CHAPTER VII

SUMMARY AND CONCLUSIONS

Targeted drug delivery was conceptualized as early as the beginning of this century by microbiologist Paul Ehrlich. However, only recently, has this concept been exploited, with the use of sophisticated carrier systems for drug molecules. Among the various macromolecular, cellular or synthetic carriers for drugs, **liposomes** have generated a great deal of interest. These tiny lipid vesicles can bind to, fuse with or be taken up by cells thus serving as a sort of microscopic Trojan horse, carrying drugs to places within the body, where they would not otherwise reach. 24 years of research into the use of liposomes in drug delivery has led to vastly improved technology in terms of drug capture, vesicle stability on storage, scaled-up production and design of formulations for specialized tasks. They are not only contributing to more efficient use of "old" drugs but are also finding applications with agents produced by recombinant DNA technology.

In recent years, liposomes have become increasingly important as vehicles for delivery of active compounds which are used for topical management. Literature reveals that topically applied liposomal products, in comparison to existing products, enhance local effect, reduce systemic effects, optimize dosage, provide prolonged release action and be cosmetically more acceptable. Hence, at present, liposomes seem to be the best carriers for dermato-pharmacotherapy.

Although considerable volume of research has been conducted in the field of liposomal corticosteroids for dermal drug delivery, lacunae exist with respect to the selection of phospholipids for preparing liposomes, the influence of varying compositions of phospholipids and cholesterol in the liposomes, selection of a proper base for preparing stable and effective formulations, variation in the kinetic parameters to assess the release of drug from liposomal topical dosage forms, impact of liposomal drug entrapment on the blanching response to corticosteroids by healthy human volunteers and clinical efficacy of liposomal corticosteroids for dermal therapy. The present study, was a modest attempt to probe into such dark zones, with a view to throw some light on these important aspects. For this, liposomes of 3 topical corticosteroids, namely, triamcinolone acetonide (TRMA), flucinolone acetonide (FLU) and clobetasol proprionte (CLO), were prepared using varying concentrations of a phospholipid - egg phosphatidyl choline (PC) and cholesterol (CHOL) and were incorporated into different gel bases. Liposomal drug gels were then compared with their respective free drug gels/creams for their ability to release drug *in vitro*, to evoke a skin blanching response *in vivo* and be clinically efficacious in patients with dry, bilateral eczema.

Reported colorimetric methods were standerdized for the estimation of the three drugs under study, PC and CHOL. The Umberger reaction involving isoniazid in acidic medium, to

form a yellow colored hydrazone with Δ^4-ketosteroids was found to be sensitive between 4-40 µg/ml for TRMA and 2-20 µg/ml for FLU and CLO. The sensitivity of the method was found to reduce in the presence of even trace quantities of water and hence it was decided to render all the samples free from water before analysing the drug content thereof. The ability of phospholipids to form a red colored complex with ferrothiocyanate in organic solutions was used to estimate PC. This method was found to be sensitive between 100-700 µg/2ml concentration of PC in chloroform. Complexation of CHOL with ferric chloride and sulphuric acid was the basis of the colorimetric method used for estimating CHOL. This method was found to be sensitive between 10-100 µg/ml of CHOL in glacial acetic acid.

21, 3 and 3 batches of liposomes of TRMA, FLU and CLO respectively, varying in their drug : PC:CHOL, millimolar ratio from 1:0.6:0.52 to 1:10.86:10.86 for TRMA and 1:2:0.24 to 1:2:2 for FLU and CLO were prepared by the lipid film hydration method. After dissolving the weighed quantities of drug, PC, CHOL and α-tocopherol(antioxidant) in a chloroform-anhydrous methanol (2:1) mixture and evaporating the solvent, under vacuum, using a rotary flash evaporator, the resulting dry lipid film was hydrated using a 25mM calcium chloride solution. Following centrifugation of the liposomal suspension and washing the pellet thrice with calcium chloride solution, off-white, discrete liposomes with negligible (<0.1%) amount of unentrapped drug were obtained.

When seen under a light microscope, liposomes appeared spherical and their particle size ranged between 0.7 μm-14μm, without a statistically significant difference in the size distribution between the batches prepared. This implies that the thickness of the PC-CHOL cover does not affect the particle size of the liposomes formed. Negative staining electron microscopy revealed that the liposomes were multilamellar.

