Chapter 11

Investigations on the in vivo performance of Olanzapine nanosuspension

11.1 INTRODUCTION

Various approaches have been tried to increase the brain delivery of centrally acting xenobiotics (Aquilante CL et al., 2000; Chekhonin VP et al., 1991; Rapoport, 1988; Ambruosi A et al., 2005; Yang CS et al., 1999; Friden PM., 1995;). Use of P Glycoprotein (Pgp) inhibitors to increase the brain delivery is a relatively new venture. Use of nanoparticles / nanosuspensions stabilized with Pgp inhibitors is not very well reported world wide. Wang and co workers determined P-gp inhibition of some commonly used excipients using an integrated high-throughput process (Wang SW et al., 2004). From the list of excipients reported by Wang SW and co workers, we have selected poloxamer 407 and d – α - Tocopherol Polyethylene Glycol Succinate 1000 for preparation of OL nanosuspension. It was hypothesized that the above excipients can inhibit the P-gp drug efflux pump present at the BBB and enhances the brain delivery of olanzapine.

Nanosuspensions of OL were prepared and characterized. The nanosuspensions were also subjected to stability evaluation at different storage conditions (Chapter 11). In the present chapter, the pharmacokinetics and biodistribution studies were performed for the nanosuspensions using radiolabeling technique to assess their potential for brain delivery of OL.

Preparation of olanzapine nanosuspension

Olanzapine (OL) nanosuspensions were prepared by pearl milling technique (Verhoff F et al., 2003). Briefly, 100 mg of poloxamer 407 was dissolved in 10 ml of distilled water in a 20 ml glass vial. 400 mg of Zirconium oxide beads of size 0.4- 0.7mm was added (40%w/v of the batch size). 300 mg of OL was added slowly to the above milling chamber. Milling was initiated by magnetic stirring at 5000 rpm for 4 hours. The nanosuspension was obtained in a powder form by lyophilization using 1:3 sucrose (with respect to total solid content) as cryoprotectant. TPGS stabilized OL nanosuspension was prepared in the same way as above by replacing poloxamer 407 with TPGS.

11.2 EXPERIMENTAL

Pharmacokinetic and biodistribution studies

Animals

Albino rats of either sex weighing about 200-250 gm were selected for the study. The rats were fasted overnight before study and were accessed to water *ad libitum.* All the animal experiments were approved by CPCSEA and local animal ethics committee.

Radiolabeling protocol, in vitro stability and challenge tests

The radiolabeling protocol utilized for radiolabeling of plain olanzapine solution, poloxamer 407 stabilized olanzapine nanosuspension (POL NS) and TPGS stabilized olanzapine nanosuspension (TOL NS) are described in detail in chapter 5. The radiolabelled complexes were also subjected to in vitro serum stability and transchelation studies to study their potential use as biomarkers for blood clearance and biodistribution studies (Chapter 5).

Blood clearance of olanzapine and POL NS and TOL NS in rabbits

White New Zealand Rabbits of either sex weighing 2.8 to 3.0 kg were selected for the blood clearance studies. Into the ear vein of rabbit, 0.5 mL of the ^{99m}Tc-labeled complexes of OL or poloxamer 407 stabilized olanzapine nanosuspension (POL NS) / TPGS stabilized olanzapine nanosuspension (TOL NS) containing 500 μCi of ^{99m}Tc was intravenously injected. The blood samples were collected at 5 minutes, 15 minutes, 0.5 hour, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours from the ear vein of rabbit and analyzed for the radioactivity in gamma ray spectrometer. The blood was weighed, and radioactivity in whole blood was calculated by considering the volume of blood as 7.3% of the total body weight.

Biodistribution studies

^{99m}Tc-labeled complexes of OL and POL NS and TOL NS were injected intravenously through the tail vein into healthy Balb/c mice weighing ~25 to 30 g. The biodistribution studies of OL, POL NS and TOL NS were performed after 1 hour, 4 hours, and 8 hours postinjection. At these time intervals, the blood was collected by cardiac puncture, the animals were humanely killed, and the organs were isolated. The organs were then weighed and measured for radioactivity in gamma ray spectrometer. The radioactivity was interpreted as percentage of injected dose per gram of organ/tissue. All the animal experiments were approved by the

Social Justice and Empowerment Committee for the purpose of control and supervision of experiments on animals, New Delhi, India.

