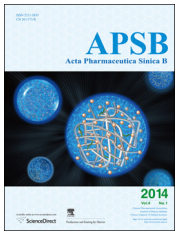
Acta Pharmaceutica Sinica B 2014;4(1):3–17

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Can formulation and drug delivery reduce attrition during drug discovery and development—review of feasibility, benefits and challenges

Basavaraj S, Guru V. Betageri*

Western Centre for Drug Development, Western University of Health Sciences, Pomona, CA 91766, USA

Received 22 November 2013; revised 6 December 2013; accepted 16 December 2013

KEY WORDS

Drug discovery and development;
Drugability;
Formulation;
Drug delivery technology

Abstract Drug discovery and development has become longer and costlier process. The fear of failure and stringent regulatory review process is driving pharmaceutical companies towards "me too" drugs and improved generics (505(b) (2)) fillings. The discontinuance of molecules at late stage clinical trials is common these years. The molecules are withdrawn at various stages of discovery and development process for reasons such as poor ADME properties, lack of efficacy and safety reasons. Hence this review focuses on possible applications of formulation and drug delivery to salvage molecules and improve the drugability. The formulation and drug delivery technologies are suitable for addressing various issues contributing to attrition are discussed in detail.

© 2014 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.

*Corresponding author.
E-mail address: gbetageri@westernu.edu (Guru V. Betageri).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

2211-3835 © 2014 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.apsb.2013.12.003

1. Introduction

The discovery of new drug is a multi-stage complex process, each stage lasting for years. Probability of molecule discovered in early stages making it to market is 1 in 10,000[1]. In addition to complexities in the science of making new safe and efficacious drugs, the political, economic factors coupled with stringent regulatory requirements and review process, the drug discovery has become even more complex and long lasting[2]. As a result, the cost of inventing a new drug has increased to staggering USD 2.6 billion from 100 million during 1979[3–5].

Pharmaceutical industry is being criticized for not bringing more innovative medicine into market for treatment of unmet medical needs. These days, industry is producing too many drugs which are similar to each other and offer marginal advantage over existing treatment. These "me too" drugs although provide alternative treatment options but led to price competition and reduced profit margins before the entry of generic versions in the market[6]. Since regulatory approval process for "me too" drugs is relatively fast and easy as these are structurally similar to approved drugs and hence the pharmaceutical companies tend to focus on analog research rather than real innovative medicine. Hence there has been innovation deficit in pharmaceutical R&D these days[7].

The sharp decline in the number of new drug approvals in the last decade can be attributed to attrition of molecules during discovery and development. The attrition rate is very high in the drug development process, only 15% of molecules entering the clinical trials receive marketing approval[8]. The success rate from phase-III clinical trial to market translation is reported to be 50–70%. The molecules are dropped during preclinical stage and withdrawn from further development during clinical studies for various reasons, such as lack of efficacy, toxicity, poor absorption, distribution, metabolism and elimination (ADME) properties, commercial interest and market competition[9,10]. The survey of molecule in clinical development from 1964–1985 revealed that poor pharmacokinetic profile contributed majorly (39.4%) for attrition of molecules in clinical development[9], however figure dropped to 10% in 2000, thanks to advancement in formulation technologies[10]. Lack of efficacy (30%) and unacceptable clinical safety and toxicity (30%) were found to be major factors for discontinuation of clinical candidates in 2000[10].

There have been several approaches discussed in the literature to reduce attrition of drug candidates in the clinical development[10], identification of right target and strong mechanism of action would reduce the failure with regard efficacy, the attrition due to toxicity and safety can be reduced by eliminating molecules with mechanism based toxicity, the identification of biomarkers, selection of appropriate animal model for efficacy testing, evaluating proof of concept at early clinical studies were suggested for reducing attrition[10].

The failure of drug candidates may not be limited to fore mentioned reasons, there are other several factors contributing to attrition, for example discovery and development of drug candidates for central nervous system (CNS) disorders face additional barriers than those intended for other therapeutic application[11]. The CNS drugs while exerting activity, may also led to unwanted changes in the brain physiology and neurochemical balance, hence the stringent safety requirements for these drugs. In addition, the blood brain barrier (BBB) also poses another barrier for development of drug candidates in CNS category. The several drug candidates reported to be dropped due to their inability to cross BBB. The Gavestinel which had completed phase III clinical trials but was failed to demonstrate clear efficacy due to its poor permeation across BBB[11]. Although there have been significant innovative solutions to address the ADME issues such as absorption by enhancing solubility and permeability of molecules. However issues such as rapid metabolism, especially first pass effects have met with limited success. Good example is resveratrol (RSV), a natural biochemical with diverse biological activity has

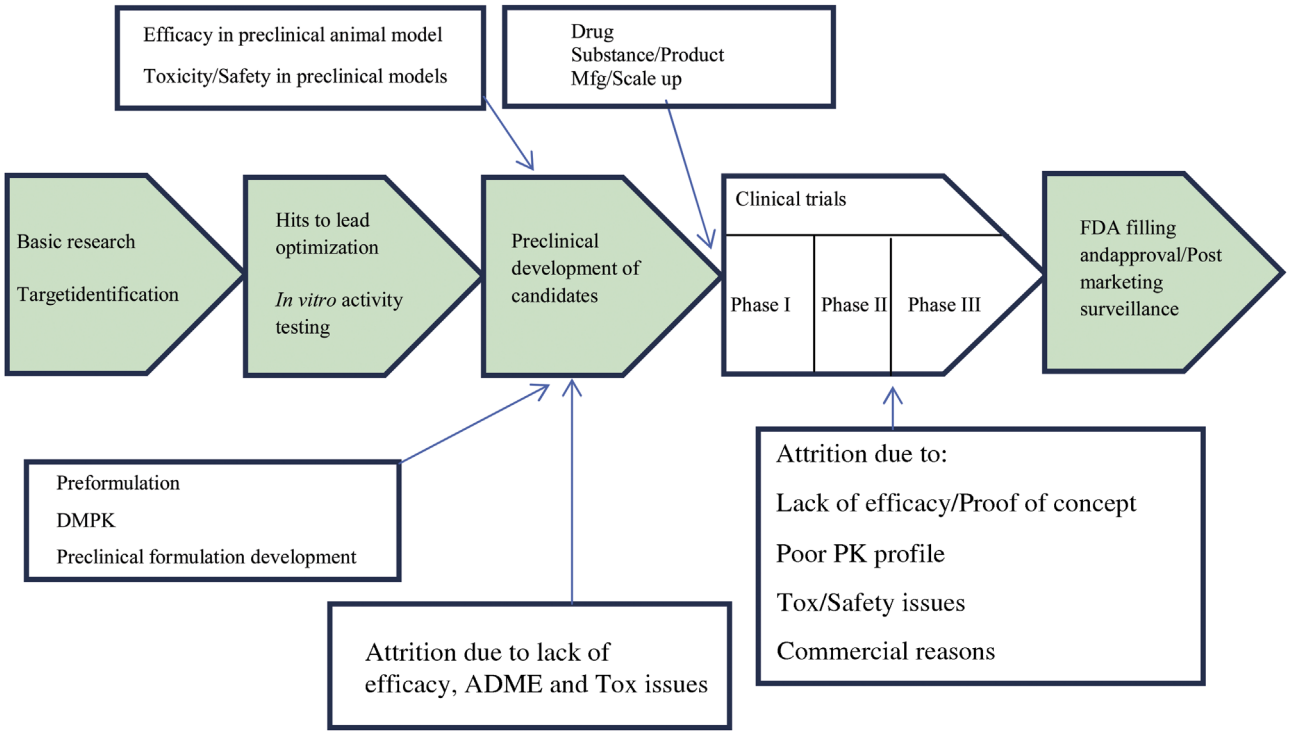


Figure 1 Representative scheme for drug discovery and development with reasons for attrition at each stage.

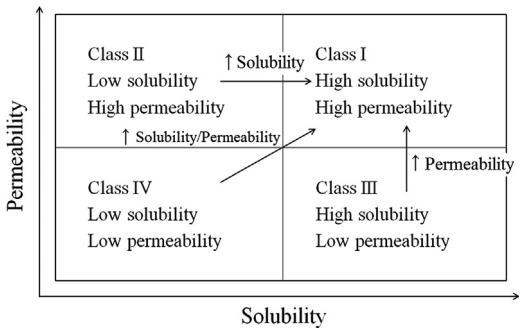


Figure 2 BCS classification system and possible ways make most of molecules into Class I.

limited clinical utility probably due to its rapid metabolism in the body (half-life of 0.13 h) and only absorbed as metabolites after oral administration due to complete presystemic metabolism[12]. The biological drug candidates such as therapeutic peptides, proteins and antibodies intended for various therapeutic applications are prone for destabilization by proteolytic enzymes in the body and also precipitate unwanted immunogenic reactions and hence specific delivery technologies are necessary to enhance chances of their drugability[13]. The excess of drug in the circulation and body tissues other than site of action may lead to toxicity. Narrow biological difference in the host and cancer cells makes chemotherapy more challenging, often antineoplastic agents are toxic chemicals and biologics designed to kill the cancer cells, while exerting desired pharmacological effects, they precipitate severe toxic effects[14]. Hence there is need for technologies to address various issues in preclinical and clinical development of drug candidates which lead to attrition. The formulation and drug delivery technologies have been tested for their utility to enable candidates at preclinical and clinical developmental stages to further stages. There are few reports suggesting how formulation strategies are explored in drug discovery and development; however, most of them tend to focus on solubility and permeability issues[15–18]. Hence this review aims to provide comprehensive information on how formulation and drug delivery technologies can be explored to overcome various challenges in drug discovery and development. The review discusses the examples of drug delivery technologies applied for enhancing therapeutic utility and safety profile of already marketed drugs, provides insight to how drug delivery can be better explored in the early stage of discovery and development to reduce the attrition. In addition, this review also briefly summarizes drug delivery research; especially proliposomes based oral delivery in our lab that can be explored for enabling drug candidates to reach market.

2. Formulation/drug delivery strategies for addressing ADMEs issues

The discovery and development of drug involves various stages. The flow chart in Fig. 1 depicts simplified drug discovery and development process[19]. The hunt for new drug starts with selecting disease and identification of target. The molecules from synthesis or biological origin would normally be screened for *in vitro* biological activity. The selected molecules then move to further preclinical testing such as *in vivo* activity in animal models, *in vitro* metabolism and pharmacokinetic profiling in animals. During this stage molecules will also be tested for physicochemical properties such solubility, ionization and partition behavior. The molecules with desired activity, ADME and physicochemical properties will undergo stringent safety and toxicity testing before they enter clinical testing. The physicochemical parameters coupled with ADME testing will assist in determining drugability of molecules. The biopharmaceutics classification system (BCS) groups molecules into four classes based on solubility and permeability as shown in Fig. 2[20]. The molecules belonging to Class I are believed to more development friendly as they possess desired characteristics which make them more drugable. The molecules belonging to Classes II–IV will have problems associated with either solubility and/or permeability. Lipinski's rule of five also provides useful information on biopharmaceutical behavior of molecules based on molecular weight H bond donors/acceptors and log *P* values[21]. The molecules which lack desired ADME profile will fail to elicit pharmacological activity *in vivo*. The importance of early ADME prediction and profiling has been reported in the literature and early ADME profiling found to improve output of discovery[22]. The many hits during discovery fail to become lead candidate for clinical development due to poor ADME properties. The formulation approaches that can improve ADME profile of molecule amenable for further development are discussed here.

