Development of Valproic Acid Niosomal *in situ* Nasal Gel Formulation for Epilepsy

Shilpa P. Chaudhari and Vibhavari M. Chatur

Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune-33.

ABSTRACT

Valproic acid is an anti-epileptic drug used in the treatment of epilepsy and seizures. The study was designed with three aims. Firstly, to enhance the solubility and bioavailability of BCS class II drug Valproic acid; secondly, to ease administration of the formulation to the epileptic patient, during an attack, and thirdly, to reduce the dose of drug for long-term treatment. In the culmination of this study, an anti-epileptic drug loaded (Valproic acid) niosomal gel for nose-to-brain-delivery using thermosensitive polymer PF 127 was formulated, which can be a controlled dose to the patient. Niosomal formulations were optimized by altering the proportions of range of Tween and Span. The formulations were prepared by ether injection method. The formulation was then evaluated for morphological characterization, encapsulation efficiency, and viscosity. Valproic acid niosomes formulated with Tween 80 and Span 60 were found to entrap high amounts of drug. Further the niosomal gel was prepared by using various concentrations of PF 127 and were optimized at effective concentrations and studied for gelation temperature, melting temperature and heat of enthalpies, mucoadhesive strength, gel strength and showed satisfactory results. The niosomes entrapped in-situ nasal gel formulations showed sufficient quantity of in-vitro drug release through the gel across the goat's nasal mucosa. In histopathological study no effect of surfactant was seen; neither had the formulations caused any damage to the nasal tissue. Hence we can conclude that the niosomal in-situ nasal gel system can be considered as a promising approach for the anti-epileptic drug Valproic Acid.

Keywords: Valproic Acid, niosome, in-situ nasal gel, Gelation study, Enthalpy study, Histopathology.

INTRODUCTION

Valproic acid is used as an antiepileptic and also used to treat bipolar disorders, migraine and schizophrenia. It is also used as a mood-stabilizing agent. Valproic acid is insufficiently delivered to the brain hence it is to be administered in high doses so as to get the clinical effect. Also, there are various drug interactions and side-effects of Valproic acid, due to long-term usage and high dose requirements. So, administration of Valproic acid in low dose is important and is one of the objectives of the present study. Distribution of Valproic acid to brain by oral administration is less compared to other anticonvulsants like phenytoin or phenobarbital. This might be due to the increased plasma protein binding of valproate and asymmetric valproate transportation so that brain-to-blood flux is more than blood-to-brain.[1] Valproic acid is a poorly water soluble, BCS class II drug. In order to increase the solubility a salt form is prepared as sodium valproate and formulated in form of capsules, sustained release tablets, enteric coated tablet, suspension and IV injections (Depakote, Abbott Pharmaceuticals). P.S. Kawtikwar *et al.*, formulated and evaluated microemulsion of Valproic acid for nose to brain delivery but as these were drops, its clearance rate was higher.[2] In previous studies, (Sharareh Eskandari *et al.*, 2011) they formulated nanostructured lipid carriers of Valproic acid for intranasal drug delivery using cetylpalmitate oil phase, pluronic, and lecithin as a penetration enhancer.[3] Since, drug expulsion is caused by on-going crystallization or transformation of the solid lipid carrier, these nanostructures improved drug loading and

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Address for correspondence
Shilpa P. Chaudhari
Marathawada Mitra Mandal's College of Pharmacy, Pune-33
E-Mail: shilpapchaudhari78@yahoo.com

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decreased drug expulsion. But at a higher oil concentration there are chances of precipitation of tiny oily nanocompartments. If the solid content is more than 50%, then the particle dispersions have very high consistency and they are cream-like or almost solid and hence difficult to administer. To increase the solubility and bioavailability niosomes can be regarded as inexpensive alternatives of non-biological origin to liposomes, as well as they are chemically stable, possess low toxicity because of their non-ionic nature. These are formed from the self-assembly of non-ionic amphiphilies in aqueous media which can entrap both hydrophilic and lipophilic drugs, either in vesicular membrane or aqueous layer.

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.[4] Niosomes can be changed or modified by the incorporation of other excipients like cholesterol into the membrane and they can possess one or more lipid bilayers encapsulating an aqueous core. A diverse range of materials have been used to form niosomes such as sucrose ester surfactants and polyoxyethylene alkyl ether surfactants. Niosomes can be effectively used in targeted and controlled drug delivery.[5] Also, they are advantageous in terms of chemical stability, low toxicity due to non-ionic nature and do not require special handling precaution or condition.[6]

The most desirable and convenient method of drug administration is the oral route because of their ease of administration. However, in many instances oral administration is not desirable when the drug undergoes significant degradation via first pass effect in liver. Hence, lack of systemic absorption through the gastrointestinal tract led to research on alternate routes of drug delivery such as parenteral, intramuscular, subcutaneous, intranasal, transdermal, etc. In case of epileptic attack, rapid onset of action of a drug to the brain is necessary and in such a case, the nasal route can be considered as an alternate route to central nervous system, as it offers some advantages like fast absorption and bypass hepatic first pass metabolism. Intranasal (IN) administration is needle free and hence an ideal alternative to the parenteral route for systemic drug delivery. Nasal mucosa has a large surface area and provides a rapid therapeutic effect for direct delivery to brain due to presence of a rich vasculature and a highly permeable structure for systemic absorption. Drug administration through the nasal cavity is easy and convenient. Avoidance of first pass metabolism is the main advantage of nasal route of drug delivery; it also eases administration, patient comfort, and compliance. By using the niosomal in-situ nasal gel formulation, an attempt was made to increase its permeation so as to increase its bioavailability.

The aim of this study was to formulate niosomal in-situ-nasal gel of Valproic Acid for lowering the dosage, improving patient compliance and to provide prolonged action. The diffusion efficiency was also studied using the bovine nasal mucosa and the formulation was further subjected to the histopathology study. Also, estimation of gelation and gel melting was carried out for the optimized formulations.

