RESEARCH PAPER

Formulation And In Vitro Evaluation of Berberine HCL Proniosomal Gel For Topical Drug Delivery

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ABSTRACT
The current paper is dedicated to the development and in vitro testing of a proniosomal gel containing berberine hydrochloride to improve topical drug delivery. Berberine hydrochloride is a bioactive alkaloid that is of great antimicrobial, anti-inflammatory and wound healing effects but limited to clinical use because of poor skin absorption, retention and release, and sustained release in traditional formulations. To address these shortcomings, non-ionic surfactants (Span series), cholesterol and lecithin were used to create a proniosomal gel system through the coacervation-phase separation system. The ready-made proniosomes were added to a Carbopol gel base in order to enhance topical application. F8 had the best properties with its vesicle size measuring about 197 nm, a low polydispersity index (0.224) and encapsulation efficiency of 98.71%. The formulation exhibited appropriate physicochemical properties including skin compatible pH, ease of spread and texture. The pseudoplastic (shear-thinning) was verified through rheological studies, which guaranteed ease of application. In vitro drug release experiments revealed a much longer release profile of up to 48 hours in comparison to the fast release of pure drug. The kinetic of drug release was based on a first-order reaction with the mechanism being diffusion-controlled (Fickian). The stability studied under ICH conditions proved the stability of the formulation with slight changes in the important parameters. Generally, the designed proniosomal gel was found to be a promising carrier system to be used in topical delivery of berberine hydrochloride, as it has better permeation, long-term drug release, stability, and patient compliance than traditional formulations.
Keywords: Berberine hydrochloride, Proniosomal gel, Topical drug delivery, Vesicular drug delivery system, Sustained release, Skin permeation enhancement.
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Introduction

Topical drug delivery systems have received an important spotlight over the last few years because of their ability to provide localized treatment, reduce systemic side effects, and enhance patient compliance. The skin is the largest organ of the human body covering about 1.5-2.0 m 2 areas and is a good shield against environmental offenses, including pathogens, chemicals and ultraviolet radiation. This protective action, however, which is mainly credited to the stratum corneum, is also a significant threat to drug permeation, especially to hydrophilic and high molecular weight drugs [1, 2]. Topical drugs can enter the skin via the transcellular, intercellular, and trans appendageal routes though the intercellular route is the most common. Nevertheless, the traditional topical preparations such as creams, ointments, and gels are usually characterized by the following shortcomings: low penetration, fast loss of drug, low ability to release drugs in a controlled manner or low therapeutic efficacy . These problems require the creation of superior drug delivery methods that can overcome the barrier properties of the skin [3, 4]. In this respect, liposomes, niosomes, transferosomes, ethosomes, and proniosomes are examples of vesicular systems of drug delivery that have been suggested as a promising method of improving dermal and transdermal delivery of drugs [8,9]. Among them, proniosomes have special benefits such as enhanced physical stability, storage convenience, less drug leakage, and controlled drug release properties . When hydrated, the proniosomes will create niosomal vesicles that will interrelate with the lipid matrix layer of the stratum corneum and thus increase drug permeation and retention into

the skin layers [5, 6]. Berberine hydrochloride is a naturally occurring quinoline alkaloid that has been shown to exhibit numerous pharmacological effects such as antimicrobial, anti-inflammatory, antioxidant, and wound healing effects . Although it has therapeutic potential, its use in the topical delivery is limited because of the low lipid solubility, low permeability and low bioavailability . Thus, the addition of berberine to more sophisticated carrier systems like proniosomal gels is a good initiative to increase its delivery to the skin and therapeutic effects. The objective of the review is to give a thorough account of the topical drug delivery systems, skin permeation processes and the use of vesicular carriers, especially the proniosomes to enhance the delivery of bioactive agents like berberine hydrochloride [7, 8].

Materials and Methods

Materials

Berberine hydrochloride was used as the model drug. Span 20, Span 40, Span 60, and Span 80 which are non-ionic surfactants and cholesterol were purchased at SD Fine Chemicals (Mumbai, India). A gift sample of Soy lecithin was acquired at Lipoid (Nattermannallee, Germany). Potassium dihydrogen orthophosphate, sodium hydroxide, ethanol (analytical grade) and orthophosphoric acid were acquired in E. Merck (Mumbai, India). The chemicals and reagents were of an analytical grade.

