Original Article

Study On Influence Of Process Parameters On Quality Of Lovastatin Microspheres By Design Of Experiments

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Abstract

Scale-up of microspheres is major challenge at large scale manufacturing. The foremost objective of this research was to study the impact of key process parameters and formulation parameters, which are significant during scale-up, on the quality characteristic of the lovastatin microspheres. Emulsion solvent evaporation technique was explored to generate microspheres. Including the amount & type of polymer, process parameters like viscosity of the internal and temperatures used for evaporation of the solvent were taken as the factors. The key quality control parameters of the microspheres viz. entrapment efficiency and drug release rate constant were considered as the response variables on which the influence of the factors to be studied. Under response surface methodology, historical data design was employed to design of experiments (DoE) analysis using Stat Ease Design Expert software. Apart from the responses, the microspheres prepared were studied for flow properties, percentage yield and surface morphology. Scanning electron microscopy (SEM) images illustrated that the microspheres were almost spherical having different surface texture for different polymers. Entrapment efficiency results were found to be in the range of 78.43 – 93.83%; and the values of drug release rate constant were to be in the range of 0.09 – 1.2 h^{-1} for all the prepared microspheres. Response surface linear model was selected to elucidate the relation between the factors and the responses. Analysis of variance (ANOVA) of the selected model indicated that all the selected factors including the key process parameters had significant influence on both the responses. Later, graphical optimization was performed with the set desirability of maximizing the entrapment efficiency and minimizing the drug release rate constant. The microspheres obtained from the optimised formula were found to have 92.4% entrapment efficiency and 0.102 h^{-1} as the drug release rate constant. These results signified that the selected factors including the process parameters had substantial influence on the quality characteristics of the prepared microspheres.

KEYWORDS: Microspheres, process parameters, Design of experiments, Entrapment efficiency, Optimization.

INTRODUCTION

Microspheres are roughly spherical solid particles, ranging in diameter of 1 – 1000µm that contain a drug dispersed in solution (or) in microcrystalline form (1). Microcapsules and microspheres are the terms that are often used synonymously. The polymeric microspheres are more stable than other particulate drug delivery systems. Upon surface modification, these microparticles can be used to deliver the contained drugs to the desired target site

efficiently and reduce the side effects (2). Microspheres are prepared by different methods; they're Solvent evaporation, Single emulsion solvent evaporation technique, Ionic gelation method, Double emulsification method, Spray drying technique, Precipitation, Freeze Drying, and Coacervation (3). The particle size, drug entrapment, drug release behaviour of the microspheres is dependent on formulation and process parameters like drug: polymer ratio, temperature, stirring rate, dispersing medium volume, polymer type, concentration of surfactant& polymer[4]. The major drawback of microspheres is difficulty in large scale manufacturing. By optimising formulation and process parameters, scale up can be increased.

Lovastatin is an anti – hyperlipidemic drug acts by competitively inhibiting the enzyme, HMG – CoA Reductase. This enzyme is responsible for the synthesis of cholesterol via mevalonic acid pathway. Lovastatin is administered in the prodrug form, in the liver it hydrolyses and converts into β – hydroxyl form, an active metabolite. This active metabolite impedes the synthesis of cholesterol in liver and therefore decreases the levels of cholesterol in the body. Lovastatin has low solubility and high permeability so that it comes under the class II of the Biopharmaceutic classification system (BCS). It is suffered from extensive intestinal and first-pass metabolism and hence it exhibits poor oral bioavailability of 5 % even though 30 – 35% is absorbed, and the elimination half-life is about 2–4h. Regular oral administration causes variations in the plasma drug concentrations because there is no control over the drug delivery. These pharmacokinetic properties of lovastatin suggest developing an extended-release formulation helps to improve the bioavailability (5-7).

