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Preparation and evaluation of vitamin A nanosuspension as a novel ocular drug delivery

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ABSTRACT:

Objective(s): The aim of this study was to prepare a nanosuspension formulation as a new vehicle for the improvement of the ocular delivery of vitamin A.

Material and Methods: Formulations were designed based on full factorial design. A high pressure homogenization technique was used to produce nanosuspensions. Fifteen formulations were prepared by the use of different combinations of surfactants Tween 80, benzalkonium chloride and Pluronic and evaluated for pH, particle size, entrapment efficiency, differential scanning calorimetry (DSC), stability and drug release. Also, Draize test was used to evaluate the irritation of rabbit eye by formulations.

Results: All formulations showed a small mean size that is well suited for ocular application. Also it was observed that the particle size decreased with increase in the amount of surfactant. Drug entrapment increased with increasing amount of surfactant. It was shown that initial and final drug release can be controlled by the ratio and the total amount of surfactants, respectively.

Conclusion: It was concluded that the use of Tween 80 and Pluronic in the formualtions with a proper ratio does not show eye irritation and could be useful to achieve a suitable nanosuspension of vitamin A as a novel ocular delivery system.

Keywords: *Benzalkonium, Nanosuspension, Pluronic, Tween, Vitamin A*

INTRODUCTION

Eye disorders can cause therapeutic discomfort in patients, with the ultimate anxiety of loss of vision or even facial derangement. Many segments of the eye are relatively inaccessible to systematically administered drugs, therefore topical delivery remains the preferred route of delivery in most disorders [1] and usually aqueous eye drops [2] which is easy to apply, less invasive in comparison to the other formulations such as ointmentand gel induce low irritation and blurred vision, is preferred [3,4].

The drug delivery system must provide high efficacy and safety, prolonged action and less invasive administration [5]. Water-insoluble drugs which have low polarity, low dissolution and instability in aqueous solution [6] are administered topically in the ointment that is not easy to apply for patient and high viscosity of this dosage form may decrease patient compliance [7]. Vitamin A (VA) is an essential vitamin which adjusts the proliferation and differentiation of corneal epithelial cells and protects conjunctival goblet cells. It has been used in the management of disorders of the ocular surface [8-10]. It is known that VA deficiency cause keratoconjunctival epithelial damage, resulting in conditions such as superficial punctate keratitis and dry keratoconjunctivitis, as well as abnormalities such as loss of conjunctival goblet cells [11,12]. VA is a water-insoluble drug and currently in management of eye disorders is used with a dose of 250 U/g as an ointment preparation [13]. VA shows poor stability and inactivates by oxidation. There are many reports concerned with side effects of retinoic acid ophthalmic ointment [9,14] whereas, it has been reported that ophthalmic solutions containing retinol palmitate (an ester of VA) are effective and cause few side effects [15]. Many physiological and anatomical limitations

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such as tear turnover, nasolachrymal drainage, reflex blinking, and ocular static and dynamic barriers decrease ocular drug permeation and lower its bioavailability [16]. To remove the mentioned difficulties and increase in bioavailability, conventional and novel drug delivery systems have been developed such as ointments, emulsion, suspensions, aqueous gels, liposomes, dendrimers, implants, contact lenses, nanomicelles, nanoparticles, nanosuspensions, microneedles, and thermosensitive gels for treatment eye diseases [17]. Ophthalmic nanosuspension can be defined as a method to increase dissolution and bioavailability of water-insoluble drug [6,18]. Drug nanosuspensions are submicron drug particles suspended in a dispersion medium (mostly water) and stabilized by polymer or surfactant [19]. Formulation with nano-size in ophthalmic drug delivery makes higher surface area for dissolution [20], enhances drug solubility [21], and represents higher bioadhesive [22] and corneal penetration characteristics [23]. Moreover it increases stabilization of drug, reduction in the amount of dose and systemic toxicity and therefore improves patient compliance and convenience [5].

