Int. J. Chem. Sci.: 10(3), 2012, 1605-1613
ISSN 0972-768X
www.sadgurupublications.com

DESIGN AND DEVELOPMENT OF METFORMIN HCl MUCOADHESIVE MICROCAPSULES DESIGN AND EVALUATION OF ALGINATE BASED MUCOADHESIVE MICROCAPSULES OF METFORMIN HCl

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ABSTRACT

In this study, an attempt was made to prepare mucoadhesive microcapsules of metformin HCl using various mucoadhesive polymers designed for oral controlled release. Metformin HCl microcapsules were prepared using sodium alginate and mucoadhesive polymer such as sodium carboxymethyl cellulose (sodium CMC), carbopol 934P or hydroxy propylmethyl cellulose (HPMC) by orifice-ionic gelation method. The microcapsules were evaluated for surface morphology and particle shape by scanning electron microscope. Microcapsules were also evaluated for their microencapsulation efficiency, *in vitro* wash-off mucoadhesion test, *in vitro* drug release and *in vivo* study. The micro capsules were discrete, spherical and free flowing. The microencapsulation efficiency was in the range of 65-80% and microcapsules exhibit good mucoadhesive property in the *in vitro* wash-off test. The percentage of microcapsules adhering to tissues at pH 7.4 after 6 h varied from 12-32%, whereas the percentage of microcapsules adhering to tissues at pH 1.2 after 6 h varied from 35-65%. The drug release was also found to be slow and extended for more than 16 h. *In vivo* testing of the mucoadhesive microcapsules in diabetic albino rats demonstrated significant antidiabetic effect of metformin HCl. The hypoglycemic effect obtained by mucoadhesive microcapsules was for more than 16 h whereas metformin HCl produced an antidiabetic effect for only 10 h suggesting that mucoadhesive microcapsules are valuable system for the long term delivery of metformin HCl.

Key words: Controlled release, Metformin HCl, Microcapsules, Mucoadhesive.

INTRODUCTION

Microencapsulation is a useful method for prolonging drug release from dosage forms and reducing adhesive effects[1-3]. Recently, dosage forms that can be precisely control

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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. Microparticles are defined as spherical polymeric particles. These microparticles constitute an important part of these drug delivery systems, by virtue of their small size and efficient carrier characteristics. However, the success of these novel microparticles is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with absorbing membranes. It can be achieved by coupling bioadhesion characteristics to microparticles and developing novel delivery systems referred as "Bioadhesive Microparticles".

Bioadhesive microparticles include microspheres and microcapsules (having a core of the drug) of 1-1000 μm in diameter and consisting either entirely of a bioadhesive polymer or having an outer coating of it, respectively. Bioadhesive microparticles have advantages such as efficient absorption and enhanced bioavailability of drugs owing to their high surface to volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[4-10.]

Metformin HCl, an effective antidiabetic that requires controlled release owing to its short biological half-life of 3.4 ± 0.7 hours, was used as the core in micro-encapsulation[11,12.] The purpose of this research was to formulate and *in vitro* evaluation of mucoadhesive microcapsules of metformin HCl with polymers like sodium CMC, carbopol 934P, sodium alginate and calcium chloride to achieve controlled release.

EXPERIMENTAL

Materials and method

Materials

Metformin HCl U.S.P was kindly gifted by M/s Aurobindo Pharmaceuticals, Hyderabad. Sodium CMC (having a viscosity of 1,500-3,000 cps of percent w/v aqueous solution at 25^{o}C), HPMC (having a viscosity of 50 cps in a 2% w/v aqueous solution at 20^{o}C), carbopol 934P, sodium alginate and calcium chloride were obtained from Central Drug House (CDH, Mumbai, India). All other reagents used were of analytical grade.

