CHAPTER 7
SUMMARY AND CONCLUSION
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In the past few decades, considerable attention has been focused on the development of colloidal drug delivery systems. The colloidal drug delivery system should ideally fulfill two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery.

Approaches are being adapted to achieve this goal, by paying considerable attention either to control the distribution of drug by incorporating it in a carrier system, or by altering the structure of the drug at the molecular level, or to control the input of the drug into the bioenvironment, or to enhance the penetration of the drug to ensure an appropriate profile of distribution.

Colloidal drug delivery system aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body. Colloidal drug delivery attempts to either sustain drug action at a predetermined rate, or by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. It can also localize drug action by spatial placement of controlled release systems adjacent to, or in the diseased tissue or organ; or target drug action by using carriers or chemical derivatization to deliver drug to particular target cell type.

Different types of colloidal carriers are present. They are lipid particles (low and high density lipoprotein-LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and vesicular like liposomes, niosomes, pharmacosomes, virosomes, etc. The vesicular systems are highly ordered assemblies of one or several concentric lipid
bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. The terms such as synthetic bilayers allude to the non-biological origin of such vesiculogenes. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies.

Niosomes are microscopic lamellar structures formed on admixture of cholesterol and single alkyl chain non-ionic surfactant with subsequent hydration in aqueous media, offering an alternative to liposomes as drug carriers capable of entrapping hydrophilic and hydrophobic drugs. Compared to phospholipids used in liposomes, the synthetic non-ionic surfactants used in the preparation of niosomes are chemically stable, precise in chemical composition and cheaper in cost. Many non-ionic surfactants like glucosyl dialkyl ethers, crown ethers, ester linked surfactants, polyoxyethylene alkyl ether, Brij and a series of spans and tweens are used with cholesterol to entrap drugs in vesicles.

The major problem in the development of vesicular systems at industrial level is their instability. Although niosomes exhibit good chemical stability during storage, there may be problems of physical instability in the dispersed form. Like liposomes, niosomes dispersion may exhibit aggregation, fusion, leaching or hydrolysis of entrapped drug, thus limiting the self-life. Different approaches have been proposed to enhance the stability of vesicles. Among them proniosomes offer a versatile delivery concept, which exhibit better stability than the niosomes. Proniosomes are dry, free flowing water-soluble carrier particles coated with surfactant mixture, which on hydration form multilamellar niosomal suspension.

Piroxicam (member of oxicam group) is one of the most potent nonsteroidal anti-inflammatory drugs, which also exhibits antipyretic activity. It is widely used in the treatment of rheumatoid arthritis, osteoarthritis, and a variety of other acute and chronic
musculoskeletal disorders. Piroxicam is well absorbed following the oral administration, however, its use has been associated with a number of gastrointestinal disorders.

Ketoprofen (propionic acid derivative) is a nonsteroidal anti-inflammatory drug with well-established analgesic and antipyretic properties. It is widely used in the treatment of rheumatoid arthritis, osteoarthritis, and a variety of other acute and chronic musculoskeletal disorders. Oral therapy of ketoprofen is very effective, but the clinical use is often limited because of the adverse effects such as irritation and ulceration of the gastrointestinal tract.

Aceclofenac (phenylacetic acid derivative) is a novel nonsteroidal anti-inflammatory drug indicated for the symptomatic treatment of pain and inflammation in acute, sub-chronic and chronic inflammatory conditions. Aceclofenac is rapidly and efficiently absorbed after oral administration. Side effects associated with the therapy are dyspepsia, nausea, abdominal pain, and diarrhoea. These side effects may be overcome by the topical administration of the drug.

All the above-mentioned drugs possess lower molecular weight and have the potential to be delivered topically. Topically these NSAIDs exhibit poor transport into the skin by simple diffusion. Vesicular systems can be used to overcome this problem of poor skin permeability, which have been reported for effective transdermal delivery of drugs.

