Chapter - III

REVIEW OF LITERATURE

The pharmacokinetic evaluation of antifungal drug miconazole was carried out by P.J.Lewi et al \(^{30}\) (1976). The pharmacokinetic profile of miconazole has been studied in normal subjects and in patients suffering from severe renal insufficiency; one group of patients was undergoing intermittent haemodialysis. A three-compartment open model was fitted to the observed plasma concentrations obtained after intravenous infusion of miconazole 522 mg over fifteen minutes. The rate constants of elimination and exchange between compartments computed for the three groups were not significantly different. The apparent volumes of distribution in the cases of renal failure not undergoing haemodialysis were significantly smaller than the corresponding control values. A computational procedure is described which reduces observations obtained after infusion to the case of a single rapid intravenous administration.

Imidazole derivatives have had a long and varied pharmacological history starting over thirty years ago. The antimicrobial potential of benzimidazole was indicated by Woolley (1944), who found that it can inhibit the growth of some fungi and bacteria, observations which were later confirmed and extended by Goldsworthy & Gertler (1949). Richard J Holt \(^{31}\) (1976) has investigated the pharmacological effects of imidazole antifungal agents when they are applied topically. During the investigation, four imidazole derivatives have undergone extensive open and comparative trials as topical agents in dermatomycoses and vaginal candidosis. They are chlornidazole (Chemic Griinentlial), clotrimazole (Bayer) miconazole (Janssen) and econazole (Janssen, CilagChemie); all also have some antibacterial activity. Many other imidazoles have been marketed, usually as
antiprotozoal or anthelminthic agents, and some of these have some antimycotic activity as well as other miscellaneous therapeutic properties. The mode of action of imidazole antimycotic agents was discussed after prolonged topical application to animals and human subjects, systemic absorption is negligible. All four agents which are available as cream, powder, lotion or vaginal tablets have many stressful studies to their credit, often with clinical and mycological of over 80% in a variety of dermatomycosis and in vaginal candidosis. The relative value of these topical agents was discussed, and it was suggested that in severe and extensive dermatomycosis consideration should be given to the systemic use of miconazole in support of topical therapy.

Hisaoka M et al (1982) studied on liposome encapsulated carboquone II (CQ), its distribution and clearance in animal models. The plasma clearance and tissue distribution of CQ was studied in rabbit and rat by intravenous administration at the same dose. CQ-liposome showed approximately three times higher plasma concentration than free CQ in rabbit and rat.

Formulation of miconazole for the treatment of vaginal candidosis was carried out by T.K.Daneshmend et al (1986). In the in vivo studies, the serum concentrations of miconazole were measured in 11 healthy adult females for 72 hours following a single 1200 mg vaginal pessary. The mean peak serum miconazole concentration was 10.4 µg/l and the mean elimination half life was 56.8 h. The mean area under the serum concentration time curve was 967 µg/l/h. The calculated mean systemic bioavailability of the vaginal pessary was 1.4%. There was large intersubject variation in serum miconazole pharmacokinetics. Based on the results formulation of miconazole was found to be effective in single dose treatment for vaginal candidosis.

Venkataram S et al (1989) studied the pharmacokinetics of cyclosporine (CsA) in two different dosage forms. CsA was formulated as liposome and intralipids, were subjected for in vivo evaluation. Selected male Newzeland white rabbits were given i.v. CsA (10 mg/kg) in three different dosage forms (1) CsA: liposomes (2) CsA: intralipid (soybean oil and phospholipids): and (3) The
commercially available sandimmune. The volume of distribution of CsA at steady state \((V_{dss})\) in sandimmune was \(2.7 \pm 0.2 \text{ L/kg}\) and was significantly lower than that of either intralipid \((10.6 \pm 2.7 \text{ L/kg})\) or liposomes \((7.4 \pm 2.3 \text{ L/kg})\). It was speculated that in addition to the benefit of removing cremophore EL, a nephrotoxic solubilization agent used in commercial CsA sandimmune, from the product, the different distribution of CsA: liposome's or CsA: intralipid may prove useful in altering the nephrotoxicity and immunosuppression of CsA during i.v. therapy.

In vitro and in vivo cutaneous penetration and antifungal activity of naftifine was studied by Stoughton R B., et al \(^{35}\) (1989). The topical antifungal agent naftifine has shown considerable potency against a broad spectrum of dermatophytes. In this study, an in vitro penetration test in human cadaver skin and an in vivo tape-stripping test were used to evaluate the penetration and antifungal activity of naftifine gel 1 percent and naftifine cream 1 percent compared with other antifungal agents. In both models, Trichophyton rubrum and T. mentagrophytes were the fungal species. Results show that naftifine gel 1 percent and naftifine cream 1 percent, in vitro and in vivo, penetrate the stratum corneum in concentrations that inhibit the growth of both fungal species. Following penetration in vitro, naftifine gel and cream were significantly more active against T. rubrum than econazole nitrate cream 1% percent.

Katare OP et al \(^{36}\)(1990) prepared proliposomes of ibuprofen using effervescent granules as solid carriers of dried phospholipids along with other lipids (soyabean lecithin, stearylamine and cholesterol). The inert atmosphere of carbon dioxide gas prevents the chance of oxidative degradation of phospholipids. The size distribution of liposomes was noted to be related to the degree of agitation provided by effervescence. Encapsulation efficiency of liposomes derived from proliposomes was shown to be nearly 100 per cent. Preparations were shown to be quite stable at \(20 ^\circ \text{C}\) when stored under an umbrella of nitrogen. The enhanced anti-inflammatory activity of ibuprofen entrapped in liposomes was exhibited
when compared with plain ibuprofen following intravenous administration using the carrageenan induced paw oedema test.

Proliposomes of indomethacin for oral administration was developed by Katare OP et al \(^{37}\)(1991). Drug was encapsulated into liposomes composed of soyabean lecithin, cholesterol and stearylamine for oral administration. Liposomes of homogenous size distribution and higher entrapment efficiency were derived from effervescent granule based proliposomes. The efficacy of the oral route was studied by measuring ulcerogenic index and anti-inflammatory activity using carrageenan induced paw oedema test in rats. The effervescent granule based liposomal products exhibited improved in vivo performance with reference to their cytoprotective and anti-inflammatory activities.

Earlier Amphotericin B was widely used as drug of choice for pediatric mucocutaneous candidiasis, but it was giving adverse reactions. Miconazole, a new imidazole antimycotic agent was considered as a safer alternative for amphotericin-B. The role of miconazole in pediatric cases with chronic mucocutaneous candidiasis was studied by Fischer et al \(^{38}\)(1991). During the in vivo study, miconazole given intravenously to five children with chronic mucocutaneous candidiasis over an 18-month period. There was marked improvement of mucosa and skin in two patients, moderate-to-mild improvement in two, and no improvement in one. Nail lesions were not improved in any patient. Adverse reactions included phlebitis, pruritus, nausea and dizziness, rash, wheezing, mild transient anemia, and mild transient transaminase (SGOT and SGPT) elevations; it was necessary to discontinue treatment in only one patient. No renal toxicity was noted. As per results, miconazole appears to be a relatively safe and promising alternative to amphotericin B in chronic mucocutaneous candidiasis.

Liposomes with clindamycin hydrochloride in the treatment of acne vulgaris was studied by Skalko N et al \(^{39}\)(1992). Liposomes with clindamycin hydrochloride using either soya lecithin and cholesterol or phosphate and cholesterol were developed and evaluated. *In-vitro* dissolution studies showed
sustained release of drug from phosphate liposomes compared to lecithin liposomes. Clinical treatment of Acne vulgaris with a lotion of liposomes, drug showed better efficiency than non-liposome lotion forms. Application of a conventional lotion solution, a non-liposome emulsion lotion and a liposomal emulsion lotion resulted in decrease of 42.9, 48.3 and 62.8 % respectively, in the total number of lesions after a 4 week treatment. The results support the possibility of developing products utilizing the liposomal dosage form that are superior to existing dosage forms for topical therapy.

Reddy N et al \textsuperscript{40}(1993) studied the possibility of improving bioavailability of flubiprofen for topical application. In the in vivo study, flubiprofen incorporated into ointment base as niosome, microspheres and beta-cyclodextrin inclusion complexes were applied transdermaly to rats. Results indicated that all the formulations (niosomes, microspheres, beta-cyclodextrin complex) demonstrated significant improvement in bioavailability and anti-inflammatory activities over the drug in ointment base.

Sharma BB et al \textsuperscript{41}(1994) prepared and evaluated the topical liposomal system containing local anaesthetic agent lignocaine. Topical multiple lamellar vesicular liposomes were prepared using the method reported by Bangham. The liposomes, composed of different lipid and charge-bearing components such as stearylamine or dicetylphosphate, were characterized for average size, size distribution, charge lamellae and drug loading. The negatively charged liposomes showed high drug loading and were incorporated in different topical vehicles, i.e. gels and ointment. These were evaluated for in vitro drug skin permeation profile. The selected formulations were evaluated for in vivo performance using the pinprick method. The results revealed localized and prolonged activity of local anaesthetic contained in liposomes when compared with equivalent conventional topical application.

In vivo pharmacokinetic and pharmacodynamics of topical Ketoconazole and miconazole in human stratum corneum was studied by L.K.Pershing et al \textsuperscript{28}(1994). In direct study, evaluating whether differential drug uptake of topical 2%
miconazole and 2% ketoconazole from cream formulations into human stratum corneum correlated with differential pharmacological activity against Candida albicans was investigated in healthy human subjects. A single 24-h topical dose of 2% ketoconazole cream or 2% miconazole cream was applied unoccluded, at the same dose (2.6 mg of formulation per square centimeter of surface area), at four skin sites on both ventral forearms of six human subjects. At the end of the treatment, residual drug was removed with a tissue from all sites and the treated site was tape stripped 11 times, either 1, 4, 8, or 24 h later. The first tape disc was discarded. The remaining tape discs, 2 through 11, were combined and extracted for drug quantification by high-performance liquid chromatography and bioactivity against C. albicans growth in vitro. Topical 2% ketoconazole produced 14-, 10-, and 7-fold greater drug concentrations in stratum corneum than 2% miconazole at 1, 4, and 8 h after a single topical dose. Ketoconazole and miconazole concentrations in the stratum corneum were similar 24 h after drug removal. Tape disc extracts from 2% ketoconazole-treated skin sites demonstrated significantly greater bioactivity in the bioassay than 2% miconazole. The increased efficacy of 2% ketoconazole compared with that of 2% miconazole in vitro reflects their differential uptake into the stratum corneum and inherent pharmacological activity. Tape stripping the drug-treated site in conjunction with a bioassay is therefore a useful approach in the determination of bioavailability of topical antifungal agents.

