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Development and Evaluation of drug loaded Eudragit RS-100 microspheres for colon specific drug delivery system

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In the present study attempt was made for colon specific drug delivery using Eudragit RS100 microspheres of Serratiopeptidase an analgesic and anti-inflammatory activities,.Eudragit RS100 microspheres of Serratiopeptidase were prepared by solvent evaporation technique using Eudragit RS100 as a rate controlling polymer and the microspheres were evaluated. Eudragit RS100 microspheres were spherical, discrete, free flowing and multinucleate monolithic type. The mean size range was found for formulation in the range of 31 to 33 µm. Entrapment efficiency was in the range of 83.827 to 95.407 %. The FTIR and DSC study confirmed that no chemical interaction took place during entrapment process. The X-ray diffraction study indicates the amorphous dispersion of the drug after entrapment in to microspheres. The effect of polymer on release profile of drug was calculated. Serratiopeptidaserelease from theEudragit RS100 microspheres was slow over 12 hr and dependant on core: coat ratio and size of microspheres. Drug release was by higuchi mechanism. Good linear relationship were observed between core : coat ratio of the microspheres and release rate.Eudragit RS100microspheres of Serratiopeptidase exhibited good controlled release characteristics and were found suitable for once a day oral controlled release products.

Keyword: Microspheres, controlled release. Eudragit RS100.

1. Introduction

Proteins and peptides represent an important class of biopharmaceuticals that exhibit increased biochemical and structural complexity compared with conventional drug-based pharmaceuticals. Thus, formulation design for delivery of therapeutic proteins remains the challenging and difficult task.

Polymeric microspheres and microcapsules have received much attention for the delivery of therapeutically useful proteins in a controlled way. Microparticulate systems can be made by various techniques involving physicochemical processes (solvent evaporation method, phase separation method) and mechanical processes (e.g. spray drying)

Colon selective drug delivery systems have been the focus of increasing interest for the last decade. This is mainly due to the recently recognized importance of this region of the gastrointestinal tract, not only for the local but also for systemic therapy. At present, the specific drug delivery to the colon is considered an important alternative for the treatment of serious local diseases such as Crohn's disease, ulcerative colitis, carcinomas and infection. Although the

human colon has a lower absorption capacity than that of the small intestine, (the colonic surface area is only 0.3 m^2 in comparison with the 120 m^2 of the small intestine), material remains in the colon for much longer time. Colonic residence time is 2-3 days, whereas food remains in small intestine for as little as 5 hr[1]

This long colonic residence time provides a significant opportunity for the slow absorption of drugs and other materials, either targeted specifically at the large intestinal mucosa or designed to act systemically. In addition, drugs which would be unstable in the small intestine may be released in the colon safely and adsorbed there to act systemically. Drugs may be delivered to the colon orally in slow release or targeted forms.

There are two main classes of bacterial enzymes, the azoreductases and the polysaccharidases, which are in a sufficient quantity as to be exploited in colonic drug targeting. Based on this idea, different natural and synthetic polymers have lately been evaluated for their susceptibility of being cleaved by these enzymes and, thus for their use as major constituent of colon-specific drug delivery systems.

Serratiopeptidase has been shown to have potent analgesic and anti-inflammatory activities, in the present investigation Serratiopeptidase and Eudragit RS 100 were used as the model water-soluble acid-labile enzyme and water-insoluble polymer, respectively, for the preparation of microspheres for oral delivery of acid-labile enzyme.

Proteolytic enzymes (eg STP) offer a powerful treatment for pain and inflammation with widespread use in arthritis, fibrocystic breast disease, chronic bronchitis, sinusitis, atherosclerosis, wound debridement and carpel tunnel syndrome. These produce pharmacological effects by absorption through the intestine into the blood stream. But oral bioavailability of these peptide drugs is generally very low, owing to the acidic condition of the stomach and poor permeability across intestinal mucosa. Enteric coated preparations are available, but they release the drug with a burst, leading to other gastrointestinal tract-related disorders thus aggravating the inflammatory conditions. In the present study, efforts have been made to prepare an oral sustained release system for these acid-labile enzymes.

Eudragit RS 100 was selected because it has been reported to release the drug at pH > 7, suitable for oral delivery of acid-labile enzyme. It is an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester exhibiting pH –dependent solubility.

