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Preparation and evaluation of nanoparticles of gum cordia, an anionic polysaccharide for ophthalmic delivery

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ABSTRACT

The purpose of present investigation was to prepare nanoparticles using gum cordia as the polymer and to evaluate them for ophthalmic delivery of fluconazole. A w/o/w emulsion containing fluconazole and gum cordia in aqueous phase, methylene chloride as the oily phase, and di-octyl sodium sulfosuccinate and polyvinyl alcohol as the primary and secondary emulsifiers, respectively, was cross-linked by ionic gelation technique to produce fluconazole-loaded nanoreservoir system. The formulation of nanoparticles was optimized using response surface methodology. Multiple response simultaneous optimizations using the desirability approach were used to find optimal experimental conditions. The optimal conditions were found to be concentrations of gum cordia (0.85%, w/v), di-octyl sodium sulfosuccinate (9.07%, w/v) and fluconazole (6.06%, w/v). On comparison of the optimised nanosuspension formulation with commercial formulation, it was found to provide comparable *in vitro* corneal permeability of fluconazole across isolated goat cornea, indicating suitability of nanosuspension formulation in ophthalmic delivery of fluconazole.

1. Introduction

Natural polysaccharides represent a group of polymers, having excellent biocompatibility, biodegradability, stability and adequate aqueous solubility. Due to these outstanding merits, they have been used extensively in design of drug delivery systems (Bhardwaj, Kanwar, Lal, & Gupta, 2000; Liu, Jiao, Wang, Zhou, & Zhang, 2008). Natural polysaccharide based hydrophilic nanoparticles have been investigated to deliver a wide range of therapeutic agents by oral, intravenous and topical administration (Cui & Mumper, 2001; Sinha & Kumria, 2001). Among the various polymers investigated so far, chitosan and its derivatives, alginate, dextran, pullulan, hyaluronic acid and chondroitin sulfate are prominent (Bodnar, Hartmann, & Borbely, 2005; Zahoor, Sharma, & Khuller, 2005; Fuente, Seijo, & Alonso, 2008). Gum cordia is one such polymer, which has an excellent potential in designing of nanoparticulate delivery systems. Gum cordia, an anionic gum is obtained from fruits of *Cordia obliqua* Willd (*Fam*: Boraginaceae). *C. obliqua*, commonly known as *lassora*, is the medium-size deciduous tree native to Indian subcontinent. The tribal population traditionally eats the ripe fruits of the plant, while the raw fruits are used as vegetable and for making pickles (Parmar & Kaushal, 1982). During earlier studies, gum cordia was evaluated as sustained release matrix formulated as tablets (Mukherjee, Dina, & Barik, 2008) and as composite beads with gum gellan (Ahuja, Yadav, & Kumar, 2010).

Aqueous eye drops are the widely accepted and most commonly used dosage forms in topical ocular delivery. About 90% of the dose applied topically in the eye from such solutions is lost due to pre-corneal losses (Schoenwald, 1985). To achieve therapeutically effective concentration of drug in ocular tissues, frequent administration of aqueous eye drops is required. In order to improve the ocular availability of drugs, enhancement of pre-corneal residence of drops by use of viscosity modifiers or bioadhesives has been tried. Ointments and suspension dosage forms also have been employed for the same purpose. The suspension for instillation into the eye must have particle size less than 10 μm, to minimize irritation, tearing and drainage of instilled dose. Nanoparticles because of their submicron size do not irritate the eye and remain gently deposited in the cul-de-sac while providing a prolonged release of drug. Earlier studies with indomethacin (Calvo, Alonso, Vila-Jato, & Robinson, 1996; Calvo, Vila-Jato, & Alonso, 1996), and ketorolac (Gupta, Madan, Majumdar, & Maitra, 2000) have demonstrated higher ocular penetration and ocular availability of drugs from nanoparticulate formulations.

In the present study, a novel polymer-surfactant nanoparticle formulation has been developed using the anionic surfactant di-octyl sodium sulfosuccinate and polysaccharide polymer gum cordia, for prolonging the contact time of water-soluble drug, fluconazole. An emulsion cross-linking technique was used to produce fluconazole-loaded submicroscopic nanoreservoir system and this technique was further optimised using design of experiment, by

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employing a three factor, 3-level Central Composite Design. Three independent variables *viz.* concentration of gum, di-octyl sodium sulfosuccinate and drug were studied for their influence on dependent variables- particle size, zeta potential and %encapsulation. Multiple response simultaneous optimizations using the desirability function were then used to find experimental conditions where the system shows the most adequate results. The optimised nanoparticulate formulation was further evaluated for *in vitro* corneal permeation characteristics and compared with the commercially available formulation of fluconazole.

