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FORMULATION AND EVALUATION OF CHITOSAN LOADED MUCOADHESIVE MICROSPHERES OF RAMIPRIL

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Abstract: The purpose of this research was to formulate and systemically evaluate in-vitro and in-vivo performances of mucoadhesive Ramipril microspheres for its potential use in the treatment of hypertension, myocardial infraction. Ramipril mucoadhesive microspheres, containing chitosan as mucoadhesive polymer and ethyl cellulose as carrier polymer, were prepared by an emulsion-solvent evaporation technique. Preformulation studies were carried out before formulation design. Total four formulations were prepared.Microspheres were discrete, spherical, free-flowing and showed a good percentage of drug entrapment efficiency. An in-vitro wash off test showed that Ramipril mucoadhesive microspheres adhered more strongly to the gastric mucous layer and could be retained in the gastrointestinal tract for an extended period of time. In-vitro dissolution test was carried out by using phosphate buffer pH 6.8. All the formulations showed good dissolution profiles. Among all the formulation F4 showed good dissolution profile with 81.0% of drug release in 12 hours. In-vitro release kinetic data of Ramipril microspheres showed that the drug release mechanism was diffusion controlled as the plots of Higuichi model was linear. All formulations exhibited Non-Fickian diffusion (n value is in between 0.5 to 1) mechanism. Stability studies were done for the selected formulation indicates that there is no change in drug content of the formulation. The results showed a sustained anti-hypertensive effect over a longer period of time in case of mucoadhesive microspheres, compared to the powder. In

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conclusion, the prolonged gastrointestinal residence time and slow release of Ramipril resulting from the mucoadhesive microspheres, could contribute to the provision of a sustained anti-hypertensive effect.

Key words: Ramipril, Chitosan, Mucoadhesive, Microspheres.

INTRODUCTION

For many drugs a well-designed drug delivery system is an important as pharmacological activities of the drug. A well designed drug delivery system can accurately deliver the drug to the site of action at desired rate and minimize its side effects by reducing exposure of drug to other tissues.[1] Microencapsulation is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material. In microencapsulation particle size is ranging from several 10 microns to 5000 microns.

Microencapsulation provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials. Several of these properties can be attained by macro packaging techniques; however, the uniqueness of microencapsulation is the smallness of the coated particles and their subsequent use and adaptation to a wide variety of dosage forms[2].Mucoadhesive drug delivery system is delivery system which utilizes the property of bioadhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time. Mucoadhesive drug delivery system could be designed for buccal, oral, vaginal, rectal, nasal and ocular route of administration.[3]

Ramiprilis a potent ACE inhibitor used in the treatment of hypertensive disease. It is a highly lipophilic (log p octanol/water, 3.32) and poorly water soluble drug, with absolute bioavailability of 28-35% and half-life 2-4 hours[4]. It undergoes significant 'first pass' metabolism. Ramipril is a prodrug and is converted into an active metabolite

ramiprilat by liver esterase enzymes. Ramiprilat is mostly excreted by the kidneys. The half-life of ramiprilate is variable, and is prolonged by heart and liver failure, as well as kidney failure.

Ramipril is marketed in India under the brand names of Cardace, Zigpril and Zorem. Single doses of Ramipril of 2.5 - 20 mg produce approximately 60 - 80 % inhibition of ACE activity 4 hours after dosing with approximately 40 - 60% inhibition after 24 hours.

MATERIALS AND METHODS

Ramipril obtained as a gift sample from Watson Pharma Pvt. Ltd., Thane. Chitosan was also obtained from Watson Pharma Pvt. Ltd., Thane. Ethyl cellulose, Span 80, Liquid paraffin(light), Ethanol, Pottassiumdihydrogen phosphate, Petroleum ether, Sodium hydroxide were obtained from Supra Pharmaceutical Pvt. Ltd., Hyderabad.

