CHAPTER 2
LITERATURE REVIEW
2.1. LITERATURE SURVEY IN THE AREA OF NOSE-TO-BRAIN (INTRANASAL) ROUTE OF DRUG DELIVERY

Perez et al., (2012) determined the brain radioactivity after intranasal administration of (32)P-small interference RNA (siRNA) complexed with poly(amidoamine) G7 dendrimers (siRNA dendriplexes) within in situ-forming mucoadhesive gels. (32)P-siRNA dendriplexes were incorporated into in situ-forming mucoadhesive gels prepared by blending thermosensitive poloxamer (23% w/w) with mucoadhesive chitosan (1% w/v, PxChi) or carbopol (0.25% w/w, PxBCP). Rheological properties, radiolabel release profile, and local toxicity in rat nasal mucosa were determined. The best-suited formulation was intranasally administered to rats, and blood absorption and brain distribution of radioactivity were measured. The gelation temperature of both formulations was 23°C. The PxChi liquid showed non-Newtonian pseudoplastic behavior of high consistency and difficult manipulation, and the gel retained 100% of radiolabel after 150 minutes. The PxCBP liquid showed a Newtonian behavior of low viscosity and easy manipulation, while in the gel phase showed apparent viscosity similar to that of the mucus but higher than that of aqueous solution. The gel released 35% of radiolabel and the released material showed silencing activity in vitro. Three intranasal doses of dendriplexes in PxCBP gel did not damage the rat nasal mucosa. A combination of (32)P-siRNA complexation with dendrimers, incorporation of the dendriplexes into PxCBP gel, and administration of two intranasal doses was necessary to achieve higher brain radioactivity than that achieved by intravenous dendriplexes or intranasal naked siRNA.

Stevens et al., (2011) studied the pharmacokinetic modeling to identify the existence of direct nose-to-brain transport in a quantitative manner. The selective dopamine-D2 receptor antagonist remoxipride was administered at different dosages, in freely moving rats, by the IN and intravenous (IV) route. Plasma and brain extracellular fluid (ECF) concentration-time profiles were obtained and simultaneously analyzed using nonlinear mixed-effects modeling. Brain ECF/plasma area under the curve ratios were 0.28 and 0.19 after IN and IV administration, respectively. A multicompartiment pharmacokinetic model with two absorption compartments (nose-to-systemic and nose-to-brain) was found to best describe the observed pharmacokinetic data. Absorption was described in terms of bioavailability and rate. Total bioavailability after IN administration was 89%, of which 75% was attributed to direct nose-to-brain transport.
Direct nose-to-brain absorption rate was slow, explaining prolonged brain ECF exposure after IN compared with IV administration. These studies explicitly provided separation and quantitation of systemic and direct nose-to-brain transport after IN administration of remoxipride in the rat.

Al-Ghananeem et al., (2010) investigated intranasal (i.n.) administration as a potential route to enhance systemic and brain delivery of didanosine (ddI). A further aim of the study was to investigate the potential use of chitosan nanoparticles as a delivery system to enhance the systemic and brain targeting efficiency of ddI following i.n. administration. Didanosine-loaded chitosan nanoparticles, were prepared through ionotropic gelation of chitosan with tripolyphosphonate anions, and characterized in terms of their size, drug loading, and *in vitro* release. The nanoparticles were administered i.n. to rats, and compared to i.n. and intravenous (i.v.) administration of ddI in solution. The concentrations of ddI in blood, CSF, and brain tissues were analyzed by ultra performance liquid chromatography mass spectroscopy (UPLC/MS). The brain/plasma, olfactory bulb/plasma and CSF/plasma concentration ratios were significantly higher (*P* < 0.05) after i.n. administration of ddI nanoparticles or solution than those after i.v. administration of didanosine aqueous solution. The ratio of ddI concentration values of the nanoparticles to the solution at 180 min post-i.n. dosing was 2.1 and 1.9 in CSF and brain, respectively. Thus, both the i.n. route of administration and formulation of ddI in chitosan nanoparticles increased delivery of ddI to CSF and brain.

Kumar et al., (2009a) formulated an olanzapine nanoemulsion that potentially delivered the drug directly to the brain following intranasal administration. The nanoemulsions were prepared using the water titration method. The mucoadhesive character was imparted by the addition of 0.5 % w/w chitosan and 0.5 % w/w polycarbophil and was characterized for drug content, pH, % transmittance, globule size, zeta potential, and polydispersity index (PDI). The composition (% w/w) of the optimized olanzapine nanoemulsion was capmul MCM®, tween 80, and a mixture of 1:1 ratio of polyethylene glycol 400 and ethanol, and aqueous phase in a ratio of 15:35:17.5:32.5. The optimized olanzapine nanoemulsion exhibited a high diffusion coefficient and no nasal cilio-toxicity. The drug released followed the Higuchi model. The optimized nanoemulsions were found to be stable for 3 months.
Kumar et al., (2009b) formulated risperidone nanoemulsion (NE) and mucoadhesive NE by the spontaneous emulsification method (titration method) using Capmul MCM as the oily phase on the basis of solubility studies. The NE formulation containing 8% oil, 44% Smix, 48% w/w aqueous phase that displayed an optical transparency of 99.82%, globule size of $15.5 \pm 2.12$ nm, and polydispersity of $0.172 \pm 0.02$ was selected for the incorporation of mucoadhesive components. The mucoadhesive formulation that contained 0.5% by weight of chitosan displayed highest diffusion coefficient, followed Higuchi model was free from nasal ciliotoxicity and stable for 3 months.

Wermeling et al., (2009) compared 2.5 mg and 5.0 mg single-dose pharmacokinetics (PK), Pharmacodynamics (PD) and tolerability of an intranasal (IN) midazolam formulation, to a 2.5-mg intravenous (IV) dose. The study was designed based on an open-label, three-way crossover, randomized PK and PD study in seventeen healthy volunteers. Twelve-hour PK parameters were determined for each treatment arm. Subjects completed serial self-ratings for sedation and other drug effects. Nurse observers made serial observations for sedation and adverse effects. An otolaryngologist conducted a nasal endoscopy, pre-dose, 2 - 4 h, and at end of study, to examine the nasal cavity for formulation induced changes in nasal anatomy. Midazolam was rapidly absorbed following IN administration, with a median $t_{\text{max}}$ of 10 min. PD responses were rapid, paralleled the PK, and in magnitude was in a rank order of IV 2.5 mg ≥ IN 5.0 mg > IN 2.5 mg doses. The formulation was well tolerated with no serious cardiovascular or respiratory complications. Fourteen subjects complained of at least one of the following: a brief and mild to moderate intensity facial flushing, nasal passage burning, sore throat or bad taste after drug administration. There were no adverse findings from the nasal endoscopic examination. Dosages of an investigational IN midazolam formulation resulted in rapid absorption and attained plasma concentrations that correlated with pharmacodynamic effects.

Khatri et al., (2008) investigated the preparation and in vivo efficacy of plasmid DNA loaded chitosan nanoparticles for nasal mucosal immunization against hepatitis B. Chitosan pDNA nanoparticles were prepared using a complex coacervation process. The prepared nanoparticles were characterized for size, shape, surface charge, plasmid loading and ability of nanoparticles to protect DNA against nuclease digestion and for their transfection efficacy. Nasal administration of nanoparticles resulted in serum Anti – Hepatitis B surface antigen (anti-HBsAg) titre that was
less compared to that elicited by naked DNA and alum adsorbed HBsAg, but the mice were seroprotective within 2 weeks and the immunoglobulin level was above the clinically protective level. However, intramuscular administration of naked DNA and alum adsorbed HBsAg did not elicit sIgA titre in mucosal secretions that was induced by nasal immunization with chitosan nanoparticles. Similarly, cellular responses (cytokine levels) were poor in case of alum adsorbed HBsAg. Chitosan nanoparticles thus produced humoral (both systemic and mucosal) and cellular immune responses upon nasal administration. The study signified the potential of chitosan nanoparticles as DNA vaccine carrier and adjuvant for effective immunization through non-invasive nasal route.

