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Research Article

FORMULATION AND EVALUATION OF NANOCARRIER DRUG DELIVERY SYSTEM FOR HYPERPIGMENTATION

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ABSTRACT

The study is focused to formulation and evaluation of nanocarrier drug delivery system for Hyperpigmentation. Aspasomes were use as a vesicle for drug delivery and Ascorbyl palmitate were explore as a bilayer vesicle forming material which formed vesicles in combination with cholesterol and Soya Phosphatidylcholine. Aspasomes were prepared by film hydration method followed by sonication in which aqueous drug solution was encapsulated in aqueous regions of bilayer. Differential scanning calorimetric data of Aspasomes dispersion and anhydrous mixtures of ascorbyl palmitate, cholesterol and Soya Phosphatidylcholine confirm the formation of bilayered vesicles with ascorbyl palmitate. The prepared Aspasomes were evaluated for drug entrapment, particle size analysis, scanning electron microscopy, differential scanning calorimetric, transdermal permeation, In vitro (release characteristics), and In vivo. Stability study of aspasomal preparation shown that the formulations were found stable for 60 days. Transdermal permeation of aspasomal preparation was much higher than the other preparations. In vivo experiments revealed that aspasomes decrease the irritation cause by model drug was found in irritation study on rat skin.

KEYWORDS: *Aspasomes, Hyperpigmentation, Differential Scanning Colorimetry, Scanning Electron Microscopy, In vitro and In vivo.*

INTRODUCTION

The novel drug delivery system is the most suitable and approachable in developing the delivery system which improves the therapeutic efficacy, drug targeting of new as well as pre-existing drugs. Thus provides controlled and sustained drug delivery to the specific site which meets the real, appropriate and well-timed drug demand of the body [1].

Normal skin colour is determined by a number of chromophores, the most important of which is melanin. Melanin Pigments produced by melanocytes are deposited throughout Epidermis, which is determinant of skin color [2]. Melanin is produced by melanocytes and is transferred to the surrounding epidermal keratinocytes. Two types of melanin pigmentation occur in humans. The first is constitutive skin colour, which is the amount of melanin pigmentation that is genetically determined in the absence of sun exposure and other influences. The other is facultative (inducible) skin colour or 'tan', which results from sun exposure. Increased pigmentation can also be due to endocrine, paracrine and autocrine factors [3]. Melanins are complex polymers derived from tyrosine and other intermediates, which are converted through a multistep process of oxidative and complexation reactions to brown black eumelanins and yellow-red pheomelanins, which create the diversity of coloration observed across the human population [4, 5].

Pigmentation is the most visible phenotypic characteristic in nature. Among the various pigments, melanin is widely distributed in living organisms such as bacteria, fungi, plants, animals and human beings [6]. Human skin color is determined principally by the amount, type and distribution of melanin in the epidermis [7].

Whitening is the elimination of the melanin that acts as a self-defense mechanism for the human skin against the exposure to Ultraviolet light. The meaning of the expression "whitening", however, has evolved to mean far more. It entails inhibiting the further synthesis of melanin [8].

Ascorbyl Palmitate is a highly bioavailable, fat-soluble derivative of ascorbic acid. Ascorbyl palmitate possesses all the benefits of vitamin C, but unlike the water-soluble form, is able to be stored in the lipid cell membrane until the body is ready to put it to use. Vitamin C offers a wide range of support for the human body. It is a potent antioxidant and free radical scavenger supporting cellular and vascular health [9, 10].

Aspasome is an ascorbyl palmitate vesicle with biological activity, Ascorbyl palmitate (ASP) was explored as bilayer vesicle forming material. It formed vesicles (Aspasomes) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Ascorbyl palmitate is Ascorbic acid ester which is amphiphilic in nature and studies delineating their surface active properties. It is capable to suppress pigmentation of the skin and decomposition of melanin; it can be used to whiten the skin [11]. It also improves elasticity of the skin by promoting the formation of collagen. Ascorbyl palmitate is more stable than ascorbic acid. Its lipophilic character is beneficial for its skin penetration [11].

