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FORMULATION, CHARACTERIZATION AND *EX-VIVO* EVALUATION OF PRONIOSOMAL GEL OF PROGESTERONE FOR TRANSDERMAL DRUG DELIVERY SYSTEM

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Abstract:

The aim of the present work is to improve the bioavailability of progesterone by formulating in to Proniosomal gel for transdermal delivery. A Proniosomal gel was prepared by using various non- ionic surfactants (tweens and spans), lecithin, cholesterol & ethanol. Proniosomes were prepared by co-acervation phase separation and were evaluated. The optimized formulation (F1) showed mean globule size of 182.6 ± 5.24 nm, PDI 0.218 ± 0.043, Zeta potential -22 ± 1.12 (mV), drug content 98.8% entrapment efficiency 97.79%. The In- vitro drug release of F1 formulation showed significantly higher drug release was 76.61 ± 2.66% compared to drug suspension (8.56 ± 3.92%) and follows zero order kinetics and Higuchi's model by Fickian diffusion. The ex-vivo permeation studies were carried out for formulation (F1) and drug suspension on rats' abdominal skin. The theoretical flux was calculated that is (41.02µg/hr/cm^2). Steady state flux value of optimized proniosomal gel formulation (67.08 ± 0.53µg/cm^2/h) was significantly high compared to drug suspension (5.78 ± 0.55µg/cm^2/h). The steady state flux of optimized proniosomal gel formulation F1 was 11.6 times to the flux of drug suspension.

Keywords: *Progesterone, Proniosomal gel, Transdermal delivery, Permeation, Entrapment efficiency*

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1. INTRODUCTION

Currently transdermal delivery is one of the most promising method for drug application as it is non-invasive, painless route that can avoid gastrointestinal side effects associated with orally administered drugs [(1)]. Progesterone is a naturally occurring progestin secreted by corpus luteum. It is a lipophilic drug used to control reproductive function and as postmenopausal therapy. It is used in the treatment of amenorrhea [(2)]. The oral delivery of progesterone is limited since it is not tolerated in higher doses and its oral bioavailability is poor due to its intense hepatic metabolism. It is belongs to BCS class II and reported oral bioavailability <2.4% [(3)]. The main objective is to develop Proniosomal gel of progesterone for Transdermal drug delivery to enhance bioavailability and to characterize the formulation for *in- vitro* andstability studies.Then *ex-vivo* permeation studies were conducted with Proniosomal gel compared with drug suspension.

2. MATERIALS AND METHODS:

2.1. Materials

Progesterone was obtained as a gift sample from MSN Laboratories, Hyderabad. Egg lecithin, Cholesterol, Tween (grades 80, 60 & 20), Span (grades 80, 60 & 20), Glycerol and Ethanol were procured from Sigma Aldrich. All the other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of proniosomal gel of Progesterone

Progesterone proniosomal gel was prepared by using various non-ionic surfactants like Tween (80, 60, & 20), Span (80, 60, & 20) by coacervation phase separation method. Progesterone with a surfactant, lecithin and cholesterol were mixed with 0.25 ml absolute ethanol in a wide mouth glass tube. The compositions of proniosomal gel progesterone formulations are listed in table 1.Then the open end of the vial was covered with cap and warmed in water bath at 65 ± 3°C for 5 min with shaking till complete dissolution of lipids. About 40 µL of 0.1% glycerol solution (65 ± 3°C) was added to the formed turbid solution, while warming in the water bath for 3-5 min till a clear solution was obtained. The mixture was allowed to cool down at room temperature till the dispersion was converted into proniosomal gel [(4)].

Table 1: Composition of various proniosomal gels of progesterone

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	30	30	30	30	30	30	30	30	30
Tween-80	450	-	-	-	-	-	-	-	-
Span-80	-	450	-	-	-	-	-	-	-
Tween-60	-	-	450	-	-	-	-	-	-
Span-60	-	-	-	450	-	-	-	-	-
Tween-20	-	-	-	-	450	-	-	-	-
Span-20	-	-	-	-	-	450	-	-	-
Tween-80 & Span-80	-	-	-	-	-	-	225+225	-	-
Tween-60 & Span-60	-	-	-	-	-	-	-	225+225	-
Tween-20 & Span-20	-	-	-	-	-	-	-	-	225+225
Egg lecithin	450	450	450	450	450	450	450	450	450
Cholesterol	50	50	50	50	50	50	50	50	50
Ethanol (µL)	250	250	250	250	250	250	250	250	250
0.1% glycerol (µL)	40	40	40	40	40	40	40	40	40

2.2.2. Characterization studies

.2.2.1. Determination of vesicle size, ZP and PDI

The proniosomal gel was diluted (1:50) with double distilled water, taken in a cuvette and placed inside the Malvern Zetasizer (Nano ZS 90). Globule size was measured at 90° light scattering angle. The polydispersity index (PDI) should be below 0.3. The zeta potential measurement was based on the electrophoretic mobility of the globules [(5)].