Method of Preparation

Emulsion Solvent Evaporaion Method

Accurately weighed quantity of polymers (chitosan and ethyl cellulose) were dissolved in 20ml of ethanol. Weighed quantity of drug is dispersed in the above polymer phase and emulsified with 100ml of liquid paraffin containing 2ml of span 80 with continuous stirring at 800 rpm using mechanical stirrer. The stirring was continued for 3.5 hrs. to ensure complete evaporation of ethanol. The microspheres were separated from liquid paraffin by filtration through watmann filter paper No.44 washed three times with petroleum ether and air dried for 12 hours**.**

Table No.1 Formulation Design

Formulation	Drug (mg)	Chitosan(mg)	Ethyl cellulose(mg)
F_1	20	460	340
F_2	20	480	320
F_3	20	530	270
F_4	20	400	400

EVALUATION

Angle of Repose

The flow characteristics are measured by angle of repose. Improper flow is due to frictional forces between the particles. Angle

of repose is defined as maximum angle possible between the surface of the pile of the powder and the horizontal plane. Lower the angle of repose the better the flow property

Tanθ = h/r

Θ = tan¯ 1 (h/r)

Where, h= height of pile

r= radius of base of pile

θ= angle of repose

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Microspheres Size Analysis

Microsphere size distribution was done by optical microscopy method.

Percentage Yelid

The total amount of microspheres obtained was weighed and the percentage yeild was calculated taking into consideration the weight of drug and polymer[6]

%Yield=(Practical yield / Theoretical yield) x100

Entrapment Efficiency

Drug entrapment efficiency of Ramipril was performed by accurately weighing 100 mg of micro particles and suspended in 100ml of simulated intestinal fluid of pH 7.4±0.1 and it was kept for 12hrs. Next day it was stirred for 15mins, and subjected for filtration. After suitable dilution, Ramipril content in the filtrate was analyzed spectrophotometrically at 210nm using Shimadzu UV-visible Spectrophotometer.

The absorbance found from the UV-spectrophotometer was plotted on the standard curve to get the concentration of the entrapped drug. Calculating this concentration with the dilution factor we get the percentage drug encapsulated in microparticles.[7]

Encapsulation efficiency = Estimated drug content/theoretical drug content × 100

***In- vitro* Wash-off Test**

The mucoadhesive property of microspheres was evaluated by an *In vitro* adhesion testing method known as wash-off method. Freshly excised piece of intestinal mucosa (2 x 2 cm) from goat were mounted on to glass slides (3 x 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support, about 100 microspheres were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of a USP

tablets disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given slow, regular up-and-down moment in the test fluid (900ml of 0.1N HCl) at 37± 0.5˚C. At the end of 30 min, at the end of one hour, and at the hourly intervals up to 5 hours, the machine was stopped and number of microspheres still adhering to tissue was calculated. The studies were carried out in triplicate[8].

***In- vitro* Dissolution Studies**

Dissolution studies were carried out for all the formulation, employing USP XXIII apparatus (Basket method) at 37± 0.5˚C rotated at constant speed of 50 rpm using 0.1N HCl as the dissolution medium. A sample of microspheres equivalent to 100mg of Ramipril was used in each test. An aliquot of the sample was periodically with drawn at suitable time interval and the volumes were replaced with fresh dissolution medium in order to maintain the sink condition. The sample was analyzed spectrophotometrically.

Kinetics of Drug Release

In order to understand the mechanism and kinetic of drug release, the drug release data of the *in vitro* dissolution study were analyzed with various kinetic model like zero order, first order, Higuchi's, Peppa`s and Coefficient of correlation (r) values were calculated for the linercurves by regression analysis of the above plots.

Scanning Electron Microscopy

The samples were dried thoroughly in vacuum desiccator before mounting on brass specimen studies. The samples were mounted on specimen studies using double sided adhesive type, and gold palladium alloy of 120A˚ knees was coated on the sample using sputter coating unit (Model E5 100 Polaron U.K) in Argon ambient of 8-10 Pascal with plasma voltage about 20MA. The sputtering was done for nearly 3mins to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current of about 80MA. The condenser lens position was maintained between 4.4-5.1.The objective lens aperture has a diameter of 240 microns and the working distance WD=39mm.

