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Original Article

STUDIES IN OXCARBAZEPINE MICROSPHERES EMPLOYING PLACKETT AND BURMAN DESIGN

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ABSTRACT

Objective: The present work was aimed to screen material and processing parameters affecting encapsulation efficiency and drug release from microspheres.

Methods: Oxcarbazepine loaded microspheres were prepared employing multiple emulsion solvent evaporation technique. Ratio of chitosan to ethyl cellulose, ratio of drug to polymer, stirring speed, ratio of dichloromethane to methanol, amount of Span 80 and the volume of aqueous phase were selected as independent variables in the Plackett and Burman design. The microspheres were characterized for percentage yield, percentage encapsulation efficiency, particle size distribution and in vitro drug release.

Results: The critical material and processing parameters affecting encapsulation efficiency were chitosan to ethyl cellulose ratio, volume of water, stirring speed and drug to polymer ratio. Initial burst release was affected by volume of water, temperature, dichloromethane to methanol ratio, amount of Span 80 and drug to polymer ratio. FTIR study showed compatibility of the drug with excipients.

Conclusion: The outcome of the study shall be used to calculate risk priority number (RPN) and for devising suitable control strategies for the critical factors at industry.

Keywords: Microspheres, Multiple emulsion solvent evaporation technique, Modified release, Plackett Burman design.

INTRODUCTION

The dosage forms that can accurately control the drug release rates and/or target the drug to specific body site will remain in high demand in future. Multiparticulate drug delivery systems (microspheres) are develop to modify drug release and for improvement of bioavailability. Microspheres can also reduce side effects, decrease dosing frequency and improve patient compliance [1, 2]. Chitosan is composed of β-(1–4)-2-deoxy-2-amino-D-glucopyranose units and of β-(1–4)-2-deoxy-2-acetamino-D-glucopyranose units [3]. Chitosan is obtained by alkaline deacetylation of chitin [4]. Chitin is obtained from protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *aspergillus* and *mucor*. Chitin is composed of β-(1,4)-linked *N*-acetyl-glucosamine units while chitosan comprises of copolymers of glucosamine and *N*-acetyl-glucosamine [5].

Chitosan has been extensively used in pharmaceutical industry for the development of modified release formulation because it is hydrophilic, nontoxic and biodegradable [6-8]. Presence of free amino groups in chitosan leads to quick solubilization in acidic media and fast release of drug. The drug release can be minimized by chemical cross-linking with aldehydes. The toxicity of aldehyde limits its use. Hydrophobic polymers can be used in combination with chitosan to modulate drug release.

Ethyl cellulose (EC) is a water insoluble polymer with excellent safety records and global compendial acceptance. It can be used for modulating drug release by coating compressed tablets or microspheres, for taste masking and even as a binder [9-11].

Oxcarbazepine, an antiepileptic drug, is used in the treatment of partial seizures and generalized tonic-clonic seizures in adults and children. Immediate release of oxcarbazepine leads to side effect such as dizziness, drowsiness, fatigue, nausea, vomiting, headache, sleeping trouble, acne, dry mouth or constipation. Short half life (about 2 h) requires frequent administration of dosage form to maintain therapeutic level of drug in body [12]. Hence, Oxcarbazepine was selected as a model drug to design modified release microspheres.

In the present investigation chitosan and ethyl cellulose were used in combination to modify oxcarbazepine release. The concept of design of experiment was employed for screening of factors. Plackett and Burman design is the best reported method for screening [13]. The basic objective of quality by design (QbD) is to develop safe, effective and customer friendly dosage form.

