Original Article

Formulation and optimization of sustained release terbutaline sulfate microspheres using response surface methodology

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ABSTRACT: The present study reports the optimization of sustained release microspheres of terbutaline sulfate (TS) and Eudragit RSPM using response surface methodology. The microspheres were prepared by the emulsion solvent evaporation process utilizing Eudragit RSPM as release retarding agent. A 3^2 full factorial design was utilized by taking the drug: Eudragit RSPM ratio (X_1), the percent of Span 80 (X_2) and the speed of rotation (X_3) as the independent variables; particle size (Y_1) and percent drug released (Y_2) were the dependent variables. The resultant microspheres were subjected to various physico-chemical analysis, *viz*., drug content, micrometrics, photo-microscopy and *in vitro* drug release. The percent of drug release at 8 h of dissolution decreased from 90.7% to 61.3% with increase in polymer concentration from 4 to 8%. It was observed that an increase in surfactant concentration from 1 to 2% and speed of rotation from 500 to 900 rpm decreased the size of microspheres (350-330 µm). The results of the present study indicate that optimized sustained release microspheres of terbutaline sulfate could be successfully prepared by the emulsion solvent evaporation method by emulsifying the drug and polymer in the ratio of 1:8, at a speed of 500 rpm, utilizing 1.5% of span 80 as emulsifying agent.

Keywords: Terbutaline sulfate, Eudragit RSPM, sustained release microspheres, response surface methodology, Box-Behnken design

1. Introduction

Much of the research effort in developing novel drug delivery systems has been focused on oral sustained release dosage forms. Among them, in the last decade, multiple unit dosage forms, such as micro particles have gained in popularity for different reasons when compared to non-disintegrating single-unit dosage forms (*1*). They distribute more uniformly in the gastrointestinal tract, resulting in more uniform drug absorption and reduced local irritation, and also avoid the unwanted intestinal retention of the polymeric material. It is also desirable to release drugs at a constant rate, thereby maintaining drug concentration within the therapeutic range and eliminating the need for frequent dosages. The rate of drug release from solid dosage forms may be modified by the technologies which, in general, are based on modifying drug dissolution by controlling access of biologic fluids to the drug through the use of barrier coatings, controlling drug diffusion rates from dosage forms and chemical interactions between the drug substance or its pharmaceutical barrier and site-specific biologic fluids (*2*).

One of the most effective techniques for preparing sustained release particles is by microencapsulation (*3-6*). This method has been employed in pharmaceutical practice for a variety of purposes. It is useful for reducing toxicity and adverse effects, separating reactive or incompatible components, controlling the rate and site of release of a drug and providing greater patient convenience and compliance. Microcapsules are small particles that contain an active agent or core material surrounded by a coating or shell. At present, there is no universally accepted size range that particles must have in order to be classified as microcapsules. However, many workers classify capsules smaller than 1 µm as nanocapsules and capsules larger than 1,000 µm as macrocapsules. Commercial microcapsules typically have a diameter between 3 and 800 µm and contain 10-90 weight percent cores. Microcapsules can have a variety of structures. Some have a spherical geometry with a continuous core region surrounded by a continuous shell; others have an irregular geometry and contain a number of small droplets or particles of core material (*7*). The micro encapsulation process in which the removal of the hydrophobic polymer solvent is achieved by evaporation has been widely reported in recent years for the preparation of microcapsules

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(*8-10*). This process is known as the emulsion solvent (ESE) technique and has been used successfully in the preparation of drug microspheres or capsules using different biocompatible polymers (*11-15*). Many formulation factors can influence the preparation of microcapsules by the ESE process (*16-20*).

In the present study, terbutaline sulfate (TS), a selective beta 2-adrenergic agonist was chosen as the model drug, as it has a short biological half-life (3-4 h). It is widely used as a bronchodilator for the treatment of bronchial asthma, chronic bronchitis and emphysema (*21-24*). Currently available treatments for asthma and bronchitis, although generally effective, are limited by the necessity for frequent drug administration and/or the possibility of unpleasant or debilitating side effects. Thus, a long acting TS formulation which would maximize the duration of active drug concentration in extra-cellular fluid is desirable to improve patient compliance. There are several studies in the literature regarding prolongation of its release using various polymers. Prolongation of TS release from all these formulations has been demonstrated by means of *in vitro* dissolution studies (*25-28*).

Modern sustained-release dosage forms require reliable excipients to ensure a release rate of the active drug which is reproducible in a narrow range. Eudragit polymers fulfill this requirements to a very high extent and enable research and development to create tailor-made solutions. In the present study, Eudragit RSPM (hydrophobic polymer) was used as the microcapsule wall-former because of its wide use as a coating material in the pharmaceutical industry. It is a copolymer with a low content of quaternary ammonium groups. Since, Eudragit RSPM film is only slightly permeable, drug release through the film is relatively retarded. Several sustained-release formulations using Eudragit RSPM, such as coated tablets and matrix type tablets, have been reported (*29-32*).

Response surface methodology, an empirical modeling technique devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results was employed in the present research work (*33*). It requires prior knowledge of the process to achieve a statistical model. Basically this optimization process involves three major steps, performing the statistically designed experiments, estimating the coefficients in a mathematical model, predicting the response and checking the adequacy of the model. This design is suitable for exploration of a quadratic response surface and constructs a second order polynomial model, thus helping in optimizing a process using a small number of experimental runs. Various formulation studies have been optimized and reported using this design. In all these studies, the observed responses were in close agreement with the predicted values of the optimized formulation (*34-38*).

There are comparatively few reports on sustained release TS microcapsules, none of which have been optimized by the factorial design approach (*25-28*). The present study was thus designed to formulate TS containing microspheres using the ESE technique by applying Box-Behnken design, multiple regression analysis and response surface modeling. The objective was to investigate the influence of independent variables on the particle size distribution and drug release properties of the TS microcapsules.