Preparation of Proniosomal Gel

The modified coacervation-phase separation technique was used to prepare berberine hydrochloride-loaded proniosomes. Drug, surfactant and cholesterol were weighed and put in clean glass vials and dissolved in small volume of absolute ethanol (~0.5 mL). The mixture was heated to 55-60 °C with periodic shaking till all the components dissolved. Phosphate buffer (pH 7.4) was then put in, and the mixture was heated further (2 minutes) to get a clear solution. The system was left to cool down to room temperature to make the proniosomal gel. The ready proniosomes were added to 1% Carbopol gel in the ratio of 1:1 to form the final proniosomal gel formulation. The preparations were refrigerated in dark containers awaiting further analysis.

Preformulation Studies

Melting Point Determination

The capillary method was used to determine the melting point of the berberine hydrochloride using a digital melting point apparatus. This was analyzed three times and the mean was taken.

FTIR Analysis

The Fourier Transform Infrared (FTIR) spectroscopy was used to assess drugexcipient compatibility. The samples were ready through the use of KBr pellet and scanned between 4000 and 500 cm -1.

Characterization of Proniosomes

Particle Size, PDI and Zeta Potential

Dynamic light scattering was used to measure the size of the vesicles, polydispersity index (PDI) and zeta potential (Malvern Zetasizer, UK). A 1:1 dilution of samples in distilled water was analyzed at 25^{o} C.

Transmission Electron Microscopy (TEM)

Morphological assessment was done using TEM. The samples were diluted and put on a grid with carbon-coated surface and stained with uranyl acetate in the presence of proper accelerating voltage.

Encapsulation Efficiency

Encapsulation efficiency (EE%) was determined by centrifugation method. The amount of free drug in the supernatant was analyzed spectrophotometrically, and EE% was calculated.

Evaluation of Proniosomal Gel

Physical Appearance and pH

Formulation was tested in terms of color, homogeneity and texture. A digital pH meter with calibration was used to measure the pH.

Rheological Studies

The rheological behavior of the samples was studied in a Brookfield viscometer with different shear rates (10-100 rpm).

Viscosity

Brookfield viscometer was used to measure the viscosity at controlled temperature (25 ± 0.5 °C).

Spreadability

The glass slide method was used to determine spreadability and standard formula was used to calculate.

In Vitro Drug Release Study

A dialysis membrane diffusion method was used to test the in vitro release of berberine hydrochloride. The formulation was put in a dialysis membrane and incubated in phosphate buffer (7.4) at 37 °C with constant stirring. The samples were sampled at specified intervals and were analyzed spectrophotometrically.

Stability Studies

The optimized formulation was subjected to stability testing according to ICH guidelines (Q1A(R2)) over 3 months in various storage conditions (25^{o} C, 60% relative humidity and 40^{o} C, 75% relative humidity). Periodic evaluation of samples was done in terms of pH, particle size, PDI and encapsulation efficiency.

3. Results

Melting Point Determination

The reported literature values of melting point of berberine range between 145 and 147°C and the melting point was found between 145 and 147°C, thus showing the purity and identity of the drug

FTIR Analysis

Berberine was found to have a melting point of 145–147°Cwhich agrees with the reported literature values and is a good indication of the purity and identity of the FTIR spectrum of berberine indicated characteristic peaks that corresponded to the functional groups of the molecule such as the aromatic and heterocyclic groups. The characteristic peaks of alkyl chains, ester, and phospholipid groups were observed in the spectra of the excipients, span 40, Span 60, lecithin, and cholesterol. No drastic change in major peaks of berberine and excipients was observed in the physical mixture, indicating the lack of chemical interaction during the mixing of the two. Equally, the optimized proniosomal gel (F8) still had the typical berberine peaks albeit with slight broadening and slight shifts that can be ascribed to physical interactions, hydrogen bonding and forming of vesicles. Notably, neither loss of significant peaks nor the formation of new peaks were found, which confirms that drug-excipient compatibility and chemical stability of berberine in the proniosomal system.

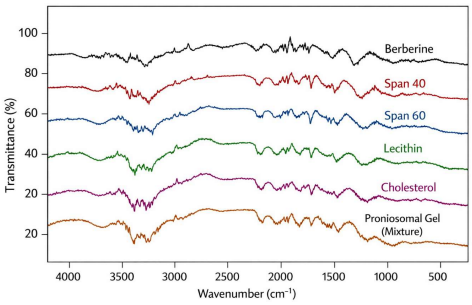

Figure:1. The spectral data of the FTIR of berberine, span 40, Span 60, lecithin, cholesterol, physical mixture and optimized proniosomal gel (F8), indicating the presence of characteristic functional group peaks and the non-existence of a significant drug-excipient interaction.

