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Research Article

OPTIMIZATION OF AN OCULAR NANOSUSPENSION FORMULATION FOR ACYCLOVIR USING FACTORIAL DESIGN

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ABSTRACT

Poorly water soluble compounds, like acyclovir (AC), are difficult to develop as drug products using conventional formulation techniques. Nanosuspensions (NS) are colloidal dispersions of pure drug particles in an outer liquid phase with an ability to enhance the dissolution rate of drugs. Ophthalmic AC delivery may benefit to the full extent from the characteristics of NS. Systematic investigations using full factorial design were carried out to identify optimal process parameters. The influence of the independent variables Pluronic® F68 concentration and Tween® 80 concentration were tested by conducting a 32 design of experiment. In the present study, the optimum NS formulation selected by the JMP® software was F7 containing 3% Pluronic® F68 and 0.5% Tween® 80. F7 showed a mean particle diameter of 21.62±1.07 nm with a polydispersity index of 0.304 and a zeta potential of -19.1 mV. It also showed an average entrapment efficiency of 85.85±3.40 with approximately 40.08% of AC being released in vitro over three hours. Optimum AC NS formulation; F7; proved itself safe when tested histopathologically on the rabbits' eyes and was capable of retaining its stability for six months.

Keywords: Acyclovir, Nanosuspension, Ocular, Factorial design

INTRODUCTION

Bioavailability of ocular drugs from conventional eye formulas (i.e, solution, suspension, and ointment) is often poor due to precorneal loss resulting from removal mechanisms (blinking, tears, and nasolacrymal drainage), non-productive absorption, transient residence time in the cul-de-sac and the relative impermeability of the drugs to the corneal epithelial membrane (1). Poorly soluble drugs are a challenging problem in ocular formulation; generally, ocular efficacy is closely related to ocular drug bioavailability, which may be enhanced by increasing corneal drug penetration, prolonging precorneal drug residence time and increasing the saturation solubility of poorly soluble drugs (2, 3). Nanosuspensions (NS) are colloidal dispersions of pure drug particles in an outer liquid phase (4) with a mean particle diameter ranging between 10 and 1000 nm (5, 6). The important and famous features of NS is their ability to increase the saturation solubility and consequently the dissolution rate of specific drug (7-9). For this feature and due to the other advantages offered by NSs over conventional ocular dosage forms, including reduction in the amount of dose, maintenance of drug release over a prolonged period of time, reduction in systemic toxicity of drug, longer residence time of nanoparticles on the corneal surface, higher drug concentrations in the infected tissue, and suitability for poorly water-soluble drugs (10), ophthalmic drug delivery, more than any other route of administration, may benefit to a full extent from the characteristics of NS stabilized by suitable stabilizers (surfactants and/or polymers) (11). Recently, NS of glucocorticoid drugs has been shown to enhance the drug absorption rate and increase the duration of drug action (12).

Acyclovir (9-(2-hydroxyethoxymethyl) guanine, AC), a synthetic analog of 20-deoxiguanosine, is one of the most effective and selective antiviral drugs. It is primarily used to treat ocular infections caused by Herpes simplex virus (HSV) (e.g. HSV epithelial keratitis), as well as in treating Varicella-zoster virus (VZV) infections (e.g. Herpes zoster ophthalmicus, HZO) (13-15). AC is commonly marketed as an ophthalmic ointment (3%), due to its poor water solubility which limits the same concentration to be reached in eye drops. Moreover, AC low lipophilicity limits its passage across the corneal epithelium and, consequently, its bioavailability. Different strategies have been applied in order to improve the ocular bioavailability of AC focusing on either its water solubility or its lipophilicity to enhance transcorneal passage. However, there is still a clear need for effective topical formulations capable of promoting drug penetration and maintaining therapeutic levels with a reasonable frequency of application. Use of polymeric NS might be one of the most interesting approaches towards achieving local controlled drug delivery.

In addition to traditional experimentation, factorial design, first reported by Box and Wilson (16) is a very useful tool for the identification of critical process parameters and to optimize the respective process conditions (17). The interaction between the two factors namely the percentage of polymer and percentage of surfactant were therefore systematically examined by applying a two full factorial design. The particle size, zeta potential, polydispersity index (PDI), entrapment efficiency and release percentage were investigated as responses describing the quality of the resulting NSs.

The aim of present study is to prepare and examine nine AC loaded NS formulations. A two-level factorial design experiment was used for obtaining a prediction of optimized formulation. The prepared NS were characterized with respect to their particle size, zeta potential, drug loading and *in vitro* drug release in order to investigate the potential of polymeric NS to deliver AC onto the ocular surface. The optimum formula was tested histopathologically and its stability in six months time was assessed.

MATERIAL AND METHODS

Materials

Acyclovir was a kind gift from Memphis Pharmaceutical Co., Egypt. Pluronic® F68, Tween® 80 (polysorbate 80) and DMSO (dimethyl sulfoxide) were purchased from Sigma-Aldrich, USA. All other chemicals and reagents used were of pharmaceutical grade.

