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6. SUMMARY AND CONCLUSIONS

6.1. Summary

By and large, formulation research in topical delivery reveals a sub-optimal utilization of the drug potential. It could make substantial difference in the treatment of diseases especially with the targets accessible by this mode of administration. In this context, the recent development of novel drug delivery systems has been quite encouraging to address the relevant issues. Two drugs, viz. ETO (a NSAID) and ITR (a retinoid) were eventually chosen for encompassing them in apt drug delivery technologies for topical route. Novel carrier systems based on lipids were chosen for the purpose, wherein phospholipids play a crucial role in working out the design and delivery of carriers. FMVs and ethosomes were chosen for ETO, while SLNs and NLCs were chosen for ITR.

Albeit substantial volume of work has been brought forth in the related aspects of the selected work in the light of prior art, yet no exclusive focus has so far been imparted to the selected molecules, despite arduous challenges. Scanty efforts have been undertaken in case of ETO so far to make it effective through topical route, while ITR is a drug with high therapeutic potential, hitherto under-explored, and under-utilized.

The major parts of the work are summarized as under:

6.1.1. Etodolac-loaded Vesicular Carriers

Drug analysis for ETO was performed using validated procedures of HPLC and UV-visible spectroscopy, as per the ICH guidelines. Calibration plot as per Steward method was developed with a coefficient of correlation of 0.9995 for the estimation of phospholipids.

Solubility studies were carried out to select the appropriate sink medium for the skin-permeation studies. For ETO, PBS pH 7.4 offered a solubility of 3.45 ± 0.27 mg/mL and could serve as sink medium for permeation studies. Octanol-water
partition coefficient was found to be $3.82 \pm 0.92$ indicating the lipophilic nature of the drug.

Screening of excipients was conducted to select the suitable components for FMVs and ethosomes. Out of a variety of phospholipids tried for ETO-loaded FMVs, Phospholipon 90 G (unsaturated) was selected for further studies. For the preparation of ethosomes, the unsaturated PL(Phospholipon 90 G) was selected. A variety of edge activators \textit{viz.} Span 80, Tween 80, sodium cholate, sodium deoxycholate and Brij 35 were tried for the purpose. Span 80 and sodium deoxycholate resulted in formation of FMVs with desired attributes; hence, selected for further studies.

FMVs were prepared by thin-film hydration technique, while cold-method was used to prepare ETO-loaded ethosomes. For the preparation of ETO-loaded ethosomes, PL and ethanol were attempted in the range of 2-5% and 20-50%, respectively. As ethanol in concentration of 50% did not form ethosomes, the concentration range was selected as 20-40% of ethanol.

FbD-based optimization studies were planned to select the optimum range of CFAs to achieve the desired limits of CQAs. Orthogonal Taguchi design was employed for the screening of CFAs substantially affecting the CQAs of FMVs. Coefficient analysis revealed that drug:PL ratio and lipid:edge activator ratio were the most influential factors. Therefore, the amounts of PL, and EgAct were selected as CFAs. For ethosomes, percentages of PL and ethanol were selected as CFAs. A $3^2$ FCCD was employed for the FbD optimization of FMVs and ethosomes. The FbD optimization studies were carried out by formulating 13 formulations as per the design and evaluating the CQAs, i.e., PDE, vesicle size, permeation flux ($J$), skin retention and amount of drug permeated after 6h ($Q_6$). The design space was demarcated, and the optimized formulation was selected by numeric and graphical techniques. Eight formulations were rationally selected amongst the domain for validating the FbD optimization studies. High values of correlation coefficients between the predicted and observed values (0.9823 to 0.9977) corroborated quite “sameness” between the observed and the predicted responses ($p < 0.001$). The corresponding residual plots
were also found to be quite regulated, with uniform, relatively narrow and random scatter around the zero-axis. The optimized FMVs contained 2% of PL and 0.12% of Span 80, while the optimized ethosomes were composed of 5% of PL and 18.6% of ethanol.

Considering the nature of drug and PL, complexation studies were carried out between Phospholipon 90G and ETO. Job’s plot revealed that the ETO:PL molar ratio of 0.6 corresponding to stoichiometric ratio of 3:2 appeared as the stable complex. Scott’s plot fetched with the association constant of 7.48 M⁻¹. FTIR spectra of the complex showed shifting and suppression of various peaks prominent in the physical mixture of PL and ETO and the ETO alone. From the IR-spectra, it can be concluded that some weak physical interactions between ETO and PL could take place during the formation of complex. The XRD pattern of the complex was significantly different from the drug and the phospholipid, indicating a substantial shift from crystalline nature towards the amorphous state. The DSC curves of the physical mixture and the complex were different as the endothermal peak of ETO was vanished from the DSC curved of the complex. It was considered that ETO had been completely dispersed in PL with the aid of interactions like vander Waals attractions and hydrogen bonding. In NMR studies, there was noticeable change in the chemical shifts of the labeled protons of ETO when complexed with phospholipid. The NMR data also confirmed the presence of complex between ETO and phospholipid.

