Formulation and Evaluation of Mucoadhesive Micropsheres of Candesartan

DILLIP KU JENA[*]**,SB.BHANJA**[1]**,BIJAYALAXMI SABAR**[2]**,UTTARABALI SABAR** [2]**,
B.B. PANIGRAHI**[3]**, NILIMA SHUKLA**[4]

*,&2. Gayatri institute of Science and Technology,Regeda,Gunupur,Odisha
1.Malla Reddy college of Pharmacy (Affiliated to Osmania University), Maisammaguda, Hyderabad,
Telangana State, India.
3. Hi-tech college of pharmacy, Bhubaneswar, Odisha
4. Sri Jaydev college of pharmaceutical Sciences,Bhubaneswar, Odisha

Abstract

Mucoadhesive microencapsulation has been accepted as a process to achieve controlled drug delivery by prolonging the residence time of the dosage form at the site of absorption thereby improving and enhancing the bioavailability of drugs. Candesartan is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. The mucoadhesive microspheres of Candesartan were formulated by orifice ionic gelation technique employing polymers like hydroxy propyl methyl cellulose, Carbopol along with Sodium alginate. The microspheres prepared were discrete, spherical and free flowing. Microspheres were evaluated for particle size, percentage yield, drug entrapment efficiency, percentage moisture loss, swelling property, *in vitro* drug release, drug release kinetics, *in vitro* wash-off test, Scanning Electron Microscopy and drug polymer interaction study by FT-IR. The microencapsulation efficiency was found relatively high with 2% polymer. Average particle size was found in the range of 5.02±0.36 to 9.45±0.43μm. Formulations F6 and F10 displayed the best results for Carbopol and HPMC based microspheres respectively. Entrapment efficiency was 71.06±0.43 and 71.26±0.67; Mucoadhesion was 94 and 92; and drug release up to 8 h was 94.97and 92.72% for F6 and F10 respectively. Drug release was diffusion controlled and followed first order kinetics. The *in vitro* wash-off test indicated that the microspheres had good mucoadhesive properties. Hence prepared mucoadhesive microspheres may be an effective strategy for the development of easy, reproducible and cost effective method for safe and effective oral drug therapy

Keywords: Candesartan, , Microspheres, Entrapment efficiency. *in vitro* wash-off test

INTRODUCTION:

In the early 1980s, the concept of Mucoadhesive was introduced into the controlled drug delivery area[1]. Many concepts have been proposed in recent years to provide a dosage form with a longer transit time and therefore a more efficient absorption. The concept of bioadhesion or more specifically Mucoadhesion is one of them to increase gastric retention of drugs. Among the various approaches for controlled systems, microencapsulation process have gained good acceptance as a process to achieve controlled release and drug targeting. Though several studies reported Mucoadhesive drug delivery systems in the form of tablets, films, patches and gels for oral, buccal, nasal, ocular and topical routes, however, very few reports on Mucoadhesive Microspheres are available [2,3]. The side effects of conventional form have been attenuated by designing the drug in the form of Mucoadhesive Microspheres which includes advantages like, maximized absorption rate due to intimate contact with the absorbing membrane, improved drug protection by polymer encapsulation, longer gut transit time resulting in extended periods for absorption. Candesartan is an Angiotensin Receptor Blocker (ARB) used mainly for the treatment of hypertension. It competes with Angiotensin II for binding at the AT1 receptor subtype. unlike ACE inhibitors and its bioavailability 10% and half Life, 9 hrs. So, the objective of this study is to prepare and evaluate the controlled release Mucoadhesive Microcapsules of Candesartan,thus reducing the frequency of dosing, side effects and increasing patient compliance. The novelty of this work is in combining the advantage of particulate system (microsphere) and mucoadhesive drug delivery system by taking Sodium alginate and Mucoadhesive polymers i.e. HPMC (K100M) and Carbopol 934.

MATERIALS AND METHODS:

Candesartan was purchased from AR Chemicals, Hyderabad. Sodium Alginate was obtained from Finar chemicals limited, Ahmadabad. Carbopol 934P was purchased from S.D. Fine chem. Ltd, Mumbai. HPMCK100M was purchased from Yarrow chemicals ltd, Mumbai. All other reagents used were of analytical grade.