Ahn B N et al (1995) designed and evaluated proliposomes containing propanolol. Developed proliposomes were characterized for surface morphology and flow ability, and following the conversion to liposomes upon hydration, size distribution, drug content and in vitro drug release of the reconstituted liposomes were examined. Multilamellar liposomes were reconstituted spontaneously from the proliposomes upon hydration. The mean diameter of the resultant liposomes was highly dependent on the PH-to-lecithin ratio, but less dependent on the lecithin-to-sorbitol ratio and sorbitol particle size (105-710 microns). Entrapment efficiency of PH in liposomes showed a maximum 10% at PH-to-lecithin ratio < 0.5 and a lecithin-to-sorbitol ratio > 0.1. Sustained release of propranolol from the proliposomes was achieved when the lecithin-to-PH ratio was > 2, and the lecithin-
to-sorbitol ratio was in the range examined (0.067-0.2). In conclusion, granular proliposomes of PH with good flowability and sustained release characteristics could be prepared by controlling the drug/lecithin/sorbitol ratio and sorbitol particle size. PH proliposomes can be potential candidates for the sustained drug delivery of propranolol when applied directly onto the mucosal membranes.

Synthesis and evaluations of newer antifungal agents with possible anticandida activity was done by Artico et al (1995). For the in vivo studies, Artico et al has proposed the following protocol using rabbit model. As per protocol, twelve male New Zealand white rabbits weighing 2.0±0.2kg were housed in separated cages, maintained in an aerated environment at 22 °C, lighted daily for 14 h (light period 5 a.m.-9 p.m.), and nourished with suitable fodder supplied by Ditta Morini (Modena, Italy), following National Institute of Health guidelines on care and use of laboratory animals. Before treatment, the hair was shorn from the backs of albino rabbits with electric clippers (Aesculap, Germany) and six areas of the skin ca. 3 cm2, each in two rows, were scarified by sandpaper type 60. These abraded portions of the skin were then infected with 0.3 mL of a suspension of C. albicans containing ca. 0.6 x 10^6 infective blastocells of the A170 strain; 48 h after the challenge, an inflammatory state ensued in each treated area of animals. The topical treatment with 0.4 mL of 1% lotion containing the test derivative was applied twice daily on 15 consecutive days beginning 48 h after C. albicans inoculation. The activity of test derivatives was assessed in comparison with a reference group treated with a 1% lotion of bifonazole and a control group treated with the vehicles only. During the treatment with the lotions, the evolutions of the lesions were evaluated daily (degree of inflammation, diameter of lesions). Evaluation of activity was performed on days 6, 10, and 18. Yeasts were removed from scales, transferred to Sabouraud agar, and incubated for cfu evaluation. Animals were considered to be cured when attempts to reisolate C. albicans after a 4 day incubation at 37 °C failed.

De Mane S et al (1996) encapsulated amphotericin B (AmB) into liposomes or binding of AmB to other lipid carriers, which results in a significant
reduction of toxicity of AmB and possible increase in therapeutic index. AmBisome, Amphocil (Amphotericin B Colloidal Dispersion) and Amphotericin B Lipid Complex (ABLC, Abelcet), these three formulations differ significantly in composition and pharmacokinetics. AmB serum levels after ABLC and Amphocil administration are low, but after AmB is much higher. Amphocil showing the highest and AmBisome the lowest rate. The optimal therapeutic dosages have not been established, but dosages as low as 1 mg/kg should be avoided in the initial treatment of fulminant fungal infections, since efficacy may be inferior to equal doses of conventional AmB.

Influence of vesicle preparation method on encapsulation of calcitonin in liposome was studied by Arien A et al 45(1997). Prepared liposomes encapsulated calcitonin by extrusion, sonication or from mixed micelles through the elimination of cholate by gel filtration. To understand the mode of calcitonin encapsulation in the vesicles, riboflavin was entrapped within the vesicles and taken as a simple model for the encapsulation of molecules in the aqueous phase. Interactions of calcitonin with the liposomal membranes were evaluated by studying the fixation of radiolabelled calcitonin to the outer surface of empty liposomes, and by preparing calcitonin-loaded LDL-like nanoparticles composed of phosphatidylcholine and cholesteryl oleate. Calcitonin entrapment in the vesicles depends largely on the vesicle preparation method. In this type of vesicle, calcitonin is exclusively embedded in the vesicle bilayer. When vesicles are prepared by extrusion or sonication calcitonin is found both in the aqueous and lipidic phases of the vesicles. Optimal calcitonin encapsulation was obtained when the liposomes were prepared by sonication.

De Logu et al 46(1997) studied the effects of in vitro activity of miconazole and ketoconazole in phospholipids formulations. Antifungal agents are often used in liposomal formulations in order to improve their pharmacological activity, but how vesicle inclusion can actually affect this is still not fully understood. The results obtained from evaluation of the in vitro activity against Candida albicans ATCC E10231 of miconazole and ketoconazole in various vesicular and non-
vesicular preparations, obtained from egg and soya phospholipids, using time–kill curves. In most cases inclusion of miconazole or ketoconazole in liposomes led to a delayed and decreased activity of the drugs, with detectable differences among the various phospholipid concentrations and different liposomal preparations (small unilamellar vesicle, liposomes, multilamellar aggregates and broken liposomal structures). The results obtained may be helpful in the study of new preparations of antifungal agents entrapped in liposomal structures.

Cortesi R et al. (1998) described the production and antiproliferative activity of liposomes containing the antitumour drug chromomycin. Liposomes were prepared by the reverse phase evaporation technique followed by extrusion through polycarbonate fillers; afterwards the vesicles were characterized in terms of dimensions, morphology and encapsulation efficacy. The analysis of the in vitro antiproliferative activity on cultured human leukemic K562 cells demonstrated that ionic and neutral liposomes containing chromomycin are 1.5 and 7-fold more effective respectively as compared to the free drug. Based on these results and taking into account the increased solubility of the drug in this system, liposomes could represent a promising drug delivery system for use in the experimental therapy using chromomycin.

Koskela RV et al. (1998) investigated the enhancement of percutaneous absorption of naproxen by phospholipids. During the investigation, it was found that presence of phospholipid decreases the skin penetration of naproxen from aqueous gels. The addition of 32% (m/m) ethanol or propylene glycol in the aqueous gel formulation with the presence of phospholipids apparently increases the percutaneous absorption of naproxen. The penetration enhancement effect of phospholipids with ethanol was, however more significant than that of phospholipids with propylene glycol. The result showed that more than 8% (w/v) ethanol is needed for the enhancing effect of phospholipids.

Interaction with P-glycoprotein and transport of erythromycin, midazolam and ketoconazole in Caco-2 cells were investigated by M. Takano et al. (1998). During the investigation, azole antifungal agents such as ketoconazole and
itraconazole reported to interact with cyclosporine A and to increase blood cyclosporine A concentrations (Campana et al., 1996). In addition, Floren et al (293) reported the marked increase in the bioavailability of another immunosuppressive agent tacrolimus, a substrate for both P-glycoprotein and CYP3A, after the co-administration of ketoconazole. The present study results showed that ketoconazole inhibited the P-glycoprotein-mediated transport of rhodamine 123 in Caco-2 cells, as reported in multidrug-resistant KB-V1 cells (Siegsmund et al., 1994). Therefore, the inhibition of drug excretion into the intestinal lumen via P-glycoprotein might play an important role in the increased absorption of cyclosporine A and tacrolimus. However, the directional transport of ketoconazole was not observed in Caco-2 cells. In addition, there was no effect of verapamil on the transport of ketoconazole. These results suggest that ketoconazole could inhibit P-glycoprotein mediated transport, but the drug itself may not be transported by P-glycoprotein.

Skin delivery of oestradiol from deformable and traditional liposomes and its mechanistic studies were carried out by Maghraby GM et al. (1999). Comparison of deformable vesicles and traditional liposomes as delivery systems for oestradiol to elucidate possible mechanisms of drug delivery through human skin was performed during the study. Lipid vesicles improved the skin delivery of oestradiol, which was compared with delivery from an aqueous control. Maximum flux (Jmax) was increased 14- to 17-fold by use of deformable vesicles and 8.2- to 9.8-fold by use of traditional liposomes. Deformable vesicles were thus superior to traditional liposomes. Drug release was negligible over the period during which skin flux was maximum. Vesicles increased drug uptake into the stratum corneum 23 to 29 fold. Relative flux values obtained from small and large vesicles were similar. The positive uptake suggested that lipid vesicles increased drug partitioning into the skin.

A clinical evaluation of a novel liposomal carrier for acyclovir (ACV) was performed by Horwitz et al. (1999). The evaluation proved the efficiency of 5 % ACV in a novel liposomal carrier (ethosome) in comparison with that of a
commercial 5% ACV cream (zovirax cream) and that of drug free vehicle in the treatment of recurrent herpes labialis in a armed, double blind, randomized clinical study and found the time to casting with the ethosomal acyclovir (1.6 days) significantly shorter than the time with the acyclovir cream (4.3 days) and the time with the drug free vehicle (4.8 days); in this arm, the shorter time to loss of crust for the ethosome (3.5 days), in comparison with the time for the cream (6.4 days) and the drug free vehicle (6.1 days), did not reach statistical significance. Horwitz et al suggested the improved efficiency of the novel liposomal preparation in comparison with zovirax cream in the treatment of recurrent herpes labialis.

Srinath P et al 51(2000) developed and performed pharmacodynamic evaluation of liposomal indomethacin. In the evaluation, Srinath P et al attempted to determine the factors influencing encapsulation of indomethacin in liposomes and to determine anti-inflammatory potential of liposomal indomethacin. The effects of method of preparation, lipid composition, charge, and cholesterol (CH) on encapsulation of indomethacin in liposomes were also investigated. With all the methods of preparation tried, the favorable lipid composition for high encapsulation of this drug was egg phosphatidyl choline:CH: stearlyamine (PC:CH:SA) at a 1:0.5:0.1 molar ratio. Inclusion of cholesterol did not affect the encapsulation efficiency of the drug in liposomes. The drug release profile from the liposomes was biphasic, and the highest percentage drug release was observed with large unilamellar vesicles (LUVs) (100 nm). Inclusion of stearylamine (PC: CH: SA 1:0.5:0.1) and phosphatidyl glycerol (PG) (PC: CH: PG 1:0.5:0.2) in the liposomes reduced the release of the drug in comparison to the neutral liposomes (PC:CH 1:1). Pharmacodynamic evaluation of the liposomes was performed by carrageenan-induced rat paw edema (acute) and adjuvant arthritis (chronic) models. The anti-inflammatory activity was increased from the first to fifth hour PC: CH: PG (1:0.5:0.2) and PC: CH: SA (1:0.5:0.1) liposomes showed the highest percentage inhibition of edema.