MATERIALS AND METHODS

Materials

Oxcarbazepine was obtained as gift sample from Torrent research centre, India. Chitosan (degree of deacetylation 85%, intrinsic viscosity 400 mPas for 1% solution in 1% aqueous acetic acid at 20°C) was obtained as gift sample from Fisaris industry, Kochi, India. The following chemicals were obtained from commercial suppliers and used as received: Ethyl cellulose (EC) (Ethocel 10) was purchased from Dow Chemical company, Switzerland; methylene chloride was purchased from RFCL, New Delhi, India; Span 80 was purchased from Loba Chemie, India; Sodium dodecyl sulfate (SDS) and acetic acid were purchased from S.D. fine chemicals, Mumbai, India.

Preparation of microspheres

Chitosan and ethyl cellulose microspheres were prepared by the multiple emulsion solvent evaporation technique [14]. Chitosan solution was prepared by dissolving chitosan in dilute acetic acid solution (2% v/v). Oxcarbazepine was dispersed in chitosan solution. Ethyl cellulose was dissolved in methylene chloride or mixture of methanol and methylene chloride containing Span 80. Chitosan solution containing drug was added drop by drop to the ethyl cellulose solution with continuous stirring on a magnetic stirrer.

The primary emulsion was then added to water phase and stirred continuously till organic solvent was evaporated. The microspheres were filtered and dried. Batches were prepared by using seven factors according to design layout shown in Table 1. Levels of factors were selected based on preliminary trials. Each batch was prepared in duplicate.

Table 1: Experimental design for various batches

Formulation	X_1	X_2	X_3	X_4	X_5	X_6	X_7
P1	0.50	01:03	1200	01:00	400	0.5	30
P2	0.25	01:03	1200	01:01	200	1	30
P3	0.25	01:01	1200	01:01	400	0.5	40
P4	0.50	01:01	500	01:01	400	1	30
P5	0.25	01:03	500	01:00	400	1	40
P6	0.50	01:01	1200	01:00	200	1	40
P7	0.50	01:03	500	01:01	200	0.5	40
P8	0.25	01:01	500	01:00	200	0.5	30

X_1: Chitosan to ethyl cellulose ratio (%), X_2: Drug to polymer ratio, X_3: Stirring speed (rpm), X_4: Dichloromethane to methanol ratio, X_5: Volume of water (ml), X_6: Amount of Span 80 (%), X_7: Temperature (°C)

Evaluation of microspheres

Drug excipients compatibility studies:

Fourier transform infrared spectroscopy (FTIR) (FTIR 8400S, Simadzu) spectra of drug, polymer, physical mixture of drug and polymer were captured. Discs consisting of 2 mg of the sample and 100 mg of potassium bromide (KBr) were prepared. Spectra were scanned between 3900 and 450 cm^{-1}.

Percentage yield

The yield was calculated by dividing the measured weight of microspheres by the total weight of all non-volatile components. The % yield of microspheres was calculated using following formula [15]:

$$\% \text{ Yield} = \frac{\text{Weight of microspheres}}{\text{Weight of Polymer + Weight of drug}} \times 100 \quad (1)$$

Percentage encapsulation efficiency

The drugs loaded microspheres (15 mg) were dissolved in the methanol. The solution was filtered through Whatman filter paper. The samples were appropriately diluted and analysed by UV spectroscopic (UV-1700, Simadzu) method at a wavelength of 254 nm [16]. The encapsulation efficiency was calculated from the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Weight of drug in microspheres}}{\text{Weight of drug initially added}} \times 100 \quad (2)$$

In vitro drug release study

Oxcarbazepine release from the microspheres was carried out as per the US FDA method using USP type II (Paddle type) dissolution apparatus. Hypromellose capsule containing microspheres, equivalent to 150 mg oxcarbazepine, was added with sinkers in 900 ml 0.3% sodium dodecyl sulphate (SDS) solution. The dissolution medium was stirred at 60 rpm and was maintained at 37±0.5°C. Samples were withdrawn periodically and same volume was replaced with fresh dissolution medium. The samples were filtered and analysed using UV spectroscopic method at a wave length of 256 nm[17].