The research work by Suhas M. Kakade suggested that process parameter (stirring rate) plays an important role in preparation of microspheres. Stirring rate influences the microspheres size i.e., higher the stirring speed lower the particle size of microspheres (8). The research work by Yang et al. suggested that process parameter (temperature) has important role in preparation of microspheres. Spheres size is dependent on temperature i.e., lower and higher temperatures result in larger spheres therefore in-between temperatures are suitable to produce smaller spheres (9). Research work by Davoud Sadeghi, suggested that concentration of surfactant plays an important role in determination of particle size as the surfactant concentration increases, mean diameter of microspheres decreases (10). Research work by S. Mao et al. reported that polymer content played an important role in size determination of the microspheres. Increasing the polymer content increases the size of microspheres (11). Research work by Hong Zhao suggested that viscosity of the polymer solution shows a very important role in particle size of microspheres. Higher viscous polymer solutions produce larger microspheres (12). Research work by P.Le Corre et al. investigated that increase in polymer molecular weight resulted in decrease in the drug release rate (13). Studies on formulation parameters are extensively reported compared to the process parameters. As both influence the preparation and characteristics of microspheres, further studies on process parameters are needed for the better development of microspheres.

Current research work was especially focussed on the optimization of process parameters (viscosity of the polymer solution and processing temperature) that have influence on preparation and characteristics of microspheres i.e., entrapment efficiency, drug release rate. The viscosity of the polymer solution was varied by taking different polymers at different concentrations. Lovastatin microspheres were generated by the technique, emulsion solvent evaporation method using Ethyl cellulose 100cps and Eudragit RSPO in a drug and polymer ratios of (1:0.5, 1:1, 1:2) at two different temperatures i.e., at 30^{o}C and at 45^{o}C. The results obtained were subjected to Design of Experiments (DoE) by applying historical data design under response surface methodology with the help of Stat Ease Design Expert software. Experimental results analysed by DoE tool are more significant and the inferences made are more valid.

MATERIALS AND METHODS

Materials

Lovastatin was acquired from Dr. Reddy's Laboratories, India as a gift sample. Ethyl cellulose 100cps and Eudragit RSPO were purchased from Sigma Aldrich, Mumbai. Acetone, Liquid heavy paraffin, Petroleum ether, and Sodium lauryl sulphate were procured from Merck Chemicals, Mumbai. The remaining chemicals utilized were analytical grade type.

Preparation of Microspheres

Lovastatin microspheres were developed in different ratios (1:0.5, 1:1, 1:2) using the polymers Ethyl cellulose 100cps and Eudragit RSPO by solvent evaporation method. Various ratios of lovastatin and polymers were liquefied in acetone and the resulted solution was dispersed slowly in heavy liquid paraffin under continuous agitation for using mechanical stirrer at high rpm at 30^{o}C and at 45^{0}C until complete evaporation of acetone. The microspheres obtained after solvent evaporation, were strained and washed with petroleum ether for 3 times & then finally washed with water and are dried and collected (14-16).

Yield of microspheres

The obtained dried microspheres were carefully weighed and the obtained weight was substituted in the below formula to calculate the yield in percentage (17-18).

$$\% \text{ Yield} = \frac{\text{Weight of the obtained microspheres x } 100}{\text{Total weight of the drug and the polymer taken}}$$

Morphological characterization

Scanning Electron Microscope (ZEOL JSM–5610) was employed to investigate shape and surface morphology of the obtained microspheres (19-20). Dried microspheres were placed on the SEM stub with double sided adhesive strip, and layered with ion sputter (200 nm thickness) under reduced pressure (0.001 torr) and photographs of the microspheres were taken by scanning the stub at different locations randomly.

Micromeritic Properties

Various micromeritic properties viz. angle of repose, Carr's index and Hausner's ratio were determined for all the prepared microspheres (21-24).

Entrapment efficiency

10 mg drug equivalent microspheres were grinded to powder and add into 100 mL of phosphate buffer pH 7.0 and subjected to mixing. At regular time intervals during mixing, samples were withdrawn and estimated for drug absorbance until constant absorbance was recorded. Using this absorbance, the amount of drug entrapped in the microspheres was estimated which was substituted in the following formula to obtain the entrapment efficiency (25-26)

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

In vitro dissolution studies

The in vitro drug release of the Lovastatin microspheres was conducted by using USP type 2 apparatus [DS 8000] by rotating paddle method (27). The test was performed by taking 900 ml of SLS phosphate buffer pH 7.0, temperature of 37 ± 0.5°C & at 50 rpm. The microspheres equivalent to 50 mg of Lovastatin were added into the dissolution medium taken in the dissolution vessel. At every 30min intervals upto 12hrs, samples were taken out from the dissolution apparatus and the same amount of fresh buffer is replaced. The absorbance of these sample solutions were detected at 238.8 nm spectrophotometrically. Drug release profiles of the formulations were analyzed by subjecting the data to the kinetic models viz. zero-order, first-order and Higuchi's models.

