Influence of Selected Formulation Variables on the Preparation of Enzyme-entrapped Eudragit S100 Microspheres

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ABSTRACT

The aim of this work is to study the influence of formulation parameters in the preparation of sustained release enzyme-loaded Eudragit S100 microspheres by emulsion solvent diffusion technique. A 3^2 full factorial experiment was designed to study the effects of the amount of solvent (dichloromethane) and stabilizers (Tween 20, 40, or 80) on the drug content and microsphere size. The results of analysis of variance test for both effects indicated that the test is significant. The effect of amount of stabilizer was found to be higher on both responses (SS_{Y1} = 45.60; SS_{Y2} = 737.93), whereas solvent concentration comparatively produced significant effect on the size of microspheres (SS_{Y1} = 0.81; SS_{Y2} = 358.83). Scanning electron microscopy of microspheres with maximum drug content (2.5 mL dichloromethane and 0.1 mL Tween 80) demonstrated smooth surface spherical particles with mean diameter of 56.83 ± 2.88 μm. The effect of formulation variables on the integrity of enzyme was confirmed by in vitro proteolytic activity. The enteric nature of microspheres was evaluated and results demonstrated ~6% to 7% release of enzyme in acidic medium. The release of enzyme from microspheres followed Higuchi kinetics. In phosphate buffer, microspheres showed an initial burst release of 20.34% ± 2.35% in 1 hour with additional 58.79% ± 4.32% release in the next 5 hours. Three dimensional response graphs were presented to visualize the effect of independent variables on the chosen response. Thus, Eudragit S100 microspheres can be successfully prepared for oral delivery of enzymes with desirable characters in terms of maximum loading and diffusion release pattern.

KEYWORDS: Enzyme delivery, factorial design, Tween 80, microspheres, 3D response graphs.

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.[1] Microparticulate systems can be made by various techniques involving physicochemical processes (solvent evaporation method, phase separation method) and mechanical processes (eg, spray drying).[2]

The solvent evaporation method is popularly used for microsphere preparation because of its simplicity, reproducibility, and fast processing with minimum controllable process variables that can be easily implemented at the industrial level.[3,4] But it is frequently used for water-insoluble drugs, as the entrapment efficiency of water-soluble drugs is low due to drug loss from the organic emulsified polymeric phase before solidification of polymer in the microspheres.[5,6] Therefore, process optimization may be advantageous for the efficient entrapment of water-soluble labile drugs like therapeutic enzymes. Moreover, a suitable emulsifier is frequently required to stabilize the emulsions during the micro-emulsification process.[7] In general, Polyvinyl alcohol (PVA) is one of the most commonly used polymeric surfactants[8]; however it is well known that it remains on the surface of particles and is difficult to remove completely.[9] PVA is also potentially carcinogenic[10] so we have tried the alternative nonionic surfactants, Tween 20, 40, and 80 as possible stabilizers for better protein encapsulation.

In the present investigation, Serratiopeptidase (STP, 52 kDa), and Eudragit S100 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 carpal tunnel syndrome.[11-13] These produce pharmacological effects by absorption through the intestines into the blood stream.[14] But, oral bioavailability of these peptide drugs is generally very low, owing to the acidic conditions of the stomach and poor permeability across intestinal mucosa.[15] Enteric coated preparations are available, but they release the drug with a burst,

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Table 1. Processing parameters used through the study

Eudragit S100	Drug	Stirring Speed	Continuous Phase	Dispersed Phase, mL (Me: Ac: DCM)	Stabilizer Concentration, mL
300 mg	100 mg	600 ± 10 rpm	100 mL aqueous medium	1. 3.5: 5.0: 2.5 2. 3.5: 5.0: 5.0 3. 3.5: 5.0: 7.5	0.05, 0.1, and 0.15

Me indicates methanol; Ac, acetone; DCM, dichloromethane.