Wang et al., (2008) prepared estradiol (E2)-loaded chitosan nanoparticles (CS-NPs) by ionic gelation of chitosan with tripolyphosphate anions (TPP). The CS-NPs had a mean size of 269.3 ± 31.6 nm, a zeta potential of +25.4 mV, and the loading capacity of E2 in CS-NP suspension was 1.9 mg/ml, whereas entrapment efficiency was 64.7% on average. Subsequently, the levels of E2 in blood and the cerebrospinal fluid (CSF) were investigated in rats following intranasal administration of CS-NPs. The E2-loaded CS-NPs were administered to male Wistar rats either intranasally or intravenously at the dose of 0.48 mg/kg. The plasma levels achieved following intranasal administration (Cmax 32.7 ± 10.1 ng/ml; tmax 28 ± 4.5 min) were significantly lower than those after intravenous administration (151.4 ± 28.2 ng/ml), while CSF concentrations achieved after intranasal administration (76.4 ± 14.0 ng/ml; tmax 28 ± 17.9 min) were significantly higher than those after intravenous administration (29.5 ± 7.4 ng/ml tmax 60 min). The drug targeting index (DTI) of nasal route was 3.2. The percent of drug targeting (DTP %) was 68.4%. These results showed that E2 was directly transported from the nasal cavity into the CSF in rats. When compared with E2 inclusion complex, CS-NPs improved E2 transport into the central nervous system (CNS).

Zhang et al., (2008) explored the potential of polyethylene glycol-grafted chitosan (PEG-g-chitosan) nanoparticles as a system for improving the systemic absorption of insulin following nasal administration. Insulin-loaded PEG-g-chitosan nanoparticles were prepared by the ionotropic gelation of PEG-g-chitosan solution using tripolyphosphate ions as the crosslinking agent. The nanoparticles were in the size range 150–300 nm, had a positive electrical charge (+16 to +30 mV). The physicochemical properties of nanoparticles were affected by the
composition of the copolymer. *In vitro* insulin release studies showed an initial burst followed by a slow release of insulin. Intranasal administration of PEG-g-chitosan nanoparticles in rabbits enhanced the absorption of insulin by the nasal mucosa to a greater extent than a suspension of insulin-PEG-g-chitosan and control insulin solution. PEG-g-chitosan nanoparticles are promising vehicles for insulin transport through the nasal mucosa.

Amidi et al., (2007) investigated the potential of N-trimethyl chitosan (TMC) nanoparticles as a carrier system for the nasal delivery of a monovalent influenza subunit vaccine. The antigen-loaded nanoparticles were prepared by mixing a solution containing TMC and monovalent influenza. The nanoparticles had an average size of about 800 nm with a narrow size distribution and a positive surface charge. The nanoparticles showed a loading efficiency of 78% and a loading capacity of 13% (w/w). It was shown that more than 75% of the protein remained associated with the TMC nanoparticles upon incubation of the particles in PBS for 3 h. The molecular weight and antigenicity of the entrapped hemagglutinin was maintained as shown by polyacrylamide gel electrophoresis and Western blotting, respectively. Single i.n. or i.m. immunization with antigen-loaded TMC nanoparticles resulted in strong hemagglutination inhibition and total IgG responses. These responses were significantly higher than those achieved after i.m. administration of the subunit antigen, whereas the IgG1/IgG2a profile did not change substantially. The i.n. administered antigen–TMC nanoparticles induced higher immune responses compared to the other i.n. antigen formulations, and these responses were enhanced by i.n. booster vaccinations. Moreover, among the tested formulations only i.n. administered antigen-containing TMC nanoparticles induced significant IgA levels in nasal washes of all mice. In conclusion, their findings demonstrated that TMC nanoparticles are a potent delivery system for i.n. administered influenza antigens.

Vyas et al., (2006) investigated clonazepam microemulsions (CME) for rapid drug delivery to the brain to treat acute status epileptic patients and evaluated the performance of CME *in vitro* and *in vivo* in rats. CME was radiolabeled with 99mTc (technetium) and biodistribution of drug in the brain was studied in Swiss albino rats after intranasal and intravenous administrations. Brain scintigraphy imaging in rabbits was also performed to ascertain the uptake of the drug into the brain. Pre and post CME formulation treated human nasal mucosa was subjected to transmission electron microscopy and investigated for mechanism of drug uptake across the
nasal mucosa. Brain/blood uptake ratios at 0.50 hour (h) following intranasal clonazepam mucoadhesive microemulsion (CMME), CME, clonazepam solution (CS), and intravenous CME administrations were found to be 0.67, 0.50, 0.48, and 0.13, respectively indicating more effective targeting with intranasal administration and best targeting of the brain with intranasal CMME. Brain/blood ratio at all sampling points up to 8 h following intranasal administration of CMME compared to intravenous was found to be two fold higher indicating larger extent of distribution of the drug in brain. Rabbit brain scintigraphy also showed higher intranasal uptake of the drug into the brain. Transmission electron microscopy revealed significant accretion of CMME within interstitial spaces and paracellular mode of transport due to stretching of the tight junctions present in the nasal mucosa. This investigation demonstrated a more rapid and larger extent of transport of clonazepam into the rat brain with intranasal CMME, which proved useful in treating acute status epileptics.

Amidi et al., (2006) investigated the potential of N-trimethyl chitosan (TMC) nanoparticles as a carrier system for the nasal delivery of proteins. TMC nanoparticles were prepared by ionic crosslinking of TMC solution (with or without ovalbumin) with tripolyphosphate, at ambient temperature while stirring. The size, zeta-potential and morphology of the nanoparticles were investigated as a function of the preparation conditions. Protein loading, protein integrity and protein release were studied. The toxicity of the TMC nanoparticles was tested by ciliary beat frequency measurements of chicken embryo trachea and in vitro cytotoxicity assays. The in vivo uptake of FITC-albumin-loaded TMC nanoparticles by nasal epithelia tissue in rats was studied by confocal laser scanning microscopy. The nanoparticles had an average size of about 350 nm and a positive zeta-potential. They showed a loading efficiency up to 95% and a loading capacity up to 50% (w/w). The integrity of the entrapped ovalbumin was preserved. Release studies showed that more than 70% of the protein remained associated with the TMC nanoparticles for at least 3 h on incubation in PBS (pH 7.4) at 37° C. Cytotoxicity tests with Calu-3 cells showed no toxic effects of the nanoparticles, whereas a partially reversible cilio-inhibiting effect on the ciliary beat frequency of chicken trachea was observed. In vivo uptake studies indicated the transport of FITC-albumin-associated TMC nanoparticles across the nasal mucosa. It was concluded that TMC nanoparticles are a potential new delivery system for transport of proteins through the nasal mucosa.
Rossa et al., (2004) examined intranasal (IN) administration of interferon (IFN\(\alpha\)-lb) as a route for targeted delivery to the rat central nervous system (CNS). Intranasal administration resulted in significant delivery throughout the CNS and cervical lymph nodes with low delivery to peripheral organs. At similar blood levels, intravenous (IV) administration of IFN\(\alpha\)-lb yielded 88–98% lower CNS levels and 100–1650% greater peripheral organ levels compared to intranasal administration. Autoradiography confirmed much greater delivery to the CNS with intranasal administration. Intranasally administered IFN\(\alpha\)-lb reached the brain intact and produced tyrosine phosphorylation of IFN receptor in the CNS. Intranasal administration showed a non-invasive method of drug delivery for multiple sclerosis (MS) that bypassed the blood–brain barrier (BBB) and directly targeted the CNS and lymph nodes.

Vila et al., (2004) investigated the potential utility of low molecular weight chitosan (CS) in the form of nanoparticles as new long-term nasal vaccine delivery vehicle. For this purpose, CS of low Mws (23 and 38 kDa) was obtained by a depolymerization process of the commercially available CS (70 kDa). Tetanus toxoid (TT) was used as a model antigen, which was entrapped within CS nanoparticles by an ionic cross-linking technique. TT-loaded nanoparticles were first characterized for their size, electrical charge, loading efficiency and \textit{in vitro} release of antigenically active toxoid. The nanoparticles were then administered intranasally to conscious mice in order to study their feasibility as vaccine carriers. CS nanoparticles were also labeled with FITC-BSA and their interaction with the rat nasal mucosa was examined by confocal laser scanning microscopy (CLSM). Irrespective of the CS Mw, the nanoparticles were in the 350 nm size range, and exhibited a positive electrical charge (40 mV). \textit{In vitro} release studies showed an initial burst followed by an extended release of antigenically active toxoid. Following intranasal administration, TT-loaded nanoparticles elicited an increasing and long-lasting humoral immune response (IgG concentrations) as compared to the fluid vaccine. Similarly, the mucosal response (IgA levels) at 6 months post-administration of TT-loaded CS nanoparticles was significantly higher than that obtained for the fluid vaccine. The CLSM images indicated that CS nanoparticles can cross the nasal epithelia and, hence, transport the associated antigen. Interestingly, the ability of these nanoparticles to provide improved access to the associated antigen to the immune system was not significantly affected by the CS Mw. Indeed, high and long-lasting responses could be obtained using low Mw CS molecules. Furthermore, the
response was not influenced by the CS dose (70–200 mg), achieving a significant response for a very low CS dose. In conclusion, nanoparticles made of low Mw CS are promising carriers for nasal vaccine delivery.