Methods

Preparation of NS

Drug-containing NS were prepared according to the o/w emulsion method (11) using probe ultrasonicator (60 Hz, 20 cycles/3 sec; Branson, Cleveland, Ohio). Briefly, AC was dissolved in a solution of copolymer pluronic® F68 dissolved in DMSO (2 mL) under stirring at 300 rpm at 70 °C. This solution was then slowly injected with a syringe into 50 mL water containing Tween® 80 and benzalkonium chloride (0.1%, w/v) kept at a low temperature in an ice water bath. During injection the mixture was vigorously mixed at an agitation speed of 800 rpm. The resulting emulsion obtained was sonicated in a probe-type sonicator and further stirring was continued for 60 minutes. The final formulation was sterilized by filtration through

0.22 µm membrane filter and filled in pre-sterilized glass vials, freezed at -80 °C and lyophilized using freeze-dryer (Novalyphe-NL 500; Savant Instruments Corp., USA).

Factorial Design

Different NS formulations containing AC were prepared based on the 3*2 factorial design. Percentage of Pluronic® F68 (*X*1) and that of Tween® 80 (*X*2) in the formulation were selected as two independent variables. Three levels of each variable were selected and nine possible batches were prepared using different levels of variables (Table 1). JMP® (version 7, SAS, USA) was used to obtain values of coefficients in the equation and *f* statistics were used to identify statistically significant terms (18).

Table 1: It shows experimental condition, design and responses of 3^2 factorial design preparation of AC NS

Variables			Code	Levels			
				1	2	3	
Pluronic® F68			X1	1%	2%	3%	
Tween® 80			X2	0.5%	1%	1.5%	
Formula code	**X1**	**X2**	**Responses**				
			Particle diameter	**PDI**	**Zeta Potential**	**Entrapment efficiency (%) [a]**	**Release percentage (%) [a, b]**
F1	1%	0.5%	149.20±8.34	0.554	-18.8	75.68±3.54	30.99±2.14
F2	1%	1%	85.15±9.11	0.507	-18.2	67.09±1.80	28.00±2.50
F3	1%	1.5%	80.69±2.21	0.612	-18.3	61.84±5.71	26.84±4.62
F4	2%	0.5%	53.16±3.65	0.317	-18.6	79.65±6.21	22.41±2.60
F5	2%	1%	60.31±5.75	0.511	-17.2	78.22±0.89	31.49±3.45
F6	2%	1.5%	100.50±8.12	0.447	-13.4	54.05±1.62	36.13±6.08
F7	3%	0.5%	21.62±1.07	0.304	-19.1	85.85±3.40	40.08±4.96
F8	3%	1%	125.60±4.93	0.647	-14.6	70.91±1.43	33.31±1.15
F9	3%	1.5%	150.00±11.34	0.453	-15	63.40±2.58	31.70±3.57

[An] Average of three experiments; [b] After three hours

Characterization of NS

Morphology using Transmission Electron Microscopy (TEM)

The morphological examination of the NSs was performed with a transmission electron microscope (TEM, JEOL, Tokyo, Japan). The samples were placed on carbon-coated copper grids for viewing by TEM.

Particle size and Zeta Potential

The mean particle diameter, polydispersity index (PDI) and zeta potential of AC-NS formulations were determined by photocorrelation spectroscopy with a Zetasizer (Malvern Instruments, Worcestershire, United Kingdom), equipped with the Malvern PCS software (version 1.27). Every sample was appropriately diluted with water filtered through a 0.45 mm filter, and the reading was carried out at a 90-degree angle in respect to the incident beam. The zeta potential (ζ) was calculated from electrophoretic mobility using Henry's equation; Eq. (1) (19):

$$U_E = 2\varepsilon\zeta f(K\alpha)/3\eta$$

Where, U_E is the electrophoretic mobility, ε is the dielectric constant of the suspending medium, η is the viscosity of the medium, K is the Debye-Hückel parameter, and f(Kα) is a correction factor that takes into account the thickness of the double layer and particle diameter (α). The K unit is a reciprocal length; 1/K is frequently described as the thickness of the electrical double layer.

Drug incorporation efficiency

Freeze-dried nanoparticles were dissolved in DMSO (10 mL). The quantity of AC in the solution was measured spectrophotometrically (UV-240 1PC, Shimadzu, Japan) at 251.5 nm against blank. Drug incorporation efficiency was expressed as drug entrapment (%); represented by Eq. (2):

$$EE\ (\%) = \frac{\text{Total amount of drug in the nanoparticles}}{\text{Initial amount of drug taken for loading studies}} X100$$

The individual values for three replicates were determined, and their mean values were reported.

Preparation of *in situ* gel base

The Pluronic gel base was prepared by dissolving 1.5% HPMC in distilled water by the aid of stirring, 10% Pluronic® F68 was then added and the solution was stored in the refrigerator at 4 °C for 24 hours. Calculated amount of each AC loaded NS formulation was added to the gel solution and used for the *in vitro* release studies.