Toutou et al 12(2000) described a novel carrier for enhanced skin delivery, the ethosomal system that was composed of phosphohpid, ethanol and water. The
skin permeation of ethosomal components, ethanol and phospholipid, was demonstrated in diffusion cell experiments. Ethosomal systems were composed of soya phosphotidyl choline 2%, ethanol 30% and water were shown by electron microscopy to contain multilamellar vesicles. $^{31}$P NMR studies confirmed the bilayer configuration of the lipids. Calorimetry and fluorescence measurements suggested that vesicle bilayers are flexible, having a relatively low $T_m$, the average vesicle size as measured by dynamic light scattering was modulated by altering the ethosome composition. Experiments using fluorescent probes and ultracentrifugation showed that the ethosomes had a high entrapment capacity for molecules of various lipophilicity.

Dayan N et al 16(2000) compared skin delivery efficiency between ethosomes and liposomes containing trihexyphenidyl HCL (THP). As the THP concentration was increased from 0 to 3%, the size of vesicles found to be decreased from 154 to 90nm, which was most likely due to the surface activity of THP. When compared with standard liposomes, ethosomes had higher entrapment efficiency and a greater ability to deliver entrapped fluorescent probe to the deeper layers of skin. The flux of THP through nude mouse skin from THP ethosomes (0.21 µg/cm$^2$/h) was 87, 51 and 45 times higher than from liposomes, phosphate buffer and hydroethanolic solution respectively.

Touitou et al 52(2001) investigated the intracellular delivery by cationic ethosomes which do not contain any positively charged phospholipids. During the intracellular delivery into Swiss albino mice skin, the molecules with various physico-chemical characterization releases from ethosomes and penetrate across the skin membrane. Confocal laser scanning micrographs showed that ethosomes facilitated the penetration of all probes into the cells, as evident from the high intensity fluorescence. In comparison when incorporated in hydroethanolic solution or classic liposomes, almost no fluorescence was detected. It was also mentioned that fibroblast viability tests showed that the ethosomal earner is not toxic to the culture cells.
Satturwiar PM et al 53(2001) carried out the work on niosomal delivery of ketoconazole, antifungal drug. Ketoconazole was encapsulated in niosomes for topical application. Ketoconazole niosomes were prepared by thin film hydration technique using surfactant (tween 40 or 80), cholesterol and drug in five different ratios (by weight). The prepared niosomes were characterized for size, shape, entrapment efficiency and in-vitro drug release (by exhaustive dialysis). Niosomes were then formulated in FAPG base and tested for in-vitro antifungal activity using cup and plate method.

Therapeutic efficacy of liposomal gentamycin in clinically relevant rat models were performed by Schiffelers et al 54(2001). Study proved that sterically stabilized liposomes were able to localize selectively at sites of infection, potentially permitting targeted drug delivery. Up to now, the majority of studies investigating therapeutic efficacy of liposomes have been conducted in animals with an intact host defense infected with high antibiotic-susceptible bacteria. In the present study, the therapeutic efficacy of gentamycin encapsulated in sterically stabilized liposomes, alone or in combination with the free drug was studied in rats with intact host defense as well as leukopenic rats. Rats were inoculated with a high gentamycin-susceptible or low-gentamycin susceptible Klebsiella pneumoniae in the left lung, resulting in an acute unilateral pneumonia. Survival rates demonstrate the valuable therapeutic properties of the liposome-encapsulated drug in these clinically relevant animal models.

Aliasgar Shahiwala et al 10(2002) studied the topical application of niosomally entrapped nimesulide (NIM). Developed niosome was extensively characterized and evaluated for in-vitro performance followed by in-vivo evaluation in rats by carrageenan induced rat paw edema method. Preparation of niosomes was optimized for highest percent drug entrapment (PDE). The prepared niosomes were incorporated into 1 percent carbopol gel base and the system was evaluated for drug diffusion across human cadaver skin (HCS) using modified validated diffusion cell. The drug retention studies in niosomes were performed at refrigerated temperature (2°C- 8°C) and at room temperature (25°C±2°C) for the
period of 2 months. *In-vivo* performance of plain drug gel, niosomally-entrapped drug in carbopol gel base and marketed formulation were evaluated using acute rat paw edema method. Highest mean percentage edema inhibition (PEI) was observed for niosomal NIM gel after 24 hours i.e. 66.68 ± 5.19 % compared to plain drug gel i.e. 12.57 ± 1.78 % and marketed NIM formulation i.e. 20.49 percent ± 0.91 percent. This investigation conclusively demonstrated the prolongation of drug release and increase in amount of drug retention into the skin and permeation across the skin after niosomal encapsulation of NIM. A.Shahiwala et al also designed a protocol to carry out in vitro diffusion study. As per protocol, a vertical type of diffusion cell was designed and validated using benzoic acid disc method and was used to carry out studies. Gel containing 5mg of drug was applied on 2.00 sq.cm area of epidermal surface of HCS tied to one end of glass permeation tube. The volume of receptor compartment was kept to 20ml. The tube was lowered in the receptor compartment in such a way that the dermal surface was just flush to surface of permeation fluid (pH 7.4 PBS,20ml) maintained at 37.5° ±0.5 °C and stirred magnetically at 50rpm. Aliquots were withdrawn and replaced with the same volume of fresh buffer, at each sampling time points and analyzed for the drug content after suitable dilutions by spectrophotometric method.

Ellaithy H M et al 55 (2002) developed the cutina lipogel and gel microemulsion for topical administration of fluconazole. The study evaluated the influence of the vehicle on the release and permeation of fluconazole, a topical antifungal drug dissolved in Jojoba oil. In-vitro drug release in Sorensen's citrate buffer (pH 5.5) and permeation through the excised skin of hairless mice, using a modified Franz diffusion cell, were performed. The rheological behavior and the apparent viscosity values for different gel bases were measured before and after storage under freezing conditions at -4 °C and were taken as measures for stability of network structure. Candida albicans was used as a model fungus to evaluate the anti-fungal activity of the best formula achieved. The results of in vitro drug release and its percutaneous absorption showed that the highest values from gel microemulsion were assured. The rheological behavior of the prepared systems showed pseudo plastic (shear-thinning) flow indicating structural breakdown of the
existing intermolecular interactions between polymeric chains. The antifungal activity of fluconazole showed the widest zone of inhibition with gel microemulsion. The gel microemulsion is an excellent vehicle for fluconazole topical drug delivery.

A concised overview on lipid based antifungal agents were carried out by Sevtap Arikan (2002). As per the review, the development of lipid formulations of antifungal drugs has made a remarkable progress in the systemic antifungal arena. The lipid-based amphotericin B formulations; amphotericin B lipid complex (ABLC), amphotericin B colloidal dispersion (ABCD), and liposomal amphotericin B (L-AMB) have been in clinical use since the 1990s. They are significantly less nephrotoxic than the parent compound and can be safely used at higher doses. The primary cost of these formulations is significantly high and the extent of data related to their head-to-head comparison remains limited. The lipid formulation of nystatin, liposomal nystatin, is another lipid-based polyene under development. Available data concerning the in vitro activity, pharmacokinetic profile, in vivo efficacy, and safety of these formulations are summarized in this overview.

Alonso-Romanowski S et al (2003) evaluated diacetylenic liposomes as carrier for oral vaccines. In order to evaluate liposomes as vehicle for oral vaccines, the characterization and stability of polymerized and non-polymerized liposomes were examined by the author. Mixtures of 1, 2-bis (10, 12-tricosadiynoyl)-sn-glycero-3 phosphocholine (DC8, 9PC) with saturated 1, 2-dimiristoyl-sn-glycero-3-phosphocholine in molar ratio 1:1 were used. Saturated and non-saturated lipids were combined to give a chemically modified membrane by UV polymerization derived from DC8, 9PC. Characterization was carried out by electronic microscopy, differential scanning calorimetry (DSC) and by hydrophobicity factor (HF). The stability towards the digestive tract (including saliva): acidic solutions, bile and pancreatic are compared to buffer pH 7.4, measuring the release of Glucose-6-phosphate or bovine plasma albumin entrapment. The polymerized liposomes showed further augmentation of the HF
and the size. DSC showed phase separation and lower Tt if compared to data obtained for DC8, 9PC. The HF, as main factor is discussed in relation to in vitro stability, suggesting that polymerized and non-polymerized liposomes would serve effectively as an oral delivery vehicle.

Liposomes have been extensively studied and suggested as a vehicle for topical drug delivery systems. However, the mechanism by which liposomes deliver drugs into intact skin is not fully understood. In the present study, D.D. Verma et al. 58(2003) have tried to understand the mechanism of transport of hydrophilic drugs into the skin using liposomes. The effect of separation of the non-entrapped, hydrophilic fluorescent compound, carboxyfluorescein (CF), from liposomally entrapped CF was investigated by measuring the penetration of CF across human skin under non-occlusive conditions in vitro using Franz diffusion cells. The fluorescent dye, CF, was incorporated into the liposomes and applied onto the skin. After a 6 and 12 h incubation period, the amount of CF in the epidermal membrane and the full thickness skin was determined by fluorescence spectroscopy or by confocal laser scanning microscopy (CLSM). The liposomal formulation containing CF both inside and outside the vesicles showed statistically enhanced penetration of CF into the human stratum corneum (SC) as compared to the formulations containing CF only outside of the liposomes and CF in Tris buffer. The CLSM results revealed that the formulation in which CF was present outside the liposomes showed bright fluorescence intensity in the SC and very weak fluorescence in the viable epidermis. However, the CF in Tris buffer failed to show any fluorescence in the viable epidermis. The results indicated that phospholipid vesicles not only carry the entrapped hydrophilic substance, but also the non-entrapped hydrophilic substance into the SC and possibly into the deeper layers of the skin.

