Expert Opinion

Cyclodextrin-poly(anhydride) nanoparticles as new vehicles for oral drug delivery

Maite Agüeros, Socorro Espuelas, Irene Esparza, Patricia Calleja, Ivan Peñuelas, Gilles Ponchel & Juan M Irache[†]

[†]*University of Navarra, School of Pharmacy, Department of Pharmaceutics and Pharmaceutical Technology, Pamplona, Spain*

Introduction: The oral administration of drugs belonging to Class IV of the Biopharmaceutical Classification System (BCS) represents a major challenge. These drugs display poor aqueous solubility and specific permeability characteristics. Most of these compounds are substrates of the P-glycoprotein and/or the cytochrome P450. Among other types of drug, various anti-cancer drugs also suffer from these drawbacks (i.e., paclitaxel), which limits the possibilities for developing oral treatments.

Areas covered: This review discusses the factors that influence the bioavailability of drugs when administered by the oral route, as well as the capabilities of cyclodextrins when associated with nanoparticles. In particular, evidence is given regarding the synergistic effect between cyclodextrins and bioadhesive nanoparticles, on the oral delivery of pharmaceuticals.

Expert opinion: This article aims to provide an overview of the multiple gains in incorporating cyclodextrins in poly(anhydride) nanoparticles, including improvement of their bioadhesive capability, the loading of lipophilic drugs and the effect on efflux membrane proteins and cytochrome P450. The combination between bioadhesive nanoparticles and P-gp inhibitors without pharmacological activity (i.e., cyclodextrins) may be useful to promote the oral bioavailability of drugs ascribed to Class IV of the BCS.

Keywords: bioadhesion, bioavailability, cyclodextrin, nanoparticles, oral delivery, paclitaxel

Expert Opin. Drug Deliv. (2011) 8(6):721-734

1. Introduction

The oral route is the preferred route for drug administration given its acceptability and convenience for patients. However, many drugs remain poorly available when administered by this route, owing to the physicochemical properties of the drug (e.g., pK_a, solubility, stability, lipophilicity, polar–nonpolar surface area, presence of hydrogen bonding functionalities and crystal form), physiological factors (e.g., gastrointestinal pH, gut blood flow, gastric emptying, intestinal transit time, colonic transit time and absorption mechanisms) and factors related to the dosage form [1].

It has been demonstrated that two of these factors, namely the aqueous solubility of a drug and its permeability across the cellular membranes, strongly determine the oral bioavailability of the drug. This has been aptly summarized in the Biopharmaceutical Classification System (BCS). The BCS categorizes drugs into one of four biopharmaceutical classes according to their water solubility and membrane permeability characteristics and broadly allows the prediction of the rate-limiting step in the intestinal absorption process following oral administration. From this system, except for Class I drugs, all pharmaceuticals will be faced with bioavailability issues owing to their poor dissolution rate (Class II), their poor permeability (Class III) or both (Class IV) [1,2].

informa
healthcare

Article highlights.

- Oral administration of drugs belonging to Class IV of the Biopharmaceutical Classification System (those characterized by both a low aqueous solubility and low permeability) still represents a major challenge.
- The encapsulation of lipophilic drugs in polymeric nanoparticles is usually hampered by its low aqueous solubility. One strategy to increase significantly the drug loading is encapsulation of the given drug as a complex with cyclodextrins.
- The incorporation of cyclodextrins in poly(anhydride) nanoparticles (made from the copolymer between methyl ether and maleic anhydride) permits modification of the distribution and, to a certain degree, the intensity of the bioadhesive properties of these carriers.
- The load of paclitaxel (Class IV of the BCS) as inclusion complex with cyclodextrins in poly(anhydride) nanoparticles improves its permeability through the intestinal mucosa and, as a consequence, its oral bioavailability (~ 80% in rat). Moreover, these nanoparticles offer sustained and prolonged paclitaxel plasma levels for 24 h.
- The reported nanoparticles would transport the drug-cyclodextrin complex to the surface of the mucosa where they would be released. Once outside the nanoparticle, the complex would dissociate and the free oligosaccharide would interact with the surface of the enterocyte, disturbing the activity of the P-gp and cytochrome P450 and, thus, permitting absorption of paclitaxel.
- Combination between cyclodextrins and poly(anhydride) nanoparticles can be an adequate strategy to facilitate oral administration of drugs belonging to Class IV of the BCS.

This box summarizes key points contained in the article.

