Is nanotechnology a boon for oral drug delivery?

Udita Agrawal, Rajeev Sharma, Madhu Gupta and Suresh P. Vyas

Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dr H.S. Gour Vishwavidyalaya, Sagar, MP 470003, India

The oral route for drug delivery is regarded as the optimal route for achieving therapeutic benefits owing to increased patient compliance. Despite phenomenal advances in injectable, transdermal, nasal and other routes of administration, the reality is that oral drug delivery remains well ahead of the pack as the preferred delivery route. Nanocarriers can overcome the major challenges associated with this route of administration: mainly poor solubility, stability and biocompatibility of drugs. This review focuses on the potential of various polymeric drug delivery systems in oral administration, their pharmacokinetics, in vitro and in vivo models, toxicity and regulatory aspects.

Introduction

Oral delivery is the most convenient and extensively used route of drug administration. Because oral dosage provides the benefit of effortless administration, most drugs are designed for oral ingestion [1]. The choice of route is driven by patient acceptability, properties of the drug, access to a disease location and effectiveness in dealing with the specific disease. It is by far the most dominant and convenient administration route with good patient compliance, especially in the opinions of patients themselves. Despite these benefits, there are also disadvantages associated with oral administration usually related to immediate release of the drug causing toxicity in practice, low aqueous solubility and low penetration across intestinal membranes [2]. Nevertheless, current knowledge on mechanism of drug absorption, gastrointestinal (GI) transit, microenvironment of GI tract and stability within the GI tract is still incomplete and challengeable [3]. Oral administration is also beset by constraints such as chemical degradation, gastric emptying and intestinal motility. Although convenient from a patient perspective, there has been demand for more-patient-compliant dosage forms.

The GI tract forms barriers to severe physiological factors (e.g. varied enzymatic activities, difference in pH, and specific transport mechanisms), which restrict intestinal drug absorption. Moreover, oral bioavailability of drugs is strongly influenced by solubility and permeability. Drugs are divided into four categories based on their solubility and permeability according to the Biopharmaceutic Classification System [4]. A drug that is administered orally must survive in the stomach.
Glossary

**Controlled release** Includes any drug delivery system from which the drug is delivered at a predetermined rate over a prolonged period of time.

**Dendrimer** Dendrimers are highly branched symmetrical macromolecules of nanosized dimensions, have well-defined molecular mass and geometry consisting of a central core, repeating units and terminal functional groups.

**Micelles** Micelles are nanoscopic aggregates of colloidal dimensions (i.e., association of colloids) formed reversibly from amphiphile molecules. They have the ability to solubilize hydrophobic drugs and bring about site-specific delivery by passive and active targeting.

**Nanosphere** Solid spherical particles in the micron range, used as matrix dosage forms.

**Nanoparticles** Nanoparticles are subnanosized colloidal structures composed of synthetic or semi-synthetic polymers.

**Nanotechnology** The design, characterization, production and application of structures where particles in the size range 0.1–100 nm play a significant part in drug delivery.

**Polymer** A chemical compound, typically formed by connecting monomer units together, that consists of repeating structures, often arranged in a chain.

**Receptor** A molecule or polymeric structure in or on a cell that specifically recognizes and binds a compound acting as a molecular messenger (neurotransmitter, peptide, folic acid, hormone, among others).

**Sustained release** Includes the drug delivery systems that achieve and ensure slow release of drugs over an extended or prolonged period of time or at a constant release (zero order) rate to attain and maintain therapeutically effective levels of drug in the circulation. Here the absorption rate is equal to the elimination rate over an extended period of time.

**Targeted drug delivery** Nanomaterials uniquely capable of localizing delivery of therapeutics and diagnostics to diseased tissues. The targeted drug delivery system has the ability to achieve high local concentration of drugs at a target site.

**Toxicity** The ability of the substance to induce harmful effects.

The harsh environment of the GI tract and should be absorbed. The bioavailability of drugs where dissolution is rate limiting becomes a challenge for effective delivery via the oral route. Hence, protective measures are required to avoid drug destruction on one hand and potentiation of absorption on the other hand in the GI tract. This objective can be accomplished by incorporating the drug into various novel drug delivery systems. A significant number of polymeric nanocarrier systems have emerged, encompassing diverse routes of drug administration, to achieve controlled and targeted drug delivery [5].

**Challenges to oral delivery**

More than 60% of conventional small molecule drug products available on the market are administered via the oral route. The physiological and anatomical barriers to bioactive absorption via the GI tract are primarily chemical, enzymatic and permeability related (e.g., mucus layer, intestinal epithelium). Poor hydrophilicity and intrinsic dissolution rate are the major factors that affect oral delivery of many existing drugs. Figure 1 compiles all the essential challenging parameters for oral bioactive delivery. Numerous drug discovery approaches such as (i) chemical entity modification, (ii) use of permeation enhancers, (iii) use of enzyme inhibitors to lower the activity of proteolytic enzymes, (iv) modulation of GI transit time, (v) minimization of hepatic first pass elimination and (vi) design of novel drug delivery systems have been screened to overcome barriers and to enhance the bioavailability of drugs. Targeted systems are expected to release drugs at the specific site of the gut, where proteolytic activity is relatively low, so that the drugs can be protected from luminal proteolytic degradation for absorption with improved bioavailability [6].

Nanotechnology presents some promotional benefits to the drug delivery field in general and oral drug delivery in particular. It permits (i) delivery of poorly water-soluble drugs, (ii) targeting of drugs to precise parts of the GI tract, (iii) transcytosis of drugs across the GI tract barrier and (iv) intracellular and transcellular delivery of large macromolecules [7]. Nanoconstruct-based oral delivery can improve efficacy, specificity, tolerability and therapeutic index of corresponding drugs. Figure 2 gives the schematic representation of various drug uptake mechanisms in the intestine.

**Polymer-based drug delivery systems addressing the challenges of oral drug delivery**

A large number of polymers are available to form various nanocarrier systems and can be categorized into either natural or synthetic polymers. Natural materials used for the nanoparticle formulation include chitosan, dextran, gelatin, alginate and agar (Fig. 3). Poly(lactide) (PLA), poly(glycolide) (PGA), poly(lactide-co-glycolide) (PLGA), poly(cyanoacrylate) (PCA), polyethylenimine (PEI) and polycaprolactone (PCL) are the synthetic polymers that are used in the design of nanocarriers [8]. Various biodegradable polymers used for oral drug delivery are shown in Fig. 4. Robust structural characteristics impart stability and a high degree of controlled release of drug molecules in the GI tract. A diverse range of pharmacophores including small molecules such as estradiol, anticancer drugs, antigens, peptide and nonpeptidic drugs such as insulin have been successfully delivered by polymeric nanoparticles [9].

The surface of the nanocarriers can be modified by adsorption or grafting of hydrophilic molecules [e.g., polyethylene glycol (PEG)], ligands (such as antibodies) and glycoproteins or peptides to increase interactions with the intestinal mucosa and to deliver medicines to target specific cells, diseases or areas of the intestine. The surface ligands that are used for the oral delivery of bioactives include: bioadhesins, mainly lectins [10]; folic acid [11]; peptidic ligands such as RGD [12]; and bile acids [13]. Prolongation of the residence time in the gut by mucoadhesion, endocytosis of the particles and/or permeabilizing effect of the polymer are the factors that govern increased uptake of the polymeric nanocarriers. Several physicochemical parameters including hydrophobicity, polymer nature and particle size influence translocation of particles across the epithelium. Table 1 shows the various nanocarriers employed for oral drug administration.

**Polymeric nanoparticles**

Nanoparticles are novel carriers that are collectively known as the colloidal drug delivery system. In vitro cytotoxicity of the
Figure 1
Diagrammatic presentation of potential advantages and barriers for the oral absorption of bioactives in the intestine.

