International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

Research Article **Open Access**

Preparation, *In-vitro* Characterization of Transdermal Proniosome Gel Containing Nifedipine

P. Anitha*, M. Purushothaman, P. Gowthami, D. Bhargavi, M. Krishnaveni, M. Rajesh, O. Sudheer Kumar

Department of Pharmaceutics, Vasavi Institute of Pharmaceutical Sciences, Kadapa, A.P, India

A B S T R A C T

As an alternative to oral route, transdermal route of drug delivery was developed because of its limitations. This delivery has limitations in permeation of most drugs. One approach to this problem has been to use lipid-based vesicles as drug carriers as proniosomes which on hydration become niosomes. In the present study transdermal Nifedipine proniosomal gels was formulated by using Lecithin, Cholesterol as encapsulating agents, Surfactant, Span, Tween and permeation enhancers. The study methodology encompasses compatibility studies using FTIR spectra, evaluation of proniosomal gels for Vesicle size analysis, encapsulation efficiency, SEM analysis and *in vitro* permeation studies. The preliminary compatibility studies conducted revealed that there no interaction between Nifedipine and exicipients which was as evident from FTIR spectral studies. The physical characterization of proniosomal gels was found to be within the acceptable limits. It was observed that the gel formulations showed good spreadability. The proniosomes showed spherical and homogenous structure in optical microscopy and SEM analysis. All formulations showed zero order drug release by diffusion mechanism. The above results indicated that the proniosomal gels of could be formulated for controlled release of Nifedipine. The proniosomal gels are suitable for Nifedipine once a day controlled release formulation.

Keywords: Nifedipine, Proniosomes, Transdermal, Skin permeation, Diffusion mechanism

A R T I C L E I N F O

CONTENTS

Article History: Received 28 June 2016, **Accepted** 09 August 2016, **Available Online** 15 September 2016

***Corresponding Author**
P. Anitha
Department of Pharmaceutics,
Vasavi Institute of Pharmaceutical Sciences, Kadapa, A.P, India
Manuscript ID: IJCTPR3129

PAPER-QR CODE

Citation: P. Anitha, *et al.* Preparation, In-vitro Characterization of Transdermal Proniosome Gel Containing Nifedipine. *Int. J. Curnt. Tren. Pharm, Res.*, 2016, 4(5): 264-269.

1. Introduction

Transdermal drug delivery system play is vital role because this route is free from invasive methods. Topical preparation is the best patient compliant manner of drug delivery in the body[1].Transdermal drug delivery system is related to drug supply as sustained release (i.e. constant plasma drug conc.), over an extended time period[2]. The rapid development of transdermal delivery formulations in the previous years is due to its ability to overcome certain problems of the conventional system of drug development[3].

Various types of transdermal therapeutic systems are utilized for long term continuous infusion of therapeutic agents, including antihypertensive, antifungal, analgesics, steroids and contraceptive drugs[4]. Although transdermal delivery is currently limited to few drugs, it has achieved considerable commercial success. Various types of transdermal drug delivery systems to overcome some limitations include liposomes, erythrosomes, liposomes, niosomes, ethosomes, and proniosomes[5].

Proniosomes and ethosomes are recent development made in transdermal therapeutic systems. These are most advance devices which ignore demerits of liposomes and niosomes such as; Liposomes require special precautions and conditions for formulation and evaluations, Complex method for routine and large scale production, Less chemical stability High cost and while niosomes possesses demerits like; Fusion, Aggregation, Sedimentation, Leakage on storage and Physical instability[6]. In order to overcome the stability problem these are characterized as proniosomes which converts into niosomes on hydration. Proniosomes provide higher stability and better skin penetration ability than the traditional lipid vesicles[7].

