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RESEARCH ARTICLE

Formulation and Optimization of Candesartan Loaded Eudragit Coated Microspheres

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Abstract:

The objective of present study is enhancement in the oral bioavailability of Candesartan using a lipid-based formulation i.e Eudragit S-100 microspheres. Candesartan is therapeutically a potent antihypertensive agent but it suffers a major drawback of poor oral bioavailability, which is estimated to be 15% due to low solubility in gastrointestinal fluid, hepatic first pass metabolism. The main emphasis of present study is on the use of new approach of lipid-based formulation to enhance the bioavailability of poorly water-soluble but highly permeable drug. The proposed formulation is aimed to enhance the bioavailability of Candesartan by using microspheres coated with eudragit S-100 polymer, which are transported through lymphatic system, thereby preventing hepatic first pass metabolism. Eudragit based microspheres were prepared by oil-in-oil solvent evaporation method using different drug- polymer ratios (1:1 to 1:4), stirring speeds (500-1500 rpm) and emulsifier concentrations (0.5%-1.25% wt/vol). Differential scanning calorimetry, study of the physical mixtures of drug and polymer revealed no drug-polymer interaction. All formulations were evaluated for particle size and shape, swell ability and percentage drug entrapment. The yield of preparation and the encapsulation efficiencies were high for all Eudragit microspheres. The in vitro drug release study of optimized formulation was also performed in simulated gastrointestinal fluids (SGF). The release profile of Candesartan from Eudragit microspheres was pH dependent.

Keywords: Eudragit S 100, Colon specific drug delivery, Microspheres, Candesartan.

INTRODUCTION:

Microspheres have been widely accepted as a means to achieve oral (1) and parenteral controlled release (2) drug delivery system. The microsphere requires a polymeric substance as a carrier and a core material. Among the various methods developed for formulation of microspheres, the solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of drug. Eudragit S 100 is a water-insoluble polymer that is widely used as a wall material for sustained release microsphere (3). This is due to its biocompatibility, good stability, easy fabrication and low cost. In recent years, microsphere dosage forms have gained increasing importance as oral controlled drug delivery systems. These systems present several advantages in comparison to unit dosage forms such as more predictable gastric emptying and less local irritation (4). Microsphere systems also minimize the possible intestinal retention of undigested polymer materials in chronic dosing (5). The drug of choice, Candesartan, is a potent antihypertensive agent belonging to the category of Angiotensine receptor antagonist. It has a short biological (6) half-life of 5.1 ± 10.5 h & low bioavailability 15% The main emphasis of present study is on the use of new approach of lipid-based formulation to enhance the bioavailability of poorly water-soluble4 but highly permeable drug by using microspheres coated with eudragit S-100 polymer, which are transported through lymphatic system, thereby preventing hepatic first pass metabolism also. In addition, the colon has a long retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. The approaches used in the colonic delivery of drugs include the use of prodrugs (7), pH-sensitive polymer coatings (8,9), time-dependent formulations (10), bacterial degradable coatings (11), time pH-controlled deliveries (12), and intestinal luminal pressure-controlled colon delivery capsules (13). The solvent evaporation method was selected as a method of choice in the present study due to its advantages .The versatility and flexibility of the

methods allow for the use of different polymers and solvents, Emulsion evaporation permits higher polymer concentration per batch production improving the microsphere yield, It can be used for entrapment of hydrophobic and hydrophilic drugs, The hydrophobic drugs use oil in water (o/w) emulsion technique, The hydrophilic drugs require the use of non-aqueous o/o emulsion or double emulsion technique, The fast evaporation rate of the solvent permits a reduction in the processing time. In addition, the colon has a long retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. The approaches used in the colonic delivery of drugs include the use of prodrugs (1), pH-sensitive polymer coatings (2, 3), time-dependent formulations (4), bacterialdegradable coatings (5), time pH-controlled deliveries (6), and intestinal luminal pressure-controlled colon delivery capsules (7).

MATERIALS AND METHOD

MATERIALS:

Candesartan was obtained as a gift sample from Matrix laboratories, Hyderabad, India. Eudragit L-100 was procured as a gift sample from Matrix laboratories, Hyderabad,India All other solvents and reagents used were of analytical grade.