DoE analysis and Optimization

Based on the experimental trials performed, historical data design was applied by utilizing Stat Ease Design Expert software. The process and the formulation variables viz. Polymer concentration in comparison to the drug (Factor A); Volume of acetone (Factor B); Temperature (Factor C) and the Polymer type (Factor D) were taken as the independent factors. The Percentage entrapment efficiency (EE %) (R1) and the drug release rate constant (k, h^{-1}) (R2) were chosen as the responses. Response surface linear model was used to elucidate the influence of the factors on these responses. The model was later diagnosed by Analysis of variance (ANOVA) and Normal plot of residuals for its suitability and to check whether the influences of these factors on the responses were significant or not. Later, graphical optimization with a desirability of maximizing the EE % and minimizing the drug release rate constant was performed to identify the best formulation.

RESULTS AND DISCUSSION

Preparation of Lovastatin microspheres

The Lovastatin microspheres were made using the emulsion solvent evaporation method in this study. The microspheres were made using 3different polymers: Ethyl Cellulose 100 cps and Eudragit RS PO, at three different polymer concentrations i.e. 1:0.5, 1:1 and 1:2. The influence of process parameters such as viscosity of the polymer phase & temperature were also investigated. The microspheres made with high viscosity polymer phase required the use of temperature for the complete removal of solvent followed by rigidization and the formation of smaller microspheres with smooth texture. Those made with a low viscosity polymer phase rigidified without use of temperature, and the surface was uneven with small pits. This could be due to the possibility of formation of small droplets at greater viscosities, as well as the controlled evaporation of the solvent from the microspheres in the first case at a moderate temperature.

Percentage yield

The results of the percentage yield were shown in Table 2. The microspheres of all the formulations exhibited more than 80% yield. This indicated that this technique was highly effective for the preparation of microspheres under the selected conditions of temperature and viscosities.

Surface morphology

The results of SEM analysis were shown in Fig 1. Images of microspheres from formulation F3 (Ethyl cellulose 100cps at 1:2ratio, 15ml acetone) revealed that the texture was not uniform and that small pits were present. The images of microspheres from formulation F7 (Ethyl cellulose 100cps at 1:2ratio, 10ml acetone) revealed that the texture was more uniform and smooth, with no pits on the surface. This could be attributed to the polymer phase's higher viscosity (28), which allowed for the formation of compact droplets during emulsification and the slow solvent evaporation caused uniform removal of the solvent in a regulated manner. The texture of the microspheres of formulation F15 (Eudragit RSPO at 1:2 ratio, 10ml acetone) was jagged and uneven, and the matrix of the polymer strands was clearly visible and complex. This could be due to the polymer molecules of Eudragit RSPO having a long chain length and a high molecular weight.

Micromeritic Properties

The microspheres were tested for various derived properties such as angle of repose, Hausner's ratio, and Carr's index, with the outcomes displayed in Table 2. The near spherical shape might be the reason for showing good flowability as evident from the results of these flow properties. The flowability studies revealed that the microspheres of all formulations had good flowability (29). These studies concluded that the microspheres were effective for compression into tablets or filling into capsules.

Entrapment efficiency

Table 2 showed the results of the entrapment efficiency. The effect of all the factors was illustrated in Fig 2(a) and 2(b). These findings indicated that increasing the concentration of the polymer improved entrapment efficiency because more amount of polymer allows more amount of drug to be entrapped in its matrix (30). The increased viscosity of the drug and polymer solution due to its increased concentration resulted in improved entrapment efficiency. Besides, the decrease in solvent volume from 15 mL to 10 mL also resulted in same effect as this could also because of the increased viscosity. But the temperature had negative effect on the EE. At higher temperatures, the diffusion rate of the solvent is high so that the drug may easily diffuse out of the microspheres along with the solvent, which can lead to decrease in the EE. EE of microspheres of different polymers was improved with the molecular weight of the polymers. Microspheres with EC 100 were found to have lesser EE than those prepared with Eudragit RSPO as the later has high molecular weight. The effects of all these factors were found to be significant at $p < 0.05$ as evidenced by the ANOVA test (shown in Table 3).