Mucoadhesive properties of the gel base

Preparation of mucin particle solution

Commercial porcine mucin was hydrated in demineralised water at 4 °C for 12 h. The mucin solution was then adjusted to pH 7.4 using 1 M NaOH. For the mucoadhesion study, the mucin solution was diluted to a final concentration of 1% (m/v) with 0.1 M phosphate buffer (pH 7.4). The solution was sonicated for 5 min using a probe sonicator followed by centrifugation at 4000 rpm for 20 min, the resulting supernatant was then filtered and the collected filtrate was used in carrying the mucoadhesive experiment.

Measurement of mucoadhesive capacity of pluronic gel base on mucin particles

In this study, the mucin-particle method was used to evaluate the mucoadhesive properties of the pluronic gel base by measuring the changes of zeta potential (20). The pluronic gel base was completely solubilized in demineralised water in a series of concentration (2.5%, 5% and 10%) and filtered before use. Equal volumes of each solution and the mucin particles solution, previously prepared, were mixed by vortexing for 1 min. The zeta potential and of the mixtures was measured by Zetasizer. Each test was performed in triplicate.

***In vitro* release study**

The *in vitro* drug release studies were performed in triplicate. Briefly, a specified amount of each AC NS formulation, containing 1% AC was accurately weighed and uniformly dispersed in 0.5 g of pluronic gel solution. The AC NS gel formulation was then secured in cellulose acetate dialysis bags (molecular weight cutoff of 12,000-14,000 Da, Sigma, USA) and suspended in 20 mL phosphate buffer of pH 7.4.

The glass beaker was placed in a mechanical shaking bath (50 cycles/min.), with temperature adjusted to 37 °C. At selected time intervals; up to 3 hrs; samples were withdrawn and replaced with fresh buffer, then analyzed spectrophotometrically at 251.5 nm versus a blank preparation.

Histopathological study

Sixteen male rabbit (weight, 2.3-2.5 kg) were used in this experiment. The animals were housed under a 12-h light-12-h dark cycle (07:00—19:00 h), with a room temperature maintained at 23±3 °C and humidity at 50±20%. Food and water were given ad

libitum. The experiment was performed in accordance with the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Institutional Animal Care and Use Committee. Animals were divided into two equal groups as follows: Group 1, animal served as control, group 2, selected gel solution of AC NS was applied topically to the right eye for successive seven days. At the end of the experiment, animals were euthanized, the eyes were enucleated and immersed in 10% neutralized buffered formalin solution, embedded in paraffin, and cut into 3µm-thick vertical sections for histopathological investigation.

Physical stability testing

The physical stability of the selected NS formula was evaluated after storage for 6 months under different temperature conditions. Exact volumes of each NS were stored in closed glass bottles and placed at 5±2 °C (refrigerator) or at 25 °C and 60% relative humidity (RH) away from direct light. Aliquots were withdrawn at 1-, 3-, and 6-month time intervals to measure particle size and entrapment efeciency, as described above.

RESULTS AND DISCUSSION

Preparation of NS

To gain insight into the formulation of NS, two stabilizers including macromolecular polymer (pluronic® F68) and a small molecular weight surfactant (tween® 80), were investigated at different concentration levels. Factorial design of the two independent variables; amount of polymer (*X1*) and amount of surfactant (*X2*); at three levels was performed.

Pluronic® F68 was reported previously as the most effective stabilizer for poorly soluble drugs (21). Poloxamers have a linear ABA triblock polymer chain (A stands for hydrophilic polyethylene oxide (PEO) segment and B stands for hydrophobic polypropylene oxide (PPO) segment). The hydrophobic PPO chains can drive the polymer to adsorb on the surface of drug particles, while the hydrophilic PEO chains surround the drug particles providing steric hindrance against aggregation. Pluronic® F68 has a lower molecular weight compared to other pluronics which may exert less kinetic restriction in the adsorption process and faster diffusion (22).

As for the surfactant, tween® 80 is a small molecule, which forms a thin adsorption layer. Its presence may markedly affect the instantaneous and the reproducible formation of nanosized homogeneously dispersed nanospheres exhibiting a high drug loading capacity (23).

The goal at this stage was not only to optimize the formulation, but to ascertain if the selected excipients alone or combined affect particle diameter, PDI, zeta potential and entrapment efficiency of AC NS as well as percentage drug released in three hours time. The concentrations of each excipient were selected based on reported values in the literature. Nine batches of different combinations were prepared by taking values of selected variables *X1* and *X2* at three levels as shown in Table 1.

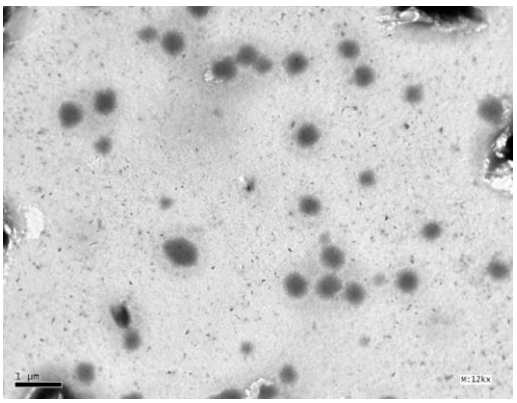


Fig. 1: It shows a TEM image of AC-loaded NS

Characterization of NS

Morphology using Transmission electron microscopy

The morphology of the prepared NS was observed using TEM as shown in Figure 1. Most of the prepared particles are spherical in shape and have smooth surfaces with diameter less than 150 nm. The TEM micrograph showed a very little number of nanoparticles with irregular or polygonal shapes. The pale areas surrounding the particles in the TEM image may be attributed to the presence of the pluronic® F68 and the surfactant molecules on the particle surfaces.

