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Research Article

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DESIGN AND EVALUATION OF SRM MICROSPHERES OF METFORMIN HYDROCHLORIDE

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ABSTRACT

The present work was envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating Sustained Release Mucoadhesive (SRM) microspheres of Metformin hydrochloride (MH) for effective control of diabetes type-2. Microspheres were prepared by emulsification solvent evaporation method using Sodium carboxy methyl cellulose (SCMC), Carbopol 934P (CP), and Hydroxyl propyl methyl cellulose K4M (HPMC) as a mucoadhesive polymers. Microspheres prepared were found discrete, spherical and free flowing. The microspheres exhibits good mucoadhesive properties and showed high drug entrapment efficiency. MH release from these microspheres was slow and extended and dependent on the type of polymer used. The mean particle size decreased and the drug release rate increased at higher Stirring speed of emulsion content. Among all the formulation, formulation F1 containing SCMC and F2 containing CP showed the best reproducible results and mucoadhesive profile with good surface morphology. The data obtained thus suggest that mucoadhesive microspheres can successfully design for sustained delivery of MH and to improve patient compliance.

Keywords: Microspheres, Diabetes type-2, Mucoadhesion, Controlled Drug Release, SCMC, CP, HPMC.

INTRODUCTION

Substantial efforts have recently been focused upon placing a drug or drug delivery system in a particular region of the body for extended period of time. From a technological point of view, an ideal Sustained Release Mucoadhesive (SRM) dosage form must have three properties. It must maintain its position in the mouth for a few hours, release the drug in a controlled fashion and provide the drug release in a unidirectional way towards the mucosa. Microspheres form an important part of such novel drug delivery systems. However, the success of these microspheres is limited owing 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 the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres.[1-6] Bioadhesive 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.[7,8] Mucoadhesive microspheres that are retained in the stomach would increase the drug absorption and decrease dosing frequency which provides better patient compliance as compared to conventional dosage forms.

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MH is an oral hypoglycaemic agent belongs to biguanides class[9]. MH 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 MH 4 times a day**.** A conventional dose of 500 mg can control glucose level 6-8 hours but not up to 12 or more hours[10]. High dose of MH may cause Vitamin B_{12} deficiency due to interference with its absorption. More ever the site of MH absorption is stomach whereas at the colon MH is poorly absorbed[9]. Owing to its short biological half life 1.5-3 hours, low bioavailability (60%) and high incidence of GI side effects (30% cases)[9-10]; it's necessary to develop a sustained release mucoadhesive dosage form of MH which adhere to the mucosa and release the drug in sustained release manner. Thus SRM microspheres of MH are suitable candidate for effective control of diabetes type-II.

Literature survey revealed that SCMC[6, 11], CP[11, 12] and HPMC[13,14] are the polymer which shows good mucoadhesive properties, high drug entrapment efficiency and release the drug in sustained release manner. Therefore in the present study MH is selected as a model drug and SCMC, CP and HPMC are chosen as a mucoadhesive polymer for design and evaluation SRM Microspheres for treatment of diabetes type-II.

MATERIALS AND METHODS

Materials

MH was a gift sample from Ranbaxy Laboratories Ltd., Hyderabad, India. CP was gifted from Colorcon Asia Pvt.

Ltd, Goa. HPMC was received as gift sample from Zydus-Cadila Healthcare Ltd, Ahmadabad, India. SCMC, n-Hexane and span 20 were procured from central drug house, New Delhi. Liquid paraffin was procured from Loba Chemie Pvt. Ltd., Mumbai. All the reagents were used of analytical grade.

Methods

Assay of Metformin hydrochloride

Metformin hydrochloride was estimate using an UV spectrophotometer method. Different solutions of MH were prepared in simulated gastric fluid (pH 1.2) and absorbance was measured on Shimazdu UV spectrophotometer at 233 nm. The method was validated for linearity, accuracy, and precision. The regression coefficient was found to be 0.996.