Drug release studies

The drug release from the microspheres of all the formulations exhibited first-order kinetics and thus the corresponding drug release rate constants were shown in Table 2. The effect of all the factors was illustrated in Fig 2(c) and 2(d). The factor A had negative effect on the drug release. Upon increasing the polymers concentration, the release rate was found to be decreased (31). This could be attributed to the more complex

polymer matrix at higher polymer levels that could hinder the drug release (32-33). The factor B had positive effect that the release rate was increased upon increasing the volume of the internal phase solvent. This could be attributed to the formation of less compact polymer matrix because of low viscosity at higher solvent volumes. This was also supported by the SEM analysis results that more compact microspheres were observed at higher viscosities. The factor C also showed positive effect on the drug release that at higher temperatures, the release rate was increased. This could be because of the formation of loose matrix as a result of rapid evaporation of the solvent at higher temperatures. The type of polymer, factor D exhibited that the release was slow in case of Eudragit RSPO than in case of EC 100 which might be due to the higher molecular weight and thus the strong matrix of Eudragit RSPO. The effects of all these factors were found to be significant at $p < 0.05$ as evidenced by the ANOVA test (shown in Table 3).

Design Validation and Optimization

The influences of all the four factors on the responses were studied by response surface linear model. The significance of this model and the effects of the factors were tested by ANOVA and the results were show in the Table 3. These results specified that the selected model and the influences of all the four factors on both the responses were significant at $p < 0.05$. Further, the normal plots of residuals shown in Fig 3 point out that all the response values were formed as a straight line but not as a sigmoid shape in case of both the responses. These plots specified that the selected linear model was significant and can be further navigated to optimization.

Graphical optimization was performed using desirability functions approach. The desirability of the responses was taken as to maximize the entrapment efficiency with a lower limit of 90%; and to minimize the drug release rate constant (which indicates more extended drug release) with an upper limit of 0.38 h^{-1} corresponding to extended release up to 12 hours. The resultant overlay plot of the graphical optimization was shown in Fig 4.

The yellow colour region was the design space inside which any combination of the factors would yield the formulation with the desired maximum entrapment efficiency and minimum drug release rate constant. One such best combination was identified by the software which was taken as the best optimized combination with predicted properties of the responses (shown in Table 4). At this combination, a new microsphere formulation was prepared and characterised to obtain results of the responses. The obtained results were reported in the Table 4 as observed values which were correlated with the predicted values as these were within the range 95% confidence intervals of the predicted values. Hence, the influence of the selected factors regarding the viscosity and temperature were found to have significant effect of the quality of the microspheres by Design of experiments analysis. Finally an optimized formulation of the microspheres was developed by graphical optimization.

CONCLUSION

The research was commenced to study the influence of processing temperature and viscosity of the polymer phase by changing compositions of the polymer and the solvent on the EE and rate of drug release from the lovastatin microspheres. From the results it was observed that at higher concentration of polymer phase, higher temperature was required for the rigidization of the microspheres after formation. Surface morphology studies (SEM analysis) indicated that at lower viscosity of the polymer (Ethyl Cellulose 100 cps) phase, microspheres of irregular surface with small pits were formed. A more uniform and smooth surfaced microspheres were formed at higher viscosity of the polymer phase. Eudragit RS PO showed the surface of the microspheres was made up of the rich complex network of the polymer chains. The release of Lovastatin from the microspheres was able to be extended until 12h at a drug to polymer ratio of 1:2 with both Ethyl Cellulose 100 cps and Eudragit RS PO. From the drug release studies an interesting finding was observed that the drug release rate was found to be reduced upon increase in the viscosity of the polymer phase even at the same amount of the polymer. All the selected factors had significant influence on the characteristics of the microspheres and thus the optimization of process parameters can help scale up of microspheres with desired characteristics.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this research work

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Table 1: Formulation of Lovastatin microspheres (F1-F16)

