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Formulation and evaluation of Oxybutynin chloride loaded proniosomal gel for transdermal drug delivery

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Abstract

Clinical utility of Oxybutynin chloride is restricted due common side effects viz., its dry mouth, and difficulty in micturition, constipation, blurred vision, drowsiness, and dizziness. This study evaluated the prospective of proniosomal gel to improve the clinical efficacy of Oxybutynin chloride and compare with oral therapy. A series of Viz., B1 to B8 Proniosomal gel were prepared by phase separation and coaccervation method using different proportions of Span (span 60, span 40, span 80, span 20): Cholesterol: Soya lecithin, further characterized for vesicle size, zeta potential and entrapment efficiency, *In vitro* drug release. The B2 formulation exhibited nano size with high entrapment efficiency, adequate zeta potential, greater transdermal flux and better stability (at refrigerated conditions). Release profile of B2 displayed anomalous behavior and release mechanism was indicative of diffusion controlled. The study concludes that transdermal route is an ideal for Oxybutynin chloride with improved clinical efficacy.

Keywords: Oxybutynin chloride; Proniosomal gel; Spans; Tweens; Cholesterol; *Ex-vivo*

1. Introduction

The International Continence Society (ICS) defines Over Active Bladder (OAB) syndrome is a symptom characterized by urinary urgency, frequency and nocturia, with or without urge urinary incontinence, in the absence of proven infection or other obvious pathology[1]. Antimuscarinic agents are currently available for the pharmacological treatment of OAB in adults[2]. Clinical studies demonstrated that various oral conventional and modified release formulations of antimuscarinic agents were effective in improving OAB related symptoms, but caused dry mouth, due to high serum concentration of the active metabolite N-desethyloxybutynin that follows hepatic first pass metabolism in the gut and liver. This property leads to need for the designing of topical transdermal drug delivery systems.

Oxybutynin chloride (OXY) is an antimuscarinic or anticholinergic agent approved by the Food and Drug Administration (FDA) for treatment of OAB[3-5]. It is a weak base with a pK_a of 9.87 and log D of 1.83, its hydrochloride salt is freely soluble in water, stable and well absorbed from the intestinal tract. These favorable properties combined with the low dose (5-15 mg/day) make it an ideal candidate for designing of oral modified release dosage form. For over 30 years, use of IR dosage forms of OXY has been limited by antimuscarinic adverse events, such as dry mouth, difficulty in micturition, constipation, blurred vision, drowsiness, and dizziness. These side effects are dose related and sometimes severe. Since the 2000s, extended release (ER) products have been investigated and developed to allow once daily administration and prolonged control of symptoms. Clinical studies have shown that ER formulations not only consistently enhanced patient compliance and efficacy but also resulted in improved tolerability including significantly higher continuation rate due to marked reduction of moderate/severe dry mouth. The latter was attributed to substantially lower intestinal metabolism because a majority of the parent drug of the ER dosage form is delivered to the distal intestinal tract where metabolic enzyme activity in the gut wall is lower or absent. As a result, a substantial increase in bioavailability relative

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to the immediate release (IR) formulation was also imparted. More recently, to totally bypass presystemic metabolism, a transdermal patch loaded with OXY was successfully developed with tolerability profile similar to that of a placebo[6-8]. Clinical trials involving a transdermal formulation of OXY have shown that this delivery method may be associated with a lower incidence of anticholinergic adverse events compared with both the immediate release and the extended release oral formulations[9,10]. Transdermal drug delivery offers potential to overcome disadvantages caused by oral administration. Unfortunately, the stratum corneum (SC) which is recognized as the primary barrier for transdermal drug delivery makes it difficult for most drugs to enter into skin by this route. Among the several approaches, one possibility to increase the amount of drug vehiculated across the SC is the use of vesicular carriers. These systems can increase skin transportation by improving drug solubilization in the formulation, drug partitioning into the skin, and by fluidizing the skin lipids[11]. Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route[12-14]. This would be possible if proniosomes form niosomes following topical application under occlusive conditions, due to hydration by water from the skin itself[15-17]. Keeping all these under consideration the present research work was aimed to prepare and evaluate OXY loaded proniosomal gel for transdermal delivery.

2. Materials and methods

Span 20, 40, 60, 80, cholesterol, soya lecithin, ethanol AR, sodium hydroxide and potassium dihydrogen orthophosphate were purchased from SD Fine Chemicals (Mumbai, India). Oxybutynin chloride is a gift sample obtained from Sun Pharma Ltd, Mumbai, India.

