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In Vitro and *In Vivo* Evaluation of Cimetidine loaded mucoadhesive microspheres

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Abstract

In the present research work mucoadhesive microspheres of cimetidine was prepared using ionotropic gelation technique. All the microspheres were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, drug entrapment, stability studies and for in vitro release kinetics and found to be within the limits. Among all the formulations M12 was selected as optimized formulation based on the physicochemical and release studies. In vitro drug release study of optimized formulation M12 showed 99.12% after 12 h in a controlled manner, which is essential for anti ulcer therapy. The innovator cimetine conventional tablet showed the drug release of 96.15% within 1 h. The drug release of cimetidine optimized formulation M12 followed zero order and Higuchi kinetics indicating diffusion controlled drug release. *In vivo* studies revealed that the optimized formulation M12 gave the highest AUC and T_{max}. The results are indicative of cimetidine as mucoadhesive microspheres for improving the oral bioavailability with controlled drug release.

Keywords: Cimetidine, mucoadhesion, chitosan, ionotropic gelation, bioavailability.

Introduction

Oral route is most sought-after for administration of drug molecules to the systemic circulation due to low cost therapy, ease of administration, patient compliance [1]. New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard novel drug delivery systems (NDDS) have many benefits, which includes improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce unwanted adverse effects [2].

Despite the problem frequently encountered with controlled release dosage forms is the inability to increase the residence time of the dosage form in the stomach and proximal portion of the small intestine, due to the rapid gastrointestinal transit phenomenon of the stomach which may consequently reduce the extent of absorption of many drugs since almost most of the drug entities are mostly absorbed from the upper part of the intestine, therefore it would be beneficial to develop a sustained release formulation which remain at the absorption site for an extended period of time so that maximum of dose is absorbed in systemic circulation. Several approaches have been immersed to prolong the residence time of the dosage forms at the absorption site and one of these is the development of oral controlled release mucoadhesive system. Various gastrointestinal mucoadhesive dosage forms, such as microspheres and tablets, have been thoroughly prepared and reported by several research groups [3,4].

Mucoadhesion is the relatively new and emerging concept in drug delivery. Mucoadhesion keeps the delivery system adhering to the mucous membrane [5].

Peptic ulcer disease is a break in the lining of the stomach, first part of the small intestine or occasionally the lower esophagus [6].

Cimetidine is histamine H_2-receptor antagonists, which is used to reduce the risk of stomach ulcers in patients treated with nonsteroidal anti-inflammatory drugs, which has less bioavailability (60%) and lesser half life of 2 h [7]. The aim of present work is to design and evaluate mucoadhesive microspheres of cimetidine *in vitro and in vivo* to enhance its bioavailability and prolong residence time in stomach.

Materials and Methods

Materials

Cimetidine pure drug was generous gift from Aurobindo Pharma Limited, Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Sodium alginate, chitosan, xanthan gum, kondagogu gum and sodium CMC were gifted from MSN Labs Ltd., Hyderabad. All other chemicals used were of analytical grade.

Formulation of Cimetidine mucoadhesive microspheres

Cimetidine mucoadhesive microspheres were prepared using different polymers like sodium alginate, chitosan, sodium CMC, xanthan gum and gum kondagogu by ionotropic gelation method. Different formulation trials of cimetidine were prepared using different concentrations of polymer and cross linking agent. Total 14 formulations were developed using different polymers in different concentrations. In this method, weighed quantity of cimetidine was added to 100 ml sodium alginate solution and thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 min the obtained microspheres were washed with water and dried at 60^{o}C – 2 h in a hot air oven and stored in desciccator [8].

Table 1: Formulation trials for Cimetidine mucoadhesive microspheres

FORMULATION CODE	CIMETIDINE (g)	SODIUM ALGINATE	SODIUM CMC(mg)	CALCIUM CHLORIDE	XANTHAN GUM	GUM KONDAGOGU
M1	2	1 %	100	7%	1%	0.5%
M2	2	1.2 %	150	7%	1.2%	0.5%
M3	2	1.4%	200	7%	1.4%	0.5%
M4	2	1.6%	250	7%	1.6%	0.5%
M5	2	1.8%	300	7%	1.8%	0.5%
M6	2	2%	350	7%	2%	0.5%
M7	2	2.2%	400	7%	2.2%	0.5%
FORMULATIN CODE	**CIMETIDINE (g)**	**SODIUM ALGINATE**	**CHITOSAN (mg)**	**CALCIUM CHLORIDE**	**XANTHAN GUM**	**GUM KONDAGOGU**
M8	2	1%	10	10%	1%	0.5%
M9	2	1.2%	15	10%	1.2%	0.5%
M10	2	1.4%	20	10%	1.4%	0.5%
M11	2	1.6%	25	10%	1.6%	0.5%
M12	2	1.8%	30	10%	1.8%	0.5%
M13	2	2%	35	10%	2%	0.5%
M14	2	2.2%	40	10%	2.2%	0.5%

Evaluation studies of Cimetidine mucoadhesive microspheres

Micromeretic parameters like particle size[9], angle of repose, bulk density, tapped density, compressibility index, Hausner's ratio [10], swelling index [11], drug entrapment efficiency and % yield [12].