2. Materials and Methods

2.1. *Materials*

Terbutaline sulfate (TS) was a generous gift from Chemical Industrial Development Co., CID (Giza, Egypt). Eudragit RSPM was kindly supplied by Rohm Pharma, and GMBH (Weitestadt, Germany) was a gift sample. Aluminum tristearate was received from Morgan Co. (Cairo, Egypt). Methylene chloride, ethanol absolute (99%) and cyclohexane were purchased from Prolabo (Briare, France). *n*-Hexane was purchased from Honil Limited (London, UK). Chloroform was bought from Labscan Ltd. (Dublin, Ireland). Light liquid paraffin was purchased from Chemaject (Egypt). Span 80 was purchased from Sigma Chemical Co. (Steinheim, Germany). Hydrochloric acid was purchased from Carloerba (Milano, Italy). Potassium chloride and monobasic potassium hydrogen phosphate were purchased from Merck (Dermstadt, Germany). All of the above materials were of analytical grade and were used without further purification. De-ionized double distilled water was used throughout the study.

2.2. *Preparation of TS microcapsules*

Microspheres were prepared by the ESE technique. Polymer solution of Eudragit RSPM was prepared in methylene dichloride. TS was dispersed in this polymeric solution to form the internal phase. Different drug-polymer ratios, *viz.*, 1:4, 1:6, and 1:8 were used to prepare the microcapsules. Known amounts of Aluminum tristearate were dispersed in the different internal phases as smoothing agent. This dispersion was added drop wise to liquid paraffin (external phase) containing several different concentrations of Span 80 as emulsifying agent. Emulsification was achieved by stirring at various rotation speeds. Stirring was continued at room temperature until complete evaporation of the solvent (methylene chloride), for approximately 2 h. Liquid paraffin was decanted and the microspheres produced were collected by filtration through Whatman No.1 filter paper. The filtrate was washed three times with *n*-hexane and three times with cyclohexane to remove the remaining oily phase and then dried overnight at room temperature.

2.3. *Optimization of TS microspheres using response surface methodology*

In the present study, a three-factors, three levels Box-Behnken design with speed (X_1), drug-polymer ratio (X_2) and concentration of span 80 (X_3) as independent variables were selected for the formulation. Three levels of speed used were 500, 700, and 900 rpm which equals to –1, 0, and +1 values for the above design. Drug-polymer ratios of 1:4, 1:6, and 1:8 reflect the –1, 0, and +1 values. While span 80 concentrations of 1, 1.5, and 2% were equal to the –1, 0, and +1 values. The various levels used are shown in Table 1. This design is suitable for exploration of quadratic response surface and constructs a second order polynomial model, thus helping in optimizing a process using a small number of experimental runs. The model constructed was as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1X_2 + a_5X_2X_3 + a_6X_1X_3 + a_7X^2_1 + a_8X^2_2 + a_9X^2_3 + E$$

Where a_0 to a_9 are the regression coefficient, X_1, X_2, and X_3 are the factors studied, Y is the measured response associated with each factor level combination and E is the error term.

2.4. *Drug content determination of TS microcapsules*

The drug content of TS microspheres was determined by an extraction method reported by Kim *et al*. (*25*). Microspheres (25 mg) were added to chloroform (20 mL) to dissolve the polymer matrix. Terbutaline sulfate was then extracted with distilled water (100 mL). The amount of terbutaline sulfate in the aqueous phase was analyzed by UV spectrophotometry (Jenway 6305 UV/Vis Spectrophotometer, Essex, UK) at 278 nm after suitable dilution.

2.5. *Evaluation of micromeritic characteristics of TS microcapsules*

The prepared TS microspheres were evaluated for the following parameters:

2.5.1. *Particle size distribution*

According to the sieve analysis method stated by U.S.P. XXIV for testing powder fineness, a definite weight of TS microspheres was placed on the mechanical sieve shaker and analyzed for particle size distribution (USP, 2002). The powder was shaken for a defined period of time (15 min) using a range of standard sieves with openings from 100 to 900 µm. The material that passes through one sieve and was retained on the next fine sieve was collected and weighed. The obtained batches were separated into different fractions based on their particle size (900, 715, 565, 407.5, 282.5, 225, and 100 µm). The logarithm of the particle size was plotted against the cumulative percent frequency on a probability scale and a linear relationship was observed. From this linear plot, both the geometric mean diameter (dg) and geometric standard deviation were measured for the TS microspheres equivalent to 50% on the probability scale. The geometric standard deviation was calculated from the slope of the line and the geometric standard deviation which was the quotient of the ratio of 50% size and 16% undersize.

Table 1. Box-Behnken design

Formula No.	Independent variables*		
	Speed (X_1)	Drug: polymer (X_2)	Span 80 (X_3)
T1	–1	–1	0
T2	0	–1	–1
T3	0	–1	+1
T4	+1	–1	0
T5	–1	0	–1
T6	–1	0	+1
T7	0	0	0
T8	0	0	0
T9	0	0	0
T10	+1	0	–1
T11	+1	0	+1
T12	–1	+1	0
T13	0	+1	–1
T14	0	+1	+1
T15	+1	+1	0
Coded values	Actual values*		
	X_1 (rpm)	X_2 (ratio)	X_3 (%)
–1	500	1:4	1
0	700	1:6	1.5
+1	900	1:8	2

2.5.2. *Density*

For determination of bulk density of the microcapsules, a sample of 50 g was poured into a 100 mL graduated cylinder (*38*). The cylinder was then dropped from a height of 1 inch onto a hard surface three times at 2 sec intervals. The volume of the powder was then read and used to calculate the bulk density by dividing the weight in (g) by the volume in (cm^3). For determination of tap density of the microcapsules, tapping onto a hard surface was carried out until a constant volume was achieved. This volume was taken and used to calculate the tap density of the microcapsules.

2.5.3. *Hausner ratio*

Hausner ratio is the ratio between bulk density and tap density. It gives an idea about the flow characteristics of powder particles. The powder has good flow when the ratio is less than 1.2, while if the ratio is more than 1.2 this indicates poor flow.

2.5.4. *Compressibility percent*

Compressibility is indirectly related to the relative flow rate, cohesiveness, and particle size of a powder. A compressible material has generally less flow, and powders with compressibility values greater than 20-21% have been found to exhibit poor flow properties (*39*). The compressibility of a material can be estimated as follows:

$$\text{Compressibility \%} = (\rho_{tap} - \rho_{bulk} / \rho_{tap}) \times 100$$

2.5.5. *Photomicroscopic observation of TS microspheres*

Photomicroscopy has the advantage of providing a direct visual representation of the particles being measured. A photomicroscope can provide details about shape, crystal habit, and homogeneity of the tested sample. In the present study a diluted suspension of TS microspheres in liquid paraffin was mounted on a slide, and then a photograph for each microsphere was taken from the prepared slide.

