CHAPTER-2

LITERATURE REVIEW
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2.1 RISPERIDONE

Study of risperidone was started in late 1980’s and Janssen Pharmaceuticals, a Belgium based Pharmaceutical Company which was later on purchased by Johnson and Johnson launched Risperdal in June 1993 [33].

Huang et al (1993) have studied the detail pharmacokinetics and pharmacodynamics of risperidone and its metabolites among poor, intermediate and extensive metabolizers. They mentioned that risperidone is metabolized by 9-hydroxylation (at alicyclic site of pyrimidinone ring), N-dealkylation (at piperidine nitrogen) and 7-hydroxylation (also at alicyclic site of pyrimidinone ring). Among all three, 9-hydroxy risperidone is equipotent as risperidone and exhibit similar pharmacological activity, other metabolites doesn’t exhibit any activity. Both risperidone and 9-hydroxy risperidone are the active metabolites and excreted with a variable t\(_{1/2}\) among extensive (3 h) and poor metabolizers (21 h) [15].

El-Barghouti et al (2005) have highlighted about pH dependent aqueous solubility of risperidone. At higher pH (>10), risperidone remains as neutral molecule with high lipophilicity. As pH decreases, mono-protonated and deprotonated form appears which increases the aqueous solubility. It has the pKa1 of 8.1 (mono-protonation) and pKa2 of 3.1 (diprotonation). At pH 3, risperidone shows maximum solubility. These findings are further confirmed by Jug M (2009). They also prepared the risperidone and HP-β-cyclodextrin inclusion complexes and showed that the aqueous solubility of risperidone can be increased by complexation and pH modulation. The pH plays a significant role in complexation of risperidone and HP-β-CD. At pH 10, complexation between neutral risperidone and HP-β-CD occurs while at pH 6.0 it occurs between mono-protonated form of risperidone and HP-β-CD. Drug ionization at pH 6.0 leads to the reduction in complex stability and indicates the lower affinity of mono-protonated risperidone (hydrophilic) for inclusion into highly lipophilic cavity of HP-β-CD. On the other hand neutral risperidone is very lipophilic and can be easily enclosed to form the inclusion complex of high stability [34].

Ould-Ouali et al (2005) have entrapped risperidone within the PEG-p (CL-co-TMC) copolymer polymeric micelle and studied the effect of copolymer on risperidone
solubility and permeability across Caco-2 cell lines. Apparent permeability of risperidone micelle was decreased when compared to the risperidone in tartaric acid solution, but the total amount of drug transported was increased as the solubility of risperidone was increased in micelle, although the mechanism was poorly understood. Since the transport was temperature dependent, presence of an active transport such as endocytosis mechanism was suggested. Due to low endocytosis at basolateral to apical side, transport of risperidone was lower at that site. Brain receptor occupancy studies and pharmacokinetic analysis on male Wistar rats indicated that both the formulations have same extent of D₂ and 5-HT₂A receptor occupancy and AUC (same bioavailability) index. Although, reduced Cₘₐₓ and increased t₁/₂ (p=0.16) was observed for polymeric micelles compared to risperidone in tartaric acid solution [35].

Mathot et al (2006) have prepared the radiolabelled polymeric micelles of risperidone and observed their fate of absorption after oral administration [36].

Samuel (2006) has reviewed the advances in various psychotropic formulations. Among antipsychotics he discussed about advantages and shortcomings of ziprasidone, olanzapine, risperidone, aripiprazole, paliperidone respectively [18].

Jug et al (2007) have prepared 1:1 hydroxypropyl-beta-cyclodextrin (HPβCD) and risperidone complex by spray-drying technique. The mucoadhesive microparticles were prepared by loading the inclusion complex into HPMC, carbomer and HPMC/carbomer interpolymer complex (IPC). Incorporation of risperidone within the microparticles, led to decrease in mucoadhesiveness with significant increase in the dissolution rate of risperidone [37].

Lu et al (2008) have prepared the sucrose acetate isobutyrate (SAIB) based in-situ sustained release system containing risperidone intended to be administered intramuscularly [38].

Muthu & Singh (2008) have developed risperidone nanoparticle using poly (€-caprolactone), a biodegradable polymer and performed their in-vitro and in-vivo study. Prolonged inhibition of psychotic behaviour by nanoparticle was observed when compared to risperidone solution [40].

Muthu et al (2009) have formulated PLGA-risperidone nanoparticles and risperidone nanoparticle containing thermo-responsive in-situ gel for parenteral (subcutaneous)
delivery. The antipsychotic activity was prolonged over a period of 72 hours when injected subcutaneously in mice, compared to risperidone solution [41].

**Muthu et al (2009)** have formulated and evaluated risperidone nanosuspensions using poly (D, L-Lactide) for parenteral use. Nanoparticles were developed by nanoprecipitation method with Pluronic F-68 and Pluronic F-127 as stabilizers. Risperidone release from nanoparticles was sustained for more than 24 hours in some batches and followed Fickian diffusion mechanism [13].

**Rahman et al (2010)** have developed the solid dispersions of risperidone with methyl-β-cyclodextrin (1:3) and formulated orally disintegrating tablets (ODT). Time taken to dissolve 50% and 90% of the drug i.e. T (50) and T (90) was decreased in formulation containing mannitol and Kollidone®CL-SF (superdisintegrant) but increased with galen1Q™-721 (diluent) and sodium starch glycolate (superdisintegrant) from ODT [42].

**Aggarwal G (2010)** has reviewed the psychotropic drugs and emphasised that transdermal drug delivery systems can be successfully employed for their administration. Selegiline (anti-depressant), rivastigmine (Alzheimer, Parkinson’s and dementia) and methylphenidate (ADHD) are few examples of the FDA approved psychotropic agents [43].

**Heemstra et al (2010)** have studied the buccal mucosa as an alternative route for delivery of risperidone. The risperidone was found to permeate by passive diffusion process. Azone (permeation enhancer) doesn’t affect the risperidone transport [44].

