Formulation, Evaluation and Optimization of Nasal pH Responsive In Situ Gel of Divalproex Loaded Invasomes

Ms. Priyanka Bhusara[1], Dr. Nishi Prakash Jain[2*], Ms. Teena Parmar[3], Dr. Parul D. Mehta[4]

Student at Lakshmi Narain College of Pharmacy[1],
Assistant Professor at Lakshmi Narain College of Pharmacy,
[2,3,] Director at Lakshmi Narain College of Pharmacy[4]
Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal, Madhya Pradesh, India-462021
drnishi51@gmail.com

Abstract

Divalproex (Sodium Valproate) is an anti-epileptic drug used for treatment of seizure disorder, migraine or a few bipolar disorders. Hair growth promoting potential of valproate has been reported in subjects of Alopecia. Topical application of valproate has been studied for promoting hair growth in Alopecia. Literature also reveals that no skin irritation is observed on topical application of Divalproex sodium in healthy subjects. Divalproex sodium is reported to have a half-life of 2h and hence is administered orally in divided doses to maximum of 1000 mg per day. Invasomes have been known to improve topical administration and prolong the release of the drug entrapped in the vesicle. The prolonged release in turn helps in reducing the dose of the drug. Considering its suitability to be applied on skin and its effectiveness in Alopecia as well as in diabetic neuropathic pain, nano-vesicles of divalproex suitable for topical application either as gel or solution were envisioned in the present work.

Keywords: *Invasomes, nano-vesicles, anti-epileptic, Divalproex (Sodium Valproate), Alopecia, in situ gel,*

1. Introduction

The drug delivery systems based on the nanocarrier have gained optimistic renovation, in particular for the drug delivery through the skin as a non-invasive route.[1] It improves essential drug/bioactive candidate penetrant properties. Invasomes are the soft liposomal vesicles embodying trivial quantities of ethanol and terpene or terpene assortments, which deed as potential transporters with amplified skin penetration.[2] Invasomes are composed of unsaturated soybean lecithin (with high % PC), small amount of ethanol, and small amount of a mixture of terpenes (cineole, citral, and d-limonene).[3] Ethanol is an effective permeation enhancer. It produces a significant effect in nano-vascular systems by giving the vesicles exclusive appearances in terms of size, zeta potential, entrapment efficacy, skin permeability and stability. Ethanol alters the structure of keratinize or lipophilic domains and decrease transition temperature of lipids. Ethanol increases the fluidity of lipids and thus ethanol based

nanovesicles are softer and less rigid as compared to liposomes.[4] The use of terpenes seems to offer the prodigious potential for use in transdermal formulations. Invasomes are deformable novel vesicles prepared by incorporating terpenes which are frolicking the penetration enhancement of active drug molecules as compared to the conventional liposomes. A complete understanding of the mucoadhesion mechanism is not entirely available. It is generally accepted, however, that inter-diffusion and interpenetration takes place between the chains of the mucoadhesive polymer and mucus gel network, which creates sufficient contact for entanglement.[5]

There are approximately 100 motile cilia covering each ciliated cell which are responsible for mucus transport, so mucociliary clearance prevails.[6] Once drugs (as particles or in solution) find their way to the mucociliary area, they are cleared from the nasal cavity and then have limited access to the absorption site.[21] There are two broadly defined mechanisms used for triggering the *in situ* gel formation of biomaterials are based on physiological stimuli (Eg., temperature and pH) & Chemical reactions (Eg., chemical and photo-initiated polymerization).[7] Photo-polymerisation is commonly used for *in situ* gel formation of biomaterials.[8] A solution of monomers or reactive macromers and initiator can be injected into a tissue site and the application of electromagnetic radiation used to form gel. Gel formation is indicated by a lack of movement of meniscus on tilting the tube. The mucoadhesive potential of each formulation can be determined by measuring the force required to detach the formulation from nasal mucosal tissue.[9]

2. Materials and Methods

2.1. Preparation of Invasomes by thin layer hydration technique

Eucalyptus oil was being used as terpenes, soy lecithin would be used as the lipid to prepare vesicles. Different divalproex-loaded invasomes were prepared using the conventional thin layer hydration technique. Accurately weighted amount of soy lecithin (200 mg) was dissolved in 10-ml ethanol and to it various concentration of terpene (eucalyptus oil) was added and stirred until a clear solution was obtained. Divalproex (1% w/v) was dissolved in a 1% v/v hydro-ethanolic solution. Both the solutions were separately sonicated at 60°C for 30 min to assure homogeneity. The organic solvent (ethanol) from the oil phase was evaporated by rotatory evaporator at 120 rpm at 60°C for 15 min to obtain a clear film on the walls of the flask. The deposited lipid film was then hydrated with aqueous phase by rotation at 120 rpm for 1 h at 60°C, followed by hand shaking for 5 min. The mixture was finally sonicated at 60°C for 30 min to obtain the invasome formulation.[10]

2.3 CHARACTERIZATION OF INVASOMES

2.3.1. Entrapment Efficiency:

Amount of divalproex in supernatant and sediment gave a total amount of divalproex in 1 ml dispersion.[11] The percent entrapment was calculated using the formula: % entrapment= amount of divalproex in sediment/amount of divalproex added ×100

2.3.2. Particle size:
The particle size of the prepared invasomes was measured using the dynamic light-scattering by Malvern Zetasizer at temperature of 25 ± 2°C.