2.2.2.2. Measurement of entrapment efficiency

The proniosomal dispersion was taken in a dialysis sac and dialyzed against 7.4 pH phosphate buffer saline at room temperature then samples were withdrawn from the medium at 24 hrs and analyzed for drug content using UV spectroscopy at λ max 249 nm [(6)].

2.2.2.3. Determination of total drug content

About 100mL of formulation was diluted with 25mL of pH 7.4 phosphate buffer solution in a volumetric flask with vigorous shaking, and amount of Progesterone was estimated by UV spectroscopy at λ max 249 nm [(7)].

2.2.2.4. Morphology studies of vesicle by SEM

The shape, surface characteristics and size of the proniosomes were observed by SEM. The weight of 0.2-0.5 g of proniosomal gel was taken in the glass tube and was mounted on an aluminum stub using double sided adhesive carbon tape. Then, the vesicles were sputter coated with gold palladium using a vacuum evaporator and examined using a scanning electron microscope S-3700N (Hitachi Ltd, Tokyo, Japan) equipped with a digital camera, at 15 kV accelerating voltage (15.0 kV 4.9mm×5.00k) [(8)].

2.2.2.5. *In- vitro* drug release studies

The drug release studies from progesterone proniosomal gel were performed by using Franz diffusion cells. Then the proniosomal gel was placed over a dialysis membrane (Himedia mol.wt 12000-14000) and sandwiched between the two compartments and fixed tightly using clamp. The whole setup was placed on magnetic stirrer. The samples (3ml) were collected up to 24hrs. Analysis was carried out using UV- Visiblespectrophotometer. Phosphate buffer pH 5.8 (15ml) was used as release media. The study was conducted at 37±0.5°C at a speed of 300 rpm. The analysis was done at 249 nm against phosphate buffer pH 5.8 as blank. The release studies were performed in triplets and mean values were considered [(9)].

2.2.2.6. *Ex-vivo* skin permeation studies

Skin was obtained from the abdominal region of male wistar rat. The skin washed with phosphate buffer and then visually inspected for its integrity. The skin was placed on Franz- diffusion cell with its stratum corneum facing upward and dermal side facing downward. 400 mg of the tested proniosomal gel (equivalent to 10) was accurately weighed and placed on the skin. The receptor compartment was filled with 15 ml phosphate buffer saline (pH 7.4). The medium was constantly stirred at 300 rpm. Three ml samples were withdrawn from the receptor compartment at various time intervals up to 24 hrs and replaced with an equal volume of fresh buffer. The samples were then analyzed by UV- Visible spectrophotometer at 249 nm [(10)].

2.2.2.7. Stability studies

The stability studies of progesterone proniosomal gel were studied at refrigeration t and room temperature conditions. Throughout the study, proniosomal formulations were stored in aluminum foil sealed glass vials. The samples were withdrawn at different time intervals over a period of 90 days [(11)].

3. RESULTS AND DISCUSSION:

3.1. Solubility studies of progesterone

Solubility of drug progesterone was estimated in different surfactants i.e., span 20, span 80, tween 20, tween 60, & tween 80, with pH 5.8, 6.8, & 7.4 phosphate buffer and water. The highest solubility of drug was observed in tweens in that mainly tween 80 grades. The results are displayed in Fig 1.