METHOD:

PREPARATION OF CORE CALCIUM ALGINATE BEADS

Different formulations of Calcium alginate beads were prepared using various drug: polymer ratio, emulsifier concentration, and various stirring speed as shown in table 1.

Emulsion of drug and Span 80 was taken in a beaker and to it solution of sodium alginate was added drop wise and stirrer well. This mixture of alginate and emulsion was then added to the solution of calcium chloride solution drop by drop using 26G syringe with continuous agitation. A gelatinous

precipitate was formed by chemical reaction between sodium alginate and calcium chloride. The prepared beads were left under continuous stirring at about 500 RPM for about 10 minutes and then removed by filtration and washed with n- Hexane 4-5 times to remove the surface drug and then dried.

Tables:

Table 1: Composition of different Candesartan Microspheres

Formulation Code	**Concentration of Sodium Alginate solution used (% w/v)**	**Concentration of Candesartan: Eudragit S 100 (%w/w)**	**Concentration of emulsifier (Span85) (% w/v)**	**Stirring Speed (rpm)**	**Stirring Time (min.)**
EMS-D_0	0	0:4	1	500	30
EMS-D_1	1	1:4	1	500	30
EMS-D_2	2	2:4	1	500	30
EMS-D_3	3	3:4	1	500	30
EMS-P_1	1	1:1	1	500	30
EMS-P_2	1	1:2	1	500	30
EMS-P_3	1	1:3	1	500	30
EMS-P_4	1	1:4	1	500	30
EMS-E_1	1	1:4	0.5	500	30
EMS-E_2	1	1:4	0.75	500	30
EMS-E_3	1	1:4	1.00	500	30
EMS-E_4	1	1:4	1.25	500	30

EMS-R_1	1	1:4	1	500	30
EMS-R_2	1	1:4	1	750	30
EMS-R_3	1	1:4	1	1000	30
EMS-R_4	1	1:4	1	1500	30

E- Eudragit, **MS-** Microspheres, **D_1-** Drug, **P_1-** Polymer, **E_1-** Emulsifier, **R_1-** Stirring speed

COATING OF CORE CALCIUM ALGINATE SPHERES

The prepared optimized batch of alginate beads were coated with polymer Eudragit S 100 using solvent evaporation method. In this method the beads were dispersed in different optimized batches of Eudragit S 100 which were dissolved in acetone and methanol in 2:1 ratio and the solvent was evaporated in a rotator evaporator using rotation of about 500 RPM and then dried.

Optimization

Various formulation variables e.g. drug concentration, polymer concentration, emulsifier concentration and process variables viz. stirring speed, which could affect the preparation and properties of microspheres were identified and studied. The compositions of formulation code of designed formulae of Candesartan microspheres are given in table 1.

Optimization of formulation & process variables:

Various formulation variables were tried to prepare microspheres viz. drug concentration: 0%, 1%, 2% and 3%, Eudragit concentrations: 1%, 2%, 3%, 4% and emulsifier concentrations: 0.5%, 0.75%, 1.0%, 1.25%, were optimized. The effects of drug concentration, Eudragit concentration and emulsifier concentration on the particle size, shape, size distribution and total drug loading efficiency are shown in table 2 respectively.

Optimization of drug

Optimization of drug ratio was the first step in the preparation of microspheres. Various ratios of drug and polymers were taken i.e.0:1, 1:1, 2:1, 3:1. Keeping stirring speed (1000 rpm), emulsifier concentration (1.25%v/v span 80), processing medium (light liquid paraffin oil, ethanol and acetone) constant, microspheres were prepared by the method and shown in table 2

From the above optimization parameters observed on the drug concentration it was observed that the batch EMS-D_3 is having the drug entrapment efficiency of 48.12 % and drug loaded in it is 40.34 %. From the above graph it was concluded that as the drug concentration is increased the DEE is increased whereas the drug loading decreases.