Preparation of microspheres[6, 15]

Mucoadhesive microspheres of MH were prepared by emulsification solvent evaporation method using various ratios of SCMC, CP and HPMC. For this, aqueous solution of drug and polymer is prepared. Then drug and polymer solution was added drop wise to the liquid paraffin containing 0.5 % span 20 as an emulsifying agent with constant stirring. The constant stirring was carried out using magnetic stirrer. The beaker and its content were heated at 80^{0}C with constant stirring for 4 hrs until the aqueous phase was completely removed by evaporation. The liquid paraffin was decanted and collected microsphere were washed 5 times with n-hexane, filtered through whattman's filter paper and dried in hot air oven at 50°C for 2 hours. Table **1** shows composition of various formulations of microspheres.

Table 1. Composition of drug loaded microspheres

Formulation	Drug (mg)	SCMC (mg)	CP (mg)	HPMC (mg)	Stirring Speed (rpm)
F1	100	900	--	--	500
F2	100	--	900	--	500
F3	100	--	--	900	500
F4	100	450	450	--	500
F5	100	450	--	450	500
F6	100	--	450	450	500
F7	100	300	300	300	500
F8	100	300	300	300	1000

Values are represented as mean ± standard deviation (n=3).
All formulations were prepared at 2% polymer concentration.

Surface morphology[16, 17]

The surface morphology and structure were visualized by scanning electron microscopy (SEM). The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to on aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. After gold coating samples were randomly scanned for particle size and surface morphology.

Particle Size[18, 19]

Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a compound microscope (Erma, Tokyo, Japan). A small amount of dry microspheres was suspended in n-hexane (10 mL). The suspension was ultrasonicated for 5 seconds. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The average particle size was determined by using the Edmondson's equation $D_{mean} = \sum nd/\sum n$, where n= number of microspheres observed and d= mean size range. The process was repeated 3 times for each batch prepared.

Drug entrapment efficacy[18]

50 mg of microsphere were taken and drug was extracted from microspheres by digesting for 24 hours with 10 ml of simulated gastric fluid (pH 1.2). During this period the suspension was agitated. After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula:

Entrapment efficiency = (Practical drug content/ theoretical drug content) ×100

The results are given in the table **2**.

In-vitro mucoadhesivity[6,7,20]

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test as reported by Lehr et al. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (∽50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following formula:

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

The observations are shown in table **2** and expressed in figure **2**, **3**, **4**.

In-vitro drug release[21, 22]

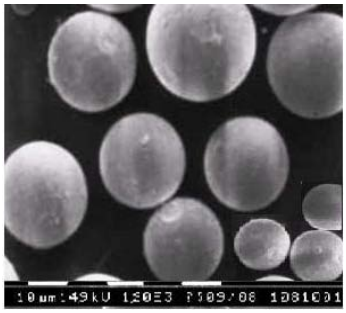
In-vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using simulated gastric fluid (pH 1.2) as dissolution medium, volume of dissolution medium was 900 ml and bath temperature was maintained at (37±1) °C throughout the study. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, 10 ml of sample was withdrawn with replacement of 10 ml fresh medium and analyzed for drug content by UV-Visible spectrophotometer at 233 nm. All the experimental units were analyzed in triplicate (n=3). Cumulative percentage drug release was calculated using an equation obtained from a standard curve. The observations are expressed in figure **5**, **6**, **7**.

RESULTS AND DISCUSSION

Surface morphology

Surface morphology of the mucoadhesive microspheres was examined by scanning electron microscopy (SEM). The SEM showed that the microspheres obtained from all the formulations are spherical with smooth surface. The SEM showed that the blend of SCMC and CP produced spherical with smooth surface microspheres due to their high solubility in water[11, 12]. The SEM of microspheres of formulation F2 is shown in figures **1**.

Figure 1. SEM of formulation F2 showing population of Microspheres



Particle size analysis

Particle size analysis of different formulations was done by optical microscopy[18, 19]. The average particle size was found to be in the range of 29.85 to 52.54 µm. The mean particle size was significantly varied according to type of polymer used for the preparation of microspheres; this may be due to fact that difference in the viscosity of the polymer solution[11]. Since high viscosity of polymer solution requires high shearing energy for breaking of droplets of the emulsion. Microspheres containing HPMC are larger as compared to SCMC microspheres because HPMC solution has more viscosity at the same concentration. Particle size decreased with increasing stirring speed due to the fact that increased in stirring speed, produce high energy, which leads to further decrease in droplets size. Results of particle size analysis are shown in table **2**.