Figure 2
Schematic representation of various drug uptake mechanisms in the intestinal epithelium.
Various natural polymers used in oral delivery of bioactives.

docetaxel-loaded nanoparticle was evaluated by the cell counting kit (CCK)-8 assay for cancer cells. In vivo pharmacokinetic parameters were measured in male Sprague–Dawley rats, and compared with the current clinical product of docetaxel (i.e. Taxotere®). There was an increase in the Caco-2 cellular uptake with increased nanoparticle concentration. It has been concluded from the in vitro and in vivo studies that tocopherol-PEG succinate (TPGS) has excellent effects in oral administration [14] and incorporation of montmorillonite (MMT) in the formulation could significantly enhance cellular adhesion and adsorption [15]. The effectiveness of biotin-functionalized nanoparticles encapsulating a combination of paclitaxel and the P glycoprotein (P-gp) inhibitor tariquidar was investigated [16]. Paclitaxel and tariquidar were encapsulated efficiently in nanoparticles. Two drug-resistant cell lines: JC and NCI/ADR-RES, were used to investigate cytotoxicity of paclitaxel and the effect of tariquidar on paclitaxel-induced cytotoxicity. Paclitaxel, either in solution or encapsulated in nanoparticles, was devoid of inducing cytotoxicity in either cell line. Addition of tariquidar significantly improved the cytotoxicity in both cell lines. Unlike in JC and NCI/ADR-RES cells, free paclitaxel and paclitaxel encapsulated in nanoparticles were observed to be cytotoxic in MCF-7 cells. Addition of tariquidar either in free form or encapsulated in nanoparticles along with paclitaxel did not alter the cytotoxicity of paclitaxel, confirming that the augmented effectiveness of dual-agent treatment in drug-resistant cells was caused by the tariquidar-mediated inhibition of drug efflux.

Tumor cell accumulation of nanoparticles also increased significantly owing to incorporation of biotin on the surface. The uptake of biotin-conjugated nanoparticles in NCI/ADR-RES cells was approximately sixfold higher than control nanoparticles, whereas in JC cells the uptake of biotin-conjugated nanoparticles was ~13-fold higher than the control nanoparticles. In a study by Davaran et al., insulin containing star-branched PLGA nanoparticles showed that star-branched PLGA nanoparticles are promising
 carriers for mitigating the burst effect and prolonging the release of insulin [17]. Nassar et al. worked on a new concept of double-coated nanocapsules to improve the oral bioavailability of P-gp substrate drugs [18]. The cellular uptake of tacrolimus using Caco-2 cells and intestinal rat segment can be markedly enhanced without affecting the physiological activity of the transporters, especially the P-gp pump. Enhanced availability and protection of tacrolimus was achieved by initially nanoencapsulating the drug-loaded oil cores with a combination of two polymethacrylate polymers followed by the microencapsulation (matrix embedding) within a bioadhesive gel of polymer hydroxyl propyl methyl cellulose (HPMC). This unique strategy was shown to release tiny drug-loaded nanocapsules rather than dissolved drug under appropriate aqueous dilution mimicking the intestinal physiological conditions [19].

Bansal et al. reported that the co-administration of P-gp inhibitors and anticancer drugs in nanoparticles offers a potential approach for circumventing P-gp-mediated efflux. It allows the drug to evade recognition by P-gp at the plasma membrane and deliver it into the cell cytoplasm or nucleus [20]. Feng et al. synthesized novel biodegradable nanoparticles using poly(lactide)-vitamin E TPGS copolymer with medical clay MMT for oral delivery of docetaxel [21]. This has also been reported that TPGS could increase in vivo oral bioavailability of cyclosporine A (CsA), vancomycin hydrochloride and talinolol [22-24]. The most frequent side-effects of cancer chemotherapy are the GI problems, which lead to mucositis and ulceration of the GI tract and diarrhea. Lopes et al. prepared nanoparticulate carriers composed of synthetic polymers, proteins and polysaccharides possessing interesting properties for oral administration of pharmaceuticals and nutraceuticals [25]. Increasing attention has been paid to the potential use of nanoparticles for peptides, proteins, antioxidants (carotenoids, Omega fatty acids, coenzyme Q10), vitamins and probiotics for oral administration.

In another study, tamoxifen-loaded PLGA nanoparticles (Tmx-NPs) were found to be significantly cytotoxic, which could be attributed to fast internalization and retention of nanoparticles within cells as compared with free drug [26]. Wu et al. designed and developed a two-stage delivery system with a multiple emulsions solvent evaporation method involving the enteric capsule and cationic nanoparticles for oral delivery of insulin [27]. For the selective release of insulin from nanoparticles in the intestinal tract, instead of the stomach, the enteric capsule was coated with pH-sensitive hydroxypropyl methylcellulose pthalate (HP55). To enhance the penetration of insulin across the mucosal surface in the intestine Eudragit® RS was also introduced to the PLGA nanoparticles. Omeprazole-containing Eudragit L 100-55 nanoparticles were prepared by Hao et al. using electrospray, which resulted in high drug loading in one step. The prepared nanoparticle showed spherical or ellipsoidal morphology and the average diameter was about 300 nm with 100% entrapment efficiency [28]. The pH-sensitive nanoparticle displayed pH-dependent release in vitro. Self-assembled nanoparticles of chitosan (CS) and over-sulfated fucoidan (OFD) with pH-sensitive characteristics were prepared by a polycation–polyanion complex method. This pH-switched nanocarrier enhanced the oral transport of antiangiogenic drug OFD, in response to simulated GI tract media. The results indicate significant inhibition of tube formation of human umbilical vein endothelial cells (HUVECs) via competitive binding of OFD and basic fibroblast growth factor (bFGF) to bFGF receptors (bFGFRs) [29].

