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Development of vitamin loaded topical liposomal formulation using factorial design approach: Drug deposition and stability

Mahesh N. Padamwar, Varsha B. Pokharkar *

Department of Pharmaceutics, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune 411 038, Maharashtra, India

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Abstract

Long-term exposure of the skin to UV light causes degenerative effects, which can be minimized by using antioxidant formulations. The major challenge in this regard is that a significant amount of antioxidant should reach at the site for effective photoprotection. However, barrier properties of the skin limit their use. In the present study, Vitamin E acetate was encapsulated into liposome for improving its topical delivery. However preparation of liposomes is very difficult due to number of formulation variables involved therein. In the present work systematic statistical study for the formulation of liposomes for topical delivery of Vitamin E using the factorial design approach was undertaken. Amount of phospholipid (PL) and cholesterol (CH) were taken at three different levels and liposomes were prepared using ethanol injection method. Liposomes were characterized for encapsulation efficiency, vesicle size, zeta potential, and drug deposition in the rat skin. Gels containing liposomal dispersion (batch with higher skin deposition of VE) were prepared in Carbopol® 980 NF and were characterized for gel strength, viscosity and drug deposition in the rat skin. Stability of liposome dispersion and gel formulation was studied at 30 °C/65% RH for 3 months. Results of regression analysis revealed that vesicle size and drug deposition in the rat skin were dependant on the lipid concentration and lipid:drug ratio. Drug deposition in rat skin had an inverse relationship with respect to PL and CH concentration. Prepared liposomal dispersion (50 mg PL:6 mg CH) showed seven-fold increase in drug deposition compared to control (plain drug dispersion). Gel formulation demonstrated six-fold and four-fold increase in drug deposition compared to control gel and marketed cream, respectively. Liposome dispersion and gel formulation were found to be stable for 3 months. Factorial design was found to be well suited to identify the key variables affecting drug deposition. Improved drug deposition from liposomal preparations demonstrates its potential for dermal delivery.

Keywords: Factorial design; Vitamins; Liposomes; Gels; Vesicle size; Skin drug deposition

1. Introduction

Continuous exposure of the skin to UV irradiations and absorption of photons by endogenous photosensitizer molecules results in formation of different reactive oxygen species, which affects appearance of the skin. It also leads to different degenerative effects and skin damages such as photoaging, sunburn, photocarcinogenesis, etc. (Wlaschek et al., 2001). Amongst the approaches used to protect skin from these degenerative effects, use of antioxidants has been adopted as an important strategy in pharmaceutical and cosmetic industry. Antioxidants are incorporated in cosmetics and pharmaceutical formulation to scavenge free radicals in skin produced by UV light and environmental pollutants (Lupo, 2001). Recently antioxidants such as Vitamins A, C and E are widely used in cosmetic products due to their obvious advantages for the skin. Antioxidants are promising in photoprotection with negligible side effects at physiological concentrations (Yamamoto, 2001). It is expected that desirable amount of antioxidant should reach at the site for effective photoprotection of the skin. However, delivery of drugs through topical preparations viz. creams, gels, lotions, emulsion, etc. limits the effectiveness of actives due to barrier properties of the skin which hinder the drug deposition and relative poor stability of vitamins due to direct exposure of actives to UV light. Thus selection of proper carrier is extremely important by considering the views in mind that they should increase drug deposition (Verma et al., 2003) and flux (Jia-You et al., 2004) and should protect drug from photodegradation.

* Corresponding author. Tel.: +91 20 25437237; fax: +91 20 25439383.
E-mail addresses: m_padamwar@yahoo.co.in (M.N. Padamwar), varshapokharkar@rediffmail.com, vbpokharkar@yahoo.co.in (V.B. Pokharkar).

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In last two decades, number of innovative microparticulate carrier systems viz. microemulsion (Spiclin et al., 2003), nanoemulsion (Jia-You et al., 2004), nanoparticles (Muller et al., 2002), liposomes (Betz et al., 2005; Kirjavainen et al., 1999), ethosomes (Lopez-Pinto et al., 2005), etc. have been reported for improving delivery of drug to the skin. Recently, liposome based formulations for topical delivery have been shown to be extremely promising for enhancement of drug penetration (Betz et al., 2005; Maestrelli et al., 2005; Cevec, 1996), improved pharmacological effects (Sharma et al., 1994; Skalko et al., 1992), decreased side effects, controlled drug release (Jia-You et al., 2004) and drug photoprotection. Arsic et al. (1999) reported encapsulation of Vitamin A palmitate into liposomes protects it from photodegradation. Foco et al. (2005) and Barry (2001) have demonstrated that delivery of sodium ascorbyl palmitate incorporated in liposomal formulation can enhance the drug deposition and helps to prevent or slowing down the complex process of photoaging in the skin. Improved delivery of drugs using liposomes is based on similarity of vesicle bilayer structure to that of natural membranes, which can alter cell membrane fluidity and fuse with cells. However the exact mechanistic basis of the same still remains unclear. The phenomenon of improved drug delivery is based on factors viz. lipid concentration, composition, lamellarity, vesicle size, surface charge, type of formulation, etc. (Betz et al., 2005; Verma et al., 2003).