Gamma scintigraphy

^{99m}Tc-labeled complexes of OL/ POL NS / TOL NS containing 500 μCi of ^{99m}Tc was intravenously injected into the ear vein of healthy white New Zealand rabbits. After 1 hour post injection, the mice were fixed an animal fixing tray board and imaging was performed with Single Photon Emission Computed Tomography (SPECT, LC 75-005, Diacam, Siemens, USA) gamma camera

Statistical Analysis

Statistical comparisons of the experimental data were performed by one way analysis of variance (ANOVA) at α level of 0.05.

11.3 RESULTS AND DISCUSSION

Blood Clearance Studies

Blood clearance studies of ^{99m}Tc-labeled complexes of OL, POL NS and TOL NS were investigated in healthy white New Zealand Rabbits. Both POL NS and TOL NS exhibited higher blood concentrations compared with free OL (Figure 11.1). The residence time of both the nanosuspension formulations in the blood was significantly higher when compared to the plain drug. However, there was insignificant difference in the residence time in blood of POL NS and TOL NS. These data indicate the long circulation capability of the formulated nanosuspension formulations. Such differential blood kinetics would also result in different biodistribution patterns.

Increase in the residence time of the POL NS formulation in the blood can be attributed to the adsorption of poloxamer 407 on the surface of the drug nanocrystals which imparts a stealth property to the drug particle, resulting in reduced protein opsonization and subsequent RES uptake. The hydrophilic polyethylene oxide (PEO) segment of the poloxamer provides a protective barrier for nanoparticles against RES uptake. Many reports (Leroux JC et al., 1996; Redhead HM et al., 2001; Peracchia MT et al., 1999) are available that protein adsorption onto nanoparticles with hydrophilic shells is decreased and the body circulation time is increased in

vivo studies. Lenaerts during his tumour targeting studies reported that poloxamer 407 copolymers would be a better choice as they are less easily desorbed from the particle surface (Lenaerts V., 1995).

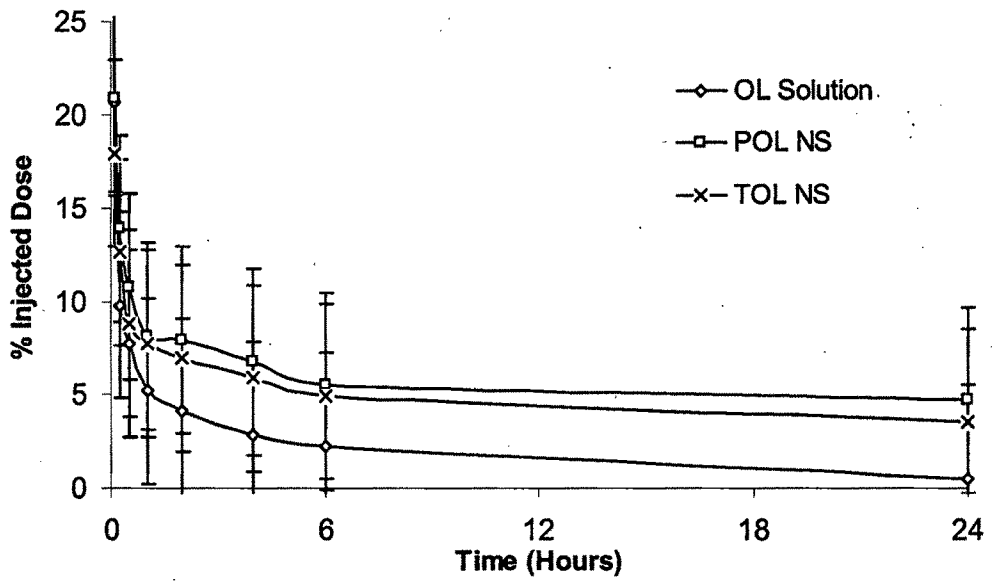


FIGURE 11.1: Pharmacokinetics of 99m Tc-labeled olanzapine and olanzapine nanosuspensions after intravenous injection into ear vein of white New Zealand Rabbits.