2.3.3. In vitro release:
The in vitro release of divalproex from different divalproex -loaded invasomes was evaluated using the dialysis bag diffusion technique.[12]

2.4 FORMULATION OF INVASOMAL *IN SITU* GEL

2.4.1 Method of preparation:

Gel formulations will be prepared by soaking varying concentration of Carbopol 934 in water for 24 h. Briefly poloxamer 407 gel was prepared by dissolving the optimized poloxamer 407 concentration in cold (4°C) water. The hazy solution formed was kept in refrigerator (2–4°C) overnight for complete dissolution resulting in a clear solution. Carbopol 934 (0.1 to 0.4 % w/v) concentration was added slowly to the optimized poloxamer 407 solution containing drug with continuous stirring at 4°C (Table 1).[15] The invasomes were dissolved in ethanol and added to this gel base under continuous stirring. Formulated gels where then finally stored at 4°C for further evaluation.

Table 1. Composition of intranasal gel formulations

Formulation Code	Invasome (% w/v)	Poloxamer 407 (%w/v)	Carbopol 934 (%w/v)
INS	Pure drug solution (0.5%)	--	--
ING1	0.5	17	0.1
ING2	0.5	17	0.2
ING3	0.5	17	0.3
ING4	0.5	17	0.4
ING5	0.5	17	0.5
ING6	0.5	17	-

2.5 EVALUATION OF IN SITU GEL

2.5.1 pH: The pH of each formulation was determined by pH meter.

2.5.2 Viscosity: Viscosity of *in situ* gel system was determined using Brook field viscometer DV-1.

2.5.3 Drug content: The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel and calculating the amount of drug present in the solution by measuring the absorbance of solution using UV spectrophotometer[13]

2.5.4 Rheological Studies: The measurement of viscosity of prepared *in situ* gel was done with Brookfield viscometer[14]

2.5.5 Gel Strength: Gel strength was determined by placing a standard weight of 35 g onto 50 g of thermoreversible gel (placed in 100 ml beaker) maintained at gelation temperature using controlled water bath. The time in seconds by the weight to penetrate 5 cm deep into the container was recorded as gel strength

2.5.6 *In vitro* drug release: Drug release from gel was determined by using Franz diffusion cell. Artificial dialysis membranes were soaked in receptor medium for 2h prior to use. Phosphate buffer saline (12 ml) pH 6.4 was added into the receptor chamber maintained at 34 ± 1°C. Gel equivalent to 2.5 mg of drug was placed into donor compartment and the setup was kept on stirring. Aliquots of 1ml were withdrawn at predetermined time intervals from receptor compartment and replaced with fresh buffer till 12 h. The samples were diluted suitably and analyzed spectrophotometrically at 254 nm and the amount of drug released was determined using calibration curve.[15]

3. RESULTS AND DISCUSSION

3.1 Physico-chemical characteristics:

Table 2. Physical characterization of sodium valproate

Sr. No.	Test	Specification	Observation
1	Color	White or off-white	White
3	Appearance	Crystalline powder	Powder
4	Melting Point	222-224°C	219-222°C
5	Solubility	slightly soluble in water, methanol, ethanol	Slightly soluble in water, and methanol, soluble in acetonitrile and DMF

3.2 Calibration curve of Divalproex sodium:

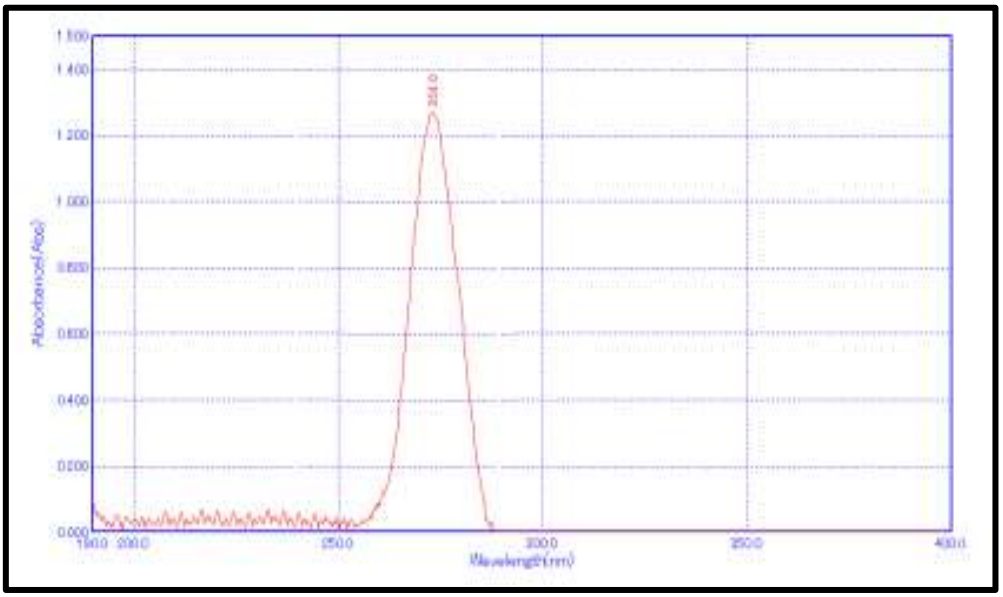


Figure 1. UV spectrum of Divalproex sodium

Table 3. Calibration data of Divalproex

Sr. No	Concentration (mg/mL)	Absorbance at 254 nm
1	5	0.195
2	10	0.418
3	15	0.615
4	20	0.798
5	25	0.996