![Biodegradable polymers used in oral drug delivery](image-url)
# TABLE 1

Various nanocarriers employed for oral drug delivery

<table>
<thead>
<tr>
<th>Nanosystems</th>
<th>Composition</th>
<th>Bioactive molecule</th>
<th>Size</th>
<th>Targeted organ or disease</th>
<th>In vitro findings</th>
<th>Cell line/animal model</th>
<th>Concluding remarks</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micelles</td>
<td>Polyethylene oxide–polypropylene oxide–polyethylene oxide (PEO–PPO–PEO)</td>
<td>Paclitaxel</td>
<td>180 nm</td>
<td>Tumor therapy</td>
<td>The extended PEO chains in the micelle shell probably enabled interactions between</td>
<td>Female C57BL/6J mice</td>
<td>The oral administration of micelles in mice showed high area under curve of micellar paclitaxel (similar to the area of i.v. Taxol®), longer mean residence time (nine-times longer than i.v. Taxol®) and high distribution volume (twofold higher than i.v. Taxol®) indicating an efficient oral absorption of paclitaxel delivered by micelles</td>
<td>[98]</td>
</tr>
<tr>
<td>N-octyl-O-sulfate chitosan (NOSC) micelles</td>
<td>NOSC</td>
<td>Paclitaxel</td>
<td>–</td>
<td>Tumor treatment</td>
<td>NOSC micelles significantly improved the oral bioavailability of PTX compared with</td>
<td>Caco-2/SD rats</td>
<td>The mechanism of P glycoprotein (P-gp) inhibition by NOSC was proved to interfere with the P-gp ATPase rather than reduce P-gp expression. In conclusion, this study suggests that NOSC micelles exhibit a potential to improve oral bioavailability of P-gp substrates</td>
<td>[99]</td>
</tr>
<tr>
<td>Mixed polymeric micelles</td>
<td>Pluronic copolymers and low molecular weight heparin-all-trans-retinoid acid LHR</td>
<td>Paclitaxel</td>
<td>140 nm</td>
<td>Enhanced oral bioavailability</td>
<td>In vitro release study showed that pluronic/LHR MPMS exhibited delayed release characteristics compared with Taxol® and faster drug release profile compared with LHR plain polymeric micelles</td>
<td>MCF-7 cells</td>
<td>PTX-loaded pluronic/LHR MPMS also showed slightly better cytotoxic effect toward MCF-7 cells and enhanced in situ intestinal absorption compared with LHR MPMS. Moreover, pluronic/ LHR MPMS achieved significantly higher oral bioavailability of PTX</td>
<td>[100]</td>
</tr>
<tr>
<td>Self-assembled β-casein micelles</td>
<td>Bovine β-casein</td>
<td>Celecoxib</td>
<td>21 nm</td>
<td>Rheumatoid arthritis and osteoarthritis</td>
<td>It enables encapsulation loads &gt;100-fold higher than other systems and long-term physical and chemical stability</td>
<td>–</td>
<td>Being a milk protein, β-casein is expected to be safe, and to release its payload, celecoxib, naturally and directly at the site of action</td>
<td>[101]</td>
</tr>
<tr>
<td>Micelles/sodium-alginate composite gel beads</td>
<td>Poly(ε-caprolactone)-block-(dimethylamino)ethyl methacrylate</td>
<td>Indomethacin</td>
<td>2.5 mm</td>
<td>–</td>
<td>The release of IND was affected not only by the pH of the medium but also the concentrations of SA and CaCl₂</td>
<td>–</td>
<td>The results suggested that the micelles/SA beads would be a good candidate for the oral drug delivery system</td>
<td>[39]</td>
</tr>
<tr>
<td>Specific 7 peptide conjugated polyethylene glycol-polycaprolactone (PEG–PCL) nanocarriers</td>
<td>PEG–PCL</td>
<td>Coumarin 6 (C6)</td>
<td>35.94 nm</td>
<td>Oral delivery</td>
<td>The developed system was nanometric in size range and spherical in shape. In vitro leakage test indicated that less than 2% of total C6 leaked from both kinds of micelles within eight hours</td>
<td>Caco-2 cells/male SD rats</td>
<td>This study demonstrated that TFR-targeted functional nanocarriers increased the intracellular uptake, altered their intracellular trafficking and enhanced their transcytosis in polarized Caco-2 cells. 7pep-M-C6 was also found to enter the cells through a specific clathrin-mediated mechanism, related to the expression of TFR on Caco-2 cells and distributed more in rat intestine in vivo</td>
<td>[102]</td>
</tr>
<tr>
<td>Dendrimers</td>
<td>G3.5 PAMAM</td>
<td>SN38</td>
<td>Oral therapy of hepatic colorectal cancer metastases</td>
<td>G3.5-balanine-SN38 was mostly stable whereas G3.5-glycine-SN38 showed 10%, 20% and 56% SN38 release in simulated gastric, intestinal and liver environments for up to 6, 24 and 48 hours, respectively</td>
<td>The designed system showed significant cytotoxicity and transport of G3.5-glycine-SN38 was highly concentration-dependent, whereas transport of G3.5-balanine-SN38 was concentration-independent. The results show that PAMAM dendrimers have the potential to improve the oral bioavailability of potent anticancer drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(amide amine) dendrimers</td>
<td>Ethylene diamine and methyl acrylate</td>
<td>Camptothecin</td>
<td>1.3 ± 0.1 nm</td>
<td>Chemotherapy G4.0 and G3.5 caused a two-to-threefold increase in oral absorption of camptothecin when co-delivered with the drug at two hours and better solubilization of the drug in simulated gastric fluid</td>
<td>This study demonstrates that cationic and anionic PAMAM dendrimers were equally effective in enhancing the oral absorption of camptothecin. Results suggest that drug inclusion in PAMAM interior controlled drug solubilization in SGF and SIF, and increased oral bioavailability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dendrimers</td>
<td>PAMAM</td>
<td>Short hairpin RNA</td>
<td>107–315 nm</td>
<td>Oral cancer delivery</td>
<td>PAMAM dendrimers are able to form compacted complexes ‘dendriplexes’ with plasmid DNA via electrostatic interaction and presented nanoscale spherical complexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-linked chitosan microspheres</td>
<td>Chitosan, 4% aqueous glacial acetic acid</td>
<td>Insulin</td>
<td>16.7–30.5 μm</td>
<td>Diabetes</td>
<td>The optimal formulation was obtained with mean particle size of 29.5 μm, and insulin encapsulation efficiency of 71.6 ± 1.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogel microspheres</td>
<td>Sodium carboxy methyl cellulose and poly (vinyl alcohol)</td>
<td>Diclofenac sodium (DS)</td>
<td>Anti-inflammatory drug</td>
<td>The in vitro drug release study was extensively evaluated depending on the process variables in acid and alkaline media. Release data indicated a non-Fickian trend of drug release from the formulations</td>
<td>The results of this study suggest that DS-loaded IPN microspheres were suitable for oral controlled release application</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyceryl monooleate-coated hollow-bioadhesive microspheres</td>
<td>Ethylcellulose, Eudragit®</td>
<td>Psoralen</td>
<td>500–1000 μm</td>
<td>Stomach diseases</td>
<td>The in vitro release test showed that the release rate of drug from the microspheres was pH-dependent, and was not influenced by the GMO coating film</td>
<td>The prepared microspheres demonstrated strong mucoadhesive properties with good buoyancy in vitro and in vivo. Pharmacokinetic analysis indicated that the elimination half-life of the hollow-bioadhesive microspheres was prolonged, and that the elimination rate was decreased. In conclusion, the hollow-bioadhesive synergic drug delivery system could be advantageous in the treatment of stomach diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanosystems</td>
<td>Composition</td>
<td>Bioactive molecule</td>
<td>Size</td>
<td>Targeted organ or disease</td>
<td>In vitro findings</td>
<td>Cell line/animal model</td>
<td>Concluding remarks</td>
<td>Refs</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td>--------------------</td>
<td>------</td>
<td>---------------------------</td>
<td>------------------</td>
<td>------------------------</td>
<td>-------------------</td>
<td>------</td>
</tr>
<tr>
<td>Poly(ester amide) (PEA) blend microspheres</td>
<td>L-lysine/-/leucine-based poly(ester amide) with pendant COOH groups and Arg-poly(ester amide)</td>
<td>Insulin</td>
<td>13.4–16.7 μm</td>
<td>Diabetes</td>
<td>The PEA–COOH/Arg–PEA blend microspheres protected the loaded insulin in simulated gastric fluid and released insulin in a fast and sustained manner in simulated intestinal fluid</td>
<td>Diabetic rats</td>
