Review Article

Formulation strategies for drug delivery of tacrolimus: An overview

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Abstract

Tacrolimus (FK 506) is a potent macrolide lactone immunosuppressive agent used for prophylaxis of organ rejection after transplantation and graft-versus-host disease after bone marrow transplantation in patients. Moreover, tacrolimus is a drug of choice in the treatment of atopic dermatitis for decreasing side effects associated with the use of topical corticosteroids. In spite of its success in ensuring graft survival, therapeutic use of tacrolimus is complicated due to its narrow therapeutic index (between 5 and 15 ng/ml). Tacrolimus has a large inter-/intra-patient variability in pharmacokinetics profile and a poor oral bioavailability because of its poor solubility, P-gp efflux, marked pre-systemic metabolism by CYP3A in the enterocytes and liver first pass effect. Several formulation approaches such as oily solution, solid dispersions, complexation with cyclodextrins, liposomes etc., have been investigated to improve oral delivery of FK 506. In this review, we have discussed various formulation approaches that have been undertaken by various researchers to solve the problems related to the drug delivery of tacrolimus.

Key words: Bioavailability, drug delivery systems, nanocapsule, self-microemulsifying drug delivery system, tacrolimus

INTRODUCTION

In large part, the success of solid organ transplantation lies in the appropriate utilization of immunosuppressive medications. Tacrolimus, a lipophilic 23-member macrolide lactone [Figure 1] isolated from *Streptomyces tsukubaensis* (molecular weight of 803.5 Da), is an important immunosuppressant widely used in transplant patients, but with a narrow therapeutic window. The half-life of tacrolimus in human is 8.7-11.3 h.[1-3] Absorption of tacrolimus has been shown to be highly variable between individuals. After oral administration, the drug is generally absorbed with mean time of peak concentrations of 1.5 to 2 hours. However, in some patients, the drug may be absorbed over a prolonged absorption period, resulting in a more flat absorption profile.[4] The mean bioavailability is approximately 21%, although there is large intersubject variability.

The adverse effects of tacrolimus includes neurotoxicity, nephrotoxicity, gastrointestinal toxicity, hyperkalemia, hypertension, and myocardial hypertrophy.[3,5,6] On the contrary, sub-therapeutic level of tacrolimus may result in acute rejection of xenografts.[7] Therefore, factors affecting the absorption or distribution of tacrolimus are of clinical importance. Tacrolimus is known as a substrate of P-glycoprotein (P-gp), a multidrug efflux transporter, and cytochrome P450 3A4 (CYP3A4).[8,9]

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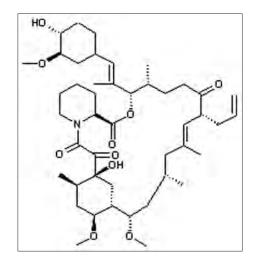E-mail: pranavrxp7@gmail.com

Figure 1: Structure of tacrolimus (http://www.zerenex-molecular.com/api.asp)

Consequently, any modulator of P-gp or CYP3A4 may alter the pharmacokinetics of tacrolimus.

Mechanism of tacrolimus involves its binding to immunophilin FK506 binding protein 12, creating a new complex that binds to calcium, calmodulin, and calcineurin, thus inactivating the phosphatase activity of calcineurin. Inactivation of calcineurin phosphatase prevents translocation of an activated T-cell transcription factor that promotes interleukin-2-mediated proliferation of helper T-cells, which plays a vital role in the immune response associated with allograft rejection.[1]

PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF TACROLIMUS

Tacrolimus has a poor solubility in water ranging from 4-12 μg/ml. The oral bioavailability of tacrolimus is poor and exhibits high intra and intersubject variability (4 to 89%, average 25%) in liver and kidney transplant recipients and in patients with renal impairment. Various factors have been reported for low and variable oral bioavailability of tacrolimus, including low solubility, high but site dependent permeability (highest in jejunum intermediate in ileum and lowest in colon), extensive first pass metabolism by CYP-450 3A4 in gut and liver, P-gp mediated drug efflux, influence of food intake and concomitant medication.[2,10] Cho *et al.,* reported that CYP3A5 genetic polymorphisms may be associated with the pharmacokinetic variation of tacrolimus in specific group of people.[11] Lampen *et al.,* also found a significant difference in the tacrolimus metabolite formation rates for males and females in human duodenal samples. Moreover, they found that tacrolimus is metabolized by both human liver and small intestinal microsomes.[12]