Characterization and evaluation of the ETO-loaded vesicular carriers were also performed. Vesicle size, PDI, zeta-potential, PDE and PDL of FMVs were found to be 476.23 nm, 0.256, -14.33 mV, 61.02% and 28.78%, respectively while that of ethosomes were 240.23 nm, 0.224, -17.56 mV, 74.32% and 14.86%, respectively. TEM photomicrographs revealed the regular nature of the vesicles formed. The vesicle density in FMVs was found to be 1.88 (± 0.431) x 10⁵ mm⁻³. The flexibility indices of FMVs and ethosomes were found to be 0.96 and 0.98, respectively.
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DSC studies of ETO-loaded FMVs and ethosomes showed the absence of drug peak indicating the interactions of the drug with the lipid components of vesicles resulting in altered endotherms.

Rheological studies and textural analysis were performed to have an insight of the rheological behavior of the developed hydrogels containing nano-carriers. The hydrogels containing ETO-loaded FMVs and ethosomes were found to be of shear-thinning nature with respective yield values and viscosities of 47.60 Pa and 49.80 Pa, and 14.24 Pa.s and 10.34 Pa.s. The textural analysis revealed that the gels are composed of uniform 3-D network without any grittiness or lumps, and with desired spreadibility attributes.

Skin permeation and retention studies were carried out on Laca mice skin employing Franz diffusion cells. Skin permeation studies revealed the supremacy of FMVs and ethosomes over the conventional gel with enhancement ratios of 3.21 and 3.23, respectively. Amount of drug permeated at the 24th hour of the study followed the order of: Ethosomal-gel > FMV-gel > Conventional gel

Order of skin retention was found to be: FMV-gel > Ethosomal gel > Conventional gel, with an enhancement ratio of 8.77 for FMVs and 4.18 for ethosomes w.r.t. conventional gel. This can be ascribed to the better interaction of the PL-based carriers with the dermal tissues. However, lower skin retention by ethosomal product can be attributed to higher skin penetration due to ethanol.

Dermatokinetic studies were carried out on excised Wistar rat skin. Studies divulged that both FMVs and ethosomes were able to transport the drug to epidermis and dermis in substantially higher amounts than that from the conventional gel. The epidermal permeation constant for FMVs was found to be 10.09 times higher than that of conventional gel, while that of ethosomes was found to be 18.04 times higher than that of the conventional gel. The dermal permeation constant for FMVs was found to be 1.30 times higher than that of conventional gel, while that of ethosomes was found to be 15.86 times higher than that of the conventional gel.
To study the biocompatibility of the optimized lipid-based nanostructured systems, skin histopathological studies were performed. Histopathological evaluation of the mice skin treated with various ETO formulations (FMVs, ethosomes and conventional gel) revealed no marked changes in the normal histology. Therefore, neither of ETO formulation was found to be irritant on the skin.

*In vivo* pharmacodynamic evaluation was performed in carrageenan-induced paw oedema and radiant-tail flick method. Ethosomes offered a rapid onset of action *vis-à-vis* FMVs in carrageenan-induced rat paw oedema, which might be ascribed to the presence of ethanol in the former. However, the inhibition profile was almost comparable for both FMVs and ethosomes. Studies revealed the supremacy of the phospholipid-based drug delivery carriers over the conventional systems owing to their better interaction with the skin components and skin-depot forming potential. In radiant tail flick method also, the percent analgesia caused by ETO-loaded vesicular formulations was found to be significantly greater than of the conventional gel. Ethosomes offered shorter onset of action, while the FMVs offered more prolonged duration of action.

Stability studies were carried out as per the ICH guidelines. Both the developed ETO carriers were found to be stable for 1 year. The formulations were able to maintain the vesicle size and PDE within the limit of 5% at the storage temperatures of 5 ± 3 °C, 30 ± 2 °C/65% ± 5% RH and 40 ± 2°C/ 75% ± 5% RH.

Clinical efficacy of ETO-FMVs was evaluated in an Open-label clinical trial. Open-label clinical studies of ETO-loaded FMVs demonstrated the topical efficacy of ETO in FMV-gel. The average improvement in pain, stiffness and physical function scores was found to be 52.78 %, 54.64 % and 42.42 %, respectively.

