Original article

Polymeric ethosomal gel loaded with nimodipine: Optimisation, pharmacokinetic and histopathological analysis

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Abstract

This study was performed with the main objective of formulating and evaluating the potential of ethosomal gel (Etho gel) to deliver nimodipine (NiM) for cardiovascular disease, a potent water insoluble anti-hypertensive drug via skin to reach the deeper layers of skin. The Box-Behnken design (BBD) was used to optimize the NiM-Eth to determine the impact of the independent and depended variables. The effectiveness of drug entrapment, vesicle size, and cumulative drug release were assessed for the NiM loaded ethosomes and NiM-Eth gel using carbopol 934 as a gelling agent. Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Power X-ray diffraction (PXRD), and scanning electron microscopy (SEM) analysis were performed and analysed their physicochemical characters. Rat abdomen skin was used to investigate drug permeability and deposition. As compared to marketed products, NiM-Eth gel produced an improved drug permeability in ex vivo experiments. The mean AUC0 to AUC0−1 of NiM-Eth gel when compared to oral formulation (Nymalize oral preparation) was found to be increased from 4.1 to 5.9 folds which was found to be resulted from first pass effect. Histopathological findings revealed that the maximum amount of NiM penetrated the stratum corneum of the skin and create drug depots in the deep layer. In summary, it can be said that NiM might be successfully prepared in NiM-Eth gel for transdermal drug delivery.

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1. Introduction

Cardiovascular diseases such as hypertension, myocardial infarction and ischemic stroke are treated by a major category of drugs called anti-hypertensives that aids in lowering the blood pressure. Anti-hypertensives have been classified based on its mechanism of action into diuretics, AT II receptor antagonists, beta-blockers, ACE inhibitors, and calcium channel blockers vasodilators (Khalil and Zeltser, 2022). Nimodipine (NiM) acts as an antihypertensive, a potent calcium channel blocker and also as a vasodilator agent owing to its employment in various cardiovascular diseases.

For transdermal and dermal drug delivery, skin serves as a barrier. It comprises of several layers through which the drug molecules penetrates and enters the systemic circulation. At times, it becomes difficult to attain the required blood-drug concentration which could be overcome by using penetration enhancers in the preparation (Alkilani et al., 2015). Liposomal delivery of drugs could possibly transport the active agents only to the superficial layers of skin which was helpful only in the local delivery of drugs, whereas ethosomes, a phospholipid vascular system have greater diffusion of drugs into the skin owing to the presence of high amounts of ethanolic content and hence found appropriate for lipophilic and hydrophilic molecules (Hussain et al., 2017).

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Transdermal or dermal route of drug administration has appreciable advantages and several novel strategies are being developed to progress the penetration ability of poorly permeable drugs. As a result, novel vesicular nanocarrier system of ethosomes has been a chief breakthrough in transdermal drug delivery. Ethosomes enhance the penetration of drugs due to the presence of ethanolic content by improving the fluidity of cell membrane (Verma and Pathak, 2010). With high deformability ethosomes can beneficially transport the therapeutic agent into the deepest layers of skin through the stratum corneum when compared to liposomes which is a crucial step for topical drug delivery systems. Furthermore, ethosomes could efficiently transport hydrophobic and hydrophilic substances intracellularly in addition to the permeation of antibiotic peptide (Zahid et al., 2018).

Novelty of the present research was to prepare an optimized ethosome formulation by optimizing various independent formulation variables with the aid of Box-behnken design. The main aim of the current work imply on the preparation and study the potential of novel polymeric ethosomal gel for the delivery of NiM and their pharmacokinetic and histopathological Investigation for the enhancement of penetration of transdermal drug delivery.

2. Experimental

2.1. Materials

Nimodipine (NiM) was received as a gift sample from Hetero laboratories, Mumbai, India. Carboprel 934 and ethanol were obtained from SD fine chemicals, Mumbai, India. Propylene glycol (PG) and soy lecithin (SL) from Research lab fine chemical industries. All other chemicals were used as received and they were of reagent grade. Institutional Animal Ethics Committee (IAEC) of Nizam Institute of Pharmacy and Research Centre, Hyderabad, India (Ref. No. I/IAEC/NIPRC/012/2022), accepted studies using animals.