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 S100 was selected because it has been reported to release the drug at pH > 7, suitable for oral delivery of acid-labile enzymes. It is an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester exhibiting pH-dependent solubility.[16]

Statistical models are extensively used in diversified areas to strengthen the art of drug formulation.[17,18] 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 (X_1) and Tween 80 (Stabilizer, X_2) 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 (Trial no. 4) was evaluated for proteolytic activity, enteric nature, and in vitro release studies.

Materials and Methods

Chemicals

Serratiopeptidase (molecular weight 52 kDa; Advanced Enzyme Technologies Ltd, Nasik, India); and Eudragit S100 (Rohm Pharma, Darmstadt, Germany); and Tween 20, 40, and 80 were purchased from Loba Chemicals (Mumbai, India) and Sdfine Chemicals Ltd (Mumbai, India_. All other chemicals used in the study were of analytical grade.

Preparation of STP-loaded Eudragit S100 Microspheres

Microspheres were prepared by a method based on the emulsion solvent diffusion technique reported by Kawashima et al[19] with few modifications. Methanol and dichloromethane were used as solvents for STP and Eudragit S100, respectively. Acetone, a water-miscible lipophilic solvent, was used to reduce interfacial tension at the dispersed droplet surface. Tween 20, 40, and 80 were tried as stabilizers in the preliminary trials. Eudragit S100 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 (Laboratory stirrer, 1NL-2116, Remi Motors Ltd, Mumbai, India). 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 10 000*g* for 10 minutes. The microspheres were resuspended in distilled water and lyophilized for 24 hours (Heto power dry LL 3000 Lyophilizer, Stuttgart, Germany). The final product was stored in a vacuum dessicator (Pharmatronic Instruments, Chennai, India) at 2 to 8ºC. 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. The trials were performed in random order.

Characterization of the Microspheres

Particle Size

Particle size analysis of drug-loaded Eudragit S100 microspheres was performed by optical microscopy using a compound microscope (Erma, Tokyo, Japan).[20] A small amount of dry microspheres was suspended in purified water (10 mL). The suspension was ultrasonicated for 5 seconds. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing Eudragit S100 microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The process was repeated for each batch prepared.

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

Serial No.	Variable X_1 DCM, mL	Variable X_2 Tween 80, mL
1.	2.5 (–1)*	0.05 (–1)
2.	5.0 (0)	0.05 (–1)
3.	7.5 (1)	0.05 (–1)
4.	2.5 (–1)	0.1 (0)
5.	5.0 (0)	0.1 (0)
6.	7.5 (1)	0.1 (0)
7.	2.5 (–1)	0.15 (1)
8.	5.0 (0)	0.15 (1)
9.	7.5 (1)	0.15 (1)

*Values in parentheses indicate coded levels of variables.

Table 3. Experimental and predicted responses obtained for the studied parameters

	Drug Content, %			Diameter of Microspheres, μm		
Trial No.	Experimental	Predicted	% Bias	Experimental	Predicted	% Bias
1.	1.05	1.19	11.76	70.87	70.60	0.38
2.	4.45	3.95	12.65	58.76	57.68	1.87
3.	2.23	2.59	13.89	49.42	50.77	2.65
4.*	9.05	8.34	8.51	56.83	58.10	2.18
5.	8.46	9.13	7.33	46.67	47.36	1.45
6.	7.56	7.61	0.65	44.59	42.63	4.59
7.	8.87	8.84	0.33	45.05	44.05	2.27
8.	8.42	8.86	4.96	35.12	35.50	1.07
9.	6.98	6.57	6.24	32.34	32.96	1.88

*Selected formulation.

Morphology

The surface morphology and shape of the microspheres were analyzed by scanning electron microscopy for selected batches (Leo, VP-435, Cambridge, UK). Photomicrographs were observed at ×200 magnification operated with an acceleration voltage of 15 kV and working distance of 19 mm was maintained. Microspheres were mounted on the standard specimen-mounting stubs and were coated with a thin layer (20 nm) of gold by a sputter-coater unit (VG Microtech, Uckfield, UK).

