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Original Article

Preparation and Evaluation of Acyclovir Liposomes by Two Techniques: A Comparative Study

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Abstract

The aim of this study was to prepare liposomes of acyclovir (ACY) by thin layer evaporation (TLE) and reverse phase evaporation (REV) methods. Twenty-seven batches of liposomes from each method were prepared using technique of three variables at three levels (3^3) factorial design. Drug/Lipid (molar ratio), hydration volume and hydration time were considered three independent variables in TLE method while that of Drug/Lipid (molar ratio), organic phase volume and aqueous phase volume in REV method. Liposomes, obtained by TLE method (TLEs) and REV method (REVs) were evaluated for geometric mean diameter, and percent drug entrapment (PDE). REVs of 3.5(2.3) µm with 77.2% and of 3.4(2.2) µm with 71.1% drug entrapment was obtained with Drug/PC/CHOL (in molar ratio) of 1:4:0.5 and 1:2:0.5 respectively while TLEs of 4.3(1.7) µm with 79.5% drug entrapment was obtained with Drug/PC/CHOL (in molar ratio) of 1:20:10. *In vitro* studies were conducted to compare drug diffusion pattern across the human cadaver skin (HCS) of promising batches of TLEs and REVs. A significantly low ($p<0.05$) flux [0.628(0.046) µg/cm^2/h] obtained by TLEs when compared with the flux [0.785(0.050) µg/cm^2/h] obtained by REVs across HCS. The flux values of ACY TLEs and REVs revealed the lamellarity. Low flux in TLEs than REVs across HCS indicated the formation of multilamellar vesicles (MLVs) in TLE method while oligolamellar vesicles (OLVs) with few lamellae surrounding the large aqueous core in REV method. Multilamellarity of the TLEs makes the liposomes to supply drug in more sustained way in the layer of HCS as indicated by high amount of drug deposition (350.7 µg) in the HCS after 72 h of application when compared with drug deposition in the HCS (321.0 µg) for the same period by REVs.

Thus, the finding of the study establishes the role of REV method producing OLVs of ACY with high PDE using 5-10 fold less amount of high costing phosphatidylcholine when compared to MLVs prepared by TLE method with insignificant change in PDE but significant change in flux, which affects the release of drug at the target site.

Keywords: Liposomes; Multilamellar vesicles; Oligolamellar vesicles; Reverse phase evaporation; Thin layer evaporation.

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Introduction

Liposomes (1) have been proposed as delivery systems for topical application, and efforts have been made in an attempt to shed potentiality of liposomes in dermatological treatment (2). Delivery systems have to be developed to impart targeted therapeutic effect topically. Unlike many parts of the body, the skin can be reached directly and hence it is assumed that drug delivery to the tissue is relatively easy. Unfortunately, the treatment of various skin diseases exhibits some difficulties, particularly when superficial layer of the stratum corneum is not the target site and drug penetration into deeper skin strata is required (3). Many skin penetration enhancers have been used to increase the drug levels within deep skin strata, i.e. deeper stratum corneum, epidermis and dermis by means of an increase of the skin permeability coefficient (4-5), a corresponding increase in transdermal penetration can lead to certain drawbacks. Thus, liposomes have been found appropriate to act as topical drug delivery systems (6-12).

Acyclovir, an antiviral drug is widely used in the treatment of herpes simplex (types 1 and 2). Unfortunately, its gastro-intestinal absorption is only 15-30% (13). Above this, it is reported to have many toxic effects if administered intravenously and penetrated poorly through skin, if delivered in conventional topical bases (14). Thus, within the scope of development of topical liposomal drug delivery devices, we focused on to develop different types of liposomes entrapping maximum drug utilizing optimum proportions of lipid and other stabilizing agents. Three variables at three levels (3^3) factorial design method was adopted considering promising variables viz; Drug/Lipid (molar ratio), hydration volume, and hydration time in TLE method while Drug/Lipid (molar ratio), organic phase volume and aqueous phase volume in REV method. Comparative evaluation of developed liposomes was conducted for their geometric mean diameter, percent drug entrapment and for *in vitro* drug diffusion studies across HCS. Results of diffusion study of both types of liposomes were related to determine the lamellarity of the liposomes considering total deposition of liposomal acyclovir in layers of HCS prepared by TLE and REV methods.

