Research Journal of Pharmaceutical, Biological and Chemical Sciences

Vesicular Carriers for Transdermal Delivery of Alfuzosin Hydrochloride.

Prasanthi D*, Kuruva Jyothirmai, and Lakshmi PK

Department of Pharmaceutics, G Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad – 500 028

ABSTRACT

Study background: Transdermal drug delivery (TDD) is the potential route for providing sustained release of drugs and offers superior patient compliance. Vesicular formulations have enhanced permeation of drugs across stratum corneum barrier. In the present study vesicular carriers are investigated for the enhancement of transdermal drug delivery of alfuzosin hydrochloride (AH). Four different types of vesicular carriers i.e., liposomes, transferosomes, flexosomes and ethosomes of AH were prepared and characterised. Vesicular carriers were prepared by using phosphotidyl choline, cholesterol, edge activator (Span80, Tween80 and sodium cholate), positively charged phospholipid (stearylamine) and ethanol. Methods: Liposomes, transferosomes, flexosomes were prepared by thin film hydration technique and ethosomes were prepared by cold method. The prepared vesicular carriers were characterised for particle size, zeta potential, entrapment efficiency, stability, *in vitro* diffusion and *ex vivo* permeation studies. Results: The vesicular size was found to be in the range of 80.09nm to 6.85μm. Zeta potential was found to be in the range of 26.9mV to 14.5mV. Ethosomes (ET) showed maximum permeation of Q_{24} (637.10 ± 1.98($\mu g/cm^2$) and transdermal flux (27.42 ±0.04$\mu g/cm^2$/hr) than flexosomes (FA) of Q_{24} (596.3±4.48$\mu g/cm^2$), flux (23.28±0.91$\mu g/cm^2$/hr) followed by transfersomes (TA) of Q_{24} (445.5±2.22$\mu g/cm^2$), flux (17.6±0.21$\mu g/cm^2$/hr) followed by liposomes (LA) of Q_{24} (183.9±2.25$\mu g/cm^2$), flux (7.45±0.25$\mu g/cm^2$/hr). Ethosomes showed 0.26±0.20hrs lag time, permeability coefficient of 5.48±0.009×10^3cm/hr and skin content of 298.01±15.4μg/g. Transdermal flux was enhanced by 6.92 times by ethosomes, 5.78 times by flexosomes, 4.44 times by transferosomes and 1.88 times by liposomes over drug solution. Vesicle skin irritation studies proved to be non-irritant and ethosomes showed fatty change in dermis. The formulations were stable at 4°C for 120 days. Conclusion: Results suggested that among four vesicular formulations, ethosomes are efficient carriers for enhancing AH transdermal delivery.

Keywords: Transferosomes, Liposomes, Flexosomes, Ethosomes, BPH, Permeation enhancement.

*Corresponding author

INTRODUCTION

Transdermal route is a potential route for delivering bioactive agents. It offers several advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patients compliance [1].

Vesicular systems are gaining importance recently owing to their ability to act as a means of sustained or controlled release of drugs [2]. These vesicles are preferred over other formulations because of their specific characteristics such as lack of toxicity, biodegradation, capacity of encapsulating both hydrophilic and lipophilic molecules, capacity of prolonging the existence of the drug in the systemic circulation by encapsulation in vesicular structures, capacity of targeting the organs and tissues, capacity of reducing the drug toxicity and increasing its bioavailability [2].

Alfuzosin hydrochloride (AH), which is an α-adrenoreceptor blocker used in the treatment of benign prostate hyperplasia has low oral bioavailability of about 64% and its physico-chemical properties like molecular weight (425.9), half-life (3-5), log P value (1.604), and low dose (2.5-10mg/day) makes it an ideal drug candidate for transdermal drug delivery(TDD). AH has been previously identified as a promising candidate for TDD [3].

In the present study four different types of vesicular formulations like liposomes, transferosomes, flexosomes and ethosomes loading AH were prepared and characterised for skin permeation studies to optimise the better vesicular carrier for the enhanced transdermal delivery of AH.