Topically Vitamin E and its derivatives are widely used to protect the body tissues from damage. It inhibits lipid peroxidation by preventing free radical generation and/or reducing malondialdehyde, and is beneficial in protecting the epidermis against UV induced damage than dietary Vitamin E (Idson, 1993). Also, presence of Vitamin E in the formulation can help to prevent lipid peroxidation.

Encapsulation efficiency (EE), drug deposition and vesicle size were the key parameters involved in formulation of topical liposomes. The number of formulation and processing variables are involved during liposome preparation may affect these parameters and hence the performance of the formulation Thus it becomes extremely difficult to study the effect of interactions between various variables and preparation of liposome by a conventional method. Factorial design and response surface methodology is an important statistical tool to study the effect of several factors influencing responses by varying them simultaneously by carrying out limited number of experiments (Kincl et al., 2005; Hirsch et al., 2005; Martínez-Sancho et al., 2004; Cochran and Cox, 1992). Literature search revealed no study carried out to formulate liposome preparation to demonstrate the influence of formulation variables on drug deposition in the skin using a factorial approach. Thus the aim of the present investigation was to carry out a systematic statistical study on preparation of liposomal formulation using factorial design approach and to explore its application for topical delivery of Vitamin E acetate (VE). Relative quantities of phospholipid and cholesterol (PL:CH) is an important parameter in light of the stability and cost consideration. Their ratio also greatly affects vesicle size and drug deposition in the skin. In the present study, liposomes were prepared by 3^2 factorial design using a modified ethanol injection method. However, VE was selected by predicting 100% encapsulation owing to its lipophillic nature, which will more effectively help in studying the effect of the formulation variables. Liposomes were characterized by EE, vesicle size, zeta potential, and drug deposition in the rat skin. Gels containing liposomal dispersion (batch with higher skin deposition of VE) were prepared using Carbopol® 980 NF and were characterized for gel strength, viscosity and drug deposition in rat skin. Stability of liposome dispersion and gel formulation was studied at 30 °C/65% RH for 3 months.

2. Materials and methods

2.1. Materials

Phospholipon 80N® (P 80N) (unsaturated phosphotidylcholine (PC) 76% and lysophopshotidylcholine 2%), was a kind gift from Phospholipid GmbH, Nattermannalee, Germany. Cholesterol (CH) and stearic acid (SA) were purchased from Qualigens Fine Chemicals, Mumbai, India and Research Lab, Mumbai, India, respectively. Vitamin E acetate (VE) was obtained as a kind gift sample from Softesule, Mumbai, India. Marketed VE cream was procured from local market. Sephadex® G25 M was purchased from Amersham Bioscience A.B. Sweden. Carbopol® 980 NF (poly acrylic acid polymer) was gift sample from Noveon, India. All other chemicals used were of HPLC or analytical grade.

2.2. Preparation of liposomes

Liposomes were prepared by modified ethanol injection method as described by Batzri and Korn (1973). In brief, required amount of P 80N, CH and SA (Table 1) were dissolved in 1 ml of ethanol containing VE (12 mg). Ethanolic solution was rapidly injected into 10 ml of double distilled water (DDW) under stirring at 500 rpm for 15 min using Teflon coated magnetic bead.

2.3. Effect of variables

To study the effect of variables on liposome performance and characteristics, different batches were prepared using 3^2 factorial design. Amount of P 80N and CH were selected as two independent variables. Vesicle size, EE and drug deposition in

Table 1
Experimental design with coded levels of variables and actual values

Batch code	Variable X_1 amount of P 80N (mg)	Variable X_2 amount of CH (mg)
LP-1	60 (+1)	12 (+1)
LP-2	60 (+1)	09 (0)
LP-3	60 (+1)	06 (−1)
LP-4	50 (0)	12 (+1)
LP-5	50 (0)	09 (0)
LP-6	50 (0)	06 (−1)
LP-7	40 (−1)	12 (+1)
LP-8	40 (−1)	09 (0)
LP-9	40 (−1)	06 (−1)

Values in parentheses indicates coded levels.

the rat skin were selected as dependent variables. Amount of SA and VE were kept constant. Values of all variables and batch codes are as shown in Table 1.