2.2. LITERATURE SURVEY IN THE AREA OF NANOSTRUCTURED LIPID CARRIER (NLC) BASED DRUG DELIVERY SYSTEMS

Wang et al., (2012) developed an aqueous dispersion of NLC containing CoQ10 prepared by hot high pressure homogenization technique. The CoQ10-NLC aqueous dispersion was prepared in order to improve the water solubility, photo-stability of Coenzyme Q10 (CoQ10). The physicochemical characterization parameters of CoQ10-NLC have been explored regarding particle size, zeta potential, polydispersity index (PDI) value and stability of CoQ10, which is an antioxidant. HPLC was employed to monitor the loading of CoQ10 encapsulated in NLC system. Morphology profile and hydrophobicity/hydrophilicity were determined and analyzed using atomic force microscopy (AFM) and water contact angle measurements, respectively. Infrared (IR) spectrometry was exploited to confirm the possible interaction and complex formation between CoQ10 and lipids. The photo-stability of CoQ10 was powerfully improved by NLC, more than 80% of CoQ10 remained unchanged in one month under natural daylight, while the retention of free CoQ10 only 33.5% under the same conditions. CoQ10-NLC offered comparatively high protection. The retention of CoQ10 in NLC system was about 65% after five-month exposure to natural daylight, the retention of free CoQ10 only 13.3%. The CoQ10 in the NLC-based formulation showed considerably enhanced photo-stability compared with CoQ10 itself, and displayed the better protection of CoQ10. The obtained results opened new perspectives on the photo-stability of CoQ10. The MTT analysis determined CoQ10-NLC' lower cytotoxicity. Thus, the study demonstrated that the CoQ10-NLC significantly improved the water solubility, especially the enhancement of photo-stability of CoQ10 under natural daylight.

Gu et al., (2011) designed the study to investigate whether a non-protein nanostructured lipid carrier (NLC) resembling high-density lipoprotein (HDL) could deliver a hydrophobic anti-atherogenic drug, lovastatin, to foam cells. Lovastatin-loaded NLC (LT-NLC) was prepared by a nanoprecipitation/solvent diffusion method. The LT-NLC-apoprotein (LT-NLC-apo) was prepared by incubating LT-NLC with native HDL. The
physicochemical parameters of LT-NLC were characterized in terms of particle size, zeta potential, morphology, entrapment efficiency, and crystallization behavior. Targeting behavior and mechanism were demonstrated by the incubation of LT-NLC-apo with a RAW 264.7 macrophage-derived foam cell model in the presence or absence of very-low-density lipoprotein (VLDL) and lipase. The results showed that LT-NLC was solid spherical or oval in shape with an average diameter of 13.8 ± 2.2 nm, zeta potential of -29.3 ± 0.2 mV and entrapment efficiency of 96.2 ± 1.3%. Phagocytosis studies showed that uptake of LT-NLC-apo by macrophages was significantly lower than LT-NLC (p < 0.01), suggesting that LT-NLC-apo could possibly escape recognition from macrophages in vivo. The uptake was increased twofold when LT-NLC-apo was incubated with transfected foam cells containing VLDL and lipase. These results indicated that non-protein NLC resembling HDL could be a useful tool to deliver lipophilic anti-atherogenic drugs to foam cells, and that uptake could be enhanced by the VLDL receptor pathway.

Eskandari et al., (2011) studied the delivery of valproic acid (VPA) to the brain by intranasal route. For this purpose, nanostructured lipid carriers (NLCs) were prepared by solvent diffusion method followed by ultrasonication and characterized for size, zeta potential, drug-loading percentage, and release. Six groups of rats each containing six animals received drug-loaded NLCs intraperitoneally (IP) or intranasally. Brain responses were then examined by using maximal electroshock (MES). The hind limb tonic extension: flexion inhibition ratio was measured at 15-, 30-, 60-, 90-, and 120-minute intervals. The drug concentration was also measured in plasma and brain at the most protective point using gas chromatography method. The particle size of NLCs was 154 ± 16 nm with drug loading percentage of 47% ± 0.8% and drug release of 75% ± 1.9% after 21 days. In vivo results showed that there was a significant difference between protective effects of NLCs of VPA and control group 15, 30, 60, and 90 minutes after treatment via intranasal route (P < 0.05). Similar protective effect was observed in rats treated with NLCs of VPA in intranasal route and positive control in IP route (P > 0.05). Results of drug determination in brain and plasma showed that brain:plasma concentration ratio was much higher after intranasal administration of NLCs of VPA than the positive control group (IP route). In conclusion, intranasal administration of NLCs of VPA provided a better protection against MES seizure.
Aditya et al., (2010) investigated artemether-loaded lipid nanoparticles (ARM-LNP) composed of 5% (w/v) lipid mass produced by a modified thin-film hydration method using glyceryl trimyristate (solid lipid) and soybean oil (as liquid lipid in a concentration ranging from 0 to 45% (w/v) with respect to the total lipid mass. The particles were loaded with 10% of the anti-malarial ARM and surface-tailored with a combination of non-ionic, cationic or anionic surfactants. ARM-LNP were further characterized for their mean particle size, zeta potential and encapsulation efficiency, reporting optimized values below 120 nm (PI < 0.250), -38mV and 97% (w/w), respectively. ARM-LNP composed of 45% soybean oil depicted a spherical-like shape by transmission electron microscopy and a biphasic release profile in phosphate buffer. Haemolytic activity was within the acceptable range (7%) revealing low toxicity risk of LNP for parenteral delivery of ARM. Biocompatibility was confirmed by hepato- and nephrotoxicity analyses. Histopathological analysis showed no significant histological changes in liver and kidney tissues in adult Swiss Albino mice treated with the selected formulations. In vivo anti-malarial activity of ARM was enhanced when formulated as LNP, in comparison to a conventional plain drug solution and to a marketed formulation which are currently in use to treat malaria patients.

Doktorovova et al., (2010) developed nanostructured lipid carriers (NLC) for topical delivery of fluticasone propionate (FP) with the aim to further improve the safety profile and decrease the adverse-side effects commonly reported in topical corticotherapy. NLC consisting of glyceryl palmito-stearate, and PEG-containing medium chain triglycerides mixture, stabilised by polysorbate 80 and soybean phosphatidylecholine were prepared. A mean particle size between 380 and 408 nm and entrapment efficacy of 95% were obtained for FP-loaded NLC. The crystallinity and polymorphic phase behaviour of FP-free and FP-loaded NLC were examined by differential scanning calorimetry and wide angle X-ray diffraction. Results revealed a low-crystalline structure and confirmed the incorporation of FP into the particles. The suitability of PEG-containing liquid lipids to form the lipid matrix of NLC was also confirmed.

Li et al., (2010) developed bufadienolides-loaded nanostructured lipid carriers (BU-NLC) for parenteral application using glyceryl monostearate as solid core, medium-chain triglyceride and oleic acid as liquid lipid material, and Lipoid E-80®, sodium deoxycholate and pluronic F68 as stabilizers. In this study, the in vitro cytotoxicity, pharmacokinetics, biodistribution, antitumor
efficacy and safety of BU-NLC were evaluated. Against human astrocytoma cell line (U87-MG) and human gastric carcinoma cell line (HGC-27) BU-NLC exhibited cytotoxicity that was similar to that of the free drug, and superior to that of the commercially available fluorouracil injection. BU-NLC exhibited a linear pharmacokinetic behavior at doses ranging from 0.25 to 1.0 mg/kg. The improved pharmacokinetic profile of bufadienolides when formulated in BU-NLC resulted in a higher plasma concentration and lower clearance after intravenous administration compared with bufadienolides solution (BU-S). A biodistribution study indicated that bufadienolides were mainly distributed in the lung, spleen, brain and kidney, and the longest retention was observed in the brain. A sarcoma-180 tumor model further confirmed the advantages of BU-NLC versus BU-S. Hemolysis and acute toxicity investigations showed that BU-NLC was safe when given by intravenous injection with reduced toxicity. In conclusion, the NLC system is a promising approach for the intravenous delivery of bufadienolides.

