

Surface morphology of microcapsules was investigated by Scanning Electron Microscopy (SEM) using JSM 6380A (JOEL, Japan). The microcapsules, coated with platinum by ion sputtering using Auto fine coater JFC-1600 (JOEL, Japan), for 20 S at 1.1 V under argon atmosphere were mounted onto metal stubs using double-sided carbon adhesive tape and the scanning electron micrographs were taken.[13]

2.4.2 FTIR Spectra

Small quantities of microcapsules were grounded with KBr and then pelletized using KBr press. Scanning was performed between the ranges of 400-4000 cm^{-1}.

2.4.3 In vitro drug release studies

Dissolution studies were carried out using USP XXIV rotating basket method. The stirring rate was 50 rpm. 900 ml of pH 7.4 phosphate buffer was used as dissolution medium and maintained at 37±1°C. Samples of 5 ml each were withdrawn at regular time intervals, filtered, diluted suitably, analyzed using double beam UV spectrophotometer at 230 nm and an equal volume of fresh medium was immediately replaced to maintain the dissolution volume. Dissolution studies were carried out up to 12 h.[14]

2.4.4 Drug release kinetic treatment

In vitro release profiles were incorporated in various kinetic models in order to find out the mechanism of drug release. The best fit with the highest correlation coefficient was shown in zero-order, Higuchi and followed by first order equations. The rate constants were calculated from the slope of the respective plots. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of microcapsules of different drug to polymer ratio was ranged between 0.45 and 0.89, indicating that the mechanism of drug release was Non-Fickian or anomalous transport.[15]

2.4.5 In vivo evaluation

In vivo evaluation studies were conducted on male wister rats of 100-150 g body weight.[16] The study protocol was approved by IAEC (Institutional Animal Ethics Committee) before the conduct of the study. Animals were made diabetic with intra-peritoneal injection of 60 mg/kg dose of freshly prepared Streptozotocin in citrate buffer. The treatments of the groups of rats were as follows:

Group I : Normoglycaemic, untreated control rats that were orally treated with 10 ml/kg of distilled water.
Group II : Hyperglycaemic, animals made diabetics by streptozotocin induction (60 mg/kg)
Group III : Hyperglycaemic rats treated with microcapsules contain Glimepiride (20 mg/kg)
Group IV : Hyperglycaemic rats treated with microcapsules contain fenugreek seed extract (200 mg/kg)
Group V : Hyperglycaemic rats treated with microcapsules contain both Glimepiride and fenugreek seed extract (Microcapsules equivalent to 8 mg of Glimepiride)

After oral administration of seed extract, drug and both containing microcapsules, the blood samples were collected from tail tip and glucose was monitored using Glucometer strips at 2 h interval for 12 h.

3. RESULTS AND DISCUSSION

3.1 Particle Size

The particle size of microcapsules varied from 879.30 ± 1.23 µm to 988.95 ± 0.33 µm (Table 2). It was observed that the size of the microcapsules was increased with increasing the seed mucilage and sodium alginate concentration. This is due to the increase in viscosity of seed mucilage and alginate, which in turn increases the droplet size during addition of the polymer dispersion to calcium chloride.

3.2 Percentage Yield, Drug Content and Encapsulation Efficiency

The percentage yield of microcapsules varied from 96.19 to 98.66 %, drug contained varied from 12.44 to 13.91 mg and the higher encapsulation efficiency was observed as the concentration of mucilage and/or alginate increased. This is due to the greater availability of active calcium binding sites in the polymeric chains and consequently the greater degree of cross linking. The highest incorporation efficiency (97.96 %) was achieved with 4% w/v seed mucilage and (97.17%) was achieved with 4% w/v sodium alginate (Table 2).

Table 2: Values of % yield, particle size, drug contain and % encapsulation efficiency for various microcapsules batches

Sr. No.	Formulation	Percentage Yield (%)	Average diameter (µm)	Drug contain (mg)	Microencapsulation Efficiency (%)
1	F1	98.66	879.30 ± 1.23	12.56	86.63
2	F2	97.22	924.09 ± 1.65	12.92	89.96
3	F3	97.14	960.99 ± 0.35	13.42	95.86
4	F4	97.91	977.49 ± 0.56	13.91	97.96
5	F5	96.19	928.14 ± 1.87	12.44	85.63
6	F6	98.33	948.71 ± 0.34	12.79	87.86
7	F7	97.64	988.95 ± 0.33	13.85	96.17

Mean ± SD, n = 5

3.3 Swelling index

The swelling index of microcapsules at pH 7.4 was higher as compared to pH 1.2. The swelling index at pH 1.2 from 34 to 150% and at pH 7.4 from 272 to 446% (Table 3). It was observed that as the concentration of seed extract increased the swelling index reduces, in F1 batch seed extract was 1 g (showed swelling index 140%) while in batch F4 seed extract was 4 g (swelling index 68 %) similarly, in sodium alginate in batch F5, where alginate was 2 g (Swelling index 150%) and batch F7, sodium alginate was 4 g (Swelling index 34%), this because in sodium alginate availability of free $COOH^-$ ions which are hydrophilic in nature but when Ca^{++} ion concentration increases the replacement of free $COOH^-$ by Ca^{++} ion and formed calcium alginate, it becomes hydrophobic.

