RESEARCH ARTICLE

Perspective Impact of Gelling Agents on the Mechanistic Behavior for the Topical Delivery of Flufenamic Acid Nano-Ethosomal Dispersion

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ABSTRACT

Objective: This work involves the preparation and *in vitro* characterization of a topical gel formulation that is capable of deep skin permeation, providing the anti-inflammatory effect for the optimum nano ethosomal dispersion formula.

Methods: The optimum ethosomal dispersion was prepared (in our laboratory) by utilizing 3% flufenamic acid (FA), 30% ethanol, 1% phosphatidylcholine (PC), 10% propylene glycol, and 1% cholesterol by cold method and 20 minutes' ultra-sonication. Four ethosomal gel formulas (G1-G4) were prepared by using carbopol 934 and carbopol 940 at 1% and 1.5% concentration at a 1:1 ratio as a gelling agent. These formulas were further subjected to *in vitro* characterization to assess their physical appearance, consistency, viscosity, spreadability, *in vitro* drug release, and *ex-vivo* skin permeation and deposition.

Results: The results revealed that the formula (G1) demonstrated the best homogeneity, consistency, and spreadability as well as an initial release of 68.53% after 10 hours, that continued up to 89.36% after 24 hours, and a significantly higher drug release percentage at pH 7.4 than pH 5.5 with substantially higher *ex vivo* abdominal rat skin permeation (60.74%) and deposition percentages (36.87%%) in comparison to flufenamic acid plain gel prepared conventionally which demonstrated 23.73% and 14.96% skin permeation and deposition percentages after 24 hours.

Conclusion: This work was successful in preparing a novel topical gel using ethosomal nanocarriers, promoting efficient topical skin delivery of the anti-inflammatory FA with a once-daily application, which improved patient compliance.

Keywords: Carbopol, Flufenamic acid (FA), Nano ethogel, Topical delivery.

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INTRODUCTION

Topical (dermal) delivery is the application of the drug to the skin itself with little or no systemic effect. On the other hand, transdermal delivery is the application of the dosage form to the skin to reach the systemic circulation to produce a therapeutic effect.[1] Topical delivery has many advantages over traditional drug delivery (oral and intravenous), such as conferment of sustained drug release, sufficient concentration of active pharmaceutical ingredients at the target site, and minimizing the dose and dose frequencies. The fluctuations in plasma drug levels and intra-individual variability can be lowered, and the first-pass metabolism, enzymatic and pH-dependent degradation can be avoided.[2,3] The lipid-based vesicles ethosomes have been developed as a novel strategy to overcome the stratum corneum (SC) barrier of the skin and to improve the topical delivery of drugs.[4] Since ethosomes are predominantly prepared in a dispersion form, their skin retention period throughout the application can be extended by integration into a carrier, for example, an ointment or gel base and a transdermal patch. As the hydrogels hold together with a cross-linked network, they can provide an efficient skin prolongation time for dispersions. Additionally, they are extremely porous, encouraging lipid vesicles to be loaded into the gel matrix and subsequently released. Moreover, they can be easily applied on the skin surface, improving patient compliance. In addition, the gel form was selected because it has better skin penetration than other semisolid dosage forms (cream and ointment). Both synthetic (carbomer and hydroxyl propyl methylcellulose) and natural (such as xanthan gum and guar gum) polymers are generally used for the preparation of hydrogel. Nevertheless, synthetic polymers are more widely utilized rather than natural ones. The most widely used synthetic polymer is CarbopoL® (carbomer) due to the superiority of its physical and rheological characteristics, and

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no skin irritation or lipid vesicle stability issues have been observed with it.[5,6]

Carbopol is a colloidal water-soluble, biodegradable polymer with mucoadhesutization and pH-sensitive properties. It is made up of poly acrylic acid chains that have been cross-linked with 0.75 to 2% of cross-linking agents, for example, polyallyl sucrose or polyallyl pentaerythritol. It is primarily used to prepare semisolid and liquid dosage forms such as gel, suspension, and emulsion employed for topical administration. It is categorized as a thickening agent, and it has been typically used to modify the kinetics of drug release.[7]

Flufenamic acid (FA) is an anthranilic acid-based non-steroidal anti-inflammatory drug with potent analgesic and anti-inflammatory effects. It is classified as class II in the bio-pharmaceutical classification system (BCS) and has a pH-dependent solubility.