MATERIALS AND METHODS

Ascorbic acid-6-palmitate, Hydroquinone (Model drug), Cholesterol and Soya Phosphatidylcholine was purchased from Merck Limited Mumbai, India. Chloroform of analytical grade, methanol (HPLC grade) and acetonitrile (HPLC grade), cellulose membrane was purchased from Hi-media Mumbai, India.

Preparation of drug loaded Aspasomes:

Formulations of Aspasomes were prepared by film hydration method under nitrogen atmosphere in which 200 µmol of lipid mixture (ascorbyl palmitate: Soya phosphatidylcholine: cholesterol in varied

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molar ratio with dicetyl phosphate included at 10 mol% of the total lipid) was dissolved in 9ml of chloroform and 1ml of methanol in a round bottom flask and was kept under reduced pressure in rotary evaporator at 50° C till it formed a thin dry film on the walls of the flask. The dried thin lipid film was hydrated with 10 ml of phosphate buffered saline (PBS, pH 7.4) containing 2 % w/v hydroquinone (HQ) at the same temperature. The vesicles were then sonicated for 2 min using ultrasonicator on which power was set at 50% of maximum output. The Aspasomes were stored in nitrogen-purged vials [11].

Table No. 1: Ingredients for Aspasomes

S. No.	Ingredients	Uses
1.	Ascorbyl palmitate	Vesicles forming component
2.	Soya phosphatidylcholine	Vesicles forming component
3.	Dicetyl phosphate	As a stabilizer
4.	Cholesterol	Provide strength to vesicle
5.	Chloroform and methanol	As an organic solvent
6.	Phosphate buffer (pH 7.4)	As a hydrating medium

Optimization of Aspasomes:

Optimization of vesicle system was done on the basis of entrapment efficiency and vesicle size by varying the concentration of ascorbyl palmitate and cholesterol.

Volume of hydration and time of hydration, solvent system and proportion of both the solvents and temperature and annealing time was optimization after the optimization of the concentration of ascorbyl palmitate and cholesterol [12, 13].

Optimization Technique:

The formulation of aspasomes is based on the concentration of ascorbic palmitate and concentration of cholesterol basically which has create a large variation on important response i.e., entrapment efficiency and vesicle size.

On the basis of concentration of independent variables and based on the trials (AP1 – AP11) in formulation (Refer table no 2), eight different formulation trials were planned. In each formulation the concentration of hydroquinone and Soya phosphatidylcholine (10 mol %) was kept constant. The study was performed by Factorial design with 2 factors at their 2 levels (2^2) [13, 14].

Table No. 2: Formulation Codes of ASP Formulations

S. No.	Ingredients (mol %)	Formulations										
		AP1	AP2	AP3	AP4	AP5	AP6	AP7	AP8	AP9	AP10	AP11
1.	Ascorbyl palmitate	50	50	33.3	25	45	50	40	60	30	70	20
2.	Cholesterol	50	--	33.3	25	45	40	50	30	60	20	70
3.	Soya Phosphatidylcholine	--	50	33.3	50	10	10	10	10	10	10	10
4.	Chloroform and methanol (9:1)	q.s. (As minimum quantity to dissolve Phospholipids: cholesterol mixture).										
5.	Phosphate buffer (pH 7.4)[2mg/ml]	q.s. (To make up volume up to 10 ml).										

Characterization of Aspasomes:

Photomicrography:

Photomicrographs of unsonicated Aspasomes were taken using LABORLUX S microscope fitted with camera at various magnifications, so as to confirm the formation and to understand the nature of vesicles.

Vesicle Shape and Surface Morphology:

Images were recorded on a Hitachi S-576 Scanning electron micrograph (magnification: 50X; accelerating voltage: 20.0 kV). Analysis was performed at 25 ± 2 °C. The aspasomal dispersion was diluted appropriately and sonicated. Few drops of the dispersion was placed on the grid and allowed to dry. After the samples dried thoroughly, the image was captured.

Determination of Vesicle Size, Zeta potential, and Polydispersity Index (PI) of Aspasomal Dispersion:

The vesicle size, zeta potential and size distribution of ASP were measured by Malvern Zetasizer 2000. Vesicular dispersion were mixed with the appropriate medium (PBS, pH 7.4) and the measurements were taken in triplicate in a multimodal mode. All measurements were performed at 25 °C after 5min of thermal equilibration. A spherical form of particles, a viscosity of the medium of 0.8872 CP and refractory index of 1.33 were assumed [15].