Drug Content

Twenty milligrams of the dried microspheres were accurately weighed. They were added to 5 mL of ethanol. After the microspheres dissolved completely, 5 mL of phosphate buffer (pH 7.4) was added to this solution and mixed thoroughly. The resulting solution was filtered using a Whattman filter (0.45-μm pore size) and analyzed for STP content by measuring absorbance in UV-spectrophotometer (Shimadzu UV-1700, Pharmaspec, Tokyo, Japan) at 229.5 nm by the first-derivative spectrophotometric method[21] using phosphate buffer (pH 7.4) and ethanol mixture (1:1) as blank. Results were expressed as mean (± SD) of 3 experiments. The measured responses are shown in Table 3.

Response Surface Analysis

The results are expressed as second order polynomial equation of the following form (Equation 1):

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{11} + b_{22}X_2^2, \quad (1)$$

where, b_i is the estimated coefficient for the factor X_i, while Y_i is the measured response. The coefficients corresponding linear effects (b_1 and b_2), interaction (b_{12}) and the quadratic effects (b_{11} and b_{12}) were determined from the results of the experiment (STAT-EASE, design expert, 7.0.3). To assess the reliability of the model, a comparison between the experimental and predicted values of the responses is also presented in terms of %Bias in Table 3.

Bias was calculated by equation 2:

$$\%\ Bias = \frac{Predicted\ Value - Experimental\ Value}{Predicted\ Value} \quad (2)$$

Enteric Nature of Microspheres

This test was performed to determine whether the drug would be released in the acidic environment of the stomach (ie, pH between 1 and 3). Twenty milligrams of microspheres were soaked in 10 mL of 0.1 N HCl that was equilibrated at 37 ± 0.5ºC in a water bath. After the immersion of the microspheres for 2 hours, the sample was centrifuged (3000 rpm, 15 minutes). The supernatant was subjected to UV-scan (Shimadzu UV-1700, Pharmaspec), which showed an absorption maxima at 229.5 nm by first-derivative spectroscopy corresponding to STP,[21] thus ensuring that the STP released had retained its native structure. Then the centrifuged sample was analyzed for STP content[21] against 0.1 N HCl blank.

In Vitro Proteolytic Activity

Prepared Eudragit S100 microspheres and plain STP solution were placed separately in 5 mL of HCl buffer (pH 1.2) or phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C and stirred constantly at 100 rpm. After 2 hours, samples were recovered by centrifugation at 3000 rpm for 15 minutes at room temperature (n = 3).

The proteolytic activity was determined by the method reported in Food Chemical Codex (2003).[22] The assay was based on a 30-minute proteolytic hydrolysis of casein at 37°C and pH 7.0. Unhydrolyzed casein was removed by

filtration and the solubilized casein was determined spectrophotometrically at wavelength of 275 nm. In this method, the protease activity is expressed as PC units of preparation derived from *Bacillus subtilis* var and *Bacillus licheniformis* var. One bacterial protease unit (PC) is defined as quantity of enzyme that produces a 1.5-μg/mL equivalent of L-tyrosine per minute under the condition of the assay.

Activity of enzyme was calculated by equation 3:

$$\frac{PC}{g} = \left(\frac{A_u}{A_s}\right)\left(\frac{0.75}{30w}\right) \qquad (3)$$

where A_u is the value obtained by subtracting blank reading from test reading, A_s is the absorption of the standard solution, 0.75 is the final volume in mL of reaction mixture, 30 is the time of the reaction in minutes, and w is the weight of the original sample in grams.