3. Method of preparation

3.1. Optimisation by Box-Behnken design (BBD)

The NiM-Eth was optimised using a BBD with three components, three levels, with twenty runs (Response surface methodology; full factorial design). The midpoints of each edge and the cubes centre, in triplicate, serve as the experimental design points. According to their levels, independent variables such as concentration of SL (5 – 15 mg; X1), volume of ethanol (1–3 %; X2), and concentration of PG (1–3 mg; X3) and the depended variables of % entrapment efficiency (EE; %, Y1), vesicle size (VS; nm, Y2), and cumulative drug released (CDR; %, Y3) were chosen (Mohamed et al., 2021a, 2021b, 2021c, 2021d, 2021e). From this design a polynomial equation is generated and is given as:

\[ Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 \]

Where, \( Y_i \) – dependent variable, \( b_0 \) – intercept, \( b_{ij} \), \( b_{ijk} \) – regression coefficients, and \( X_i \) – the number of independent variables that were picked from earlier research.

By employing this experimental design, NiM loaded ethosomes was prepared and stored in air-tight containers for further evaluation studies and the optimized ethosomal batch has been considered for the gel formulation.

3.2. NiM loaded ethosomes (NiM-Eth)

The NiM-loaded ethosomal dispersions were prepared using a technique modified slightly from that proposed by El-Shenawy et al. (2019). The aqueous and organic phases were prepared independently. The organic phases containing SL and NiM were dissolved in ethanol at room temperature while being vigorously stirred (at 650 rpm) using a magnetic stirrer (REMI 2). To prepare the essential ethosomal suspension, drop wise addition of PG previously dissolved in water using a syringe. Both the phases were then mixed then magnetically stirred at 650 rpm for 30 min. The obtained suspension was sonicated (Sonics and Materials VCX 750, Inc., USA) for 5 min at a frequency of 20 kHz at 4 °C using a probe sonicator ultrasonic processor (UP50H, Hielser, Teltow, Germany). For additional investigation, formulations were kept chilled at 4 °C.

3.3. Preparation of NiM-ethosomal gel (NiM-Eth gel)

In a beaker, 1 g of carbopol 934 and 2 mL distilled water was added with continuous stirring for 2 h. To this mixture, 1 mg of NiM (equivalent weight of NiM-Eth), 2 mL PG, and 0.5 mL tri-ethanolamine and 5 mL of buffer saline (pH 7.4) was added under a homogenizer (Ultra Turrax T25, IKA, USA), at 12,000 rpm for 5 min (Sakran et al., 2022). The screened ethosomes were mixed with the obtained gel to form ethosomal gel (NiM-Eth gel).

4. Characterization of ethosomes

4.1. Vesicle physical analysis

Physical appearances of the NiM-Eth such as vesicle shape, size, and surface characteristics were analysed by Zetasizer, Nano ZS90 (Malvern Instruments ltd., UK) according to the method described by Mohamed et al. (2021a, 2021b, 2021c, 2021d, 2021e).

4.2. Entrapment efficiency (% EE)

Centrifugation process was used to determine the % EE of NiM ethosomal vesicles. In this method, ethosomal vesicles were separated using centrifugation (Eppendorf centrifuge 5430R, Germany) with a rotating speed of 15,000 rpm for 60 min (Sguizzato et al., 2020). The resulted sediment was extracted from the supernatant liquid whereby the vesicles liberate the drug which was analyzed by UV spectrophotometer (UV S.220 V, 2401 (PC), Shimadzu Corporation, Japan) for the drug content using ethanol at 238 nm and the % EE was given by,

\[ \text{EE} \left( \% \right) = \frac{\text{Amount of NiM incorporated} \times 100}{\text{Total concentration}} \]

4.3. Fourier transform infrared spectroscopy (FTIR)

Samples of NiM ethosomal vesicles weighing 2 mg were blended with 200 mg of potassium bromide to produce disks that are analyzed using Fourier transform IR spectra at a range of 400 to 4000 cm\(^{-1}\) and 4 cm\(^{-1}\) repreparation to evaluate the drug and the excipients are compatibility (Mohamed et al., 2022).