2. Materials and methods

2.1. Materials

The fresh and raw fruits of *C. obliqua* Willd. (*fam: Boraginaceae*) were procured locally from Hisar (India) and were authenticated by taxonomists of National Institute of Science Communication and Information Resources (NISCAIR, New Delhi, India) (Authentication voucher No: NISCAIR/RHMD/Consult/-2008-09/1158/190). Fluconazole was obtained as gift sample from Aurobindo Pharma India Ltd. (Mandal, AP, India). Di-octyl sodium sulfosuccinate (AOT) was purchased from Sigma–Aldrich (St. Louis, USA). All other chemicals used were of reagent grade and were used as such.

2.2. Methods

2.2.1. Extraction of gum

Gum cordia was extracted from the fruits as reported earlier (Mukherjee et al., 2008). Briefly, mucilage was expressed from the fruits by tincture press and dissolved in distilled water followed by precipitation with 1% (v/v) hydrochloric acid. The precipitated gum so obtained was dried at room temperature, grounded and sieved.

2.2.2. Experimental design

A central composite design with $\alpha = 1$ was employed as per the standard protocol (Singh, Kumar, & Ahuja, 2005). The concentration of the polymer (gum cordia), amount of AOT and percentage of drug loading were selected as formulation variables on the basis of previous trials, and studied at 3 levels (*i.e.* −1, 0, +1). The central point (0, 0, 0) was studied in sextet. All other formulation and processing variables were kept invariant throughout the study. Table 1 summarizes an account of the 20 experimental runs studied, their factor combination, and the translation of the coded levels to the experimental units employed during the study. Encapsulation efficiency, particle size and zeta potential were taken as response variables. The experimental design and statistical analysis of the data were done using the Design Expert Software (Version 7.1.6, Stat-Ease Inc., Minneapolis, MN).

2.2.3. Preparation of gum cordia nanoparticles

The nanoparticles of gum cordia were prepared employing the emulsion cross-linking technique (Mahesh et al., 2007). To optimize formulation and process variables employed in formulation of nanoparticles, various formulations of nanoparticles were prepared in triplicate employing the Central Composite Design as depicted in Table 1. Briefly, a solution of gum cordia and drug was prepared in deionised water (pH 8.0). One ml of this solution was emulsified into AOT solution (3 ml) in methylene chloride by sonication (Power Sonic 410, Cyber Lab) for 1 min over ice bath. The resulting primary emulsion was further, emulsified into aqueous solution (15 ml) of polyvinyl alcohol (PVA) (3.5%, w/v) by sonication for 3 min over ice bath, in order to form a secondary w/o/w emulsion. The emulsion was stirred using a magnetic stirrer, and 5 ml of aqueous calcium chloride solution (60%, w/v) was added gradually to the above emulsion with stirring. Resulting emulsion was stirred further for 18 h. To remove methylene chloride, the emulsion was rotated under vacuum in Rotavapor (Steroglass, Italy) at 60 rpm for 1 h. Nanoparticles so formed were recovered by centrifugation (C-24 BL, Remi Equipments Pvt. Ltd, Mumbai, India) at 20,000 rpm for 1 h. To remove PVA and unentrapped drug, nanoparticles were washed twice with deionized water and centrifuged. The residue so obtained was resuspended in water and lyophilized (Alpha 2–4 LD Plus, Martin Christ, Germany) using mannitol (1%, w/v) as cryoprotectant at −90 °C and 0.0010 mbar pressure for 24 h.

2.2.4. Evaluation of gum cordia nanoparticles

The nanoparticles of gum cordia were evaluated for their particle size, zeta potential, encapsulation efficiency, and *in vitro* corneal permeation characteristics.

2.2.5. Transmission electron microscopy

The morphology of the fluconazole-loaded nanoparticles was observed with transmission electron microscope (268 D JEOL, JEM-1230, Japan) at 100 kV.

Table 1
Central composite design using formulation variables influencing % encapsulation (Y_1), particle size (Y_2) and zeta potential (Y_3).

