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Formulation and evaluation of transdermal delivery of beclomethasone dipropionate via ethosomes

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Abstract

This study tested beclomethasone dipropionate for maximal solubility in aqueous buffers to formulate and evaluate beclomethasone dipropionate ethosomes for transdermal delivery. Compared to traditional liposomes, ethosomal systems can deliver greater concentrations of beclomethasone dipropionate via mice's skin at a regulated release rate. Ethosomal formulations had much greater drug flow values (P G.01). This would suggest that ethanol improves the way drugs pass through the layers of the skin. Of all the generated ethosomal formulations, 22.83 ± 0.56 µg/cm2/h was the most significant drug flow obtained. The release kinetics mechanism was assessed by fitting the permeation data to the zero-order, first-order, and Higuchi diffusion models. A linear link between the quantity of drug released and the square root of time was discovered, and all permeation profiles followed the Higuchi diffusion model. The primary barrier to drug penetration is bypassed when the medication is transported by ethosomes into the stratum corneum, greatly enhancing skin delivery. However, the amount that this function enhances transdermal flux may depend on several variables, including medication release from vesicles in the stratum corneum.

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INTRODUCTION

After topical application to the skin, transdermal medication distribution allows localized skin tissue treatment and makes systemic therapy easier. However, this administration method has many difficulties because the skin naturally prevents medicinal chemicals from penetrating it. Since people have applied substances to their skin for therapeutic purposes for thousands of years, transdermal delivery offers an appealing substitute for oral drug delivery. It is well-positioned to provide an alternative to

hypodermic injection [1]. In the present era, numerous topical formulations have been developed to treat specific local medical conditions. There are currently 19 transdermal delivery systems available for medications including testosterone, fentanyl, lidocaine, and oestradiol; combination patches that combine multiple medications for hormone replacement and contraception; and iontophoretic and ultrasonic delivery systems for pain relief [2]. Effective transdermal medications with existing delivery systems have molecular weights of only a few hundred Daltons, have lipid-heavy octanol-water partition coefficients, and need milligrams or less dosage per day1-4. The transdermal administration of peptides and macromolecules, including novel genetic treatments utilizing DNA or small-interfering RNA6, has presented unique hurdles. It has been challenging to use this route to deliver hydrophilic medicines [3].

METHODOLOGY:

FTIR study: Fourier transform infrared spectroscopy, or FTIR, was used in drug-polymer compatibility investigations to verify that the drug's entrapment within polymeric systems is solely a physical process and that there is no ongoing interaction between the drug and polymer combination.

To verify the identity of the drug and identify any interactions between it and the excipients, FTIR absorption spectra of the pure drug, all of the polymers utilized, and the combination of drug and polymers were obtained. [4] The drug's compatibility with the formulation was verified using FTIR spectrum analysis. Using the Shimadzu FT-IR 8300 spectrophotometer and the potassium bromide pellet method, the FTIR spectra of beclomethasone dipropionate and the formulation, including all polymers, were determined in the wavelength range of 4,000 to 400 cm-1.

Formulation of Liposomes

Liposomes were synthesized via solvent evaporation. In a tiny volume of diethyl ethanol: chloroform (1:1.5) mixture, precisely 50 mg of phosphatidylcholine from soybean lecithin, cholesterol, and diacetyl phosphate were dissolved in a round-bottom flask. The organic phase was mixed with the aqueous phase containing 15 mg of beclomethasone dipropionate, resulting in a 5:1 ratio between the two phases. [5] After that, the mixture was sonicated for ten minutes.

After creating a stable white emulsion, the water and organic solvent mixture were progressively evaporated using a rotary vacuum evaporator at 55ºC until a thin layer developed on the flask wall. The resultant film was vacuum-sealed to remove any remaining organic solvent residue.

After that, this film was hydrated with an aqueous solution and kept in a thermostatically controlled water bath at 55ºC for an hour. After an hour at room temperature, the resultant liposomal suspension was sonicated for 20 minutes.

Formulation of Ethosomes

Ethosomes were made using the method previously outlined, but a hydroalcoholic solution (20%–40% [vol/vol] ethanol) was used to hydrate the resultant film.