Formulation of niosomes and proniosomes entrapped with drug is affected by many formulation and process variables i.e. choice of the surfactant, nature of membrane additives, nature of drug, amount of drug, molar ratio of surfactant to cholesterol, total amount of lipid, hydration temperature, hydration time and time of sonication. For an optimal response all these variables must be carefully controlled in the design of a niosomal drug delivery system.

In the present study different niosome-based gel formulations were developed with a view to improve the permeation and effectiveness of the piroxicam, ketoprofen and
aceclofenac. For this purpose, niosomes and proniosomes prepared with different compositions were incorporated into polymer gel matrix and extensively characterized and evaluated both for in-vitro followed by in-vivo tests.

**Experimental**

**Preparation and optimization of niosomes**

Calibration curve for nonsteroidal anti-inflammatory drugs were prepared by spectrophotometric method, where piroxicam, ketoprofen and aceclofenac give $\varepsilon_{\text{max}}$ at 353.5 nm, 261 nm and 275 nm respectively in PBS pH 7.4. Each time, a blank containing blank niosomes was treated in the same manner to account for any absorbance due to the surfactant component.

Thin film hydration technique was selected for the preparation (among the different methods) and optimization of the niosomes containing nonsteroidal anti-inflammatory drugs due to its simplicity and reproducibility. Thin film hydration technique involves solubilization of the surfactant, cholesterol and drug in an organic solvent and subsequently the solvent evaporated to obtain a thin film followed by hydration to obtain niosomes. Span 60 was selected for the preparation of piroxicam and aceclofenac niosomes and Span 40 for the ketoprofen niosomes. The optimization of various formulation variables namely; molar ratio of Span60 to cholesterol and amount of drug for piroxicam, total lipid concentration and amount of drug for ketoprofen niosomes and molar ratio of drug to lipid concentration and volume of hydration for aceclofenac niosomes were carried out as per the $3^2$ factorial design.

Niosome batches were prepared as per experimental design and were characterized for percentage drug entrapment and vesicle size. Polynomial equations (quadratic) were developed by multiple regression analysis technique for measured responses, and $P$ value of coefficient of each term help in predicting whether the effect is significant or not.
Check point analysis was performed to validate the polynomial equation in predicting the responses.

Significant effect of molar ratio of span 60 to cholesterol and amount of drug was observed on the PDE of piroxicam niosomes and for MVD only molar ratio of span 60 to cholesterol was found to be significant. Optimum value of span 60 to cholesterol (58:42) and amount of drug (3.3 mg) shows PDE value of $79.9\% \pm 2.34$ and MVD of $4.92 \mu m$.

In case of ketoprofen niosomes both the selected independent variables total lipid concentration and amount of drug have significant effect on the PDE and only total lipid concentration show a significant effect on the MVD. Optimum value of total lipid concentration (10 mmol) and amount of drug (6.17 mg) shows PDE value of $65.98 \% \pm 1.94$ and MVD of $5.08 \mu m$.

Similarly, for aceclofenac niosomes, both the selected independent variables, molar ratio of drug: total lipid and volume of hydration show a significant effect on PDE and volume of hydration has a significant effect on the MVD. Optimum value of molar ratio of drug to total lipid (1:28) and volume of hydration (5.92 ml) shows PDE value $66.72\% \pm 2.88$ and MVD value $4.88 \mu m$.

**Preparation and optimization of proniosomes**

Niosomes were found to be instable during the stability studies for the one month hence proniosomes were prepared in order to make them stable in the form of dry granules, which can be rehydrated, immediate before use by simply vortexing it with hot water. The slurry method was used for the preparation of the proniosomes and maltodextrin was selected as a carrier. For the preparation of proniosomes, surfactant, cholesterol and drug were taken in a round bottom flask containing maltodextrin as carrier. The flask was attached to rotary evaporator under reduced pressure until the mass in the flask
resulted in a dry, free flowing product. Then proniosomes were transformed to niosomes by hydrating with PBS pH 7.4 at 80 °C using a vortex mixer. The composition of the proniosomes influence their characteristics such as, size, and percentage drug entrapment and therefore need to be optimized to obtain the desired values. Various experimental designs were used for preparation and optimization studies of proniosomes containing piroxicam, ketoprofen and aceclofenac.