2.4. High performance liquid chromatography (HPLC)

Estimation of VE was carried out using HPLC (JASCO, Japan) equipped with a UV detector. Detection was carried out at 280 nm. Column used was C-18 (0.5 μm, Hypersil®, 25 mm × 4.6 mm, Thermo) attached with guard column (ODS, Hypersil®, 20 mm × 4 mm, Thermo). Drug was eluted using methanol as mobile phase at a flow rate of 1.6 ml/min.

2.5. Characterization of liposomes

2.5.1. Size distribution

Mean vesicle size and size distribution of empty and drug-loaded liposomes was determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK) based on photon correlation spectroscopy. Analysis ($n = 3$) was carried out for 100 s at room temperature by keeping angle of detection at 90°.

2.5.2. Zeta potential (ζ) determination

Charge on empty and drug loaded vesicles surface were determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s and average ZP, charge and mobility of liposomes were determined.

2.5.3. Encapsulation efficiency (EE)

EE was determined by minicolumn centrifugation method as described by Fry et al. (1978). Sephadex® G25 M solution (10%, w/v) was prepared in DDW and was kept aside for 24 h for swelling. To prepare minicolumns, Whatman filter pad was inserted in 1 ml syringe and swelled Sephadex was added slowly to it. Care was taken to avoid air entrapment in the column. Excess amount of water was removed by spinning the column at 2000 rpm for 3 min using Eppendorff centrifuge 5810 R (Hamburg, Germany). Liposome dispersion (100 μl) was slowly added on prepared column and centrifuged as earlier. Procedure was repeated on the same column by adding 100 μl of DDW. Free drug remained bound to the gel, while vesicles travelled the gel and were collected from first and second stage of centrifugation. Obtained eluted liposomes were ruptured using sufficient volume of methanol and percent encapsulation was calculated from total amount of VE present in 100 μl of liposomes by HPLC using Eq. (1). Method was validated by applying free drug solution instead of liposomes (Essa et al., 2002).

$$\text{encapsulation efficiency (EE)} = \left(\frac{Q_e}{Q_t}\right) \times 100 \quad (1)$$

where Q_e is the amount of encapsulated VE and Q_t is the amount of VE present in 100 μl of liposomes.

2.6. Drug deposition studies

Deposition study ($n = 3$) was performed on the excised skin of sacrificed albino rats (225–250 g) using Franz Diffusion cells. Abdominal skin of rat was shaved and skin was carefully separated. Subcutaneous fat was carefully removed using a scalpel. Skin section thus obtained was mounted on Franz Diffusion cells having surface area of 3.91 cm^2 and receptor compartment having a capacity of 22 ml. Epidermal side of the skin was exposed to ambient condition while dermal side was kept facing to receptor solution. Receptor compartment was filled with DDW as diffusion medium (37 ± 0.5 °C). Tween 80 (0.5%, v/v) was added to maintain the sink condition. Reservoir solution was stirred at 500 rpm. Diffusion cells were protected from light. Skin was saturated with dissolution medium for 1 h before the application of sample. A dose equivalent to 300 μg of VE encapsulated in liposomes was applied on donor compartment. For determination of drug deposited in the skin, cell was dismantled after a period of 24 h and skin was carefully removed from the cell. Drug present on the surface of the skin was removed by using method described by Plessis et al. (1994) using Scotch Tape (Scotch Magic Tape, 810, Birla 3M Ltd., Banglore, India). After strippings the skin was cut into small pieces and drug present in the skin was extracted in methanol under sonication and estimated by HPLC. Similarly drug deposition from VE dispersion in water was carried out as control preparation to find out weather entrapment of vitamin in liposomes improves the skin drug deposition. Analysis of data was done using 'PCP Disso V 3' software (IIPC, PCP, Pune, India).

2.7. Preparation of liposome gels

On the basis of factorial design approach, liposomal batch (LP-6) was selected for further formulation studies of liposomal gel. Gels were prepared using Carbopol® 980 NF at different concentrations. Briefly, required amount of Carbopol® 980 NF was added into water and kept overnight for complete humectation of polymer chains. Liposome dispersion (LP-6) was added to hydrated carbopol solution with stirring to give final concentration of 0.1, 0.3 and 0.5% (w/w). Gelling was induced by neutralizing the dispersions to pH 6.8–7.0 using 18% (w/v) NaOH solution. Prepared gel formulations were characterized for following parameters.

2.7.1. Content uniformity

Drug content uniformity was determined by analyzing drug concentration in gel taken from three to four different points using HPLC.

2.7.2. Viscosity of gel

Viscosity behaviour ($n = 3$) of Carbopol® 980 NF and liposomal gel formulations (0.1, 0.3 and 0.3%, w/w) was measured using Brookfield LV-DV III Programmable rheometer (cone-plate viscometer) equipped with Spindle CP-40 (Brookfield Engineering Laboratory, Incorporation, Middleboro). A cone and plate sensor having a diameter of 2.4 cm was used with an angle of 0.8° was used. Thickness of sample in the middle of sensor was 5/1000 in. Viscosity behaviour of liposome free gel (control) was also measured similarly.