Statistical models are extensively used in diversified areas to strengthen the art of drug formulation. A 3^2 factorial design is an established method to study the effect of selected parameters. The amount of dichloromethane (DCM) in the organic phase and Tween 80 (Stabilizer) were selected as independent variables while the drug content and the diameter of microspheres were chosen as the dependent variables in the present investigation. The levels for these 2 parameters were determined from the preliminary trials. Furthermore selected formulation with maximum entrapment was evaluated for proteolytic activity, enteric nature and in vitro release studies.

2. Materials and Methods

2.1 Materials

Serratiopeptidase was received as a gift sample from Advanced Enzyme Technologies Ltd., Nashik. Eudragit RS 100 was received as a gift sample from Evonik Degussa India Pvt. Ltd. All the reagents and solvents used were of analytical grade satisfying Pharmacopoeial standards.

2.2 Methods

Microspheres were prepared by a method based on the emulsion solvent diffusion technique reported by with few modification[5]. Methanol and dichloromethane were used as solvent for STP and Eudragit RS-100 respectively. Acetone a water-miscible lipophilic solvent was used to reduce interfacial tension at the dispersed droplet surface. Tween 20, 40, 80 were tried as stabilizers in the preliminary trials. Eudragit RS-100 and STP were dissolved in an organic solvent blend consisting of methanol, acetone, and dichloromethane. The resultant solution was

emulsified with aqueous medium containing a stabilizer while stirring at 600 rpm. The emulsion was then stirred at room temperature (RT) for 3 to 5 hours for solvent evaporation. The collected microspheres were washed 3 times with demineralized water (500 ml) by centrifugation at 10000 for 10 minute. The microspheres were resuspended in distilled water and lyophilized for 24 hours. The final product was stored in a vacuum desiccators at 2 to 8 ^{0}C. Parameters for all the preparations are summarized in table 1. The full factorial design and layout with coded and actual values of variables for each batch are shown in table 2.

Table 1: Processing Parameters used through the study.

Sr.No	Drug	Polymer	Stirring Speed	Continuous Phase	Dispersed Phase Me:Ae:DCM	Stabilizer Concentration
1	STP 100 mg	300 mg	600 ± 10 rpm	100 ml methanol	3.5: 5: 5	0.05, 0.1, 0.15
					3.5: 5: 10	
					3.5: 5: 15	

Table 2: Full factorial experimental design layout with coded levels and actual values of variables.

Sr.No	Variable X_1 DCM, ml	Variable X_2 Tween 80, ml
1	5 (-1)	0.1 (-1)
2	10 (0)	0.1 (-1)
3	15 (1)	0.1 (-1)
4	5 (-1)	0.15 (0)
5	10 (0)	0.15 (0)
6	15 (1)	0.15 (0)
7	5 (-1)	0.20 (1)
8	10 (0)	0.20 (1)
9	15 (1)	0.20 (1)

2.3 Evaluation of microspheres

The prepared microspheres were evaluated for percentage yield, encapsulation efficiency, particle size and in vitro drug release.

1. Percentage yield :

The percentage yield of microspheres was determined from the ratio of the weight of solidified microspheres obtained to the weight of solid materials used in the inner phase.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

2. Encapsulation efficiency :

20 mg of microspheres were accurately weighed and crushed by using mortar and pestle. Crushed microspheres were suspended in 10 ml methanol and stirred for half an hour. Then the suspension was filtered through whatman filter paper No. 44. Then 1 ml of this solution was diluted to 100 ml with distilled water and absorbance was measured at 280 nm against distilled water as a blank. The drug content was determined from the standard curve. Encapsulation efficiency was calculated from following relationship.

$$\text{Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100$$

3. Particle size analysis of microspheres:

Average particle diameter and size distribution of microspheres were determined by laser diffractometer using a Mastersizer Micro Version 2.19 (Malvern Instruments, Malvern,UK) Approximately 10 mg of microspheres were stirred in 10 ml distilled water.Then aliquot of the microspheres suspension was added into recirculation unit, which was subsequently

circulated 3500 times per minute. Particle size was expressed as equivalent volume diameter. The particle size distribution was also expressed in terms of SPAM factor determined as:

$$SPAM = \frac{d_{90} - d_{50}}{d_{10}}$$

Where d_{10}, d_{50} and d_{90} are the diameter sizes and the given percentage value is the percentage of particles smaller that that size. A high SPAM value indicates a wide size distribution.