Particle size and Zeta Potential

All of the nine AC NS preparations containing different concentrations of stabilizing polymer (Pluronic® F68) and surfactant (Tween® 80) were assessed for their particle diameter, PDI and zeta potential (Table 1). The mean particle diameter for all tested NS formulations was found to be less than 150 nm which was reported suitable for ocular applications (19), PDI was less than 0.65 and zeta potential values were sufficient for maintaining stable NS preparations in the presence of pluronic® F68 (24, 25). Stabilizers are meant to wet the surfaces of the particles and retard Ostwald ripening and agglomeration to increase the stability of the preparation by providing a steric barrier (26).

Drug incorporation efficiency

The entrapment efficiency of AC in the prepared NS was determined and listed in Table 1. The entrapment efficiency was found to be in the range of 54% to 85%. It could be noticed that, the entrapment efficiency increased with increasing the pluronic® F68 concentration. The highest entrapment was obtained with formula F7 containing 3% of pluronic® F68 whilst the lowest entrapment efficiency was found with formula F6 containing 2% of the polymer. In contrast, the entrapment of the drug in the NS formulations generally decreased as the concentration of tween® 80 increased (from 0.5 to 1.5), this may be due to an increase in the concentration of tween 80 above its critical micelle concentration.

Preparation of AC in pluronic *in situ* gel base

In order to increase the effectiveness of AC in the NS formulations, the selection of a suitable dosage form for its ocular application represents a major importance. Therefore, a pluronic based in situ gel was prepared in order to increase the contact time of AC NS formulation in the eye and thus enhance its ability to release the drug in a sustained manner which will in turn enhance its bioavailability. Pluronic gel base was tested for its gellation temperature (data not shown) and mucoadhesive properties. All of the AC NS formulations were incorporated into the pluronic gel solution before testing their release properties.

Mucoadhesive properties of pluronic gel base

Mucin-particle method was used to evaluate the mucoadhesive properties of the pluronic gel base by measuring the changes of zeta potential. A significant change in surface properties of mucin particles by pluronic compared to mucin without pluronic at pH 7.4 was reported (Table 2).

The zeta potential of mucin particles mixed with different concentrations of pluronic was shifted towards and exceeded zero with increasing concentration of pluronic. It was observed that the higher the concentration of pluronic, the more extensive the changes were found in the zeta potential of mucin particles. These results suggested that pluronic had a high affinity to mucin particles and they postulate that the interaction between pluronic and mucin particles were responsible for the changed surface properties of the mucin particles (20).

Mucoadhesion results confirm the mucoadhesive properties of pluronic and points out that the formulated AC nanoparticulate system presented provides a promising system for ocular drug delivery of AC for a prolonged time.

Table 2: It shows the effect of the interaction of pluronic F68 and mucin on Zeta potential

	Pluronic percentage concentration (% wt/wt)	Zeta potential (mV)
Mucin	-	-19.76
Mucin + pluronic	2.5% pluronic	4.41
Mucin + pluronic	5% pluronic	6.13
Mucin + pluronic	10% pluronic	8.56

In vitro release study

The *in vitro* drug represents a very important parameter in the prediction of drug bioavailability form different formulations. As indicated in Table 1 and Figure 2, dissolution of AC showed a greater rate and extent from NSs compared to untreated drug. Approximately 25% of AC were released from NS incorporated into Pluronic gel base in 3 hours time compared to less than 3% dissoluted of the pure drug. The enhancement of dissolution rate of AC from NS could be attributed to increased surface area of the nano-sized preparations, the presence of Pluroinc® F68 and the addition of tween® 80.

With the decrease in particle size, a high energy state is achieved which increases the extent to which the particle can dissolve due to the increase in dissolution pressure. These parameters collectively increase the hydrophilic character and improve the wettability of the drug (27, 28).

Factorial Design

The results of analysis of variance (ANOVA) of the factorial design are presented in Table 3. Based on the results of ANOVA, the particle diameter was found to be significantly ($p < 0.01$) affected by the interaction of *X1* and *X2* as shows the surface plot for particle diameter in response to the investigated factors; polymer amount and surfactant amount (Figure 3a). In contrast to the particle diameter the zeta potential and the loading efficiency were affected significantly and exclusively by the amount of surfactant ($p < 0.01$), (Figure 3b, 3c). The factor amount of polymer showed a very limited influence on the zeta potential and entrapment efficiency. There is no significant interaction suggested between the two factors by JMP®, i.e. the amount of polymer influence on the zeta potential and entrapment efficiency is independent from the surfactant amount. Neither of the other tested responses seemed to be influenced by any of the tested factors.