Compatibility Studies by IR-Spectroscopy:

The drug polymer and polymer-polymer interaction was studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to a potassium bromide disc was mixed with dry KBr.The mixture was grind into a fine powder using an agate mortar and then compressed into a KBr disc in a hydraulic press at a pressure of 1000psi. Each KBr disc was scanned 16times at 2 mm/sec at a resolution of 4 cm^{-1} using cosine apodization. The characteristic peaks were recorded.

Preparation of Irbisartan Mucoadhesive Microspheres by Orifice-Ionic Gelation Method:

Sodium alginate (1%) and the Mucoadhesive polymer Carbopol 934 and HPMC K100M (1%) were dissolved in Distilled water to form a homogeneous polymer solution. The active core material Irbisartan (100mg) was added to the polymer solution and mixed thoroughly with a stirrer to form a smooth viscous dispersion. The resulting

dispersion was then added drop wise into calcium chloride (2%w/v) solution through a syringe with a needle of size No: 18. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45°C for 12 hours. The composition of Candesartan Mucoadhesive Microspheres shown in Table 1.

Evaluation of Candesartan Mucoadhesive Microspheres:
Micromeritic properties :
Bulk density, Tapped density and Hausner's ratio and Carr's index, were determined to assess the flow ability of the prepared microspheres.
Bulk density[4]:
The product was tapped using bulk density apparatus for 1000 taps in a cylinder and the change in volume was measured. Bulk density of the formulations was determined by using the following formula

$$\text{Bulk Density} = \frac{\text{Total Weight}}{\text{Total Bulk Volume}}$$

Tapped density [5]:
Tapped density is used to investigate packing properties of microcapsules into capsules. The tapped density was measured by employing the conventional tapping method using a 10mL measuring cylinder and the number of tappings was 100 as sufficient to bring a plateau condition. Tapped density was calculated using the following formula:

$$\text{Tapped Density} = \frac{\text{Total Weight}}{\text{Total Tapped Volume}}$$

Hausner's ratio [6]:
It is another parameter for measuring flow ability of the microspheres. It is calculated using the following formula, H = Bulk Density/ Tapped Density,   Where, H = hausner's ratio

Compressibility index[7]:
It is indirect measurement of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials since all of them can influence the consolidation index. It is also called as compressibility index. It is denoted by CI and is calculated using the formula below.
Compressibility index = $(1 - V_o/V) * 100$
Where, V_o = volume of microspheres before tapping
V = volume of microspheres after 100 tappings.
Production yield (%)[8]:
The production yield of microspheres of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres and % production yields were calculated as per the formula mentioned below.
 % PY = W0 / WT X 100

 PY = Production Yield; WO=Practical mass (microspheres); WT = Theoretical mass (Polymer + Drug).

Encapsulation efficiency and drug loading [9]
To determine the amount of drug encapsulated in microspheres, a weighed amount (50 mg) of microspheres was suspended into 0.1N Hcl and sonicated for 15 min in order to extract the entrapped drug completely. The solution was filtered through whatman filter paper and further dilutions were made. This solution was assayed for drug content by UV spectrophotometer at 244 nm.
EE (%) = ED/AD X 100
EE= Encapsulation efficiency; ED= Amount of encapsulated drug; AD= Amount of drug   added.
DL (%) = WD / WT X 100
DL= Drug loading; WD = Weight of drug loaded in microspheres; WT = Total weight of microspheres.
Particle size analysis [10]:
Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. The average particle size of the microspheres was expressed as diameter
Swelling Index [11]:
The dynamic swelling property of microspheres in the dissolution medium was determined. Microspheres of known weight were placed in dissolution solution for 8 hr and the swollen microcapsules were collected by a centrifuge and the wet weight of the swollen microspheres was determined by first blotting the particles with filter paper to remove absorbed water on surface and then weighing immediately on an electronic balance. The percentage of swelling of microspheres in the dissolution media was then calculated by using
Swelling index: SI = $(W_t - W_O)/W_O \times 100$
Swelling ratio: W_t/W_O
Where SI = percentage of swelling of microspheres, W_t = weight of the microspheres at   time t, W_O = initial weight of the microspheres
Loose surface crystal study [12]:
The Candesartan encapsulated microspheres prepared were evaluated for surface associated drug content on the surface of microspheres. From each batch, 100 mg of microspheres were shaken in 20 ml of 0.1N Hcl for 5 min and then filtered through Whatman filter paper. The amount of drug present in filtrate was determined spectroscopically and calculated as a percentage of total drug content
Moisture loss [13]:
The Candesartan loaded microcapsules was evaluated for % of moisture loss which sharing an idea about its hydrophilic nature. The microcapsules weighed initially kept in desiccators containing calcium chloride at 37°C for 24 hour. The final weight was noted when no further change in weight of sample
% Moisture loss= initial weight-final weight/final weight x 100

