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FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF METFORMIN HYDROCHLORIDE

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ABSTRACT

Mucoadhesive microspheres of metformin hydrochloride were prepared by orifice ionic gelation method to target the drug absorption in the stomach and upper part of intestine by increasing the residence time of drug in upper part of gastro intestinal tract and to control the drug release in therapeutic range for a longer period of time. Here sodium alginate was used as crosslinking agent and carbopol 934P and hydroxypropyl methylcellulose were used as mucoadhesive polymers in the formulation step. The surface morphology of the microspheres was characterized by scanning electron microscopy. The prepared microspheres were discrete, spherical in shape and showed free flowing properties and exhibited good mucoadhesive property in the *in vitro* wash-off test. Among all the formulation, Formulation F4 containing carbopol 934P and F8 containing hydroxy propyl methyl cellulose (HPMC K-100M) showed a high drug entrapment efficiency of 84.15 ± 0.01% and 78.21 ± 0.09% and a percentage swelling index of 148.1 ± 1.45% and 197.4 ± 1.20%. Percentage mucoadhesion after 10 h was 40 ± 2 % and 35 ± 2 % for F4 and F8 respectively .The drug release from the formulations F4 and F8 controlled for more than 12 h. The data obtained thus suggest that mucoadhesive microspheres can successfully design for controlled delivery of metformin hydrochloride and to improve patient compliance. The mechanism of drug release was evaluated using the linear regression coefficient.

Keywords: Microspheres, Diabetes Type-2, Mucoadhesion, Controlled Drug Release, Orifice Ionic Gelation Technique.

INTRODUCTION

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulations. However, this approach is associated with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans. This normally averages of 2-3 hrs through the major absorption zone, mainly stomach and upper part of the intestine. This results in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose [1].

The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e. release from the dosage form should follow zero-order kinetics [2].

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems [3-5]. Microspheres have varied applications and are

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prepared using assorted polymers[6]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. So, various attempt have been made to increase the bioavailability as well as prolong the gastric residence time of dosage form in the stomach resulted in development of bio adhesive drug delivery system which will provide an intimate contact of the drug delivery system with the absorbing membranes [7-10]. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site [11-14]. Gastric mucoadhesive drug delivery offers a number of applications for drugs having poor bioavailability because of narrow absorption window in the upper part of gastrointestinal tract. It retains the dosage form at the site of absorption and thus enhances the bioavailability.

Carbopol 934P, hydroxy propyl methyl cellulose (HPMC K100M, HPMC K4M) was selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive and biodegradable properties and sodium alginate was used as crosslinking agent in the preparation.

Metformin HCl is an oral hypoglycaemic agent belongs to biguanide class. Metformin HCl has been reported to control glucose level and improve lipid profile in type-II diabetics. The effective control of diabetes type-II requires administration of 500 mg of metformin HCl more than three times a day**.** A conventional dose of 500 mg can control glucose level 6-8 hours but not up to 12 or more hours. High dose of metformin HCl may cause Vitamin B12 deficiency due to interference with its absorption. More ever the site of metformin HCl absorption is stomach and upper part of intestine whereas at the colon metformin HCl is poorly absorbed. Owing to its short biological half life of 1.5-3 hrs and low bioavailability of 50-60%. It is necessary to develop a mucoadhesive dosage form of metformin HCl which adhere to the mucosa and release the drug in slow and controlled manner [16-17]. So, that development of oral controlled release dosage forms thus, would clearly advantageous. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency and decrease dose requirements.

MATERIALS AND METHODS

Metformin HCl was obtained as gift sample from Strides acrolabs Pvt. Ltd, Bangalore. Sodium alginate and Carbopol 934P was purchased from Rolex Chemical Industries, Mumbai. HPMC K-100M, HPMC K-4M was obtained from NR Chem. Mumbai. Calcium chloride was purchased from S.D. Fine Chem Ltd. Mumbai. Hydrochloric acid was purchased from Reachem Laboratory Chemicals Pvt. Ltd, Chennai. All other Chemicals were of analytical grade.