2.1. Preparation of proniosomal gel

The proniosomal gel were prepared as per formula (table 1) by coacervation phase separation method[18,19]. In glass vials accurately weighed amounts of the surfactant, cholesterol, soya lecithin and OXY were mixed with absolute alcohol (about 1.5 ml) and warmed in water bath (55-60 ^{o}C) for 5 min while shaking until complete dissolution of cholesterol. Then phosphate buffer pH 7.4 (1.5 ml) was added and the mixture was further warmed in the water bath for about 2 min so that a clear solution was obtained. The mixtures allowed to cool at room temperature and observed for the formation of transparent, translucent, or white creamy proniosomal gel[20]. OXY loaded proniosomal gel was then incorporated into 1% w/w carbopol gel at 1:1 ration. During incorporation initially, plain carbopol gel is prepared by transferring accurately weighed amount of carbopol in measured amount of distilled water, the mixture was sonicate for 6-8 min to dissolve carbopol completely and avoid any air drops. Further the mixture is neutralized with triethanolamine to attain the desire pH. In later stage carefully incorporate OXY loaded proniosomal gels in carbopol gel with constant stirring until desired consistency was obtained. The gel obtained was preserved in dark until characterization[21]. In order to optimize and evaluate various OXY loaded proniosomal formulations, different grades of nonionic surfactants such as sorbitan esters viz., span 20, span 40, span 60 and span 80 at different proportions were used in addition to cholesterol and soya lecithin. The formation of proniosomal gel was observed after 24 h[22].

Table 1 Formula of OXY loaded proniosomal gel formulations

Ingredients	**Batches**							
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**
OXY	100	100	100	100	100	100	100	100
Span-60 (mg)	330	525	-	-	-	-	-	-
Span-40 (mg)	-	-	330	525	-	-	-	-
Span-80 (mg)	-	-	-	-	330	525	-	-
Span-20 (mg)	-	-	-	-	-	-	330	525
Cholesterol (mg)	110	105	110	105	110	105	110	105
Lecithin (mg)	160	130	160	130	160	130	160	130
Ethanol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
pH 7.4 buffer (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

2.2. Evaluation of proniosomal gels

2.2.1. Encapsulation efficiency (% EE)23-25

The proniosomal gel encapsulating OXY were separated by hydrating with 10 ml of phosphate buffer pH 7.2 and the solution was sonicated for 10 min. The drug containing niosomes separated from the unentrapped drug by centrifugation at 15,000 rpm for 30 min. The supernatant was recovered and properly diluted using phosphate buffer pH 7.2 and assayed spectrophotometrically at 225 nm. The other method viz., exhaustive dialysis also performed to compare the efficiency. The amount of OXY encapsulated was determined by subtracting the amount of unencapsulated (free) drug in the supernatant from the total drug incorporated as shown by the formula,

$$\% \text{ Encapsulation effeciency (EE)} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \times 100$$

2.2.2. Size and morphology

Spread a thin layer of formed proniosomes on a glass slide and observe the structure of vesicle under microscope, collect the images using digital camera[26]. In each case vesicle size analysis was carried out using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The freshly prepared hydrated niosomes were dispersed in double distilled water (DDW) and was used to characterize the vesicle size. Polydispersity Index (PDI) was also determined as a measure of homogeneity. Zeta potential of the niosome formulations was determined to estimate stability of the formulations.

2.2.3. In vitro drug release study[27,28]

In vitro release studies on OXY loaded proniosomal carbopol gel were performed using Franz-diffusion cell. The dialysis cellophane membrane was mounted between the donor and receptor compartment. The receptor medium was phosphate saline buffer pH 7.2. A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37 ±1 ^{o}C. A Teflon-coated magnetic bead operated to a magnetic stirrer stirred the receptor fluid. Periodically remove the samples and replaced immediately with an equal volume of receptor media, further drug content was determined spectrophotometrically at 225 nm. Compute the *In vitro* drug release data by using PCP Disso V3.