Exp. no.	X_1 (%)	X_2 (%)	X_3 (%)	Y_1 (%)	Y_2 (nm)	Y_3 (mV)
1	−1 (0.5)	−1 (1.0)	−1 (5)	41.11	594.4	−41.3
2	1 (1.0)	−1 (1.0)	−1 (5)	29.13	470.3	−39.3
3	−1 (0.5)	1 (10)	−1 (5)	93.00	510.2	−51.6
4	1 (1.0)	1 (10)	−1 (5)	83.00	395.6	−47.8
5	−1 (0.5)	−1 (1.0)	1 (15)	42.08	828.4	−41.3
6	1 (1.0)	−1 (1.0)	1 (15)	27.20	847.1	−37.9
7	−1 (0.5)	1 (10)	1 (15)	60.00	489.2	−53.2
8	1 (1.0)	1 (10)	1 (15)	57.00	506.7	−47.0
9	−1 (0.5)	0 (5.5)	0 (10)	56.20	378.8	−53.4
10	1 (1.0)	0 (5.5)	0 (10)	43.50	365.5	−49.5
11	0 (0.75)	−1 (1.0)	0 (10)	28.50	596.2	−38.9
12	0 (0.75)	1 (10)	0 (10)	75.60	388.8	−47.5
13	0 (0.75)	0 (5.5)	−1 (5)	46.54	315.7	−49.9
14	0 (0.75)	0 (5.5)	1 (15)	42.90	428.9	−45.0
15	0 (0.75)	0 (5.5)	0 (10)	43.10	359.9	−46.7
16	0 (0.75)	0 (5.5)	0 (10)	42.40	336.4	−49.7
17	0 (0.75)	0 (5.5)	0 (10)	43.10	329.5	−49.9
18	0 (0.75)	0 (5.5)	0 (10)	38.46	344.7	−45.7
19	0 (0.75)	0 (5.5)	0 (10)	39.00	353.7	−46.7
20	0 (0.75)	0 (5.5)	0 (10)	34.81	328.8	−48.4

X_1—concentration of gum cordia, X_2—concentration of AOT, X_3—concentration of drug.

Table 2
Model summary statistics of the quadratic response surface model.

Response factor	Model							Lack of fit	
	F-value	Prob > *F*	R^2	Adj. R^2	Pred. R^2	Adeq prec.	C.V. (%)	*F*-value	Prob > *F*
Y_1	51.31	<0.0001	0.9595	0.9408	0.8986	25.40	8.87	2.14	0.208
Y_2	154.59	<0.0001	0.9929	0.9864	0.9584	42.58	3.95	2.96	0.1294
Y_3	16.31	<0.0001	0.9362	0.8788	0.7496	13.63	3.47	0.71	0.6404

2.2.6. Size analysis and zeta potential

Particle size of nanoparticles was determined by photon correlation spectroscopy (PCS) with a Zetasizer (Malvern Instruments Ltd. UK).

2.2.7. Encapsulation efficiency

Encapsulation efficiency is the percentage of the actual mass of drug encapsulated in the polymeric carrier, relative to the initial amount of loaded drugs (Leonardi, Lamas, & Olivieri, 2008), and was calculated using the following equation:

$$\%\text{Encapsulation Efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100 \quad (1)$$

For actual drug loading an accurately weighed quantity of each formulation (equivalent to 2 mg of drug) was taken, sonicated in 10 ml of methanol for 5 min, filtered through 0.45 μm syringe filter, and diluted appropriately. The contents of fluconazole in the samples were determined spectrophotometrically by measuring the absorbance at 260 nm in UV–vis Spectrophotometer (Cary 5000, Varian, Australia).

2.2.8. In vitro corneal permeation study

The optimized formulation of fluconazole-loaded gum cordia nanoparticles was compared with the commercial formulation of fluconazole eye drops for their corneal permeation characteristics using an isolated goat cornea mounted over modified Franz-diffusion cell (Ahuja, Dhake, Sharma, & Majumdar, 2007). Whole eyeball of goat was transported from the local butcher shop to the laboratory in cold (4 °C) normal saline within one hour of slaughtering of the animal. The cornea was carefully excised along with 2–4 mm of surrounding sclera tissue and was washed with cold normal saline till the washing was free from proteins. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments of an all-glass modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.95 cm^2. The receptor compartment was filled with 11 ml of freshly prepared phosphate buffer saline (pH 7.4). One milliliter of ophthalmic formulation was placed on the cornea and the opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained at 35 °C with constant stirring, using a Teflon-coated magnetic stir bead. One-millilitre sample was withdrawn from the receptor compartment at various time intervals up to 120 min and was analyzed for fluconazole content spectrophotometrically by measuring absorbance at 260 nm. Each withdrawn sample was replaced with equal volume of phosphate buffer saline. At the end of the experiment, each cornea (free from sclera) was weighed, soaked in 1 ml methanol, dried overnight at 90 °C and reweighed. From the difference in weights corneal hydration was calculated. The study was designed with paired corneas, *i.e.*, one cornea of an animal received aqueous solution while the contra lateral cornea received nanosuspension.