In Vitro Drug Release

In vitro release of STP from microspheres was evaluated in phosphate buffer (pH 7.4). Amount of microspheres equivalent to 20 mg of STP were transferred to the prewarmed dissolution media (20 mL) and maintained at 37 ± 0.5°C under stirring at 50 rpm. Samples were withdrawn every hour up to 6 hours and the volume was replaced immediately by fresh phosphate buffer. The sample withdrawn was centrifuged (3000 rpm, 15 minutes). The supernatant solution was filtered and analyzed for STP content by measuring absorbance in a UV-spectrophotometer (Shimadzu UV-1700, Pharmaspec) at 229.5 nm by the first-derivative spectrophotometric method[21] using phosphate buffer (pH 7.4) as blank. Results were expressed as mean (± SD) of 3 experiments.

Results and Discussion

Rapid diffusion of solvents into the continuous aqueous phase is responsible for complete precipitation of the polymer present in the microdroplets. The process of diffusion is dependent on the solubility of the organic solvent in the aqueous phase, as the more aqueous soluble the solvent, the lower will be the interfacial tension between the more hydrophilic organic solvent and outer aqueous phase leading to faster diffusion of solvent and rapid precipitation of the polymer.[23] The solubility of the dichloromethane is 2% wt/wt that is reported to be significant for rapid diffusion of solvent and better polymer precipitation.[24] A part of the DCM could also move with acetone and methanol into the outer aqueous phase, contributing to faster precipitation of polymer and greater drug loading. The rapid diffusion of acetone into the aqueous phase causes a remarkable decrease in interfacial tension between the organic and aqueous phase and hence finer microspheres are obtained.[5] The droplets may remain in the liquid state if the solvent has very low aqueous solubility and this condition results in greater leaching of water-soluble drug in the outer aqueous phase. Eudragit S100 dissolved completely at each of the selected levels of DCM.

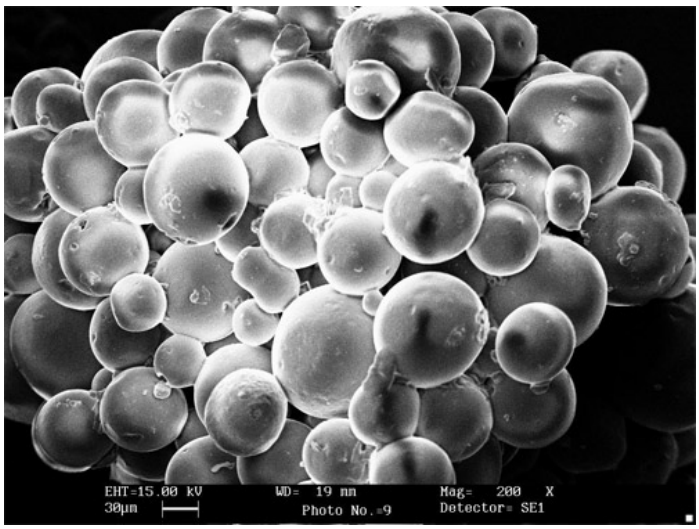

Figure 1. Scanning electron micrograph of microspheres of trial no. 4 with maximum drug loading (Tween 80, 0.10 mL; DCM, 2.5 mL).

At least one stabilizer is necessary for microsphere formation and suspension stabilization. Tween 20, Tween 40, and Tween 80 were used as surfactants to prepare blank microspheres; however, using Tween 20 and Tween 40, microsphere formation was not achieved successfully independent of their concentration. Microspheres with superior topographical characteristics were obtained when Tween 80 was used as the emulsifier (Figure 1). This might be due to better emulsification capability of Tween 80 as compared with Tween 20 or Tween 40.[25]

Serratiopeptidase is a neutral protease with isoelectric point of 6.1.[26] Drug loading has been reported to improve by adjusting the pH of the aqueous phase by suppression of ionization.[27] The solubility of STP and hence leaching into the outer aqueous phase increases as the pH of the solution increases above its isoelectric point. The pH of Tween 80 aqueous solution is in the range of 6.0 to 6.5 and hence did not warrant pH adjustment.[26] The limit for Tween 80 solutions was selected in the range of 0.05 mL and 0.15 mL through preliminary trials.

