Statement of Research Achievements, if any, on which any Award has already been received by the Applicant.

[I] Received TATA Innovation Fellowship 2018-19 by Department of Biotechnology (DBT) Govt of India for outstanding contribution and commitment to find innovative solution in healthcare in the area of translational research

This award has been conferred for contribution in the area of translational research based on controlled and targeted drug delivery technologies for enhanced therapeutic efficacy with special emphasis on osteoporosis and parasitic diseases. The main focus is on (a) development of strategy for cost-saving, patient-friendly and evidence-based products for industry; and (b) bringing good to patients suffering from osteoporosis and parasitic diseases.

(a) Development of nano-therapeutics for bone health

In the area of osteoporosis he has developed Layer-by-layer technology bearing kaempferol which has impacted on product development by enhancing bioavailability of kaempferol by an order of magnitude with and enhanced osteogenic efficacy **(Gupta et al Nanomedicine 2013; IF 4.73 and Eur. J. Pharm. Biopharm 2012; IF 4.2; Granted US, European, Australia Patents)**. Quercetin-based solid lipid nanoparticles (QSLNs) was developed to enhance the bioavailability of quercetin to evaluate its effects on bone health. The developed quercetin loaded solid lipid nanoparticles inhibited bone loss in osteopenic rats. QSLNs inhibited the receptor activator of nuclear factor-kappa B ligand (RANKL)-induced osteoclast cells differentiation and the expression of osteoclast-specific genes in in vitro experiments using bone marrow cells treated with RANKL and M-CSF. The data from this study suggest that, overall, QSLNs treatment recovers bone loss **(Ahmad et al RSC Adv 2016 IF 2.94**). Developed gelatin based chemically cross linked cryogel system embedded with CaCO3microspheres and ciprofloxacin hydrochloride was incorporated in both the microspheres and the 3D matrix of cryogel for therapeutic intervention in osteoporosis and associated osteomyelitis **(Pandey et al Eur. J. Pharm. Sci 2016; IF 3.46)**. His contribution in translational research, he has successfully licensed and commercialized two products as **Joint Fresh™ and Reunion ™** for secondary osteoporosis and osteoarthritis.

(b) Development of smart nanocapsules bearing chemotherapeutic drugs for better management of parasitic diseases

In the area of **leishmaniasis** he identified that sulfated derivatives of a common sugar (4-SO4GalNAc) could be used as a high-performance targeting ligands to target tissue-resident macrophages especially *Leishmania*-infected macrophages, suggesting an alternative strategy for the treatment of leishmaniasis. Developed oil templated nanocapsules bearing doxorubicin for macrophage targeting through Phosphatidylserine ligand and established that the ligand can provide a new insight for efficient drug delivery to specialized macrophages through “eat me” signal **(Kansal et al J. Antimicrob. Chemother. 2012; IF 5.22)**. He developed stable AmB bearing lipo-polymerosome formulation with low toxicity that showed synergistic efficacy in case of leishmaniasis (**Gupta et al Mol. Pharm. 2014; IF 4.384)** and further explored targeting potential of Lectin functionalized lipo-polymerosme bearing AmB and found that it has improved pharmacokinetic and pharmacodynamic profile than commercial formulation Fungizone **(Bioconjugate Chem. 2014; IF 4.7)**. He also established that lipoteichoic acid functionalized lipo-polymerosome has potential to target and stimulate antigen presenting cells as chemo-immunotherapeutic approach against intracellular infectious disease **(Gupta et al Biomacromolecules 2015; I.F. 5.74)**. He also developed self-assembled ionically sodium alginate cross-linked amphotericin B encapsulated nanoparticles for better chemotherapy and non-toxic delivery in Visceral Leishmaniasis (**Singodia et al Pharm Res 2015; I.F. 3.34)**

In the area of **Malaria** he has developed Arteether nanoemulsion for enhanced efficacy against Plasmodiumyoelii nigeriensis malaria. The nanoemulsion showed significantly high anti-malarial efficacy and survival rate of mice giving 80% cure rate at 12.5 mg/kg for 5 days in comparisonto 30% cure rate of arteether in oil at the same daily dose and it was also comparable to the 100% cure rate at 12.5 mg/kg for 5 days for ART given intramuscularly **(Dwivedi et al RSC Adv 2014; Colloids and Surface: Biointerfaces 2015 I.F 3.99; Int J. Phar 2014 I.F. 3.86).** He has also developed novel combination kit bearing arteether and sulfadoxine for the treatment of *P.falciparum* Malaria. In this study, the nano-formulation showed 100% curative effect with nearly $1/4^{th}$ of curative dose of α/β-arteether in combination of $1/32^{th}$ of SP curative dose **(1983/DEL/2014)**. This formulation is at TLR 4 and is ready for licensing.

[II] INSA-DFG fellowship award under Bilateral Exchange Programme by Indian National Science Academy, New Delhi and Deutsche Forschungsgemeinschaft, Germany) 2008.