4.4. Differential scanning calorimetry (DSC) analysis

Differential Scanning Calorimetry was operated to conduct thermal behavior study of NiM loaded NiM-Eth. About 5 mg samples were placed into the porcelain crucible and were analyzed.
from 30 to 40 °C at a rate of 10 °C/min under continuous nitrogen atmosphere (Mohamed et al., 2022).

4.5. Power X-ray diffraction (PXRD) analysis

NiM ethosomal vesicle samples were analyzed for wide-angle X-ray scattering by means of an X-ray diffractometer employing copper potassium radiation source 100 mA at 40 kV. The PXRD was carried out at a wavelength of 1.5405 Å (0.154 nm) with an incremental temperature from 10 to 70 °C at a rate of 1 °C/s (Mohamed et al., 2022).

4.6. Morphology

NiM and NiM-Eth was examined for by scanning electron microscopy (SEM) to characterise the morphology of surface. In this study, a single drop of formulation was fixed on a clear glass stub which is then dried and coated with Polaron E 5100 Sputter coater and observed under SEM (Moideen et al., 2020).

4.7. In vitro NiM release study

The in vitro NiM release from NiM ethosomal vesicles was tested in Franz diffusion cell that is unjacketed and vertical type with a surface area of 6.154 cm². The donor partition was filled with 5 mg NiM formulation while the receptor partition was filled with 6.8 pH buffer and the dialysis membrane was pre-treated with pH-5 mg NiM formulation while the receptor partition was filled with 6.8 phosphate buffer. This system was maintained at 37 ± 2 °C with continuous stirring for 24 h (Albash et al., 2019). 0.5 mL of aliquot was syringe out at prescheduled time intervals while the same amount was replaced at each time of withdrawal to retain the receptor volume. Finally, the samples were diluted to determine the quantity of NiM using spectrophotometer.

4.8. Skin irritation study

NiM and NiM-Eth gels was tested for skin irritation by means of erythema and edema scaling system. Erythema scale stands as 0-none; 1–2-mild; 3 stands for moderate; 4 stands for severe; 5 stands for scar formation. Edema scale 0 stands for none; 1 stands for slight; 2 stands for well defined; 3 stands for moderate; 4 stands for severe (Paliwal et al., 2019). Composite of erythema and edema scores 0 stands for none; 1 to 2 stands for mild; 3 to 5 stands for moderate; 6 to 8 stands for severe irritation.

4.9. In vivo pharmacokinetics study

Albino male Wistar rats were used to study the relative absorption of transdermal NiM formulation with an oral NiM ethosomal suspension. Experimental animals were maintained in animal house with standard laboratory conditions. Twenty rats weighing between 180 and 250 g were split into two groups of ten each for this in vivo investigation. Group A was given a marketed NiM preparation (Nymalize oral preparation, Arbor Pharmaceuticals LLC, Columbus, OH) orally where group B received NiM ethosomal gel (Vijayanand et al., 2016). The gel was applied on the shaved region on dorsal side of animals. The heparinized capillaries were used to draw blood samples (500 mL) from the retinovascular plexus and the plasma was obtained after centrifugation at 5000 rpm for 10 min which is then stored at −20 °C for further HPLC analysis (Al-Joufi et al., 2021). The combination of supernatant was evaporated to dry it, and the dried deposit was reconstituted by adding 200 μL of the mobile phase, which was a 60:40 mixture of acetonitrile and 2 % acetic acid. The aforementioned combination was added in 100 μL increments to the HPLC system (Prominence, Shimadzu, Japan). Using a C-8 column (5 m, 4.6 mm i. d. 250 mm) and a flow rate of 1 mL/min, reversed-phase HPLC (RP-HPLC) was utilised to evaluate the samples. Signal detection at 425 nm was recorded in triplicates (He et al., 2012). Pharmacokinetic parameters of the formulation were estimated by plasma concentration versus time profile obtained from non-compartmental analysis using Kinetica® 5.0 software.

