The current research work aimed at development of PN delivery system for cosmetic and therapeutic applications by using statistical experimental design based methodologies. CoQ$_{10}$ was selected as a cosmetic agent for treating photo-induced skin aging. Whereas, KT was used as a therapeutic agent to treat periodontitis. Topical PN formulations of both the actives were developed for percutaneous absorption.

### 7.1 SUMMARY

#### 7.1.1 CoQ$_{10}$ PN gel formulation for anti-aging potential
PN gel delivery system of CoQ$_{10}$ was developed for its antiaging potential for topical application. The available oral dosage forms of CoQ$_{10}$ results in poor bioavailability problems associated with CoQ$_{10}$ are due to its large molecular weight and high lipophilic nature. Moreover, available topical formulations have problems of permeation and repeat dosing due to short duration of action. Topical application of CoQ$_{10}$ provides antioxidant effect and effective against photo induced skin aging. A considerable increase in population with aging symptoms and growing concern about appearance has created a rising demand for treatments to correct the visible signs of aging. With the advancement in technology and increasing awareness in the population created significant demand to treat and reverse the signs of aging caused by photo damage. Therefore, despite its strong antioxidant property and great potential for antiaging its therapeutic potential remains massively underutilized till date.

Hence, administration of CoQ$_{10}$ using combination of targeted topical drug delivery with novel PN formulation system could be an efficient and fruitful approach against anti-aging. The present work was aimed at developing a novel CoQ$_{10}$ PN gel formulation for anti-aging treatment to combat the percutaneous absorption and solubility issues. The use of statistical approach in the formulation development of PN gel system results in optimized formulation. The developed formulation enhanced CoQ$_{10}$ transport and retention into skin and thus, results in enhanced and prolonged effects against aging.

A brief summary of the research work is as follows:

- Available analytical method using RP-HPLC was used and validated as per ICH guidelines. Developed method was utilized for quantitative determination of CoQ$_{10}$ in solubility studies, drug content in the formulation, *in vitro* and *ex vivo* evaluation experiments.
Media for *ex vivo* permeation study was optimized to provide maximum CoQ\textsubscript{10} solubilization. The medium comprises a combination of phosphate buffer (pH 7.5) and ethanol (70:30 ratio).

Drug-excipient interaction study was performed in order to evaluate the compatibility of CoQ\textsubscript{10} with solid excipients, intended to be used in development of the PN formulation. DSC and FTIR spectroscopy technique was used for evaluating drug-excipient interactions and results in no interaction between studied excipients.

Various pre-formulation experiments were performed to select the components of the formulation and other variables (surfactant selection, lecithin selection, solvent selection, % drug load). Out of all studied non-ionic surfactants, Span 20, Span 80 and Span 85 were found to exhibit niosome formation ability with gel nature. However, based on higher entrapment Span 85 was selected for PN formation. Lecithin from soyabean (Leciva S35 and Leciva S70) were investigated for their ability to form vesicles and Leciva S35 was selected amongst them based on high entrapment and low vesicle size. Similarly, n-butanol and pH 7.5 phosphate buffer were selected as organic solvent and aqueous phase respectively for PN formulation. Drug pay load study results lead to the selection of 1.0% drug load in the formulation of CoQ\textsubscript{10} PN gel.

CoQ\textsubscript{10} loaded PN gel was prepared using simple method reported in literature. The method lead to solubilization of all components using n-butanol with heat and arrest of micelle formation step by using a minimal quantity of aqueous phase to form gel.

The ranges of the formulation components were selected from previous studies available in literature and was optimized using statistical experimental design.

Concentration of surfactant, cholesterol and soya lecithin were selected as independent variables.

I-optimal mixture design was used to optimize the formulation components and evaluated for two responses, viz, percent drug entrapment and drug release in 12 h.
Individual effect and interactions between various factors were studied and their effects were evaluated. Concentration of cholesterol and soya lecithin are amongst the most influential factors.

Validation of statistical model was performed on three formulations selected from random points of software provided solutions. The experimental and predicted values of selected response were compared and prediction error was calculated. The overall prediction error for three formulations was found less than 5% (actual value between -3.30% to +3.07%), showed good prognostic ability of the postulated model.

Based on the outcome of experimental design three optimum formulations (PNC1, PNC9 and PNC11) were further characterized and evaluated using ex vivo permeation and retention studies using mice excised skin.