MATERIALS AND METHODS

Alfuzosin hydrochloride (AH) was obtained as a gift sample from Dr.Reddy's Laboratories Ltd (Hyderabad, India). Phospholipon 80 H and Phospholipon 90 H was obtained as a gift sample from Lipoid GmbH (Germany). Soyaphosphotidyl choline (SPC) was purchased from Otto Chem. Ltd. (India). Ethanol, Methanol, Span80, Tween80, cholesterol and chloroform were purchased from S.D. Fine-Chem. Ltd. (India). Sodium cholate was purchased from NR Chem Ltd. (India). Stearyl amine was purchased from Hi Media Laboratories Pvt. Ltd.

Preparation of vesicular carriers

Liposomes, transferosomes and flexosomes were prepared with different ratios and combinations of phospholipid as shown in table 1 by thin film hydration technique. Liposomes were prepared by using phosphotidyl choline and cholesterol. In the preparation of transferosomes instead of cholesterol, edge activators (EA) like span 80, tween 80 and sodium cholate were used. Along with EA positively charged lipid (stearyl amine) is used in the preparation of flexosomes. In thin film hydration technique, phospholipid and cholesterol were

taken in round bottomed flask (RBF) and dissolved in sufficient amount of solvent. RBF was connected to rotary flash evaporator (Supervac, Mumbai, India) and continously rotated at 60°C until a thin layer forms. Drug (AH) solution in phosphate buffer saline (pH 7.4) was added to the thin film and again rotated in rotary flash evaporator for about 30 mins at 60°C. The preparation was left to cool at room temperature for 30 mins and then it was sonicated at 4°C for five cycles of 3 minutes each with a 1-minute rest between cycles using a probe sonicator (Sonics Vibra cell) [4].

Table 1: Formulation table for vesicular formulations.

Formulation code	Drug(%)	SPC:CH (w/w)	EA	SPC:EA (w/w)	SPC (% w/w)	Ethanol (%v/v)	Stearyl amine (mg/ml)	CHCL$_3$: methanol (v/v)	PBS pH 7.4 (ml)
LA	0.5	450:150	-	-	-	-	-	2:1	10
TA	0.5	-	Tween 80	85:15	-	-	-	2:1	10
FA	0.5	-	Tween 80	85:15	-	-	3.25	2:1	10
ET	0.5	-	-	-	3	20	-	-	10

AH (alfuzosin hydrochloride); SPC (Soya Phosphotidyl choline); CH (cholesterol); EA (Edge activator); CHCL$_3$ (chloroform); PBS (phosphate buffer saline).

Ethosomes were prepared by cold method [5]. The ethanolic vesicular system was composed of phospholipid, ethanol, drug in the concentrations as shown in table 1. Phospholipid was dissolved along with the drug in ethanol. This mixture was heated to 40°C±1°C and a fine stream of double distilled water was added slowly, with constant mixing at 700 rpm with mechanical stirrer (Remi instruments, Vasai, india) in a house-built closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 40°C± 1°C. The preparation was left to cool at room temperature for 30 mins and then it was sonicated at 4°C for five cycles of 3 minutes each with a 1-minute rest between cycles using a probe sonicator (Sonics Vibra cell) [4].

Thirty percent of hydroethanolic solution of drug and 0.5% drug solution in distilled water were prepared to compare with liposomes, transferosomes, flexosomes and ethosomes.

Characterization of prepared vesicular formulations

Vesicular formulations were analyzed for % entrapment efficiency study, vesicle size analysis, surface morphology, *in vitro* diffusion study, *ex vivo* permeation study, skin deposition study, skin irritation study and stability study.

Vesicle shape and surface morphology

Scanning electron microscopy (SEM) was used to characterize the surface morphology of optimised vesicles. One drop of vesicular suspension was mounted on a clear-glass stub, air-dried, gold coated with polaron E5100 sputter coater (Polaron, United kingdom), and visualised under scanning electron microscope (Jeol 5400, Japan).

Vesicle size and zeta potential

The vesicle size and zeta potential were measured by photon correlation spectroscopy (Delsa Nano, Beckman Coulter Inc.UK).

Entrapment efficiency

Prepared ethosomal vesicles were separated from the free (unentrapped) drug by centrifugation technique [5]. 2 ml of the ethosomal suspension was diluted with distilled water upto 5 ml and centrifuged at 20,000 rpm for 45 min at 4°C using a cooling centrifuge (Eltek centrifuge). After centrifugation, the supernatant and sediment were recovered, and sediment was lysed using methanol and filtered through a 0.45 μm nylon disk filter. The concentration of AH in the supernatant and sediment was analysed by UV-VIS double beam spectrophotometer (Chemito Spectrascan UV2600, India) at 245nm. The percentage drug entrapment was calculated using the following equation:

$$\% \text{ drug entrapment} = \frac{\text{Amount of entraped drug recovered}}{\text{Total amount of drug}} * 100$$

The determination of entrapment efficiency was repeated three times per sample at 25°C.