Preparation and comparative analysis of two podophyllotoxin liposomes were carried out Hou GR et al. 59(2003). Hou GR et al prepared two different liposome gels containing podophyllotoxin (PPT) by reverse phase evaporation technique, and optical and electron microscopy were performed respectively for
examining the appearances of the 2 liposomes and the diameter of the particles. Under optical microscope, PPT-DPPC liposome was observed to be composed of spherical particles with diameter range of 2.05 to 0.825 micron (average 1.45 micron). PPT-DPPC liposome appeared multivesicular under electron microscope, with the embedding ratio of 73.8%, and 79.1% for PPT-soya lecithin liposome (n=3). PPT-DPPC liposome had a embedding ratio of 65.2%, 58.8% and 56.4% after preserved 1, 3, 6 months respectively. As for PPT-soya lecithin liposome, the embedding ratios were 70.3%, 60.4%, and 51.3% respectively. The PPT in the liposomes prepared by reverse phase evaporation technique is evenly distributed throughout the gel. The preparation is relatively simple with high embedding ratio.

Lodzki M et al (2003) developed a transdermal delivery system for cannabidiol (CBD), a new candidate for treatment of rheumatic disease. Objective of the study was to design the ethosomal system for CBD. CBD ethosomes were characterized by transmission electron microscopy, con local laser scanning microscopy and differential scanning calorimetry. Result indicated that CBD and phosphatidylcholine form an eutectic mixture. In-vivo application of ethosomal CBD to nude mice produced a significant accumulation of the drug in the skin and in the underlying muscle. Upon transdermal application of the ethosomal system to the abdomen of mice for 72 h, steady-state levels were reached at about 24 h and lasted at least until to end of the experiment, at 72 h. In conclusion, ethosomes CBD's skin permeation and it's accumulation in a depot at level that demonstrate the potential of transdermal CBD to be used as an anti-inflammatory treatment.

Niosomal acetazolamide was developed for topical application and was evaluated extensively by Aggarwal D et al (2004). As per findings bioavailability of acetazolamide gets enhanced by the topical route and to improve the corneal permeability of the drug, niosomes of acetazolamide were prepared (employing span 60 and cholesterol) by different methods. It was found that the reverse-phase evaporation method (REV) gave the maximum drug entrapment efficiency (43.75%) as compared with ether injection (39.62%) and film hydration (31.43%) techniques. Drug entrapment efficiency varied with the charge and the percent
entrapment efficiency for the REV method was 43.75, 51.23 and 36.26% for neutral, positively charged and negatively charged niosomes, respectively. Corneal permeability studies, however, showed that the percent permeation and the apparent permeability coefficient for the charged niosomes were less than for the neutral ones. The developed niosomal formulations of acetazolamide showed a comparable physiological effect (33% reduction of IOP in REV and 37% reduction in dorzolamide) with duration of up to 6 h (the duration being 3 h for dorzolamide). Results of the study indicate physiologically active topical niosomal formulation of acetazolamide relative in efficiency to the newer local carbonic anhydrase inhibitor, dorzolamide.

Dendrisomes are vesicular structures derived from cationic lipidic dendrons. The behavior of this novel synthetic lipidic cationic lysine-based dendron (partial dendrimer) in aqueous media and its ability, with and without cholesterol, to self-assemble into higher order structures was studied by Al Jamal K. T et al (2004) to gain an understanding of these structures as potential drug carriers. The dendron was prepared by solid-phase peptide synthesis. A reverse-phase evaporation (REV) technique was used to prepare cationic vesicular aggregates of the dendron with different molar ratios of cholesterol. The size and zeta potential of these supramolecular aggregates or "dendrisomes" was determined by photon correlation spectroscopy (PCS). Dendrisome morphology and thermotropic properties were studied by transmission electron microscopy (TEM) and differential scanning calorimetry (DSC). Radiolabeled penicillin G was used as a model of a negatively charged water-soluble compound to investigate the encapsulation efficiency of the dendrisomes. In vitro release of the drug was determined using as a comparator a REV liposome formulation. Dendrisomes of all compositions have higher encapsulation efficiencies and slower release rates compared to the comparator. Cholesterol was found both to increase the size of the aggregates from around 310 to 560 nm and to increase shape irregularities, but did not change the positive zeta potential, in the order of +50 mV, of the dendrisomes. Cholesterol decreases penicillin G entrapment efficiency but increases solute leakage at 25 degrees C.
Albertini B et al \(^{63}\) (2004) investigated the effect of aerosil \(^{®}\) on the properties of lipid controlled microparticles. As part of the investigation, theophylline-loaded microparticles of a lipid carrier, Precirol \(^{®}\) ATO 5, were prepared by the ultrasonic spray-congealing method. The goal of the work was to investigate the effect of different concentrations and kind of colloidal silicon dioxide (Aerosil \(^{®}\) 90, 200 and 300) on the microparticle characteristics (particle size, drug loading, morphology and kinetics of release). The results showed that the introduction of Aerosil® improved the drug distribution in the different particle sizes and that the mean diameter of the microparticles decreased with the viscosity of the suspension to be nebulized, especially that with Aerosil® 300. Whatever the microparticles formulation is, SEM and image analysis did not reveal any remarkable difference of the microparticle shape and surface area, suggesting that other parameters could influence the dissolution behavior. Actually, the dissolution profiles of all the formulations appeared to be closely related to the physico-chemical properties of Aerosil®, especially to its gelation properties, which are a function of its specific surface area. In particular, microparticles having high concentration of Aerosil® 200 and 300 approached a zero order release kinetics, while Aerosil® 90 Theophylline-loaded microparticles of a lipid carrier, Precirol® ATO 5, were prepared by the ultrasonic spray-congealing method. The goal of the work was to investigate the effect of different concentrations and kind of colloidal silicon dioxide (Aerosil® 90, 200 and 300) on the microparticle characteristics (particle size, drug loading, morphology and kinetics of release). The results showed that the introduction of Aerosil® improved the drug distribution in the different particle sizes and that the mean diameter of the microparticles decreased with the viscosity of the suspension to be nebulized, especially that with Aerosil® 300. Whatever the microparticles formulation is, SEM and image analysis did not reveal any remarkable difference of the microparticle shape and surface area, suggesting that other parameters could influence the dissolution behavior. Actually, the dissolution profiles of all the formulations appeared to be closely related to the physico-chemical properties of Aerosil®, especially to its gelation properties.
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Tamoxifen encapsulated in liposomes for topical application was developed by Bhatia A et al (2004). Prepared liposomes were characterized for various physical properties. Characterization was performed using Malvern Mastersizer, optical microscope and micromeritic attributes were also done. Stability of liposomes was assessed for a period of 5 weeks in terms of their drug holding capacity. Liposomal formulations were also evaluated for in-vitro skin permeation, using mice skin. The results thus obtained were compared with that of aqueous solution and carbopol gel, containing tamoxifen in equal amount. Obtained result showed that amongst different storage conditions, the liposomes stored at 2 to 8°C were found to be most stable with only 5% drug loss over the storage period of 5 weeks. Liposomal formulations showed significantly higher skin permeation of tamoxifen (flux value 63.67 µg/cm/hr for liposomal suspension and 59.87 µg/cm/hr for liposomal gel) than solution (21.65 µg/cm/hr) and carbopol gel (24.55 µg/cm/hr). Conclusion was given as the phospholipids enriched amphiphillic nature of the vesicles can be responsible for modifying the properties of keratinized layer. The findings supported the improved and localized drug action on the skin thus providing a better option to deal with skin cited problems.

Souto E B et al (2004) carried out investigations on development of a controlled release formulation based on solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for topical delivery of clotrimazole. SLN and NLC are colloidal carrier systems providing controlled release profiles for many substances. Clotrimazole-loaded SLN and NLC were prepared by the hot high-pressure homogenization technique in order to evaluate the physical stability of these particles, as well as the entrapment efficiency of this lipophilic drug and its in vitro release profile. The particle size was analyzed by PCS and LD showing that the particles remained in their colloidal state during 3 months of storage at 4, 20 and 40 °C. For all tested formulations the entrapment efficiency was higher than
50%. The obtained results also demonstrate the use of these lipid nanoparticles as modified release formulations for lipophilic drugs over a period of 10 h.

J.M.Aiache et al (2004) performed a comparative study of miconazole based formulations against candida species. As per the results of the study, in all subjects the tablets gave higher and more prolonged salivary miconazole concentrations than the gel. Thus salivary miconazole AUC(0,24 h) was 37.2 times greater for the 100 mg tablet (90% confidence interval [CI] 22.9, 60.5) and 18.9 times greater for the 50 mg tablet (CI 11.7, 30.6) compared with the gel. Similarly, \( C_{\text{max}} \) was 17.2 times greater (CI 11.8, 25.2) and 7.8 times greater (CI 5.3, 11.4) for the 100 mg tablet and 50 mg tablet, respectively. Comparison of the 100 mg and 50 mg tablets gave ratios of 2.2 and 2.0 for \( C_{\text{max}} \) and AUC (0, 24 h), respectively (CI 1.5, 3.2 and 1.2, 3.2). The mean time that salivary miconazole concentrations were above 0.4 \( \mu \text{g ml}^{-1} \) (the concentration reached 3 h after application of the oral gel according to published data) or above 1.0 \( \mu \text{g ml}^{-1} \) (the MIC of some Candida species) was greater for both bioadhesive tablets than for the oral gel (10–14 h vs 1.5 h and 7 h vs 0.6 h). Only 19 plasma samples from eight subjects had concentrations of miconazole above 0.4 \( \mu \text{g ml}^{-1} \). Ten of these were taken from five subjects after administration of the gel and nine from three subjects after administration of the tablets.