This work aims to prepare a topical drug delivery system for flufenamic acid utilizing ethosomal technology with suitable rheological properties, effective permeability, and prolonged release to be given once daily to treat topical inflammation.

MATERIALS AND METHODS

Materials

Cholesterol was purchased from Alpha Chemika, India. Soya lecithin (purity 98%) was purchased from Baoji Guokang Bio-Technology Co., Limited, and FA (purity 98%) was purchased from Shanghai Ruizheng Chemical Tech Co., Ltd. Sodium lauryl sulfate, propylene glycol, sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Thomas baker, India. Carbopol 934 was purchased from Xi'an Prius bioengineering, China, and carbopol 940 from Hangzhou hyper chemicals. Triethanolamine was purchased from Hopkins and Williams Ltd, England, and ethanol from Scharlau, Germany.

Methods

Preparation of the Gel Base

Carbopol 934 gel and carbopol 940 gel (1%) and (1.5%) w/v: Accurately 1 and 1.5 gm respectively of each carbopol powder were dispersed in 80 mL deionized water with continuous stirring using a magnetic stirrer at 700 rpm and 30°C for about 30 to 60 min. The volume was completed to 100 ml by deionized water. A few drops of triethanolamine (TEA) were added to neutralize the pH to 7. A transparent viscous gel was formed, which was stored in the refrigerator for one night to remove all the air bubbles.[8,9]

Preparation of Nano Ethosomal Gel (Nano Ethogel)

The optimum nanoethosomal dispersion formula (which was prepared in our laboratory) containing 1% phosphatidylcholine (PC), 30% ethanol, 10% propylene glycol (PG) and 1% cholesterol was selected to prepare four nano ethogel formulas (G1-G4) containing 3% w/w FA using two different percentages (1 and 1.5%) of each gelling agent (carbopol 934 and carbopol 940). This was accomplished by the incorporation of the dispersion drop by drop to the gel base (gelling agents) in a ratio of 1:1 (ethosomal dispersion: gel base) with continuous gentle stirring by a spatula until a creamy white gel was achieved.[10] The content of the nano ethogel formulas is recorded in Table 1.

The prepared ethogel formulas were compared with the conventional plain gel (containing no ethosomal vesicles) of FA, which was prepared by dissolving the pure drug (3% w/w) in a minimum volume of ethanol (10% v/w), and then this solution was incorporated drop by drop to 1% w/v carbopol 934 gel base with continuous mixing by spatula until a gel was achieved.[11]

Characterization of the Prepared Nano Ethogel

Physical Appearance

All the prepared nano ethogel formulas (G1-G4), as well as the plain FA gel, were characterized by visual inspection for any phase separation or precipitation, color, homogeneity, grittiness, and consistency.[12]

pH Determination for Ethogels

The pH of all nano ethogels (G1-G4), as well as the plain FA gel, were investigated. This was accomplished by immersing the pH-meter electrode tip in 20 gm of each gel for one minute and recording the pH reading.[13]

Ethogels Viscosity Determination

This study was accomplished by Brookfield Digital viscometer. A sample of 25 gm of each ethogel formulas (G1-G4), as well as the plain FA gel, were appropriately weighted and placed in a glass vial, S-64 spindle was positioned inside the glass (without touching the bottom), and the viscosity in centipoise was measured at room temperature after 30 seconds at 1, 2, 2.5, 5, 10, 12, 20, 50, 60, and 100 rpm.[14]

Spreadability Determination of Nano Pathogens

In this study, the specific weight of 1-gm of each ethogel formula (G1-G4) and the plain FA gel were weighted and placed between two 20×20 cm glass slides. The diameter of the circle formed from the spread gel was measured. Then, 500 gm weight was applied over the outer glass; the gel will spread more, forming a circle with a larger diameter. After 5 minutes, when no further spreading was expected, the weight was removed and the diameter of the circle was measured. The difference between the two diameters was estimated as the spreadability in cm.[15]

Drug Content Determination

A sample of about 0.5 gm of nano ethogel formulas (G1-G4) and the plain FA gel were weighed and placed in a 100 mL volumetric flask with methanol up to 100 mL, then placed in the bath sonicator for 1 hour. Ten ml was taken and centrifuged