Formulation of mucoadhesive microspheres

Mucoadhesive microspheres containing anti-diabetic drug as a core material were prepared by orifice ionic gelation technique [18]. Sodium alginate and the mucoadhesive polymers like Carbopol 934P, HPMC K-100 M and HPMC K-4M were dissolved in 40 mL purified water to form a homogeneous polymer solution. The active substance, metformin hydrochloride (500mg) was added to the polymeric solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into 10 % w / v calcium chloride solution (40 mL) through a syringe (no.20). The added droplets were retained in the calcium chloride solution for 15 min to complete the curing reaction and to produce spherical rigid microspheres. The microspheres were collected by decantation and the product thus separated was washed repeatedly with water and dried at 40 °C for 3 h in hot air oven. Stored in a desiccator over fused $CaCl_2$ until further study. The prepared batches of mucoadhesive microsphere are as shown in following Table 1.

EVALUATIONS

1. **Percentage yield [18]**

The percentage yields of microspheres were calculated by the weight of final product after drying with respect to the initial total weight of the drug and polymer. The percent yields were calculated by the formula given below.

Percentage Yield = Practical mass (microspheres) / Theoretical mass × 100

2. **Drug content and Drug Entrapment Efficiency (DEE) [18-19]**

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 50 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 1800, Shimadzu, Japan) at 233 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula.

% DEE = (Amount of drug actually present / Theoretical drug load expected) × 100

3. Particle Size analysis [18]

The particle size of the microspheres was determined by using optical microscopy method. Approximately 10 microspheres were counted for particle size using a calibrated optical Microscope.

4. Swelling Index [20-22]

Swelling index was determined by measuring the extent of swelling of microspheres in 0.1N HCl (pH 1.2) buffer. To ensure the complete equilibrium, exactly weighed amount of microspheres were allowed to swell in 0.1N HCl (pH 1.2) buffer. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The microspheres then dried in an oven at 60 °C for 5 hr until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula.

% Swelling index = (mass of swollen microspheres - Mass of dried Microspheres) /

Mass of dried microspheres x 100

5. *In vitro* wash-off test (% Mucoadhesion) [20-21]

A 1 cm x 1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch x 1 inch) using a thread. Microsphere was spread onto the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groves of the USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen regular up and down movements in a beaker containing the simulated gastric fluid (0.1N HCl). At the end of every time interval, the number of microsphere still adhering on to the tissue was counted and there adhesive strength was determined by following formula.

% mucoadhesion = (no. of microspheres remains / no. of applied microspheres) ×100

6. *In vitro* drug release [20-21]

Dissolution studies on all the 12 formulations of Metformin HCl microspheres were carried out using a USP dissolution apparatus Type II (paddle- Electro labs, TDT-08 L). 0.1N HCl was used as the dissolution medium. To carry out *In-vitro* drug release, accurately weighed 50 mg of loaded microspheres were dispersed in dissolution fluid in a dissolution jars and maintained at 37±2 °C under continuous stirring at 100 rpm. At selected time intervals 1 mL samples were withdrawn through a hypodermic syringe fitted with a 0.45 µm Millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analysed spectrophotometrically after suitable dilution. The released drug content was determined from the standard calibration curve of given drug.

7. Scanning Electron Microscopy [18]

A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 6.0 kV, original magnifications × 300.

8. Release kinetics [23]

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equations namely zero order (% drug release vs. time), first order (log% unreleased drug vs. time), and Higuchi matrix (% drug release vs. square root of time). In order to define a model which will represent a better fit for the formulation, drug release data further analysed by Peppas equation, $Mt/M\infty=kt^n$, where Mt is the amount of drug released at time t and M∞ is the amount released at time ∞, the Mt/M∞ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusion exponent, a measure of the primary mechanism of drug release. Regression co-efficient (r^2) values were calculated for the linear curves obtained by regression analysis of the above plots.

9. Accelerated Stability Studies [24]

Stability studies were carried out on most satisfactory formulation as per ICH guidelines Q1A at 40 ± 2°C (75 ± 5% RH). The most satisfactory formulation stored and sealed in aluminium foil. These were stored for 3 months. After 1, 2, and 3 month interval % drug entrapment efficiency, Particle size, % mucoadhesion of most satisfactory formulation was determined. *In vitro* release study was also carried out of best formulation.

RESULTS AND DISCUSSION

Mucoadhesive microspheres of Metformin HCl with coat consisting of various concentration of sodium alginate and mucoadhesive natural and synthetic polymers in ratio of 1:1, 1:1.5, 1:2 and 2:1 (Alginate: Mucoadhesive polymer) could be prepared by orifice ionic gelation technique. The microspheres were found to be discrete, almost spherical, free flowing and of the monolithic matrix type. The microspheres were completely covered with coat polymer.