2.7.3. Gel strength

Gel strength ($n = 6$) of Carbopol® 980 NF and liposomal gels were determined using Ultra Test, Mecmesin Tester (Mecmesin, West Sussex, England). Instrument was modified as per requirement. A piston having diameter of 7.98 mm with plane surface at base was attached to Advanced Force Guage (AFG). AFG measures force in Newton (produced due to resistance) required to travel down the piston in gel up to a predetermined specific distance. Vertical movement of the piston at a constant speed was achieved using motor. Gels were filled in measuring cylinder (i.d. 14.2 cm) up to 7 ml and shaft was moved down at a constant speed of 2 in./min. Force required for piston to travel a distance of 5 cm down in gel was measured.

2.8. Drug deposition from liposomal gels

Deposition of drug in the rat skin ($n = 3$) from liposomal gels, control gel and from marketed cream containing VE were determined as described earlier using Franz diffusion cells.

2.9. Stability of liposomes

Stability of liposome dispersion (LP-6) and 0.3% (w/w) gel formulation (LG-6) were carried out at 30 °C/65% RH for 3 months. Effects of temperature and RH on the vesicle size, EE, drug deposition in rat skin were studied for liposomal dispersion and content uniformity, gel strength, viscosity, drug deposition in rat skin were studied for liposomal gel during stability period.

3. Results and discussion

Preliminarily the optimum concentrations of P 80N, CH, and SA were determined to obtain stable liposomes devoid of aggregation, fusion and sedimentation (visual observation) using a ternary phase diagram (data not shown). Ten micromoles (3 mg) of SA was found to be optimum to prevent aggregation of liposomes. Amount P 80N and CH were found to be critical in preparation and stabilization of liposomes and hence selected as variables in the 3^2 factorial design (Table 1).

3.1. Statistical data

Responses of different batches obtained using factorial design are shown in Table 2. Obtained data were subjected to multiple regression analysis using Unistat software (Megalon, USA). The data were fitted in Eq. (2).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1 X_1 + \beta_{22} X_2 X_2 + \beta_{12} X_1 X_2 \quad (2)$$

Results of multiple regression analysis for all parameters studied are summarized in Table 3. Insignificant variables were removed, and adequacy of fitted model was checked by analysis of variance (ANOVA). Response surface plots were generated using 'PCP Disso V 3' software (IIPC, PCP, Pune, India).

3.2. Effect on encapsulation efficiency

EE is expressed as fraction of drug incorporated into liposomes relative to total amount of drug used (Kim et al., 1998). EE for all batches was found to be in the range of 98–101% (Table 2). The process variables were found to be insignificant in case of EE. However observed high EE may be attributed to high lipophilicity of the drug.

3.3. Effect on size and size distribution

Ethanol injection method was found to produce unilamellar and homogeneous population of liposomes as indicated by the low polydispersity index (Table 2). Relative amount of P 80N, CH and VE play an important role in the determining of vesicles size through interactions between them. Size of empty vesicle was found to be in the range of 124–206 nm. However vesicle size of drug loaded liposomes was found to be decreased as compared to empty vesicle size in case of LP-4 to LP-9 batches. Reduction in vesicle size of drug-loaded liposome may be attributed to degree of association of drug with lipid. Hydrophobic VE intercalate into the lipid bilayer leading to appreciable cohesion among the apolar portion of the membrane, causing reduction in the vesicle size (Manconi et al., 2002). However batches having higher amount of P 80N (LP-1 to LP-3) did not demonstrate significant reduction in vesicle size as compared to empty one. This lack in reduction of vesicle size in case of higher lipid level may be attributed to insufficient drug molecules for complete degree of association with the lipids. To confirm our hypothesis we prepared few additional batches of liposomes having same lipid composition to

Table 2
Responses obtained for studied parameters from experimental batches ($n = 3$)