The drug content (Y_1) and the mean diameter of microspheres (Y_2) from the 9 experiments were used to generate predictor equations for Eudragit S100 microspheres with independent variables as dichloromethane (DCM) concentration and stabilizer (Tween 80) concentration. The results

Table 4. Regression analysis data for measured responses

	Drug Content, Y_1		Mean Diameter, Y_2	
Coefficients	Full Model	Reduced Model	Full Model	Reduced Model
b_0	9.13	6.34	47.36	48.85
b_1	–0.37	–0.37	–7.73	–7.73
b_2	2.76	2.76	–11.09	–11.09
b_{11}	–1.15	–	3.00	–
b_{22}	–3.02	–	–0.77	–
b_{12}	–0.77	–	2.19	–
R^2	0.9559	0.6364	0.9908	0.9574
Significance	0.030	–	0.003	–
F	13.02	5.25	64.93	67.46

of multiple regression analysis and analysis of variance test (ANOVA) are summarized in Table 4.

The percent drug content and mean diameter of STP microspheres showed R^2 values of 0.9559 and 0.9908 (Table 4), respectively; indicating good fit, and it was concluded that the second-order model adequately approximated the true surface. Furthermore, low value of %bias for all batches showed good agreement between predicted and experimental values as shown in Table 3.

The fitted model is shown in Equation 4 and Equation 5:

$$Y_1\,(\%\,Drug\ Content) = 9.13 - 0.37X_1 + 2.76X_2 - 0.77X_1X_2 - 1.15X_1^2 - 3.02X_2^2 \quad (4)$$

$$Y_2\,(\%\,Mean\ Diameter) = 47.36 - 7.73X_1 - 11.09X_2 + 2.19X_1X_2 + 3.00X_1^2 - 0.77X_2^2 \quad (5)$$

For the drug content and mean diameter of microspheres, the calculated F values of 13.02 and 64.93, respectively, were found to be greater than the critical (statistical table value) value of $F_{5,3,95\%}$ (9.01) and hence it may be concluded that 1 variable contributes significantly in the regression. The break up of source sum of squares (Source SS) in ANOVA indicated that the contribution of factor X_2 (Tween concentration) (SS_{Y1} = 45.60; SS_{Y2} = 737.93) is much higher than factor X_1 (DCM concentration)(SS_{Y1} =0.81; SS_{Y2} = 358.83) for optimizing the drug content as well as the mean diameter of microspheres. The interaction terms X_1X_2 and the polynomial terms X_1X_1 and X_2X_2 indicated insignificant values of individual source sum of squares. The reduced model shows an insignificant value of F for drug content, indicating a small role of interaction between selected variables on the response Y_1. But, in the case of mean diameter (Y_2), interaction terms are significant depicting the combined effect of DCM and Tween 80 concentration on the response Y_2. This argument is further investigated by testing the model in portions.[28] The calculated value of F for effect on drug content and mean diameter were found to be 7.25 and 3.64, respectively. The critical (statistical table value) value of $F_{3,3,95\%}$ is 9.28. Since the calculated value of F is lower than the critical table value, we conclude that the interaction does not significantly contribute for the prediction of drug content and mean diameter in the microspheres. Response surface plots also indicate (Figure 2) the negative effect of DCM concentration on mean diameter of microspheres. This effect is also supported by Cheu et al,[29] who reported that the increase in particle size of microspheres with decrease in solvent concentration (DCM) might be due to an increase in polymer concentration and viscosity. With the increase in the DCM concentration, the mean