1. Percentage yield

The percentage yield of microspheres of all formulations was shown in Table 2. Percentage yield of all formulations are in the range of 60.22 ± 2.06% to 96.50 ± 1.01%. As the concentration of sodium alginate was increased, the % yield was also found to be increased. So, the F4, F8 and F12 batches showed good % yield of 82.65±1.24%, 86.75±1.09% and 96.50± 1.01%.

2. Drug content and Drug Entrapment Efficiency (%DEE)

The drug content of the dried microspheres of all formulations shown in Table 2. Drug content of all formulations are varied between 25.24 ± 1.06 mg to 42.07 ± 0.79 mg and drug entrapment efficiency varied from

50.48 ± 1.2 to 84.15 ± 0.01. This result will indicate that increase in sodium alginate concentration will increase the drug content as well as drug entrapment efficiency. The Formulation containing Carbopol 934P as a mucoadhesive polymer will shows the maximum drug content as well as drug entrapment efficiency. So, the drug content and entrapment efficiency of formulation based on mucoadhesive polymers are as

Carbopol 934P > HPMC K-100M > HPMC K-4M.

3. Particle size analysis

The particle size of the dried microspheres of all formulations shown in Table 2. Particle sizes of all formulations are varied between 94 ± 0.30 and 144 ± 0.5 µm. This was agreeing with the finding that there was lower particle size obtained when carbopol 934P is used as mucoadhesive polymer and highest particle size was observed when HPMC K-100M used as mucoadhesive polymer. While intermediate particle size observed when HPMC K-4M used as mucoadhesive polymer in preparation of mucoadhesive microspheres.

4. Swelling index (SI)

Swelling increase as the time passes because the polymers gradually absorb water due to hydrophilic nature of polymer. The outer most polymers hydrate and swell resulting in formation of gel barrier at the outer surface. As the gelatinous layer progressively dissolves and / or dispersed, the hydration swelling release process is continuous towards new exposed surface. Swelling index is increase as the polymer concentration increase. Carbopol 934 P shows the less swelling while the HPMC swelled rapidly at the beginning because of its high viscosity. So, swelling properties of different formulation is depends upon the viscosity of polymer used. Swelling index is as:

Carbopol 934 P > HPMC K4M > HPMC K 100M.

Swelling properties of all formulations after 10 h is shown in Figure 1.

5. *In-vitro* wash-off test for Microsphere

To assess the mucoadhesive property of microspheres, *In-vitro* wash-off test was performed for all the formulations. Adhesion of polymer with the mucus membrane is mediate by hydration in the case of hydrophilic polymer. Upon hydration these polymers becomes sticky and adhere to mucus membrane. The higher the concentration of sodium alginate higher will be the mucoadhesion. Formulation F4 containing two parts of sodium alginate and one part of Carbopol 934 P shows the maximum mucoadhesion. The order of % mucoadhesion for all the formulations, after 10 hours was found to be as follows:

F4 > F8 > F12 > F1 > F5 > F7 > F6 > F9 > F2 > F3 > F10 >F11

A result of mucoadhesion of all formulation after 10 h is shown in Figure 2.

6. *In-vitro* drug release study

Drug release study of all the formulations containing drug were performed in simulated gastric fluid (pH 1.2) at 37 ± 2°C. Drug release form these microspheres were slow, extended and dependent on the type of polymer and concentration of polymer used. Viscosity of polymer increase in the formulation, the release of drug from formulation is decrease which may be due to increase in strength of gel matrix of the polymer. HPMC K-100M having high viscosity which will shows the least drug release from the microsphere then Carbopol 934P and HPMC K-4M. Carbopol 934P is a cross linked polymer with high molecular weight and viscosity. So, it would swell and hold water inside its microgel network. These properties responsible for retarding the drug release. While increase in sodium alginate concentration in the formulation containing Carbopol 934P shows the slow and longest release making formulation more effective. In the case of HPMC K-4M will show the faster release of drug from the formulation because of its less viscosity. After the end of 10 hrs, the drug release were found to be 92.26±1.23, 75.21±0.98, 64.49±0.65, 79.69±0.80, 71.71±1.20, 59.18±0.54, 52.04±1.02, 60.95±1.20, 97.73±1.30, 99.48± 1.21, 97.66±1.34 and 97.65±0.97 % of formulation F1,F2,F3,F4,F5,F6,F7,F8,F9,F10,F11 and F12 respectively.