For the optimization of the proniosomes independent variables selected were molar ratio of span 60 to cholesterol, surfactant loading and amount of drug for piroxicam, total lipid concentration, surfactant loading and amount of drug for ketoprofen and molar ratio of drug to total lipid, surfactant loading and volume of hydration for aceclofenac.

Surface characteristics of the proniosomes batches were studied by the scanning electron microscopy (SEM). Pronosome-derived niosomes were characterized for the PDE and MVD. SEM study of pure maltodextrin (carrier particle) showed a porous surface, which makes it an effective carrier and provides more surface area for the coating of the surfactant mixture. SEM images of the proniosomes showed coating of the surfactant mixture on the surface of carrier particles.

Proniosomes were prepared as per the experimental design and were characterized for percentage drug entrapment and vesicle size after transforming to niosomes. The responses measured were fitted to quadratic model (polynomial equation) by carrying out multiple regression analysis and F value obtained by performing ANOVA to identify statically significant terms. Lack of fit was checked for all the experimental designs as all these selected designs include replicated center points. Contour plots were plotted for different independent variable to know the effects on responses. A check points analysis was performed to confirm the utility of established polynomial equations and contour plots in the prediction of responses.
For the optimization the piroxicam proniosomes Box-Behnken experimental design was used. It was found that molar ratio of span 60 to cholesterol and amount of drug has a significant effect on the PDE of proniosome-derived piroxicam niosomes and surfactant loading was found to be significant in determining the MVD value. Optimum value selected for the molar ratio of span 60 to cholesterol, surfactant loading and amount of drug was 1:1, 2.68x and 6.38 mg respectively, which gives PDE value 82.16 % ± 2.48 and MVD value 4.86 μm.

For the optimization of the ketoprofen proniosomes central composite Box-Wilson design was used. It was found that the total lipid concentration and amount of drug has a significant effect on the PDE of proniosome-derived ketoprofen niosomes. Effect of total lipid concentration was found to positive while of amount of drug had a negative effect on the PDE. All the independent variables including surfactant loading have a significant effect on the MVD of ketoprofen niosomes. Optimum value obtained for total lipid concentration, surfactant loading and amount of drug was 10mmol, 2.55x and 7.84 mg respectively, which shows PDE value 54.82% and MVD value 4.98 μm.

For the optimization of the aceclofenac proniosomes a central composite design was used. It was found that the molar ratio of drug to total lipid and volume of hydration having a significant effect on the PDE of proniosome-derived aceclofenac niosomes. Molar ratio of drug to total lipid has a negative and volume of hydration having a positive effect on the PDE. Also, it was found that molar ratio of drug to total lipid and surfactant loading having a significant effect on the MVD of aceclofenac niosomes. Optimum value selected for the molar ratio of drug to total lipid, surfactant loading and volume of hydration was 4.7 mg aceclofenac/10mmol, 1.8x and 5 ml respectively, which shows PDE value 70.28% ± 1.86 and MVD value 5.12 μm.
Proniosomes of all the drugs in dry form were found to be stable at refrigerated and room temperature for period of 3 months.

**Preparation and evaluations of niosome-based gel formulations**

Niosome-based gel formulations of piroxicam, ketoprofen and aceclofenac were prepared, characterized and investigated for in vitro release, permeation and in vivo studies on rats.

Niosome-based gel formulations were prepared by two different methods. First, selected batches of the niosomes and proniosomes were centrifuged to obtain pellets, which were incorporated in to carbopol dispersion and pH was raised to 5.9 – 6.2 with triethanolamine solution. Second, Surfactant, cholesterol and drug were dissolved in chloroform for piroxicam and aceclofenac and diethyl ether for ketoprofen.

