Development and in vitro/in vivo evaluation of controlled release provesicles of a nateglinide–maltodextrin complex

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Abstract The aim of this study was to characterize the provesicle formulation of nateglinide (NTG) to facilitate the development of a novel controlled release system of NTG with improved efficacy and oral bioavailability compared to the currently marketed NTG formulation (Glnate™ 60). NTG provesicles were prepared by a slurry method using the non-ionic surfactant, Span 60 (SP), and cholesterol (CH) as vesicle forming agents and maltodextrin as a coated carrier. Multilamellar niosomes with narrow size distribution were shown to be successfully prepared by means of dynamic laser scattering (DLS) and field emission scanning electron microscopy (FESEM). The absence of drug-excipient interactions was confirmed by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies. In vitro release of NTG in different dissolution media was improved compared to pure drug. A goat intestinal permeation study revealed that the provesicular formulation (F4) with an SP:CH ratio of 5:5 gave higher cumulative amount of drug permeated at 48 h compared to Glnate™ 60 and control. A pharmacodynamic study in streptozotocin-induced diabetic rats confirmed that formulation F4 significantly (P < 0.05) reduced blood glucose levels in comparison to Glnate 60. Overall the results show that controlled release NTG provesicles offer a useful and promising oral delivery system for the treatment of type II diabetes.

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1. Introduction

Since the 1980s, non-ionic surfactant vesicles (niosomes) have been shown to possess distinct advantages over conventional dosage forms and play an increasingly important role in drug delivery. Compared to phospholipids, nonionic surfactants form vesicles that are more stable, easier to handle and less expensive to produce. In recent years, provesicular (proniosomal) derived niosomes have received considerable attention as an oral dosage form with the potential to improve therapeutic activity, reduce side effects and enhance stability of drugs to chemical degradation or transformation. This is because niosomes themselves have limitations for oral delivery due to poor integrity at the site of absorption, physicochemical instability to hydrolysis, separation of drug and their tendency to sediment and aggregate.

A proniosomal formulation is a dry formulation of a liquid crystalline niosomal hybrid which converts to niosomes upon hydration with aqueous media. It offers a versatile drug delivery system that is not only capable of encapsulating drug but can also minimize drug degradation after administration, prevent undesirable side effects and increase drug bioavailability. In addition, it is convenient to transport, distribute and store and less subject to the high cost and variable purity problems of phospholipid based formulations. This makes proniosomes (or 'dry niosomes') a promising, commercially valuable product.

Nateglinide [N-(trans-4-isopropylcyclohexylcarbonyl)-o-phenylalanine] is a novel non-sulfonylurea oral hypoglycemic agent which has outstanding clinical effectiveness in the treatment of type II diabetes mellitus. Its mechanism of action involves increasing insulin release from pancreatic β-cells through inhibition of potassium-ATP channels. After oral administration, NTG is rapidly absorbed from the gastrointestinal tract and rapidly eliminated from plasma with a half-life of approximately 1.5 h. As a result, a dose of NTG of 20–40 mg must be administered thrice a day. In addition, NTG has low bioavailability and poor dose proportionality probably resulting from its limited absorption through the gastrointestinal tract consequent on its low water solubility (8 mg/L) and/or wettability.

Maltodextrins (MLTs) are complex mixtures of high and low molecular weight carbohydrates obtained by acid and/or enzymatic hydrolysis of starch. They contain linear amylose and branched amylopectin degradation products and are considered as β-glucose polymers joined by α-(1,4) and α-(1,6) linkages. MLTs are endowed with the capability to form complexes with various classes of compounds usually of the host–guest type. Complex formation depends on the size of the complexing molecule and is believed to require a conformational change from a flexible coil to a helix form in the presence of the guest molecule. Because NTG probably exhibits dissolution rate limited absorption, we anticipated that its dissolution rate would be improved through the preparation of an MLT complex. The aim of this study was therefore to examine a provesicular system based on the MLT complex of NTG.