Release studies of all formulations are shown in Figure 3.

7. Scanning electron microscopy (SEM)

Morphology of microspheres of optimized formulation F4 and F8 was examined by scanning electron microscopy. The view of the microspheres showed a hollow spherical structure with a smooth surface morphology. The outer surface of the microspheres was smooth and dense, while the internal surface was porous. The shell of the microspheres also showed some porous structure.

Scanning electron photograph of optimized formulation F4 and F8 are shown in Figure 4.

8. Release kinetics study

The kinetics investigations of the release profile gave us useful insight into the drug release rate and mechanism of drug release. All the formulation dissolution data was subjected to regression analysis and were fitted to kinetic models, visualized Zero order, First order, Higuchi square root and Korsemeyers- peppas. The r- value of zero order of the above 12 formulation were in the range of 0.9020 to 0.9964 Among 12 formulations some formulation F1, F2, F3, F4, F9, F10, F11, F12 release the drug by Zero-order kinetics and some are F5, F6, F7, F8 release the drug by First order kinetics. The results suggest that the drug was released by mixed order kinetics.

To ascertain, the drug release mechanism the in vitro release data were also subjected to Higuchi's diffusion equation ($Q=kt^{0.5}$). The r- values of all the

formulation of higuchi's equation were lowest 0.9113 to highest 0.9955. It suggests that the higuchi's diffusion plots of all formulations were fairly linear.

The formulation also subjected to Korsemeyers-peppas plot by taking log % CDR vs. log Time. The plots are found to be linear and the regression values ranges from 0.9607 to 0.9970. The n – values of all formulation was found to be in the ranges of 0.4243 to 1.3327. It suggests that the Korsemeyers-peppas plots of all formulations were linear. The formulations F1,F5,F6 and F8 will release the drug by non-fickian mechanism and the formulation F7 shows the drug release by fickian mechanism while the formulations F2,F3,F4,F9,F10,F11 and F12 will shows the release of drug from microspheres by Super Case II transport mechanism were erosion of polymer followed by drug release occurs.

So, optimized formulation F4 shows the drug release by Case II transport mechanisms which involve the release of drug by erosion and F8 release the drug by non fickian diffusion mechanism.Release kinetics data of optimized formulation is shown in Table 3.

From above all the formulations F4 and F8 was selected as optimized formulation based on % drug entrapment efficiency, % swelling, % mucoadhesion and in vitro drug release. These two formulations will release the drug in controlled manner then other formulation. So, F4 and F8 were subjected to the accelerated stability studies for 3 months.

9. Accelerated Stability studies

The best formulation F4 and F8 stored and sealed in aluminium foil. Accelerated Stability studies were carried out on most satisfactory formulation as per ICH guidelines Q1A at 40 ± 2°C (75 ± 5% RH) for 3 months. After 30 days, 60 days and 90 days intervals % drug entrapment efficiency, % mucoadhesion, particle size, *In vitro* release study of most satisfactory formulation was determined. The results of accelerated stability studies are given in Table 4.

Table 1. Compositions of mucoadhesive microspheres

Formulation Code	Drug (mg)	Sodium Alginate (mg)	Carbopol 934 P (mg)	HPMC K-100M (mg)	HPMC K-4M (mg)	Ratio
F1	500	500	500	-	-	1:1
F2	500	500	750	--	-	1:1.5
F3	500	500	1000	-	-	1:2
F4	500	1000	500	-	-	2:1
F5	500	500	-	500	-	1:1
F6	500	500	-	750	-	1:1.5
F7	500	500	-	1000	-	1:2
F8	500	1000	-	500	-	2:1
F9	500	500	-	-	500	1:1
F10	500	500	-	-	750	1:1.5
F11	500	500	-	-	1000	1:2
F12	500	1000	-	-	500	2:1

Table 2 Results of Percentage Yield, Particle Size, Drug Content and Drug Entrapment