Experimental

Materials

Acyclovir was received as a gift sample from Cadila Pharma, Ahmedabad and Phosphatidylcholine (type-E 80) from Lipoid Gmbh, Germany. Cholesterol was purchased from S. D. Fine chemicals, Mumbai, α-tocopherol from Himedia, Mumbai, and Dialysis sacks [Mol wt. 12,000 (cut off)] from Sigma Chemical Co. St Louis, MO. All other chemicals and solvents were of analytical reagent grade.

Methods

Preparation of liposomes

Thin Layer Evaporation Method (TLE): Twenty-seven batches of liposomes of ACY were prepared by TLE method (15) by taking three variables at three levels as shown in Table 1. Batches were designed as shown in Table 2. PC, CHOL and α-tocopherol (1% of PC by weight) were dissolved in solvent mixture (2:1, chloroform: methanol) in 250 ml round bottom flask (Quick fit neck B-24). Flask was rotated in a rotary flash evaporator at 120 rpm under vacuum of 500 mm of Hg at 37 °C to remove the organic solvent using a nitrogen gas bleed to form smooth, uniform and dried film. Hydration of film was carried out by aqueous drug solution of 9 μmol/ml under nitrogen atmosphere at room temperature. Liposomal suspension was sonicated in ice bath for 5 minutes using probe sonicator (Rolsonic, Mumbai). Annealing of liposomes at room temperature was carried out for 1 hour after sonication. Each batch was prepared three times on three different days. The prepared liposomes were evaluated for PDE and mean geometric diameter. Results are shown in Table 2.

Reverse Phase Evaporation Method (REV): Twenty-seven batches of ACY were prepared by REV method as previously reported (16). Three variables at three levels were taken as shown in Table 1. Batches were designed as shown in Table 2. Results of PDE and mean geometric diameter are shown in Table 2.

Statistical analysis

The results obtained in terms of PDE and flux through HCS were compared using student t-

Table 1. Coded units of 33 factorial design.

Method	Variables	Levels		
		Low	Medium	High
TLE	x_1^a	1:6.6:3.4	1:13:7	1:20:10
	x_2^b	3.0 ml	6.0 ml	9.0 ml
	x_3^c	1 hr	2 hrs	3 hrs
REV	x_4^d	3.0 ml	6.0 ml	9.0 ml
	x_5^e	1.0 ml	1.5 ml	2.0 ml
	x_6^f	1:2:0.5	1:3:1.5	1:4:0.5
	Transformed values	-1	0	1

[a & f] Drug/PC/CHOL (in molar ratio), [b] volume of hydration medium, [c] hydration time
[d] organic phase volume, [e] aqueous phase volume

test, and differences were considered significant at $p<0.05$.

Characterization of liposomes

Dialysis technique was carried out for removing unentrapped ACY from ACY liposomal suspension to determine PDE.

Dialysis Time: Dialysis time was decided by taking highly concentrated ACY solution (5 mg/10 ml) in the dialysis sack as a donor compartment in 200 ml water in a beaker put on magnetic stirrer as a receiver compartment. Samples from receiver compartment were taken periodically till the whole drug was dialyzed from the dialysis sack. The maximum time for the completion of dialysis of ACY from the dialysis sack to the receiver compartment was found to be 4 h. Thus, the dialysis time was decided 4 h to ensure that there is no chance to remain unentrapped drug in the donor compartment.

Determination of PDE: ACY within liposomes was estimated after removing unentrapped ACY by the method of dialysis for 4 h as previously reported (16).

The solution of receptor compartment was estimated for unentrapped ACY at 250 nm using Hitachi U-2000 spectrophotometer. The entrapped drug was separated from the liposomal suspension of donor compartment by modified Bligh-dyer two-phase extraction method (20), which was conducted as follows:

Accurately measured 0.1 ml of liposomal suspension was transferred to 15 ml calibrated centrifuge tube and the volume was made up to 2.5 ml with a 5% w/v sodium chloride solution. 5.0 ml of chloroform was added to the contents of the centrifuge tube followed by vortexing for 2 minutes and centrifuged at 3000 rpm for 15 minutes. The lower chloroform layer was separated using a glass syringe with a long needle and transferred into a 10 ml volumetric flask after passing it through a bed of anhydrous sodium sulphate. The extraction procedure was repeated by adding 2.5 ml chloroform and 2.5 ml of 5%w/v sodium chloride solution in the centrifuge tube already containing 2.5 ml of aqueous solution. The content was vortexed and then centrifuged as earlier and the lower chloroform layer was separated and collected in the same way done after first extraction. 2.5 ml of chloroform was further added to the centrifuge tube already containing 5.0 ml of aqueous solution. The content of centrifuge tube was further extracted in the similar manner and the chloroform layer was separated and collected in the same volumetric flask. The volume of combined chloroform extract was made up to 10 ml, if necessary, with chloroform. The ACY content was estimated in the aqueous layer of the extract after suitably diluting with 5% w/v sodium chloride solution by measuring the absorbance at 250 nm against the reagent blank. The mean PDE of all the batches is recorded in Table 2.