In vitro diffusion studies

Diffusion study of vesicular formulations was performed using Franz diffusion cell. The cell was locally fabricated and volume of receptor compartment was 20ml. The dialysis membrane was mounted between the donor and receptor compartments. Vesicular formulations equivalent to 5mg of drug were taken on the dialysis membrane and the compartment clamped together. The receptor compartment was filled with phosphate buffer saline pH 7.4 and the hydrodynamics in the receptor compartment was maintained by stirring on a magnetic stirrer at 600rpm. At pre-determined time intervals, 1ml of sample were withdrawn and an equal volume of buffer was replaced. The samples were analyzed after appropriate dilution for drug content spectrophotometrically [6].

Ex vivo permeation study

Institutional animal ethical committee (IAEC) approved skin permeation studies. Locally fabricated Keshary Chein diffusion cell was used for permeation studies. Studies were conducted using dermatomed and prepared rat skin. 20ml of PBS 7.4 was taken in receptor compartment and was continuously stirred with magnetic stirrer and equilibrated at $37^{o}C\pm1^{o}C$ with a recirculating water bath. 1ml of vesicular formulation was taken in donor compartment and 1ml sample was withdrawn through the sampling port at predetermined time intervals for 24hours and drug content was analyzed by UV-VIS double beam spectrophotometer (Chemito Spectrascan UV2600, India) at 245nm. Similar studies were performed with hydroethanolic solution and drug solution.

Skin deposition studies

The amount of AH retained in the skin was determined by skin deposition studies. At the end of the permeation studies (24hrs), the skin was washed 10times with a cloth immersed in methanol. A sample of skin was weighed and homogenized with methanol for 5mins using an electric stirrer. The resulting solution was centrifuged at 7000rpm for 10mins and supernatant was analyzed by UV-VIS double beam spectrophotometer (Chemito Spectrascan UV2600, India) at 245nm.

Vesicle Skin interaction study

Interaction studies were studied on the basis of structural changes in stratum corneum, epidermis and dermis [7]. The formulations were applied topically to the dorsal portions of rat for 8hrs. Later, the rats were killed by cervical dislocation; the skin was excised and fixed by immersion in 50% neutral formalin solution in saline for 24hrs. The samples were subjected to histological processing by dehydration and rehydration with graded alcohols, paraffin block processing and stained with haemotoxyllin-eosin. Microscopic evaluation using dark-light microscope was performed by blinded assessor.

Stability studies

Vesicles were stored at $4^{o}C\pm0.5^{o}C$ for 120 days. To determine their stability, entrapment efficiency of vesicles were measured using the method described earlier.

RESULTS AND DISCUSSION

Liposomes, transferosomes and flexosomes were prepared by thin film hydration technique and ethosomes were prepared by cold method. On characterization, the surface morphology of all the vesicular formulations was found to be spherical shape which was studied using scanning electron microscopy.

The vesicle size of liposomes(LA) was found to be lower (80.9nm) than transfersomes (TA), cationic flexosomes(FA) and ethosomes(ET) shown in table 2. This result might be attributed to the presence of cholesterol which causes the bilayer to be more compact [8]. Cationic flexosomes, FA showed lower particle size (119.9nm) than transfersomes(TA) and ethosomes(ET) because of the addition of positive charged lipid. This result could be attributed to the existence of charging agent in vesicle bilayer, which increases its affinity to be curved, hence reducing the size of the vesicles [8]. Transfersomes(TA) showed higher particle size (149.5nm) than liposomes(LA) and cationic flexosomes(FA) because of the incorporation of edge activator into conventional phosphatidylcholine vesicles, had an influence on the particle size, i.e., edge activator increased the vesicles particle size [5]. Ethosomes (ET) showed maximum particle size (6.85μm) than liposomes(LA), transferosomes(TA) and cationic flexosomes(FA).