Nifedipine, a calcium channel blocker used in the treatment of hypertension and angina pectoris. Nifedipine (20–60 mg) once-daily, orally given in the treatment of hypertension. Its solubility is poor in both lipophilic and hydrophilic media. The treatment requires a constant release of the drug into systemic circulation. Since, its half life is 2-4 hrs requires frequent dosing of the drug. Even though Nifedipine is rapidly and almost completely absorbed from GI tract but it undergoes extensive first pass metabolism (around 60%) resulting in a poor bioavailability (45%) after oral administration. Hence, to improve its therapeutic efficacy, patient compliance and to reduce the frequency of dosing and side effects as well as to avoid its extensive first pass metabolism, transdermal drug delivery approach was considered to be better suitable for Nifedipine[8]. An objective in treatment of chronic disease like hypertension is to provide greater therapeutic effect, overcome the side effects by complex therapeutic regimen, to simplify the treatment regimen and to improve patient compliance upon administering Proniosomal transdermal delivery of Nifedipine. Hence, in the present investigation, it is aimed to Design and evaluate the Proniosomal transdermal delivery of drug namely Nifedipine with two different surfactants like Tween and Span and Stabilizers like Lecithin and cholesterol.

2. Materials and Methods

Nifedipine was provided by Hetero drugs Pvt. ltd. Soya lecithin was purchased from Hi media-Bombay, Cholesterol was purchased from Hi media chemicals Bombay. All other chemicals used throughout this investigation were of analytical grade and no additional purification was carried out. Double distilled water was used throughout the study.

Preparation of calibration curve of Nifedipine

The standard calibration curve yields a straight line, which shows that the drug follows Beer's law in the concentration range of 1to 25µg/mL by using phosphate buffer pH 7.4 at 238nm. A standard graph was plotted by keeping the known concentration on X – axis and obtained absorbance on Y-axis[9].

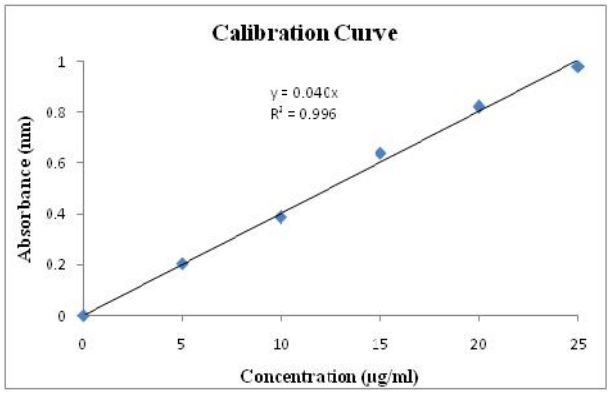

Figure 1: Calibration Curve of Nifedipine

Drug – polymer compatibility studies

One of the requirements for the selection of suitable exicipients (or) carrier for pharmaceutical formulations is its compatibility. Therefore in the present work, a study was carried out using FTIR to confirm the absence of any possible chemical interaction between the Drug and Exicipients namely Span, Tween, Lecithin and Cholestrol[10].

Development of Proniosomal Gel

Proniosomal gel was prepared by a Coacervation-phase separation method. Precisely weighed amounts of surfactant, lecithin, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (0.5 ml) was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (0.1% glycerol solution) was added and warmed on a water bath till a clear solution was formed which was converted into proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization [11, 12]. Compositions of proniosomal gel formulations are given in Table 2. Drugs incorporated in Gel –Nifedipine-20mg, Carbopol 934- 2%

Characterization of proniosomal gel:

The Characterization of Proniosomal Transdermal gel of Nifedipine was done by using the following evaluation methods.

Vesicle size analysis:

Hydration of proniosomal gel (100mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 100 x magnification. The sizes of 200-300 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope [13]. The Results for vesicle size were shown in Table No.3

% Entrapment efficiency

To evaluate the loading capacity of proniosomal systems for Nifedipine, proniosomal gel (100mg) was dispersed in distilled water and warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 18000 rpm for 40min at 5ºC (Remi CPR-24 centrifuge). The clear fraction was used for the determination of free drug at 238.0 nm spectrophotometrically. The percentage encapsulation efficiency was calculated from Equation[14]. The Results for % entrapment Efficiency were shown in Table No.3

% Encapsulation Efficiency = [1- (Unencapsulated drug / Total drug)] x 100

Scanning Electron Microscopy (SEM) Analysis:

Best formulation of proniosomes NF3 were coated uniformly with gold palladium by using Sputter coater under vacuum (0.1 mm Hg), after fixing the sample in individual stabs and was randomly examined [15,16]. SEM analysis of NF3 was shown in Figure No. 5.