Formulation Code	Ratios	Lovastatin (g)	Ethyl cellulose 100cps (g)	Eudragit RSPO (g)	Acetone	Temperature
F1	1:0.5	0.5	0.25	-	15ml	30ºC
F2	1:1	0.5	0.5	-	15ml	30ºC
F3	1:2	0.5	1.0	-	15ml	30ºC
F4	1:2	0.5	1.0	-	15ml	45ºC
F5	1:0.5	0.5	0.25	-	10ml	45ºC
F6	1:1	0.5	0.5	-	10ml	45ºC
F7	1:2	0.5	1.0	-	10ml	45ºC
F8	1:2	0.5	1.0	-	10ml	30ºC
F9	1:0.5	0.5	-	0.25	15ml	30ºC
F10	1:1	0.5	-	0.5	15ml	30ºC
F11	1:2	0.5	-	1.0	15ml	30ºC
F12	1:2	0.5	-	1.0	15ml	45ºC
F13	1:0.5	0.5	-	0.25	10ml	45ºC
F14	1:1	0.5	-	0.5	10ml	45ºC
F15	1:2	0.5	-	1.0	10ml	45ºC
F16	1:2	0.5	-	1.0	10ml	30ºC

Table 2: Flow Properties of Lovastatin Microspheres of formulations F1 – F16

Formulation code	Angle of repose	Carr's Index	Hausner's ratio	Yield (%)	Entrapment efficiency (%)	Drug release rate constant (h^{-1})
F1	17.103 ± 0.12	3.875 ± 0.01	1.040 ± 0.01	90.13 ± 2.15	78.43 ± 2.44	1.28 ± 0.08
F2	16.921 ± 0.11	3.258 ± 0.02	1.034 ± 0.01	92.56 ± 3.06	81.41 ± 1.36	0.86 ± 0.05
F3	16.537 ± 0.09	3.539 ± 0.01	1.037 ± 0.01	86.30 ± 1.59	85.09 ± 2.09	0.57 ±0.04
F4	16.909 ± 0.13	3.429 ± 0.02	1.036 ± 0.01	81.88 ± 2.47	80.67 ± 3.14	0.98 ± 0.07
F5	16.812 ± 0.12	3.102 ± 0.02	1.032 ± 0.01	83.01 ± 2.68	85.98 ± 2.92	1.12 ± 0.11
F6	21.170 ± 0.12	3.611 ± 0.01	1.037 ± 0.01	80.23 ± 1.34	87.41 ± 3.16	0.46 ± 0.03
F7	17.181 ± 0.13	2.917 ± 0.02	1.031 ± 0.01	93.21 ± 3.06	89.25 ± 3.74	0.31 ± 0.04
F8	16.926 ± 0.13	2.570 ± 0.01	1.026 ± 0.01	90.37 ± 2.67	92.68 ± 1.68	0.15 ± 0.02
F9	17.108 ± 0.15	3.459 ± 0.02	1.034 ± 0.01	83.15 ± 1.59	77.83 ± 2.08	0.58 ± 0.04
F10	20.120 ± 0.12	2.610 ± 0.01	1.026 ± 0.01	90.42 ± 1.38	85.39 ± 3.32	0.3 ± 0.02
F11	23.942 ± 0.15	4.309 ± 0.03	1.045 ± 0.01	88.34 ± 2.61	90.26 ± 2.55	0.22 ± 0.01
F12	20.321 ± 0.16	3.571 ± 0.02	1.037 ± 0.01	83.10 ± 2.05	85.74 ± 1.83	0.45 ± 0.03
F13	20.162 ± 0.11	3.210 ± 0.01	1.033 ± 0.01	88.91 ± 3.41	84.06 ± 2.45	0.47 ± 0.05
F14	22.371 ± 0.13	4.309 ± 0.02	1.048 ± 0.01	84.12 ± 2.53	88.17 ± 3.29	0.29 ± 0.04
F15	22.461 ± 0.15	4.406 ± 0.01	1.046 ± 0.01	82.66 ± 1.94	91.45 ± 2.63	0.17 ± 0.02
F16	19.324 ± 0.19	3.95 ± 0.02	1.041 ± 0.01	90.13 ± 2.26	93.83 ± 2.15	0.09 ± 0.01

Table 3: Results of the ANOVA for response surface linear model for both the responses