Adsorption of TPGS on the surface of TOL NS can very well contribute to the increase in the in vivo circulation time of the TPGS stabilized nanoparticles. The chemical structure of TPGS is similar to other amphiphiles comprising lipophilic alkyl tail and hydrophilic polar head portion. Its bulky structure and large surface area characteristics make it an excellent emulsifier (Zhang Z Feng SS., 2006). Moreover, it has been found that co-administration of vitamin E TPGS could enhance cytotoxicity, inhibit P-gp mediated multi-drug resistance, and increase the oral bioavailability of anticancer drugs (Yu L et al., 1999; Dintaman JM et al., 1999). It is well known that nanoparticles coated by PEG segments prevent opsonization and thus avoid the MPS uptake (Peracchia MT et al., 1999; Storm G et al., 1995). Gref and co workers reported that the surface density and the chain length of PEG on the surface of nanoparticles plays a vital role in protection of the particle from protein opsonization (Gref R et al., 2000).

Biodistribution Study

Table 11.1 and figure 11.4 represents the organ/tissue concentrations after intravenous injection of ^{99m}Tc- OL and ^{99m}Tc radiolabeled POL NS and TOL NS. Plain OL solution exhibited higher concentrations in the organs of the reticuloendothelial system (RES), such as liver, spleen, and lung when compared to the nanosuspension formulations. The overall uptake of POL NS and TOL NS by liver and spleen was significantly lower than the free OL ($p < 0.005$). The uptake of both plain OL solution and the nanosuspension formulations was initially high but decreased with time. However, in the spleen, there was a gradual increase in the accumulation of plain OL solution and both the nanosuspension formulations with time. There was a significant uptake ($p < 0.001$) of plain OL solution in the kidney when compared to the nanosuspension formulations at all the three time points studied. The concentration of TOL NS was relatively higher in brain (9-fold and 2.42-fold higher than plain OL solution and POL NS at 1 hour respectively) (Figure 11.3). Hydrolyzed technicium tends to accumulate in the stomach and intestine. Insignificant radioactivity was recovered from stomach and intestine and showed constancy with time indicating the in vivo stability of the radiolabeled complexes.

The increased brain concentrations obtained with both the nanosuspension formulations can be explained as follows. Pluronic block copolymers are reported to inhibit the P-glycoprotein (P-gp) drug efflux system at the BBB and increase the permeability of a broad spectrum of drugs (Batrakova EV et al., 2002). Alterations in intracellular ATP levels and simultaneous membrane fluidization in the BBB by poloxamer co polymers are believed to be efflux inhibition (Batrakova EV et al., 2001).

Dintaman and coworkers (Dintaman JM et al., 1999) during their investigation on P-gp inhibition by TPGS concluded that TPGS can increase the drug absorption by decreasing the transport back into the intestinal lumen. The authors felt that the above mentioned activity of TPGS is primarily due to inhibition of P-gp present in the intestinal lumen. Varma and co workers (Varma MVS et al., 2005) observed enhanced bioavailability of paclitaxel and concluded that this increase in bioavailability is primarily due increase in solubility and permeability of the drug. Use of TPGS alone or in conjunction with Labrasol has been shown to increase the C_{max} value of vancomycin hydrochloride (Prasad YVR et al., 2003).

Many studies are going on to study the exact mechanism by which TPGS affect drug permeability. However, there still lot to learn. TPGS action is attributable to its ability to increase solubility through micelle formation and subsequent enhancement of permeability across the cell membrane inhibiting the drug efflux pump P-gp. However, TPGS showed P-gp inhibitory activity in Caco -2 cells at a concentration (0.005%w/v) which is below its critical micellar concentration (0.02%w/v). These data indicate that the surfactant monomer molecules are involved in the inhibition of the polarized pump (Nerurkar MM et al., 1997). TPGS has been shown to rigidize cell membranes, this does not appear to be the primary means of P-gp inhibition (Rege et al., 2002).