Touitou E et al (2004) investigated the dermal and intracellular delivery of bacitracin, a model poly peptide antibiotic from ethosomes. Bacitracin and fluorescently label bacitracin (FITC - Bac) ethosomes were characterized for shape, lamellarity, fluidity, size distribution and entrapment capacity by scanning electron microscopy (SEM), transmission electron microscopy (TEM), differential scanning calorimetry (DSC) and ultracentrifugation, respectively. These studies demonstrated that the antibiotic peptide was delivered into deep skin layers through intercorneocyte lipid domain of stratum corneum. Occlusion had no effect on the permeation profile of the drug from ethosomes in in-vivo experiments.
Jain S et al \(^{20}(2004)\) encapsulated the zidovudine, in recently developed novel vesicular carrier ethosomes, for its enhanced transdermal delivery as an anti-HIV agent. The results obtained from ethosomal zidovudine were compared with other prepared formulations. Ethosomes of zidovudine were prepared and characterized \textit{in-vitro} and \textit{in-vivo}. The effect of different variables on skin permeation of ziduvudine was studied using locally fabricated keshryn-chien type of diffusion cell. To confirm better skin permeability of ethosomes fluorescence microscopy using rhodamine-123 as fluorescence probe was performed. The optimized ethosomal formulation showed transdermal flux \(78.5 \pm 2.5 \, \mu g/cm^2/hr\) across the rat skin as compared to \(5.2 \pm 0.5\) for control hydroethanolic solution of drug, and \(7.2 \pm 0.6 \, \mu g/cm^2/hr\) for ethanolic drug solution. It was included that these lamellar stacks disrupted the organization of skin bilayers leading to increased skin permeability and this was further confirmed by fluorescence microscopy. Jain S et al concluded that ethosomes can increase the transdermal flux, proving the better release pattern of ziduvudine.

Magdy I M et al \(^{67}(2004)\) tried to optimize the chlorphension (CHL) containing emulgel formulation using 2 types of gelling agents hydroxypropylmethyl cellulose (HPMC) and Carbopol 934. The prepared emulgels were evaluated for their physical appearance, rheological behavior, drug release, antifungal activity, and stability. Commercially available CHL topical powder was used for comparison. All the prepared emulgels showed acceptable physical properties concerning color, homogeneity, consistency, spreadability, and pH value. They also exhibited higher drug release and antifungal activity than the CHL powder. The drug release from all the emulgels was found to follow diffusion-controlled mechanism. Stability studies showed that the physical appearance, rheological properties, drug release, and antifungal activity in all the prepared emulgels remained unchanged upon storage for 3 months. It was suggested that the CHL emulgel formulation prepared with HPMC with the oil phase concentration in its low level and emulsifying agent concentration in its high level was the formula of choice since it showed the highest drug release and antifungal activity.
Abeer A W et al \(^{68}(2004)\) formulated cetrizine as topical phosphatidylcholine-hydrogenated liposomes; the developed formulation was evaluated for peripheral antihistaminic activity and systemic absorption in rabbit model. Prepared cetrizine vesicles were small unilamellar vesicles (SUV) and multilamellar vesicles (MLV) using L-a-phosphatidylcholine hydrogenated (HPC), and into Glaxal Base (GB) as the control. In a randomized, crossover study conducted in rabbit model, each formulation, containing 10 mg of cetrizine, was applied to the depilated backs of 6 rabbits (3.08 ± 0.05 kg). Histamine-induced wheal tests and blood sampling were performed before cetrizine application and at designated times for up to 24 hours afterwards. Compared with baseline, histamine-induced wheal formation was suppressed by cetrizine in SUV only at 24 hours, in MLV from 0.5 to 24 hours, and in GB from 0.5 to 8 hours \((P = .05)\). Wheal suppression by cetrizine in SUV at 24 hours (91.7% ± 5.2%) and in MLV from 1 to 24 hours (93.8% ± 2.2% to 76.2% ± 6.5%) was greater than in GB (36.5% ± 7.4% to 60.6% ± 14.2%) from 1 to 24 hours \((P =-.05)\). Faster onset, as well as greater and more persistent suppression was obtained from cetrizine in MLV. Plasma cetirizine concentrations from MLV (area under the curve [AUC] of 221.2 t 42.3 mg.hr/mL) were lower than from GB (AUC of 248.3 ± 34.6 mg.hr/mL). In this model, cetirizine from MLV had excellent topical antihistamine activity, while systemic exposure was reduced, compared with cetirizine from GB.

In vitro skin penetration study of dazmegrel with a bioelastic matrix system was performed by Barbara W.K et al \(^{69}(2004)\). For the in vitro skin penetration study a full-thickness greyhound skin used, which was obtained from dogs that had recently (<1 h) been utilized as part of other ongoing research projects. Hair on the dorsolateral aspect of the trunk of the greyhound dogs was clipped; the skin was washed, and depilatory cream was used to remove the remaining stubble. The fat was removed from the dermal side and the skin was placed horizontally between a donar (epidermal) and receptor (dermal) chamber of the percutaneous penetration cells (Kemppainen, 1993). The receptor fluid was continually stirred by a motor driven, Teflon-coated magnetic stir bar. The epidermal surface was exposed to room air, had a surface area of 1.9cm\(^2\), and the volume of the receptor chamber...
was 2 ml. The receptor fluid was tissue culture media, (RPMI media, GIBCO BRL, Grand Island, NY, USA) supplemented with sodium bicarbonate, HEPES buffer, and ciprofloxacin antibiotic (Miles, West Haven, CT, USA), and had a pH of 7.4. The solubility of dazmegrel in the receptor fluid was 0.143 mg/ml. It has been shown that there is good correlation between in vitro and in vivo skin absorption of lipophilic compounds when skin penetration is calculated by summing the penetrant in the dermis and receptor fluid.

Topical application of antigen and adjuvant directly on intact skin, termed as Topical Immunization (TI) or Transcutaneous Immunization (TCI), which is a novel and emerging method of vaccine delivery because of its safety and convenience. Gupta et al. (2004) has developed a lipid based vesicular formulation which can be used for topical immunization. This research work by Gupta et al. covers the brief immunology of skin and a mechanistic insight into delivery concepts of topical immunization with an emphasis on vesicular systems. Skin being a potentially rich site for immunization, Immune response elicited by TI depends upon structure and composition of the skin of target species. TI induces potent, functional immune responses vis-à-vis offers significant practical advantages for vaccine delivery. Various routes of carrier entry into the skin include the intercellular pathway, the transcorneocyte pathway and the trans-appendageal pathway. Among various approaches for topical immunization, namely physical, chemical and vesicular, latter is gaining wide attention. Vesicular carriers, i.e. liposome, niosome, transfersomes and virosome, elicit immune response by different mechanisms. Some lipids directly lower the skin permeability barrier, which resides primarily in the stratum corneum. Hence, specially designed lipid vesicles, used as topical delivery system, are attracting increasing attention and can be used for TI.

Sang-Chul S et al. (2005) developed the new gel formulations containing tretinoin which showed enhanced transdermal delivery. The tretinoin gel formulation was prepared using bioadhesive carbopol gels. The release characteristics of drug from the carbopol gel were studied according to
temperature, receptor medium and drug concentrate. For the enhancement of its percutaneous absorption, some kind of penetration enhancer was used. As the concentration of drug increased, the release of drug form the gel increased, showing concentration dependency. The increase of temperature showed the increased drug release, depending upon activation energy of permeation. Among the enhancers used such as the glycols and the non-ionic surfactants, polyoxyethylene-2-oleyl either showed the best enhancing effect. The carbopol gels of tretinoin containing enhancers could be developed for the enhanced transdermal delivery of drug.

Lecithin based organogels are potential phospholipids structures for improved transdermal drug delivery. The purpose of the study by Kikwai L et al.\textsuperscript{2} (2005) was to develop and evaluate topical lecithin organogel formulations of Spantide II, a neurokinin-1 receptor (NK-1R) antagonist, for the treatment of inflammatory skin disorders. Spantide II lotion and gel was formulated with and without n-methyl-2-pyrrolidone (NMP) as a penetration enhancer. The release of Spantide II from gels was evaluated using microporous polyethylene and polypropylene membranes in a Franz Diffusion cell setup. In vitro percutaneous absorption of Spantide II from lotion and gel formulations was evaluated using the above setup by replacing the membranes with hairless rat skin. Among different gels studied, PF127 gel showed highest (70-fold) release of Spantide II compared with hydroxypropyl methylcellulose (HPMC) and hydroxypropyl cellulose (HPC) gels. Lotion and gel formulations with or without NMP showed no detectable levels of Spantide II in the receiver compartment of the Franz diffusion cell until 24 hours. However, Spantide II showed significant retention in epidermis and dermis from lotion and gel formulations at 24 hours. In conclusion, Spantide II was stable as a topical formulation and delivered to target skin tissue (epidermis and dermis) for the treatment of ACD.

Lopez-Pinto JM et al.\textsuperscript{23} (2005) studied the effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. All the systems were characterized for shape, lamellarity, particle size and entrapment efficiency percentage (EE), by
transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM), laser diffraction and ultracentrifugation or dialysis methods, respectively. CLSM studies showed that ethosomal systems were much more efficient at delivery the fluorescent substance into the skin in terms of quantity and depth, than either liposomes or hydro-alcoholic solutions.

Erythromycin ethosomal systems were developed by Godin et al.\textsuperscript{21}(2005). Physicochemical characterization and their antibacterial efficiency was evaluated of developed ethosomes were carried out by the Godin et al. TEM, CLSM, DLS, DSC and ultracentrifugation tests indicate that erythromycin ethosomes are soft vesicles encapsulating 78.6\% erythromycin. Ethosomes are efficient carriers for erythromycin delivery to bacteria localized within the deep skin strata for eradication of staphylococcal infection. Developed ethosomes showed enhanced antibacterial efficacy.

Ammonium glycyrrhizinate is a useful drug for the treatment of various inflammatory-based skin diseases. Drug was encapsulated in ethosomal vesicles for the enhanced skin penetration. In vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers proved the improved performance of ethosomal formulations consisting of ammonium glycyrrhizinate. Esposito E et al.\textsuperscript{72}(2005) has evaluated various ethosomal suspensions made up of water, phospholipids and ethanol at various concentrations for their potential application in dermal administration of ammonium glycyrrhizinate, The percutaneous permeation of ammonium glycyrrhizinate ethosomes was evaluated in vitro through human stratum corneum and epidermis membranes by using Franz's cells and compared with the permeation profiles of drug solutions either in water or in a water-ethanol mixture. Reflectance spectrophotometry was used as a non-invasive technique to evaluate the earner toxicity, the drug permeation and the anti-inflammatory activity of ammonium glycyrrhizinate in a model of skin erythema in vivo on human volunteers. Ethosomal suspensions had mean sizes ranging from 350 run to 100 nm as a function of ethanol and lecithin quantities, i.e., high amounts of ethanol and a low
lecithin concentration provided ethosome suspensions with a mean size of approximately 100 nm and a narrow size distribution. In vitro and in vivo experiments were earned out by using an ethosome formulation made up of ethanol 45% (v/v) and lecithin 2% (w/v). The ethosome suspension showed very good skin tolerability in human volunteers, also when applied for a long period (48 hr). Ethosomes were able to significantly enhance the anti-inflammatory activity of ammonium glycyrrhizinate compared to the ethanolic or aqueous solutions of this drug.