2.2.9. Statistical analysis of the data and validation of optimization model

Various RSM computations for the current optimization study were performed employing Design Expert software (Version 7.1.6, Stat-Ease Inc, Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis (MLRA) approach. The general form of the MLRA model is represented as Eq. (2).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \quad (2)$$

where β_0 is the intercept representing the arithmetic average of all quantitative outcomes of 20 runs; β_1 to β_9 are the coefficients computed from the observed experimental values of Y; and X_1 and X_2 are the coded levels of the independent variables (Singh et al., 2005). The terms X_1X_2 and X_i^2 ($i = 1$–2) represent the interaction and quadratic terms, respectively. The interaction terms show how the response changes when two factors were simultaneously changed. The second-degree term (X_i^2) is included to investigate non-linearity. Statistical validity of the polynomials was established on the basis of ANOVA provisions of the Design Expert software. To study the combined effect of different variables on the response, 3-D response surface plots were generated using the Design Expert software. The optimum values of dependent variables to achieve the desired response were calculated using the numerical optimization tool along with desirability approach.

3. Results

Gum cordia is an anionic gum, which dissolves in water on addition of sodium hydroxide forming sodium salts. The water-soluble sodium salt of gum cordia precipitates on addition of divalent ion such as calcium ion. Di-octyl sodium sulfosuccinate, commonly known as AOT is an anionic surfactant, having a polar sulfosuccinate head group and large branching, di-octyl side chain. AOT forms reverse micelles in non-polar solvents such as methylene chloride, hexane etc. AOT being a double chain amphiphilic forms bilayer structure in multiple emulsions. Further, anionic AOT also interacts with cationic calcium ions to form insoluble salts. In the present investigation we have used the surfactant-polymer system composed of gum cordia and anionic surfactant AOT. Each analyzed variables were evaluated at three levels, high level (+1) and low level (−1). The aqueous core of gum cordia entrapped into the reverse micelles formed by the AOT in methylene chloride was further emulsified in the aqueous phase using PVA as a secondary emulsifier. On addition of calcium ion, gum cordia and AOT are cross-linked forming a core comprising of gum cordia and AOT head groups, and surrounded by hydrophobic matrix comprising of AOT side chain with the drug encapsulated in the core. Earlier studies on the preparation of nanoparticles using emulsion cross-linking technique had demonstrated that the particle size, encapsulation efficiency and zeta potential of nanoparticles are significantly influenced by the concentration of polymer, surfactant and drug (Mahesh et al., 2007). Particle size of the nanoparticles is an important factor, which governs their body distribution. Zeta potential of nanoparticles is an important parameter, which determines their stability. For a stable nanosuspension, the zeta potential of the nanoparticles should be more than ±30 mV (Patravale, Abhijit, &

Table 3
Summary of each factor effect and its p-values for, responses Y_1, Y_2 and Y_3.

Factor	Y_1		Y_2		Y_3	
	Factor effect	p-value	Factor effect	p-value	Factor effect	p-value
X_1	−5.26	0.0021	−21.58	0.0036	1.93	0.0036
X_2	20.06	<0.0001	−104.6	<0.0001	−4.84	<0.0001
X_3	−6.36	0.0005	81.41	<0.0001	0.55	0.3063
X_1^2	5.29	0.0480	40.03	0.0043	−3.06	0.0103
X_2^2	7.49	0.0086	160.3	<0.0001	5.19	0.0003
X_3^2	0.00	0.0000	40.18	0.0042	0.94	0.3585
X_1X_2	0.00	0.0000	1.04	0.8743	0.58	0.3372
X_1X_3	0.00	0.0000	34.36	0.0003	0.48	0.4244
X_2X_3	−7.26	0.0094	−65.09	<0.0001	−0.27	0.6401