Percent Entrapment Efficiency:

The hydroquinone entrapment capacity by ASP was determined by centrifugation method with cooling microcentrifuge (Remi instruments ltd Mumbai) at 4^0C at 12000 rpm for 15 min. The supernatant (free drug in PBS pH7.4) and sediment (vesicle entrapping the hydroquinone) were collected separately. Supernatant and sediment (lysis of vesicles by Triton X-100 0.5% v/v or methanol) was removed and analyzed for drug quantity. The entrapment efficiency was calculated using the following equation [16].

$$EE\ \% = [(T-S)/\ T] \times 100$$

Where,

T is the total amount of NS detected both in supernatant and sediment;

S is the amount of NS detected only in the supernatant.

Lyophilization and Reconstitution:

Each 1 ml aspasomes dispersion with lyoprotectant (trehalose) was filled into 5 ml glass vials, covered with special stoppers for lyophilization and placed in an Epsilon 2-4 freeze dryer. For freezing the samples, the sample plate was cooled with 0.2 °C/min from 20 to −50 ◦ C under atmospheric pressure. After 2 h, the pressure was reduced to 0.04 mbar and the plate was heated to −10 °C. The samples were dried under these conditions for 25 h. Afterwards the plate was slowly heated (0.125 °C/min) to 20 °C and the pressure was reduced to 0.001 mbar (17-18). These conditions were kept for 10 h to remove residues of water. Finally, the samples were flushed with nitrogen gas and the vials were closed directly inside the freeze dryer [19].

Differential Scanning Calorimetry:

Aspasome dispersions (Lyophilized) and anhydrous mixtures were studied by Differential Scanning Calorimeter (Mettler DSC 821e, Mettler-Toledo, Switzerland) to understand the thermotropic properties and phase transition behavior. Analysis was carried out in conventional aluminium pan with a heating rate of 5° C/min [19, 20].

Drug content:

The drug content of optimized Aspasomes was determined by using UV method, 1g of Aspasomae equivalent to 5mg of hydroquinone was diluted with 10 ml distilled water, mixed until a slightly opaque

dispersion was obtained and afterwards the dispersion was centrifuged at 3000 rpm for 30 min. The supernatant was collected separately while the sediment was discarded. The supernatant was filtered through the polycarbonate membrane filter (0.20 µm) and was diluted with 10 ml of distilled water and analysed for drug quantity. The concentration of hydroquinone was determined by measuring the absorbance at 289nm using spectrophotometer [22].

In vitro release:

Aspasome preparation was subjected to release studies using dialysis apparatus Prior to release study, maximum extent of unentrapped HQ was removed by dialyzing the original preparation. Five milliliters of dialyzed aspasome preparation was subjected to release studies. One hundred milliliters of PBS was placed in receptor cell. Dialyzed aspasome preparation or HQ solution (in PBS, pH 7.4) was transferred to donor cell. Five-milliliter samples were drawn from the receptor cell at preset intervals over a period of 18 h.

At each time immediately after removal of sample, the medium was compensated with PBS. The samples were analyzed for HQ by UV spectrophotometer. HQ solution (in PBS, pH 7.4) served as control. Similar experiments were performed with aqueous HQ solution. All experiments were performed in triplicate and values are reported as mean ± standard deviation [23, 24].

Skin irritation studies:

The albino rats of either sex weighing 20-22gms were used for this test. The intact skin was used. The hair was removed from the mice 3 days before the experiment. The animals were divided into two batches and each batch was again divided into two groups. Aspasomal HQ was used on test animal. A piece of cotton wool soaked in saturated drug solution was placed on the back of albino mice taken as control. The rats were treated daily upto seven days and finally the treated skin was examined visually for erythema and edema [25, 26].

Stability study:

The optimized Aspasome dispersion and lyophilized formulation were kept in sealed vials (30 ml capacity) and stored at 2-8 ^{0}C (refrigerated temperature), 25 ± 2^{0}C (room temperature, RT) for 3 months.

