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Comparing *in vitro* Permeability of a Nanocarrier-Hydrogel Hybrid System with an Alcoholic Hydrogel for Sustained Transdermal Drug Delivery

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Abstract **–** Nanocarriers are commonly used as various types of drug delivery systems. However, the topical application of aqueous dispersions of nanocarriers is limited due to high fluidity of the formulations. In contrast, the topical application of various types of hydrogels are common for the good consistency and spreadability properties. For example, most marketed topical preparations of oestradiol (E2), which are used as hormone replacement therapy in menopausal women, are alcoholic hydrogels. Though, such formulations must be applied daily and chronic usage of alcohol can be damaging to the skin. In this paper, a lipid nanocapsule (LNC)-hydrogel hybrid system containing E2 was developed without the use of any alcohol or other organic solvents with an aim to achieve sustained transdermal drug delivery. In the LNCs, more than 95% of the drug was encapsulated and the dispersion was used to prepare a hydrogel using carboxypolymethylene. Moreover, an alcoholic hydrogel containing E2 was formulated to compare the *in vitro* transdermal E2 permeability using synthetic model STRAT-M®. Additionally, an apparatus for the permeability study was developed by modifying USP dissolution apparatus-I and its suitability was realized by comparing its results with the permeability in Franz diffusion cell apparatus. The LNC-hydrogel hybrid system showed more stable and sustained delivery of E2 compared to the alcoholic hydrogels. Therefore, the E2-LNC-hydrogel hybrid system can be a promising formulation for hormone replacement therapy in menopausal women with the potential to increase dosing intervals, reduce hormone label fluctuations by its more stable flux, and eliminate the damaging effects of chronic alcohol application on the skin.

Keywords**:** Nanocarrier-hydrogel hybrid, Lipid nanocapsule, Hydrogel, Transdermal drug delivery, Oestradiol

1. Introduction

Nanocarriers are simple colloidal systems with size between 1 to 100nm, and that are widely used for drug delivery purposes [1]. Nanocarriers can be safe mediums of drug delivery and sustain release of medications can be easily achieved [2]. By modification of physicochemical properties of the efficacy of nanocarriers can be greatly improved [3], although they are usually not suitable to apply topically as an aqueous dispersion due to their too fluid characteristics. In contrast, hydrogels are crosslinked three dimensional networks of polymers [4] with a tendency to swell by absorbing water and have good consistency, which is ideal for topical formulations [5]. Recently, tendency of combining nanocarriers with hydrogels are increased to make them suitable for transdermal application [6]-[7]. In this current study, two formulations containing oestradiol hemihydrate (E2) i.e., an alcoholic hydrogel and a lipid nanocapsule-hydrogel hybrid system, were prepared and evaluated as possible transdermal drug delivery systems for hormone replacement therapy in menopausal women.

2. Methodology

2.1. Preparation of Lipid Nanocapsules (LNCs) of E2

To prepare the LNCs as per previously described method [8], all ingredients (except water for thermal shock) were taken to a 20ml scintillation vial along with 5 mg E2 (Table 1). The vial was then placed to a magnetic stirrer (Torrey Pines Scientific, USA) with a Teflon coated magnetic stirrer bar. Afterwards, the mixture was slowly heated to 90°C to make it water in oil (w/o) emulsion and then cooled at 60°C to convert it to oil in water (o/w) emulsion. In between this two cycles

phase inversion zone was noticed at around 76°C. The heating-cooling cycle was repeated 5 times, and during the 6th cooling cycle, thermal shock was given around the phase inversion temperature by adding ice cold water to form the LNCs.

Table 1: Ingredients of LNC Formulation

Ingredients	Concentration (% w/w)
Kolliphor HS15	32.00 %
Labrafac® WL1349	21.00 %
Water for Dispersion	46.00 %
Lipoid® S PC-3	1.05 %
NaCl	1.25 %
Water for Thermal Shock	195% of water for dispersion

2.2. Determination of Size of LNCs

The particle size of the prepared LNCs was determined by dynamic light scattering technique (Zetasizer Nano ZS, Malvern). Briefly, 3 µl of LNC sample was taken and diluted to 9 ml with ultrapure water to prepare the final sample for size determination by the mentioned technique.