Kulkarni, 2004). In addition, zeta potential has been shown to affect the intra-cellular distribution of the nanoparticles (Panyam, Zhou, Prabha, Sahoo, & Labhasetwar, 2002) Thus, considering the same, concentration of gum cordia, concentration of AOT and concentration of drug were selected as the variables for optimization, while particle size, zeta potential and encapsulation efficiency were selected as dependent variable. The optimization study was conducted using response surface methodology employing central composite design (Table 1). The results of response generated using the experimental design was fitted into polynomial models and ANOVA test was applied to the polynomial models to estimate the significance of the model. The results of this analysis revealed that the response encapsulation efficiency (Y_1) fitted best into the quadratic model with backward elimination, while the response for particle size (Y_2), and zeta potential (Y_3) fitted best into quadratic model. The polynomial models for the responses Y_1, Y_2 and Y_3 can be represented by Eqs. (3), (4) and (5) respectively.

$$Y_1 = 41.94 - 5.26X_1 + 20.06X_2 - 6.36X_3 - 7.26X_2X_3 + 5.29X_1^2 + 7.49X_2^2 \quad (3)$$

$$Y_2 = 338.15 - 21.58X_1 - 104.59X_2 + 81.41X_3 + 1.04X_1X_2 + 34.36X_1X_3 - 65.09X_2X_3 + 40.03X_1^2 + 160.30X_2^2 + 40.18X_3^2 \quad (4)$$

$$Y_3 = -48.06 + 1.93X_1 - 4.84X_2 + 0.55X_3 + 0.58X_1X_2 + 0.48X_1X_3 - 0.27X_2X_3 - 3.06X_1^2 + 5.19X_2^2 + 0.94X_3^2 \quad (5)$$

The polynomial equations comprise the coefficients for intercept, first-order main effect, interaction terms, and higher order effects. The sign and magnitude of the main effects signify the relative influence of each factor on the response. Also a negative sign signifies antagonistic effect while a positive sign indicates a synergistic effect.

Table 2 presents the result of ANOVA test on the quadratic regression model and details the model summary statistics for the selected significant models, which indicated that the response surface model developed for three responses, were significant and adequate, without significant 'lack of fit'. It can be observed that, all responses bearing R^2 value >0.9, which indicates a good correlation between the experimental and predicted responses. In addition, the predicted R^2 value is in reasonable good agreement with adjusted R^2 value, resulting in reliable models. Further, the higher values (>4) of Adequate Precision indicate adequate signal. The relatively lower values of coefficient of variation indicated better precision and reliability of the experiments carried out.

Table 3 presents the results of factor effects and associated p-values for the responses Y_1, Y_2 and Y_3. The data reveals that significant factors affecting the response Y_1 were the synergistic effects of linear contribution of X_2 and quadratic contributions of X_1 and X_2. On the other hand Y_1 was antagonistically affected by the linear contributions of X_1, X_3 and interaction effects of X_2 and X_3. The response Y_2 was significantly affected by the synergistic effects of linear contribution of X_3, quadratic contributions of X_1, X_2 and X_3 and by interaction effects of X_1 and X_3, while Y_2 was antagonistically affected by significant factor effects of linear contributions of X_1, X_2 and interaction effects of X_2 and X_3. In case of zeta potential (Y_3), the linear effects of X_1 and quadratic effects of X_2 contributed to significant increase in zeta potential, while the linear effects of X_2 and quadratic effects of X_1 contributed towards significant reduction in zeta potential.

Figs. 1–3, portray the 3-D response surface plots constructed using the models generated by response surface methodology. Fig. 1 shows the combined effect of concentration of AOT (X_2) and concentration of drug (X_3) on % encapsulation. It can be observed that the effect of AOT is more pronounced than the effect of drug concentration. Thus, concentration of AOT is the limiting factor; any subtle variation in AOT concentration will greatly influence the %encapsulation. In the present technique, AOT forms reverse micelles, which along with gum act as nanoreservoir for encapsulation of drug. Increasing the amount of AOT allows encapsulation of more drugs within the matrix. The maximum encapsulation of drug within the matrix occurs in nanoparticles prepared using lower drug concentration and higher AOT concentration.