Whenever a dosage form is administered orally, first the drug should be released and dissolve in the surrounding gastrointestinal fluid to form a solution. This process is solubility limited. A low solubility and very low solubility of the compound result in low dissolution rate in the mucosal fluids and elimination of a fraction of the drug before absorption. Once the drug is in the solution form, it passes across the membranes of the cells lining the gastrointestinal tract. This process is permeability limited. The adsorption of orally administered drugs is restricted to transport through the enterocytes (transcellular transport) [3] or between the enterocytes (paracellular transport) [4] and transport through M cells. Drugs absorbed by the transcellular pathway are generally low-molecular-mass hydrophobic entities that are able to diffuse through the membrane, either on their own or associated with a specific membrane transporter. In both instances, the rate is determined by the concentration gradient across the intestinal membrane, with the blood acting as a sink. In some particular instances, drugs may be absorbed by fluid-phase endocytosis (pinocytosis), an energy-dependent saturable process in which the molecule travels inside membrane vesicles. However, once inside the cell, a fraction of these vesicles may fuse with enzyme-rich lysosomes, leading to degradation of the drug. Alternatively, endocytosis of the drug may be triggered by its binding to a receptor, given sufficient structural analogy with the natural substrate. As with pinocytosis, this pathway may lead to degradation of the absorbed substances.

Typically, hydrophilic molecules cannot diffuse freely through the intestinal membrane, owing to their low affinity for the lipidic constituents. Therefore, in the absence of an appropriate membrane transporter, the paracellular route or transport through M cells are the only ones available for their absorption. In the paracellular space, the presence of tight junctions between adjacent cells restricts the passage of large molecules (< 350 kDa) through the intercellular space. On the other hand, M cells are phagocytic enterocytes found in the follicle-associated epithelium of the Peyer's patches [5]. They are specialized in the capture and transport of bacteria, viruses, macromolecules and particles from the gut lumen to immune cells across the epithelial barrier, and thus are important in stimulating mucosal immunity. Unlike their neighboring cells (enterocytes), they have the unique ability to take up compounds from the lumen of the small intestine by means of endocytosis or phagocytosis and then deliver them via transcytosis to dendritic cells and lymphocytes located in a unique pocket-like structure on their basolateral side. In any case, the total contribution of the paracellular pathway or transit through M cells to general drug transport is, in general, very discrete. The surface of intercellular pores and M cells represents only 0.01 – 0.1% and < 1% of the intestinal epithelial cells, respectively [5].

Apart from the solubility and absorption pathways, an extensive presystemic metabolism is also a handicap that limits the oral bioavailability of many drugs. Compounds such as proteins, peptides and nucleic acids are prone to rapid inactivation in the acidic environment of the stomach and digestion by secreted enzymes that produce their degradation before absorption. Furthermore, during and after the adsorption many drugs can be degraded by the cytochrome P450 (CYP) system. Enzymes of the CYP family are expressed at high levels in the villus tip enterocytes of the small intestine at concentrations equal to or higher than in the liver [6,7].

Finally, in addition to the first-pass metabolism, active secretion of absorbed drug is now recognized as a significant factor in oral drug bioavailability. Of particular interest is the MDR1 gene product P-glycoprotein (P-gp), a multidrug efflux pump. As CYP3A, P-gp is located in the intestinal villus enterocytes. This colocalization and the sharing of a remarkable number of substrates and inhibitors suggest that CYP3A and P-gp may form a concerted barrier to drug absorption and that intestinal drug metabolism and countertransport processes are a major determinant of oral drug bioavailability and variability [7].

Several strategies have been proposed to circumvent these limitations, including reduction of drug particle size, salt

formation, administration with P-gp [8] or CYP inhibitors, or pro-drug synthesis [9]. Another interesting approach relies on encapsulation of the drug inside nanosized carriers such as polymeric nanoparticles, micelles, liposomes and other lipid carriers [10]. They can protect the drug against gastrointestinal degradation. Moreover, the polymers and their physicochemical properties such as size and surface can be modulated to increase the strength of their interactions with either mucosal surface or, after diffusion within the mucus, with the intestinal cells.

This transitory immobilization results in a delay in the transit time of the particles in the gastrointestinal tract and, in this way, an increase in the drug concentration gradient from the lumen to the epithelia (mucoadhesion) or direct contact with intestinal cells (bioadhesion), which is the first step before drug adsorption [11]. In a few cases, nanoparticles themselves can cross the epithelial membrane and transport the drug cargo from the lumen to the blood or lymph. As described before, particles should be taken up by M cells or translocate between epithelial cells, both pathways with a minor contribution to the whole drug adsorption.

After describing the structure and physicochemical and biological properties of cyclodextrins (CDs) [12-14], this work analyzes cyclodextrin-based colloidal drug carriers previously reported in the literature [15-18]. Therein, the bioadhesive properties of cyclodextrin-poly(anhydride) nanoparticles and their performance for enhancing the oral bioavailability of lipophilic drugs such as paclitaxel [19], ciclosporin A [20] or atovaquone were investigated [21].