RESULTS AND DISCUSSION

In this present work efforts have been made to develop mucoadhesive microspheres of Ramipril by emulsion solvent evaporation method using chitosan and ethyl cellulose. Total four formulations were prepared and the detailed composition is shown in **Table No. 1.** The prepared microspheres were subjected to determine angle of repose, particle size, drug entrapment efficiency, *In-vitro* dissolution and scanning electron microscopy. Flow properties of the prepared microspheres were determined by conducting **Angle of repose**determinations shown in **Table No. 2.** All the formulations showed angle of repose within the range of 24˚15 to 26˚51. Results indicating that they are having good flow properties. Particle size was determined by optical microscopy method. Results were showed in **Table No.2**. The **Percentage yield** of microspheres of all the formulations was in the range of 78.90% to 90.95% shown in the **Table No. 3.** The **Entrapment efficiency** was in the range of 62.68 to 72.59 shown in **Table No. 3**. The test results of wash-off test were showed in the **Table No. 4, 5**. Microspheres showed good mucoadhesive properties in the *In-vitro* wash-off test. Formulation F4 has more mucoadhesive strength than others.

In vitro drug release studies

The results of the *in vitro* dissolution studies of formulations F1 to F4 are shown in **Table No.6**. Among all the formulations F4 showed good dissolution profile with 81.0% of drug release in 12 hours.

Drug release kinetic data for microspheres was shown in **Table No. 7**. All the formulations exhibited anomalous (Non-Fickian) diffusion (n value is in between 0.5 to 1.0) mechanism. The **drug release mechanism** was diffusion controlled as the plot of Higuichi model is linear**.**Morphology of the microspheres was investigated by scanning electron microscopy. The photograph of formulations were taken by **scanning electron microscope** shown in the **Figure No.2.** The microspheres prepared by this method were found to be spherical, free flowing and it was observed by Scanning electron microscopy

Table No.2 Flow Properties of Ramipil Microspheres

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S.No.	Formulation	Angle of Repose	Particle size(µm)
1	F1	24º 15’	224.732
2	F2	25º 39’	320.216
3	F3	25 º23’	268.611
4	F5	22º 56’	371.886

Table No.3Percentage Yield and Entrapment Efficiency of Ramipril Microspheres

Formulation	Percentage Yield	Entrapment efficiency
F_1	82.64	67.43
F_2	78.90	63.10
F_3	80.50	62.68
F_4	90.95	72.82

Table No.4*In–vitro* Wash-off Test for Ramipril Microspheres in 0.1N HCL

Formulation	Mean Percentage of Microspheres Adhering to Tissue in 0.1N HCL				
	1hr	2hr	4hr	6hr	8hr
F_1	78.3	70.2	57.6	34.8	20.4
F_2	80.7	71.4	58.6	36.4	21.8
F_3	81.6	73.5	59.7	37.1	23.7
F_4	84.1	75.2	64.1	40.8	27.1

Table No.5*In- vitro* Wash off Test for Ramipril Microspheres in PBS pH 6.8

Formulation	Mean percentage of Microspheres Adhering to Tissue in PBS pH 6.8			
	1hr	2hr	4hr	6hr
F_1	68.4	58.3	29.2	14.2
F_2	70.3	60.1	31.1	16.1

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F_3	71.6	61.3	32.4	17.3
F_4	74.2	64.9	35.8	20.5

Figure No.1 Dissolution Profile For Formulations F1-F4

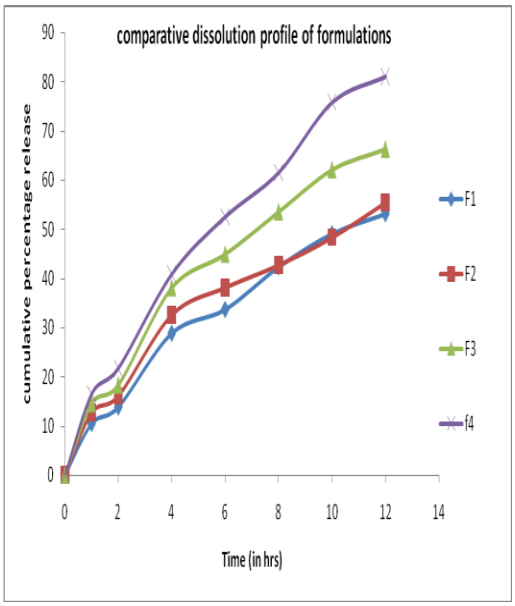

Table No.6 Cumulative Percentage Release Profile of Formulations F1-F4

Time(hrs)	Cumulative percentage release			
	F1	F2	F3	F4
1	10.50	12.75	14.62	16.50
2	13.87	16.12	18.37	21.75
4	28.88	32.62	38.17	40.87
6	33.71	38.24	44.99	52.50
8	42.37	42.74	53.62	61.50
10	49.12	48.37	62.20	75.75
12	53.17	55.49	66.37	81.00