2.6. *Preparation and assay of TS capsules*

Capsules were prepared by putting the equivalent of 7.5 mg of TS in the microsphere form into hard gelatin capsule of zero size. The procedure was done for each suggested formula based on their drug content. For assay, each TS capsule was emptied and its microsphere content was dissolved in methylene chloride and the drug was determined spectrophotometrically at 278 nm. The assay was done in triplicate.

2.7. *In vitro dissolution and kinetic studies of TS capsules*

In vitro drug release was studied according to the USP XXIV basket method using Dissolution Apparatus Type I (Pharma Test PTWII, Hamburg, Germany). The dissolution medium employed was 900 mL of HCl (pH 1.2) for 2 h which was then changed to 900 mL phosphate buffer (pH 6.8) at 37 ± 0.5ºC. The basket speed was 50 rpm. At appropriate time intervals, 5 mL of each sample was taken and replaced by fresh dissolution media. The samples were analyzed at 278 nm by UV/VIS spectrophotometry.

3. Results and Discussion

3.1. *Optimization results for preparation of microspheres*

Box-Behnken design was used for formulating TS microspheres. This design deals with optimization of formulation variables to improve *in-vitro* release of TS capsules. A three-factors, three levels Box-Behnken design with speed (X_1), drug-polymer ratio (X_2) and percent of span 80 (X_3) as independent variables were selected for the formulation.

TS microspheres were prepared by the ESE technique. This method is generally known to be simple, reproducible and economical. Eudragit RSPM was used to control the release of TS from the microcapsules. The drug polymer ratios employed were 1:4, 1:6, and 1:8. Microspheres were formed in the presence of a small amount of aluminum tristearate. Flocculation was clearly recognized when no aluminum tristearate was added to the system. Specifically, with 5% aluminum tristearate, the microspheres were nearly uniform and free flowing with good reproducibility. Aluminum tristearate reduces the interfacial tension and prevents electrification and flocculation during the preparation of microspheres. Addition of excess aluminum tristearate (10-20%) to the system resulted in a large amount of aggregates. The action of aluminum tristearate as smoothing agent and liquid paraffin as external phase were used as part of the ESE technique while the surfactant (Span 80) was used as emulsifying agent.

3.2. *Drug content determination*

The production yield is a measure of accuracy of the microencapsulation technique, since it measures the actual weight of the prepared microspheres (drug and other excipients). This value was calculated by dividing the actual weight of the prepared TS microspheres by the theoretical weight. While the drug content determination measures the actual weight of TS itself inside the microspheres. The range of the production yield of the prepared microspheres was found to be between 46.7% and 98.7% as shown in Table 2. The greatest yield appeared in formula T_{14} (98.7%) while

the lowest yield appeared in formula T_2 (46.7%). Formulae T_3, T_4, T_6, T_7, T_8, T_9, T_{10}, T_{11}, and T_{12} showed a production yield above 85%. Formulae T_1 and T_5 had a production yield of 78.7 and 78.2%, respectively. The rank order of the drug content was measured by the deviation from the theoretical weight. Formula T_{15} gave the best drug content of the prepared TS microspheres (140.1%), while formula T_2 showed the lowest value (51.8%).

3.3. *Micromeritic properties*

3.3.1. *Particle size distribution*

The particle size distribution of different formulae of TS microspheres was determined by the sieve analysis method. Formulae T_3, T_4, T_7, T_8, and T_9 exhibited the best distribution pattern as the largest weight determined lied between 500-315 µm. While, formulae T_7, T_{10}, and T_{11} gave the second best group distribution as largest weight calculated lied between 800-500 µm. The remaining formulae exhibited either low or high distribution as the largest weight determined lied either below 200 µm or above 800 µm. The sieve analysis data of each formula was used to determine the average arithmetic mean diameter. The results obtained were in agreement with the weight distribution. The arithmetic mean diameters of low distribution gave values between 250-330 µm. while that of high distribution gave values between 500-610 µm. At the same time, the arithmetic mean diameter of the good distribution formulae lied between 330-500 µm. Probability scales were used to calculate the geometric mean diameter and geometric standard deviation. These values were obtained by plotting the particle size in micrometers on the x-axis versus the cumulative percent frequency under size (probability scale) on the y-axis. The geometric mean diameter for each formula was determined at the 50% size while the geometric standard deviation was calculated by dividing the 50% size / 16% undersize.

3.3.2. *Density, Hausner ratio, and compressibility index*

The bulk densities of TS microspheres ranged from 0.190 g/cm^3 to 0.467 g/cm^3. While, the tap densities ranged from 0.333 g/cm^3 to 0.666 g/cm^3. Formula F_{15} gave the lowest values for both bulk and tap densities. While, formulae T_5 and T_{12} gave the highest values. The values of Hausner ratio below 1.2 indicated good flow while the values above 1.2 indicated poor flow properties. Formulae T_1, T_5, T_6, T_9, and T_{10} showed good flow while the remaining formulae exhibited a values higher than 1.2. The values of percent compressibility below 20-21% exhibit good flow while the values greater than 21% indicate poor flow. Formulae T_1, T_5, T_6, T_9, and T_{10} showed good flow while the remaining formulae exhibited values higher than 21%. It is obvious from the data in Table 3 that there was an inverse proportionality between the particle size and the particle number for TS microcapsules studied. The best formulae in terms of micromeritic properties were found to be T_2, T_1, T_{15}, T_{13}, and T_{12}.

3.3.3. *Photo-microscopic determination of TS microcapsules*

Photo-microscopic technique was used to get a clear view of the surface morphology of the prepared TS microspheres. Also, this technique reflects the efficiency of the ESE process. It was found that the majority of TS microcapsules were irregular in shape except formulae T_3 and T_9 in which the shape of the particles were semi-spherical, as observed in Figure 1.