***In vitro* drug release studies**

The *in vitro* drug release studies of proniosomal gel were carried out by means of treated cellophane membrane. *In-vitro* release studies on proniosomal gel were performed using Franz-diffusion cell. The capacity of receptor compartment was 10 ml. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the membrane [17, 18]. The receptor medium was saline phosphate buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1ºC. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrerr[19]. At each sampling interval, (1 ml) were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectro photometrically (Systronics-2200) at 238 nm [20, 21].

Release kinetics: The dissolution data is fitted to popular release models such as Zero order, (Higuchi) Diffusion and (Peppa's & Korsemeyer) Erosion equations [22].

3. Results and discussions

Recently, there has been resurgence in development of transdermal system for therapeutic use because of its better safety profile, better bioavailability, and better patient compliance. Different techniques have been developed to enhance the penetration of drug across the skin to avoid the major problem of traditional transdermal patch. One of the techniques to enhance the penetration technique is through vesicle formation. In the present work efforts have been made to prepare proniosomal transdermal drug delivery system of Nifedipine using different exicipients such as Span 80, Tween 80, Lecithin and Cholesterol then the results were compared for all the formulations. The drug reservoir was prepared by using Carbopol 934 and combination of above mentioned proniosomal gel and permeation enhancer used was Propylene glycol. Nifedipine solution was scanned in the UV range of 200-400nm using systronic u.v-visible spectrophotometer.

The Spectrophotometric method of analysis of Nifedipine at λ_{max} 238 nm was found to be reproducible and highly sensitive. The standard curves of Nifedipine were prepared in ethanol and phosphate buffer solution (pH 7.4), at λ_{max} 238 nm. The data were regressed to obtain the straight line. The correlation coefficient greater than 0.996 was observed in all the cases, which indicated that, the drug follows Beer-Lambert's law in the concentration range of 10mg/10ml (Table 1 and Figure 1).

The Preformulation studies are the first step in the development of any formulation. The major goal of this study is to establish compatibility of drug with that of the polymers used. The development of a successful formulation depends only on suitable selection of exicipients. Hence the physical state of the drug, Nifedipine using different exicipients such as Span 80, Tween 80, Lecithin and Cholesterol individually and the admixture of drug and exicipients used were studied by FTIR to know the drug – polymer compatibility after interpretation and IR spectra in Figure 2-4.

The physicochemical compatibility of the drugs and the Exicipients was established through FTIR studies (Figure 2-4). IR spectral analysis of Drug Nifedipine showed the peaks at wave numbers of 3398 (N-H stretching), 1685 (C =O Stretching), 1527 (aromatic NO2), 1435 (C – N Stretching) confirming the purity of drug with standard respectively. In the physical mixture of Drug with Exicipients the major peaks show the wave numbers of drug. However, additional peaks were absorbed in physical mixtures which could be due to presence of polymers and indicated that there was no chemical interaction between drug and other exicipients.