**Rahman et al (2010)** have studied the effect of composition variation in risperidone solid lipid nanoparticles (SLN) using Box-Behnken Design of Experiment (DOE). Results of the study reveals that drug loading have significant effect on SLN characteristics [45].

**Amann et al (2010)** have developed PLGA-risperidone implants using scalable single-screw extrusion system, using 40% of risperidone and 60 % PLGA in different lactide to glycolide ratio (50:50, 65:35, 75:25 and 85:15). Release of risperidone was analysed and found better at *in-vitro* and *in-vivo* level and bioavailability was further verified through locomotor testing in rodents. IVIVC showed level B correlation and
found that release of risperidone was significantly increased with glycolide concentration and reduced when lactide concentration was increased [46].

**Patel et al (2011)** reported that solid-lipid nanoparticles (SLN) containing risperidone improves blood brain barrier permeability in mice when administered via intra nasal route. After 1 hour of intranasal administration of SLN in mice, nearly 10 fold and 5 fold higher blood brain barrier permeability was observed as compared to marketed risperidone. Localization of risperidone in brain was further confirmed by gamma scintigraphy imaging [47].

**Selmin et al (2012)** have shown that although an FDA approved long acting formulation of risperidone is available as Risperidal® CONSTA® (risperidone encapsulated in PLGA microspheres), the presence of tertiary amine/basic drug (e.g. risperidone) have significant influence on the biodegradation of PLGA microspheres. They observed that degradation of microspheres occurred at early stage during the preparation of microspheres due to the availability of risperidone in solution. In addition, higher lactide content in (85:15) in polymer chain decreases the onset and rate of degradation whereas increase in glycolide content (65:35), increased rate of hydration and diffusion of water, that lead to increased hydrolysis. Results of the study was found in agreement with previous reports that claims that hydrolysis of ester bonds occurs faster in presence of basic environment [48].

**Silva et al (2012)** have developed hydrogels containing solid lipid nanoparticles for the oral transmucosal delivery of risperidone. Release of risperidone was on higher side when risperidone was entrapped in hydrogel containing SLN in comparison to conventional preparation available in dispersion. Release was found to be pH dependent and followed the Fickian diffusion mechanism [49].

**Kozielska et al (2012)** used population based approach to develop PK-PD model, which highlighted the effect of risperidone and paliperidone concentration in brain and their affinity towards dopamine D2 and serotonin 5HT2A receptors. Kinetic parameter of different metabolites and binding pattern of risperidone to 5HT2A was also studied. It was concluded that 2-compartment model was best fitted to plasma pharmacokinetics, and binding of drug to 5HT2A have strong role on brain to plasma ratios [50].
Krishnamoorthy et al (2012) have prepared the solid dispersions of risperidone using mannitol as carrier in different ratios using dispersion method and also evaluated them for different parameters. Linear increase in solubility was observed when the amount of carrier and temperature was increased. Solid dispersion showed high dissolution rate in comparison to pure risperidone and followed Korsemeyer-Peppas model. Reduction in drug crystallinity, particle size reduction and compatibility of drug and carrier was examined using XRD, DSC, FT-IR, Near Infra red, Raman technique [51].

Yardi et al (2012) have successfully established the Level “A” IVIVC of risperidone immediate release tablets, using GastroPlus™, (the gastrointestinal simulation based on advanced compartmental absorption and transit model) to predict gastrointestinal absorption profile of risperidone using plasma data and in-vitro dissolution data in different dissolution media [52].

Bagratashvili et al (2013) compared rapid expansion of supercritical solution (RESS) and supercritical anti-solvent (SAS) methods for efficient release risperidone. Both method produced variable size of risperidone from an initial particle size of 50-100µm to 5-20µm in size. SAS method was found efficient than RESS as it produced risperidone, free from any contamination like solvent traces. Alteration in the shape and size of particles can be easily achieved. During SAS micronization polymorphic form of risperidone changes from triclinic to monoclinic [53].

Aggrawal et al (2013) have prepared transdermal patches of risperidone by varying concentration of eudragit RL-100 and RS-100, penetration enhancers, like olive oil, groundnut oil and jojoba oil using solvent casting method. The 3:2 mixtures of ERL-100 and ERS-100, using a mixture of olive oil and jojoba oil as penetration enhancer showed highest permeation rate in 72 hours. Transdermal patches caused prolonged release of risperidone with relative bioavailability of 115.20 % in rabbits. The neuroleptic activity was also studied and compared with control and marketed oral preparation [54].

Navitha et al (2014) have developed risperidone implants using poly-(caprolactones) and determined in-vitro in-vivo correlation (IVIVC) with the optimized formulation. The implants were found to release the drug for a period 3 months [55].
D’Souza et al (2014) have established level “A” in-vitro-in-vivo correlation (IVIVC) using four long-acting subcutaneous risperidone formulations by fractional AUC method [56].

Imam et al (2014) have employed $4^3$ factorial design to investigate the effects of formulation variables on risperidone proniosome formulations via transdermal route. Test formulation displayed higher permeation rate (ER=4.4 times) compared to conventional liposomes across rat skin. Compared to oral dosage forms, mean AUC value was 1.31 times higher for proniosomes whereas the $C_{\text{max}}$ value was reduced significantly (p<0.001) to 87.45 ± 18.84 [57].

Prieto et al (2014) have prepared dendrimers using polyamidoamine (PAMAM) polymer loaded with risperidone and the in-vivo toxicity study was also performed on Zebrafish model (used to study developmental neurobiology). Significant changes in heart rate and brain development was observed when larvae were treated with free risperidone whereas no effect was observed with risperidone dendrimers. So it was concluded that side effects were reduced when risperidone was administered as complexes [58].