Methods

Preparation of microcapsules

Mucoadhesive microcapsules containing metformin HCl were prepared employing sodium alginate in combination with three mucoadhesive polymers- sodium CMC, carbopol 934P and HPMC as coat materials. Orifice-ionic gelation method was employed to prepare

the microcapsules[13]. Sodium alginate and the mucoadhesive polymer were dissolved in 50 mL of purified water to form a homogeneous polymer solution. The active substance metformin HCl was added to the polymer solution (in a ratio of metformin HCl: polymer solution 1 : 1) and mixed thoroughly to form a viscous dispersion. The resulting dispersion was then added manually dropwise into calcium chloride (10% w/v) solution (100 mL) through a syringe with a 26 gauge needle. The addition of dispersion in the calcium chloride solution was completed within 3 h of the preparation of the dispersion. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microcapsules. The time of gel formation influences the drug loading efficiency and also the cohesion of the gel[14]. The microcapsules were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45^{o}C for 12 h. The microcapsules prepared with their coat composition are listed in Table 1.

Table 1: Polymer admixture ratios, amount of polymer, metformin HCl micro-encapsulation efficiency and viscosities of polymer solutions

S. No.	Micro-capsules	Polymers admixture composition (mg/mg)	Viscosity of polymer solution	Microencapsulation efficiency (%)
1.	MC1	Alginate/Sodium CMC (1000 : 1000)	1,800 cps	80.00 (0.20)
2.	MC2	Alginate/Carbopol (1,000 : 1,000)	35,680 cps	72.00 (1.35)
3.	MC3	Alginate/HPMC (1000 : 1000)	45.8 cps	65.50 (0.76)
4.	MC4	Alginate/Sodium CMC (1,666.6 : 333.3)	1,609 cps	75.68 (0.80)
5.	MC5	Alginate/Carbopol (1,666.6 : 333.3)	35,670 cps	73.43 (2.50)
6.	MC6	Alginate/HPMC (1,666.6 : 333.3)	41.8 cps	68.20 (2.70)
7.	MC7	Alginate/Sodium CMC (1,800 : 200)	1,448 cps	78.00 (2.05)
8.	MC8	Alginate: Carbopol (1,800 : 200)	35,630 cps	75.56 (2.72)
9.	MC9	Alginate: HPMC (1,800 : 200)	37.6 cps	71.72 (2.23)

Figures in parentheses are coefficient of variation values.

Estimation of metformin HCl

Metformin HCl content in the microcapsules was estimated by a UV-spectrophotometer (UV-1700, Shimadzu, Japan). Actual drug content and encapsulation efficiency of the microcapsules was determined by the following method. 100 mg of microcapsules were crushed using mortar and pestle. The crushed microcapsules were placed in 100 mL of 0.1 N HCl (pH 1.2) and shaken for 1 h at 37 ± 0.5°C in mechanical shaker. The samples were then filtered to obtained clear solution and analyzed for the drug content spectrophotometrically at 233 nm. It gives drug content for 100 mg of microcapsules from that calculate drug content for total quantity of micro capsules. From actual drug content, the value of encapsulation efficiency was determined using the formula given below. The method was validated for linearity, accuracy and precision. The method obeyed Beer's law in the concentration range 1 to 10 µg/mL. When a standard drug solution was assayed repeatedly (n = 6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6% and 0.8%, respectively.

Microencapsulation efficiency

An appropriate amount of microcapsules was first crushed, then weighed and suspended in methanol to extract the drug from microcapsules while assuring that there was no loss of material in the process. After 24 h, the filtrate was assayed spectrophotometrically at 233 nm for drug content against methanol as blank. Microencapsulation efficiency was calculated using the formula:

$$\text{Microencapsulation efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100 \qquad \ldots(1)$$

Particle size analysis

Particle size distribution of microcapsules was done by sieve analysis procedure. The microcapsules were shaken on a mechanical shaker, using a nest of British standard sieves, for 15 min.

Scanning electron microscopy (SEM)

SEM was performed for morphological characterization of microcapsules using scanning electron microscope (SEM-LECIA, 5430, London, U.K). They were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film (thickness, 200 nm) under reduced pressure (0.001 mm Hg).

In vitro drug release study

The drug release was performed using USP 24 (paddle type) apparatus at 37 ± 0.5°C and at 100 rpm in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) as dissolution medium. Microcapsules (100 mg) of metformin HCl were used for the test. Five mL of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 μm membrane filter, diluted suitably and analyzed spectrophotometrically at 233 nm. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of test sample. The drug release experiments were conducted in triplicate (n = 3).