Table 1: Composition of the nano ethogel formulas prepared using the selected nanoethosomal formula

Formulas	*Gelling agent w/v*	*Ratio**
G1	Carbopol 934 1%	1:1
G2	Carbopol 934 1.5%	1:1
G3	Carbopol 940 1%	1:1
G4	Carbopol 940 1.5%	1:1

*Ratio: Ratio of ethosomal dispersion: gel base.

at 3000 rpm for 15 minutes. The supernatant was collected and diluted appropriately. To estimate the drug content, the absorbance was examined in the UV-visible spectrophotometer at 288 nm λ max.[16]

In vitro Release of the Drug from the Prepared Ethogels

The *in vitro* release study of FA from all nano ethogels (G1-G4) as well as the plain FA gel was done using a dialysis membrane (3500 MWCO) in 100 mL phosphate buffer (PB) pH 7.4 containing 0.1% w/v sodium lauryl sulfate (SLS) by taking one gram of each formula and placing it in the dialysis bag which was sealed from both ends and then immersed in the release medium which was magnetically stirred at 100 rpm at 37 ± 0.5°C. At predetermined time intervals, accurately three mL aliquots were taken and replaced immediately with an equal volume of the fresh medium. The absorbance of each sample was determined using a UV-visible spectrophotometer at 288 nm λ max, and the assessment of the percentage of release was determined according to the calibration curve equation.[17,18] Furthermore, the release study was also determined in 100 mL of PB pH 5.5 containing 0.1% SLS for the nano ethogel formula (G1) in comparison to FA plain gel.

Selection of Optimum Nano Ethogel Formula

The optimum nano ethogel formula (G1) was selected on the basis of its percentage of release, spreadability, homogeneity, drug content, and viscosity.

Drug and excipient compatibility study by FTIR

A FTIR spectrophotometer was used to determine the compatibility of the drug (FA) with other additives in the selected formula. It was applied for the pure drug, 1:1:1:1 physical mixture of carbopol 934: cholesterol: PC: FA, and nano ethogel formula (G1), KBr was used to compress the samples into pellets, while for ethogel (G1), the KBr cell was used by dripping numerous drops of the sample on the cell and covering it with another cell (sandwiching the sample between two KBr cells) to ensure no trapped air. Both the pellets and the cells were scanned between (4000–400) cm^{-1}.[19]

Human Skin Irritation Test (*In vivo* Study)

The skin irritation potential of selected nano ethogel formula G1 was performed on 20 healthy volunteers who were not taking any topical medication by applying one gram of the ethogel (G1) to a 5 cm^2 area of the back of the hand, then covered by a piece of gauze and kept for 10 hours. The gel was removed, and the volunteers were examined for any sign of redness or irritation. The identical procedure was repeated daily for 7 days, and the scores of erythema and edema were recorded.[20,21]

Ex vivo studies

In *ex vivo* studies, abdominal rat skin was used to study ex-vivo permeation and ex-vivo deposition percentages of the drug-using Franz cell.

(A) Preparation of Rat Skin

A female albino rat, about 210 gm and 8 weeks old, was selected to perform the *ex vivo* permeation study. The abdominal skin was excised, a razor blade carefully removed the hair without damage to the skin surface. The excess of subcutaneous fat tissue was withdrawn by treating the skin with diethyl ether solvent. The skin was washed with a 0.9% w/v NaCl solution, aluminum foil was used to wrap it, and it was stored in a freezer. Before starting the experiment, the skin was hydrated with PB (pH 7.4) for 60 minutes at 37 ± 0.5 °C.[22]

(B) Ex vivo Permeation Study

This study was carried out using a glass Franz cell having a 50 mL capacity receptor compartment and an 8 cm^2 diffusion area. The rat skin separated the two cell compartments in such a way that the SC layer of the skin faced the donor compartment and the dermal layer faced and touched the receiver compartment, which contained 50 mL of PB pH 7.4 (with 0.1% SLS) and was magnetically stirred at 100 rpm at 37 ± 0.5°C. One gem of the selected nano ethogel G1as well as the FA plain gel were applied (each one separately) on the skin surface in the donor compartment, which was covered with parafilm to prevent ethanol evaporation. Over a period of 24 hours, 3 mL was collected from the receiver medium at a regular interval and replaced immediately with the same fresh medium to maintain the sink condition. Care was taken to prevent bubble formation during sample collection. The absorbance for each sample was estimated at 288 λ max.[23]