MATERIALS AND METHODS

Materials

Valproic acid was a gift sample from Centaur Pharmaceuticals Ltd. Pune (India). Polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan monopalmitate (Tween 40), polyoxyethylene sorbitan monostearate (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monolaurate (Span 20), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan monooleate (Span 80), Methanol and Chloroform were purchased from S.D. Fine Chemicals, Mumbai. Cholesterol was purchased from Loba Chemi, Mumbai. Polymer Pluronic F-127 was purchased from Sigma Aldrich (USA).The Phosphate buffer saline (pH 7.4) was prepared as described in Indian Pharmacopoeia (1996). All other solvents and reagents used for the study were of analytical grade.

Methods

Formulation of niosomes

Niosomes containing Valproic were prepared by modified ether injection technique[7] using non-ionic surfactants (Span 20, 40, 60, 80 and Tween 20, 40, 60, 80) and cholesterol at different ratios.[8] Cholesterol and surfactants were dissolved in 6 ml diethyl ether mixed with 2 ml methanol, containing weighed quantity of Valproic Acid. The obtained solution was slowly injected by using micro syringe, at a rate of 1 ml/min via 14-gauge needle into 15 ml of aqueous phase maintained at temperature 60°C.[9] The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60–65°C. Then, the formulations were sonicated three times at 50 Hz in a bath-sonicator (Ralsonics model RP 120, Mumbai, India) for 15 min with a 5 min interval in between. Different batches of niosomes were prepared in order to select an optimized formula as per the general method described above. The proportion of surfactants and the fixed proportion of cholesterol for the preparations of niosomes are given in Table 1 and optimized niosomal formula is given in Table 2.

Table 1: Various Ratios in which the Niosomal Formulations are Made

Sr. No	Batch	CHOL:SURFACTANT (μM)
1	Span 20 (1a-1f)/Span 40 (2a-2f)/ Span 60 (3a-2f)/Span 80 (4a4f)/ Tween 20 (5a5f)/Tween 40 (6a-6f)/ Tween 60 (7a-7f)/Tween 80 (8a-8f) (Amount of drug incorporated 400 mg)	1:1.5
2		1:2.5
3		1:30
4		1:3.5
5		1:4.5
6		1:6.0

Table 2: Optimized Concentration of Pluronic F127

Formulation	Cholesterol:Surfactant	Gel%
Span 80 (IV-C)	1:30	18.0%
Tween 20 (V-A)	1:1.5	18.0%

Characterization of sonicated vesicles

Determination of vesicle diameter

The size, shape, and lamellar nature of vesicles in sonicated formulations were observed by optical microscopy (Kandasamy Ruckmani *et al*., 2010) using a calibrated eyepiece micrometer and photographs were taken at ×400 magnification with a digital camera (Motic, 8.1 megapixel, Japan).

Determination of entrapment efficiency

Valproic acid niosomal formulations were centrifuged at 16,700 rpm for 90 min at 4°C (Kandasamy Ruckmani *et al*., 2010) using a refrigerated centrifuge (Eppendorf, 5415 R, Germany), so as to separate niosomes from non-entrapped drug. The concentration of the free drug in the supernatant was determined by measuring absorbance at 211 nm with a UV spectrophotometer (Shimadzu, UV 1650PC, Kyoto, Japan). The percentage of drug entrapment in niosomes was calculated by the following formula.

$$\text{Percent drug entrapment} = \text{Total drug} - \text{drug in supernatant}/\text{Total drug} \times 100$$

This process was repeated thrice to ensure that free drug was completely removed. Percent drug entrapment was confirmed by lysing the niosomes with n-propanol after centrifugation and measuring absorbance at 211 nm.

Determination of viscosity

Viscosity of the formulations was determined using an Ostwald viscometer (Yash Enterprises, Pune, India) at 34°C.

Formulation of niosomal in situ *nasal gel*

Aqueous gels of PF-127 were prepared containing 16–24% w/w of polymer slowly added to cold distilled water with continuous stirring.[10] Mixture was kept overnight at 4°C to ensure complete dissolution. After estimating the gelation temperature, the optimized concentration of PF-127 was used for further niosomal gel formulation. Niosomal gels were prepared using the same formula and the resultant dispersion was stored at 4°C in a refrigerator for further studies.

Evaluation of niosomal in situ *nasal gel*

The above formulated PF-127 niosomal in-situ-nasal gels of Valproic acid were subjected to evaluation for the following parameters.

Evaluation by FTIR, DSC and SEM

Interaction between drug, polymer, and the surfactants was performed by Differential Scanning Calorimetry and FTIR spectroscopy and also SEM imaging.

Visual appearance, clarity and pH

Visual appearance and clarity were observed for the presence of any particular matter. The pH of in-situ gels was measured using digital pH meter.[11]

Drug content analysis

It was carried out using UV-Spectrometric method and sufficient amount of methanol was added to lyse the vesicles. Then 0.1 ml of formulation was diluted to 100 ml of simulated nasal fluid pH 7.4 and the absorbance was measured at 211 nm using simulated nasal fluid pH 7.4 as blank.[12]

Rheological studies

These studies were carried out in the Brookfield Viscometer LV DV2+ Pro with spindle SC 18 at 34°C in a small sample adaptor.

The mucoadhesive forces

The mucoadhesive forces of the gels were determined by means of modified analytical two- pan balance.[13] The nasal mucosa of goat was taken in saline solution to 37°C before use. At the time of testing a section of tissue was attached, keeping the mucosal side out, on to upper glass vial using a rubber band. Next, one vial with a section of tissue was connected to the balance and the other vial was fixed on a height-adjustable pan. To the lower vial, a niosomal nasal gel was applied. The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of upper vial. After which the upper vial was then connected to the balance. Weights were added at a constant rate to the pan on the other side of the modified balance of the used device until the gel gets detached from tissue. The mucoadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights

required for the detachment using the following formula,

$$\text{Detachment stress (dynes/cm}^2) = mg/A$$

Where,
m = the weight added to the balance in gram.
g = acceleration due to gravity taken as 980 cm/sec^2.
A = area of tissue exposed.
(The surface area of mucosa was 1.12 cm^2)