MLT-based provesicular powders offer a simple and stable carrier for efficient oral delivery of lipophilic or amphiphilic drugs since they allow the production of provesicles with greater drug loading. Due to its high surface area and porous structure, MLT forms provesicles with high surfactant/carerrier mass ratios. MLT-based NTG provesicles prepared with the nonionic surfactant Span 60 (SP) have been previously reported. SP with its longer saturated alkyl chains and high phase transition temperature shows higher entrapment efficiency (EE) in comparison with those of other nonionic surfactants. Cholesterol (CH) is also commonly included not only to improve the stability and EE of a vesicular formulation but also to impart rigidity and orientational order to the niosomal bilayer.

Many formulation approaches have been investigated to improve the bioavailability of NTG including various solvent systems, solid dispersions, complexes with β-cyclodextrins, floating microspheres, polymeric nanoparticles and solid lipid nanoparticles. To date, no study has evaluated an MLT-based provesicular drug delivery system of NTG for diabetic therapy. In this study, controlled release provesicles of NTG–MLT complex were prepared and evaluated with the aim of producing an NTG formulation that would provide decreased dosing frequency, fewer side effects and increased bioavailability.

2. Material and methods

2.1. Materials

NTG (purity 99.87%) was a gift from Alembic Pharmaceutical Ltd. (Vadodara, India). A commercial sample of Glinate™-60 (Glenmark Pharmaceuticals Ltd., Mumbai, India) was procured from a retail pharmacy. MLT and SP were purchased from Loba Chemical Pvt. Ltd. (Mumbai, India). CH was obtained from Sigma Chemical Co., India. Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride and potassium chloride were all of analytical grade from S.D. Fine Chem. Ltd. (Mumbai, India). All other chemicals and solvents were of analytical grade and used as received.

2.2. Preparation of NTG–MLT provesicular powders

Provesicular powders of the NTG–MLT complex were prepared containing different ratios of CH and SP according to a literature method with slight modifications. The compositions of the NTG provesicles are given in Table 1. In brief, 100 mg MLT was placed into a 100 mL round-bottomed flask followed by a solution of NTG (60 mg), SP and CH (total lipid 100 μmol/L) in 10 mL chloroform. The mixture was vortexed for 5–10 min to obtain a slurry with additional chloroform being added in the case of mixtures with lower surfactant loading. Chloroform was then removed by rotary evaporation under reduced pressure at 50–60 °C over 15–20 min. After drying, powders were placed in a desiccator overnight to ensure complete evaporation of solvent. The final “provesicular powders” were stored in sealed glass containers at room temperature until characterization.

2.3. Formation of niosomes from provesicular powders

Niosomes were prepared from provesicular powders by hydration with phosphate buffered saline (PBS) pH 7.4 at 80 °C using a vortex mixer for 2 min. The resultant dispersion was then subjected to determination of particle size, zeta potential, EE and morphology.

2.4. Characterization aspects

2.4.1. Field emission scanning electron microscopy (FESEM)

The morphology of pure NTG, blank and the optimized formulation (F4) was examined by FESEM using a JSM 6360 electron
microscope (Jeol, UK) at an accelerating voltage of 17 kV. A small amount of sample was placed on the FESEM holder with double sided adhesive tape and coated with a layer of gold of 150 Å for 2 min under argon at a pressure of 0.3 atm. Each experiment was performed in triplicate.

2.4.2. Size and distribution analysis
Vesicle size (VS) and polydispersity index (PI) were determined at 25 °C by dynamic light scattering using a Zetasizer NanoZS90 analyzer (Malvern Instruments, Malvern, UK) at 90° scattering angle. Zeta potential was measured using Laser Doppler Micro-electrophoresis. NTG provesicular powder (2 mg) was mixed with 10 mL PBS pH 7.4 and vortexed for 3 min in a glass tube. The dispersion was then filtered through a syringe filter (0.45 μm) before determination. PI < 0.3 was taken to indicate a homogenous and monodisperse population; PI > 0.3 was taken to indicate high heterogeneity. Each experiment was performed in triplicate.