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value	Inference[d]
R1: Entrapment Efficiency						
Model	314.60	4	78.65	24.83	< 0.0001	**Significant**
A-Polymer Conc.	133.06	1	133.06	42.01	< 0.0001	**Significant**
B-Internal Phase Volume	162.10	1	162.10	51.19	< 0.0001	**Significant**
C-Temperature	21.86	1	21.86	6.90	0.0235	**Significant**
D-Type of polymer	15.62	1	15.62	4.93	0.0483	**Significant**
Residual	34.84	11	3.17			
Cor Total	349.43	15				
R2: Drug release rate constant						
Model	1.67	4	0.42	15.58	0.0002	**Significant**
A-Polymer Conc.	0.61	1	0.61	22.71	0.0006	**Significant**
B-Internal Phase Volume	0.43	1	0.43	16.19	0.0020	**Significant**
C-Temperature	0.14	1	0.14	5.18	0.0438	**Significant**
D-Type of polymer	0.62	1	0.62	23.33	0.0005	**Significant**
Residual	0.29	11	0.027			
Cor Total	1.96	15				
Note: [a]-Sum of Squares; [b]-Degrees of Freedom; [c]-Mean Sum of Squares; [d]-p-Value less than 0.05 indicates model terms are significant						

Table 4: Comparison of the predicted and observed values of the responses for the optimized formulation

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: Polymer conc. (66.49% w/w) B: Int. Phase Vol. (10.02 mL) C: Temperature (34.11^{o}C) D: Type of polymer (Eudragit RSPO)	R1: EE%	93.91	91.72	96.09	92.4
	R2; k (h^{-1})	0.09	0.08	0.11	0.102

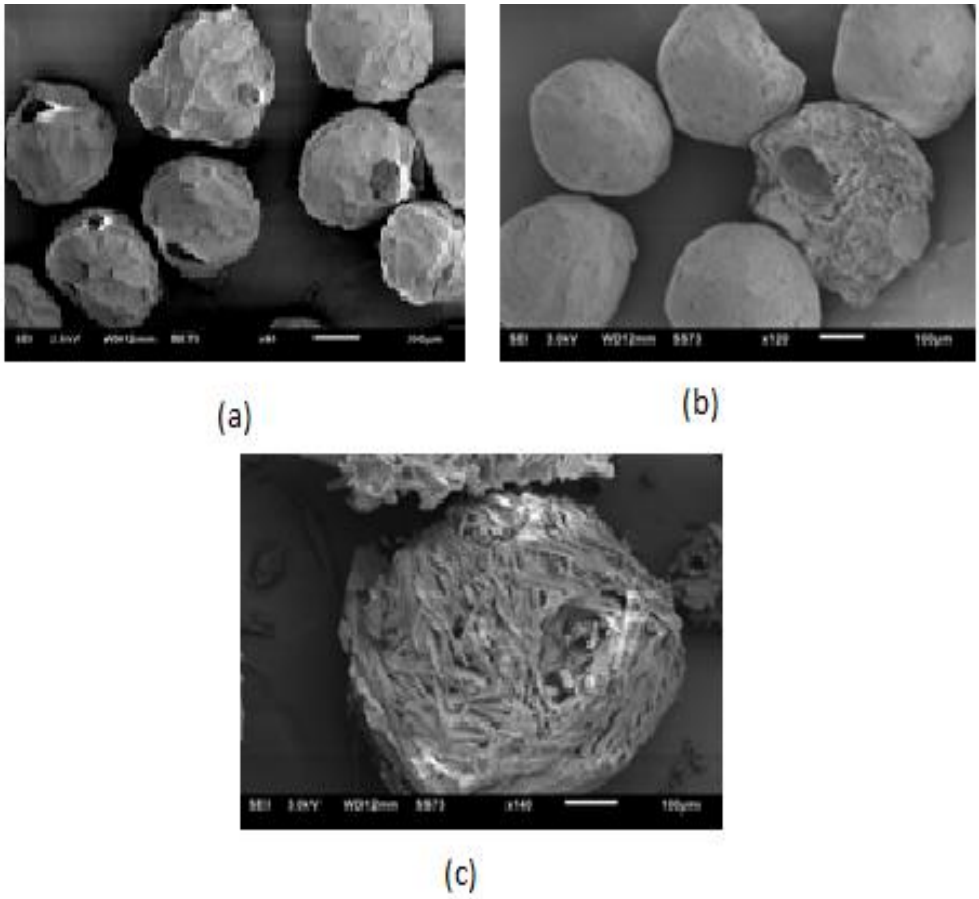

Figure 1: SEM images of Lovastatin microspheres of formulations (a) F3, (b) F7, (c) F15.