The zeta potential of the liposomes(LA), transfersomes (TA) and ethosomes(ET) was found to be negative, (-23.0mV, -26.9mV and -8.14) shown in table 2 due to the net charge of the lipid composition in the formulations [8]. The zeta potential of the cationic flexosomes, FA was found to be positive (14.5mV), because of the addition of positive charged lipid, stearylamine. The presence of positive charge indicates the formation of cationic vesicles and the obtained higher zeta potential may overcome flocculation and aggregation of vesicles [9].

As per the results shown in table 2 ethosomes(ET) showed maximum entrapment efficiency of 91.86±3.25%, followed by cationic flexosomes(FA) 71.06±1.03%, then transfersomes(TA) 55.51±1.68% and liposomes(LA) 48.94±0.25% . TA showed higher entrapment than LA because of the addition of edge activator, which allows growth in vesicle size [10]. FA showed still more entrapment efficiency than LA and TA due to the presence of stearylamine, which tend to increase the interlamellar repeat distances between successive bilayers in the vesicle, swelling the structure with the greatest proportion of the aqueous phase results in increased overall entrapped volume [7]. EA showed maximum entrapment efficiency than LA, TA and FA due to high concentration of ethanol (20%). Previous studies reported that increase in ethanol concentration results in increased entrapment [12].

Ethosomes (ET) showed maximum permeation of Q_{24} of 637.10±2.95μg/cm^2, flux of 27.42±0.04μg/cm^2/hr, permeability coefficient of 5.48±0.009×10^{-3}cm/hr, followed by cationic flexosomes (FA) of Q_{24} of 596.3±4.48μg/cm^2, flux of 23.28±0.91μg/cm^2/hr, permeability coefficient of 4.65±0.006×10^{-3}cm/hr, followed by transfersomes (TA) of Q_{24} of 445.5±2.22μg/cm^2, flux of 17.6±0.21μg/cm^2/hr, permeability coefficient of 3.52±0.009×10^3 cm/hr, followed by liposomes (LA) of Q_{24} of 183.9±2.25μg/cm^2, flux of 7.45±0.25μg/cm^2/hr, permeability coefficient of 0.14±0.05×10^{-3}cm/hr, followed by hydroethanolic solution of Q_{24} of 167.1±1.85μg/cm^2, flux of 6.66±0.13μg/cm^2/hr, permeability coefficient of 0.13±0.08×10^{-3}cm/hr and pure drug of Q_{24} of 108.3±1.25μg/cm^2, flux of 3.96±0.04μg/cm^2/hr, permeability coefficient of 0.079±0.04×10^{-3}cm/hr. It indicates, four vesicular formulations of LA, TA, FA & ET showed maximum permeation than pure drug and hydroethanolic solution, shown in figure 1.

Upon comparing with pure drug and hydroethanolic solution, LA of liposomes showed less permeation than TA, FA and ET because of the presence of cholesterol, which could lead to increase rigidity of the vesicle and decrease the permeability of the lipid bilayer, which may cause lower permeation of AH through the skin [8].

TA showed higher permeation than LA because of the presence of edge activator, which provides characterisitic fluid membrane with high elasticity so, they can squeeze through the pores in the SPC, and can also adsorb onto or fuse with the SPC, and the intact vesicle can penetrate into and through the intact skin [10]. FA showed higher permeation than LA and TA because of the presence of positive charged lipid, stearylamine on the bilayer of the vesicle, which could bind to negative charges of the SPC enhancing the AH through the skin [11].

