Formulation and Optimization of Mucoadhesive Microspheres of Metoclopramide

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Metoclopramide microspheres containing a mucoadhesive polymer chitosan were prepared and investigated with a view to develop mucoadhesive microspheres. The chitosan microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a crosslinking agent. Results of preliminary trials indicate that volume of crosslinking agent, time for crosslinking and speed of rotation affected characteristics of microspheres. Microspheres were discrete, spherical and free flowing. The microspheres exhibited good mucoadhesive property in the *in vitro* wash off test. Metoclopramide release from these mucoadhesive microspheres was slow, extended and depended on the polymer to drug ratio. Drug release was diffusion controlled and followed non-Fickian diffusion. Microspheres prepared using polymer to drug ratio of 4:1 were found suitable for oral controlled release with good mucoadhesion up to 8 h. The microspheres exhibited good swelling index and 72 % drug entrapment efficiency.

Metoclopramide hydrochloride is a potent antiemetic and prokinetic, effective even for preventing emesis induced by cancer chemotherapy[1]. It is also used in the treatment of certain disorders of the digestive tract, including gastricstasis and gastroesophageal reflux[2]. It is highly water-soluble and is rapidly absorbed after oral administration[3]. Its short biological half-life (4 to 6 h) necessitates the need to be administered in three or four doses of 10-15 mg per day. Therefore the development of controlled release dosage forms would clearly be advantageous. Moreover, the site of absorption of metoclopramide is in the stomach. Dosage forms that are retained in the stomach would increase the absorption, improve drug efficiency and decrease dose requirements.

Dosage forms that can precisely control the release rates and target drugs to a specific body site have created enormous impact in formulation and development of novel drug delivery systems. Microspheres form an important part of the novel drug delivery systems. They have varied applications and are prepared using various polymers.

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Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery[4-6]. However, the success of these microspheres 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 the absorbing membranes[7-9]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres.

Bioadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due 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[10-13]. Chitosan was selected as a polymer in the production of bioadhesive microspheres due to its mucoadhesive and biodegradable properties. Chitosan (obtained by deacetylation of chitin,) is a cationic polymer that has been proposed for use in microsphere systems by various authors[14-17]. Thus, the aim of the present study was to optimize, characterize and evaluate mucoadhesive microspheres of metoclopramide.

MATERIALS AND METHODS

Metoclopramide was a gift sample from Zydus Cadila, Ahmedabad. Chitosan was obtained as a gift sample from Central Institute of Fisheries Technology, Cochin. Dioctyl sodium sulfosuccinate (DOSS) and petroleum ether 80:20 were procured from Willson Laboratories, Mumbai and S. D. Fine Chemicals Ltd., Mumbai. Liquid paraffin and glutaraldehyde were purchased from Loba Chemie Pvt. Ltd., Mumbai. All other reagents used were of analytical grade.

Preparation of microspheres:

Mucoadhesive microspheres of chitosan were prepared by simple emulsification phase separation technique. Chitosan was used as a polymer and was crosslinked using glutaraldehyde as per method described by Thanoo et al.[14]. Two hundred milligrams of chitosan was dissolved in 20 ml of 2% w/v aqueous acetic acid solution. Fifty milligrams of drug was dissolved in the polymer solution. In batches P1 to P15 the polymer to drug ratio was kept constant at 4:1. It was changed in formulations A1 to A6 from 1:1 to 6:1. The resultant mixture was extruded through a syringe (No. 20) in 100 ml of liquid paraffin (heavy and light 1:1 ratio) containing 0.2% DOSS and stirring was carried out using a Remi stirrer at 1000 rpm. After 15 min, glutaraldehyde (25% v/v aqueous solution) was added and stirring was continued. The amount of cross-linking agent and cross-linking time were varied in batches P1 to P15 from 1 to 6 ml and 1 to 3 h respectively. In batches A1 to A6, 5 ml glutaraldehyde was used and cross-linking time was kept to 1 h. Microspheres thus, obtained were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. They were finally washed with water to remove excess of glutaraldehyde. The microspheres were then dried at 45° for 12 h in hot air oven. The effect of formulation variables on characteristics of the microspheres is summarized in Table 1 and 2.