2. Cyclodextrins

Cyclodextrin is a common name for cyclic α-1,4-glucans, which are a family of cyclic oligosaccharides produced as a result of the transformation of starch by the cyclodextrin glucanotransferase enzyme through an intramolecular transglycosylation reaction [22]. Chemically, these molecules are macrocycles composed by 6, 7 or 8 α-D-glucopyranose units linked by α-(1,4) glycosidic bonds (α-, β- and γ-CDs, respectively) [12]. Cyclodextrins with fewer than 6 units cannot be formed due to steric hindrances, whereas oligosaccharides with 9 or more glucose units are difficult to purify, although large-ring CDs (δ-CD, ε-CD and τ-CD) have been isolated [13,14] and references to CDs with > 60 [23] and several hundred [24] glucose units have been made.

For common oligosaccharides, and as a consequence of the conformation of these glucopyranose units, the ring that constitutes the CDs is a wreath-shaped truncated cone [25]. In this structure, the central cavity is characterized by the presence of skeletal carbon, hydrogen atoms and glycosidic oxygen bridges, which confer it a lipophilic character [25]. On the contrary, the external part of the oligosaccharide is hydrophilic owing to the presence of secondary hydroxyl groups of the sugar residues at the wider edge of the ring and primary hydroxyl groups at the narrow edge of the cone [26]. Owing to this architecture, cyclodextrins have an apolar internal cavity, defined as a 'micro heterogeneous environment' [22], and a hydrophilic outer surface. This special feature allows the cyclodextrin to host on its internal cavity a wide variety of hydrophobic guest molecules by no covalent bonds, forming what is known as an inclusion complex. The formation of these complexes consists basically of a replacement of the water molecules included in the hydrophobic cavity of the CD by the less polar guest. Then, van der Waals forces, hydrophobic interactions and hydrogen bonds hold the CD and its guest together [25]. This phenomenon may induce significant modifications in the physicochemical properties of the included molecules [27,28], such as solubility and bioavailability enhancements, stabilization against the degradative effects of oxidation, control of volatility and sublimation, physical isolation of incompatible compounds and controlled release of drugs, among others [29].

Each type of cyclodextrin has a different ability to form inclusion complexes with specific guests. This capacity depends on a proper fit of the guest molecule in the hydrophobic cavity. Thus, to improve the drug carrier properties with respect to the natural CDs, many derivatives, different from their parent cyclodextrin, have been synthesized [22]. These modifications can improve the solubility (of the CD derivative and its complexes), the association between the CD and its guest with concomitant stabilization against light or oxygen, and help control the chemical activity, reactivity and mobility of the guest molecule [22,28].

From a physicochemical point of view, these chemically modified CDs can be classified into three categories: hydrophobic, ionizable and hydrophilic derivatives [30,31]. The first group has the ability to decrease the solubility of guest molecules, so this type of cyclodextrin is appropriate for use as a sustained release drug carrier of water-soluble drugs [31,32]. The ionizable CDs can be used to modify the release rate of the drug, depending on the pH of the solution. Finally, hydrophilic CDs (methylated, hydroxyalkylated or branched oligosaccharides) are characterized by their high solubility in water, making them suitable for use as solubilizers for poorly water-soluble drugs [30].

In the last few years, several types of amphiphilic cyclodextrin have also been synthesized, including the so-called 'lollipops' (resulting from the grafting of only one aliphatic chain on a 6-amino-β-cyclodextrin [33,34]), medusa-like cyclodextrins (obtained by grafting of hydrophobic chains on all the primary hydroxyl groups of β-cyclodextrin [35,36]), skirt-shaped cyclodextrins (esterified hydroxyl groups on C2 and C3-cyclodextrins [37,38]) and bouquet-shaped oligosaccharides (alkyl-cyclodextrins [39]). Among them the skirt-shaped cyclodextrins are particularly interesting [40] because they are susceptible to being biodegradable [40].

Another interesting property of cyclodextrins is their ability to disturb and inhibit the activity of P-gp [41,42]. This transmembrane protein transports a broad range of

chemically diverse hydrophobic compounds, including chemotherapeutics, to confer multi-drug resistance on cells [41]. In the gut, the activity of P-gp results in an extrusion or excretion of drugs and other compounds from the epithelium, before reaching the general circulation, back to the intestinal lumen. This effect also permits an increase of the drug exposition to metabolizing enzymes within the enterocyte (i.e., CYP3A4) [43,44]. As a consequence of the effect of P-gp and/or CYP3A4, the absorption and bioavailability of several drugs is poor. P-gp recognizes its substrates within the lipid bilayer, and may be involved in the relocation of cholesterol from the cytosolic to the exoplasmic leaflet of the plasma membrane and in stabilization of the cholesterol-rich microdomains [41]. Therefore, there is a strong dependence of the ATPase activity of P-gp on the amount of cholesterol incorporated into native membrane vesicles [45]. In this context, it was hypothesized that some hydrophilic cyclodextrins would be capable of solubilizing the cholesterol and, therefore, inhibiting the efflux pump activity of P-gp [46]. Arima and collaborators, working with Caco-2 cells, concluded that dimethyl-β-cyclodextrin (DMβCD) would inhibit the P-gp activity by allowing release of P-gp from the apical membrane of cells to the supernatant buffer. This finding was supported by the results of flow cytometric analysis, which indicated that the P-gp level on the Caco-2 cell surface was decreased by pretreatment with the oligosaccharide, although the P-gp expression on the cell surface was recovered within 12 h. Therefore, the inhibitory effect of DMβCD on the P-gp activity seemed to be non-cytotoxic and transient [47].