***In-vitro* wash off test [14,15]:**
The mucoadhesive property of microspheres was evaluated by an *in vitro* adhesion testing method known as the wash-off test. Freshly excised pieces of intestinal mucosa from sheep were mounted onto glass slide. About 100 microspheres were spread onto wet rinsed tissue specimen and immediately thereafter the slides were hung onto the arm of a tablet disintegrating machine. Then the machine was operated. The tissue specimen was given a slow, regular up and down movement in the test fluid at about 37°C contained in a vessel of the machine. At the end of 1, 2, 3, 4, 5, 6, 7,8 hrs the machine was stopped and the number of microspheres still adhering to the tissue was counted. The test was performed at 0.1N hydrochloric acid solution.

% Mucoadhesion = (N_a-N_l) / N_a X 100

Where, N_a = number of microspheres applied; N_l = number of microspheres leached out.

In-vitro* drug release studies [16,17]*:
900mL of 0.1N HCL was placed in the dissolution vessel and the USP dissolution apparatus I (Basket method) was assembled. The medium was allowed to equilibrate to temperature of 37°C ±0.5°C. Microspheres were placed in the dissolution vessel and the vessel was covered, the apparatus was operated for 8hrs at 50 rpm. At definite time intervals the 5mL of the dissolution fluid was withdrawn, filtered and again 5mL blank sample was replaced. Suitable dilutions were done with the dissolution fluid and the samples were analyzed spectrophotometrically at 211 nm using a UV-spectrophotometer (Lab India).The cumulative drug release was calculated by using standard curve.

Scanning Electron Microscope (SEM).
The surface morphology of the microspheres was studied with the aid of a Scanning Electron Microscope (SEM).

In-vitro* drug release kinetics [18]*:
In order to study the exact mechanism of drug release from microcapsules, drug release data was analyzed according to Zero order, First order, Higuchi square root and Korsemeyer-Peppas model.The analysis of the drug release mechanism from a pharmaceutical dosage form is an important but complicated process and is practically evident in the case of mucoadhesive controlled release systems. The order of drug release from mucoadhesive controlled release systems was described by using Zero order kinetics or First orders kinetics. The mechanism of drug release from the mucoadhesive controlled systems was studied by using the Higuchi equation and the Korsemeyer - Peppa's equation

Zero order release:
 It defines a linear relationship between the fractions of drug released versus time

Q = ko t

Where, Q is the fraction of drug released at time t and ko is the zero order release rate constant. A plot of the fraction of drug released against time will be linear if the release obeys zero order release kinetics.

First order release:
Wagner assuming that the exposed surface area of a tablet decreased exponentially with time during dissolution process suggested that drug release from most of the slow release tablets could be described adequately by apparent first-order kinetics. The equation that describes first order kinetics is

In (1-Q) = - K1t

Where, Q is the fraction of drug released at time t and k1 is the first order release rate constant. Thus, a plot of the logarithm of the fraction of drug un dissolved against the time will be linear if the release obeys the first order release kinetics.

Higuchi equation:
It defines a linear dependence of the active fraction released per unit of surface (Q) and the square root of time.

 $Q=K_2t^{\frac{1}{2}}$

Where, K_2 is the release rate constant.

A plot of the fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. This equation describes drug release as a diffusion process based on the Fick's law, square root time dependant.

Korsemeyer - Peppas equation
In order to define a model, which would represent a better fit for the formulation, dissolution data was further analyzed by Peppa's and Korsemeyer equation (Power law).

M_t/M_α = K.tn

The drug release, the value of n can be used as abstracted. A plot between logs of M_t/M_α against log of time will be linear if the release obeys Peppa's and Korsemeyer equation and the slope of this plot represents "n" value.

