5. SUMMARY AND CONCLUSIONS

Both of the selected drugs viz. tamoxifen citrate (TAM) and diclofenac diethylamine (DLF), have been struggling to date with various shortcomings on several fronts. TAM, hitherto, has only been prescribed through oral route, while DLF has not been utilized optimally on account of varied delivery hurdles. The current research project was undertaken to address these hiccups in the drug delivery into and across the skin barriers. The innovative formulation strategies employed during the studies have been conceived and contemplated upon after noticing the recent developments in preserving and promoting the pharmacotherapeutics potential of drugs to cater to the needs of the patients as well as the physicians. A brief summary of the work, and the conclusions drawn from it, are presented as under:

Vesicular (FMVs) and Non-vesicular (LOs) systems of TAM

In the first instance, analytical methodologies (i.e., HPLC and UV spectroscopy) were established and validated for the quantitative estimation of TAM. Equilibrium solubility of drug indicated TDW as the most appropriate aqueous medium for the preparation delivery systems and TDW (with 5% methanol) as that for the receptor phase fluid. Partition and solubility studies revealed the lipophilic character of TAM.

TAM-loaded FMVs were prepared by TFH technique, owing to its advantages of higher entrapment efficiency and ease of formulation. For separation of unentrapped drug from vesicles, Sephadex mini-column technique was found to be the most suitable, reproducible and efficient.

Taguchi design was employed for screening of the various process and formulation variables. PL 90H and Span 80 were selected for preparation of FMVs. The ratios of TAM:PL and PL:EgAct were found to be most influential factor variables, while probe sonication (3 min) was finalized as the sonication method for further optimization studies. Implementation of DoE through FCCD, revealed that maximum entrapment efficiency and drug permeation, as well as smaller vesicle size were obtained at the intermediate levels of both of
the factors i.e., TAM:PL and EgAct. Optimium search employing various search methods, i.e., brute-force, graphical and numeric, located TAMOPT4 (i.e., PL 90H at 1:10 weight ratio with TAM and 15.2% of Span 80 w.r.t. to PL) as the “optimum formulation” over the entire experimental domain. Linear correlations obtained between the predicted and observed values of response variables, and the randomized residuals validated the high prognostic ability of the polynomial models for optimizing TAM-FMVs.

Morphology and micromeritic studies ratified the formation of positively charged SUVs (D50 = 125 nm) having sufficient flexibility to cross the narrow pores of skin without losing its shape and drug contents. The intercalation between EgAct and TAM in the lipid bilayers was further confirmed through DSC and XRD studies.

To make the rheological characteristics favorable for on-site topical application, Carbopol gel was selected as the secondary base for incorporation of optimized TAM-FMV suspension. The pH and organoleptic character of TAM-lipogel were found to be suitable for application on the skin tissue. The rheological and textural characterization of TAM lipogel indicated the attainment of desirable rheological properties viz. fairly good gel strength, ease of spreading, extrusion from tube and adequate cohesiveness.

For development of TAM LO, based on the comprehensive studies, PL 90G and IPP were selected as organogelator and organic solvent, respectively. To improve the stability of drug-loaded LOs, Pluronic and Span 80 were included as secondary gelator and auxiliary gelator, respectively.

D-optimal mixture design was employed to obtain an optimized template PLO gel w.r.t. gel strength, viscosity, spreadability and consistency. The optimized PLO (i.e., PLGOPT1) was composed of organic phase and aqueous phase in the weight ratio of 1:4.

TAM-PLO was prepared by incorporating TAM in organic phase of template PLO. The phase transition behaviour indicated the fairly appreciable robustness along with desired softening of gel on application of TAM-PLO. The developed PLO was found to have appropriate rheology and gel strength.
Summary and Conclusions

The enhanced permeation of TAM was obtained with both of the developed systems (i.e., FMVs and PLOs), though FMVs (i.e., ~1.5 times) were found to be superior.

In vitro cytotoxicity studies on HEK and MCF-7 cell lines by MTT assay revealed the superior efficacy of vesiculated TAM (i.e., up to 2 times) vis-à-vis pure drug. The chemopreventive and therapeutic effect of TAM studied on DMBA-TPA induced mice skin carcinogenesis, showed significant increase (i.e., 37%) in tumor latency with the application of TAM lipogel, attributable to the delay in the promotion phase of carcinogenesis. Significant decrease in the incidence of tumors was seen with TAM lipogel (i.e., ~3 fold) and TAM PLO (i.e., ~1.6 fold) compared to TAM hydrogel at the completion of study. Evaluation of anti-psoriatic activity on mice tail also revealed analogous results with the highest activity observed with TAM lipogel (i.e., ~5 fold) and TAM PLO (i.e., ~3 fold) vis-à-vis TAM hydrogel. The enhanced efficacy of the vesicularly entrapped drug may be ascribed to its favorable interaction with the skin tissue. Hair growth retarding study on mouse skin revealed the appreciable pharmacodynamic activity of externally applied TAM in FMVs, justifying the development of its topical gel formulations.