Assay of metoclopramide:

Metoclopramide was estimated using an UV/Vis spectrophotometric method. Aqueous solutions of

TABLE 1: EVALUATION OF PRELIMINARY TRIAL BATCHES

Batch No.	Volume of Crosslinking agent (ml)	Crosslinking time (h)	In vitro wash of test (% Mucoadhesion after 1 h)	% Drug Entrapment Efficiency	Characteristics of microspheres
P1	1	1	82.5	36.4(±2.4)	
P2	1	2	80.0	39.1(±1.5)	Very irregular
P3	1	3	75.2	40.0(±3.1)	
P4	2	1	79.6	51.1(±3.0)	
P5	2	2	76.4	55.2(±1.8)	Slightly
P6	2	3	70.8	58.0(±1.6)	irregular
P7	4	1	70.5	58.3(±1.4)	
P8	4	2	65.8	59.6(±3.1)	Spherical free
P9	4	3	62.1	60.8(±2.0)	flowing
P10	5	1	72.0	72.2(±1.4)	
P11	5	2	64.2	72.9(±2.1)	Spherical free
P12	5	3	61.1	73.0(±2.6)	flowing
P13	6	1	62.5	72.0(±4.1)	
P14	6	2	55.1	73.9(±2.6)	Spherical free
P15	6	3	49.2	72.4(±1.5)	flowing

All the batches were prepared at polymer to drug ratio 4:1.

TABLE 2: POLYMER TO DRUG RATIO, PARTICLE SIZE, DRUG CONTENT AND DRUG ENTRAPMENT EFFICIENCY OF THE MICROSPHERES

Batch No.	Polymer to Drug ratio	Particle size ($\mu m \pm S.D$)	Practical Drug Content (mg)	Drug Entrap- ment Effi- ciency (%)	T_{90} (min)
A1	1:1	56.6±1.35	26.2 (0.88)	52.4	370
A2	2:1	62.4±2.19	27.9 (1.26)	55.9	395
A3	3:1	65.1±0.88	32.3 (1.08)	64.7	369
A4	4:1	70.3±1.62	36.1 (0.78)	72.3	427
A5	5:1	79.3±1.09	34.3 (0.65)	68.6	429
A6	6:1	94.8±3.48	35.3 (1.94)	70.7	442

The theoretical drug content of microspheres of all the batches is 50 mg. All the batches were prepared using 5 ml glutaraldehyde and cross linking time 1 h.

metoclopramide were prepared in distilled water and absorbance was measured on a Shimadzu UV/Vis spectrophotometer at 272 nm[18]. The method was validated for linearity, accuracy and precision. The method obeys Beer's Law in the concentration range of 2-50 µg/ml. When a standard drug solution was analysed repeatedly (n=5) the mean error (accuracy) and relative standard deviation (precision) were found to be 0.9% and 1.8%, respectively.

Drug content:

Fifty milligrams of accurately weighed microspheres were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10 ml of distilled water. After 24 h the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula, practical drug content/theoretical drug content x 100. The drug content and the drug entrapment efficiency for batches P1 to P15 and A1 to A6 have been reported in Tables 1 and 2, respectively.

Particle Size and Swelling Index of microspheres:

The particle size of the microspheres was determined by using optical microscopy method. One hundred microspheres were counted for particle size using a calibrated optical microscope. The average particle size of microspheres of batches A1 to A6 is depicted in Table 2. For estimating the swelling index, the microspheres were suspended in 5 ml simulated gastric fluid (pH 1.2). The particle size was monitored every 1 h using an optical microscope. The increase in particle size of the microspheres was noted for upto 8 h and the swelling index was calculated[20]. The results are shown in Table 3.