Similarly, it has been described that some cyclodextrins may influence the activity of cytochrome P450. CYP consist of ~ 40 P450s, among which the isoenzyme CYP3A4 is of special interest because it shows very broad substrate specificity [48] and it is involved in the presystemic metabolism of several drugs [49-51]. Thus, hydroxypropyl-β-cyclodextrin (HPβCD) and methyl-β-cyclodextrin (MβCD) would inhibit the activities of CYP2C19 and CYP3A4 [52].

3. Combination between cyclodextrins and colloidal carriers

One of the major problems encountered with colloidal carriers (i.e., liposomes and nanoparticles) appears during their preparation, and results from low water solubility of the drug leading to either a low yield in drug loading, or a slow or incomplete release rate of the drug. To overcome these drawbacks, several authors have proposed the use of cyclodextrins. Thus, the association between cyclodextrins and liposomes was first proposed by McCormack and Gregoriadis [15,16]. Its main purpose was to combine some advantages of cyclodextrins (such as increase in drug solubility) with some advantages of liposomes (such as targeting of drugs to their activity site) into a single dosage form circumventing problems associated with each system.

This new concept was applied to dexamethasone [53], dehydroepiandrosterone, retinol and retinoic acid included in HPβCD [15,16,53]. The drug-cyclodextrin complex was entrapped in liposomes, allowing improvement of the drug loading and its retention without destabilization of the lipid bilayers [38,54]. In addition, it was suggested that, owing to the resulting increase in the drug/lipid ratio, it would be possible to reduce the size of the resulting liposomes [55]. Apart from the effect of cyclodextrins on drug entrapment, it was demonstrated that encapsulation of photolabile drug/α- or γ-cyclodextrin inclusions in liposomes containing light absorbers increased their stability [56]. Moreover, complexation with cyclodextrins was able to increase drug solubility and permeation across the skin, thus improving drug bioavailability through the topical route [57]. The effectiveness of such a combined approach has been demonstrated recently by using both classic [58-60] and deformable [61,62] liposomes.

On the other hand, the incorporation of cyclodextrins into polymer nanoparticles was also proposed in order to increase drug solubility in the aqueous solutions in which poly(alkyl cyanoacrylate) nanoparticles are prepared by a polymerization reaction [17]. Thus, poly(isobutyl cyanoacrylate) nanoparticles were prepared by anionic polymerization in 0.01 M HCl containing 1% w/v poloxamer 188 and cyclodextrins. Their size was found to be dependent on the cyclodextrin type, the smallest particles being obtained with hydroxypropyl-β- or hydroxypropyl-γ-cyclodextrin, 103 and 87 nm, respectively [17]. In 2001, following this strategy, Boudad and collaborators incorporated HPβCD–saquinavir inclusion complex into poly(alkyl cyanoacrylate) nanoparticles in order to increase drug loading [63]. It was found that large amounts of cyclodextrins remained associated with the particles, resulting in a 20-fold increase in saquinavir loading compared with nanoparticles prepared in the absence of cyclodextrins. This increased loading capacity has been reported to be a consequence of two different mechanisms. The former would be related to the increase of drug concentration in the polymerization medium in which the nanoparticles are formed, resulting from the addition of the drug:cyclodextrin complex. The latter would be due to the presence of immobilized cyclodextrins in the structure of the nanoparticles. This fact would create numerous lipophilic sites that would be available for the complexation of a lipophilic drug [54]. More recently, Çirpanli and co-workers proposed an association between cyclodextrins and poly(lactide-*co*-glycolide) (PLGA) or poly-ε-caprolactone (PCL) nanoparticles to increase the stability of camptothecin against hydrolysis [18].

Another possibility for combining cyclodextrins and nanoparticles may be based on polymerization between the oligosaccharide and the monomer in order to obtain a copolymer, which would be used to prepare the nanoparticles. In this context, Mooguee and collaborators synthesized PLGA-βCD copolymer by reacting L-lactide, glycolide and β-cyclodextrin in the presence of stannous octoate as a catalyst. The resulting copolymer was used to load adriamicin

(doxorubicin) within PLGA-βCD nanoparticles by a modified double emulsion method [64]. The resulting carriers displayed high entrapment efficiencies as well as adequate *in vitro* release profiles for anticancer therapy.

In the same way, Wang and co-workers synthesized a new biodegradable and amphiphilic copolymer based on HPβCD, lactic acid monomers and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine segments (HPβCD-PLA-DPPE) [65]. In an aqueous medium, the copolymer forms micelles that can be used for the encapsulation of poorly soluble drugs (i.e., doxorubicin), yielding devices with superior cytotoxic activity [65].