2.3. Determination of Encapsulation Efficacy of LNCs

The weight of the prepared LNC was calculated and theoretical amounts of drugs per volume was determined. The LNC preparation was vortexed, 1 ml of LNC was taken and centrifuged at 3000 rpm for 5 minutes. The required amount of supernatant was collected and taken into a 5ml volumetric flask the rest was filled with methanol and mixed well to break the LNCs and dissolve the contents. The theoretical concentration of taken volume was noted. The mixture was filtered and taken into a 1.5ml HPLC vial and analyze on HPLC (Prominence-I, LC-2030C 3D Plus; Shimadzu Corporation, Japan) according to USP protocol for E2 (described in 2.8).

2.4. Preparation of LNC Embedded Hydrogels

Total weight of LNC was measured and noted as initial weight (about 4 gm). At first, 0.3% l-ascorbic acid was added and stirred for 10 minutes, which will served as an antioxidant [9]. After addition of ascorbic acid, 0.2% methyl paraben and 0.1% propyl paraben (both were dissolved in propylene glycol) were added and stirred for 5 minutes. Afterwards, 2.5% Carbopol 974P NF was gradually added for one hour under continuous stirring. Then, propylene glycol was added to make up its concentration 9.1% and stirred for 15 minutes. Finally, triethanolamine (TEA) was gradually added at 25µl aliquots until the LNC-Carbopol mixture converts to viscus and thick consistency. The formulation was labelled by F1

2.5. Preparation of Alcoholic Gel

Alcoholic gels were prepared by using ethanol and water (1:1), propylene glycol (9.1%), Carbopol 974P NF (2.5%), and TEA for the comparison of transmembrane drug permeability. The ingredients addition procedure and sequence were same as the hydrogel preparation procedure discussed under 2.4. The formulation was labelled as SA1.

2.6. Evaluation of the Prepared LNC Embedded Hydrogel

The prepared hydrogels were inspected visually (Figure 1a) for the presence of any lumps, color consistency and homogeneity [10]. For the measurement of pH, 0.5 g of prepared hydrogels were taken into a 20 ml beaker and 10 ml water was added to it. This mixture was stirred until it mixed uniformly. The mixture was transferred into a 10 ml beaker and pH was measured by an electronic pH meter (S220, Mettler Toledo) [11]. Spreadability was measured by using glass slides (Figure 1b). Two 10’’/10’’ 5mm glass slides was taken and cleaned by using 70% isopropyl alcohol. A circle of 1 cm diameter was marked on the center of a slide and 0.5 g of gel was placed on the circle. The other slide was placed on top of the gel and 500 g of standard weight was placed on top of that for 5 minutes. The diameter of the cycle

was measured using a scale [12]. Along with LNC embedded hydrogels, two commercially available gels were evaluated. Spreadability index was calculated by the following equation.

$$\text{Spreadability index (SI)} = d^2 \times \frac{\pi}{4} \quad (1)$$

Here, d= diameter of the circle (cm).

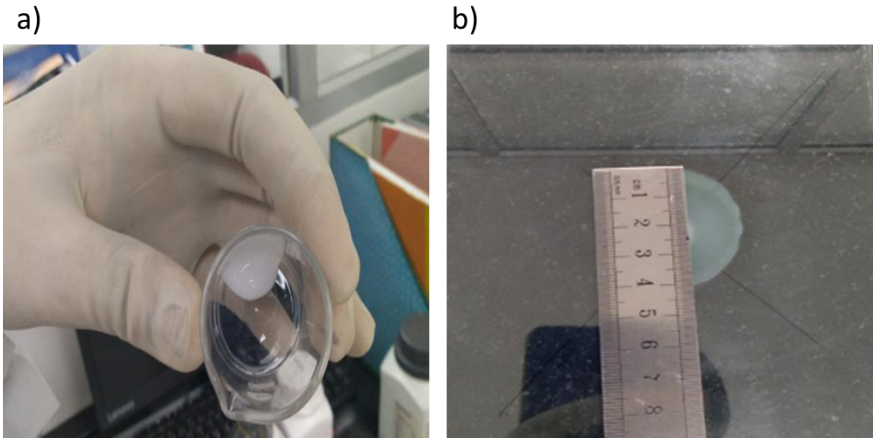

Figure 1: Properties analysis of Hydrogels; a) Visual Inspections; b) Spreadability Measurement

2.7. *In Vitro* Transmembrane Drug Permeability Study

2.7.1. Solubility Study for Media Selection

For the proper dissolution of the drug in the media, the solubility of E2 in different concentrations of sodium lauryl sulphate (SLS) solution in phosphate buffer (PB) of pH 7.4 was studied. In brief, 4 gm of E2 into 4 ml SLS in PB solutions of various concentrations i.e., 0.3%, 0.5%, 0.75%, 1%, 2%, 3%, 4%, and 5% (w/v) and the mixtures were stirred for 3 hours. Afterwards, the mixtures were centrifuged at 3000 rpm for 5 minutes and the supernatants were collected and diluted with equal amounts of methanol and assayed on HPLC as per method described in 2.8.