Gel strength

Formulated gels were placed in the test tubes and gelled in a thermostat at 37°C. The apparatus for measuring gel strength (Weight: 27 gm.) was then placed onto the niosomal gel formulation. The time taken by the apparatus to sink to a depth of 5 cm through the prepared gel was measured for Span and Tween niosomal gel formulations.[14]

In vitro drug diffusion studies

The horizontal diffusion chamber was used for the present study using goat nasal mucosa. Phosphate buffer solution of pH 7.4 was used in the receptor chamber. Before starting the study, the mucosa was pre-incubated with phosphate buffer solution of pH 7.4 so as to saturate the mucosa; so that there should not be any change in permeability. The niosomal nasal gel formulation solution was taken into the donor compartment. The quantity of the niosomal gel formulation was approximately about 1–1.5 ml of gel. The speed of the magnet was adjusted at an optimum speed. Sampling was done at regular intervals, i.e. for 15, 30, 45, 60, 75, 90, 120 and 150 min. The sink condition was maintained with phosphate buffer solution. The samples were diluted with methanol and further measurements were carried out on the UV spectrophotometer at 211 nm.[15]

Data analysis of permeability study

The diffusion co-efficient under the steady state flux across the goat's nasal mucosa for optimized gel formulations of Span as well as Tween was calculated by following equation and recorded.[16]

$$Peff = (dC/dt)_{ss} \times V/AC_D$$

Where,
$(dC/dt)_{ss}$ (μg mL^{-1} s^{-1}) = Steady state flux
A (cm^2) = Permeation area
V (mL) = The volume of receiver compartment
C_D (μg mL^{-1}) = Initial donor concentration.

Evaluation of gels for gelation and gel-melting

The optimized niosomal gel formulations of Span 80 and Tween 20 with the various concentrations of PF-127 (i.e. 16–245 w/w) were further studied for the gelation and gel-melting. The enthalpy of gelation and enthalpy of gel melting was also carried out. (Shilpa Chaudhari *et al*., 2006)

Estimation of gelation and gel melting

The gelation temperature was measured by heating the gel formulation (about 1–2°C) in a test tube with gentle stirring till the gel is formed. Gelation was considered at the point where there was no flow seen when the test tube was overturned.

The gel-melting temperature was recorded at a point when the gel starts flowing upon tilting the tube through an angle of 90°.

Enthalpy of gelation and gel melting

The enthalpies of gelation and gel-melting for the pluronic gel (plain) and with the drug formulation were calculated by using following equations:

$$\text{Lm } C = \Delta H°_{gel}/RT_1 + \text{Constant} \quad (1)$$

$$\text{Lm } C = \Delta H°_{mel}/RT_2 + \text{Constant} \quad (2)$$

Where,
$\Delta H°_{gel}$ and $\Delta H°_{mel}$ = Enthalpy of gelation and gel melting respectively
T_1 and T_2 = Gelation and gel melting temperature respectively.

Histopathology study

To study the mucosal toxicity in terms of the effect of surfactants and polymer in the present formulation, the goat nasal mucosa was placed in contact with the drug-containing niosomal gel for about 24 hours as well as 48 hours. (P. S. Kawitkar *et al*).

The slides were prepared and the histopathology study was done in the pathological laboratory.

RESULTS AND DISCUSSION

Characterization of niosomes in terms of vesicle size, entrapment efficiency, and viscosity

Among the various methods reported for niosome preparation, Valproic Acid niosomes were prepared by modified ether injection method. The influence of different surfactants, and Drug : Surfactant : Cholesterol ratio was studied on Valproic acid entrapment in niosomes. Experiments were designed using Tweens and Spans keeping cholesterol concentration fixed as shown in Table 1.

The formulation of niosomes from Span and Tween range was optimized on the basis of entrapment efficiency and drug release. The small unilamellar, spherical shaped vesicles in the range of 50–100 nm were observed by optical microscopy using Motic microscope and photographs were taken at 400 X magnification

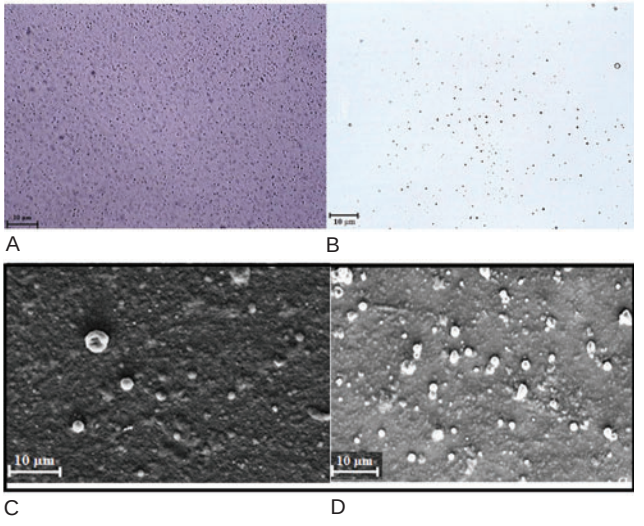


Figure 1: Photo micrographs of Valproic acid-loaded niosome. (A) Span 80 niosomes microscopic image, (B) Tween 20 niosomes microscopic image, (C) SEM Image of Span 80 niosomes, (D) SEM Image of Tween 20 niosomes.

with a digital camera (Motic, 8.1 megapixel, Japan) as shown in the Figure 1.

Scanning Electron micrographs of lyophilized non-ionic surfactant vesicles of Span 80, cholesterol and Tween 20, cholesterol in ratio of 1:3 and 1:1.5 respectively, appear similar in appearance and the size distribution of niosomes tends to be fairly wide. Nearly all the niosomes of Tweens and Spans showed the vesicular diameter in the range as shown in above Figure 1.