Formulation Code	% Yield ± S.D*	Average particle Size (µm) ± S.D*	Drug content (mg) ± S.D*	% DEE ± S.D*
F1	62.60 ± 1.21	94 ± 0.30	34.40± 0.40	68.81±0.7
F2	70.91 ± 1.30	113 ± 0.83	37.37±1.05	74.75±0.01
F3	70.95 ± 1.31	139 ± 0.1	39.85±1.20	79.70±0.05
F4	82.65 ± 1.24	115 ± 0.67	42.07±0.79	84.15±0.01
F5	75.33 ± 1.56	141 ± 0.61	29.20±1.09	58.40±1.03
F6	82.74 ± 1.37	138 ± 0.29	32.17±1.80	64.35±0.05
F7	63.40 ± 1.87	113 ± 0.7	35.14±1.47	70.29±1.04
F8	86.75 ± 1.09	144 ± 0.5	39.10±0.99	78.21±0.09
F9	67.80 ± 2.10	119 ± 0.33	25.24±1.06	50.48±1.20
F10	60.22 ± 2.06	103 ± 0.31	32.67±0.56	65.34±0.09
F11	66.10 ± 2.54	106 ± 0.4	33.16±1.90	66.20±1.21
F12	96.50 ± 1.01	108 ± 0.49	36.88±0.23	73.76±0.77

Table 3. Release mechanism of optimized formulation

Kinetic Profile of various formulations	1st order equation		Zero order equation		Higuchi equation	Korsemeyers-peppas		Release mechanism
	K_1	r^2	K_0	r^2	r^2	n	r^2	
F4	0.199	0.8963	7.9447	0.9964	0.9113	0.9625	0.9892	Zero order / case II transport
F8	0.092	0.9913	5.3251	0.9802	0.9771	0.6670	0.9967	First order / non fickian

Table 4. Results of Stability study of optimized formulation

Evaluation parameters	Formulation code	0 days	30 days	60 days	90 days
Particle Size(µm)	**F4**	115 ± 0.67	115 ± 0.45	115 ± 0.30	115 ± 0.15
	F8	144 ± 0.50	144 ± 0.38	144 ± 0.10	143 ± 0.67
% Drug Content	**F4**	84.15 ±0.01	84.09 ±0.09	83.50 ±0.15	82.90 ± 0.67
	F8	78.21 ±0.09	78.12 ±0.37	78.00 ±0.67	77.53 ± 1.02
% Mucoadhesion	**F4**	40 ± 2	39 ± 3	38 ± 4	37 ± 2
	F8	35 ± 3	34 ± 3	34 ± 3	32 ± 4
%In – Vitro Drug Release	**F4**	93.20 ±1.20	93.00 ±1.10	92.00 ±1.30	91.80 ± 0.98
	F8	69.40 ±0.98	69.20 ±0.98	69.05 ±1.45	68.86 ±1.20

