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PREPARATION OF SKIN FRIENDLY TOPICAL GEL CONTAINING BETAMETHASONE DIPROPIONATE LOADED ETHOSOMES AND SALICYLIC ACID FOR THE TREATMENT OF ECZEMA

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ABSTRACT

Ethosomes, a lipid-based vesicular system, are a cutting-edge method for delivering medicinal drugs transdermally for either local or systemic action. Betamethasone Dipropionate ethosomes were fabricated and then these were incorporated into the final gel containing salicylic acid. Betamethasone is an anti-inflammatory agent and salicylic acid acts as a keratolytic agent. The increased drug entrapment and permeation are caused by the high ethanol content of ethosomes. By changing the proportions of ethanol and lipoid S100 in a cold method, ethosomes were created. The concentration of ethanol utilized ranged from 30 to 40%, whereas the concentration of phospholipids ranged from 3 to 5%. The characteristics of ethosomes made with 40% ethanol and 3% Lipoid S100 were improved. The size, zeta potential, entrapment efficacy, *in-vitro* release profile, ex-vivo skin permeation study, plus *in-vitro* stability investigations of the prepared Betamethasone Dipropionate ethosomes were all assessed. With an average size of 220-240nm, entrapment efficiency was 81.42-91.65%%, zeta potential was -12.91 to -14.42, and its *in-vitro* cumulative drug release over 12 hours was 96%. The drug penetration from the ethosomes gel formulation through the rat skin was two times more than that of the Betamethasone control gel. Ethosomes gel's rheological behaviour was evaluated, and pseudo plastic flow behaviour suggested that the formulation was suitable for topical use. Moreover, the formulations were stable at 8 °C and the ethosomes formulation demonstrated improved drug penetration through the epidermal membrane, improving the medication's bioavailability.

Keywords: Ethosomes, Gel, *In-vitro*, Salicylic acid, Phospholipids

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INTRODUCTION

The skin is a multilayer external organ that serves as a permeability border and a defensive tissue, blocking the passage of foreign substances from the surroundings. It is the largest biological organ, measuring 1.8 m^2 in total surface area of a human. Skin is a peripheral sensing organ that regulates heat, pressure, coldness, discomfort/pain, and touch stimuli and aids in organ-to-organ information exchange. The skin as a route for delivery can offer numerous benefits, such as avoiding first-pass metabolism, less volatility in plasma levels of drug, focusing therapeutically active components for a targeted effect, and improved patient compliance. It is an effective and selective barrier to chemical permeation. Water-soluble compounds and medications are generally unable to pass through the skin, which acts as a natural water barrier **[1]**. The epidermis (particularly the stratum corneum) is the principal regulatory layer in general **[2]**.

Several techniques have been developed to date to overcome this hindrance. Many of these are complicated enough to compromise patient compliance such as microneedle and iontophoresis etc. Some of the chemical use may cause skin damage. One approach that does not possess these kinds of complications is the vesicular system **[3]**. Elastic vesicles, such as ethosomes, are among this noninvasive vesicular structure that allow for deeper drug permeation into the skin **[4]**. Ethosomes have already been shown to improve drug delivery in vivo and in vitro in both non-occlusive and occlusive conditions **[5].** It is effective at transporting chemicals from the skin to blood circulation. The ethosomal carrier had previously been used to

transport the antiviral medication acyclovir to the skin.

Medical science continuously strives to bring improvements and to expand the frame of treatment options. Traditional treatment approaches involve avoiding irritants and prospective allergens, as well as keeping the skin hydrated with thick emollients. The therapeutic approach is topical corticosteroids, which can only be administered safely for a short time. Steroid-free therapy, such as topical immune modulators, has been demonstrated to be quite effective in the maintenance of eczema. In some cases, various therapies such as antimicrobials, systemic immunosuppressants, and phototherapy may also be used **[6, 7]**.

The most prescribed medications for treating dermatological disorders are topical corticosteroids. They are mostly used as monotherapy or in conjunction with other drugs to improve efficacy. Ever since the first introduction, several better preparations have become available **[8]**. Betamethasone dipropionate is a chemically synthesized fluorinated corticosteroid with a class 3 intermediate potency that is effective and well tolerated when used topically **[9]**. Betamethasone dipropionate is known for its characteristic antiproliferative, immunomodulatory, and anti-inflammatory activity. Due to its poor penetration through the skin, its topical use causes a barrier in treatment options **[10]**.

Salicylic acid has taken a unique, now legendary route from tree bark to the human stratum corneum. It was first identified as a helpful aid in hyperkeratinization problems. From treatment to research, it has delivered a series of nuanced developments, serving as both a therapeutic agent and a research tool. It has good tolerance for human skin. Salicylic acid is a keratolytic agent. Its keratolytic effect has been used most often for the treatment of dermatosis presented with a hyperplastic corneal layer. Anti-scaling activity of 0.05% betamethasone dipropionate has been noticed to be potentiated by the addition of 2% salicylic acid **[11]**.

The precedence of transdermal drug delivery has been widely reported in the literature **[12]**. Reduced potential adverse reactions, as well as the avoidance of first-pass metabolism and intestinal disintegration, are among the benefits. The barrier characteristic of the skin, which functionally hampers the admission of exogenous materials, prevents most molecules from being delivered percutaneously. Numerous mods of skin permeability augmentation, documented in multiple scientific publications and patents, have been employed to overcome this hurdle **[11]**. Ethosomes are vesicular transports that are used to transport chemicals into and through the skin. The mechanism of action to penetrate the drug into the deeper layer of skin can be attributed to ethanol effect and ethosome effect. It comprises of interaction of ethanol with a polar head of the lipophilic region. This interaction lowers the transition temperature of lipid molecules present in the stratum corneum of the skin. Thus, ultimately enhancing the fluidity and decreasing the density of the lipid bilayer. In this second step lipid molecules penetrate and fuse with the skin lipids layer opening new channels through which drugs leave into the deep skin layer.

Phospholipids, rather high concentrations of ethanol (20–50%), and water make up the majority of ethosomal systems. Investigations have demonstrated that phospholipid vesicles can coexist with ethanol at high concentrations and generate an enhanced delivery carrier called ethosomes **[13]**. The ethanol's hydrating effect on phospholipid bilayers aids in the formation of vesicles with a soft flexible structure **[14]**. Ethosomes come in a variety of sizes ranging from tens of nanometers to micrometers. The current work is aimed to enhance the betamethasone dipropionate absorption through the stratum corneum using ethosomal carrier technology. And salicylic acid is used as a keratolytic agent. The combination of these two drugs can lead to sustained delivery and better treatment of eczema.

MATERIAL AND METHODS

Materials

Betamethasone dipropionate (Sigma Aldrich, Germany), LIPOID S 100 (Lipoid, Germany), Ethanol, Propylene Glycol (Sigma Aldrich, Germany). Triethanolamine (Dae-Jung, Korea). Carbopol 940 (VWR), Distilled water (Department of pharmacy, IUB, Pakistan).