Evaluation of Ethosomes

Vesicle Morphology

Table 1 Composition of Various Liposomes (1-5), Ethosomes (6-8) Solutions

Batches	Cholesterol (mg)	Phosphati-dylcholine (mg)	Ethanol(% vol/vol)	Dicetylphosphate (mg)	Beclomethasone Dipropionate(mg)
F1	10	50	-	-	15
F2	30	50	-	-	15
F3	20	50	-	2.5	15
F4	25	50	-	5	15
F5	25	50	-	7.5	15
F6	25	50	20	5	15
F7	25	50	30	5	-
F8	25	50	40	5	-

Visualization by image processing and optical microscopy: After appropriately diluting samples of the produced vesicles, they were inspected under an optical microscope. The vesicles ' pictures were uploaded to an IBM-compatible computer using a video camera (JVC, Victor Co., Yokohama, Japan). At least 300 vesicles were measured for every sample, and image processing software was utilized to analyze the shape [6] automatically.

Vesicle Size Distribution

Using laser diffraction at 25 degrees Celsius, the vesicle sizes of each liposomal suspension (formulas 1-5) and each autosomal suspension (formulas 6-8) were measured in triplicate. The homogeneity was calculated using the polydispersity index (PI). A homogeneous population is indicated by tiny PI (G0.1) values, but PI values of 9 0.3 show considerable heterogeneity. When using the SPSS statistics software (SPSS Inc., Release 14.0 for Windows, Chicago, IL), data were entered, and a 1-way analysis of variance (ANOVA) test with multiple comparisons using the least squared difference (LSD) was applied [7].

Determination of Entrapment Efficiency

The percentage of the vesicle preparations was ultra centrifuged for two hours at 15,000 rpm after being stored at 4oC overnight. The concentration of free (unentrapped) beclomethasone dipropionate was measured by spectrophotometry (Shimadzu UV–1601 PC Double Beam, Kyoto, Japan) at λmax 216 nm in the supernatant [8]. The following calculation was used to get the SS entrapment percentage:

$$EE\,\frac{1}{4}\frac{1}{2} < \frac{Q_t - Q_s}{Q_t} > X\ 100$$

***In Vitro* Permeation studies**

Franz diffusion cells with an effective permeation area of 4.90 cm2 were used for the experiments. A constant 37°C ± 0.5°C was maintained in the temperature. A magnetic stirrer spinning at 50 rpm continuously agitated the receptor compartment's 20 mL of phosphate-buffered saline (pH 7.4). The initial step looked at medication penetration via the skin. An isotonic phosphate buffer (pH 7.4) with 0.11% (wt/vol) formaldehyde added as a preservative served as the receptor media [9]. The skin of newborn mice or artificial semipermeable membranes (six days or less) was used for the permeation investigations. The initial studies used a synthetic barrier to illustrate the impact of several variables, such as the quantity of diacetyl phosphate and cholesterol. For additional research on drug permeation via the biological barrier, the formula with the best drug penetration through the synthetic barrier was selected [10].

Mathematical modeling for drug release profile

Zero-order kinetics

It explains the mechanism wherein the medication release rate is unaffected by concentration [11], [12].

$$Q_{ts} = Q_0 + k_0\,t$$

First order kinetics

It explains how drugs are released from systems when the release rate varies with concentration.

$$\text{Log}\ Q_t = \text{Log}Q_0 + k_1\,\frac{t}{2.303}$$

Higuchi model

It describes how the proportion of drug release from a matrix is determined by the square root of time.

$$\frac{Mt}{M\alpha} = k_H\,t^{\frac{1}{2}}$$

Korsemayer-Peppas model

The potent law effectively characterizes the release of drugs from slabs, cylinders, and spheres by asserting that the relationship between the fractional amount of drug release and the release time is exponential.

$$\frac{Mt}{M\alpha} = Kt^n$$

$$Log\,[\frac{Mt}{M\alpha}] = log\,K + n\,Log\,t$$

RESULTS AND DISCUSSION

Drug polymer compatibility

These investigations are critical to verifying the drug's structure, activity, rate of degradation, and release pattern with different polymeric materials utilized in the formulation. It was noted that each

of the study's primary peaks did not significantly change. This suggests no problems with Beclomethasone Dipropionate's compatibility with the drugs Cholesterol, Phosphatidylcholine, and Dicetylphosphate. **Figure 1** and **Table 2** display the interpreted values and FTIR spectra, respectively.