Figure No.2 Zero Order Plot For Formulation F1

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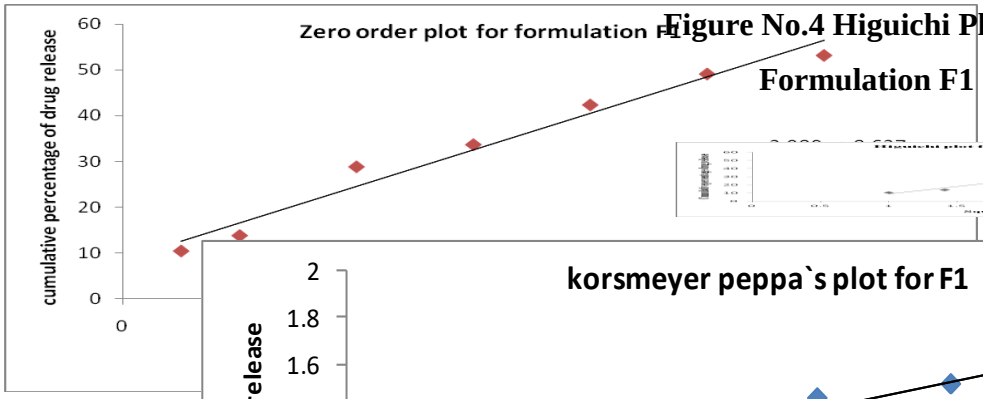