The overall aim of this investigation was to formulate water-insoluble drug vitamin A in the nanosuspension form and to prepare a novel ocular drug delivery of this vitamin with proper physicochemical characteristics and less irritation.

MATERIALS AND METHODS

Materials

Vitamin A (Darupakhsh, Iran) and Pluronic F68 (Sigma, U.S.A) were purchased from indicated sources. Tween 80, benzalkonium chloride, EDTA, ethanol, acetone, and hydroxtethylcellulose were supplied from Merck Co., Germany. All chemicals were of analytical grade.

Design of formulations

3^2 full factorial designs were used for the design of formulations. Two independent factorial designs were selected to design of formulations containing Tween 80 and benzalkonium chloride, and Tween 80 and Pluronic, separately. Therefore, 15 different formulations were prepared. Formulations and their components were shown in Table 1. Independent variables were the amount of surfactant(s) in formulations (with the levels of 0.1, 0.5 and 1%) and ratio of Tween 80:benzalkonium chloride or Tween 80:pluronic (with the levels of 0, 50 and 100%). Dependent variables (responses) were particle size, drug entrapment, and drug release at 8 and 24 hrs.

Preparation of nanosuspension

Nanosuspensions were produced by high pressure homogenizer (HPH) (EmulsiFlex-C3, Avestin, Canada) technology. Initially, Drug was dissolved by heating and sonication, in 20 mL of acetonand:ethanol (3:1). Ethanol was used as a co-solvent to solubilize the drug. The solution was slowly injected into 80 mL of water (nonsolvent) containing different surfactants ratio under magnetic stirring and processed using Ultra Turax (IKA T-25,Germany) at 14,000 rpm for 3 min. Then the obtained pre-mix was homogenized by HPH at 5000 bar for 3 cycles.

pH measurements

pH of formulations was measured by using pH meter (MettlerTolendo Model).

Differential scanning calorimetry (DSC)

DSC was performed using Mettler Toledo Thermal analyzer. Samples of materials and formulation were placed in a standard aluminum pan and fitted with a perforated lid for scanning so that material should not spill outside. For heating scans of prepared sample, a heating rate of 10 °C/min was employed in the range of 0-200 °C.

Particle size analysis

The mean particle size of formulations was measured by Particle size analyzer (Scatterscope 1 Qudix model, South Korea). All assays were carried out in triplicate and the mean value was reported.

Determination of drug entrapment

Entrapment efficiency (the percentage of incorporated drug) was determined spectrophotometrically at 362 nm. The formulations were centrifuged, after which sediment was found amount of the free drug was detected in upper layer and amount of incorporated drug was determined by following equation;

Entrapment efficiency

$$(EE)\ (\%) = \frac{W\ initial - W\ free\ drug}{W\ initial} \times 100 \quad \text{Eq. 1}$$

Table 1. Components of formulations

Formulation	VA (mg)	Tween (%)	Benzalkonium (%)	Pluronic (%)	HEC (%)	EDTA (%)	Ethanol (ml)	Acetone (ml)	Water (ml)
F_1	350	-	0.1	-	0.35	0.1	5	15	80
F_2	350	-	0.5	-	0.35	0.1	5	15	80
F_3	350	-	1	-	0.35	0.1	5	15	80
F_4	350	0.05	0.05	-	0.35	0.1	5	15	80
F_5	350	0.25	0.25	-	0.35	0.1	5	15	80
F_6	350	0.5	0.5	-	0.35	0.1	5	15	80
F_7	350	0.1	-	-	0.35	0.1	5	15	80
F_8	350	0.5	-	-	0.35	0.1	5	15	80
F_9	350	1	-	-	0.35	0.1	5	15	80
G_1	350	-	-	0.1	0.35	0.1	5	15	80
G_2	350	-	-	0.5	0.35	0.1	5	15	80
G_3	350	-	-	1	0.35	0.1	5	15	80
G_4	350	0.05	-	0.05	0.35	0.1	5	15	80
G_5	350	0.25	-	0.25	0.35	0.1	5	15	80
G_6	350	0.5	-	0.5	0.35	0.1	5	15	80

Drug release studies

The release of VA from prepared formulations was evaluated using diffusion cells (cut off:12000). The donor and acceptor compartment were separated by a dialysis membrane. The donor compartment contained 20 ml of formulation and the acceptor compartment was filled with 20 ml freshly prepared phosphate buffer with pH 6.8 under magnetic stirring at 37 °C. At regular time intervals within 24 hr samples of 1 ml withdrew from acceptor compartment and replaced by the same volume of fresh phosphate buffer. The samples were diluted with phosphate buffer and analysis spectrometrically at 362nm [24].