<td>The in vivo test demonstrated that the oral administration of insulin-loaded PEA blend microspheres could effectively suppress the blood glucose level in diabetic rats for 10 h, and the oral bioavailability was improved to 5.89 ±1.84% in healthy rats. These results indicate that the PEA blend microspheres are promising vehicles for the oral delivery of insulin</td>
<td>[43]</td>
</tr>
<tr>
<td>Alginate–chitosan microspheres</td>
<td>Alginate solution and chitosan</td>
<td>Insulin</td>
<td>7.5 μm</td>
<td>Diabetes</td>
<td>Under the pH conditions, only 32% of insulin released during the simulated transit time of drug (two hours in the stomach and four hours in the intestine). Whereas under the pH conditions of the blood environment insulin release was stable and sustained for a long time (14 days)</td>
<td>Male SD rats</td>
<td>The blood glucose level of diabetic rats could be effectively reduced and stably kept for a long time (60 hours) after oral administration of the insulin-loaded alginate–chitosan microspheres. Therefore, the alginate–chitosan microspheres were found to be promising vectors showing a good efficiency in oral administration of protein or peptide drugs</td>
<td>[109]</td>
</tr>
<tr>
<td>Self-assembled nanocarrier (TSN)</td>
<td>Teniposide, TPGS, MCT and HS-15</td>
<td>Teniposide</td>
<td>31.2 ± 4.1 nm</td>
<td>Tumor therapy</td>
<td>The intestinal absorption of teniposide from TSN was improved 4.09-fold and 6.35-fold in duodenum and jejunum at 0.5 hours after oral administration, then significantly decreased with the prolongation of time</td>
<td>Caco-2 cell monolayer and MCF-7 cells</td>
<td>The cytotoxicity of teniposide in MCF-7 cells was significantly increased after its encapsulation into TSN. After oral administration, TSN was mainly absorbed into the systemic circulation via the intestinal lymphatic pathway, and the oral bioavailability of TSN was greatly improved over 5.41-fold that of teniposide solution. In particular, the teniposide concentration in the tumor was obviously increased over sevenfold that of teniposide solution and TSN could specifically accumulate in the tumor site in xenograft model after oral administration</td>
<td>[60]</td>
</tr>
<tr>
<td>Mixed polymeric micelles</td>
<td>Pluronic copolymers and LHR conjugate</td>
<td>Paclitaxel</td>
<td>140 nm</td>
<td>Oral drug delivery system</td>
<td>In vitro release study showed that pluronic/LHR MPMS exhibited delayed release characteristics compared with Taxol® and faster drug release profile compared with LHR plain polymeric micelles (PPMs).</td>
<td>MCF-7 cells</td>
<td>PTX-loaded pluronic/LHR MPMS also showed slightly better cytotoxic effect toward MCF-7 cells and enhanced in situ intestinal absorption compared with LHR PPMs. Moreover, pluronic/LHR MPMS achieved significantly higher oral bioavailability of PTX. Therefore, pluronic/LHR MPMS could be promising drug carriers for PTX oral administration</td>
<td>[100]</td>
</tr>
<tr>
<td>BmpB-CKS9-WSC-PLGA MPs vaccine</td>
<td>Porous PLGA microparticles coated with M cell homing peptide-coupled chitosan</td>
<td>Brachyspira hydysenteriae (BmpB) as a model vaccine</td>
<td>3.36 μm</td>
<td>Targeted oral delivery</td>
<td>The release study showed that release of BmpB from the BmpB-PLGA and BmpB-WSC-PLGA MPs was higher at pH 7.2 compared with pH 1.2</td>
<td>Female BALB/c mice</td>
<td>BmpBCKS9-WSC-PLGA MPs demonstrated to induce Th1- and Th2-type responses based on elevated IgG1 and IgG2a titers. The present study showed that this approach would be applicable for swine dysentery vaccine delivery to induce mucosal and systemic immune responses in piglets</td>
<td>[110]</td>
</tr>
<tr>
<td>Polymeric micelles</td>
<td>Amphiphilic polyallylamine (PAA)</td>
<td>siRNA</td>
<td>150–300 nm</td>
<td>Gastrointestinal diseases</td>
<td>The quaternised PAA formed stable nanocomplexes with siRNA, which were resistant to aggregation and degradation in simulated gastric and intestinal fluids</td>
<td>Caco-2 cells</td>
<td>In addition, substantial levels of cellular uptake followed by endosomal release and subsequent successful reporter gene silencing in Caco-2 cells indicate that the quaternized amphiphilic PAA derivatives have potential to achieve delivery in the GI tract</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------------------</td>
<td>-------</td>
<td>-------------</td>
<td>--------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Micelles</td>
<td>Tocopherol succinate glycol chitosan (GC-TOS) conjugates</td>
<td>Ketoconazole</td>
<td>101 nm</td>
<td>oral delivery of poorly soluble drugs</td>
<td>The conjugates spontaneously formed micelles in aqueous solution with a critical micelle concentration of 2 μg/ml and spherical micelles were formed. The GC-TOS increased water solubility of two model class II drugs</td>
<td>Caco-2 cell monolayer</td>
<td>GC-TOS increased the solubility of poorly soluble drugs and enhanced the intestinal permeation of a BCC class II drug in vitro with no toxicity</td>
<td></td>
</tr>
<tr>
<td>Transferrin receptor (TfR) specific 7peptide nanocarrier</td>
<td>PEG-b-PCL, NHS-PEGb-PCL solution of 7 pep</td>
<td>Coumarin 6</td>
<td>30 and 40 nm</td>
<td>Oral drug delivery</td>
<td>The micelle systems were spherical in shape. The CMC for PEG-b-PCL micelles and 7peptide-modified PEG-b-PCL micelles are 0.87 mg/ml and 1.23 mg/ml, respectively</td>
<td>Caco-2 cells</td>
<td>The functional nanocarriers could specifically interact with gastrointestinal endothelial cells, increase their transport and alter their pathway as a result</td>
<td></td>
</tr>
<tr>
<td>Vesicles</td>
<td>PLA-P85-PLA</td>
<td>Insulin</td>
<td>178 nm</td>
<td>Oral insulin delivery</td>
<td>It was observed that insulin was released gradually from PLA-P85-PLA vesicles and almost all insulin was released 7.5 hours later and cytotoxicity studies indicate that PLA-P85-PLA block copolymer has good biocompatibility</td>
<td>OVCAR-3 cells/ diabetic mice</td>
<td>These results proved that PLA-P85-PLA vesicles could be promising polymeric carriers for oral insulin delivery application owing to their sustained and enhanced hypoglycemic effect</td>
<td></td>
</tr>
<tr>
<td>Multifunctional polymeric nanoparticles</td>
<td>Galactose-modified trimethyl chitosan-cysteine (GTC) conjugates with various galactose grafting densities</td>
<td>shRNA and siRNA</td>
<td>130–160 nm</td>
<td>Targeted treatment of hepatoma</td>
<td>GTC NPs with moderate galactose grafting density, termed GTC2 NPs, were superior in facilitating cellular uptake, promoting nuclear distribution, and silencing target genes, leading to notable inhibition of cell growth</td>
<td>Caco-2 monolayers/ tumor-bearing mice</td>
<td>In tumor-bearing mice, orally delivered GTC2 NPs could effectively accumulate in the tumor tissues and silence the expression of Survivin and vascular endothelial growth factor (VEGF), evoking increased apoptosis, inhibited angiogenesis and thus the most efficient tumor regression. Moreover, compared with single gene delivery, co-delivery of iSur-pDNA and siVEGF showed synergistic effects on inhibiting in vitro cell proliferation and in vivo tumor growth. This study could serve as an effective approach for synergistic cancer therapy via oral gene delivery, and highlighted the importance of ligand grafting density in the rational design of targeted nanocarriers</td>
<td></td>
</tr>
</tbody>
</table>
In another study, Du et al. explored the potential of transferrin receptor (TfR)-specific 7peptide (7pep) to increase the interaction of a nanocarrier system with GI epithelial cells. The peptide was conjugated to PEG-b-PCL copolymer and the functional nanocarriers were developed that exhibited higher intracellular uptake compared with unmodified nanocarriers [30]. A confocal microscope was used to analyze TfR expression in Caco-2 cells. Confocal images were obtained of Caco-2 cells incubated with unmodified micelles (M-C6) or coumarin 6 (7pep-M-C6) for different time periods and at different temperatures. TfR-mediated endocytosis is mainly dependent on clathrin-mediated endocytosis [31]. The treatment of chlorpromazine exhibited a significant cellular uptake inhibition of 7pep-M-C6, whereas no significant change in cellular uptake was observed in the M-C6 group, suggesting that the endocytosis of M-C6 might not be specifically clathrin-mediated. This confirms 7pep-M-C6 uptake is TfR-endocytosis mediated.