Table 3: Values of % swelling index for microcapsules of Glimepiride

Formulation	% Swelling index	
	pH 1.2	pH 1.2
F1	140 ± 1.45	437 ± 4.67
F2	87 ± 2.43	386 ± 5.78
F3	79 ± 1.78	303 ± 4.24
F4	68 ± 1.46	272 ± 5.89
F5	150 ± 2.99	446 ± 6.68
F6	74 ± 2.86	394 ± 4.89
F7	34 ± 1.70	314 ± 5.23

Mean ± SD, n = 5

3.4 Morphology of Microcapsules

The SEM photomicrographs of drug-loaded microcapsules showed that the microcapsules were almost spherical in shape with rough and nonporous surface (Figure 1).

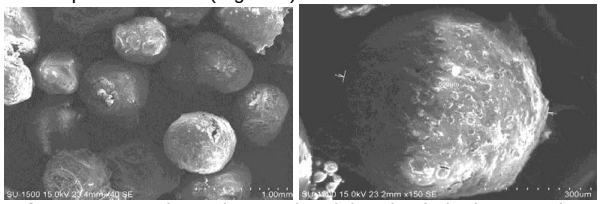

Figure 1: SEM photomicrographs of drug-loaded microcapsules with seed mucilage and sodium alginate

3.5 FTIR Spectra

In the IR Spectra of Glimepiride showed numbers of peak at 3344 cm^{-1}, 2900 cm^{-1}, 1627 cm^{-1}, 1427 cm^{-1}, 1342 cm^{-1} and 1251 cm^{-1} wave numbers were due to N-H stretching, -O-H- (acid), C = C stretching, -C-H- (alkanes), SO_2-NH, -C-N- stretching, respectively. IR spectra of microcapsules containing combination of Glimepiride, seed mucilage and sodium alginate were showed all these peaks unchanged. The above interpretational data clearly states no interaction between the pure drug Glimepiride, seed mucilage and sodium alginate.

3.6 In vitro drug release studies

The drug release from the microcapsules decreased as the concentration of polymers increased suggesting that drug release could be controlled by varying the concentration of polymers coat (Figure 2). It can be attributed to increase in the densities of the polymer matrix resulting in larger microcapsules and this in turn increase the diffusion path. The formed calcium-alginate precipitate is hydrophobic so release of drug was delayed.

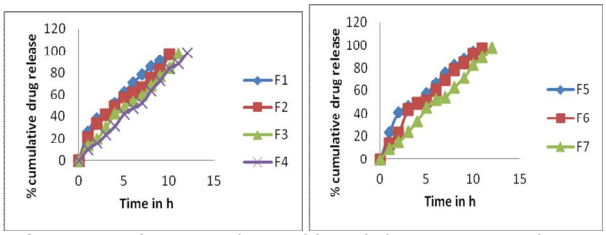

Figure 2: *In vitro* Drug release of formulations F1 to F4 and F5 to F7

3.7 Drug release kinetics

In vitro drug release data was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equations, Higuchi and Korsmeyer models to ascertain the mechanism of drug release. The results of linear regression analysis of data including regression coefficient are summarized in Table 4. Regression coefficient 'R^{2}' value indicating drug release from all the formulations was found to follow zero order kinetics. The all microcapsules formulations suggested that diffusion was the predominant mechanism limiting drug release since the 'R^{2}' values of Higuchi's plots were nearer to unity, and the drug release was by non-fickian diffusion mechanism.

Table 4: Kinetic treatment of drug release data of various batches

Formulation Code	Zero order equation	First order equation	Higuchi's equation	Korsmeyer Peppas equation	Diffusion coefficient (n)
	R^2				
F1	0.969	0.871	0.964	0.992	0.68
F2	0.988	0.886	0.961	0.998	0.68
F3	0.965	0.872	0.957	0.986	0.65
F4	0.961	0.817	0.991	0.994	0.68
F5	0.988	0.884	0.959	0.997	0.65
F6	0.979	0.965	0.973	0.997	0.68
F7	0.971	0.943	0.983	0.993	0.68

3.8 *In vivo* study

In *in vivo* study, microcapsule F4 batch containing Glimepiride and fenugreek seed extract were challenged to rats, showed the blood glucose level of Glimepiride microcapsules was 198 mg/dl and % reduction in blood glucose level was 94.22% after 2 h, and in microcapsules containing both Glimepiride and seed mucilage, the blood glucose level and % blood glucose reduction was found 173 mg/dl, 83.25% respectively, after 2 h. This showed the synergistic effect and also sustained effect was found over a period of 12 h (Figure 3).

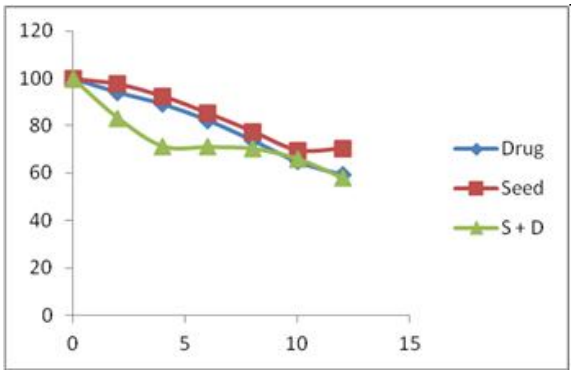

Figure 3: % reduction in blood glucose level in animals

4. CONCLUSION

Thus, large spherical microcapsules with a coat consisting of fenugreek seed extract and alginate could be prepared by an ionic gelation process. Glimepiride release from these microcapsules was slow and extended over 12 h, depends on composition of the coat. Drug release was diffusion controlled and followed zero-order kinetics. In the *in vivo* evaluation, F4 batch alginate-seed mucilage (1:4) microcapsules could sustain the hypoglycemic effect of Glimepiride over a period of 12 h, and showed the synergistic effect. These microcapsules were suitable for oral controlled release of Glimepiride.

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