Ocular tolerability test (Draize test)

To determine ocular irritancy and damaging effects formulations were evaluated according to a modified Draize test. Male albino rabbits were used in the experiment. They were hold in according to National Institutes of Health guidelines. A drop of formulations was instilled directly into the cornea in the right eye every 30 min for 6 hr and a drop distilled water as a control instilled into Left eye. Condition of the eyes was checked after 10 min, 6 hr, and 24 hr after the end of the treatments. The swelling of conjunctiva was graded on a scale from 0 to 4. Discharge and redness were graded on a scale from 0 to 3 [25].

Stability study

Each of the formulations maintained in closed amber glass bottles and placed at 25 °C (room temperature) and away from direct light. After 2 weeks drug contents and drug release from all the formulations were determined by the method discussed previously.

Statistical analysis of data

The effects of independent variables upon the responses were modeled using following second order polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{12}X_1X_2$$

Eq. 2

The modeling was performed using SPSS (Version 20.0) with a backward, stepwise linear regression technique and significant terms ($P<0.05$) were chosen for final equations. Response surface plots and contour plots resulting from equations obtained by Statgraphics Centurion XVI.

RESULTS AND DISCUSSION

pH measurements

The pH value is an important factor in the ophthalmic formulation process. The pH must be such that formulation does not induce any irritation to eyes upon administration and be stable at this pH. pH values for all the formulations were within adaptable range 6.1 - 6.4 and hence would not cause any irritation upon administration of the formulations. The results showed that the change of surfactants does not have any special effect on pH.

Differential scanning calorimetry (DSC)

The DSC thermograms are represented in Figs 1 and 2. As shown in Fig 1, VA powder represents three endothermic peaks at 55, 78 and 128 °C. Meanwhile, in the thermograms of produced formulations (Fig 2) the peaks at 55°C and 78°C were disappeared. Peak at 128 °C was shifted to 113 °C in formulations containing benzalkonium chloride while it did not significantly change in nanosuspensions composed of Tween and Pluronic. Therefore, the latter surfactants exhibited less possible interactions with the other components of formulations compared with benzalkonium.

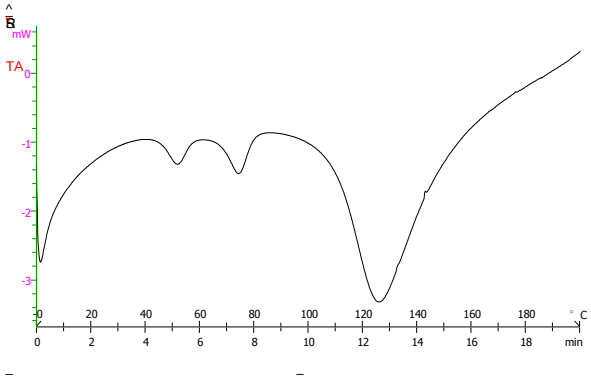

Fig. 1. DSC thermogram of vitamin A powder

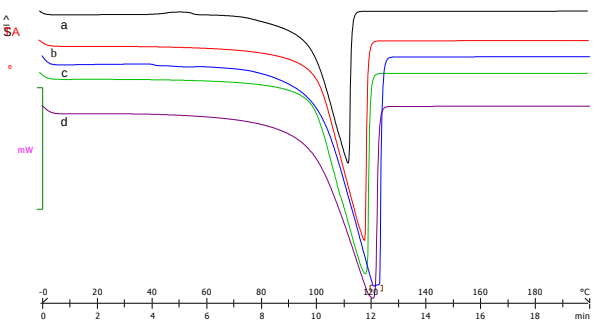

Fig. 2. DSC thermograms of (a) formulation containing benzalkonium (F3), (b) formulation containing Pluronic (G3), (c) formulation containing Tween and benzalkonium (F6), (d) formulation containing Tween and Pluronic (G6) and (e) formulation containing Tween (F9)