The % TRMA entrapped in all its liposomal batches prepared, except that with TRMA:PC:CHOL ≡ 1:0.6:0.54 (TRMA1), ranged between 70-90% indicating that once the PC-CHOL cover exceeded that, provided by TRMA1, entrapment efficiency remained almost similar. All the batches of liposomes of FLU and CLO showed an entrapment efficiency between 72-88%.

Liposomal gels of the batches containing liposomes of the drugs under study were prepared by incorporating a calculated amount of the pelleted liposomal suspensions into a 5% HPMC K4M gel base. Phenyl mercuric nitrate (0.001%) was used as a preservative in this base. The gel base was also used to prepare free drug gel for each drug under study. The prepared gels were analysed for their drug content and were used for further studies only if their drug content was 90-110% of the claimed amount.

In vitro permeation of the 3 drugs, across albino rat skin, from their gels was studied using a validated vertical in vitro set up. The permeation fluid, pH 7.4 phosphate

buffered saline I.P., was analysed, at five time intervals for 24 hours, for its drug content. Based on these results the release profiles and meaningful kinetic parameters for predicting drug release patterns and screening formulations with maximum therapeutic activity were calculated. Drug release profiles were expressed in terms of amount released as a function of time and of the square root of time. An apparent linear relationship ($0.966 \leq r \leq 0.999$) was found to exist between the amount released and square root of time data for all the gels studied. This indicated that the membrane was highly permeable to the drugs under study and that sink conditions prevailed. It also suggested that drug release from the gels followed the simplified Higuchi's diffusion model.

The kinetic parameters calculated from the results of the in vitro studies included release rate (R), permeability coefficient (P), diffusion coefficient (D), partition coefficient (K), quantity of drug entering in the membrane till 6 hours $Q^i(6)$, quantity of drug in the membrane Q^m, factor for quantity of drug entering into rat blood stream Csf and the ratio Q^m/Csf. In general, incorporation of drugs into liposomes caused a reduction in the R,P,D and Csf values and an increase in the K, $Q^i(6)$, Q^m and Q^m/Csf values as compared to those for respective free drug gels. Low 'P' and 'D' values are indicative of the decrease in the ability of the drug to cross the membrane and enter the sink-this being supported by the low 'Csf' values. A high 'K' value is

indicative of the ease of entering into the horny layer- this being supported by the high '$Q^i(6)$' value. A high 'K' value coupled with a low 'R' value is indicative of the greater affinity of the drug for the cornified tissue with the transport towards the deeper tissues being adversely affected. In such a case, thermodynamic activity of the diffusing species immediately below the horny layer would approach that of the source and the rate determining step would be clearance from the barrier rather than penetrating the barrier. A high 'K' and low 'R' value are also indicative about the fact that the drug might not be getting cleared from the barrier quickly - the fact being supported by a high Q^m value. The increased Q^m/Csf ratio is indicative of increased effect and reduced side effects with the liposomal form.

Comparing the various liposomal gels of TRMA amongst themselves, those gels with TRMA:PC≡1:1.81 showed the maximum percent reduction in the R(71.25%), P(73.59%), D(93.29%) and Csf(71.00%) values and maximum percent enhancement in the K(293.75%), $Q^i(6)$(316.81%), Q^m(328.64%) and Q^m/Csf (1378.08%) values. Hence, 3 gels containing liposomes with this TRMA:PC ratio and TRMA:PC:CHOL ratios of 1:1.81:0.46(T_7), 1:1.81:0.68(T_8) and 1:1.81:0.91(T_9) were subjected to an accelerated stability study at room temperature for 45 days. Although these batches did not show any changes in the drug content and <u>in vitro</u> permeation characteristics, the liposomes in the gels T_8 and T_9 exhibited a tendency to

coalesce and seperate out from the gel base thus compromising on the aesthetic appeal of the formulations. Hence narrowing down the field to just one composition i.e. 1:1.81:0.46, gels of these liposomes were prepared using 5% HPMC E4M gels base, 0.8% Carbopol 941 gel base and 20% PVA gel base in order to study the effect of the nature of the gel base on the release of TRMA from its free and liposomal drug gels. The results of the in vitro permeation studies conducted for these gels showed that for the free drug gel, the preference of gel base would be in the order of Carbopol 941>HPMC E4M> HPMC K4M while that for the liposomal TRMA gel would be in the order of HPMC K4M> Carbopol 941>HPMC E4M indicating that the nature of the gel base does influence the release of drug from it. Free and liposomal TRMA was released at a higher rate and to the same extent from the PVA gel base indicating the possibility of disruption of the liposomal structure in this base.