The glass tube was attached to rotary flash evaporator to evaporate the organic solvent under vacuum resulting in a formation of a gel. This obtained gel was subjected to vigorous mechanical agitation for collapsing gel to fluid. The tube again was attached to a rotary flash evaporator for the removal of the remaining solvent. Then absolute ethanol and phosphate buffer pH 7.4 were added in the tube and the mixture was further warmed in the water bath to obtain dispersion, which on cooling gives provesicles. This obtained provesicles was mixed in to an aqueous dispersion of carbopol for the preparation of provesicular surfactant gel. pH of the gel was raised to 5.9 – 6.2 by using triethanolamine solution.

All the selected formulations were subjected to in vitro release and rat skin permeation studies.

For piroxicam niosome-based gel formulations, initial in vitro release from provesicular surfactant gel was found to be a higher compared to niosomal gel formulation. This may
be due to presence of unentrapped drug in vesicular surfactant gel. Increase in the cholesterol proportion in niosome retards the release of the drug from the niosomal gel. A high proportion of the span 60 in the niosomes leads to more release of drug initially but overall less cumulative release (45.68%) compared to niosomes prepared with equal ratio of the span 60 and cholesterol (54.64%). Release of the drug from the vesicular gel containing soya and egg lecithins was less as compared to the vesicular surfactant gel prepared only with the span 60 and cholesterol which may be due to the affinity of drug to the lecithins.

In vitro permeation of drug through rat skin was found to considerably lower than its release through synthetic membrane indicating barrier property of the skin. Plain gel was also prepared by simply mixing the drug with carbopol dispersion and evaluated for in vitro permeation. The transdermal permeation of piroxicam from different niosomal and vesicular surfactant gel was significantly higher than that from the plain gel which indicates the effect of niosomes on the permeation of drug.

The carrageenan induced rat paw edema method was used as a tool to compare the efficacy of the niosome-based gel formulation, with plain drug formulation and marketed formulation. Increase of paw thickness of rats was measured at different intervals of time. Increase in paw thickness of rats treated with niosomal gel batches NP4 and NP5 were found to be significantly lower than the plain and marketed gel.

Niosome-based gel formulations containing ketoprofen, the drug release study showed that the release of the drug from the vesicular surfactant gel formulations was significantly higher than the niosomal gel formulations. Higher release of the ketoprofen from the vesicular surfactant gel may be due to solubilization and penetration enhancing effect of ethanol present in the formulation. Among the different niosomal gel formulations less drug release was observed from the formulations (NK5 and NK6),
which were prepared with high lipid concentration. In vitro skin permeation studies of the
gel formulations also, showed the similar results as in vitro release studies. Results of
the in vivo studies revealed that the provesicular surfactant gel formulation PGK showed
significant reduction in increase in paw thickness after carrageenan injection compared
to niosomal gel and \( \text{PGM} \) gel. Provesicular surfactant gel PGK was found to be
superior among all the formulation studied according to both in vivo and in vitro
permeation studies.

In case of the aceclofenac niosome-based gel formulations, in vitro release of the drug
from various gel formulations revealed a higher release of the drug from the plain gel
and provesicular surfactant gel obtained. This may be due to presence of free drug in
plain gel and provesicular surfactant gel compared to niosomal gel in which drug is
entrapped within niosomes. Results of the in vitro permeation studies showed that the
higher drug permeated from the batches NA6 and NAO, which may be due to more total
amount of encapsulated drug in these batches.

Results of the in vivo studies for the different niosome-based gel formulations were
compared. The group treated with NA6 showed highest reduction in the paw thickness
for all points of time.

For all the niosome-based formulations after 4 h, in vitro release profile shows a linear
relationship with the time indicating zero-order drug release kinetics. This suggests that
the niosomes act as reservoirs for continuous drug release.

In conclusion, topically applied, NSAIDs loaded niosomes can substantially improve drug
permeation; thereby offering clear-cut advantages over conventional dosage forms.
Before findings of this investigation can be commercially realized, the detailed clinical
investigations with special emphasis on efficacy and side effects are to be accomplished
for success in market.