Optimization of drug polymer ratio

Optimization of drug polymer ratio was the first step in the preparation of microspheres. Various ratios of drug and polymers were taken i.e. 1:1, 1:2, 1:3, 1:4. Keeping stirring speed (1000 rpm), emulsifier concentration (1.25%v/v span 80), processing medium (light liquid paraffin oil, ethanol and acetone) constant, microspheres were prepared by the method and shown in table 2

Table 2: PREPARATION OF CORE CALCIUM ALGINATE BEADS

parameters		Formulation code	Average diameter	DRUG LOADING	DEE
Drug :Polymer	0:4	EMS-D_0	225.40	0	0
	1:4	EMS-D_1	234.58	69.99	12.25
	2:4	EMS-D_2	240.99	45.86	41.02
	3:4	EMS-D_3	247.05	40.34	48.12
Drug :Polymer	1:1	EMS-P_1	290.21	62.75	37.68
	1:2	EMS-P_2	331.20	67.22	44.20

	1:3	EMS-P_3	440.52	70.46	60.02
	1:4	EMS-P_4	579.32	84.64	81.78
Emulsifier conc.(%v/v)	0.5	EMS-E_1	580.24	70.24	80.01
	0.75	EMS-E_2	461.40	69.14	65.12
	1.0	EMS-E_3	391.52	50.20	49.60
	1.25	EMS-E_4	287.22	41.02	29.87
Stirring Speed (rpm)	500	EMS-R_1	295.21	62.95	62.45
	750	EMS-R_2	263.24	64.74	69.22
	1000	EMS-R_3	231.02	73.31	76.85
	1500	EMS-R_4	182.12	58.72	57.20

Optimization of Emulsifier Concentration

Concentration of emulsifier is an important parameter which needs to be optimized because it is responsible for optimum particle size and stability of the microsphere. Span 80 was selected as an emulsifier and various concentrations of span 80 were taken. Microspheres were prepared by same method as described a with optimized ratio of drug polymer (1:4), keeping stirring speed (1000 rpm), and volume of processing medium constant as shown in table 2.

From the above optimization parameters observed on the emulsifier concentration it was observed that the batch EMS-E_3 is having the drug entrapment efficiency of 49.60 % and drug loaded in it is 50.20 %. From the above graph it was concluded that as the emulsifier concentration is increased the DEE

and loading is decreased. So the optimum emulsifier concentration recommended for best DEE and loading is found to be 1%.

Optimization of stirring speed

Stirring speed plays an important role in the microspheres size distribution and drug loading. Microspheres were prepared by same method as described above with optimized ratio of drug polymer (1:4), keeping emulsifier concentration (1%v/v span 80) volume of processing medium constant, at different speeds i.e.500, 750, 1000, 1500 rpm as shown in table 2

From the above optimization parameters observed by the change in stirring speed it was observed that the batch EMS-R_3 is having the drug entrapment efficiency of 76.85 % and drug loaded in it is 73.31 % as shown in figure 4. From the above graph it was concluded that as the stirring speed is increased the DEE and loading also increases but decreases after a certain point. So the optimum stirring speed recommended for best DEE and loading is found to be 1000 RPM.

Drug Content

The amount of Candesartan associated with the microspheres was analyzed in terms of surface adsorbed drug and entrapped drug.

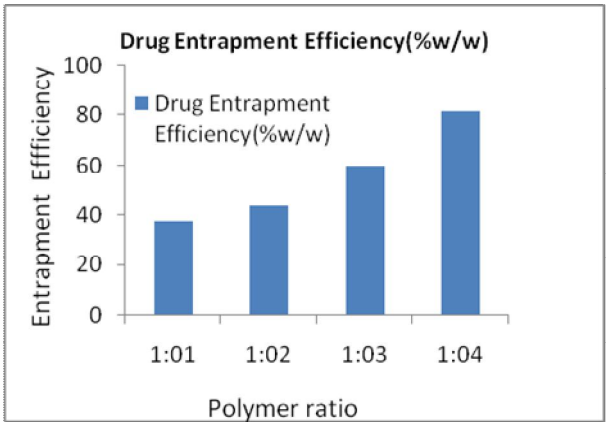
Estimation of surface drug in microspheres:

100 mg of microspheres was dispersed in 10 ml of PBS (pH 7.4) and shaken vigorously for 10 minutes and supernatant was kept aside. Similarly, the sediment was again treated in the same manner and second supernatant was mixed with first supernatant and analyzed for candesartan content spectrophotometrically as described previously. The amount of prednisolone in the mixed washings gave the amount of drug adsorbed on the surface of the microspheres.

Estimation of entrapped drug in microspheres:

The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of simulated gastric fluid pH 7.4 repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using simulated gastric fluid pH 7.4. The solution was

filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV-1800, Shimadzu) against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula: **D.E.E. = (Amount of drug actually present /Theoretical drug load expected) × 100**.