- Bulk density and tapped density
- Both loose bulk density (LBD) and tapped bulk density (TBD) was determined. A quantity of 2 g of microspheres from each batch was introduced in a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall on to a hard surface from a height of 2.5 cm at 2 sec intervals. The tapping was continued till no volume change was noted. LBD and TBD were determined by following formula.
- LBD = weight of powder in gms/ volume of packing in ml
- TBD = weight of powder in gms/ tapped volume of packing in ml

4. Compressibility index

Carr developed this method of measuring powder flow from bulk densities. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and is calculated according to following equation.

$$\% \text{ Compressibility} = \frac{LBD - TBD}{LBD} \times 100$$

5. In vitro drug release study of STP microspheres

Microspheres equivalent to 10 mg of Serratiopeptidase were filled in a capsule and in vitro drug release was studied using USP Apparatus II with 900 ml of dissolution medium at 37.5 ± 0.1 ^{0}C for 12hrs at 100 rpm. Phosphate buffer (pH 6.5) was used as dissolution medium. 10 ml of sample was withdrawn after every hour, and was replaced with an equal volume of fresh dissolution medium. Collector sample were analysed at 280 nm by spectrophotometrically. The study was performed in triplicate. Dissolution study was also conducted for marketed capsule dissolution data for different formulations.

Table 3*: In-vitro* drug release study for Eudragit RS100 microspheres of microspheres are reported in Table 4.

Time (hr)	Cumulative % drug release (mean ± SD., n=3)								
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RS9
1	39.17 ±2.14	28.62 ± 1.04	23.48 ± 1.36	21.12 ± 1.50	24.12 ± 0.98	26.69 ± 1.22	25.44 ± 2.12	30.58 ± 0.68	26.21 ± 1.23
2	49.97 ± 1.62	34.11 ± 1.12	26.43 ± 1.53	23.51 ± 1.30	34.74 ± 1.22	36.88 ± 1.77	33.96 ± 1.71	37.41 ± 1.36	30.61 ± 1.21
3	60.94 ± 1.29	43.12 ± 1.28	35.77 ± 2.68	39.78 ± 1.46	43.32 ± 0.95	44.22 ± 1.33	45.33 ± 1.22	48.27 ± 0.79	37.63 ± 1.78
4	64.12 ± 1.50	55.50 ± 0.71	42.80 ± 0.60	48.95 ± 2.17	50.35 ± 0.85	50.68 ± 1.55	52.99 ± 0.44	54.91 ± 2.79	44.84 ± 1.30
5	71.35 ± 1.55	59.47 ± 2.07	47.84 ± 1.56	56.71 ± 1.08	59.49 ± 1.28	56.08 ± 1.72	58.88 ± 1.72	62.42 ± 1.90	51.33 ± 1.78

6	76.66 ± 1.55	64.87 ± 1.02	55.72 ± 1.92	66.49 ± 0.81	67.84 ± 0.99	64.30 ± 0.88	66.45 ± 0.70	66.10 ± 1.76	59.74 ± 2.30
7	85.23 ± 0.81	70.17 ± 0.79	63.33 ± 2.16	72.63 ± 0.92	74.59 ± 1.01	72.43 ± 0.77	71.60 ± 1.70	71.09 ± 1.31	66.54 ± 2.71
8	93.01 ± 1.08	78.59 ± 0.79	70.92 ± 1.62	78.71 ± 1.67	82.51 ± 0.88	79.65 ± 0.56	80.13 ± 2.19	75.94 ± 0.78	71.51 ± 1.06
9	96.87 ± 0.37	85.97 ± 1.05	75.44 ± 0.80	86.21 ± 0.57	88.40 ± 1.32	83.045 ± 0.67	84.50 ± 1.30	83.91 ± 1.64	78.85 ± 2.14
10	100.54 ± 0.45	91.37 ± 1.74	84.97 ± 1.86	90.42 ± 1.11	93.67 ± 1.33	89.30 ± 0.40	90.70 ± 2.22	88.90 ± 2.30	86.53 ± 1.72
11	-------	95.62 ± 0.27	90.84 ± 1.80	95.73 ± 0.37	96.54 ± 1.44	94.13 ± 0.27	95.55 ± 1.11	95.16 ± 0.93	91.05 ± 0.93
12	------	100.26 ± 0.52	95.86 ± 0.54	97.83 ± 0.22	99.00 ± 0.36	97.45 ± 0.11	98.92 ± 0.11	100.27 ± 0.24	95.55 ± 1.12

2.4 Micromeritics study:

Particle size of drug affects flow characteristics, tableting as well as release from delivery system. Micromeritics study of the drug powder was done by Malvern master sizer which works on the principle of laser diffraction. The study was conducted using chloroform. The drug was found to be sparingly soluble in chloroform hence the study could be conducted in chloroform. Obscuration of laser beam by the particle was found to be 12.8% which was sufficient obscuration for the study.