Particle size analysis

In order to determine the effect of the independent variables on different responses, mathematical relationships were generated between the dependent and independent variables using the statistical software SPSS. The equations of the responses are given below:

$$Y_{1(tw/bzm)} = 215.998 - 93.538X_1 + 10.500X_2 - 5.646X_1X_2 - 0.037X_2X_2 \quad \text{Eq. 3}$$

$$Y_{1(tw/plc)} = 858.624 - 453.670X_1 - 9.046X_2 + 0.083X_2X_2 \quad \text{Eq. 4}$$

$$Y_{2(tw/bzm)} = 56.854 + 0.345X_2 + 17.490\ X_1X_1 - 0.003X_2X_2 \quad \text{Eq. 5}$$

$$Y_{2(tw/plc)} = 56.438 + 19.179X_1 + 0.136X_2 - 0.001X_2X_2 \quad \text{Eq. 6}$$

$$Y_{3(tw/bzm)} = 43.288 + 0.157X_2 + 6.405X_1X_1 - 0.001X_2X_2 \quad \text{Eq. 7}$$

$$Y_{3(tw/plc)} = 49.231 - 0.221X_2 - 9.123X_1X_1 + 0.220X_1X_2 + 0.001X_2X_2 \quad \text{Eq. 8}$$

$$Y_{4(tw/bzm)} = 70.411 - 0.032X_2 + 10.909\ X_1X_1 \quad \text{Eq. 9}$$

$$Y_{4(tw/plc)} = 66.978 + 10.645X_1 \quad \text{Eq. 10}$$

Analysis of variance (ANOVA) (Table 2) indicated that the assumed regression models were significant and valid for different responses.

The three-dimensional response surfaces were drawn to predict the effects of the independent variables on each response.

All formulations showed a small mean size that is suitable for ocular application. The particle size of formulations was shown in Table 3.

Accordingly, formulations F2 and F3 had the minimum particle size. These formulations contained 0.5 and 1% benzalkonium. On the other hand, G1 and F7 with the maximum particle size composed of 0.1% Pluronic and Tween, respectively. Fig 3 represents the effect of independent variables on particle size.

As shown in Fig 3, the particle size reduction depends on the amount of surfactant and surfactants ratio. Particle size decreased with increasing the amount of surfactant. Surfactants reduce the surface tension and thus by improving dispersion of particles in the water can reduce the size of nanosuspension [26]. The particle size decreasing effect of surfactants was mostly observed with benzalkonium and Tween and Pluronic in the ratio of 1:1. In fact, the other ratios of latter surfactants increased the mean particle size of nanosuspensions.

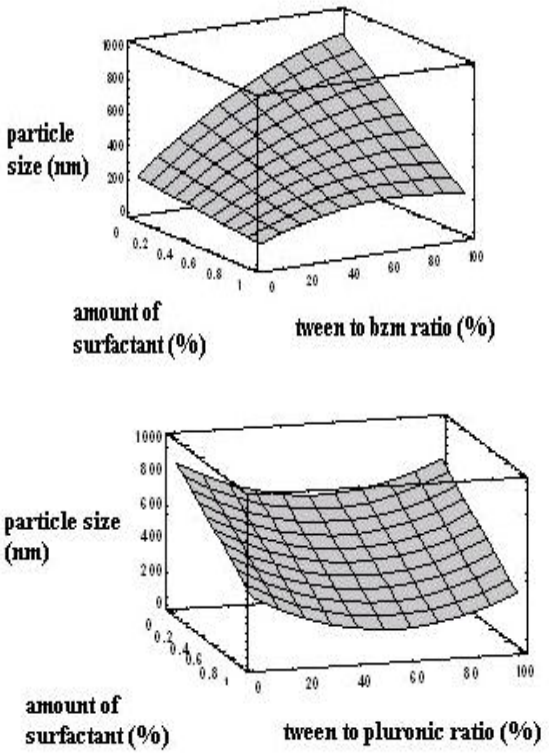

Fig. 3. Response surface plots of Y1 (particle size) for formulations containing (a) Tween and benzalkonium and (b) Tween and Pluronic