2.2.4. Ex-vivo permeation study

Ex-vivo permeation study was carried out for optimized formulation using male Wistar rat skin as reported by Ibrahim et al[29] as shown in figure 1. The study was undertaken under the permission of the University Animal Ethics Committee IAEC of V.L.College of Pharmacy, Raichur, and Karnataka, India (BU/BT/394/13-14). The rat skin was mounted between the donor and receptor compartment with the stratum corneum facing upper side on the diffusion cell. The efficient diffusion area of the cell was 1.41 cm^2 and 20 ml phosphate buffer pH 6.8 was taken in receptor compartment. The temperature was maintained at 37±1 °C. Optimized OXY loaded proniosomal carbopol gel equivalent to 5 mg was spreaded over the rat skin. The receptor compartment content was stirred with the help of magnetic beads. The samples were collected at different time intervals and were immediately replaced with the fresh media. The samples were analyzed for drug content using UV spectrophotometer at 225 nm. The amount of drug permeated at different time intervals and relevant parameters was calculated [30,31].

Figure 1 *Ex-vivo* permeation studies

2.2.5. Irritation/sensitivity studies

Albino male Wistar rats (200–250 g) were selected for the study as described by Draize et al[32]. All the animals used in the study were caged and maintained according to the guidelines of CPCSEA or principles established for care and use of laboratory animals. The rats were divided into three groups (n = 4). Group I: Control (Plain placebo gel); Group II:

OXY loaded Carbopol gel; Group III: OXY loaded Proniosomal Carbopol gel (S-60) as shown in figure 2. The rats were anesthetized and the dorsal area was shaved and wiped with 70% alcohol swab. Control and proniosomal gel groups were applied to the respective groups. The skins were scored according to erythema and edema scale as 0 (none); 1(slight); 2 (well defined); 3 (moderate); 4 (scar formation by visual observations).

Figure 2 Grouping of animals for skin irritation studies

2.2.6. Stability studies

The short stability study was performed according to ICH guidelines. The optimized OXY loaded proniosomal carbopol gels were filled in tightly closed glass vials and subjected to stability testing. The formulations were kept at refrigerated conditions (4±1°C) and at room temperature (25±2°C) and were analyzed for vesicle size, drug content and entrapment efficiency after 3 months.

3. Results and discussion

3.1. Appearance

The physical appearance was found that the formulae B1, B2, B3 and B4 were white creamy gel, B5, B6, B7 and B8 white to pale viscous gel, pH was measured by using digital pH meter and was found to be 5.5 to 6.8 (Table 2). The production yield was found to be in the range of 87.26 ± 0.1528 to 91.55 ± 0.2155 indicate the adapted method for the preparation proniosomal gel was reproducible. The percent drug content was found to be in the range of 97.23 ± 0.1464 to 99.15 ± 0.0712 with low SD values indicate the drug distribution was uniform within the formulations.

3.2. FTIR studies

FTIR spectra of OXY and Optimized OXY loaded proniosomal gel were analyzed by Brucker FTIR spectrophotometer and the spectra were recorded in the region of 4000-1000 cm^{-1}. The spectra were shown in figure 3. FTIR spectra of OXY showed the characteristic peak at 3305.83 cm^{-1} (-OH stretching), 3038.49 cm^{-1}(C≡C stretching), 2993.61 cm^{-1} (CH=CH stretching, aromatic), 2930.42 cm^{-1}(CH_2 = CH_2 stretching, cyclohexyl) and 1737.81 cm^{-1} (C=O ester). The characteristics peak of OXY corresponding to (CH_2=CH_2, cyclohexyl) was observed at 2921.14cm^{-1} and (C=O ester) at 1733.12 cm^{-1} in Optimized OXY loaded proniosomal carbopol gel. The other characteristics peak of OXY is also observed with slight shifting towards lower wavelength. The comparative FTIR spectra suggest no detectable changes, which confirmed the absence of any chemical interactions between them[33].