The sample from either batch at each temperature was taken at definite time intervals and observed visually under microscope for change in consistency (crystalline structure) and the ability of vesicles to retain the drug (i.e., drug-retentive behaviour) was assessed. For determination of the drug retention the sample were centrifuged at 12000 rpm for 30 min, and then the supernatant was analyzed for free drug concentration. All measurements were performed in triplicate *(n=3)* [27].

RESULTS AND DISCUSSION

Optimization of Aspasomes (ASP):

Amount of ascorbic palmitate and concentration of cholesterol were found to be critical parameters in preparation of aspasomes hence they selected as independent variables in the 2^2 factorial designs.

Effect of variables on particle size (PS):

The most important parameter, which needs to monitor during ASP preparation its best performance, is the vesicle size and size distribution of ASP. To understand the effect of lipid concentration on vesicle size, coefficient observed for EE fitted in Eq. is

Final Equation in Terms of Coded Factors:

$$PS = +552.28 + 243.35 * A - 128.64 * B$$

A positive correlation was observed for the both variables. The term A and B are for the respected variables if new product is to be designed ,the values should be putted in the equation at the place of A and B. This could give the same repittabilty in the product at any quantity.

Effect of variables on entrapment efficiency (EE):

Determination of EE is an important parameter in case of aspasomes as it may affect the drug release and skin deposition. EE is expressed as the fraction of drug incorporated into aspasomes relative to total amount of drug used. In the present study, the observed EE for all batches were in the range of 32.60–53.56 %. To understand the effect of lipid concentration on vesicle size, coefficient observed for EE fitted in Eq. is

$$EE= +41.92 -1.16 * A - 23.61 * B$$

A positive correlation was observed for both variables A and B

Thus with equal molar concentration of ASP and CHOL was used in the formulation the entrapment efficiency found to be increased. The batch with less particle size and less polydispersity index was selected as optimized batch ahead of the batch with more % EE.

Photomicrography:

The Presence of vesicles in aspasome dispersion was confirmed by viewing unsonicated system using an optical microscope under 100 X magnifications**.** Vesicles were seen in all the preparation containing 25-75 mol% of CHOL. The vesicles were spherical and majority of them were multi-lamellar. Very few large unilamellar vesicles were also seen.

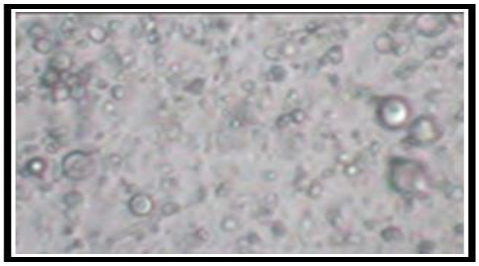

Fig. 1: Photomicrographs of Unsonicated Aspasomes Dispersion Formed with under 100 X Magnifications

Entrapment Efficiency:

The hydroquinone entrapment capacity by the optimized formulation of ASP was found to be maximum in the formulation AP1 (ASP: CHOL) in which the concentration of ascorbyl palmitate and cholesterol are equal. The drug encapsulation efficiency within the ASP was found to be 53.52% of optimized formulation. The low encapsulation is mainly due to the hydrophilic nature of drug.

Table No. 3: Entrapment efficiency of different Aspasomal formulation of hydroquinone

S. No.	Parameters	Results
1	Ascorbyl palmitate : cholesterol : Soya phosphatidylcholine molar ratio	45 : 45 : 10 (mol%)
2	Solvent system	Chloroform: methanol (9:1)
3	Hydration temperature	50-55 $_0$C
4	Hydration volume	10 ml
5	Hydration time	1 hour
6	Annealing time	3 hour
7	Percentage drug entrapment	55.52
8	Average vesicle size (nm)	396.8± 0.63

SEM studies:

The nature of the aspasomal dispersion particles was further confirmed by SEM studies. Moreover, findings of the SEM study also suggested a spherical nature of ASP (Fig. 2).

Vesicle Size, Zeta potential, and Polydispersity Index (PI) of Aspasomal Dispersion:

Effect of composition on vesicle size, zeta-potential and percent entrapment of HQ in Aspasomes is shown in below table. Vesicle size and zeta potential shows aspasome form stable vesicles with less agglomeration of dispersion (Table 4).