The combination between cyclodextrins and nanoparticles can also be of interest for the delivery of proteins and peptides. In fact, protein encapsulation in polymer nanoparticles may be associated with aggregation phenomena mediated by the interaction of hydrophobic residues with polymer surfaces. These interactions are often accompanied by a drastic reduction of biological potency, creating serious problems in formulating drug delivery systems [66]. Cyclodextrin complexation represents an effective strategy for improving protein therapy by stabilizing them against aggregation, thermal denaturation and degradation. Proteins are mostly hydrophilic and too bulky to be wholly included in the cavity of cyclodextrins. Nevertheless, the hydrophobic side chains in the peptides may penetrate into the cavity of the oligosaccharide, leading to the formation of non-covalent inclusion complexes, which improves the stability of proteins [67]. In an interesting work, Sajeesh and Sharma described the preparation and evaluation of an oral insulin delivery system based on hydroxypropyl-β-cyclodextrin-insulin complex encapsulated in polymethacrylic acid-chitosan-polyether (polyethylene glycol-polypropylene glycol copolymer) nanoparticles [68]. The presence of the cyclodextrin decreased the inactivation of the entrapped protein. In a similar study, insulin was complexed with cationic β-cyclodextrin polymers and then encapsulated into alginate/chitosan nanoparticles. The presence of the oligosaccharide allowed the insulin structure to be maintained and preserved during the nanoparticles' preparation and release process. In addition, the cumulative release of insulin was much higher when the cyclodextrin was presented because the protein was retained mainly in the core of the nanoparticles and was well protected against hydrolysis [69].

In another interesting work, chitosan/cyclodextrin nanoparticles were used as carriers for oral delivery of the peptide glutathione (GSH) [70,71]. The presence of sulfobutyl-ether-β-cyclodextrin (SBEβCD) was found to be more useful for promoting encapsulation of the peptide in the core, minimizing its presence at the surface of nanoparticles [70]. In addition, the resulting chitosan/SBEβCD nanoparticles provided absorption-enhancing properties of GSH in all segments of the duodenum, whereas the effect of conventional nanoparticles was restricted to the first segment of the duodenum.

4. Cyclodextrin-poly(anhydride) nanoparticles

Some years ago Gantrez® AN, or the copolymer between methyl vinyl ether and maleic anhydride, was proposed as material for the preparation of nanoparticles for mucosal drug delivery [72]. The different copolymers (commercialized as Gantrez™ from ISP, USA) are widely used for pharmaceutical applications as denture adhesives, thickening and suspending agents and as adjuvants for the preparation of transdermal patches. In addition the ester derivatives are also used as film-coating agents. The oral toxicity of these polymers is quite low (i.e., for Gantrez® AN the LD_{50} in guinea-pigs is ~ 8 – 9 g/kg *per os*). In addition, the copolymer is considered as GRAS (generally recognized as safe) and it can be used for the preparation of non-parenteral medicines licensed in the UK [73].

This copolymer permits the preparation of nanoparticles under 'soft' conditions and the resulting carriers have demonstrated a high ability to develop bioadhesive interactions within the gastrointestinal tract [74]. This phenomenon may be related with the formation of carboxylic groups during the hydrolysis of the anhydride residues of the copolymer. These carboxylic groups would develop hydrogen bonds with components of the mucosa [74]. More importantly, the surface of these poly(anhydride) nanoparticles can be easily modified by simple incubation with different excipients or ligands in order to modify their physicochemical and biological properties as well as their fate within the gastrointestinal tract.

Cyclodextrin-poly(anhydride) nanoparticles can be prepared by solvent displacement, in which the selected cyclodextrin (or inclusion complex) is incubated with the copolymer (Gantrez AN 119) in acetone for 1 h at room temperature. The nanoparticles can be obtained after the addition of water [75] or a hydroalcoholic mixture [72]. Then the suspensions can be purified (if necessary) and dried by either lyophilization [72] or spray-drying [75]. In a recent study, Agüeros and collaborators studied the influence of the type of cyclodextrin on the physicochemical characteristics and bioadhesive properties of the resulting nanoparticles [76]. Different oligosaccharides were assayed including β-cyclodextrin (βCD), HPβCD and 6-monodeoxy-6-monoamino-β-cyclodextrin (NHβCD).

When nanoparticles were obtained by mixing the selected oligosaccharide with the copolymer in acetone, desolvated with a mixture of ethanol and water (1:1 by vol.) and lyophilized, the resulting particles showed an average size of ~ 150 nm. The amount of cyclodextrin associated with the nanoparticles was found to be dependent on the type of oligosaccharide used [76]. Surprisingly, β-cyclodextrin was incorporated in a more effective way to the poly(anhydride) nanoparticles than other oligosaccharides. This fact was related with the relatively lower aqueous solubility of βCD (1.85 mg/ml) compared with the hydroxypropyl and amino derivatives (> 40 mg/ml).