In vitro wash-off test for microspheres:

The mucoadhesive property of the microspheres was evaluated by *in vitro* adhesion testing method known as wash-off method as reported by Chowdary et al.[19]. A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (3*1") using a thread. Approximately 100 microspheres were spread 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 whereby the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, which contained the gastric fluid (pH 1.2). At the end of 30 min, 1 h and at hourly intervals upto 10 h, the numbers of microspheres still adhering to the tissue were counted. The results of the *in vitro* wash off test are shown in Table 4.

Drug release study:

The drug release study was carried out using USP XXIV basket apparatus at 37±0.5° and at 50 rpm using 900 ml of distilled water as a dissolution medium (n=3) as per USP XXVI dissolution test prescribed for metoclopramide extended release tablet[18]. Microspheres equivalent to 10 mg of metoclopramide were used for the test. Five millilitres of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µ membrane filter, diluted suitably and analyzed spectrophotometrically. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percent drug dissolved at different time intervals was calculated using the Beer Lambert's standard curve. The time required for 90% (t_{90}) drug to re-

TABLE 3: SWELLING INDEX OF MUCOADHESIVE MICROSPHERES

Time (h)	Swelling index*					
	A1	A2	A3	A4	A5	A6
0.5	0.398±0.21	0.374±0.05	0.274±0.13	0.118±0.06	0.225±0.12	0.245±0.20
1.0	0.654±0.18	0.543±0.14	0.299±0.09	0.238±0.14	0.328±0.25	0.345±0.19
2.0	0.724±0.08	0.652±0.28	0.594±0.15	0.461±0.29	0.526±0.24	0.574±0.13
3.0	0.795±0.16	0.778±0.32	0.654±0.34	0.605±0.35	0.636±0.21	0.687±0.07
4.0	0.882±0.31	0.802±0.07	0.704±0.41	0.624±0.24	0.820±0.10	0.875±0.32
5.0	0.890±0.09	0.820±0.04	0.729±0.28	0.768±0.30	1.184±0.12	1.203±0.26
6.0	-	0.997±0.21	0.902±0.08	1.150±0.24	1.230±0.09	1.348±0.16
8.0	-	1.025±0.26	1.162±0.16	1.284±0.11	1.342±0.15	1.412±0.24

*The values are represented as mean±standard deviation (n=3).

lease was calculated. The values of t_{90} for the batches A1 to A6 are shown in Table 2.

RESULTS AND DISCUSSION

The mucoadhesive microspheres of chitosan were prepared by simple emulsification phase separation technique. Chitosan was selected as a polymer for the preparation of mucoadhesive microspheres due to its biodegradable and mucoadhesive properties. The formulation of the present microspheres is based on the solubility behavior of chitosan, which is poorly soluble in water. In the presence of acetic acid, chitosan shows good aqueous solubility. Different concentration of acetic acid from 1% w/v to 6% w/v were tried but no significant effect of acetic acid was observed on % mucoadhesion or drug entrapment efficiency, therefore 1% w/v of acetic acid was selected for the study.

One of the important factors related to microspheres as reported by Li et al.[21] is viscosity of the polymer solution. Polymer concentrations of 0.5%, 1% and 2% w/v were selected for preliminary trials. Flake formation was observed when chitosan concentration was used at a level of 0.5%, whereas maximum sphericity was observed at 1% level. The chitosan solution was found to be a too viscous to pass through the syringe when use at 2% level. Therefore 1% w/v of chitosan in 1% acetic acid was found to be the optimum concentration. The addition of 0.2% w/v of DOSS to the dispersion medium was found to be essential to minimize aggregation of microspheres.