Figure 1: Comparision of release profile of vesicular formulations with pure drug solution and hydroethanolic solution of drug
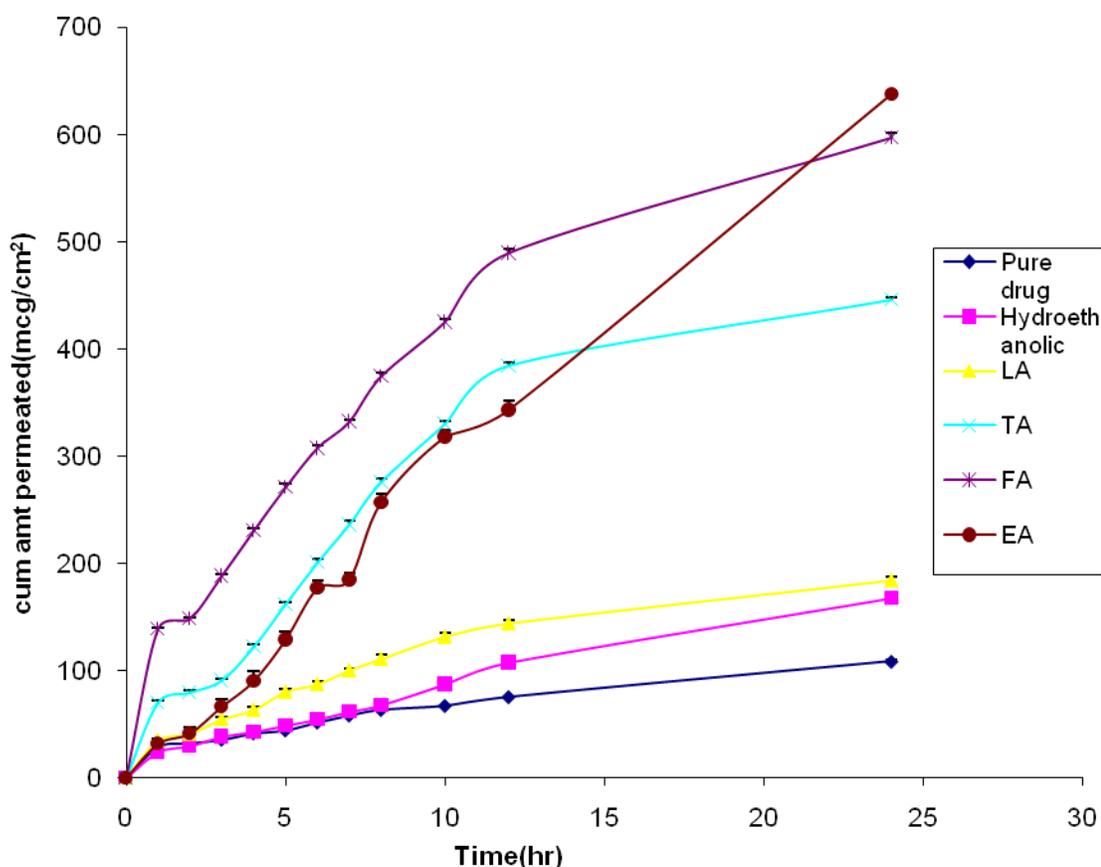
ET showed maximum permeation than LA, TA and FA because of the presence of high concentration (20%) of ethanol. wherein it has been already reported [4] that ethanol enhances permeation through various permeation mechanisms like push and pull effect, fluidization of lipids and vesicular bilayers of stratum corneum providing malleability to vesicles to penetrate skin pores smaller than their diameter [4, 13, 14].

Enhancement ratio is in the order of ethosomes, ET (6.92) > cationic flexosomes, FA (5.87) > transfersomes, TA (4.44) > liposomes, LA (1.88) > hydroethanolic solution (1.68) over drug solution shown in table 2.

Table 2: Comparison of physical and permeability parameters of liposome, transfersome, cationic flexosome and ethosomal formulations with pure drug and hydroethanolic solution of drug.

Permeability parameters	Pure drug	Hydro ethanolic solution	LA	TA	FA	EA
EE(%)	-	-	48.94±0.25	55.51±1.68	71.06±1.03	91.86±3.25
Zeta potential(mV)	-	-	-23.0	-26.9	14.5	-8.14±0.62
Particle size(nm)	-	-	80.9	149.5	119.9	6.85±1.35 (μm)
$Q_{12}(\mu g/cm^2)$	75.27±0.91	107.23±1.4	143.92±3.21	384.16±3.25	489.09±3.98	343.18±8.94
$Q_{24}(\mu g/cm^2)$	108.3±1.25	167.1±1.85	183.9±2.25	445.5±2.22	596.3±4.48	637.10±2.95
Flux($\mu g/cm^2/hr$)	3.96±0.04	6.66±0.13	7.45±0.25	17.6±0.21	23.28±0.91	27.42±0.04
Permeability coefficient (cm/hr×10^{-3})	0.079±0.04	0.13±0.08	0.14±0.05	3.52±0.009	4.65±0.006	5.48±0.009
Lag time(hr)	4.02±0.31	3.09±0.25	2.86±0.14	0.84±0.15	0.41±0.22	0.26±0.20
Skin content(μg/g)	94.35±11.1	86.33±8.5	80.44±12.1	61.86±11.3	52.21±10.5	298.01±15.4
Enhancement ratio	1	1.68	1.88	4.44	5.87	6.92