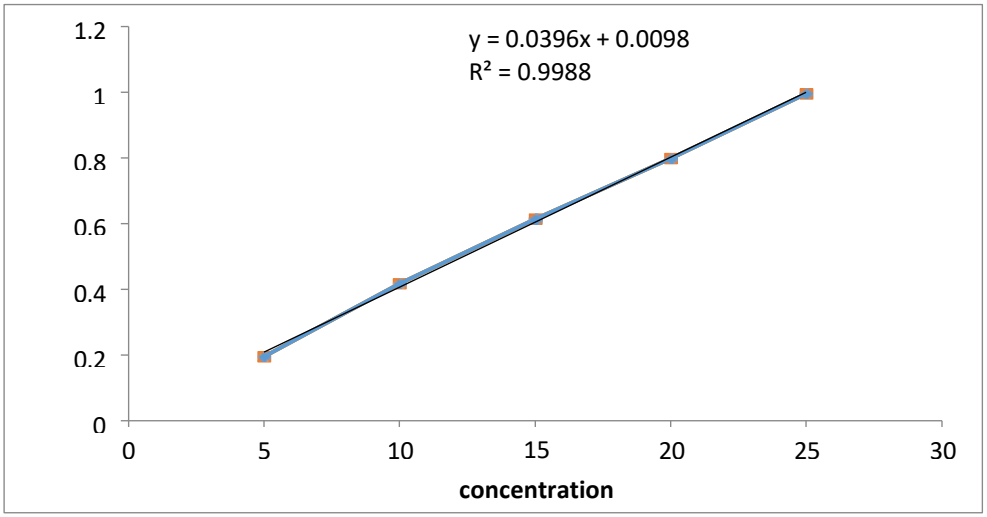


Figure 2. Calibration curve of sodium valproate

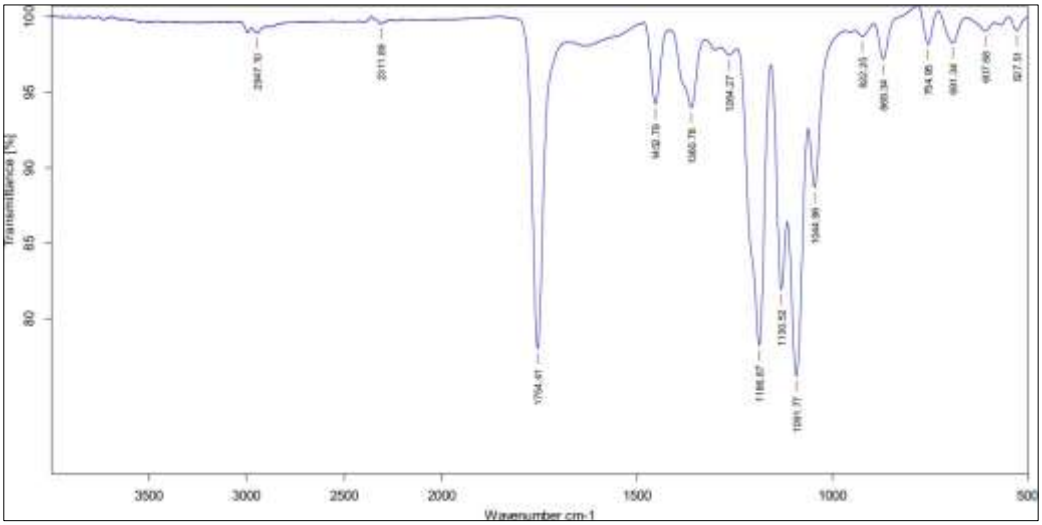
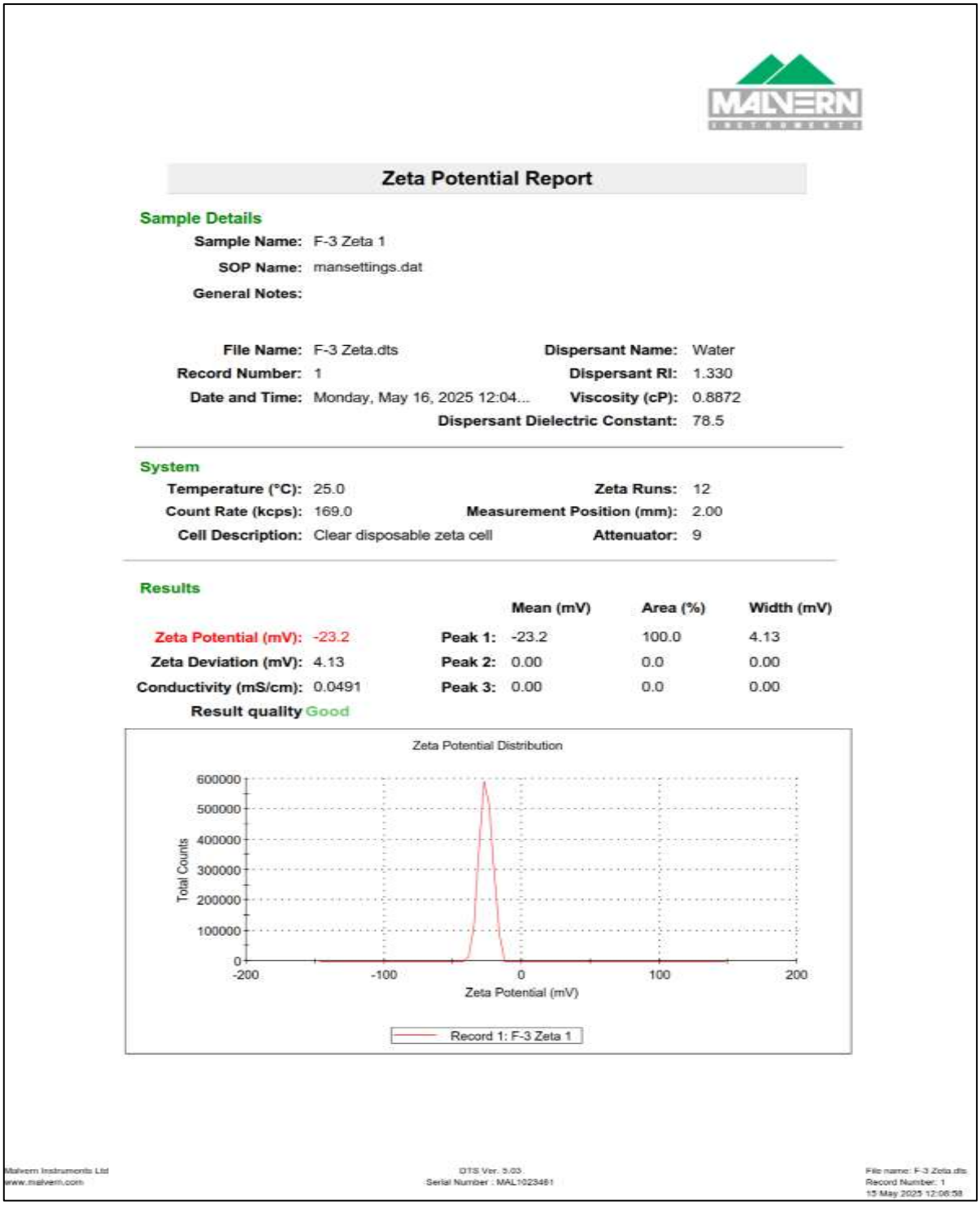


