INFORMATION ABOUT TEN BEST PAPERS HIGHLIGHTING THE IMPORTANT DISCOVERIES/CONTRIBUTIONS DESCRIBED IN THEM

1. Amit K. Jain, Kaushik Thanki, **Sanyog Jain**, Solidified self-nanoemulsifying formulation for oral delivery of combinatorial therapeutic regimen: Part II. In vivo pharmacokinetics, antitumor efficacy and hepatotoxicity. **Pharmaceutical Research,** 31(4), 2014: 946-58. **(3.9)**

Tamoxifen (TMX) is a chronically administered gold line drug for the treatment of estrogen receptor positive breast cancer. However, extensive P-gp efflux and first pass metabolism in liver lead to the poor oral bioavailability (10-20 %). Extensive hepatic first pass metabolism of TMX also enforces the generation of free radicals and causes lipid peroxidation in hepatic cells, which eventually leads to the severe hepatotoxicity. Quercetin (QT), a polyphenol antioxidant, acts as a free radical scavenger is reported to combat the liver damage against drug or chemically induced toxic free radicals. Additionally, QT possesses inhibitory action on P-gp efflux pump as well as anticancer property by virtue of its multiple molecular mechanisms in cancer cells. However, poor oral bioavailability of QT (~2%) owing to poor aqueous solubility, pre-systemic metabolism and first pass hepatic metabolism limits its oral deliverability and utility in combination with Tmx. The present work focused on the development of TMX and QT combination loaded into solid self nano-emulsifying drug delivery system (s-Tmx-QT-SNEDDS) for improving oral bioavailability and reducing hepatotoxicity of the TMX. SNEDDS represents one of the most popular, commercially meaningful and readily scalable delivery vehicles for improving the oral bioavailability of poorly aqueous soluble or highly lipophilic drugs. Further, we have converted conventional liquid SNEDDS into solid form (s-SNEDDS) that offers the combined advantages of conventional lipid based drug delivery system (i.e. enhanced solubility and bioavailability) with those of solid dosage forms (e.g. low production cost, convenience of process control, high stability and reproducibility, better patient compliance). The developed formulation showed higher cellular uptake, cytotoxicity and nuclear co-localization in MCF-7 cells revealing higher efficiency of the formulation. At the same time, higher Caco-2 cell uptake revealed its potential for oral delivery which was well corroborated with *in vivo* pharmacokinetics; that suggested ~8- fold and ~4-fold increase in oral bioavailability as compared to the commercial Tmx citrate and free QT respectively. s-Tmx-QT-SNEDDS could suppress tumor growth in breast cancer animal model by ~80% in contrast to ~35% observed with Tmx citrate. The safety profile of s-Tmx-QT-SNEDDS was also established and no measurable hepatotoxicity and oxidative stress were observed in contrast to free drug(s) and their combinations. The IPR of this work has been protected by Indian Patent number **296411 granted on May 02, 2018 and technology has been licensed to VAV Life Sciences, Mumbai for further development and commercialization.**

2. Chander Parkash, Varun Kushwah, Sameer S. Katiyar, Pradeep Kumar, Viness Pillay, Sarasija Suresh, **Sanyog Jain**, Improved metabolic stability and therapeutic efficacy of a novel molecular gemcitabine phospholipid complex. **Int. J. Pharm.**, 530(1-2), 2017: 113-127 **(IF:5.8)**