(C) Ex vivo Skin Deposition Study

The percentage of FA (from G1 and FA plain gel, each one separately) deposited in skin rat was determined after the completion of the 24 hours skin permeation study. The excess nano ethogel G1 and the plain FA gel on the skin surface were removed by wiping it with PB-soaked cotton and washing it three times with the same solution. The skin was cut into 16 small pieces and soaked in 10 mL of methanol for 8 hours, then sonicated in a bath sonicator for 2 hours. Each sample was taken, filtered by 0.22 μm Millipore, suitably diluted, and the absorbance was analyzed by using a UV-visible spectrophotometer, and the percentage of deposition was estimated.[24]

Stability Study

Stability testing according to ternational Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines was used to determine the stability of the selected nano ethogel formula (G1). The experiment was conducted by keeping the selected formula at three different temperatures 4°C, 25, and 40 °C, for 1-month. The formula was stored in a glass vial covered with parafilm, and its physical appearance, pH, drug content, and *in vitro* release were examined.[25]

Statistical Analysis

The experimental data were presented as standard deviation (SD) mean. For statistical analysis, a one-way ANOVA with Tukey's test and paired sample test were used at 95 percent significance (pairwise comparisons), at which significant

results are equal to ($p < 0.05$) and non-significant results are equal to ($p > 0.05$).

RESULTS AND DISCUSSION

Characterization of the Prepared Nano Ethogel

Physical Appearance

All the nano ethogel formulas (G1-G4) presented as a creamy white and homogenous ethogel with no precipitation and acceptable consistency. The plain FA demonstrated a less homogenous appearance with no precipitation.

pH Determination

The pH values of all nano ethogels formulas were (G1 = 6.35 ± 0.03, G2 = 6.17 ± 0.1, G3 = 6.18 ± 0.07 and G4= 6.01 ± 0.3) and FA plain gel was 6.16 ± 0.1, indicating that they have a low risk of causing skin irritation.[26]

Ethogels Viscosity Determination

The viscosity of each nano ethogel formula (G1-G4) and FA plain gel were assessed at various speeds (shear stress), and the results were presented in Table 2. The results revealed that all nano ethogel exhibited a pseudoplastic flow. In other words, as the shear rate increased, the apparent viscosity decreased immediately because the normally disorganized molecules of a gelling agent were forced to align their long axes in the flow direction, reducing internal resistance and resulting in lower viscosity.[27]

Formulas containing 1.5% gelling agent (G2 and G4) are significantly ($p < 0.05$) more viscose than formulas with 1% (G1and G3) of the same polymer (means as the concentration of gelling agent increased, gel viscosity increased accordingly). This is because the polymer concentration increased the degree of cross-linking, which caused more water molecules to be trapped and held in the carbopol structure, which resulted in a vicious and rigid structure.[28,29] Similar results were observed with *Quercus infectoria* extract herbosomal gel.[30]

Furthermore, formulas containing carbopol 940 (G3 and G4) are more viscous than formulas containing carbopol 934 (G1 and G2) with the same polymer concentration. This could be attributed to the difference in the cross-linking density between the two grades of carbopol polymer (carbopol 940 has a higher cross-linking density than carbopol 934).[31] The same results were reported with topical emulgel of clarithromycin.[32] The FA plain gel (containing 1% carbopol 934) exhibited significantly ($p < 0.05$) higher viscosity than the nano ethogel G1 formula containing the same concentration of carbopol 934.

