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Research Article

Development and Optimization of Fluvastatin Sodium Loaded Biodegradable Microspheres

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ABSTRACT

Fluvastatin sodium is a hypolipidemic agent that reduces cholesterol synthesis by inhibiting HMG-COA reductase. The drug has a comparatively short biological half-life (1.2 hours) and low bioavailability (24–29%), making it an appropriate candidate for a sustained-release drug delivery system. This study aimed to formulate biodegradable microspheres of fluvastatin sodium by optimization through an experimental design approach. Microspheres containing fluvastatin sodium were prepared by o/w emulsification solvent evaporation method using poly (lactic-co-glycolic acid) (PLGA 50:50) as a biodegradable polymer. 3^2 full factorial design was applied to study the effect of drug to polymer ratio and stirring speed on dependent variables, i.e. particle size, entrapment efficiency, Q_{1h}, $t_{80\%}$. Prepared formulations were subjected to evaluate physicochemical properties and release characteristics. DSC and FTIR proved no interaction between the drug and excipients. Microspheres possessed size in the range of 193 to 344 µm and entrapment efficiency varied from 63.1 to 85.6%. Formulations showed drug release up to 23% within 1-hour. while $t_{80\%}$ was found in between 3–9 hours. Regression analysis and ANOVA results suggested a significant effect ($p<0.05$) of variables on responses. The results of the present study suggested that biodegradable microspheres of fluvastatin sodium prepared using poly (lactic-co-glycolic acid) can be a promising alternative for conventional delivery and suitable for sustained drug release.

Introduction

The traditional approach to dosage form design and development involves the method of change in one variable at a time which is very time-consuming as well as has no consideration of the combined effects of variables. The design of the experiment approach can be used to study the complex effects of independent variables with their interaction on product qualities during pharmaceutical dosage form development.[1,2] Lots of research have been reported utilizing the design of experiment concept in designing dosage forms.[3] Factorial design is one such proven technique in studying the relative influence of selected individual variables as well as their interactions on some critical quality parameters of pharmaceutical products.[4]

The sustained release systems have made significant progress in terms of clinical efficacy and patient compliance.[5] Multi-particulate drug delivery systems gained significant importance among the several drug delivery systems.[6,7] The use of multi-particulate-based drug delivery allows cautious tailoring of drug release to the specific site through the selection of appropriate formulation variables. These systems tend to release drugs more uniformly through uniform spreading over the entire GIT and prevent high local drug concentration and risk of toxicity, thereby releasing the drugs more uniformly.[8] Developing microspheres using biodegradable polymers is a general practice used in producing sustained-release dosage forms.[9]

Biodegradable polymers have been utilized as carriers for controlled release drug delivery of numerous drugs and

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biotechnological products.[10] Biodegradable polymers, either natural or synthetic, possess a greater extent of swelling properties when come in contact with aqueous medium and thus prolong residence time. They are also able to be cleaved via chemical or enzymatic degradation into by-products that are biocompatible. Biodegradable microspheres as drug delivery systems have lots of advantages over conventional systems as in later drug is instantaneously released and shows no effect after a short period. This leads to increased dosing frequency. Biodegradable microspheres provide sustained release of drug over a prolonged period, thereby eliminating the administration of frequent multiple doses.[11] The rate and extent of drug release from microspheres depend on the type and concentration of the polymer. The major drawback of the system is the drug-loading efficiency of biodegradable microspheres, which is very complex and makes it difficult to control the release of drug. The rate of drug release can be controlled by several characteristics, including biodegradation kinetics of polymers, thermodynamic compatibility between drug and polymer, physicochemical characteristics of polymer and drug as well as shape of devices.[12]

Amongst several available biodegradable polymers, poly (lactic-co-glycolic acid) (PLGA) is extensively used in designing numerous controlled release formulations due to their good biocompatible and biodegradation characteristics in physiological environments.[13] PLGA is a copolymer of poly (lactic acid) (PLA) and poly (glycolic acid) (PGA). It is also very easy to shape PLGA into drug delivery systems of almost all scales as well as to encapsulate a variety of drugs.[14] Products of biodegradation of PLGA are lactic acid and glycolic acid, both of which are biologically inert and can be eliminated from the body by normal metabolic and excretion routes.[15-17]