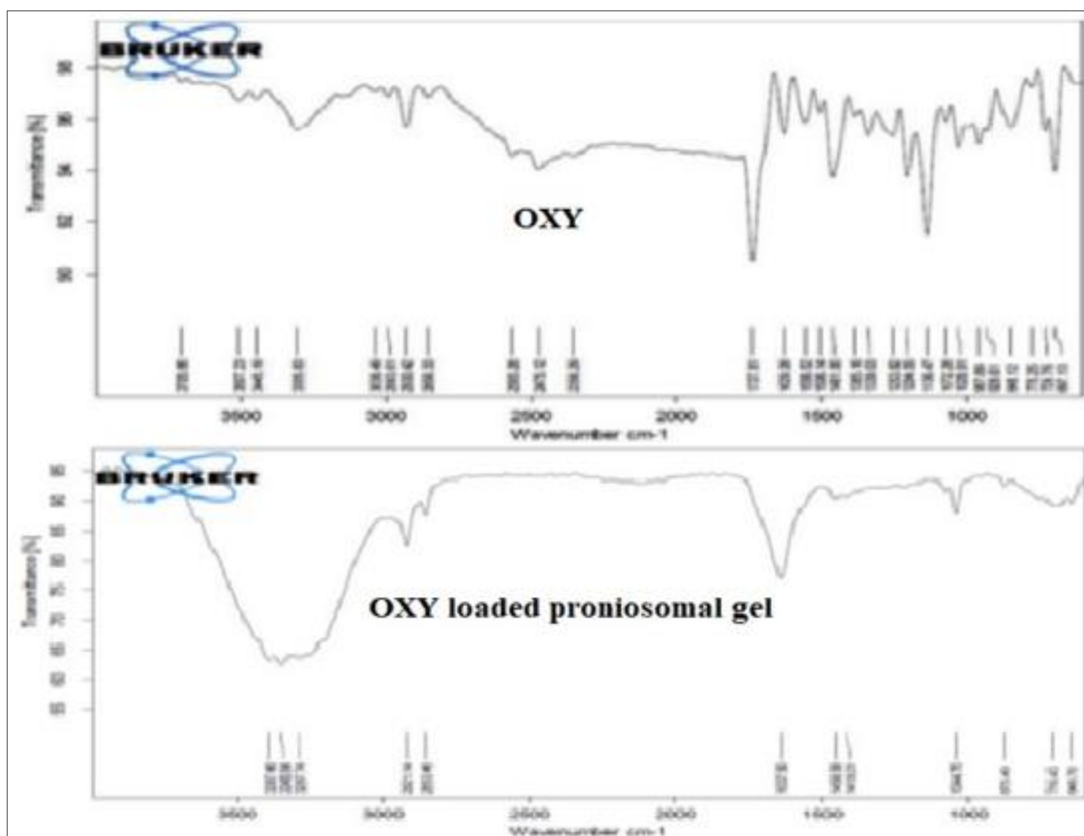

Figure 3 FTIR spectra of OXY and OXY loaded proniosomal gel

Table 2 The physical appearance, pH, % entrapment efficiency percentage (% EE) and, particle size, OXY loaded proniosomal gels

Batches	Physical appearance	pH (n=3)	EE% ± SD (n=3)	Size (µm)	PDI
B1	White creamy gels	5.5	96.24±0.312	2.937	0.613
B2	White creamy gels	5.6	92.91±0.512	3.238	0.574
B3	White creamy gels	5.9	88.36±0.978	4.146	0.471
B4	White creamy gels	6.1	83.24±1.100	4.791	0.421
B5	Pale white viscous gel	5.9	80.91±0.889	5.363	0.358
B6	Pale white viscous gel	6.8	76.82±0.987	5.442	0.332
B7	Pale white viscous gel	6.4	72.48±0.798	5.774	0.307
B8	Pale white viscous gel	5.8	70.57±0.698	5.861	0.288

3.3. Entrapment efficiency

The entrapment was expressed as the percentage of the total amount of OXY utilized in the preparation of proniosomal gels (table 2). The encapsulation efficiency depends on the type of surfactants, HLB value, their alkyl chain, critical packing parameter (CPP) and the phase transition temperature. The surfactants used in the preparations were span 60, span 40, span 80 and span 20. Span series have the same head group with different alkyl chain and has HLB values 4.7, 6.7, 5.3 and 8.6 respectively and CPP ranges from 0.5 to 1. The lower the HLB of the surfactant, the higher will be the drug entrapment efficiency as in the case of proniosomes prepared using span series[34]. The span 60 has lower HLB value and longer alkyl chain allows well closed uniformly packed bilayer structure within which OXY gets completely packed,

these results are supported by Hao T[35], Alsarra et al[36]. Span 60 has highest phase transition temperature (50°C) as compared to both span 20, span 40 and span 80 results increased % EE, because hydration temperature used to make niosomes should usually be above the gel-to-liquid phase transition temperature of the system that results in niosomes that are less leaky and have high entrapment efficiency[37,38]. This could also be attributed to the structure, orientation, and packing behavior of the surfactants. Hence, Proniosomal gel prepared from span 60 showed higher encapsulation efficiency of OXY followed by span 40, span 80 and span 20.