Micelles

Micelles are molecular aggregates of approximately 20–100 nm formed from surfactants or amphiphilic polymers above their critical micellar concentration (CMC) in colloidal dispersions. The systems contain hydrophobic cores that act as a reservoir for lipophilic drug molecules surrounded by the hydrophilic corona (usually PEG) that provides steric stabilization assuring the integrity of the system in an aqueous environment holding the adequate amount of guest drug molecules [32]. Oral bioavailability of poorly water-soluble drugs is increased one to three times after encapsulation into micelles made up of monomethyl PEG750-poly(caprolactone-co-trimethylcarbonate) [33]. TPGS (PEGylated vitamin E) provides water-solubility and surfactant properties to vitamin E and allows the formation of micelles. Polymeric micelles made up of pH-sensitive polymers contain in their hydrophobic block a pH-sensitive unit such as acrylic acid (AA) moieties and hydrophobic non-ionizable units for self-assembly [34].

Yao et al. studied dequalinium-functionalized micelles for intracellular targeting via the oral route of administration for co-localization in the mitochondria, guided by transmembrane electric potential within the cell. The cell culture experiments revealed selective accumulation of the system in the mitochondria and endoplasmic reticulum within the cells with strong inhibitory action against MCF-7 and MCF-7/Adr cell lines. The permeability studies also revealed higher transport of dequalinium across Caco-2 cells [35]. Moa et al. investigated the effect of N-octyl-O-sulfate chitosan (NOSC) micelles on the oral absorption of paclitaxel in vivo and in vitro [36]. The oral bioavailability of paclitaxel loaded in NOSC micelles (Ptx-M) was six-times higher in comparison with that of an orally dosed Taxol®. Significantly higher amounts of paclitaxel were accumulated in Caco-2 cells via clathrin- and caveolae-mediated endocytosis. Polyionic copolymer mPEG-grafted alginic acid (mPEG-g-AA)-based polion complex (PIC) micelles were also exploited to enhance the oral absorption of salmon calcitonin (sCT). The higher Caco-2 permeability of sCT without significantly affecting transepithelial electrical resistance (TEER) values suggests that the transport of PIC micelles across Caco-2 cell monolayers chiefly involves a transcytosis mechanism via endocytosis rather than the paracellular pathway [37].

Zohra et al. studied mixed polymeric micelles of pluronic copolymers and low molecular weight heparin-all-trans-retinoid acid (LHR) conjugate to enhance paclitaxel oral bioavailability [38]. Permeability, area under the curve (AUC) and Cmax of paclitaxel were higher with mixed micelles than Taxol®. Huang et al. prepared indomethacin (IND)-loaded six-arm block copolymer poly(ɛ-caprolactone)-block-(dimethylamino)ethyl methacrylate micelles and concluded that developed micelles were able to protect IND from being released under acidic conditions in the stomach and therefore enabled delivery to the small intestine and colon [39]. In a recent study, a supersaturated polymeric micelle (super-PM) system was designed to increase molecularly dissolved drugs and gain an insight into the effect of the degree of supersaturation on oral absorption of CsA in rats. The transport flux of CsA across a Caco-2 monolayer was increased with initial supersaturation but further increase in supersaturation actually resulted in decreased CsA transport [29].

Microspheres

Polymeric microspheres present a flexible platform for applications in drug delivery, diagnostics and bioseparations and can be administered via different routes. They can be coated with peptides, antigens, antibodies and nucleic acid probes, and can be loaded with hydrophobic bioactives. Microsphere drug delivery systems can be fabricated by techniques including combinations of phase separation or precipitation, spraying methods and emulsion or solvent evaporation. Microspheres of alginate-containing antitubercular drug served as efficient drug carriers. Weekly oral administration into infected guinea pigs for 6–8 weeks resulted in complete bacterial clearance [40]. Alginate, owing to its bioadhesive nature, binds to the intestinal epithelium and thus enhances absorption of drug. Clinical use by the oral route is the additional plus point with alginate. Oral bioavailability of CsA is usually very low owing to its poor absorption, which is related to its high molecular weight, high lipophilicity and poor aqueous solubility [41].

Furtado et al. fabricated insulin-containing poly(fumaric-co-sebacic) anhydride microspheres by the phase inversion nanoencapsulation method. In vivo performance was evaluated in a type 1 diabetic rat as well as type 1 diabetic dog model [42]. It was found that blood glucose levels were suppressed in the fasted and fed state. Recently, He et al. developed novel oral insulin-containing microspheres made of a blend of biodegradable poly(ester amide) (PEA). In the formulation, pH-responsive material was used for the protection of insulin from the harsh environmental conditions of the stomach. Arginine-based PEA (Arg-PEA) improved the intestinal absorption of the drug. The PEA-COOH/arginine-based PEA microspheres sheltered the loaded insulin in simulated gastric fluid. The in vivo test demonstrated that the oral administration of the formulation could effectively suppress the blood glucose level in diabetic rats. These results indicate that the PEA-blend microspheres are promising vehicles for the oral delivery of insulin [43].

Dendrimers

Dendritic architecture holds unique physical and chemical properties that make it a potential carrier particularly in the field of drug delivery. Oral delivery of many drugs via plain as well as...
surface-modified dendrimers has been widely investigated. Poly (amido amine) (PAMAM) dendrimers have shown promise as transepithelial permeability enhancers, intestinal-penetration enhancers, drug solubilizers and drug carriers for oral delivery. It was found that generation size, concentration of dendrimers, pH, core, internal branching units, surface groups as well as temperature effect the dendrimer-mediated oral delivery of bioactives. The enhancement of oral bioavailability of P-gp substrate potential using PAMAM-drug complex was studied [44]. The release of doxorubicin from doxorubicin–PAMAM complex appears to be slow and lower (74.5% during 24 hours) compared with that from the doxorubicin solution (more than 95% during 3 hours). The higher accumulation of doxorubicin in Caco-2 cells was observed in the presence of CsA, as compared to that treated with free doxorubicin. The absorbptive amount in Caco-2 cells was significantly increased over all time points using doxorubicin–PAMAM complex. The time-dependent mucosal-to-serosal transport of doxorubicin was observed in dissimilar segments of small intestines of rat. At 90 min, the transport efficiency of the doxorubicin–PAMAM complex from the mucosal side to the serosal side was four to seven times higher than that of the doxorubicin solution. A slight increase in permeability of 3H-mannitil across the small intestinal mucosa of all segments upon incubation with doxorubicin–PAMAM complex was observed. The doxorubicin–PAMAM complex has a bioavailability 700-fold higher than that of free doxorubicin.

7-Ethyl-10-hydroxy-camptothecin (SN-38) is a potent topoisomerase I poison and a biologically active metabolite of irinotecan hydrochloride (CPT-11). SN-38 has potent antitumor activity, approximately 1000-fold more active than CPT-11, but has poor aqueous solubility. To enhance the solubility, 4.0 G dendrimers were used because of their appreciable permeability and higher number (i.e. 64) of amine groups on their surface. Increased oral bioavailability associated with the observed increased solubility as well as permeability of SN-38–PAMAM dendrimer complexes helped to reduce dose-related toxicity of SN-38. Studies suggested that there was a linear correlation between the number of free amine groups and cellular toxicity; thus cytotoxicity of higher generation (4.0 G) amine-terminated PAMAM dendrimers can be reduced by conjugation to drug molecules while maintaining their transepithelial permeability. PAMAM dendrimers (4.0 G) are toxic at concentrations higher than 10 μM. No appreciable toxicity (cell viability >90%) was observed for the complexes after incubation for 2 hours with Caco-2 cells at 0.1 μM. The uptake values for complexes were significantly higher (P < 0.05) than those for free SN-38 and three-to-four-fold higher compared with unmodified dendrimers [45]. Increase in permeability could be caused by an increase in the hydrophobicity associated with the complexing of SN-38 to PAMAM dendrimer.