The effect of different concentration of Tweens (20, 40, 60 and 80) on the entrapment is depicted in Table 4. Entrapment efficiency for niosomes prepared with Tween 20 was higher than that with Tween 80. Similarly, entrapment of Tween 80 was more than Tween 40 and Tween 60. The results obtained for Tween 20 indicate, that longer the alkyl chain length of the surfactant, lesser the drug entrapment. The results were somewhat similar to previous studies (Kandasamy Ruchmani *et al.*, 2010). For Tween 80 the results obtained indicate that lower the HLB, higher the entrapment compared to Tween 40, and 60, similar to results reported earlier.[17] According to explanation of (Malgorzata Graca *et al.*, 2007), the shortest surfactant, Tween 20, formed the thinnest layer compared to Tweens 40 and 60.[18] One sees that thickness increased with increasing length of the alkyl moiety. As Tween 80 possesses one unsaturated carbon–carbon double bond in the alkyl moiety, on physical grounds one expects unsaturation to force the molecule to lie more nearly parallel to the hydrophobic substrate. This is probably the reason that despite the similar extended lengths of the Tween 80 molecule and the Tween 60 molecule, the thickness at full surface coverage was measured to be approximately half that of Tween 60 and also it was observed that viscosity of Tween 20 < Tween 80 < Tween 40 < Tween 60. So, the viscosity and the film thickness of Tweens may be playing an important role in entrapment, as our results of entrapment are in the same order of viscosity.

The entrapment efficiency of Spans was more than Tweens for all Spans, but within it Span 80 showed more entrapment than Span 20, 40 and 60. This might be due to low HLB and lower the HLB, higher the entrapment for Spans.

Viscosity for niosomes was high for spans and was 349 ± 0.41 cp to 420 ± 0.61 cp than tweens 235 ± 0.01 to 272 ± 0.12 might be due to particle size variation of the niosomes.

In vitro release

Niosomal Valproic acid release was slightly more for spans than tween Tables 3 and 4 for Span 80 (61.32 ± 0.33%) than Tween 20 (54.50 ± 0.33%) in first 2 hour. Span 80 has highest entrapment and drug release in Span range and Tween 20 has highest entrapment and drug release in Tween range.

Based on the entrapment efficiency, viscosity and drug release Span 80 was optimized but to study the effect of surfactant on the gelation and mucosal diffusion along with Span 80, Tween 20 with higher entrapment and drug release was selected for further studies.

Gelation study

As the temperature of nasal cavity is 34°C, our aim was to formulate the preparation that may gel at this temperature. Out of range of PF127 from 13% to 24% gelling temperature 34°C was found for 18% w/w of PF127. If the gelation is below 34°C or at room temperature it may lead to difficulty in manufacturing, handling and administration. Similarly, if the temperature is above 34°C than it would lead to rapid clearance of the drug from nasal cavity.[19] Keck T *et al.*, suggested mechanism of gelation due to progressive dehydration of the polymer micelles of polyoxypropylene chains as temperature increase leading to increased chain entanglement due to conformational changes in the orientation of methyl groups in the side chains. As discussed in the earlier studies (Kabanov AV *et al.*, 2002), discussed the mechanism of gelation based on micelles packing and entanglements.[20] The number of micelles formed increases as a consequence of negative coefficient of solubility of block polymer micelle with increase in temperature. Formulation ranging from 13% to 24% gelled at temperature 29.3°C–38.1°C. These temperatures were found to be optimum for in-situ gelling of formulation with minimum loss of administered dose by clearance from the site of application.

Table 3: Mean Particle Size, Viscosity, Percent Entrapment Efficiency, *in-vitro* Release of Span Niosomes

Code	PARTICLE SIZE	VISCOCITY	%ENTRAPMENT	%RELEASE
1-a	0.85 ± 0.8	420 ± 0.61	98.43 ± 0.12	50.24 ± 0.13
1-b	0.91 ± 0.6	390 ± 0.26	98.41 ± 0.43	49.29 ± 0.17
1-c	0.82 ± 0.15	384 ± 0.32	98.60 ± 0.46	46.12 ± 0.23
1-d	0.81 ± 0.15	419 ± 0.23	97.98 ± 0.31	48.34 ± 0.17
1-e	0.82 ± 0.17	392 ± 0.26	97.82 ± 0.43	46.92 ± 0.13
1-f	0.79 ± 0.25	412 ± 0.32	98.01 ± 0.32	45.73 ± 012
2-a	0.88 ± 0.13	390 ± 0.37	98.92 ± 0.09	54.02 ± 0.09
2-b	0.82 ± 0.26	393 ± 0.38	98.56 ± 0.10	51.16 ± 0.12
2-c	0.83 ± 0.3	412 ± 0.39	98.62 ± 0.11	49.54 ± 0.31
2-d	0.80 ± 0.14	394 ± 0.50	98.48 ± 0.20	53.56 ± 0.42
2-e	0.81 ± 0.51	399 ± 0.41	98.56 ± 0.26	51.68 ± 0.24
2-f	0.80 ± 0.46	352 ± 0.42	98.56 ± 0.23	48.10 ± 0.32
3-a	0.93 ± 0.2	348 ± 0.43	99.32 ± 0.26	55.45 ± 0.43
3-b	0.95 ± 0.36	362 ± 0.39	98.45 ± 0.15	54.26 ± 0.54
3-c	0.89 ± 0.42	353 ± 0.45	98.86 ± 0.46	53.08 ± 0.45
3-d	0.91 ± 0.25	352 ± 0.56	98.82 ± 0.19	53.56 ± 0.76
3-e	0.92 ± 0.36	366 ± 0.27	99.06 ± 0.39	53.68 ± 0.47
3-f	0.97 ± 0.21	353 ± 0.38	99.92 ± 0.36	47.00 ± 0.48
4-a	0.87 ± 0.22	350 ± 0.39	99.82 ± 0.27	50.20 ± 0.37
4-b	0.91 ± 0.23	359 ± 0.20	99.92 ± 0.38	57.55 ± 0.48
4-c	1.1 ± 0.34	349 ± 0.41	100.47 ± 0.29	61.13 ± 0.35
4-d	0.85 ± 0.15	352 ± 0.12	99.08 ± 0.40	60.14 ± 0.40
4-e	0.91 ± 0.26	360 ± 0.23	99.18 ± 0.31	58.97 ± 0.21
4-f	0.90 ± 0.17	356 ± 0.44	99.8 ± 0.42	58.39 ± 0.32