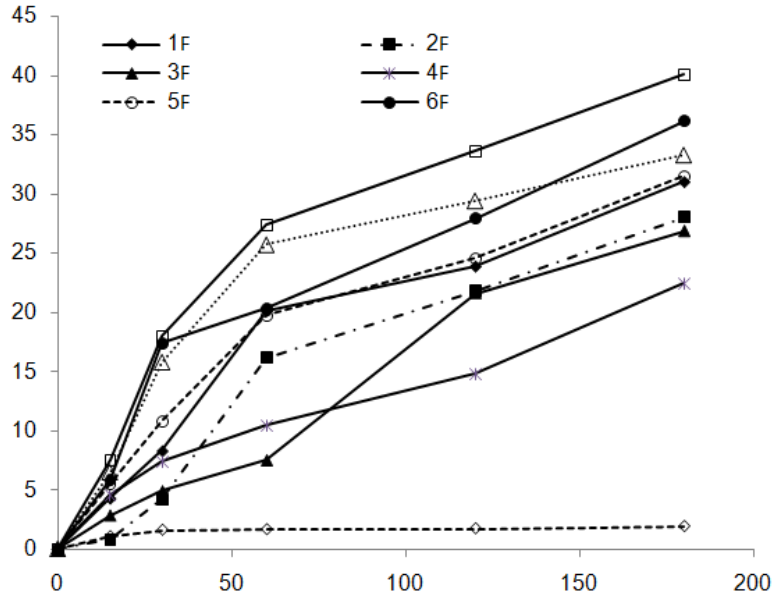
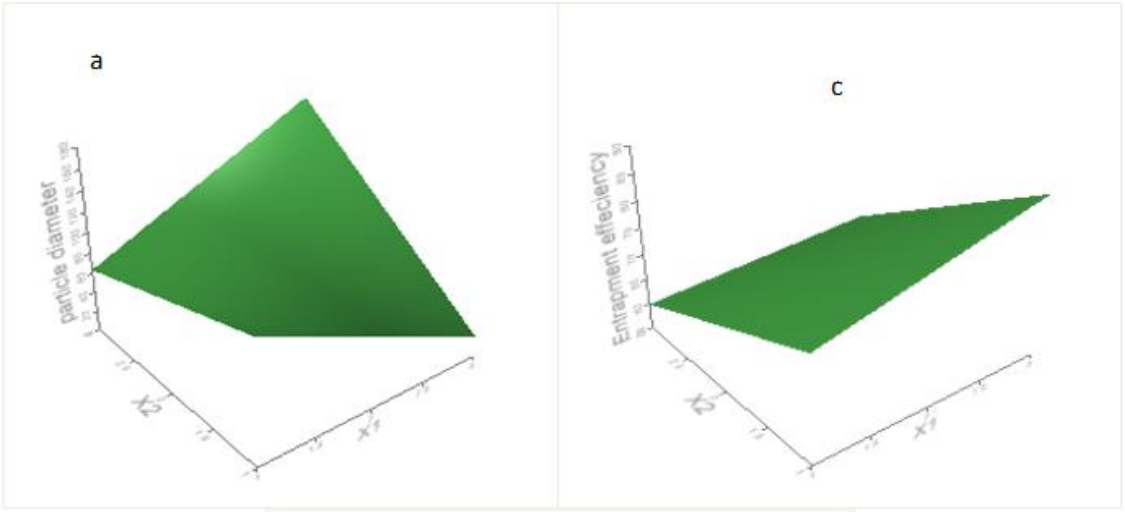


Fig. 2: It shows the *In vitro* release profile of AC from different NS formulations



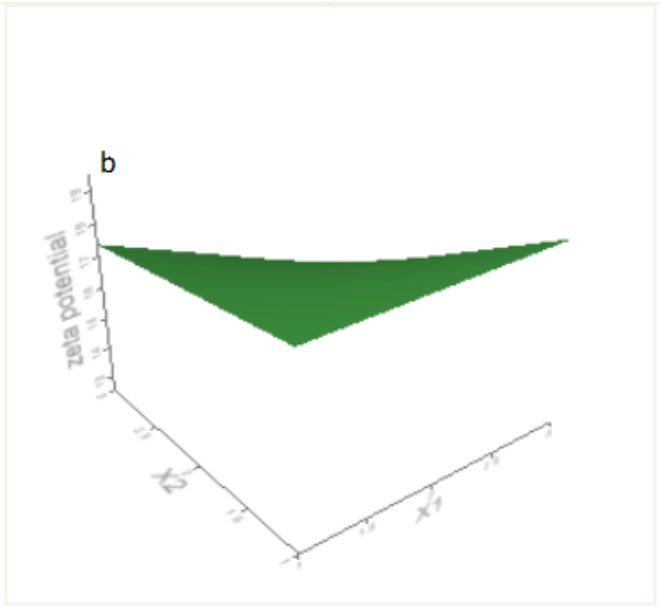


Fig. 3: It shows asurface profiler showing the influence of the independent variables amount of polymer and amount of surfactant on the quality attributes particle diameter (a), zeta potential (b) and entrapment efficiency (c).