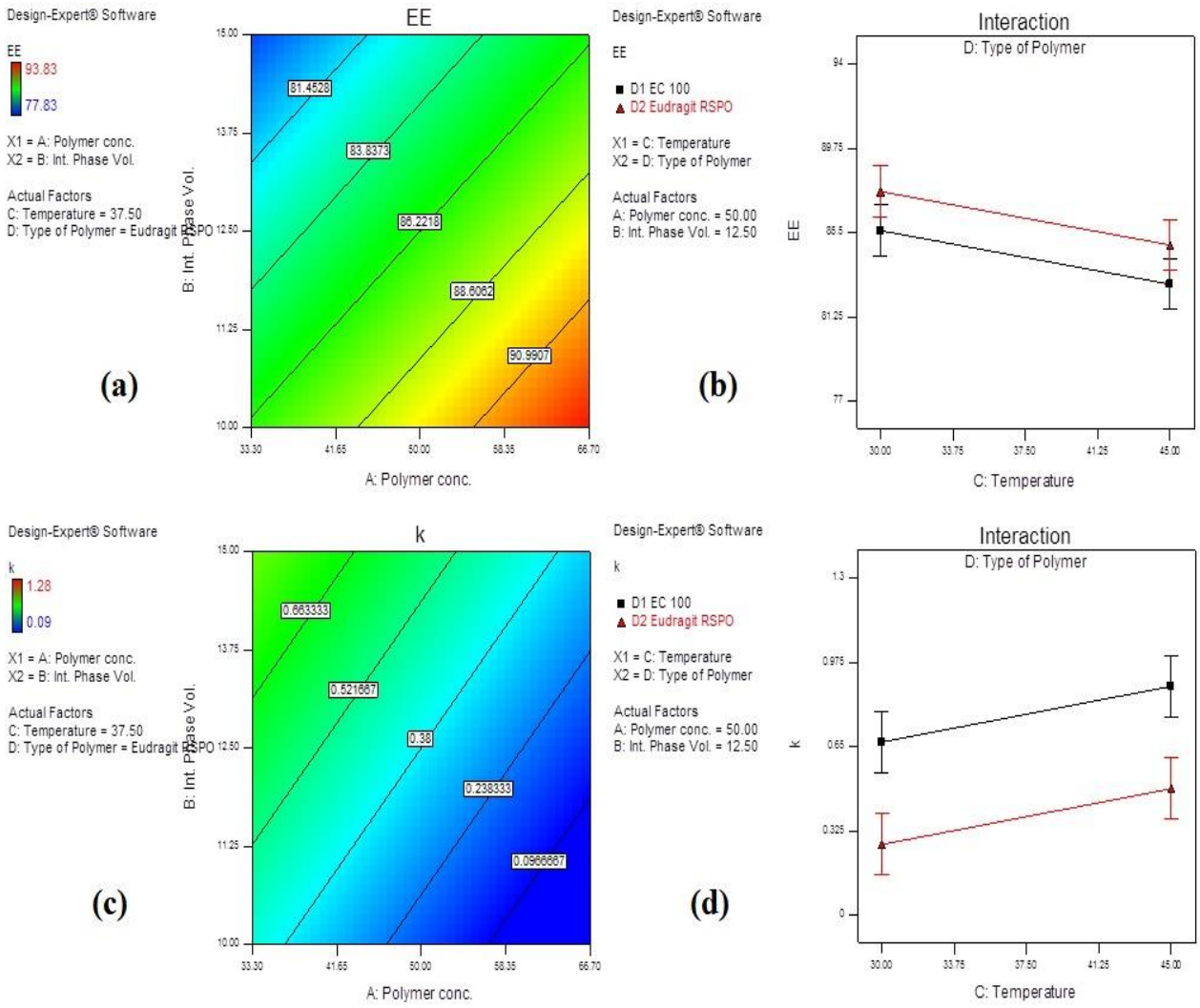

Fig 2: (a) Contour plot illustrating the influences of the factors A & B on the response EE; (b) Interaction plot illustrating the influences of the factors C & D on the response EE; (c) Contour plot illustrating the influences of the factors A & B on the response k; and (d) Interaction plot illustrating the influences of the factors C & D on the response k