RESULTS AND DISCUSSIONS:
Drug compatibility studies:
The IR spectral studies of pure Candesartan, Hydroxy Propyl Methyl Cellulose, Carbopol, Sodium alginate and combination of drug and polymers containing highest proportion were carried out. When the characteristic peaks of Candesartan were compared with the combination of Candesartan and polymers, it was found that the same fundamental peaks were also present in the drug-polymer combinations indicating there was no interaction between Candesartan and polymers used and the spectral data are presented in Fig 1-5

Micromeritic properties:
The Micromeritic studies revealed that the microspheres have better flow property which indicates the microspheres produced are spherical and non-aggregated. The, Bulk density, Tapped density, Carr's index and Hausner's ratio for all formulations i.e.F1to F10 were found to be in the range of 0.27±0.07 to 0.48±0.05, 0.41±0.08 to 0.59±0.03, 1.15 to 1.18 and 11.55 to 15.21 respectively. All the formulations showed excellent flow ability as expressed in term of Micromeritic parameters. The results are shown in Table 2.

Percentage yield:
It was observed that percentage yield of all formulations i.e.F1 to F10 was ranging from 85.19% to 89.15%. The formulation F9 showed maximum yield i.e. 89.15%. Due to higher concentration of polymers which indicates that

this orifice ionic gelation method was very useful for adoption in the formulation of Candesartan Mucoadhesive Microspheres.The results are shown in Table 3.

Drug encapsulation efficiency:

The drug content was determined by UV spectrophotometric method. The standard deviations among the values were found to be less. This indicates that the drug was distributed almost uniformly throughout the batch of microspheres. The microencapsulation efficiency was in the range of 71.06±0.43% to 86.32±0.46 %. This improved encapsulation efficiency simply by due to the greater proportion of polymer with respect to amount of drug. The results are shown in the Table 3.

Particle size:

The particle size of Candesartan Microsphere was analyzed by optical microscopy. The average particle size was found to be in the range of 5.02±0.36 to 9.45±0.43 μm. The average particle size of microspheres was found to be increased as the concentration of the polymer was increased. This may be due to increased coat thickness with increasing polymer proportion. Particle size of the microspheres was large. The results are shown in Table 3 .

Swelling Index*:*

The degree of swelling of formulations F1, F2, F3, F4 and F5 were 160±1.52%, 180±2.68%, 184±3.64%, 176±1.98% and 182±2.88 % respectively and for formulations F6,F7,F8,F9 and F10 were 194±3.65%,132±2.48% ,162±1.68% ,178±3.20% and 190±2.51% respectively which indicates the hydrophilicity property of the polymers with establishing the fundamentals that the increase in degree of swelling depends on the polymer concentration in formulation. The formulation F6 showed good degree of swelling. The results are shown in the Table 3.

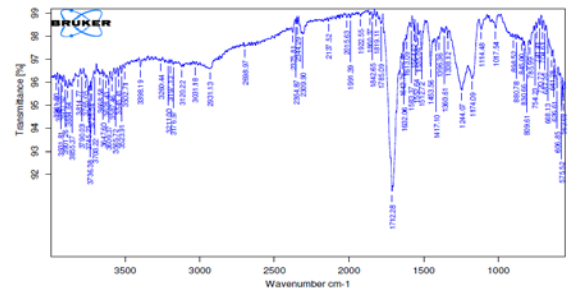**Fig 3: IR spectrum of pure HPMC k 100m**

Fig 4: IR spectrum of pure Candesartan drug
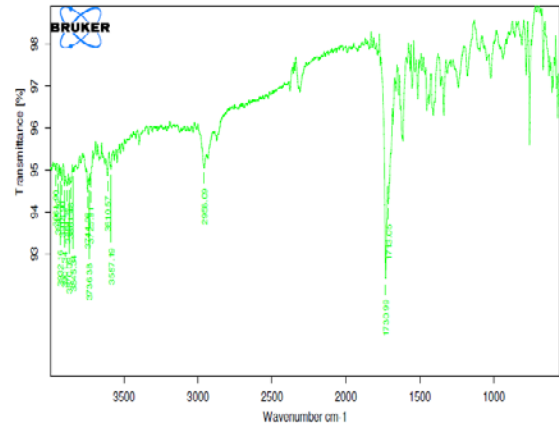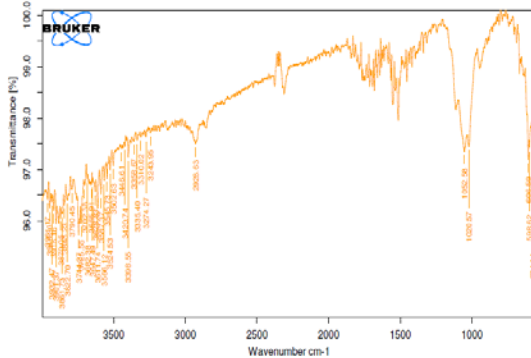