Differential scanning calorimetry (DSC):

The DSC thermograms of pure hydroquinone, ascorbyl palmitate, cholesterol and physical mixture of hydroquinone, ascorbyl palmitate, cholesterol and lyophilized formulation of ASP-HQ. The pure hydroquinone showed a sharp single endothermic peak at 178.95 ^{0}C. A depressed endothermic peak was also found almost at the same temperature from the curve of physical mixture. A broad small endothermic peak was observed in ASP-HQ formulation showed that the hydroquinone loaded in ASP was not in a crystalline state but in an amorphous form. Ascorbyl palmitate and cholesterol showed melting endotherms at 116.73 ± 2 ^{0}C and 149.84 ± 2 ^{0}C, respectively and when the material was formulated as ASP, the temperature was much lower 59.84 ± 2 ^{0}C than original values. However, there was no obvious change when the raw material was mixed in the physical mixture and the phase transition temperature of vesicle dispersions were always much lower than anhydrous lipid mixtures and the smaller peak is due to rearrangement of the individual lipid molecules within the bilayer (Fig. 3).

Drug content:

Drug content was found to be in the range of 92.5-84.5. There was no significant difference observed in the % drug at various locations.

In Vitro studies:

In *In-Vitro* studies the Percent HQ released versus time plots of Aspasomes are shown in (Fig. 4). Significant changes in release were observed with change in CHOL content in the bilayer of aspasome.. In 20 hours released study up to 78 % of drug was released rapidly and then linear in this range, after it drug releases slowly and in Comparative % *In-Vitro* Drug Release of Plane HQ, Aspasomal HQ, and Aspasomal HQ Gel were show that aspasomal HQ showed much slower release rate than HQ solution (Fig. 4).

Kinetics of Release:

The drug release data from the aspasomal gel was fitted into different models. The value of r^2 was found to be highest for the Korsmeyer-peppas (r^2 = 0.9997). This indicates that the magnitude of the release exponent "n" indicate the release mechanism (fickian diffusion,case II transport, or anomalous transport). In the present study the limit consider n=0.45 which indicate fickian diffusion controlled release (Table 5).

Skin Irritation Test:

The albino rats were treated daily and finally the treated skin was examined visually for erythema and edema and there was no visual evidence of erythema and edema was found on test subject, up to two week of treatment period.

Stability study:

After 3 months of storage at 4^{0}C and RT, no significant changes in the size of all the three form of ASP formulations were found. The entrapment efficiency of the ASP formulations had fallen by about 5% in ASP-HQ dispersion. This result may be due to the crystal transition of cholesterol which was caused by the temperature and storing. ASP-HQ lyophilized and ASP-HQ gel exhibited a good stability during the period of 3 months. No obvious change in clarity of prepartion was found (Table 6).