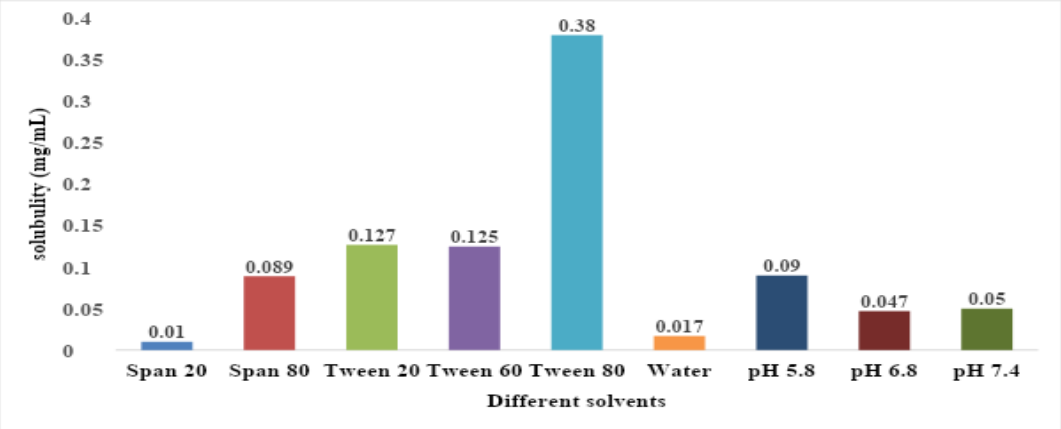

Fig 1: Solubility studies of progesterone in different surfactants and buffers

3.2. Determination of vesicle size, ZP, PDI, drug content and EE

The prepared proniosomal gel formulations were evaluated for vesicle size, PDI, ZP, EE and drug content. The results are given in Table 2. The drug content of all progesterone proniosomal gel formulations was found between in the range of 93.81 to 98.82%. There was no significant difference in the entrapment efficiency of all the developed formulations. The optimized formulation (F1) showed vesicle size 182.6 nm, PDI was found 0.128; ZP was -22.4 mV sufficient for stabilization of vesicles. The drug content was 98.82% and entrapment efficiency was 84.12%.

Table 2: Vesicle size, PDI, ZP, drug content and EE of formulations (Mean ± SD; n=3)

Formulations	Vesicle size (nm)	PDI	ZP (mV)	Drug content (%)	EE (%)
F1	182.6 ± 5.24	0.120 ± 0.043	-22.4 ± 1.12	98.82 ± 2.31	84.12 ± 1.73
F2	452.8 ± 7.66	0.913 ± 0.091	-41.1 ± 2.78	94.79 ± 2.89	79.63 ± 2.12
F3	338.7 ± 5.67	0.128 ± 0.038	-26.3 ± 2.08	95.64 ± 1.74	76.48 ± 2.35
F4	298.4 ± 9.86	0.073 ± 0.052	-18.6 ± 1.18	97.62 ± 2.05	80.39 ± 2.76
F5	344.2 ± 10.59	0.258 ± 0.073	-17.4 ± 2.68	96.75 ± 2.65	78.26 ± 1.92
F6	527.2 ± 8.16	0.458 ± 0.078	-13.6 ± 1.98	93.81 ± 2.42	81.67 ± 2.85
F7	340.8 ± 8.71	0.248 ± 0.017	-25.8 ± 2.67	96.87 ± 2.59	80.21 ± 3.11
F8	385.2 ± 11.83	0.263 ± 0.089	-23.4 ± 2.79	95.35 ± 2.84	81.92 ± 2.97
F9	448.5 ± 6.98	0.370 ± 0.067	-37.1 ± 1.85	94.61 ± 1.97	80.38 ± 2.85

3.3. *In- vitro* drug release studies

The drug release studies were performed for all the prepared formulations. The formulation are developed by tweens, they showed good release pattern compare to spans. The formulation like F1, F3 and F5 fabricated by tween 80, tween 60 and tween 20; they showed percentage of drug release were 76.61%, 69.40% and 60.18%. The formulations like F7, F8 and F9 made by combination of surfactants are tweens and spans grades; they showed 72.16%, 66.02% and 61.12%, these are showed better release compare spans. The formulations F2, F4 and F6 developed by various grades of span 80, 60 and 20 and observe the poor drug release. The optimized formulation (F1) compared with pure drug suspension. The results are represented in Fig 2.

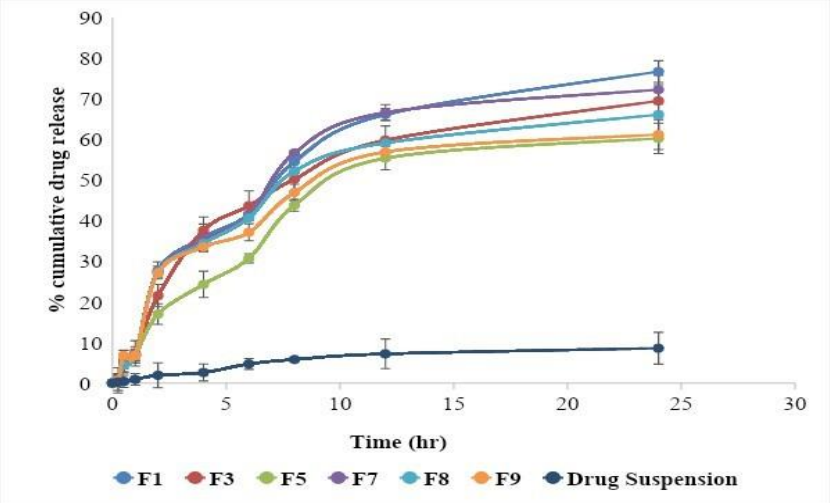

Fig 2: *In vitro* release profile of progesterone proniosomal gel formulations

3.4. Drug release kinetics

The drug release profiles of drug from F1 seems to follow zero order kinetics as it is evidenced by correlation coefficient (R^2= 0.9509) and Higuchi's equation (R^2 =0.9624), n values (0.5331)indicates that mechanism of drug release was by Fickian diffusion. The results of release kinetics showed in Table 3.