3.4. *In vitro release of TS capsules*

In vitro release studies of TS capsules containing different drug-polymer ratios were evaluated by

Table 2. Production yield and percentage recovery of TS microcapsules

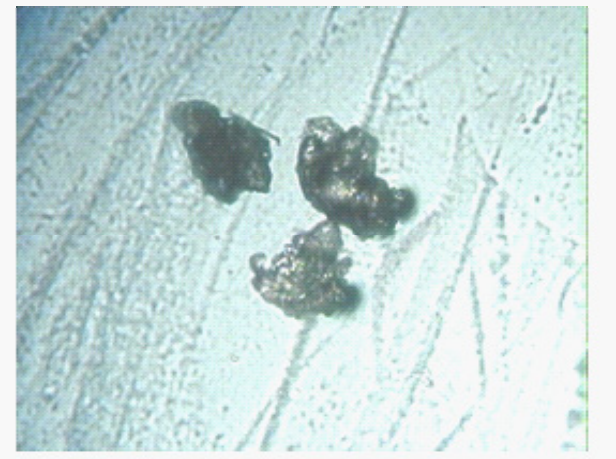
Formula No.	Core/coat ratio	Production yield (%)	Theoretical drug content (mg)	Actual drug content (mg)	Drug content (%)
T_1	1:4	78.7	20.00	26.76	133.80
T_2	1:4	46.7	20.00	10.36	51.80
T_3	1:4	91.2	20.00	13.76	68.80
T_4	1:4	87.6	20.00	15.84	79.20
T_5	1:6	78.2	14.26	9.42	66.05
T_6	1:6	91.2	14.26	11.41	80.01
T_7	1:6	92.2	14.26	13.53	94.88
T_8	1:6	88.2	14.26	8.89	62.34
T_9	1:6	90.3	14.26	13.44	94.24
T_{10}	1:6	86.3	14.26	10.25	71.87
T_{11}	1:6	96.3	14.26	9.36	56.63
T_{12}	1:8	95.7	11.11	11.33	101.90
T_{13}	1:8	80.3	11.11	13.07	117.60
T_{14}	1:8	98.7	11.11	8.83	79.47
T_{15}	1:8	83.3	11.11	15.56	140.10

Table 3. Determination of density, Hausner ratio, % compressibility and specific surfaces of TS microcapsules

Formula No.	Density (g/cc)		Hausner ratio	Compressibility %	Specific surfaces	
	Bulk	Tap			S_W†	S_V††
T_1	0.285	0.333	1.16	14.4	775	221
T_2	0.400	0.533	1.33	24.9	458	183
T_3	0.363	0.500	1.37	27.4	343	124
T_4	0.200	0.307	1.54	34.8	907	181
T_5	0.467	0.540	1.15	13.5	210	98
T_6	0.414	0.462	1.11	10.3	218	90
T_7	0.300	0.480	1.60	37.5	421	126
T_8	0.375	0.502	1.33	25.2	366	137
T_9	0.400	0.470	1.12	14.8	369	148
T_{10}	0.446	0.480	1.07	07.1	244	109
T_{11}	0.285	0.400	1.40	28.8	510	145
T_{12}	0.421	0.666	1.58	36.8	339	143
T_{13}	0.300	0.429	1.43	30.1	666	200
T_{14}	0.401	0.546	1.36	26.5	288	115
T_{15}	0.190	0.333	1.75	42.9	1162	221

† Surface area per unit weight; †† Surface area per unit volume.

A: TS microcapsule (T_3)



B: TS microcapsule (T_0)

Figure 1. Photomicroscope images of TS microspheres.

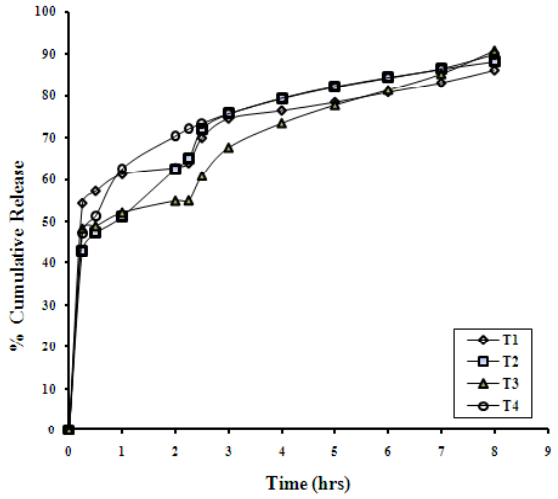


Figure 2. *In vitro* release of TS capsules containing drug: polymer ratio 1:4.

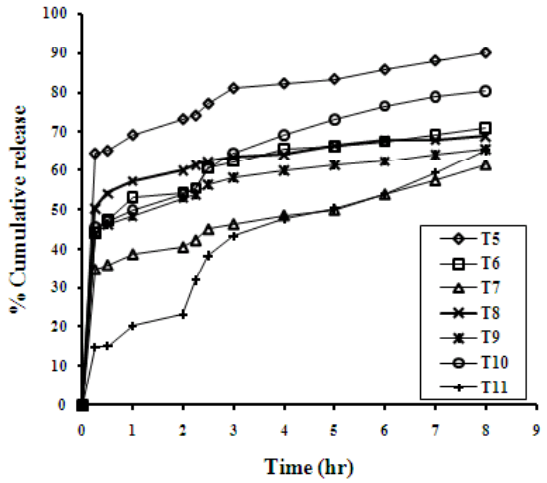


Figure 3. *In vitro* release of TS capsules containing drug: polymer ratio 1:6.

measuring the cumulative percent release. Figures 2-4 show the *in vitro* release of TS from capsules containing the drug and Eudragit in the ratios of 1:4, 1:6, and 1:8, respectively. The best formulae for *in vitro* release after a period of 8 h were observed to be T_{11}, T_7, T_{12}, T_9, and T_{14}. The investigated formulae containing different drug-polymer ratios (1:4, 1:6, and 1:8) were arranged, in ascending order, in terms of micromeritic properties and i*n vitro* release. The best formulae in terms of both *in vitro* release and micromeritic properties were found to be T_{11}, T_{12}, T_9, T_2, and T_7.