Figure 3. FTIR spectra of Divalproex

3.2 Evaluation of Invasomes

Table 4. Particle size, zeta potential and entrapment efficiency of invasomes

Formulation	Particle size	Zeta Potential	% EE
F1	221.7 nm	-18.9 mV	69.45
F2	209.8 nm	-20.4 mV	80.17
F3	131.5 nm	-23.2 mV	86.08



Zeta Potential Report

Sample Details

Sample Name: F-3 Zeta 1
SOP Name: mansettings.dat
General Notes:

File Name: F-3 Zeta.dts
Record Number: 1
Date and Time: Monday, May 16, 2025 12:04...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 169.0
Cell Description: Clear disposable zeta cell
Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 9

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -23.2	Peak 1:	-23.2	100.0	4.13
Zeta Deviation (mV): 4.13	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0491	Peak 3:	0.00	0.0	0.00

Result quality Good

Malvern Instruments Ltd
www.malvern.com
DTS Ver. 5.03
Serial Number : MAL1023481
File name: F-3 Zeta.dts
Record Number: 1
16 May 2025 12:06:58

Figure 4: Zeta potential of F3

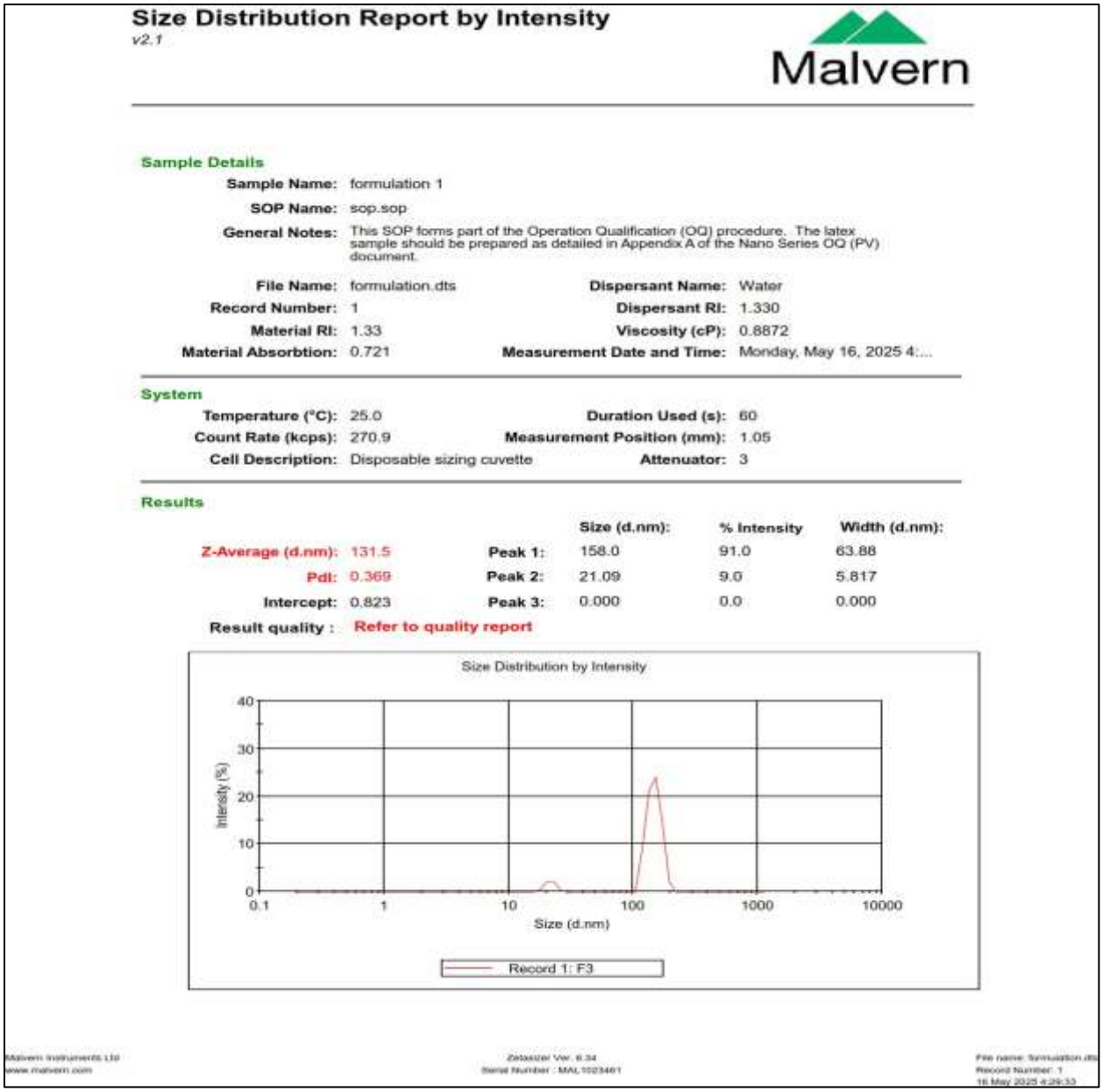


Figure 5. Particle size of F3

3.3.3 In vitro drug release

The release of divalproex from invasomes was studied for a period of 12 hours and the invasomes were able to sustain the release of divalproex for duration of 12 hours. The results of release study are presented in Table 5.