Table 4: Mean Particle Size, Viscosity, Percent Entrapment Efficiency, *in-vitro* Release of Tween Niosomes

Code	PARTICLE SIZE	VISCOCITY	%ENTRAPMENT	%RELEASE
5-a	**0.9 ± 0.21**	235 ± 0.01	**99.98 ± 0.29**	54.50 ± 0.33
5-b	0.8 ± 0.10	236 ± 0.10	99.78 ± 0.32	53.10 ± 0.24
5-c	0.82 ± 0.15	237 ± 0.32	98.89 ± 0.37	52.60 ± 0.15
5-d	0.86 ± 0.17	239 ± 0.26	99.14 ± 0.47	51.60 ± 0.26
5-e	0.89 ± 0.06	237 ± 0.10	99.42 ± 0.34	53.50 ± 0.37
5-f	0.85 ± 0.21	239 ± 0.06	99.52 ± 0.14	52.14 ± 0.28
6-a	0.85 ± 0.15	246 ± 0.10	98.32 ± 0.39	46.91 ± 0.39
6-b	0.85 ± 0.15	242 ± 0.26	98.20 ± 0.43	43.03 ± 0.20
6-c	0.84 ± 0.10	241 ± 0.21	97.23 ± 0.42	43.61 ± 0.31
6-d	0.83 ± 0.10	240 ± 0.10	97.92 ± 0.40	47.61 ± 0.42
6-e	0.82 ± 0.15	252 ± 0.12	97.18 ± 0.42	42.41 ± 0.23
6-f	0.79 ± 0.15	253 ± 0.38	97.15 ± 0.43	40.55 ± 0.34
7-a	0.87 ± 0.25	237 ± 0.26	98.92 ± 0.19	51.43 ± 0.25
7-b	0.76 ± 0.21	241 ± 0.15	96.77 ± 0.45	50.24 ± 0.36
7-c	0.79 ± 0.20	241 ± 0.55	96.84 ± 0.23	49.50 ± 0.37
7-d	0.84 ± 0.21	237 ± 0.21	97.70 ± 0.27	49.04 ± 0.48
7-e	0.83 ± 0.10	239 ± 0.06	97.89 ± 0.33	47.65 ± 0.29
7-f	0.86 ± 0.10	236 ± 0.10	98.26 ± 0.44	47.65 ± 0.50
8-a	0.83 ±0.21.	268 ± 0.21	98.10 ± 0.49	38.21 ± 0.43
8-b	0.81 ± 0.10	269 ± 0.26	97.96 ± 0.25	36.80 ± 0.41
8-c	0.82 ± 0.15	272 ± 0.12	97.94 ± 0.22	41.00 ± 0.35
8-d	0.84 ± 0.06	261 ± 0.38	97.95 ± 0.30	40.10 ± 0.26
8-e	0.85 ± 0.10	259 ± 0.06	97.91 ± 0.44	32.80 ± 0.37
8-f	0.82 ± 0.15	253 ± 0.10	97.12 ± 0.34	30.73 ± 0.48

Gelation studies on plain pluronic gels and in presence of Span 80 formulation and Tween 20 formulation showed that with increase in concentration of pluronic, the gelation temperature decreases and gel melting temperature increases as shown in Figure 2. This might be due to higher number and volume occupied by micelles at lower temperature. At higher temperatures disruption in micelle arrangement occurs which are indicated by negative value of enthalpy of gel melting as shown in Figure 3. The enthalpy of gelation depends on the type and extent of interaction occurred like its solubility in water as shown in Figure 4 and Figure 5. As such enthalpy of gelation and gel melting for plain, niosomal gel with Span 80 and niosomal gel will Tween 20 are given in Table 5.

As such not much effect is seen on enthalpy of gelation and gel melting. A negligible difference of 0.5 Kcal/mol increase in gel melting and 0.02 Kcal/mol increase in gelation is seen which implies that there is no interaction between the niosomal formulation and the gel when incorporated in the system.

Mucoadhesive strength was found for two minutes of contact time as per the study carried out by Rita J. Majithiya *et al.*, 2006. Analysis of mucoadhesive strength for PF127 and other gel formulations showed that the detachment stress was more i.e. PF127, and it possessed adhesive properties as well as gel strength increased with incorporation of span Niosomal formulation in it. Whereas mucoadhesive strength decreased slightly with incorporation of Tween niosomal formulation. This increase in mucoadhesive strength for spans and decrease for tweens might be due to surface activity of the surfactant, surface activity depends on the balance between it hydrophilic and hydrophobic properties. Pluronic has both polyoxyethylene and polyoxypropylene groups and it shows both hydrophilic and hydrophobicactivity. With the increase of the length of the ethylene oxide chain (hydrophilic) of a polyoxyethylene non-ionic surfactant (Tweens) results in a decrease of

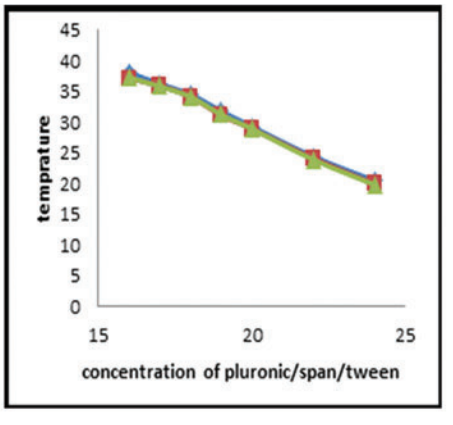


Figure 2: Gelation Study of in-situ niosomal nasal gel containing (♦) PF 127, (■) Span 80, (▲) Tween 20.