Spreadability Determination of Nano-Ethogels

When applied to the skin or affected part, spreadability refers to the size of the area over which the gel spreads easily. The spreading value of a gel affects its bioavailability and the bioavailability of a gel is influenced by its spreading value.[33] The spreadability of a gel is highly dependent on its viscosity, firmness, formulation temperature, shear time, and application area. The results of the nano ethogel formulas (G1 = 4.1 cm ± 0.12, G2 = 3.0 cm ± 0.17, G3 = 3.6 cm ± 0.09 and G4 = 2.7 cm ± 0.3) showed good spreadability for topical delivery. The lowest spreadability (2.7 cm) was expressed by the most vicious nano ethogel formula (G4), while the highest spreadability was displayed by the lowest viscosity nano ethogel formula (G1) (4.1 cm). Similar results were observed with loxoprofen sodium transferosomal gel.[34] Since the FA plain had a higher viscosity than G1 (both of them containing 1% carbopol 934), it demonstrated a lower spreadability (3.1 cm ± 0.11) than G1.

Drug Content Determination

The results of the drug content of all nano ethogel formulas were (G1 = 99.61% ± 1.8, G2= 99.53% ± 2.1, G3= 98.89% ± 2.6 and G4= 100.03% ± 1.1) as well as for FA plain was 95.22% ± 2.3. This corresponded to the acceptable range of USP (85-115 percent), demonstrating that the preparations had high adequacy and high content uniformity.[35]

In vitro Release of the Prepared Nano Ethogels

The *in vitro* release study was done for all nano ethogel formulas (G1-G4) as well as the FA plain gel in PB pH 7.4 prepared with 0.1% SLS and the results are depicted in Figure 1.

The nano ethogel formulas (G1-G4) prepared by nano-ethosomal technology exhibited a significantly ($p < 0.05$) higher *in vitro* release than the FA plain gel (where the plain gel released 3.44, 33.64, and 51.58% of the drug within 1, 10 and 24 hours, respectively) because they were prepared with a higher percentage of ethanol (30%). They contained 10% PG,

Table 2: Viscosity in centipoise of nano pathogens (G1-G4) and FA plain gel measured at room temperature in various shear stress values are mean (n = 3)

Speed (rpm)	*G1 ± SD*	*G2 ± SD*	*G3 ± SD*	*G4 ± SD*	*FA plain gel ± SD*
1	32404 ± 1.4	77106 ± 7.6	44177 ± 2.2	79412 ± 3.5	75306± 9.2
2	28161 ± 2.4	47243 ± 5.6	27014 ± 8.6	47176 ± 5.8	45422 ± 6.9
2.5	24125 ± 1.7	42634 ± 5.9	21722 ± 9.4	45598 ± 7.7	38800 ± 1.6
5	15665 ± 1.2	25843 ± 8.2	15218 ± 9.8	28707 ± 6.4	23146 ± 3.3
10	10757 ± 4.7	14372 ± 7.7	10146 ± 1.7	16952 ± 2.3	13615 ± 5.3
12	9266 ± 3.2	12706 ± 6.8	8370 ± 8.9	15156 ± 6.2	11936 ± 9.0
20	6777 ± 1.9	8660 ± 1.4	5388 ± 4.3	9460 ± 5.1	8071 ± 5.9
50	2830 ± 3.9	5100 ± 8.4	3358 ± 8.1	4642 ± 2.2	4220 ± 7.1
60	2462 ± 6.8	4210 ± 5.2	2952 ± 6.4	4304± 6.5	3742 ± 8.8
100	1840 ± 4.9	3199 ± 1.5	2109 ± 2.8	3964 ± 1.8	2682 ± 7.8

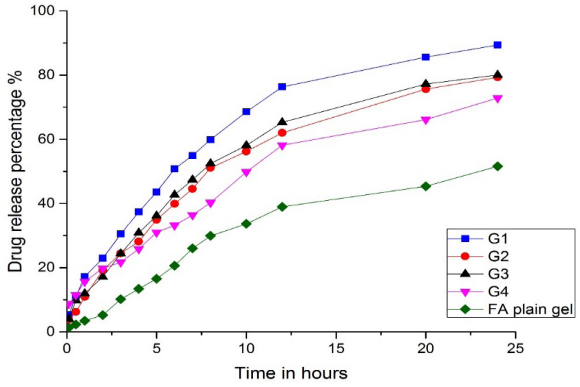

Figure 1: *In vitro* release profile of flufenamic acid from nano ethogel formulas (G1-G4) and flufenamic acid plain gel in phosphate buffer solution (pH 7.4) with 0.1% sodium lauryl sulfate.