Ex vivo studies using excised skin of swiss albino mice depicted that three selected formulations showed flux value (ranging from 42.34 to 46.69 µg cm\(^{-2}\) h\(^{-1}\)), against 17.54 µg cm\(^{-2}\) h\(^{-1}\) for conventional carbopol gel. Flux value is significantly higher in case of PN formulations compared to carbopol gel. Enhancement ratio of 2.66, 2.41 and 2.53 for PNC1, PNC9 and PNC11, respectively suggested better permeation of PN formulations across mice skin. Skin retention was found 18.03±0.32, 22.02±0.42, 16.44±0.35 and 4.32±0.33% for PNC1, PNC9, PNC11 and carbopol gel respectively. Results of retention studies clearly indicate that the amount of CoQ\(_{10}\) retained in skin was considerably higher in case of PN formulations, than with carbopol gel.

pH measurement result showed that the developed formulation was having pH close to neutral and suitable for topical application. Whereas active content result indicated uniform nature of developed formulations.

Based on the characterization of three PN gel formulations (PNC1, PNC9 and PNC11), PNC9 was selected as the best optimized formulation showing higher skin retention and flux values.

The average vesicles size of optimized PN formulation (PNC9) was found to be 1.87 ± 0.34 µm, indicating a submicron size of vesicle in the studied formulation.
The morphology study using optical microscope showed spherical shape of the vesicles and TEM revealed that the vesicles appeared dark with bright surroundings. This corroborates the spherical and uniform nature of the prepared vesicles.

The optimized CoQ\textsubscript{10} PN gel (PNC9) exhibited percent drug entrapment of 82.52\% and drug release in 12 h was 42.62\%. Vesicle size of the formulation was 1.87±0.34 µm, flux value of 42.34±0.90 µg cm\textsuperscript{-2} h\textsuperscript{-1} and skin retention value in 12h was 22.02±0.42 \%.

The stability results indicated that PN gel formulation for 60 days is more stable in refrigerated condition (2 - 8ºC) as compared to room temperature (25 ± 2ºC). Any increase in temperature is therefore critical to the formulation in terms of vesicle size and entrapment.

Rheological studies of the optimized formulation (PNC9) showed ‘n’ value of 0.871, clearly indicate that the system is shear thinning in nature. Viscosity and yield values of the prepared system were found within the limits indicate, lesser fluidity, higher plasticity and good rigidity of gel structure.

Texture analysis of the optimized formulation (PNC9) showed fairly good cohesiveness, which is essential to keep the formulation adhered to application site. The Uniformity of texture curve confirmed the smoothness and absence of any grittiness or lumps in the PN gel.

Antiaging potential of optimized formulation was performed using photo induced aging in an animal model (swiss albino Mice) and evaluated using visual skin scoring of animals, skin sagging, histopathology and biochemical estimations.

Data indicates that significant reduction in visual score is observed with CoQ\textsubscript{10} PN gel (p<0.05) as compared to alone UV treated group. Moreover, a comparison between PN gel and conventional gel suggested a superior effect of PN gel formulation.

Skin sagging data indicates that CoQ\textsubscript{10} PN gel formulation showed a significant reduction in recovery time compared to UV treated group and also significantly better than conventional formulation.
Histology of mice skin treated with CoQ_{10} PN gel resembled closely with control group showing adequate fat in dermis, regularly distributed hair follicles, distinct epidermis and dermis layers with negligible inflammation. These pictures confirmed that PN gel formulation significantly reduced aging. Significant reduction in MDA levels was observed with CoQ_{10} PN gel (p<0.001) compared to UV treated group. MDA enhancement factor for CoQ_{10} PN gel and CoQ_{10} conventional gel treated group w.r.t. control was approximately 1.4 and 1.8 respectively. Significant difference (p<0.01) was observed in terms of protection against lipid peroxidation between PN gel formulation and conventional gel formulation, where PN gel formulation offered better protection.

SOD levels were restored to 81.3% and 66.8% as that of control group by PN gel and conventional gel respectively. Insignificant difference in SOD levels between control and CoQ_{10} PN gel indicated the restoration of normal SOD levels.

CA levels were restored to 72.1% and 60.6% as that of control group by PN gel and conventional gel, respectively. These results showed superior photoprotective effect of PN gel formulation as compared to conventional gel.