Fig. 2 exhibits the combined effect of concentrations of AOT/gum, drug/gum and drug/AOT, respectively on particle size of nanoparticles. The plot (Fig. 2a) show an antagonistic curvilinear relationship between the AOT/gum and particle size, with the effect of AOT more pronounced than the gum. AOT, an anionic surfactant

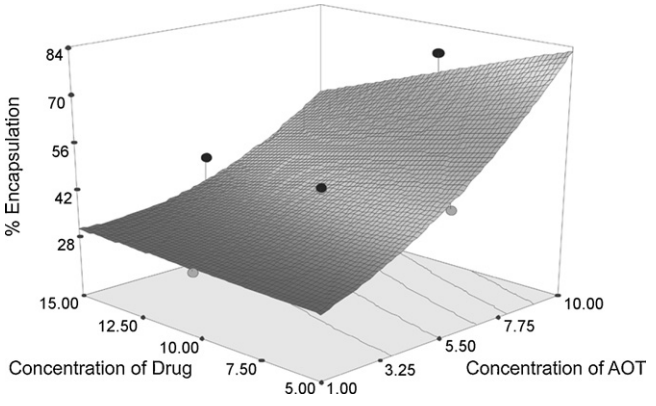

Fig. 1. Response surface plot showing combined effect of concentrations of AOT and drug on %encapsulation of drug within the nanoparticles.

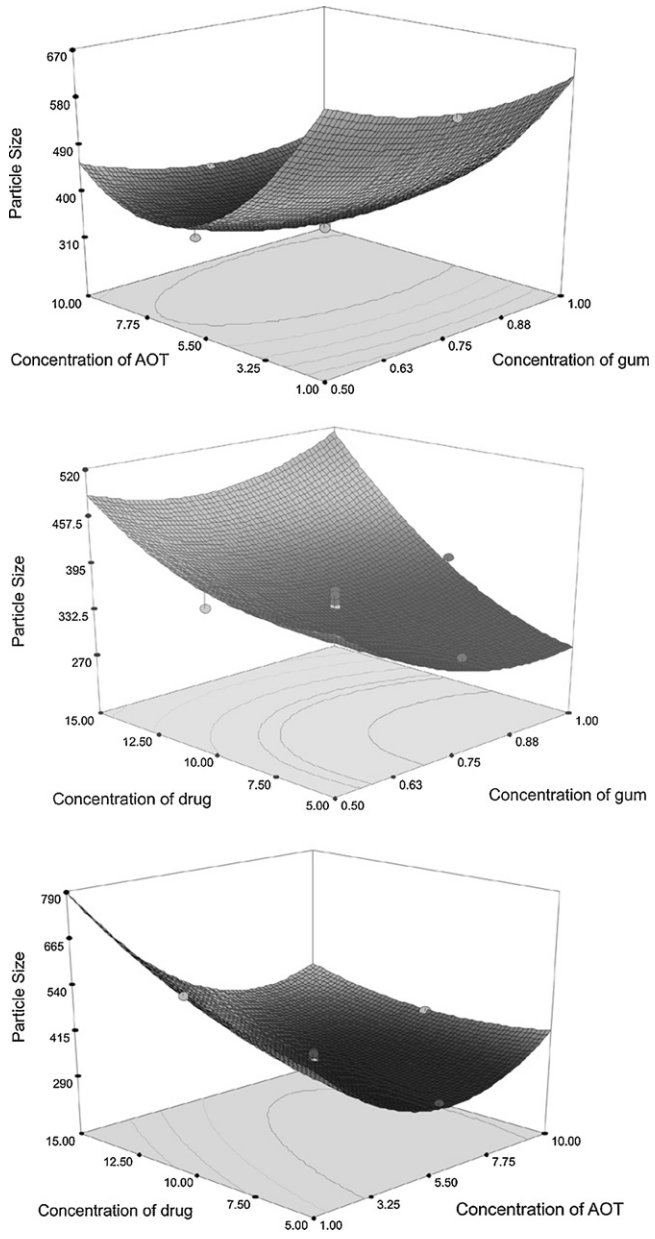

Fig. 2. Response surface plots showing combined effect of concentrations of (a) AOT and gum, (b) drug and gum, and (c) drug and AOT, on particle size of nanosuspension.

forms reverse micelles, increasing the concentration of AOT in the system decreases the size of reverse micelles and thus the particle size. A curvilinear synergistic relationship between the drug and particle size, while a curvilinear antagonistic relationship between the gum and particle size can be observed (Fig. 2b). The effect of drug concentration is more pronounced than the effect of gum on particles size. Increasing the concentration of drug increases the size of the core matrix encapsulating the drug, leading to increase in particle size. Fig. 2(c) displays the combined effect of drug concentration and concentration of AOT on particle size, with the effect of AOT more pronounced than the drug concentration.