3.5. *Kinetic study of the in vitro release of TS capsules*

The data of the *in-vitro* release from TS capsules were treated by different kinetic orders or systems to explain the release mechanism for each formula. The formulations were subjected to zero, first and second-order kinetic equations, as well as, to Higuchi's diffusion model, Hixson-Crowell cube root law and Baker-Lonsdale equation. Table 4 shows the kinetic parameters for each formula according to the suitable order or system.

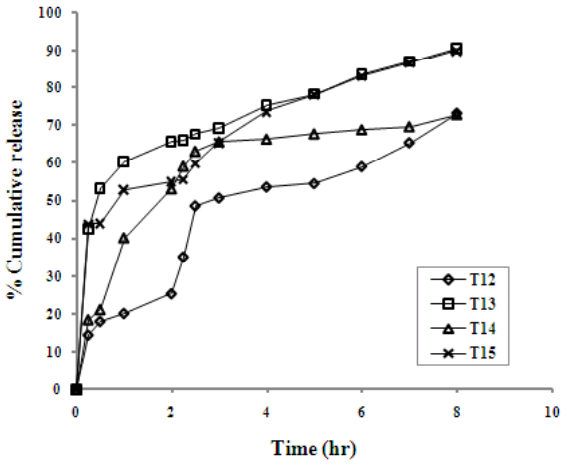


Figure 4. *In vitro* release of TS capsules containing drug: polymer ratio 1:8.

3.6. *Data correlation with in vitro release and particle size*

It was found that the particle size of the prepared microcapsules was an important factor affecting the *in vitro* release of the drug. Also the technique parameters had a great effect on *in vitro* release. So, the three levels of the Box-Behnken design studied were correlated with both *in vitro* release from TS capsules after 8 h on one side and particle size of TS microcapsules in the range 500-315 µm on the other side. These relationships are illustrated in Figure 5. It shows the factor range of the three levels (–1, 0, and +1) which indicate the minimum and maximum level of each item used. For example, –1 speed equals up to 500 rpm. Zero equals to 700 rpm and +1 equals to 900 rpm. It was found that increasing the speed of the apparatus would decrease and then increase the *in vitro* release after 8 h. The best speed for preparation of TS microspheres may be either 500 rpm or 900 rpm. The same effect was observed using the second level *i.e.* the drug-polymer ratio) but the increase of the *in vitro* release in the 1:4 ratio was found to be less than the 1:8 drug-polymer ratio. The best drug-polymer ratio was found to be 1:8, while, the effect of Span 80% was decreased gradually from l% to 2%. The best percentage of Span 80 was found to be the lowest concentration, *i.e.* 1%.

Based on these figures it could be interpreted that the optimum *in vitro* release of TS after 8 h was obtained at a speed of 500 or 900 rpm using a 1:8 drug-polymer ratio and 1% of Span 80. On correlating the factor range versus the obtained particle size percent in the range from 500 µm to 315 µm of the prepared TS microspheres as seen in Figure 5B, the particle size percent studied would increase and then decrease by increasing the speed, drug-polymer ratio and the Span 80%.

Table 4. Kinetic parameters for *in vitro* release of TS capsules

Formula No.	Intercept	Slope	Correlation coefficient	Specific Rate constant (h^{-1})	$t_{1/2}$ (h)	Order of reaction
T_1	1.662	0.065	0.985	0.150	4.608	First
T_4	1.673	0.083	0.983	0.192	3.592	First
T_2	0.012	0.008	0.995	0.008	1.132	Second
T_{10}	0.014	0.004	0.991	0.004	2.237	Second
T_{14}	0.015	0.002	0.924	0.002	3.403	Second
T_5	58.37	11.33	0.989	11.33	19.46	Diffusion
T_6	39.83	11.52	0.979	11.52	18.82	Diffusion
T_8	48.88	7.668	0.979	7.668	42.50	Diffusion
T_9	39.85	9.488	0.990	9.788	27.76	Diffusion
T_3	0.768	0.209	0.989	0.209	4.561	H-C†
T_7	0.606	0.077	0.993	0.077	12.22	H-C
T_{15}	0.713	0.223	0.995	0.223	4.271	H-C
T_{11}	0.004	0.012	0.985	0.012	4.294	B&L*
T_{12}	0.005	0.016	0.969	0.016	3.248	B&L
T_{13}	0.044	0.028	0.993	0.028	1.919	B&L