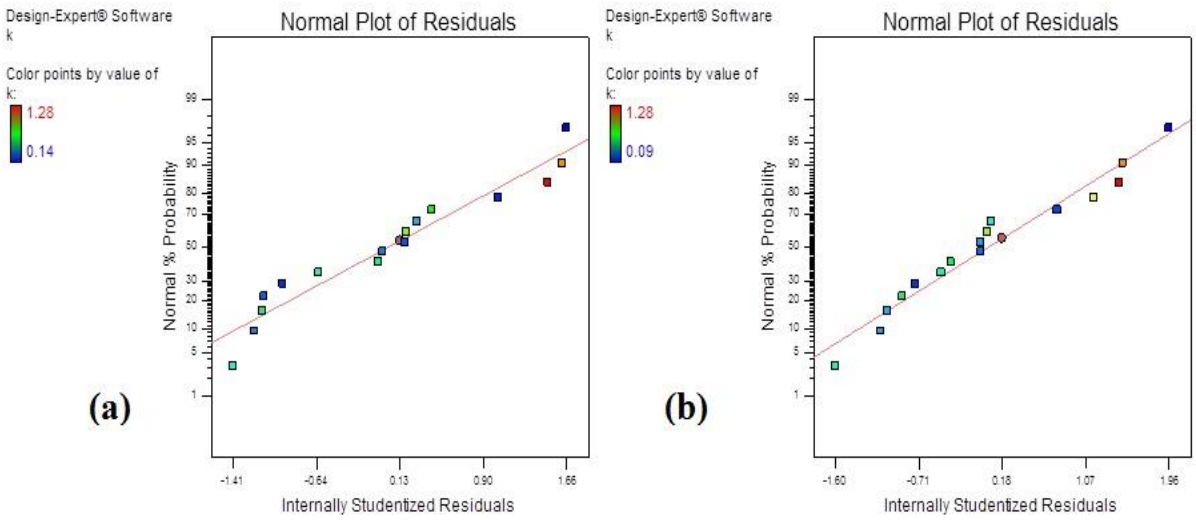

Fig 3: Normal plots of residuals for the responses (a) Entrapment efficiency; and (b) Drug release rate constant

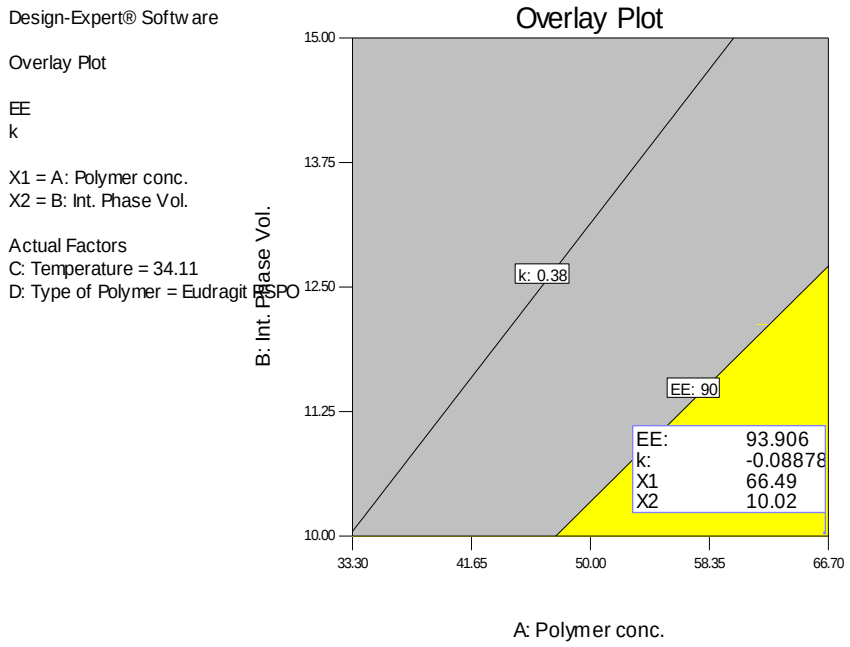

Fig 4: Overlay plot showing the design space (the yellow colour region)