Fig. 3 shows the combined effect of concentrations of AOT/gum, drug/gum and drug/AOT on the zeta potential. It can be observed that AOT, which coats the core comprising of matrix of drug and gum, had the most pronounced effect on zeta potential. AOT being anionic imparts negative charge on the nanoparticles, which increases in magnitude with increasing the concentration of AOT.

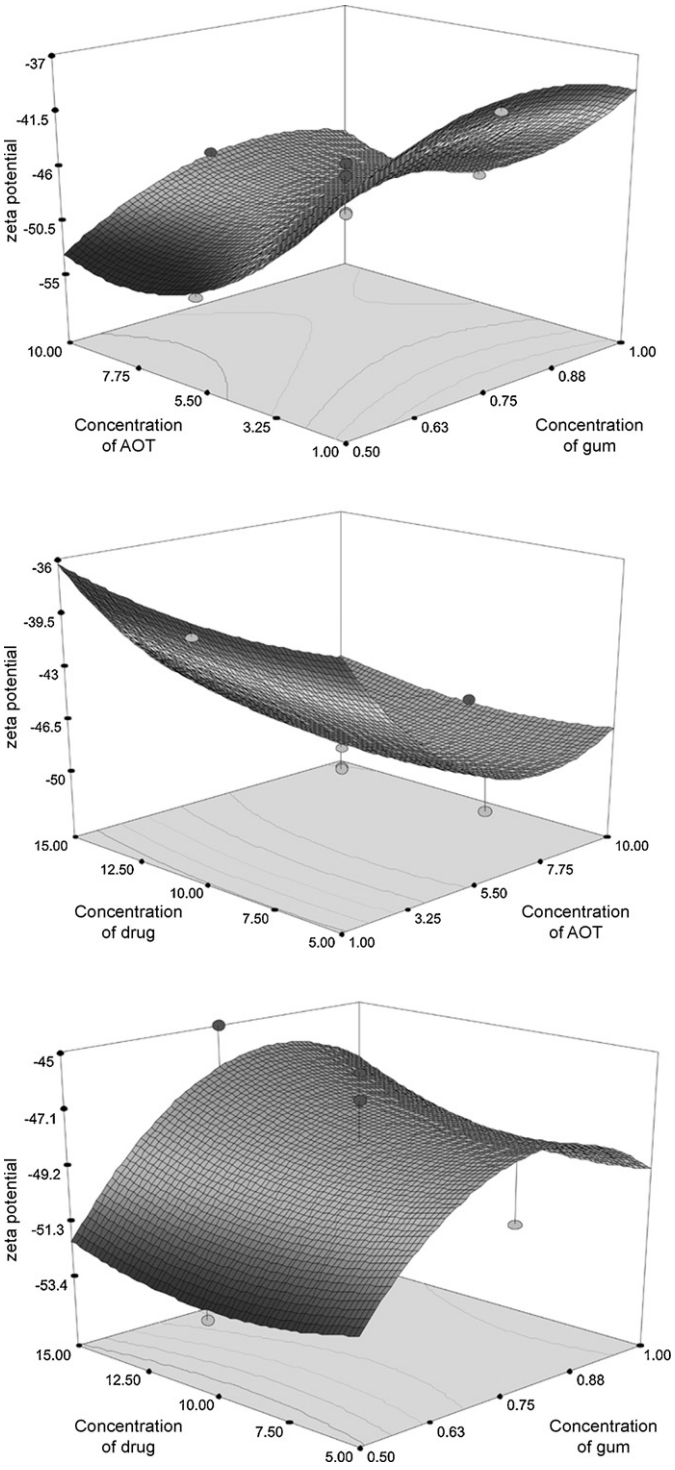

Fig. 3. Response surface plots showing combined effect of concentrations of (a) AOT and gum, (b) drug and AOT, and (c) drug and gum, on zeta potential of nanosuspension.

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. The optimization was done with constraints for %encapsulation, Y_1 in the range of 65–93%, particle size Y_2 in the range 315–394 nm, and numerical value of zeta potential, Y_3 −55 to −48 mV as the goals to locate the optimum setting of independent variables in the new formulation. The optimal calculated parameters were- concentrations of gum cordia (0.85%, w/v), AOT (9.07%, w/v) and fluconazole (6.06%, w/v).