2.4.3. EE of niosomes
EE was determined by centrifugation to separate non-entrapped drug from niosomes. In this method, 1 mL aliquots of drug-loaded niosomal dispersions were centrifuged (Remi CPR-24) at 18,000 rpm for 40 min at 4 °C. The niosomal pellet was resuspended in PBS pH 7.4 and then centrifuged again to ensure removal of free drug from the void volume between niosomes. After each centrifugation, drug content was determined in the supernatant by UV–Vis spectrophotometry (JASCO V-560) at 210 nm using 1-cm quartz cells against PBS pH 7.4 as blank. The amount of encapsulated drug was obtained by subtracting the amount of free drug from the total drug added. Each experiment was carried out in triplicate. EE (%) is calculated as follows:

\[
EE(\%) = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug}} \times 100\%
\]

2.4.4. Osmotic shock studies
The effect of osmotic shock on niosome formulations was investigated by measuring the change in VS following incubation of vesicular suspensions in media of different tonicities. A hypertonnic medium was simulated using 1 mol/L sodium iodide solution; isotonic medium was normal saline (0.9% NaCl); hypotonnic medium was 0.5% NaCl. Vesicular suspensions were incubated in these media for 3 h before VS was measured.

2.4.5. Fourier transform infrared (FTIR) spectroscopy
FTIR spectra of pure NTG, blank and F4 formulations were recorded in KBr discs using a Jasco FTIR spectrophotometer (Model FTIR-4100, Jasco, USA) to examine interactions between drug and excipients. FTIR measurements were performed at ambient temperature at a constant resolution of 0.9 cm⁻¹ in the scanning range 4000–500 cm⁻¹.

2.4.6. Differential scanning calorimetry (DSC)
The physical state of NTG (whether crystalline or amorphous) in provesicular powders was investigated by DSC using a Perkin-Elmer differential scanning calorimeter (Model Pyris Diamond, DSC, USA). Samples were placed in flat bottomed aluminum pans and heated from 35 to 350 °C using a platinum crucible and alpha alumina powder as reference material. Heat flow rate was kept at 12 °C/min with a nitrogen stream at 20 mL/min.

2.4.7. X-ray diffraction (XRD)
XRD analysis was conducted using an Ultima IV Multipurpose X-ray diffractometer (Rigaku, Japan). The samples were measured with a Cu target, nickel filtered, graphite diffracted beam monochromator using 40 kV voltage and 30 mA current. The rate of scanning was 1 °/min over a diffraction angle of 2θ in the range 3–45°.

2.5. In vitro release
The release of NTG from provesicular powders was determined in HCl (pH 1.2) and PBS, pH 7.4 using a vertical Franz diffusion cell. The receptor compartment contained 100 mL dissolution medium maintained at 37 ± 0.5 °C by means of a thermostatically controlled water bath and magnetic stirring at 50 rpm. Aliquots (1 mL), after 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h using a syringe, were filtered through 0.2 μm membrane filter and determined by UV–Vis spectrophotometry (JASCO V-560) at 210 nm. Each sample was replaced with fresh medium to maintain sink conditions. Dissolution experiments were carried out in triplicate. In order to understand the barrier effect of the dialysis membrane, in vitro release of NTG from the same amount of pure NTG was investigated in the same way. Release data were evaluated according to zero order, first order and Higuchi diffusion models. The correlation coefficients (r) of the fits were subjected to statistical evaluation using ANOVA at 5% level of significance.

### Table 1 Composition, entrapment efficiency (EE %) and particle size analysis of various provesicular formulations of nateglinide.

| Formulation | Lipid composition | EE (%) | Mean vesicle size (nm) | PI |
|-------------|-------------------|--------|-----------------------|----|
|             | SP | CH |  |  |  |
| F1 | 9 | 1 | 66.56 ± 1.41 | 364.9 ± 43.7 | 0.55 |
| F2 | 7.5 | 2.5 | 71.03 ± 2.25 | 405.1 ± 53.1 | 0.49 |
| F3 | 6 | 4 | 77.73 ± 1.50 | 382.6 ± 47.3 | 0.45 |
| F4 | 5 | 5 | 84.76 ± 1.13 | 262.4 ± 76.2 | 0.27 |
| F5 | 4 | 6 | 80.23 ± 2.20 | 333.2 ± 54.0 | 0.40 |
| F6 | 2.5 | 7.5 | 74.65 ± 2.18 | 374.8 ± 61.2 | 0.49 |
| F7 | 1 | 9 | 68.71 ± 1.27 | 331.1 ± 44.8 | 0.45 |

SP, Span 60; CH, Cholesterol; and PI, Polydispersity index.