Evaluation Parameters

Vesicle Morphology

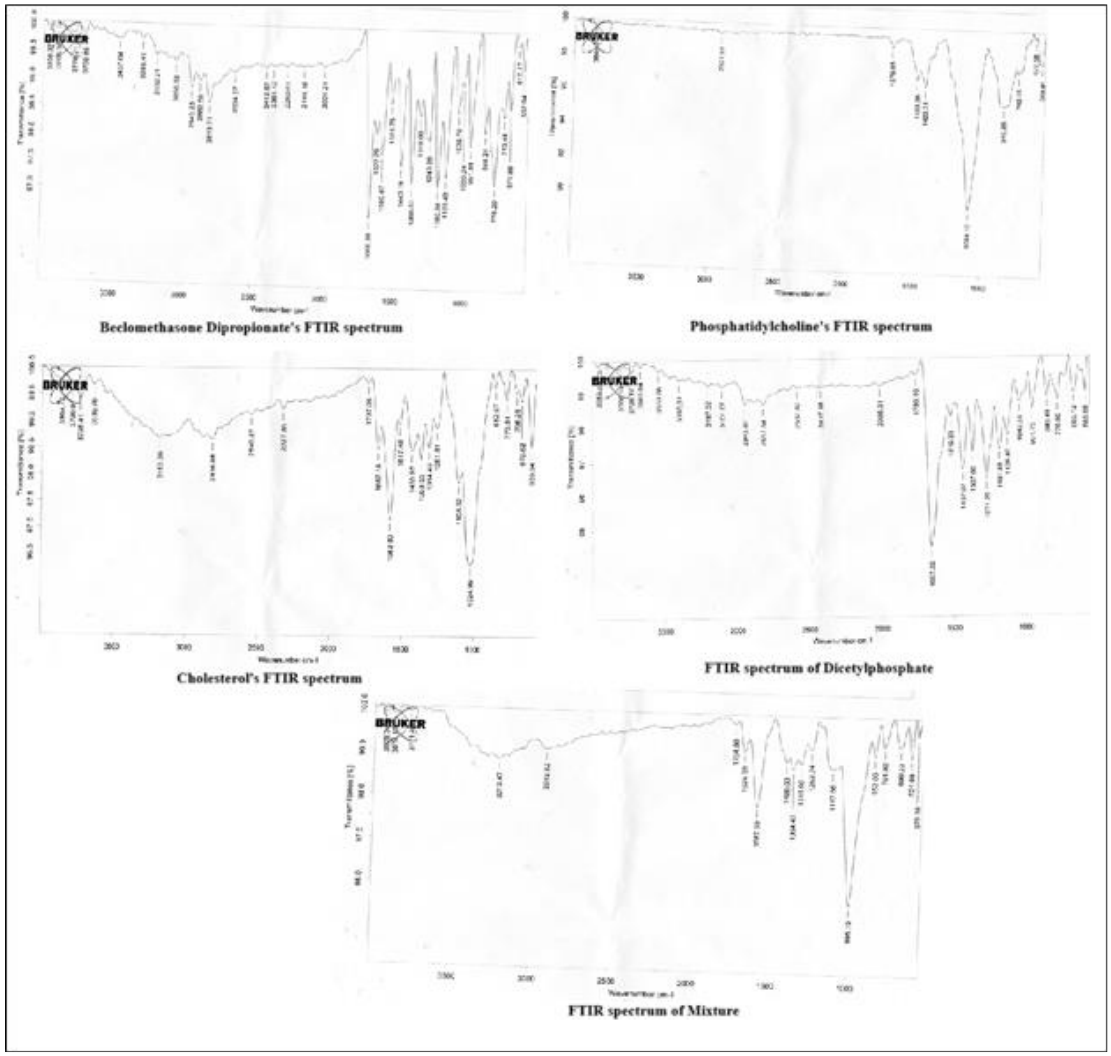

Figure 1 FTIR spectrums of Drug and Polymers

Table 2 Interpretation of FTIR spectral data

Functional Group	Drug	Phosphati-dylcholine	Cholesterol	Dicetyl-phosphate	Mixtures
O-H stretch (Carboxylic acids)	2927	2816	3396	1587	1364
C=O stretch (Carbonyl (general) a, β-unsaturated aldehydes)	1670	1737	2817	1587	1364
N-O asymmetric stretch (Nitro compounds)	1483, 1423	1517	1437	1587	1364
C-N stretch (esters, ethers Aliphatic amines)	1094	1433	2817	1409	1587

Spherical-shaped vesicles predominated when the produced formulations were examined with a Leica optical microscope and a SEM [**Figure 2**]. The vesicles have a consistent size and seem to have several layers.