2.1. *Formulation approaches for improving solubility*

Poor solubility leads to incomplete absorption and solubility of molecules becomes problem in early stages of discovery[23]. An estimated 70% of molecules in the developmental pipeline are believed to be poorly soluble and 40% of already approved drugs are poorly soluble[24]. There have been several inventions in the formulation science that led to viable technologies for the formulation of poorly soluble drugs to improve delivery. The buffering/salt formation, cyclodextrin complexation, nanosuspensions, emulsion/microemulsion systems, cosolvency and surfactant solubilization are commonly used technologies for delivery of insoluble drugs.

Salt formation has been considered simple and most effective method for improving solubility and dissolution rate of weakly acidic and basic drugs[25]. The salt formation provides viable strategies for molecules with solubility as rate limiting in their development. There are several drugs on market as salt forms such as nelfanavir mesylate, atazenavir sulfate, ziprasidone hydrochloride and mesylate, imatinib mesylate, indanvir sulfate, metformin hydrochloride, amlodipine besylate, losartan potassium, diclofenac sodium and potassium, sodium valproate, and ketrolac tromethamine. For many of these molecules, selection of appropriate salt forms had enhanced their developability into clinical candidates and finally to marketing approval. Apart from salt approach, the buffering/pH adjustment is widely used in the preclinical formulation development of insoluble molecules[26]. Nearly 85% of molecules from one of the Pfizer research facilities were formulated with pH adjustment and cosolvency in the year 2000[26]. Similarly many of parenteral formulations on the market contain counter ions for pH modifications, examples include Ciprofloxacin Injection, Leurolide, Vincristine, Trimethoprim/Sulfamethaxazole and Methylprednisolone Injections[27].

Cyclodextrins have been explored extensively for formulation of insoluble molecules. The cyclodextrins are versatile excipients used in oral and parenteral formulations and choice of excipient for solubilization of new chemical entities (NCEs) in the preclinical

development[28,29]. The DRF-4367 a weakly acidic NCE had an oral bioavailability of 20% after oral administration as suspension to rats; however, solubilized formulation with hydroxy propyl-β-cyclodextrin and meglumine enhanced bioavailability of molecule nearly to 100%, thus enabling molecule for further development[29].

The administration of molecules in the form of nanosuspension has shown to increase the oral bioavailability. The formulation of molecules as nanosupension in the early preclinical development offers simple, safe and cost effective formulation strategies for formulation of insoluble NCEs. There are many simple lab based instrumentation and techniques described in the literature to produce nanosuspension for the preclinical applications[30]. The nanosuspension of DRF-4367 with mean particle size of 100–300 nm prepared using bead milling followed by high pressure homogenization has showed significant improvement in the oral bioavailability in rats (65% at 30 mg/kg) as against plain suspension (35%)[31].

Emulsions/microemulsions based drug delivery systems have been found useful in enhancing oral bioavailability of insoluble molecules[32]. The microemulsions have also been used in preclinical formulation development of insoluble NCEs[33]. The torcetrapib which was considered to be blockbuster molecule for treatment of elevated cholesterol inherently had poor absorption due to limited solubility in GI fluids. The development of self microemulsifying drug delivery system (SMEDDS) formulations consisting of oils/surfactants/cosurfactants has led to increased absorption of molecule in the dogs as compared to aqueous suspension[33]. There was 30–40-fold increase in C_{max} and AUC when administered as SMEDDS under fasted conditions[33]. The poor solubility was considered to be one of the problems in the preclinical development and appropriate formulation development ensured progress of molecule to clinical testing. However the molecule was discontinued from late stage clinical testing due to safety reasons[34].

The cosolvent and surfactant based formulation approach has been widely used to address the solubility issue in the preclinical development[35,36]. The *N*-Epoxymethyl-1,8-naphthalimide (ENA) a novel antiproliferative drug candidate with potent anticancer activity was found to have poor solubility and stability in aqueous systems; however, the use of ethanol and Cremophore® EL has resulted in a viable formulation necessary for further preclinical development of the molecule. Similar solvent/surfactant based system was used for formulation of taxols[27]. The general template for screening of formulation for insoluble NCEs at discovery stage has been described in the literature[36]. These examples have clearly demonstrated that formulation/drug delivery technologies can overcome issue of solubility in drug discovery/development. However in our experience, given advent of these many technologies for solubilization, it is not always possible to formulate all insoluble molecules. Certain rock solid molecules may still pose problem and go undeveloped.

2.2. *Formulation approaches for improving permeability*

Permeability of drugs across the GI membrane is one of rate limiting step in the absorption of drugs. The solubility and permeability of drugs together determine extent of oral absorption. The physicochemical factors such as log *P*, molecular weight, polar surface area, charge/ionization, number of hydrogen bond donors and acceptors determine permeability of molecule[37]. In general, hydrophobic small molecules with higher log *P*, lesser polar surface area, will have greater permeability across biological membrane. Unionized species are more favored for absorption than ionized species. In addition, drug efflux mediated mainly by transports such as P-glycoprotein (Pgp), a multidrug resistant proteins (MRP) and organic cation transporters (OCT) lead to poor absorption of drug molecules[38]. The low permeability of molecules may hinder their development and several NCEs are reportedly dropped during the discovery stage.

There are several methods reported in the literature for enhancement of permeability and prevent the efflux of drug molecules by transporters. The permeation of molecules occurs by two pathways, transcellular uptake across epithelial cells and paracellular passage through intercellular space. The improvements in both paracellular and transcellular absorption has been accomplished by the use of chemical enhancers. Diverse classes of compounds including detergents, surfactants, bile salts, Ca^{2+} chelating agents, fatty acids, medium chain glycerides, acyl carnitine, alkanoyl cholines, *N*-acetylated a-amino acids, *N*-acetylated non-a-amino acids, chitosans, mucoadhesive polymers, and phospholipids have been investigated for permeation enhancement[37,39]. Many of these agents act as detergent disturb structure of lipid bilayer leading to increased membrane fluidity and permeability. On other hand, certain agents act as calcium chelators and disturb tight junction between epithelial cells. The trimethyl chitosan reported to bind to components of tight junction and widen the intercellular space[39].

Various excipients which are commonly used in the pharmaceutical formulations are reported to be potent inhibitors of Pgp mediated efflux of drugs. The cosolvents such as polyethylene glycol, surfactants such as polysorbates, Vitamin E TPGS, Cremophores, Poloxamers and dendrimers have been reported to significantly reduce Pgp efflux of drugs[40]. Coadministration of cyclosporine, a Pgp substrate with paclitaxel has resulted in enhanced absorption of paclitaxel in human[41]. Increased and consistent absorption of cyclosporine from SMEDDS formulation, Neoral® could be partially due to bypassing of Pgp efflux during the absorption[42]. The scientific efforts in enhancing permeability of molecules have been successful to considerable extent. Several techniques described in this section can be utilized in early stage of drug discovery for salvaging of molecules with poor permeability. However, these techniques have limitations and not all molecules can be benefited.

2.3. *Formulation/drug delivery approaches for addressing metabolism and elimination*

Fate of drug in the body depends on its susceptibility to metabolizing enzymes. Most of drugs undergo metabolism before excretion and very few drugs are excreted unchanged. Phase I metabolism includes reactions such as hydroxylation and oxidation. The phase II metabolism involves dervitization reactions such as glucuronidation and sulfation[43]. The metabolism renders the molecules more hydrophilic and amenable for excretion. Rate of metabolism determines plasma half-life of molecule. Faster metabolism leads to shorter half-life. Optimum plasma residence of molecules is essential to elicit pharmacological action. Molecules which show significant activity *in vitro* often fail to produce meaningful activity in *in vivo* conditions due to rapid metabolism and resveratrol is one such molecule[12]. Molecule best in class, in terms of activity may not progress to further development various issues such as ADME, rather molecules with balanced properties

will move forward[44]. In this section, formulation approaches to address metabolism are discussed.

Many of the commonly used excipients have shown to reduce metabolism of drugs in *in vitro* conditions. The surfactants such as Tween 80, Cremophore® EL and Solutol® HS have reduced metabolism of midazolam when coincubated with rat liver microsomes. The reduction of metabolism was up to 50% in case of Solutol® HS and Cremophore® EL at 0.3% *w/v* surfactant concentration[45] and similar results were reported for colchicine with these surfactants[46]. Intravenous pharmacokinetic studies revealed that Solutol® HS had significant effect on clearance of colchicine, clearance was significantly reduced and C_{max} was increased, however effects were minimal on midazolam. The weak cytochrome p450 (CYP) inhibitory effect of surfactant was sufficient to affect moderately metabolized colchicine[47]. In contrast, pharmacokinetic profile of midazolam was unchanged because of its rapid rate of clearance through metabolism[48]. The amount of surfactant below the CMC after dilution with blood was also attributed to lack of effects on metabolism as against *in vitro* results. The surfactants have been reported to affect pharmacokinetic profile of anticancer drugs such as paclitaxel, docetaxel, etoposide and doxorubicine[45]. The intravenous administration of 2.5 mL/kg Cremophore® EL, 10 min before dosing of either doxorubicine or doxorubicinol has resulted in doubling of AUC and C_{max} and reduction of clearance to half for both the drugs[49]. Hence coadministration of surfactants can be considered to reduce metabolism of molecules during discovery stages, however hypersensitivity reactions towards surfactants needs to be considered. Surfactants with better clinical tolerability must be used.

Another simple, safe and effective approach to stabilize the molecule against metabolism is polymer conjugation. Reversible or irreversible covalent attachment of polymer to small molecule non-peptide drugs can result in reduced metabolism, increased circulation time, improved activity and reduced toxicity[13]. The shielding effects of polymer on drug molecules against enzymes/chemicals result in reduced degradation. However, all molecules may not be feasible for polymer conjugation. The molecules with functional groups such as OH, COOH, NH_2, SH can be explored for polymer conjugation[49]. Various polymers, such as dextrans, polyethylene glycol (PEGs), *N*-(2-hydroxypropyl)methacrylamide (HPMA) and polyglutamic acid, have been explored for conjugation with drugs.

The polymer conjugation approach has been successfully applied for improving safety, efficacy, and the pharmacokinetic profile of small molecule drugs such as doxorubicin (DOX), norfloxacine (NOR), campothecine (CAMP) and paclitaxol (PTX)[50–56]. Numerous reports have been published on polymer conjugation of doxorubicin. Doxorubicin-dextran polymer conjugates were extensively investigated. In mice, conjugate was found to have a higher plasma level, which persisted for a long time, and thus provided very effective passive targeting[51]. However, phase I clinical studies of the conjugate showed that the conjugate was slightly more toxic than DOX itself. WHO grade IV hepatotoxicity, grade III cardiotoxicity and grade III thrombocytopenia were some of the major clinical signs noticed. However, polymer conjugate had a prolonged circulation time, with a dose dependent elimination half-life of $t_{1/2\beta}$ of 2.69 to 11.58 h and $t_{1/2\gamma}$ of 41.44 to 136.58 h (doses 12.5–40 mg/m^2).