TACROLIMUS DRUG DELIVERY SYSTEMS

Various formulation approaches have been investigated for resolving the problems of tacrolimus. Researchers have gone through possible approaches for better pharmacokinetic profile and enhancing the efficacy of drug formulation. Fundamental mechanism of different formulations was to either increase water solubility of tacrolimus, thereby ensuring proper absorption from GI-tract, or to reduce P-gp efflux and CYP-450 mediated metabolism for enhancing oral bioavailability. Here, we have discussed several formulation approaches like SMEDDS,[13,14] pro-drug,[15] oily solution,[16] solid dispersions,[17] complexation with cyclodextrins,[18] pH-sensitive microspheres,[19] nanocapsule,[20] micelles,[21] nanosomes,[22] nano-liposomes of dry powder inhaler,[23] and liposomes[24] for delivery of tacrolimus.

Self emulsifying or self-microemulsifying drug delivery system

Self emulsifying or micro emulsifying formulation contains oil (synthetic or natural) with hydrophilic or lipophilic surfactants and co-solvents. This mixture is isotropic in nature and emulsify when exposed to GI media under mild agitation and forms oil in water emulsion.[25,26] Based on the type of surfactants used and presence or absence of oil, they are classified in four different types. Among these, type – III are known to form SMEDDS that is self-microemulsifying oil in water emulsion having globule size less than 200 nm in most cases.[27] Lipid-based formulations, especially SEDDS/SMEDDS, improve as well as normalize drug absorption, which is particularly beneficial for low therapeutic index drug like tacrolimus. These formulations enhance absorption by a number of mechanisms like improving drug solubility and maintaining drug in solution state throughout GI tract, inhibition of P-glycoprotein-mediated drug efflux and pre-absorptive metabolism by gut membrane-bound cytochrome enzymes,[28,29] promotion of lymphatic transport, which delivers drug directly to the systemic circulation while avoiding hepatic first-pass metabolism,[30] and also by increasing GI membrane permeability.[31]

Vivek *et al.,*[13] developed SMEDDS (Self-microemulsifying drug delivery dystem) of tacrolimus by using capmul MCM or capryol 90 as oil, cremophore EL or tween 20 as surfactants and carbitol as a co-surfactant. Formed SMEDDS showed improved solubility and dissolution rate. The optimized formulation (which was selected on the basis of drug loading efficiency, larger micro-emulsification area with high amount of oil incorporation, fast dispersion and minimum effect of pH, and dilution on mean globule size) of tacrolimus SMEDDS was superior to commercial formulation (pangraf capsule) with respect to *in vitro* dissolution profile and *in vivo* immunosuppressant activity. Optimized SMEDDS showed 100% drug release in 15 minutes compared to marketed formulation, which required 2 hr for complete drug release. Again, selected SMEDDS were stable over the period of 3 months at 40^0C and 75% RH, in terms of drug content, drug dissolution profile, and self micro emulsifying efficacy. *In vivo* study was carried out in mice and lymphocyte count was used as a measuring tool for therapeutic efficacy. Magnitude of reduction of lymphocyte showed by tacrolimus SMEDDS was significantly greater than Pangraf capsules. This clearly indicates the superiority of tacrolimus SMEDDS compared to marketed product. Authors assume that this improved therapeutic efficacy could be primarily because of increased solubility and dissolution rate of FK 506 leading to its rapid and efficient dispersion in GI tract. In addition, microemulsion formed was sufficiently stable and had very small globule size which provides a large interfacial surface area for drug diffusion.

Wang *et al.,*[14] developed SMEDDS by using bioenhancing excipients. Here, word bio-enhancing excipient covers the list of excipients that can promote the absorption of drug by changing normal functioning of P-gp or CYP enzyme system. TPGS and Cremophor can inhibit both pre-systemic drug metabolism and intestinal efflux mediated by P-gp. The researchers prepared two optimized formulations by using Miglyol 840 as oil phase, Transcutol *P* as co-surfactant, TPGS as surfactant (TPGS-SMEDDS) or Cremophor EL40 as surfactant (Crem-SMEDDS). Out of these two formulations, Crem-SMEDDS showed better dissolution profile than

TPGS-SMEDDS. Crem-SMEDDS showed 90% drug release in 20 minutes whereas TPGS-SMEDDS took 100 minutes to release the same amount of drug. *In vivo* bioavailability study revealed that both Crem-SMEDDS and TPGS-SMEDDS showed significantly higher extent of oral absorption, approximate AUC value 4000 ng.h/ml and 4500 ng.h/ml, respectively, than the homemade solution-600 ng.h/ml AUC value. The relative bioavailability of TPGS-SMEDDS and Crem-SMEDDS was almost same 735.3% and 802.0%, respectively. It could be assumed that in this case, bioenhancing ability (reducing P-gp mediated efflux or CYP450 mediated metabolism of tacrolimus) of excipients, and not the dissolution, plays an important role for improving bioavailability because dissolution of both SMEDDS was significantly different.