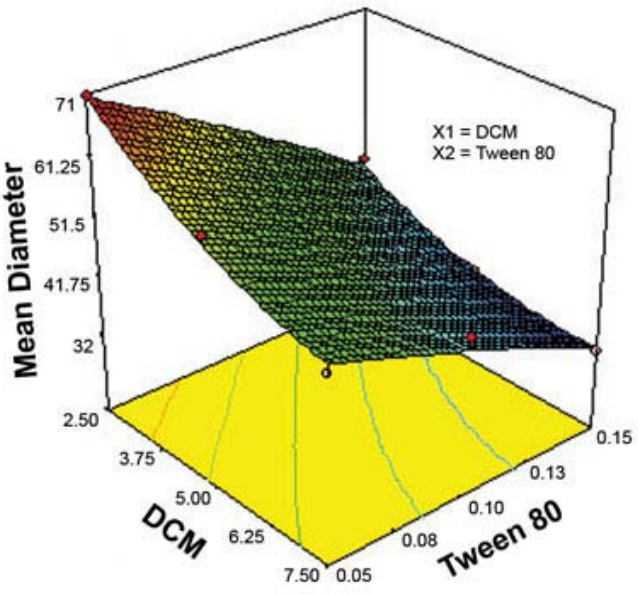

Figure 2. 3D surface curve for the effect of selected variables on the mean diameter of microspheres.

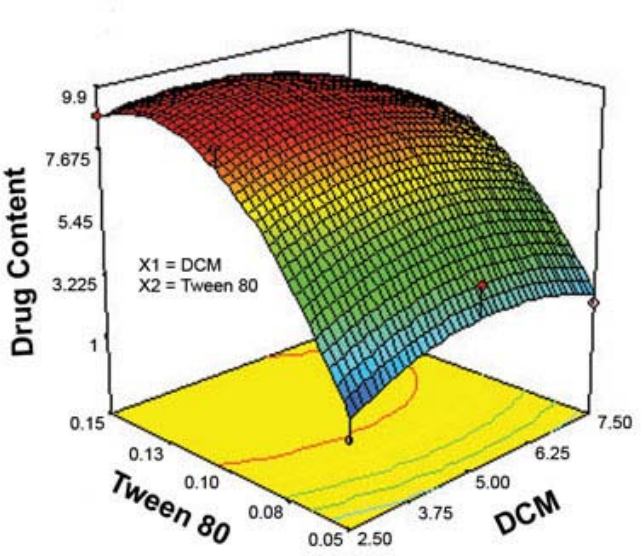

Figure 3. 3D surface curve for the effect of selected variables on the drug content of microspheres.

diameter of microspheres decreased significantly due to a decrease in the concentration of the polymer. Recently, Gao et al[30] also reported the increase in the size of microspheres prepared by emulsion solvent diffusion technique with the decreasing concentration of DCM. They reported that an increase in the size of microspheres with the decreasing amount of DCM might be due to a comparative increase in the amount of cosolvent (acetone). Acetone is a good solvent for both drug and polymer and can mix with the bridging liquid, ie, DCM, and the comparative increase in its concentration being responsible for conglutination of microspheres with no effect on drug loading. Our work is also in good agreement with the aforementioned research.

DCM at low level (X_1, –1) and Tween 80 concentraltion at medium level (X_2, 0) yielded microspheres with the highest drug content (9.05%), which may be due to additive effect of viscosity at low DCM level and better dispersion obtained at medium 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 9.05% 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 high level of (Tween 80) X_2, lower drug content was found and maximum loading was found at medium level. The viscosities of 0.05%, 0.10%, and 0.15% Tween 80 were found to be 1.28 mPa, 1.30 mPa and 1.32 mPa, respectively (Brookefield viscometer, LVDV-I+, Brookfield Engineering Laboratories, Inc., Middleboro, MA). So, a possible reason for decreased drug loading at a low level is decreased viscosity; at a higher 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, which was also supported by Zhang and Zhu.[31] As for Tween 80, its CMC is ~0.014 mol/L.[31]

Figure 2 and Figure 3 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 4) refers to the decreased drug loading as the concentration of DCM was increased. Similarly, in Equation 5, for the size of microspheres depicts the negative coefficients for X_1 and X_2 indicating the negative effect of X_1 and X_2 on the size of microspheres (Figure 2).