Pardeike et al., (2010) compared Cutanvoa Nanorepair Q10 cream, the first NLC containing cosmetical product introduced to the market in October 2005, to an identical o/w cream without NLC with regards to particle size, melting behaviour, rheological properties and the in vivo effect on skin hydration. The consistency, the spreadability on the skin and the subjective feeling of increase in skin hydration were evaluated using a standardized questionnaire, and compared to hydration data measured. Furthermore, it was shown by epicutaneous patch test that Cutanova Nanorepair Q10 cream had no irritating effects on the skin. By laser diffraction (LD) and differential scanning calorimetry (DSC) measurements it was shown that NLC were physically stable in Cutanova Nanorepair Q10 cream. After 7 days application of Cutanova Nanorepair Q10 cream and NLC negative control cream an increase in skin hydration was confirmed by measurements in vivo. From day 28 on the skin hydration measured in the test areas of Cutanova Nanorepair Q10 cream was significantly higher than the skin hydration in the test areas of the NLC negative control cream (p = 0.05). The subjective feeling of increase in skin hydration was also rated from the volunteers as superior for Cutanova Nanorepair Q10 cream. The rheological properties of Cutanova Nanorepair Q10 cream contributed to a better subjective impression of consistency and spreadability on the skin than found that for NLC negative control cream.

Shen et al., (2009) developed a thiolated non-ionic surfactant, cysteine-polyethylene glycol stearate (Cys-PEG-SA), for the assembling of nanoparticulate ocular drug delivery system with
mucoadhesive property. Cys-PEG-SA was synthesized in two steps reaction involving a new
derivative intermediate formation of p-nitrophenylcarbonyl-PEG-SA (pNP-PEG-SA). Up to
369.43±25.54 μmol free thiol groups per gram of the conjugates was reached. The
nanostructured lipid carrier (NLC) loaded cyclosporine A (CyA) was prepared by melt-
emulsification method. The mucoadhesive NLC (Cys-NLC) was obtained by incubating NLC
emulsion with Cys-PEG-SA. The mucoadhesive properties of these nanocarriers were examined
by using mucin particles method. The particle size or zeta potential of the porcine mucin
particles were changed with the added concentration of Cys-PEG-SA, and the disulphide bond
breaker cysteine significantly reduced the adhesion of Cys-NLC to mucin particles (P < 0.05),
whereas PEG-SA and NLC did not alternate the properties of the mucin particles. When Cys-
NLC was administered topically to the rabbit eye, the encapsulated cyclosporine was found to
remain on the ocular surface in the cul-desac for up to 6 h, both precorneal retention time and
concentration were dramatically increased (P < 0.05), compared with the NLC without thiomer
modification.

Han et al., (2008) investigated the influence of surfactants on properties of NLC. Four types of
surfactants and their mixtures were used in the absence of model drugs thereby avoiding the
interaction between surfactants and drugs. The hot high-pressure homogenization method was
employed to produce NLC and the physicochemical properties of NLC, such as particle size
distribution, zeta potential and DSC analysis were investigated. The results indicated that ionic
surfactants such as sodium deoxycholate (SDC), showed obviously low emulsification efficiency
in the preparation. However, it increased the zeta potential of nanoparticles leading to improve
the physical stability of the system. Non-ionic emulsifier, especially Poloxamer 188, offered
additional steric stabilization effect avoiding aggregation of the nanoparticles in the colloidal
system. The formulation in the study combined four types of additives including ionic surfactant
(SDC), non-ionic emulsifier (Poloxamer 188 and Tween-80), and Lecithin to obtain favorably
stable NLC drug delivery system, which could stabilize for more than 1 year without phase
separation at 4 °C.

Joshi et al., (2008) explored the potential of nanostructured lipid carriers (NLC) for the
intravenous delivery of artemether (ARM), a poorly water-soluble antimalarial agent. The NLC
of ARM (Nanoject) were formulated by employing a microemulsion template technique. The
NLC were evaluated for particle size, encapsulation efficiency, *in vitro* drug release and *in vitro* hemolysis. The antimalarial activity of the Nanoject and conventional ARM injectable formulation was evaluated in *Plasmodium berghei* infected mice. The average particle size of Nanoject was 63±28nm and the encapsulation efficiency was found to be 30±2%. The Nanoject released ARM in a sustained manner. *In vitro* haemolytic studies showed that Nanoject had lower haemolytic potential (~ 13%) as compared to all the components when studied individually. Nanoject showed significantly higher (*P* < 0.005) antimalarial activity as compared to the marketed injectable formulation. The antimalarial activity of Nanoject lasted for a longer duration (more than 20 days) indicating that Nanoject may be long-circulating *in vivo*. Nanoject showed significantly higher survival rate (60%) even after 31 days as compared to marketed formulation which showed 0% survival (100% mortality). This clearly indicated that Nanoject offered several advantages over the currently marketed oily intramuscular formulation (Larither®).

Liu et al., (2008) developed two types of 10-hydroxycamptothecin (10-HCPT) loaded nanostructured lipid carriers (NLC-F 68 and NLC-Brij 35) intended for use as the alternative formulation of 10-HCPT for parenteral administration using an emulsification-ultrasonication method and fully characterized from physicochemical and in vitro release standpoint. The particle size was measured by laser diffraction, being 108 nm and 126 nm for NLC-F 68 and NLC-Brij 35, respectively. Zeta potentials of two NLCs were 28.5 mV and 32.1 mV analyzed by photon correlation spectroscopy. The incorporated efficiency was more than 85%. It is observed that NLCs are homogeneous and spherical in shape by transmission electron microscopy. Differential scanning calorimetry analysis of NLCs showed that 10-HCPT was dispersed within NLC in an amorphous state. The combination of trehalose and mannitol as cryoprotectant was most suitable for HCPT-NLC lyophilization. The in vitro release behavior for two types of NLC was similar and displayed biphasic drug release pattern with rapid release at the initial stage and prolonged release afterwards. These results suggest that NLC could be exploited as a carrier of 10-HCPT with high incorporation efficiency and controlled release and that NLC may serve as the alternative delivery system for parenteral administration of 10-HCPT.

Teeranachaideekul et al., (2008) characterized the physicochemical properties and studied the *in vitro* release of ascorbyl palmitate from semi-solid lipid nanoparticles based on nanostructured...
lipid carriers (NLC gels) systems with the desired viscosity for dermal delivery. NLC gels were obtained by a one-step production procedure employing a high pressure homogenization technique using different solid lipid matrices. Ascorbyl palmitate (AP) was selected as a lipophilic active ingredient due to its range of cosmetic applications. After the production, particles within the size range 170–250 nm having polydispersity index lower than 0.3 were obtained from all formulations. After the AP incorporation into the NLC gels, the zeta potential increased to values higher than ±30 mV. Almost 100% encapsulation efficiency was observed. The obtained SEM and AFM data revealed nonspherical shaped nanoparticles. From DSC and X-ray diffraction studies, it was shown that the lipid recrystallized in the solid state possessing a less ordered structure as compared to the bulk material. The release study of active-loaded NLC gel formulations using Franz diffusion cells revealed that the type of lipid matrix affects both the rate and the release pattern. The viscoelastic measurements revealed a more elastic than viscous behaviour of NLC formulations indicating a typical gel-like structure.

Bondi et al., (2007) developed nanostructured lipid carriers (NLC) as colloidal carriers for two antitumor compounds that possess a remarkable antineoplastic activity. But their limited stability and low solubility in water could give a very low parenteral bioavailability. Results revealed an enhancement of the cytotoxicity effect of drug-loaded NLC on human prostate cancer (PC-3) and human hepatocellular carcinoma (HuH-6, HuH-7) cell lines with respect to that of both free drugs. Results of characterization studies strongly supported the potential application of these drugs-loaded NLC as prolonged delivery systems for lipophilic drugs by several administration routes, in particular for intravenous administration.

Teeranachaideekul et al., (2007a) studied the formulation parameters affecting the stability of AP after incorporation into NLC. These were evaluated including types of lipids, types of surfactants, storage conditions, i.e. temperature and nitrogen gas flushing, the effects of drug loading as well as types of antioxidants. After storage for 90 days, the mean particle size analyzed by photon correlation spectroscopy (PCS) was lower than 350 nm. The zeta potential measured by the Zetasizer IV was higher than ±30 mV in all developed AP-loaded NLC formulations which varied according to the types of lipid and surfactant. Concerning the chemical stability of AP, it was found that AP-loaded NLC prepared and stored in non-degassing conditions, a higher percentage of AP loading in NLC, lower storage temperature (4 °C),
addition of antioxidants as well as selection of suitable surfactants and solid lipids improved the chemical stability of AP. Moreover, an improvement of long-term chemical stability of AP was achieved by addition of antioxidants with nitrogen gas flushing as compared to those without antioxidant. The percentage of drug remaining at both 4 °C and room temperature (25 °C) was higher than 85% during 90 days of storage.