Najlah et al. assessed the dendrimer–naproxen (NAP) link for stability, cytotoxicity and transport across Caco-2 monolayers and the influence of surface modifier (lauroyl chain) on the transport properties [46]. It was found that stability of NAP prodrugs in enzymatic-rich media (80% human plasma and 50% rat liver homogenate) varied according to the medium and the linker between the dendrimer. The direct linkage of NAP to the 0.0 G dendrimer (0.0-G–NAP) resulted in a very stable amide prodrug. Attaching the lactic acid linker to PAMAM dendrimer decreases the rate of hydrolysis. The degradation rate of the lactic ester prodrugs increases with an increase in the esterase activity of the medium (liver > plasma), suggesting that the diethylene glycol linker produces esters that are more susceptible to enzymatic hydrolysis than lactate esters. The results indicated that dendrimer prodrugs with diethylene glycol linkers have the ability to release the parent drug in plasma once absorbed. The MTT assay suggested that attachment of NAP directly or via a linker to the PAMAM dendrimer had no influence on the viability of Caco-2 cells at concentrations up to 3.0 μm for an incubation time of up to 180 min. The permeability of 0.0-G–NAP and 0.0-G–lact–NAP across Caco-2 monolayers, especially in the A–B direction, was significantly higher than that of NAP itself. The low aqueous solubility of NAP facilitated the conjugation to 0.0-G–PAMAM dendrimer either directly by an amide bond (0.0-G–NAP) or by ester bonds using lactic acid (0.0-G–lact–NAP) or diethylene glycol (0.0-G–deg–NAP) as bioalike linkers. The stability of the 0.0-G–lact–NAP and 0.0-G–deg–NAP ester conjugates were high with approximately 90% of the conjugates remaining at all pH values after 48 hours. 0.0-G–lact–NAP was more stable than 0.0-G–deg–NAP, possibly owing to the greater spacer length in the diethylene glycol linker prodrug. The design, synthesis, characterization and stability studies of NAP conjugates with 0.0-G–PAMAM dendrimer using several covalent linkers were reported. Conjugates formed by direct amide linkage of NAP to the 0.0 G dendrimer were not suitable for the development of prodrugs because of their high chemical and enzymatic stability. By contrast, NAP was gradually released from the dendrimer conjugate formed using a lactic acid ester linkage, and such conjugates could have potential as controlled release systems or prodrugs for drug targeting. Conjugates in which NAP was linked to the 0.0 G dendrimer through a diethylene glycol linker showed high chemical stability in buffers, but readily released NAP in plasma. Such conjugates have potential as carriers for low solubility drugs such as NAP [47].

Najlah et al. formulated PAMAM dendrimer (1.0 G)-based prodrugs of the water-insoluble P-gp substrate terfenadine (Ter) using succinic acid (suc) or succinyl-diethylene glycol (suc-deg) as a linker or spacer to yield Gly-suc-Ter and Gly-suc-deg-Ter, respectively. In addition, the permeability of Gly-suc-deg-Ter was enhanced by attaching two lauroyl chains (L) to the dendrimer surface (L2-Gly-suc-deg-Ter). All of the 1.0 G dendrimer-terfenadine prodrugs were more hydrophilic than the parent drug. The LDH assay indicated that the dendrimer prodrugs had no impact on the viability of Caco-2 cells up to a concentration of 1 mM. However, the IC50 of the prodrugs was lower than that of 1.0-G–PAMAM dendrimer because of the high toxicity of terfenadine. Dendrimer prodrugs showed an increase of the apparent permeability coefficient (Papp) of terfenadine in apical-to-basolateral (A–B) and basolateral-to-apical (B–A) directions across monolayers of Caco-2 cells following conjugation to 1.0-G–PAMAM dendrimer. It was also found that the A–B Papp of the dendrimer prodrugs was significantly greater than B–A Papp and the surface-modified dendrimer prodrug L2-G1-suc-deg-Ter showed highest A–B permeability among the conjugates [48]. Effects of PAMAM dendrimers on the intestinal absorption of 5(6)-carboxyfluorescein (CF), fluorescein isothiocyanate-dextrins (FDs), calcitonin and insulin were assessed. Concentration- and generation-dependent increase in the absorption of CF was observed in the presence...
of 0.5% (w/v) 2.0-G–PAMAM dendrimer. However, no absorption-enhancing effect on macromolecular drugs including FD and insulin absorption was identified. The findings suggested that PAMAM dendrimers at lower concentrations could enhance the absorption of poorly absorbable drugs from the small intestine [49].

In a study by Yandrapu et al., novel thiolated dendrimers were developed for mucoadhesive acyclovir delivery. The thiolated dendrimers showed sustained release of acyclovir and higher mucoadhesion [50]. Cohen et al. explored the potential of linear poly(amide amine) (PAAs) which form self-assembled cationic nanocomplexes with oppositely charged proteins for the delivery of human serum albumin (HSA) protein. The nanocarriers were highly mucoadhesive and stable under neutral (extracellular) conditions. The results showed higher uptake of the system when exposed to human-derived intestinal Caco-2/TC7 cells and HT29-MTX mucus-secreting cells [51].

Teow et al. reported the ability of paclitaxel-containing third-generation (3.0 G) PAMAM dendrimer-based carriers to enhance the permeability across cellular barriers. The surface of the dendrimers was modified with lauryl chains and conjugated with paclitaxel via a glutaric anhydride (glu) linker. Permeability studies of dendrimer-drug conjugates demonstrated an increase in the Papp in both directions across both cell monolayers compared with unmodified 3.0-G–PAMAM and free drug. Moreover, lauryl-conjugated dendrimer had ~12-fold greater permeability across human colon adenocarcinoma cell line (Caco-2) and porcine brain endothelial cell (PBEC) monolayers than that of paclitaxel alone [52]. In another study, camptothecin was co-delivered with cationic, amine-terminated 4.0-G–PAMAM dendrimer and anionic, carboxylate-terminated PAMAM dendrimer (3.5 G) in CD-1 mice. Camptothecin solubilization was improved in simulated gastric fluid with PAMAM (4.0 G and 3.5 G) and caused a two-to-threefold increase in oral absorption at 2 hours. Results suggest drug inclusion in PAMAM interior-controlled solubilization in simulated gastric and intestinal fluids, and increased oral bioavailability without modulation of the tight junctions [53].

Thiagarajan et al. investigated the potential of 6.5-G–PAMAM as a carrier for oral drug delivery in CD-1 mice and concluded that dendrimers can permeate the gut epithelial barrier [54]. Later, the same group reported the effect of surface charge and size of dendrimers on the permeability through the epithelial barrier and acute toxicity after oral administration in CD-1 mice. Authors concluded that the maximum tolerated dose for anionic dendrimers was tenfold higher than for cationic dendrimers [55]. From the results it can be concluded that polymeric nanocarriers are an efficient system for the oral administration of therapeutics and are of paramount importance to medical application and advancement. The studies provide relevant information about improved mucosal penetration and cellular uptake through these advanced drug delivery systems.

**Solid lipid nanoparticles**

Lipid nanocarriers have been widely employed for improving the oral bioavailability of various drugs. Solid lipid nanoparticles (SLNs) and self-emulsifying drug delivery systems have been extensively beneficial in enhancing oral drug delivery. SLNs are devices made from lipids that are solid at room temperature. Because they are derived from physiologically compatible lipids such as fatty acids (e.g. stearic acid), fatty acid esters (e.g. glyceryl monostearate, glyceryl behenate), triglycerides (e.g. tristearin, trilaurin), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate), SLNs present a safe and effective alternative for drug delivery. They can be eroded and degraded by bile salts and pancreatic lipase in the body [56]. Silva et al. studied the oral delivery of SLNs containing risperidone (a poorly water-soluble drug) [57]. The system was found to be stable with high entrapment efficiency. Drug transport studies indicated passive redistribution of drug following uptake by the cells indicating the delivery potential of the formulations. Candesarren cilexetil-loaded SLNs were successfully developed to improve the oral bioavailability. It is reported that the formulation could be internalized into the enterocytes and then transported into the systemic circulation via the portal circulation and intestinal lymphatic pathway. The pharmacokinetic study revealed higher oral bioavailability of candesartan after incorporation into SLNs [58].