The campothecine–PEG conjugates were prepared using glycine linker. The electronegativity of linker was found to increase the stability of conjugates[52]. The alkylated glycine linker further enhanced stability of conjugates. The activity of these conjugates was similar to campothecine-20-PEG without glycine linker which hydrolyzed rapidly in plasma. These results demonstrate that despite being stable in plasma, campothecine–glycine–PEG conjugates demonstrated good activity because of probable hydrolysis of conjugates in cells to release parent molecule. This way circulation half-life can be increased without affecting activity and improving overall efficacy.

Norfloxacin (NOR)–dextran conjugates were prepared and evaluated for the targeted delivery to macrophages in liver to maximize anti-tuberculosis treatment[53]. Norfloxacine was conjugated to dextran *via* tetrapeptide spacer, such as Gly–Phen–Gly–Gly (GFGG), Gly–Phe–Gly–Gly (GFAL). The conjugates were relatively stable in plasma and buffers, and slowly hydrolyzed in the presence of cathepsin. The NOR–peptide–dextran–mannose conjugates were also prepared to enhance targeting to macrophages. Although conjugates were less active against mycobacterium than norfloxacin, it was envisaged that under *in-vivo* conditions, conjugates would hydrolyze to native norfloxacin, resulting in the desired therapeutic effect.

Paclitaxol has been conjugated to soluble polymers such as polyethylene glycol[54], micelle forming diblock polymer polyethylene glycol–polylactide (PEG–PLA)[55]. The paclitaxol–PEG conjugated made *via* ester link, was found to undergo rapid hydrolysis in plasma with $t_{1/2}$ of 30 min. The PTX–PEG ester conjugates were considered ideal for prodrugs and for parenteral formulation to overcome solubility issues. However, PTX–PEG prodrugs were found unsuitable for this use as long circulating drug delivery systems. Paclitaxol was conjugated to PLA–PEG with an ester bond, the conjugate was found to be active against H7402 liver cancer cell line[55]. The authors concluded that paclitaxol was released in the cells, and that polymer conjugation did not affect the activity of paclitaxol. However, data on pharmacokinetic and *in vivo* activity was not reported.

Recently, polymer conjugates of a metabolically very unstable molecule resveratrol were prepared and evaluated[56–58]. It was observed that RSV–PLA–PEG ester conjugates were found to be relatively stable in plasma, displayed better pharmacokinetic profile with multifold higher C_{max} and AUC than plain RSV.

3. Proliposomal drug delivery: solution for drug delivery needs in drug discovery and development

The lipid based drug delivery systems are suitable for both oral and parenteral applications. Emulsions systems have been in use for decades, the liposomal parenteral formulations were additions. The lipid based drug delivery has been investigated extensively in recent times. The proliposomal drug delivery is becoming increasingly popular (Table 1). Proliposomes are basically powder mixture of drug and lipid, intended to produce multilamellar liposomes upon contact with aqueous media entrapping adequately hydrophobic drugs[59,60]. The proliposomes have distinct advantages over liposomes, as they are free flowing powder can be incorporated into solid dosage forms such as capsules or tablets. In addition, simple process of preparation makes them more suitable for industrial scale manufacturing[59,60]. The multilamellar liposomes containing drug molecules formed from proliposomes mixture are believed to be absorbed *via* lymphatic absorption. Based on lipid composition and type of lipid, there can two possible ways for absorption of entrapped drug. Firstly, certain investigations suggest that intact liposomes may be absorbed *via* endocytosis by enterocytes[61]. Due to considerable size, the

Table 1 Pharmaceutical applications of proliposomal formulations in drug discovery and development with examples.

Formulation	Drug	Developmental issues addressed	Reference
Proliposomes beads with DSPC	Glyburide	Dissolution	63
Proliposomes beads with DSPC	Halofrantine	Bioavailability	64
Proliposomal beads with DSPC, cholesterol and Tween 80	Cromolyn sodium	Permeability	65
HSPC/cholesterol/stearylamine	Zaleplon	Bioavailability and first pass effect	66
Lipid formulation with phosphatidylcholine/cholesterol	Vinpocetine	Solubility limited bioavailability	67
Lipid formulation with phosphatidylcholine	Silymarin	Poor solubility in water/oils, poor bioavailability	68

absorbed liposomes may be transported *via* lymphatic system into system circulation[62]. The formation of mixed micelles consisting of lipids and bile salts would lead to passive diffusion of vesicles across villi followed by chylomicron formation during the passage. The chylomicrons are believed to be taken up by lymphatic system[62]. It can be anticipated that sufficiently hydrophobic drug molecules would remain in the hydrophobic core of micelles and not exposed to external environments. Hence proliposomes can be solution to issues related to solubility, permeability, Pgp efflux, presystemic metabolism of drugs and can be considered as candidate enabling technique during drug discovery. We briefly summarize some of proliposomal work carried out in our laboratory and other research organizations.

The proliposomal formulations of glyburide were evaluated to improve dissolution. The enteric coated proliposomal beads of glyburide with distearoyl phosphatidylcholine (DSPC), showed remarkable improvement in dissolution of glyburide, the dissolution in phosphate buffer pH 7.4 was more than 95% as against 37% from marketed formulation[63].

Halofrantine, a highly lipophilic drug used to treat chloroquine resistant plasmodium falciparum infection, has low and variable bioavailability probably due to its lipophilicity. The enteric coated proliposomal formulation of halofrantine with DSPC showed more than 40% increase C_{max} and 90%–100% increase in area under curve (AUC) in rats compared to suspension of halofrantine[64]. This increased and consistent absorption is believed to be because of facilitated absorption and lymphatic uptake in the presence of lipids bypassing solubility barrier.

Cromolyn sodium is an anti-inflammatory drug used in prophylactic treatment of bronchial asthma and allergic rhinitis. It has oral bioavailability of $<1\%$. The proliposomal formulation with lipid DSPC, cholesterol and Tween 80 showed nearly 7.5-fold increase in Caco-2 permeability as against control cromolyn sodium in phosphate buffered saline[65]. These investigations in our laboratory clearly demonstrated applications of proliposomal formulations in overcoming drug development barriers. These observations have been clearly corroborated by results of investigation from other labs.

Zaleplon is a hypnotic drug indicated in insomnia and is a potential anticonvulsant, reported to be poorly soluble in water and has high first pass effect. As a result, it has 30% oral bioavailability after oral administration. However, there was 2–5-fold increase in oral bioavailability was observed when administered as proliposomal formulation[66]. Similarly, vinpocetine which is indicated for cardiovascular disease was also found to be poorly soluble and had suffered high first pass effect. The oral bioavailability was reported to be mere 7% in human. The proliposomal formulation in rabbits showed remarked improvement in the bioavailability with 3.5-fold increase compared to pure drug[67]. Silymarin is poorly soluble both in water and oil, making it difficult to formulate as emulsion formulation, but it was successfully formulated as proliposomes with an improved bioavailability of 3.5-fold as against pure drug in beagle dogs[68].

These examples have clearly demonstrated the potentials of proliposomal formulation for ADME issues. It can be opined that such formulation approaches can be very helpful in salvaging molecules in the discovery stages. However, major limitations of proliposomal drug delivery are, only hydrophobic molecules which have greater affinity towards lipids can be formulated as proliposomes, mechanism of absorption is not clearly established, and lack of *in vivo-in vitro* correlation. Effect of lipid digestion and lipolysis on *in vivo* performance of delivery system needs greater research attention. The hydrophilic drugs with intrinsic permeability and metabolism issues may not be benefited from this technique. Nevertheless, proliposomes seems to be versatile delivery system for BCS classes II and III drugs.

4. Drug delivery strategies for improving drugability of biologics

4.1. *Protein formulation and stabilization*

Formulation of biologics presents unique problems than non-peptide small molecules. The inactivation of proteins, peptides and DNAs happens not just by chemical degradation but also by slight physical changes[69,70]. The physical changes that affect activity of protein and peptide drugs include aggregation, precipitation, adsorption onto surface and denaturation due to changes in quaternary, tertiary and secondary structures. The chemical pathways involved in inactivation of biologics include deamidation, oxidation, hydrolysis, racemization, beta elimination, and disulfide exchange[69,70].

The formulation efforts have been successful in addressing various stability issues associated with proteins and peptides. Several approaches have been reported to stabilize protein formulations. Exclusion of water from the formulation can lead to greater stability of products. The most common method to remove water from product is freeze drying/lypholization. However, freeze drying not always leads to greater stability, phenomenon called “freeze concentration” would lead to drastic changes in microenvironmental pH conditions during the freezing may lead to cold denaturation of proteins[70–72]. However, inclusion of stabilizer, such as mannitol, trehelose and sucrose, which acts as cryoprotectant coupled with optimized freeze drying cycle, can lead to greater stability of protein based products. Oily suspension for injections was also reported for stabilization.

Additives: There are several excipients reported to affect positively the stability of proteins. The inclusion of sugars in the formulation increases the stability of proteins by minimizing the thermodynamic activity of proteins which lead to aggregation. List of sugars include mannitol, trehelose, maltose and fructose.

Table 2 List of PEGylated protein products.

Product	Original biologic	Therapeutic indication	Purpose of PEGylation
Adagen® (pegademase bovine)	Bovine adenosine, deamidase	Severe combined immunodeficiency (SCID)	Increased serum half-life
Oncaspar® (pegaspargase)	Asparaginase	Acute lymphoblastic leukemia	Increased serum half-life, less allergic reactions
PEG-Intron® (PEGylated IFN-α2b)	IFN-α2b	Hepatitis C	Increased serum half-life
Pegasys® (PEGylated IFN-α2a)	IFN-α2a	Hepatitis C	Increased serum half-life
Neulasta® (pegfilgrastim)	G-CSF	Neutropenia	Increased serum half-life
Somavert® (pegvisomant)	hGH mutein	Acromegaly	Increased serum half-life
MIRCERA® (PEGylated epoetin-β)	Epoetin-β	Anemia associated with chronic renal failure	Increased serum half-life
Cimzia® (certolizumab pegol)	Anti TNF Fab	Rheumatoid arthritis and Crohn′s disease	Increased serum half-life
Macugen® or Macuverse® (pegaptanib)	Anti-VEGF aptamer (an RNA oligo-nucleotide)	Treatment of ocular vascular disease	Longer residence at site of action, improved efficacy

The polyols such as glycerin increases the solvation of proteins and reported to prevent aggregation[73]. Cyclodextrins are reported to encapsulate proteins and stabilize[74]. Surfactants such as poloxamers, polysorbate 80 and polysorbate 20 have been reported to prevent aggregation of proteins. The surfactants have been also found to prevent aggregation during the freeze drying process and adsorption onto surfaces[75–78]. The amino acids such as glycine, lysine and arginine have prevented the aggregation, adsorption of proteins and improved thermal stability of proteins[79–82]. Inclusion of salts/buffering agents has resulted in increased solubility and thermal stability, and minimized aggregation of proteins[83,84]. The use of chelating agents such as edetic acid (EDTA), Tris (tromethamine) has shown to stabilize proteins against chemical degradation[69]. Similarly, the fatty acids and lipids such as phosphatides are reported to stabilize the nucleic acids against chemical degradation[85,86]. These studies have clearly demonstrated role of formulation in retaining activity of biologics.