Teeranachaideekul et al., (2007b) developed nanostructured lipid carriers (NLC) composed of cetyl palmitate with various amounts of caprylic/capric triacylglycerols (as liquid lipid) and Coenzyme Q10 (Q10) was incorporated in such carriers due to its high lipophilic character. A nanoemulsion composed solely of liquid lipid was prepared for comparison studies. By photon correlation spectroscopy a mean particle size in the range of 180–240 nm with a narrow polydispersity index (PI) lower than 0.2 was obtained for all developed formulations. The entrapment efficiency was 100% in all cases. The increase of oil loading did not affect the mean particle size of NLC formulations. NLC and nanoemulsion, stabilized by the same emulsifier, showed zeta potential values in the range - 40/- 50 mV providing a good physical stability of the formulations. Scanning electron microscopy studies revealed NLC of disc-like shape. With respect to lipid polymorphism, a decrease in the ordered structure of NLC was observed with the increase of both oil and Q10 loadings, allowing therefore high accommodation for Q10 within the NLC. Using static Franz diffusion cells, the in vitro release studies demonstrated that Q10-loaded NLC possessed a biphasic release pattern, in comparison to Q10-loaded nanoemulsions comprising similar composition of which a nearly constant release was observed. The NLC release patterns were defined by an initial fast release in comparison to the release of NE followed by a prolonged release, which was dependent on the oil content.

Li et al., (2008) developed an ocular drug delivery system based on nanostructured lipid carrier and investigated its in vitro and in vivo characteristics. Ibuprofen was chosen as the model drug. Four different formulations of Ibuprofen nanostructured lipid carriers were prepared by melted-ultrasonic methods; Gelucire 44/14 was screened as one of the solid lipid matrix materials due to the good particle size dispersion and excellent contribution to the corneal permeability of the model drug. The modified Franz-type diffusion cells and isolated corneas were used in the test of drug corneal permeability and the in vivo releasing tests were carried out using microdialysis method. Ibuprofen nanostructured lipid carriers displayed controlled-release property. The AUC
of the optimized formulation of ibuprofen nanostuctured lipid carriers was 3.99 times more than that of ibuprofen eye drops.

Souto and Muller (2006) investigated the effectiveness of clotrimazole (fungicidal) incorporated into nanostructured lipid carriers (NLC) for the local treatment of cutaneous and mucosal infections compared with solid lipid nanoparticles (SLN). The aim was to increase its dermal bioavailability and to control drug release, thereby potentially reducing its side effects. Prior to the release studies, the carrier was optimized and characterized by using different techniques. Laser diffractometry (LD), photon correlation spectroscopy (PCS) and scanning electron microscopy (SEM) indicated that SLN were spherical in shape with a mean size of 400 nm. Some aggregation phenomena occurred during preparation of SEM samples due to the lipid character of the carriers. No physico-chemical instability of the drug-loaded lipid nanoparticles was detected during 2 years of storage at different temperatures. X-ray and DSC results suggested that during storage time the drug remained molecularly dispersed in the lipid matrix.

Souto et al., (2006) studied the suitability of Compritol 888 ATO for the production of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for the entrapment of a lipophilic model drug. This study assesses the crystalline structure of the bulk lipid, as well as the changes that occur in its crystal lattice with the addition of ‘impurities’, such as oil (\(\alpha\)-tocopherol) and drug (ketoconazole), using DSC and X-ray diffraction analysis before and after thermal stress. Aqueous SLN and NLC dispersions were produced using an appropriate surfactant/co-surfactant system and their physicochemical stability was assessed by PCS, LD, DSC and by WAXS. It was found that the crystalline lattice of Compritol 888 ATO is composed of very small amounts of the unstable \(\alpha\) polymorphic form characteristic of triacylglycerols, which disappears after thermal stress of bulk lipid. Mixing oils and drug molecules which are soluble in this lipid decreased its lattice organization and, thus, was revealed to be suitable for production of lipid nanoparticles containing ketoconazole. However, particle growth could not be avoided during shelf life.

Hu et al., (2005) developed stearic acid (SA) nanostuctured lipid carriers with various oleic acid (OA) content by solvent diffusion method in an aqueous system. The size and surface morphology of nanoparticles were significantly influenced by OA content. As OA content
increased up to 30 wt%, the obtained particles showed pronounced smaller size and more regular morphology in spherical shape with smooth surface. Compared with solid lipid nanoparticles (SLN), NLC exhibited improved drug loading capacity, and the drug loading capacity increased with increasing OA content. These results were explained by differential scanning calorimetry (DSC) investigations. The addition of OA to nanoparticles formulation resulted in massive crystal order disturbance and less ordered matrix of NLC, and hence, increased the drug loading capacity. The drug in vitro release behavior from NLC displayed biphasic drug release pattern with burst release at the initial stage and prolonged release afterwards, and the successful control of release rate at the initial stage can be achieved by controlling OA content.

Jores et al., (2003) compared the structure and performance of colloidal dispersions of nanostructured lipid carriers (NLCs) with solid lipid nanoparticles (SLNs). Colloidal lipid dispersions were produced by high-pressure homogenization and characterized by laser diffraction, photon correlation spectroscopy, wide-angle x-ray scattering, and differential scanning calorimetry. Proton nuclear magnetic resonance spectroscopy and electron spin resonance experiments were performed to investigate the mobility of the components and the molecular environment of model drugs. Furthermore, a nitroxide reduction assay with ascorbic acid was conducted to explore the accessibility of the lipid model drug from the outer aqueous phase. Proton nuclear magnetic resonance spectra clearly demonstrated that NLC nanoparticles differ from nanoemulsions and from SLNs by forming a liquid compartment that is in strong interaction to the solid lipid. The electron spin resonance model drug was found to be accommodated either on the particle surface with close water contact (SLN) or additionally in the oil (NLC). The oil compartment must be localized on the particle surface, because it can be easily reached by ascorbic acid. Neither SLN nor NLC lipid nanoparticles showed any advantage with respect to incorporation rate or retarded accessibility to the drug compared with conventional nanoemulsions. Based on the experimental data it was concluded that NLCs are not spherical solid lipid particles with embedded liquid droplets, but they are rather solid platelets with oil present between the solid platelet and the surfactant layer.
2.3. REVIEW OF PATENTS RELATED TO DRUG DELIVERY TO BRAIN

Nnochiri et al., (2005/6956051 B1) invented amphiphilic drug-oligomer conjugates that were capable of traversing the blood-brain barrier ("BBB") and methods of making and using such conjugates. An amphiphilic drug-oligomer conjugates was comprised of a therapeutic compound conjugated to an oligomer, wherein the oligomer was comprised of a lipophilic moiety coupled to a hydrophilic moiety. The conjugates of the invention was further comprise of therapeutic agents such as proteins, peptides, nucleosides, nucleotides, antiviral agents, antineoplastic agents, antibiotics, etc., and prodrugs, precursors, derivatives and intermediates thereof, chemically coupled to amphiphilic oligomers.

Reinhard C. and Frey II WH (2006/0216317 A1) invented a method for delivering polynucleotide agents (DNA or mRNA) that poorly cross or are unable to cross the BBB to the central nervous system of a mammal by way of a neural pathway originating in either the olfactory region of the nasal cavity, or in an intranasal or extranasal tissue that is innervated by the trigeminal nerve. The disclosed method precluded the drug delivery obstacle imposed by the mammalian blood-brain barrier. More specifically, the invention provided a method for the delivery of polynucleotides to the CNS of a mammal through or by way of neural pathway associated with the olfactory or trigeminal nerves. Agents delivered according to the method of the invention circumvent the BBB and were delivered directly to the CNS. The delivery method provided for the direct transport of exogenous agents into the CNS. In this manner, a polynucleotide agent might be transported along a neural pathway to the CNS, or by way of a perivascular channel, a prelymphatic channel, or a lymphatic channel associated with the brain and/or spinal cord.

Heppe et al., (2006/0051423 A1) disclosed a chitosan-based transport system for overcoming the blood-brain barrier. This transport system can convey active agents or markers into the brain. The transport system contains at least one substance selected from the group consisting of chitin, chitosan, chitosan oligosaccharides, glucosamine, and derivatives thereof, and optionally one or more active agents and/or one or more markers and/or one or more ligands. A particular problem was the transport of hydrophilic substances through the BBB. Pharmaceutical researchers therefore were looking for ways to encapsulate such hydrophilic substances in lipophilic particles or bind them to particles with substances that permit receptor-mediated
transport across the BBB. Chitosan has some interesting properties and has been studied in many areas of medicine and pharmaceutics. It is known that nanoparticles with chitosan coats or nanocapsules can transport pharmaceuticals into the body or overcome the skin-blood or intestine-blood barrier. These barriers are overcome relatively easily. However the blood-brain barrier (BBB) is one of the most problematic barriers to overcome as it has highly selective transport systems and as the cells are very tightly joined. Thus, the invention was related to provide a chitosan-based transport system for overcoming the blood-brain barrier. This transport system is to convey active agents or markers into the brain.