4.10. Histopathological study

Rats skin which was treated with ethosomal suspension, hydroethanolic preparation and NiM ethosomal gel for 24 hrs and untreated rat skin (control) were fixed onto the diffusion cell. The skin was made formulation free by wiping it using a tissue paper and these skin tissues were fixed in saline containing 10 % v/v formalin preparation for about 72 h (Zhang et al., 2014). Treated skin tissues were made into vertical sections, hematoxylin and eosin stained and observed under microscope. Histopathology studies reveal the penetration ability, mechanism of penetration enhancing ability and the ethosomal vesicle interaction with the skin.

4.11. Ex vivo permeation study

For ex vivo skin penetration investigations, Albino male wistar rats around 7 to 9 weeks of age and the weight between 120 and 150 g were used. A knife was used to remove the hair from the abdomen skin, and the skin was then detached from the underlying connective tissue and placed on aluminium foil. Gently removing the dermis layer allowed researchers to examine the skin for fat or surface irregularities (Abdellatif et al., 2017). This study was carried out in Franz diffusion cell in which 200 mL of ethosomal suspension filled in the donor compartment to prevent evaporation the compartment was fenced with aluminum foil whereas, the receptor compartment comprises phosphate buffer of pH 6.8. In between the two compartments rat abdominal skin was fixed where it was just in interaction with the receptor surface. The system was maintained at 37 ± 1 °C with 100 rpm continuous magnetic stirring. To keep the sink condition, 0.5 mL aliquots were taken out and refilled with phosphate buffer at predefined intervals (1, 2, 3, 4, 5, 6, 7, 8, 20 and 24 h). At 238 nm, the collected samples were subjected to spectrophotometric analysis.

5. Result and discussion

5.1. BBd

For the purpose of NiM-Eth formulation variable optimization for statistical analysis by ANOVA, model equation generation, contour plot construction, and 3D response surface plotting for each response, BBD was used. BBD is a second-order dynamic experimental design with a minimal number of trials (Mohamed et al., 2022).

5.2. EE, vS and CDR

Develop the link between various parameters and NiM-Eth EE; the response surface methodology (RSM) indicated that the quadratic models were significantly snug for the dependent variable. The following equation represents the polynomial model for a response (EE %; Y1), vS (nm), and CDR (%).

\[
Y_1 = 88.19 + 5.55X_1 - 1.77X_2 + 2.44X_3 + 2.75X_4X_5
- 3.35X_1X_3 - 2.45X_2X_5 - 11.26X_3^2 - 4.21X_4^2 - 5.85X_5^2. \tag{2}
\]
Y2 = 98.95 – 48.79X1 – 90.76X2 – 22.45X3 + 40.75X1X2 + 75.75X1X3 – 30X2X3 + 114.59X12 + 119.62X22 + 71.08X32, \quad (3)

Y3 = 97.54 + 3.83X1 + 4.07X2 – 1.37X3 – 0.98X1X2 – 1.06X1X3 – 2.21X2X3 – 10.02X12 – 12.73X22 – 3.85X32. \quad (4)

The percent entrapment, vesicle size and %CRD of factorial formulations were presented in Table 1. The influence of variables can be evaluated by the presented equations. The regression coefficient ideals are required to validate the model suitability (Mohamed et al. (2021a, 2021b, 2021c, 2021d, 2021e)). In this study, regression coefficient was high indicating an appropriate quadratic fitting model for entrapment and flux response.