The purity and identity of the drug were confirmed by the fact that the melting point of Berberine was 145°C -147 °C as compared to the reported literature values.

Particle size and polydispersity index of berberine proniosomes

The size and size distribution of the berberine-loaded proniosome particles were examined using dynamic light scattering (DLS). The improved formulation was found to have a standard deviation of 1.3 nm and a mean vesicle size of 197.22 nm. Polydispersity index (PDI) was estimated to be 0.224 and it was observed that the vesicular system was rather narrow in size and well homogeneous. The PDI value of less than 0.3 indicates that the formulation is monodispersed and stable and there is no significant aggregation of vesicles.

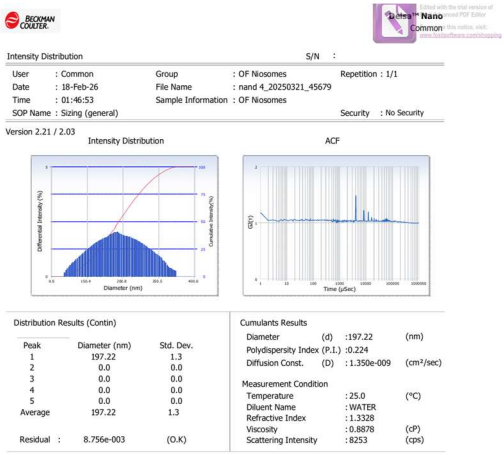

Figure 2. Polydispersivity Index and Size of Berberine proniosomes

Transmission Electron Microscopy (TEM) analysis of berberine proniosomes

Transmission electron microscopy (TEM) was used to analyze the morphology of the berberine-loaded proniosomes. The micrograph showed that the vesicles were mostly round in shape and had distinct boundaries which means that the niosomal vesicles were formed successfully. The particles were observed to be well dispersed, and there was very little aggregation, which is indicative of the homogeneity of the formulation. The vesicle size was observed to be of the order of nanometer (approximately 200 nm), which is quite similar to the size of the particles determined by DLS. The vesicles also had a characteristic core-shell arrangement indicating that there was a bilayer membrane as found in niosomal systems. The vesicular system stability and integrity are also shown by the smooth surface morphology. All in all, the TEM analysis establishes that stable, nanosized and uniformly distributed berberine-loaded proniosomes are formed and can be used in the delivery of topical drugs.

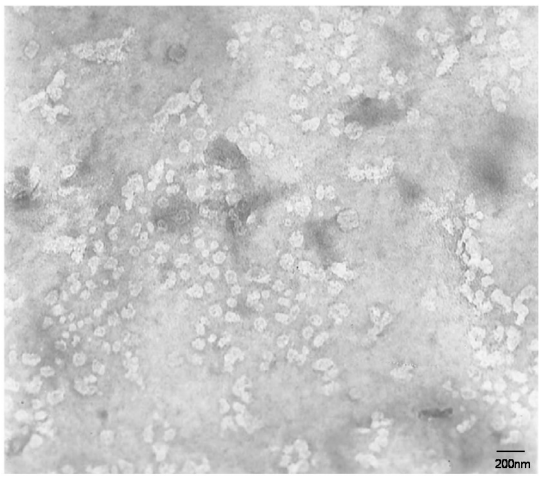

Figure 3. Tem analysis of berberine proniosomes

Encapsulation efficiency

Table 2. Particle Size, Polydispersity Index (PDI), and Encapsulation Effectiveness of Proniosomal Formulations (F1-F10) Loaded with Berberine HCl

Formulation Code	Particle Size (nm)	PDI	Encapsulation Efficiency (%)
F1	>250	>0.500	87.2 ± 1.3
F2	>240	>0.520	88.5 ± 1.4
F3	>260	>0.550	86.9 ± 1.2
F4	>270	>0.580	87.8 ± 1.5
F5	>230	>0.510	89.6 ± 1.3
F6	>220	>0.530	90.8 ± 1.4
F7	>210	>0.520	91.5 ± 1.2
F8	197.2 ± 1.3	0.224	98.71 ± 1.1
F9	>215	>0.540	90.2 ± 1.3
F10	>225	>0.560	± 1.4

Characterization of proniosomal gel

Appearance and pH

The pH of semi-solid formulation is important in ensuring the stability of the active pharmaceutical ingredient and the physicochemical characteristics of the formulation. It might also have an impact on the product's viscosity and the effectiveness of the additional preservatives. The proniosomal gel loaded with berberine had a pH of 5.5–6.0, which is consistent with the skin's typical physiological pH. As a result, the formulation is appropriate for topical treatments and is not likely to irritate or cause discomfort.