Mucoadhesiveness

The *in vitro* mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segments of jejunum were averted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the averted sac from the position of 2 cm above. Then the sac was suspended in a 50 ml tube containing 40 ml of saline by the wire, to immerse in the saline completely. The sacs were incubated at 37^{0}C and agitated horizontally. The sacs were taken out of the medium after immersion for 1, 2, 3, 4, 5, 6, 7 and 8 h, immediately repositioned as before in a similar tube containing 40 ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation [13].

Mucoadhesion= (No. of microspheres adhered/ No. of microspheres applied) x 100

In vitro drug release studies

In vitro drug release studies for developed cimetidine microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 0.1 N HCl at 37±0.5^{0}C temperature at 100 rpm. The amount of drug release was determined at different time intervals of 0, 1, 2, 3, 4, 6, 8, 10 & 12 h by UV-visible spectrophotometer (Shimadzu UV 1800) at 218 nm [14].

Drug excipient compatibility studies

The drug excipient compatibility studies were carried out by Fourier transmission infrared spectroscopy (FTIR) method, Differential

Scanning Calorimetry (DSC), SEM and release order kinetics along with stability studies [12].

In vivo bioavailability studies

Twelve New Zealand white rabbits of either sex were (weighing 2-3 kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25^0C, RH 45% and 12 h alternate light and dark cycle with 100% fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and *water ad libitum.* The protocol of animal study was approved by the institutional animal ethics committee with IAEC No: 37/VCP/IAEC/2015/9/DBP/AE12/Rabbits. Rabbits were randomly divided into two groups, each group contained six animals. The group A rabbits were fed with cimetidine mucoadhesive microspheres (optimized formulation M12), group B fed with marketed product cimetine with equivalent dose to animal body weight. Blood samples (approximately 0.5 ml) were obtained with syringes by marginal ear vein at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 20 and 24 h post dose. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5 min and stored frozen at 20 C until analysis.

Preparation of plasma samples for HPLC analysis

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µl of 70% of acetonitrile and 30% water was injected for HPLC analysis.

Determination of Cimetidine in rabbit plasma by HPLC method

Determination of cimetidine using internal standard ranitidine by high performance liquid chromatography with a RP-C18 chromatographic column, Phenomenex Kinetex (150 mm 4.6 mm i.d) and a mobile phase consisting of methanol: water (60:40 v/v) at a flow rate 0.8 ml/min and the wavelength detection was 225 nm.

Pharmacokinetic Analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 h, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity. The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant. The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ±SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

Results and Discussion

Mucoadhesive microspheres

Figure 1: Cimetidine mucoadhesive microspheres

All fourteen formulations were evaluated for various micromeretic and physicochemical parameters and found to be within the limits. Among all the formulations, M12 shown best results of particle size, bulk density, tapped density, angle of repose and Carr's index. The percentage yield and entrapment efficiency of all the formulations were measured by assay method and found to be within the limits. The formulation M12 showed good percentage yield and entrapment efficiency, swelling index and mucoadhesiveness.

In vitro drug release studies

Cimetidine microspheres were evaluated for *in vitro* drug release studies in 0.1N HCl and the results were depicted in **Table 2**. The formulation M12 showed best drug release of 99.12% within 12 h. The drug release of optimized formulation M12 was in controlled manner when compared with innovator product cimetine i.e., 96.12% within 1 h.

Table 2: *In vitro* cumulative % drug release of Cimetidine Mucoadhesive microspheres Formulations.

Time in (h)	M1	M2	M3	M4	M5	M6	M7	Innovator (Cimetine 200 mg)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	16.81±0.22	18.62±0.52	16.44±0.45	12.06±0.22	10.08±0.98	10.27±0.14	10.6±0.22	96.15±0.12
2	35.59±0.23	32.97±0.16	29.61±0.16	26.69±0.21	21.35±0.78	18.5±0.18	24.36±0.11	---
4	57.97±0.32	50.16±0.13	46.38±0.22	43.48±0.11	36.73±0.76	27.75±0.16	35.92±0.21	----
6	78.61±0.16	71.06±0.22	59.34±0.52	58.95±0.13	48.64±0.66	45.31±0.33	60.81±0.13	----
8	94.04±0.32	83.2±0.23	72.61±0.34	70.53±0.21	57.08±0.44	68.06±0.12	72.36±0.33	----
10	93.24±0.12	96.78±0.32	81.65±0.22	84.71±0.22	68.34±0.12	75.93±0.22	86.89±0.41	----
12	91.69±0.23	93.56±0.16	93.18±0.23	90.65±0.16	80.19±0.32	88.72±0.11	92.13±0.11	----

