Contents

11.1 INTRODUCTION

Decline of memory function or dementia is usually caused by degeneration in the cerebral cortex, the part of the brain responsible for thoughts, memories, actions and personality. There are a number of drugs available today for improving brain function. The more recent anti-dementia agents belong to the so-called acetylcholinesterase inhibitors such as Tacrine, Rivastigmine, Donepezil, Galanthamine, Dihydroergotamine, Piracetam and **Noopept** etc. A growing number of herbal remedies, vitamins and other dietary supplements such as Fisetin, L-Theanine and **Vinpocetine** etc. are also promoted as treatments for Alzheimer's dementia and related diseases. They can be appealing to some people as they come from natural ingredients.

Vinpocetine (VPN) is a semisynthetic derivative alkaloid of vincamine, an extract from the periwinkle plant. VPN is widely marketed as a supplement for vasodilation and as a nootropic for the treatment of cerebrovascular disorders and age-related memory impairment. However, the clinical use of its marketed commonly used oral formulations is limited by its poor dissolution profile, and extensive first pass metabolism that results in very low bioavailability (~7%). This **poor oral bioavailability** together with the **short $t_{1/2}$** (2.54 ± 0.48 hrs) implies the necessity of **frequent drug dosing (three times daily)**, a situation that

is inconvenient for patients of dementia and results in poor compliance. The 15-30mg/day of VPN in three divided dose is generally recommended in treating dementia or cerebrovascular disorders.

Noopept (NPT) is a synthetic dipeptide molecule derived from the endogenous dipeptide cycloprolylglycine, a combination of the amino acids glycine and proline in a cyclic configuration. It is commonly prescribed as cognitive enhancer in Russia while readily available and legal in USA. It is well-known for its anti-oxidant, anti-inflammatory, anxiolytic effect as well as mild psychostimulant-like effects. However, preliminary findings based on serum concentrations and excretion kinetics suggested that oral doses of 50mg/kg are roughly equivalent to injected dosages of 5mg/kg. This implies that **oral bio-availability is only 10%** compared to injections.

To overcome the aforesaid concerns associated with oral administration of the VPN and NPT, consideration was given to non-invasive, user-friendly **transdermal route** that offers a number of inherent advantages viz., avoiding first-pass metabolism, achieving infusion like zero order drug delivery profile, improving patient compliance by reducing the frequency of administration for short half-life medications and providing opportunity to cease absorption in the event of an overdose or other problems. However, the outermost skin layer, stratum corneum (SC), composed of dead keratinized tissue of about 10–20 μm in thickness constitutes a major obstacle and severely limits the delivery of molecules. A variety of physical and chemical methods have been explored to breach SC including use of penetration enhancers, lasers, electrical energy, ultrasound, radio frequency, thermal energy and microneedles.

Microneedles provide a minimally invasive, painless way of creating microchannels in skin which can then allow the transport of drug delivery vehicle across impervious SC barrier, deeper into the skin and systemic circulation. The structures are small enough that they do not reach the nerve endings rich region of dermis and thereby avoid sensation

of pain. The dimensions of the pore created in skin by microneedles are typically around 50-200μm through which even nanosized particulate drug carriers can easily be delivered transepidermally. Among a variety of microneedles, silicon or metal microneedles suffers the problems of requiring dedicated and costly clean room facilities and chances of accidental needles break off in the skin which may arise complications. In contrast, **dissolving microneedles patch (MNP)** made up of hydrophilic biodegradable polymers avoid such drawbacks and can be made by a simple and cheap micromolding technique, get dissolved in the skin after creating microchannels.

Another obstacle during transdermal delivery of lipophilic drugs is imposed by relatively higher hydrophilicity of dermal region as compared to epidermal region. Being lipophilic, VPN and NPT both were expected to get deposited in skin epidermis. Hence, VPN and NPT were further envisaged to be incorporated in to suitable nanocarriers that can easily carry the payload to the vicinity of papillary area having rich capillary network while controlling their delivery to meet therapeutic need. To serve the aforementioned purpose, **polymeric nanoparticles (PNP)** and **ultradeformable liposomes (UDL)** were selected for present study.