   Gemcitabine (GEM) is a deoxycytidine nucleoside analogue with a wide spectrum of cytotoxic activity against pancreatic, breast, lung, and ovarian cancer. It is rapidly converted into the inactive metabolite 2'-deoxy-2', 2'-diflurouridine (dFdU) by cytidine deaminase in blood, liver, kidney and other tissues. The biggest hurdle associated with the current clinical treatment with GEM is its very short plasma half-life (~45 min). To overcome this hurdle, it is usually administered at higher dose via i.v route which increases dose dependent toxicity that includes severe haematological toxicity, and toxicity related to highly perfused organs such as liver and kidney. Here, we demonstrated the formation of molecular complex of phospholipid with water soluble drug. So far, this is the first ever attempt to improve the metabolic stability of GEM by modulating its aqueous solubility. The present work was aimed to develop molecular complex of phospholipid and GEM (GEM-PLC) as a sustained delivery platform by promoting lipid solubility and thus, plasma stability of drug molecule. Initially, the GEM-PC was prepared by solvent evaporation method, which further was suitably characterized by different techniques like DSC, FT-IR, ^{1}H-NMR, ^{31}P-NMR, P-XRD SEM and TEM. TEM images of diluted aqueous dispersion of GEM-PC showed micellar structure. *In silico* study also revealed the significant interaction between drug and phospholipid. GEM-PC demonstrated sustained drug release pattern and high plasma stability (~2.2 fold) *in vitro* as compared to GEM. Increased *In vitro* cytotoxicity and apoptosis was observed with GEM-PC, when incubated with human pancreas adenocarcinoma cell lines. *In vivo* pharmacokinetics showed the almost 2 fold increase in $AUC_{0-\infty}$ (area under curve) with phospholipid complex as compared with GEM and GEMITA (marketed formulation). Toxicity studies also signify the safety of GEM-PC over GEMITA. Pharmacodynamics studies in pancreatic tumor model further revealed higher efficacy of GEMPC than GEMITA. These findings suggested the higher potential of phospholipid based technology for the enhancement of metabolic stability and therapeutic efficacy of GEM.

3. Kaushik Thanki, Rameshwar Prajapati, Abhay T Samgamvar, **Sanyog Jain**, Long chain fatty acid conjugation remarkably decreases the aggregation induced toxicity of Amphotericin B. **Int. J. Pharm.**, 544(1), 2018: 1-13 **(5.8)**

   Amphotericin B is an antimicrobial membrane-acting drug used in the treatment of systemic fungal infections. However, the clinical utility of AmB is often low as a result of (i) dose-limiting toxicity which is closely associated with its aggregation wherein the selectivity for its target i.e. ergosterol in fungal membranes is diminished and (ii) limited oral bioavailablity. The latter is attributed to the unfavorable physicochemical properties of the AmB e.g., low solubility,

gastrointestinal instability, and poor intestinal permeability. The hypothesis of present work was that by applying a lipid conjugation approach the aggregation induced toxicity of AmB vis-à-vis permeability can be overcome. From the array of fatty acids, the oleic acid (OA) was selected for conjugation due to its great impact on increasing the Caco-2 permeability of AmB. AmB-OA conjugate was synthesized using standard carbodiimide chemistry and characterized thoroughly. Due to the reported strong correlation between the self-aggregation of AmB and toxicity, the aggregation behavior of AmB and AmB-OA was studied by in silico modeling and confirmed experimentally. In vitro hemolytic studies and viability assays in kidney cells (HEK 293 cells) suggested that AmB in aggregated was highly toxic but not AmB-OA. In silico modeling suggested possible aggregation conformation of AmB-OA dimers that retains the selectivity for cholesterol even in aggregated state when embedded in in silico generated lipid bilayers. The results were further confirmed by assessing the interactions of monomeric and aggregated state of AmB and AmB-OA with that of cholesterol and ergosterol containing liposomes employing circular dichroism spectroscopy. The findings were subsequently corroborated by in vivo nephrotoxicity studies. To conclude, the lipid conjugation approach may be a promising strategy for reducing the dose-limiting toxicity of AmB. We further demonstrated that oil solubility of the AmB-OA was increased to ~1000 fold that paves the way to develop some lipid based formulation with higher drug loading.