EF10 showing maximum EE 88.9 %, vS 95 nm, and CDR 98.5 % was chosen from the experimental trials by BBD optimization cube plot. ANOVA presented less than 0.05p value at 9 5 % assurance frontier by means of student t-test. With BBD three factors were estimated and the variables of the study were formula mg of SL, % of ethanol and PG (Table 2). The dependent parameter was NiM EE (%), vS (nm) and CDR (%). Experimental design is a precision tool that is set up with the main aim to obtain the required information efficiently (Table 2). Factorial experimental designs are beneficial in terms of least trials and efficient estimation of statistical significance of independent variables on dependent variables (Cuzick et al., 2013).

Also, from the obtained polynomial equations it was concluded that the +ve coefficient of ethanol shows positive contribution to EE and vS and indicates to improvement of NiM release at all response points but, the –ve coefficient of independent variable SL signifies the effect of flux ad entrapment. ANOVA also presented ethanol as significant variable with p value < 0.01 at % CDR. As ethanol response point is insignificant at entrapment results, PG and SL was insignificant at all response points which suggests the significance of ethanol in the ethosomal drug release (Alqahtani et al., 2022).

From the linear model that is obtained from regression analysis, 3D response plots were setup where, the resulted responses were depicted as bars as a function of independent variables which is presented in Fig. 1. These plots convey the correlation between response and independent variables on a whole and also perhaps at its margin (Fagir et al., 2015). 3D response and countor plots displaying the effects of (Fig. 1a and d) EE (%), (Fig. 1b and d) vS (nm), and (Fig. 1c and e) CDR (%).

The optimised formulation (EF21) validated for response variables set as a maximum (Y1) and minimum (Y2 and Y3) range generated by design noted as SL was 5 mg, 2 % of ethanol, and 3 % of the PG, with the 5 min for the time of sonication. The experimental value of EE, vS and CDR (89.22 ± 43.12 %, 96.45 ± 5.47 nm and 97.78 ± 7.90 % respectively) when associated with that of the prediction value (88.23 %, 95.76 nm and 98.48 % respectively) and the prediction error as calculated as 0.81, 3.2, and 2.99 % respectively. From this technique, the negligible percentage of errors might concluded that the independent and depended variables were ideal.

5.3. FTIR

The FTIR spectrum of NiM, polymers, and NiM-Eth gel confirmed surface modification of ethosomal gel as shown in Fig. 2a. FTIR spectrum of pure NiM indicated the characteristics peak of The characteristic peaks of NiM were observed in the spectra at 3289 cm⁻¹ (N–H stretching), 3087 cm⁻¹ for C–H aromatic stretching, 2943 cm⁻¹ (C–H aliphatic stretching), 1688 cm⁻¹ for C=O stretching in ester, 1671 cm⁻¹ (C=C stretching), 1630 cm⁻¹ (C=C aromatic), 1531 cm⁻¹ for NO2, 1390 cm⁻¹ for C-CH₃, and 1129 cm⁻¹ for –C=C–ester.

The NiM-Eth gel of all spectrum showed similar characteristic peaks from NiM, SL, ethanol, and PG, for C–H vibrations, and C=O stretching from EF21. For NiM-Eth gel formed with carbopol, the intensity bands of amide (1545 cm⁻¹) increased. Likewise, the intensity and peak location of amine groups at 671–912 cm⁻¹ areas caused by the twisting vibration were changed in the gel formation, demonstrating the presence of N–H groups (Mohamed et al. (2021a, 2021b, 2021c, 2021d, 2021e)). Hence, concluded that the C=O stretching vibration reduced in peak respective NiM, was suggested that the strong complexation of the SL with the PG formed. A moderate alteration of the peak corresponding to NiM and SL was observed at NiM-Eth gel due to the drug being finely complexes into the carbopol core, whereas the SL peak was shown with reduced intensity (2888 and 2893 cm⁻¹, respectively).