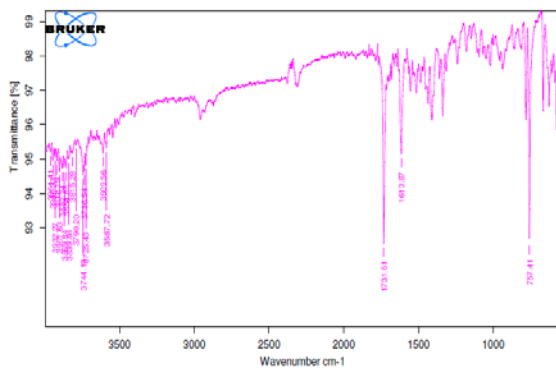**Fig 5: IR spectrum of Candesartan Mucoadhesive Microspheres**
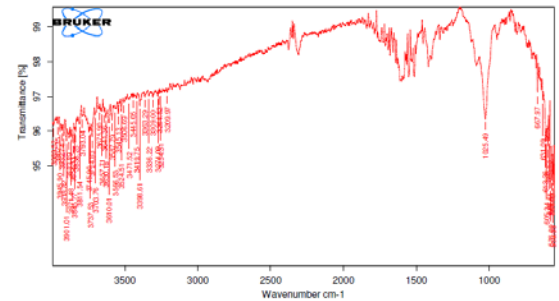
Fig 1: IR spectrum of pure Sodium alginate

Fig 2: IR spectrum of pure Carbopol 934

Fig 6: *invitro* **wash off test for Mucoadhesive Microspheres of Candesartan**

Table 1: Preparation of Candesartan Mucoadhesive Microspheres

FORMULATION CODE	DRUG(mg)	SODIUM ALGINATE	CARBOPOL(934)	HPMC(K100)
F1	100	1	0.25	0.75
F2	100	1	0.5	0.5
F3	100	1	0.75	0.25
F4	100	1	0	1
F5	100	1	1	0
F6	100	1	1	0.5
F7	100	1	0.5	1
F8	100	1	0.75	0.75
F9	100	1	0.25	1.25
F10	100	1	1.25	0.25

Table 2: micrometric properties of Candesartan Microspheres for formulations F1-F10

FORMULA	BULK DENSITY	TAPPED DENSITY	COMPRESSIBILITY INDEX	HAUSSNER'S RATIO
F1	0.32±0.02	0.52±0.06	1.15	13.46
F2	0.28±0.05	0.47±0.09	1.16	14.28
F3	0.45±0.08	0.59±0.03	1.15	13.20
F4	0.32±0.01	0.49±0.02	1.17	15.21
F5	0.42±0.03	0.55±0.05	1.16	14
F6	0.34±0.04	0.49±0.05	1.18	11.55
F7	0.48±0.05	0.52±0.09	1.17	12.58
F8	0.31±0.08	0.47±0.04	1.15	13.46
F9	0.27±0.07	0.41±0.08	1.15	13.72
F10	0.38±0.06	0.42±0.05	1.18	11.55

n=3±S.D.

Table3: Evaluation parameters of Candesartan Mucoadhesive Microspheres for formulations F1-F10

| FORMULATION	PERCENTAGE	DRUG	PARTICLE	DEGREE OF	LOOSE	MOISTURE
F1	85.19	75.76±0.67	5.02±0.36	160±1.52	34.32±0.12	10.76±0.32
F2	88.64	74.68±0.56	6.21±0.46	180±2.68	35.52±0.31	8.68±0.41
F3	84.91	75.02±0.48	6.00±0.55	184±3.64	34.13±0.22	7.02±0.56
F4	86.33	76.58±0.64	5.57±0.49	176±1.98	28.69±0.15	11.58±0.28
F5	87.29	74.16±0.51	6.23±.0.39	182±2.88	38.69±0.28	9.16±0.31
F6	88.12	83.06±0.43	7.65±0.47	194±3.65	22.32±0.34	7.06±0.45
F7	83.43	75.45±0.65	8.36±0.51	132±2.48	30.95±0.18	9.45±0.25
F8	85.65	86.32±0.46	7.68±0.38	162±1.68	26.63±0.16	8.32±0.36
F9	89.15	77.91±0.58	9.45±0.43	178±3.20	26.35±0.32	7.91±0.38
F10	86.89	78.26±0.67	8.52±0.46	190±2.51	27.59±0.16	11.26±0.43

n=3±S.D.