Size analysis and morphology

The formations of microspheres were monitored during preparation steps by an optical microscope with transmitted light at 40 X magnification. The mean particle size of microspheres was calculated by measuring size of hundred particles with the help of a calibrated ocular micrometer [18]. The shape and surface morphologies of the selected batch microspheres was examined using scanning electron microscope (SEM). SEM images of the microspheres were recorded using a Philips FE1 scanning electron microscope at the required magnification.

RESULTS

Multiparticulate sustained release system offers advantages like no dose dumping, minimal potential side effect and low inter person variability. Chitosan, a positively charged biodegradable polymer, was used to formulate oxcarbazepine microspheres. Chitosan microspheres showed rapid drug release in acidic dissolution medium. To retard the drug release, ethyl cellulose coated chitosan microspheres were prepared according to method described by the researchers [19]. The encapsulation efficiency was decreased when an effort was made to coat the chitosan microspheres with ethyl cellulose. The reason for this occurrence could be formation of few blank ethyl cellulose microspheres during the process. To address this problem, chitosan ethyl cellulose complex microspheres were prepared by selecting multiple emulsion solvent evaporation technique [14]. Preliminary trials showed that the aggregates were formed. Efforts were made to stabilize primary emulsion because it has been reported that stability of emulsion affects the morphology and porosity of microspheres and there by affect encapsulation and drug release [20]. Drug encapsulation and release properties were affected by various other factors like drug to polymer ratio, amount of external phase and internal phase, amount of surfactant, temperature, stirring speed and type of organic solvent [21-24]. Seven factors were selected for screening i.e. chitosan to ethyl cellulose ratio, drug to polymer ratio, stirring speed, dichloromethane to methanol ratio, amount of Span 80 and volume of water phase. The design layout is shown in Table 1. The levels of these factors were decided by preliminary trials or from literature survey. Percentage encapsulation efficiency and burst release were selected as dependent variable.

Drug excipients compatibility studies

FTIR of oxcarbazepine (Figure 1) shows that drug is in crystalline form and the characteristic peaks of drug are shown at 3469.70 cm^{-1} and 3342.41 cm^{-1}. The physical mixture of chitosan and drug showed similar peaks while physical mixture of ethyl cellulose and drug showed 3469.70 cm^{-1} peak was slightly sifted to 3467.77 cm^{-1}.

Percentage yield

The % yield was varied from 70.00 to 93.40%. For all the investigated factors the p value > 0.05.

Influence of investigated parameters on % encapsulation efficiency (Y_1)

Encapsulation efficiency varied from 75.00% to 99.90%. The result shown in Table 2 and Figure 2 depicts that significant factors affecting encapsulation efficiency were chitosan to ethyl cellulose ratio (X_1), volume of water (X_5), stirring speed (X_3) and drug to polymer ratio (X_2).

Influence of investigated parameters on burst release (Y_2)

The in vitro release of oxcarbazepine in 0.3% SDS (Figure 3) showed biphasic release pattern, i.e. initial burst release followed by slow release. The burst effect was probably due to quick release of drug present on the surface of microspheres. The result shown in Table 2 and Figure4 depicts that significant factors affecting burst release were volume of water (X_5), temperature (X_7), dichloromethane to methanol ratio (X_4), amount of Span 80 (X_6) and drug to polymer ratio (X_2).

DISCUSSION

Drug excipients compatibility studies

Stability of formulation based on selection and quality of raw material hence it is advisable to check drug excipient compatibility. From the result of FTIR we can conclude that there is no interaction between drug and polymers.

Percentage yield

Any process can be economic to be used for industrial scale production only if yield is high. The p value > 0.05 shows that the % yield was not significantly affected by investigated factors.