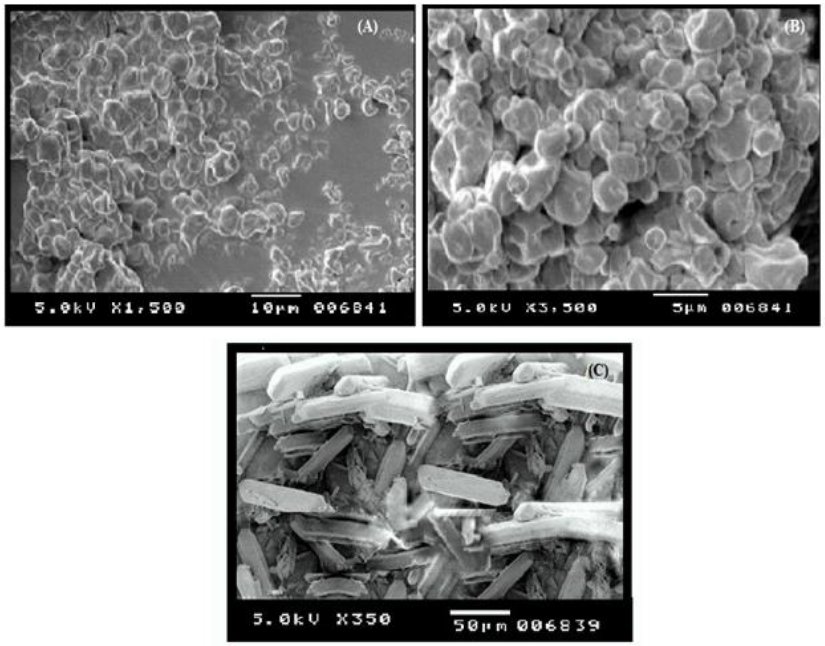

Fig. 2: SEM Image

Table No. 4: Vesicle Size, Zeta potential, and Polydispersity Index (PI) of Aspasomal Dispersion

S. No.	Formulation code	Average vesicle size (nm) (Average±S.D)	Zeta potential (mv) (Average ± S.D)	Polydispersity index (PI) (Average ± S.D)
1.	AP26	396.8±06.3	-55.2±2.4	0.441±0.2
2.	AP27	420.7±08.4	-52.5±2.8	0.531±0.4
3.	AP28	448.0±12.3	-48.2±3.5	0.670±0.3