Wang *et al.*,[32] developed a novel gastro-retentive drug delivery system based on a self-microemulsifying (SME) lipid mixture for improving the oral absorption of the immunosuppressant tacrolimus. Liquid SME mixture composed of Cremophor RH40 and monocaprylinglycerate, was blended with polyethylene oxide, chitosan, polyvinylpyrrolidone, and mannitol, and then transformed into tablets via granulation, with ethanol as the wetting agent. The tablets were characterized with respect to swelling, bioadhesive, and SME properties. *In vitro* dissolution was conducted using HCl buffer at pH 1.2. Oral bioavailability of the tablets was examined in fasted beagle dogs. The tablet could expand to 13.5 mm in diameter and 15mm in thickness during the initial 20 min of contact with the HCl buffer at pH 1.2. The bioadhesive strength was as high as 0.98 ± 0.06 N/cm². The SME gastroretentive sustained-release tablets preserved the SME capability of the liquid SME formations under transmission electron microscope. When compared with the commercially available capsules of tacrolimus, the relative bioavailability of the SME gastroretentive sustained-release tablets was $553.4\% \pm 353.8\%$.

Solid dispersion
Recently in 2012, modified Eudragit E (polymer was dissolved in 10% HCL and final solution was spray dried and used) and HPMC TC-5E were used for preparing solid dispersion of tacrolimus by solvent evaporation method and Eudragit E was found to be superior than HPMC based solid dispersion. Solid dispersion prepared with Eudragit showed approximately 15 times higher solubility (1300 μl/ml) compared to HPMC solid dispersion (85 μl/ml). Moreover, eudragit solid dispersion inhibited reprecipitation of tacrolimus for 24 hrs.[33]

To improve the solubility of tacrolimus, solid dispersion was prepared by Park *et al*. Unlike traditional solid dispersion where the drug particle is converted to amorphous forms from crystalline forms[34] which may decrease the physical stability of drug molecules,[35] second proposed mechanism is reduction in particle size to provide larger surface area for dissolution.[36] In this technique, Young and co workers developed new surface attached carrier solid dispersion for enhancing the solubility of tacrolimus and, thereby, improving the dissolution rate of the

same. To overcome the drawback of chemical decomposition of drugs in melting method,[37,38] authors utilized the spray drying technique for preparation of surface attached carrier solid dispersion. Moreover, this technique is also environmental friendly because it utilized water instead of organic solvent.[39] Two different solid dispersions were prepared at different ratio of Na-CMC (carboxymethyl cellulose- sodium). Dispersion II which contained 3/24/3 Tacrolimus/Na-CMC/SLS, respectively, shows better solubility enhancement (2,000-fold solubility) and dissolution (10 folds) compared to drug powder than dispersion I, 3/1.2/0.3, which contained less amount of Na-CMC and surfactant. SEM, XRD, and DSC studies confirmed the presence of crystalline nature of drug after preparation of dispersions. Enhanced solubility and dissolution could be because of attachment of hydrophilic carrier and surfactant on the surface of drug crystal, which converts the hydrophobic surface of drug to hydrophilic surface.

In 2010, other investigators investigated that solvent evaporation was a more efficient technique than solvent wetting and surface attached solid dispersion. Solid dispersion prepared by solvent evaporation technique showed 600 μg/ml solubility, while solvent wetting and surface attached technique showed around 450 and 70 μg/ml solubility, respectively. In case of dissolution study, less than 10 % drug was released in 2 hr from the powder formulation. Approximate 75%, 60%, and 30 % drug was released from dispersion prepared by solvent evaporation, solvent wetting, and surface attached method, respectively. Enhanced solubility and dissolution rate could be explained by the conversion of crystal forms to amorphous from or conversion of hydrophobic to hydrophilic surface. It was, thus, concluded that solid dispersion prepared by means of solvent methods convert the drug from crystalline to amorphous state rather than converting drug particle surface from hydrophobic to hydrophilic, which is a common mechanism of surface attached solid dispersion.[40]