Data are expressed as means ± SD, n = 3.
2.6. Goat intestinal permeation study

An intestinal permeation study was performed using a vertical Franz diffusion cell with an effective diffusion area of 3.14 cm². The experiment was carried out using mucosal sheets of freshly killed goat intestine obtained from the local slaughterhouse and stored at −18 °C. Intestines were first flushed with physiological solution at room temperature for 2 h to remove any intestinal contents. A circular piece of intestine about 3 cm diameter was then threaded into the donor compartment of the vertical diffusion chamber and 2 mL drug loaded with niosomal dispersion was added to the mucosal side and sealed with Parafilm. The receptor compartment was filled with 25 mL PBS pH 7.4, maintained at 37.5 ± 0.5 °C and magnetically stirred to prevent any boundary layer effects. Aliquots (1 mL) were withdrawn from the receptor compartment after 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 36 and at 48 h, each withdrawal being immediately replaced with fresh PBS to maintain sink conditions. Drug content of samples was analyzed by UV–Vis spectrophotometry (JASCO V-650) at 210 nm. Permeation studies of NTG from Glinate 60 and pure NTG (control) were also investigated in the same way. Each experiment was performed in triplicate.

2.6.1. Calculation of permeation parameters

The permeation of NTG through goat intestine was constructed by plotting the total cumulative amount of drug permeated per unit surface area (dM/A µg/cm²) versus time t (h). NTG steady state flux, Jss (µg/cm²/h) is calculated as the slope of the linear regression line.

\[ J_{ss} = \frac{dM}{A dt} \] (2)

The permeability coefficient (Kp) is calculated using the relation derived from Fick’s first law of diffusion as follows:

\[ K_p = \frac{J_{ss}}{C_0} \] (3)

where \( C_0 \) is the initial drug concentration in the donor compartment.

The enhancer ratio (En) is also calculated from the following equation:

\[ E_n = \frac{J_{enh}}{J_{ctrl}} \] (4)

where \( J_{enh} \) is the flux from the formulation and \( J_{ctrl} \) is the flux of drug from control (pure NTG).

2.7. Stability study

Provesicular powder formulations were packed and sealed in amber glass vials and subjected to stability studies as per ICH guidelines and kept in refrigerator (2–8 °C) and room temperature (25 ± 2 °C) for a period of 3 months. Samples were withdrawn and hydrated with PBS after 1, 2 and 3 months and examined for any drug precipitation by optical microscopy. The EE and mean VS of each sample were determined and compared to freshly prepared provesicular formulations. Each experiment was performed in triplicate. Samples are also evaluated for retention of NTG which is calculated as:

\[ \text{Retention of NTG (\%)} = \frac{\text{Entrapped NTG after storage}}{\text{Entrapped NTG before storage}} \times 100 \] (5)

2.8. Pharmacodynamic study

Male Wistar albino rats (age 8 weeks; weight 180–200 g) were kept in clean polypropylene cages and maintained at 25 °C under a dark/light cycle (12 h/12 h) for 10 days prior to the experiment. Standard laboratory diet and water were provided ad libitum. The animal study protocol was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Central Government of India and the Institutional Animal Ethics Committee of Jadavpur University, Kolkata. A total of 18 rats were divided into three equal groups and following an overnight fast (18 h) were given a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (65 mg/kg) dissolved in PBS pH 7.4 to induce diabetes. Diabetes was verified 72 h later by evaluating blood glucose levels using a one-touch glucometer (Accuchek); rats showing blood glucose > 250 mg/dL were considered to be diabetic and used in the study. Group I was diabetic control, Group II was given an oral dose of NTG 15 mg/kg as Glinate™ 60 and Group III (Test) was given an oral dose of 15 mg/kg NTG as provesicular formulation F4. Blood samples were collected from the retro orbital plexus prior to the dose and after 1, 2, 4, 8, 12, 18 and 24 h. Blood glucose was determined immediately using the one-touch glucometer.