Table 2. % yield, % drug entrapment, Particle size, % mucoadhesion and R^2 value of microspheres

Formulation code	% yield	% Drug entrapment	Particle size (µm)	% Mucoadhesion after 1 hr	R^2 value for *In vitro* drug release
F1	69.45±3.20	71 ± 2.45	39.54±2.43	94.33±2.33	0.9835
F2	67.56±2.80	80 ± 3.23	36.20± 1.88	92.22±2.43	0.9947
F3	75.10±2.95	64 ± 2.86	52.54 ±3.24	90.23±2.83	0.9976
F4	72.52±2.84	78 ± 2.75	43.62 ±2.78	85.20±1.90	0.9828
F5	68.12±3.10	67 ± 2.84	48.12 ±2.84	96.33±2.95	0.9843
F6	70.44±2.60	62 ± 3.68	46.85 ±3.15	84.44±2.38	0.9967
F7	71.32±2.45	61 ± 2.54	42.54 ±2.75	89.44±2.32	0.9984
F8	66.44±2.60	58 ± 2.64	29.85 ±3.15	88.44±3.12	0.9983

Values are represented as mean ± standard deviation (n=3). All formulation were prepared at 2% polymer concentration

Drug entrapment efficiency

Drug content in different formulations was estimated by UV Spectrophotometric method. Percent drug loading efficiency of microspheres was found in the range of 58.00 to 80.00 (table **2**).

Formulation F2 containing CP showed maximum % drug loading about 80 % whereas formulation F8 containing Blend of SCMC, CP and HPMC showed minimum % drug loading about 58% because these microspheres are small in size which results more loss of drug from surface during washing of microspheres. Rank order of % drug loading of various formulations was found to be as follows:

F2 > F4 > F1 > F5 > F3 > F6> F7> F8

In-vitro mucoadhesivity test

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.

Formulation F1 containing SCMC showed the highest mucoadhesivity. The greater mucoadhesivity of SCMC microspheres were due to anionic nature of the polymer which is desirable characteristics of adhesion to the mucus layer[11]. Formulation F8 containing Blend of SCMC, CP and HPMC showed the shortest mucoadhesion time to the small size of microsphere which take short time for solubilization. The order of % mucoadhesivity for all the formulations, after 8 hours was found to be as follows (figure-**2, 3, 4** and table **2**):

F1 > F5> F2 > F3 > F7> F8 > F4 > F6

Figure 2. Comparative % mucoadhesion of microspheres of formulations F1-F3

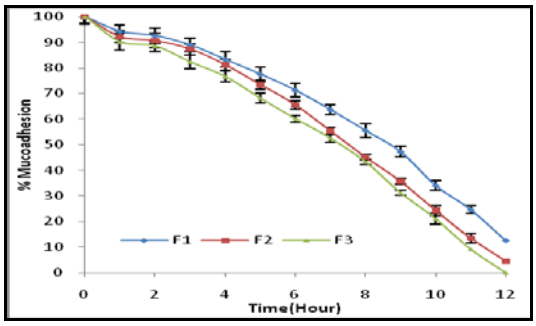


Figure 3 Comparative % mucoadhesion of microspheres of formulations F4-F6

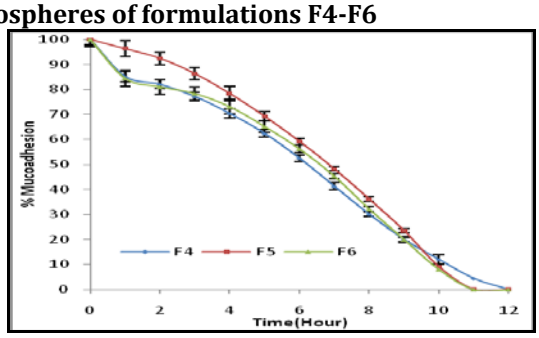
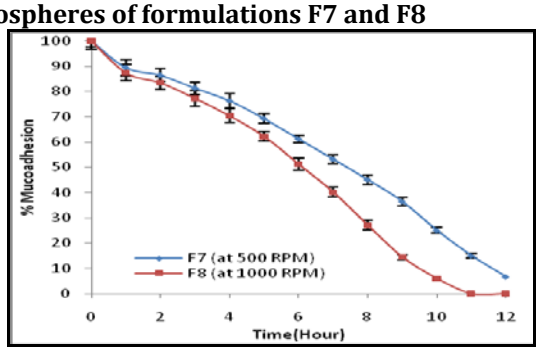