Mucoadhesion testing by *in vitro* wash-off test

The mucoadhesive properties of the microcapsules were evaluated by *in vitro* wash-off test as reported by Lehr et al.[15] A 2 cm wide and 2 cm long (2 x 2) piece of rat intestinal mucosa was tied onto a glass slide (3 in. long and 1 in. wide) using thread. About fifty microcapsules were spread onto the wet, rinsed, tissue specimen, and allowed to hydrate for 30 s. The prepared slide was hung onto one of the grooves of a USP 24 tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the test fluid at 37°C contained in one liter vessel of the machine. At the end of 1 h, and at hourly intervals up to 6 h, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed at both gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4).

RESULTS AND DISCUSSION

Microcapsules of metformin HCl with a coat consisting of sodium alginate and a mucoadhesive polymer-sodium CMC, carbopol 934P or HPMC in 1 : 1, 5 : 1 and 9 : 1 ratio could be prepared by the orifice-ionic gelation method. Microcapsules with a coat of mucoadhesive polymer alone could not be prepared by this method because of their water soluble nature[16].

Twenty formulations with different polymers admixtures were prepared. The ratio of mucoadhesive polymer was kept constant and the proportion of only sodium alginate was increased because of the gelling property of alginate to form the beads. These formulations were tested for particle size and an *in vitro* mucoadhesion test. From the study on these formulations, we found that there was no effect of polymer type and ratio mixtures of

polymer solution. Only extrusion device and viscosity of the polymer solution affected the particle size. Out of these 20 formulations, 9 formulations with alginate : mucoadhesive polymer ratio of 1 : 1, 5 : 1 and 9 : 1 were selected for *in vitro* study on the basis of these formulation (ratio 1 : 1, 5 : 1 and 9 : 1) was in the range of 35-68% after 6 h. Since the main aim of our study was to improve the bioadhesive strength of microcapsules so these formulations were selected for the *in vitro* release study. From the *in vitro* release study, formulations MC7 and MC8 have shown good controlled release for more than 16 h and thus, they were selected for the *in vitro* study.

Calibration curve of metformin HCl, Y = 0.042 x - 0.01 (r = 0.999), showed the good linear relationship with concentration ranged between 2-20 µg/mL. The lowest measurable concentration was 2 µg/mL and percentage coefficient of variation was from 0.2 to 3.5 and therefore, the result was sufficiently acceptable.

Size of extrusion device and the viscosity of polymer solution were found to affect the particle size of microcapsules prepared by orifice-ionic gelation method. Decreasing the viscosity of polymer solution caused the mean particle size to shift towards a lower particle size. Increasing the viscosity of polymer solution, formed larger droplets and consequently, microcapsules with large particle size. Increasing the size of extrusion device increased the particle size of microcapsules. Needle No. 26 was found suitable for the formulation of microcapsules. The mean particle size was not affected by the polymer type and the ratio of mixture of polymers for all formulations. The SEM photographs indicated that the microcapsules were spherical and rough in nature. Surface morphology also revealed presence of cracks on the surface. The microencapsulation efficiencies were high for all microcapsules obtained. The micro encapsulation efficiencies were found to be affected by the type of polymer. The microencapsulation efficiency for sodium alginate-sodium CMC was found higher compared to sodium alginate-HPMC and sodium alginate-carbopol 934P. The micro encapsulation efficiencies were found unaffected by different ratios of polymer mixture. The micro encapsulation efficiency was in the range of 65-80% (Table 1).

Metformin HCl release from the microcapsules was studied at pH 7.4 for 16 h, Metformin HCl release from microcapsules was slow and depended on the composition of the coat. Microcapsules of alginate-carbopol gave relatively slow release when compared to others. The order of increasing release rate observed with various microcapsules was sodium alginate-carbopol 934 P < sodium alginate-sodium CMC < sodium alginate-HPMC.

Metformin HCl release from microcapsules MC7 and MC8 was slow and extended over a period of 16 h, and these microcapsules were found suitable for oral controlled release formulation. The microcapsules of batch MC7 and MC8 were also evaluated at pH 1.2. Metformin HCl release from microcapsules MC7 and MC8 at pH 1.2 was slow as compared to release at pH 7.4. The reason for this low release might be the acid resistant nature of sodium alginate and metformin HCl is a weak acid drug and its solubility is higher at high pH, as expected.