Batch code	Encapsulation efficiency (%EE) ±S.D.	Vesicle size of empty liposomes (nm) ±S.D.	Vesicle size of drug loaded liposomes (nm) ±S.D.	Polydispersity index (PI) of drug loaded liposomes	VE deposited in the skin (μg/cm^2) ±S.D.	ζ of empty liposomes (mV) ±S.D.	ζ of drug loaded liposomes (mV) ±S.D.
LP-1	100.45 ± 3.44	206.50 ± 12.56	208.22 ± 15.89	0.280	2.85 ± 1.09	−52.00 ± 0.99	−53.87 ± 0.51
LP-2	99.65 ± 1.69	198.25 ± 15.30	199.45 ± 16.74	0.242	5.72 ± 2.09	−50.13 ± 1.10	−52.93 ± 0.85
LP-3	98.68 ± 0.76	189.15 ± 15.16	192.13 ± 18.68	0.233	6.94 ± 2.62	−47.00 ± 1.13	−48.70 ± 0.62
LP-4	98.37 ± 2.09	201.64 ± 14.81	189.00 ± 14.81	0.189	5.01 ± 1.45	−42.50 ± 0.72	−50.27 ± 0.42
LP-5	98.66 ± 1.93	169.15 ± 13.32	161.75 ± 13.89	0.204	6.33 ± 2.79	−30.67 ± 4.81	−51.07 ± 1.57
LP-6	100.95 ± 3.25	164.95 ± 14.46	136.00 ± 13.38	0.211	13.31 ± 1.66	−27.37 ± 1.46	−35.33 ± 2.71
LP-7	99.78 ± 2.11	158.40 ± 16.22	142.15 ± 13.18	0.198	6.85 ± 1.30	−26.90 ± 2.71	−29.30 ± 1.87
LP-8	98.71 ± 4.94	136.05 ± 13.89	123.90 ± 16.65	0.201	8.77 ± 1.49	−30.47 ± 3.72	−33.57 ± 1.16
LP-9	100.55 ± 3.82	124.25 ± 12.33	116.55 ± 13.32	0.200	11.24 ± 2.43	−39.67 ± 0.74	−45.33 ± 1.00

Table 3
Summary of regression analysis results for measured responses

Parameters	Coefficients							
	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	r^2	p
Empty vesicle size	174.34	29.217	14.708	−9.786	6.342	–	0.9716	0.0001
Loaded vesicle size	161.044	37.716	16.142	–	–	–	0.9567	0.0001
Drug deposition	7.443	−1.893	−2.796	–	–	–	0.6602	0.0003

that of LP-1 to LP-3 but the amount of drug was doubled. We found that size of vesicles was lower (157.1, 145.3, 140.4 nm, respectively) than size of LP-1 to LP-3 batches. These results indicate that size of liposomes also depends on lipid:drug ratio. Our results are in accordance with Bradford et al. (2003) who also demonstrated that the dimensions of hexagonal phases of the lipid micelle decreases with increasing tocopherol content. Manconi et al. (2002) also demonstrated reduction in vesicle size after addition of tretinoin in liposomes.

To understand the effect of lipid concentration on vesicle size, coefficient observed for the both empty and drug loaded liposomes size was fitted in Eq. (2) to generate Eqs. (3) and (4), respectively.

$$Y = 174.34 + 29.217X_1 + 14.708X_2 - 9.786X_1X_1 + 6.342X_2X_2 \quad (3)$$

$$Y = 161.044 + 37.716X_1 + 16.142X_2 \quad (4)$$

A positive correlation was observed for both variables X_1 (P 80N) and X_2 (CH) in vesicle size of both empty ($r^2 = 0.9716$) as well as drug loaded liposomes ($r^2 = 0.9567$). However empty vesicle size showed a curvilinear equation. Thus with increase in P 80N and CH vesicle size was found to be increased (Figs. 1 and 2). Lopez-Pinto et al. (2005) also demonstrated increase in vesicle size with cholesterol content. In both cases effect of P 80N was more prominent than the effect of CH as indicated by respective coefficient values (Table 3).

3.4. Determination of zeta (ζ) potential

Values of ζ potential for empty and drug loaded liposomes are shown in Table 2. Values of ζ potential showed prepared liposome have sufficient charge and mobility to inhibit aggregation of vesicles. It was observed that ζ potential of vesicles was increased after the addition of drug to liposomes.

3.5. Effect on skin drug deposition

Number of methods has been reported to determine drug deposition in the skin such as diffusion, microdialysis, microscopic studies, etc. (Foldvari et al., 1990; Yamashita and Hashida, 2003). In the present study, drug deposition was determined by diffusion method. After 24 h the amount of drug deposited in the skin was determined by HPLC. Drug depositions in the skin for different batches are depicted in Table 2. There was only 6.91 ± 0.31 μg/cm^2 of VE was deposited from the control preparation (plain drug dispersion in water). However, deposition of VE found to be increased in case of liposomal batches. As shown in Table 2 comparatively less amount of VE was deposited from batches (LP-1 to LP-3) with higher level of P 80N (+1). This may be due to comparatively higher particle size and high level of P 80N. Verma et al. (2003) reported with increase in vesicle size penetration of carboxyfluorescein