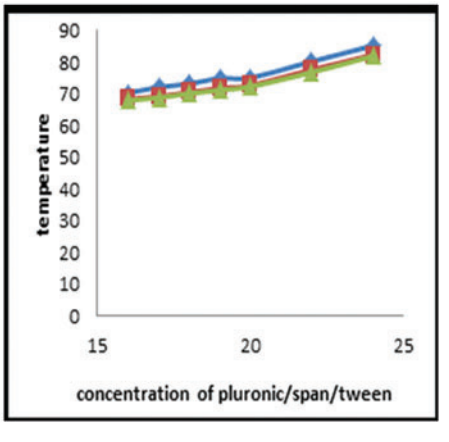


Figure 3: Melting Temperature study of in-situ niosomal nasal gel containing (♦) PF 127, (■) Span 80, (▲) Tween 20.

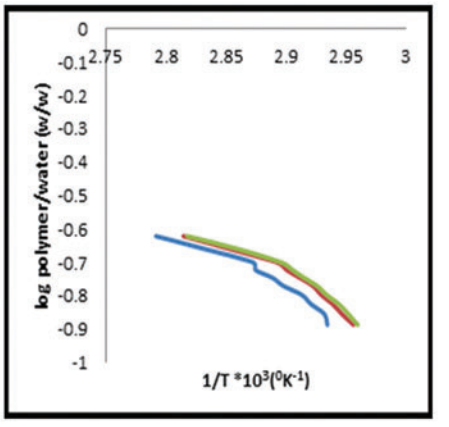


Figure 4: Enthalpy of Gelation – (—) PF 127, (—) Span 80, (—) Tween 20.

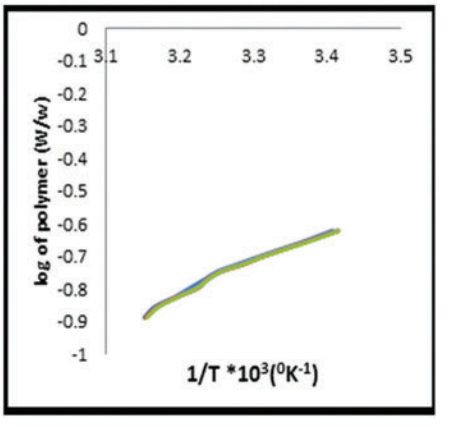


Figure 5: Enthalpy of Melting – (—) PF 127, (—) Span 80, (—) Tween 20.

Table 5: Enthalpies of Gelation and Gel Melting of PF-127 Gel, S-80 Gel, T-20 Gel

Formulations	ΔHgel (kcal/mol)	ΔH melting (kcal/mol)
PF-127	1.987524 ± 0.354	–3.573108 ± 0.254
S80	1.988118 ± 0.325	–3.674682 ± 0.145
T20	2.011086 ± 0.286	–3.67587 ± 0.488

Data are means ± SD (n = 3)

surface activity.[21] Out of all the three gel formulations that containing span niosomes in gel showed highest mucoadhesive force and gel strength indicating a significant improvement in the drug residence time.

Evaluation of Span 80 and Tween 20 niosomal gels by FTIR, DSC and SEM

FTIR

The IR spectrum of Valproic acid indicated the appearance of sharp –COOH peak at 3257.77 cm^{-1}, in addition to –COOH of acid a peak at 2929.87 corresponding to $–CH_2CH_3$ and 1710.92 stretch for carbonyl group (C=O), 942.43 for OH group. (Figure 6)

The IR spectra Span 80 shows peak at 1742 cm^{-1}, 1716 cm^{-1} corresponding to C=O stretch while a broad peak at 3415 cm^{-1} indicated for the OH group.[22] The IR spectra for Tween 80 shows peak at 1710 cm^{-1} representing C=O stretch, 2900 cm^{-1} for COOH, 3030 cm^{-1} for CH_3CH_2.

The Valproic acid Tween 20 and Valproic acid Span 80 formulation exhibited spectra where in some characteristic peaks of Valproic acid at 1710 cm^{-1}, 2929 cm^{-1}, 3257 cm^{-1} are missing while the peak at 942.47 cm^{-1} shift slightly to 947.05 cm^{-1} which indicate the entrapment of Valproic acid in the vesicles. Same effect was observed with Valproic acid and Tween 20 formulation as shown in Table 6. (Figure 6)

DSC

DSC analysis was carried out on pure drug, drug polymer, and formulations with Span 80 and Tween 20. Valproic acid exhibited a sharp endothermic peak at 221°C while drug polymer DSC showed two peaks at 52°C and 198.7°C which was a decrease in temperature. Similarly, DSC formulation with Span 80 and Tween 20 exhibited two endothermic sharp peaks at 175.1°C and 174.8°C which again showed that it did not have defined melting point and was in the range of 180°C–200°C which is not much deviated from endotherm of pure drug but again two peaks showed that there was an entrapment of drug within vesicles. (Figure 7) And broadening of peak indicates amorphous nature of that due to lyophilisation process and as such they did not interact to form any additional chemical entity.

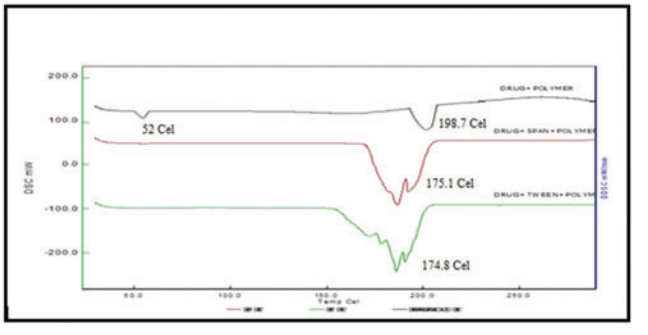


Figure 7: DSC thermogram of (—) Valproic acid, (—) Span 80 Niosomal Gel (—) Tween 20 Niosomal Gel.