4.2. *Drug delivery systems to improve efficacy of biologics*

Although the stability of proteins has been improved by conventional formulations, the fate of biologics in the body remained challenge. There have been several innovations to improve tolerability and enhance therapeutic effects of proteins in the body. The injection of protein products in the body may precipitate unwanted biological responses leading to early clearance of the products. The immunological and antigenic response may lead to early clearance of proteins from the circulation. The proteins are also prone to degradation by proteolytic enzymes[13]. The small molecular weight proteins are eliminated by kidney ultrafiltration[13]. The issue of rapid clearance of proteins from body has been addressed successfully by conjugating with polymers. The protein-polymer conjugation has reported to prevent immunological and antigenic responses, stabilize against proteolytic enzymes, improve solubility and decrease elimination by kidney ultrafiltration[13,50]. The conjugation of polymer to drug/protein reported to shield the molecule against chemical/enzymatic degradation and larger molecular weight of polymer reduces kidney ultrafiltration[50]. In addition, the polymer conjugates improve cellular uptake of conjugated *via* adsorption-endocytosis mechanism[50]. Although there have been observations where the activity of protein reduced after polymer conjugation, however the increased plasma concentration and prolonged circulation time would counter the reduced activity and result in overall improved efficacy[87]. The polymer conjugation was also successful in passive and active targeting, which will be discussed in subsequent sections. The covalent linkage of polyethylene glycols to proteins is commonly known as PEGylation of proteins. The PEGs are considered to be safe and PEGylation is the most common technique used to improve therapeutic benefits of protein drugs. The list of PEGylated proteins approved in the United States is given in Table 2. The various multiparticulate drug delivery systems, such as liposomes, microparticles and nanoparticles, have been developed to release proteins and peptides slowly for longer time but there is no commercial success yet[88]. However, given the amount of research work being undertaken, there would no surprise if the particulate delivery system with protein hits market in coming years. There are several reports on vaccines loaded nanoparticle formulations. The formulation of new castle disease virus (NDV) vaccine in the form of nanoparticles with mucoadhesive polymer chitosan showed 100% protection of chickens against NDV infection after oral and nasal administration[89]. The plain NDV vaccines showed 40–80% protection. Similarly, IgA loaded chitosan nanoparticles containing pertussis toxin improved nasal absorption and increased immune response[90]. The same nanoparticles showed multi-fold increase in the immune response after subcutaneous injection as against pure pertussis toxin[91]. These preliminary studies showed promise of nanoparticles in improving efficacy of vaccines. Further, exploration is necessary to achieve significant success and to demonstrate proof of concept. Most widely used protein drug is insulin and it has attracted lot of researches in last couple decades, mainly on noninvasive delivery. The inhalation product, Exubera was approved by FDA and was later withdrawn from the market. The oral liposomal formulation of insulin, PEGylated insulin (IN-105, Oral), Oral-Lyn (buccal) are being tested in clinics. The IN-105 for oral delivery and Oral-Lyn for buccal delivery seems to promising in small clinical trials[92]. However, larger clinical trials are necessary to prove their effectiveness. Their successful completion of clinical trials would lead to dramatic change in research and development of protein based drugs. This would revolutionize drug development program and shift paradigm of drug discovery towards therapeutic proteins.

5. Drug delivery strategies for improving efficacy of CNS drugs

The number of people suffering from CNS disorders is on increasing trend, yet very few drugs getting approved for the

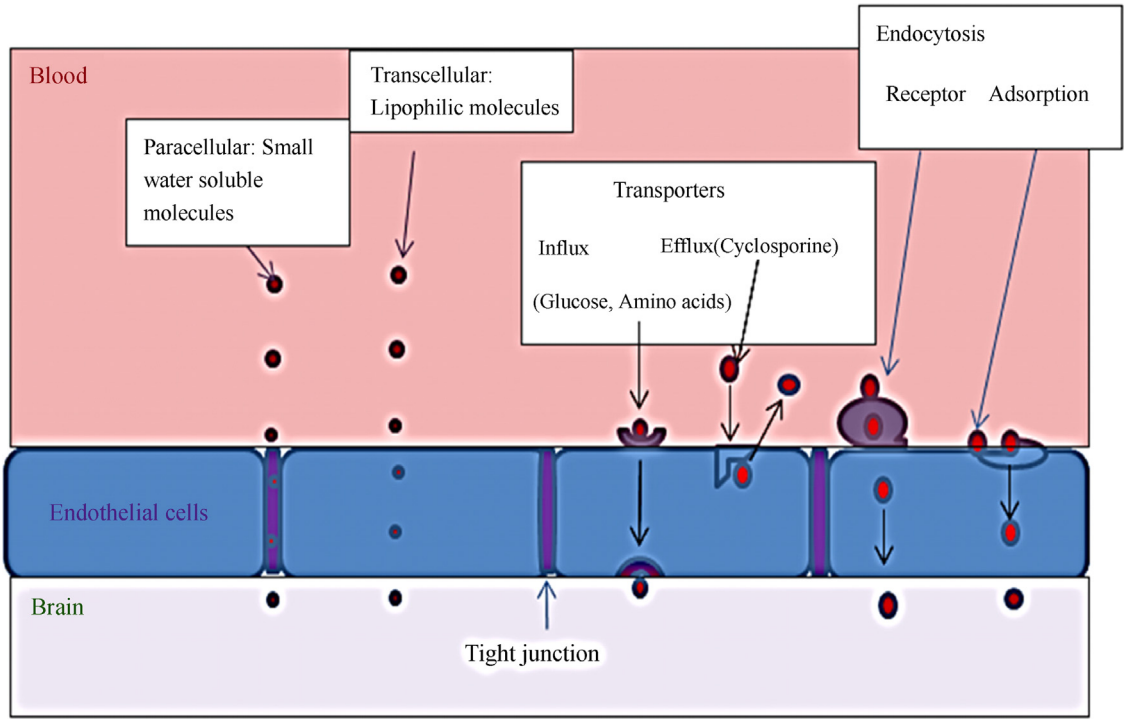


Figure 3 Representative diagram of blood brain barrier and major pathways of drug transport across the BBB. Modified from Ref. 100.

treatment. Staggering 35% of total disease burden in the Europe is from CNS disorders[93]. The global burden from CNS diseases is expected to rise to 14% in 2020[94]. It has been reported that most of the CNS drug development efforts (99%) are directed towards finding new molecule, only meager 1% of efforts are on delivery of molecules to brain[95]. The main problem encountered in the delivery of drugs to brain is the presence of various barriers in the brain which prevent entry of drugs, other chemicals and toxins. The main barrier is BBB. The BBB is situated at brain and blood interface, is mainly composed of brain vascular endothelial cells and is barely penetrated (Fig. 3). Other cells, such as pericytes, astrocytes and neuronal cells, also play an important role in the functioning of BBB. Main function of BBB is to maintain homeostasis of the brain. The other barriers, blood-cerebrospinal fluid (CSF) barrier, blood-spinal barrier, choroid plexus and brain-CSF barrier also restrict movement of drug molecule into brain[96–98]. The molecules with favorable physicochemical properties will reach the brain. The molecular weight, log *P*, polar surface area and charge play the important role in brain permeation of drugs[99]. The ideal drug candidate should have MW < 450 Da, log *P* 1.5–2.5, polar surface are of 65–70 Å^2. The molecules bearing positive charge at pH 7–8 and those with tertiary nitrogen in the structure will have greater BBB permeability[99].

Based on physicochemical properties there are several absorptive pathways available for drug transport across the BBB. The hydrophobic small molecules with log *P* with MW < 450 are absorbed by passive transcellular diffusion, some of water soluble small molecules reported to be transported *via* paracellular route across intercellular spaces. The essential nutrients, such as glucose, amino acids and nucleosides, are taken up by facilitated/carrier mediated transport. Lipid soluble amphiphilic molecules, such as cyclosporine and azidothymidine, are reported to be substrate of Pgp and undergo efflux process after passive diffusion to be back into system circulation. Other pathway for transport of essential proteins and peptides is endocytosis, the molecules, such as insulin, transferrin and leptin, are internalized by receptor mediated endocytosis, whereas histone and cationic albumin undergo adsorption mediated endocytosis (Fig. 3). Despite these many pathways for absorption, the delivery of drugs to brain is limited because of selective nature of BBB for passive and facilitated diffusion, rigid tight junction, active transport proteins for efflux of amphiphilic molecules[100].

Apart from the drug design tool to increase the CNS delivery of drugs by increasing lipophilicity, there are several drug delivery techniques described in the literature for the CNS delivery of drugs and are described in following sections.

5.1. *Chemical delivery systems: prodrugs/lipid conjugates*

This mode of drug delivery system involves modification of drug physicochemical properties, especially the lipophilicity of molecule by prodrug formation or conjugation with lipidic material. This technique is based on fact that codeine and heroine acetylated prodrugs of morphine show greater activity than the morphine because of increased lipophilicity and brain penetration. In the brain, these prodrugs are hydrolyzed back to morphine, a more polar molecule, which gets locked in the brain[100]. The delivery of horseradish peroxide to brain was enhanced 2–3-fold after conjugation with stearic acid[101]. However, increased lipophilicity may also increase the distribution of drugs to other body tissues leading to faster elimination of drugs from systemic circulation[102].

5.2. *Nasal delivery*

The olfactory organs, such as nasal cavity, have reasonably good penetration of olfactory neurons surrounded by a sleeve of arachnoid membrane containing CSF. This connection enable

uptake of nasally administered drugs into brain[100]. The study involving the nasal perfusion of hydrophilic sulfonamides in the rats revealed that there was significant amount of sulfonamides in CSF compared to IV administration. The plasma levels of sulfonamides after IV and nasal administrations were not significantly different, indicating direct transfer of nasally administered drugs into brain *via* olfactory neuronal connection[103]. Similar findings were reported for cephalexin, where the CSF concentration of cephalexin was significantly higher when administered intranasally compared to IV and intradueodanal dosing despite similar plasma concentration[104]. This study once again revealed nose–brain direct delivery. In addition to simple nasal formulations, the formulations with mucoadhesive polymers, nanoparticles can be considered to increase residence time of formulation in the nasal cavity in order to increase brain uptake of drugs.

5.3. *Modulation of BBB*

Another approach for enhancing the brain permeation is to modulate BBB. There have been reports suggesting use of hypertonic mannitol solution to open up the BBB. The injection of 25% mannitol solution *via* carotid artery has resulted in opening of BBR and subsequent administration of drugs *via* same artery resulted in the enhanced penetration into brain. The enhanced permeation could be due to shrinkage of cells leading to opening of paracellular route. This osmotic opening has resulted in 10–100-fold increase in methotrexate delivery to brain. The 1-*O*-heptyltriglycerol and bradykinin were also found to open up BBB. The modulation of BBB may come with risk, potential toxins and viruses may get entry into brain[100].