A significant (p<0.01) increase in GSH levels in CoQ_{10} PN gel compared to CoQ_{10} conventional gel, where GSH levels were restored to 74.8% and 60.6% of the control group by PN gel and conventional gel, respectively. This study concluded marked increase in the protection of animal skin against oxidative stress in terms of increased GSH level by CoQ_{10} PN gel formulation vis-à-vis CoQ_{10} conventional gel.

A significant increase in total protein levels was observed with CoQ_{10} PN gel (p<0.001) and CoQ_{10} conventional gel (p<0.01) as compared to UV treatment group, where total proteins were restored to 77.1% and 63.4% of the control group by PN gel and conventional gel, respectively.

### 7.1.2 Ketoprofen (KT) PN gel formulation for periodontitis treatment

KT is one of the propionic acid derivatives and is a potent non-steroidal anti-inflammatory drug (NSAID) with a high therapeutic value in the treatment of periodontitis. Many preclinical and clinical studies demonstrated the use of KT in effective treatment of periodontitis. But associated side effects using oral delivery of
KT is a drawback and results in decreasing interest amongst the health practitioners. Delivery of KT at periodontal pockets is a right approach which ensures the availability of the active at target site along with negligible side effects. Use of novel dosage forms like vesicular delivery system is an added advantage, which ensures better availability of KT at specific site for longer time along with controlled effect. Based on the therapeutic suitability for KT in periodontitis a novel topical KT PN gel formulation for periodontitis treatment was developed using experimental design methodology.

A brief summary of the research work is as follows:

- A quality by design (QbD)-based simple, precise, cost effective and stability indicating RP-HPLC method was developed and validated as per ICH guidelines for estimation of KT.
- The method development included the defining of CAAs, whereas Taguchi design was employed for factor screening. Two parameters were selected based on their significant effect on CAAs and were optimized using central composite design.
- The chromatography separation was finally achieved using C-18 column, pH 6.8, phosphate buffer–methanol (50:50, v/v) as a mobile phase at a flow rate of 1.0 mL/min and UV detection at 258 nm.
- Media for *ex vivo* permeation study was selected to provide maximum KT solubilization, and the media selected was pH 6.8 phosphate buffer.
- The physical and chemical interactions between drug and excipients was studied to evaluate the compatibility of KT with solid excipients, intended to be used in development of the PN formulation. DSC and FTIR spectroscopy technique were used for evaluating drug-excipient interactions. The spectra and thermogram confirmed that there was no chemical interaction.
- Pre-formulation trials were conducted in order to select the excipients (surfactant selection, lecithin selection, solvent selection, % drug load) for PN formulation. Based on these experiments Span 80 and Leciva S35 was selected as non-ionic surfactant and lecithin, respectively. Cholesterol and oleic acid was amongst other components of the formulation. Similarly, n-butanol and pH 6.8 phosphate buffer was selected as organic solvent and aqueous phase, respectively for the PN formulation. Drug pay load experiments were also conducted to determine the
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The mean vesicle size of all 20 PN formulations used in optimization experiments was found between 3.76 - 12.16 µm, indicating a narrow distribution of vesicle in the studied formulations.

Based on the outcome of experimental designs, three KT PN formulations (N5, N11, N20) with acceptable response parameters [high entrapment efficiency (not
less than 80%), maximum drug release (not less than 60%) and adequate vesicle size] were selected and mixed in 1:1 ratio with secondary vehicle, Carbomer (Carbopol® 974P) to gel rheologically acceptable formulation for application.

- pH of the studied formulation was found in the range of 6.39 to 6.72 which can be considered good for topical application in buccal cavity. Drug content of the formulations was found to be 97.58 to 101.62 for studied formulation and suggest a good homogeneity for formulation gel.

- KT PN gel coded as PNG2, PNG5 and PNG8 (prepared using 1.0% Carbopol® gel) were considered suitable and taken forward for further studies. Mucoadhesive strength of these formulations ranges from 14.27 – 17.29 g and offer a good strength for application.

- Stability studies at refrigerated condition (2 - 8°C) and room temperature (25 ± 2°C) for a period of 90 days suggested that PN formulations are more stable in refrigerated condition compared to room temperature.

- Ex vivo permeation studies using porcine buccal mucosa of the three selected formulation showed more than double cumulative drug permeated at 12 h (ranging from 540.56 to 667.16 µg/cm²), against 250.85 µg/cm² for conventional gel. Cumulative permeation at 12 h is significantly higher in case of PN formulations compared to conventional gel. Flux value of 0.059, 0.044, 0.061 and 0.017 mg cm⁻² h⁻¹ was obtained for PNG2, PNG5, PNG8 and conventional gel respectively, suggested significantly higher (more than 3 times for PNG2 and PNG8) values compared to conventional gel.