Fluvastatin sodium is a 3-hydroxy-3 methyl glutaryl co-enzyme (HMG-COA) reductase inhibitor that acts on plasma lipids and reduces cholesterol synthesis in the liver by inhibition of HMG-COA reductase, resulting in decreased cholesterol concentrations. The drug has a comparatively short biological half-life (1.2 hours) and low bioavailability (24–29%), making it an appropriate candidate for a sustained-release drug delivery system.[18,19]

Several products have been approved for use on the market using PLGA-based biodegradable microspheres in the past decades. Biodegradable microspheres based on PLGA are biocompatible, stable, and degradable, making them suitable for a wide range of applications. It is also possible to customize drug release kinetics from such microspheres and provide sustained drug release for a prolonged period to reduce dosing frequency. Future applications of biodegradable microspheres will expand with their improved design and efficacy.[12]

An emulsion solvent evaporation method using 50:50 PLGA was used by Soni *et al.*[20] to prepare microparticles containing gefitinib. Formulation optimization was performed employing a 3^2 factorial design. In line with the Fickian diffusion and first-order kinetics, the prepared microparticles provide sustained release of the drug for 72 hours. Thus, sustained drug release can be obtained by loading them efficiently into the biodegradable polymer PLGA. Similarly, using a polycationic pH-sensitive polymer, Kharb *et al.*[4] prepared taste-masked, ondansetron-loaded microspheres and optimized using 3^2 full factorial design. In phosphate buffer pH 6.8 medium, optimized taste-masked microsphere formulations retarded drug release significantly, resulting in improved taste masking and palatability. Therefore, the present work was intended to develop and evaluate sustained-release biodegradable microspheres of fluvastatin sodium with a synthetic polymer. Optimization of the formulation was carried out by employing 3^2 factorial design to study the effect of various formulation and processing variables like drug-to-polymer ratio and stirring speed on particle size, entrapment efficiency, and release of drug from the microspheres.

Materials and Methods

Materials

Drug (fluvastatin sodium) was acquired from Suven Life Sciences Ltd., Hyderabad, India and Polymer (PLGA 50:50) was obtained from Sigma-Aldrich Chemicals Private Limited, Bangalore (India). Polyvinyl alcohol, acetone and dichloromethane were purchased from Merck Pvt. Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade and purchased from Loba Chemie Pvt. Ltd., Mumbai (India).

Preparation of Microspheres

Microspheres were prepared by the o/w emulsification solvent evaporation method.[21,22] drug and polymer in proportion to the concentration dissolved in the organic solvent mixture containing dichloromethane and acetone. The solution was added dropwise to 50 mL aqueous dispersion of polyvinyl alcohol (2.0%) being stirred on a magnetic stirrer. This resulted in the formation of emulsion which on evaporation, converted into microspheres. The resultant emulsion was allowed to stir on a magnetic stirrer for about 3 hours at room temperature for evaporation of the solvent and, consequently, the formation of microspheres. The reaction mixture was allowed to be set aside for the settling of formed microspheres. After the decantation of the supernatant solvent, prepared microspheres were collected by filtration. The microspheres were then kept for 12 hours at room temperature for drying and eventually stored in desiccators containing fused calcium chloride till further study. The amount of polymer and stirring speed were varied according to the batch, as shown in Table 1.

Optimization of Mwicrospheres

Factorial designs are time-saving approaches to optimize the dependent variables to obtain the desired outcome

in pharmaceutical product development.[20] Moreover, it saves time as minimum experimentation gives maximum output by the use of multiple regression equations.[23] 3^2 factorial design was employed using Design Expert® software to optimize the formulation. Here, two factors were evaluated each at three levels (-1, 0 and +1) and experimental trials were performed using all possible 9 combinations. Based on the literature survey, drug-to-polymer ratio (X1) and stirring speed (X2) were selected as independent variables in the present study. By preliminary trials, drug-to-polymer ratio and stirring speed were selected in the 1:1 to 1:3 and 500 to 1000 rpm range, respectively.

Entrapment efficiency (Y1), mean particle size of microspheres (Y2), drug release within 1-hour (Y3) and time required to release 80% of drug (Y4) were selected as dependent variables. A statistical model was utilized to evaluate the response with interactive and polynomial terms.[24] Design Expert® software was used to generate polynomial equation as follows:

$$Y = b_0 + b_1X1 + b_2X2 + b_{11}X1^2 + b_{22}X2^2 + b_{12}X1X2 \quad (1)$$

In the above equation, Y represents the dependent variable; b_0 represents the arithmetic mean response while b_1 and b_2 are the expected coefficient for the factors X1 and X2, respectively. X1 and X2 show the main effect which represents the average result by changing one variable at a time from its low to high value. The change in responses due to simultaneous change in two factors is shown by the interaction term (X1X2). To investigate nonlinearity, polynomial terms ($X1^2$ and $X2^2$) were included.[25] Regression analysis was used for further data analysis and analysis of variance (ANOVA) was employed to confirm no substantial deviation between the developed full model and the reduced model.[26] Design expert® was used to draw response surface plots to study response variation alongside two independent variables.