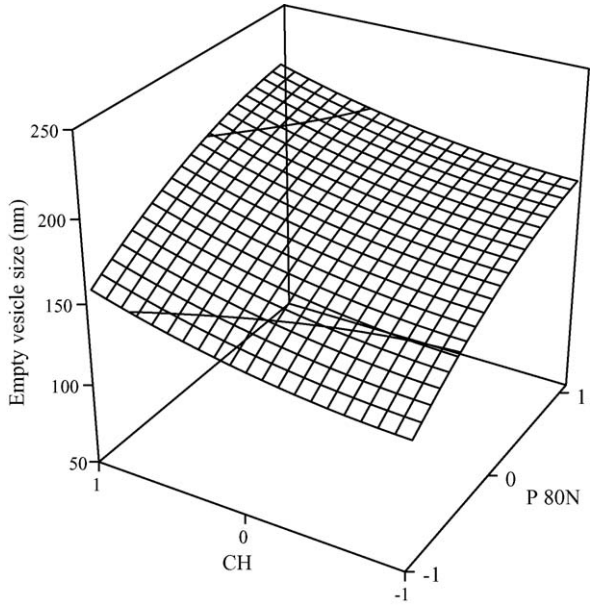

Fig. 1. Effect of lipid concentration on vesicle size of empty liposomes.

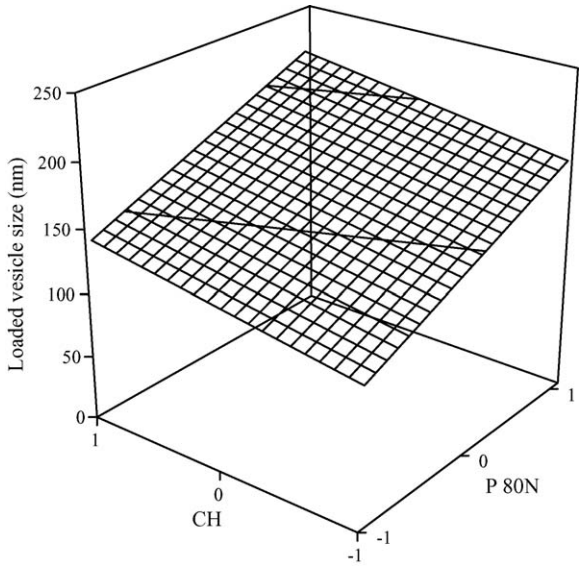

Fig. 2. Effect of lipid concentration on vesicle size of drug loaded liposomes.

decreases and found that intermediate particle size gave better penetration. Secondly, larger vesicles and formulations with high lipid content may not penetrate well into the deeper layers of the skin and remain on skin surface forming a lipid layer, which can further strengthen barrier properties of the stratum corneum and thus hinder diffusion of molecule in the skin. Among all batches, LP-6 which had intermediate vesicle size showed maximum drug deposition of $13.31 \pm 1.66\ \mu g/cm^2$, and was selected for the further study of gel formulation. To study the effect of lipids on drug deposition in the rat skin Eq. (5) was generated after fitting the observed coefficient in Eq. (2).

$$Y = 7.443 - 1.893X_1 - 2.796X_2 \quad (5)$$

Drug deposition was found to be an inverse function of X_1 (P 80N) and X_2 (CH). Drug deposition increased with decreasing amount of P 80N and amount of CH (Fig. 3). The inhibitory effect of X_2 on drug deposition was found to be more prominent than X_1 as indicated by the observed respective coefficient (Table 3). The planar and rigid ring system of cholesterol is thought to reside in outer layer part of the fatty acyl chain region where it tends to restrict the motion of chains in liquid crystalline bilayers and may sterically hinder the diffusion of drug molecules (Robinson et al., 1995; Lagerquist et al., 2001). A good degree of fitness was obtained for this parameter. But the obtained degree of fitness was lower as compared to other two parameters, i.e. empty vesicle size and drug loaded vesicle size. This could be due to introduction of some additional bio-variables, which varies from skin to skin and are beyond precise control. Due to this the obtained data get scattered which ultimately results in the comparatively lower r^2 value.

Improved deposition of drug from the liposome preparation into the skin than control preparation may be attributed to either penetration of intact liposome or due to molecular mixing of phospholipids with skin lipids. However, Ganesan et al. (1984) and Ho et al. (1985) stated that size of liposomes would not allow diffusion of liposomes through densely packed outer layer of the skin. In contrast to this Kirjavainen et al. (1999) stated that phospholipid enhances drug deposition by temporarily changing skins ultra structural properties. Lipids present in the stratum corneum are in tightly ordered solid or gel phases, with traces of material in a fluid liquid crystal phase where the freedom of movement of individual molecule is higher. When liposomes come in contact with the skin lipids some budding will occur which results in fusion and molecular mixing of liposomes with closely packed lipids of the stratum corneum. This would elevate proportion of fluid lipid relative to solid or gel arrangements. Thus, a temporarily less well-packed lipid structure forms, allowing drug to penetrate through more easily, which will act as a typical penetration enhancer effect (Vrhovnik et al., 1998; Essa et al., 2003).