† Hixson Crowell cube root law; * Baker-Lonsdal equation

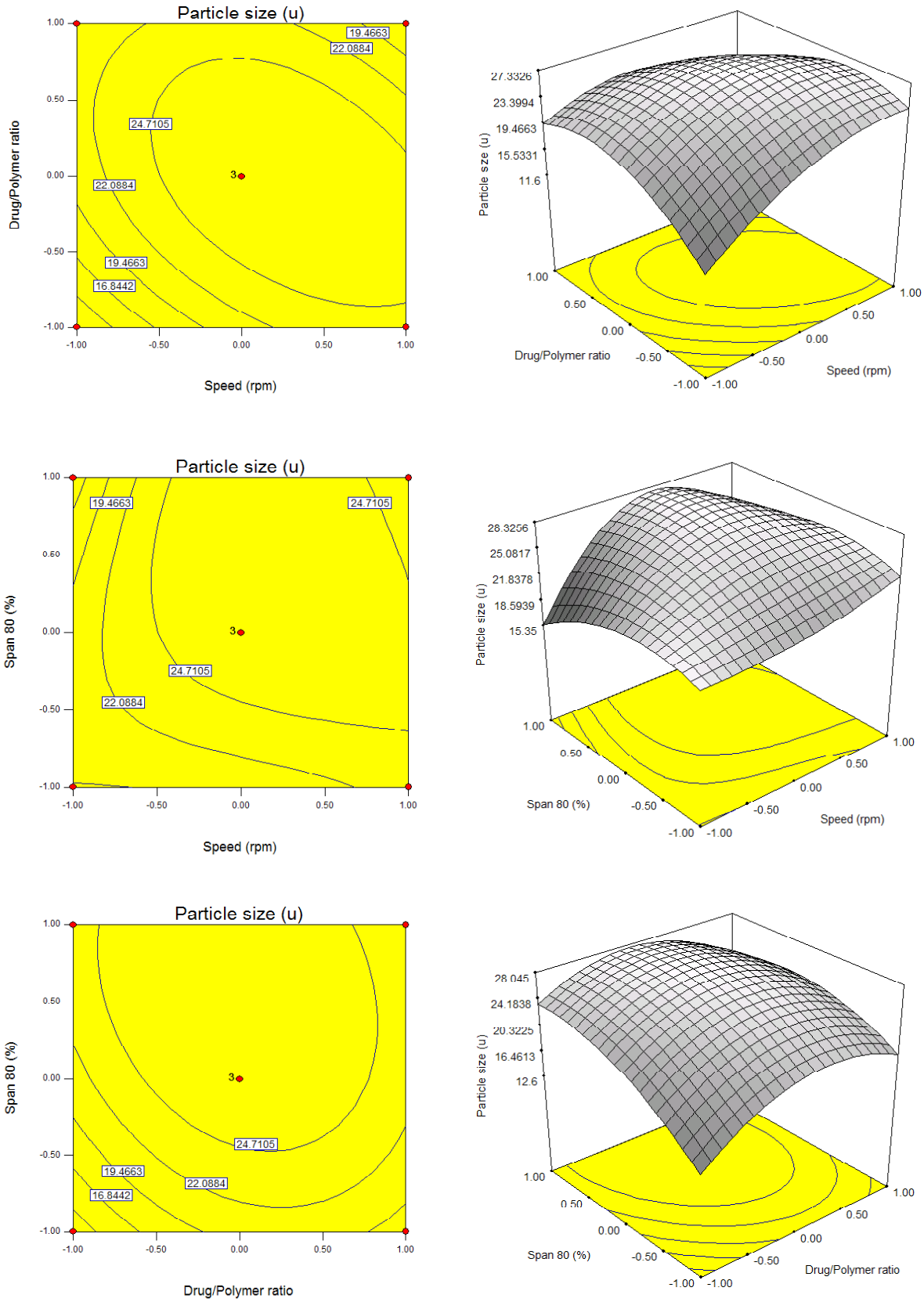


Figure 5. Response surface plots showing the effect of different levels of independent variable (X) on particle size (Y_1). X_1 = speed (rpm); X_2 = drug : polymer; X_3 = % span 80.

3.7. *Characterization of TS microspheres and formulated capsules using response surface methodology*

The dependent variables studied were Y_1 (percent particle size range between 500-315 µm) and Y_2 (cumulative percent released after 8 h). Based on the experimental design, the factor combination resulted in different TS release rates. The range of response for Y_1 was 29.60% in T_7 (maximum) and 11.6% in T_1 (minimum). The range of response for Y_2 was 90.7% in T_3 (maximum) and 61.3% in T_7 (minimum). The dependent and independent variables were related using mathematical relationships obtained from the statistical package. The polynomial equation obtained was:

$$Y_1 \text{ (Particle size)} = 26.95 + 1.58X_1 + 1.46X_2 + 1.09X_3 - 4.95X_1^2 - 5.82X_2^2 - 2.15X_3^2 - 1.96X_1X_2 + 0.55X_1X_3 - 1.79X_2X_3$$

$$Y_2 \text{ (Dissolution after 8 h)} = 62.33 - 0.55X_1 - 3.50X_2 - 7.38X_3 + 5.72X_1^2 + 16.87X_2^2 + 6.42X_3^2 + 3.27X_1X_2 - 1.13X_1X_3 - 5.02X_2X_3$$

The equations represent the quantitative effect of process variables (X_1, X_2, and X_3) and their interactions on the responses (Y_1 and Y_2). The values of X_1, X_2, and X_3 were substituted in the equation to obtain the theoretical values of Y_1 and Y_2. The theoretical (predicted) values were compared with the observed values and were found to be in reasonably close agreement. Table 5 shows the observed, predicted and residual values for particle size, while Table 6 shows the observed, predicted and residual values for the *in vitro* release after 8 h.

The relationship between the dependent and independent variables were further elucidated using contour plots and response surface plots. In Figure 6 are the contour plots showing the effect of factors X_1, X_2, and X_3 on the response Y_1, where the small circles indicate levels at which maximum response would be observed. Figure 7 shows the response surface plots for the independent variables and their influence on the response Y_1 (particle size). At low levels of X_3 (Span 80), Y_1 increased from 19.36 to 23.26% when the speed (X_1) was increased from 500 to 900 rpm. At high levels of X_3, Y_1 was increased from 15.35 to 21.45% when the speed (X_1) was increased from 500-900 rpm. At low levels of X_1 (Speed), Y_1 increased from 15.3 to 19.36% when the span 80% (X_3) was decreased from 2 to 1%. At high levels of X_1, Y_1 was increased from 21.45 to 23.26% when the span 80 (X_3) was decreased from 2 to 1%. At low levels of X_3 (Span 80), Y_1 was increased from 12.6 to 18.1% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At high levels of X_3, Y_1 was decreased from 23.45 to 21.78% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At low levels of drug-polymer ratio (X_2) Y_1 was decreased from 23.4 to 12.6 when the span 80 (X_3) was decreased from 2 to 1%. At high levels of X_2, Y_1 was decreased from 21.78 to 18.1% when the span 80 (X_3) was decreased from 2 to 1%. At low levels of X_1 (Speed), Y_1 was increased from 11.6 to 19.45% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At high levels of X_1, Y_1 was decreased from 23.75 to 16.85% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At low levels of drug-polymer ratio (X_2) Y_1 was decreased from 23.75 to 11.6% when the speed (X_1) was decreased from 900 to 500 rpm. At high levels of X_2, Y_1 was increased from 16.85 to 19.45% when the speed (X_1) was decreased from 900 to 500 rpm.