Figure No.4 Higuichi Plot For Formulation F1

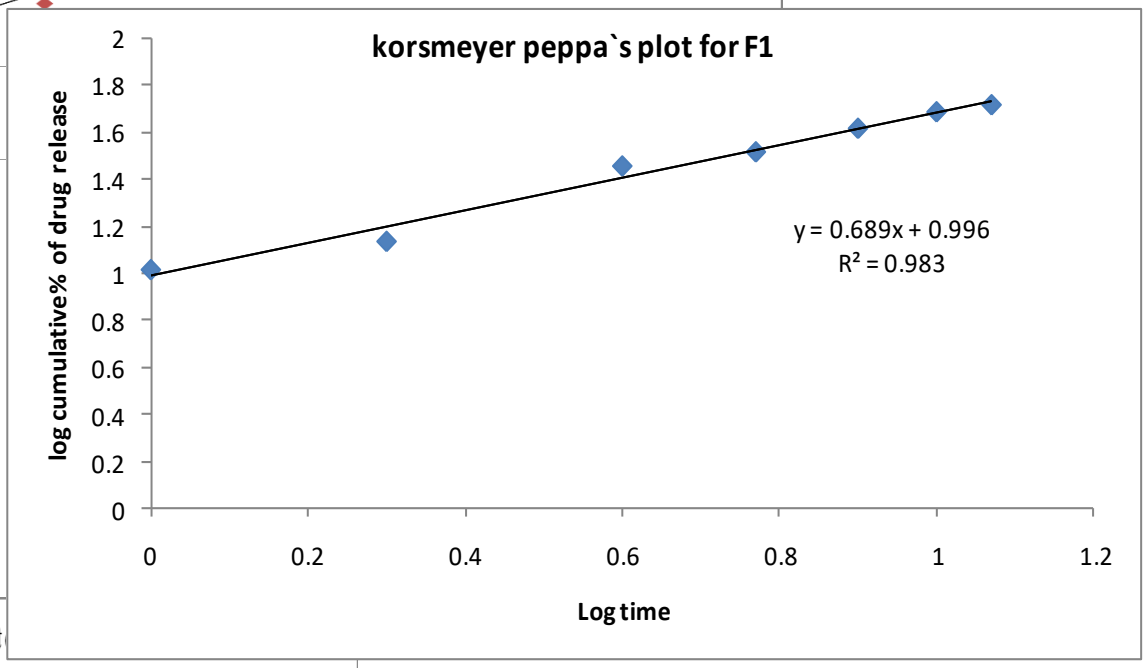

Figure No.3 First Order Plot For Formulation F1

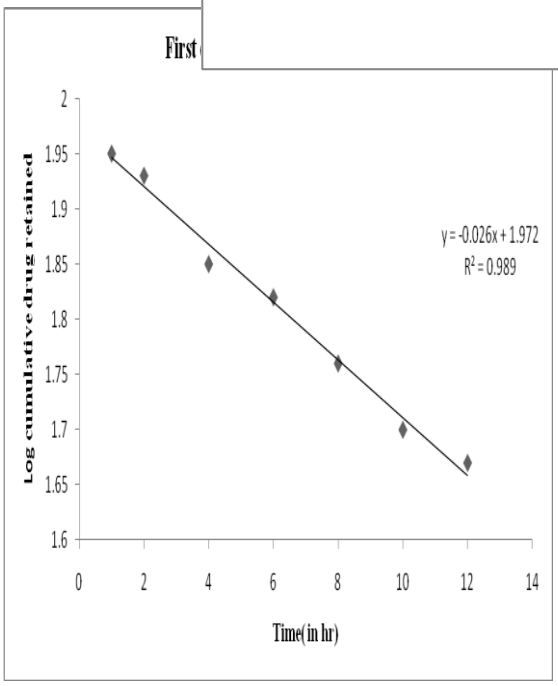

Figure No.5 KorsmeyerPeppa`s Plot For Formulation F

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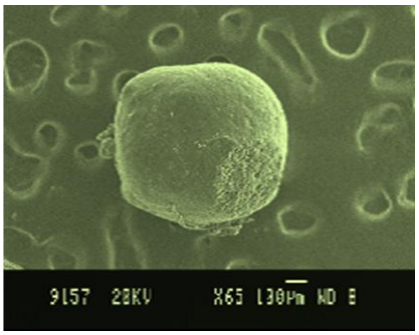
Formulation	Zero order plots	First order plots	Higuichi plots	Korsemeyerpeppa`s plot		Possible drug release mechanism
	R^2	R^2	R^2	R^2	n	
F1	0.9718	0.9899	0.9908	0.9836	0.6892	**Non-Fickian, Higuichi**
F2	0.9543	0.976	0.983	0.974	0.6194	**Non-Fickian, Higuichi**
F3	0.9604	0.9913	0.9882	0.9782	0.6547	**Non-Fickian, First order**
F4	0.9821	0.9861	0.9916	0.9886	0.6773	**Non-Fickian, Higuichi**

Table No.8 *In- Vitro* Release Kinetic Data For Ramipril Microspheres

Figure No. 2 Surface View of Microspheres of Ramipril



CONCLUSION

Ramipril mucoadhesive microspheres were prepared successfully using chitosan as a mucoadhesive polymer and ethylcellulose as a release retardant in different proportions. Preformulation studies of ramipril were done initially and results directed for the further course of formulation. Based on the preformulation studies different batches were prepared using selected excipients. Prepared microspheres were evaluated for the percentage yield, drug content, entrapment efficiency, particle size determination, *in-vitro* wash- off test, *in-vitro* dissolution test. The drug content and entrapment efficiency were good. Among all the formulations F4 showed better results. Mucoadhesive property of F4 was better than the other formulations.

Dissolution was carried out in phosphate buffer pH 6.8 at 210nm. All the formulations were evaluated using different kinetic models i.e. Zero order kinetics, First order kinetics, Krosmeyerpeppa`s model and Higuichi kinetics. All the formulations exhibited Non-Fickian diffusion mechanism. The drug release was diffusion controlled as the plot of Higuichi model was found to be linear. The formulation F4 was selected as an optimized formulation with 81.00 % of drug release in 12 hours.

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