Selected liposomal gels of TRMA and all the liposomal gels of ~~FLU~~ and CLO were compared with their respective conventional drug creams, for their ability to evoke a skin blanching response during the human skin blanching assay. The test was conducted, on both forearms of 8 volunteers who had proved to be average blanchers after a preliminary screening test. The blanching reponses were graded on a 0-4 scale rating by three blinded observers and the blanching scores were expressed for each formulation as the % of total possible score (%TPS). Statistical analysis of the results of

these studies showed that the observers did not differ significantly in their ability to grade the blanching response. Besides, no significant differences were found between the blanching scores on the right arm and left arm of all volunteers indicating that no arm bias exists. The volunteers too did not differ significantly in their ability to elicit the blanching response except for the less potent TRMA.

The different liposomal gels of TRMA in HPMC K4M gel base did not differ significantly amongst themselves and with the free TRMA gel with respect to the % TPS. However, among the liposomal gels, gel T_7 showed maximum blanching potential.

When liposomal and free drug gels prepared using different gel bases were compared with the conventional TRMA cream (in Cetomacrogol cream base), statistically significant differences were observed in the % TPS between the liposomal TRMA gels and conventional TRMA cream but no statistically significant difference existed for this parameter between free TRMA gels and conventional TRMA cream. Although all the liposomal TRMA gels gave a higher % TPS as compared to their respective free drug gels, the differences were not statistically significant. Among the free and liposomal TRMA in different gel bases, no statistically significant differences were seen in the % TPS values; however, the values were in the order of K4M>E4M>Carbopol 941 for the free TRMA gel and K4M=E4M>Carbopol for the liposomal TRMA gel.

The liposomal gels of FLU with the composition of liposomes being 1:2:0.48 (F_2) and 1:2:2 (F_3) showed a statistically higher blanching response as compared to the conventional FLU cream (in Cetomacrogol cream base) with F_2 showing marginally higher % TPS values as compared to F_3. The liposomal FLU gels exhibited higher % TPS values as compared to the free FLU gel but the difference was not statistically significant. The free FLU gel gave a higher % TPS as compared to the conventional cream but the difference was not statistically significant.

No statistically significant differences were observed in the % TPS between the free and liposomal CLO gels and conventional CLO cream. However, the liposomal CLO gels gave a % TPS higher than that given by free CLO gel which in turn gave a higher % TPS as compared to that of conventional CLO cream.

One liposomal gel per drug under study, found promising after in vitro and in vivo tests, was analysed for its efficacy as compared to its free drug gel in double blind clinical trials on patients with dry, bilateral eczema. The liposomal composition in the gel in terms of Drug:PC:CHOL was 1:1.81:0.46 for TRMA and 1:2:0.48 for FLU and CLO. Percentage improvement in the eczematous condition, over a 4-week period with weekly assessment, was found comparable between the liposomal and free drug gel, for all the 3 drugs. A longer duration of clinical trials over a large number of patients may be required to generate a convincing data for making a

significant comparison with the in vitro and in vivo results obtained in this study.

The following conclusions can be drawn from this study :

(1) It is confirmed by in vitro studies that incorporation of corticosteroids into liposomes increases the quantity of drug in the skin (site of action) and restricts the amount of drug entering systemic circulation (site of side effects). However, in vivo experimentation to estimate the radio-labelled corticosteroid in the blood and other organs, needs to be performed to confirm the validity of this result.

(2) The best liposomal composition in terms of Drug:PC:CHOL for topical preparation of TRMA was found to be 1:1.81:0.46 while that for FLU and CLO as 1:2:0.48.

(3) HPMC K4M gel base seems to be the most promising one for preparing gels of liposomes containing corticosteroids.

(4) The blanching potential of liposomal corticosteroid gels is greater than that of their conventional creams.

(5) Liposomal corticosteroid gels are comparable to free corticosteroid gels in their efficacy in patients with dry bilateral eczema.

Promising results of this study bring us closer in realizing the reality of liposomal topical preparations of corticosteroids in near future.