3.4. Size analysis

The photomicrograph of OXY loaded proniosomal gels showed the presence of homogenous population of vesicles with spherical shape as shown in figure 4. Smaller vesicle size is advantageous to decrease the irritation and improve the penetration of the vesicles into the skin. The particle size of proniosomal gel prepared with span 60 were found to be lower as compared to the other grades like span 40, span 20 and span 80 (table 2). The size of proniosomes showed a regular increase with an increase of the surfactant HLB values. It was observed that the incorporation of spans and cholesterol in the formulation interacts with the bilayer (lecithin present) and resulted in the increased vesicle size. As the concentration of spans increases the vesicle size increases, which is in argument to the findings of Imam et al[39]. Zeta potential (ZP) was found to be -38.1 mV with a negative charge (figure 5). Wen *et al*[40] stated that the high value of ZP (more than 30 mV) showed greater repulsion between charged particles, therefore, reducing aggregation or flocculation and electrically stabilized the colloidal particles. Junyaprasert *et al*[41] obviated that the preferential adsorption of hydroxyl ions at the vesicle surface was responsible for the negative surface charge of vesicles made from nonionic surfactants. The result showed a good physical stability of the OXY within the prepared gel was obtained, as ZP increased, also the repulsion between the vesicles has increased which prevent their reaggregation and provided electrical stability of the system[42,43]. The average particle diameter of OXY loaded proniosomal gel (figure 4) was found to be in the range of 2.937 μm to 5.861 μm, vesicles were discrete and separate with no aggregation or agglomeration. It was observed that the vesicles were smaller with the presence of span 60 (B1, B2) as compared to Span 40 (B3, B4). As the ratio of span 60 decrease in the formulation (B1, and B2), the vesicle size increased followed by span 40, span 20 and span 80 which is in an agreement to the findings of Pankaj *et al*[44].