Hesperetin nano-suspensions with a mean PCS diameter of 300 nm was produced with the three stabilizers Poloxamer, Plantacare 2000 and Inutec after 30 homogenization cycles at 1500 bar. Obtaining the same size independent on the stabilizers used is in line with the theory that the final crystal size depends only on the power density (pressure) during the homogenization process and on the hardness of the crystals but not on the stabilizer. Tween 80 yielded slightly larger PCS diameters (around 350 nm) indicating that this stabilizer is not able to similarly efficiently stabilize the produced crystals at the end of the homogenization process, very slight aggregation seems to occur. Interpretation of the zeta potential data predicted Inutec and Plantacare stabilized nanosuspensions as the most stable ones, which was confirmed by the short-term stability study. However, for dermal products also the Poloxamer and Tween stabilized nano-suspensions appear still usable, presumed that the slight aggregation does not continue. A long-term stability study over a period of up to 2 years is presently going on to finally assess the stabilizing ability of the stabilizers. After addition of the nanocrystals to dermal formulations, the nanocrystals are additionally stabilized by the high viscosity of the formulation. Despite this additional stabilizing effect, it is advantageous to incorporate a priori the most stable nanocrystals, therefore also the long-term stability of the nanosuspensions themselves is important. Nanosuspensions are also sold as concentrates for admixture to cosmetic and pharmaceutical products. Of course these products need to possess a sufficient shelf live. In addition, nanosuspensions are also of interest as oral pharmaceutical suspensions (e.g. product Megace ES) **(Mishra et al Int. J. Pharm 2009; Shaal et al 2014 Pharmazie).**

[III] Received Fast Track Young scientist award by Department of Science and Technology, India 2006.

Cyclosporine A is the only analog to have been extensively used in the clinical practice. It is the prototype of a new generation of the immunosuppressive agent that selectively suppresses the activation of T lymphocytes, primarily by impairing the autocrine production of T lymphocytes growth factors. Low oral bioavailability of systemically acting drugs is often associated with variable plasma concentrations and poorly controlled pharmacologic and toxic effects. In addition, incomplete oral bioavailability results in the wasting of much of an oral dose, and adds to the cost of drug therapy, especially when the active drug substance is expensive. Furthermore, P-glycoprotein (P-gp), an ATP-dependent efflux transporter has been proposed as a factor in the poor absorption of CsA via various experiments, including single nucleotide polymorphism of P-gp.

The work comprises the delivery Cyclosporine A (CsA) using various nonionic surfactant based formulations composed of pharmaceutically acceptable ingredients. It has been envisaged that the tendency of CsA to get effluxed (a main reason for low bioavailability)

from P-gp transporters can be minimized if the CsA is encapsulated within the structured vehicle. Moreover, the increases in the CsA concentration at absorption site in solubilized form will down regulate the flip-flop mechanism of CsA efflux. The preparations of CsA available in the market contains cremophor and other solvents which is reported to be potentially toxic and therefore it has been envisaged that having a formulation of CsA devoid of cremophor and solvents would be more desirable. It is also envisaged that the market formulations are not independent of bile flow and its absorption and bioavailability is affected by variability in bile flow within gastrointestinal tract.

The apparent permeability coefficient (P_{app}) of BL formulation was found to be much higher at all time points compared to control. It was interesting to note that fraction of CsA was effluxed in BL formulation as well, but the degree of permeation was much higher indicating better absorption by BL formulation. The permeation profile of all the formulations was found in order of BL>ME>NV2>Control. It is evident that BL formulation is able to enhance permeation which could be due to reduction in efflux and improvement in solubility and permeability of CsA. The formulations was able to prevent P-gp mediated efflux and moreover formulations especially BL are contributing in improving solubility and permeability. It has been observed that order of efflux in all formulations was in order of ileum>jejunum>duodenum. This data reflects that P-gp is playing its vital role in effluxing CsA back into GI lumen, but the formulations are able to push CsA from lumen to portal blood. When studied in animals the pattern of concentration–time curve of CsA from BL formulation was very similar to that of (marketed preparation) Neoral®, showing two maxima indicating that both of the formulations are undergoing enterohepatic circulation. It is evident that highest relative bioavailability was achieved with BL formulation i.e. 173% compared to Neoral®. BL formulation increased C_{max} and AUC_{0-24} to almost 1.9 and 1.7 times respectively compared to Neoral without impacting MRT oral, suggesting an effect primarily during cyclosporine absorption.

In nutshell this work comprises the delivery CsA using various nonionic surfactant based formulations composed of pharmaceutically acceptable ingredients. The formulations NV2, ME and BL with these compositions resulted in improved bioavailability compared to control formulation. A bioavailability study in rats showed that ME formulation is equally effective with respect to marketed Neoral and exhibited similar bioavailability. However BL formulation is more effective in improving relative bioavailability to a degree of 173% with respect to marketed formulation.

Patent Granted

P.R Mishra, Vure Prasad, A.K. Dwivedi and S Singh “Composition and methods of nonionic surfactant based vesicular formulation for improved delivery of cyclosporine **Indian Patent** (2013) 258311.

Dr Prabhat Ranjan Mishra