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FORMULATION AND *IN-VITRO* EVALUATION OF CANDESARTAN MUCOADHESIVE MICROSPHERE

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ABSTRACT

Mucoadheshive microsphere of candesartan were prepared by Ion-gelation technique to achieve increase retention time reduce the frequency of daily administration whose main target is to absorb in the stomach and upper part of intestine by increase the residence time of drug in upper part of GIT and to control the release of drug for longer period of time. Here different mucoadheshive polymer and release retard polymers are used for extend release of drug. Sodium Alginate is used as cross linking agent, carbopol, HPMC are used as mucoadheshive polymer. Mucoadheshive candesartan microsphere were evaluated for the parameters like, Drug content and entrapment efficiency, swelling index, Micrometric property, the surface morphology of microsphere was characterized by SEM, the prepared microsphere were spherical with varied surface roughness, free flowing property, *In-vitro* wash off test showed prepared microspheres exhibits for mucoadheshive properties, dissolution studies of all 9 formulations were performed for all batches. Formulation 7 contains HPMC based mucoadheshive microsphere showed maximum Drug content and efficiency as compared to other batches.

KEYWORDS: Candesartan, Mucoadhesion, Mucoadhesive polymers, Sodium Alginate.

INTRODUCTION

The drug delivery system (DDS) is defined as formulation or a device that enables the therapeutic substance to introduce into the body, which improves its efficacy and safety by controlling the rate, time and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product and subsequent transport of active ingredients across the biological membranes to the site of action. The pharmaceutical research is being steadily shifted from development of new

chemical entities to development of Novel Drug Delivery System (NDDS) of existing drug molecule to maximize their effectiveness in terms of therapeutic action and patient protection. Drug those easily absorbed from the gastrointestinal tract (GIT) and have a short half-life are eliminated quickly from the blood circulation, require frequent dosing. To avoid this, extensive efforts have been focused on controlled drug delivery system (CRDDS) and sustain release drug delivery system (SRDDS). Amongst the various routes of drug delivery, oral route is perhaps the most preferred to the patient. The oral controlled release (CR) formulation has been developed in an attempt to release the drug at a predetermined rate into the GIT and maintain a constant drug concentration in the serum for longer period of time. Similarly in case of sustain release dosage form slow release of drug occurs over an extended period of time. But not particularly at a predetermined rate. Such oral drug delivery devices have a restriction due to a short Gastric Retention Time (GRT), a physiological limitation. Therefore devices which prolong gastric retention by retaining the CR system in the stomach for a longer time have been developed and explored over the past couple of years. Gastro retentive system is one of the approaches for such controlled release drug delivery system. This system delivers drug for local or systemic efforts. Due to prolonged residence time in stomach the duration of drug release could increase. Gastro retentive system includes bioadhesive system, buoyant system, swell-able /expandable system, high density. Microsphere constitute an important part of these particular drug delivery system by virtue of their small size and efficient carrier capacity. Microsphere are carrier linked drug delivery system in which particle size ranges from (1-1000micrometer)in diameter having a core of drug and entirely outer layers of polymers as coating material. However the success of microsphere is limited due to their short residence time at site of absorption. It would be therefore advantageous to have means for providing an intimate contact of drug delivery system with the absorbing membrane. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. A mucoadhesive microsphere is one potential strategy for prolonging G.R.T. Mucoadhesive delivery system get popularized as novel drug delivery system as mucous membranes are relatively permeable, allowing for rapid uptake of a drug into the systemic circulation and avoiding first pass metabolism. Bioahesion can be defined as the process by which a natural or a synthetic polymer can adhere to a biological substrate. The substrate bioadhesive property can help in devising a delivery system capable of delivering a bioactive agent for a prolonged period of time at a specific delivery site. Bioadhesive characteristics can be given by the implementation of mucoadhesive polymers. Mucoadhesive polymers improving drug

delivery by promoting dosage form residence time and contact time with the mucous membranes. Mucoadhesion properties mainly influenced by polymeric based properties like degree of cross linking, chain length and various functional groups in polymer structure. Mucoadhesive system have widely used through-out many mucosal covered organelles for active ingredients delivery for local or/systemic effect. Bioadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drug 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. The objective of the work is to improve the oral bioavailability of Candesartan Cilexetil by formulating mucoadhesive microspheres which will provide protection from intestinal milieu using various mucoadhesive polymers.