The bioadhesive properties of nanoparticles were evaluated after quantification of the adhered fraction in the different segments of the gastrointestinal tract of rats. For this purpose, cyclodextrin-poly(anhydride) nanoparticles were labeled with rhodamine B isothyocyanate [76]. Overall, all the cyclodextrin-poly(anhydride) nanoparticles displayed a higher ability to develop bioadhesive interactions with the gut mucosa than control ones. However, as control carriers, the association of cyclodextrins to poly(anhydride) nanoparticles did not confer specific targeting properties. In any case, these nanoparticles showed an initial ability to concentrate in the stomach mucosa and the upper regions of the small intestine (duodenum and proximal jejunum). About 12 – 20% of the given dose of cyclodextrin nanoparticles adhered to the stomach and ~ 14 – 22% in the small intestine. These values were up to two times higher than the values found for control nanoparticles. Three hours after administration, the adhered fraction of cyclodextrin nanoparticles in the gastrointestinal mucosa decreased significantly in the stomach and appeared to move to the distal part of the gastrointestinal tract. Thus, the adhered dose in the small intestine was calculated to be ~ 26% (for HPβCD NP (nanoparticles)) and 19% (for βCD NP and NHβCD NP), whereas for control nanoparticles only 12% of the given dose remained adhered within the intestinal mucosa. These data were confirmed by calculating the area under the curve of bioadhesion (AUC_{adh}) [77,78], which was found to be 1.8 times higher for HPβCD NP and NHβCD NP than for nanoparticles ($p < 0.01$ and $p < 0.05$, respectively). For βCD NP, these parameters were only slightly higher than for conventional nanoparticles [76].

From fluorescence microscopy visualization studies, cyclodextrin-poly(anhydride) nanoparticles were mainly found broadly and homogeneously distributed along the ileum mucosa, whereas conventional nanoparticles were found mainly in the outer layer (mucus) of the ileum. On the other hand, after radiolabeling of these nanoparticles with technetium, it was observed that after oral administration the nanoparticles remained in the stomach during the first hour. Then, they were slowly discharged in the small intestine and continued to move along the gut during the time of the experiment (**Figure 1**). In any case, from *in vivo* imaging studies, cyclodextrin-poly(anhydride) nanoparticles remained in the gut with no evidence of translocation of distribution to other organs of the body of animals [79]. These cyclodextrin-poly (anhydride) nanoparticles have been evaluated for the oral delivery of lipophilic compounds, including paclitaxel [19], cyclosporine A [20] and the antimalarial atovaquone [21,75].

The case of paclitaxel (PTX) is particularly challenging. Paclitaxel is a potent anticancer agent approved for the treatment of a large number of solid tumors. However, its oral administration is severely hampered owing to the low aqueous solubility, P-glycoprotein efflux and first-pass metabolism by cytochrome P450 located in the gut and the liver (CYP2C8 and CYP3A4). As a result, the oral bioavailability of paclitaxel with conventional formulations is very low. To solve these drawbacks, the combination between bioadhesive nanoparticles and cyclodextrins, as 'soft' inhibitors of biological transporters and cytochrome P450, can be an interesting strategy to increase the oral bioavailability of this anticancer drug [19].

For the preparation of these nanoparticles, the first step was the preparation of an inclusion complex between the cyclodextrins and paclitaxel. This inclusion complex displayed a globular morphology with an average size of ~ 30 nm (**Figure 2B**). Then, the inclusion complex was mixed with the polymer and nanoparticles were obtained by the desolvation process described above. The resulting nanoparticles displayed a size of ~ 300 nm and the drug loading was dependent on the type of cyclodextrin used to prepare the inclusion complex. Thus, the highest drug loading was obtained when HPβCD was used (~ 160 μg/mg). For PTX-NHβCD NP and PTX-βCD NP the drug loadings were ~ 90 and 40 μg/mg, respectively. In all cases, the nanoparticles displayed a homogeneous size and an irregular and granular surface, as observed by scanning electron microscopy (**Figure 2C**). This special morphology would result from encapsulation of some of the inclusion complex globules observed in **Figure 2B** into a single carrier particle.

It is important to note that when nanoparticles were prepared in the absence of cyclodextrin, the paclitaxel loading was found to be very low. In fact, during the preparation of nanoparticles large aggregates of drug occurred and only a small fraction of the given dose appeared to be encapsulated within the matrix of poly(anhydride) nanoparticles. However, when these preparations were observed by transmission electron microscopy (see **Figure 2A**), small paclitaxel aggregates were observed outside the nanoparticles.