Vesicle Size Distribution

Studies using laser diffraction revealed that liposomes and ethosomes differed significantly in size (P G.05). For ethosomal systems, different liposomes ranged from 382.3 to 736.6 nm, as shown in **Table 3**. The majority of formulations' lower PI values may be a sign of their homogeneity. Ethosomal formulations 7 and 8 exhibited the lowest values (0.11). Conversely, formula 6's PI value of 0.24 may suggest that the dispersion is heterogeneous.

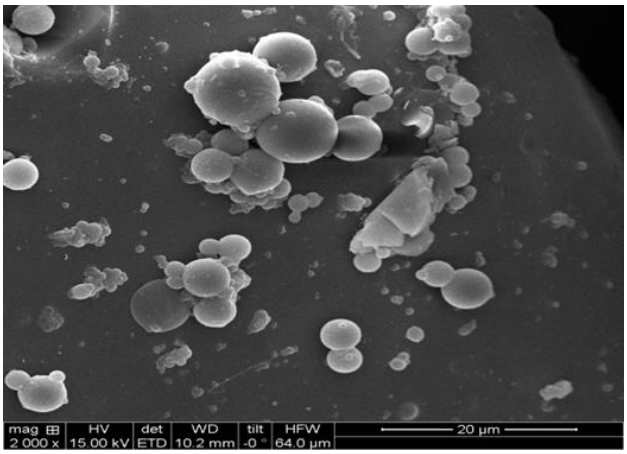

Figure 2 Optical micrographs of Beclomethasone Dipropionate

Table 3 Physical Characteristics of Vesicle Size Distribution

Batches	PI	Vesicle Diameter (nm)	Entrapment Efficiency (%)
F1	0.32 ± 0.08	742.6 ± 34.7	42.6 ± 2.1
F2	0.28 ± 0.05	662.2± 41.5	38.1 ± 1.6
F3	0.35 ± 0.07	581.8 ± 32.7	49.8 ± 2.2
F4	0.32 ± 0.06	533.4 ± 33.5	43.6 ± 2.2
F5	0.24 ± 0.08	481.5 ± 35.5	48.2 ± 4.1
F6	0.41 ± 0.05	454.9 ± 38.2	41.8 ± 5.2
F7	0.11 ± 0.07	382.4 ± 36.4	45.9 ± 4.3
F8	0.22± 0.07	378.8 ± 17.5	43.7 ± 4.8

These outcomes support the conclusions drawn by other investigators, who found that the presence of ethanol in the vesicles is the cause of the generalized decrease in mean vesicle diameters. It was proposed that ethanol likely modifies the system's net charge and provides some steric stabilization, which could ultimately result in a drop in the mean vesicle size.

Entrapment Efficiency

At 40% (vol/vol) ethanol, the highest entrapment percentage of 49.7% was attained (formula 8). It was shown that at increasing ethanol concentrations, liposomes exhibited more excellent stability. Because of the electrostatic repulsions, ethanol, like diacetyl phosphate, may stabilize the formulation, avoiding or postponing the development of vesicle aggregates. Two elements determine the stability and, thus, the entrapment efficiency of the vesicles. The first is the concentration of ethanol; vesicles with high ethanol concentrations have thinner membranes, which indicates the establishment of a phase in which hydrocarbon chains are interpenetrating. The presence of cholesterol is the second factor that affects vesicle stability. It does this by giving the lipid layers more rigidity, which increases system stability, decreases the chance of vesicle fusion, and increases resistance to the high rotational energy that ultracentrifugation exerts.

***In Vitro* Permeation and Skin Deposition Studies**

Based on the earlier findings, liposomal formula five was chosen for additional in vitro drug penetration and mouse skin deposition investigations. It was contrasted with ethosomal formulations (formulas 6, 7, and 8, respectively) that included 20%, 30%, and 40% ethanol. **Figure 3** illustrates how Beclomethasone Dipropionate from the formulations mentioned above permeates the skin of newborn mice. Analyzing the drug flux of the identical formulations to the receiver compartment (**Table 4**) could provide similar results. Compared to liposomal formula 5, the drug flux values of ethosomal formulae 6, 7, and 8 were significantly higher (P G.01). This

would suggest that ethanol improves the way drugs pass through the layers of the skin. Formula 7 produced the maximum drug flux (22.83 ± 0.56 µg/cm2/h) among all the generated ethosomal formulations.