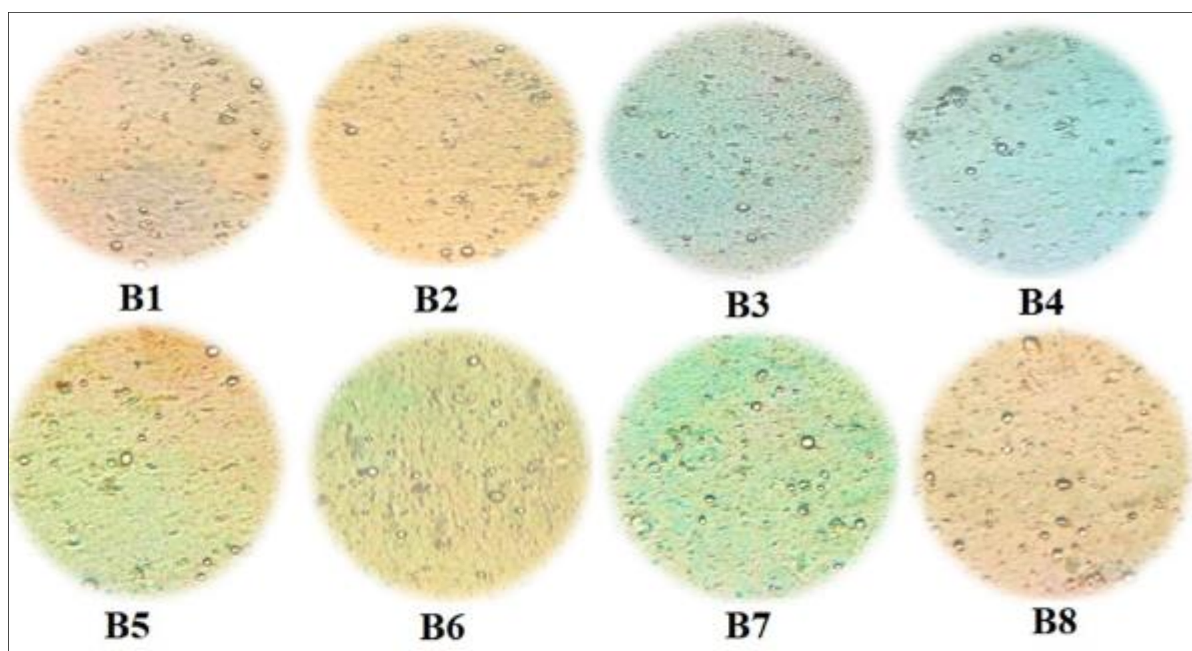

Figure 4 Microphotographs of OXY loaded proniosomal gel formulations

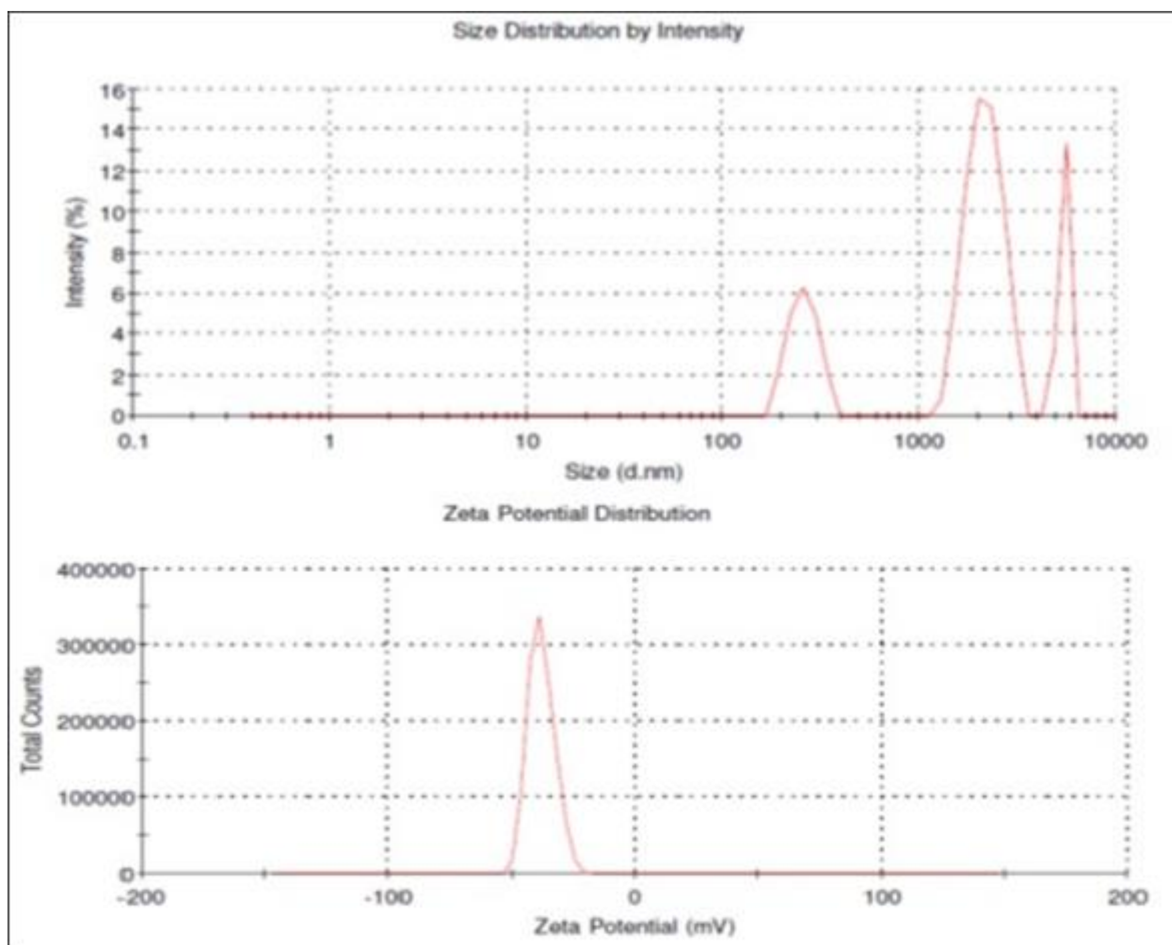

Figure 5 Average diameter and Zeta potential B1 proniosomal gel

3.5. *In vitro* release of OXY from proniosomal gel

The drug release from proniosomal gel was indicative of influence of nature and type of surfactant, and was shown in figure 6. The cumulative percent drug release was found to be 21.41 ± 1.172, 16.77 ± 0.2855 for B1 and B2 formulations; 26.52 ± 0.407, 22.56 ± 0.3101 for B3 and B4 formulations; 28.48 ± 0.349, 24.65 ± 0.1813 for B5 and B6 formulations; 30.39 ± 0.182, 26.6 ± 0.1554 for B7 and B8 formulations after 2hr. In all the formulations the faster drug release about 20 -30 % was observed after 2hr it is mainly due to release of drugs from the adhered niosomes and improperly formed niosomes. The cumulative percent drug release was found to be 68.27 ± 0.995, 55.61 ± 0.1422 for B1 and B2 formulations; 73.4 ± 0.326, 56.68 ± 0.3551 for B3 and B4 formulations; 76.76 ± 0.821, 59.63 ± 0.3732 for B5 and B6 formulations; 77.31 ± 0.168, 57.63 ± 0.3706 for B7 and B8 formulations after 6hr. The drug release was steady after 6hr was observed in all the formulations due complete exhaust of adhered drug particles from the niosomes and dissolved improperly formed niosomes. The cumulative percent drug release was found to be 87.77 ± 0.995, 65.61 ± 0.3706 B1 and B2 formulations; 88.21 ± 0.627, 71.65 ± 0.2058 for B3 and B4 formulations; 88.93 ± 0.820, 76.73 ± 0.1926 for B5 and B6 formulations; 89.22 ± 0.248, 77.54 ± 0.2217 for B7 and B8 formulations after 12hr. The order of slow and controlled release from the formulations are B2 < B4 < B6 < B8 < B1 < B3 < B5 < B7. The *In vitro* drug release data for B1 to B8 formulations were fitted into various kinetic equations to find out the order and mechanism of drug release. The correlation coefficient showed that the release profile followed the first order, also from korsemeyer peppas model, the release exponent 'n' was found to be greater than 0.5 indicated the drug release followed anomalous behavior and release mechanism was indicative of diffusion controlled[45,46]. Proniosomal gel prepared with span 60 showed significant lowest drug release at $p < 0.05$ compared to span 40, span 60 and span 