Cabaleiro et al (2014) performed genotyping study on 70 healthy volunteers and proposed that poor CYP2D6 metabolizers have high $C_{\text{max}}$, AUC, $t_{1/2}$ and lower clearance for risperidone whereas lower $C_{\text{max}}$, AUC, $t_{1/2}$ and high clearance for 9-hydroxyrisperidone. These differences in pharmacokinetics were due to the CYP2D6, COMT, and VKORC1 polymorphism whereas adverse effect occurs due to gender and polymorphism in CYP2C19, AGTR1 and NAT2 [59].

Saddar et al (2014) have prepared radioiodinated risperidone and lamotrigine with $^{125}$Iodine by direct electrophilic substitution reaction with chloramine-T (CAT) as oxidizing agent with high labeling yields of 89±3.75 and 97.5±1.0%. Formulation showed good in-vitro and in-vivo stability. Brain uptake of radiolabelled risperidone and lamotrigine were higher as compared to $^{99m}$Tc-HMPAO (currently used radiopharmaceutical for brain imaging) and found stable. They concluded radiolabelling of both the drugs could be used as better brain imaging radiopharmaceuticals [60].
Mudhakir et al (2014) have developed chitosan based nanoparticles to encapsulate risperidone using tripolyphosphate (TPP) as cross-linking agents via ionic gelation method. pH of chitosan, sodium TPP, concentration and surface charge of risperidone have significant effect on the encapsulation efficiency. Sodium dodecyl sulphate (SDS) an anionic surfactant was used to modify the surface charge of risperidone from cationic to anionic. An increase in entrapment efficiency up to 25.62% was observed with 0.05% SDS. When the concentration of SDS was increased beyond 0.1% the particle size increases from nanometres to few micrometres. So it was concluded that chitosan was better carrier for anionic drugs, since it causes ionic interaction with anionic drug [61].

Abu Bakr Mohammed et al (2014) have used full factorial design to prepare solid dispersion based effervescent tablets of risperidone using various carriers like trehalose, inulin, pregelatinized starch, sodium carboxymethyl cellulose and eudragit E-100. Presence of risperidone in amorphous form within the carrier matrix was confirmed by DSC, XRD, FTIR and SEM. The optimized formulation exhibited better bioavailability of 161.41% with higher extent of absorption compared to marketed conventional tablets [62].

D’Souza et al (2015) have advocated that when basic nucleophilic drugs like risperidone and olanzapine are encapsulated in biodegradable polymers like PLGA, they accelerate the degradation of PLGA. pH and temperature also influences the degradation reaction with respect to increased drug loading [63].

Lin et al (2015) have prepared the hybrid depot of PLGA microspheres with SAIB system (sucrose acetate isobutyrate) to reduce the burst release (since risperidone was entrapped in microspheres) associated with Risperidone-SAIB depot (30 fold reduction) prepared by Lu et al (2007). Higher risperidone plasma level were maintained (1.55-16.3 ng/ml) for a period of 4 to 78 days, as compared to Risp-SAIB depot. The half-life of Risp-SAIB depot was 54.6 days with good in-vivo biocompatibility [64].

El-say et al (2015) have applied Box-Behnken design to develop an orally disintegrating –mini-tablet of high quality and less variability. The optimized formulation exhibited mannitol-to-avicel mixture (6:4 m/m), 2mm diameter, 5kN crushing strength, minimum friability, good flowability, no drug interactions, short
disintegration time of 8.4 sec and 53.7 sec in tongue and skimmed milk. They concluded that Box-Behnken design provides ready-to-use excipient combination and considered suitable for paediatric population [65].

Yerrangunta et al (2015) have developed long-acting microsphere formulations of risperidone using polycaprolactone of different molecular weights and blends using emulsion solvent evaporation technique. Both in-vitro and in-vivo studies showed that blend (1:1) of PCL-45000 and PCL-80000 resulted in drug release over a period of 90 days and followed zero order release mechanism without lag time [66].

Saha et al (2015) have prepared and evaluated risperidone loaded electro-conductive hydrogels (an implantable system) containing polyacrylamide and poly-pyrrole as carrier to provide controlled release of risperidone. Physical and chemical change in the conducting network was induced by swelling and altering the oxidation state of materials. Alteration in the composite properties will led to the electro-liberation of the risperidone. Cytotoxicity studies on HepG2 and C6 cells confirmed the biocompatibility of the hydrogels. They concluded hydrogels can be used as an implantable drug delivery system [4].

Bera et al (2015) have developed oil entrapped alginate beads containing risperidone as gastro-retentive system using ionotropic gelation technique. Various parameters like polymer: drug ratio, oil: water ratio on entrapment efficiency and cumulative release were optimized by $3^2$ factorial designs. The developed system exhibited excellent buoyancy, better ex-vivo mucoadhesion. System caused slower release of risperidone and followed Korsemeyer-peppas model with Fickian diffusion mechanism [67].

Panda et al (2016) have co-entrapped clozapine and risperidone within the PLGA nanoparticles using spray-drying method. Entrapment efficiency was 94.74% for clozapine and 93.12% for risperidone. The low molecular weight PLGA released almost 80% of the drug in 10 days. The drug available inside the nanoparticles had amorphous form and did not show any chemical interaction with PLGA [68].

2.2 VESICULAR SYSTEMS

Bangham et al (1965) have introduced the term liposomes for the first time and defined them as the microscopic vesicles (25 nm to 10 µm) enclosing an aqueous
space surrounded by membrane composed of lipid bilayer structures and considered them as a cell membrane model [69,70].

**Gregoriadis et al (1976)** have discussed that liposomes are inert in nature and exhibits strong potential for drug delivery to site of action. The drug is enclosed either in the aqueous core or are intercalated within the lipid bilayers depending on their physicochemical properties and are stable under the physiological conditions [71].

**Okahata et al (1981)** has reported that most of the amphiphiles (ionic) are toxic and are unsuitable as drug carriers [72].

**Payne et al (1986)** have reviewed the limitations of liposomes namely chemical and physical instability, sterility issues, drug incompatibility, immunologic and toxicological concerns. They suggested that stability of liposomes can be increased via preparation of proliposomes which upon hydration gives liposomes. These proliposomes can be administered intravenously or given via other routes. They have also investigated about the suitability of sodium chloride and sorbitol as carrier materials [73,74].