Testosterone ethosomes for enhanced transdermal delivery was developed by Ainbinder D et al (2005). The ethosomal formulation was characterized by transmission electron microscopy and dynamic light scattering for structure and size distribution. Ultracentrifugation method was used for determining entrapment capacity. To evaluate the feasibility of this delivery system to enhance testosterone permeation through the skin, first the systemic absorption in rats was compared with a currently used gel (AndroGel). Further, theoretical estimation of testosterone blood concentration following ethosomal application in men was made. For this purpose, in vitro permeation experiments through human skin were performed to establish testosterone skin permeation values. In the design of these experiments, testosterone solubility in various solutions was measured and the effect of the receiver medium on the skin barrier function was assessed by confocal laser scanning microscopy. Theoretical estimation shows that testosterone human plasma concentration value in the upper part of the physiological range could be achieved by application of the ethosomal formulation on an area of 40 cm. This area is about 10 times smaller than required with current non-patch formulations. It shows that the ethosomal formulation could enhance testosterone systemic absorption and also be used for designing new products that could solve the weaknesses of the current testosterone replacement therapies.

Zeljka P et al (2005) developed a bioadhesive liposomal gel for local therapy of vaginitis. Physical characterization and in vitro evaluation of the developed liposomal formulation was carried out to prove its efficiency for the
treatment of vaginitis caused by fungi. Liposomes containing two commonly applied drugs in the treatment of vaginal infections, namely clotrimazole and metronidazole, were prepared by the proliposome and the polyol dilution methods. Both types of liposomes were characterised and compared for particle size, polydispersity, entrapment efficiency, and tested for in vitro stability in media that mimic human vaginal conditions (buffer, pH 4.5, and vaginal fluid simulant). To achieve application viscosity and to further improve their stability, liposomes containing drugs were incorporated in a bioadhesive gel made of Carbopol 974P NF resin. In vitro release studies have demonstrated that even after 24 h of incubation in vaginal fluid stimulant (at 37 °C) more than 30% of the originally trapped clotrimazole (or 50% of metronidazole) was still retained in the gel. Storage stability studies have proved the ability of Carbopol 974P NF gel to preserve original size distributions of incorporated liposomes. All the performed experiments confirm the applicability of bioadhesive liposome gels as a novel delivery system for local therapy of vaginal infections.

There is considerable interest in the skin as a site of drug application both for local and systemic effect. However, the skin, in particular the stratum corneum, poses a formidable barrier to drug penetration thereby limiting topical and transdermal bioavailability. Skin penetration enhancement techniques have been developed to improve bioavailability and increase the range of drugs for which topical and transdermal delivery is a viable option. This review by Heather AE et al. (2005) describes enhancement techniques based on drug/vehicle optimization such as drug selection, prodrugs and ion-pairs, supersaturated drug solutions, eutectic systems, complexation, liposomes, vesicles and particles. Enhancement via modification of the stratum corneum by hydration, chemical enhancers acting on the structure of the stratum corneum lipids and keratin, partitioning and solubility effects are also discussed. The mechanism of action of penetration enhancers and retarders and their potential for clinical application is thoroughly discussed in the review.
Jain S et al. (2006) in their research work for identifying best vesicular approaches for drug delivery across the skin, has discussed the significance of differential scanning colorimetry (DSC) technique. Stratum corneum (SC) of skin is mainly comprised of lipids, protein and low molecular weight water-soluble components. Changes in these skin micro constituents can be understood by instrumental methods like (DSC) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The former provides information about changes in thermotropic behavior of SC lipids and proteins, whereas the latter provides data about alterations at molecular and conformational level. Most of the DSC thermograms of intact mammalian SC show two reversible and two irreversible transitions in the temperature range of 25-125 degrees C. The reversible endotherms are ascribed to lipid melting transitions, whereas the irreversible endotherms are ascribed to protein denaturation. Similarly, the FTIR spectral bands of SC occurring between 2920-2850 cm\(^{-1}\) and between 1650-1550 cm\(^{-1}\) have been suggested to arise from lipid and protein molecular vibrations, respectively. Treatment of skin with solvents or permeation enhancers alters the composition of lipids or their molecular arrangement in the skin microenvironment, which leads to changes in permeability of drug molecules. Furthermore, inhibition of lipid synthesis in epidermis with concomitant decrease in enthalpy of lipid endothermic transitions and reduction in height and area of asymmetric and symmetric C-H stretching peaks have been found to be directly correlated with enhanced permeation of drugs. In addition, method of skin preparation, type of skin, types of enhancer etc. also influence both the nature and intensity of responses recorded in spectrographs and thermograms. Therefore, the modification in spectrographs and thermograms of skin samples treated with various enhancers, vehicles etc. are expected to provide better insight into their mechanism of action on the skin. This review article shall critically evaluate the thermotropic and infrared spectroscopic data of SC/epidermis after various treatments.
Luigi Mondello et al. (2006) have evaluated the in vivo antifungal activity of bergamot essential oil by-product, named ‘Peratoner’. It was evaluated through applications to male Wistar rats’ back skin, previously infected with Candida albicans, following the treatment samples were taken to evaluate the fungal load and punch biopsies were carried out for morphological studies. In infected rats without Peratoner treatment, skin detachment with infiltrating cells was observed. The presence of C. albicans cells was evident on the surface strata of the epidermis, which was detached from the basal cells. After 24 h, in the case of Peratoner treatment, the epidermic strata were still few in number, while the infiltrating elements in the dermis were fewer in quantity and a small cluster of C. albicans cells, above the stratum corneous, was also visible. After 48 h of treatment, the skin revealed proliferation of the strata, while in the dermis infiltrating cells were still evident. Following this period and up to a week after treatment, a full recovery of the cutaneous structure was observed.

In vitro cutaneous delivery of niosomal vesicles incorporated with tretinoin III was evaluated by Manconi M et al. (2006). Influence of drug thermodynamic activity and niosomal characteristics on the transdermal delivery of tretinoin (TRA) was also evaluated during the study. Positively and negatively charged vesicular formulations were prepared using either stearylamine or dicetylphosphate as a charge inducer. Niosomes made with polyoxyethylene (4) lauryl ether and liposomes made with soy phosphatidylcholine were also prepared and studied. The effect of the vesicular incorporation of tretinoin on its (trans) dermal delivery through the newborn pig skin was investigated in vitro using Franz cells, in comparison with a commercial formulation of the drug (RetinA). The amount of tretinoin delivered through and accumulated in the several skin layers was detected by HPLC. Overall, obtained results showed that tretinoin cutaneous delivery is strongly affected by vesicle composition and thermodynamic activity of the drug. In particular, small, negatively charged niosomal formulations, which are saturated with tretinoin, have shown to give higher cutaneous drug retention than both liposomes and commercial formulation.
Vesicular delivery systems like liposomes, niosomes, and ethosomes are preferred for the enhanced skin delivery of various drug molecules. In their research, Mustafa M A et al \(^{78}\) (2006) tried to find out the mechanism behind the enhanced skin delivery by deformable liposomes and ethosomes. Despite intensive research, the mechanisms by which vesicular systems deliver drugs into intact skin are not yet fully understood. In this current study, possible mechanisms, by which deformable liposomes and ethosomes improve skin delivery of ketotifen under non-occlusive conditions, were investigated. In vitro permeation and skin deposition behavior of deformable liposomes and ethosomes, having ketotifen both inside and outside the vesicles (no separation of free ketotifen), having ketotifen only inside the vesicles (free ketotifen separated) and having ketotifen only outside the vesicles (ketotifen solution added to empty vesicles), was studied using rabbit pinna skin. Results suggested that both the penetration enhancing effect and the intact vesicle permeation into the stratum corneum might play a role in improving skin delivery of drugs by deformable liposomes, under non-occlusive conditions, and that the penetration enhancing effect was of greater importance in case of ketotifen. Regarding ethosomes, results indicated that ketotifen should be incorporated in ethosomal vesicles for optimum skin delivery. Ethosomes were not able to improve skin delivery of non-entrapped ketotifen.

Enhanced dermal and transdermal delivery of methotrexate (MTX) using ethosomal carrier system was studied by Vaibhav D et al \(^{79}\) (2006). MTX loaded ethosomes were studied for vesicular size, shape and surface morphology, entrapment efficiency, stability, in vitro human cadaver skin permeation (using modified Franz diffusion cell) and MTX deposition. Confocal laser scanning microscopy (CLSM) was performed to study the mechanism and depth of MTX loaded ethosomes. Fluidity of ethosomal carriers was further assessed using DSC. An enhanced transdermal flux (57.2±4.34 mg/cm²/h) of MTX was observed that was 3-4 fold higher than conventional liposomes and plain drug solution (P<0.05). Also, a greater skin drug deposition was observed in case of MTX loaded ethosomes as compared to other formulations. These results could be ascended to nanometric size, enhanced fluidity and high entrapment efficiency of ethosomal
carrier. CLSM data further confirmed the better penetration of ethosomal carriers to deeper skin layers (200 mm). Our results of the present study demonstrated the feasibility of ethosomal carriers for dermal and transdermal delivery of MTX, which provides better transdermal flux, higher entrapment efficiency, and possesses the ability of a self-penetration enhancer as compared to conventional liposomes.

Pallavi V et al (2006) developed and evaluated the topical formulation containing solid lipid nanoparticles (SLN) of vitamin A. The nanoparticulate dispersion and its gels were evaluated for various parameters such as particle size, in vitro drug release, in vitro penetration, in vivo skin hydration, and skin irritation. In vitro release profile of vitamin A palmitate from nanoparticulate dispersion and its gel showed prolonged drug release up to 24 hours, which could be owing to embedment of drug in the solid lipid core. In vitro penetration studies showed almost 2 times higher drug concentration in the skin with lipid nanoparticle-enriched gel as compared with conventional gel, thus indicating better localization of the drug in the skin. In vivo skin hydration studies in albino rats revealed increase in the thickness of the stratum corneum with improved skin hydration. The developed formulation was nonirritant to the skin with no erythema or edema and had primary irritation index of 0.00. From the study, it can be concluded that SLN represents a promising participate carrier having controlled drug release, improved skin hydration, and potential to localize the drug in the skin with no skin irritation.