- Skin retention was found to be 11.49, 8.99, 15.43 and 3.82 % for PNG2, PNG5, PNG8 and conventional gel, respectively. Results indicates that conventional gel showed significantly lower skin retention in comparison to all PN gel formulations.

- Based on the results of permeation and retention studies of selected three PN gel formulations (PNG2, PNG5 and PNG8), one best formulation PNG8 was selected as optimum formulation and evaluated for further critical studies.

- DSC results indicated interactions at large extent between KT and PN components and confirms the entrapment of KT into bilayer vesicle structure.
The morphology study of KT PN formulation using optical microscope and TEM revealed spherical shape and submicron size of the vesicles.

Rheological studies of the optimized formulation (PNG8) showed ‘n’ value of 0.67, which indicated shear thinning nature of the systems. Viscosity and yield values indicated acceptable fluidity and non-Newtonian behavior of optimized formulation. Texture analysis of the optimized formulation (PNG8) showed fairly good cohesiveness and smoothness for application.

Pharmacodynamic studies using experimentally induced periodontitis in wistar rat resulted in improved diseased condition. GI score as obtained from macroscopic images of exposed rat periodontium treated under various groups showed that optimized PN gel formulation significantly reduced gingival inflammation. However, animals treated with conventional gel results in non-significant reduction of inflammation.

Histopathological study of animals reveled that animals treated with optimized KT PN gel improved the disease condition, whereas slight inflammatory cellular infiltration observed along with preserved alveolar process, cementum with no osteoclasts and with intact cementum. Whereas, periodontal score of animals treated with conventional gel showed non-significant improvement in the diseased condition.

7.2 CONCLUSION

7.2.1 CoQ_{10} PN gel formulation for anti-aging potential

To conclude, the present work utilized the immense potential of pro-vesicular carrier approach in enhancing the anti-aging potential of CoQ_{10} in topical application by incorporating it in PN gel formulation. The formulation development part was governed by experimental design methodology using I-optimal mixture design for desired carrier characteristics. Selected compositions were evaluated for skin permeation studies and showed better skin permeation. More than two fold increase in the permeation flux was observed in PN formulations as compared to conventional gel. The submicron vesicle size and 22% skin retention of CoQ_{10} make the formulation suitable for topical applications. Assessments of the formulations for various enzymatic and non-enzymatic estimation in animal skin after UV irradiations proved...
the efficacy of developed formulation, whereas the results showed a significant improvement \textit{vis-a-vis} conventional formulation. Histopathology studies of animal skin treated with PN gel formulation showed better healing and regeneration process compared to conventional gel.

Antiaging benefits of CoQ\textsubscript{10} are already established and can be further enhanced by incorporation into stable and skin friendly proniosome gel formulation. The benefits includes penetration enhancement, increased residence time and prolonged action by bringing biochemical and biomechanical changes in the skin layer. The study can be considered revealing for usefulness of the tested ‘\textit{novel proniosomal gel formulation of CoQ}_{10}’ as more effective and safer than conventional formulation.

7.2.2 KTPN gel formulation for periodontitis treatment

The current study highlights the development and characterization of a novel PN gel formulation for periodontal treatment, employing systematic optimization. \textit{I}-optimal mixture design was utilized to achieve optimum composition of KT PN gel with desired responses. The results support the fact that optimized formulations have desired \textit{in vitro} release, entrapment efficiency, stability, mucoadhesiveness and viscosity results. DSC study revealed interactions between KT and PN components, whereas entrapment studies proved high entrapment of KT in vesicles. The release characteristics of optimized formulations showed extended release of drug with a negligible lag time. Selected PN gel formulations showed high skin permeation flux and skin retention of KT, compared to conventional marketed gel formulation. Animal study using experimentally induced periodontitis and treatment with optimized KT PN gel resulted in marked improvement of gingival inflammation and periodontal scores when evaluated using visual and histopathological studies. Optimized proniosomal gel composition containing ketoprofen was found to be safe and more effective than conventional gel formulation. Current findings suggested better delivery system established as ‘\textit{proniosomal ketoprofen gel}’ for periodontal treatment and it is suggested that the optimized formulation can be further considered for pharmacodynamic efficacy studies on human subjects in clinical situations.