Microcapsules with a coat consisting of sodium alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the *in vitro* wash-off test. The mucoadhesion test for microcapsules was performed at gastric pH 1.2 and intestinal pH 7.4 continuously for 6 h. The wash-off was faster at intestinal pH (7.4) than at gastric pH (1.2). The results of the wash-off test indicated that microcapsules had fairly good mucoadhesive property. The percentage of microcapsules adhering to tissue at 7.4 after 6 h varied from 12 to 32, whereas the percentage of microcapsules adhering to tissue at pH 1.2 after 6 hours varied from 35 to 68 (Tables 2 and 3).

Table 2: Percentage of microcapsules adhering to tissue in 0.1 N HCl, pH 1.2

Micro-capsules	Time (hrs.)					
	1	**2**	**3**	**4**	**5**	**6**
MC1	75 (1.8)	70 (2.1)	65 (1.2)	62 (0.6)	55 (31)	51 (1.1)
MC2	78 (0.8)	72 (1.0)	70 (1.5)	68 (1.1)	65 (1.6)	60 (2.3)
MC3	82 (0.9)	78 (2.1)	75 (1.1)	72 (1.6)	70 (2.0)	68 (2.3)
MC4	70 (1.6)	68 (1.2)	60 (2.8)	55 (2.8)	50 (1.8)	42 (3.3)
MC5	76 (2.2)	72 (1.5)	70 (0.6)	65 (2.8)	56 (1.4)	52 (1.5)
MC6	77 (1.7)	70 (2.1)	65 (1.7)	63 (2.8)	60 (1.6)	55 (0.7)
MC7	78 (1.8)	68 (2.1)	65 (0.9)	52 (3.1)	48 (2.3)	42 (1.4)
MC8	80 (2.3)	68 (1.8)	60 (2.0)	55 (1.6)	50 (3.8)	40 (4.0)
MC9	82 (1.1)	72 (1.1)	65 (3.3)	52 (1.3)	48 (3.1)	35 (2.8)

Figures in parentheses are coefficient of variation values

Table 3: Percentage of microcapsules adhering to tissue in phosphate buffer pH 7.4

Micro-capsules	Time (hrs.)					
	1	**2**	**3**	**4**	**5**	**6**
MC 1	68 (0.6)	62 (2.5)	55 (2.0)	45 (1.1)	35 (1.7)	28 (28)
MC 2	70 (1.1)	66 (1.8)	60 (3.0)	42 (1.6)	32 (2.5)	30 (2.4)
MC 3	66 (2.1)	55 (0.9)	50 (3.6)	40 (2.0)	35 (3.1)	25 (2.4)
MC 4	70 (1.3)	62 (1.9)	55 (2.5)	50 (2.8)	45 (1.3)	32 (3.4)
MC 5	72 (2.5)	55 (3.2)	48 (2.5)	32 (3.1)	25 (2.8)	18 (3.3)
MC 6	68 (1.6)	42 (2.6)	35 (2.2)	28 (1.4)	20 (2.0)	12 (3.3)
MC 7	71 (2.5)	54 (0.4)	36 (2.8)	30 (2.2)	25 (4.0)	18 (2.2)
MC 8	76 (0.8)	65 (2.3)	55 (2.0)	42 (0.9)	35 (2.8)	25 (0.8)
MC 9	70 (1.6)	52 (1.7)	45 (1.7)	35 (1.7)	20 (3.0)	12 (1.6)

Figures in parantheses are coefficient of variation values

CONCLUSIONS

The spherical microcapsules with a coat consisting of alginate and a mucoadhesive polymer (sodium CMC, carbopol 934P or HPMC) could be prepared by an orifice-ionic gelatin process. The microcapsules exhibited good mucoadhesive properties in an *in vitro* test. Metformin HCl release from these mucoadhesive microcapsules was slow and extended over longer periods of time (12-16 h) and depended on compositions of the coat. Developed mucoadhesive microcapsules are suitable for controlled release effect after oral administration of metformin HCl.

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Revised : 26.12.2011 *Accepted : 27.12.2011*