Nanoparticles have been extensively utilized to tailor the drug release as per therapeutic requirements. However, their poor penetrability across stratum corneum often limits their transdermal use. Here, skin microporation with microneedles could be of great use to assist nanoparticles to overcome the SC barrier. In contrast to nanoparticles, ultradeformable liposomes have the ability to penetrate the skin intact owing to their vesicle deformability. and act as a drug reservoir to continuously transport drug through the skin. Additionally, solubility of poorly water-soluble drugs can be significantly improved in both the nanocarriers than in aqueous solution, which leads to a greater concentration gradient across the skin and subsequently improves permeation. An attempt was also made to incorporate these nanocarriers in MNP to combine skin microporation and drug administration in single

step. This may also be beneficial for delivering a constant and calculated fraction of drug each time, providing occlusive condition to prolong the time for which the pore remains opened, better handling and storage of the formulation, providing environmental protection to the ingredients and avoiding any microbial invasion through such pores.

11.1.1 Aims of the study

The present study was aimed at formulation and development of PNP and UDL loaded fast dissolving MNP of both VPN and NPT and their exhaustive *in vitro* and *in vivo* characterization to achieve following objectives:

- ✓ Maximizing drugs' permeation
- ✓ Sustaining drug release for prolonged period
- ✓ Improving bioavailability
- ✓ Reducing dose and dosing frequency
- ✓ Patient compliance
- ✓ Effective treatment/management of dementia

11.2 ANALYTICAL METHOD DEVELOPMENT

UV and HPLC methods for quantitative estimation of VPN in various *in-vitro, ex-vivo and in-vivo* experimental samples were developed and validated. The calibration plots, using UV-1800 spectrophotometer (Shimadzu, Japan), in acetonitrile at 274.6 nm λmax, in a mixture of acetonitrile and methanol (ratio, 2:8) at 273.8 nm λmax and in a mixture of ethanol and double distilled water (ratio, 3:7) at 271 nm λmax were generated and used to estimate the drug entrapped in polymeric nanoparticles, drug entrapped in ultradeformable liposomes and drug released in *in-vitro* diffusion media, respectively. For VPN estimation in *ex-vivo* and *in-vivo* samples, a more sensitive LC-20AT/SPD-20A HPLC system (Shimadzu, Japan) with Supelco® C18 column (Sigma-Aldrich, India) was used. A mixture of ammonium acetate buffer (1.54%) and acetonitrile [ratio, 40:60 v/v] was used as mobile phase at a flow rate of 1 mL/minute. The chromatogram was generated at 280 nm wavelength and calibration plot was generated. Analytical interference

study was also performed to ensure that the selected formulation excipients were not interfering with drug estimation methods.

HPLC methods were developed and validated for quantitative estimation of NPT in various *in-vitro, ex-vivo and in-vivo* experimental samples owing to less sensitivity of UV spectrophotometers towards NPT.

11.3 PREFORMULATION STUDIES

Authentication of drugs and evaluation of their compatibility with other formulation components of interest was done as a part of preformulation studies. VPN and NPT both were authenticated based on the comparison of their melting point, UV absorption spectra, FTIR spectra and DSC thermograms with that available in literatures. The UV absorption spectrum of VPN sample in 96% ethanol showed all characteristic peaks at 229 nm, 275 nm and 315 nm wavelengths. The presence of characteristic aromatic, carbonyl and ether stretching peaks of VPN was observed in the FT-IR spectrum of drug sample received. A sharp endothermic peak was observed at 151.35 °C which was in-line with its melting point that was found to be 151 °C. The UV absorption spectrum of NPT sample in 95% ethanol showed all characteristic peaks at 253 nm, 259 nm and 265 nm wavelengths. The presence of characteristic ester, peptide and N-acyl stretching peaks of NPT was observed in the FT-IR spectrum of drug sample received. A sharp endothermic peak was observed at 96.4 °C which was in-line with its melting point that was found to be 97 °C. These results confirmed the authenticity of the API samples received.