**Guzman et al (1987)** have reported that drugs with high or low octanol-water partition coefficients are better incorporated within the vesicles than those with intermediate partition coefficients [75].

**Martin et al (1990)** have discussed various factors that must be considered during manufacturing of liposomes e.g. choice of solvent, sterility requirements, pyrogen control, and stability of raw and finished products [76].

### 2.2.1 Niosomes

**Handjani-vila et al (1979)** have reported applications of niosomes in cosmetics for the first time. They proposed hydration of mixture of cholesterol and single chain non-ionic surfactant will lead to the formation of niosomes. Niosomes displays low toxicity and permits close contact of drug substances with stratum corneum [77].

**Baillie et al (1985)** have discussed the preparation and properties of non-ionic surfactant vesicles (niosomes) and suggested it can be used as alternative to liposomes. They prepared osmotically active niosomes using carboxyfluorescein that
causes low release of the drug. They concluded physical characteristics of the niosomes are affected by the method of preparation [78].

Azmin et al (1985) have prepared niosomes entrapped with methotrexate and studied pharmacokinetic parameters after intravenous administration of niosomes on mice. Niosomes prolonged the methotrexate level in blood and increased its BBB permeability. The metabolic profile of methotrexate was also altered by the niosomes which possibly occurred due to delayed formation of 7-hydroxy methotrexate in liver [79].

Florence et al (1985) have used non-ionic surfactants to prepare chemically stable, osmotically active niosomes as a better alternative to phospholipid vesicles. Certain ionic amphiphiles like dicetylphosphate and stearylamine were also used to form the stable niosomes [80].

Baillie et al (1986) have used niosome as drug carrier for sodium stibogluconate for better targeting property. Niosome was found more active than free drug against murine visceral leishmaniasis [81].

Rogerson et al (1988) have prepared niosomes containing doxorubicin with C_{16} triglycerol ether with and without cholesterol. Niosomes containing cholesterol showed delayed release at in-vitro level whereas there was little difference between the plasma profiles of the two preparations, when administered by bolus injection to S180 tumour bearing mice. The half-life of the drug in niosomes was prolonged when compared to free solutions. Niosomes containing cholesterol showed more effective reduction in tumour growth [82].

Chandraprakash et al (1990) have prepared niosomes containing methotrexate using Span 40, Span 60 and Span 80. The entrapment efficiency was higher with Span 60 in comparison to tween 80, which may be due to the increased lipophilicity and tissue distribution [83].

Udupa et al (1993) have encapsulated methotrexate in niosomes containing spans and tweens as non-ionic surfactants and performed their physicochemical characterization, pharmacokinetic studies and analysed their effect on tumour remission of mice transplanted with S-180 sarcoma. The t_{1/2} of methotrexate was prolonged which ensured prolonged circulation of niosomes in the blood. They concluded sustained
release of the methotrexate from niosomes may decrease the toxicity of anticancer drugs [84].

Yoshioka et al (1994) have prepared niosomes containing carboxyfluorescein using series of sorbitan esters (Span 20, 40, 60, 80 and 85). Entrapment efficiency was significantly increased with increased concentration of lipids and cholesterol in the formulation. Span 60 formulation has the highest entrapment efficiency. They advocated size of the vesicles increases with increase in the HLB from Span 85 (HLB 1.8) to span 20 (HLB 8.6) and the release rate also get altered with type of surfactant used [29].

Erdogan et al (1998) have performed the comparative evaluation of liposomes and niosomes containing iopromide with respect to characterization, stability and in-vitro release. Both the formulations were prepared in gel and liquid crystalline state. Phospholipon-100 was used in preparing liquid crystalline state and distearyl phosphatidylcholine for gel state of liposomes. Similarly hexadecyl poly-(3)-glycerol was used for preparing gel state and dialkyl poly-(7)-glycerol ether for liquid crystalline state. Formulations followed the Higuchi release kinetics and niosomes were found more stable than liposomes. No significant difference was observed with gel and liquid crystalline states. In-vivo studies revealed that both liposomes and niosomes interact with plasma proteins which resulted in loss of lipids and leakage of drugs [85].

Arunothayanun et al (2000) have studied the effect of temperature and sonication on the physical property and phase transition of niosomes, containing hexadecyl diglycerol ether (C\textsubscript{16}G\textsubscript{2}). They concluded that on heating or sonication these vesicles undergo a reversible transition from polyhedral to spherical shape [86].

Hao et al (2002) have used evaporation-sonication method for the entrapment of colchicines within the niosomes and concluded that these niosomes have high entrapment efficiencies as well as very few side effects. They also suggested that soluble drugs can be easily entrapped by this method [87].

Manconi et al (2002) have prepared niosomes containing tretinoin using polyoxyethylene lauryl ether, sorbitan esters and commercial mixture of octyl/decylpolyglucosides with cholesterol and dicetyl phosphate. Results reveal that
in presence of cholesterol all the amphiphiles were able to form the stable vesicular dispersion. multilamellar vesicles (MLVs) were larger than large unilamellar vesicles (prepared by extrusion) and small unilamellar vesicles (prepared by sonication). Entrapment efficiency was high with MLVs (91-99%) than extruded vesicles (88-98%). *In-vitro* release study showed that release of drug was faster with niosomes and liposomes. In addition, it was concluded that release was mainly affected by the vesicular structure and the drug release increases significantly from MLVs to LUVs to SUVs [88].

**Jain et al (2006)** have worked on the lymphatic delivery of rifampicin via niosomal system and concluded that the drug effectively reached the lymphatic system after intraperitoneal administration of niosome. They concluded that niosomes loaded with rifampicin can be used for the effective management of tuberculosis [89].