4. Kaushik Thanki, Tushar Date, **Sanyog Jain**, Enabling oral amphotericin B delivery by merging the benefits of prodrug approach and nanocarrier-mediated drug delivery. **ACS Biomaterials Science & Engineering**, 9(6), 2023: 2879-2890 **(5.8)**

   Amphotericin B (AmB) is gold standard therapy for leishmaniasis and fungal infections. All currently marketed AmB products are administered through an intravenous (i.v.) route and involve high treatment costs. Designing an orally effective AmB formulation can substantially reduce the cost of therapy and improve patient compliance. However, it is a challenging task because of the distinctive physicochemical properties of AmB. Previously, we developed a lipid-based prodrug of AmB, AmB-oleyl conjugate (AmB-OA), which showcased remarkable stability in the gastrointestinal (GI) environment and improved intestinal permeation. Hereby, we have developed self-nanoemulsifiying drug delivery system (SNEDDS) of AmB-OA to further enhance the oral bioavailability of AmB and potentiate its therapeutic benefits. SNEDDS was developed by screening a wide range of oils, surfactants, and co-surfactants, and formulation composition was optimized using extreme vertices design. AmB-OA SNEDDS possessed the ability of quick self-nanoemulsification on dilution (droplet size ~56 nm) along with remarkable stability in the GI environment. Accelerated stability (40 °C/75% relative humidity) studies and freeze–thaw cycling studies proved that the formulation was stable at tropical conditions as well

as temperature cycling stress. Drug transport analysis in Caco-2 cells revealed a remarkable increase in drug transport for AmB-OA SNEDDS compared to AmB along with minimal cellular toxicities. AmB-OA SNEDDS showcased ~8.9-fold higher AUC_{Tot} than AmB in in vivo pharmacokinetic study, proving the effectiveness of formulation to enhance oral bioavailability. In vivo toxicity analysis highlighted the ameliorated toxicity risk associated with SNEDDS formulation. Therefore, the AmB-OA SNEDDS formulation may provide a cost-friendly and effective strategy to resolve the oral AmB drug delivery challenge.

5. Shivani Sahu, Sameer S. Katiyar, Varun Kushwah, **Sanyog Jain***. Active natural oil-based nanoemulsion containing tacrolimus for synergistic antipsoriatic efficacy. **Nanomedicine,** 13(16), 2018: 1985-98. **(IF: 5.5)**

   It is well known that psoriatic skin possesses a commendable barrier for the delivery of topical therapeutics due to its tough, horny and scaly skin morphology. Thus, to prevail over the faced challenges, the present investigation was focused on developing a system loaded with anti-psoriatic agent with effective skin permeation. Tacrolimus is a calceneurin inhibitor with immunosuppressant action and has shown potential for managing psoriatic condition. However it exhibits several drawbacks in its topical application like severe skin irritation (topical), nephrotoxicity and hepatotoxicity. Thus, we develop a novel formulation which utilizes herbal oil as a functional excipient to form nanoemulsion which can exhibit dual advantage in formulation and in improving the efficacy. In doing so, kalonji oil, a naturally occurring oil with proven anti-psoriatic activity was used as a functional excipient for formulating the tacrolimus loaded nanoemulsion using Design of Expert® (Stat-Ease Inc., MN, USA). The nanoemulsion prepared revealed a size of 89.41 ± 4.60 nm with narrow PDI of 0.209 ± 0.045 with desirable dispersibility and biphasic sustained release pattern. The nanoemulsion was then formulated into nanioemulsion gel by optimizing the gelling agent based on spreadability, stability and rheological behavior. The prepared nanoemulsion gel at in vitro level revealed 4.33 fold enhanced dermal bioavailability and significantly ($P < 0.001$) higher percentage of growth inhibition of A431 cells in comparison to free tacrolimus suspension. Similarly, investigation done at in vivo level significantly reduced the levels of serum cytokines i.e. IL-6 and TNF-α was observed in the animals treated with the prepared nanogel in comparison to free drug and marketed formulation (Tacroz Forte, Glenmark Pharmaceuticals Ltd, Maharashtra, India). Thus, as a result a safe and effective nanogel formulation comprising of functional oil and drug with minimal systemic exposure compared with conventional formulation, which have limited skin access and subsidiary side effects was successfully developed. The established strategy also opens a new avenue in the functional excepient-based nanoemulsion system for better management of topical diseases and presents a selective and safe option for clinical applications.