Table 4:*In-vitro* wash off test of Candesartan Mucoadhesive Microspheres for formulations F1-F10

FORMULATION/TIME(hr)	1hr	2hr	4hr	6hr	8hr
F1	78	74	68	61	55
F2	81	82	73	68	63
F3	88	87	84	72	77
F4	79	75	71	64	52
F5	74	68	63	58	50
F6	93	89	86	83	81
F7	94	87	82	76	74
F8	93	88	83	79	77
F9	92	86	81	77	77
F10	93	85	81	78	75

Table 5: *invitro* drug release kinetics studies of prepared Candesartan microspheres

FORMULATION/R2	FIRST	ZERO	HIGUCHI	PEPPAS		Hixson Crowell
				R^2	n	
F1	0.797	0.699	0.961	0.997	0.35	0.797
F2	0.906	0.715	0.923	0.970	0.25	0.851
F3	0.944	0.813	0.958	0.973	0.42	0.907
F4	0.856	0.664	0.893	0.960	0.21	0.792
F5	0.885	0.697	0.912	0.997	0.25	0.830
F6	0.833	0.581	0.849	0.851	0.21	0.743
F7	0.925	0.770	0.968	0.975	0.35	0.862
F8	0.679	0.544	0.816	0.962	0.14	0.629
F9	0.922	0.783	0.962	0.998	0.32	0.887
F10	0.959	0.747	0.944	0.998	0.29	0.907
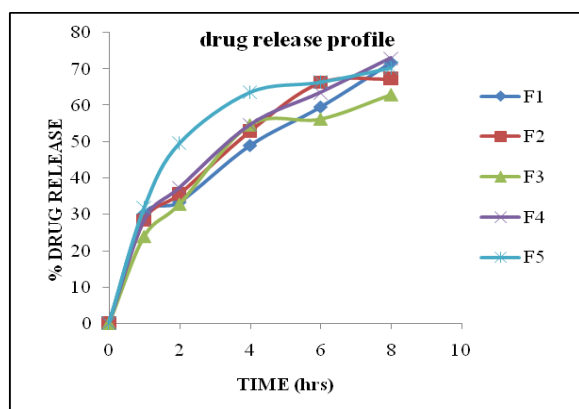
Fig 7: Cumulative % drug release of formulations F1-F5
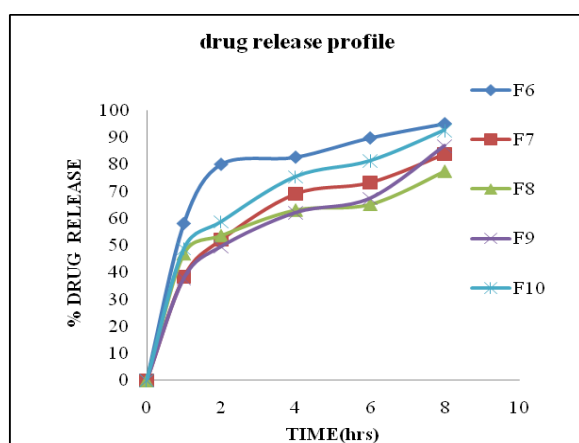
Fig 8: Cumulative % drug release of formulations F6-F10
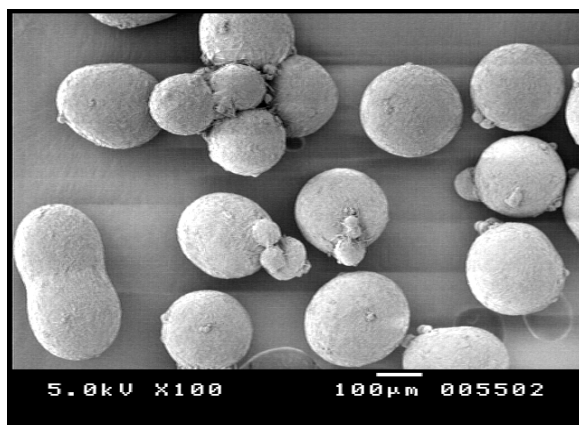
Fig- 9: SEM photograph of Candesartan microspheres at 100x and 1000x magnification.