2.5 FTIR Analysis

The Infra-red spectroscopy analysis was performed by Fourier Transformation Infrared Spectrophotometer 8400 (Shimadzu), with a resolution of 8 cm^{-1} , in the range of 4000-5000 cm^{-1} , KBr pellet.

2.6 Differential scanning calorimetry (DSC):

The DSC analysis of pure drug, drug-loaded microspheres, and polymer were carried out using Shimadzu DSC 60 to evaluate any possible drug-polymer interaction. The analysis was performed at a rate 10.0^0 C min^{-1} to 300 ^{0}C temperature range under nitrogen flow of 25 ml min^{-1}

2.7 X-Ray diffraction studies (XRD)

Drug, polymer formulated microspheres were analysed by XRD in order to check effect of compression on crystallinity of ingredients as well as to check any interaction between the excipients. Powder X- ray diffraction patterns were obtained by a diffractometer (PW 3710, Philips).

2.8 Scanning electron microscopy (SEM)

The shape and surface morphologies of the drug - loaded microspheres were investigated using scanning electron microscopy (XL 30 ESEM Philips).

3. Results And Discussion

3.1 Effect on Drug Encapsulation Efficiency

The method showed good encapsulation efficiency. Percent drug encapsulated was found to be in a range of 83-95% for Eudragit RS-100 microspheres. It was observed that with increase in polymer concentration drug encapsulation efficiency was increased. Drug encapsulation

efficiency was slightly increased as the concentration Tween 80 was increased because dispersing agent decrease the interfacial tension between the lipophilic and hydrophilic phases of the emulsion and simplify the formation of microspheres also this dispersing agent provides a thin protective layer around the droplets and reduces the extent of their collision and coalescence.

3.2 Effect on particle size

Particle size for Eudragit RS-100 microsphere was found in the range of 31-33µm with SPAN factors ranging between 8-9.

3.3 Kinetic treatment of dissolution data

All the formulations followed Higuchi's kinetics with R^2 ranging from 0.9633-0.9992.

3.4 Effect on drug release

In vitro dissolution results showed that the microspheres prepared with a different core-coat ratio gave better-sustained action.Eudragit RS-100 gave sustained action over 12 hrs Table 3 clearly illustrates that the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices. An inverse relationship was observed between polymer content and drug release rate from the prepared microspheres. In all cases of polymers it was seen that microspheres containing 25% polymer released the drug more rapidly, while those with 100% polymers exhibited a relatively slower drug release profile.

3.5 Physical properties of optimized microspheres

It was found that all batches of microspheres were discrete, free flowing and spherical.

Bulk density was found to be 0.4788, which showed good flow characteristics of microspheres.

Tapped density was found to be 0.574 which showed good flow characteristics of microspheres.

A value of angle of repose was 30.02indicated good flow properties of microspheres.

Compressibility index was found to be 16.66 resulted in good to excellent flow properties of microspheres is due to the fact that the microspheres membrane a more open structure.

Surface topography of the optimized microspheres

Optimized microspheres RS2 were analyzed for surface characterization using Scanning Electron Microscopy.

RS2 microspheres were found to be spherical, discrete, non-porous structure with rugged polymeric surface. (Figure 4)

RS2 microspheres were found to be spherical, discrete, with distinct pores on the surface.

3.6 Compatibility study of optimized microspheres.

The compatibility study of Serratiopeptidase with excipients was done by UV spectroscopy, X ray diffractometry, FTIR, and DSC.

I. By UV Spectroscopy**:**

The UV spectrum of pure drug solution of Serratiopeptidase was obtained at 209 nm RS microspheres were found at 209 nm, which showed that there was no interaction between drug and excipients.

II. By X-ray diffractometry:

Characteristic crystalline peaks of Serratiopeptidase were observed at 2θ of 10.087, 12.412, 13.488, 14.123, 15.296, 17.749, 19.505, 22.445, 24.731, 26.222, 28.467, 30.845, 32.538 (Figure 15) indicating the presence of crystalline. Peaks of Serratiopeptidase are also present in Eudragit RS-100, microspheres even if reduced in intensity. Typical diffraction patterns of Serratiopeptidase loaded Eudragit RS-100 microspheres are shown in Figure 3. The decreased intensity of peaks is due to decrease in drug crystallinity. This indicates that Serratiopeptidase is present in the Eudragit RS-100, microspheres with reduced crystallinity.