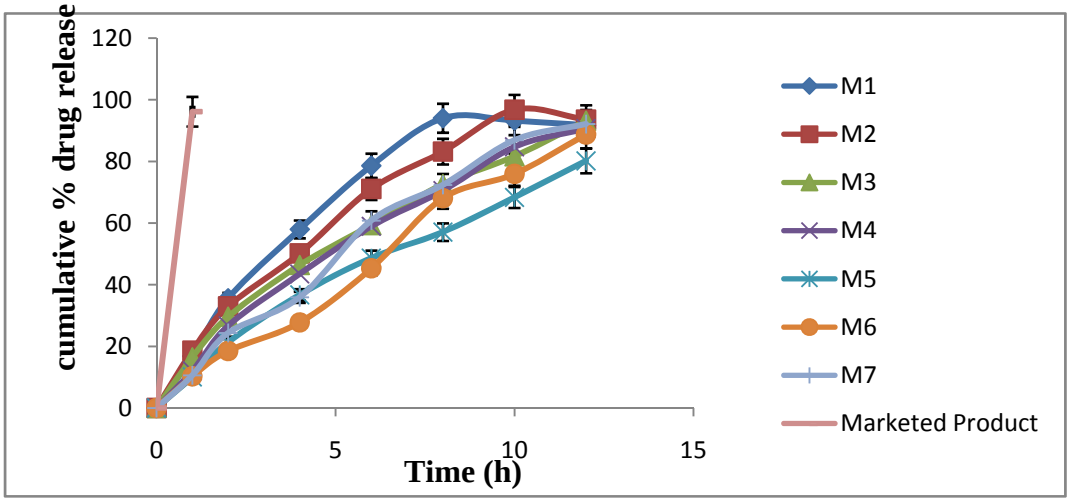

Figure 2: *In-vitro* cumulative % drug release of Cimetidine Mucoadhesive microspheres formulations

Table 3: *In vitro* cumulative % drug release of Cimetidine mucoadhesive microspheres formulation

Time (h)	M8	M9	M10	M11	M12	M13	M14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	9.31±0.33	8.4±0.12	13.96±0.32	11.17±0.16	12.41±0.22	9.67±0.12	8.22±0.12
2	16.48±0.52	17.79±0.22	24.73±0.16	19.78±0.15	20.76±0.23	17.41±0.32	14.08±0.22
4	26.76±0.33	30.61±0.43	37.62±0.11	32.12±0.11	35.82±0.32	24.36±0.16	20.7±0.22
6	37.72±0.56	40.53±0.44	53.29±0.21	45.27±0.16	50.62±0.34	31.76±0.17	37.02±0.32
8	50.24±0.52	47.56±0.52	62.4±0.12	60.29±0.32	67.73±0.16	45.63±0.22	58.79±0.87
10	63.21±0.51	61.95±0.33	72.59±0.33	75.85±0.16	81.09±0.22	51.53±0.32	71.84±0.32
12	78.05±0.55	76.82±0.22	81.23±0.32	83.69±0.52	99.12±0.13	80.64±0.16	85.39±0.22

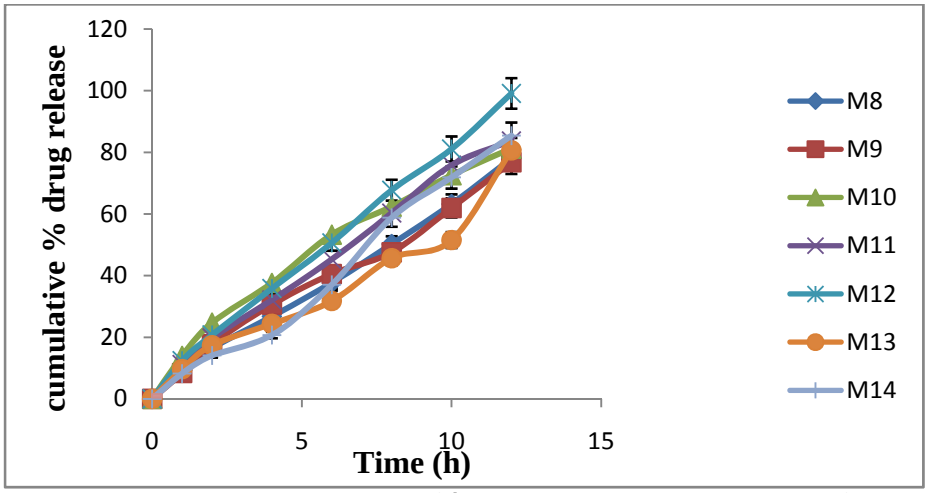

Figure 3: *In vitro* cumulative % drug release of Cimetidine mucoadhesive microspheres formulation