Contour plots show that various combinations of X_1 and X_2 may satisfy any specific requirement (in this case, minimum 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 DCM (X_1) and Tween 80 concentrations were 2.5 mL and 0.13% for desirable response (Figure 4).

The enteric nature of the coating for trial no. 4 was evaluated. Absolute enteric coating could not be achieved as 6.56% of the drug was released in 0.1 N HCl medium (pH

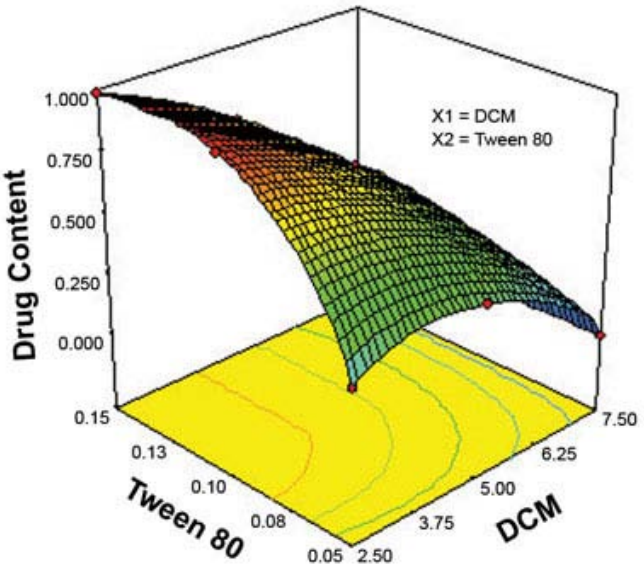

Figure 4. 3D surface curve for the desirable output in terms of maximum drug content and low level of DCM.

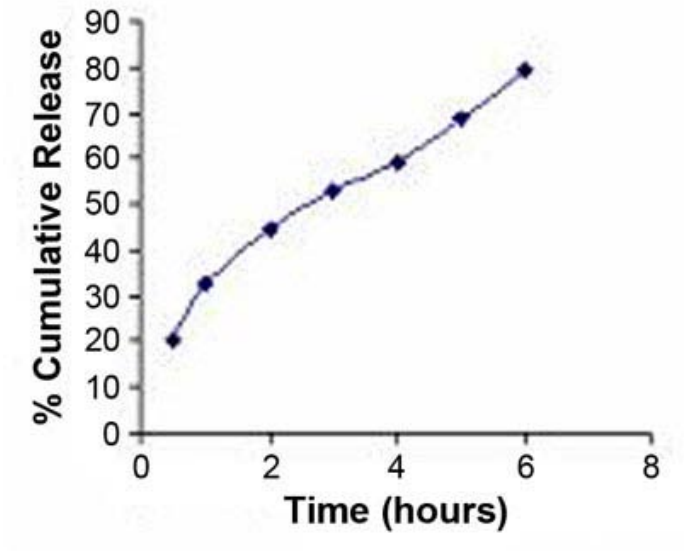

Figure 5. In vitro release profile of serratiopeptidase from Eudragit S100 microspheres of trial no. 4.

1.2) in 2 hours. This could be attributable to the adsorption of STP on the surface of microspheres. Another reason could be leaching of drug from the microspheres where inefficient coating could have occurred. The formulation could protect the acid-labile enzyme from gastric degradation and would release its drug load slowly at pH 7.4 in the ileum (last part of small intestine).[32]

The proteolytic activity of STP encapsulated in microspheres was evaluated separately before and after treating them for 2 hours in acidic (0.1 N HCl, pH 1.2) and basic media (phosphate buffer, pH 7.4). The Eudragit S100 microspheres showed ~2.20% ± 0.06% and 0.89% ± 0.32% loss of proteolytic activity in acidic medium and basic medium respectively. Meager loss of activity in alkaline media may be due to the processing steps involved in formulation preparation. At the same time, plain STP solution exhibited almost complete loss of activity in acidic medium and 86.84% ± 4.43% activity was retained in alkaline medium. But microspheres exhibited much better retention of proteolytic activity as compared with plain solution. A possible explanation for the improved physical and chemical stability of proteolytic enzymes in microspheres may be due to the effective protection of enzymes from the acidic environment.