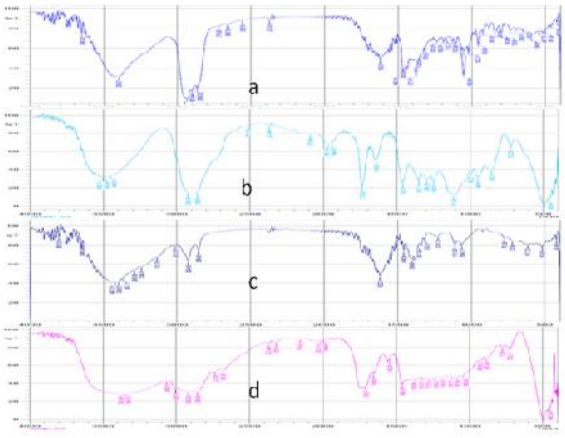

Figure 2: FTIR Spectra of a) Span 80 b) Tween 80 c) Cholesterol d) Lecithin

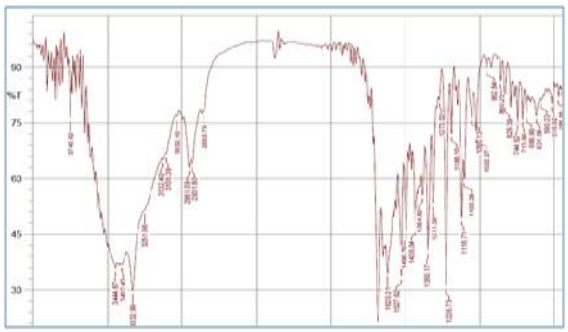

Figure 3: FTIR spectra of Pure Nifedipine

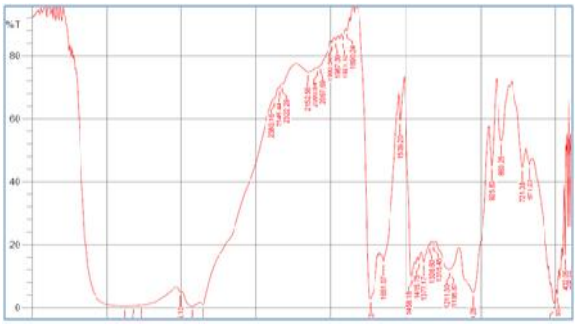

Figure 4: FTIR spectra of Formulation NF3

Results of Vesicle size of Nifedipine proniosome are presented in (Table No.3), which indicated that Vesicle formed with Span is smaller in size than vesicle formed with Tweens; this is due to greater hydrophobicity of Spans than Tweens. It is indicated that increasing in hydrophobicity decreases surface energy of surfactants resulting in smaller vesicle size. Size of vesicle was reduced when dispersion was agitated. The reason for this is the energy applied in agitation which results in breakage of larger vesicles to smaller vesicles. The size range was found to be 3.26±0.024µm to 5.80±0.25µm. Entrapment efficiency was found to be higher in case of proniosome prepared with Span80 than proniosome prepared with Tween80 this is due to fact that Span 80 is more hydrophobic than Tween 80, which act as solid at room temperature and showed higher phase transition temperature (Tc), low HLB value and long alkyl chain length and results are shown in Table 3.

Surface morphological studies (SEM) revealed that proniosomes formed were spherical and homogeneous as shown in Figure 5.

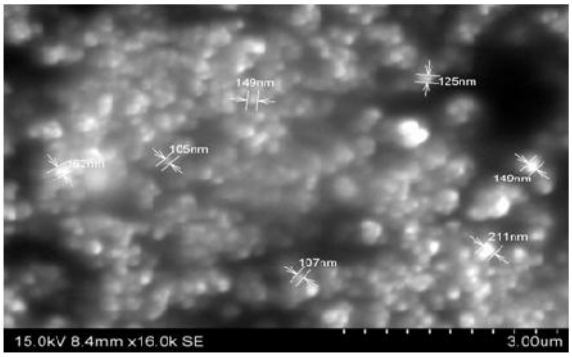

Figure 5: SEM Analysis of NF3

In vitro release studies are often performed to predict how a delivery system might work in an ideal situation as well as give some indications of its *in vivo* performance since drug release dictates the amount of drug available for absorption. The amount of drug released from different proniosomal gel formulation was found in the order of NF3 > NF1 > NF2 > NF4> NF6 > NF5 as shown in (Figure.6). It was found that NF3 showed controlled release property from 10 to 24 hrs. The cumulative release found to 98.43% at the 24th hrs, respectively. The release rate was constant from 10thto 24th hrs. From kinetic release studies, it was confirmed that the release of drug follows zero order shown in Table.4. Thus the formulation exhibited zero order release over this period.