The mathematical modeling of AC NS was carried by the following equations:

Eq. (3): *Y= 62.067+ (-2.97*X1) +17.8683*X2+ [(X1-2)*(X2-2)*49.2225]*

Eq. (4): Y_1*= 22.489+ (-1.1*X1) + (-1.63*X2) + [(X1-2)*(X2-2)*-0.9]*

Eq. (5): Y_2*=86.182+ (2.593*X1) + (-10.313*X2) + [(X1-2)*(X2-2)*-2.155]*

Where Y is the dependent variable of particle diameter, Y_1 is the dependent variable of zeta potential and Y_2 is the dependent variable of entrapment efficiency.

The optimum NS formulation selected by the JMP® software was F7. The calculated desirability factor offered for the formulation was 0.57. No significant deviations between theoretical and experimental values of tested responses were found (21.74±59.19 nm *vs* 21.74±59.19 nm for particle diameter, -18.45 mV *vs* -19.1 mV for zeta potential and 85.80±10.31% *vs* 85.85±3.40 %, respectively).

Histopathological study

The systematic experiments of the factorial design enabled the successful identification of AC NS formulation F7 as a formula with improved particle diameter, zeta potential and entrapment efficiency. The next logical step was to confirm that F7 formulation is perfectly tolerated with no evidenced symptoms of ocular irritation.

Histopathological examination of the cornea of the left eye of control rabbit showed the normal structure of cornea, anterior corneal epithelium consisted of stratified squamous epithelium, corneal stroma and posterior endothelium. The keratocyte nuclei in the cornaeal stroma were present (Figure 4a). Histopathological investigation showed the characteristic cell layers of control retina: choroid; retinal pigment epithelium; photoreceptor outer segments; photoreceptor inner segments; cone inner segments; outer nuclear layer; outer plexiform layer; inner nuclear layer; inner plexiform layer; ganglion cell layer; nerve fiber layer; vitreous chamber (Figure 4b). Examination of the cornea and retina of rabbit treated with AC loaded NS F7 showed normal structure (Figures 4c, 4d).

Table 3: It shows the analysis of variance for a 3^2 Factorial design experiment for evaluation of variables' effect on the AC NS formulations

Response	Variable	Sum of squares	df[a]	F value	Prob>F
Particle Diameter	X1	52.9254	1	0.0693	0.8028
	X2	1915.6640	1	2.5089	0.1741
	X1*X2	9691.4180	1	12.6928	0.0162[b]
PDI	X1	0.01206017	1	0.7665	0.4214
	X2	0.01892817	1	1.2030	0.3227
	X1*X2	0.00207025	1	0.1316	0.7316
Zeta Potential	X1	7.260000	1	3.7856	0.1093
	X2	16.006667	1	8.3465	0.0342[b]
	X1*X2	3.240000	1	1.6895	0.2504
Entrapment efficiency (%)	X1	40.35227	1	1.7419	0.2441
	X2	638.18907	1	27.5483	0.0033[b]
	X1*X2	18.57610	1	0.8019	0.4116
Release percentage (%)	X1	61.824600	1	2.0891	0.2080
	X2	0.236017	1	0.0080	0.9323
	X1*X2	4.473225	1	0.1512	0.7134

[a] degrees of freedom.

[b] Significance level based on 1 df; $p < 0.01$.