$$\text{PDE} = \frac{\text{Drug entrapped}}{\text{Total initial drug added}} \times 100 \qquad (1)$$

Determination of geometric mean diameter: Samples of ACY liposomes were evaluated for geometric mean diameter after suitable dilution

Table 2. Experimental design, PDE and mean geometric diameters of liposomes.

Batch No.	Variables			PDE (±SEM) in liposomes		dg (avg) (μm)	
	X_1	X_2	X_3	REVs	TLEs	REVs	TLEs
1	-1	-1	-1	34.3(0.05)	24.3 (0.176)	5.0 (1.5)	3.6 (2.0)
2	1	-1	-1	71.1 (0.188)	24.1 (0.346)	3.4 (2.2)	3.6 (1.8)
3	1	-1	-1	48.3 (0.094)	37.3 (0.037)	3.3 (2.0)	4.2 (2.0)
4	-1	0	-1	31.3 (0.094)	37.6 (0.066)	4.3 (1.9)	4.0 (2.2)
5	1	0	-1	71.0 (0.047)	55.8 (0.113)	3.4 (2.0)	3.7 (2.1)
6	1	0	-1	48.8 (0.094)	63.4 (0.176)	4.5 (1.9)	4.1 (1.7)
7	-1	1	-1	48.6 (0.094)	26.2 (0.037)	3.5 (1.9)	3.2 (2.3)
8	1	1	-1	68.3 (0.283)	34.4 (0.130)	4.4 (1.6)	3.8 (2.0)
9	1	1	-1	41.4 (0.333)	41.4 (0.000)	4.9 (1.8)	3.9 (1.9)
10	-1	-1	0	33.2 (0.094)	34.7 (0.330)	4.7 (1.8)	3.4 (2.1)
11	1	-1	0	76.0 (0.094)	48.3 (0.130)	3.5 (2.1)	3.5 (1.9)
12	1	-1	0	43.9 (0.094)	36.3 (0.000)	3.9 (1.8)	4.3 (1.6)
13	-1	0	0	34.8 (0.283)	41.7 (0.037)	3.6 (2.0)	3.8 (2.1)
14	1	0	0	74.2 (0.188)	63.2 (0.133)	3.9 (2.0)	4.2 (2.0)
15	1	0	0	40.6 (0.377)	79.5 (0.857)	3.7 (2.1)	4.3 (1.7)
16	-1	1	0	38.2 (0.283)	36.0 (0.360)	3.6 (2.2)	3.7 (2.1)
17	1	1	0	72.0 (0.141)	39.1 (0.366)	3.2 (2.0)	4.1 (1.9)
18	1	1	0	38.9 (0.141)	67.6 (0.000)	3.5 (2.3)	4.2 (1.9)
19	-1	-1	1	67.8 (0.094)	27.9 (0.308)	3.6 (2.3)	3.5 (1.9)
20	1	-1	1	77.2 (0.330)	32.4 (0.033)	3.5 (2.3)	3.7 (1.8)
21	1	-1	1	40.6 (0.047)	44.9 (0.448)	3.3 (2.2)	4.3 (1.7)
22	-1	0	1	69.6 (0.188)	36.6 (0.145)	3.3 (2.2)	3.8 (2.0)
23	1	0	1	73.8 (0.141)	38.0 (0.130)	4.5 (2.0)	4.0 (1.8)
24	1	0	1	38.6 (0.094)	67.3 (0.340)	3.9 (2.0)	4.1 (1.8)

PDE- % drug entrapment, dg- mean geometric diameter, σg-geometric standard deviation, TLEs and REVs - liposomes prepared by TLE and REV method respectively.

by optical microscopy as previously reported (16).

Volume mean diameter of potential batches: The volume mean diameter of liposomes of the potential batches of each of method (Batch-15 and Batch-2 of TLE and REV method respectively) was determined by laser light diffraction using Malvern Mastersizer. The results are shown in Table 2.