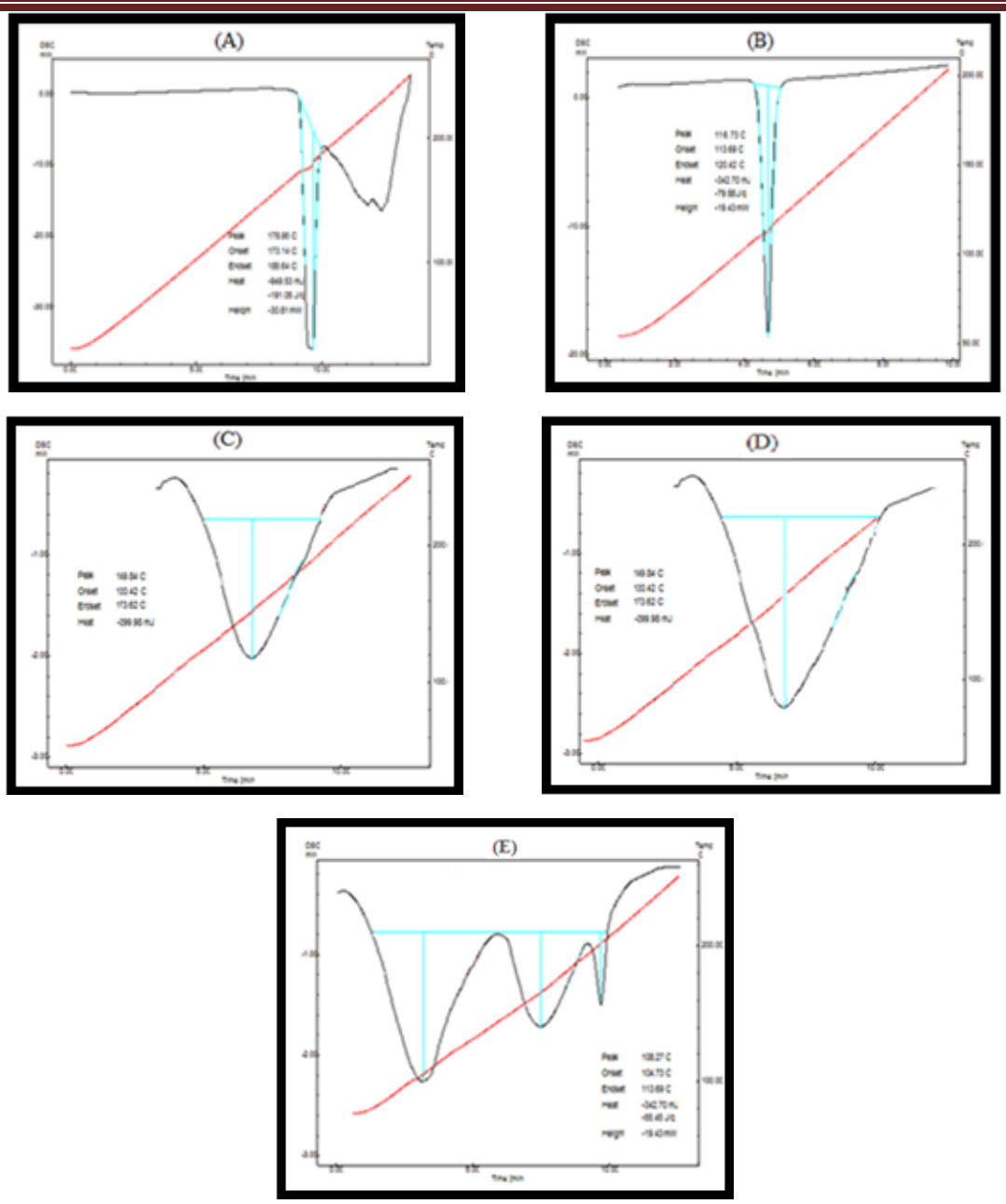

Fig. 3: DSC Thermogram of (A) Hydroquinone (B) Ascorbyl Palmitate (C) Cholesterol (D) Soya phosphatidylcholine (E) Mixture of API+ Excipients

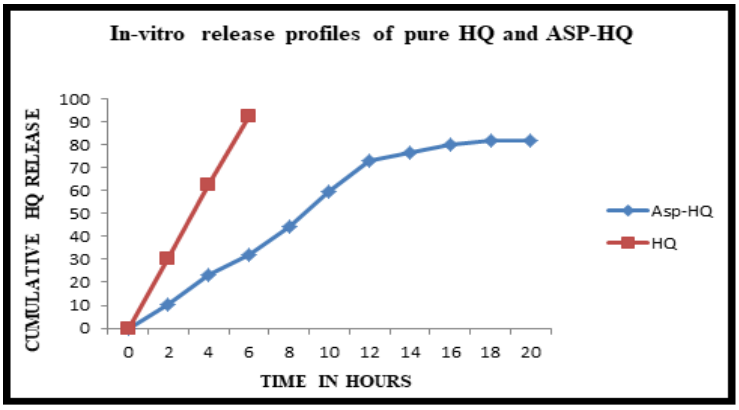

Fig. 4: Comparative % *In-Vitro* Drug Release of Plane HQ and Aspasomal HQ

Table No. 5: Release kinetics

S. No.	0 order	1 order	2 order	Korsmeyer-peppas	Hickson-crowell
Asp-HQ	0.946	0.324	0.260	0.999	0.737

Table No. 6: Stability studies of ASP-HQ dispersion

No. of days	ASP-HQ dispersion			
	Entrapment efficiency (%)		Vesicle size (nm)	
	4-8^{0}C	RT	4-8^{0}C	RT
0	53.56	53.56	348.6	348.6
30	52.24	50.45	350.8	352.4
60	50.18	48.68	352.5	360.9

CONCLUSION

Ascorbyl palmitate formed vesicles (Aspasomes) in presence of cholesterol, encapsulating HQ solution. The antioxidant potency of aspasome and it is capable to suppress pigmentation of the skin and decomposition of melanin; it can be used to whiten the skin. Thus, they may find applications as drug delivery system which is use for whitening the pigmented skin. Aspasomes enhanced the transdermal permeation of HQ. The antioxidant property and lipophilic character indicate it is a promising tool for transdermal drug delivery system.It also improves elasticity of the skin by promoting the formation of collagen.

In the present study we have shown that aspasomes hydroquinone was highly efficient in the treatment of skin pigmentation disorders and produce whitening effect on skin, because of its promising feature it was explored as a promising carrier for drug delivery especially in case when the antioxidant activity and whitening activity is required.