Dichloromethane free solid dispersion of tacrolimus was prepared in 2003 by using different carrier HPMC and ethanol as a solvent. Unlike conventional technique where drug and hydrophilic polymers (HPMC/PVP/PEG6000) were dissolved in DCM and ethanol (solvent evaporation technique), the new method was tried using only ethanol to swell hydrophilic polymer HPMC, and the drug was dissolved in swelling polymer. Physicochemical properties of new solid dispersion were same as the solid dispersion prepared by conventional method. XRD and DSC data confirmed that tacrolimus was in amorphous state in all solid dispersions. All prepared solid dispersion showed supersaturated dissolution profile (50 μg/ml), which was maintained for 24 hr in HPMC solid dispersion only, while in other two carriers it was decreased (30 μg/ml), which might be because of re-crystallization of the dissolved drug. *In vivo* studies for comparison of oral absorption between solid dispersions with two preparation methods were conducted in monkey. Five mg of tacrolimus containing solid dispersion was given with 20 ml of water. New method and conventional method showed almost equal AUC value 578 and 551 ng.h/ml, respectively. Prepared

HPMC solid dispersion without use of DCM was stable over the period of three months.[35]

An interesting study has been undertaken by Ahmed S and his team,[41] where different techniques namely NIR, PXRD, DSC, and Raman spectroscopy were evaluated for their ability to detect the trace crystallinity of tacrolimus in solid dispersion formulations. Solid dispersion was prepared by HPMC using solvent evaporation technique and spiked with different amount of drug crystal. The team found following order for sensitivity of method to detect drug crystal in formulations. NIR ($r^2 = 0.99$) > PXRD (0.97) > Raman (0.95) > DSC (0.89). Partial least square regression analysis was employed for evaluating different models.

Tacrolimus with *Schisandrasphenanthera* extract

In 2007, Xin and co workers[42] investigated the preliminary role of *Schisandrasphenanthera* extract on the pharmacokinetic profile of tacrolimus in healthy volunteers. In China, it is a common practice that *Schisandrasphenanthera* extract (SchE) and tacrolimus are co-administrated to treat renal and liver transplant recipients. Simultaneous administration of SchE in healthy volunteers increased the mean AUC (164%), AUMC T_{max} (around 36%) and C_{max} (approximate 200%) of tacrolimus, whereas its CL/F (49%) and V/F (53%) decreased significantly. It was assumed that interaction of herbal extract with P-gp and CYP 3A4 may be (later confirmed by Xiao *et al.*,) the factors that might increase the oral AUC of tacrolimus.

In 2010, Qin *et al.*,[43] investigated *in vitro* and *in vivo* effects of *Schisandrasphenanthera* extract (Wuzhi Tablet) on the absorption and first-pass intestinal and hepatic metabolism of tacrolimus. In this study, the herbal extract, when co-administered with tacrolimus, increased the AUC and F_{oral}.$F_{abs} \times F_g$ and F_h were increased by 111% and 21%, respectively, suggesting that the reduction in the intestinal first pass effect was responsible for enhanced oral absorption of tacrolimus. In $CaCo_2$ cell line studies, WZ extract was found to inhibit P-gp mediated efflux of tacrolimus. Moreover, this herbal extract was also responsible for inhibiting tacrolimus metabolism in human liver and rat microsomes.

Nanosomes

To reduce the side effects associated with polyoxyl 60 hydrogenated castor oil and organic solvents, nanosomes were prepared in aqueous media without the use of any organic solvent.[22] Use of aqueous phase in the preparation of nanosomes makes it more suitable for an industrial scale up because there is no necessity to remove organic solvent and the strict air-quality controls instituted by the law-enforcing, potential toxicity and explosion of organic solvents. Current, marketed preparation of tacrolimus contains polyoxyl 60 hydrogenated castor oil (HCO-60) and ethanol. Among these two excipients, polyoxyl 60 hydrogenated castor oil is known to produce side effects like hyperpyrexia, leukocytosis.[44] In high dose, it causes neurotoxicity and nephrotoxicity (Prograf®). Nanosomes dispersion was prepared by using comparatively low toxic lipids like Soy

Phosphatidylcholine and alpha-tocopherol. Final suspension was lyophilized and reconstituted in water for injection before use. Fourteen days a toxicity study of prepared formulation was conducted in mice and rats and was found to be immortal. Prepared dispersion showed almost similar pharmacokinetic profile in healthy humans as compared to marketed HCH-60 based product (AUC value- 572721 pg.h/ml of HC0-60; 627391 pg.h/ml of nanosomals) and was also equally effective which was confirmed by percentage fall in lymphocyte. A total of two adverse events were reported during the clinical trial phase. Both the adverse events (upper respiratory tract infection and burning epigastric pain) were mild in nature and resolved without requiring the withdrawal of the subject.