2.7.2. Transmembrane Permeability Study in Franz Diffusion Cell Apparatus

Freshly prepared media was poured into the acceptor compartment and membrane was placed on top of that with donor compartment. The acceptor chamber volume of Franz diffusion cell was 7 ml and the surface area for drug permeation was 0.64 cm2. The water bath was started which was set at 37°C. The stir bar was placed, and machine was runed for 30 minutes for the uniform heating of media. After that 0.5 g of alcoholic gels were placed on the donor compartment and the sealed with paraffin film to prevent evaporation. 0.9 ml of sample was withdrawn at 1, 2, 3, 4, and 5 hour and replaced with fresh media. The samples were analyzed by HPLC as per USP method (described in 2.8) [13]–[15].

2.7.3. Transmembrane Permeability Study in Modified USP Dissolution Apparatus-I

A hole with 17 mm diameter was made on the plastic caps of the 20 ml scintillation vials (Figure 2) and a small pore was created at its bottom for air pass. A 2x2 inch 4mm glass slide was placed on its bottom with silicon glue and dried for 24 hours. From the USP dissolution apparatus-I (TDT-08L, Electrolab), baskets were removed, and this glass setup was placed and tied by rubber bands [16]. Strat M® membrane was cut to 20mm diameter and placed inside the cap, with the shiny part towards the inside of the vials (donor compartment) for putting the hydrogels. The total surface area in contact with gel was 2.26 cm^2. About 1 g of the prepared gels was placed on the membrane and the cap was attached with the vial. 100 ml of freshly prepared media was placed to the vessels of dissolution apparatus and the equipment was run at 50 rpm at 37°C. The apparatus was downed in such way that the membrane just touched the top of the dissolution media. 2.5 ml of

media was withdrawn at 1, 2, 3, 4, 5, 6, 7, 24, 28, 31, 48, 52, 54, 72, 75, 77, 96, 100, 103, and 105 hours and replaced with fresh media. Equal amount of methanol was added to all withdrawn samples and kept for 5 minutes with gentle shake to mixed properly.

Flux of the drug permeation was calculated by the following formula-

$$\text{Flux} = \frac{dQ}{dt} \times \frac{1}{S} \quad (2)$$

Here, dQ/dt is the permeation of the drug per hour (slope); S is the contact area of the membrane.

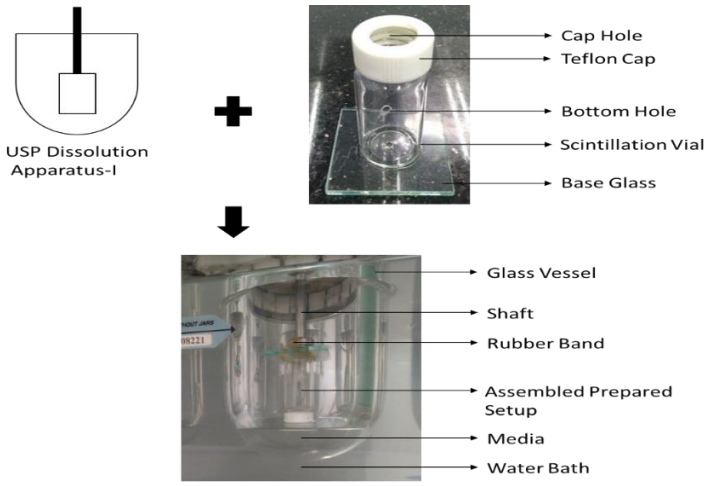

Figure 2: The Modified USP Dissolution Apparatus-I

2.8. E2 Quantification by HPLC

All the mixture was filtrated by a PVDF filter (0.45 µm) and placed to a 1.5 ml HPLC vial for analysis. The analysis was done according to USP procedure for E2. Acetonitrile (Dae Jung, Korea) in ultrapure water (55:45) was the mobile phase, flow rate was 1 ml/minute, and detection was done with UV detector at 205nm wavelength. A 3.9 mm × 30 cm, 5 µm, C18 column (Kromasil®, Sweden) was used for separation. The sample injection amount was 25 µl and runtime was 15 minutes per samples.

3. Results

3.1. Results of Particle Size Analysis & Encapsulation Efficacy of LNCs

The mean size of the prepared LNCs was 36.2 ± 2.4 nm (mean ± SEM; n=4). The mean encapsulation efficiency of the LNC batches was 96.9 ± 5.1%.