REFERENCES:

1. Robinson JR and Lee VHL. In Controlled Drug Delivery: Fundamental Applications. **1987**;2(N4):7.
2. Jablonski NG. The evolution of human skin and skin colour. J Cosmet Derm **2004**;33:585-623.
3. Hearing VJ. Biochemical control of melanogenesis and melanosomal organization. J Invest Derm **1999**;4:24-28.
4. Parvez S. Kang M. Chung H. S. Cho C. Hong MC. Shin MK. Bae H. Survey and mechanism of skin depigmentation and lightening agents. Phytother Res **2006**;20:921-934.
5. Nikolaou V. Stratigos A J. and Katsambas AD. Established treatments of skin hypermelanoses. J Cosmet Derm **2006**;5:303-308.
6. Nudelmanl A. Ben-Ishail Z. Ruse M. Schamrothz J. Skin-depigmenting prodrugs of hydroquinone. Eur J Med Chem **1993**;2:159-164
7. Engasser PG. and Maibach HI. Cosmetics and dermatology: Bleaching creams. J. of the Am. A of Derm **1981**;5:143-147
8. Mills OH. and Kligman AM. Further experience with a topical cream for depigmenting human skin. J Soc Cosmet Chem **1978**;29:147-154.
9. Spicilin P. Gasperlin M. Kmetec V. Stability of ascorbyl palmitate in topical microemulsion. Int J Pharm **2001**;222:271-279.
10. Darr D. Combs S. Dunston S. Manning T. Pinell S. Tropical vitamin C protects porcine skin from ultraviolet radiation-induced damage. Br J Derm **1992**;127:247-253.
11. Gopinath D, Ravia D, Raoa BR, Apte SS, Renuka D. and Rambhaua D. Ascorbyl palmitate vesicles (Aspasomes): formation, Characterization and application. Int J Pharm **2004**;71:95-113.
12. Wehlrle P. Benita S. Magnenhein B. The influence of process parameters on the PLA size distribution evaluated by means of factorial design. Eur J Pharm Biopharm **1995**;41:19-26.
13. Mowafy H. Response surface methodology for the development of self-nanoemulsified drug delivery system (SNEDDS) of indomethacin. J Appl Sci Res **2009**;5:65-79.
14. Raissi S. Developing new processes and optimizing performance using response surface methodology. World Acad of Sci Eng and Tech **2009**;49:10-42.
15. Doijad R. Manvi F. Godhwani B. Joseph R. Formulation and targeting efficiency of cisplatin engineered solid lipid nanoparticles, Ind J Pharm Sci **2008**;70:7-20.
16. Lau KG. Hattori Y. Chopra S. Ultra-deformable liposomes containing bleomycin: In vitro stability and toxicity on human cutaneous keratinocyte cell lines. Int J Pharm **2005**;300:12-4.
17. Ewoud CA. Winden V. Crommelin DJA. Short term stability of freeze-dried, lyoprotected liposomes. J Control Rel **1999**;58: 69–86.
18. Mehnert W. Schwarz C. Freeze drying of drug free and drug loaded solid lipid nanoparticles. Int J Pharm **1997**;157:171-79.
19. Castelli F. Sapietro GM. Puglia C. Rizza L. Bonina F. Characterization of indomethacin loaded lipid nanoparticles by differential scanning calorimetry. Int J Pharm **2005**;304:231-238.
20. Dragicevic-Curic N. Winter S. Stupar M. Temoporfin-loaded liposomal gels: Viscoelastic properties and in vitro skin penetration. Int J Pharm **2009**;373:77-84.
21. Mourtas S. Haikou M. Theodoropoulou M. The effect of added liposomes on the rheological properties of a hydrogel: A systematic study. J Colloid Interf Sci **2008**;317:611-9.
22. Abdel-Mottaleb MMA. Mortada ND. Elshamy AA. Awad GAS. Preparation and evaluation of fluconazole gels. Egypt J Biomed Sci **2007**;23:266-286.
23. Mohamed MN. In vitro release of hydrophilic and hydrophobic drugs from liposomal dispersions and gels. Act Pharm **2006**;56:311-324.
24. Dubey V. Mishra D. Jain NK. et al. Dermal and tarnsdermal delivery of an anti –psoriatic agent via ethanolic liposomes. J Control Rel 2007;123:148-54.
25. Reddy MS. Mutalik S. Rao GV. Preparation and evaluation of minoxidil gels for topical application in alopecia. Ind J Pharma Sci **2006**;2:432-436.
26. Dragicevic-Curic N. Winter S. Krajisnik D. Stability evaluation of temoporfin-loaded liposomal gels for topical application. J Liposome Res **2010**;20:38-48.

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