Table 1: Formulation of microspheres by 3^2 full factorial design

Batch	*Actual values*		*Coded values*				
	Drug to polymer ratio (X1)	*Stirring speed (rpm) (X2)*	*X1*	*X2*	*$X1^2$*	*$X2^2$*	*X1X2*
F1	1:1	500	-1	-1	1	1	1
F2	1:1	750	-1	0	1	0	0
F3	1:1	1000	-1	1	1	1	-1
F4	1:2	500	0	-1	0	1	0
F5	1:2	750	0	0	0	0	0
F6	1:2	1000	0	1	0	1	0
F7	1:3	500	1	-1	1	1	-1
F8	1:3	750	1	0	1	0	0
F9	1:3	1000	1	1	1	1	1

The model was further validated for reliability by comparison of experimental values with predicted values of responses and % bias was calculated using the following formula:

$$\%\ Bias = \frac{Predicted\ value - Experimental\ value}{Predicted\ value} \times 100 \quad (2)$$

Entrapment efficiency and yield

Entrapment efficiency is important for the assessment of drug loading and drug release characteristics. Generally, increased drug loading leads to an acceleration of the drug release. Drug entrapment efficiency represents the proportion of the initial amount of drug, which has been incorporated into the microspheres.[27] The efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

$$\%\ Entrapment = \frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100 \quad (3)$$

Theoretical drug content was determined by calculation assuming that the entire drug present in the polymer solution gets entrapped into microspheres. An accurately weighed amount (50 mg) of microspheres was dissolved in 50 mL of 0.1 N sodium hydroxide solution and complete dissolution of drug was achieved by placing them in a rotary shaker bath overnight. After appropriate dilution with same solution, absorbance was measured at 304 nm using UV Spectrophotometer[28] and practical drug content was calculated using a calibration equation. The % yield was calculated by determining the weight of output (formed microspheres) against weight of employed raw materials (drug and polymer) using the formula:

$$\%\ yield = \frac{Total\ weight\ of\ microspheres}{Total\ weight\ of\ drug\ and\ polymer} \times 100 \quad (4)$$

Particle size

Determination of the mean particle size of drug-loaded microspheres was carried out by optical microscopy in which an ocular micrometre, calibrated with a stage micrometre, was utilized. A sufficient quantity of microspheres was dispersed in 1% solution of SLS in water and a few drops of this solution were spread on a clean glass slide, then observed under an optical microscope. Size of 100 microspheres was determined randomly and the appropriate mean diameter was calculated for each batch.[29]

Surface Morphology

The shape and size of microspheres were observed under the optical microscope with 10X magnification and photomicrographs were taken for comparison with different batches of microspheres. The preliminary observation of the size and surface of prepared

microspheres was done and the batches for further study were selected based on microscopic observations. Surface morphology of drug-loaded microspheres was observed by optical microscope. Photomicrographs of the dried microspheres were taken at 40X magnification. The surfaces of the microspheres were observed for surface morphology. Further, the surface morphology of selected batches was observed in the SEM photographs.[30]

Scanning electron microscopy

Scanning electron microscopy (SEM) has been used to examine the morphology of microspheres' intact and fractured or sectioned surfaces. SEM studies were carried out using an SEM (Make: Zeiss, Germany; Model: EVO-18-13-04). Samples of dried microspheres were placed on an electron microscope brass stub and coated with a sputter coater (Make: Emitech, model no. SR7620) for 4 minutes and the process current applied was 10 mA. SEM images of microspheres were taken by random scanning of the stub.[31]

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of prepared microspheres were recorded and compared with that of pure drug to evaluate possible drug-polymer interaction and structural changes during processing. FTIR spectroscopy was performed on (FTIR 8400 Spectrophotometer, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 minutes on KBr-press and the spectra were scanned in the wave number range of 4000–600 cm^{-1}. FTIR spectra of fluvastatin sodium and drug-loaded microspheres were recorded and observed to compare identical peaks of drug.[32]