Influence of investigated parameters on % encapsulation efficiency (Y_1)

It is worth while to note that design of experiment is an important element of QbD. In order to identify critical material attributes (CMA) and critical process parameters (CPP), multiple regression analysis was carried out. A linear model describing the relationship between the independent variables and % encapsulation efficiency was evaluated.

$$Y_1 = 89.03 - 6.32X_1 - 4.20X_2 - 5.08X_3 + 0.77X_4 + 5.30X_5 + 0.23X_6 - 1.08X_7 \quad (3)$$

The effect plot for encapsulation efficiency (Figure2) shows the impact of evaluating factors on encapsulation efficiency. Positive value for a coefficient indicates that the independent variable favours the response and a negative value indicates an inverse relationship between response and factor [25]. Chitosan to ethyl cellulose ratio (X_1) showed negative effect on % encapsulation efficiency. It is worth while to note that chitosan enhances the dissolution of oxcarbazepine [26].

The probable reason for decrease in encapsulation efficiency is dissolution enhancement of oxcarbazepine in water phase. The volume of water (X_5) has positive effect on drug encapsulation efficiency.

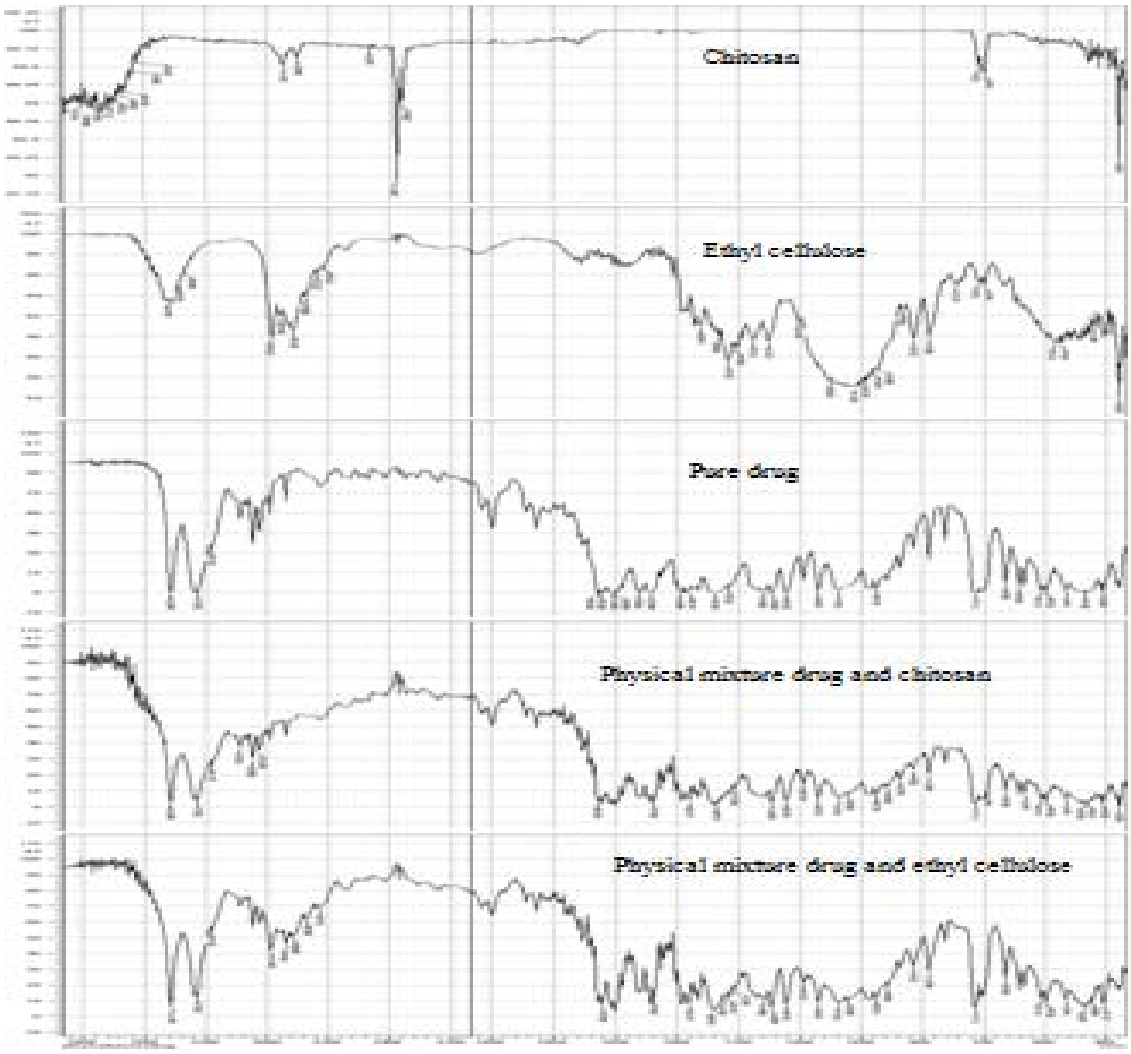

Fig. 1: FTIR of chitosan, ethyl cellulose, pure drug and physical mixture of drug and chitosan and physical mixture of drug and ethyl cellulose

Table 2: Statistical analysis of encapsulation efficiency (Y_1) and burst release (Y_2)

Factor	Encapsulation efficiency (Y_1)		Burst effect (Y_2)	
	Coefficient	*p*- value	Coefficient	*p*- value
Chitosan to Ethyl cellulose ratio (X_1)	-6.3166	0.0005	0.7987	0.4986
Drug to Polymer ratio (X_2)	-4.1953	0.0061	-2.7362	0.0413
Stirring speed (X_3)	-5.0803	0.0021	-1.4675	0.2291
Dichloromethane to Methanol ratio (X_4)	0.7703	0.517	-3.1812	0.0224
Volume of water (X_5)	5.3034	0.0016	-5.1125	0.0019
Amount of Span 80 (X_6)	0.2253	0.8478	-3.0575	0.0265
Temperature (X_7)	-1.0784	0.3704	3.6188	0.0124

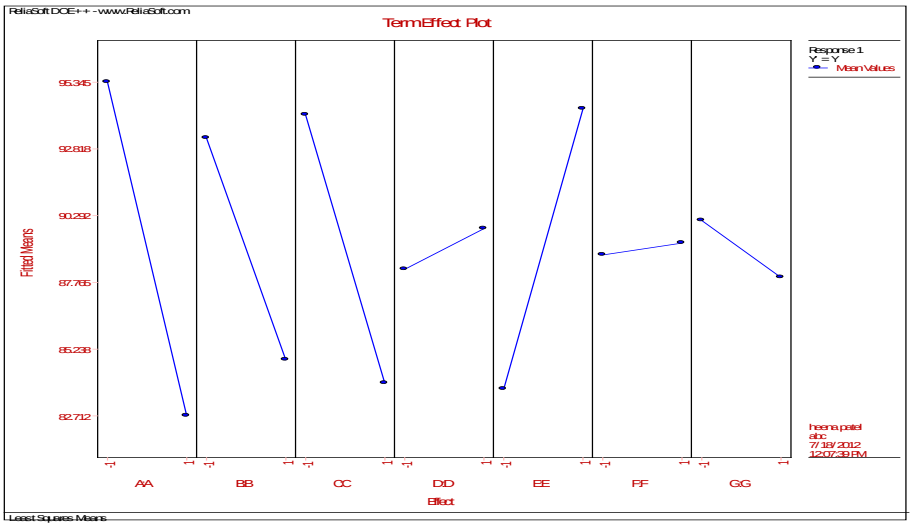

Fig. 2: Term effect plot for % encapsulation efficiency

A:A= Chitosan to ethyl cellulose ratio, B:B= Drug to polymer ratio, C:C= Strring speed, D:D= Dichloromethane to methanol ratio, E:E= Volume of water, F:F= Amount of Span 80, G:G= Temperature