Table 1
BBD with calculated responses of NiM-Eth.

| Code (EF) | SL (mg) | Ethanol (%) | PG (%) | EE (%) | vS (nm) | CDR (%) |
|----------|---------|-------------|--------|--------|---------|---------|
| 1        | 1       | 1           | 0      | 87.9   | 430     | 61.9    |
| 2        | 0       | 1           | 1      | 73.5   | 539     | 63.7    |
| 3        | 0       | 0           | 0      | 88.7   | 129     | 98.7    |
| 4        | 1       | 0           | −1     | 89.9   | 102     | 98.3    |
| 5        | 0       | 1           | −1     | 76.9   | 283     | 85.1    |
| 6        | 0       | 0           | 0      | 47.2   | 483     | 63.8    |
| 7        | 1       | 0           | 1      | 71.3   | 330     | 70.3    |
| 8        | 0       | −1          | 1      | 54.2   | 681     | 61.5    |
| 9        | 0       | 0           | −1     | 85.9   | 85      | 95.5    |
| 10       | −1      | 0           | 1      | 88.9   | 95      | 98.5    |
| 11       | 0       | 0           | 0      | 68.3   | 376     | 71.7    |
| 12       | 0       | 0           | 0      | 75.2   | 289     | 81.2    |
| 13       | −1      | 1           | 0      | 88.3   | 108     | 96.8    |
| 14       | −1      | 0           | −1     | 68.5   | 485     | 72.7    |
| 15       | 0       | 0           | 0      | 55.9   | 483     | 71.9    |
| 16       | 1       | −1          | 0      | 59.7   | 169     | 68.3    |
| 17       | 0       | 0           | 0      | 68.5   | 285     | 89.6    |
| 18       | −1      | −1          | 0      | 85.5   | 95      | 97.8    |
| 19       | 0       | 0           | 0      | 71.6   | 285     | 72.5    |
| 20       | 0       | 0           | 0      | 68.5   | 320     | 76.8    |
5.4. DSc

A distinctive representative endothermic melting peak at the starting temperature of 125.3 °C was visible on the pure NiM showing in the DSC thermogram (Fig. 2b). A dehydration process may be to blame for the large endothermic impact that the DSC trace of NiM-Eth gel displayed between 35 and 120 °C. Despite considerable size reduction and widening of the NiM endothermic peak, NiM-Eth gel still exhibits the distinctive endothermic peak of NiM, which was 125.3 °C. The typical NiM melting point peak

| Parameter | source | DF | Sum of squares | Mean of squares | F Value | P Value |
|-----------|--------|----|---------------|----------------|---------|---------|
| EE        | Model  | 9  | 3036.91       | 337.43         | 29.92   | < 0.0001|
|           | Residual | 10 | 112.76        | 11.28          |         |         |
|           | Lack of fit | 5  | 97.21         | 19.44          | 6.25    | 0.0329  |
|           | Pure error | 5  | 15.55         | 3.11           |         |         |
| VS        | Model  | 9  | 5.661E+05     | 62898.64       | 42.38   | < 0.0001|
|           | Residual | 10 | 14843.04      | 1484.30        |         |         |
|           | Lack of fit | 5  | 13691.71      | 2738.34        | 11.89   | 0.0084  |
|           | Pure error | 5  | 1151.33       | 230.27         |         |         |
| %CDR      | Model  | 9  | 3610.75       |               |         |         |
|           | Residual | 10 | 57.70         | 5.77           |         |         |
|           | Lack of fit | 5  | 50.10         | 10.02          | 6.59    | 0.0295  |
|           | Pure error | 5  | 7.60          | 1.52           |         |         |

Fig. 1. 3D response and contour plots displaying the effects of (a and d) EE (%), (b and d) VS (nm), and (c and e) CDR (%).

Fig. 2. (a) FTIR analysis of (i) Pure NiM, (ii) PG, (iii) SL (iv), EF21, and (v) NiM-Eth gel and (b) DSC Analysis of pure NiM and (b) NiM-Eth gel.