Preliminary trials were carried out to optimize the process of preparation. Batches P1 to P15 were prepared to study the effect of volume of cross-liking agent (glutaralde-

TABLE 4: RESULTS OF IN-VITRO WASH OFF TEST TO ASSESS MUCOADHESIVE PROPERTIES OF THE MICROSPHERES

Batch No.	% Mucoadhesion to stomach mucosa (h)				
Time (h)	1	2	4	8	10
A1	54 (0.8)	48 (2.6)	38 (1.9)	18 (2.3)	03 (2.5)
A2	58 (1.4)	50 (2.4)	39 (1.8)	20 (1.6)	11 (3.0)
A3	61 (2.2)	55 (3.0)	41 (2.2)	22 (1.5)	12 (1.7)
A4	72 (2.8)	61 (3.5)	52 (2.7)	25 (1.9)	10 (2.1)
A5	74 (3.0)	60 (2.4)	45 (1.6)	17 (0.9)	04 (1.8)
A6	64 (1.6)	43 (0.9)	24 (2.5)	09 (2.2)	-

Figures in parentheses are coefficient of variation (CV) values.

hyde), time for cross-linking and stirring speed on the % mucoadhesion, drug entrapment efficiency and particle size.

The volume of glutaraldehyde was varied from 1, 2, 4, 5 and 6 ml. Discrete spherical microspheres (Batches P7 to P15) were obtained using 4, 5 and 6 ml of glutaraldehyde. Batches P1 to P6 prepared using 1 and 2 ml yielded irregular microspheres. The higher amount of glutaraldehyde appears to favour the cross-linking reaction and hence spherical free flowing microspheres were obtained. The microspheres of batches P7 to P15 also showed significant effect on the % mucoadhesion and drug entrapment efficiency (Table1). Batches P7 to P9 prepared using 4 ml glutaraldehyde showed good % mucoadhesion but % drug entrapment efficiency was below 60%. Batches P10 to P12 also showed good mucoadhesion as well as 70% drug entrapment efficiency. In the microspheres of batches P13 to P15 the % drug entrapment efficiency was above 70% but mucoadhesion decreased. Thus, we can conclude that 5 ml of glutaraldehyde was the optimum amount. Increase in the cross-linking time (1 to 3 h) inversely affected the % mucoadhesion. The cross-linking polymer probably becomes more rigid and thus mucoadhesiveness decreases. The cross-linking time did not have a significant effect on the % drug entrapment efficiency.

To investigate the effect of stirring speed, batches were prepared at 500 and 1000 rpm. The results were in general agreement with general theory of microspheres that the particle size of the microspheres prepared at 1000 rpm was smaller than those prepared at 500 rpm. Increase in the volume of glutaraldehyde (4 to 5 ml) slightly increases the particle size as seen in size comparison of batches P7 and P10. Comparison of microspheres of batches P7 to P9 prepared at same speed reveals that the cross-liking time did not have a significant effect on particle size. (fig. 1). The microspheres of batches P1 to P7 were irregular so were not taken up for particle size study. Moreover, microspheres of batches P13 to P15 had a particle size of above 100 µm. Thus, on the basis of above studies further batches were prepared using 5 ml of glutaraldehyde, the cross-linking time was kept at 1 h and speed 1000 rpm.

The polymer to drug ratio was varied from 1:1 to 6:1 (batches A1 to A6) and its effect was studied on the characteristics of microspheres. On increasing the concentration of polymer the particle size increases (Table 2). The results of in vitro wash off test shown in the Table 4 indicate that as the polymer to drug ratio increases, the % mucoadhesion also increases. The more amount of polymer results in higher

amount of free $-NH_2$ groups which are responsible for binding with sialic acid groups in mucus membrane and thus result in increase in mucoadhesive properties. In batches A2 to A4 the % mucoadhesion was above 20% even after 8 h.

Swelling studies data reveal that the amount of polymer plays an important role in solvent transfer. It can be concluded from data shown in Table 3 that with an increase in polymer concentration, the time for maximum swelling index increases. Thus, we can conclude that amount of polymer directly affects the swelling index.