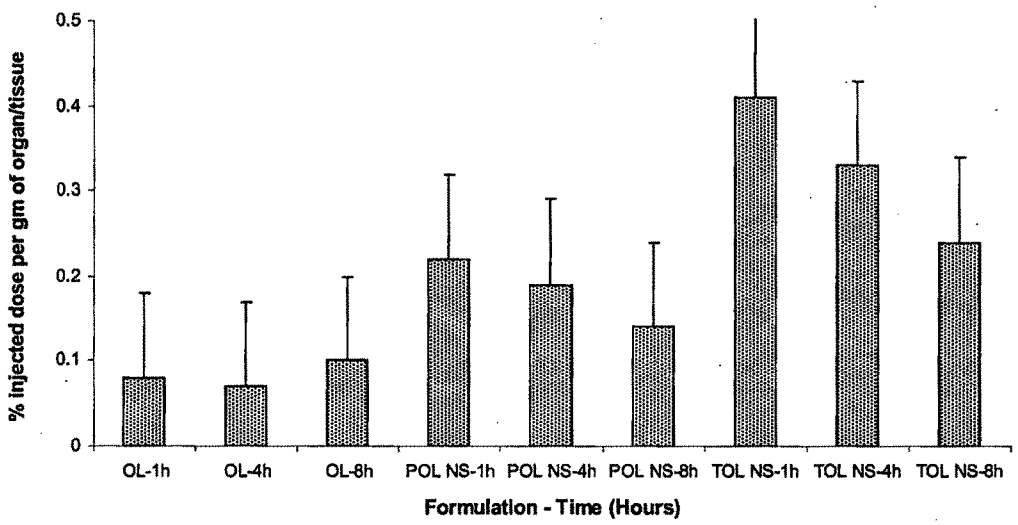
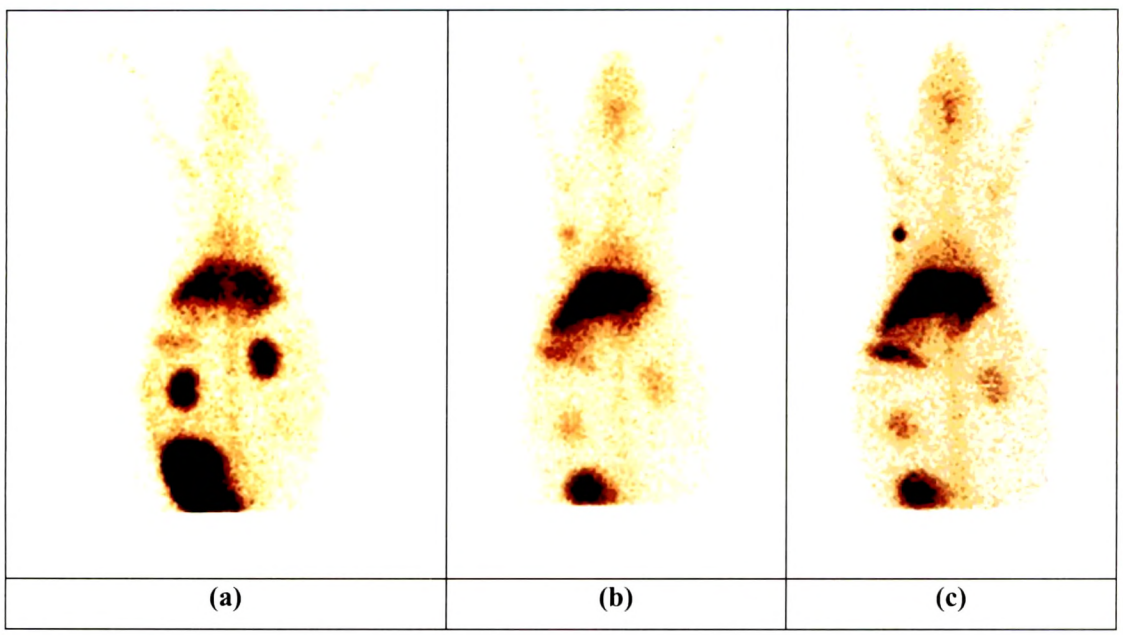


FIGURE 11.2: Brain Concentrations of ^{99m}Tc-labelled olanzapine and ^{99m}Tc-labelled olanzapine nanosuspension after intravenous injection into Balb/c mice. The values plotted are the mean ± S.D of 3 experiments

The gamma scintigraphic images taken after 1 hour post intravenous injection of plain OL – ^{99m}Tc radiolabelled complex into New Zealand rabbits are shown in figure 11.3a. The images clearly shown the difference in the concentration of the radiolabelled complex in the brain after intravenous injection of POL NS (Figure 11.3b) and TOL NS (11.3c)



(a)	(b)	(c)

FIGURE 11.3: Gamma Scintigraphic image of plain olanzapine (a), POL NS (b), TOL NS (c), after 1 h of intravenous injection in white New Zealand Rabbits. The black portion in the figure represents radiolabelled complex.