Table 5: In vitro release of divalproex from invasomes

Time (h)	% Release		
	F1	F2	F3

0	0.00	0.00	0.00
1	5.68	4.90	6.30
2	18.78	14.20	14.50
3	26.31	24.30	25.60
4	31.93	37.50	40.30
5	41.45	43.71	45.32
6	52.03	49.82	54.73
7	65.14	61.23	70.82
8	72.54	76.3	78.28
9	88.14	86.28	82.81
10	93.37	96.6	85.8
11	98.51	98.77	91.53
12	91.46	92.51	98.63

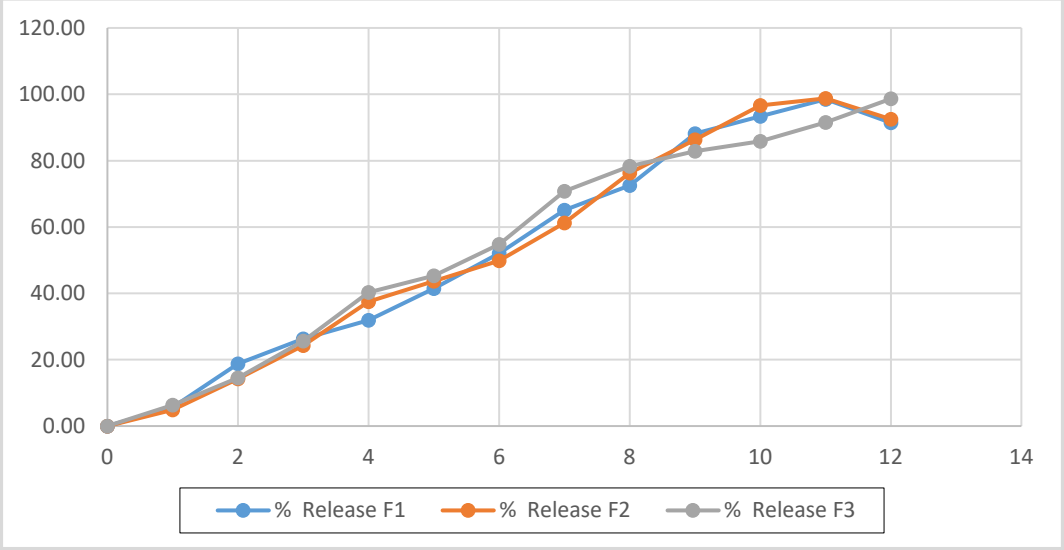


Figure 6: *In vitro* release of divalproex from invasomes

F3 with lowest particle size and highest release of Divalproex was selected as the best formulation of *in situ* intranasal gel.

3.4 Determination of gelation temperature

Phase transition temperature determination is a preliminary step in the formulation of the *in situ* gel. Gelation temperature of gel formulations is shown in Table 7. which suggests that Poloxamer 407 in the concentration of 17% w/v showed best results for phase transition at 31-33°C. As the concentration of poloxamer increased -from 18 to 20 %, transition temperature decreased from 34 to 25 °C.

Table 6: Gelation temperature of Poloxamer 407

Sr. No.	Poloxomer 407 (%w/v)	Gelation Temperature (°C)
1	15	No gelling till 40
2	16	No gelling till 40
3	17	31-33
4	18	30-32
5	19	26-27
6	20	24-25

In situ intranasal gels must transform to gel form at nasal temperature and exist in solution form when stored at room temperature. If the gelation temperature of is lower than 25°C, a gel may be formed at room temperature whereas when the gelation temperature is higher than 34°C, solution form will not show phase transition at the nasal temperature resulting in the nasal clearance of the administered drugs at an early stage.

The addition of carbopol 934 also affected the gelation behavior of the formulations. The effect of varying concentration of carbopol 934 on gelation temperature revealed that all the formulations were able to transform to gel form at temperature from 25-32°C. Increasing the concentration of carbopol 934 led to a decrease in gelation temperature of the formulations (Figure 7). A concentration of 0.4% and higher of carbopol 934 decreased the gelation temperature to 23°C making the concentrations unsuitable for *in situ* intranasal gel delivery.