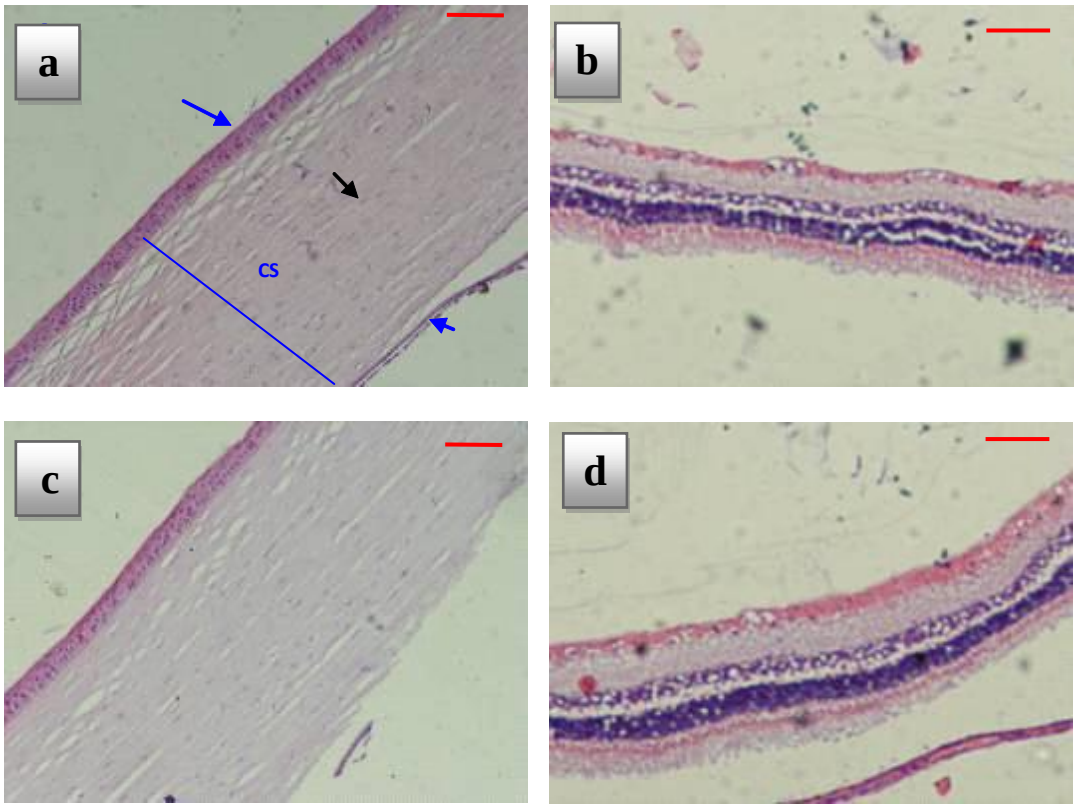


Fig. 4: It shows microphotographs of the left eye of control rabbit showing the structure of cornea, anterior corneal epithelium consisted of stratified squamous epithelium (arrow), corneal stroma (CS) and posterior endothelium (arrowhead). Notice the keratocyte nuclei in the cornaeal stroma (black arrow) (H & E, Bar = 20 µm) (a), Microphotographs of the left eye of control rabbit showing the characteristic cell layers of control retina. choroid; retinal pigment epithelium; photoreceptor outer segments; photoreceptor inner segments; cone inner segments; outer nuclear layer; outer plexiform layer; inner nuclear layer; inner plexiform layer; ganglion cell layer; nerve fiber layer; vitreous chamber.(H & E, Bar = 20 µm) (b). Microphotographs of the left eye of control rabbit treated with AC NS showing Cornea structure that appeared as control one (H & E, Bar = 20µm) (c), Microphotographs of the left eye of control rabbit showing the characteristic cell layers of control retina that appear like normal (H & E, Bar = 20 µm) (d).

Table 4: It shows the physical stability testing of NS

Factor	Refrigerated			25 C & RH 40%		
	1M	3M	6M	1M	3M	6M
Particle size	22.17	21.95	23.20	23.17	23.53	24.16
Entrapment efficiency	84.12	82.34	83.18	85.12	80.34	81.91

Physical stability testing

The stability of AC NS F7 was investigated by determining changes in particle size and entrapment efficiency after storing for a period of six months at different temperatures. Visual identification was done on the basis of color changes or agglomeration of NS. The appearance of formula F7 did not change when refrigerated or stored at room temperature for six months. The mean particle size and the entrapment efficiency showed very slight changes at the end of the studying period (Table 4). The results of the stability studies proved that NS formulation could preserve the stability of AC in various storage conditions. This finding could be explained as follows; during the process of nanosization, hydrophobic/hydrophilic interactions of surfactant molecules and drug would keep stabilizers anchoring on the surface of the newly formed particles (29), therefore, the molecules of stabilizers suited on the surface of the nanoparticles are expected to hide the drug inside. This means that, for the NS only the particle surface is exposed to the external media including water and light, therefore, in case degradation is to occur, it will take place in only the outer layer of molecules protecting the inner part of the drug nanoparticles (30).

CONCLUSION

This study investigated a combination of two independent variables; concentration of polymer and concentration of surfactant; in an attempt to develop a promising ocular delivery system to enhance the therapeutic efficacy of AC. Application of experimental design allowed the optimization of different factors to yield spherical and stable NS with small particle size, low polydispersity index and high entrapment efficiency. The entrapment efficiency was directly proportional to the concentration of Pluronic® F68 and inversely proportion to the concentration of Tween® 80. AC NS exhibited higher dissolution rate relative to the free drug. The histopathological studies on the cornea and the retina of rabbits' eyes after treatment with the optimized AC NS revealed normal structure, which means that the prepared NS did not exhibit any harmful effect on the animals' eyes. The optimum NS formulation retained its stability for 6 months.

REFERENCES

1. W. Zhang, M.R. Prausnitz and A. Edwards. Model of transient drug diffusion across cornea. Journal of Controlled Release 2004; 99 (2): 241-258.