Figure 1. Swelling behaviour of F1 to F12 formulation after 10 hrs

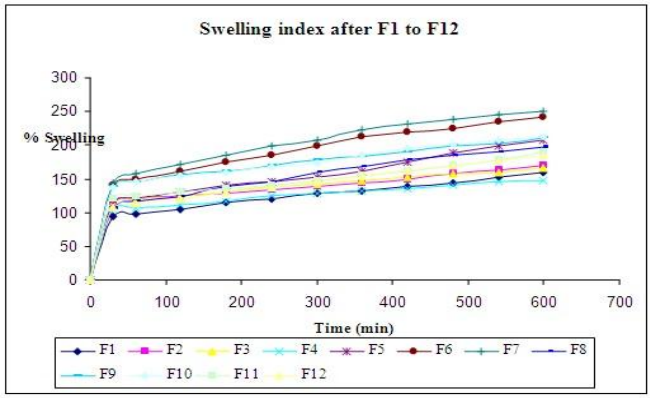


Figure 2. Mucoadhesion of F1 to F12 after 10 hrs

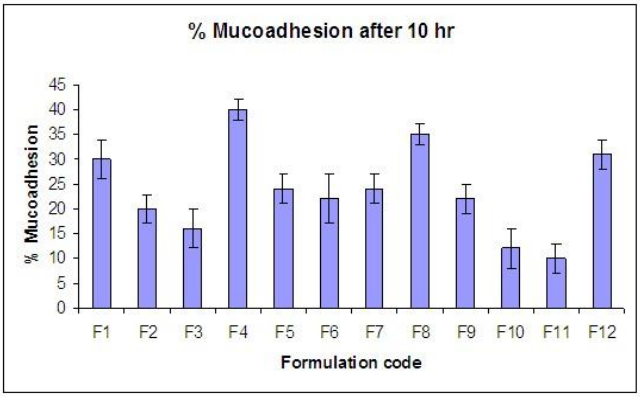


Figure 3. In vitro drug release profile of F1 to F12 formulation

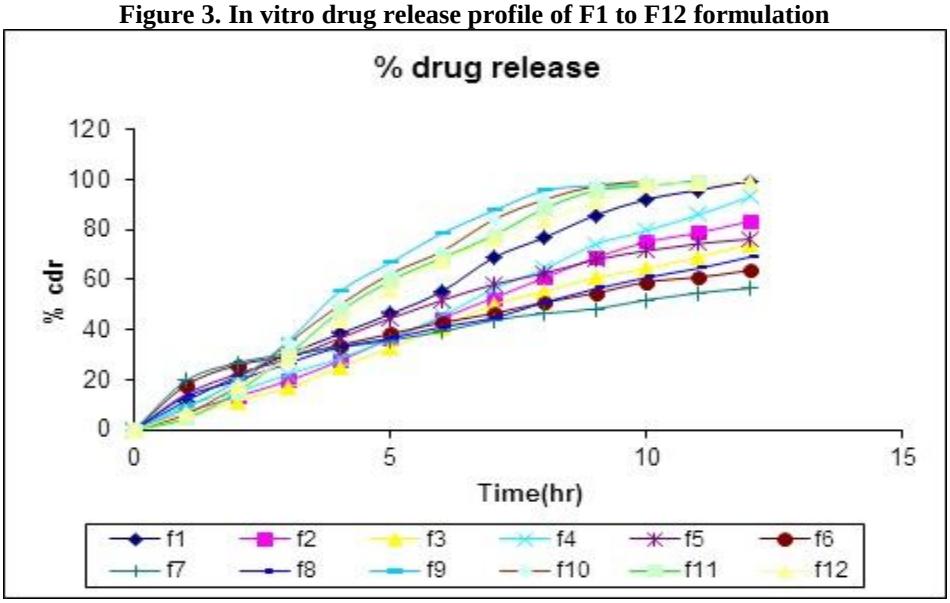
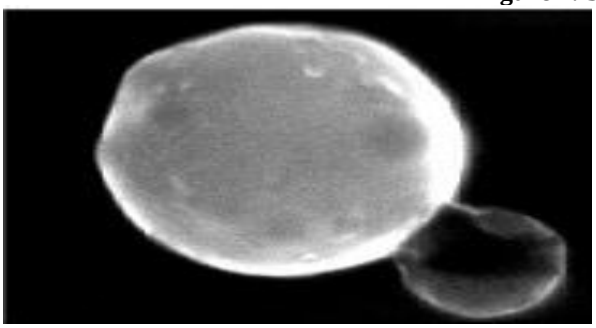
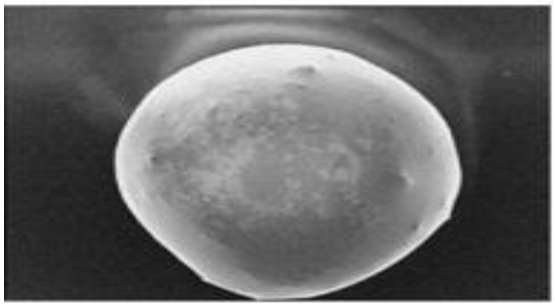


Figure 4. SEM of F4 and F8



(F4)



(F8)

CONCLUSION

A successful attempt has been made to formulate mucoadhesive microspheres of metformin hydrochloride using sodium alginate and a mucoadhesive polymer (Carbopol 934P, HPMC K100M, HPMC K4M) could be prepared by Orifice Ionic gelation technique. The microspheres exhibit good mucoadhesive properties in an *in-vitro* wash off test. Metformin hydrochloride release from these mucoadhesive microspheres was slow and longer period of time and depends on the composition of coat. Drug release from optimized formulation by diffusion mechanism and mixed order kinetics. So, these Mucoadhesive microspheres are thus suitable for oral control release of metformin hydrochloride.

REFERENCES:

1. Rouge N, Buri P, Doelker E. Drug absorption sites in the gastrointestinal tract and Dosage forms for site specific delivery. *Int J Pharm*, 136, 1996, 117-139.
2. Thomas Wai-Yip Lee and Joseph R. Robinson. Controlled / Release Drug-Delivery Systems 'Remington's Pharmaceutical Sciences', 20th Edn, Vol 1, Mack Publishing Company, 2000, 903-929.
3. Woo B, Jiang G, Jo Y, DeLuca P. Preparation and characterization of composite PLGA and Poly (acryloyl hydroxyl methyl starch) microsphere system for protein drug delivery. *Pharm Res*, 2001, 18, 1600-1606.
4. Capan Y, Jiang G, Giovagnoli S, DeLuca PP. Preparation and characterization of poly (D, L-lactide-co-glycolide) microsphere for controlled release of human growth hormone. *AAPS Pharm Sci Tech*, 4, 2003, E28.
5. Gohel MC, Amin AF. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. *J Control Release*, 51, 1998, 115-122.
6. Vasr JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. *Int J Pharm*, 255, 2003, 13-32.
7. Ikeda K, Murata K, Kobayashi M, Noda K. Enhancement of bioavailabity of dopamine via nasal route in beagle dogs. Chem Pharm Bull (Tokyo), 40, 1992, 2155-2158.
8. Nagai T, Nishimoto Y, Nambu N, Suzuki Y, Sekine K. Powder dosage form of insulin for nasal administration. *J Control Release*, 1, 1984, 15-22.
9. Ilium L, Farraj NF, Critechley H, Davis SS. Nasal administration of gentamicin using a Novel microsphere delivery system. *Int J Pharm*, 46, 1988, 261-265.
10. Schaefer MJ, Singh J. Effect of isopropyl myristic acid ester on the physical characteristics and in vitro release of etoposide from PLGA microspheres. *AAPS PharmSciTech*, 1, 2000, E32.
11. Rao SB, Sharma CP. Use of chitosan as biomaterial, studies on its safety and haemostatic Potential. *J Biomed Mater Res*, 34, 1997, 21-28.
12. Lehr CM, Bouwstra JA, Schacht EH, Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymer. *Int J Pharm*, 78, 1992, 43-48.
13. Henriksen I, Green KL, Smart JD, Smistad G, Karlsen J. Bioadhesion of hydrated Chitosans, an in vitro and in vivo study. *Int J Pharm*, 145, 1996, 231-240.
14. Chowdary KPR, Rao YS. Design and in vitro and in vivo evaluation of mucoadhesive Microcapsules of glipizide for oral controlled release, a technical note. *AAPS PharmSciTech*, 2003, 4, E39.
15. Yellanki shiva kumar, Deb sambit kumar, Goranti sharada, Nerella Naveen kumar. Formulation Development of Mucoadhesive Microcapsules of Metformin HCL Using Natural and Synthetic Polymers and In vitro Characterization. *Int J Pharm*, 2(2), 2010, 321-329.

16. Tripathi KD. Essential of medical pharmacology. 5th Edn, JayPee Brothers medical publishers (P) Ltd New Delhi, 2003, 245-248.
17. Complete Metformin Hydrochloride information, drug information online, and www.drug_com.mht.
18. Yellanki Shiva Kumar, Deb Sambit Kumar, Goranti Sharada, Nerella Naveen Kumar. Formulation Development of Mucoadhesive Microcapsules of Metformin HCL Using Natural and Synthetic Polymers and In vitro Characterization. *Int J Pharm*, 2(2), 2010, 321-329.
19. Kalyankar TM, Nalanda T. Rangan, Mubeena khan, Avinash Hosmani and Arvind Sonwane. Formulation and Evaluation of Mucoadhesive Pioglitazone HCL Microspheres. *IJPWR*, 2010, 1.
20. Najmuddin M, Ahmed A, Shelar S, Patel V, Khan T. Floating Microspheres of Ketoprofen, formulation and evaluation". *Int J Pharm and pharmaceutical sciences*, 2(12), 2010, 83-87.
21. Kakkar AP. Characterization of Ibuprofen Loaded Microcapsules Prepared by Ionotropic Gelation. *Ind. J. Pharm. Sci*, 57(2), 1995, 56-60.
22. Manna A, Ghosh I, Goswami N, Ghosh LK, Gupta BK. Design and Evaluation of an Oral Controlled Release Microparticulate Drug Delivery System of Nimesulide by Ionotropic Gelation Technique and Statistical Optimization by Factorial Analysis. *J Sci Ind. Res*, 58(9), 1999, 717-722.
23. Yadav KS, Satish CS, Shivkumar HG. Preparation and evaluation of chitosan-Poly (Acrylic acid) Hydrogels as Stomach specific delivery for amoxicillin and Metronidazole. *Indian J. Pharm. Sci*, 69, 2007, 91-95.
24. Note for guidance on stability testing. Stability testing of new drug substances and products 2003. [Cited 2008 Dec 02], Available from URL, http,//www.tga.gov.