Differential scanning calorimetry

The physical state of the drug in the microspheres was analyzed by differential scanning calorimeter (DSC-60, Shimadzu, Tokyo, Japan). The DSC thermograms of samples of fluvastatin sodium drug and drug-loaded microspheres were recorded using aluminum pans, heated at a scanning rate of 10°C/min, and carried out within a temperature range of 45 to 300°C.[33]

In-vitro dissolution studies

Drug release from plain drug samples, as well as microspheres, was studied by *in-vitro* dissolution test performed in basket type dissolution apparatus (USP Apparatus-I; USP, TDT-08L) using 900 mL deaerated water as dissolution medium was maintained at constant temperature 37 ± 0.5°C with constant stirring at 50 rpm. At pre-determined time intervals, aliquots of 5 mL samples were withdrawn. The volume of the dissolution medium was maintained constant by replacing the withdrawn sample with a fresh medium every time. Samples were sufficiently diluted with 0.1 N NaOH and analyzed by UV-spectrophotometer at 304 nm.[28] The amount of drug release was calculated using a calibration equation, and the cumulative %drug release was calculated, plotted against time to generate the dissolution profile.

Results and Discussion

Emulsion solvent evaporation is one of the promising techniques used in the preparation of microspheres. The technique involved the formation of emulsion followed by evaporation of solvent from internal phase which solidifies the internal phase containing drug and polymer to convert them into microspheres.[34]

The present study involved the dispersion of drug and polymer in organic solvent which can be easily evaporated. The preliminary studies were performed to select the operating range for the stirring speed and other parameters. Drug-loaded microspheres were initially prepared at different stirring speed and size of prepared microspheres was measured. It was found that particle size decreased with increased stirring speed up to 1000 rpm. Below 500 rpm speed, there was a problem of sticking and above 1000 rpm, aggregation occurs. From this, it was concluded that the size of microspheres was satisfactory between stirring speeds of 500–1000 rpm. Preliminary trials also suggested a significant influence of polymer concentration on the quality of microspheres. Based on the literature survey and preliminary trials, the drug-to-polymer ratio was selected in the range of 1:1 to 1:3. Microspheres in all the factorial design batches were observed and found spherical in shape without aggregation, making them free-flowing.

Microspheres formed were spherical in shape with smooth surfaces as observed under a microscope and portrayed in photomicrographs (Fig. 1). These can also be correlated with flow characteristics of microspheres and could be confirmed with SEM photographs (Fig. 2). As depicted in SEM photographs, microspheres possess spherical shape with smooth surfaces.

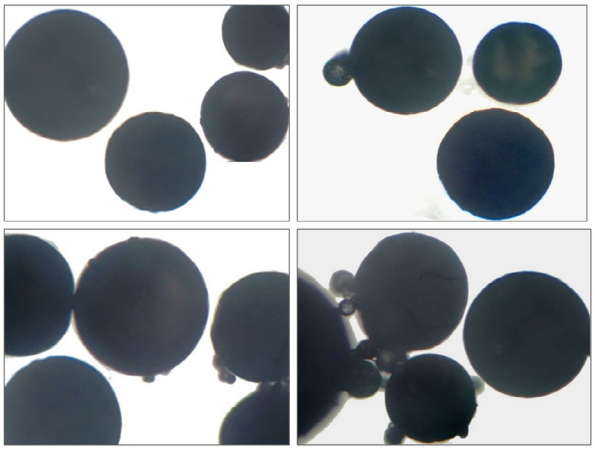

Fig. 1: Photomicrographs of microspheres

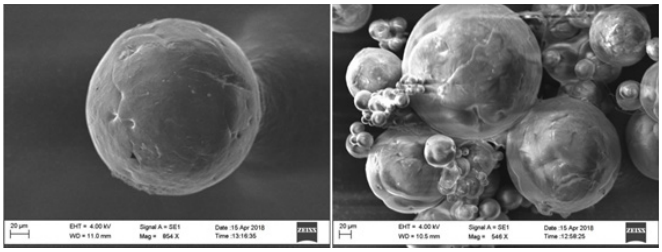

Fig. 2: SEM Photographs of microspheres

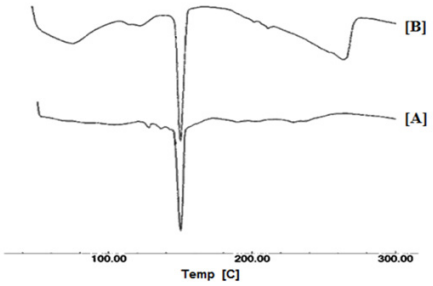

Fig. 3: DSC thermogram of [A] fluvastatin sodium drug; and [B] drug loaded microspheres

DSC thermograms of fluvastatin sodium drug and drug-loaded microspheres were shown in Fig. 3. As observed endothermic peaks corresponding to the melting point of the drug in all thermograms were in close proximity to each other, there was no significant change in thermal behavior of the drug in the presence of polymer as well as formulation and processing parameters. This suggests that there was no significant impact of the polymer used or the formulation and processing parameters on the drug's thermal behavior.