Cataldo et al., (2008/0131409 A1) disclosed the use of marrow-derived adult progenitor cell (MAPCs) in methods of treating various medical disorders typically caused by or involving loss of cells or loss of cell functions. These cells when administered intranasally can cross the blood brain barrier and terminally differentiate into cholinergic neurons or otherwise localize and terminally differentiated. It was believed to be the first report claiming mammalian cells, specifically MAPCs, to be delivered by intranasal administration were capable of entering the olfactory bulb and brain. Several transport pathways exist from the nasal epithelium to brain and include: 1) the olfactory nerve pathway; 2) the olfactory epithelial pathway; and 3) the systemic pathway (Mathison et al, 1998; Illium, 2000). The olfactory nerve pathway and the olfactory epithelial pathway provide direct passage to brain. The systemic pathway utilizes the systemic circulation, with substances or cells entering capillaries in either the respiratory or olfactory epithelium. Passage of materials through this route is generally slow due to limited movement through the BBB. The findings indicated the rapid appearance of MAPCs within the olfactory bulb and the neocortex, which was consistent with the cells entering through the intercellular clefts in the olfactory epithelium, i.e. the glomerular cell layer, or extracellularly along the neurons to reach the CSF and the brain. The interpretation was also supported by the appearance of GFP-positive MAPCs in the order: nasal cavity—>olfactory mucosa—>olfactory bulb—>CNS.

Sung et al., (2009/0155374 A1) invented nanoparticles with pharmaceutical composition of chitosan and polyglutamic acid with bioactive agents and delivered by means of via oral or nasal absorption with enhanced permeability for the purpose of medical uses. It was one object of the invention to provide a novel nanoparticle system and methods of preparation for paracellular...
transport drug delivery using a simple and mild ionic-gelation method upon addition of, for example, a polyy-glutamic acid (y-PGA) solution, into regular molecular weight chitosan solution. In one embodiment, the chitosan employed was N-trimethyl chitosan (TMC). In an alternate embodiment, the chitosan employed was low molecular weight chitosan (low-MW CS). In one embodiment, the molecular weight of a low-MW CS of the invention was about 80 kDa or less, preferably at about 40 kDa, adapted for adequate solubility at a pH that maintains the bioactivity of protein and peptide drugs. It was stipulated that a chitosan particle with about 30-50 kDa molecular weight was kidney inert. The particle size and the zeta potential value of the prepared nanoparticles were controlled by their constituted compositions. The results obtained by the TEM (transmission electron microscopy) and AFM (atomic force microscopy) examinations showed that the morphology of the prepared nanoparticles was generally spherical or spheroidal in shape. Evaluation of the prepared nanoparticles in enhancing intestinal paracellular transport was investigated \textit{in vitro} in Caco-2 cell monolayers. Some aspects of the invention provided the nanoparticles with CS dominated on the surfaces to effectively reduce the transepithelial electrical resistance (TEER) of Caco-2 cell monolayers. The confocal laser scanning microscopy (CLSM) observations confirmed that the nanoparticles or fragments thereof with CS dominating on the surface are able to open the tight junctions between Caco-2 cells and allowed transport of the nanoparticles via the paracellular pathways.

Bentz et al., (2009/0136505 A1) revealed the pharmaceutical compositions and methods for delivering a polypeptide to the central nervous system of a mammal via intranasal administration. The polypeptide can be a catalytically active protein or an antibody, antibody fragment or antibody fragment fusion protein. The polypeptides are formulated with one or more specific agents. It has been discovered that globular protein molecules, such as an antibody fragment linked to a therapeutic peptide or protein, can be delivered directly to the central nervous system of a mammal, thereby bypassing the blood brain barrier. Accordingly, methods of delivering a therapeutic composition to the central nervous system of a mammal are provided. The methods are advantageous in treating a wide variety of disease or conditions. Methods of delivering therapeutic compositions to the CNS, including the brain and spinal cord and cervical nodes, of a mammal by a non-systemic route are provided. The delivery method therefore allows for localized and targeted of the therapeutic compositions to the brain via the nasal passage.
Consequently, the relates to delivery of the composition by a route other than intravenous, intramuscular, transdermal, intraperitoneal, or similar route which deliver the composition through for example the blood circulatory system. It has been discovered that antibody fragments conjugated or otherwise linked to a therapeutic polypeptide may be delivered to the CNS including the brain and spinal cord and cervical nodes, of a mammal by administration of fusion molecule intranasally.
2.4. REVIEW OF PATENTS RELATED TO LIPID NANOCARRIERS

Bondi’ et al., (2010/0247619 A1) invented nanoparticulate lipid vector (i.e., NLC) consisting of riluzole trapped in lipids, and the preparation and characterization and their use to prepare medicinal products for the treatment of Amyotrophic Lateral Sclerosis and Multiple Sclerosis. The systems obtained exhibited a different biodistribution from the free drug in vivo, and could be used to prepare pharmaceutical formulations. A mixture of mono-, di- and triglycerides with behenic acid with the use of surfactants such as phosphatidylcholin and co-surfactants such as taurocholic acid sodium salt was employed for the preparation of nanoparticles. The system was subjected to in vivo studies on rats to evaluate the differences in biodistribution between the drug carried by the nanoparticles and the free drug. The trial was also designed to establish whether the neuroprotective action of the drug delivered by the system was more effective than that of the free drug. In particular, tests were performed to establish whether the rats treated with the carried riluzole manifested clinical signs of allergic encephalomyelitis, the experimental model of Multiple Sclerosis, more slowly than those treated with free riluzole. The results demonstrated that the nanoparticulate systems according to the invention cross the blood brain barrier (BBB) more easily, thus enabling the drug to reach higher concentrations in the CNS. These results were verified on rats in both acute and chronic tests.

Shastri et al., (2006/0083781 A1) disclosed solid lipid nanoparticles comprising a neutral lipid and a first functionalized polymer comprising at least on ionic or ionizable moiety and methods for providing same. In a further aspect, the invention related to tumor targeting therapeutic systems multimodel diagnostic therapeutic systems, thermoceptive payload delivery systems, magnetic driven targeting systems, therapeutic diagnostic systems, stabilized ink compositions, and cosmetic formulations comprising the solid lipid nanoparticles of the invention. In a further aspect, the invention related to methods of delivering one biologically active agents, pharmaceutically active agent, magnetically active agent or imaging agent across the blood brain barrier, across a cellular lipid bilayer and into a cell, and to a subcellular structure.

Nazzal and Sylvester (2011/0052704 A1) disclosed compositions containing tocotrienol, non-tocotrienol lipids and surface active agents; compositions containing particles having a statin and a tocotrienol wherein the particle size is less than 1000 nm; and microemulsions containing a statin and a tocotrienol were disclosed. Methods relating to the creation of such composition and
the use of such composition are further disclosed. NLC loaded with tocotrienol-rich-fraction of palm oil were prepared. The anti-proliferative effects of those NLC was evaluated against neoplastic +SA mammary epithelial cells. Melt emulsification method was used in the preparation of NLC containing surfactant to lipid ratio of about 0.5:1, with a total lipid concentration of about 0.25% w/v, from sonication at about 60% pulsar rate for about 10 minutes. The nanoparticles evaluated showed stability after several months of storage and exhibited potent-antiproliferative effect against neoplastic +SA mammary cells.
2.5. REVIEW OF PATENTS RELATED TO DULOXETINE

Jain et al., (2010/0209498 Al) invented the methods to address and overcome the commonly encountered degradation problems with the formulations of duloxetine. Duloxetine is acid labile and acid hydrolysis of its ether linkage results in a thienyl alcohol and 1-naphthol. 50% of the dosage is hydrolyzed to 1-naphthol within one hour at a pH of 1.0, which is achieved under fasting conditions. At a pH of 2.0, 10% of the dosage degrades to 1-naphthol in one hour and at a pH of 4.0, 10% degradation would take up to 63 hours. Typically, such acid sensitive compounds are formulated as enteric-coated pellets to protect them from degradation. A stable formulation of duloxetine was prepared by encapsulating duloxetine or a salt thereof in a capsule and coated the capsule with an enteric coat polymer. Thus, the duloxetine did not react with the enteric polymer and the enteric coating further prevented the duloxetine from degradation and provided a stable formulation. In another embodiment, a stable formulation of duloxetine was prepared by melt granulating the duloxetine or a salt thereof with a pharmaceutically acceptable carrier. The granules was further coated with a seal coat polymer and enteric coat polymers. The carrier coats the duloxetine or a salt thereof and hence prevented the exposure of duloxetine or a salt thereof to the enteric polymer and thus provided a stable formulation. The inventors also discovered that duloxetine could be stabilized against acid degradation by conjugating it with ion exchange resins. The conjugation between duloxetine and the ion exchange resin particles resulted from ionic bonds between oppositely charged species because of their mutual electrostatic attraction. The resulting stabilization was sufficient to prevent degradation of duloxetine in acidic pH.