Table 3. Organoleptic Properties of Berberine-Loaded Proniosomal Gel

Organoleptic Parameter	Characters observed
Colour	Yellow to pale yellow
Grittiness	No grittiness
Greasiness	Non-greasy
Stickiness	Non-sticky
Texture	Smooth and homogeneous
Stiffness	No stiffness (easily spreadable)

Rheological behavior of berberine HCl proniosomal gel

The rheological behavior of the proniosomal gel loaded with berberine HCl was evaluated using shear stress as a function of shear rate. The results obtained revealed that there was an increase in shear stress as shear rate rose, showing that the two parameters had no linear relationship. The formulation's non-Newtonian shear-thinning (pseudoplastic) behavior was confirmed by the progressive drop in viscosity with an increase in shear rate. The gel was comparatively more difficult to flow at low shear rates, while the system could flow easily at high shear rates. Topical preparations with this property are preferable because They maintain enough viscosity while at rest, yet they become simple to apply and disseminate on the skin. Also, the curve does not show any sharp change or hysteresis, which indicates high structural integrity and reliability of the formulation. This is due to the smooth and continuous flow pattern which implies that the drug is uniformly dispersed in the proniosomal gel matrix. All in all, the rheological

profile identifies that the gel formulated has an appropriate flow property to deliver topically to guarantee enhanced patient compliance and successful topical application.

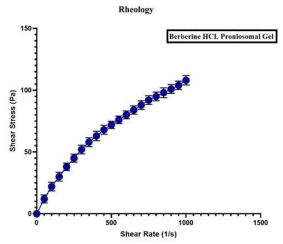

Figure 4. Proniosomal gel rheological profile loaded with berberine HCl and reliant on shear rate (s-1) and shear stress (Pa). The formulation shows non-Newtonian pseudoplastic (shear-thinning) behavior, where the shear stress increases as the shear rate increases.The information is presented as means and standard deviations (n = 3).

Viscosity

A time dependence of viscosity of berberine HCl loaded proniosomal gel was carried out on a constant shear condition. These data have shown a progressive reduction of the viscosity between about 0.7 Pa s and 0.16 Pa s during the period of study. The decreasing viscosity with time suggests that the formulation is shear-thinning, i.e., whereby the internal resistance to flow reduces as the shear is applied continuously. This high viscosity at the beginning indicates that the gel is well organized in gel network which gradually disintegrates under shear stress resulting in an increase in the flowability. The gradual and steady decrease in viscosity with no sudden variations indicates the consistency and consistency of the formulation. This is beneficial in topical applications because it allows the topical to be easily spread at the time of application and at rest to have adequate viscosity to prevent running off. Overall, it is possible to state that the viscosity profile has confirmed that the developed berberine proniosomal gel has the desirable rheological properties that can be utilized in the effective delivery of topical drugs.

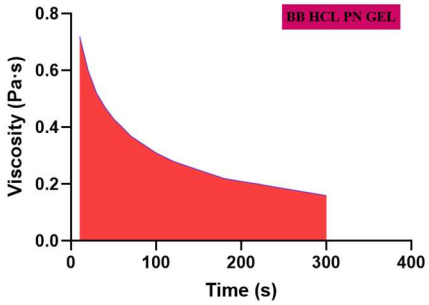

Figure 5. Time dependence of viscosity profile of berberine HCl-loaded proniosomal gel. The development of the formulation shows gradual reduction of viscosity with time holding constant shear implying shear-thinning behavior. Such a decrease in viscosity corresponds to the destruction of the internal gel network during constant shear that is positive in terms of better spreadability and topical application.