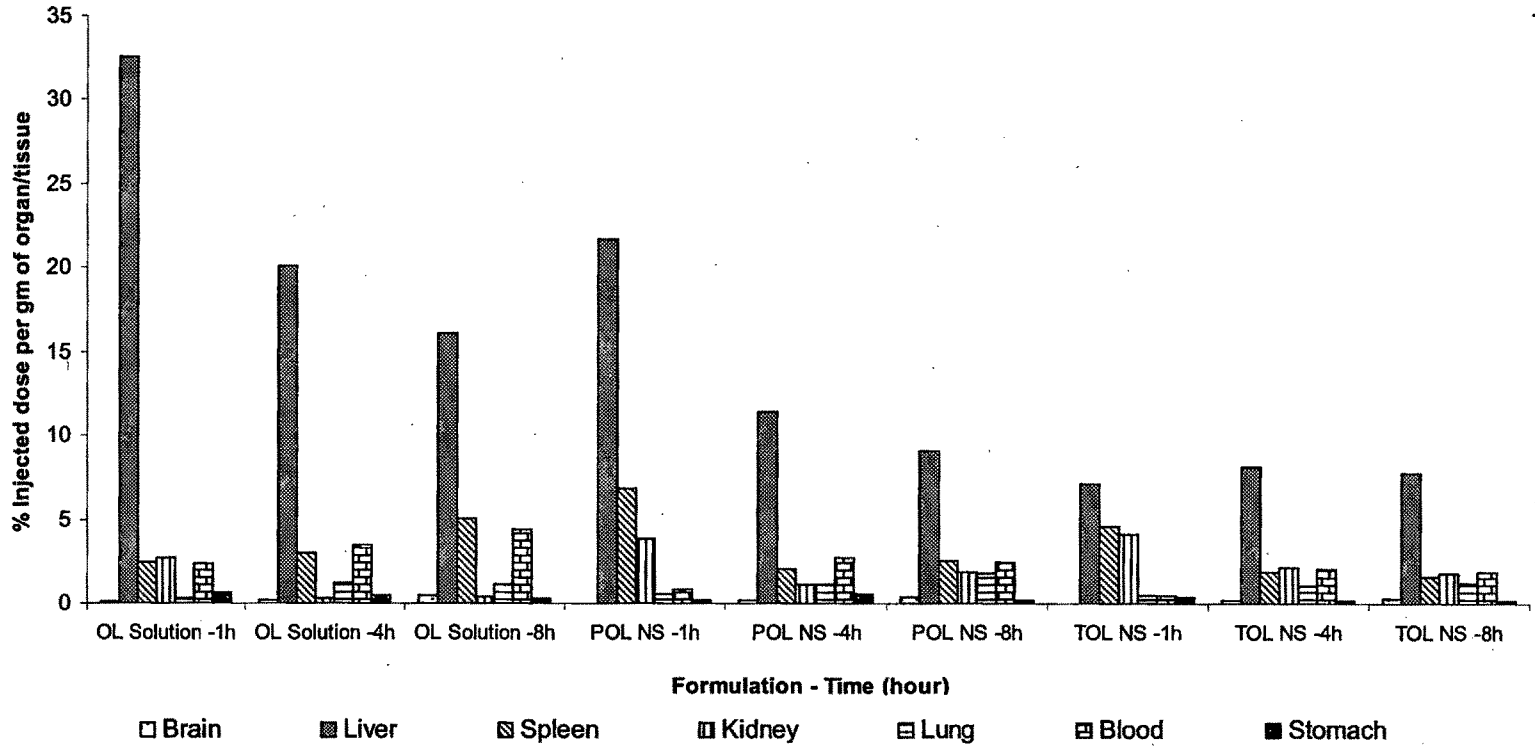


Figure 11.4: Tissue distribution of olanzapine after intravenous injection as plain solution, poloxamer 407 stabilized nanosuspension (POL NS) and TPGS stabilized nanosuspension (TOL NS). The values plotted are the mean ± S.D of 3 experiments