6. **Sanyog Jain**, Dnyaneshwar P. Kale, Rajan Swami, Sameer S. Katiyar, Co-delivery of Benzoyl Peroxide and Adapalene using Modified Liposomal Gel for Improved Acne Therapy, **Nanomedicine,** 13(12), 2018:1481-93 **(IF:5.5)**

   Acne is a chronic disease related with inflammatory and noninflammatory lesions resulting from androgen induced increased sebum production, altered keratinization, inflammation and bacterial colonization by *Propionibacterium acne*. Combination therapy including retinoid (Adapalene, AD) and antimicrobial agent (Benzoyl peroxide, BPO) has been a choice of treatment for acne. However, the problem that arises from this combination (BPO-AD) is related to its side effects and dermal availability. Both the drugs show scaling, dryness, erythema, burning/stinging, contact dermatitis, pruritus, peeling, sunburn and so forth, as side effects. Other performance issues are related to their poor physicochemical characteristics affecting their utility and poor patient compliance. Addressing these problems is major challenge in topical formulation development and thus in effective treatment of acne. The present work emphasizes on the development and characterization of modified liposomal delivery system of BPO-AD loaded in gel (BPO-AD-mLipo gel) for effective treatment of acne. The strategy exploits the utilization of multiple auxiliary components such as terpenes, ether, esters, glycols etc. to modify the properties of liposomes for improving the stability, loading capacity of the system as well as skin penetration potential. Extensive optimization was carried out to evaluate the effect of different excipients and their respective concentration and various process variables. E*x vivo* skin permeation study revealed that nondetectable amounts of BPO and AD were permeated across the skin. However, skin dermatokinetics suggested that BPO-AD mLipo gel showed higher bioavailability in both SC and deeper skin layers. Appreciation in dermal bioavailability was 2.1- and 5.4-fold for AD and 3.0- and 7.83-fold higher for BPO as compared with Epiduo gel (2.5% BPO, 0.1% AD) and free drug gel, respectively. Thus, the formulation would strictly consign to the epidermal, dermal and subcutaneous (SC) layers of the skin. The mechanistic pathway of mLipo dermal penetration was also studied using confocal laser scanning microscopy that reveled maximum amount of mLipo reached at dermis (high green fluorescence) and permeated through SC via transcellular and intercellular route. Anti-acne efficacy and safety profile of formulation was evaluated in animal model. Significantly enhanced dermal bioavailability and reduction in skin irritation and papule density in animal model were observed with BPO-AD-mLipo-gel as compared with free drugs and Epiduo® (marketed formulation), respectively.

7. Ashish K. Agrawal, Harshad Harde, Kaushik Thanki, **Sanyog Jain.** Improved stability and antidiabetic potential of insulin containing folic acid functionalized polymer stabilized multilayered liposomes following oral administration. **Biomacromolecules**, 15 (1), 2014: 350-360. **(IF:6.2) (Article met attention of global media and it became front page news in almost all leading newspapers during 18-21 December 2013)**

Oral insulin is a challenge for scientists and dream of patients. Major obstacles to oral delivery of insulin are poor permeability, instability in acidic gastric environment as well instability in presence of various proteolytic enzymes. Thus, the study was aimed towards resolving these issues wherein insulin loaded liposomes coated with anionic poly(acrylic acid) (PAA) followed by cationic folic acid-poly(allyl amine) hydrochloride conjugate were prepared to form layerosomes. The insulin entrapped within the freeze-dried layerosomes was found stable both chemically as well as conformationally and developed formulation exhibited excellent stability in simulated biological fluids. Caco-2 cell and ex- vivo intestinal uptake studies revealed higher uptake of folic acid functionalized layersomes in comparison with their plain counterparts. *In vivo* pharmacodynamic and pharmacokinetic studies further revealed almost double hypoglycemia and approximately 20% relative bioavailability in comparison with subcutaneously administered standard insulin solution. Overall the proposed strategy is expected to contribute significantly in the field of designing ligand-anchored, polyelectrolyte-based stable systems in drug delivery. The work was selected as one of the best work among the other published articles in other ACS journals and **has been highlighted in various science newsletters and applauded in different newspapers across the globe. ACS itself has done wide publicity of that article.**