Table 2
Observed and Predicted values with Residuals of the Response Y1, Y2, and Y3 for NiM-Eth.
almost entirely vanished in the freeze-dried formulation, although there was a faint endothermic peak that might have been caused by some drug-SL interaction and reduced carbopol crystallinity, indicating that the complex was not fully formed. The DSC thermogram of the co-precipitated formulation, on the other hand, demonstrated the persistence of the NiM endothermic peak, proving that an ideal complex had not formed at a 1:1 M ratio in the solid state (Mohamed et al., 2020).

5.5. PXRD

The PXRD spectrum of NiM showed sharp defined peaks (Fig. 3a) on 2 theta values of 21.1°, 18.9°, and 13.2°, and many tiny peaks at 25.44°, 23.98°, 24.76°, and 27.61° indicating the crystalline nature of the sample. When complex formation (Fig. 3b) was suggested, the diffraction pattern of a NiM-Eth gel clearly diverges from that of pure NiM. The characteristic NiM peaks are seen at 19.6, 16.1, and 11.4° in the NiM-Eth gel diffractograms, respectively, even if diminished intensities of 591, 312, and 754. This could be accounted for by the reduction in particle size during physical mixing and some interaction between NiM and carbopol that causes a decrease in NiM crystalline nature. Some of the diffractograms in both Fig. 3a and b show a peak of high intensity at around 4.33, which was most likely the result of diffraction from the planes of the aluminium sample holder. This is a typical mistake made while recording X-ray diffraction patterns and is not a sign of a compound’s possible crystalline properties (Mohamed et al., 2022). The PXRD results showed that the medication and gel that had been integrated persisted in the NiM-Eth gel and that the lipid matrix had a higher gel EE.

5.6. SEM

As seen in Fig. 4, the SEM has been extensively employed to give surface and morphological information of pure NiM and NiM-Eth gel. The crystalline structure of pure NiM had a characteristic prism-surface and had an average particle size of 5 μm (Fig. 4a). The vesicle surface seems to be smoother, more spherical, and disseminated as a uniform and homogeneously formed (Mohamed et al. (2021a, 2021b, 2021c, 2021d, 2021e)). This could be due to the vesicles were molecularly formed a complex of both carbopol and NiM has vanished as a result of the topological alterations seen in the NiM-Eth gel (Fig. 4b). These outcomes matched the diffractogram obtained from XRD data.

5.7. In vitro NiM release

The in vitro profiles of NiM from NiM-Eth gel in PBS, pH6.8 are shown in Fig. 5a. There was no initial burst release of NiM from both EF21 (about 4.64 ± 0.43 %) and NiM-Eth gel (about 3.22 ± 0.23 %) was found in the first 1 h, followed by release of 79.86 ± 2.98 and 48.14 ± 3.11 % in 28 h (Fig. 5a). The initial least release might be attributed to the fact that NiM molecules were tightly bound onto SL in prepared ethosomes by ionic interaction.

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**Fig. 3.** PXRD analysis of (a) Pure NiM and (b) NiM-Eth gel.

**Fig. 4.** SEM images of (a) pure NiM and (b) NiM-Eth gel.
which could be easily adsorbed in an ionic aqueous environment (Senthivel et al., 2022). NiM-Eth gel gradually increased the constant release of NiM (39.98 ± 2.89 % release in 24 h) but to a lesser extent than that by (48.14 ± 3.11 %release in 28 h). Overall rate of NiM release was found to be higher from EF21 than from NiM-Eth gel, but the extent of NiM released after 30 h was found to be the same (around 90 %) from both the EF21 and NiM-Eth gel.

5.8. Skin irritation study

The free NiM and the NiM-Eth gel were applied to rat skin for the skin irritation test, which was then monitored for 72 h. The drug and NiM-Eth gel produces a moderate to severe erythema in terms of the Primary Irritation Index (PII). For free NiM preparation, PII was seen at 1.7, which limits the rat skin appropriateness and tolerance. On the other hand, the NiM-Eth gel formulation had a PII of 1.1 and produced less discomfort (Imam et al., 2017). Thus, patients cannot use it directly. Due to the improved encapsulation effectiveness of the drug, NiM-Eth gel might ideally be able to lessen/diminish the irritation and increase patient acceptance.