Preparation of Ethosomes loaded with Betamethasone Dipropionate

The traditional cold method was used to prepare betamethasone ethosomal vesicles (Touitou et al., 2000). Ethosomes were prepared by dissolving different concentrations of Lipoid (S100) in varying concentrations of ethanol using a magnetic stirrer while keeping the solution temperature at 30 degrees Celsius as shown in **Table 1**. While continuously stirring, a measured amount of propylene glycol was introduced to the alcohol-phospholipid solution. The above solution was designated as the organic phase. A weighed amount of 30°C water was added to another vial to create aqueous betamethasone dispersion. Keeping the amount of Betamethasone constant in all formulations, the above-prepared drug solution was introduced dropwise into the organic phase. Closed containers were used, and the stirring speed was kept at 1700rpm. The mixture was stirred for more than five minutes, and then it was sonicated in a bath sonicator for five minutes.

Table 1: Compositions of different betamethasone formulations.

Code	Betamethasone %	Lipoid S100 %	Ethanol %	Propylene Glycol %	Cholesterol %	Water up to 100%
B1	0.5	2.5	30	20	1.25	q.s.
B2	0.5	3.0	30	20	1.50	q.s.
B3	0.5	3.5	30	20	1.75	q.s.
B4	0.5	2.5	40	20	1.25	q.s.
B5	0.5	3.0	40	20	1.50	q.s.
B6	0.5	3.5	40	20	1.75	q.s.

Preparation of Betamethasone Dipropionate Ethosomal Gel

Carbopol 934 in a 2% concentration was added to the distilled water while continuously stirred to create the gel. Triethanolamine was gradually added to the water after carbopol had thoroughly dissolved until a clear, translucent gel with a pH of 6–6.5 was formed. A 0.5 percent concentration of the optimized ethosomal formulation was gently added to the gel and homogenized to provide a consistent formulation.

Characterization

Particle Size, Zeta Potential and Poly Dispersity Index

A Zetasizer Nano ZS (Malvern Instruments, UK) was utilized for monitoring the mean particle size and poly-dispersity index (PDI) and zeta potential of each formulation at the 25°C after proper dilution. All experiments were performed in triplicate.

Entrapment Efficiency

The next step was calculating the EE percent after the six different experimental formulations had been prepared. Using a tiny pipette, 1 ml of the formulation was injected into the Eppendorf tubes to conduct this test. The ultra-homogenizer was filled with tubes. For 30 minutes, the formulation was homogenized at 12,000 rpm. The supernatant was removed from the compact sediment following homogenization, and it was then diluted with pH 7.4 phosphate buffers. Then, a UV-visible spectrophotometer was used to detect the supernatant's absorbance at 240 nm. The quantity of drug in the supernatant was determined using the values of the linear equation. Using a liquid supernatant, an indirect method was used to measure the EE percent **[15]**.

The following eq. provides the formula used to calculate the EE percent.

$$EE\% = \frac{Entrapped\ drug\ mg}{total\ amount\ of drug\ added\ mg} \times 100$$

FTIR Analysis All six formulations and individual formulation components underwent FTIR analysis. Wavenumbers between 500 and 4000 cm^{-1} were searched for in the samples. FTIR spectra revealed the absorbance of infrared radiation at a particular wavenumber, a sign of the presence of functional groups in the samples.

In-vitro Diffusion Study

Studies on drug diffusion or release were carried out utilizing a dialysis membrane. A dialysis tube with a molecular weight cutoff ratio of 12000–14000 Dalton was used. The membrane was sliced into a specific size and let soak in a buffer solution of pH 7.4 for 24 hours. The membrane can be activated by immersing it in a solution that causes the pores to open. The membrane's one side was knotted with thread once activated. Following the addition of 1 ml of formulation, the other edge of the dialysis membrane was knotted with the aid of thread to create a tiny size pocket.

All six test formulations were identically put into the membrane pockets. The paddle of the USP paddle equipment was then used to secure these formulation-containing pouches. 7.4 pH buffer was used as the release medium, and the temperature of the medium was maintained at 37°C throughout the process. All Baskets of the dissolution apparatus were filled to the mark. The equipment was used for 12 hours. At predetermined intervals, samples were collected, and a new medium was added in their place.

Using a UV-visible spectrophotometer, samples were examined at 240 nm. The readings were taken in a triplicate manner and Calculated drug diffusion over time. Statistical kinetic models were employed to release data to ascertain the rate and mechanism of drug release. Higuchi, Korsmeyer Peppas, zero order, and first order models were used.

Ex-vivo Permeation Analysis

On the skin of the rats, the betamethasone-loaded ethosomal gel was allowed to permeate. For the investigation, male albino rats were chosen, and the abdomen skin was meticulously dissected. Male rats were chosen because they had less adipose tissue under their abdomen skin than female rats do. Following the dissection, ethanol was used to wash away any adipose tissues that had stuck to the abdomen skin. Skin was stored in the refrigerator until it was needed for a test analysis. The Franz diffusion cell was used to conduct a permeation study in pH 7.4 buffer at 32 °C.

The buffer was placed in the Franz cell, which had rat skin attached between the donor and receiver compartments so that the dermal layer of the skin faced the receiver medium. The test was done on the skin for 24 hours at a speed of 300 rpm using 0.5 g of the test sample. At regular intervals, 2ml of each sample was removed from the receiver chamber and replenished with fresh buffer. The control sample underwent the same process. A gel containing 0.05% of the drug was mixed immediately with pure betamethasone dipropionate to create the control sample. The penetrated medication was examined using a UV-visible photometer in triplicate on both the test and control samples. Results were plotted against the amount of cumulative medicines that soaked via rat skin over time. The enhancement ratio of penetration was computed using the test sample's and the control sample's flux over the membrane.

Stability Studies of Ethosomal Suspension

To determine the temperatures at which the formulation parameters stay stable during the two-month investigation, a suspension containing Betamethasone dipropionate ethosomes was held at 8°C and 25°C. The drug suspension's physical characteristics, Entrapment efficacy, and pH were investigated **[16]**.

Entrapment Efficiency

The stability analysis employed the effectiveness of drug entrapment as a metric. EE percent was measured throughout the period of a two-month study utilizing the aforementioned ultracentrifugation method at varied time intervals. Samples were taken at various intervals and then evaluated for EE percent at time o, day 1, day 2, day 3, day 7, day 14, day 21, day 28, day 45, and day 60. The variation in the amount of drug entrapped from 0 to 60th days was recorded and presented graphically for both temperature settings **[17].**

pH

Calculating pH provides further information on the stability of the suspension. The pH of a freshly made solution was measured, and the suspension was then stored at 8°C and 25°C for later examination. The samples were obtained at regular intervals, and the analysis was carried out over two months. Data from the experiment was tallied and plotted against a line graph **[16]**.

Physical Appearance

The suspension was seen for observable changes in color, smell, and consistency. During two months of stability tests, these characteristics were tracked by visual inspection of the pharmaceutical suspension.

Stability Studies of Ethosomal Gel

For three months following storage at four distinct temperatures 8°C, 25°C, 40°C and 40°C+75 percent RH, the Betamethasone dipropionate ethosomal gel formulation was tested for several stability criteria. Both formulations had their gel stability properties assessed before being stored for three months at the aforementioned temperatures and getting periodic analyses **[18]**.

Organoleptic Assessment

Throughout the course of the investigation, changes in color, smell, liquefaction, and microbiological growth were examined in control and ethosomal gel preparations held at four different temperatures. For comparison, the results were tabulated.

pH

After retaining both formulations at °C, 25°C, 40°C and 40°C+75 percent RH for two months, a pH analysis of the control and ethosomal gel was performed.

Pre-calibrated laboratory scale pH meters were used to take measurements in a triplicate manner. To determine how temperature affected the pH of each formulation of the control and ethosomal gels, data was organized into tabulated and graphical forms **[18]**.