The transport and permeability of paclitaxel included in cyclodextrin poly(anhydride) nanoparticles through the ileum mucosa were determined '*ex vivo*' in Ussing chambers with jejunum rat portions [80]. **Figure 3** summarizes the main absorption enhancement ratios of paclitaxel when loaded as inclusion complex with HPβCD in poly(anhydride) nanoparticles.

To find evidence for the possible secretion of paclitaxel in the gastrointestinal tract, permeability coefficients were determined in the two directions mucosal-to-serosal (absorptive direction) and serosal-to-mucosal (secretory direction) [81,82]. In jejunum tissue and in the mucosal-to-serosal direction, PTX formulated as Taxol® (Bristol-Myers Squibb, USA) (the commercially parenteral formulation) showed a low absorptive apparent permeability ($P_{app} \sim 1.4 \times 10^{-6}$ cm/s). Similar low values of permeability were found when paclitaxel was formulated as an inclusion complex with the cyclodextrins. On the contrary, when the paclitaxel complexes were loaded in poly(anhydride) nanoparticles, the intestinal permeability of paclitaxel increased up to 12-fold higher than the reference formulation (Taxol). However, this permeability improvement was not observed when the PTX–HPβCD complex was physically mixed with empty poly(anhydride) nanoparticles (not shown). When the P_{app} of paclitaxel was studied from the serosal-to-mucosal

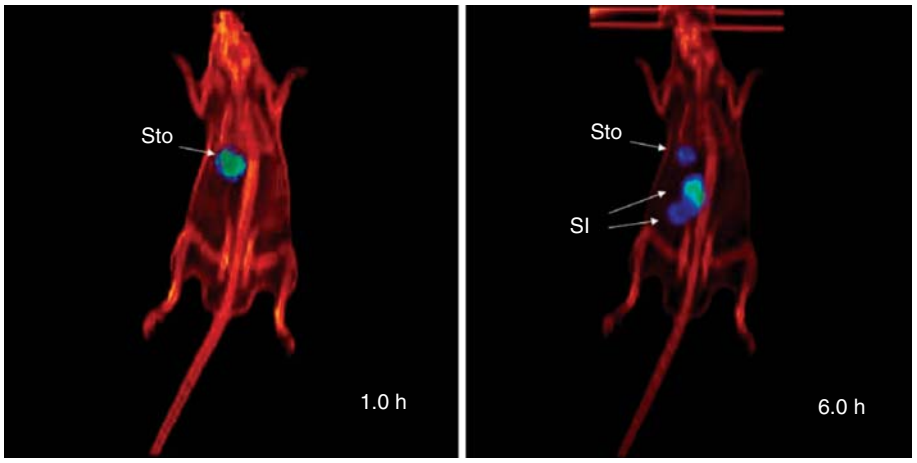

Figure 1. Biodistribution study of cyclodextrin-poly(anhydride) nanoparticles by gammacamera images 1 and 6 h after oral administration of 10 mg of ^{99} ^{m}Tc-labeled HPβCD NP.
HPβCD NP: Hydroxypropyl-β-cyclodextrin-loaded-poly(anhydride) nanoparticles; SI: Small intestine; Sto: Stomach.

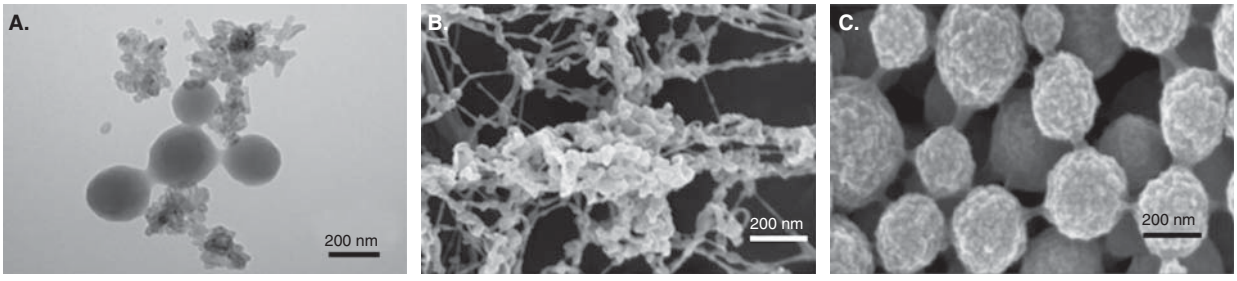

Figure 2. Morphology of nanoparticles. A. Transmission electron microscopy image of PTX NP. **B, C.** Scanning electron microscopy images for **(B)** PTX-HPβCD inclusion complex and **(C)** PTX-HPβCD.
PTX-HPβCD NP: Paclitaxel-hydroxypropyl-β-cyclodextrin-loaded-poly(anhydride) nanoparticles; PTX NP: Paclitaxel-loaded-poly(anhydride) nanoparticles.