2.9. Statistical analysis

Statistical analysis of differences was carried out by one way ANOVA and Student’s t-test using Origin Pro 8.0 (Origin Lab Corporation, Northampton, USA). Data are reported as mean ± SD. Differences for which \( P < 0.05 \) were considered statistically significant.

3. Results

3.1. Morphology

FESEM images of pure NTG, blank and the F4 formulation are shown in Fig. 1. The niosome size distribution was in the range 25.0–410 nm. The average diameter of the niosomes correlated well with values obtained by DLS.

3.2. EE and VS

EE of NTG provesicular powders is given in Table 1. Fig. 2D represents the intensity – size distribution histogram of provesicular formulations as obtained from DLS measurements at 25.0 ± 0.1 °C. The mean VS of formulations with NTG molar ratios from 9:1 to 1:9 was found to be between 260 and 410 nm indicating all have narrow size distributions.

3.3. Osmotic shock studies

The effects of osmotic shock on provesicular formulations are presented in Table 2. It was found that shrinkage occurred for all formulations incubated in hypertonic medium whereas an increase in VS occurred in hypotonic medium. When incubated in normal saline (0.9% NaCl), formulations showed a small increase in VS.
3.4. FTIR spectroscopy

FTIR spectra of pure NTG, blank and the F4 formulation (Fig. 2A) were very similar indicating that no significant interaction occurred between NTG and the other excipients.

3.5. DSC

DSC data (Fig. 2C) reveal that pure NTG shows a sharp endothermic peak at 132.10 °C corresponding to its melting point. Thermographs of the other two formulations show only slight differences in the endothermic peak of NTG suggesting that no significant interaction occurs between NTG and the other constituents of the formulations.

3.6. XRD

XRD patterns are presented in Fig. 2B. Pure NTG exhibited a strong and characteristic XRD pattern consistent with a crystalline powder whereas the blank and formulation F4 showed loss of peaks and more diffuse peaks indicative of an amorphous form of NTG.

3.7. In vitro release study

The in vitro release profiles of NTG from the provescicular formulations and pure NTG are shown in Fig. 3. It was found that provescicular formulation F4 (SP:CH molar ratio 5:5) exhibited the highest rate of dissolution of NTG in both PBS pH 7.4 and HCl (pH 1.2).

3.8. Goat intestine permeation study

The permeation of NTG from the three formulations as shown in Fig. 4 reveals that formulation F4 gives the greatest permeation of NTG through goat intestine followed by Glinate™ 60 and control (Table 3). This may be due to the ability of the non-ionic surfactant to act as a penetration enhancer through the gut wall.

3.9. Pharmacodynamic study

The hypoglycemic activity of the three formulations in STZ-induced diabetic rats is shown in Fig. 5. It was observed that formulation F4 produced a significant reduction in blood glucose levels compared to Glinate™ 60 (P<0.05) over 24 h.

3.10. Stability study

Fig. 6 shows the results of stability testing of formulation F4 stored under refrigeration and at room temperature for three months. Fig. 6C shows that the retention of NTG (%) in formulation F4 was relatively unchanged under refrigerated conditions for up to 3 months (P<0.05).