The compatibility among drug and other formulation components were analyzed using a multi-component 'prototype' formulation method. Herein, a blend of drug and other excipients, at their maximum anticipated level in the formulations, was prepared and its DSC thermogram was compared with that of individual formulation components. No shifting, addition or deletion of endothermic peaks was observed in DSC thermograms of formulation prototypes as compared to

individual excipients' thermogram. The result suggested the compatibility among the mixture components.

11.4 VPN AND NPT LOADED UDL

Out of several available methods for preparation, ethanol injection method was chosen for UDL preparation of both the drugs. Ethanol injection method involves use of non-harmful organic solvent and offers easy scalability. A blend of soya phosphatidylcholine (Phospholipon 90G; P90G; Tg - 0 °C) and hydrogenated soya phosphatidylcholine (Phospholipon 90H; P90H; Tg - 55 °C) was used as bilayer components together with sodium deoxycholate (SDC) as edge activator. When stored between the glass transition temperatures (Tg) of the two lipids, the lipid with higher Tg forms a gel state while lipid with lower Tg forms a liquid crystal state. The presence of several gel and liquid crystal phase in lipid bilayers improves entrapment of hydrophobic drugs and stabilize liposomes by restricting lateral movement and aggregation of these drugs.

The quality by design (QbD) approach was adapted for formulation development. Based on the scientific, therapeutic, industrial and regulatory aspects, quality target product profile (QTPP) elements and their targets were established. Similarly, based on the prior knowledge, literature review and experiment trials, two response variables viz., vesicular size and drug entrapment were selected as critical quality attributes (CQA). All the possible factors that could influence the CQA were identified to build a fish bone diagram and qualitative evaluation was done to categorize these factors in to low, medium and high risk factors. Factors with low to medium risk were controlled by assigning a constant level during preliminary trials. Seven factors including amount of P90G, P90H, SDC, VPN/NPT, stirring speed, injection rate and ethanol volume were qualitatively identified as high risk factors. Fraction factorial screening design was applied to statistically evaluate the impact of these high risk factors on CQA. Minitab® 17.1.0 software was used to generate randomized batch matrix with 17 experimental runs. Batches

were prepared as per the run order suggested by software and formulations were evaluated for Vesicular size and drug entrapment. The data obtained were entered in software for statistical processing. The Pareto and Normal plots generated for both VPN UDL and NPT UDL showed that amount of Lipids, SDC and Drugs had a significant effect on vesicular size of resulting liposomes considering $P < 0.05$ as a level of significance. Similarly, amount of lipids and VPN showed significant impact on drug entrapment of VPN UDL while amount of lipids showed significant impact on drug entrapment of NPT UDL. Owing to these observations, amount of lipids, drug and surfactant were selected as critical material attributes (CMA) for both VPN UDL and NPT UDL and exhaustively evaluated during final optimization to obtain the design space. Based on the main effect plots, the other three non-critical factors viz., injection rate, stirring speed and organic solvent volume were assigned a constant level of 0.5 mL/min, 500 rpm and 1 mL, respectively for both VPN UDL and NPT UDL.

Design-Expert® 7.0.0 was used to apply combined D-optimal response surface design for final optimization. Amount of P90G and P90H in lipid mixture was varied in such a way to keep the total lipid level constant at 20 mM. A randomized batch matrix of 28 experimental runs was generated by the software. Batches were prepared, evaluated for CQA and data obtained were entered in software for statistical processing. Software suggested quadratic model for mix order and linear model for process order for both VPN UDL and NPT UDL. Model terms with a p-value less than or equal to 0.05 (α-level) were considered as significant while hierarchy based removal of insignificant model terms (p-value > 0.1) was done to simplify the model. ANOVA of both CQA showed low standard deviation (SD) and high correlation coefficients (R^2) value indicating better prediction of responses by the model. A good agreement of Predicted R^2 with other R^2 also supported the prediction potential of the model. Diagnostic plots further demonstrated the normal distribution of data, constant variance, absence of lurking variable and