Fig. 4 depicts FTIR spectra of fluvastatin sodium drug and drug-loaded microspheres. The characteristic peaks of fluvastatin sodium in FTIR spectra were compared with that of the physical mixture of the drug with polymer and drug-loaded microspheres. FTIR spectra of pure drug showed the peaks for corresponding functional groups at wave numbers 1155.36 cm^{-1} (C-O stretching), 1215.15 cm^{-1}, (C-N), 1581.63 cm^{-1} (C=O stretching), 3419.79 cm^{-1} (very broad, O-H stretch), 966.34 cm^{-1} (aryl-F), while that of drug loaded microspheres showed characteristic peaks at wave numbers 1153.43 cm^{-1} (C-O stretching), 1213.23 cm^{-1}, (C-N), 1581.63 cm^{-1} (C=O stretching), 3429.43 cm^{-1} (very broad, O-H stretch), 964.41 cm^{-1} (aryl-F). The characteristic peaks of pure drug in all FTIR spectra indicated no chemical interaction between fluvastatin sodium with PLGA and other materials used in processing.

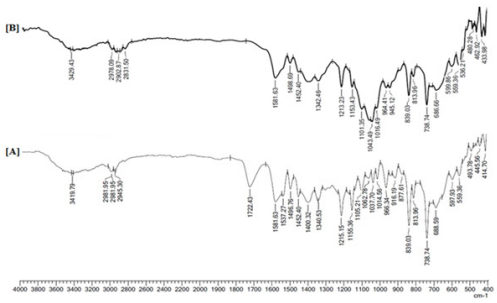

Fig. 4: FTIR spectra of [A] fluvastatin sodium drug; and [B] drug loaded microspheres

The data of the experimental run of factorial batches were analyzed by Design Expert® software. Table 2 shows predicted values calculated from the obtained model and actual values measured through experiments for various dependent variables. The lower values for %bias were obtained due to close agreement between actual and predicted values indicating the validity of the model. The results showed that the model was highly accurate and reliable. The model provided a good approximation of actual values and was able to predict the experimental outcome very accurately. The mathematical relationship indicating the quantitative influence of factors (independent variables) on responses (dependent variables) generated using multiple linear regression analysis could be adequately characterized by the following polynomial equations:

Table 2: Experimental and predicted values for responses

Batch	*Entrapment efficiency (%)*			*Particle size (µm)*			Q_{1hr} *(%)*			$t_{80\%}$ *(min)*		
	Actual	*Pred*	*% Bias*	*Actual*	*Pred*	*% Bias*	*Actual*	*Pred*	*% Bias*	*Actual*	*Pred*	*% Bias*
F1	67.7	68.42	1.05	213.84	209.94	-1.86	18.56	19.55	5.04	253	252.58	-0.16
F2	65.2	64.81	-0.60	199.32	212.25	6.09	21.27	20.62	-3.14	235	223.33	-5.22
F3	63.1	62.77	-0.53	193.75	184.72	-4.89	23.31	22.97	-1.48	198	210.08	5.75
F4	78.6	77.78	-1.06	266.36	261.66	-1.80	15.45	13.82	-11.77	373	391.00	4.60
F5	74.2	73.74	-0.62	275.57	274.73	-0.31	14.36	14.96	4.03	354	343.00	-3.21
F6	70.0	71.28	1.79	252.43	257.98	2.15	16.35	17.37	5.89	318	311.00	-2.25
F7	85.6	85.70	0.12	299.63	308.23	2.79	8.45	9.09	7.05	528	510.42	-3.44
F8	80.4	81.24	1.04	344.16	332.08	-3.64	10.25	10.29	0.42	421	443.67	5.11
F9	79.3	78.35	-1.21	322.61	326.09	1.07	13.45	12.77	-5.36	398	392.92	-1.29