In vitro diffusion studies

Preparation of skin: Human cadaver skin (HCS) was obtained from autopsy at the Faculty of Medicine, M. S. University, Baroda. Doctor of autopsy was requested to supply HCS cleared from subcutaneous fat from the dermal side. It was washed with distilled water, shaved and then stored at-4 °C. Full thickness skin pieces of approximately 6.0 cm^2 area were punched out and were hydrated with diffusion medium (distilled water) 24 h prior to use.

Methodology: A modified diffusion test apparatus (17) with a diffusion area of 5.3 cm^2 was used for the diffusion studies. It was validated by benzoic acid disc method (18). Skin specimens were mounted with the epidermal side up at the bottom of the single-chamber. The mounting of the skin was done using Fevi-quick glue at the brim of the diffusion tube to avoid leakage of the test sample and supported with thread crossover the cell. The temperature of the receiver chamber, containing 50 ml of diffusion medium was maintained by thermostatically controlled magnetic stirrer at 37±0.5 °C, under continuous stirring at the rate of 50 rpm, in a way that the dermal surface just flushes to the surface

of the diffusion fluid. The epidermal surface was left exposed to ambient conditions. The purified liposomal suspensions prepared by TLE and REV methods were added to the epidermal surface. Drug concentration in the receiver chamber was determined by withdrawing samples over time and measuring drug by UV spectrophotometer (Hitachi, Japan) at 250 nm against purified water as a reference. Drug flux (J: in microgram per square centimeter per hour) was calculated from the slope of a plot of regressed drug concentration (µg/ml) versus time, the area of treated skin and volume of the receiver chamber (19). Each study was continued for 72 h, during which the drug in receiver chamber (µg/ml) across HCS was calculated at each sampling time point. The results of the skin penetration of drug by liposomes prepared by TLE and REV methods are shown in Figure 1.

Determination of drug deposition in HCS layers: After completion of diffusion studied for 72 h, the diffusion tube cell was carefully removed and the samples of purified liposomal suspension prepared by TLE and REV remained in the donor compartment was diluted with 10 ml of 5% w/v sodium chloride solution. 1.0 ml of this dispersion was taken in 15 ml centrifuge tube. The drug was extracted by two-phase extraction method as described by Bligh-dyer (20). The concentration of drug was measured at 250 nm against proper reagent blank. The drug estimated on epidermal layer was considered as still available in donor compartment and from the mass balancing of the drug; the drug retained in the skin was calculated.

Results and Discussion

Twenty-seven batches were prepared using three variables at three levels (3^3) factorial design by TLE and REV methods. The three major variables selected for TLE method were Drug/PC/CHOL (x_1^a) in molar ratios, volume of hydration medium (x_2^b) and hydration time (x_3^c) while organic phase volume (x_1^d), aqueous phase volume (x_2^e) and Drug/PC/CHOL (x_3^f) in molar ratios in REV method. All batches were evaluated for PDE and geometric mean diameter. Results are recorded in Table 2. A significant ($p<0.05$) variation in PDE was observed in liposomes prepared by both the methods. Liposomes prepared by TLE method entrapped maximum 79.5% (batch 15) drug using Drug/PC/CHOL (in molar ratios) of 1:20:10 while 77.2% (batch 20) and 71.1% (batch 2) PDE were entrapped in liposomes using Drug/PC/CHOL (in molar ratios) of 1:4:0.5 and 1:2:0.5 by REV method respectively. This result revealed that REV method for the preparation of ACY liposomes is better than TLE because it reduces the PC requirement 5 to 10 fold giving nearly same PDE within liposomes. This can be explained on the basis of drug's moderate solubility in water and insolubility in most of all organic solvents. TLE method, which produces multilamellar vesicles (MLVs) possessing small layers of interstitial water. Thus, a large amount of PC can improve the number of lamellae and thereby layers of interstitial water are also increased to entrap more drug or it may also be due to increase in number of liposomes per ml. On the other hand, REV method is suitable to produce either Large unilamellar vesicles (LUVs) or oligolamellar vesicles (OLVs) possessing a large aqueous core in the liposomes. Thus, drug being moderately soluble in water can entrapped in the large aqueous core area surrounded by either single or three or four lipid bilayers with less amount of PC as compared to PC consumed in TLE method.