Spreadability

The capacity of control and ethosomal gel formulations stored at various temperatures to maintain the required spreadability during the stability research was examined. To measure spreadability, 0.5g of the prepared gel was applied to a glass slide in a circle with a diameter of 1 cm. A second coverslip was used on top of the first slide, and a load of about 500g was put on the upper slide. After 5 minutes, the weight was taken down, and the spreadability of the gel was determined by measuring the change in the diameter of the circle after spreading **[17]**.

Rheological Analysis

A programmed Rheometer at 25+0.5°C was used to measure the viscosity of control and ethosomal gel compositions at various temperatures. Sample measurements were taken at 0 time, 30 days, 60 days, and 90 days. Different gels' viscosities were measured at speeds between 40 and 85 rpm (in 5 steps), and shear rates between 80 and 170 (in 10 steps) were applied to each sample. Shear rates were used to ensure the quality of prepared gels after applying shear pressures. Readings were taken three times **[16, 19]**.

RESULTS AND DISCUSSION

Entrapment Efficacy

Due to the presence of ethanol in ethosomes, both lipophilic and hydrophilic compounds can be more effectively entrapped within them. This makes ethosomes a superior skin delivery mechanism over liposomes **[15]**. Betamethasone dipropionate is a water-soluble steroid whose trapping in the ethosomes is improved by the effect of ethanol's

solubility enhancement on the ethosomes' flexible phospholipid layer, which facilitates the drug's penetration into the vesicles. The formulation with the higher concentration of ethanol (40 percent) among the six experimental formulations exhibits greater entrapment efficiency than the formulations with lower alcohol concentrations **[20]**. The positive influence of ethanol on encapsulation efficacy is restricted to a specific concentration (40 percent) .Any further increase in ethanol content causes a decline in entrapment, which the vesicles' leakiness may cause **[15]**. The B5 formulation, which contains 40% ethanol, has the highest Betamethasone dipropionate entrapment efficiency, at 91% as shown in **Table 2**.

Particle Size, Zeta Potential and Poly Dispersity Index

The ethosomal system contains ethanol, which accounts for the smaller particle sizes compared to liposomes, which contain less ethanol. Because higher ethanol contents aid in developing small, compact vesicles, the smaller the particle size, the easier the absorption via skin berries. The vesicles shows the size in the range of 220.2-240.32 nm, which may promote skin permeability owing to the tiny size of the particles **[20]**. One factor that indicates a formulation's stability is its zeta potential. High zeta potential values indicate excellent system stability because higher charges have a more substantial repelling effect and prevent particle aggregation **[21]**. In addition to giving the vesicles a negative net charge, ethanol also offers the ethosomes electrostatic stability **[20]**. Zeta potential in our study was -12.91 to -14.42 indicating that vesicles would not assemble quickly and would be suspended for a prolonged period of time.

FTIR Analysis

The FTIR study aids in understanding how a drug and formulation components could interact. The acquired spectra show bond creation, bond breaking, functional group transition, etc. As shown in **Fig. 1** the FTIR spectra of BMD ethosomes. O-H stretch at 3300 cm^{-1}, C-H stretch at 3000 cm^{-1}, C=O stretch at 1755 cm^{-1}, C=O stretch at 1660 cm^{-1}, C=C stretch at 1620 cm^{-1}, C=O stretch at 1620 cm^{-1}, 1189 cm^{-1}, C=O stretch at propionate ester at 1189 cm^{-1}, and C=O stretch at 1068 cm^{-1} are the characteristic peaks of BMD in the (C-O stretch, 11-hydroxyl). The FTIR spectra of BMD ethosomes showed all of the unique BMD peaks.

Table 2: Different characteristics of the developed ethosomal formulation.

Formulation	Entrapment efficacy %	Vesicle size	Zeta potential	PDI
B1	81.42	238.76	-14.20	0.260
B2	90.95	235.12	-13.34	0.255
B3	82.37	225.50	-15.34	0.270
B4	84.13	230.45	-12.97	0.276
B5	91.65	220.20	-12.91	0.251
B6	86.79	240.32	-14.42	0.262

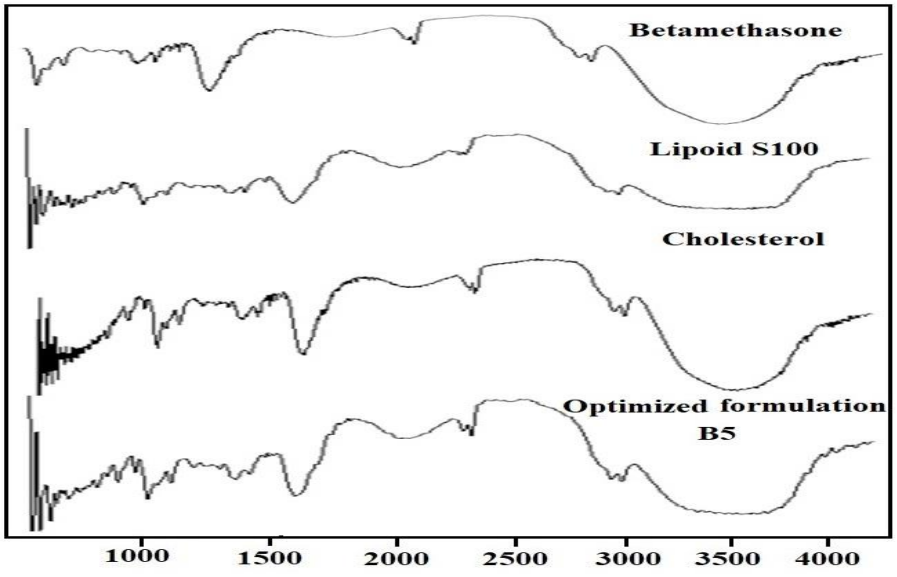


Figure 1: FTIR spectra of betamethasone dipropionate, lipoid S100, cholesterol and B5 formulation.

***In-Vitro* Release Studies**

The release behavior of the drug in the physiological system is indicated by dissolution experiments of the ethosomal formulation. To undertake release studies on the Betamethasone dipropionate ethosomes, 7.4 pH buffers were used as a substitute medium for the skin condition. The drug was released in the following study in a range of 85 to 99% as shown in **Fig. 2**. Compared to formulations B4 to B6, formulations B1 to B3 exhibit less drug release over 12 hours. This effect may be explained by the last three formulations' increased ethanol concentrations, which make the bilayer more flexible and thus make it easier for drugs to exit the vesicles. The drug's release from the first three formulations ranged from 85 to 90 percent, while the drug's release from the final three formulations ranged from 89 to 99 percent **[22]**. The drug release from the dialysis was high, and virtually all drug was released in under 4 hours, which may be because more of the medication is available in soluble form, according to the drug solution in the buffer solution. On the other hand, ethosomal formulation distributes the medicine gradually over 12 hours due to the ethosomal membrane's phospholipid barrier, which slows the drug release rate from the system. These findings suggested that ethosomes release drugs over a long period, giving the system its sustained activity **[23]**.

Release Kinetics

DDsolver.xla was used to apply kinetic models to each of the six formulations displayed in **Table 3**. Zero order, first order, the Higuchi model, and the Korsmeyer-Peppas model were among the models used. R^2 values were used to get the correlation coefficient.