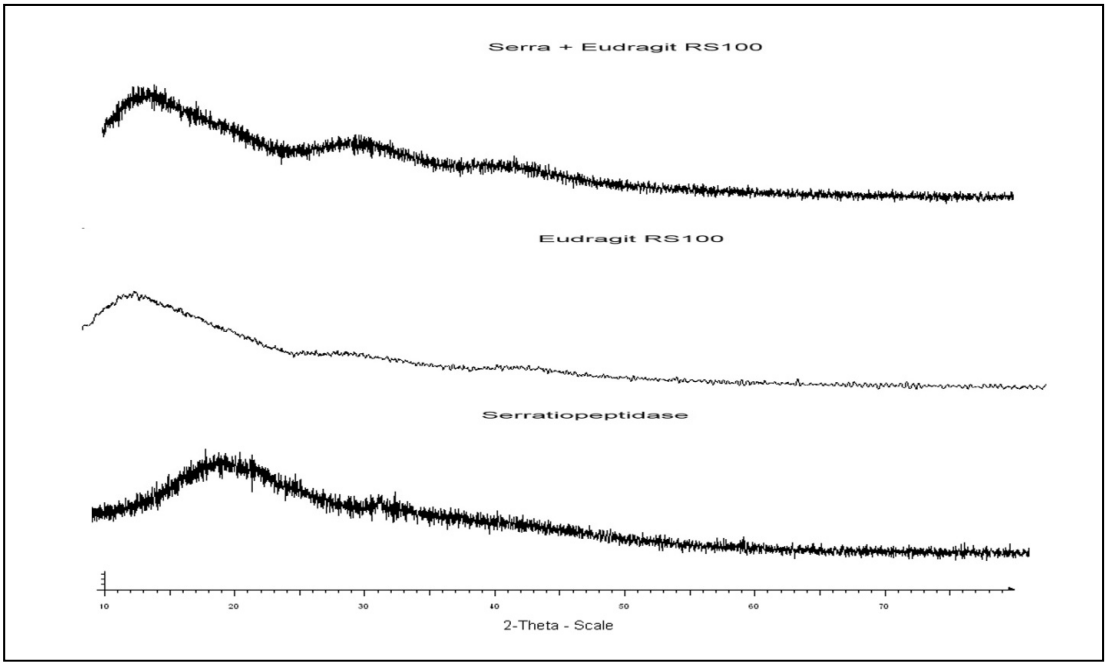

Fig 1: XRD spectrum of Serratiopeptidase, Drug loadedEudragit-100 microspheres

III. By FTIR

The characteristic peaks of aromatic NH2, aliphatic NH, aliphatic OH and aromatic c=c of pure drug were almost identical with those of, Eudragit RS-100 microspheres which indicated that absence of any polymer drug interaction. Typical FTIR patterns of Serratiopeptidase loadedEudragit RS-100, microspheres are shown in Figure 2.

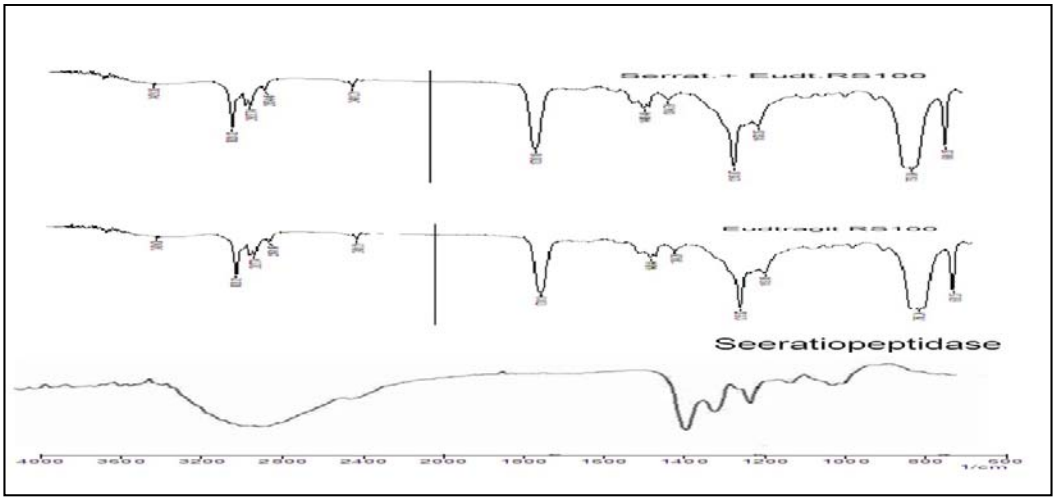

Fig 2: FTIR Spectrum of Serratiopeptidase, Eudragit RS-100 and drug loaded Eudragit RS-100 microspheres