80. The prolonged and slow OXY release when compared to proniosomal gel formulations prepared with other span series, it may be due the HLB value of the surfactant. This could be related to the condensed vesicular structure composed of span 60, and cholesterol, which is considered a great barrier to drug diffusion and a retardant to its release. Thakur *et al*. obviated that the delayed release of proniosomal gel formulations is due to the slow release of drug from proniosomes and this may be attributed to the need of proniosomes for a time to be hydrated to form niosomal vesicles before starting release of drug across the

cellophane membrane[47]. Thus, the formulation exhibited zero-order release over this period. Gupta *et al*. attributed this fact as the molecules of spans 60 ordered gel state at the *In vitro* permeation condition of 25°C[48].

3.5.1. In vitro skin permeation study

For the *In vitro* permeation experiment, animal skin was used because human skin is more difficult to obtain. The *In vitro* permeation study gives the information about the behaviour of the molecule *in vivo.* The amount of the drug permeated gives the information about the amount of drug absorbed into the blood[49]. It is reported that proniosomes will be hydrated to niosomes vesicles before the penetration through the skin. The surfactant and cholesterol amount showed a significant variation in the permeation of drug through the skin. Figure 7 shows the percentage drug release of OXY from rat skin in phosphate buffer pH 7.4. Viswanad et al., reported that increased permeation flux due to increase in surfactant concentration may be due to the non-ionic surfactant present in it, which modifies the structural composition of stratum corneum and increases the thermodynamic activity of the drug as well as skin vesicular partitioning[50]. Moreover, the presence of unsaturated double bond in the oleate side chain of Span 60 was responsible for the significant enhancement of OXY permeation. The packing nature of unsaturated fatty acids changes the stratum corneum lipid structure upon binding to the keratin filament, hence increase drug permeability across skin.

3.5.2. Skin irritation test

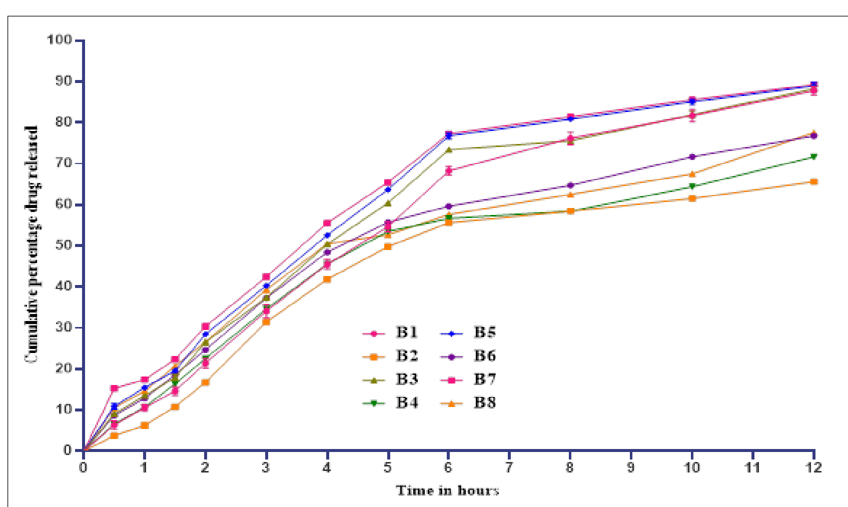

Figure 6 Comparative *In vitro* dissolution profiles of OXY loaded proniosomal gels