easy prediction of values from the model. Numerical optimization for achieving minimum vesicular size and maximum drug entrapment resulted optimization solutions with composite desirabilities of 0.949 and 0.936 for VPN UDL and NPT UDL, respectively. The optimized composition of VPN UDL was predicted to have 15.17 mM P90G, 4.83 mM P90H, 15 mole% SDC and 5 mole% VPN with mean vesicle size of 76.3 nm and drug entrapment of 87.21 %. Similarly, the optimized composition of NPT UDL was predicted to have 15.14 mM P90G, 4.86 mM P90H, 15 mole% SDC and 5 mole% NPT with mean vesicle size of 66.59 nm and drug entrapment of 83.49 %. Three verification batches with optimized composition showed mean vesicle size and drug entrapment within 95% confidence interval indicating the validity of the model.

Optimized VPN UDL and NPT UDL were characterized *in vitro* for shape and surface morphology, zeta potential and drug release. Transmission electron microscopic images of both VPN UDL and NPT UDL showed spherical shape with smooth surface while their zeta potentials were found sufficient enough (-31.7 mV and -29.5 mV, respectively) to keep the particles dispersed via repulsive forces. *In vitro* drug release data of both VPN UDL and NPT UDL showed >50 % drug release in first 8 hours and > 80 % drug release in 24 hours. Drug release kinetic modelling showed higher R^2 values for Higuchi as well as first order model suggesting a diffusion controlled system where release rate was dependent on remaining drug concentration within the carrier.

11.5 VPN AND NPT LOADED PNP

Out of several available methods for preparation, nanoprecipitation method was chosen for PNP preparation of both the drugs owing to its easy scalability. Poly (lactic-co-glycolic acid), PLGA 50:50 was used as biodegradable polymer matrix and Poloxamer 188 (P188) was used as stabilizer. The quality by design (QbD) approach was adapted for formulation development. Based on the scientific, therapeutic, industrial and regulatory aspects, quality target product

profile (QTPP) elements and their targets were established. Similarly, based on the prior knowledge, literature review and experiment trials, three response variables viz., particle size, drug loading and drug entrapment were selected as critical quality attributes (CQA). All the possible factors that could influence the CQA were identified to build a fish bone diagram and qualitative evaluation was done to categorize these factors in to low, medium and high risk factors. Factors with low to medium risk were controlled by assigning a constant level during preliminary trials. Six factors including amount of PLGA, VPN/NPT, P188, stirring speed, injection rate and organic solvent volume were qualitatively identified as high risk factors. Fraction factorial screening design was applied to statistically evaluate the impact of these high risk factors on CQA. Minitab® 17.1.0 software was used to generate randomized batch matrix with 17 experimental runs. Batches were prepared as per the run order suggested by software and formulations were evaluated for predefined CQA. The data obtained were entered in software for statistical processing. The Pareto and Normal plots generated for both VPN PNP and NPT PNP showed that amount of polymer, stirring speed and organic solvent volume had a significant effect on particle size of resulting PNP considering $P < 0.05$ as a level of significance. Similarly, amount of drugs showed significant impact on drug loading and drug entrapment of VPN PNP and NPT PNP both. Owing to these observations, amount of polymer and amount of drug were selected as CMA while stirring speed and volume of organic solvent were selected as critical process parameters (CPP) for both PNP and exhaustively evaluated during final optimization to obtain the design space. Based on the main effect plots, the other two non-critical factors viz., amount of P188 and injection rate were assigned a constant level of 0.25 %w/v and 0.5 mL/min, respectively for both VPN PNP and NPT PNP.