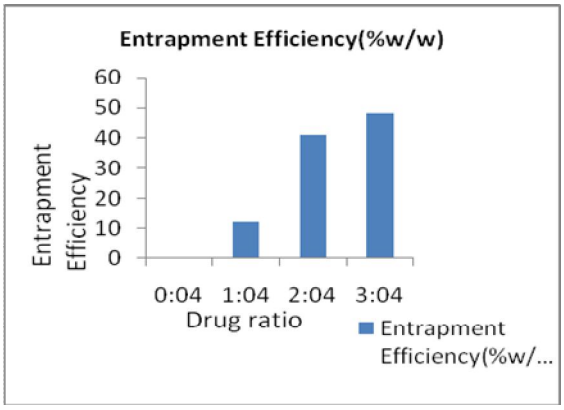


Fig 1 Optimization of Drug w.r.t. % D.E. **Fig 2 Optimization of Drug Polymer Ratio w.r.t.% D.E.E**

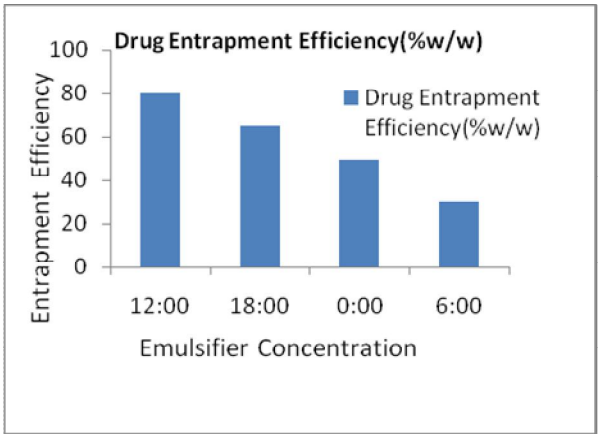


Fig 3 Optimization of Emulsifier Concentration w.r.t. % D.E.E.

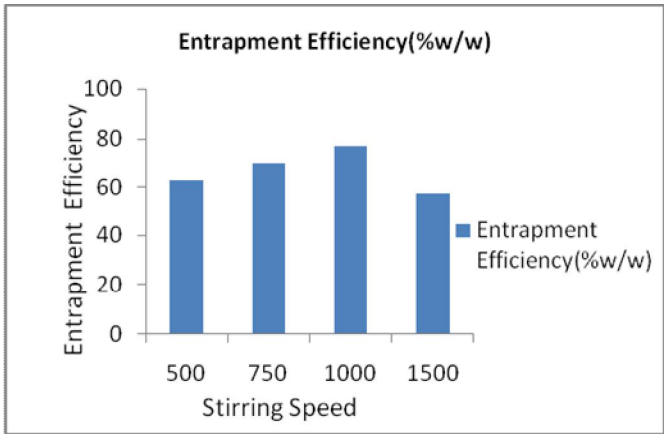


Fig 4 Optimization of Stirring speed w.r.t % D.E.E

CHARACTERIZATION OF PREPARED MICROSPHERES

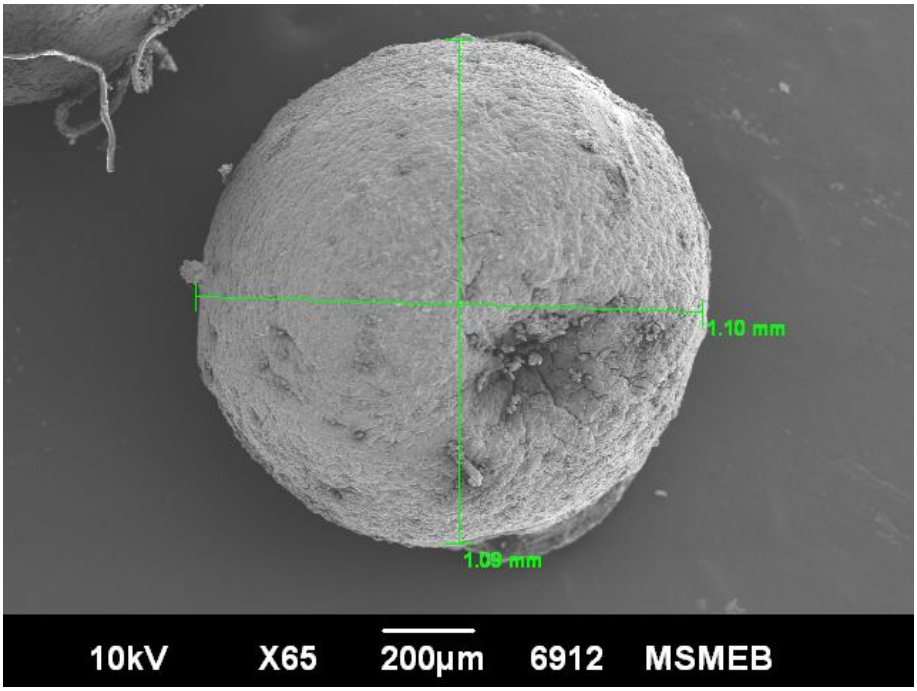
The prepared microspheres were characterized for shape , size and size distribution, percent drug loading and total drug loading efficiency, swellability, and in vitro drug release.