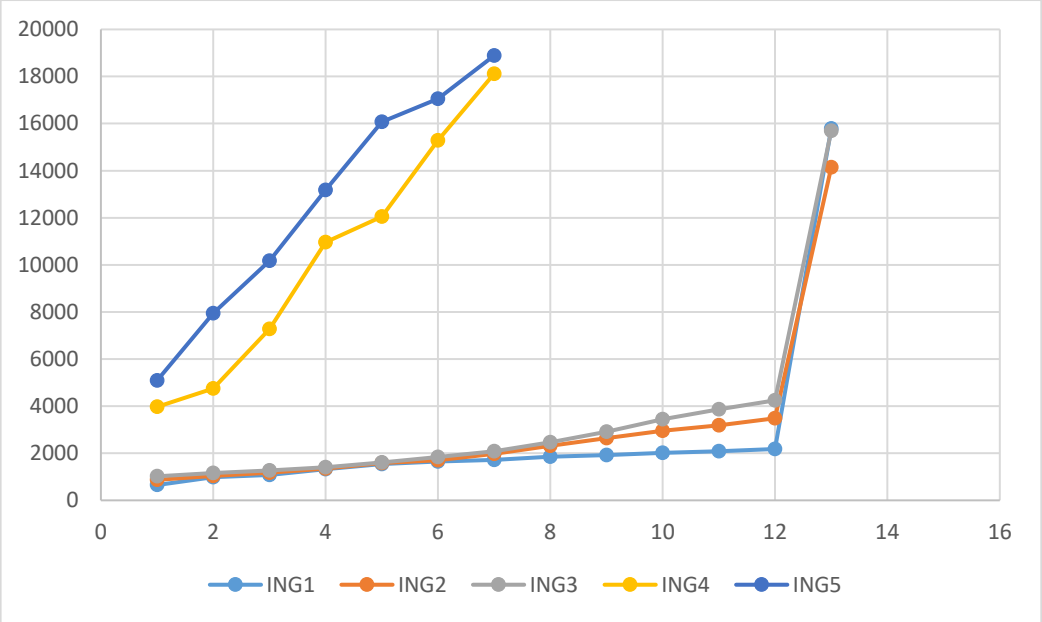


Figure 7: Effect of carbopol 934 on gelation temperature

3.5 Evaluation of the *in situ* gel formulations

3.5.1. Physicochemical properties

The pH of all the formulations was found to be from 5.85 to 6.08 which is in between nasal pH (5.5 to 6.5). This ascertains that all the formulations are compatible with nasal mucosa. The formulations ING1, ING2 and ING3 were found to be clear while ING4 and ING5 were turbidity appearance revealing that clarity of the gel is inversely proportional to the concentration of Carbopol 934. The results of pH, clarity, drug content, viscosity, gelling time and gel strength are presented in Table 7.

Table 7. Physicochemical properties of the *in situ* gel formulations

Formulation code	pH	Clarity	Drug content (%)	Viscosity (cps)		Gel Strength (g)	Gelling time (sec)
				Sol	Gel		
ING1	5.85	++	92.4	250	1080	4.6	11
ING2	6.06	++	95.2	320	1220	5.3	10
ING3	6.08	++	95	450	1640	6.1	8
ING4	5.93	+	95.8	560	1780	6.8	7
ING5	5.98	+	96.7	650	2020	7.5	5

The most important feature for intranasal *in situ* gel is viscosity of the formulation. A formulation suitable for application to the nasal cavity should ideally have a low viscosity when applied and after administration should have a high viscosity in order to stay at the application site. All the formulations exhibited a carbopol 934 concentration dependent increase in viscosity. Viscosity of the both *in situ* sol and *in situ* gel was examined at 100 rpm. ING5 formulation was having maximum viscosity. The viscosity of ING3 (450 in sol to 1640 in gel) was taken as optimum. Viscosity of the sol formulation ranged between 250 to 650 cps while that of the *in situ* gel ranged between 1080 to 2020 cps.

3.5.2 Rheological Studies

The flow curve (viscosity against speed / rpm) of the formulations indicated that for the all the polymer concentrations, the formulations exhibited the properties of pseudoplastic systems with shear thinning. The prepared formulations tend to thin when being exposed to shearing force and therefore tend to be easily syringeable and spreadable. The effect of agitation speed on viscosity is presented in Table 8 and Figure 8.

Table 8. Rheological behaviour of the gel formulations

rmulation code	Viscosity (cps)					
	10 rpm	20 rpm	40 rpm	60 rpm	80 rpm	100 rpm

ING1	1480	1360	1120	880	650	470
ING2	1530	1410	1200	920	810	630
ING3	1610	1520	1310	1080	930	770
ING4	1720	1640	1400	1250	1010	890
ING5	1810	1720	1490	1360	1180	1010

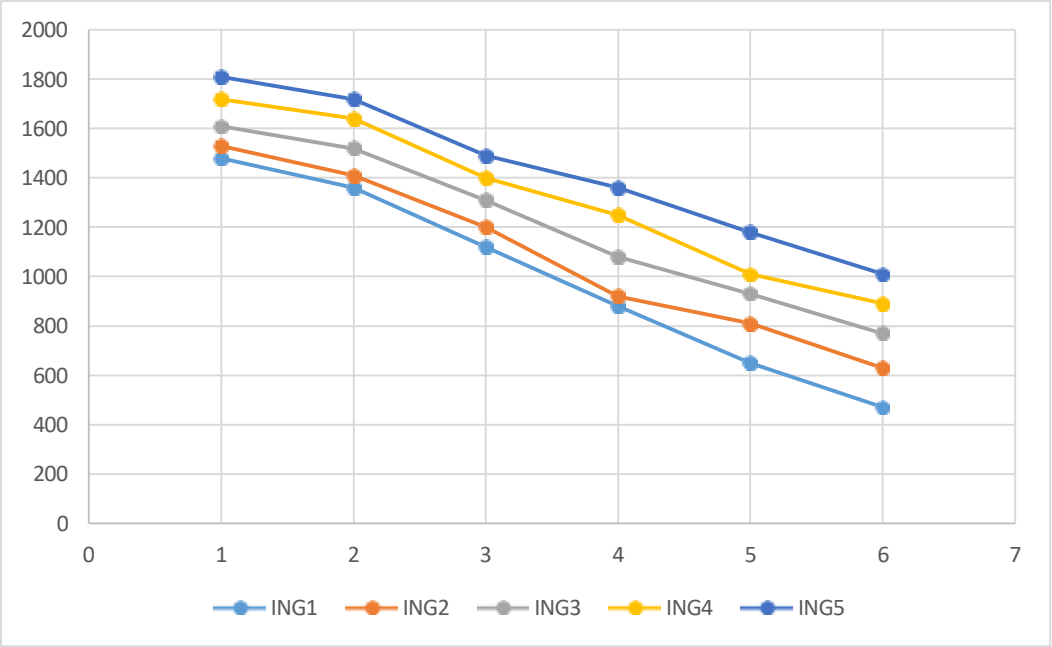


Figure 8. Rheological behaviour of *in situ* gel

3.5.3 *In vitro* drug release from *in situ* gel

The *in vitro* release studies of different formulations of drug loaded *in situ* gels were carried out for 20 min in PBS pH 6.8. PBS of pH6.8 was selected as medium for drug absorbance since it resembles nasal pH. Throughout the study the pH and temperature were kept constant.