Box Behnken response surface design was applied for final optimization using Minitab® 17.1.0 software. A randomized batch matrix

of 29 experimental runs was generated by the software. Batches were prepared, evaluated for CQA and data obtained were entered in software for statistical processing. Software suggested quadratic model consisting of Linear, quadratic and two-way interaction terms for both VPN PNP and NPT PNP. Model terms with a p-value less than or equal to 0.05 (α-level) were considered as significant while hierarchy based removal of insignificant model terms (p-value > 0.1) was done to simplify the model equations. ANOVA of all three CQA showed low standard deviation (SD) and high correlation coefficients (R2) value indicating better prediction of responses by the model. A good agreement of Predicted R2 with other R2 also supported the prediction potential of the model. Diagnostic plots further demonstrated the normal distribution of data, constant variance, absence of lurking variable and easy prediction of values from the model. Numerical optimization for achieving minimum particle size with maximum drug loading and drug entrapment resulted optimization solutions with composite desirabilities of 0.977 and 0.998 for VPN PNP and NPT PNP, respectively. The optimized composition of VPN PNP was predicted to have 20 mg PLGA, 2 mg VPN and 0.25 %w/v P188 with mean particle size of 105.24 nm, drug loading of 7.10 % and drug entrapment of 71.11 %. Software predicted similar composition for NPT PNP also with mean particle size of 80.23 nm, drug loading of 6.71 % and drug entrapment of 70.07 %. Three verification batches with optimized composition showed mean particle size, drug loading and drug entrapment within 95% confidence interval indicating the validity of the model.

Optimized VPN PNP and NPT PNP were characterized in vitro for shape and surface morphology, zeta potential and drug release. Transmission electron microscopic images of both VPN PNP and NPT PNP showed spherical shape with smooth surface while their zeta potentials were found sufficient enough (-38.5 mV and -36.5 mV, respectively) to keep the particles dispersed via repulsive forces. In vitro drug release data of both VPN PNP and NPT PNP showed >50 % drug

release in first 8 hours and >80 % drug release in 24 hours. Drug release kinetic modelling showed higher R^2 values for Higuchi as well as first order model suggesting a diffusion controlled system where release rate was dependent on remaining drug concentration within the carrier.

11.6 UDL AND PNP LOADED FAST DISSOLVING MNP

A simplified micromold casting method was used for fabrication of fast dissolving microneedle patches that requires less production time and cost and holds promise for large scale production. Dermastamp (L4001) having 35 titanium microneedles were utilized as master templates to prepare poly-dimethylsiloxane (PDMS) micromolds. Poly vinyl alcohol (PVA, MW 6kD) and poly vinyl pyrollidone (PVP, MW 3.5 kD) were used as binder and filler, respectively for the preparation of microneedle matrix. The quality by design (QbD) approach was adapted for formulation development of blank microneedle patch. Based on the scientific, therapeutic, industrial and regulatory aspects, quality target product profile (QTPP) elements and their targets were established. Similarly, based on the prior knowledge, literature review and experiment trials, axial needle fracture force (ANFF) was selected as critical quality attribute (CQA). All the possible factors that could influence the CQA were identified to build a fish bone diagram and qualitative evaluation was done to categorize these factors in to low, medium and high risk factors. Factors with low to medium risk were controlled by assigning a constant level during preliminary trials. Three factors including amount of PVA, PVP and total solid content were qualitatively identified as high risk factors. Combined D-optimal design was applied to exhaustively evaluate the impact of these high risk factors on CQA. Design-Expert® 7.0.0 software was used to generate randomized batch matrix with 19 experimental runs. Batches were prepared as per the run order suggested by software and formulations were evaluated for ANFF. The data obtained were entered in software for statistical processing. Software suggested quadratic model for mix order and linear model for process order. Model terms with a p-value less