Table 2. Analysis of Variance (ANOVA) of dependent variable Y_1 for formulations containing Tween and Pluronic

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1048473.212	5	209694.642	36.455	.000[b]
	Residual	120793.528	21	5752.073		
	Total	1169266.741	26			
2	Regression	1047940.221	4	261985.055	47.505	.000[c]
	Residual	121326.520	22	5514.842		
	Total	1169266.741	26			
3	Regression	1036741.823	3	345580.608	59.976	.000[d]
	Residual	132524.918	23	5761.953		
	Total	1169266.741	26			

a. Dependent Variable: Y1
b. Predictors: (Constant), X2X2, X1X1, X1X2, X2, X1
c. Predictors: (Constant), X2X2, X1X2, X2, X1
d. Predictors: (Constant), X2X2, X2, X1

Table 3. Particle size of the experimented formulations

Formulation	Particle size (nm)
F_1	207.33 ± 13.01
F_2	156.66 ± 18.03
F_3	140.33 ± 14.84
F_4	614.00 ± 14.52
F_5	497.00 ± 21.16
F_6	231.66 ± 21.03
F_7	846.33 ± 51.92
F_8	502.66 ± 12.09
F_9	272.00 ± 15.09
G_1	813.68 ± 18 .00
G_2	655.00 ± 27.22
G_3	371.33 ± 21.38
G_4	468.66 ± 40.41
G_5	387.33 ± 14.97
G_6	258.33 ± 11.01

Drug entrapment results

The results of drug entrapment efficiency showed that formulations F1 and G1 had the minimum entrapment with the amounts of 58.13% and 58.26%, respectively and maximum entrapment was belonged to F6 (87.20%). As for Fig 4, entrapment efficiency increased by increasing the total amount of surfactant. This could be obviously due to reducing the surface tension and increasing the tendency of drug to aqueous phase in presence of surfactant [27]. Thus particles migrate out of the solvent phase and are dispersed in water. Also it was observed that using the 1:1 ratio of surfactants can improve drug entrapment compared with the other ratios and separate using each of surfactants.

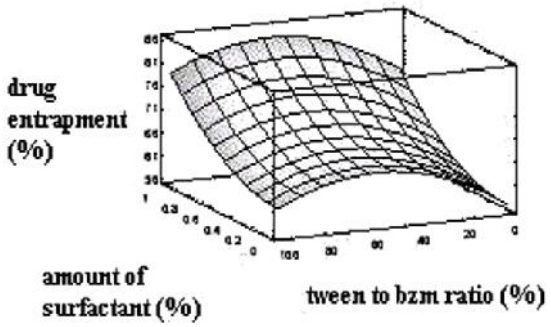

Fig. 4. Response surface plot of Y2 for formulations containing Tween and benzalkonium

Drug release studies

In vitro drug release from nanosuspensions in the phosphate buffer media with pH 6.8 has been shown in Fig 5. As shown, an initial burst effect was observed

from all of the formulations and 20-40% of drug released at the first hour. Afterwards the drug release followed an approximate steady pattern, and cumulative percent drug released for formulations after 24 hr was 70-80 %. The burst release in the first hour can be attributed to the drug loaded on the surface of nanosuspensions [28]. In effect, some parts of drug may deposits on the surface of nanoparticles which dissolves when the formulation encounters the dissolution media. Increase of drug release was observed as a function of the total amount of surfactants. Also dissolution data showed that drug release at the end of 8 hours increased with increase in the ratio of tween to benzalkonium (data not shown). As shown in Fig 6, drug release increased in the presence of Tween; Meanwhile, Pluronic caused a decrease in drug release. Final drug release at the end of 24 hr mostly depended on the total amount of