Table 4 Values of Drug Flux

Drug Flux (µg/cm2 /h)	Batches
9.51 ± 0.44	F5
22.83 ± 0.56	F7
15.53 ± 0.94	F8

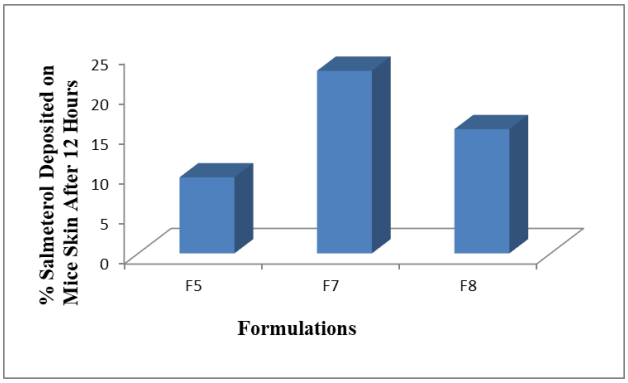

Figure 3 After extracting different liposomal, autosomal, and control formulations using a 50% hydroalcoholic solution at 37ºC ± 0.5ºC for 12 hours, the percentage of SS that was deposited on the skin of mice in vitro.

These results could help to explain Figure 5's in vitro skin deposition outcomes from various formulae. The proportion of autosomal formulas 6 and 7 and liposomal formula five beclomethasone dipropionate deposited in the skin. The primary barrier to drug penetration is bypassed when the medication is delivered to the stratum corneum via liposomes or ethosomes, greatly enhancing skin delivery. Skin deposition may be significantly improved by drug encapsulation. However, the amount that this function enhances transdermal flux may depend on several variables, including medication release from vesicles in the stratum corneum. Drug solubility in the lipids of the stratum corneum and drug affinity for vesicles determine the rate and amount of drug released.

Formulas 7 and 8 are more effective than formula seven at delivering beclomethasone dipropionate transdermally, according to statistical analysis of the prior results (i.e., the percentage of SS permeated through mice skin after 24 hours and the percentage of Beclomethasone Dipropionate deposited in the skin). The results of formulas 7 and 8 did not significantly differ (P 9.05); as a result, formula 8, which has a lower ethanol concentration (30% vol/vol), was selected for additional research.

Release Order Kinetics

Fitting the permeation data to the zero-order, first-order, and Higuchi diffusion models allowed for evaluating the release kinetics mechanism [**Table 5**]. A linear link between the quantity of drug released and the square root of time was discovered, and all permeation profiles followed the Higuchi diffusion model. The vesicles may

Table 5 Beclomethasone Dipropionate Ethosomes formulation F1 to F8 release kinetics

Model	F1		F2		F3		F4	
	m	r^2	m	r^2	m	r^2	m	r^2
Zero-order	69.3	0.722	1132	0.894	15.87	0.008	72.99	0.313
First order	0.058	0.513	0.076	0.674	0.042	0.324	0.033	0.434
Higuchi's Matrix	5432	0.632	8734	0.876	212.0	0.032	515.4	0.176
Korsmeyer-Peppar	2.453	0.789	2.456	0.992	1.897	0.564	1.763	0.554

Table 6 Beclomethasone Dipropionate Ethosomes formulation F1 to F8 release kinetics (continued)

Model	F5		F6		F7		F8	
	m	r^2	m	r^2	m	r^2	m	r^2
Zero-order	1414	0.876	15.51	0.823	154.5	0.878	2314	0.843
First order	0.074	0.567	0.044	0.543	0.048	0.578	0.072	0.488
Higuchi's Matrix	7865	0.765	1057	0.678	1198	0.768	11409	0.788
Korsmeyer-Peppar	2.461	0.887	2.028	0.887	2.044	0.982	2.675	0.867

have served as reservoirs for the ongoing supply of encapsulated medication.

CONCLUSION:

Because ethanol, ethosomal vesicles, and skin lipids work in concert, the presence of ethanol in the aqueous compartment of the vesicles promoted the encapsulation of beclomethasone dipropionate. It improved its penetration through the skin of newborn mice. Compared to traditional liposomes, ethosomal systems can deliver greater concentrations of beclomethasone dipropionate via mice's skin at a regulated release rate. Compared to liposomal formula 5, the drug flux values of ethosomal formulae 6, 7, and 8 were significantly higher (P G.01). This would suggest that ethanol improves the way drugs pass through the layers of the skin. Formula 7 produced the maximum drug flux (22.83 ± 0.56 µg/cm2/h) among all the generated ethosomal formulations. The release kinetics mechanism was evaluated by fitting the permeation data to the zero-order, first-order, and Higuchi diffusion models. All permeation profiles adhered to the Higuchi diffusion model and found a linear relationship between the amount of medication released and the square root of time.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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