It takes 9 to 12 hours for drug transit through the absorptive area of the gastrointestinal (GI) tract,[33] whereas γ scintigraphy studies confirm a short GI transit time from mouth to cecum of 4 to 6 hours.[34] Thus, assuming maximum GI tract transit time of 12 hours, a formulation in the small intestine is expected to release its drug load within 6 hours. Considering the same, in vitro drug release for trial no. 4 microspheres was studied for a duration of 6 hours. An initial burst of 20.34% ± 3.53% was observed in the first hour due to the drug located on or near the surface of the microspheres. Microspheres are matrix systems in which the drug molecules are dispersed throughout the particles. The rate of release of drug dispersed in an inert matrix has been described by Higuchi, where a plot of amount of drug released versus square root of time should be linear if the release of the drug from the matrix is diffusion controlled.[35] The release mechanism of drug from microspheres (Trial no. 4) was evaluated.

The (%) cumulative release of STP from microspheres increased up to 6 hours (Figure 4). Thus, the amount of drug remaining in the microspheres would decrease over the same period. Accordingly, a plot of amount of drug remaining versus the square root of time (ie, 100 − Amount released versus $\sqrt{t}$) for the formulations showed a linear decline, indicating a diffusion-controlled release, following Higuchi kinetics (Figure 5). These results are consistent with those obtained by Jain et al,[16] who studied the release of insulin, a potent peptide hormone, from microspheres prepared using Eudragit S100 as the microsphere matrix. The release of drug from these microspheres followed the Higuchi matrix model (Figure 6).

Further parameters can be identified by a systemic approach for optimum formulation in terms of better long-term stability that is the key need toward highly fragile moieties like proteins and peptides. This could also be used for oral delivery of other classes of proteolytic enzymes with prophylactic and therapeutic significance.

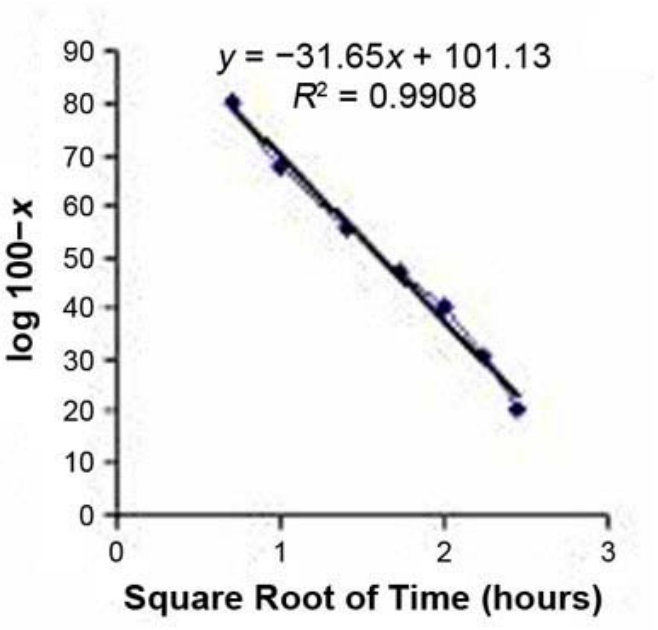

Figure 6. Higuchi plot of enzyme release from Eudragit S100 microspheres of trial no. 4.

CONCLUSION

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 S100 microspheres. DCM concentration had a negative effect on size and drug loading, 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, concentration of stabilizer had a significant effect on both microsphere size and drug content.

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