Loose surface crystallography:
Loose surface crystal study done showed relative amount of drug encapsulated in outer layers. Formulations F1,F2,F3,F4andF5showed34.32±0.12%,35.52±0.31%,34.13±0.22% , 28.69±0.15% and 38.69±0.28% respectively and F6,F7,F8,F9 andF10 showed 22.32±0.34%,30.95±0.18%, 26.63±0.16%, 26.35±0.32% and27.59±0.16% respectively. Surface drug content of microspheres decreased with increase in the concentration of the polymer. Initially in batches with low polymer concentration the surface associated drug content was more due to the lower encapsulation efficiency. As the polymer concentration increased from F1-F5, F6-F10 it showed increased encapsulation efficiencies and hence decreased surface drug contents. The results are shown in the Table 3.

Moisture loss:
The percentage moisture loss of formulations F1 to F5 were 10.76%,8.68%,7.02%,11.58%,and 9.16 % respectively and formulations F6 to F10 were 7.06%,9.45%,8.32%,7.91%and 11.26% respectively. The results ensure the presence of diminutive water content which can be due to the involvement of water in process and hydrophilic property of mucoadhesive polymers shown in Table 3.

In-vitro wash off test:
Microspheres with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive property in the *in vitro* wash off test. The rapid wash-off, observed may be due to ionization which increases their solubility and reduces adhesive strength. The results of wash off test indicated that the microcapsules had fairly good mucoadhesive properties. The *in vitro* study results revealed that Candesartan release from the microspheres was slow and spread over extended period of time shown in Table 4 and Fig 06.

In-vitro drug release studies:
The percentage drug release from formulations, F1-F10 was observed for 8 hours in 0.1 N HCl[19].
The formulations F1-F5 drug release was found to be 77.3% to 82.75%,by using 2% polymer. The maximum drug release was found in F2 due to equal proportion of concentration of polymers i.e. Carbopol 934 p and HPMC K100 m (0.5:0.5).the formulations F6-F10 was found to be 77.45% to 94.97% by using 2.5% polymer. The maximum drug release was found in f6 due to increase in

concentration of primary polymer and decrease in concentration of secondary polymer Carbopol 934p to HPMC k100m (1:0.5). Among all formulation F6 was found to be best i.e. 94.97%.the results of *in-vitro* dissolution studies are shown in the fig 7 and fig 8.

Scanning Electron Microscopy (SEM)

SEM photograph of optimized microspheres at 100× magnification, at 1000× magnification. SEM photographs showed discrete, spherical microspheres. SEM photographs also showed the presence of drug crystal on the surface of microspheres revealing that the microspheres were having some rough surface**.** The drug crystals on microspheres were may be due to the presence of un entrapped drug in dispersion medium. The results are shown in fig 9.

Kinetics of Drug Release:

The drug release data was subjected for mathematical treatment to check the release order kinetics. Plots of log cumulative percent drug remaining Vs time were found to be linear with all the microsphere formulations indicating that the drug release was according to the first order kinetics. To evaluate the drug release mechanism from microsphere Peppa's plot were constructed and these plots were found to be linear with all microspheres indicating that the drug release mechanism from the microspheres was diffusion controlled. The results of all microspheres showed 'n' values less than 0.5 which indicates that it follows fickian diffusion with first order.The Kinetic data of release profiles of Candesartan microspheres are shown in table 5.

CONCLUSION:

The Mucoadhesive Microspheres of Candesartan were successfully prepared by orifice Ionic Gelation Technique using polymers Sodium alginate, Carbopol and HPMC and confirmed that it is a best method for preparing Candesartan Mucoadhesive Microspheres from its higher percentage yield. The percentage of encapsulation of all formulations was found to be in the range of 71 % to 86%. Higher percentage of entrapment was obtained by increasing the concentration of polymer. The particle size of a microsphere was determined by optical microscopy and all the batches of microspheres show uniform size distribution. The *in-vitro* dissolution studies showed that Candesartan Mucoadhesive Microspheres formulation F6 (94.97%) showed better sustained effect over a period of 8 hours than other formulations.

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