which increased the solubility of FA in these formulas since it is highly soluble in these solvents. In addition, the ethogel contained elastic vesicles (ethosomes) in nano-size, which increase the solubility by increasing the surface area.[36,37] While the plain gel was prepared by dissolving the drug is only 10% ethanol without introducing the vesicles. This was inconsistent with the reported superior *in vitro* release property of ethosomal gel over plain melatonin gel.[38]

The nano ethogel formula G1 (containing 1% carbopol 934) showed a significantly ($p < 0.05$) higher drug release than G3 (containing 1% carbopol 940) where G1 gave 17.24, 68.53, and 89.36% within 1, 10, and 24 hours, respectively, while G3 gave 11.93, 58.08, and 80.02% within 1, 10, and 24 hours, respectively as shown in Figure 1. This is because carbopol 940 has a higher cross-linking density than carbopol 934, resulting in a higher gel viscosity, which makes the matrix more tortuous for the drug to diffuse through, causing lower drug release.[39] The same results were observed with G2 and G4 containing 1.5% of two different carbopol grades. Where G2 exhibited a significantly ($p < 0.05$) higher FA release after 10 and 24 hours than G4. These results agreed with the reported release of anti-fungal itraconazole from carbopol 934 emulgel and carbopol 940 emulgel.[40] Also, they agreed with the higher release of meloxicam from the niosomal carbopol 934 hydrogel.[41]

The results revealed that as the concentration of gelling agent increased from 1% (G1) to 1.5% (G2), a significant ($p < 0.05$) decrease in the drug release occurred where the formula G2 (1.5% carbopol 934) gave a release of 56.20% after the initial 10 hours and 79.32% after 24 hours. The same results were observed with G3 (containing 1% carbopol 940) and G4 (containing 1.5% carbopol 940). This is due to increasing gel viscosity upon increasing gel base concentration and the formation of a more rigid structure, leading to lower FA release.[42] Similar results were observed with fluconazole topical gel.[43]

The results revealed that the nanoethogel formula (G1) showed the highest release profile at PB pH 7.4 than all ethogel formulas as well as the FA plain gel. The release of

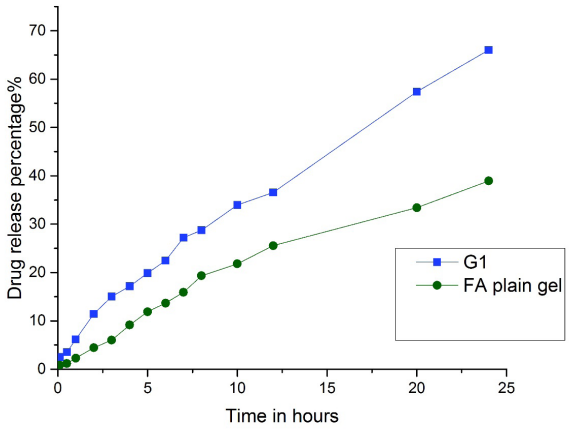

Figure 2: *In vitro* release behaviors of nano ethogel G1 and flufenamic acid plain gel in phosphate buffer pH 5.5 containing 0.1% sodium lauryl sulfate.

the drug from the G1 formula was also followed in PB pH 5.5 in comparison to the release of the drug from the plain gel for further investigation of the contribution of ethosomes to drug release. Figure 2 shows the *in vitro* release profiles of the nano ethogel formula (G1) and the FA plain gel (both of them prepared using 1% carbopol 934 as a gel base) in PB pH 5.5 containing 0.1% SLS. The G1 formula outperformed the plain FA gel in *vitro* release at pH 5.5 when the plain gel showed 2.28% (within 1 hour) and 15.90% (within 10 hours) and 38.94% (after 24 hours) while G1 gave 6.15% (within 1-hour), 27.22% (within 10 hours), and 65.99% (after 24 hours) respectively. This could be attributed to the significant ($p < 0.05$) high viscosity of plain gel that may delay the diffusion of the drug.[44]

In general, FA release from (G1) formula and plain gel at pH 5.5 was significantly ($p < 0.05$) lower than that at pH 7.4. This is related to the pH-dependent solubility of weak acid FA (pKa 4.17).[45]

The *in vitro* release study for formula G1 exhibited a significantly ($p < 0.05$) higher release profile than the FA plain gel formula in both PB pH 7.4 and PB pH 5.5, which reflects the significant contribution of the presence of ethosomes included in the G1 formula in comparison to the plain gel prepared by the conventional method containing the same gelling agent.