Table 3: Stability study data for vesicular formulations

Formulation	Initially (% EE)	After 120 days (%EE)	
	4^oC	4^oC	25^oC
LA	48.76	45.69	32.41
TA	55.45	52.02	37.35
FA	71.46	68.46	54.78
EA	91.86	90.98	78.35

Lag time is in the order of pure drug (4.02±0.31hr) > hydroethanolic solution (3.09±0.25hr) > liposomes, LA (2.86±0.14hr) > transfersomes, TA (0.84±0.19hr) > cationic flexosomes, FA (0.41±0.22hr) > ethosomes, ET (0.26±0.20) shown in table 2.

Skin content is in the order of pure drug (94.35±11.1μg/g) > hydroethanolic solution (86.33±8.5μg/g) > liposomes, LA (80.44±12.1μg/g) > transfersomes, TA (61.86±11.3μg/g) >

cationic flexosomes, FA (52.21±10.5µg/g) > ethosomes, ET (298.01±15.4) shown in table 2. Upon comparing the results of vesicular formulations shown in table.no.2, Q_{12} of ET (343.18±8.94µg/cm^2) was less when compared with Q_{12} of TA (384.16±3.25µg/cm^2) and FA (489.09±3.98µg/cm^2) but the entrapment efficiency, flux and enhancement ratio of EA were more when compared with LA, TA, FA, hydroethanolic solution of drug and pure drug solution due to high concentration of ethanol used (20%). Concentration of phospholipid and ethanol effects entrapment efficiency and flux [12].

Vesicle-skin interaction studies did not exhibit any major alterations except the slight fatty change in dermis revealing a decrease in resistance of skin as a barrier by fluidization of lipids, by ethanol [4]. Stability studies performed at 4°C±1°C for 120 days showed good storage stability and the results were shown in table 3.

CONCLUSION

By this study it is concluded that, soft malleable vesicles consisting of phospholipids and higher concentration of ethanol exhibited synergitic effect of phospholipids and ethanol on permeation proving ethosomes are better carriers for AH transdermal delivery than liposomes, transferosomes and cationic flexosomes.

REFERENCES

[1] Roopesh Sachan, Tarun Parashar, Soniya, Vishal Singh, Gaurav Singh et al. Int J Res Develop Pharm Life Sci 2013; 2(2): 309-316.
[2] Shailesh T. Prajapati, Charmi G. Patel and Chagan N. Patel. Asian J Biochem Pharm Res 2011; 2(1): 508-524.
[3] Prasanthi D, Khatry S, Shastri N, Sadanandam M. Indian Drugs 2010; 47(2): 25-33.
[4] Prasanthi D, Lakshmi P K. Int Curr Pharm J 2012; 1(11): 370-375.
[5] Verma P, Pathak K. Nanomed Nanotechnol Biol Med 2012; 8(4): 489-496.
[6] Prasanthi. D and P.K. Lakshmi. ISRN Pharm. 2012; 1-8.
[7] Jain S, Tiwary AK, Sapra B, Jain NK. AAPS PharmSci Tech 2007; 8(4): E1-E8.
[8] Sureewan Duangjit, Praneet Opanasopit, Theerasak Rojanarata and Tanasait Ngawhirunpat. J Drug Del 2011;1-9.
[9] Alladi Saritha, D. Rambhau, S.Srinivasan, K.Mahalingan. J Global Trends Pharm Sci 2010; 1(1): 7-14.
[10] Ghada M. El Zaafarany, Gehanne A.S. Awad, Samar M. Holayel, Nahed D. Mortada. Int J Pharm 2010; 397: 164-172.
[11] Nina Dragicevic-Curic, Susanna Grafe, Burkhard Gitter, Sven Winter, Alfred Fahr. Int J Pharm 2010; 384: 100-108.
[12] Dubey V, Mishra D, Nahar M, Jain V and Jain NK. Nanomed Nanotechnol Biol Med 2010; 6: 590-596.
[13] Panchangula P, Salve PR, Thomas NS, Jain AK, Ramarao P. Int J Pharm 2001; 219: 95-105.
[14] Komatsu H, Okada S. Biochim Biophys Acta 1996; 1283: 73-79.