Table 3: Release kinetic data of proniosomal gel

Formulations	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Peppas (R^2)	Peppas(n)
F1	0.9509	0.9289	0.9624	0.9510	0.5331
F3	0.9286	0.9176	0.9276	0.9241	0.3919
F5	0.8814	0.8856	0.0.8301	0.8194	0.2532
F7	0.9441	0.9213	0.9409	0.9340	0.4351
F8	0.8932	0.9015	0.8889	0.8896	0.3402
F9	0.8692	0.8969	0.8393	0.8325	0.2742

3.5. Ex-vivo permeation studies

The formulation F1 showed highest amount of drug permeation through rat abdominal skin (8156.09 µg) compared todrug suspension (DS) (2201.28 µg). The results are showed in Fig 2.

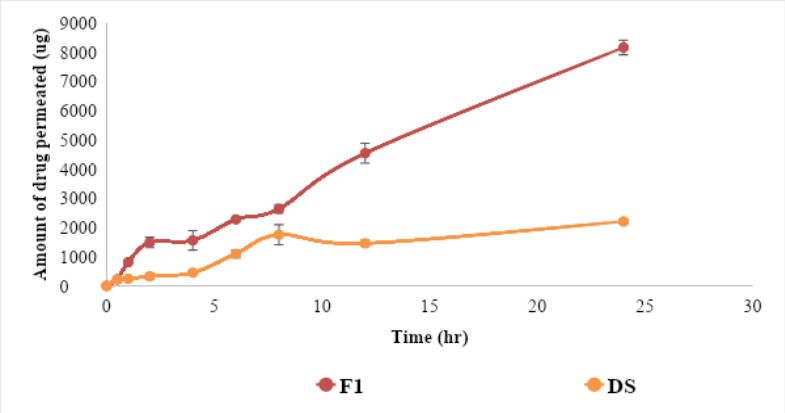

Fig 2: Amount of drug permeate through rat abdominal skin

3.6. Steady state flux calculation

The proniosomal gel formulation (F1) showed significantly high permeation (flux) at P<0.0001 compared to drug suspension. It was also observed that formulation F1 (67.08 ± 0.53µg/cm^2/hr) showed significantly high steady state flux compared to other formulations and drug suspension (5.78 ± 0.551µg/cm^2/hr). The formulation F1 showed 11.6 times enhancement in the flux compared to drug suspension.

3.7. Stability studies

The optimized formulation was stored in vails at room temperature (25^{o}C) and refrigerated temperature (4^{o}C) for a period of three months and observed for size and zeta potential [12]. There are neglible changes in the size, PDI and ZP during the storage conditions, these changes are indicates no significant differences were observed on aging of progesterone proniosomal gel. The results are depicted in Table 4.

Table 4: Stability studies of progesterone proniosomal gel (Mean ± SD; n=3)

Day	Conditions	Zeta size ±SD (nm)	PDI ± SD	ZP (mV)
1	Room temperature (25^{o}C)	182±5.24	0.218±0.043	-22±1.12
	Refrigerated temperature (4^{o}C)	178±4.61	0.209±0.041	-23±1.61
30	Room temperature (25^{o}C)	191±3.45	0.224±0.036	-21±1.08
	Refrigerated temperature (4^{o}C)	187±3.23	0.213±0.045	-22±1.45
60	Room temperature (25^{o}C)	196±2.15	0.231±0.062	-23±1.78
	Refrigerated temperature (4^{o}C)	190±1.34	0.224±0.034	-20±0.98
90	Room temperature (25^{o}C)	195±2.14	0.231±0.162	-23±1.68
	Refrigerated temperature (4^{o}C)	192±1.33	0.224±0.134	-20±0.99

4. CONCLUSION:

From the presented study, it is clear that the progesterone proniosomal gels were formulated using coacervation phase separation method by using various surfactants. The formulation (F1) having the smaller size of vesicles, lower PDI, sufficient ZP for stabilization, higher entrapment efficiency, drug release and stability was maintained during storage period. F1 showed a significantly high amount of drug permeated, flux compared to the drug suspension.

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