Data revealed that all medication formulations and suspensions adhered to the Korsmeyer-Peppas model, which had the highest R^2 value. When the exponent for the Korsmeyer-Peppas model is positive ($n > 0.1$), it means that the release of the drug followed non-Fickian super case-II transport. The rate and patterns of drug release from the system are calculated using these models **[20]**.

Ex-Vivo Permeation Study

The ethosomal system is made up of phospholipids and ethanol, this combination allows for the drug to penetrate the skin of berries more effectively. This is the primary property of ethosomes and the reason they are used in skin preparations. Ethanol promotes drug penetration across dermal membrane due to its impact on the stability of the lipid bilayer of the skin membrane and the deformability of the ethosomes. Ethanol and other alcohols are utilized in a biphasic ethosomal system, which improves the drug's ability to permeate the body. Propylene glycol is the alcohol that is most frequently utilized for this purpose (PG).

In comparison to ethanol, propylene glycol has a higher viscosity and is more hygroscopic, which prolongs the time that ethosomes remain in the epidermal layer and results in continuous activity. PG helped keep the formulation stable as well. In this investigation, the ethosomal gel formulation demonstrated more penetration through the rat abdomen skin than the ascorbic acid control gel. In a 24-hour test utilizing a Franz diffusion cell, the ethosomal formulation demonstrated drug permeation up to 69% while the control gel only permeated 37% as shown in **Fig. 3**. The amount of medicine that permeated through the rat skin was measured, and the results showed that the drug's ethosomal gel permeated the skin twice as much as the control gel did.

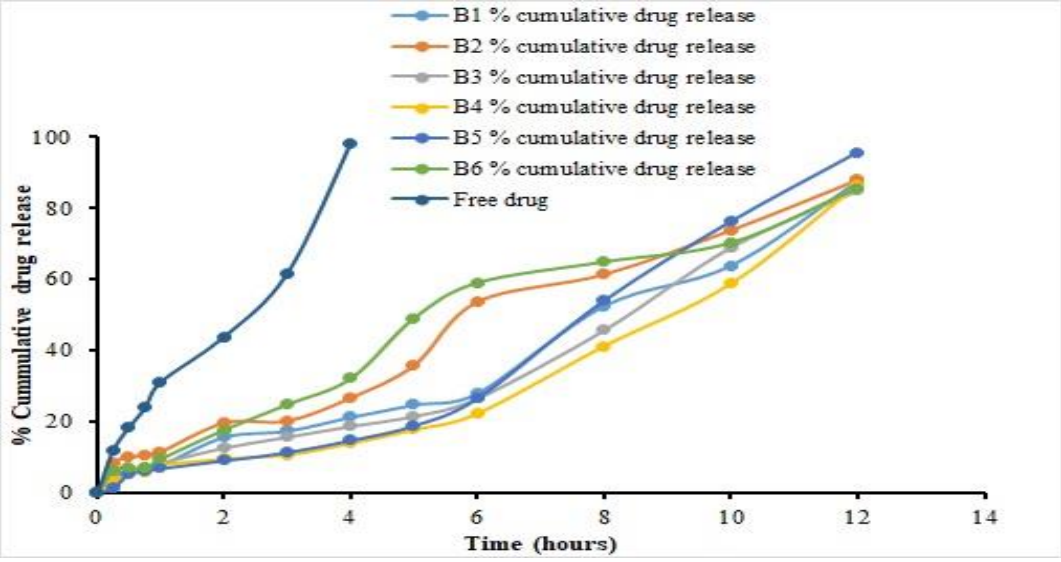


Figure 2: *In-vitro* release profile of betamethasone dipropionate ethosomes in phosphate buffer pH 7.4.

Table 3: Korsmeyer Peppas kinetics of In-vitro drug release

Formulation	Korsmeyer peppas Rate constant(k_{KP})	n(Exponent)	R^2
B1	3.707	1.255	0.9764
B2	1.662	1.640	0.9848
B3	2.767	1.374	0.9735
B4	1.222	1.702	0.9733
B5	9.382	0.899	0.9753
B6	11.884	0.801	0.9771
Drug Suspension	27.707	0.852	0.9682

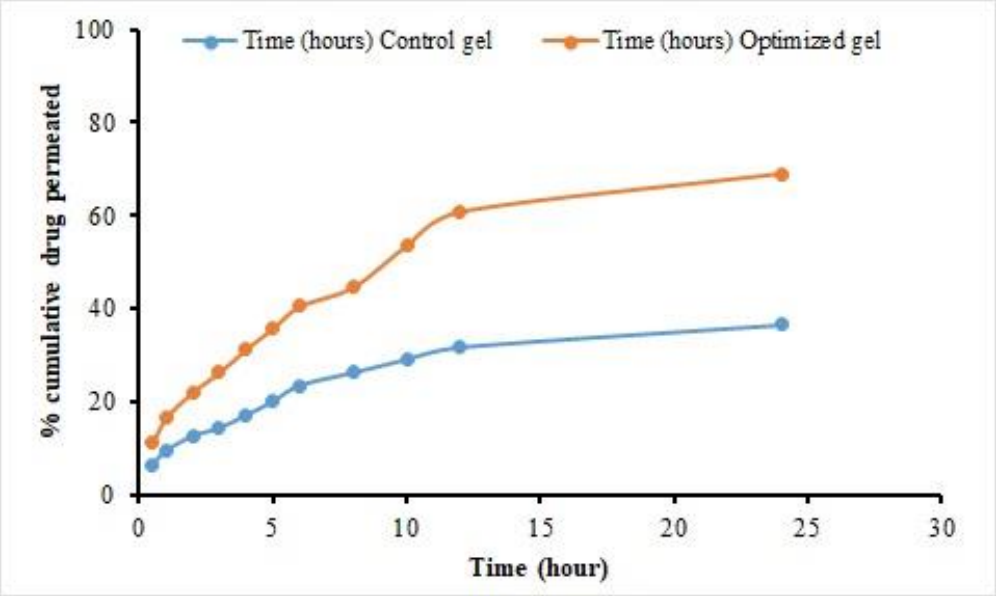


Figure 3: Comparative permeation analyses of control gel and optimized gel.

Stability Study of Ethosome Suspension

pH

The long-term stabilization of ethosomal suspension necessitates the examination of various formulation factors, one of which is pH. The drug and ethanol are the two most significant variables that change the transition temperature of the phospholipid bilayer. The optimal Betamethasone dipropionate suspension formulation was kept at two different temperatures, 8ºC and 25°C, for around two months while the pH of the formulation was tracked. The data suggests that the pH of the suspension stored at 8°C will vary less than the suspension stored at 25°C. The pH of the suspension was 4.585 at time zero, following storage at two temperatures. However, the change was negligible in the case of 8°C, while the pH decline was more noticeable in the formulation when it was held at 25°C as shown in **Fig. 4** and **Table 4**. This may be because high temperatures cause an increase in lipid permeability and the release of Betamethasone dipropionate, which lowers pH. According to these results, the formulation is more stable at 80°C than at higher temperatures [24].

Entrapment Efficiency

The formulation's stable storage conditions were established by maintaining the optimized ethosomes suspension at two temperatures. After two months of research, it was discovered that the data showed that the suspension held at 8ºC was more stable and had a more minor change in entrapment efficiency than the formulation stored at 25ºC as shown in **Fig. 4** and **Table 4**. According to this research, the shift of vesicles lipid into liquid form, which results in drug leakage from ethosomes, may be the cause of the variation in drug entrapment under higher temperature circumstances. Another potential reason for reduced entrapment is high-temperature lipid breakdown. The statistics revealed that 4-8ºC was the most stable storage temperature for the ethosomes **[24, 25]**.