1. Shape

Microspheres were suspended in water; a drop was placed on a glass slide, covered with a cover slip and viewed under the optical microscope to examine their shape.

2. Scanning electron microscopy

Scanning electron microscopy (SEM) is one of the most commonly used method for characterizing drug delivery systems, owing in large part of simplicity of sample preparation and ease of operation. Scanning electron microscopy was carried out in order to characterize surface morphology of the microspheres. In this study the morphological observations were carried out to study the surface morphology of microspheres. SEM micrographs and typical surface morphology of the microspheres are given in figures 3.



3. Swellability/Degree of Swelling

From the swelling study as shown in table 15 and figure 19 it was observed that as compared to other polymer like pectin, guar gum etc which are having a swelling phenomena to a greater extent, Eudragit S 100 is not having a swelling phenomena and when placed in phosphate buffer

pH 7.4 Eudragit S 100 does not show a swelling phenomena to a greater extent as compared to various gums.

4. In Vitro Drug Release Studies in Simulated Gastrointestinal Fluids of Different pH

In vitro drug release studies were performed in simulated gastric fluid pH 1.2 for 2 h and then studied in pH 4.5 i.e simulated gastric fluid and simulated intestinal fluid from 3^{rd} to 6^{th} hour and then finally release observed in phosphate buffer pH 7.4. The comparison of different dissolution media are shown in tables 6. The Percentage cumulative drug release was higher at phosphate buffer 7.4 and Acetate buffer 4.5 when compared to 0.1N HCl because of the higher solubility of drug in between pH 4.0-7.0.The cumulative percentage drug released from the formulations was found to be 83.62% in phosphate buffer 7.4. From the release profile it is clearly evident that the drug release increased as the pH of the dissolution media increased.

The cumulative release of drug significantly Increased with increasing drug and polymer concentration but after a certain extent release was found to decrease as shown in figures 6, 7. The increased density of the polymer matrix at higher concentrations resulted in an increased diffusional pathlength. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.

With increase in emulsifier concentration from 0.5 to 1.2 5%, drug release was also found to increase from 75.14 to 82.22 %. Whereas as with the change in stirring speed the release was found to increase but after a above 1000 rpm the release was found to decrease as shown in table and figure.

The results are recorded in table 6 and graphically shown in Fig. 6-9.

RESULT AND DISCUSSION:

Eudragit microspheres of candesartan were successfully prepared by solvent evaperation technique.The microspheres produced were generally spherical, discrete upon dispersions in an aqueous medium and having uniform size ranges from 25 to 32 µm.

The microspheres were coated with Eudragit S100 oil-in-oil solvent evaporation method. Different core: coat ratios were taken to optimize the final formulation. The final product was finally dried for 24 hours.

The effect of various process variables viz.stirring speed and formulation variables e.g. drug concentration, polymer concentration and emulsifier concentration were studied. The results suggested that these variables influence the shape, size, size distribution, swellability, total drug loading efficiency and in vitro drug release of the final preparation. Hence these parameters were optimized to prepare microspheres of small size with narrow size distribution, good drug loading efficiency and good drug release at colonic Ph. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of simulated gastric fluid pH 7.4 repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using simulated gastric fluid pH 7.4. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV-1800, Shimadzu) against appropriate blank.

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