**Mukherjee et al (2007)** have developed liposomes and niosomes containing soya lecithin, cholesterol and non-ionic surfactant, span 20 loaded with acyclovir. Higher loading was observed with niosomes in comparison to liposomes. *In-vitro* release study revealed that 90% of drug was released within 150 min in case of liposomes whereas from niosomes only 50% of drug was released in 200 min. Niosomes showed better stability than liposomes, so it was concluded that niosomes are more suitable for the parenteral administration of acyclovir [90].

**Attia et al (2007)** have prepared niosomes loaded with acyclovir to improve its poor and variable oral bioavailability. Niosomes was prepared using Span 60, cholesterol and dicetyl phosphate in 65:60:5 molar ratios. The entrapment efficiency of acyclovir was approximately 11%. Niosomes with unilamellar spherical shape exhibited retarded release of acyclovir via Higuchi release mechanism. The relative bioavailability of the niosome formulation was increased to more than two folds compared to free drug solution in rabbits after administration of single oral dose at 40mg/kg. There was also an increase in mean residence time (MRT), which was indicative of sustained release of acyclovir. Authors suggested that niosomes are the promising delivery system for acyclovir to improve the oral bioavailability and to achieve prolongs release profile [91].

**Mokhtar et al (2008)** have developed proniosome gels using different types of spans with and without cholesterol, which upon hydration immediately formed the
niosomes. They have also evaluated the effect of different processing and formulation variables on proniosomes formulation. The entrapment efficiency observed was in the order of Span 60 > Span 40 > Span 20 > Span 80. Maximum loading efficiency achieved was 94.61% in proniosomes. They concluded that %EE was directly influenced by total lipid, drug and cholesterol content. Dicetylphosphate induces the negative charge and stearylamine induces the positive charge on proniosomes. *In-vitro* release study showed that niosomes followed the biphasic behaviour. Author concluded proniosomes as a stable precursor for the immediate preparation of niosomal carrier systems [92].

**Abd-Elbary et al (2008)** have formulated the proniosome containing cromolyn sodium using sucrose stearates (non-ionic surfactant). They found that drug release rate was retarded in case of niosomes obtained by the hydration of proniosomes as compared to drug solution. The nebulisation efficiency and the stability were also higher for the niosomes. They concluded that proniosomes are better alternative to niosomes since they overcome the problems of physical instability of niosomes [93].

**Jadon et al (2009)** have prepared niosomes containing griseofulvin to improve its poor and variable oral bioavailability, using different types of spans, cholesterol and dicetyl phosphate. Results of the study showed that span 60 have the highest entrapment efficiency and exhibited retarded *in-vitro* drug release when compared to free drug. *In-vivo* study on albino rats showed significant improvement in the oral bioavailability after single oral dose. Niosomes exhibited sustained drug release with almost double $C_{\text{max}}$ and increased AUC when compared to free drug [94].

**Nasr et al (2009)** have prepared celecoxib proniosomes to improve its oral bioavailability and performed their *in-vitro* and *in-vivo* evaluation. The entrapment efficiency found was about 95% with relative bioavailability 172.06%. Extent of dissolution of proniosomes and absorption was increased when compared to free drug. $T_{\text{max}}$ was prolonged in case of proniosomes whereas no significant difference was observed in $t_{1/2}$ and elimination rate. They concluded that oral bioavailability of celecoxib can be improved with the proniosome formulations [95].

**Hashim et al (2010)** have prepared and characterized the ribavirin niosomes for liver targeting. They found that niosomes (molar ratio 4:2:1) have higher encapsulation
efficiency and exhibits sustained release profile. The concentration of drug in the liver increased to 6 folds when compared to ribavirin solution [96].

Ruckmani et al (2010) have analyzed the effect of hydration time, sonication time, rotation speed of evaporation flask, effects of charge-inducers and centrifugation on % entrapment and release of zidovudine from niosomes [97].

Hao et al (2011) have investigated the effect of hydrogen bonding between various components of niosome. They concluded that the release of p-hydroxyl benzoic acid in simulated intestinal fluid is much slower than in simulated gastric fluid, and the release rate of salicylic acid (SA) in simulated gastric fluid is lower than in simulated intestinal fluid [98].

Pardakhty et al (2011) have encapsulated human insulin in the vesicular system to prevent its enzymatic degradation and bioavailability improvement. It was found that niosomes containing Brij 92 and span 60 exhibit lower release rate than Brij 52 niosomes. Niosomes have successfully protected the insulin when compared to free insulin against the proteolytic enzymes. Rats showed significant hypoglycaemic effect which was evident from the decreased blood glucose level and increased serum insulin level. Niosomes were also found to be stable against bile salt solution and prolonged the insulin release, so it can be concluded that niosomes are better carriers for oral delivery of insulin [99].

Alam et al (2011) have reviewed the applications and limitations of everted gut sac model in studying the drug absorption, metabolism and interactions and also highlighted the effect of various factors like age, sex, species, chronic therapy and diseased state on drug absorption. They have concluded that this technique is very much useful for studying the in-vitro drug absorption, metabolism; drug transport mechanism and to understand the effect of drug transporters in the absorption and bioavailability [100].

Sezgin-Bayindiret et al (2013) performed preparation, characterization, pharmacokinetics and biodistribution studies of paclitaxel niosomes for its improvement of oral bioavailability. They showed that niosomes coated with carbopol 974 is more stable in presence of bile salt. The drug release was extended and high plasma drug concentration was observed in rats after oral administration in comparison to drug in suspension. They suggested that paclitaxel accumulates in liver
and intestine, hence it can be used for the treatment of intestine and liver carcinomas [101].

Junyaprasert et al (2013) have developed niosomes containing ellagic acid for dermal delivery and studied the effect of penetration enhancers on EA-loaded niosome. They analysed that drug distribution of niosomes with the help of confocal laser scanning microscopy. The niosomes were found at the penetration depth of 30-90 µm (the epidermis layer) with dimethylsulfoxide (DMSO) and 90-120 µm (the dermis layer) under the skin with N-methyl-2-pyrrolidone (NMP). They concluded that the DMSO is suitable penetration enhancer for epidermal delivery and NMP for dermal delivery of EA [102].