3.2. Results of Hydrogel Evaluation

All the prepared hydrogels were uniform, white in color and opaque, no lumps were present, and homogeneity of hydrogels was noticed. The consistency of the prepared gels was thick. The market preparations also had similar characteristics. The average pH of the prepared hydrogels was 5.90 ± 0.02 (mean ± SEM; n=4). The pH of the market preparation CP1 was 4.91 and pH of CP2 was 6.98. Average spreadability index of the prepared gels of F1 was 15.46 ± 0.83 cm^2 and the spreadability index of the market preparations was 12.56 cm^2 and 15.02 cm^2 respectively for CP1 & CP2. Therefore, the prepared nanocarrier-hydrogel hybrid was uniform, had a pH and spreadability index comparable to the market preparations.

3.3. Results of *In Vitro* Transmembrane Permeability Assay

3.3.1. Results of Solubility Analysis

E2 showed concentration depended solubility on SLS-PB solutions. Table 2 represent the data of the solubility analysis. analysis. For the better results and as the media capacity of Franz Diffusion Cells were 7 ml, 3% SLS concentrations were were selected for the media.

Table 2: Solubility E2 in SLS-PB (mean ± SEM; n=4)

SLS Concentrations (% w/v)	Average Conc. of E2 (mg/ml)	Volume Required to solubilize the E2 (ml)
0.30	0.01 ± 0.007	100.0
0.50	0.011 ± 0.004	93.8
0.75	0.012 ± 0.002	81.1
1.00	0.023 ± 0.002	43.5
2.00	0.166 ± 0.006	6.0
3.00	0.367 ± 0.002	2.7
4.00	0.525 ± 0.015	1.9
5.00	0.660 ± 0.010	1.5

3.3.2. Results of *In Vitro* Transmembrane E2 Permeation Study comparing Franz Diffusion Cell Apparatus and Modified USP Dissolution Apparatus-I

From the data of Franz diffusion cell apparatus, drug permeation from prepared alcoholic gels were increased with time. Table 3 represents the data of drug permeation assay. Besides, assay from USP apparatus showed higher % of permeation on same time intervals.

Table 3: *In vitro* drug permeation in Franz diffusion cell apparatus and modified USP dissolution apparatus-I (mean ± SEM; n=4)

Time (hour)	Permeation of E2 from SA1 in Franz Diffusion Cell Apparatus (%)	Permeation of E2 from SA1 in Modified USP Dissolution Apparatus-I (%)
1	0.135 ± 0.005	0.6235 ± 0.1605
2	0.61 ± 0.03	2.261 ± 0.146
3	1.115 ± 0.065	3.673 ± 0.345
4	1.585 ± 0.085	5.6975 ± 0.5665
5	1.91 ± 0.16	7.698 ± 0.48

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Figure 3a represents the data of accumulative permeation per hour of Franz diffusion cell apparatus and modified USP dissolution apparatus-I. For the difference in the area, permeation rate was different. But in compared to their cumulative permeation quantity (figure 3b) both were similar. That means same amounts of drugs were passed through the membrane in both apparatuses.