Variables other than Drug/PC/CHOL in molar ratios in TLE method were volume of hydration medium and hydration time, which were optimized to 6.0 ml and 2.0 h respectively. However, volume of hydration medium less than or more than 6.0 ml may lead to insufficient hydration of lipid bilayers forming fragile liposomes from which drug leaks rapidly during sonication. Similarly, hydration time less than or more than 2.0 h resulted into incompletely hydrated or excessively hydrated liposomes may

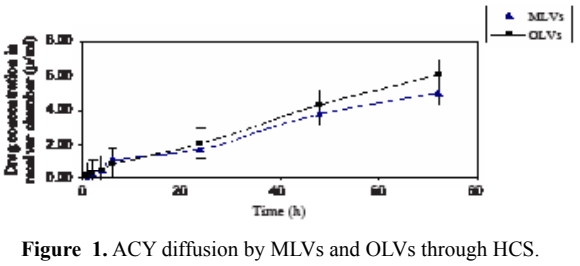

Figure 1. ACY diffusion by MLVs and OLVs through HCS.

Table 3. Deposition of ACY in layers of HCS.

TLEs		REVs	
Total entrapped drug (µg)	Drug deposited in HCS after 72 h (µg)	Total entrapped drug (µg)	Drug deposited in HCS after 72 h (µg)
1590	350.7	1422	321.0

lead to diffusion of drug from the liposomes and thus affected in decrease of PDE.

The critical evaluation of PDE within liposomes reveals efficiency of REV method. It was observed that high PDE (>70%) was obtained at all levels of Drug/PC/CHOL (in molar ratios) with appropriate ratio of organic to aqueous volumes, which play very crucial role in the formation of emulsion (w/o). The step needs appropriate ratio of aqueous to organic phase to obtain uniformly distributed globules of aqueous phase that are surrounded by layers of lipid on evaporation of organic phase, thereby entrapping large aqueous solution in the core area of the liposomes. Thus, this method was found to be efficient in selecting appropriate ratios of aqueous volume to organic phase volume using high Drug/Lipid ratio to obtain high PDE. The appropriate ratio of organic phase to aqueous phase in the present investigation was found to be 6:1 at all levels of Drug/PC/CHOL (in molar ratios), resulting in slight variation in PDE (batches 2, 11 and 20). Diverting the ratio of organic phase to aqueous phase from 6:1 in other batches reduces the drug entrapment less than 70% and increases more than the 70% on the consumption of more PC. The results signify the importance of the formation of proper emulsion (w/o) before evaporation of organic phase without being affected by the Drug /PC/CHOL (in molar ratios). Thus, REV method can be utilized for the preparation of liposomes with high entrapment of drug with high Drug/lipid ratio as in case of batch-2 in which nearly equivalent amount of drug is entrapped with minimum consumption of PC. This result persuaded us to select batch-2 prepared by REV method for further skin penetration studies. The range of mean geometric diameters of liposomes prepared by TLE method was found to be 3.2(2.3) µm to 5.4(1.6) µm. It was observed that in TLE method, increase in the proportion of PC increases the size of the vesicles, may be due to increase in the lamellae. But, this was not found in liposomes prepared by REV method where the range of mean geometric diameters was found to be 3.2(2.0) µm to 5.0(1.5) µm. The size of vesicles prepared by REV method was not affected by the increase or decrease of PC proportions but seems to be affected by the factors like organic phase volume to aqueous phase volume resulting in different globule size of aqueous phase in the emulsion.

The results of skin penetration studies with ACY TLEs (batch-15) and REVs (batch-2) are shown in Figure 1 and the flux values are calculated. Each drug formulation was studied six times with HCS. The delivery of ACY through HCS from TLEs was slow, and drug concentration in the receiver chamber reached to 5.4 µg/ml after 72 h; while it was found to be 7.4 µg/ml in same period by REVs. Drug delivery was 1.4 fold more by the REVs than that obtained with TLEs ($p<0.05$). The flux calculated for TLE liposomes across HCS was less [0.628(0.046) µg/cm^2/h] than the flux calculated for REV liposomes [0.785(0.050) µg/cm^2/h], although the total drug present in the liposomal suspension of TLEs and REVs were 1590 µg and 1422 µg respectively (Table 3). The lower flux across HSC by TLEs may be due to the drug being present in the interstitial water layers of the vesicles, which retains the drug in skin layers equal to 350.7 µg after 72 h (Table 3) but the higher flux across HSC in REVs may be due to the drug being present in the central aqueous core surrounded by single or three to four lipid bilayers. Thus, less quantity of lipids in REVs retains less amount of drug in HSC layers equal to 321 µg (Table 3) for the same period of time. This observation confirms that the TLEs are multilamellar while the REVs are either unilamellar or oligolamellar.