IV. By DSC

The characteristic endothermic peak for Serratiopeptidase was obtained at 241.81 °C, which was also obtained inEudragit RS-100, microspheres with slight change.which showed, that drug is dispersed in microspheres. Typical. DSC patterns of Serratiopeptidase loaded Eudragit RS-100 microspheres are shown in Figure 3.

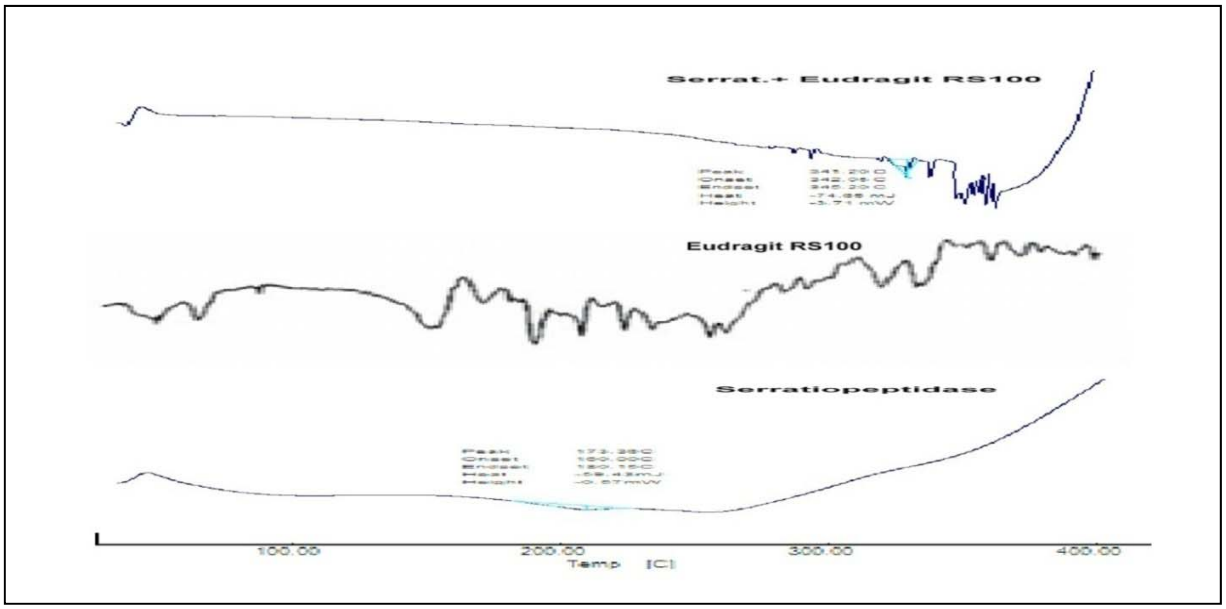

Fig 3: DSC curves of Serratiopeptidase, drug loaded Eudragit RS-100 microspheres

4. SEM

Prepared microspheres were spherical and completely covered with polymer coat. The surface of the drug –loaded microspheres had small pores on the their surfaces, which will be responsible for control drug release Typical sem photograph of Serratiopeptidase loaded Eudragit RS-100 microspheres are shown in Figure 4.