Liposomes are tiny spheres ranging in diameters from 50 nm to several microns in size range. These nano or submicron sized liposomes can be considered as one of the best carriers for the drug delivery. The scope of this mini review by Jia-You Fang (2006) is to introduce the concept of liposomes and to describe some aspects and mechanisms of stimulating topical and injectable products with liposomes. Two examples discussed in this article are topical delivery across skin and injectable formulations for anticancer drugs. Classic liposomes are of little value as carriers for drug delivery via the skin because they do not penetrate it.
deeply. Only specially designed liposomes have been shown to be capable of achieving enhanced delivery. The incorporation of additives, such as anionic surfactants and ethanol, can fluidize the phospholipid bilayers, thus increasing the depths to which liposomes can penetrate the intercellular pathways of the skin. Also, liposomes that have been conjugated with PEG or antibodies can increase the residence time of anticancer drugs in the circulation and enhance drug accumulation in tumors.

Cortesi R et al \(^{82}\)(2007) described the production and characterization of liposomes and micellar dispersions for benzoheterocyclic derivatives of distamycin (DD). Cortesi R et al tried to design all the formulations to increase the solubility of DD in an aqueous environment and to reduce the possible toxicity problems related to the administration of these drugs. For instance, liposomes were prepared by reverse phase evaporation technique followed by extrusion through polycarbonate filters, then characterized in terms of dimensions, morphology, and encapsulation efficacy. The analysis of their in vitro antiproliferative activity on cultured human and mouse leukemic cells demonstrated that liposomes and micellar dispersions containing DD exert quite different effects. These effects were compared with those shown by the free drug depending on type of drug and also cell line used.

E.R. Bendas et al \(^{83}\)(2007) developed the ethosomal formulations of sulbutamol sulfate for enhanced transdermal delivery. The main objective of the work was to compare the transdermal delivery of salbutamol sulfate (SS), a hydrophilic drug used as a bronchodilator, from ethosomes and classic liposomes containing different cholesterol and dicetylphosphate concentrations. All the systems were characterized for shape, particle size, and entrapment efficiency percentage, by image analysis optical microscopy or transmission electron microscopy, laser diffraction, and ultracentrifugation, respectively. In vitro drug permeation via a synthetic semipermeable membrane or skin from newborn mice was studied in Franz diffusion cells. The selected systems were incorporated into Pluronic F 127 gels and evaluated for both drug permeation and mice skin depositions. In all systems, the presence of spherical-shaped vesicles was
predominant. The vesicle size was significantly decreased by decreasing cholesterol concentration and increasing dicetylphosphate and ethanol concentrations. The entrapment efficiency percentage was significantly increased by increasing cholesterol, dicetylphosphate, and ethanol concentrations. In vitro permeation studies of the prepared gels containing the selected vesicles showed that ethosomal systems were much more efficient at delivering SS into mice skin (in terms of quantity and depth) than were liposomes or aqueous or hydroalcoholic solutions.

Jain S et al. 84(2007) have formulated and evaluated the ethosomes for enhanced transdermal delivery of lamivudine. The purpose of the present research was to investigate the mechanism for improved intercellular and intracellular drug delivery from ethosomes using visualization techniques and cell line study. Ethosomal formulations were prepared using lamivudine as model drug and characterized in vitro, ex vivo and in vivo. Transmission electron microscopy, scanning electron microscopy, and fluorescence microscopy were employed to determine the effect of ethosome on ultrastructure of skin. Cytotoxicity and cellular uptake of ethosome were determined using T-lymphoid cell line (MT-2). The optimized ethosomal formulation showed 25 times higher transdermal flux (68.4 +/- 3.5 microg/cm(2)/h) across the rat skin as compared with that of lamivudine solution (2.8 +/- 0.2 microg/cm(2)/h). Microscopic studies revealed that ethosomes influenced the ultrastructure of stratum corneum. Distinct regions with lamellar stacks derived from vesicles were observed in intercellular region of deeper skin layers. Results of cellular uptake study showed significantly higher intracellular uptake of ethosomes (85.7% +/- 4.5%) as compared with drug solution (24.9% +/- 1.9%). The results of the characterization studies indicate that lipid perturbation along with elasticity of ethosomes vesicles seems to be the main contributor for improved skin permeation.

Fluconazole gels were formulated in various polymer bases and developed gels were evaluated by Jain et al. 85(2007). Fluconazole, antifungal drug in the form of gels were formulated using polymers like HPMC, Carbopol 934,
Methylcellulose and Sodium alginate. The gels were evaluated for various physicochemical parameters like pH, viscosity, rheology, drug content, spreadibility and skin irritation test. In addition, in vitro drug release by diffusion using cellophane membrane and permeation through hair less rat skin using modified Kiesery Chien Diffusion cell was performed. The rheological behaviour and apparent viscosity values for different gel bases were measured before and after storage under freezing condition at 2-8 accelerated stability testing at 45±2 °C and were taken as measure for stability of gel network structure. Also 0C and 75%±5% R.H for 3 months were performed. Among the four formulations, gel prepared using HPMC shows desired properties and exhibit better release pattern when compared with other formulations prepared with Carbopol, Sodium alginate and Methylcellulose.

Gan Hu et al 86(2007) have investigated on the advances made by phospholipids as carriers in topical application of various drugs. During the investigations, the main difference observed between conventional liposomes and ethosomes is that ethanol, instead of cholesterol, is encapsulated in ethosomes. Phospholipids are the main components in ethosomes, especially phosphatidyl choline (also known as lecithin). Paolino et al evaluated percutaneous permeation of ammonium glycyrrhizinate (an effective drug for skin inflammation) in ethosomes by using Franz s cells. Ethosomes formulation, compared with water and ethanol solutions, led to an increase of in vitro percutaneous permeation of both methylnicotinate and ammonium glycyrrhizinate and enhanced the anti-inflammatory activity of ammonium glycyrrhizinate.

Mechanism behind the in vitro percutaneous absorption enhancement of carvedilol by penetration enhancers was investigated by Amin S et al 87(2008). The effect of penetration enhancers like tulsi (basil) oil, eucalyptus oil, clove oil, black cumin oil, oleic acid and Tween 80 on the percutaneous absorption of model lipophilic drug carvedilol was investigated using excised rat abdominal skin. Transdermal flux, permeability coefficient and enhancement factor were calculated for each penetration enhancer. Black cumin oil (5% v/v) was selected on the basis
of its highest enhancement in permeation and was evaluated further for its mode of action using DSC, FTIR and histological studies. The results indicated that the oil shows its action by extraction of lipids from stratum corneum as well as by loosening the hydrogen bonds between ceramides subsequently leading to fluidization of the lipid bilayer.

Differential Scanning Calorimetry was used as the tool to study the thermal behavior of lipid bilayers and liposomal stability by Demetzos C 88 (2008). Thermodynamical techniques are applied for determining the thermal stress of medicinal compounds of the excipients as well as their interactions during the formulation process. The physicochemical properties and the stability of the medicinal products could be measured as a function of temperature or time using thermal analysis. Differential Scanning Calorimetry (DSC) is a suitable thermal analysis technique for determining the purity, the polymorphic forms and the melting point of a sample in the Pharmaceutical Industry. It is also considered as a tool to study the thermal behavior of lipid bilayers and of lipidic drug delivery systems, like liposomes by measuring thermodynamic parameters (i.e. DeltaH and Tm), which affect the stability of the liposomal suspension under given storage conditions.

In vitro percutaneous permeation and skin accumulation of finasteride using vesicular ethosomal carriers was investigated by Rao et al 89 (2008). In order to develop a novel transdermal drug delivery system that facilitates the skin permeation of finasteride encapsulated in novel lipid-based vesicular carriers (ethosomes) finasteride ethosomes were constructed and the morphological characteristics were studied by transmission electron microscopy. The particle size, zeta potential and the entrapment capacity of ethosome were also determined. In contrast to liposomes ethosomes were of more condensed vesicular structure and they were found to be oppositely charged. Ethosomes were found to be more efficient delivery carriers with high encapsulation capacities. In vitro percutaneous permeation experiments demonstrated that the permeation of finasteride through human cadaver skin was significantly increased when ethosomes were used. The
finasteride transdermal fluxes from ethosomes containing formulation (1.34±0.11 µg/cm²/h) were 7.4, 3.2 and 2.6 times higher than that of finasteride from aqueous solution, conventional liposomes and hydroethanolic solution respectively (P<0.01). Furthermore, ethosomes produced a significant (P<0.01) finasteride accumulation in the skin, especially in deeper layers, for instance in dermis it reached to 18.2±1.8 µg/cm². In contrast, the accumulation of finasteride in the dermis was only 2.8± 1.3 µg/cm² with liposome formulation. The study demonstrated that ethosomes are promising vesicular carriers for enhancing percutaneous absorption of finasteride.

Study on the interaction of ketoconazole with human and bovine serum albumin by fluorescence spectroscopy was carried out by Guo et al.\textsuperscript{90}(2008). The binding of ketoconazole to human serum albumin and bovine serum albumin was studied by using fluorescence and ultraviolet spectroscopy. The measurements were performed in 0.1 mol/L phosphate buffer solution at pH=7.40±0.1. Decreasing of quenching constant was observed in association with temperature increase. Our findings show that the quenching mechanism of fluorescence of serum albumins by ketoconazole was static quenching because of compound formation. The thermodynamic parameters ∆G, ∆H, and ∆S at different temperatures were calculated, showing that the electrostatic interactions and hydrophobic interaction are the main forces for the binding of ketoconazole to serum albumins. The distance r between the donor (Trp-214) and acceptor (ketoconazole) was obtained according to fluorescence resonance energy transfer theory.