Waddad et al (2013) have prepared the niosomes using different spans and tweens containing Morin hydrate which is a bioflavonoid with anticancer and antioxidant activity. Tween 60 niosomes showed the maximum entrapment efficiency, release rate and was found most stable than other formulations. The molecular modelling studies showed the hydrogen binding occurs between the drug and human serum albumin. The AUC of the drug from niosomes was increased (1.3-2.7 fold) compared to drug solution. The images from ex-vivo studies showed that the drug was accumulating in the brain which indicates the permeability of tween 60 niosomes across the blood brain barrier [103].

Muzzalupo et al (2013) have used alkyl glucopyranoside surfactant to prepare niosomes containing methotrexate. Higher entrapment efficiency and delayed release was observed with niosome formulations. Haemolytic tests showed that sugar based surfactants are more haemolytic and their activity increases with increase in alkyl chain length. They concluded vesicle formation decreases the surfactant toxicity [104].

Hasan et al (2013) have used Span 40 and cholesterol to prepare niosomes containing metformin. Dicetylphosphate (-ve) and 1, 2-dioleoyl-3-trimethylammonium-propane chloride salt (+ve)) was used as charged inducers. The entire formulations showed the high entrapment efficiencies and sustained release, particularly with positively charged niosomes. Hypoglycemic effect was prolonged with niosome formulations. Area time curve (AUC), maximum hypoglycemic response and time of maximum response (T_{max}) were higher in niosomes than in drug solution. Authors concluded
that extended release and better hypoglycaemic activity can be obtained from the niosome formulation [105].

Wilkhu Jitinder et al (2014) have studied the effect of cholesterol in niosome formation by thermal analysis and molecular dynamics. Langmuir studies showed that cholesterol gets intercalated in the monolayer and helps in the bilayers formation. Molecular simulation studies also showed that cholesterol is essential for niosome formation and the absence of which lead to unsuccessful bilayers formation [106].

Bragagniet al (2014) have developed niosomes containing dynorphin-B for brain targeting and found that the niosome showed pronounced antinociceptive effect upon intravenous administration to mice. They proposed that niosomes can be used as potential carrier for the delivery of neuroactive peptides across the BBB [107].

Abdelkader et al (2014) have investigated the role of both surfactant type and cholesterol level on *in-vitro* characteristics and *in-vivo* performance of the niosomes loaded with timolol maleate for ocular administration. The lowering of intraocular pressure in rabbits was found in the order of Span 40> Span 20 > Span 60. The AUC was higher for span40: cholesterol (7:3) due to good compromise between thermo responsiveness and efficient loading of drug compared with Span 20 and span 60 [108].

Sezgin-Bayindiret al (2015) worked on the development and characterization of mixed niosomes containing candesartan cilexetil to improve its aqueous solubility and oral bioavailability. *In-vitro* drug release was improved with niosomes containing Span 60 and Pluronic P85 and with better stability against bile salts and sedimentation behaviour. DSC study showed the conversion of crystal structure of drug to the amorphous form which resulted in the enhanced drug release from the niosomes [109].

Moghassemi et al (2015) worked on uptake and transport of insulin across intestinal membrane using trimethyl chitosan coated insulin niosomes and concluded that the particles were between 100-180 nm in diameter and were stable for over 60 days at 4°C. Insulin permeability through Caco-2 cell monolayer was enhanced 4-fold by niosome compared with insulin alone [110].
Arzani et al (2015) have formulated and characterized the niosomes containing carvedilol to overcome the poor oral bioavailability issue. They also studied the effect of bile salt inclusion within the vesicles (bilosome) and effect of type of charge on the niosomes. They showed that bilosomes containing 20% sodium cholate and 30% sodium taurocholate gave the greatest enhancement in the intestinal absorption and relative bioavailability. Peak plasma concentration was 2.3 fold for positively charged and 1.7 fold higher for negatively charged than suspension. The increased bioavailability was due to the involvement of lymphatic absorption [111].

Khan et al (2015) have prepared the niosomes loaded with diacerein using different types of spans and tweens together with cholesterol and performed their compatibility studies using ATR-FTIR techniques. They suggested that there were no significant interactions in the characteristics peak of diacerein after combining with non-ionic surfactants in niosomes [112].

Shaker et al (2015) have developed the tamoxifen citrate niosomes and evaluated their potential for localized cancer therapy in breast cancer and solid tumor. The niosomes prepared with Span 60 and cholesterol (1:1 molar ratio) showed the nanospherical shape with 92.3 % entrapment efficiency. Prolonged release of the drug was observed which was based on diffusion mechanism. The niosomes showed enhanced cellular uptake upto 2.8 folds and exhibited significant cytotoxic activity against MCF-7 breast cancer cell line. Tumor volume was remarkably reduced with niosomes compared to free drug. Author concluded that niosomes are suitable delivery system which provides local and sustained delivery of tamoxifen citrate cancer therapy [113].

Mokale et al (2016) have developed proniosomes containing famotidine and performed their in-vitro and ex-vivo study. It was prepared by coacervation-phase separation method using different ratios of span 60 and cholesterol. Entrapment efficiency was 78-89% and found proportional to cholesterol and surfactant concentration and almost 96% of drug was released within 24 hours. Ex-vivo study performed on stomach mucosa by using Franz diffusion apparatus showed that proniosomes have significantly higher flux than at the same dose of marketed preparation. Higher drug retention was observed when the stability study was performed at refrigerated temperature. Hence author advocated that proniosomes containing famotidine is a promising prolonged delivery system with good stability characteristics [114].
2.2.2 Proniosomes in TDDS

Fang et al (2001) have developed and studied in-vitro permeation of proniosomes containing estradiol. Encapsulation efficiency of estradiol with spans was very high and was approximately 100%. Span 40 and span 60 have the higher permeation rate across the rat skin which may be due to the penetration enhancer effect of the non-ionic surfactant and vesicle-skin interactions. Increased permeation of estradiol was not observed with that of the niosome suspension while the proniosomes efficiently delivered the drug across the skin. No significant difference in encapsulation efficiency and skin permeation of estradiol was observed in proniosomes with and without cholesterol. Hence author concluded that type and content of non-ionic surfactant in proniosomes are important factors in transdermal estradiol delivery [115].