Figure 4 Comparative % mucoadhesion of microspheres of formulations F7 and F8



Drug release study

Drug release study of all the formulations containing drug were performed in simulated gastric fluid (pH 1.2) at 37^{0}C ± 1. Drug release form these microspheres were slow, extended and dependent on the type of polymer and concentration of polymer used. Formulation F1 containing SCMC showed the maximum release due to rapid swelling property and high dissolution of SCMC in dissolution environment (0.1 N HCl). Dissolution medium permeation in to the microspheres is facilitated due to high swelling action of the SCMC which leads to more medium for the transport of the drug is available. While HPMC microspheres showed the least drug release. Drug release is significantly affected by the size of microspheres. Formulation F8 shown fastest drug release among all the formulation due to fact that these microspheres are small in size. After the end of 10 hrs, the drug release were found to be 95.45±2.55, 91.43±2.55, 72.23±2.10, 86.22±3.33, 82.54±3.44, 76.76±2.33, 78.54 and 96.22 % for formulations F1, F2, F3, F4, F5, F6, F7 and F8 respectively(figure **5**, **6** and **7**).

Figure 5. Cumulative % drug release from microspheres of formulation F1-F3

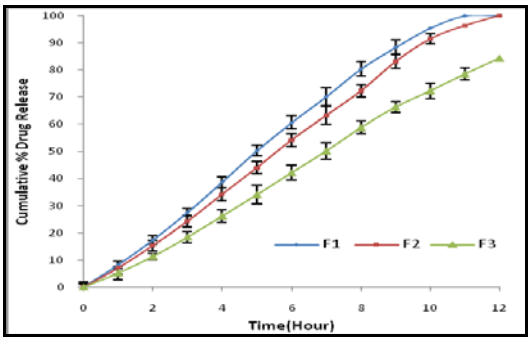


Figure 6. Cumulative % drug release from microspheres of formulation F4-F6

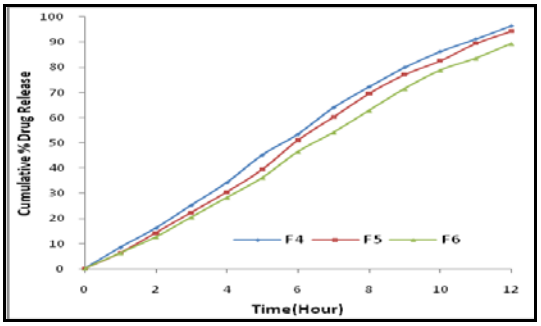
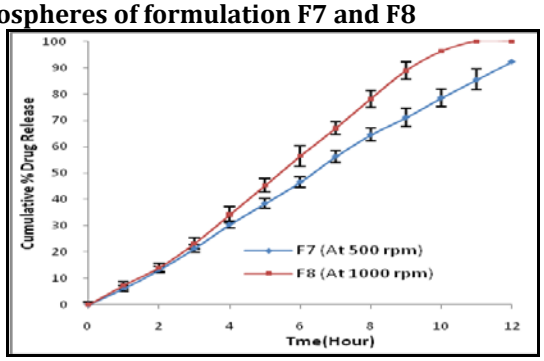


Figure 7. Cumulative % drug release from microspheres of formulation F7 and F8



FUTURE PROSPECTS

While the control of drug release profiles has been a major aim of pharmaceutical research and development in the past two decades, the control of GI transit profiles could be the focus of the next two decades and might result in the availability of new products with better therapeutic possibilities and substantial benefits for patients. Mucoadhesive microspheres would become the promising candidate for delivery various drugs in sustained release manner. Dosing frequency and loss of drug also reduced by use of such type of formulations. Thus SRM microspheres of MH would become a promising candidate for therapy of diabetes type-II in the future.

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