Thus it may be concluded that REV method is better than TLE method for the preparation of ACY liposomes in terms of PDE within the liposomes for the reason drug being moderately water soluble trapped in the aqueous central core of liposomes prepared by REV method. We could achieve higher PDE within liposomes with significantly low Lipid/Drug ratio, saving high

costing phosphatidylcholine. About 5-10 fold reduction in PC amount with marginal change in PDE compared to liposomes prepared by TLE method and also with marginal increase in mean flux across HCS. Hence, ACY liposomes can be prepared commercially by REV method at much lower cost without sacrificing the drug skin deposition substantially. It is also suggested that a sustained delivery of ACY across the skin appears to be achievable through topical application of ACY load liposomes prepared by REV method.

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References

(1) Mezei M and Gulasekharan V. Liposomes a selective drug delivery system for the topical route of administration. *Life Science* (1980) 26: 1473-1477

(2) Ceve G. Rationale for production and dermal application of lipid vesicles. In: Braun Faleo O, Korting HC and Maibach HI. (eds.) *Liposome Dermatics.* Springer-verleg, Berlin (1992) 82-90

(3) Loth H. Percutaneous absorption and conventional penetration enhancers. In: Braun Faleo O, Korting HC and Maibach HI. (eds.) *Liposome Dermatics* Springer-verleg, Berlin (1992) 3-10

(4) Gummer C. Vehicles as penetration enhancers. In: Bronaugh R and Maibach H. (eds.) *Percutaneous Absorption*. Marcel Dekker, Switzerland (1985) 571-575

(5) Loth H. Vesicular influence on transdermal drug penetration. *Int. J. Pharm.* (1991) 68: 1-10

(6) Mezei M and Gulasekharam V. Liposomes: a selective drug delivery system for topical route of administration. I. Lotion dosage form. *Life Sci*. (1980) 26: 1473-1477

(7) Gesztes A and Mezei M. Topical anesthesia of the skin by liposome encapsulated Tetracaine. *Anesth. Analg*. (1988) 67: 1079-1081

(8) Egbaria K, Ramachandran C and Weiner N. Liposomes as topical drug delivery system. *Adv. Drug Del. Rev.* (1990) 5: 287-300

(9) Fresta M and Punglisi G. Corticosteroid dermal delivery with skin lipid liposomes. *J. Controlled Release*. (1997) 44: 141-151

(10) Lasch J, Laub R and Whlrab W. How deep do intact Liposomes penetrate into human skin? *J. Controlled Release* (1991) 18: 55-58

(11) Touitou E, Levi-Schaffer F, Dayan N, Alhaique F and Riccieri F. Modulation of caffeine skin delivery by carrier design: Liposomes verse permeation enhancers. *Int. J. Pharm*. (1994**)** 103: 131-136

(12) Masini V, Bonte F, Meyback A , Wepierre J. Cutaneous bioavailability in hairless rats of tretinoin in liposomes or gel. *J. Pharm. Sci*. (1993) 82: 17-21

(13) Martindale. *The Extra Pharmacopoiea.* 31st (eds.) Royal Pharmaceutical Society, London (1996) 652.

(14) Freeman DJ, Sheth NV and Spruance S. Failure of topical acyclovir in ointment to penetrate human skin. *Antimicrob. Agents Chemother*. (1986) 29: 730-732

(15) Szoka F and Papahadjopoules D. Liposomes: Preparation and Characterization. In: Knight CG. (ed.) *Liposomes: from Physical Structure to Therapeutic Application*. Elsevier, Amsterdam (1981) 51-82

(16) Seth AK and Misra AN. Mathematical modeling of preparation of acyclovir liposomes: reverse phase evaporation method. *JPPS* (2002) 5: 294-300

(17) Franz JJ. Percutaneous absorption on the relevance of *in vitro* data. *J. Invest. Dermato*. (1975) 67: 190-196

(18) Keshary PR and Chien YW. Mechanism of transdermal controlled nitroglycerin administration: Development of finite-dosing skin permeation system. *Drug. Dev. Ind. Pharm*. (1984) 10: 883

(19) Spruance SL, McKeough MB and Cardinal JR. Penetration of guinea pig skin by acyclovir I different vehicles and correlation with the efficacy of tropical therapy of experimental cutaneous herpes simplex virus infection. *Antimicrob. Agent Chemother*. (1984) 25: 10-15

(20) New RRC. (ed.) *Liposomes: a Practical Approach*, Oxford University Press. London (1990) 33: 105 *Book, editor as author.*

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