Development, characterization and in vitro skin penetration studies of temoporfin (mTHPC) loaded invasomes were performed by Nina Dragicevic-Curic et al.\textsuperscript{91}(2008). The mTHPC is a highly hydrophobic second generation photosensitizer with low percutaneous penetration. In order to enhance its percutaneous penetration it was necessary to develop a mTHPC-loaded drug carrier system for enhanced skin delivery. mTHPC-loaded invasomes were developed, characterized and investigated for the in vitro percutaneous penetration.
of mTHPC into abdominal human skin using Franz diffusion cells. mTHPC-loaded invasomes were prepared using non-hydrogenated soybean lecithin (10% w/v), ethanol (3.3% w/v) and a mixture of terpenes (0.5 and 1% w/v). The invasomes obtained were of a sufficiently small particle size (b150 nm) and polydispersity index (b0.3). The particle size of invasomes increased following an increase in the amount of terpenes in the invasomes. All invasomes possessed a negative surface charge. The vesicles appeared to be unilamellar and oligolamellar, spherical and oval in shape. An interesting phenomenon was the finding that with increasing the amount of terpenes, the number of deformed vesicles in the dispersion increased. In vitro skin penetration data revealed that the invasome dispersion with 1% of the mixture of terpenes showed a significantly enhanced deposition (p<0.05) of the drug in the SC compared to liposomes without terpenes and the ethanolic solution.

Formulation and evaluation of liposomes containing ketoconazole was carried out by Patel et al.\(^9\text{2}\) (2009). The liposomal product of ketoconazole was prepared with the view to improve therapeutic response and reduce the possible adverse symptoms. Here liposomes of ketoconazole were prepared using thin film hydration technique. Percentage entrapment efficiency was optimized after studying the effect of various process and formulation variables. Maximum entrapment efficiency was found to be 54.41±0.19 %. The prepared liposomes were found to have good morphological properties and size distribution. From DSC thermograms of liposomes it can be concluded that significant interaction occur between drug and lipid components of the vesicles that lead to higher entrapment efficiency. The percentage cumulative drug release from the optimized batch i.e. F4 was found to be 34.96±0.86 % after 12 hours of diffusion studies. Stability studies showed maximum percent drug retention at refrigerated temperature (2-8°C). The drug entrapment efficiency can be attributed to phospholipids’ ability to vesiculate independently because they carry two bulky nonpolar lipid chains and a polar head group, which helps them spontaneously form into closed bilayer systems.
A.N.Misra et al \(^9^3\)(2009) have developed novel delivery system “ethosomes” containing fluconazole for the treatment of topical fungal diseases. Aim of this work was to prepare and characterize fluconazole (FLZ) encapsulated ethosomes, incorporate it in suitable dermatological base, and assess its comparative clinical efficacy in the treatment of Candidiasis patients against liposomal gel, marketed product and hydroethanolic solution of the drug. Drug encapsulated ethosomes and liposomes were prepared and optimized by “Hot” method technique and lipid film hydration technique. Vesicular carriers were characterized for % entrapment efficiency, particle size and shape, in vitro drug diffusion study, mean % reduction in dimension of Candidiasis lesion and stability study by using suitable analytical technique. Vesicle size and drug entrapment efficiency of the optimized ethosomes and liposomes were found to be 144±6.8nm and 82.68% and 216±9.2 nm and 68.22% respectively. Microscopic examinations suggest ethosomes to be multilamellar spherical vesicles with a smooth surface. The differential scanning calorimetry results suggest high fluidity of the ethosomes than liposomes. In vitro drug diffusion studies demonstrated that % drug diffused from ethosomes was nearly twice than liposomes and three times higher than the hydroethanolic solution across rat skin. From the clinical evaluation, the developed novel delivery system demonstrated enhanced antifungal activity compared to liposomal formulation, marketed formulation and hydroethanolic solution of the drug.

Yi-Ping Fang et al \(^9^4\)(2009) have investigated on the topical delivery of ethosomal 5-aminolevulinic acid in a hyperproliferative skin animal model using the CLSM technique to evaluate the penetration behavior for the treatment of psoriasis. Psoriasis, an inflammatory skin disease, exhibits recurring itching, soreness, and cracked and bleeding skin. Currently, the topical delivery of 5-aminolevulinic acid-photodynamic therapy (ALA–PDT) is an optional treatment for psoriasis which provides long-term therapeutic effects, is non-toxic and enjoys better compliance with patients. However, the precursor of ALA is hydrophilic, and thus its ability to penetrate the skin is limited. Also, little research has provided a platform to investigate the penetration behavior in disordered skin. We employed
a highly potent ethosomal carrier (phosphatidylethanolamine; PE) to investigate the penetration behavior of ALA and the recovery of skin in a hyperproliferative murine model. We found that the application of ethosomes produced a significant increase in cumulative amounts of 5–26-fold in normal and hyperproliferative murine skin samples when compared to an ALA aqueous solution; and the ALA aqueous solution appeared less precise in terms of the penetration mode in hyperproliferative murine skin. After the ethosomes had been applied, the protoporphyrin IX (PpIX) intensity increased about 3.64-fold compared with that of the ALA aqueous solution, and the penetration depth reached 30–80 lm. The results demonstrated that the ethosomal carrier significantly improved the delivery of ALA and the formation of PpIX in both normal and hyperproliferative murine skin samples, and the expression level of tumor necrosis factor (TNF)-a was reduced after the ALA–ethosomes were applied to treat hyperproliferative murine skin. Furthermore, the results of present study encourage more investigations on the mechanism of the interaction with ethosomes and hyperproliferative murine skin.

H.Khambete et al. (2010) developed gellified emulsion for sustained delivery of itraconazole for topical fungal diseases. Gellified emulsion (Emulsion in gel) have emerged as one of the most interesting topical drug delivery systems as it has dual release control system i.e. emulsion and gel. Also the stability of emulsion is increased when it is incorporated in gel. Itraconazole is an orally or topically active antifungal agent with a broad spectrum of activity. The objective of this project was to develop a gellified emulsion for control delivery for Itraconazole. In present work we prepare emulsion and then incorporated in carbapal gel. The prepared formulation was evaluated on basis of pH, spreadability, Viscosity, drug content, in vitro release and Stability Studies. The microbial assay and Skin irritation studies on rabbit was also performed. The result of studied reveled that the optimized batch shows 95.08% release in 48 hours and stable for around three. The result of microbial assay compared with marketed product, the result shows 46.6% inhibition of optimized batch where as marketed preparation shows only 32.3% inhibition. While result of skin irritation test shows
no edema and erythema. Hence it can be concluded that emulsion based system is more effective and safe system for sustain delivery of antifungal agent(s).

Comparative evaluation of hepatitis B surface antigen–loaded elastic liposomes and ethosomes for human dendritic cell uptake and immune response was performed by D.Mishra et al. (2010). The aim of the present study was to evaluate two vesicular carrier systems, ethosomes and elastic liposomes loaded with hepatitis B surface antigen, for in vitro qualitative and quantitative uptake by human dendritic cells (DCs) and ability to stimulate T lymphocytes. Quantitative uptake of antigen-loaded carriers was documented by flow cytometry, and internalization of the systems by the DCs was studied using spectral bioimaging. Ability of antigen-pulsed DCs to stimulate autologous peripheral blood lymphocytes and levels of TH1/TH2 cytokines were also examined using flow cytometry. Both vesicular carrier systems as antigen delivery modules and DCs as antigen-presenting cells were able to generate a protective immune response. However, ethosomes were found to have higher internalizing ability and immunogenicity in comparison with elastic liposomes. These properties of ethosomes coupled with their skin-navigating potential, make it an attractive vehicle for development of a transcutaneous vaccine against hepatitis B in preference to elastic liposomes.

Y-C.Ah et al. (2010) have developed a novel transdermal patch incorporating meloxicam, in vitro and in vivo characterization of developed patches were performed during the study. In vitro skin permeation tests of samples were performed using a vertical Franz diffusion cell whose diffusion area was 0.785cm2, and hairless mouse skin. The skin was excised and the subcutaneous fat and other extraneous tissues were trimmed. The skin was mounted on the Franz diffusion cells with the stratum corneum (SC) facing the donor compartment. The receptor compartment was 5ml in volume, and filled with pH 7.4 phosphate buffer solution (PBS) whose temperature was maintained as 32°C. The receptor solution of a Franz diffusion cell was fully replaced with fresh PBS at 8 and 24h, while stirring at 600rpm. The collected PBS was subjected to high performance liquid
chromatography (HPLC) to determine the content of the MX in PBS. The size of each patch sample for skin permeation was 1.5cm×1.5cm.

In vitro skin penetration and stability studies of surface charged temoporfin-loaded flexible vesicles was carried out by Nina Dragicevic-Curic et al (2010). In order to increase topical delivery of temoporfin (mTHPC), a highly hydrophobic photosensitizer with low percutaneous penetration, neutral, anionic and cationic flexible liposomes (i.e. flexosomes) were prepared and investigated for their penetration enhancing ability. The in vitro skin penetration study was performed using human abdominal skin mounted in Franz diffusion cells. Besides the effect of surface charge of flexosomes on skin penetration of mTHPC, also its effect on physical properties (particle size, polydispersity index, lamellarity) and physicochemical stability of vesicles was investigated. Photon-correlation spectroscopy revealed that vesicles had after preparation a small particle size and low polydispersity index, while cryo-electron microscopy confirmed that these vesicles were mostly unilamellar and of a spherical shape. Regarding stability, contrasting to anionic flexosomes showing lack of long-term stability, neutral and cationic flexosomes were stable during 9 months storage at 4 °C. As to the penetration enhancing ability, cationic flexosomes possessed the highest, i.e. they delivered the highest mTHPC amount to stratum corneum and deeper skin layers compared to conventional liposomes, neutral and anionic flexosomes. In conclusion, mTHPC-loaded cationic flexosomes could be a promising tool for delivering mTHPC to the skin, which would be beneficial for the photodynamic therapy of cutaneous malignant or non-malignant diseases.

S.D.Maurya et al (2010) have formulated and evaluated ethosomes of stavudine. The entrapment efficiency of different vesicular formulation and in traditional liposomes was calculated as percent total drug entrapped. The greatest entrapment of stavudine in ethosomes (50.26±2.7) is compared to conventional liposomes (40.25±3.8) could be attributed to the greater retentivity of stavudine in ethanol present in ethosomal core. The data indicate that entrapment efficiency depends on ethanol concentration, as the concentration increases up to 30%, results
in increase in entrapment efficiency of ethosomal formulation. With further increase in ethanol concentration entrapment efficiency decreases, owing to increase fluidity of membrane and vesicles become more permeable that leads to decrease in entrapment efficiency of ethosomal formulation. The elasticity of ethosomal vesicle membrane (38.6±2.5) was found to be 5.5 fold higher than liposome (6.94±2.1). Higher concentration of ethanol present in ethosomes perhaps provided elasticity to vesicle membrane by reducing the interfacial tension of the vesicle membrane.