Table 4
The experimental and predicted values for response Y_1, Y_2 and Y_3 along with percentage prediction error observed for the optimum test condition and random checkpoints.

Checkpoint Conditions $X_1/X_2/X_3$	Y_1			Y_2			Y_3		
	Obser	Pred	Error (%)	Obser	Pred	Error (%)	Obser	Pred	Error (%)
0.85/9.07/6.06[a]	73.54	70.88	3.62	343.64	345.1	−0.42	−46.4	−48	−3.45
1/10/5	83.00	83.14	−0.17	395.6	402.77	−1.81	−47.8	−48.08	−0.59
0.5/1/15	42.08	40.82	2.99	828.4	817.93	1.26	−41.3	−41.16	0.34
0.75/10/10	70.6	69.52	1.52	388.8	393.86	−1.30	−47.5	−47.75	−0.53

[a] Represents optimum composition.

Table 5
Comparison of corneal permeation characteristics of marketed formulation (Zocon) and nanosuspension of fluconazole.

Formulation	Cumulative permeation (%)			%Corneal hydration
	60 min	90 min	120 min	
Nanosuspension	16.78 ± 2.75	21.04 ± 3.22	23.5 ± 4.29	80.12 ± 0.823
Zocon	18.56 ± 5.81	22.26 ± 3.26	23.92 ± 4.31	80.34 ± 1.100

Values are mean ± sd ($n = 3$).

3.1. Validation of RSM results

To check the reliability of the developed mathematical models, the response of the optimal formulation of nanoparticles and three additional checkpoint formulations covering the entire range of experimental domain was recorded. For each of these test runs the experimentally determined response was compared with the response predicted by the mathematical models. Table 4 lists the test conditions of the optimum and the random checkpoints, their experimental and predicted values for both the response variables, along with the calculated percentage prediction error. On linear correlation of the observed and predicted response variables, the value of correlation coefficient r^2 was found to be 0.9961, 0.9998 and 0.9575, for % encapsulation, particle size and zeta potential, respectively. Thus, the lower magnitude of percentage prediction error (−0.17 to 3.62 for Y_1, −1.81 to 1.26 for Y_2 and −3.45 to 0.34 for Y_3) as well as significant values (>0.9) of r^2 in the current study indicate the robustness of the mathematical model and high prognostic ability of response surface methodology. The optimized nanoformulation had maximum %encapsulation, minimum particle size and adequate zeta potential.

3.2. In vitro corneal permeation study

Table 5 compares the corneal permeation characteristics of nanosuspension formulation of fluconazole with commercial formulation of fluconazole (Zocon). It can be observed that there was no significant difference between the %cumulative permeation of fluconazole from the nanosuspension formulation in comparison to commercial aqueous formulation. Even though the test formulation is in suspension form, it provided permeation comparable to solution dosage form, which may be attributed to nanometric size of suspended particles. Earlier studies conducted using nanoparticles demonstrated endocytic uptake of drug by cells of corneal endothelium (Calvo, Vila-Jato, et al., 1996). Further, it is expected that during *in vivo* use the nanosuspension-based formulation will provide higher ocular concentration of fluconazole as compared to solution dosage forms because of prolonged residence time of nanoparticles in the *cul-de-sac*.

4. Discussion

Fluconazole-loaded gum cordia-AOT nanoparticles were successfully prepared by emulsion-cross-linking technique. The emulsion-cross-linking technique reported here results in nanometric size particles with narrow particle size distribution. Further, the nanoparticulate formulation was optimized by statistical screening design considering gum cordia, AOT and fluconazole concentration as independent variables. Optimization study revealed that, concentration of AOT had more pronounced effect on % encapsulation, particle size and zeta potential as compared to concentration of gum cordia and drug. It was observed that on increasing the concentration of AOT in system, results in smaller particle sizes with higher negative zeta potential and higher %encapsulation. Further, at lower concentration of drug and polymer higher %encapsulation and smaller particle size was observed. The optimized formulation was further compared with marketed formulation of fluconazole (Zocon) for corneal permeation characteristics, which revealed that there was no significant difference between the percentage cumulative permeation of fluconazole from the nanosuspension formulation in comparison to commercial aqueous formulation. However, further *in vivo* studies employing animals are required to confirm whether enhanced *in vitro* corneal permeation also translates into higher ocular concentration of fluconazole during *in vivo* studies.

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