Table 9. *In vitro* drug release from the *in situ* gel formulations

Time (min)	ING1	ING2	ING3	ING4	ING5	ING6
2	6.1	4.2	3.8	2.5	2	7.8
4	17.5	12.4	10.4	8.7	6.8	19.2
6	32.6	26.2	22.8	22.5	18.3	34.3
8	48.4	37.8	31.3	30.3	23.2	50.1
10	66.7	46	42.4	38.6	35.1	68.4
12	86.1	53.1	49.2	45.8	40.7	87.8
14	100.1	67.3	61.7	57	46.1	101.5
16	98.4	78.6	74	63.7	55.8	100.3
18	97.1	87	84.9	72.5	62.4	98.8
20	96.5	95.7	96.2	82.8	75.6	97.2

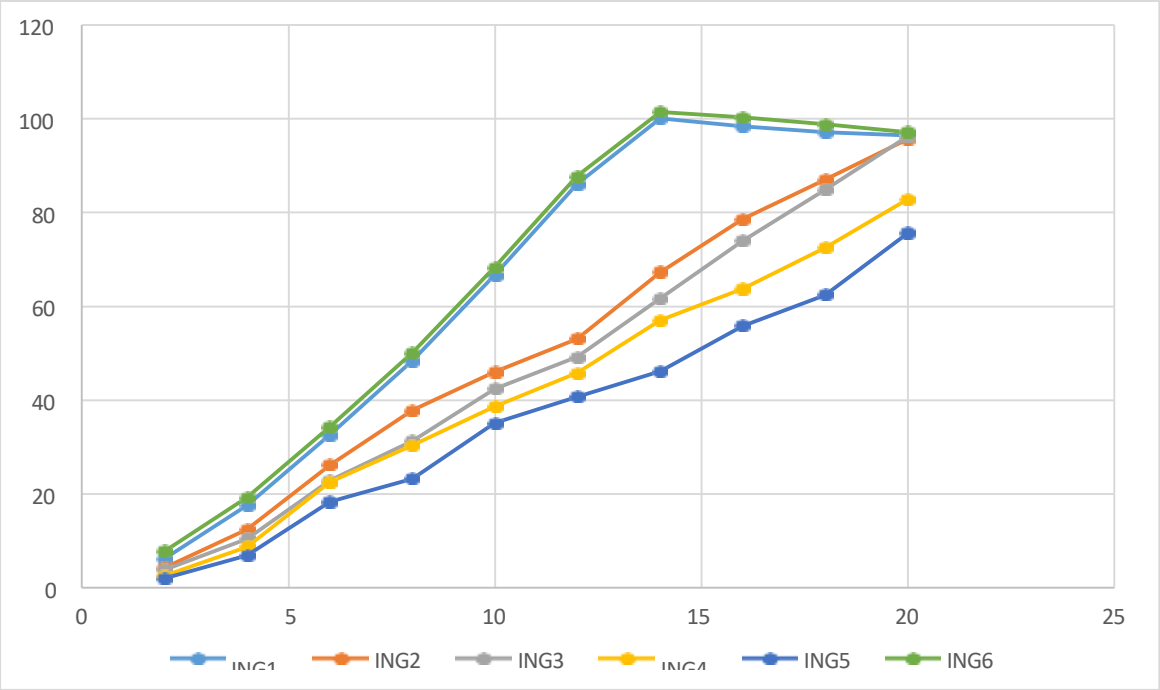


Figure 9. Comparative drug release profile from the formulations

4. Discussion

The *in situ* intranasal gel delivery system loaded with invasomes of Divalproex was prepared using cold stirring method and evaluated for various parameter. The identity of the drug sample was confirmed by observing its organoleptic characters, melting point, solubility profile and FT-IR spectroscopy. Invasome formulation **F3** with lowest particle size and highest release of Divalproex was selected as the best formulation of *in situ* intranasal gel.
The concentration of poloxamer 407 to be used was optimized by gelation temperature study and it was found that 17% w/v of the same was able to gelify at temperature equivalent to the nasal temperature. Formulations with increasing concentration of carbopol 934 were prepared and evaluated. The higher concentration of carbopol 934 increased the gel characteristics so much that they may not be pleasant for administration.

The pH of all the formulations was found to be from 5.85 to 6.08, viscosity of the sol formulation ranged between 250 to 650 cps while that of the *in situ* gel ranged between 1080 to 2020 cps. The drug content ranged from 92.4 to 96.7 %. The flow curve of the formulations indicated that for the all the polymer concentrations, the formulations exhibited the properties of pseudoplastic systems with shear thinning.

The *in vitro* release studies of different formulations of drug loaded *in situ* gels were carried out for 20 min in PBS pH 6.8. The maximum drug release from the formulations ranged from 75.6 to 100.1 % over the duration of in vitro release study.