3.6. Liposomal gel formulation

Liposomal gels were formulated using Carbopol® 980 NF by considering its ease of application. Liposomal gels (0.1, 0.3 and 0.5%, w/w) showed uniform distribution of drug through out batch (data not shown). Addition of Carbopol® 980 NF to liposomal dispersion showed decrease in viscosity as compared to control gel for 0.1% (w/w) liposomal gel. However viscosity was found to be increased as compare to control for 0.3 and 0.5% (w/w) liposomal gels (Fig. 4). Contradictory findings on changes in the viscosity after addition of liposomes are identified from the literature. Boulmedarat et al. (2003) reported shear-thinning effect on Carbopol® 974P with incorporation of sterically stabilized and positively charged liposomes and methyl β-cyclodextrin while viscosity was found to be increased after addition of liposomes at higher lipid concentration (10 mM). Whereas Pavelic et al. (2001) did not report any change in viscosity of Carbopol® 974P NF after addition of liposomes. Thus the exact mechanistic role of liposomes on modifying the rheological behaviour of gel forming polymers needs to be further exemplified.

Preliminarily the gel strengths of plain Carbopol® 980 NF gels were determined. Increasing the Carbopol® 980 NF concentration from 0.1 to 0.5% (w/w) resulted in increased gel strength (indicated by increase in force). However, the behaviour of gels

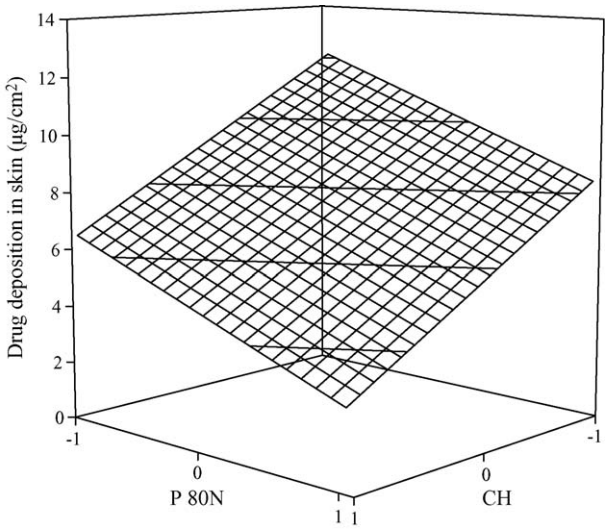

Fig. 3. Effect of lipid concentration on VE deposition in rat skin after 24 h.

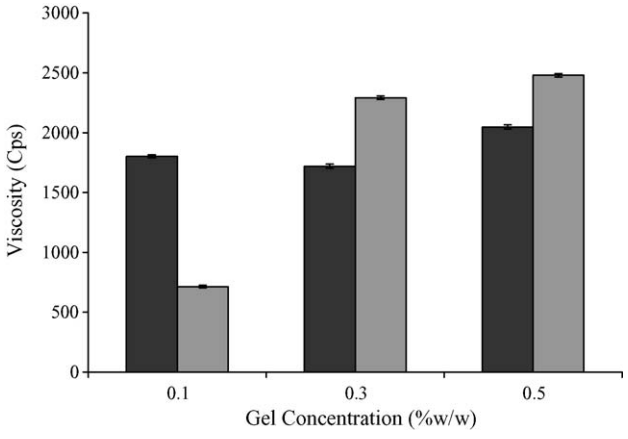

Fig. 4. Effect of liposome on gel viscosity ($n = 3$). Key: (■) Carbopol® 980 NF gel; (▩) liposome gel.

Table 4
Effect on vesicle size and skin drug deposition during stability ($n = 3$)

Parameters	LP-6			LG-6 (0.3%)			
	Initial	1 month	3 months	Initial	15 days	1 month	3 months
Vesicle size (nm ± S.D.)	132.4 (±0.21)	144.5 (±0.48)	151.2 (±1.1)	NA	NA	NA	NA
Drug deposition ($\mu g/cm^2$ ± S.D.)	13.31 (±1.66)	12.39 (±2.58)	12.30 (±1.91)	5.46 (±1.20)	4.98 (±0.98)	4.10 (±0.84)	4.12 (±0.75)

at different concentrations varies after the addition of liposomal dispersion. Results obtained for gel strength are in agreement with viscosity results (Fig. 5).