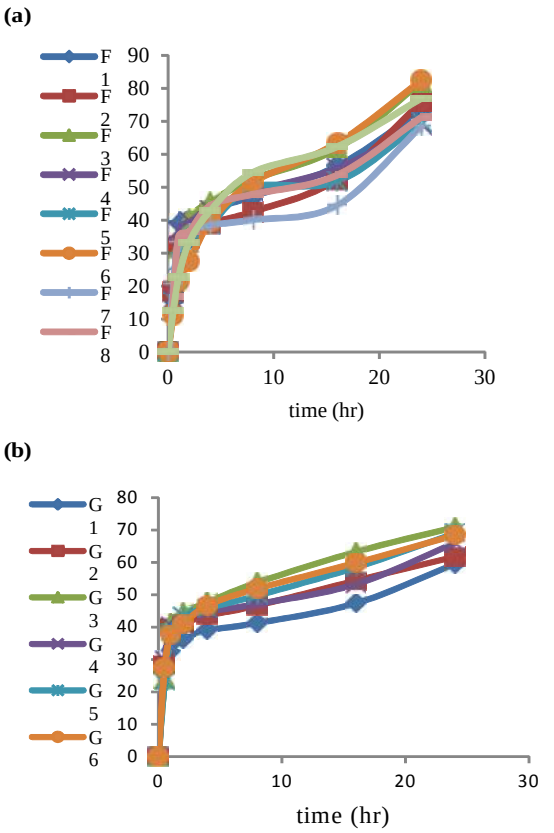

Fig. 5. Drug release profiles from formulations containing (a) Tween and benzalkonium and (b) Tween and Pluronic

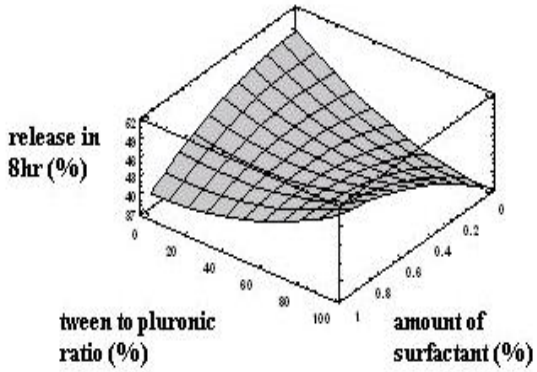

Fig. 6. Response surface plot of Y3 (drug release at 8 hr) from formulations containing Tween and Pluronic

Ocular tolerability test (Draize test)

The data of Draize tests did not showed any sign of swelling of conjunctiva in rabbit eyes. It was also demonstrated that formulations containing benzalkonium caused a little discharge and redness in rabbit eyes. But this phenomenon was not observed by Tween and Pluronic. Benzalkonium is a cationic surfactant and may induce more irritation compared with other surfactants [29]. However, non-ionic surfactants Tween and Pluronic could be less irritable and therefore use of these surfactants for producing nanosuspension with favorite characteristics will be more helpful.

Stability study

Stability studies showed that decrease in drug entrapment after 1 month was rare and drug release was not significantly changed after this period. Therefore, all of the formulations showed a stable manner and drug entrapment and release was not affected in different periods.

CONCLUSION

According to the results of this study all formulations showed a small mean size that is a perfect size for ocular application. Also it was observed that the total amount of surfactant lowered the particle size and increased drug entrapment.

Formulations containing benzalkonium had minimum particle size. On the other hand, the ratio of surfactants

was an important parameter on the size control in nanosuspensions made by Tween and Pluronic. Also, it was concluded that initial and final drug release can be controlled by the ratio of surfactants.

Use of Tween 80 and Pluronic in the formulations with a proper ratio did not show eye irritation and could be useful to achieve a suitable nanosuspension of vitamin A as a novel ocular delivery system.

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