Vesicular (FMVs) and Non-vesicular (LOs) systems of DLF

Analogous to TAM, analytical methodologies (i.e., HPLC and UV spectroscopy) for quantitative estimation of DLF were also established and validated. Equilibrium solubility of drug indicated TDW as most appropriate aqueous media for the preparation delivery systems and as receptor phase fluid for DLF. Partition along with solubility studies revealed the amphiphilic character of DLF.

DLF-loaded FMVs, composed of PL 90H and Span 80, were prepared employing TFH technique. A FCCD was employed to obtain the optimum composition for DLF-loaded FMVs with appropriate balancing of the levels of lipid (i.e., drug:l lipid ratio; 1:10.26), and EgAct content (20.45%).
Summary and Conclusions

Morphology and micromeritic studies ratified the formation of negatively charged SUVs (D_{50} = 120 nm). Fluorescence microscopy revealed that the prepared FMVs could find the improved accessibility into the skin tissues. The placement of DLF within the lipid bilayers was confirmed through DSC and XRD studies. The pH and organoleptic characteristics of DLF lipogel were found to be suitable for application on skin. The rheological and textural characterization of DLF lipogel indicated the attainment of desirable rheological properties viz. fairly good gel strength, ease of spreading and extrusion from tube along with adequate cohesiveness.

DLF-loaded PLO was prepared using optimized template PLO (i.e., PLGOPT1). Microscopy of PLOs revealed organization of micelles, differentiating them from vesicles. The prepared PLO possessed suitable stability and softening for easy application. The rheological characters of DLF-PLO were analogous to that of TAM-PLO. The drug permeation for lipogel was found to be higher (i.e., -1.6 times) compared to PLO.

Stability studies indicated appreciable stability of the all the developed formulations (i.e., FMVs, lipogels and PLOs). Storage in refrigerator was found to be most appropriate for the developed formulations to achieve their prolonged shelf-life.

The developed formulations of DLF, when subjected to in vivo pharmacodynamic anti-inflammatory activity evaluation employing carrageenan-induced rat paw edema model, showed significantly higher efficacy of DLF lipogel compared to DLF in PLO (p<0.005) and Emulgel® (p<0.002). Radiographic evaluation of rat paws affirmed the same.

The clinical efficacy of topically applied DLF lipogel was significantly more than the placebo and Emulgel® for all outcome measures i.e., pain (p=0.002); physical function and stiffness (p=0.01). The improvement in WOMAC index after completion of study was found be higher with DLF-FMVs (i.e., 36%) compared to Emulgel® (i.e., 23 %) and placebo gel (i.e., -13.03 %).

Overall the work conducted on the sound hypothesis of the afore-said drug delivery approaches, entailed optimized phospholipid-based systems viz.
vesicles and reverse micelles structures, (i.e., organogel) revealing the improved dermatological performance of the drugs. Herein, the phospholipids, in association with other components, played a pivotal role in providing the requisite direction towards their delivery while providing them with conducive physicochemical mantle. The central point of the success of these developed systems is stemmed forth from the very presence of phospholipid, which by virtue of its unique molecular architecture and its association with water, provides exquisite drug-modifying characteristics. The interactions with biological membranes of phospholipids in its membranous, non-covalently linked self-assemblies offers the unique advantage. Moreover, the presence of aqua-lipoidal solubility of the drug further helps in its transport and interactions for improved pharmacological action. Application of systematic optimization techniques based on recently recognized experimental design models viz. FCCD and D-OMD, further played a stellar role in getting the delivery systems with “the best attributes”. The intensive and extensive characterization of the optimized systems with a high degree of reproducibility can claim such systems as the “standardized” ones. Comprehensive evaluation of these drug delivery formulations, in vitro and in vivo, provided adequate proof for their impact on biological systems, corroborating their importance in real life situations. On stability front too, the developed systems were found to be quite robust and stable. Finally, the clinical studies on patients ushered in an unquestionable confidence in the importance of overall approach.

To conclude, the current studies successfully embarked upon the development of “optimized” phospholipid-based vesicular and non-vesicular drug delivery systems with improved therapeutic efficacy, safety and patient compliance. Nevertheless, the promising systems developed during these research endeavors need to be evaluated beyond the laboratory precincts to the production milieu. This calls for further studies using newer animal models, multi-centric clinical trials, scale-up studies, patent filling and finally, technology transfer to the industrial houses.