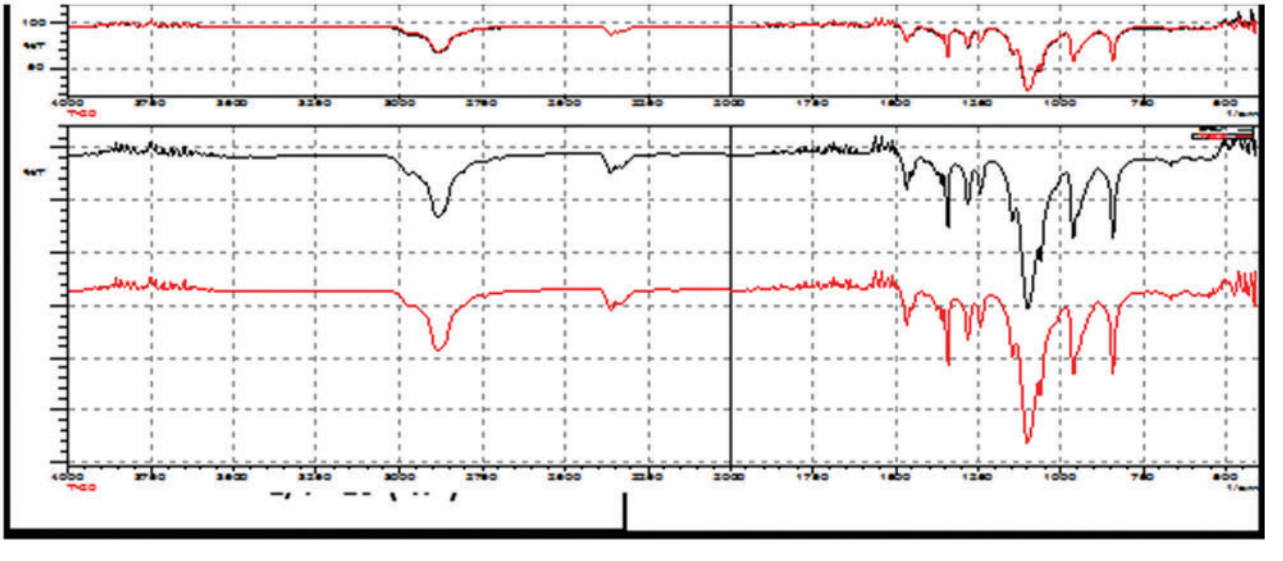


Figure 6: FTIR Spectra of (—) Valproic acid, (—) Span 80 Niosomal Gel (—) Tween 20 Niosomal Gel.

Table 6: Evaluation Parameters of Optimized Niosomal Gel Formulations

Formulations	Particle size (μ)	Viscosity (cp)	Mucoadhesive strength (dyne/cm²)	Gel strength (sec)	pH	Drug content	Percent Release
Span 80	0.9 ± 0.3	3300 ± 0.3	4552 ± 0.5	102 ± 0.5	4.74 ± 0.01	88.67 ± 02	48.18 ± 0.3
Tween 20	0.8 ± 0.1	2150 ± 0.2	4443 ± 0.5	98 ± 0.5	4.83 ± 0.005	87.37 ± 03	45.27 ± 0.3

Data are means ± SD (n = 3)

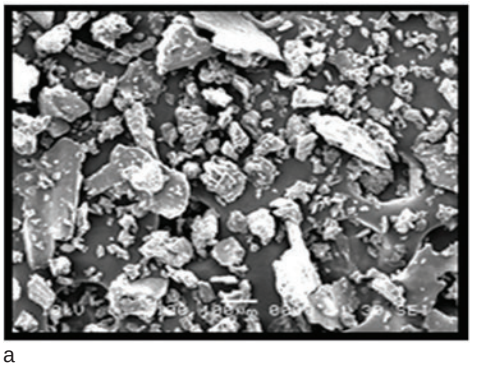
SEM

SEM photomicrographs of freeze dried niosomal gels containing Span 80 and Tween 20 were taken with ×2500 magnification, as seen in Figure 8 a and b showed closed structures in the gel. The Span 80 gel lyophilized structure showed more amorphous nature of the particles than Tween 20 gel. Both the pictures were indicative of homogenous matrix formation compared to that of plain SEM study of niosomes as shown in Figure 8 (a and b).

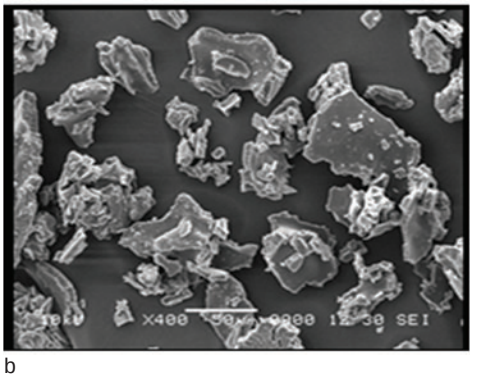
All the studied parameters that were done to check the compatibility of drug with the polymer as well as with the surfactants, showed satisfactory results and were found to be compatible with each other.

In- vitro permeation study

Valproic acid showed increased permeability of span containing niosomal gel across goat's nasal mucosa than tweens (Figure 9). This might be due to surface activity of surfactants. The flux for Span 80 niosome is 161.84 and Tween 20 niosome is 134.02 similarly the diffusion coefficient values of Span 80 and Tween 20 niosomes were 1.50×10^{-3} and 1.24×10^{-3} respectively as shown in Table 7. But in form of gel the flux and permeation values were somewhat same for Span 80 and Tween 20 niosomal gel i.e., 73.16 and 73.10 value of flux and 6.80×10^{-4} and 6.75×10^{-4} value of effective permeation coefficient for Span 80 and Tween 20 respectively. These lower mean flux and diffusion coefficient values of niosomal gel are suggestive of prolong drug release. According to Schreier H *et al.*, the higher drug retention in skin may be due to creation of reservoir effect for drug in skin and deposition of components of niosomes with drug into the skin, thereby increasing the drug retention capacity into the skin.[23] This helped us to achieve our third objective of controlled drug release.



a



b

Figure 8: SEM images of lyophilized formulations of span and tween in-situ niosomal nasal gel. (a) Span Gel, (b) Tween Gel.