MATERIALS AND METHODS

Materials

Candesartan was obtained as a gift sample from Dr. Reddys Lab. Hyderabad. Sodium alginate, HPMC, CMC & carbapol were obtained from Cipla Pharma ltd. Goa. Calcium chloride was procured from.

Instruments

Shimadzu FTIR, USP disintegration test apparatus (Shreeji), Dissolution apparatus USP24 paddle type (Electrolab), Bulk density apparatus (Dolphin) Scanning electron micrograph – Diya labs Mumbai, Brooke field viscometer (LVDVE).

Method of Preparation of microspheres

Candesartan mucoadhesive microspheres were prepared by ionic gelation technique. Microsphere containing Candesartan where prepared employing sodium alginate alone and in combination with HPMC, CMC and carbopol. The homogenous polymer solution was prepared in distilled water and stirred magnetically with gentle mix. The drug and cross-linking agent (sodium alginate) were added to the polymer solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with middle size no.18 into calcium chloride 5% solution kept under magnetic stirrer at 100rpm. The microspheres were retained in calcium chloride solution for 30mins to produce rigid discrete particles. They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of calcium chloride. Then the microsphere were dried at 40% under vacuum for 12hrs.

Table 1: Formulation of Candesartan using different polymer.

Formulation	Drug(mg)	Sod. Alginate(mg)	HPMC (mg)	CMC(mg)	Carbopol (mg)	Cal. Chloride (mg)
F1	300mg	500mg	200mg	___	___	5%
F2	300mg	500mg	___	200mg	___	5%
F3	300mg	500mg	___	___	200mg	5%
F4	300mg	500mg	300mg	___	___	5%
F5	300mg	500mg	___	___	300mg	5%
F6	300mg	500mg	___	300mg	___	5%
F7	300mg	500mg	400mg	___	___	5%
F8	300mg	500mg	___	400mg	___	5%
F9	300mg	500mg	___	___	400mg	5%

Evaluation of Mucoadhesive Microspheres

1. Percentage Yield

The prepared microspheres were assessed for the yield value. The batch was weighed after total drying and the yield % was calculated using the formula given below. Each batch was formulated in triplicate batches (n=3) to get a reproducible yield.

$$\% \text{ Yield} = \frac{\text{The amount of microspheres practically obtained (g)}}{\text{The theoretical amount (g)}} \text{ X } 100$$

2. Entrapment Efficiency

Initially the mucoadhesive microspheres were powdered by mortar & pestle. Then powder equivalent to 100mg was dissolved in 100ml of 0.1N HCl. Then from the prepared solution 1ml of solution was taken and volume make up was done by 0.1N HCl solution. The solution was filtered through the Whatman filter paper No.41 to obtain stock solution. The absorbance of resulting solution was taken at ƛmax 259.5nm by using UV-Spectrophotometer. The % of encapsulation efficiency can be estimated by following formula:

$$\%\text{Entrapment} = (\text{Actual content}/\text{Theoretical content}) \times 100$$

3. Swelling Index

Swelling index illustrate the ability of mucoadhesive microspheres to get swelled at the absorbing surface by absorbing fluids available at the site of absorption, which is primary requirement for initiation of mucoadhesion.

Procedure: - The dynamic swelling property of microcapsules was determined by placing the microspheres in 100ml of distilled water for 24 hours. Further, the swollen microcapsules

were dried by keeping on a filter paper and the weight was noted down. The percentage swelling was then calculated by using following formula:

$$\%Swelling=\{(D_T-D_O)/D_O\}\times100$$

Where, D_O=Weight of dried microsphere

D_T=Weight of swelled microsphere

4. Scanning Electron Microscopy (SEM)

The morphology of microspheres was examined by scanning electron microscopy(SEM). The outer surface of the microspheres was observed by SEM. By the SEM study the size, shape, outer structure of microspheres can be determined. A small amount of microspheres spread on gold stub. After that the stub containing microspheres placed in SEM. A scanning electron photomicrograph is taken at an acceleration of 5KV & chamber pressure of 0.6mmHg.

5. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR Spectral measurement was performed using thermo electron FTIR spectrometer to confirm the presence of any interaction between the polymer and drug. The IR spectra of the free drug, physical mixture, formulation & empty microspheres were recorded. The identical peaks corresponding to the functional groups features confirm that neither the polymer nor the method of the preparation has affected the drug stability.