3.7. Drug deposition study from gel formulations

Drug deposition from liposomal gels of different concentrations was carried out as described previously. Drug deposited in the rat skin from liposomal gels at different concentration from 0.1, 0.3 and 0.5% (w/w) was found to be 6.65, 5.54 and 2.74 $\mu g/cm^2$, respectively. Decrease in the deposition from gels as compared to liposomal dispersion might be due to increased viscosity and block co-polymer of carbopol, which retards the release from its structure. Liposomal gel (0.1%, w/w) showed maximum deposition of VE in the skin, however viscosity of this system was very low to handle. Therefore 0.3% (w/w) liposomal gel, which has optimum viscosity and drug deposition, was selected for the stability study. Drug deposition from control gel and marketed cream containing VE was found to be 0.44 ± 0.11 and 1.15 ± 0.21 $\mu g/cm^2$, respectively (Fig. 6). Fig. 6 showed comparative deposition of VE in the skin from different formulations.

3.8. Stability study

Stability of liposome dispersion as well as 0.3% (w/w) liposomal gel was carried out for 3 months at 30 °C/65% RH. Responses obtained for different parameters during stability are as shown in Table 4. Insignificant ($p > 0.001$) effect was observed

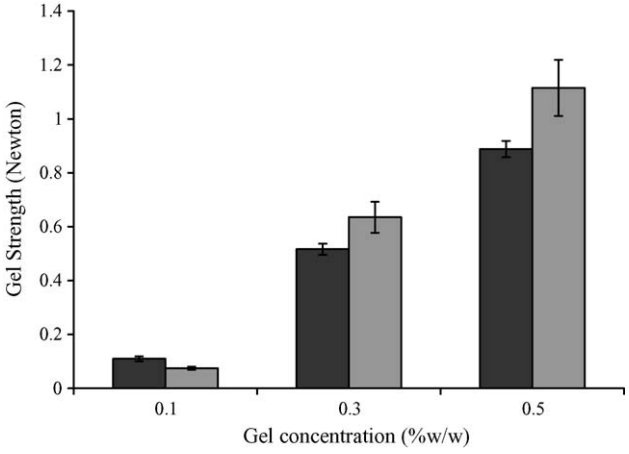

Fig. 5. Effect of liposomes on gel strength ($n = 6$). Key: (■) Carbopol® 980 NF gel; (▩) liposome gel.

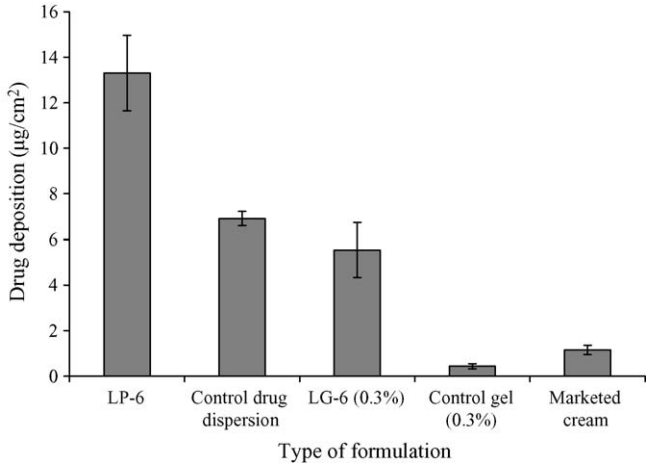

Fig. 6. Amount of VE deposited in the rat skin after 24 h from different formulations ($n = 3$).

on EE and content uniformity throughout the stability period for liposome dispersion and liposomal gel, respectively. Similarly insignificant ($p > 0.001$) increase in vesicle size was observed (Table 4) with time, which might be attributed to very slight fusion of the liposomes. This insignificant increase in vesicle size might be due to presence of surface negative charge on liposomes, which either avoids or delays formation of liposome aggregates due to electrostatic repulsion.

Drug deposition from liposomal dispersion and gel was found to be slightly decreased with time (Table 4). Deposition of VE in the skin from liposomal dispersion and gel was found to insignificantly decreased ($p > 0.001$) during stability period indicating prepared liposomes and gels are stable for 3 months. No effect was observed on viscosity and gel strength (data not shown) during stability period, which indicates gels were stable at 30 °C/65% RH for 3 months.

4. Conclusions

Preparation of liposomes using factorial design was found to be well suited and sound approach to obtain stable liposomal formulation of Vitamin E acetate. Loading of the highly lipophillic drug such as Vitamin E in the liposomes greatly influences the micromeritic properties of the vesicle. Variables such as amount of phospholipid, amount of stabilizer and lipid:drug ratio have a profound effect on the vesicle size and drug deposition in the rat skin. Increased drug deposition in rat skin as compared to control drug dispersion, control gel and marketed cream suggest that liposomal formulation promotes drug deposition in the

rat skin thus has potential for dermal delivery. Liposomal dispersion and liposomal gel were found to be stable for 3 months at 30 °C and 65% RH.

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