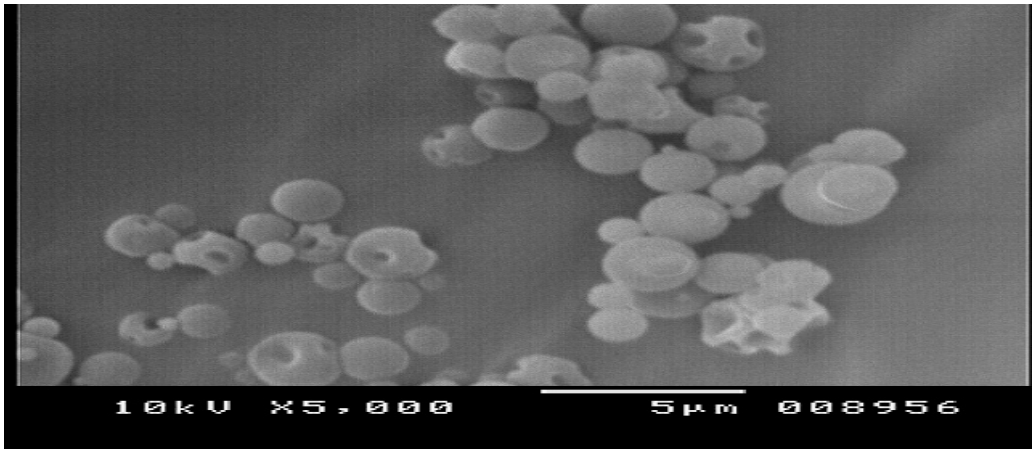

Fig 4: scanning electron microscopy of Serratiopeptidase, drug loaded Eudragit-100 microspheres at 2000 magnification

Table 4: Summary of results of A) model analysis B) lack of fit C) R-square analysis for measured responses

Source	(% Drug Content)			
	Sum of Squares	**Mean Square**	**F Value**	**P >F**
Model Significant	152.02	76.01	63.58	0.0001
A-A DCM	10.69	10.69	8.94	0.0243
B-B Tween 80	141.33	141.33	118.21	0.001
Residual	7.17	1.20		
Cor Total	159.20			

The Model F-value of 63.58 implies the model is significant. There is onlya 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms.Values greater than 0.1000 indicate the model terms are not significant.

Table 5: Summary of results of A) model analysis B) lack of fit C) R-square analysis for measured responses

Source	Particle Size			
	Sum of Squares	**Mean Squares**	**F Value**	**P value Prob> F**
Model Significant	8.58	1.72	14.65	0.0256
A-A DCM	0.089	0.089	0.76	0.4480
B-B Tween 80	2.55	2.55	21.75	0.0186
AB2 06	1	17.58	0.0247	
A^2	1	1.80	0.2719	
B^2	1	31.35	0.0113	
Residual	0.35	0.12		
Cor Total	8.93			

The Model F-value of 14.65 implies the model is significant. There is onlya 2.56% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, AB, B2 are significant model terms.Values greater than 0.1000 indicate the model terms are not significant.

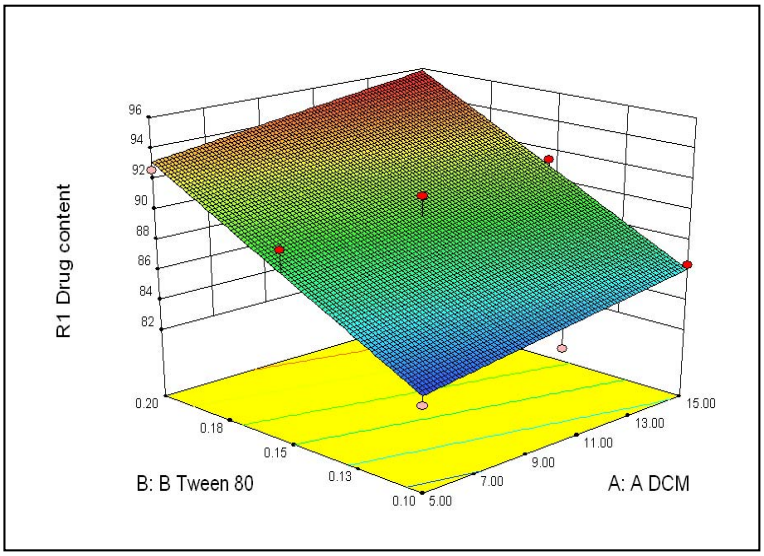

Fig 5: 3D surface curve for the effect of selected variables on the drug content of microspheres.