5.4. *Carrier mediated transport*

There are several transporters identified in the BBB which facilitate solute transport across the BBB. Medium chain fatty acid transporters, large neutral amino acid carrier, monocarboxylic acid carrier, organic cation transporter, purine carrier, nucleoside carrier, hexose/glucose transporter are identified in the brain. Based on understanding of stereochemical requirements for transporters, the drugs/drug delivery systems can be constructed to target these transporter proteins for uptake into brain[100]. However, glucose transporter such as GULT1 is believed to be very stereoselective in picking up molecules, in such case designing of false/pseudosubstrate for brain transport is complicated. The antiepileptic drug valproic acid is reported to be transported across *via* medium chain fatty acid transporter. The *in situ* brain perfusion study in rats demonstrated that valproic acid is transported through medium chain fatty acid transporter, presence of short chain fatty acids and ketoacids did not inhibit brain uptake of valproic acid[105]. However, the medium chain fatty acids (C6–C12) inhibited the brain uptake of valproic acid suggesting medium chain fatty acid transporter involvement in the uptake of valproic acid.

Large neutral amino acid transporter (L system, LAT1) is reported to have wide selectivity in transporting molecules[100]. The *in situ* rat brain perfusion studies carried out for tyrosine conjugated ketoprofen, showed concentration dependent uptake of conjugate. The ketoprofen itself is not substrate for LAT1; however, conjugation of tyrosine enhanced the brain uptake of drug. The coperfusion of LAT1 inhibitor reduced the brain uptake of conjugated ketoprofen suggesting LAT1 mediated mechanism of uptake[106]. In another study, the phenylalanine prodrugs of valproic acid have shown greater affinity for LAT1. Meta-substituted phenylalanine prodrugs displayed 10-fold higher affinity for LAT1 and 2-fold higher brain uptake than parasubstituted analogues[107]. Interestingly, the valproic acid has been reported to be substrate for medium chain fatty acid transporter.

Despite stringent selectivity of GULT1 transporters, there are studies suggesting possible GULT1 mediated uptake of glucose prodrugs. The glucose prodrugs of ketoprofen and indomethacin in the brain perfusion studies displayed inhibitory effect on glucose uptake by brain and prodrugs were able to cross BBB in temperature dependent manner[107].

Endocytosis mechanism has been explored for brain delivery of drugs. The receptor mediated and adsorption mediated endocytosis are reported for brain delivery. The abundance of transferrin receptors on brain vascular endothelial cells provides opportunity for delivery of drugs to brain. Role of transferrin in brain delivery has been elegantly summarized in the literature[108]. The transferrin itself has limited application for brain targeting due to saturation of receptors due to endogenous transferrin. The Antitransferrin receptor (OX26) monoclonal antibodies against the rat transferrin receptor have displayed greater affinity for protein and larger application in the drug delivery. OX26-drug conjugates can be prepared using linker based direct conjugation of suitable drug to OX26, The drug can also be conjugated to OX26 *via* PEG spacer or biotin/avidin spacer[108,109]. Perfusion of OX26 conjugated gold nanoparticles through carotid artery revealed endocytosis of nanoparticles in brain capillary endothelium[109]. In another study, pharmacokinetic and brain distribution studies of ^{3}H-daunomycin in liposomes, PEG–liposomes and OX26-PEG–liposomes was evaluated in rats[110]. The delivery of daunomycin to brain has increased 3-fold in case of OX26 coupled liposomes compared to free daunomycin and daunomycin in plain liposomes. The PEG conjugated liposomes did not deliver the drug to brain. The proteins drugs were also targeted to brain *via* receptor mediated endocytosis. ApoB conjugated proteins were successfully taken into brain *via* low density lipoprotein receptor mediated endocytosis[110]. The cationized albumin was found to undergo adsorption mediated endocytosis into brain. After intravenous injection of cationized and plain rat serum albumin to rats, the mean brain volume of distribution of cationized albumin was found to be 46 μL/g for cationized albumin compared to 9.3 μL/g for plain serum albumin[111]. These results indicate adsorption of cationized albumin to endothelial cells followed by endocytosis. The cationized albumin can be considered as vehicle for brain delivery of drugs.

Coadministration of drugs inhibiting efflux proteins has been found to increase the brain delivery of substrate drugs. The clinical study was conducted to determine effect Pgp inhibitor drug on distribution of substrate drug to brain. This study revealed that there was enhanced supply of drug to brain when dosed with Pgp inhibitor. The antidiarrheal drug loperamide is potent opiate. However, its inability to cross BBB makes the drug safe without any CNS side effects and abuse potential. However, when 16 mg of loperamide was administered with 600 mg of quinidine, a Pgp inhibitor, there were significant opiate like effects observed[112]. The respiratory response to carbon dioxide rebreathing indicated that there was indeed respiratory depression. In contrast, there was no respiratory depression observed when the same dose of loperamide was given with placebo[112]. These investigations revealed that it possible to enhance BBB penetration of drugs if the Pgp efflux is the barrier for BBB penetration.

However, potential side effects of Pgp inhibitor drugs needs to be taken into account and intravenous administration of safe excipients such as poloxamers can be considered for inhibiting efflux.

Direct injection of drugs into brain parenchyma, inraventricularly or intrathecally, has been reported; however, risk of damaging brain tissues is high[100]. In conclusion, there are several drug delivery systems available for improving the delivery to brain. The LAT1 targeting, OX26 targeting and inhibition of efflux are promising. However, further exploration and clinical investigations are essential for their application in improving efficacy of drugs. Nevertheless, it is good to divert more attention to brain drug delivery to salvage many clinical candidates with promising therapeutic potential.

6. Drug delivery strategies for addressing efficacy/safety *via* targeting

The ability of drug to exert pharmacological actions depends on rate and extent at which the drug reaches site of action[113]. The desired biological action can be achieved if adequate amount of drug is available in the affected tissues. Presence of drug molecule beyond tolerable level in the body tissues other than site of action would precipitate unwanted biological responses leading to toxicity. Discontinuance of many molecules in the discovery and development program could be attributed to poor accumulation of molecules in the site of action and rather wide distribution to other parts of the body leading to lack of efficacy and increased toxicity. In this section, we discuss briefly the examples of successful drug targeting and their possible application during discovery/developmental stages. The drug targeting is usually achieved by either passive targeting or active targeting. The passive targeting involves spontaneous accumulation drug delivery systems in the diseased parts of the body. On other hand, active targeting involves targeting moiety to drug or drug carrier to enhance pick up by target cells. The tumor is highly vascularized in response to increased demand for nutrition supply from rapidly growing tumor cells. The tumor vascular endothelium is more porous, this allows leakage of nano-drug carriers into tumor through pores. The inefficient lymphatic drainage and slow venous blood return will allow drug carriers to stay longer time in the tumor[114]. This process is regarded as enhanced permeation and retention (EPR). The cancer drug candidates are usually more toxic than other therapeutic molecules and hence drug targeting has been extensively applied to cancer treatment[114].

The Doxil® was first liposomal formulation approved by United States Food and Drug Administration (FDA), the formulation is PEGylated liposome encapsulating doxorubicin. The free doxorubicin has circulating half-life of 0.2 h and AUC of 4 $\mu g \cdot h/mL$; in contrast, Doxil® has half-life of 55 h and AUC of 900 $\mu g \cdot h/mL$[115]. More than 90% of doxorubicin remained in liposomes during the circulation[116]. The liposomal formulation avoided repeated administration of drug and showed reduced clinical cardiotoxicity[116,117]. Myocet® is another doxorubicin liposomal formulation with circulating half-life of 2.5 h. It is reported to be safer than doxorubicin[118]. DaunoXome® and Onco-TCS® are approved non-PEGylated liposomal formulations of daunorubicin and vincristine. The Genexol-PM, a novel polymeric micelles encapsulating Paclitaxel, exhibited significant efficacy and better safety profile than Cremophore® EL contained formulations[119]. The Preclinical studies in mice revealed that the concentration of paclitaxel in tumor for micelle formulation were higher than plain paclitaxel[120].

Active targeting to tumor has also been investigated. Abraxane®, albumin bound nanoparticles containing the paclitaxel, demonstrated higher response rates, a better safety profile compared with conventional paclitaxel, and improved survival in patients receiving it as second-line therapy[121]. The albumin nanoparticles were reported to be actively transported into cancer cells *via* secreted protein acidic rich in cysteine (SPARC) mediated transport[122]. The oxaliplatin is novel cisplatin derivative with reduced kidney toxicity; however, this drug is only effective when given with 5-flurouracil. The oxaliplatin is reported to partition rapidly into erythrocytes and this was attributed to lack of anticancer activity when given alone. The transferrin–PEG–liposomes containing oxaliplatin showed improved circulation time, reduced partitioning, and increased localization of oxaliplatin in tumor[123]. Intravenously administered transferrin–PEG–liposomes containing oxaliplatin suppressed the tumor growth more effectively than PEG–liposomes, bare liposomes and free drug[123]. The increased efficacy with transferrin liposomes could be due to increased circulation time and transferrin mediated endocytosis of liposomes into cancer cells.

Drug-polymer conjugation was also explored for tumor targeting. The doxorubicin conjugated to HPMA polymer lysosomally cleavable tetrapeptide linker. The polymer conjugate was labeled with galactosamine. After intravenous administration to 32 patients, 16.9% of dose was accumulated in liver and 3.3% of dose was seen tumor after 24 h post dosing[124]. However, there was no targeting seen with conjugates without galactosamine. The amphotericin B is potent antifungal drug but has narrow therapeutic index due to its toxicity. The liposomal formulation (AmBisome) was successfully delivered drug to fungi cells without causing systemic toxicity[125]. Various other targeting systems and delivery to other organs such as heart, lungs and atherosclerotic lesions are described in the literature[113]. These studies suggest that novel targetable drug delivery systems can be designed to enhance the developability of molecules in the discovery and development stage.

7. Timed release drug delivery and applicability in drug discovery and development

The efficacy and safety of drugs is again dependent on the concentration of drug in the systemic circulation. The level above maximum safety concentration would lead to toxicity and level below the minimum effective concentration results in lack of efficacy. In order to deliver the drug at desired level, timed release/modified release formulations proven effective. Usually the modified release formulations are designed for existing drugs for better efficacy and safety profile; however, proper application timed release formulations will certainly help the clinical candidates in reaching desired clinical endpoints. Some examples of timed release formulations are discussed here.