Table 4: pH and entrapment efficiency of ethosomal formulation at different temperatures.

Time (Days)	pH		Entrapment efficiency (EE) %	
	8 ˚C	25 ˚C	8 ˚C	25 ˚C
Fresh	4.558	4.558	88.32	88.32
1	4.464	4.402	85.10	85.87
2	4.396	4.084	84.37	84.95
3	4.287	3.984	83.74	84.02
7	4.240	3.956	81.96	83.70
14	4.235	3.803	81.16	81.50
21	4.199	3.784	81.42	78.11
28	3.924	3.486	81.16	75.79
45	3.876	3.278	81.40	73.83
60	3.803	2.987	80.09	66.08

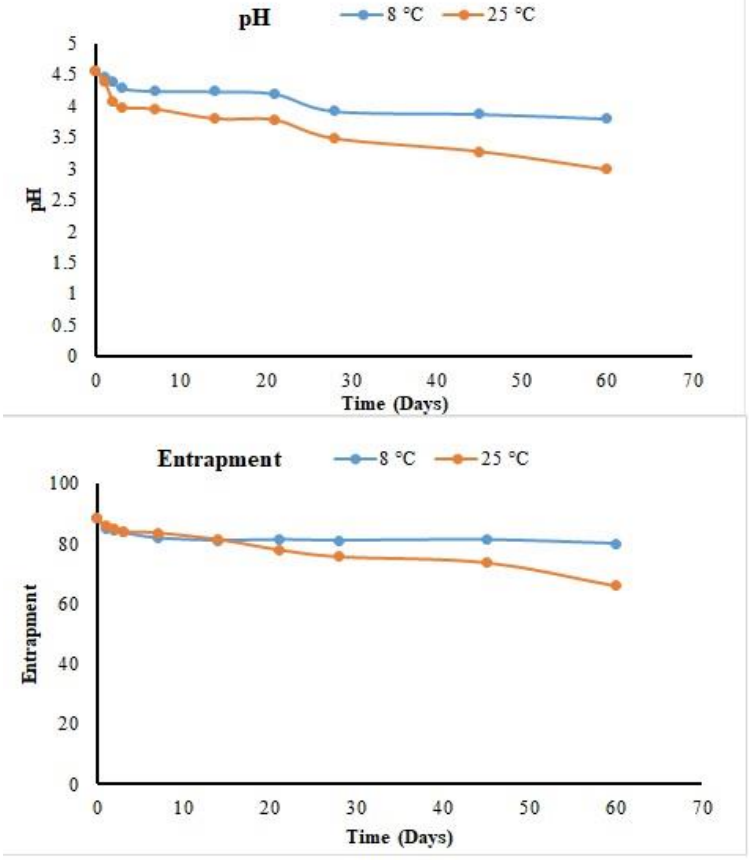


Figure 4: pH and entrapment efficiency of ethosomal formulation at different temperature.

Stability Study of Ethosomal Gel

Organoleptic Evaluation

As ethosomal suspension could not be applied to the skin for medicinal purposes, optimized ethosomes suspension was added into a gel formulation. Prepared ethosome gel and controlled ethosome gel were preserved for around three months in four separate storage settings. Under the following conditions: 80C, 250C, 400C, and 400C+75%RH, the preparations' color, smell, and degree of liquefaction were examined in both the optimal gel and the cool gel **Table 5**. For the specified storage period, neither the control nor the optimized gel stored at 8C displayed any color or odor changes or signs of liquefaction. The optimized gel's color changed to orange at the end of the trial, developed a slight odor, and to some extent, liquefied during the study. In contrast, the control gel remained unchanged at higher temperatures **[25]**. Since no antioxidants were included in the gel formulation to preserve the medication from oxidation, the color change in the optimized gel may be the result of Betamethasone dipropionate being released from the ethosomes at higher temperatures **[26]**.

pH Analysis of Ethosomal Gel Formulation

Since higher acidic and alkaline pHs can irritate the skin, the dermal gel's pH is a crucial criterion for assessing its therapeutic efficiency. Determining the pH of the gel over two months at various temperatures was done for this reason. The pH of the optimized and control gel was monitored for changes throughout the storage time under four different settings. The formulations were created with a pH range of 6.6 to 6.7 and held at various humidity and temperature levels. Since it does not irritate the skin, this pH is suitable for application to the skin **[27]**. At all temperatures, the pH of the control gel increased; the shift was less pronounced at lower temperatures but more remarkable at room and higher temperatures. In the case of the optimized gel, the pH increased, but the change was less than the control at all temperatures, which may be because the Betamethasone dipropionate was released from the vesicles and prevented the pH from rising **Table 6**. Because the pH of both the control and the optimized gels stayed within the physiologic pH range of the skin during the whole research, the outcomes were satisfactory **[26]**. The data's statistical analysis revealed appreciable variations in the outcomes for the pH change.

Spreadability

The therapeutic success of dermal gels depends on their capacity to disseminate, which also helps patients accept the drug. Gels exhibiting high-spread properties are advantageous because they facilitate easy, consistent drug application to the skin **[28]**. Spreadability has an impact on how gel behaves when it is packaged in tubes. The manufactured gel exhibited good Spreadability, as seen by the Spreadability values reported in **Table 7**, and the values stayed within acceptable bounds during the gel's storage duration. Whereas the Spreadability of the optimized gel rose during storage while remaining acceptable, the Spreadability of the control gel exhibited just a slight change. The Spreadability of carbopol gel was quite good **[17]**. Throughout the investigation, there was a considerable shift in the values of the four groups.

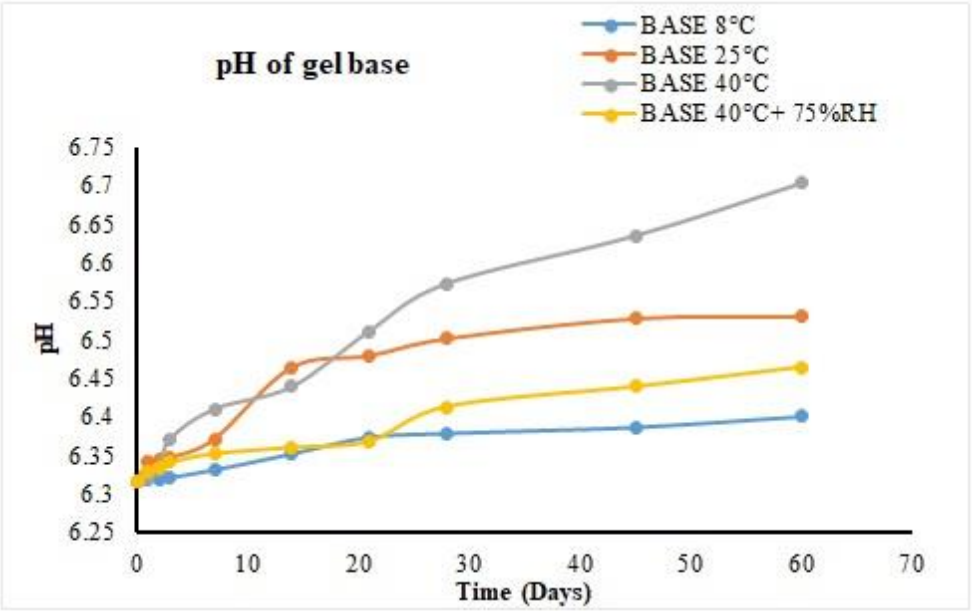
Table 5: Physical characterization of ethosomal gel kept at different temperatures.