Y_1 (DC) = +89.64 + 1.34 X_1 + 4.85 X_2

DCM at higher level (X_1, 1) and Tween 80 concentration at higher level (X_2, 1) yielded microspheres with the highest drug content (95.42%) which may be due to additive effect of viscosity at high DCM level and better dispersion obtained at high level of Tween 80 stabilizer. When $X_2$was set at a high level (+1 and X_1 was set either at a medium (0) or high level (+1), less than 95.42 % of the drug was loaded in the microspheres.This might be due to drug leakage in the continuous aqueous phase as at a high or medium level of DCM, droplets may have remained in the liquid form for a relatively longer duration of time. At both a low and medium level

of (Tween 80) X_2 maximum loading was found at higher level. The viscosities of 0.1%, 0.15%, and 0.20% Tween 80 were found to be 0.30 mPa, 0.32 mPa and 0.35 mpa respectively (Brookfield viscometer). So, a possible reason for decreased drug loading at a low level is decreased viscosity; at a high level it might be due to the formation of sphere- shaped micelles at a higher concentration of Tween 80 than its Critical Micelle Concentration (CMC) whereby sphere- shaped micelles are further transformed into cylinder-shaped micelle structures.

Figure 4 and figure 5 represent the response surface plot, which shows the effects of the X_1 and X_2 on the size of microspheres and drug loading. As can be seen through the response surface graphs X_2 is the most significant factor effecting drug content. As expected from the estimated design the size of microspheres decreased as the DCM (X_1) increased. The negative coefficient of X_1 in the case of Y_1 response (Equation 1) refer to the decreased drug loading as the concentration of DCM was increased. Similarly, in Equation 2 for the size of microspheres depicts the negative coefficient for X_1 and X_2 indicating the negative effect of X_1 and X_2 on the size of microspheres

Contour plots show that various combinations of X_1 and X_2 may satisfy any specific requirement (in this case, maximum DCM and maximum drug loading) while taking into consideration other factors such as cost, stability, and so forth. The results from the estimated ridge of maximum response in terms of desirability revealed that optimum DMC (X_1) and Tween 80 concentration were 15 mL and 0.20% for desirable responses

Figure: 4 represent the response surface plot, which shows the effects of the X_1 and X_2 on the drug content of microspheres. As can be seen through the response surface graphs, X_1 is the most significant factor effecting drug content whereas X_2 affects size as well as drug content of microspheres significantly.

The positive coefficient of X_1 and positive coefficient of X_2 in case of Y_1response (equation) refers to the positive influence of drug amount and Tween 80 on the size of microspheres.

$$Y2\ (PS) = 31.48 + 0.12\ X1 + 0.65\ X2 + 0.72\ X1X2 - 0.33\ X12 + 1.36X22$$

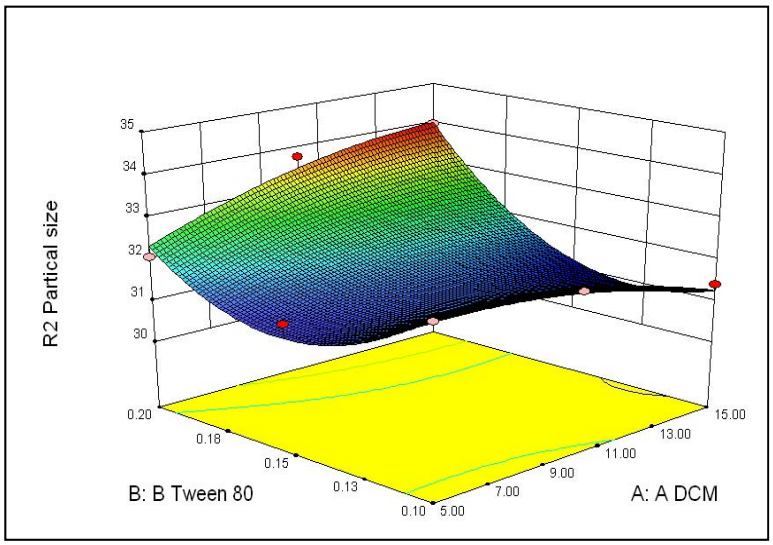

Fig 6: 3D surface curve for the effect of selected variables on the particle size of microspheres

5. Conclusion

Eudragit RS-100 polymer was found suitable as microencapsulating agent and the Eudragit RS-100 coated microspheres exhibited good controlled release characteristics and were found suitable for oral controlled release products. The solvent evaporation technique found to be an excellent approach in the design of controlled release microspheres of Serratiopeptidase.

The size of microspheres and the loading of protein in carrier was highly dependent on the solvent and stabilizer concentration for the preparation of Eudragit RS-100 microspheres DMC concentration had a positive effect on size and drug content whereas Tween 80 concentration had a positive effect on drug content and a negative effect on the size of microspheres. As compared with solvent concentration of stabilizer showed significant effect on both microspheres size and drug content.

6. Acknowledgement

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