RESULTS AND DISCUSSION

The present research was carried to develop mucoadhesive drug delivery system. Loaded microspheres containing sodium alginate, HPMC polymers prepared by ionic gelation techniques. The influence of the formulation and dosage parameters in formulation of microsphere was studied with respect to percentage yield value, entrapment efficiency, in vitro drug release, in vitro wash off test, stability study .In case of in vitro dissolution F7 release the drug in controlled manner up to 16 hours; indicating promising potential of the over conventional dosage form.

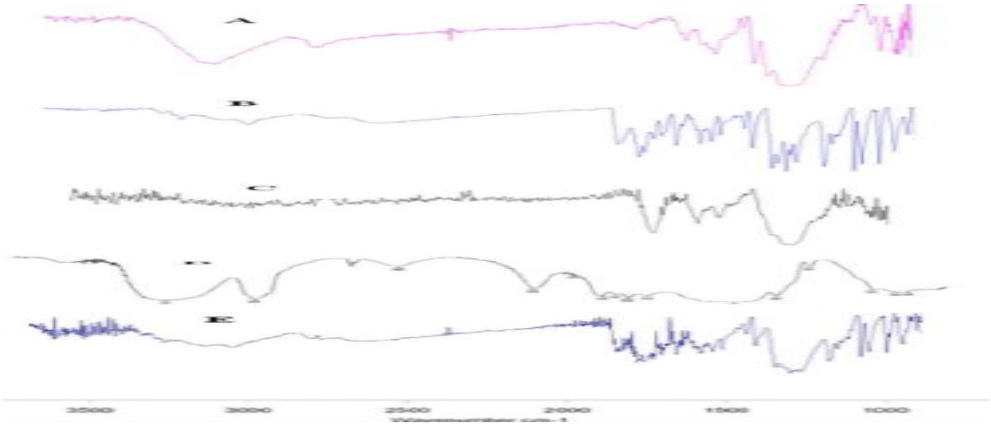

Fig. 1: FTIR spectra of (A) Candesartan, (B) Sodium alginate, (C) Sodium CMC, (D) HPMC K4M and (E) Optimized formulation.

1. Percentage Yield

Percentage yield of the formulations were carried out and was found to be within the range of 66.30 to 95.23%.

2. Percent Encapsulation Efficiency

Percent Encapsulation Efficiency of the formulations were found to be within the range of 60% to 92.3%.

3. Stability Study

Accelerated stability studies were carried out for the optimized formulation as per the ICH guidelines. Optimized formulation (F7) was packed and stored 40±2ºc, 75%RH up to two month. In the specified time interval the in-vitro drug release rate was determined. The accelerated stability studies of the optimized formulation (F7) showed that the prepared microspheres were stable up to three months. The dissolution rate of the optimized formulation show no significant change upon storage.

4. Micrometrics property

For the prepared formulation carr's index came in between 7,646 to 19.37%, hausner's ratio in between 1.07 to 1.30, angle of repose in between 18 to 30°. This results confirms good flow property of microsphere.

5. SEM Data

Microspheres were found to be discrete non aggregated, free flowing and monolithic matrix type Figure depicts the SEM photograph which indicating that the microsphere were

spherical and completely covered with coating polymer. SEM photograph of the optimized formulation (F7) is given below.

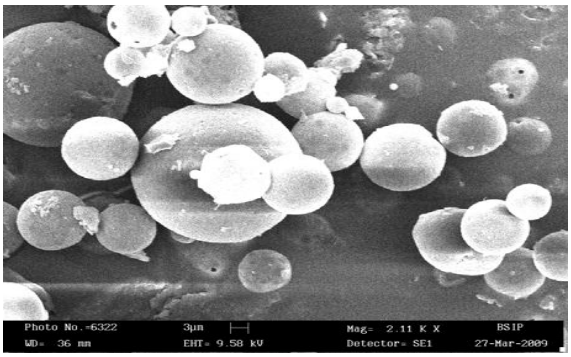

Fig. 2: S.E.M of optimized formulation.

6. Differential Scanning Calorimetry(DSC)

DSC analysis was carried out to identify the compatibility between the drug and excipients. DSC analysis of pure drug 1:1 physical mixture of drug excipients were carried out. Sample (2-8mg) were accurately weighed and heated in sealed aluminum pans at a rate of 10°c/min between 0-30°c temperature ranges under nitrogen atmosphere.