Polymeric micelle for intravenous delivery

Recently, polymeric micelle was prepared for intravenous delivery of tacrolimus by Wang *et al.*[21] In the last few years, it has been reported that polymeric micelles can be a good carrier for hydrophobic drug delivery. Basically, in polymeric micelles, hydrophobic drug is entrapped in a core surrounded by hydrophilic polymeric shell 9.[45] Besides the obvious advantage of improved solubility, this system has good stability, long *invivo* circulating properties, passive targeting, minimized cytotoxicity, reduced drug degradation, minimized adverse effects of the drug on visceral organs and has the potency to be site-specific. According to authors, current marketed preparation of pangraf IV injection contains Cremphor EL as excipient, which can cause hemolysis *in vivo*. Author and his team synthesized biodegradable polymer poly(_-caprolactone)– poly(ethyleneglycol)–poly(_-caprolactone) (PCEC) copolymers which has reasonable potential application in drug delivery system due to its characterized core–shell structure and good biocompatibility. Tacrolimus loaded micelle were prepared by means of solid dispersion technique. The prepared micellar solution was filtered and the filtrate was stored for further investigation and lyophilized. Prepared micelle was evaluated for different tests like drug release, cytotoxicity, hemolytic test, stability and XRD. Results showed that the prepared micelles had excellent stability and dispersibility. XRD studies confirmed that the drug was entrapped in core of formed micelles. Drug loaded micelles showed sustained release (up to 192 h) of entrapped drug. According to cytotoxicity and hemolytic tests, the PCEC micelles were confirmed to be low-toxic (50% inhibiting concentration (IC_{50}) of PCEC micelles was higher than 3 mg/ml) and suitable for intravenous injection tests (no sign of hemolysis up to concentration of 8 mg/ml).

Nanocapsule

Interesting approach has been undertaken by Benita *et al.*,[20] who developed a novel double coated nanoparticulate delivery system, which can reduce intestinal degradation and thus enhance uptake of tacrolimus without changing the physiological function of P-gp. Inhibition of active efflux is one of the strategies to improve oral absorption of tacrolimus. Some active pharmaceutical ingredients have succeeded in markedly improving the oral bioavailability of P-gp substrate

drugs by P-gp inhibition in animals.[46-48] However, their clinical applicability has been limited since they lacked specificity and inhibited two or more ABC transporters. Their administration was also associated with marked adverse effects. Secondly, P-gp can be inhibited by inert pharmaceutical excipients for enhancing intestinal absorption of P-gp substrate drugs. Until now, only encouraging *in vitro* and animal study results have been published,[49-50] although toxic effects resulting from chronic administration of these excipient inhibitors cannot be excluded. To overcome the above mentioned drawbacks, researchers utilized the concept of two drug delivery systems that are swellable microspheres and nanocapsules which swelled and adhered to the mucosa due to the presence of HPMC, while the Eudragit L dissolved creating large pores in the microsphere matrices allowing the diffusion of nanocapsules. Argan oil, oleolylpolyoxyglycerides, Edragit RS, Edragit L, and tacrolimus were dissolved in Acetone: Ethanol (95:5) mixture and water was added to produce o/w emulsion. 0.5% HPMC solution was added in above dispersion prior to spray drying. Delivery system consisting of lipid nanocapsules of tacrolimus was embedded in a specific blend of Eudragit polymers together with HPMC. Optimized formulation with or without verapamil showed equivalent uptake as compared to tacrolimus solution combined with verapamil, a well known P-gp inhibitor in Caco-2 monolayer studies, thereby, confirming that verapamil was not playing a role in tacrolimus absorption elicited by double coated nanocapsule drug delivery system. Intestinal jejunum permeation studies confirmed that the novel delivery system enhanced tacrolimus absorption by escaping the P-gp efflux pump effect and protecting the drug from the degradation effect of the CYP450 enzymes.