4. Discussion

The size and diameter of the vesicles observed by FESEM were found to correlate well with corresponding values obtained by DLS measurements. FESEM images of formulation F4 showed distinct spheres with smooth surfaces and revealed the absence of native crystals of NTG in the provescicular powders. The change from a highly porous surface in the blank to a plain surface in formulation F4 undoubtedly indicates an efficient drug loading of NTG into the MLT matrix. In terms of the EE, it was found to be in the range 66%–84% and to be significantly higher in the formulation with an SP:CH molar ratio of 5:5 (84.76%) than in the other formulations (P<0.05). It was also found to increase as the SP:CH molar ratio changed from 9:1 to 5:5 suggesting that niosomes with relatively higher CH content have reduced bilayer permeability to NTG. However, when the SP:CH molar ratio changed from 4:6 to 1:9, it resulted in a significant decrease in EE suggesting that the CH content beyond a certain level led to disruption of the bilayer structure. Vesicles produced from provescicular formulations were found to have VS in the range 260–410 nm (Fig. 2D) and to be negatively charged as reflected in the zeta potentials (E/C0 = −45 mV). Moreover, vesicles produced from formulation F4 were found to have PI<0.3 indicating a homogeneous and monodisperse distribution of colloidal vesicles. DLS measurements also showed a narrow size distribution further supporting an almost uniform distribution of provesicle-derived niosomes. VS was found to be inversely proportional to EE.

The FTIR spectrum of pure NTG showed an intense peak at 3356 cm^{-1} assigned to –NH stretching. Another three distinct peaks were observed at 1600 cm^{-1}, 1740 cm^{-1} and 2861–3063 cm^{-1} assigned to –C=O, –COOH and –CH stretching vibrations, respectively. In terms of interaction between NTG and excipients, these four peaks showed no tangible shift indicating the absence of any interaction.

In the DSC profiles, pure NTG showed a sharp characteristic endotherm at 132.10 °C equating to its melting point. However, the DSC thermogram of formulation F4 showed a small blunt
Figure 2  (A) FTIR spectra, (B) XRD profiles and (C) DSC thermograms of (a) nateglinide (b) the blank formulation and (c) formulation F4. (D) Size distribution intensity of provesicular formulations (F1–F7) (data are means ± SD, n=3).

Table 2  Effect of osmotic shock on nateglinide provesicular formulations.

| Formulation | Average vesicle size (nm) after incubation with | PBS pH 7.4 | 1 mol/L NaI | 0.9% NaCl | 0.5% NaCl |
|-------------|-----------------------------------------------|------------|-------------|------------|------------|
| F1          |                                               | 364.9 ± 43.7 | Shrunk      | 376.4 ± 31.3 | 412.7 ± 67.8 |
| F2          |                                               | 405.1 ± 53.1 | Shrunk      | 415.5 ± 35.7 | 566.1 ± 67.5 |
| F3          |                                               | 382.6 ± 47.3 | Shrunk      | 395.2 ± 79.7 | 476.5 ± 53.2 |
| F4          |                                               | 262.4 ± 76.2 | Shrunk      | 270.3 ± 108.4 | 309.5 ± 115.0 |
| F5          |                                               | 332.2 ± 54.0 | Shrunk      | 334.2 ± 68.1 | 476.0 ± 92.3 |
| F6          |                                               | 374.8 ± 61.2 | Shrunk      | 387.0 ± 116.5 | 398.3 ± 56.3 |
| F7          |                                               | 331.1 ± 44.8 | Shrunk      | 333.1 ± 73.1 | 562.0 ± 84.5 |

Data are expressed as means ± SD, n=3.

Figure 3  In vitro release profile of nateglinide-loaded provesicle formulations in (A) PBS pH 7.4 and (B) HCl pH 1.2.
endotherm over the range 125–135 ºC which may have resulted from a change in NTG structure from a crystalline to an amorphous form. This may lead to an increase in the dissolution profile of NTG13 because amorphous drug does not require energy to break up the crystalline lattice15.

In terms of XRD data, the intensity of the diffraction pattern of pure NTG was higher at 4.3º, 13.9º and 20.1º over a diffraction angle of 2θ indicating that it mainly exists as a crystalline material. However, the diffractograms of the blank and formulation F4 showed some loss of peaks and more diffused peaks suggesting its presence in an amorphous state. These results reveal that NTG changes from a crystalline form to an amorphous form when formulated in proniosomes consistent with DSC studies.