2. G.G. Liversidge, K.C. Cundy, J.F. Bishop and D.A. Czekai. Surfactant modified drug nanoparticles. US Patent5. 1992; 145: 684.
3. H. Sucker, in R.H. Muller and G.E. Hildebrand (Eds.), Pharmaceutical Technology: Modern Dosage forms, second ed., WVG mbh, Stuttgart, 1998, p. 383.
4. J. Möschwitzer, G. Achleitner, H. Pomper and R.H. Müller. Development of an intravenously injectable chemically stable omperazole formuation using nanosuspension technology. European Journal of Pharmaceutics and Bio-pharmaceutics 2004; 58: 615-619.
5. C.M. Keck and R.H. Müller. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. European Journal of Pharmaceutics and Bio-pharmaceutics 2006; 62 (1): 3-16.
6. L. Gao, D. Zhang and M. Chen. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. Journal of Nanoparticle Research 2008; 10: 845-862.
7. B.H.L. Böhm and R.H. Müller. Lab-scale production unit design for nanosuspensions of sparingly soluble cytotoxic drugs. Pharmaceutical Sciences Technology Today 1999; 2 (8): 336-339.
8. E. Merisko-Liversidge, G.G. Liversidge and E.R. Cooper. Nanosizing: a formulation approach for poorly-water-soluble compounds. European Journal of Pharmaceutical Sciences 2003; 18 (2): 113-120.
9. P. Kocbek, S. Baumgartner and J. Kristl. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. International Journal of Pharmaceutics 2006; 312 (1): 179-186.
10. V.H.L. Lee and J.R. Robinson. Topical ocular drug delivery: recent developments and future challenges. Journal of Ocular Pharmacology 1986; 2: 67-108.
11. V.B. Patravale, A.A. Date and R.M. Kulkarni. Nanosuspensions: a promising drug delivery strategy. Journal of Pharmacy and Pharmacology 2004; 56 (7): 827-840.
12. M.A. Kassem, A.A. Abdel Rahman, M.M. Ghorab, M.B. Ahmed and R.M. Khalil. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. International Journal of Pharmaceutics 2007; 340 (1-2): 126- 133.
13. D.M. Richards, A.A. Carmine, R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery. Acyclovir. A review of its pharmacodynamic properties and therapeutic efficacy. Drugs 1983; 26 (5): 378-438.
14. J.J. Sanitato, P.A. Asbell, E.D. Varnell, G.E. Kissling and H.E. Kaufman. Acyclovir in the treatment of herpetic stromal disease. American Journal of Ophthalmology 1984; 98: 537-547.
15. J.J. O'Brien and D.M. Campoli-Richards. Acyclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drugs 1989; 37 (3): 233-309.
16. G. Box and K.B. Wilson. On the experimental attainment of optimum conditions. J. R. Stat. Soc. Ser. B. Methodol 1995; 13 (1): 1-45.
17. S. Verma, Y. Lan, R. Gokhale and D.J. Burgess. Quality by design approach to understand the process of nanosuspension preparation. International Journal of Pharmaceutics 2009: 377 (1): 185-198.
18. S. Bolton (3rd Ed.), Pharmaceutical Statistics: Practical and Clinical Applications, Marcel Dekker, New York, 1997.
19. S.M. Agnihotri and P.R. Vavia. Diclofenac-loaded biopolymeric nanosuspensions for ophthalmic application. Nanomed, Nanotech. Biol. Med. 2009; 5: 90-95.
20. H. Takeuchi, J. Thongborisute, Y. Matsui, H. Sugihara, H. Yamamoto and Y. Kawashima. Novel mucoadhesion tests for polymers and polymer-coated particles to design optimal mucoadhesive drug delivery systems. Adv. Drug Deliv. Rev. 2005; 57 (3) 1583-1294.
21. P. Liu, X. Rong, J. Laru, B. van Veen, J. Kiesvaara, J. Hirvonen, T. Laaksonen and L. Peltonen. Nanosuspensions of poorly soluble drugs: preparation and development by wet milling. Int. J. Pharm. 2011; 411 (1-2): 215-222.
22. J. Lee, J.Y. Choi, C.H. Park. Characteristics of polymers enabling nano-comminution of water-insoluble drugs. Int. J. Pharm. 2008; 355 (1-2): 328-336.
23. H. Fessi, F. Puisieux, J. P. Devissaguet, N. Ammoury, and S. Benita. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 1989; 55: R1-R4. H. Fessi, F. Puisieux, J.P. Devissaguet, N. Ammoury and S. Benita. Int. J. Pharm., (1989) R1.
24. T. Venkatesh, Avinash Kumar Reddy , J. Uma Maheswaria, M. Deena Dalithb and C.K. Ashok Kumarb. Nanosuspensions: Ideal Approach for the Drug Delivery of Poorly Water Soluble Drugs. Der Pharmacia Lettre 2011; 3 (2): 203-213.
25. H. P. Thakkar, B. V. Patel, and S. P. Thakkar. Development and characterization of nanosuspensions of olmesartan medoxomil for bioavailability enhancement. J. Pharm. Bioallied Sciences 2011; 3 (3): 426-434.
26. J. Malakar, A. Basu and A. Ghosh. Nanosuspension: A Nano-Heterogeneous Carrier for Drug Delivery System International Journal of Pharmaceutical & Biological Archives 2012; 3 (1) 4-13.
27. V. M. Pandya, J. K. Patel and D. J. Patel. Formulation and Optimization of Nanosuspensions for Enhancing Simvastatin Dissolution Using Central Composite Design. Dissolution Technologies. August 2011; 40-45.
28. N. Arunkumar, M. Deecaraman, C. Rani, K.P. Mohanraj and K. Venkates Kumar. Preparation and Solid State Characterization of Atorvastatin Nanosuspensions For Enhanced Solubility And Dissolution. International Journal of PharmTech Research 2009; 1 (4): 1725-1730.
29. R.H. Müller and C. Jacobs. Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. Int. J. Pharm. 2002; 237 (1-2): 151-161.
30. R.H. Müller and C.M. Keck. Challenges and solutions for the delivery of biotech drugs - a review of drug nanocrystal technology and lipid nanoparticles J. Biotechnol. 2004; 113: 151-170.