Q_{1hr} = drug released in 1 hr; $t_{80\%}$ = time to release 80% of drug; Pred = Predicted

Entrapment efficiency

$(Y1) = + 73.744 + 8.217\ X1 - 3.25\ X2 - 0.425\ X1\ X2 - 0.717\ X1^2 + 0.783\ X2^2$ (5)

$\text{Particle Size}\ (Y2) = + 274.73 + 59.915\ X1 - 1.84\ X2 + 10.768\ X1\ X2 - 2.568\ X1^2 - 14.913\ X2^2$ (6)

$Q_{1hr}\ (Y3) = + 14.963 - 5.165\ X1 + 1.775\ X2 + 0.0625\ X1\ X2 + 0.495\ X1^2 + 0.635\ X2^2$ (7)

$t_{80\%}\ \text{release}\ (Y4) = + 343 + 110.167\ X1 - 40\ X2 - 18.75\ X1\ X2 - 9.5\ X1^2 + 8\ X2^2$ (8)

A conclusion about the effect of variables on responses can be drawn using the above equations considering the magnitude and mathematical sign of the coefficients. The positive or negative sign of the coefficients is an indication of whether the effect of factors on responses is synergistic or antagonistic, respectively. Response surface plots and contour plots can demonstrate effect of independent variables on responses. Response surface and contour plots for all responses were generated using Design Expert® software and represented in Figs 5 and 6, respectively.

Entrapment efficiency is important for the assessment of drug loading in microspheres and is dependent on several formulation and process variables. As shown in Table 2, the percentage of drug entrapment in microspheres was found in the range of 63 to 86% and the percentage yield of microspheres was The entrapment efficiency and yield of microspheres were found to be dependent on amount of polymer as indicated by higher values for both at higher polymer concentrations. The results of this study suggest that the amount of polymer and stirring speed significantly influence the entrapment efficiency and yield of microspheres.

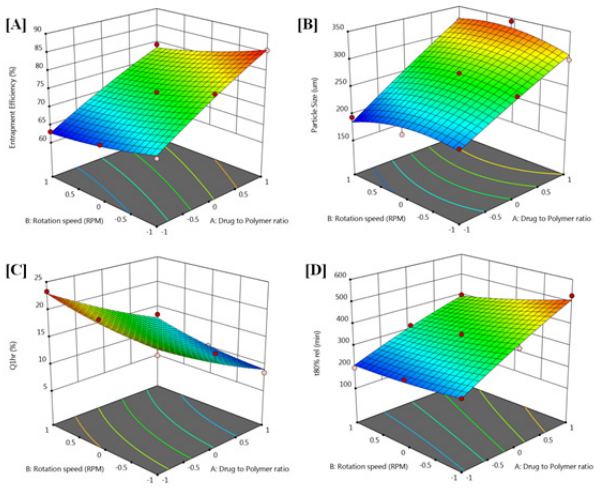

Fig. 5: Response surface plots showing influence of selected variables on [A] entrapment efficiency; [B] particle size; [C] drug release in 1 h; and [D] time required for 80 % drug release

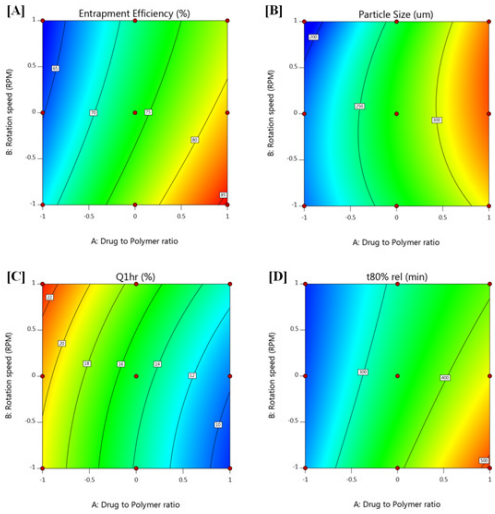

Fig. 6: Contour plots showing relationship between various levels of independent variables with [A] entrapment efficiency; [B] particle size; [C] drug release in 1 h; and [D] time required for 80 % drug release