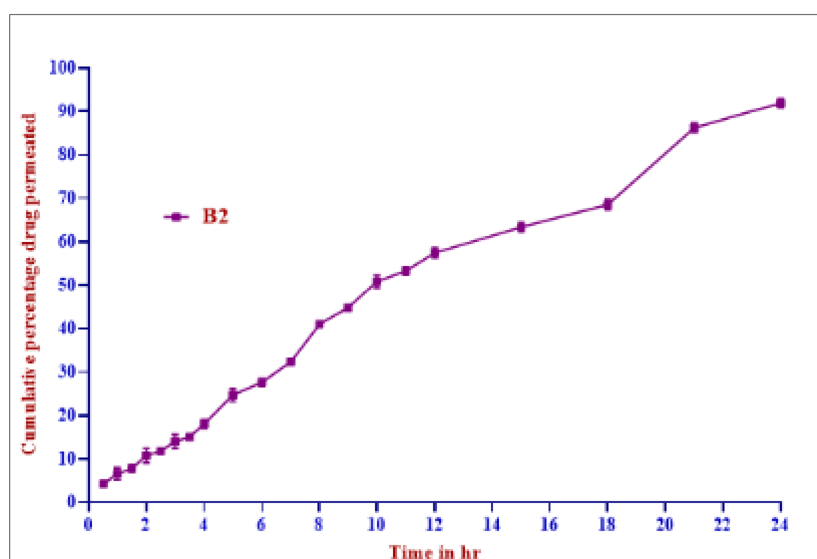

Figure 7 Cumulative amount drug permeated of optimized proniosomal gel formulation (B2)

The Albino rats received B2 (optimized proniosomal gel) as well as Control gel were free from of any irritation and there were no signs of erythema. According to Draize et al, the optimal proniosomal gels and plain gels considered to be non-irritant and the optimal proniosomal gel formula is safe to be applied on the skin for the intended period of time (table 3). The phospholipids, cholesterol, span series surfactant and ethanol used in the preparation of proniosomal gel were mild and no Oedema was observed.

Table 3 The irritation scores of B2 and Control gels on normal rat skin after administration of 24hr to 7 Days

Formulation	**Application period**						
	Erythema scores						
	Day-1	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Day-6**	**Day-7**
B2	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0

3.5.3. Physical stability studies

In the present study, stability studies were performed on optimized formulation (B2). It was stored at 4°C ± 1°C for 6 months and observed for the change in % EE, average PS of the vesicles. It was observed that there was no change in the physical appearance as there was neither change in the formulation consistency nor aggregation of vesicles. Furthermore, there was no significant change in EE%, PS of OXY proniosomal gel after the storage for 6 months. Our findings were in accordance with the study done by Sandeep *et al*. who explained the nonsignificant change in size and EE% in span 60 surfactant-based formulation due to its high phase transition temperature and low permeability[51]. This result showed the high stability and suitability of proniosomal gel for the topical OXY delivery.

4. Conclusion

OXY-loaded proniosomal gel was prepared successfully by coacervation phase separation method. The prepared proniosomal gel exhibited desired entrapment efficiency and Particle size. It was found that B2 which chosen as a best formula according to entrapment efficiency, PS, and *In vitro* release study. It was found that B2 which contains high concentration of span 60 is the most appropriate surfactant for the preparation of proniosomes. *In vitro* release studies proved that the prepared proniosomal gel contains Oxy considered to be a successful topical transdermal drug delivery system and provide a sustained release of encapsulated drug. Furthermore, there was no significant change in EE% and PS of OXY proniosomal gel after storage for 6 months.

Compliance with ethical standards

Acknowledgments

We wish to thank to the principal and management of V.L.College of pharmacy for providing the facilities to carry out the work.

Disclosure of conflict of interest

There are no conflicts of interest.

Statement of ethical approval

The study was undertaken under the permission of the University Animal Ethics Committee IAEC of V.L.College of Pharmacy, Raichur, and Karnataka, India (BU/BT/394/13-14).

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