Singh et al. aimed to treat liver cirrhosis with SLNs loaded with thyroquinone (THQ–SLNs). In vivo studies revealed a significant increase in the bioavailability of THQ–SLNs as compared with THQ suspension [59]. Pharmacodynamic data also showed a significant decrease in the serum biomarker enzymes after oral administration of THQ–SLNs as compared with control and marketed (i.e. SILY-BON®) formulations against paracetamol (PCM)-induced liver cirrhosis. Zhang et al. demonstrated that the oral bioavailability of simvastatin, a cholesterol-lowering agent, after its incorporation into the lipid nanoparticles was improved significantly [60]. Müller et al. reported SLNs as a safe and better carrier for the oral delivery of cyclosporine than drug nanocrystals [61].

**Self-emulsifying drug delivery systems**

Self-emulsifying drug delivery systems (SEDDS) enhance the oral bioavailability of poorly soluble drugs by maintaining the drug in a dissolved state and enhancing the rate and the extent of drug absorption. They consist of a mixture of oil and surfactant that is capable of forming emulsion (oil-in-water) upon gentle agitation provided by the GI motion. Abdul et al. concluded a gliempiride-containing solid self-nanoemulsifying drug delivery system increases in vitro drug release and therapeutic efficacy as compared with free drug [62]. SMEDDS enhance the oral bioavailability of the poorly water-soluble compound 20(S)-25-methoxydammarane-3β;12β;20-triol (25-OCH3-PPD). Optimized SEDDS formulations containing Cremophor® EL glycerin as the co-surfactant and Labrasil® M1944 (30%) improved the solubility, lymphatic transport and thus the bioavailability [63]. SMEDDS-containing phyllanthin consisted of phyllanthin:Capryol™ 90:Cremophor® RH 40:Transcutol® P (1:38:39.45:44.38:14.79) in % w/w. The formulation was stable for at least 6 months under accelerated conditions, and oral bioavailability of phyllanthin in rats was significantly enhanced as compared with plain phyllanthin [64]. Sermkaw et al. reported that an oral self-microemulsifying formulation of an Andrographis paniculata extract in liquid and pellet forms enhanced the AUC by 15-fold for liquid SEDDS and 13-fold for SEDDS pellets compared with the extract in aqueous suspension [65]. In a study on pharmacokinetics and bioavailability of procolubol suspension, oil solution and SEDDS were evaluated and compared in rats. It was concluded that relative bioavailability of
SEDDS was noticeably enhanced through lymphatic transport as compared with oil solution and suspension [66].

**Polymeric nanocarriers in oral gene delivery**

Currently, numerous biotechnological efforts are being made to attain goals toward oral and/or mucosal gene delivery. The principal benefits offered by oral gene delivery are the ease of target approachability, patient compliance owing to noninvasive delivery and the feasibility of local and systemic gene therapy [67]. In the cases of short hairpin RNA (shRNA) or small interfering RNA (siRNA), denoted RNA interference (RNAi) and DNA vaccine, oral delivery might attract extensive attention owing to long-lasting silencing of target genes or satisfactory therapeutic efficacy when presented in the proper structure [68]. Han et al. synthesized multifunctional galactose-engineered trimethyl-chitosan-cysteine (GTC) conjugates. Synergistic antitumor efficacy against hepatoma was obtained via oral co-delivery of survivin shRNA-expression pDNA (iSur-pDNA) and vascular endothelial growth factor (VEGF) siRNA (siVEGF) [69]. In another study, He et al. reported mannose-modified trimethyl-chitosan-cysteine (MTC) nanoparticles as highly efficient polymeric carriers for oral delivery of tumor necrosis factor (TNF)-α siRNA [70]. Bowman et al. studied the oral gene delivery efficacy of Factor-VIII-encoded plasmid DNA encapsulated chitosan nanoparticles. The concentration of functional Factor VIII protein in plasma was assayed by chromatogenic and thrombin generation technique, reaching a peak level of 2–4% Factor VIII at day 22 after oral administration [71].

Zhang et al. reported enhanced intestinal absorption of siRNA using ternary polymeric nanoparticles prepared by ionic gelation of chitosan, N-trimethyl chitosan (TMC) or thiolated trimethyl chitosan (TTMC) with triply phosphate (TPP). It was concluded that permeability was enhanced across ex vivo rat ileum and cellular uptake was improved in Raw 264.7 cells after oral delivery [72].

**Importance of micro- versus nano-particles in oral delivery**

Particle size for oral drug delivery is an important factor because it is concerned with their adhesion and interaction with the cell and drug release dynamics. The mechanism enabling the particles to pass through GI barriers includes paracellular passage of the particles (<50 nm), particles endocytosed by intestinal enterocytes (<500 nm) and uptake by M cells of the Peyer’s patches (<5 μm) [73]. Studies have shown higher uptake of particles with mean diameters of 50–100 nm in the rat intestine as compared with larger particles. Particles of more than 1 μm in diameter were found to be exclusively localized in Peyer’s patches with low uptake, whereas the passage to associated lymphoid tissues of particles with >3 μm diameter could not be observed [74].

**Biofate of nanocarriers and release of drug in the GI tract**

Nanoparticles can pass through the GI tract by passive and active transport and are rapidly eliminated through feces and urine indicating their absorption across the GI tract barrier and entry into the systemic circulation [75]. Oral delivery leads to exposure to the bacterial population present in the GI tract. Polymeric drug delivery materials are capable of converting into less-complex products via chemical degradation. Alterations in the polymer side groups and destruction of macromolecular backbone results in low molecular weight products. Biodegradable polymers leave no residue in the body and undergo complete degradation after drug release. Biodegradable materials employed in drug delivery are accomplished by polymer dissolution after drug release. PLGA nanoparticles degrade into glycolic acid and lactic acid via hydrolytic cleavage. Poly(alkyl 2-cyanoacrylate) nanoparticles degrade into alkyl cyanoacetate and formaldehyde [76]. The release of the encapsulated drug from the nanoparticles could be a result of bioerosion or biodegradation of the polymer or, alternatively, by swelling of the particles resulting in the diffusion of the drug [77].

**Pharmacokinetics of orally administered nanocarriers**

Nanocarrier stability and uptake by enterocytes or M cells depends upon size, composition, surface characteristics and architecture. Nanocarriers made up of water-soluble polymers that form stable carriers are absorbed as particles, whereas polymers forming less-stable particles like polyelectrolyte complexes (e.g. chitosan) or polymeric micelles partly dissociate and are not completely absorbed as a particle. Absorption of the polymer depends upon its physicochemical characteristics (e.g. its molecular weight, conformation and hydrophobicity). Nanoparticles, micelles or drugs are delivered to blood when absorbed via enterocytes, whereas the carrier system is delivered to the gut-associated lymphoid tissue (GALT) and lymphoid cells after being transcytosed close to the immune system when M cell uptake occurs [78]. After oral absorption the chemical and physical properties of the nanoparticles affect pharmacokinetics and biodistribution in the body. The factors influencing pharmacokinetics include size, charge and surface modification with PEG to avoid uptake by the reticuloendothelial system (RES), prolongation of circulation half-life and diffusion in the tissues [79].

**In vitro, in vivo and ex vivo techniques or models for the study of oral delivery of polymeric nanocarriers**

In vitro techniques are useful in interpretation of the ability of the nanocarrier system to overcome the barriers in the GI tract in the in vivo environment. Models such as simulated gastric fluids and membrane analysis can be used to correlate the in vivo environment without the use of human cell lines. Because the GI tract presents a unique microenvironment of enzymes and ionic strength it affects the chemical and colloidal stability of the nanocarrier. Simulated gastric (pH 1.2) and intestinal (pH 6.8) fluids are better systems to study and analyze the release profiles of controlled release nanocarrier systems compared with phosphate buffer saline. These release mediums are prepared according to USP XXVI recommendations or the British Pharmacopoeia [80], and the experiments are performed at 37°C with agitation (100 rpm) [81].