### 6.1.2. Isotretinoin-loaded Non-vesicular Carriers

Analysis of the ITR was performed as per the ICH guidelines by HPLC and UV-visible spectroscopy.
Solubility studies were carried out to select the appropriate sink medium for the skin-permeation studies. Various solvent systems were tried for the solubility studies and 2.0% (w/w) T80 along with 20% (w/v) ethanol in IPB was selected as the sink for permeation studies. The log P value of ITR was found to be 5.61 ± 0.46. High log P value indicated that the drug needed to be manipulated in terms of physicochemical properties to achieve the target of topical delivery.

Selection of solid lipid and blend of solid and liquid lipid is important for the development of SLNs and NLCs, respectively. Solubility of ITR, as observed in various lipids, discerned the following order: Stearic acid > Glyceryl monostearate > Compritol > Palmitic acid > Cetyl palmitate.

With the aim to prepare reservoir-type SLNs and increase the photostability, the lipid with moderate solubility, i.e., Compritol was finally selected for further studies. Solubility of ITR in various oils (mg/g) followed the order as under: IPM > Soyabean oil > Tocopherol > Sesame oil > Olive oil, IPM, therefore, was selected as the oil phase owing to the high solubility offered as well as due to its reported penetration enhancement properties.

FbD-based optimization studies were planned to select the optimum range of CFAs to achieve the desired limits of CQAs. Orthogonal Taguchi design was employed for the screening of CFAs substantially affecting the CQAs of SLNs. Coefficient analysis revealed that amounts of Compritol and PL were the most influential factors. A 3^2 FCCD was employed for the FbD optimization of SLNs. The FbD optimization studies were carried out by formulation 13 formulations as per the design and evaluating the CQAs, i.e., PDE, particle size, permeation flux (J) and skin retention. The design space was demarcated and the optimized formulation was selected by numeric and graphical techniques. Eight formulations were rationally selected amongst the domain for validating the FbD optimization studies. High values of correlation coefficients between the predicted and observed values (0.9832 to 0.9940) corroborated quite "sameness" between the observed and the predicted responses (p < 0.001). The corresponding residual plots were also found to be quite regulated, with uniform, narrow and random scatter around the zero-axis. The optimized SLNs
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contained 0.676 % of Compritol and 0.208 % of phospholipid. The NLCs were composed of 0.2 % of IPM, 1.2 % of Compritol, and 0.6 % of phospholipid.

Characterization and evaluation of the ETO-loaded vesicular carriers were also performed. Particle size, PDI, zeta-potential, PDE and PDL of SLNs were found to be 76.6 nm, 0.334, -22.4 mV, 89.49 % and 5.06 %, respectively while that of NLCs were 82.2 nm, 0.312, -17.50 mV, 78.6 % and 1.96 %, respectively. TEM photomicrographs revealed the regular nature of the nanocarriers formed.

DSC studies of ITR-loaded SLNs and NLCs showed the absence of drug peak indicating interactions resulting in the dispersion of drug molecules within the lipid molecules resulting in absence of drug endothermal peak in drug-loaded SLNs and NLCs.

Rheological studies and textural analysis were performed to have an insight of the rheological behavior of the developed hydrogels containing nano-carriers. The hydrogels containing ITR-loaded SLNs and NLCs were found to be of shear-thinning nature with respective yield values and viscosities of 145.08 Pa and 115.54 Pa, and 33.74 Pa.s and 27.18 Pa.s. The textural analysis revealed that the gels are composed of uniform 3-D network without any grittiness or lumps, and with desired spreadibility attributes.

Skin permeation and retention studies were carried out on Laca mice skin employing Franz diffusion cells. Skin permeation studies revealed the supremacy of lipid-based nanocarriers over commercial gel with enhancement ratios of 2.07 and 1.84, respectively. Amount of drug permeated at the 24th hour of the study followed the order of: SLN-gel > NLC-gel > Commercial gel, Order of skin retention was found to be: SLN-gel > NLC gel > Commercial gel, with an enhancement ratio of 3.62 for SLNs and 3.01 for NLCs w.r.t. commercial gel. This can be ascribed to the better interaction of the lipid based carriers with the dermal tissues.