Figure 5 represents the contour plots showing the effects of factors X_1, X_2, and X_3 on the response Y_1 (particle size), where the small circles indicate levels at which maximum response would be observed. Figure 6 shows the response surface plots for the independent variables and their influence on the response Y_2

Table 5. Actual values, predicted values and residuals for particle size

Formula No.	Variable levels*			Particle size			Dissolution after 8 h		
	X_1	X_2	X_3	Actual	Pred.	Residual†	Actual	Pred.	Residual†
T_1	–1	–1	0	11.60	11.18	0.42	86.20	92.25	–6.05
T_4	0	–1	–1	12.60	14.64	–2.04	88.30	91.48	–3.93
T_2	0	–1	1	23.45	20.40	3.05	90.70	86.77	3.93
T_{10}	1	–1	0	23.75	20.27	3.48	89.90	84.60	5.30
T_{14}	–1	0	–1	19.36	17.74	1.62	90.50	81.27	9.23
T_5	–1	0	1	15.35	18.81	–3.46	70.90	68.78	2.13
T_6	0	0	0	29.60	26.95	2.65	61.30	62.33	–1.03
T_8	0	0	0	23.60	26.95	–3.35	69.00	62.33	6.67
T_9	0	0	0	27.65	26.95	0.70	65.70	62.33	3.37
T_3	1	0	–1	23.26	19.80	3.64	80.30	82.43	–2.12
T_7	1	0	1	21.45	23.08	–1.63	65.20	65.43	–0.23
T_{15}	–1	1	0	19.45	18.03	1.42	73.40	78.70	–5.30
T_{11}	0	1	–1	18.10	21.15	–3.05	90.60	94.52	–3.92
T_{12}	0	1	1	21.78	19.74	2.04	72.90	69.73	3.18
T_{13}	1	1	0	16.85	17.27	–0.42	90.20	84.15	6.05

* X_1 = speed (rpm); X_2 = drug: polymer; X_3 = % span 80; † Residual value = actual value – predicted value.

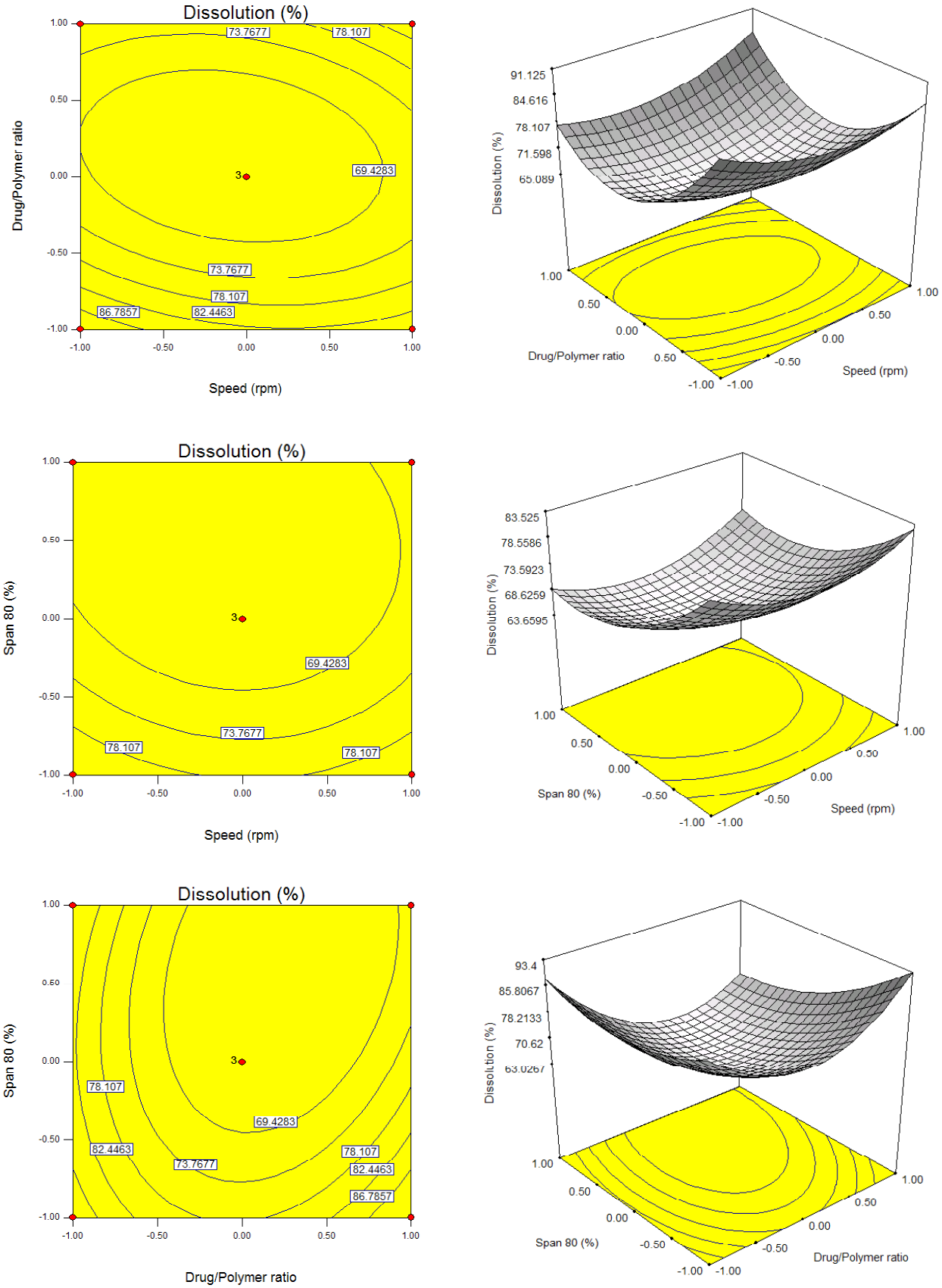


Figure 6. Response surface plots showing the effect of different levels of independent variable (X) on percent drug release from TS microspheres after 8 h (Y_2). X_1 = speed (rpm); X_2 = drug: polymer; X_3 = % span 80.