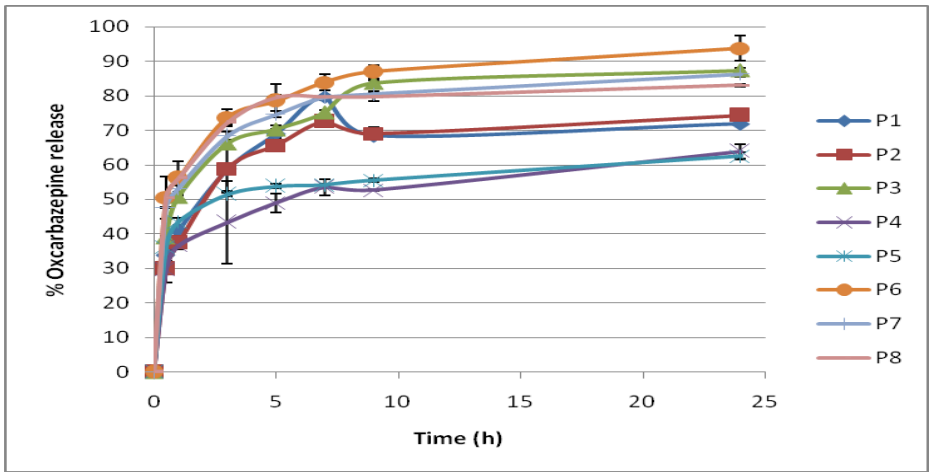

Fig. 3: Mean percentage oxcarbazepine release (n=3)

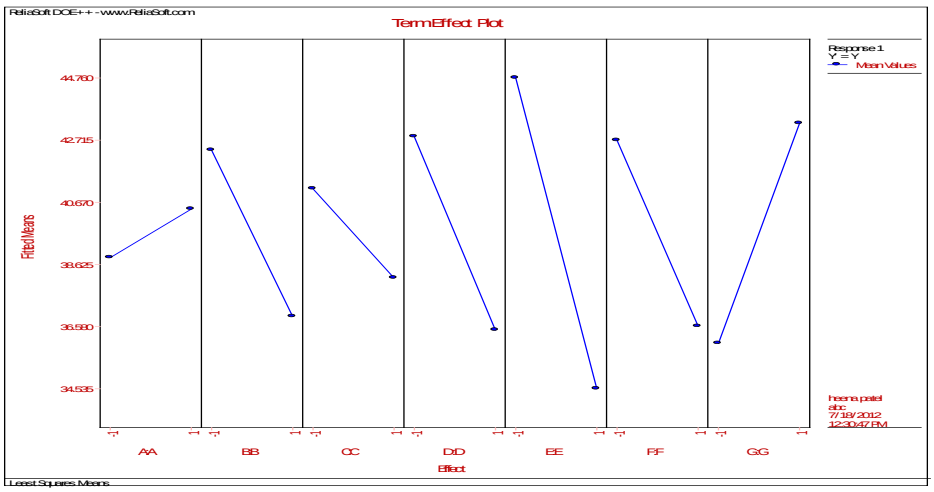

Fig. 4: Term effect plot for burst release at 0.5 h

A:A= Chitosan to ethyl cellulose ratio, B:B= Drug to polymer ratio, C:C= Strring speed, D:D= Dichloromethane to methanol ratio, E:E= Volume of water, F:F= Amount of Span 80, G:G= Temperature

Size analysis and morphology:

Figure 5 shows the particle size distribution of the eight batches of microspheres. Microspheres were poly dispersed. Particle size ranged from 13.3 to 159.6 µm. The p value for all the independent variable is >0.05. SEM study of microspheres is shown in Figure 6.