Spreadability

The spreadability of the berberine HCl loaded proniosomal gel was measured and compared to the unloaded gel formulation. The data showed the plain gel (PG) had a spreadability of about 11.5 gcm/sec, compared to berberine HCl proniosomal gel (BHPG) whose resultant had a slightly lower reading of about 10.2 gcm/sec. The small loss of the spreadability of the proniosomal gel can be explained by the addition of lipid-based vesicular ingredients, which enhance the internal consistency and structural integrity of the formulation. This decrease, however, did not affect the spreadability of BHPG significantly, and it is possible to state that the given formulation can be applied with ease and spread uniformly on the skin surface. The developed proniosomal gel, in general, has sufficient spread capacity, which guarantees convenient topical application and high patient compliance.

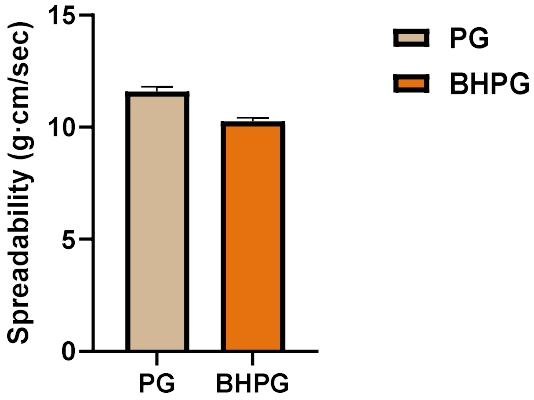

Figure 6. Comparative Spreadability of Plain Gel (PG) and Berberine HCl-Loaded Proniosomal Gel (BHPG)

In vitro drug release study

In the in vitro drug release profile, proniosomes, proniosomal gel, and pure berberine HCl all showed noticeably different release characteristics. The free berberine HCl demonstrated high drug release rate with the drug being released by about 95-98% in 6 hours and on the other hand the proniosomal formulation took a maximum of 8 hours to achieve the same degree of drug release. It shows that the proniosomal formulation had nearly 1.3 times slower release rate than the pure drug, which confirms the significance of vesicular encapsulation in regulating the diffusion of drugs. Comparatively, the proniosomal gel showed much longer drug release as the release was about 95% within 48 hours. The proniosomal gel showed a significantly longer duration of extended release, of almost 8 folds compared to the pure drug, indicating a significant retardation of diffusion of the drug. Moreover, the proniosomal gel was found to have the release time increased about 6-folds relative to that of the proniosomal formulation and is attributable to the extra barrier properties of the gel matrix and vesicular entrapment. Overall, the results clearly demonstrate that incorporating berberine HCl into proniosomes and subsequently gel-formulating it significantly enhances the sustained-release profile, making it more suitable for prolonged topical drug delivery.

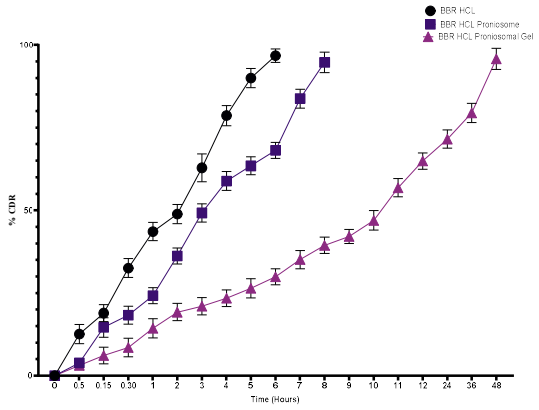

Figure 7. Comparative in vitro drug release profiles of proniosomes loaded with berberine HCl, proniosomal gel filled with berberine HCl, and berberine HCl (BBR HCl). The proniosomal gel had ~6–8-fold extended release, suggesting improved sustained release behavior, although the proniosomal formulation showed ~1.3-fold slower release than the pure drug. The information is displayed as mean ± standard deviation (n = 3).

Drug release kinetics of berberine HCl proniosomal gel

The in vitro drug release data of the proniosomal gel loaded with berberine HCl were fitted into several kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, in order to shed light on the release process. Regression coefficient (R^2) values of 0.8622 (zero-order), 0.8927 (first order), 0.8363 (Higuchi), and 0.5651 (Korsmeyer–Peppas model) were obtained from the models. The highest R2 value (0.8927) for the first-order kinetic model indicates that drug release from the proniosomal gel is mostly concentration-dependent. Additionally, the Higuchi model demonstrated a reasonably excellent linearity (R2 = 0.8363), indicating that drug release from the gel matrix is significantly influenced by diffusion. However, the Korsmeyer–Peppas model's relatively lower R2 value suggests that it is less appropriate for explaining this formulation's release behavior. The Korsmeyer-Peppas model's release exponent (n value ≈ 0.48) shows that drug diffusion through the gel matrix, a Fickian diffusion mechanism, is primarily responsible for controlling drug release. Overall, the findings support the berberine HCl proniosomal gel's suitability for long-term topical distribution by showing that it follows first-order kinetics with diffusion-controlled drug release.