Organ/ tissue	Percent injected dose per gm of organ/tissue (± SEM)								
	1 hour			4 hour			8 hour		
	OL Soln	POL NS	TOL NS	OL Soln	POL NS	TOL NS	OL Soln	POL NS	TOL NS
Brain	0.05 ± 0.01	0.19 ± 0.02	0.46 ± 0.03	0.04 ± 0.01	0.15 ± 0.01	0.33 ± 0.02	0.02 ± 0.01	0.17 ± 0.02	0.24 ± 0.03
Liver	32.53 ± 1.81	20.12 ± 1.12	16.11 ± 0.98	21.68 ± 1.87	11.41 ± 1.34	9.04 ± 0.87	7.12 ± 0.57	8.16 ± 0.99	7.77 ± 1.01
Spleen	2.47 ± 0.54	3.01 ± 0.37	5.03 ± 0.47	6.81 ± 0.74	2.03 ± 0.24	2.54 ± 0.45	4.63 ± 0.82	1.87 ± 0.28	1.61 ± 0.31
Kidney	2.76 ± 0.15	0.29 ± 0.03	0.36 ± 0.03	3.85 ± 0.77	1.16 ± 0.11	1.88 ± 0.44	4.11 ± 0.55	2.19 ± 0.33	1.76 ± 0.21
Lung	0.29 ± 0.06	1.20 ± 0.24	1.11 ± 0.17	0.58 ± 0.11	1.12 ± 0.20	1.82 ± 0.44	0.51 ± 0.05	1.06 ± 0.16	1.23 ± 0.33
Blood	2.31 ± 0.11	3.47 ± 0.29	4.41 ± 0.41	0.83 ± 0.09	2.71 ± 0.19	2.43 ± 0.29	0.49 ± 0.10	2.10 ± 0.45	1.86 ± 0.24
Stomach	0.66 ± 0.08	0.49 ± 0.09	0.31 ± 0.06	0.23 ± 0.06	0.54 ± 0.13	0.22 ± 0.04	0.42 ± 0.07	0.21 ± 0.05	0.19 ± 0.03

TABLE 11.1: Organ/tissue concentrations of 99mTc-labeled olanzapine and olanzapine nanosuspensions in Balb/c mice

11.4 CONCLUSION

Radiolabeling of plain OL solution and both the OL nanosuspensions was performed with ^{99m}Tc, with high labeling efficiency and in vitro and in vivo stability. In vivo, the nanosuspensions showed higher blood residence time and brain concentration compared to the OL solution, indicating the potentiality of nanosuspension forms in brain delivery of olanzapine. There was also a significant reduction in the uptake by the RES organs of OL after administration in nanosuspension form. These data substantiate the potential of the above formulated poloxamer 407 or TPGS stabilized OL nanosuspension as a long circulating system in blood and higher brain deposition.

11.5 REFERENCES

Ambruosi A, Yamamoto H, Kreuter J, Body distribution of polysorbate-80 and doxorubicin-loaded [14C] poly (butyl cyanoacrylate) nanoparticles after i.v. administration in rats, *Journal of Drug Targeting*, 2005, 13, 535–542

Aquilante CL, Letrent SP, Pollack GM, Brouwer KL, Increased brain P- glycoprotein in morphine tolerant rats, *Life Sci.*, 2000, 64, 47–51.

Batrakova EV, Li S, Alakhov VY, Miller DW, Kabanov AV, Optimal Structure Requirements for Pluronic Block Copolymers in Modifying P-glycoprotein Drug Efflux Transporter Activity in Bovine Brain Microvessel Endothelial Cells, *Journal of Pharmacology and Experimental Therapeutics*, 2002, 304,845–854

Batrakova EV, Li S, Vinogradov SV, Alakhov VY, Miller DW, Kabanov AV, Mechanism of Pluronic Effect on P-Glycoprotein Efflux System in Blood-Brain Barrier: Contributions of Energy Depletion and Membrane Fluidization, *Journal of Pharmacology and Experimental Therapeutics*, 2001, 299, 483–493.