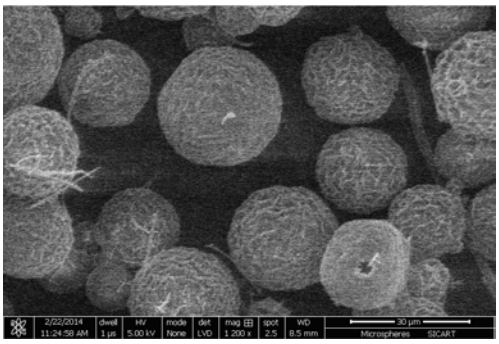

Fig. 5: Particle size distribution of oxcarbazepine microspheres

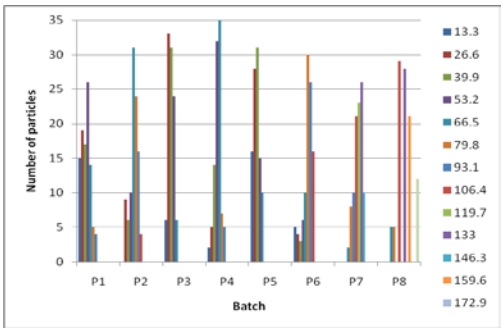

Fig. 6: Scanning electron micrographs of microspheres (batch P6)

This may be due to quick solidification of polymer, which prevents diffusion of drug to the external phase. Stirring speed (X_3) has negative effect on encapsulation efficiency. As the stirring speed increases, the mean particle size of microspheres decreases which leads to increases in surface area. The final impact is increased drug diffusion in continuous phase during microspheres preparation. It is reported that factors favouring the increase of mean size could increase encapsulation efficiency due to a reduced surface area [27]. The drug to polymer ratio (X_2) has negative effect on encapsulation efficiency. It may be due to increase in chitosan amount as the polymer increase. From the result it was found that the dichloromethane to methanol ratio (X_4), amount of Span 80 (X_6) and temperature (X_7) had no significant effect ($p > 0.05$) on % encapsulation efficiency of microspheres.

Influence of investigated parameters on burst release (Y_2)

A linear model describing the relationship between the independent variable and burst drug release at 0.5 h was evolved.

$$Y_2 = 39.65+0.80X_1-2.74X_2-1.47X_3-3.18X_4-5.11X_5-3.06X_6+ 3.62X_7 \quad (4)$$

The effect plot for burst release (Figure4) shows the impact of evaluating factors on burst release. The significant factors (*p value < 0.05**) affecting burst release were volume of water (X_5), temperature (X_7), dichloromethane to methanol ratio (X_4), amount of Span 80 (X_6) and drug to polymer ratio (X_2) (Table 2). The volume of water used in preparation of microspheres has negative effect on burst release. Quick diffusion of organic solvent in the outer phase and rapid solidification results in decreased burst release. Rapid evaporation of solvent at higher temperature may results in increased porosity of microspheres and hence quick drug release. Due to the formation of stable primary emulsion in presence of methanol, the drug might be well entrapped in the microspheres and burst drug release was retarded. Also, miscibility of methanol in external phase leads to fast solidification of polymer. Span 80 as a surfactant increases primary emulsion stability and therefore the effect is similar to previous factor. From the result it was found that the chitosan to ethyl cellulose ratio (X_1) and stirring speed (X_3) had no significant effect (p value > 0.05) on burst release from microspheres.

Size analysis and morphology

The p value for all the independent variable showed that no factor had significant effect on size of microspheres. SEM study of microspheres (Figure 6) show that microspheres were spherical with smooth surface.

CONCLUSION

Oxcarbazepine loaded chitosan and ethyl cellulose microspheres, prepared without using cross-linking agent, showed acceptable physical properties and bi-phasic drug release. The use of design of experiment is demonstrated to meet the primary requirements of quality by design (QbD). The study indicates the use of Placket and Burman Design to identify the important variables for further optimization. The volume of water and temperature were selected as most important factors affecting the formulation of Oxcarbazepine microspheres. Further optimization can be done using factorial, box behnkem or central composite design.

CONFLICT OF INTEREST STATEMENT

I do not have any financial or personal relationship exist that may create conflict of interest or any bias in my work.

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