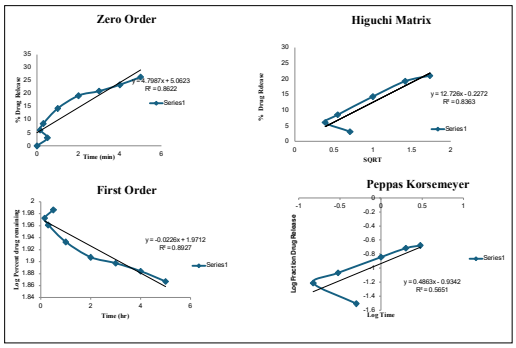

Figure 8. Berberine HCl-loaded proniosomal gel drug release kinetic modeling fitted to Higuchi, Korsmeyer-Peppas, zero-order, and first-order models. The first-order model (R^2 = 0.8927) demonstrated the strongest association, indicating concentration-dependent release, while the Higuchi model suggested diffusion-controlled drug release.

Stability studies

Table 4. **Over the course of three months, a short-term stability analysis of optimized berberine HCl proniosomal gel (F8) at 25 ± 2°C and 60 ± 5% Changes in pH, particle size, encapsulation effectiveness, and polydispersity index (PDI) were all revealed by RH**

Time	pH	Particle Size (nm)	PDI	EE (%)
0	5.58	197.2	0.224	98.71
1	5.57	198.5	0.228	98.10
2	5.56	200.1	0.232	97.60
3	5.55	202.0	0.238	97.10

Table 5. Short-term stability research was conducted on optimized berberine HCl proniosomal gel (F8) at accelerated conditions (40 ± 2°C/75 ± 5% RH) for three months to ascertain the effects of storage on pH, particle size, polydispersity index (PDI), and encapsulation efficiency.

Time	pH	Particle Size (nm)	PDI	EE (%)
0	5.58	197.2	0.224	98.71
1	5.55	201.5	0.240	97.80
2	5.52	205.8	0.255	96.90
3	5.49	209.6	0.270	96.00

Conclusion

To improve topical drug delivery, the current study has focused on the creation and in vitro testing of a proniosomal gel impregnated with berberine hydrochloride. The primary objective was to address the limited skin penetration of the conventional topical berberine formulations, low retention, and absence of prolonged release. Proniosomal formulations containing lecithin, cholesterol, and several non-ionic surfactants (Span 20, 40, 60, and 80) were prepared using the coacervation-phase separation method. F8 exhibited the best physicochemical properties such as the smallest vesicle size (~197 nm), the lowest polydispersity index (0.224), and the highest encapsulation efficiency (98.71 ± 1.1%), which showed the homogenous vesicle distribution and the high encapsulation efficiency of the compound. The optimized proniosomal formulation was then added to a Carbopol gel base yielding a non-irritant, homogeneous, and smooth topical formulation which had a skin compatible pH (5.560). The rheological analyses proved that the gel was non-Newtonian pseudoplastic, which is desirable in topical application because it provides easy spreadability and satisfactory retention in the place of application. The proniosomal gel was able to release the medication throughout 48 hours, but the pure drug only released after a brief period of time, according to the in vitro drug release experiment, which demonstrated the proniosomal gel's significantly larger release profile. Kinetics of drug release showed that the formulation conformed to first-order kinetics and diffusion-controlled (Fickian) release mechanism, which validates the importance of vesicular encapsulation and gel matrix to control drug release. In addition, it was found that the optimized formulation was stable within three months under the recommended conditions of ICH, with a slight change of the particle size, PDI, pH, and encapsulation efficiency, which proves

that the optimized formulation is robust and can be stored. In general, Proniosomal gel is determined to be a promising and practical carrier system of topical application of berberine hydrochloride, providing better drug permeation, longer release, greater stability, and patient adherence, as opposed to traditional preparations.

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