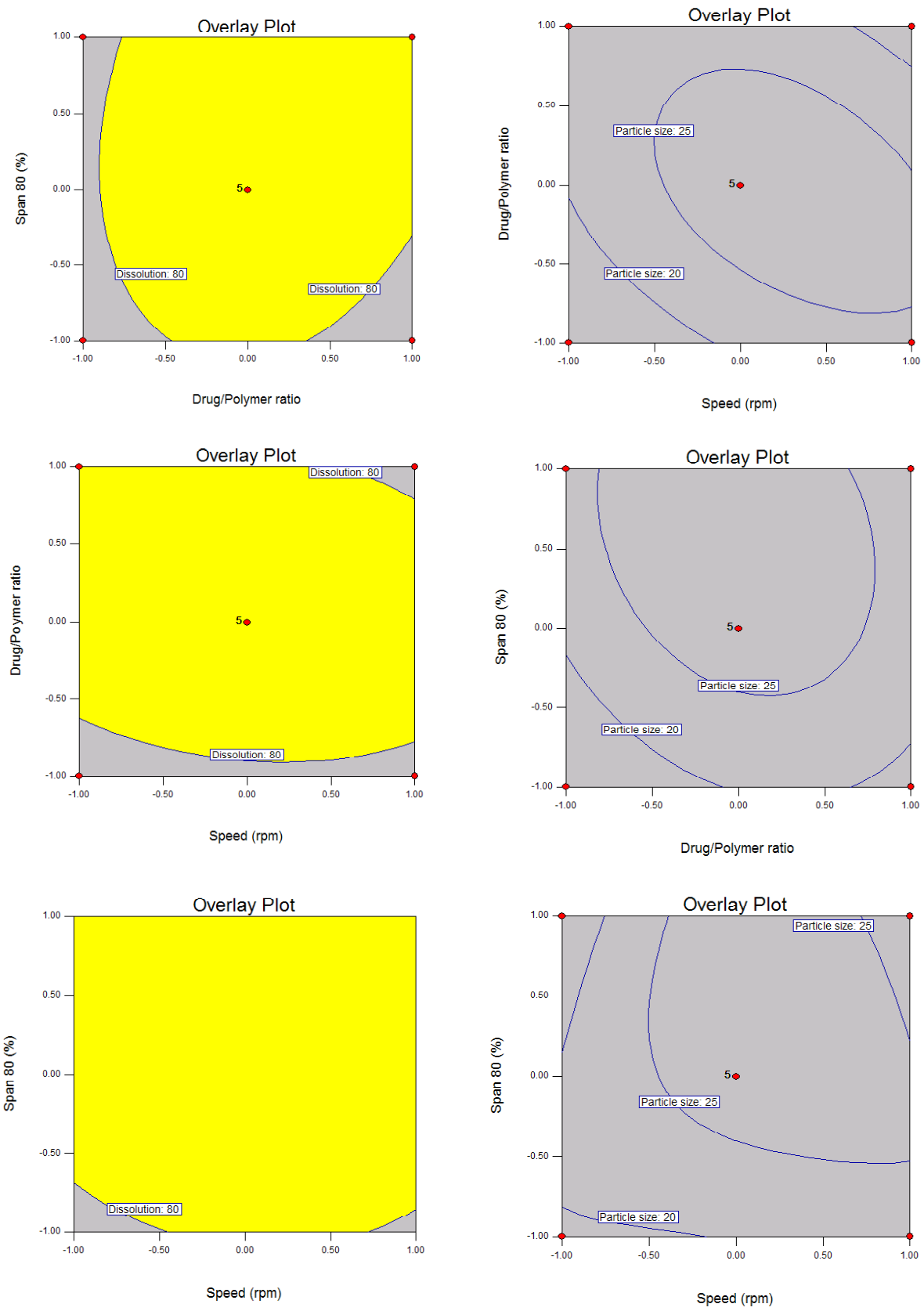


Figure 7. Overlay plots showings the effect of different levels of independent variable (X) on the dependent variable; Y_1 (right) particle size and Y_2 (left) % drug release. X_1 = speed (rpm); X_2 = drug: polymer; X_3 = % span 80.

(dissolution after 8 h). At low levels of X_3 (Span 80%), Y_2 was decreased from 90.5 to 80.3% when the speed (X_1) was increased from 500 to 900 rpm. At high levels of X_3, Y_2 was decreased from 70.9 to 65.2% when the speed (X_1) was increased from 500-900 rpm. At low levels of X_1 (Speed), Y_2 was increased from 70.9 to 90.5% when the span 80% (X_3) was decreased from 2 to 1%. At high levels of X_1, Y_2 was increased from 90.5 to 89.5% when the span 80 (X_3) was decreased from 2 to 1%. At low levels of X_3 (Span 80%), Y_2 was increased from 88.3 to 90.6 % when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At high levels of X_3, Y_2 was decreased from 90.7 to 72.9% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At low levels of drug-polymer ratio (X_2), Y_2 was decreased from 90.7 to 88.3% when the span 80% (X_3) was decreased from 2 to 1%. At high levels of X_2, Y_2 was increased from 72.9 to 90.6% when the span 80 (X_3) was decreased from 2 to 1%. At low levels of X_1 (Speed), Y_2 was decreased from 86.2 to 73.4% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At high levels of X_1, Y_2 was increased from 89.5 to 90.2% when the drug-polymer ratio (X_2) was increased from 1:4 to 1:8. At low levels of drug-polymer ratio (X_2), Y_2 was decreased from 89.9 to 86.2% when the speed (X_1) was decreased from 900 to 500 rpm. At high levels of X_2, Y_2 was decreased from 90.2 to 73.4% when the speed (X_1) was decreased from 900 to 500 rpm. By superimposing the contour plots of both responses we can delimit the optimal zone (Figure 7). We consider that the average particle size is optimum 20 to 25 u and a satisfactory drug release will be achieved with a value more than 80%. This zone has been verified with an experimental point (speed 700 rpm, drug/polymer ratio 1:5.6, span 0.85%) which leads to optimal particle size and maximum drug release.

4. Conclusions

From the present investigations it can be concluded that the emulsion solvent evaporation technique is an effective method for the preparation of terbutaline sulfate microspheres using Eudragit RSPM as a release retardant. The application of a factorial design approach helped in identifying the critical factors in the preparation and optimization of microcapsules. The results of the experimental study confirm that the polymer and emulsifier concentration as well as the speed of emulsification, significantly influence the dependent variables, *i.e.*, particle size and *in vitro* release. The total rank-order of terbutaline sulfate concerning the micromeritic parameters and the *in-vitro* dissolution could be arranged in a descending order as follow: $T_{12} > T_{11} > T_9$. An accelerated stability study of the TS microspheres will be a continuation of this work.

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