Kumhar et al (2003) have prepared proniosomal gel containing ethinyl estradiol and levonorgestrel for transdermal drug delivery. It was prepared by coacervation phase separation technique using span 20, 40, 60 and 80 with egg lecithin, Brij 58, dicetyl phosphate, soya lecithin and cholesterol. Results showed that formulations with span 20 and span 40 (3:1) have better in-vitro and in-vivo performance [116].

Alsarra (2005) has prepared and evaluated proniosomes containing ketorolac for transdermal drug delivery. Proniosomes showed improved drug permeation across the excised rabbit skin and also reduced the lag time. Span 60 formulations provided higher ketorolac flux across the skin than with tween 20 formulations (7 and 4 folds than control respectively). No significant decrease in flux was observed with change in cholesterol content and decrease in lecithin content. Almost 99% of the drug was encapsulated in the proniosomes and the vesicles size was highly dependent on the compositions of the proniosome formulations. Proniosomes can act as promising carrier for transdermal delivery due to simple production and scale up [117].

Alsarra (2008) has evaluated the transdermal potential of niosomes containing piroxicam. The proniosomes were prepared using different types of spans and tweens, lecithin and cholesterol and were evaluated for various parameters. Proniosomes exhibited high encapsulation efficiency. Span 60 formulations showed higher flux across the skin than tween 80 and it also showed higher release rate in comparison to span 20 and span 80, while formulations bearing tween showed higher release rate
than with spans. These formulations also reduced the lag time. The results suggested that proniosomes can act as an alternative approach for transdermal delivery of piroxicam [118].

**Thakur et al (2009)** have developed the proniosomes containing losartan potassium for transdermal drug delivery. It was prepared using different types of non-ionic surfactants like Span 20, Span 40, Span 60, Span 80, Tween 20, Tween 40 and Tween 80. Best *in-vitro* skin permeation profile was observed with proniosomes containing span 40 in 24 hours and significant increase in permeability parameters like flux, permeability coefficient and enhancement ratio were also observed for the same formulation. HPMC was found most suitable base than carbopol for the fabrication of transdermal patch containing proniosomes as it gave better release and better permeation in a steady state manner over a desired period of time (24 hours) across the rat skin. In *in-vivo* study proniosomes showed significant increase in bioavailability (1.93 times) compared to oral formulations of losartan potassium. The proniosome formulations were stable when stored at room temperature (30±2°C) and at refrigeration temperature (4±2°C) for 45 days [119].

**Aboelwafa et al (2010)** have investigated the effect of formulation variables on the preparation of carvedilol proniosome formulations for its efficient transdermal drug delivery. Polyoxyethylene alkyl ethers like Brij 78, Brij 92 and Brij 72 and sorbitan fatty acid esters (span 60) were used for the preparation of carvedilol containing proniosomal gels. 2³ full factorial designs was employed to study the effect of formulation variables, like cholesterol content, weight of proniosomes and amount of drug loaded. Proniosomes with Brij 72 and span 60 exhibited higher entrapment efficiency. %EE was increased with increase in weight of proniosomes and the amount of carvedilol. On the other hand increasing cholesterol content in span 60 formulations, significantly decreased the release rate of drug. Brij 72 proniosomes containing high amount of carvedilol showed higher release rate. Proniosomes with span 60 showed higher permeation across the hairless mice skin, so this can be used as a promising approach for transdermal delivery of carvedilol [120].

**El-Laithy et al (2011)** have developed a proniosomes system containing sugar esters as non-ionic surfactants to overcome the poor bioavailability of vinpocetin associated with its marked hepatic first pass. These proniosomes were converted into the niosomes upon hydration with skin moisture when applied under occlusive
conditions. Entrapment efficiency was higher with all the formulations and the vesicle size ranges from 0.63 \( \mu \text{m} \) to 2.52 \( \mu \text{m} \) which favoured efficient transdermal delivery. Extent of drug permeation across the rat skin was higher in case of proniosomes. Extent of absorption of vinpocetin from proniosomes was higher compared to oral tablet with a relative bioavailability of 206%. Histopathological studies indicated moderate skin irritation with sugar esters, hence it can be concluded that sugar esters can be used as a promising carrier for vinpocetin [121].

**Abbas et al (2013)** have prepared proniosome gel containing metoprolol tartrate. It was prepared by coacervation phase separation method using spans and tweens of different HLB value, lecithin and cholesterol. Higher entrapment efficiencies were obtained with span 40 and span 60 (86.6 and 78.09 % respectively). Only 31.18 % of drug was released from span 40 formulations in 11 hour, hence it can be concluded that proniosomes could be a promising transdermal delivery systems for metoprolol tartrate for prolonged release profile [122].

**Rahman et al (2014)** have formulated the proniosomes loaded with tretinoin for topical application in the treatment of acne and also performed the *in-vitro* characterization, skin irritation test and clinical studies. The developed proniosomes showed higher efficacy and very low irritancy compared to marked product in human volunteers [123].

**Wen et al (2014)** have developed proniosomes containing mefenamic acid for transdermal delivery, since its oral administration is associated with severe gastrointestinal side effects. Proniosomes were developed using cholesterol, lecithin (different sources) and different types of non-ionic surfactants. Results showed that entrapment efficiency is greatly affected by type of surfactants rather than lecithin types and were higher with Span 80. Release of the drug was significantly affected by the type of lecithin used. The addition of cholesterol increased both the drug release and skin permeation flux. Significant inhibition of rat paw edema was observed with proniosomes compared with the gel of the same drug. Hence, they suggested that proniosomes provides safe alternative to enhance the transdermal delivery of mefenamic acid [124].