The metformin, an anti-diabetic drug, is suggested be taken orally twice a day up to 1500 mg for maintaining blood glucose levels within acceptable limits. The common side effects of metformin are stomach upset and nausea. The extended release oral formulations have been tested clinically to improve efficacy and safety[126]. The extended formulations containing the 2000 mg of metformin showed significant decrease in glycated hemoglobin (A1C) levels compared to immediate release formulation (1500 mg

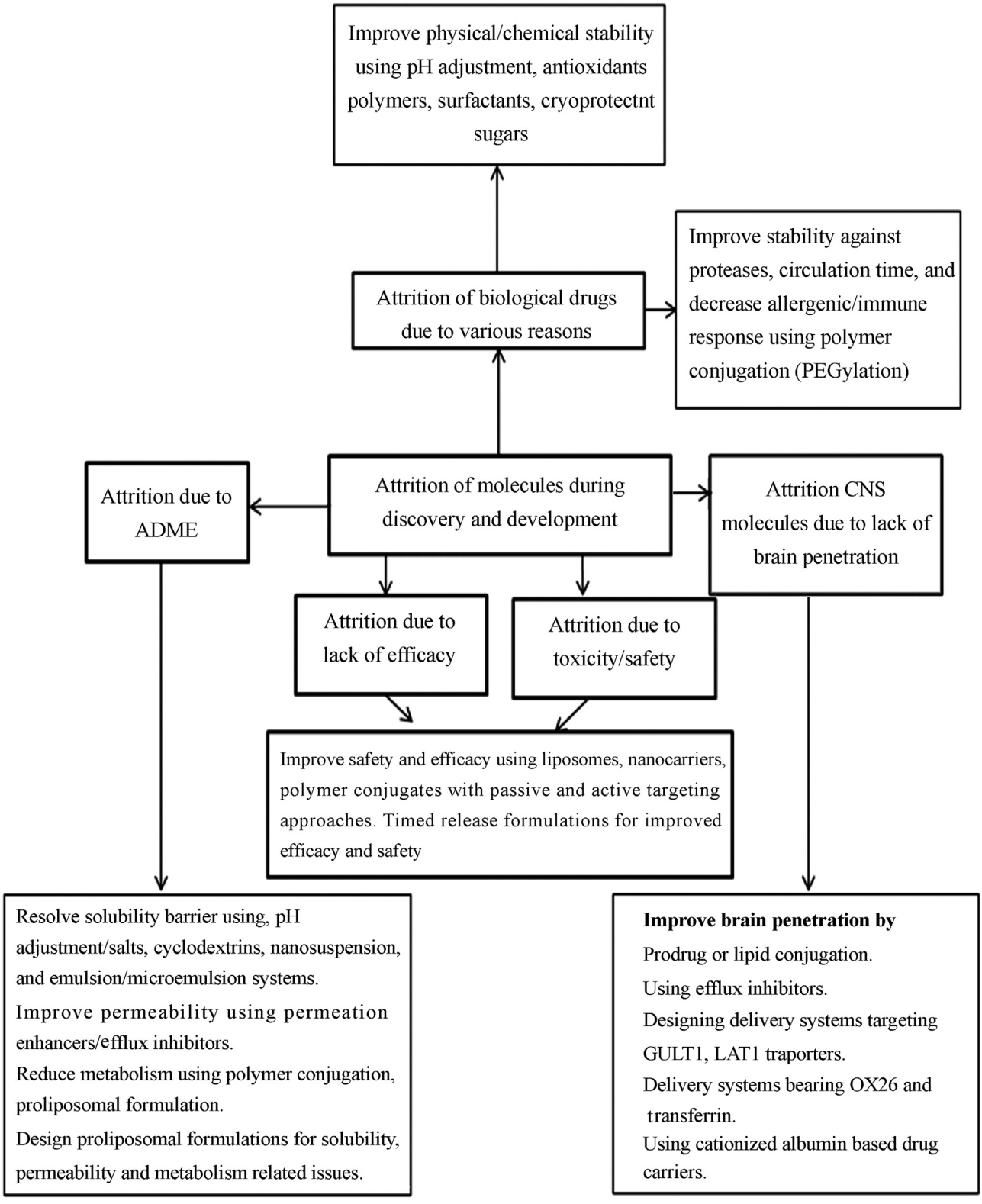


Figure 4 Flow chart for showing various formulation/drug delivery solutions for drug attrition during discovery and development.

twice a day). The A1C values were 6.94% and 6.73% for drug naive patients and >65 years old patients (7.33% and 7.23% for immediate release formulation). The GI side effects were either reduced or not observed in case of extended release formulations. This could be due to slow and steady release of drug without over shooting plasma level while maintaining the desired therapeutic concentration over the time.

Minocycline is oral antibacterial drug used to treat variety of infection and commonly prescribed for treatment of acne. The immediate release formulations reported to produce vestibular

adverse effects. The extended release formulation of minocycline was tested clinically for treatment of acne and incidence of vestibular side effects. The extended release formulation at 1 mg/kg body weight significantly reduced the number of inflammatory lesions. The reports of vestibular adverse events were comparable to placebo group[127]. The formulation was found effective in treating acne without significant adverse effects. Similarly, extended release formulation of niacin (NIASPAN®) was reported to provide consistent and desired plasma level required for pharmacological activity and incidence of liver toxicity were reported to be significantly less than sustained release formulations[128]. The extended release formulation was found efficacious in treating hyperlipidemia and liver related adverse effects were comparable to placebo group[128]. The delayed release formulations of fenofibric acid was found effective in treating hyperlipidemia alone or in combination with statins[129]. Another nice example of formulation playing important role in developability of molecule is didanosine formulation. Initially, didanosine was administered as solution/suspension with antacids/buffered tablet. The didanosine was believed to degrade in acidic pH conditions of gastric fluid and cause side effects. Buffered formulations were reported to reduce the toxicity of molecule. Recently, enteric coated formulation has been approved and has displayed comparable pharmacokinetic profile with improved tolerability[130]. Briefly, these examples clearly demonstrate that timed release formulations have greater potential in salvaging molecules with toxicity/safety concerns. Overall, summary of formulations/drug delivery systems contributing to improved clinical applications or overcoming developmental barriers of drug molecules is shown in Fig. 4.

8. Exploring non-oral and non-parenteral formulations/drug delivery to improve drugability of molecules during discovery and development stages

The various issues hindering development of molecules into drug candidate can be overcome by using drug delivery rout alternative to oral and parenteral applications. Nasal delivery route for vaccination and enhancement of brain delivery has been discussed in previous sections. Routes such as buccal and transdermal can be explored during discovery and development. Routs such as sublingual, buccal, rectal and nasal can be difficult to establish in preclinical animal models, such as rat and mice. In addition, establishing pharmacokinetic and pharmacodynamic correlation in animal model will be little difficult. Alternative routes can minimize ADME related issues; however, delivery of drugs to specific organ and stabilizing the drug in circulation cannot be achieved. Moreover, protein/peptide drugs would not be benefited from alternative routes of delivery because of permeability issues. Transdermal drug delivery has been explored to improve efficacy, tolerability and compliance of several drugs belonging to various classes[131]. Some of the drugs were available as oral formulations. Interesting example is development of transdermal and buccal formulations of testosterone[132,133]. The testosterone, which undergoes complete metabolism when given orally, needs injection. Development of transdermal patch overcome need of repeated injection and provided needed drug levels for almost 24 h[132]. Similarly, sustained release buccal tablets were found useful with just twice a day dosing but economical than transdermal patch[133]. When new molecules suffer from drawbacks of first pass metabolism or have shorter plasma half-life than alternative delivery, routes such as transdermal can be considered.

9. Conclusions

It is evident from the review of literature and clinical reports that formulation and drug delivery systems offered viable solution to drug molecules with inherent ADME barriers, safety and efficacy issues. Protein based drugs have greatly benefited drug delivery systems such as polymer conjugation. The physical and chemical stability of protein drugs in conventional injectable formulations has been addressed successfully with inclusion of stabilizing excipients, change of process or pH of formulation. The targeting of drugs to different organs and tumors has been proved clinically. The literature data suggest limited but reasonable success of drug delivery systems in enhancing the drug supply to brain. Timed release formulations have demonstrated favorable effects on safety and efficacy of drugs. These observations suggest that formulation/drug delivery approaches can be very useful tool in reducing attrition of molecules during the drug discovery and development. There needs increased emphasis on the application of formulation/drug delivery at early stages of drug development path. The research in drug delivery should be directed more towards improving developability of clinical candidates rather already approved drugs. There are many challenges that formulation science has not dealt with and hence leave a lot of scopes for further investigations. Oral delivery of proteins and peptides has not proven successful in clinics. Despite significant progress in drug targeting, clinical success of targeted delivery is limited expect to those system with passive targeting to tumor such has liposomes. There needs concerted efforts from formulation and molecular biology scientists to identify targetable cellular components and respective targeting markers for delivery devices. Research on particulate oral drug delivery has not yet successful completely. The oral delivery of drugs *via* nanoparticles and liposomes needs greater research attention. Identifying targeting ligands to improve uptake of nanoparticles/liposomes *via* lymphatic and endocytosis/carrier mediated transport across GIT is necessary. Even prodrug design did not completely explore the possibility of active transport across GIT. Success in these areas would almost solve problems with oral delivery.

References

1. ⟨http://www.innovation.org/drug_discovery/objects/pdf/RD_Brochure.pdf⟩ [accessed on 06.06.12].
2. Dimasi JA. New drug development in the United States from 1963 to 1999. *Clin Pharmacol Ther* 2001;**69**:286–96.
3. Dimasi JA, Hansen RW, Grabowski HG. The price of innovation: new estimates of drug development costs. *J Health Econ* 2003;**22**:151–185.
4. DiMasi JA, Grabowski HG. The cost of biopharmaceutical R & D: is biotech different? *Manage Decis Econ* 2007;**28**:469–79.
5. Light DW, Warburton R. Demythologizing the high costs of pharmaceutical research. *Biosocieties* 2011;**6**:34–50.
6. Gagne JJ, Choudhry NK. How many "me-too" drugs is too many? *JAMA* 2011;**305**:711–2.
7. Drews J, Ryser S. Innovation deficit in the pharmaceutical industry. *Drug Inform J* 1996;**30**:97–108.
8. Kermani Faiz. Future challenges of ADME studies, business briefing. *Future Drug Discov* 2006.