Chekhonin VP, Kabanov AV, Zhirkov YA, Morozov GV, Fatty acid acylated Fab-fragments of antibodies to neurospecific proteins as carriers for neuroleptic targeted delivery in brain, *FEBS Lett.*, 1991, 287,149–152.

Dintaman JM, Silverman JA, Inhibition of P-glycoprotein by D alpha- tocopheryl polyethylene glycol 1000 succinate (TPGS), *Pharm Res.*, 1999, 16, 1550–1556.

Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Muller RH, Stealth corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain

length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption, *Colloid. Surf. B*, 2000, 18, 301–313.

Lenaerts V, Nanocapsules with a reduced liver uptake: targeting of phthalocyanines to EMT-6 mouse mammary tumour in vivo, *Eur. J. Pharm. Biopharm.*, 1995, 41, 38–43.

Leroux, JC, Allemann E, Jaeghere FD, Doelker E, Gurny R, Biodegradable nanoparticles from sustained release formulations to improved site specific drug delivery, *J. Control Release,* 1996, 39, 339–350.

Mishra P, Chuttani K, Mishra AK, Sharma RK, Radiolabelling of diltiazem with ^{99m}Tc and its evaluation for tumor targeting, Ind J Nucl Med., 1999, 14, 249-254.

Nerurkar MM, Ho NFH, Burton PS, Vidmar TJ, Borchardt RT, Mechanistic roles of neural surfactants on concurrent polarized and passive membrane transport of a model peptide in Caco – 2 cells, *J. Pharm. Sci.*, 1997, 86, 813-821.

Peracchia MT, Fattal E, Desmaele D, Besnard M, Noel JP, Gomis, JM, Appel M, Angelo J, Couvreur P, Stealth PEGylated polycyanocarylate nanoparticles for intravenous administration and splenic targeting, *J. Control. Release*, 1999, 60, 121–128.

Prasad YVR, Puthli SP, Eaimtrakarn S, Ishida M, Yoshikawa Y, Shibata N, Takada K, Enhanced intestinal absorption of vancomycin with Labrasol and D-alpha-tocopherol PEG 1000 succinate in rats, *Int J Pharm.*, 2003, 250, 181-190.

Rapoport SI, Osmotic opening of the blood–brain barrier, *Ann Neurol,* 1988, 24,677–684.

Redhead HM, Davis SS, Illum L, Drug delivery in poly (lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: in vitro characteristics and in vivo evaluation, *J. Control. Release*, 2001, 70, 353–363.

Rege BD, Kao JP, Polli JE, Effects of non ionic surfactants on membrane transporters in Caco -2 cell monolayers, *Eur J Pharm Sci.*, 2002,16,237-246

Storm G, Belliot SO, Daemen T, Lasic DD, Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system, *Adv. Drug Deliv. Rev.*, 1995, 17, 31–48.

Varma MVS, Panchagula R, Enhanced oral paclitaxel with vitamin E-TPGS: effect on solubility and permeability in vitro, in situ and in vivo, *Eur. J Pharm. Sci.*, 2005, 25, 445-453

Verhoff F, Pace GW, Snow RA, Millar F, Milled particles, *US patent no 6,634,576*, 2003.
Wang SW, Monagle J, Mcnulty C, Putnam D, Chen H, Determination of P-glycoprotein Inhibition by Excipients and Their Combinations Using an Integrated High-Throughput Process, *Journal of Pharmaceutical Sciences*, 2004, 93

Yang CS, Lu FL, Cai Y, Body distribution in mice of intravenously injected camphotothericin solid lipid nanoparticles and targeting effect on the brain, *J. Cont. Rel.*, 1999, 59, 299–307
Yu L, Bridgers A, Polli J, Vitamin E-TPGS increases absorption flux of an HIV protease inhibitor by enhancing its solubility and permeability, *Pharm Res.*, 1999, 16, 1812–1817.

Zhang Z, Feng SS, Nanoparticles of poly (lactide) /vitamin E TPGS copolymer for cancer chemotherapy: Synthesis, formulation, characterization and in vitro drug release, *Biomaterials*, 2006, 262–270.