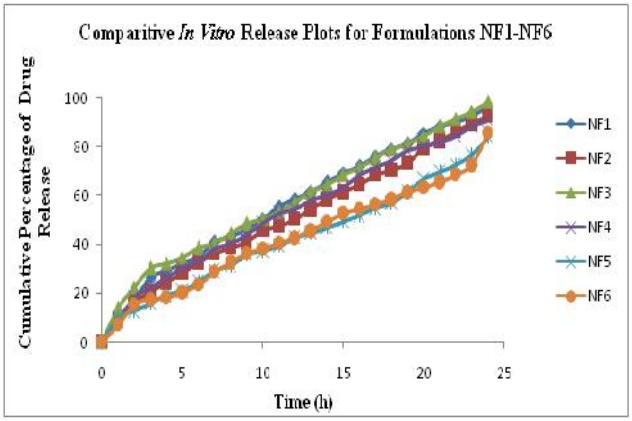

Figure 6: Comparison of *In vitro* Release studies for formulations NF1-NF6

4. Conclusion

The results of investigation revealed that proniosomes offers an alternative colloidal carrier approach in transdermal drug delivery. Even though Nifedipine is rapidly and almost completely absorbed from GI tract but it undergoes extensive first pass metabolism resulting in a poor bioavailability after oral administration. Hence, to improve its therapeutic efficacy, patient compliance and to reduce the frequency of dosing and side effects as well as to avoid its extensive first pass metabolism, proniosomal transdermal drug delivery approach was considered to be better suitable for Nifedipine. The more medications prescribed, the fewer patients adhere to the full treatment regimen. In contrast, the total number of medications taken per day could be reduced if the drug given through proniosomes. The results obtained from the present study clearly revealed that proniosomal gel containing Nifedipine which is prepared by using Coacervation phase separation method are capable of releasing drug for the extended period of time.

5. Acknowledgement

The author wishes to thank the management of Vasavi Institute of Pharmaceutical sciences for allowing carrying out the research and for providing all facilities to carry out the remaining part of the research work. The author is grateful to Hetero drugs, India for the gift samples of Nifedipine.

Table 1: Calibration Curve of Nifedipine

S.No.	Concentration (µg/mL)	Absorbance (nm)
1	5	0.205
2	10	0.386
3	15	0.642
4	20	0.821
5	25	0.982

Table 2: Composition of Proniosomal gel using Nifedipine

F. Code	Span 80 (mg)	Tween 80 (mg)	Lecithin (mg)	Cholesterol (mg)	Alcohol (ml)
NF1	900	-	900	100	0.5
NF2	900	-	900	200	0.5
NF3	900	-	450	100	0.5
NF4	-	900	900	100	0.5
NF5	-	900	900	200	0.5
NF6	-	900	450	100	0.5

Table 3: Characterization Techniques of Proniosomal gel

F. Code	Vesicle Size (µm) ±S.D.	% Entrapment Efficiency
NF1	3.26±0.24	81.42
NF2	4.26±0.46	79.27
NF3	3.36±0.196	85.43
NF4	4.80±0.14	80.34
NF5	5.80±0.25	76.86
NF6	3.80±0.025	78.32

Table 4: Diffusion characteristics for all formulations NF1-NF6

Batch code	Regression for *In-vitro* plot (r^2)	Regression for Higuchi's plot (r^2)	Slope for Peppa's plot (n)
NF1	0.933	0.955	0.686
NF2	0.963	0.929	0.710
NF 3	0.904	0.959	0.591
NF 4	0.938	0.952	0.705
NF 5	0.973	0.900	0.698
NF 6	0.963	0.913	0.725

6. References

[1] Kanitakis J. Anatomy, histology and immune histochemistry of normal human skin. *Eur J Dermatol*. 2002, 12(4), 390-399.

[2] Basak SC, Vellaiyan K. Transdermal drug delivery system, *Eastern Pharm.* 1997, 40(1), 63-67.

[3] Finnin CB. Transdermal drug delivery-What to expect in the near future. Business briefing: *Pharmaceutical technology*. 2003, 192-93.