Time		**Fresh**	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 45**	**Day 60**
Color	8°C	W	W	W	W	W	W	W	W
	25°C	W	W	W	W	W	W	SY	SY
	40°C	W	W	SY	SY	SY	SY	Y	Y
	40°C+ 75% RH	W	W	W	SY	SY	Y	Y	Y
Odor	8°C	NC	NC	NC	NC	NC	NC	NC	NC
	25°C	NC	NC	NC	NC	NC	NC	*	*
	40°C	NC	NC	*	*	*	*	*	**
	40°C+ 75% RH	NC	NC	*	*	*	*	**	**
Liquefaction	8°C	NC	NC	NC	NC	NC	NC	NC	NC
	25°C	NC	NC	NC	+	+	NC	NC	NC
	40°C	NC	NC	+	+	NC	NC	NC	NC
	40°C+ 75% RH	NC	NC	+	+	+	NC	NC	NC

RH=Relative Humidity, W= white, YW= Yellowish White, Y=Yellow, NC= no change Observed, += Slight Phase Separation/ liquefaction, *= A little Bit of Smell

Table 6: pH of base and ethosomal gel formulation stored at different temperatures.

Time (Days)	**BASE**				**FORMULATION**			
	8°C	**25°C**	**40°C**	**40°C+ 75%RH**	**8°C**	**25°C**	**40°C**	**40°C+ 75%RH**
Fresh	6.317	6.317	6.317	6.317	6.306	6.306	6.306	6.306
Day 1	6.318	6.341	6.321	6.329	6.310	6.309	6.315	6.322
Day 2	6.319	6.344	6.332	6.334	6.328	6.310	6.324	6.326
Day 3	6.321	6.347	6.371	6.341	6.354	6.321	6.328	6.332
Day 7	6.331	6.371	6.410	6.352	6.368	6.335	6.336	6.343
Day 14	6.352	6.464	6.439	6.360	6.377	6.354	6.344	6.349
Day 21	6.374	6.479	6.511	6.368	6.385	6.359	6.357	6.353
Day 28	6.379	6.501	6.573	6.413	6.416	6.366	6.365	6.373
Day 45	6.387	6.527	6.635	6.439	6.425	6.371	6.372	6.394
Day 60	6.401	6.530	6.703	6.464	6.432	6.381	6.380	6.425



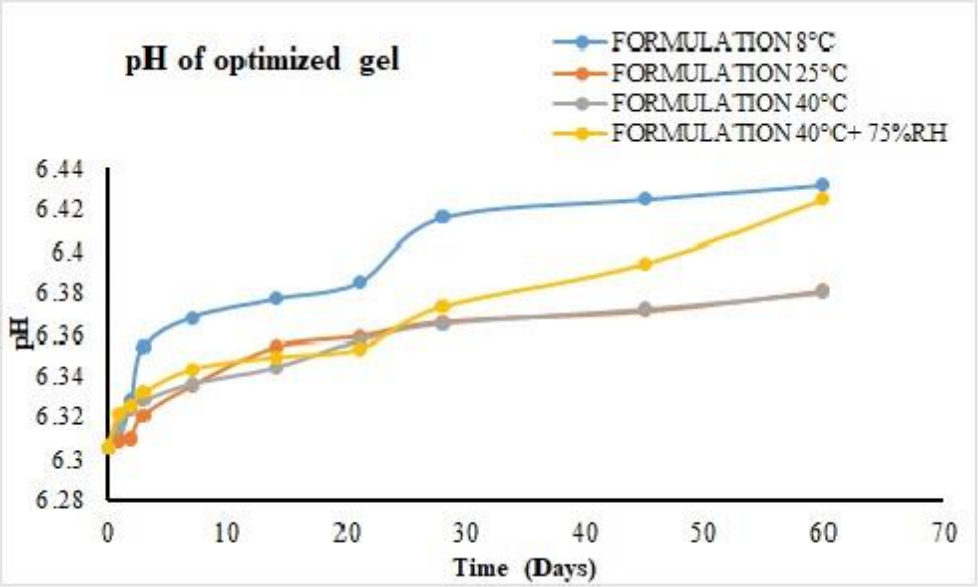


Figure 5: pH of the gel base and optimized gel at different temperatures.

Table 7: Spreadability studies of ethosomal gel at different temperatures.

Time	Spreadability (cm) BASE				Spreadability (cm) FORMULATION			
	8°C	25°C	40°C	40°C+ 75%RH	8°C	25°C	40°C	40°C+ 75%RH
Fresh	3.4	3.4	3.4	3.4	3.1	3.1	3.1	3.1
Day 1	3.6	3.6	3.5	3.6	3.5	3.6	3.7	3.8
Day 7	3.6	3.6	3.6	3.6	3.6	3.8	4.0	4.3
Day 14	3.7	3.6	3.7	3.7	3.6	3.9	4.2	4.4
Day 21	3.7	3.6	3.8	3.7	3.6	4.3	4.4	4.5
Day 28	3.7	3.6	3.9	3.8	3.7	4.4	4.5	4.3
Day 45	3.6	3.5	3.6	3.5	3.7	4.7	4.6	4.3
Day 60	3.5	3.3	3.5	3.3	3.8	4.8	4.6	4.6