than or equal to 0.05 (α-level) were considered as significant. Considering the hierarchy, no insignificant model terms were removed by the software during model reduction. ANOVA of ANFF showed low standard deviation (SD) and high correlation coefficients (R^2) value indicating better prediction of responses by the model. A good agreement of Predicted R^2 with other R^2 also supported the prediction potential of the model. Diagnostic plots further demonstrated the normal distribution of data, constant variance, absence of lurking variable and easy prediction of values from the model. Numerical optimization for achieving maximum ANFF resulted optimization solutions with a desirability of 0.998. The optimized composition of blank MNP was predicted to have 45 %w/w of total polymer content comprising PVA and PVP in 90:10 ratio with mean ANFF of 0.71 N. Three verification batches with optimized composition showed mean ANFF within 95% confidence interval indicating the validity of the model. Optimized blank MNP was characterized in vitro for shape and morphological features, skin penetrability and pore closure study. The scanning electron microscopic (SEM) images showed smooth surfaced, conical microneedles with 1.5 mm length, around 200 μm base diameter. The microneedle array geometry observed in SEM images was suitable for skin piercing with minimal damage. Skin penetrability study showed presence of 35 trypan blue stained pores in pig ear skin indicating penetration of all 35 microneedles of MNP with ANFF around 0.7 N. Pore closure study revealed that under occlusive condition, pores created by fast dissolving MNP were remained open (Average pore diameter 47.9 μm) for 24 hours.

Optimized VPN/NPT loaded UDL/PNP were concentrated under vacuum and mixed with concentrated PVA-PVP blend in such a way to keep the total solid content levels at 45 %w/w. Resulting dispersions were casted in to MNP and characterized *in vitro* for morphological features, axial needle fracture force, dissolution profile, physical stability of nanocarriers in MNP and drug release. A similar geometry with

smooth, conical microneedles were observed in all four formulation loaded MNP as compared to blank MNP. The ANFF of PNP/UDL loaded MNP showed insignificant difference as compared to blank MNP indicating that nanocarriers loaded MNP possessed enough strength to easily breach the skin surface as observed with blank MNP. The microneedle length of each MNP was reduced to base within 2 minutes indicating fast dissolving nature of these MNP. No significant difference in dissolution profile of nanocarriers loaded MNP was observed when compared to blank MNP. Nanocarriers loaded MNP were re-dispersed in prefiltered double distilled water and the nanocarriers were evaluated for surface characteristics, size and drug retained as their physical stability indicators. Transmission electron microscopic images of nanocarriers' dispersions obtained after their MNP dissolution showed retention of morphological characteristics by respective nanocarriers indicating their stability within MNP. Further, no significant difference was observed in mean vesicle/particle size as well as entrapment efficiency of re-dispersed nanocarriers when compared to their initial values. *In vitro* drug release data of nanocarriers loaded MNP showed similar observations (~50 % drug release in first 8 hours and >80 % drug release in 24 hours) as compared to their respective nanocarriers' dispersions. Such observation indicated that even after increase in viscosity, the drug release from nanocarriers remained rate limiting. Release kinetics showed higher R^2 values for Higuchi as well as first order model suggesting a diffusion controlled system where release rate is dependent on remaining drug concentration within the carrier.

11.7 SAFETY & PERMEABILITY EVALUATION

Full thickness pig ear skin was utilized to demonstrate the permeability behavior of developed MNP. Permeability data of VPN PNP MNP showed 8-fold increase in permeation (J_{SS} – 9.753 µg/cm^2/h) while VPN UDL MNP showed 9.1-fold increase in permeation (J_{SS} – 11.091 µg/cm^2/h) as compared to VPN suspension (J_{SS} – 1.218 µg/cm^2/h). Similarly, permeability data of NPT PNP MNP showed 5.6-fold increase in

permeation (J_{ss} –9.285 µg/cm²/h) while NPT UDL MNP showed 6.5-fold increase in permeation (J_{ss} – 10.858 µg/cm²/h) as compared to NPT suspension (J_{ss} – 1.665 µg/cm²/h). Comparing the deposition data revealed higher deposition of VPN/NPT through PNP as compared to UDL indicating a better permeation potential of ultradeformable liposomes over PLGA nanoparticles. FITC loaded formulations were also prepared by simply replacing the drugs with FITC to demonstrate the permeation potential of developed formulation. The fluorescence microscopic data were found in-line with the ex vivo permeation and deposition data. Maximum fluorescence was observed in section of skin treated with FITC PNP MNP and FITC UDL MNP confirming the enhanced permeability through developed nanocarriers loaded fast dissolving microneedle patches.