Dermatokinetic studies were carried out on excised Wistar rat skin. Dermatokinetic studies divulged that both SLNs and NLCs were able to transport the drug to epidermis and dermis in substantially higher amounts than that from the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India
commercial gel. The epidermal permeation constant for SLNs was found to be 90.42 times than that of commercial gel while that of ethosomes was found to be 19.68 times that of the commercial gel. The dermal permeation constant for SLNs was found to be 0.61 times than that of commercial gel while that of ethosomes was found to be 0.46 times that of the commercial gel.

To study the biocompatibility, skin histopathological studies were performed. Histopathological evaluation of the mice skin treated with lipid-based ITR formulations (SLNs and NLCs) revealed no marked changes in the normal histology, however, marked signs of skin disruption were seen in the commercial gel-treated skin. Hence, the developed systems were found to be biocompatible and were able to circumvent the irritation caused by ITR.

*In vivo* pharmacodynamic evaluation of ITR was performed in testosterone-induced acne model and UV-induced photoaging in mice. The percent papule reduction in testosterone-induced acne model was found to be 91.1 % for SLNs, 88.1 % for NLCs and 69.2 % for commercial gel. The results unequivocally demonstrate the significant superiority of the nano-encased ITR to the marketed product. Thus, the nano-encapsulated ITR was found to be more efficacious and biocompatible vis-à-vis the marketed product, however, there was no marked difference between the two selected nanocarriers. In anti-photoaging studies, NLC-gel and SLN-gel treated group offered minimal visual skin grades, significantly lower than the tested marketed formulation. No marked difference was perceptible in the visual scores of both SLN-gel and NLC-gel treated groups. The MDA concentration in the Group 2 animals (no treatment) was found to be approx. 4.3 times higher than that of the control group (no UV-exposure), indicating elevated levels of lipid peroxidation and free radical production under the influence of UV-irradiation. The MDA enhancement factors for SLN-gel, NLC-gel and commercial gel treated groups w.r.t. the control group were observed to be approx. 1.6, 1.9 and 2.5, respectively. The developed SLN-gel and NLC-gel were able to significantly enhance the GSH levels vis-à-vis the marketed product-treated groups. As decreased values of reduced glutathione construes marked increase in the oxidative stress, the studies
unequivocally vouch the markedly superior photoprotective potential of nano-
colloidal gel over the commercial products.

Anti-microbial activity of the developed systems and plain ITR was evaluated
against P. acnes. The studies revealed that ITR also possesses its own antimicrobial
activity with MIC value of 125 μg/mL vis-a-vis 31.75 μg/mL for the control
antibiotic, i.e., clindamycin phosphate. Incorporation of ITR in lipid-based
nanocarriers resulted in marked decrease in MIC values, i.e., 62.5 μg/mL for both
ITR-SLNs and ITR-NLCs. The two-fold enhancement in the antimicrobial activity of
the nano-entrapped ITR can be ascribed to the better interaction of the lipid-based
nanocarriers with the bacterial cell wall resulting in increased contact time and
sustained delivery of the drug to the bacteria.

Stability studies were carried out as per ICH guidelines. Both the developed ITR
carriers were found to be stable for 1 year as per ICH guidelines. The formulations
were also able to maintain the particle size and PDE within the limit of 5% at the
storage temperatures of 5 ± 3 °C, 30 ± 2 °C/65% ± 5% RH and 40 ± 2°C/ 75% ± 5%
RH.

6.2. Conclusions

The overall outcome of the studies, as planned to accomplish the aims and
objectives, points towards the successful development of optimized drug-bearing
carrier-based novel formulations of the drugs, ETO and ITR. A systematic and
scientific approach involving all the best and feasible investigative studies was
conducted for the two drugs, while keeping the key-targets always in the sight. It
included pre-developmental and developmental aspects such as analytical studies,
drug-excipient compatibility and interactions, FbD-based systematic approach of
choosing the variables and delivery-targets to be achieved, formulation method
development, characterization of FbD-optimized formulation for drug delivery
specific attributes, evaluation for in-vitro and in-vivo parameters, and stability
aspects. Specific attempts were also made to evaluate ETO formulations for
preliminary clinical assessment in patients. The best formulation for ETO has been
advised as ethosomes on account of its deeper penetration and the resultant
pharmacokinetic and pharmacodynamic actions on various strata of skin. In case of ITR, NLCs and SLNs were found to be more or less equivalent to each other in the \textit{in vivo} performance in animals.

Despite all the positive indications stipulated in the work, the scalability on industrial scale production as well as mass level clinical trials need to be conducted in order to make these novel products available to the suffering patients.

In a nutshell, the project work has been endeavored systematically on strong scientific grounds with outcomes on a highly satisfactory note, thus furnishing hopes and promises to be employed as safe, effective and economical healthcare tools.