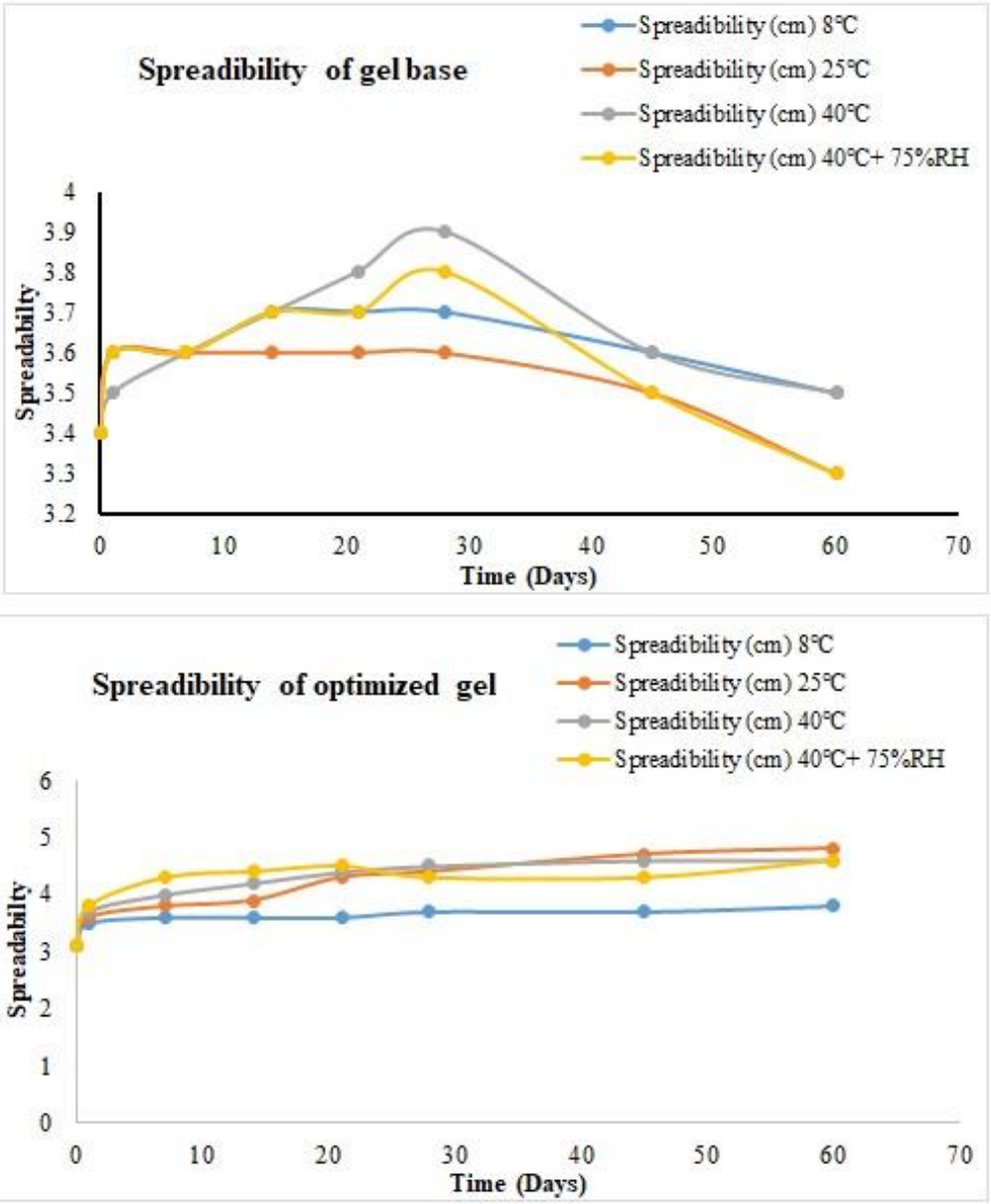


Figure 6: Spreadability of gel base and optimized gel at different temperatures.

Rheology

The viscosity of topical preparations is another crucial component that aids product distribution during topical administration and is also used to gauge drug penetration across the skin **[16]**. Thixotropic systems are characterized by a decrease in viscosity in response to an increase in shear rate **[19]**. The rheological changes occurred when optimized ethosomes gel and control gel was held for three months under varied storage settings. The ethosomes gel's initial viscosity value was higher than the control gel's, which could be explained by the ethosomes formulation's phospholipid component, which thickens the product **[16]**. However, as the gel formulation liquefies at higher temperatures, the viscosity of the ethosomes gel decreased while that of the control gel increased after the study. As the shear rate continued to increase, the formulations' viscosity dropped. Both the control gel and the gel containing ethosomes had flow index values that were smaller than unity, indicating the gel's ability to thin under shear. Throughout the stability period, the gel behaved consistently, which makes it the ideal trait for topical applications **[19]**.

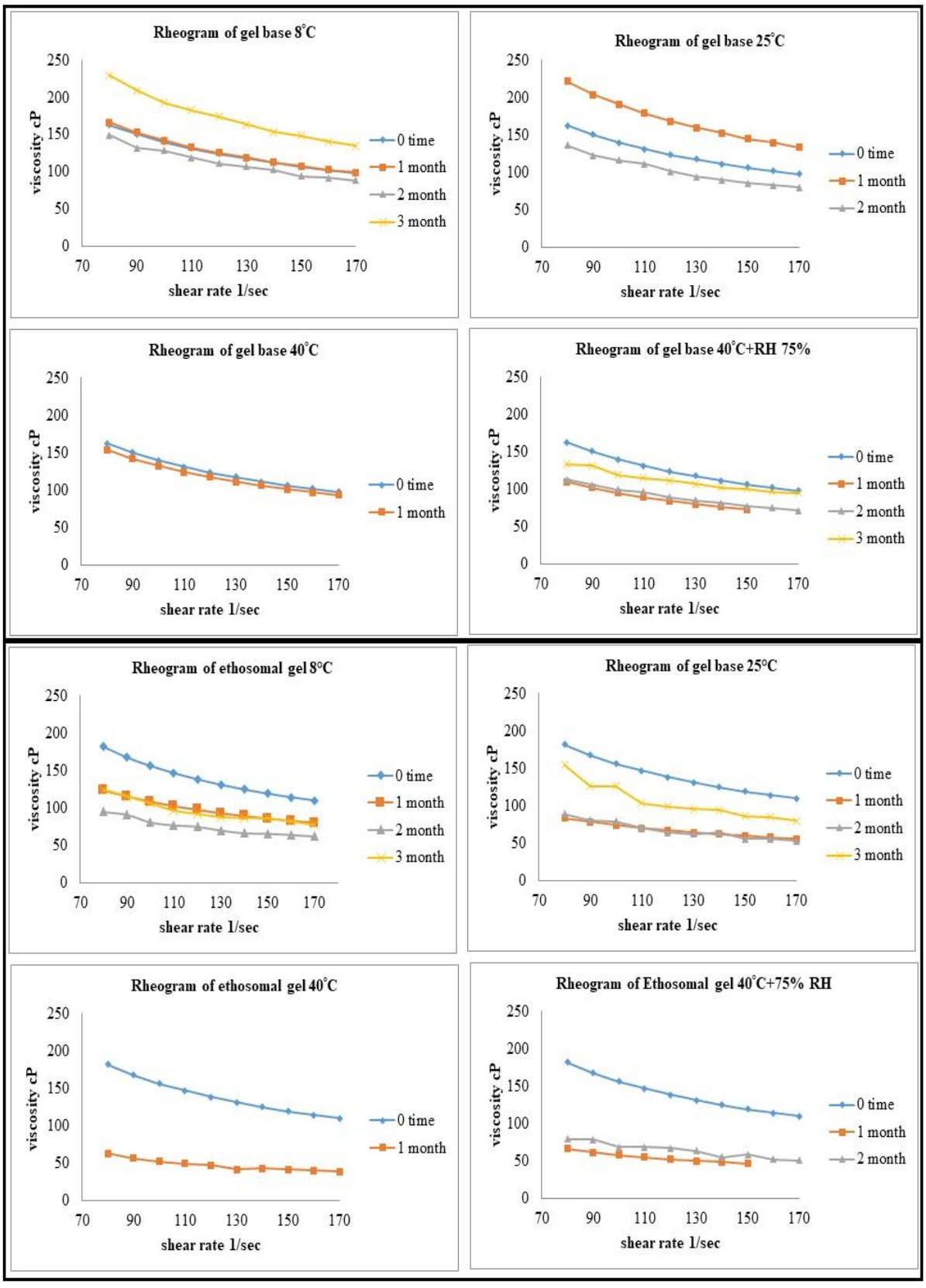


Figure 7: Rheogram of gel base and optimized ethosomal gel formulation at different temperatures.