[4] Touitou E. Compositions for applying active substances to or through skin. *US Patent*, 5540934, 1996.

[5] Perrett S, Golding M, Williams WP. A Simple Method for the Preparation of Liposomes for Pharmaceutical Applications: Characterization of the Liposomes. *J. Pharm. Pharmacol.* 1991, 43(3), 154-61.

[6] Bouwsta JA, Honeywell-Nguye PL. Skin structure and mode of action of vesicles. *Adv Drug Del Rev.* 2002, 54, S41-S55.

[7] Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. *Eur J Pharm Biopharm*. 2000, 50, 161-177.

[8] Dubey V, Mishra D, Nahar M, Jain NK. Vesicles as tools for the modulation of skin permeability. *Expert Opin. Drug Deliv*, 2007, 4(6), 579-593.

[9] Neeraja P, Amaleshwari M, Ravali G. Formulation and evaluation of multiple emulsion. *Int J pharm, cheml and bio sci.* 2014, 4(3), 673-680.

[10] Pankaj S, Rini T, Dandagi PM. Formulation and evaluation of proniosome based drug delivery system of Anti fungal drug Clomtriazole. *Int J pharm sci nano tech.* 2013, 6(1), 1946-1951.

[11] Gupta A, Prajapathi S, Bala Murugan K. Design and development of a proniosomal trandermal drug delivery system of captopril. *Tropical J Pharm Res.* 2007, 6, 687-93.

[12] Ramandeep Kaur, Meenu Nagpal, Rupinder Sidhu, Sukhdev Singh, Upendra Kumar Jain. A review on proniosome, a promising carrier for delivery of drug across membranes. *World J Pharm and Pharm Sci.* 2014, 3(9), 714-727.

[13] Azeem A, Ahmad FJ, Talegaonkar S. Exploration of skin permeation mechanism of frusemide with proniosomes. *Pharmazie.* 2009, 64, 735-40.

[14] Shamsheer Ahmad S, Sabareesh M, Patan Rafi Khan, Sai krishna P, Sudheer B. Formulation and Evaluation of Lisinopril Dihydrate Transdermal Proniosomal Gels. *J App Pharm Sci*. 2011, 1(8), 181-185.

[15] Jeevana Jyothi B, Guru Lakshmi G. *In-Vitro* and *In-Vivo* evaluation of Transdermal prolonged release proniosomal gel formulations of Propranolol HCL. *Int J Drug Del & Res*. 2014, 6 (3), 7-17

[16] Prakash Goudanavar, Ashish Kute, Doddayya Hiremath, S.R. Reddy. Development and Characterization of Perindopril Erbumine Loaded Proniosomal Gel. *Asian J Pharm and Tech.* 2012, 2(2), 54-58.

[17] Sheth PR, Tossounian J. The hydrodynamically balanced system (HBSTM): A novel drug delivery system for oral use. Drug *Dev and Ind Pharm.* 1984, 10(2), 313-339.

[18] Prajapati SK, Kumar S, Sahu VK, Prakash G. Proniosomal gel of flurbiprofen: formulation and evaluation. *J Drug Del & Therap.* 2012, 2(1), 1-5.

[19] Chandrasekhar N. Physico chemical and Pharmacokinetic Parameters in drug selection and Loading for Transdermal Delivery. *Indian J Pharm Sci.* 2008, 70, 94-96.

[20] Hemanth Patil N, sharwaree R Hardikar, Ashok V. Bhosale. Formulation development & evaluation of proniosomal gel of carvedilol. *Int J Pharm and Pharm Sci.* 2012, 4(1): 191-197.

[21] Abdelbary.G, El-gendy N. Niosome-Encapsulated Gentamicin for Ophthalmic Controlled Delivery. *AAPS Pharm Sci Tech*. 2008, 1058-1059.

[22] Kapil Kumar,A. K. Rai. Development and evaluation of proniosomes as a promising drug carrier to improve transdermal drug delivery. *Int res J pharm.* 2011, 2(11), 71-74.