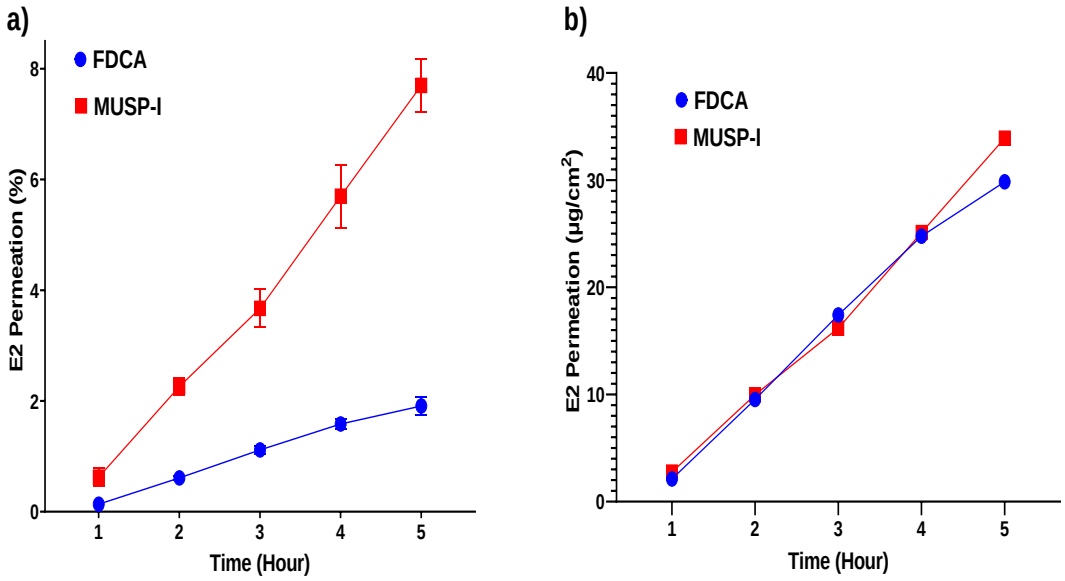

Figure 3: a) Accumulative E2 permeation of SA1 from Franz diffusion cell apparatus (FDCA) and modified USP dissolution apparatus-I (MUSP-I); b) Comparison of E2 Permeation Quantity per cm^2

3.3.3. Results of *In Vitro* transmembrane E2 Permeation from Hydrogels in Modified USP Dissolution Apparatus-I

The results of the transmembrane E2 permeation are shown in Figure 4. In flux analysis (Table 4), we can observe that the flux from SA1 was 4.4-folds higher than F1 between 1-7 hours. The drug permeation was much faster for SA1 up to 24 hours, after which the flux was reduced. The flux of 1-7 hour was 8.28-folds higher than the flux between 24-105 hour for SA1. In comparison, the flux from F1 was more sustained with only 2.1-folds difference between the time points. Therefore, drug permeation from F1 was much sustained and less fluctuating compared to SA1.

Table 4: Flux calculated from the Drug Permeation Assay

Formulation	Time (hour)	Flux (µg/cm^2 h)
SA1	1 - 7	2.303
	24 - 105	0.278
F1	1 - 7	0.520
	24 - 105	0.247

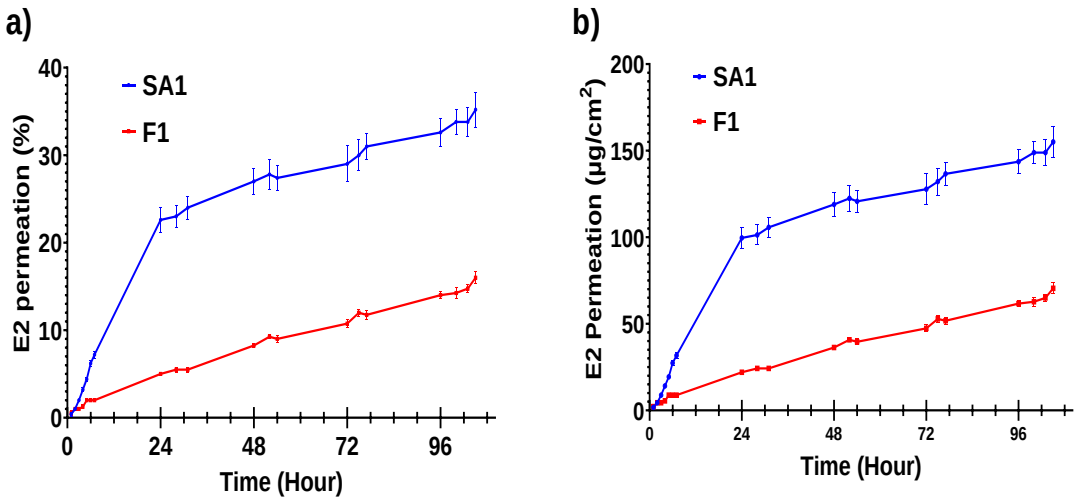

Figure 4: Comparison of- a) E2 transmembrane Permeation (%) from SA1 and F1; b) E2 transmembrane permeation (µg/cm^2) from SA1 and F1

4. Conclusion

Hybrid hydrogels can benefit from many advantageous features of both nanocarriers and hydrogels. In our study, drug permeation study of alcoholic gel on both Franz Diffusion Cell Apparatus and modified USP Dissolution Apparatus-I have shown almost similar drug permeation per square centimetre. As it is commonly available on most of the pharmaceuticals and universities it can be a good alternatives of Franz diffusion cell apparatus. Besides, the lipid nanocapsule-hydrogel hybrid system showed much sustained and stable transmembrane permeation of E2 compared to the alcoholic gel. Therefore, it has the potential to increase the dosing interval from once per day, reduce fluctuations of plasma drug circulation by providing a stable flux, and prevent the negative effects of chronic alcoholic formulation use on the skin and improve patient compliance.

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