This may be due to enhancement of NTG solubility by the non-ionic surfactant or to a change in NTG structure from the crystalline to the amorphous form in provesicular formulations40.

In the in vitro release study, values of drug release from the formulation F4 with an SP:CH molar ratio of 5:5 were 93.32% and 66.20% in dissolution media with pH 7.4 and 1.2, respectively. Compared to other provesicular formulations and control, formulation F4 showed a significant enhancement of NTG dissolution and a higher EE (84.76%) in both dissolution media (P<0.05). This may be due to enhancement of NTG solubility by the non-ionic surfactant or to a change in NTG structure from the crystalline to the amorphous form in provesicular formulations40. In fact, all provesicular formulations exhibited significantly higher dissolution than control in both dissolution media.

In the goat intestinal permeation study, the mean cumulative amount of drug permeated from formulation F4 at 48 h (30995.08 µg) was significantly higher (P<0.05) than from Glinate™ 60 and control as reflected in its higher steady state flux (626.3 µg/cm²/h) and permeability coefficient (62.63 cm/h). Furthermore, the enhancement ratio of formulation F4 was double than that of control indicating that the order of increasing permeation enhancement was F4>Glinate™ 60>control. In summary, the permeability of NTG was significantly enhanced (P<0.05) through the provesvesicular approach suggesting that provesicles might also enhance its oral bioavailability.

In the pharmacodynamic study, Fig. 5 shows that the groups of STZ induced diabetic rats treated with formulation F4 (test group) and Glinate™ 60 (standard group) both showed a significant (P<0.05) reduction in blood glucose levels at 4 h after the dose. Subsequently the group treated with Glinate™ 60 showed increasing blood glucose levels whereas the group treated with formulation F4 showed gradually decreasing levels up to 18 h. This difference was sufficient for the blood glucose level in the formulation F4 treated group to be significantly less (P<0.05) than in the Glinate™ 60 treated group.

In the stability study, provesicular formulations stored in the refrigerator (2–8 ºC) and at room temperature (25 ± 2 ºC) for three months showed no evidence of drug precipitation and retained their loose and uniform appearance. However, after 3 months formulation F4 was more stable under refrigerated conditions than at room temperature. Moreover, in terms of drug retention, Fig. 6C shows that the retention in formulation F4 is unchanged during storage under refrigerated conditions for 3 months (P<0.05). In summary, the results reveal that formulation F4 is more stable under refrigerated conditions than at room temperature with the ability to assign an extended shelf-life.

**Table 3** Permeation parameters of nateglinide from formulation F4 in comparison with Glinate™ 60 (Marketed) and control across goat intestine.

| Formulation | M (µg)a | Jₘ (µg/cm²/h)a | Eᵣ | Kᵣ (cm/h) |
|-------------|---------|----------------|-----|-----------|
| F4          | 30,995 ±117 | 626.3 ± 2.5 | 1.44 | 62.63     |
| Marketed    | 27,061 ±134 | 510.5 ± 2.1 | 1.17 | 51.05     |
| Control     | 22,576 ±192 | 433.0 ± 3.7 |  –   | 43.30     |

M, cumulative amount of drug permeated in 48 h; Jₘ, steady state flux; Eᵣ, enhancement ratio; and Kᵣ, permeability coefficient. P<0.05.

aData are expressed as means ± SD, n = 3.
produced a significantly greater reduction in blood glucose. It appears likely that these NTG provesicles will give higher bioavailability than pure NTG and provide a more effective treatment for type II diabetes.

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5. Conclusions

The results of the present study show that a provesicle formulation of an NTG–MTL complex with SP and CH produced free flowing, homogeneous and smooth vesicles. Of a number of formulations containing different SP:CH ratios, formulation F4 with an SP:CH molar ratio of 5:5 provided the highest EE and stability and gave significantly higher release of NTG (P < 0.05) in PBS pH 7.4 and HCl (pH 1.2) than from pure NTG. Furthermore, in a goat intestinal permeation study, formulation F4 gave significantly higher mean cumulative amount of drug permeated at 48 h than Glinate™ 60 and control and, in a pharmacodynamic study,
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