The drug release from batches A1 to A6 (fig. 2) indicates that as the polymer to drug ratio is increased the T_{90} also increases. Batch A6 showed highest T_{90} of 441 min. However, batch A6 showed low % mucoadhesion as compared to batches A4 and A5. Batch A4 prepared at 4:1 polymer to drug ratio showed highest i.e. 72% mucoadhesion after 1 h, 72.3% drug entrapment efficiency and good swelling properties. The particle size of microspheres of batch A4 is 70.3 µm. The T_{90} of batch A4 is 427 min. No significant difference in % mucoadhesion and T_{90} was observed in batches A4 and A5, thus, batch A4 was considered as the best batch. Drug release from microspheres of batch A4 was diffusion controlled and followed non-Fickian diffusion (kinetic exponent (n)=0.75). Thus, we can conclude that metoclopramide release from the mucoadhesive
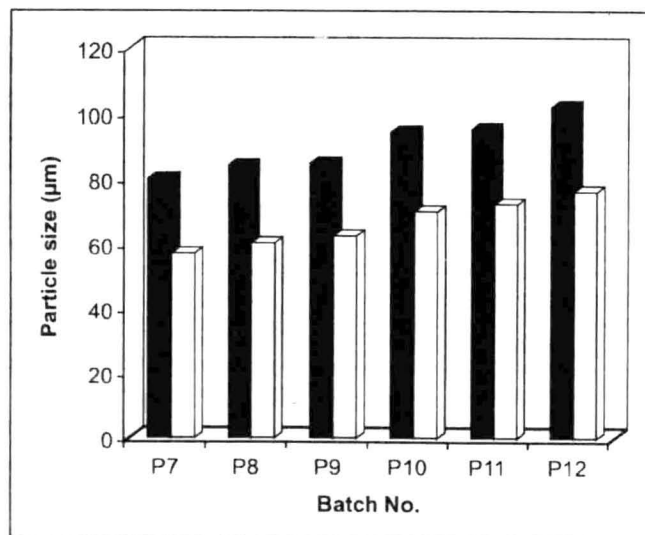
Fig 1: Comparison of particle size distribution of preliminary trials at different speed.

Particle size distribution of preliminary trials at different speed ■ 500 RPM and □ 1000 RPM.
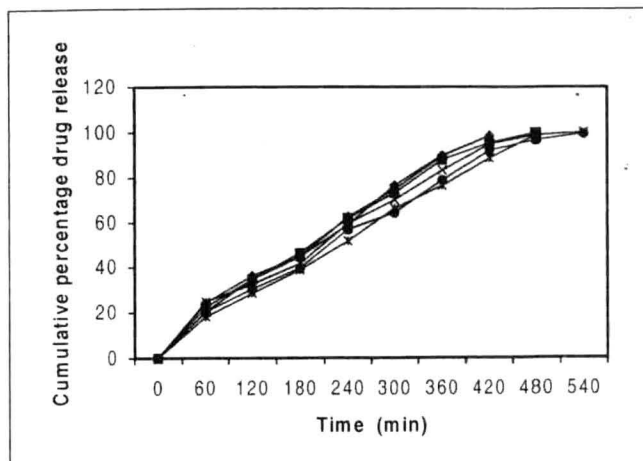
Fig 2: *In vitro* drug release profiles from different mucoadhesive microspheres.

In vitro release of Metoclopramide from mucoadhesive microspheres formulations, A1 (-♦-), A2 (-■-), A3 (-▲-), A4 (-x-), A5 (-*-), A6 (-●-).

microspheres was slow and extended and it depended on the processing parameters and polymer to drug ratio.

The study demonstrates the preparation of mucoadhesive microspheres of metoclopramide using chitosan as a polymer. Glutaraldehyde was used as a cross-linking agent. The volume of cross-liking agent, polymer to drug ratio and stirring speed had a significant effect on the microsphere characteristics. The microspheres also showed good mucoadhesive properties. Chitosan microspheres were able to give extended drug release of metoclopramide.

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