9. Prentis RA, Lis Y, Walker SR. Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964-1985). *Br J Clin Pharmacol* 2012;**25**:387–96.
10. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* 2004;**3**:711–5.
11. Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *NeuroRx* 2005;**2**:554–71.
12. Walle T, Hsieh F, DeLegge MH, Oatis JE, Walle UK. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos* 2004;**32**:1377–82.
13. Monfardini C, Veronese FM. Stabilization of substances in circulation. *Bioconjug Chem* 1998;**9**:418–50.
14. DeGeorge JJ, Ahn C-H, Andrews PA, Brower ME, Giorgio DW, Goheer MA, et al. Regulatory considerations for preclinical development of anticancer drugs. *Cancer Chemother Pharmacol* 1997;**41**:173–85.
15. Hodgson J. ADMET-turning chemicals into drugs. *Nat Biotechnol* 2001;**19**:722–6.
16. Davis SS. Formulation strategies for absorption windows. *Drug Discov Today* 2005;**10**:249–57.
17. Chaubal MV. Application of formulation technologies in lead candidate selection and optimization. *Drug Discov Today* 2004;**9**:603–9.
18. Kwong E, Higgins J, Templeton AC. Strategies for bringing drug delivery tools into discovery. *Int J Pharm* 2011;**412**:1–7.
19. US FDA. Innovation or stagnation: challenge and opportunity on the critical path to new medical products. Challenges and opportunity report; 2004 March. Available from: ⟨http://www.fda.gov/scienceresearch/specialtopics/criticalpathinitiative/criticalpathopportunitiesreports/ucm077262.htm⟩.
20. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm Res* 1995;**12**:413–20.
21. Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discov Today* 2004;**1**:337–41.
22. Wang J, Urban L. The impact of early ADME profiling on drug discovery and development strategy. *Drug Discov World* 2004;**5**:73–86.
23. Stegemann S, Leveiller F, Franchi D, de Jong H, Lindén H. When poor solubility becomes an issue: from early stage to proof of concept. *Eur J Pharm Sci* 2007;**31**:249–61.
24. Loftsson T, Brewster BE. Pharmaceutical applications of cyclodextrins: basic science and product development. *J Pharm Pharmacol* 2010;**62**:1607–21.
25. Serajuddin AT. Salt formation to improve drug solubility. *Adv Drug Deliv Rev* 2007;**59**:603–16.
26. Lee YC, Zocharski PD, Samas B. An intravenous formulation decision tree for discovery compound formulation development. *Int J Pharm* 2003;**253**:111–9.
27. Powell MF, Nguyen T, Baloian L. Compendium of excipients for parenteral formulations. *PDA J Pharm Sci Technol* 1998;**52**:238–311.
28. Gopinathan S, Nouraldeen A, Wilson AG. Development and application of a high-throughput formulation screening strategy for oral administration in drug discovery. *Future Med Chem* 2010;**2**:1391–8.
29. Basavaraj S, Sihorkar V, Shantha Kumar T, Sundaramurthi P, Srinivas NR, Venkatesh P, et al. Bioavailability enhancement of poorly water soluble and weakly acidic new chemical entity with 2-hydroxy propyl-β-cyclodextrin: selection of meglumine, a polyhydroxy base, as a novel ternary component. *Pharm Dev Tech* 2006;**11**:443–51.
30. Niwa T, Miura S, Danjo K. Universal wet-milling technique to prepare oral nanosuspension focused on discovery and preclinical animal studies—development of particle design method. *Int J Pharm* 2011;**405**:218–27.
31. Shanthakumar T, Prakash S, Basavraj R, Ramesh M, Kant R, Venkatesh P, et al. Comparative pharmacokinetic data of DRF-4367 using nanosuspension and HP-B-CD formulation. In: Proceedings of the international symposium on advances in technology and business potential of new drug delivery systems. India, Mumbai; 2004, p. 75.
32. Pouton CW. Formulation of self-emulsifying drug delivery systems. *Adv Drug Deliv Rev* 1997;**25**:47–58.
33. Perlman M, Murdande S, Gumkowski M, Shah T, Rodricks C, Thornton-Manning J, et al. Development of a self-emulsifying formulation that reduces the food effect for torcetrapib. *Int J Pharm* 2008;**351**:15–22.
34. Yeboah J. Flaws in current clinical trial design: need for intermediate markers. *J Postgenom Drug biomark Dev* 2011;**1**:102e.
35. Dong Y, Ng WK, Surana U, Tan RB. Solubilization and preformulation of poorly water soluble and hydrolysis susceptible *N*-epoxymethyl-1, 8-naphthalimide (ENA) compound. *Int J Pharm* 2008;**356**:130–6.
36. Mansky P, Dai WG, Li S, Pollock-Dove C, Daehne K, Dong L, et al. Screening method to identify preclinical liquid and semi-solid formulations for low solubility compounds: miniaturization and automation of solvent casting and dissolution testing. *J Pharm Sci* 2007;**96**:1548–63.
37. Salama NN, Eddington ND, Fasano A. Tight junction modulation and its relationship to drug delivery. *Adv Drug Deliv Rev* 2006;**58**:15–28.
38. Wagner D, Spahn-Langguth H, Hanafy A, Koggel A, Langguth P. Intestinal drug efflux: formulation and food effects. *Adv Drug Deliv Rev* 2001;**50**:S13–31.
39. Thanou M, Verhoef J, Junginger HE. Oral drug absorption enhancement by chitosan and its derivatives. *Adv Drug Deliv Rev* 2001;**52**:117–26.
40. Werle M. Natural and synthetic polymers as inhibitors of drug efflux pumps. *Pharm Res* 2008;**25**:500–11.
41. Terwogt JMM, Malingré MM, Beijnen JH, Wim W, Rosing H, Koopman FJ, et al. Coadministration of oral cyclosporin A enables oral therapy with paclitaxel. *Clin Cancer Res* 1999;**5**:3379–84.
42. Mueller EA, Kovarik JM, van Bree JB, Tetzloff W, Grevel J, Kutz K. Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharm Res* 1994;**11**:301–4.
43. Kumar GN, Surapaneni S. Role of drug metabolism in drug discovery and development. *Med Res Rev* 2001;**21**:397–411.
44. Beresford AP, Selick HE, Tarbit MH. The emerging importance of predictive ADME simulation in drug discovery. *Drug Discov Today* 2002;**7**:109–16.
45. Bravo González RC, Huwyler J, Boess F, Walter I, Bittner B. *In vitro* investigation on the impact of the surface-active excipients Cremophor EL, Tween 80 and Solutol HS 15 on the metabolism of midazolam. *Biopharm Drug Dispos* 2004;**25**:37–49.
46. Bravo González RC, Boess F, Durr E, Schaub N, Bittner B. *In vitro* investigation on the impact of Solutol HS 15 on the uptake of colchicine into rat hepatocytes. *Int J Pharm* 2004;**279**:27–31.
47. Bittner B, González RCB, Walter I, Kapps M, Huwyler J. Impact of Solutol HS 15 on the pharmacokinetic behaviour of colchicine upon intravenous administration to male Wistar rats. *Biopharm Drug Dispos* 2003;**24**:173–81.
48. Bittner B, González RCB, Isel H, Flament C. Impact of Solutol HS 15 on the pharmacokinetic behavior of midazolam upon intravenous administration to male Wistar rats. *Eur J Pharm Biopharm* 2003;**56**:143–6.
49. Webster LK, Cosson EJ, Stokes KH, Millward MJ. Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. *Br J Cancer* 1996;**73**:522–4.
50. Veronese FM, Morpurgo M. Bioconjugation in pharmaceutical chemistry. *Farmaco* 1999;**54**:497–516.
51. Danhauser-Riedl S, Hausmann E, Schick H-D, Bender R, Dietzfelbinger H, Rastetter J, et al. Phase I clinical and pharmacokinetic trial

of dextran conjugated doxorubicin (AD-70, DOX-OXD). *Invest New Drug* 1993;**11**:187–95.
52. Greenwald RB, Pendri A, Conover CD, Lee C, Choe YH, Gilbert C, et al. Camptothecin-20-PEG ester transport forms: the effect of spacer groups on antitumor activity. *Bioorg Med Chem* 1998;**6**: 551–562.
53. Roseeuw E, Coessens V, Balazuc A-M, Lagranderie M, Chavarot P, Pessina A, et al. Synthesis, degradation, and antimicrobial properties of targeted macromolecular prodrugs of norfloxacin. *Antimicrob Agents Chemother* 2003;**47**:3435–41.
54. Greenwald RB, Gilbert CW, Pendri A, Conover CD, Xia J, Martinez A. Drug delivery systems: water soluble taxol 2′-poly (ethylene glycol) ester prodrugs design and *in vivo* effectiveness. *J Med Chem* 1996;**39**:424–31.
55. Zhang X, Li Y, Chen X, Wang X, Xu X, Liang Q, et al. Synthesis and characterization of the paclitaxel/MPEG–PLA block copolymer conjugate. *Biomaterials* 2005;**26**:2121–8.
56. Basavaraj S, Benson HAE, Brown DH, Chen Y. Novel formulation strategies to improve solubility and pharmacokinetic profile of Resveratrol. Controlled Release Society, Product Development Forum, Poorly Soluble Drugs. 2011; Jan 26–29, Miami, FL, USA.
57. Basavaraj S, Ann Elizabeth Benson H, Havelock Brown D, Chen Y. Application of solvent influenced Fluorescence-quenching and enhancement to develop a highly sensitive HPLC methodology for analysis of resveratrol-PEG conjugates. *Curr Pharm Anal* 2013;**9**:199–207.
58. Basavaraj S, Benson HA, Cruickshank C, Brown DH, Chen Y. Development of a liquid chromatography/mass spectrometry methodology to separate, detect, characterize and quantify PEG-resveratrol prodrugs and the conjugation reaction precursors and intermediates. *Rapid Commun Mass Spectrom* 2011;**25**:1543–51.
59. Betageri GV. Proliposomal drug delivery system. EP Patent 2474307; 2012.
60. Shaji J, Bhatia V. Proliposomes: a brief overview of novel delivery system. *Int J Pharm Biosci* 2013;**4**:150–60.
61. Chia-Ming C, Weiner N. Gastrointestinal uptake of liposomes. I. *In vitro* and *in situ* studies. *Int J Pharm* 1987;**37**:75–85.
62. Charman WCN, Stella VJ. *Lymphatic transport of drugs*. Boca Raton: CRC Press LLC; 1992.
63. Kumar R, Gupta RB, Betageri GV. Formulation, characterization, and *in vitro* release of glyburide from proliposomal beads. *Drug Deliv* 2001;**8**:25–7.
64. Hiremath PS, Soppimath KS, Betageri GV. Proliposomes of exemestane for improved oral delivery: formulation and *in vitro* evaluation using PAMPA, Caco-2 and rat intestine. *Int J Pharm* 2009;**380**:96–104.
65. Brocks DR, Betageri GV. Enhanced oral absorption of halofantrine enantiomers after encapsulation in a proliposomal formulation. *J Pharm Pharmcol* 2002;**54**:1049–53.
66. Janga KY, Jukanti R, Velpula A, Sunkavalli S, Bandari S, Kandadi P, et al. Bioavailability enhancement of zaleplon *via* proliposomes: role of surface charge. *Eur J Pharm Biopharm* 2012;**80**:347–57.
67. Xu H, He L, Nie S, Guan J, Zhang X, Yang X, et al. Optimized preparation of vinpocetine proliposomes by a novel method and *in vivo* evaluation of its pharmacokinetics in New Zealand rabbits. *J Control Release* 2009;**140**:61–8.
68. Yan-yu X, Yun-mei S, Zhi-peng C, Qi-neng P. Preparation of silymarin proliposome: a new way to increase oral bioavailability of silymarin in beagle dogs. *Int J Pharm* 2006;**319**:162–8.
69. Manning MC, Patel K, Borchardt RT. Stability of protein pharmaceuticals. *Pharm Res* 1989;**6**:903–18.
70. Parkins DA, Lashmar UT. The formulation of biopharmaceutical products. *Pharm Sci Technol Today* 2000;**3**:129–37.
71. Swarbrick J, Boylan JC. *Encyclopedia of pharmaceutical technology*. Vol. 20, Suppl. 3. Boca Raton: CRC Press LLC; 2001.
72. Tang XC, Pikal MJ. The effect of stabilizers and denaturants on the cold denaturation temperatures of proteins and implications for freeze-drying. *Pharm Res* 2005;**22**:1167–75.
73. Gekko K, Timasheff SN. Mechanism of protein stabilization by glycerol: preferential hydration in glycerol–water mixtures. *Biochemistry* 1981;**20**:4667–76.
74. Lovatt M, Cooper A, Camilleri P. Energetics of cyclodextrin-induced dissociation of insulin. *Eur Biophys J* 1996;**24**:354–7.
75. Österberg T, Fatouros A, Mikaelsson M. Development of a freeze-dried albumin-free formulation of recombinant factor VIII SQ. *Pharm Res* 1997;**14**:892–8.
76. Takeda K, Sasa K, Nagao M, Batra PP. Secondary structural changes of non-reduced and reduced ribonuclease A in solutions of urea, guanidine hydrochloride and sodium dodecyl sulfate. *Biochim Biophys Acta* 1988;**957**:340–4.
77. Gonzalez G, MacRitchie F. Equilibrium adsorption of proteins. *J Colloid Interface Sci* 1970;**32**:55–61.
78. Wang PL, Johnston TP. Enhanced stability of two model proteins in an agitated solution environment using poloxamer 407. *J Pharm Sci Technol* 1993;**47**:183–9.
79. Johnston TP. Adsorption of recombinant human granulocyte colony stimulating factor (rhG-CSF) to polyvinyl chloride, polypropylene, and glass: effect of solvent additives. *PDA J Pharm Sci Technol* 1996;**50**:238–45.
80. Takagi T, Matsuo T [Inventors]. Gamma-globulin preparation for intravenous administration, process for production thereof and process for preparation of gamma-globulin of low anticomplementary activity. EP Patent 0,025,719; 1981.
81. Quinn R, Andrade J. Minimizing the aggregation of neutral insulin solutions. *J Pharm Sci* 1983;**72**:1472–3.
82. Taneja S, Ahmad F. Increased thermal stability of proteins in the presence of amino acids. *Biochem J* 1994;**303**:147–53.
83. Foster TM, Dormish JJ, Narahari U, Meyer JD, Vrkljan M, Henkin J, et al. Thermal stability of low molecular weight urokinase during heat treatment. III. Effect of salts, sugars and Tween 80. *Int J Pharm* 1996;**134**:193–201.
84. Ahmad F, Bigelow CC. Thermodynamic stability of proteins in salt solutions: a comparison of the effectiveness of protein stabilizers. *J Protein Chem* 1986;**5**:355–67.
85. Vologodskii AV, Cozzarelli NR. Conformational and thermodynamic properties of supercoiled DNA. *Annu Rev Biophys Biomol Struct* 1994;**23**:609–43.
86. Pisal DS, Kosloski MP, Balu-Iyer SV. Delivery of therapeutic proteins. *J Pharm Sci* 2010;**99**:2557–75.
87. Jevševar S, Kunstelj M, Porekar VG. PEGylation of therapeutic proteins. *Biotechnol J* 2010;**5**:113–28.
88. Tan ML, Choong PF, Dass CR. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. *Peptides* 2010;**31**:184–93.
89. Zhao K, Chen G, Shi XM, Gao TT, Li W, Zhao Y, et al. Preparation and efficacy of a live newcastle disease virus vaccine encapsulated in chitosan nanoparticles. *PloS One* 2012;**7**:e53314.
90. Sharma S, Benson HA, Mukkur TK, Rigby P, Chen Y. Preliminary studies on the development of IgA-loaded chitosan–dextran sulphate nanoparticles as a potential nasal delivery system for protein antigens. *J Microencapsul* 2012;**30**:283–94.
91. Sharma S, Mukkur TK, Benson H, Chen Y. Enhanced immune response against pertussis toxoid by IgA-loaded chitosan–dextran sulfate nanoparticles. *J Pharm Sci* 2012;**101**:233–44.
92. Heinemann L, Jacques Y. Oral insulin and buccal insulin: a critical reappraisal. *J Diabetes Sci Technol* 2009;**3**:568–84.
93. Pardridge WM. Why is the global CNS pharmaceutical market so under-penetrated? *Drug Discov Today* 2002;**7**:5–7.
94. World Health Organization. The Global Burden of Disease Project. Available from: 〈http://www.who.int/healthinfo/global_burden_disease/2004_report_update/en/〉[acessed on 07.10.12].
95. Pardridge WM. Drug delivery to the brain. *J Cereb Blood Flow Metab* 1997;**17**:713–31.
96. Krewson CE, Klarman ML, Saltzman WM. Distribution of nerve growth factor following direct delivery to brain interstitium. *Brain Res* 1995;**680**:196–206.