The safety of developed formulations was assessed using viability evaluation of HaCaT cells *in vitro*. These cells were exposed to developed formulations and MTT assay was performed to quantify the percent viable cells as an indicator of safety. The viability of cells treated with VPN/NPT loaded nanocarriers were found significantly higher (>85 %) than positive control (Triton X 100, 26.61 %) and near to negative control (PBS 6.8, 100 %) indicating the least toxic nature of developed formulations.

11.8 *IN VIVO* STUDIES

The *in vivo* studies were performed for pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of VPN and NPT via their newly developed formulations. Study protocols were approved by Institutional Animal Ethics Committee. Female Sprague-Dawley rats were used and administered with formulations containing animal equivalent dose of 1.54 mg/Kg and 1.03 mg/Kg for VPN and NPT, respectively. Kinetica software was used to process plasma drug concentration data obtained at various time points to calculate various pharmacokinetic parameters. The optimized UDL/PNP loaded MNP of both VPN and NPT exhibited $t_{1/2}$ ranging from 11-16 hours and MRT ranging from 17-25 hours signifying a

better controlled plasma levels for prolonged duration as compared to their oral suspensions. Further, around 3-5-fold rise in relative bioavailability was observed which could owe to avoidance of first-pass metabolism together with better penetrability and controlled drug release behavior of developed formulations and also advocated the possibility of dose reduction.

Pharmacodynamic study was performed to demonstrate the effectiveness of VPN and NPT via developed MNP. Tukey's multiple comparison test was applied using Prism software to statistically compare the mean escape latencies observed in different treatment groups. An insignificant difference in mean latency of Model control and vehicle control groups indicated that excipients used in the developed formulations did not have any pharmacological effect on selected disease model. Animals treated with optimized MNP of VPN/NPT loaded nanocarriers showed a significant reduction in latency as compared to model control group. The same was found comparable to latency of standard control group animals administered with their oral suspensions.

11.9 STABILITY STUDY

Three months' stability data of all four nanocarriers loaded MNP stored in hermetically sealed containers with desiccants was generated at 30 °C/65 %RH. Any significant change in ANFF of MNP, size of loaded nanocarriers or drug retained within loaded nanocarriers were selected as indicators of stability owing to their direct impact on transdermal performance of these systems. No significant change in these parameters were observed during three months of storage advocating the stability of developed MNP when stored appropriately.

11.10 CONCLUSION

The aim of this study was to overcome the limitations of oral delivery of VPN and NPT via development and characterization of their suitable transdermal formulations. The UDL and PNP of these drugs were successfully prepared and optimized to have minimum vesicle/particle size and maximum drug entrapment. Their *in vitro* characterization

revealed spherical shape, uniform size distribution and favorable zeta potential as well as prolonged drug release over 24 hours. Optimized nanocarriers were incorporated in fast dissolving MNP to facilitate their transdermal delivery. Optimized MNP of PVA/PVP showed rapid dissolution, sufficient needle strength and successfully penetrated the pig ear skin. Characterization of nanocarriers loaded MNP demonstrated similar needle strength, similar drug release behavior and physical compatibility of nanocarriers within MNP, Cell viability study showed non-toxic nature of these developed formulations. *Ex vivo* study revealed enhanced flux via nanocarriers loaded MNP. The results of pharmacokinetic and pharmacodynamic studies revealed that nanocarriers loaded MNP of both drugs were able to maintain plasma drug concentration above MEC for prolong period of time and therefore seems suitable for once daily application.

In a nutshell, the final optimized UDL and PNP loaded MNP of both VPN and NPT were found to possess quality attributes in desired range as defined in QTPP indicating the success of utilizing QbD approach for formulation development.

11.11 FUTURE PERSPECTIVE OF PRESENT STUDY

Present study could be utilized as a platform technology for development of other potent lipophilic drugs where infusion-like delivery is required with better patient compliance.