In vivo bioavailability studies

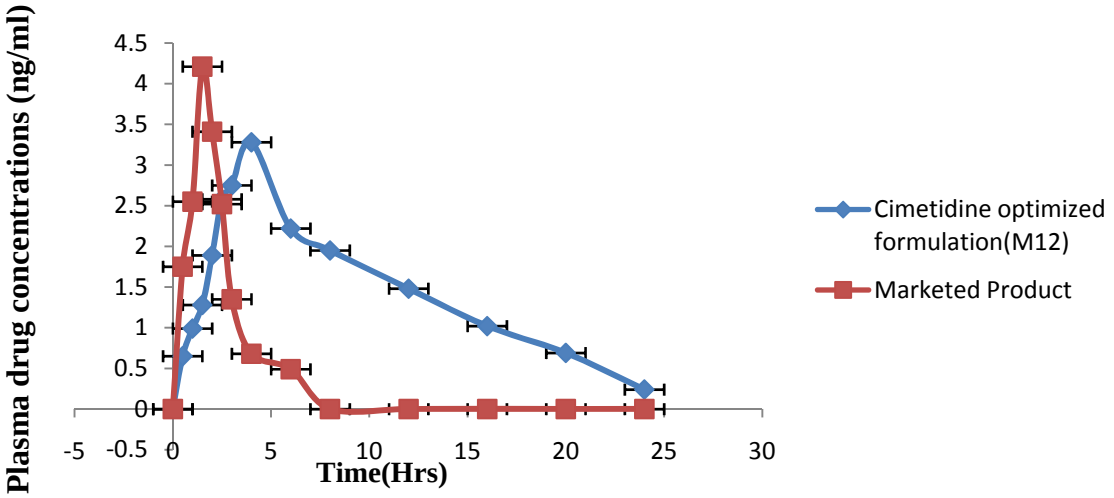

Figure 4: Plasma concentrations at different time intervals for Cimetidine optimized formulation (M12) and Marketed Product Cimetine.

Table 4: Comparison of pharmacokinetic parameters of Cimetidine optimized formulation (M12) and Marketed Product

Parameters	Cimetidine Optimized formulation (M12)	Marketed Product
C_{max}(ng/ml)	3.28±0.03	4.21±0.05
AUC_{0-t}(ng hr/ml)	28.15±1.14	20.21±1.26
AUC_{0-} (ng hr/ml)	33.22±1.24	25.15±0.05
T_{max} (hr)	4.00±0.03	1.50±0.04
$t_{1/2}$ (hr)	7.751 ± 0.41	3.244 ± 0.01
Kel (hr^{-1})	2.013 ± 0.11	1.122 ± 0.33

Bioavailability parameters

Mean plasma concentration profiles of prepared cimetidine optimized formulation and marketed product was presented in Figure 4, cimetidine optimized formulation exhibited as sustained release *in vivo* when compared with innovator tablet. All the pharmacokinetics parameters displayed in Table 4, cimetidine marketed drug was available in plasma within an hour after its oral administration. The T_{max} of the test cimetidne was significantly different ($p<0.05$) from that of the standard. Low T_{max} value for the reference drug (1.50±0.04 h) indicated rapid absorption while the higher T_{max} of the test drug (4.00±0.03 h) suggested slower absorption. This delayed absorption of test preparation was most likely due to the sustained release of the drug. In order to estimate the amount of drug absorbed from the test formulation, the relative bioavailability was calculated from the AUC of the reference and test formulations (20.21±1.26 ng. h/ml for the reference product versus. 28.15±1.14 ng. h/ml for the test formulation). These results indicated that the test formulation increased the bioavailability of cimetidine in rabbits effectively.

Conclusion

In conclusion, it could be said that the cimetidine mucoadhesive microspheres developed, showed a high percentage of mucoadhesion and drug entrapment efficiency. Among all the formulations, M12 was selected as optimized formulation based on the physicochemical parameters and release studies. In vitro *drug* release study of formulation M12 showed 99.12% after 12 h in a controlled manner, which was essential for disease like peptic ulcer. The innovator cimetine conventional tablet showed the drug release of 96.15% within 1 h. This would indicate the potential of mucoadhesive cimetidine microspheres for use in the provision of a sustained anti ulcer effect. The proposed optimized formulation M12 depicts an effective way to prolong drug release. The in-vivo mucoadhesive efficiency of optimized formulation was excellent and microspheres were retained in rabbit stomach for longer period of time.

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