97. De Boer AG, Gaillard PJ. Drug targeting to the brain. *Annu Rev Pharmacol Toxicol* 2007;**47**:323–55.
98. Serwer LP, James CD. Challenges in drug delivery to tumors of the central nervous system: an overview of pharmacological and surgical considerations. *Adv Drug Deliv Rev* 2012;**64**:590–7.
99. Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx* 2005;**2**:541–53.
100. Begley DJ. Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. *Pharmacol Therapeut* 2004;**104**:29–45.
101. Batrakova EV, Vinogradov SV, Robinson SM, Niehoff ML, Banks WA, Kabanov AV. Polypeptide point modifications with fatty acid and amphiphilic block copolymers for enhanced brain delivery. *Bioconjug Chem* 2005;**16**:793–802.
102. Juillerat-Jeanneret L. The targeted delivery of cancer drugs across the blood–brain barrier: chemical modifications of drugs or drug-nanoparticles? *Drug Discov Today* 2008;**13**:1099–106.
103. Sakane T, Akizuki M, Yamashita S, Nadai T, Hashida M, Sezaki H. The transport of a drug to the cerebrospinal fluid directly from the nasal cavity: the relation to the lipophilicity of the drug. *Chem Pharm Bull* 1991;**39**:2456–8.
104. Sakane T, Akizuki M, Yoshida M, Yamashita S, Nadai T, Hashida M, et al. Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. *J Pharm Pharmacol* 1991;**43**:449–51.
105. Adkison K, Shen DD. Uptake of valproic acid into rat brain is mediated by a medium-chain fatty acid transporter. *J Pharmacol Exp Ther* 1996;**276**:1189–200.
106. Gynther M, Laine K, Ropponen J, Leppänen J, Mannila A, Nevalainen T, et al. Large neutral amino acid transporter enables brain drug delivery *via* prodrugs. *J Med Chem* 2008;**51**:932–6.
107. Peura L, Malmioja K, Laine K, Leppänen J, Gynther M, Isotalo A, et al. Large amino acid transporter 1 (LAT1) prodrugs of valproic acid: new prodrug design ideas for central nervous system delivery. *Mol Pharm* 2011;**8**:1857–66.
108. Qian ZM, Li H, Sun H, Ho K. Targeted drug delivery *via* the transferrin receptor-mediated endocytosis pathway. *Pharmacol Rev* 2002;**54**:561–87.
109. Bickel U, Kang YS, Yoshikawa T, Pardridge WM. *In vivo* demonstration of subcellular localization of anti-transferrin receptor monoclonal antibody-colloidal gold conjugate in brain capillary endothelium. *J Histochem Cytochem* 1994;**42**:1493–7.
110. Huwyler J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. *Proc Natl Acad Sci U S A* 1996;**93**:14164–9.
111. Pardridge WM, Triguero D, Buciak J, Yang J. Evaluation of cationized rat albumin as a potential blood-brain barrier drug transport vector. *J Pharmacol Exp Ther* 1990;**255**:893–9.
112. Sadeque A, Wandel C, He H, Shah S, Wood A. Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin Pharmacol Ther* 2000;**68**:231.
113. Torchilin VP. Drug targeting. *Eur J Pharm Sci* 2000;**11**:S81–91.
114. Danhier F, Feron O, Préat V. To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *J Control Release* 2010;**148**:135–46.
115. Hofheinz R-D, Gnad-Vogt SU, Beyer U, Hochhaus A. Liposomal encapsulated anti-cancer drugs. *Anti-Cancer Drugs* 2005;**16**:691–707.
116. ⟨http://www.doxil.com/hcp/mechanism-of-action⟩ [accessed on 23.05.13].
117. Safra T, Muggia F, Jeffers S, Tsao-Wei DD, Groshen S, Lyass O, et al. Pegylated liposomal doxorubicin (doxil): reduced clinical cardiotoxicity in patients reaching or exceeding cumulative doses of 500 mg/m^2. *Ann Oncol* 2000;**11**:1029–33.
118. Theodoulou M, Campos SM, Batist G, Winer E, Norton L, Hudis C, et al. TLC D99 (D, Myocet) and Herceptin (H) is safe in advanced breast cancer (ABC): final cardiac safety and efficacy analysis. In: Proceedings of the American Society of Clinical Oncology 2002; p. 216.
119. Lee K, Chung H, Im S, Park Y, Kim C, Kim S-B, et al. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. *Breast Cancer Res Tr* 2008;**108**:241–50.
120. Kim SC, Kim DW, Shim YH, Bang JS, Oh HS, Wan Kim S, et al. *In vivo* evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. *J Control Release* 2001;**72**:191–202.
121. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, et al. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *J Clin Oncol* 2005;**23**:7794–803.
122. Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A, et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. *Clin Cancer Res* 2006;**12**:1317–24.
123. Suzuki R, Takizawa T, Kuwata Y, Mutoh M, Ishiguro N, Utoguchi N, et al. Effective anti-tumor activity of oxaliplatin encapsulated in transferrin–PEG–liposome. *Int J Pharm* 2008;**346**:143–50.
124. Seymour LW, Ferry DR, Anderson D, Hesslewood S, Julyan PJ, Poyner R, et al. Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. *J Clin Oncol* 2002;**20**:1668–76.
125. Adler-Moore J. AmBisome targeting to fungal infections. *Bone Marrow Transpl* 1994;**14 suppl 5**:S3–7.
126. Schwartz S, Fonseca V, Berner B, Cramer M, Chiang Y-K, Lewin A. Efficacy, tolerability, and safety of a novel once-daily extended-release metformin in patients with type 2 diabetes. *Diabetes Care* 2006;**29**:759–64.
127. Fleischer Jr AB, Dinehart S, Stough D, Plott RT. Safety and efficacy of a new extended-release formulation of minocycline. *Cutis* 2006;**78**:21–31.
128. Goldberg A, Alagona Jr P, Capuzzi DM, Guyton J, Morgan JM, Rodgers J, et al. Multiple-dose efficacy and safety of an extended-release form of niacin in the management of hyperlipidemia. *Am J Cardiol* 2000;**85**:1100–5.
129. Wachter K. Trilipix approved for lipid management in combo with a statin. *Int Med News* 2009;**42**:4.
130. Damle BD, Kaul S, Behr D, Knupp C. Bioequivalence of two formulations of didanosine, encapsulated enteric-coated beads and buffered tablet, in healthy volunteers and HIV-infected subjects. *J Clin Pharmacol* 2002;**42**:791–7.
131. Prausnit MR, Langer R. Transdermal drug delivery. *Nat Biotechnol* 2008;**26**:1261–8.
132. ⟨http://www.rxlist.com/androderm-drug.htm⟩ [accessed on 09.12.13].
133. ⟨http://www.rxlist.com/striant-drug.htm⟩ [accessed on 09.12.13].