CONCLUSION

A formulation of Betamethasone dipropionate was made using the traditional cold process demonstrated good stability. The developed formulations showed increase entrapment efficiency of betamethasone greater than 90%. The *in-vitro* release revealed that the drug released from the formulation in 12 hours. Betamethasone dipropionate ethosomes gel demonstrated more drug permeability across skin than the Betamethasone dipropionate control gel, according to investigations on the skin of male albino rats. The formulation variables were shown to be more stable at 8°C than at 25°C after two months of in-vitro stability tests on the suspension of ethosomes. For two months, a stability test on the ethosomes gel was carried out at 8°C, 25°C, 400°C, and 400°C+75%RH. The highest stability was seen for the parameters under investigation at 8°C. The formulations' rheological data indicated that they were suitable for topical application because they displayed thixotropic activity. In the future, the current study may be extended to the in-vivo animal study and humans based clinical evaluation for management of eczema.

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REFERENCES

1. Barry, B.W., Novel mechanisms and devices to enable successful transdermal drug delivery. European journal of pharmaceutical sciences, 2001. 14(2): p. 101-114. https://doi.org/10.1016/S0928-0987(01)00167-1
2. Hadgraft, J., Passive enhancement strategies in topical and transdermal drug delivery. International journal of pharmaceutics, 1999. 184(1): p. 1-6. https://doi.org/10.1016/S0378-5173(99)00095-2
3. Vinoth Kumar, S., Design and In-Vitro Evaluation of Simvastatin Ethosomal Gel. 2021,
4. Kassem, A.A., A. El-Alim, and S. Hosam, Vesicular Nanocarriers: a potential platform for dermal and transdermal drug delivery, in Nanopharmaceuticals: Principles and Applications Vol. 2. 2021, Springer. p. 155-209. https://doi.org/10.1007/978-3-030-44921-6_5
5. Touitou, E. and H. Natsheh, Topical Administration of Drugs Incorporated in Carriers Containing Phospholipid Soft Vesicles for the Treatment of Skin Medical Conditions. Pharmaceutics, 2021. 13(12): p. 2129. https://doi.org/10.3390/pharmaceutics13122129
6. Souto, E.B., Elastic and ultradeformable liposomes for transdermal delivery of active pharmaceutical ingredients (APIs). International Journal of Molecular Sciences, 2021. 22(18): p. 9743. https://doi.org/10.3390/ijms22189743
7. Richardson, L.D. and M. Norris, Access to health and health care: how race and ethnicity matter. Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine: A Journal of Translational and Personalized Medicine, 2010. 77(2): p. 166-177. https://doi.org/10.1002/msj.20174
8. Mehta, A.B. Topical corticosteroids in dermatology. Indian journal of dermatology, venereology and leprology, 2016. 82: p. 371. doi: 10.4103/0378-6323.178903
9. Lebwohl, M. Twice-weekly topical calcipotriene/betamethasone dipropionate foam as proactive management of plaque psoriasis increases time in remission and is well tolerated over 52 weeks (PSO-LONG trial). Journal of the American Academy of Dermatology, 2021. 84(5): p. 1269-1277. https://doi.org/10.1016/j.jaad.2020.09.037
10. Khurana, B., D. Arora, and R. Narang, Topical delivery of nanoemulsion for antipsoriatic drugs. Journal of Drug Delivery and Therapeutics, 2018. 8(5-s): p. 1-11. https://doi.org/10.22270/jddt.v8i5-s.1914
11. Lévêque, J.L. and D. Saint-Léger, Salicylic acid and derivatives, in Skin Moisturization. 2002, CRC Press. p. 377-388.
12. Lane, M.E., Skin penetration enhancers. International journal of pharmaceutics, 2013. 447(1-2): p. 12-21. https://doi.org/10.1016/j.ijpharm.2013.02.040
13. Dwivedi, D., et al., Investigating the potential of Quercetin enthused nano lipoidal system for the management of dermatitis. Research Journal of Pharmacy and Technology, 2021. 14(12): p. 6516-6526. http://dx.doi.org/10.52711/0974-360X.2021.01127
14. Benson, H.A. and J.C. McElnay, Topical non-steroidal anti-inflammatory products as ultrasound couplants: their potential in phonophoresis. Physiotherapy, 1994. 80(2): p. 74-76. https://doi.org/10.1016/S0031-9406(10)61010-3
15. Li, G., et al., Preparation and in vitro evaluation of tacrolimus-loaded ethosomes. The Scientific World Journal, 2012. 2012. https://doi.org/10.1100/2012/874053
16. Tiwari, R., et al., Development, characterization and transdermal delivery of dapsone and an antibiotic entrapped in ethanolic liposomal gel for the treatment of lapromatous leprosy. The Open Nanomedicine Journal, 2018. 5(1). http://dx.doi.org/10.2174/1875933501805010001
17. Ubaid, M., et al., Formulation and in vitro evaluation of carbopol 934-based modified clotrimazole gel for topical application. Anais da Academia Brasileira de Ciências, 2016. 88: p. 2303-2317. https://doi.org/10.1590/0001-3765201620160162
18. Patravale, V.B. and A.G. Mirani, Preparation and characterization of solid lipid nanoparticles-based gel for topical delivery, in Pharmaceutical Nanotechnology. 2019, Springer. p. 293-302.
19. Arora, D. and S. Nanda. Quality by design driven development of resveratrol loaded ethosomal hydrogel for improved dermatological benefits via enhanced skin permeation and retention. International journal of pharmaceutics, 2019. 567: p. 118448. https://doi.org/10.1016/j.ijpharm.2019.118448
20. Bisht, D., et al., Development of ethosomal gel of ranolazine for improved topical delivery: in vitro and ex vivo evaluation. Journal of molecular liquids, 2017. 225: p. 475-481. https://doi.org/10.1016/j.molliq.2016.11.114
21. Iizhar, S.A., et al., In vitro assessment of pharmaceutical potential of ethosomes entrapped with terbinafine hydrochloride. Journal of advanced research, 2016. 7(3): p. 453-461. https://doi.org/10.1016/j.jare.2016.03.003
22. Mushtaq A, Zulfiqar H, Arif S, Adnan M, ul Haq I, Hussain T. Formulation and evaluation of transdermal patch of lidocaine HCl for insect bite. Journal of Contemporary Pharmacy. 2023 Dec 31;7(2):50-6. https://doi.org/10.56770/jcp2023722
23. Sattar M, Sarfraz S, Liaquat U, Shoukat I, Ahmad B, Hussain T. Formulation and evaluation of topical piroxicam microemulgel for arthritis. Journal of Contemporary Pharmacy. 2023 Jun 30;7(1):16-23. https://doi.org/10.56770/jcp.2023713
24. David, S.R.N., et al., Formulation and in vitro evaluation of ethosomes as vesicular carrier for enhanced topical delivery of isotretinoin. International Journal of drug delivery, 2013. 5(1): p. 28.
25. Akhtar, N. and K. Pathak, Preclinical and clinical aspects of antimicrobial drugs delivered through ethosomal vesicles. Anti-Infective Agents, 2012. 10(1): p. 15-25.
26. Ramadon, D., E. Anwar, and Y. Harahap, In vitro penetration and bioavailability of novel transdermal quercetin-loaded ethosomal gel. Indian Journal of Pharmaceutical Sciences, 2018. 79(6): p. 948-956.
27. Akhtar, N. and K. Pathak. Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. AAPS pharmscitech, 2012. 13(1): p. 344-355. https://doi.org/10.1208/s12249-012-9754-y.
28. Sakran, W., et al., Ethosomal gel for rectal transmucosal delivery of domperidone: design of experiment, in vitro, and in vivo evaluation. Drug Delivery, 2022. 29(1): p. 1477-1491. https://doi.org/10.1080/10717544.2022.2072542.