pH sensitive microsphere

In 2005, the use of pH sensitive microsphere containing nanoparticle loaded tacrolimus for colon delivery was investigated in inflammatory bowel disease. The study was focused to minimise the adverse effects of tacrolimus during the treatment of inflammatory bowel disease that has been achieved by targeting the drug at desired site of action. To achieve this objective, authors Lamprecht *et al.*,[19] prepared tacrolimus loaded nanoparticles for selective drug accumulation and penetration at the site of inflammation, for minimising loss of drug because of nanoparticles degradation. During passage to colon these nanoparticles were entrapped in pH sensitive microsphere, thereby, avoiding undesirable effects. For making NPMS (nanoparticle loaded microsphere), the drug was incorporated in PLGA and this nanoparticle was embedded in Eudragit P-4135F polymer. Prepared pH sensitive microsphere containing nanoparticle loaded tacrolimus showed 100 % nanoparticles and tacrolimus release at pH 7.4, and at pH 4, approximate 30% nanoparticles and tacrolimus were released in 4 hrs. NPMS formulations showed better therapeutic value (calculated from colitis activity score, colon/body weight, and myeloperoxidase activity) than nanoparticle loaded tactolimus, microsphere loaded tacrolimus, and solution of tacrolimus.

Prodrug

To reduce the side effects of surfactant used in intravenous delivery of tacrolimus, highly water soluble tacrolimusprodrug was synthesized. Marketed tacrolimus injection contains cremophor EL, which is nephrotoxic[51] and can produce anaphylactoid reactions,[52] while hydrogenated castor oil can cause allergic symptoms such as leukocytosis, hyperpyrexia, eruption,[53] and immunological suppression of the growth of erythroid progenitors in humans.[54] Authors developed the novel tacrolimus conjugated compound (can be usefor oral, parenteral, intranasal, intrabronchial, transdermal, and topical drug delivery systems), which can be dissolved in water, formed by chemically binding the sparingly soluble drug, tacrolimus, with the water soluble polymer, mPEG. Firstly, tacrolimus was esterified by initially acylating in presence of iodoacetic acid and a coupling reagent, such as dicyclohexylcarbodiimide (DCC) and a base such as dimethylaminopyridine (DMAP) at the 24-, 32-, or 24, 32-positions. In the presence of base (sodium bicarbobate), this acylatedtacrolimus reacted with mPEG (methoxy poly (ethylene glycol)). These conjugates were converted again into tacrolimus by the action of enzymes in human liver homogenate. Prepared conjugates were subjected for enzymatic hydrolysis test and converted back to tacrolimus by the action of enzymes in human liver homogenate with a short half-life of the hydrolysis reaction while the derivatives were stable for longer period of time in aqueous media, which allowed reconstitution of preparation without the concern for any significant chemical degradation in the formulation.[15]

Nano liposomal dry powder inhaler

To achieve site specific drug delivery subsequently reducing systemic toxicity for preventing refractory rejection of lungs after transplantation, nano-liposomal dry powder inhaler of tacrolimus was formulated and characterized. Liposomes were prepared by using lipid film hydration method using HSPC and cholesterol. Tacrolimus loaded liposomes were dispersed in phosphate buffer saline (PBS) pH 7.4 containing different additives like lactose, sucrose, and trehalose, and L-leucine as anti-adherent. The dispersion was spray dried and spray dried powders were characterized. Developed formulations were found to have *in vitro* prolonged drug release up to 18 hours, following Higuchi's Controlled Release model. *In vivo* studies revealed maximal residence of tacrolimus in the lungs for 24 hours, suggesting slow clearance from the lungs.[23]

CONCLUSION

Due to high potency and better success ratio compared to cyclosporine, different formulation approaches of tacrolimus have been investigated *in vitro* and *in vivo* in animals. However, the *in vitro* findings have not translated efficiently into human clinical trials, with very few researchers working on human trials. Ahmad and group prepared polyoxyl 60 hydrogenated castor oil free nanosomes dispersion and found them to be pharmacokinetically equivalent to marketed products. Secondly, in China, traditional *S. sphenanthera*extract (SchE), containing Schisandrin could enhance the *in vivo* whole blood concentration

of tacrolimus, which might be due to the inhibition of CYP3A and/or P-gp. These findings were confirmed by Huang *et al.* The majority of work has been focused on enhancement of *in vitro* solubility and absorption. There is no doubt that development of new formulations or analogues of tacrolimus with better bioavailability and having low inter/intra-subject variability will be critical for future development of tacrolimus formulation. This will make the use of drug more effective and safe. In this regard, SEDDS can be effective which is known for the improvement of absorption and can also reduce the variability associated with *in vivo* absorption. Care should be taken while selecting the excipients, which can block P-gp efflux and inhibit CYP metabolism. At the same time, the surfactants used should be safe.

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