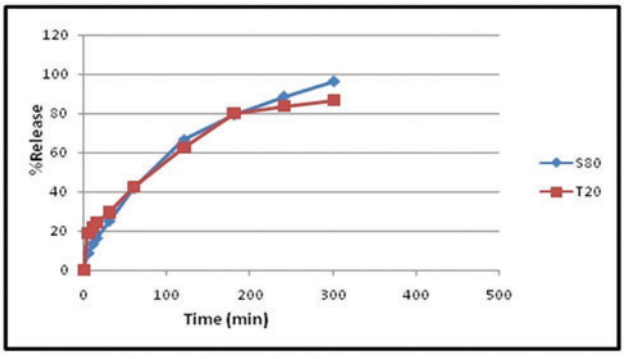


Figure 9: *In vitro* release of in-situ niosomal nasal gel of optimized formulation of Span (–) and Tween (–) across goat nasal mucosa.

Histopathological evaluation of mucosa after *in vitro* permeation study

In the histopathological study, the microscopicaly observations indicated that with the optimized formulations there was a significant drug diffusion and has no significant effect on the microscopic structure of mucosa and also no cell necrosis was observed. No effect of surfactant was seen; neither had the formulations caused any damage to the nasal tissue.

The histopathological study results were confirmed by the pathological evaluation study and reports found are as shown in Figure 10.

Table 7: Viscosity, Particle Size, Entrapment, Percentage Release, Flux, Diffusion Coefficient of Optimized Formulations

Code	Viscosiy (cp)	Particle Size	Entrapment Percent	Percent Release	Flux (µg/min)	Diffusion coefficient (cms^{-1})
5-a	235.3 ± 0.34	0.97 ± 0.21	99.78 ± 0.29	61.32 ± 0.33	161.82 ± 0.274	1.50×10^{-3}
4-c	420 ± 0.61	0.67 ± 0.15	98.63 ± 0.22	54.50 ± 0.35	134.12 ± 0.885	1.24×10^{-3}

Data are means ± SD (n = 3)

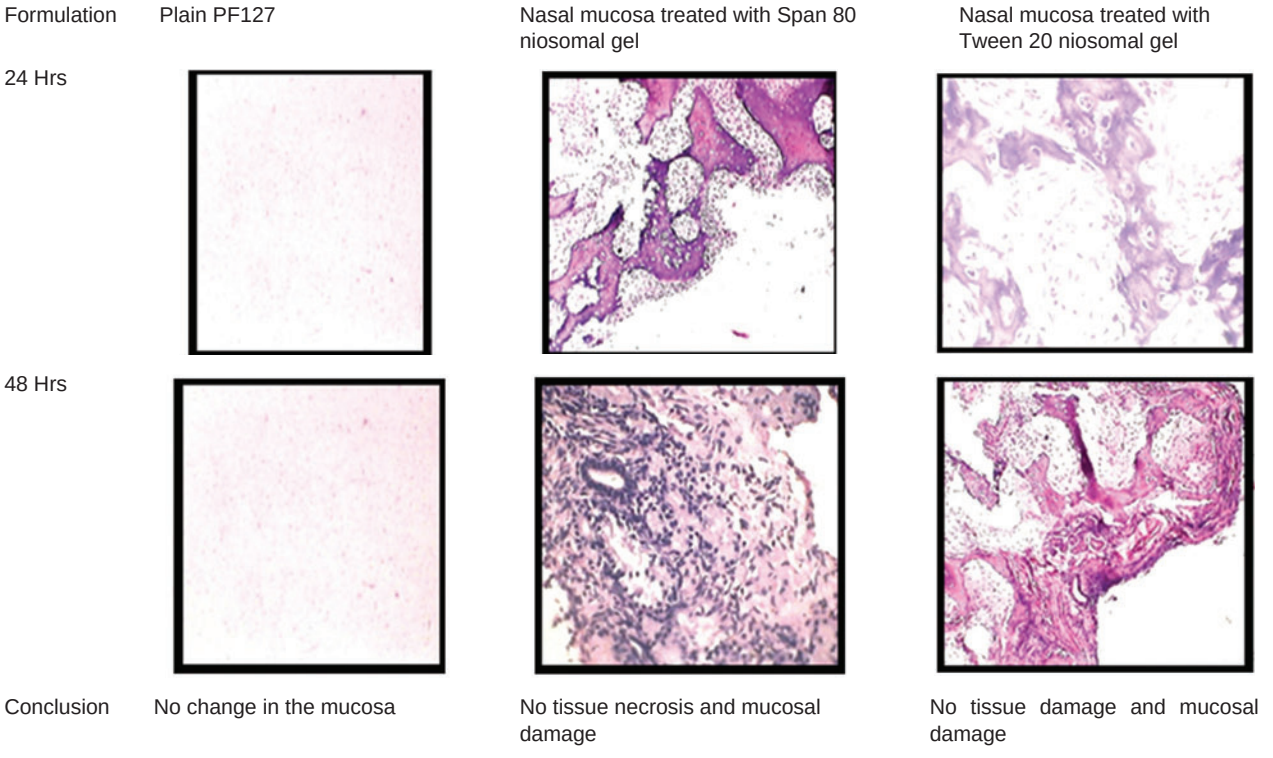


Figure 10: Histopathological evaluations of section of Goat nasal mucosa incubated with and without drug after 24 and 48 Hrs.

CONCLUSION

The present study was an attempt to develop and evaluate the niosomal in-situ nasal formulations of Valproic Acid by using different surfactants in Span as well as Tween range in different concentration and keeping the cholesterol content constant. The niosomes entrapped in-situ nasal gel formulations were able to release the sufficient quantity of drug in order to provide immediate relief for epilepsy which was one of the objectives of the present study. The niosomal gel prepared with PF127 was evaluated for gelation study and the other parameters like mucoadhesive strength and gel strength, and showed satisfactory results. Most importantly, the *in-vitro* release of the drug through the gel across the goat's nasal mucosa was satisfactory and showed the controlled pattern release. There was drastic increase in the viscosity of formulation at the temperature of the nasal cavity indicating the occurrence of in-situ gelling phenomenon. Moreover, this formulation also provides the ease of administration as it is in the liquid form at non-physiologic conditions and thus helps in increasing patient compliance. Hence we can conclude that the niosomal in-situ nasal gel system can be considered as a promising approach for the anti-epileptic drug Valproic Acid.

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