Biswas and Karamjai (2010/0261775 Al) invented duloxetine hydrochloride Form I, which is suitable for preparing pharmaceutical dosage forms, a process for preparing Form I of duloxetine hydrochloride and also developed a simple and efficient process for preparing duloxetine hydrochloride from duloxetine maleate. The process for the preparation of Form I of duloxetine hydrochloride comprised of dissolving duloxetine hydrochloride in a solvent, treating the solution obtained with an anti-solvent and isolating Form I of duloxetine hydrochloride from the reaction mixture. The process consists of taking a suspension of duloxetine maleate (20 g) in water was basified to about pH 12 using 30% aqueous sodium hydroxide solution at about 25°C. The reaction mixture was extracted with toluene (2x200 mL). The toluene layer was washed with water till the pH was between 7 and 8 and then concentrated under reduced pressure to obtain
duloxetine hydrochloride as an oily mass which was dissolved in ethyl acetate (90 mL). The pH of the solution was adjusted to between 1.5 and 2.0 using a solution of hydrochloric acid in ethyl acetate [Assay -8% (w/w)] at 5-10° C. to attain the pH of 1.5 to 2.0. The reaction mixture was stirred at 5°-10° C. for 2 h. The resultant solid was filtered, washed with ethyl acetate (2x20 mL) and dried under vacuum at 45°-50° C. for 8-10 h to obtain duloxetine hydrochloride as an off-white solid. The Form 1 of duloxetine Hydrochloride was obtained by making a mixture of duloxetine hydrochloride in absolute ethanol (30 mL) was stirred at 65°-70° C. for 15 minutes to obtain a clear solution. Activated charcoal (1.0 g) was added to the solution so obtained and stirred at 65°-70° C. for further 30 minutes. The charcoal was filtered and washed with absolute ethanol (3x15 mL) at about 25° C. Diisopropyl ether was added (35 mL) to the combined filtrate and washed at 40°-45° C. The reaction mixture was reheated to 65°-68° C. for 15 minutes and cooled to 25°-30° C. to obtain a white solid as a precipitate. The mixture was stirred at 25°-30° C. for 2 h and further at 5°-10° C. for 2 h. The solid was filtered, washed with a mixture of absolute ethanol (7.5 mL) and diisopropyl ether (7.5 mL) at about 25° C. and dried under vacuum at 45°-50° C. for 8 to 10 h to obtain the Form 1 of duloxetine Hydrochloride.

In this process, duloxetine hydrochloride was chemically and/or enantiomerically pure pharmaceutically acceptable salts of duloxetine or pure duloxetine hydrochloride. The terms "chemically pure duloxetine HCl" and "chemically pure pharmaceutically acceptable salt of duloxetine" refer to duloxetine hydrochloride/ pharmaceutically acceptable salt of duloxetine, containing less than about 0.14 percent area by HPLC of the DLX-ISO3 impurity. Preferably, the level of DLX-ISO3 is less than about 0.07 percent area by HPLC, and, most preferably, is less than about 0.02 percent area by HPLC. A chemically pure duloxetine HCl/pharmaceutically acceptable salt of duloxetine in accordance with the invention may be substantially free of DLX-ISO3, such that the DLX-ISO3 is below the detection limit; i.e., the chemically pure duloxetine HCl/pharmaceutically acceptable salt of duloxetine preferably contains essentially 0.0 percent DLX-ISO3 within the error limits of the detection. The term "enantiomerically pure duloxetine HCl" and "enantiomerically pure pharmaceutically acceptable salt of duloxetine" refer to a duloxetine HCl/pharmaceutically acceptable salt of duloxetine, containing less than about 0.04 percent area by HPLC of the duloxetine R-enantiomer. Preferably, the level of the duloxetine R-enantiomer is less than about 0.03 percent area by HPLC, and, more preferably, is less than about
0.02 percent area by HPLC. An enantiomerically pure duloxetine HCl/ pharmaceutically acceptable salt of duloxetine in accordance with the invention may be substantially free of the R-enantiomer, such that the R-enantiomer is below the detection limit; i.e., the enantiomerically pure duloxetine HCl/pharmaceutically acceptable salt of duloxetine preferably contains essentially 0.0 percent R-enantiomer within the error limits of the detection. This process comprises dissolving duloxetine in water or a solvent selected from the group consisting of acetone, methyl ethyl ketone (MEK), methyl t-butyl ether (MTBE), ethanol, isopropanol, and n-butanol, and mixtures thereof with water, and crystallizing duloxetine HCl. Preferably, the solvent is a mixture of acetone and water or isopropanol. The duloxetine HCl obtained after the crystallization contains less than about 0.14 percent area by HPLC DLX-1S03 and less than about 0.04 percent of the R-enantiomer of duloxetine. The crystallization process may be repeated in order to increase the chemical and enantiomeric purity even further either with the same or a different solvent that was used for the first crystallization.
2.6. LITERATURE SURVEY IN THE AREA OF METHOD DEVELOPMENT FOR THE ANALYSIS OF DULOXETINE

Samanidou et al., (2012) developed a novel and simple high-performance liquid chromatography method for the simultaneous determination of two selective serotonin reuptake inhibitors (fluoxetine and paroxetine) and two serotonin-norepinephrine reuptake inhibitors (venlafaxine and duloxetine) in alternative samples of toxicological interest such as hair, nail clippings, and cerebrospinal fluid (CSF). The separation was achieved on a Hichrom Kromasil 100-5C(18) (250 x 4.6 mm) 5 μm column by using ammonium acetate (0.05 M)-acetonitrile (59:41% v/v) as the mobile phase, delivered isocratically at a flow rate of 1.3 mL/min. Ultraviolet detection at 235 nm was used for monitoring the eluting analytes. Validation was performed in terms of linearity, selectivity, accuracy, precision, and stability. Correlation coefficients were greater than 0.9954. The limits of quantitation ranged between 0.3 and 2.1 ng/μL for all analytes in the liquid matrix (CSF), while the respective values were in the range of 0.3-3.6 ng/mg for solid matrices (hair and nail clippings), with an injection volume of 20 μL. Repeatability and intermediate precision (relative standard deviation, RSD%) were less than 16.6%. The method was successfully applied to actual hair and nail samples from a patient under fluoxetine treatment.

Chhalotiya et al., (2010) developed a stability-indicating RP-HPLC method for duloxetine hydrochloride (DUL) in the presence of its degradation products generated from forced decomposition studies. The drug substance was found to be susceptible to stress conditions of acid hydrolysis. The drug was found to be stable to dry heat, photodegradation, oxidation and basic condition attempted. Successful separation of the drug from the degradation products formed under acidic stress conditions was achieved on a Hypersil C-18 column (250 mm × 4.6 mm id, 5μm particle size) using acetonitrile: 0.01 M potassium dihydrogen phosphate buffer (pH 5.4 adjusted with orthophosphoric acid) (50:50, v/v) as the mobile phase at a flow rate of 1.0 ml/min. Quantification was achieved with photodiode array detection at 229 nm over the concentration range 1-25 μg/ml with range of recovery 99.8-101.3 % for DUL by the RP-HPLC method. Statistical analysis proved the method to be repeatable, specific, and accurate for estimation of DUL.
Patel et al., (2010) described the development of a stability-indicating RP-HPLC method for duloxetine hydrochloride (DLX) in the presence of its degradation products generated from forced decomposition studies. The drug substance was found to be susceptible to stress conditions of acid, base, oxidation, wet heat, dry heat, and photodegradation. The drug was found to be stable to the dry heat condition attempted. Successful separation of the drug from the degradation products formed under stress conditions was achieved on a Phenomenex Cl8 column (250 x 4.6 mm id, 5 micron particle size) using acetonitrile-methanol-0.032 M ammonium acetate buffer (55:05:40, v/v/v) as the mobile phase at a flow rate of 1.0 mL/min at 40 °C temperature. Quantification was achieved with photodiode array detection at 290 nm over the concentration range 0.2-5 microg/mL with mean recovery of 101.048 ± 0.53% for DLX by the RP-HPLC method. Statistical analysis proved the method was repeatable, specific, and accurate for estimation of DLX.