5. Conclusion

The present study represents formulation of *in situ* intranasal gel for sumatriptan using poloxamer 407 and carbopol 934. Formulation (ING3) was found to be optimized due to its desirable gelation temperature, gelling time and gel strength. *In-vitro* release studies suggest that carbopol not only acts as mucoadhesive agent but also as a penetration enhancer whereas poloxamer acts as thermoreversible polymer leading to sustained release of drug for longer time. In conclusion, intranasal gel of Dilvaproex loaded invasome could be better alternative to existing conventional dosage form to improve drug bioavailability and patient compliance.

6. References

[1] *Kumar P, Sankar C and Mishra B. Delivery of macromolecules through skin. The IndianPharmacist 2004, 7-17.*

[2] *Rizwan M, Aqil M, Talegoankar S, Azeem A, Sultana Y and Ali A, Enhanced transdermal drug delivery techniques: an extensive review Delivery of macromolecules through skin, The Indian Pharmacist, 2004, 7-17.*

[3] *Kumar R and Philip A, Modified Transdermal Technologies: Breaking the Barriers of Drug Permeation via the Skin, Trop J Pharm Res. 2007, 6(1):633-644.*

[4] *Jain S, Bhandra D, Jain S and Jain N K, Transfersomes- A Novel carrier foreffective transdermal drug delivery controlled and novel drug delivery 1stEdition, CBS Publishers and Distributors New Delhi 1997: 426-451.*

[5] *Jain N, Talegonkar S and Jain N K, New ways to enter the blood stream:Emerging strategies in transdermal drug delivery, The Pharma Review.Sep-Oct 2004,1- 60.*

[6] *Kumar P, Sankar C and Mishra B, Delivery of macromolecules through skin. The indianPharmacist 2004, 7-17.*

[7] *Touitou E., Dayan N., Bergelson L., Godin B and Eliaz M..Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Control. Rel. 2000, 65, 403-418.*

[8] *Manosroi A., Jantrawut P., Khositsuntiwong N., Manosroi W. and Manosroi J., Novel Elastic nanovesicles for Cosmeceutical and Pharmaceutical Applications. Chiang Mai. J. Sci. 2009; 36(2), 168-178.*

[9] *Maestrelli F., Capasso G., Maria L., Rodríguez G., Rabasco A.M., Ghelardini C. and Mura P., Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. J. Lipo.Resear. 2009, 1-8.*

[10] *Bhalaria M.K., Naik S. and Mishra A.N., Ethosomes: A novel delivery system for antifungal drug in the treatment of topical fungal diseases. Indian J. Exp. Biology. 2009; 47, 368-375.*

[11] *Verma D.D. and Fahr A., Synergistic penetration effect of ethanol and phospholipids on the topical delivery of Cyclosporin A. J. Control Rel. 2004; 97, 55-66.*

[12] *Sultana SS,Sailja A,Ethosomes: A Novel approach in the design of transdermal drug delivery system,International Journal of MediPharm Research,Vol.02, , pp 17-22, 2015*

[13] *Touitou E., Compositions for applying active substances to or through the skin. US Patent 1995 538, 934.*

[14] *Jain S, Umamaheshwari R B, Bhadra D and Jain N K., Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti HIV agent. Indian J. Pharm Sci 2004; 66(1), 72- 81.*

[15] *Tauitou E, Dayan M, Bergelson L, Godin B and Eliaz M., Ethosomes- novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Con Release 2000, 65, 403- 413.*

[16] *Touitou E. Compositions for applying active substances to or through the skin. US Patent 1995,38, 934.*

[17] *El-Maghraby G.M.M., Williams A.C. and Barry B.W., Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int. J. Pharm. 2000, 196(1), 63-74.*

[18] *Fry D.W., White J.C. and Goldman I.D., Rapid secretion of low molecular weight solutes from liposomes without dilution. Anal. Biochem. 1978, 90, 809-815.*

[19] *New RRC., Preparation of liposomes and size determination, In:Liposomes A Practical Approach, New RRC (Ed.), Oxford University Press, Oxford. 1990, 36-39.*

[20] *Cevc G., Schatzlein A. and Blume G., Transdermal drug carriers: Basic properties, optimization and transfer efficiency in case of epicutaneously applied peptides. J. Control. Rel. 1995, 36, 3-16.*

[21] *Berge V., Swartzendruber V.B. and Geest J., Development of an optimal protocol for the ultrastructural examination of skin by transmission electron microscopy. J. Microsc. 1997, 187(2), 125-133.*

[22] *Toll R., Jacobi U., Richter H., Lademann J., Schaefer H. and Blume U., Penetration profile of microspheres in follicular targeting of terminal hair follicles. J. Invest. Dermatol. 2004; 123:168-176.*

[23] *Lopez-Pinto JM, Gonzalez-Rodriguez ML and Rabasco AM., Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. Int J Pharm. 2005, 298,1-12.*

[24] *Lauer AC, Ramachandran C, Lieb L.M, Niemiec S and Weiner ND., Targeted delivery to the pilosebaceous unit via liposomes. Adv. Drug Delivery 1996, 18, 311-324.*

[25] *Johnsen SG, Bennett EP, Jensen and VG Lance, Therapeutic effectiveness of oral testosterone. 1974, 2, 1473-1475.*

[26] *Jain S, Vesicular approaches for transdermal delivery of bioactive agent. Ph.D thesis, Dr. H.S. Gour University, Sagar, India, 2005*

[27] *Dayan N and Touitou E., Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs liposomes. Biomaterials 200; 21: 1879- 1885.*

[28] *Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A and Levi-Schaffer F. Intracellular delivery mediated by an ethosomalcarrier, Biomaterialds 2001, 22, 3053.*

[29] *Jain S, Umamaheshwari R B, Bhadra D and Jain N K., Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti HIV agent, Indian J. Pharm Sci 2004, 66(1), 72- 81.*