Table 3 summarizes regression analysis and imparts the magnitude of the synergistic/antagonistic effect of independent variables on responses. As revealed in equation (5), the positive value for the co-efficient of X1 suggested the synergistic effect of the drug-to-polymer ratio on drug entrapment, while the negative value for the co-efficient of X2 suggested the antagonistic effect of stirring speed. The equation also indicated that the effect of the drug-to-polymer ratio is more significant compared to stirring speed. Thus, an increase in polymer concentration resulted in increased drug entrapment which can be attributed to reduced diffusion of drug from microspheres at higher polymer concentration.[1] Furthermore, the higher polymer concentrations can create a more stable matrix for drug entrapment, thus enabling better drug entrapment within microspheres. Higher polymer concentrations also create a more robust matrix, leading to higher yields of microspheres.[35] Further, an increase in stirring speed caused a reduction in particle size and increased surface area resulting in more diffusion of the drug and ultimately decreased drug entrapment. This is because the increased stirring speed caused the polymeric particles to break down into smaller pieces, increasing their surface area and allowing for more

Table 3: Summary of regression analysis

Coefficient	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
Entrapment efficiency (%)	73.74	8.22	-3.25	-0.425	-0.717	0.783
Particle size (µm)	274.73	59.92	-1.84	10.768	-2.568	-14.913
Q_{1hr} (%)	14.96	-5.17	1.775	0.0625	0.495	0.635
$t_{80\%\ (min)}$	343.00	110.17	-40	-18.75	-9.5	8

Q_{1hr} = drug released in 1 hr; $t_{80\%}$ = time to release 80% of drug

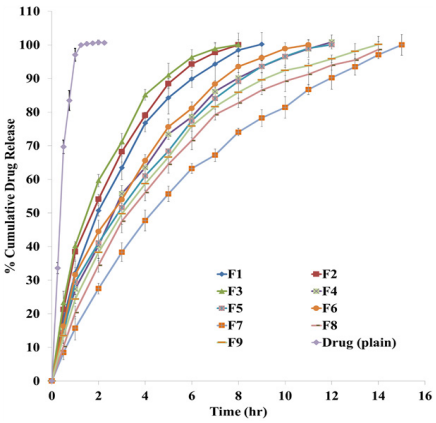

Fig. 7: *In-vitro* drug release profiles of plain drug and various factorial batches

of the drug to diffuse into the particles. Due to this, less drug got entrapped in microspheres.[36] As displayed in Figs 5[A] and 6[A], the response surface and contour plot clearly exhibited non-linear dependency of entrapment efficiency on each variable. The findings are similar to that of reported previously.[37,38]

The particle size of microspheres was determined in terms of mean diameter using the optical microscopy method and depicted in Table 2. Size of microspheres varied between 193 to 345 µm. The results indicated a profound effect of the drug-to-polymer ratio on particle size. An increase in the amount of polymer resulted in an increase in particle size, which could be attributed to the formation of larger globules due to higher viscosity of fluid.[39] The increase in particle size was also attributed to the increased coalescence of globules due to the lower surface tension caused by the increased amount of polymer.[40] On the other hand, microspheres with smaller size were formed at higher stirring speed compared to slower stirring.[41] Further, the combined effect of both factors can be predicted from coefficients in polynomial equations.

Equation (6) suggested the synergistic effect of X1 (drug-to-polymer ratio) and the antagonistic effect of X2 (stirring speed) on particle size. However, higher values of the co-efficient of X1 compared to that of X2 indicated a more intense effect of polymer concentration on particle size than that of stirring speed. Thus, particle size increased with an increase in polymer concentration and a decrease in stirring speed. These findings can be correlated with larger droplet formation during emulsification due to higher viscosity at higher polymer concentrations. At higher stirring speed, droplet size of the emulsion is reduced resulting in the formation of microspheres with smaller size.[42] The smaller size was attributed to the increased shear force which caused the globules to break up into smaller droplets. The increased rate of coalescence of these droplets further enhanced this phenomenon.[43] Figs 5[B] and 6[B] also exhibited a non-linear trend of particle size with increments of each variable. These also indicated that drug-to-polymer ratio was more influenced by particle size than stirring speed.