**Yongtai et al (2015)** have developed niosomes containing salidroside to improve the cutaneous absorption. Niosomes with span 40 and cholesterol (4:3) showed good
biocompatibility with skin tissue and also provided maximum transdermal flux and skin deposition of the drug than aqueous solution. The study also revealed that internalization of niosomes by HaCaT cells (human epidermal immortal keratinocytes) may be achieved through pinocytotic vesicles and macropinocytosis, which consumes energy, rather than via lysosomes. In CCC-ESF cells (human embryonic skin fibroblasts), pinocytotic vesicles and lysosomes were both important mediators of endocytosis. So it can be concluded that niosome formulations could improve the dermal and transdermal salidroside delivery [125].

El-Maghraby et al (2015) have investigated the effect of penetration enhancers in proniosomes to increase the transdermal drug delivery of nisoldipine. Plain proniosomes were prepared using sorbitan monostearate, cholesterol; ethanol and small amount of water. Proniosomes were also prepared using lecithin, oleic acid, oleic acid and propylene glycol and isopropyl myristate. The transdermal flux of the proniosomes was observed to be high in comparison to aqueous solution of nisoldipine. The increase in transdermal flux followed the order of P/OA-PG 1.5 > P/OA-PG 1 > P/OA-PG 0.5 > P/OA >P/lec> P/IPM > P > control (aqueous solution of drug). They advocated that proniosomes can be used to enhance the transdermal drug delivery and also highlighted the role of penetration enhancers and their mechanisms to increase the proniosomes skin delivery [126].

2.3 RATIONALE AND OBJECTIVE OF WORK

Poor water solubility, high first-pass metabolism, poor BBB penetration of 9-hydroxy risperidone (active metabolite) and other pharmacokinetic problem restricts the usage of risperidone which is considered as superior antipsychotic drug. In order to increase the bioavailability and to prolong the duration of action, several formulations like cyclodextrin complexes, solid dispersions, nanoparticles, solid-lipid nanoparticles, nanoemulsions, microspheres etc. have been formulated for parenteral and intra-nasal route. However, parenteral route is invasive and less desirable. Similarly, abundance of Cytochrome P-450 enzymes in nasal mucosa and mucocilliary clearance are the major limiting factor affecting bioavailability of drug through nasal route, thus lesser amount of parent drug is available to cross BBB. Hence, preferred route for administration of risperidone is oral and transdermal. A palatable fast disintegrating tablet, M-Tab, a marketed preparation of risperidone better suits to paediatric and
geriatric patients having swallowing problems, to manage acute psychosis. But duration of action is short, thus requires repetitive dosing. Risperdal Consta, a long acting depot preparation is also available in market. The use of Risperdal Consta therapy requires the initial use of oral delivery of drug, because the release of drug has a lag phase of 3 weeks from depot. Hence, repetitive administration of risperidone is required which is usually associated with increase in dose dependent adverse effects.

Earlier researchers have formulated different drug delivery systems using PLGA, polycaprolatone, PLA, Compritol 888 ATO etc, which are expensive and require special treatments before use. Thus to formulate a cheaper, palatable and effective formulations, methods need to be developed, using economical and GRAS (generally recognized as safe) ingredients. Among these, non-ionic surfactants play an important role. They help to solubilize the drug as well as act as permeation enhancers in intestine (in case of oral route) and through the stratum corneum (in case of transdermal route). This action of non-ionic surfactants appears to be linked to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components. Low concentrations of surfactants may also reversibly increase permeation via tight junctions. Xiuhua et al [127] have shown that non-ionic surfactants are strong inhibitors of Cytochrome P-450 enzyme in both in-vitro and in-vivo, although the mechanism is largely unknown. Based on the above evidences, we envisage that non-ionic surfactant based vesicles (niosomes) can prevent the early transformation of risperidone to its metabolite 9-hydroxy risperidone which would result in better bioavailability across the BBB. In view of this, we proposed to formulate the niosome formulations of risperidone for oral use and proniosomes based transdermal formulations. The objectives of the present work are:

1. Formulation and evaluation of Risperidone niosomes for oral use.
2. Formulation and evaluation of Risperidone proniosomes (gel) for transdermal use.
3. Comparison of optimized formulations with suitable marketed preparation of risperidone.
2.4 PLAN OF WORK

I. Pre-formulation studies of risperidone

i. Physical appearance

ii. Solubility

iii. Partition coefficient

iv. Melting point & DSC

v. FTIR spectroscopy

vi. Preparation of standard curve (UV spectroscopy & HPLC)

II. Formulation and Development of Niosomes (oral use)

i. Selection of method of preparation

ii. Optimization of various formulation ingredients

iii. Evaluation of different niosomal formulations

   a. % Entrapment efficiency

   b. Vesicle size, polydispersity index and zeta potential

   c. In-vitro release study

   d. Stability studies (in presence of bile salt)

   e. Morphology

   f. Drug-excipient compatibility studies

      • Fourier transform infra-red (FTIR) spectroscopy

      • Differential scanning calorimetry (DSC)

   g. Drug release kinetics

   h. Stability studies (effect of temperature)

   i. Ex-vivo absorption study
III. Formulation and development of proniosomes (transdermal use)

i. Preparation of proniosomal gel

ii. Optimization of proniosome compositions

iii. Evaluation of proniosomes

   a. % Entrapment efficiency
   
   b. Vesicle size, polydispersity index and zeta potential
   
   c. *In-vitro* release study
   
   d. Morphology
   
   e. Drug-excipient compatibility studies
      
      • Fourier transform infra-red (FTIR) spectroscopy
   
   f. Drug release kinetics
   
   g. Stability studies (effect of temperature)
   
   h. Irritation/sensitivity studies
   
   i. *Ex-vivo* permeation study
   
   j. Occlusion studies
   
   k. *In-vivo* absorption studies