8. Harshad Harde, Krupa Siddhapura, Ashish Kumar Agrawal, **Sanyog Jain**. Development of dual toxoid-loaded layersomes for complete immunostimulatory response following peroral administration. **Nanomedicine**, 10(7), 2015: 1077-1091. **(IF:5.5)**

   Conventional vaccination has never served the crucial actualities of ‘expanded program on immunization,’ this is because it is associated with the risk of pain, trauma and needle born infectious diseases resulting in poor patient compliance. Oral vaccination can eliminate aforementioned drawbacks associated with conventional vaccination. However, degradation of antigen in harsh gastric environment and poor permeability across gastrointestinal membrane continues to be a major challenge for their practical utilization as oral vaccination. Thus, the present study deals with the development of dual toxoid i.e. tetanus toxoid (TT) and diphtheria toxoid (DT)-loaded layersomes for oral immunization. The lyophilized toxoids loaded layersomes were prepared by layer-by-layer coating of polyacrylic acid (PAA) and polyallylamine hydrochloride (PAH) on liposome template followed by lyophilization. This ‘numero uno’ strategy provides adequate protection to liposomes as well as the entrapped antigens in harsh biological milieu. The TT and DT loaded layersomes were prepared using thin film hydration method which revealed a size of 173 ± 80 nm with narrow PDI and zeta-potential of +57.7 ± 1.5 mV and also exhibited 83.10 ± 2.27 % and 80.74 ± 2.21 % entrapment efficiency of TT and DT respectively. The layersomes revealed no significant changes ($P > 0.05$) in any of

their attributes like size, PDI and % EE when they were incubated in simulated biological fluid thus, indicating their stability. For qualitative and quantitative uptake in antigen presenting cells, FITC conjugated BSA was used as an indicator, which revealed that layersomes exhibited 8.62-fold higher uptake in APC's in comparison to free BSA-FITC. Similar results were observed in case of enterocyte mimicking cells i.e. Caco-2 cells in which, layersomes exhibited 3.78 fold higher cell uptake in comparison to free BSA-FITC. Finally the immunization study showed that the mean anti-TT and anti-DT IgG titer of per orally administered toxoid-loaded layersomes and im [intramuscular administration] administered commercial 'Dual antigen' vaccine was significantly higher ($p < 0.001$) in comparison with orally administered commercial 'Dual antigen' vaccine, liposomes and single-coated liposomes. Similar increase in anti-TT and anti-DT sIgA titer was observed in saliva, intestinal secretion and fecal matter in comparison to single-coated liposomes and commercial 'Dual antigen' administered through respective routes (oral and i.m). Also, the orally administered layersomes demonstrated significantly higher IL-2 and IFN-γ levels than other counterparts. To sum it up in brief, the layersomes exhibited complete and protective (>0.1 IU/ml) immunostimulatory response which included serum IgG titer, mucosal sIgA titer and cytokines (IL-2 and IFN-γ) levels following the peroral administration. Thus, the aim of developing robust toxoid-loaded stable liposomes (layersomes) via layer-by-layer tuning of polyelectrolytes was successful. The system exhibited excellent stability in simulated biological fluids without interfering the integrity and conformational based biological activity of antigen. However, the developed platform technology can also be exploited for improvement of oral bioavailability of various therapeutic proteins or peptides such as insulin and many more in coming future

9. Minal Bathara, Tushar Date, Dasharath Chaudhari, Rohan Ghadi, Kaushik Kuche, **Sanyog Jain**, Exploring the Promising Potential of High Permeation Vesicle - Mediated Localised Transdermal Delivery of Docetaxel in Breast Cancer to Overcome the Limitations of Systemic Chemotherapy. **Molecular Pharmaceutics,** 17(7), 2020: 2473-2486 **(4.9)**