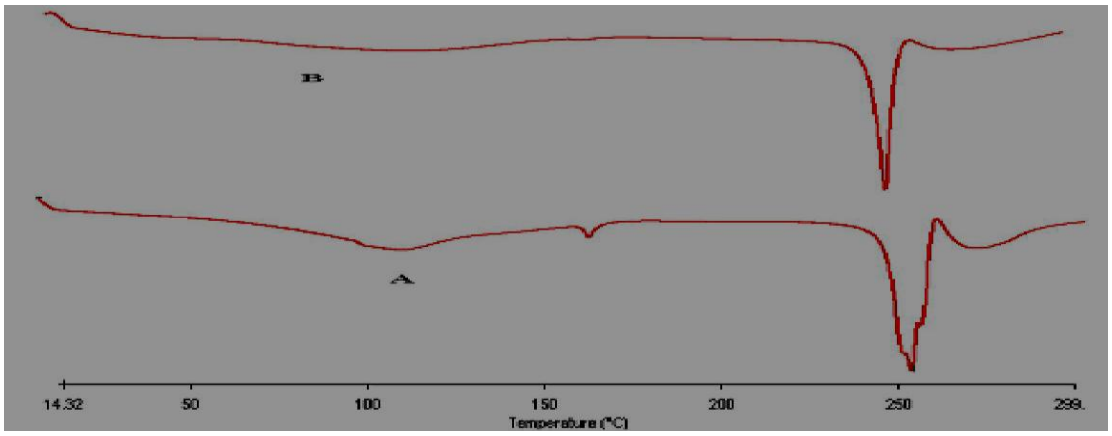

Fig. 3: DSC thermogram of (A) Candesartan and (B) Optimized formulation.

7. *In vitro* wash-off test

4cm×4cm piece of goat stomach mucosa was tied onto a glass slide (3inch × 1inch) using a thread. Microspheres were spread onto the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groove of the USP tablet disintegrating test apparatus. The disintegrating apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid. At the end of every time

interval, the number of microspheres still adhering onto the tissue was counted and there adhesive strength was determined using the formula given below.

Mucoadhesive property = (No. of microsphere adhered/ no. of microsphere applied) ×100

Table 2: *In vitro* wash off test.

Formulation code	% Mucoadhesion						
	Time in hour						
	0.5h	1h	2h	3h	4h	5h	6h
F1	65	51	49	45	38	28	10
F2	63	52	47	43	32	27	7
F3	70	65	53	47	37	23	8
F4	72	63	59	45	32	30	12
F5	78	64	58	46	34	28	6
F6	73	62	53	43	38	20	7
F7	90	86	75	65	55	35	15
F8	82	76	65	59	48	39	11
F9	84	75	69	55	43	30	13

8. *In–vitro* drug release study

Drug release studies were carried out in USP paddle type dissolution test apparatus. A quantity of microsphere equivalent to 100mg of drug was used for the test 0.1N HCl was used as dissolution medium. The volume of the dissolution medium was 900ml & the bath temperature was maintained at 37±0.5°C. The microspheres were placed in the dissolution vessel & the vessel was covered, the apparatus was operated for 12hours at 100rpm. At definite time intervals 1ml of the dissolution fluid was withdrawn, filtered & volume make up was done to 10ml. 1ml of blank sample was replaced to the dissolution vessel, so as to maintain the volume. The samples withdrawn were analyzed spectrophotometrically at a ƛmax 259.5nm using UV- spectrophotometer.

Table 3: *In-vitro* drug release.

			% drug release		
			Time in hour		
Formulation code	**4h**	**8h**	**12h**	**16h**	**24h**
F1	25.23	38.57	55.65	82.22	90.27
F2	23.54	39.23	56.32	56.72	95.55
F3	30.72	38.54	69.52	58.23	90.23
F4	20.55	35.76	68.57	59.43	99.52
F5	15.75	30.63	69.84	58.95	97.12
F6	20.21	31.54	65.43	56.57	90.11
F7	15.18	28.92	42.72	59.44	98.23
F8	22.15	32.54	55.26	69.43	91.25

CONCLUSION

The present research was carried to develop mucoadhesive drug delivery system. Candesartan cilexetil loaded microspheres containing sodium alginate, HPMC polymers prepared by ionic gelation techniques. The influence of the formulation and dosage parameters in formulation of Candesartan Cilexetil microsphere was studied with respect to percentage yield value, entrapment efficiency, in vitro drug release, in vitro wash off test, stability study. In case of *in vitro* dissolution F7 release the drug in controlled manner up to 16hours; indicating promising potential of the Candesartan Cilexetil over the conventional dosage form.

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