Choong et al., (2009) developed a simple and sensitive LC-MS method and validated for the simultaneous quantification of aripiprazole (ARI), atomoxetine (ATO), duloxetine (DUL), clozapine (CLO), olanzapine (OLA), sertindole (STN), venlafaxine (VEN) and their active metabolites dehydroaripiprazole (DARI), norclozapine (NCLO), dehydrosertindole (DSTN) and O-desmethylvenlafaxine (OVEN) in human plasma. The above mentioned compounds and the internal standard (remoxipride) were extracted from 0.5 mL plasma by solid-phase extraction (mix mode support). The analytical separation was carried out on a reverse phase liquid chromatography at basic pH (pH 8.1) in gradient mode. All analytes were monitored by MS detection in the single ion monitoring mode and the method was validated covering the corresponding therapeutic range: 2-200 ng/mL for DUL, OLA, and STN, 4-200 ng/mL for DSTN, 5-1000 ng/mL for ARI, DARI and finally 2-1000 ng/mL for ATO, CLO, NCLO, VEN, OVEN. For all investigated compounds, good performance in terms of recoveries, selectivity, stability, repeatability, intermediate precision, trueness and accuracy, was obtained. Real patient plasma samples were then successfully analysed.

Srinivasulu et al., (2009) described the development of a reversed phase liquid chromatographic (RPLC) analytical method for duloxetine HCl in the presence of its impurities and degradation products generated from forced decomposition studies. The drug substance was subjected to
stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of duloxetine HCl was observed under acid hydrolysis. The drug was found to be stable in other stress conditions studied. Successful separation of the drug from the synthetic impurities and degradation products formed under stress conditions was achieved on a Zorabax XDB C18, 50 mm x 4.6 mm, 5.0 micron column using a mixture of aqueous 0.1% trifluoroacetic acid, methanol, tetrahydrofuran (60:20:20, v/v/v) as mobile phase. The HPLC method developed was validated with respect to linearity, accuracy, precisions, specificity and ruggedness.

Melo et al., (2009) developed a new polymeric coating consisting of a dual-phase, polydimethylsiloxane (PDMS) and polypyrrole (PPY) for the stir bar sorptive extraction (SBSE) of antidepressants (mirtazapine, citalopram, paroxetine, duloxetine, fluoxetine and sertraline) from plasma samples, followed by liquid chromatography analysis (SBSE/LC-UV). The extractions were based on both adsorption (PPY) and sorption (PDMS) mechanisms. SBSE variables, such as extraction time, temperature, pH of the matrix, and desorption time were optimized, in order to achieve suitable analytical sensitivity in a short time period. The PDMS/PPY coated stir bar showed high extraction efficiency (sensitivity and selectivity) toward the target analytes. The quantification limits (LOQ) of the SBSE/LC-UV method ranged from 20 ng mL(-1) to 50 ng mL(-1), and the linear range was from LOQ to 500 ng mL(-1), with a determination coefficient higher than 0.99. The inter-day precision of the SBSE/LC-UV method presented a variation coefficient lower than 15%. The efficiency of the SBSE/LC-UV method was proved by analysis of plasma samples from elderly depressed patients.

Dhaneshwar et al., (2008) described a simple, precise and accurate HPTLC method for its estimation as bulk and in tablet dosage form. The chromatographic separation was carried out on precoated silica gel 60 F254 aluminium plates using mixture of chloroform:methanol (8:1 v/v) as mobile phase and densitometric evaluation of spots was carried out at 235 nm using Camag TLC Scanner-3 with win CAT 1.3.4 version software. The experimental parameters like band size of the spot applied, chamber saturation time, solvent front migration, slit width etc. were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved with Rf value 0.11±0.01. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity (40-200 ng/spot), precision (intra-day
Chapter 2

Literature review

RSD 0.46-0.75%, inter-day RSD 0.46-1.59%), accuracy (98.72±0.20) and specificity according to ICH guidelines. The proposed method can analyse ten or more formulation units simultaneously on a single plate and provides a faster and cost-effective quality control tool for routine analysis of duloxetine hydrochloride as bulk drug and in tablet formulation.

Patel et al., (2008) developed a simple, specific, accurate and precise method, namely, reverse phase high performance liquid chromatography for estimation of duloxetine HCl in pharmaceutical formulations. For the high performance liquid chromatography method, Phenomenox C-18, 5 μm column consisting of 250×4.6 mm i.d. in isocratic mode, with mobile phase containing 0.01M 5.5 pH phosphate buffer: acetonitrile (60:40 v/v) and final pH adjusted to 5.5±0.02 with phosphoric acid was used. The flow rate was 1.2 ml/min and effluent was monitored at 231 nm. The retention time was 5.61 min. The method was validated in terms of linearity, accuracy and precision. The linearity curve was found to be linear over 0.25-4 μg/ml. The limit of detection and limit of quantification were found to be 0.10 and 0.25 μg/ml respectively. The proposed method was successfully used to determine the drug content of marketed formulations.

Satohin et al., (2007) developed a sensitive bioanalytical method for the measurement of two major circulating metabolites of duloxetine [4-hydroxy duloxetine glucuronide (LY550408) and 5-hydroxy-6-methoxy duloxetine sulfate (LY581920)] in plasma. This method produced acceptable precision and accuracy over the validation range of 1-1000 ng/mL. Several issues were addressed in order to develop an LC/MS/MS assay for these metabolites. First, 4-hydroxyduloxetine glucuronide required chromatographic resolution from the 5-, and 6-hydroxy duloxetine glucuronide isomers. Second, the glucuronide conjugate is readily ionized under positive ESI conditions, while the sulfate conjugate required negative ESI conditions to obtain adequate sensitivity. Finally, the chromatographic conditions needed to separate the glucuronide isomers were not suitable for the analysis of the sulfate conjugate. The present method addressed these challenges, and was successfully applied to multiple human pharmacokinetic studies in which subjects received oral doses of duloxetine hydrochloride.
Mercolini et al., (2007) developed a new reliable analytical method for the determination of duloxetine plasma levels in depressed patients. The present paper dealt with the development of a rapid and sensitive high-performance liquid chromatographic method for duloxetine analysis in human plasma. The assays were carried out using a C8 reversed-phase column and a mobile phase composed of 60% aqueous phosphate buffer containing triethylamine at pH 3.0 and 40% acetonitrile. The UV detector was set at 230 nm and loxapine was used as the internal standard. An original pre-treatment of plasma samples was developed, based on solid-phase extraction (SPE) with mixed-mode reversed phase-strong cation exchange cartridges (30 mg, 1 mL). The extraction yields values were higher than 90%. Linearity was found in the 2-200 ng mL\(^{-1}\) duloxetine concentration range; the limit of quantitation was 2.0 ng mL\(^{-1}\) and the limit of detection was 0.7 ng mL\(^{-1}\). The method was applied to plasma samples from depressed patients undergoing therapy with duloxetine. Precision data and accuracy results were satisfactory and no interference from other drugs was found. Thus, the method was considered to be suitable for the therapeutic drug monitoring of duloxetine in depressed patients' plasma.

Kamila et al., (2007) developed a simple, sensitive and accurate UV spectrophotometric method for the assay of duloxetine hydrochloride in raw material and capsules. Validation of the method yielded good results concerning range, linearity, precision and accuracy. The absorbance was measured at 290 nm for duloxetine capsule solution. The linearity range was found to be 5-50 microg/mL for the drug. It was found that the excipients in the commercial formulation did not interfere with the methods.

Ma et al., (2007) developed and validated a rapid and sensitive liquid chromatography-mass spectrometric (LC/MS) method for the determination of duloxetine in human plasma using flupentixol as the internal standard (I.S.). Sample preparation of the plasma involved deproteination with acetonitrile twice, repeatedly. Samples were then analyzed by HPLC on a Thermo Hypersil-Hypurity C18 column (150 x 2.1 mm, 5 microm). A single-quadrupole mass spectrometer with an electrospray interface was operated in the selected-ion monitoring mode to detect the \([M+H]^+\) ions at 298 m/z for duloxetine and at 435 m/z for the internal standard. Pharmacokinetics was measured in 12 healthy Chinese male volunteers (6 males and 6 females) who received a single regimen with 3 different dosages at 22.4, 44.8 and 67.2 mg.
Chapter 2  

Literature review

of duloxetine enteric-coated capsules. The developed method was sensitive and specific for quantifying duloxetine levels in human plasma and successfully applied to a clinic pharmacokinetic study of an enteric-coated capsule of duloxetine hydrochloride administered as a single oral dose.