5.9. Pharmacokinetics

Pharmacokinetic parameters of Nymalize oral preparation after the oral administration resulted with Cmax of 8.1 ng/mL and tmax of 5 h whereas, NiM-Eth gel formulation has sustained plasma concentration for about 36 h which was shown by decrease in Cmax.
and delayed tmax of 3.61 ng/mL and 12 h respectively (Table 3). The mean AUC0 to AUC0-\infty of NiM-Eth gel when compared to oral formulation (Nymalize oral preparation) was found to be increased from 4.1 to 5.9 folds which was found to be resulted from first pass effect (Fig. 5b).

The optimized NiM-Eth gel formulation showed prolonged plasma profile and enhanced bioavailability suggested a reduction in both dose and frequency of administration influencing patient compliance, cost and dose dependent side effects (Ramadon et al., 2017).

5.10. Histopathological studies

Histopathological findings of earlier studies, It was hypothesised that the nano drug delivery system may enable more drugs to penetrate the stratum corneum of the skin and create drug depots in the deep layer, thus enhancing the efficacy of wound healing. Fig. 6 shows images of hematoxylin and eosin staining for healed wound because new capillaries were observed in groups of the free NiM and NiM-Eth gel. Fig. 6b reveals the enhanced NiM to promote healing, as indicated by the black arrow in the group of the NiM-Eth gel, which also displayed embolization and localised parakeratosis (Akhtar et al., 2022).

5.11. Ex vivo permeation studies

Fig. 7 shows the effectiveness of the NiM-Eth gel in enhancing NiM penetration via transdermal membrane by displaying the percent cumulative NiM penetrated through Albino rat belly skin membrane. Compared to pure NiM, which was roughly 17.20 ± 2.36 percent after 24 h, the optimal formulation had a cumulative NiM permeation rate of 63.72 ± 3.99 percent. The increase in permeation supported the ethosomal vesicles’ superior deformability (El-Menshawe et al., 2017). The addition of PG and ethanol increased the vesicle’s flexibility and improved their capacity to distort, permitting them to squeeze through the skin and boost the generated NiM-Eth gels penetration influence (Arora and Nanda, 2019). The remarkable and distinctive structure of NiM-Eth gel, which allows for improved NiM penetration via deep transdermal membranes, may be responsible for the encouraging outcomes. Ethanol makes the SL in cell membranes fluid and causes the SL intercellular matrix to partially dissolve.

Additionally, NiM-Eth gels SL membranes become more elastic while ethanol was present, enhancing the gel’s capacity to deliver the medication into deeper abdominal layers. The use of NiM-Eth gel as a NiM delivery system was strongly believed by the findings of the experiments due to their profound impact on increasing the percent penetration of the extremely hydrophobic drug (NiM), which can increase up to fourfold over free NiM.

6. Conclusion

According to the results of this study, NiM can be successfully prepared in gel-based ethosomal TDDS, which may be employed to provide a quicker onset of action while also extending the drug delivery for up to 24 h. The goal of the current study was to construct, improve, and develop nimodipine ethosomal system in order to have a quick start of action that would endure for a long time. NiM-Eth EF21 comparative in-vitro drug release pattern was tested for around 24 h. For pharmacokinetic parameter and ex vivo permeation tests, ethosomal gel comprising ethosomal suspension and carbopol 940 as a gelling agent was created and compared with marketed formulations carrying the same drug concentration. It was discovered that the ethosomal gel had improved penetration in comparison to the commercial formulation. However, more research on human cadaver skin, in vivo research, and the formation of in vitro relationships are necessary.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 7. Ex vivo permeation sketches of NiM from prepared NiM-Eth gel compared to free NiM.
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