   The currently available systemic chemotherapy for treating breast cancer often results in serious systemic side effects and compromises patient compliance. The distinct anatomical features of human breasts (e.g., embryological origin of breast skin, highly developed internal lymphatic and venous circulation, and the presence of mammary fat layers) help in preferential accumulation of drugs into breasts after topical application on breast region. This unique feature is termed as localized transdermal delivery which could be utilized for effectively delivering anticancer agents to treat breast cancer and reducing the systemic side effects by limiting their presence in blood. However, the clinical effectiveness of this drug delivery approach is highly limited by barrier properties of skin reducing the permeation of anticancer drugs. In the present work, we have developed high permeation vesicles (HPVs) using phospholipids and synergistic combination of permeation enhancers (SCOPE) to improve the skin permeation of drugs.

Docetaxel (DTX) was used as a model drug for hypothesis testing. The optimized SCOPE mixture composed of sodium oleate/sodium lauryl ether sulfate/propylene glycol in 64:16:20% w/w ratio. DTX HPVs were prepared using phospholipid: SCOPE, 8:2% w/w ratio. DTX HPVs exhibited as a uniform deformable vesicles with size range 124.2 ± 7.6 nm, significantly improved skin permeation profile, and sustained drug release until 48 h. Superior vesicle deformability, better vesicle membrane fluidization, and SCOPE mediated enhancement in skin fluidization were the prime factors behind enhancement of DTX permeation. The improved cellular uptake, reduced IC50 values, and higher apoptotic index of DTX HPVs in MCF-7 and MDA-MB-231 cells ensured the therapeutic effectiveness of HPV based therapy. Also, HPVs were found to be predominantly internalized inside cells through clathrin and caveolae-dependent endocytic pathways. Bioimaging analysis in mice confirmed the tumor penetration potential and effective accumulation of HPVs inside tumors after topical application. In vivo studies were carried out in comparison with marketed intravenous DTX injection (Taxotere) to compare the effectiveness of topical chemotherapy. The topical application of DTX HPV gel in tumor bearing mice resulted in nearly 4-fold tumor volume reduction which was equivalent to intravenous Taxotere therapy. Toxicity analysis of DTX HPV gel in comparison with intravenous Taxotere dosing showcased remarkably lower levels of toxicity biomarkers (aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), and creatinine), indicating improved safety of topical chemotherapy. Overall results warranted the effectiveness of topical DTX chemotherapy to reduce tumor burden with substantially reduced risk of systemic toxicities in breast cancer.

10. Krupa Siddhapura, Harshad Harde, **Sanyog Jain**. Immunostimulatory Effect of Tetanus Toxoid Loaded Chitosan Nanoparticles Following Microneedles Assisted Immunization. **Nanomedicine: Nanotechnology, Biology and Medicine,** 12(1), 2016: 213-222 **(5.4)**

    The use of skin as a route for vaccination has been a clinically important topic for some time. In this article, we have investigated the efficacy of both solid microneedles and hollow microneedles as methods for topical delivery of tetanus toixoid. The study investigated potential of tetanus toxoid loaded chitosan nanoparticles (TT-Ch-NPs) following bare topical and microneedles assisted immunization. The TT-Ch-NPs were prepared by ionotropic gelation method using poly(sodium-4-styrene sulfonate) (PSS) as crosslinking agent which exhibited ~208 nm size and ~99% entrapment efficiency. The manufacturing process did not have any detrimental effect on integrity and conformation of antigen. The in vitro analysis demonstrated higher skin penetration following microneedles assisted immunization. In vivo immunization studies exhibited that TT-Ch-NPs delivered through microneedles induced comparable IgG and IgG1 titer, yet higher IgG2a titer than commercial TT vaccine. Similarly, microneedles assisted

administration of TT-Ch-NPs generated higher Th1 cytokines, albeit no significant alteration in Th2 cytokines levels than commercial TT vaccine. In conclusion, microneedles assisted administration of TT-Ch-NPs especially via hollow microneedles (HMN) could be considered as best preferred route for immunization due to induction of more balanced Th1/Th2 biased immune response. The positive finding in the experiments could provide a better method for vaccination in the clinical setting in the future