In vitro peptide and drug transport analysis is performed by the use of a dialysis membrane model to determine drug transport. Here, nanocarrier dispersion is placed in dialysis bags or a dialysis tube and suspended in the buffer with the desired ionic strength at 37°C and movement is facilitated by shaking at 100 rpm [82,83]. The human epithelial colorectal adenocarcinoma cell line, Caco-2, typically represents only gut epithelial cells; however, there are...
many other cell types including mucosal cells and M cells that play major parts in permeability of a substance via oral delivery. Caco-2 monolayers can either be grown in a monolayer on a single cell culture well plate or in a Transwell®. In the Transwell® model different cell lines can be seeded at the bottom chamber below the Caco-2 monolayer grown on an inserted porous membrane. Confluence and viability of the monolayer is measured by TEER. A TEER value of 260–450 Ω cm² is appropriate for the experimental period [84]. Furthermore, the mucus lining of the epithelial layer limits the transport of nanocarrier from the gut fluids to the epithelial cells. A co-culture model of Caco-2 and HT29, a human adenocarcinoma cell line, provides a model for the mucus-secreting goblet cells. Presence of methotrexate induces the differentiation of HT29 cells into mature goblet cells making this cell line important in mucus layer formation. The viability of the Caco-2 monolayer with the mucus secretion is determined using TEER or by staining with 0.25% w/v toluidine blue for microscopic examination [85]. Lymphocytes taken from the Peyer’s patches of mice mixed with the Caco-2 cell line are involved in the formation of M cells in the Transwell® system. Because this model does not solely use human cell lines and also lacks uniformity, another method using human Burkitt’s lymphoma Raji B cells is used. A more appropriate model to study M cell transport mechanism is the inverted co-culture model that encourages closer contact between two cell lines. A new technique termed gut-on-a-chip microdevice is fabricated with upper and lower microchannels using polydimethylsiloxane, providing an in vitro model that mimics the intestinal structure and transport properties [86,87].

The mucus-hygroscopic properties, as well as transport and cellular uptake studies, are carried out on everted rat intestine. In the case of lipid-based particles, these studies were found to be inaccurate because ex vivo permeation studies failed to correlate in vivo performances. This could presumably be due to the differences in the ex vivo and in vivo extracellular environment as well as the difference in intestinal segment cells ex vivo, thus showing the potential inaccuracy of this model [88].

In vivo evaluation is mandatory to validate the true performance of an oral delivery system. Drug-release kinetics and biodistribution of the nanocarrier reveal most of the important information about the system. Many of the imaging techniques that focus on the stomach, small intestine and the colon are also helpful because oral delivery of a system is primarily observed in the GI tract. In vivo techniques include organ analysis (histological), symptom reversal (changes in enzyme or protein levels, behavior changes), antibody analysis (immune response), DNA and/or RNA extraction, protein extraction, blood analysis, radioactive labeling and IVIS (an optical imaging technique that uses fluorescence) [89,90].

Toxicity issues
Local toxicity associated with oral delivery of chemotherapeutics is a matter of concern so that potent compounds can be delivered orally without causing damage to the gut epithelium. Toxicity studies are vital to establish the potential of nanocarriers [91]. Further studies are needed to determine the physicochemical and molecular properties as well as biodistribution of nanoparticles. Despite the research in recent years in nanotoxicology, precise information about the behavior and biokinetics of nanoparticles is not known. Nanoparticles reach the GI tract after mucociliary clearance from the respiratory tract through the nasal region, or can be ingested directly [92]. Only a few studies are reported to evaluate the toxicity of nanoparticles following oral ingestion. Acute toxicity of copper particles and nanocopper was measured in mice and results indicate higher toxicity of nanocopper than copper particles [75]. Pathological damage to liver, kidney and spleen was also observed with nanocopper. By delivering the anticancer drugs with other drugs and by controlling the initial burst release of the drug, damage to the gut system can be minimized.

Dong and Feng designed paclitaxel-containing PLGA-montmorillonite (detoxifier of paclitaxel) nanoparticles for oral delivery. Further studies on GI lymphatic uptake, transport and toxicological effects on the GI tract need to be explored [93]. The in vitro screening of polymers can predict general cytoxicity, hemocompatibility and complement activation. In general, nanotoxicological studies can be assessed by employing all the current experimental techniques of cellular biology and toxicology. The techniques that can be used to assess toxicity of nanomaterials include assays for cell viability and/or proliferation, microscopic evaluation of intracellular localization, in vitro hemoysis and genotoxicity [94].

Regulatory aspects
The main governmental body that is entitled to regulate nanocarriers through the Center for Drug Evaluation and Research (CDER), the Center for Biologics Evaluation and Research (CBER), and the Center for Devices and Radiological Health (CDRH) is the FDA [95]. In 1996, the National Nanotechnology Initiative (NNI), a federal research and development program, was established to coordinate governmental multiagency efforts in nanoscale science, engineering and technology [75]. There are insufficient data available regarding the toxicology of nanocarrier systems, which hinders governmental regulation [96] and thus no regulatory requirement to test nanoparticle safety and environmental impacts has been formalized. Research on nanotechnology should be focused on understanding the effects on the environment, health and safety. The behavior of nanomaterials in the environment and the human body should be studied. Instruments and methods to measure, characterize and test nanomaterials should be designed and safety of technology that uses nanoparticles should be assessed. The exclusive biological properties of polymeric nanocarriers and the associated probable risks that might differ from the bulk material of the same chemistry should be considered. Multidisciplinary studies including toxicology, material science, medicine, molecular biology and bioinformatics should be encouraged [97].

Concluding remarks and future prospects
The increasing relevance of the potential of various nanocarriers in drug delivery emphasizes the need to explore the routes by which they can be administered. Theoretically, nanocarriers should be able to overcome several of the possible problems relating to the solubilization and bioavailability of drugs. From the vast amount of knowledge about developments and advancements on the GI barriers for therapeutic macromolecules and nanotechnology over
the past few decades, it is now essential to integrate and utilize this knowledge as a guideline to make an oral drug delivery system that can reach the patients. Multifunctional nanocarriers that can specifically protect the loaded bioactives from enzymatic degradation, enhance drug absorption by use of specific targeting ligands, prolong GI retention and contain diagnostic agents to detect the disease hold the basis for improved nanocarrier efficiency in oral delivery. Furthermore, conjugates and complexes of the drug or proteins that retain their activity and are resistant to proteolytic degradation can be explored for their efficiency. By taking advantage of such nanocarrier systems and development of multifunctional particles, the benefits associated with the oral route of administration can be maximized.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

Udita Agrawal and Rajeev Sharma are grateful to the Council of Scientific and Industrial Research (CSIR) and New Delhi and Indian Council of Medical Research (ICMR) India, respectively, for financial support in the form of Senior Research Fellowship.

References

30 Du, W. et al. (2013) Transferrin receptor specific nanocarriers conjugated with functional 7 peptide for oral drug delivery. *Biomaterials* 34, 794–806
37 Li, N. et al. (2012) The use of poloyon complex micelles to enhance the oral delivery of salmon calcitonin and transport mechanism across the intestinal epithelial barrier. *Biomaterials* 33, 8881–8892
43 He, P. et al. (2013) Polysorb amide: blend microspheres for oral insulin delivery. *Int. J. Pharm.* 455, 259–266
DRUDIS 1393 1–17

Drug Discovery Today • Volume 00, Number 00 • May 2014

Please cite this article in press as: U. Agrawal, et al., Is nanotechnology a boon for oral drug delivery?, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.03.011

16

www.drugdiscoverytoday.com
112 Duhem, N. et al. (2012) Tocol modified glycol chitosan for the oral delivery of poorly soluble drugs. Int. J. Pharm. 423, 452–460
113 Du, W. et al. (2013) Transferrin receptor specific nanocarriers conjugated with functional 7peptide for oral drug delivery. Biomaterials 34, 794–806