Selection of Optimum Nano Ethogels Formula

The formula G1 was selected as an optimum nano ethogel formula based on its highest percentage of release, acceptable viscosity and highest spreadability, good homogeneity, suitable pH, high drug content (99.61%). This formula was subjected for further study.

Drug and Excipient Compatibility Study by FTIR

The FTIR spectrum of pure FA powder was identical to the reported spectrum indicated the drug used is of high purity.[46] The same characteristic peaks of FA were presented in the physical mixture (FA, PC, cholesterol and carbopol 934 in ratio 1:1:1:1) with less intensity and in the selected nano ethogel

G1 with little or no shifting (as demonstrated graphically in Figure 3 with the absence of new peaks formation, proving the compatibility and uniformity of the FA with the excipients in selected ethogel formula.[47,48]

Human Skin Irritation Test (*In vivo* study)

The *in vivo* investigation of the optimized nano ethogel formula (G1) revealed that there was no evidence of skin irritation such as erythema, edema, or ulceration following the application of the optimal formula. Additionally, after a week of daily application, there were no signs of irritation with 0 edema score achieved by the 15 volunteers, confirming that no irritation was observed from single or repeated applications and no skin sensitivity reaction. This indicates that ethanol in the nano ethogel formula (G1), even with its high concentration (30%), is incapable of inducing skin erythema, and this formulation is safe for topical delivery.[49] This result is identical to the previously reported no skin irritation and 0 edema score achieved by volunteers who were subjected to an ethosomal gel formulation containing the antioxidant rutin.[50]

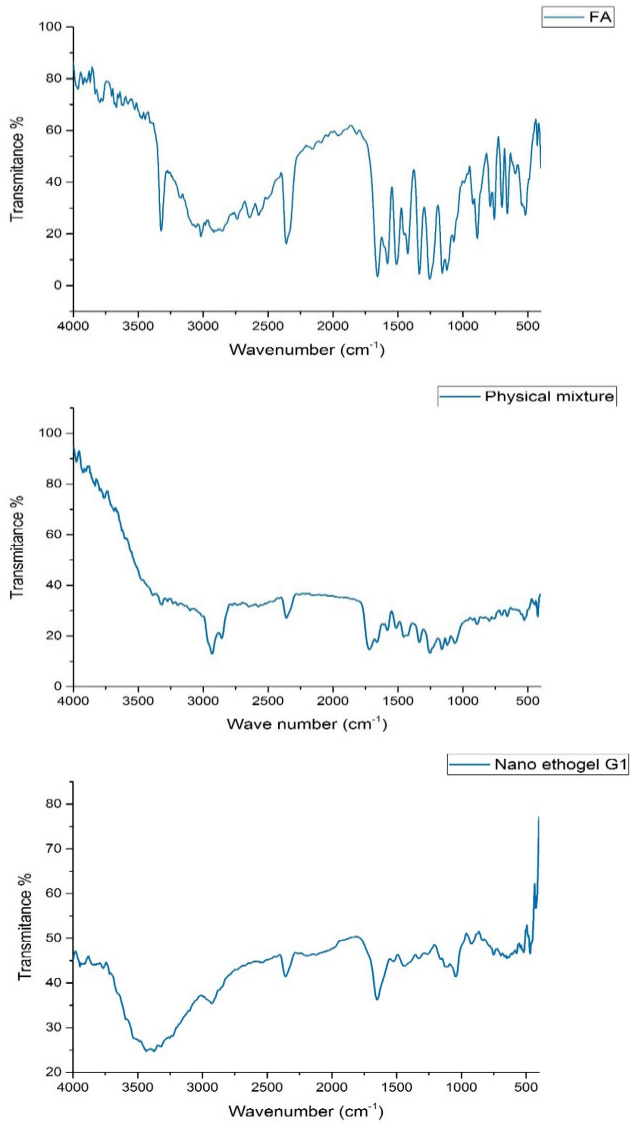

Figure 3: FTIR spectrums of pure flufenamic acid, physical mixture 1:1:1:1 of carbopol 934: cholesterol: PC: FA, and nano ethogel G1, respectively.