The drug release profiles of plain drug and various formulations of microspheres are shown in Fig. 7. The drug release rate from microspheres directly depends on the polymer concentration. As revealed from dissolution profiles, drug release seems to be slow with negligible burst effect. The time required to release nearly 100% of the drug from microspheres ranges from 8 to 15 hours, indicating sustained release of the drug compared to plain drug, which released nearly 100% within 1.25 hours. Further, $t_{80\%}$ (time required to release 80% of the drug) is given in Table 2, which shows the significant influence of the amount of polymer on release rate of the drug from microspheres.[40]

Antagonistic effect of X1 (drug to polymer ratio) and synergistic effect of X2 (stirring speed) on Q_{1hr} (drug release in 1-hour) were revealed from the co-efficient in equation (7), suggesting dependency of drug release on polymer concentration and stirring speed. Similarly, equation (8) indicated a synergistic effect of X1 (drug-to-polymer ratio) and an antagonistic effect of X2 (stirring speed) on $t_{80\%}$ (time to release 80% of drug). Moreover, both equations suggested a greater influence of polymer concentration on drug release compared to stirring speed. The findings are also supported by response surface and contour plots (Fig. 5C,D, and 6C,D) which indicated non-linear dependency of drug release on selected variables. These results designated substantial potential of polymer to retard drug release from microspheres. As stirring speed increased, Q_{1hr} increased while $t_{80\%}$ decreased. These can be attributed to the effect of stirring speed on

Table 4: ANOVA results for full quadratic model for all responses

Response	*SS*	*df*	*MS*	*F-value*	*p-value*	*SD*	*Mean*	*CV*	R^2	*Adj-*R^2	*Pred-*R^2	*Adeq Precision*
Entrapment efficiency	471.43	5	94.29	57.55	0.0035	1.28	73.79	1.73	0.9897	0.9725	0.8789	21.943
Particle size	22480.92	5	4496.18	24.55	0.0123	13.53	263.07	5.14	0.9761	0.9364	0.7094	13.334
$Q_{1\,hr}$	180.28	5	36.06	16.79	0.0211	1.46	15.72	9.32	0.9655	0.9080	0.6002	11.600
$t_{80\%}$	84134.92	5	16826.98	31.06	0.0087	23.27	342.00	6.81	0.9811	0.9495	0.7841	15.804

SS = sum of square; df = degree of freedom; MS = mean of square; SD = standard deviation;
CV = coefficient of variation; Adj-R^2 = adjusted R^2; Pred- R^2 = predicted R^2; Adeq = adequate;
Q_{1hr} = drug released in 1 hr; $t_{80\%}$ = time to release 80% of drug

the particle size of microspheres. At higher stirring speed, particle size decreased, resulting in increased surface area exposed to the drug's dissolution, which consecutively increased rate of drug release from microspheres, resulting in a shortening of $t_{80\%}$ and an increase in Q_{1hr}.[44] On the other hand, an increase in polymer concentration leads to increased matrix formation within microspheres, resulting in increased path length for the diffusion of drug and thereby reducing the rate of drug release from microspheres. Moreover, drug release from microspheres was also influenced by particle size, which depends on polymer concentration. As polymer concentration increased, particle size also increased and thereby resulted in decreased surface area available for diffusion and dissolution of drug, leading to retarded release of drug.[45] A similar conclusion can also be drawn from Fig. 7, depicting the *in-vitro* drug release profile.

The analysis of variance (ANOVA) was performed to estimate the model's significance. Statistical models for each response were generated and tested for significance. ANOVA results are shown in Table 4 where the model F-values for Y1, Y2, Y3 and Y4 were 57.55, 24.55, 16.79 and 31.06, respectively. The large F-values for all dependent variables indicated that the fit of a model to the data was significant. This indicates that the model is statistically significant and can be used to predict the dependent variables. The R^2 value for all variables were >0.96 indicating a good correlation. Moreover, Adj-R^2 and Pred-R^2 values were in good agreement and suggested that the used mathematical model had described data adequately. The results of the model showed that the data was properly described and was statistically significant. Thus, the model can be used for predictive purposes with high accuracy. Results also indicated that the model generated was significant at $p<0.05$ for all variables.

Conclusion

Biodegradable microspheres for sustained release of fluvastatin sodium were prepared using PLGA 50:50 as a biodegradable polymer by emulsification solvent evaporation technique and optimized by 3^2 full factorial design. The independent variables (drug-to-polymer ratio and stirring speed) were found to significantly affect particle size, entrapment efficiency and drug release from microspheres. Statistical analysis of data suggested a greater influence of polymer concentration compared to stirring speed. Observed responses of optimized formulation were in close agreement with the predicted value, indicating excellent predictability of the optimization procedure. FTIR and DSC analysis showed compatibility without any significant interaction between drug and polymer, while sphericity and surface smoothness were revealed in SEM photographs. Depending on polymer concentration, microspheres showed prolonged release of the drug up to 15 hours. The approach can be utilized as an alternative to conventional delivery of drug for prolonged duration of action.

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