Ex vivo Studies

Ex vivo Permeation Studies

The percentage of FA from nano ethogel (G1) and FA plain gel permeated through abdominal rat skin were estimated up to 24 hours and plotted as a function of time and the results are depicted in Figure 4. The results demonstrated that the nano ethogel (G1) exhibited a 2.5 times higher percentage of skin permeation than plain gel after 24 hours with the values of 60.74% versus 23.73%, respectively.

Ex vivo Deposition Study

The FA-loaded ethosomal gel facilitated a higher rate of skin drug deposition after 24 hours (36.87%) when compared to plain gel (14.96%).

The superior ex vivo performance of ethogel over plain gel in permeation and deposition studies could be attributed to two factors: first, the fluidity and elasticity of ethosomes as a carrier with a small particle size of less than 300 nm that facilitates FA perpentation and fusion in the deeper skin layer, resulting in its accumulation and serve as a reservoir allowing continuous drug delivery by interaction of the biocompatible and biodegradable PC in the binary ethosome (G1) with the phospholipids of the epidermal layer.[51-53] Second, the penetration enhancing effect of ethanol and PG ensures vesicle elasticity and results in a higher percentage of FA permeate due to SC layer hydration, increasing the fluidity of the SC lipid bilayer and intracellular lipid extraction. Moreover, they have the ability to increase the thermodynamic activity of FA.[54,55] These results correspond to previous research that found griseofulvin ethosmal gel had better skin permeation and deposition than conventional one.[56]

Stability Study

The effect of different storage temperatures on the physical properties of selected nano ethogel (G1) was determined by measuring its physical appearance, pH, drug content, and

Table 3: Stability testing of the selected nano ethogel Formula (G1) at various temperatures (4°C, 25°C and 40°C after 1 month), values are mean (n = 3):

Parameters	*Initial results*	*4 °C ± SD*	*25 °C ± SD*	*40 °C ± SD*
Physical appearance	+++ good	+++ good	+++ good	++ good
pH	6.35 ± 0.03	6.31 ± 0.3	6.27 ± 0.2	6.18 ± 0.03
Drug content %	99.61 ± 0.1%	99.53 ± 0.2%	99.30 ± 0.7%	98.85 ± 0.3%
Release %	89.36 ± 0.5%	89.20 ± 0.7%	88.17 ± 0.5%	86.54± 0.4%

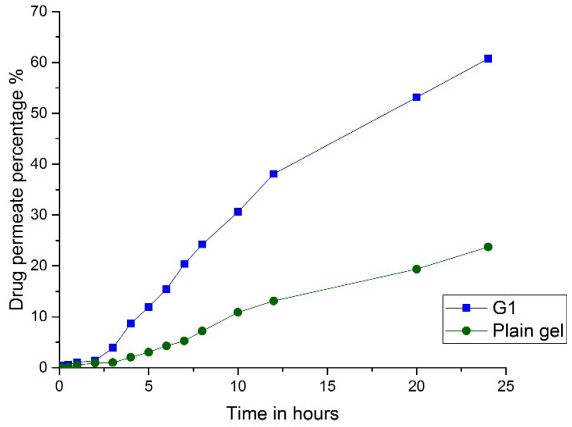

Figure 4: The *ex vivo* rat skin permeation profile of flufenamicc acid from the selected nano ethogel (G1) and plain gel in phosphate buffer pH 7.4 containing 0.1% sodium lauryl sulfate.

in vitro release, and the results were depicted in Table 3. The results showed that there is a non-significant ($p > 0.05$) difference in all the parameters after one month storage period at 4, 25 and 40 °C, demonstrating that the prepared nano ethogel has a high degree of stability.

CONCLUSIONS

This study was successful in preparing a topical gel using nanoethosomal technology with natural lipid (soya lecithin) and carbopol 934 as a gelling agent in 1:1 ratio that provided a sustained release of flufenamic acid for up to 24 hours with superior abdominal rat skin permeation and deposition, spreadability, and *in vitro* release in comparison to the prepared flufenamic acid plain gel, which provided an effective topical delivery that can be given once daily leading to Improve patient compliance.

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