direction, that is, in the secretory direction (S–M), the drug permeability was much higher than observed in the absorptive direction (M–S). Interestingly, in this case no statistical differences were found in the P_{app} values of paclitaxel when the drug was assayed as Taxol or was formulated in HPβCD complexes loaded in poly(anhydride) nanoparticles. This asymmetric permeation found for free paclitaxel (Taxol) is strong evidence of an active efflux mechanism. Obviously, for paclitaxel this efflux would be mediated by the P-gp localized at the surface of enterocytes [83]. This phenomenon was corroborated by experiments of co-administration of paclitaxel and verapamil, which is a well-known inhibitor of the P-gp efflux pump [84]. In this case, the apparent permeability coefficient of paclitaxel in the M–S direction was found to be ~ 20 times higher than in the absence of verapamil. Similar values were found when experiments were performed at 4°C. Under those conditions, the activity of the ATP-dependent efflux pump is disabled and paclitaxel is absorbed only by passive diffusion [85].

Surprisingly, when contact between PTX-HPβCD NP and the intestinal tissue was hampered by addition of a semipermeable membrane, paclitaxel was not able to permeate through the intestinal tissue. In fact, under these circumstances, in which the bioadhesive interactions between nanoparticles and the mucosa were prevented, the apparent coefficient of paclitaxel was found to be 25-fold lower than for the control Taxol.

All of these results were confirmed by pharmacokinetic studies. In this case, a single dose of 10 mg/kg was administered by the oral route to laboratory animals. When paclitaxel as inclusion complex with cyclodextrins was loaded in poly (anhydride) nanoparticles and then orally administered to rats, the anticancer plasma concentrations increased for the first 4 – 6 h until the C_{max} was reached. Then, the paclitaxel plasma levels were maintained until 24 h post-administration. The C_{max} value was similar for PTX-HPβCD NP and PTX-βCD NP and five times higher than for NHβCD NP. Similar plasmatic curves were observed when the nanoparticle formulations were evaluated in mice (see **Figure** 4). In any case, very low plasma levels of paclitaxel were found when the drug was administered as complex with HPβCD alone

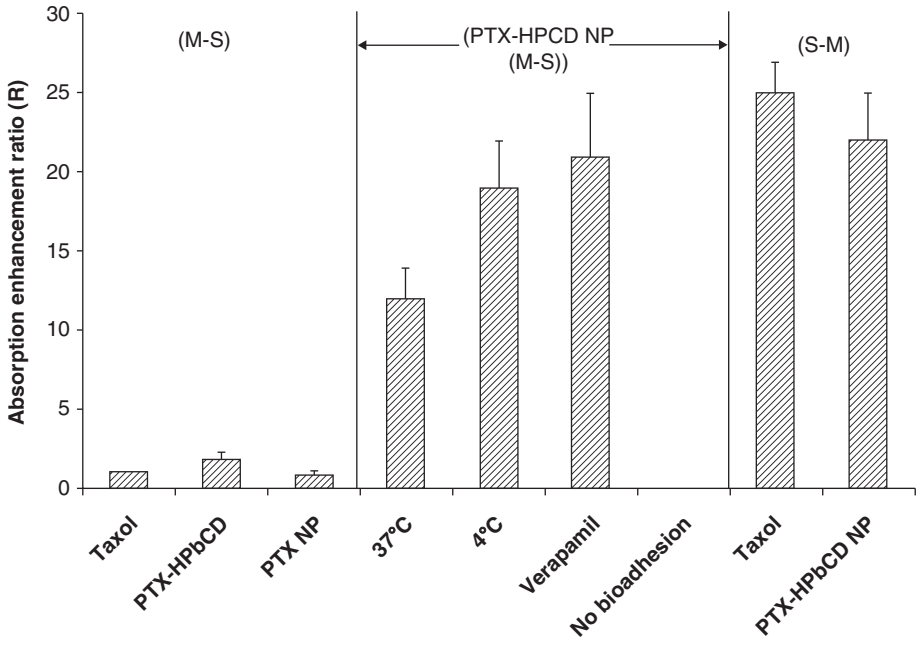

Figure 3. Absorption enhancement ratio (R) of paclitaxel under the different conditions tested in jejunum tissue in Ussing chamber experiments. Data expressed as the mean ± s.d. (n = 3). R was calculated as the quotient between the apparent permeability of jejunum to paclitaxel when included in the formulation tested and the apparent permeability to the drug when formulated in the reference formulation (Taxol®, M–S, 37°C). PTX-HPCD NP were assayed under the following experimental conditions. M–S: mucosal-to-serosal direction; S–M: serosal-to-mucosal direction; Verapamil: in the presence of verapamil 0.2 mM as inhibitor of the P-gp; No bioadhesion: addition of a dialysis membrane (cutoff molecular mass 100,000 Da) to avoid interaction between nanoparticles and the mucosa; 4°C M–S: the activity of the ATP-dependent efflux pump was disabled and the transport of paclitaxel was due only to passive diffusion.

PTX NP: Paclitaxel-loaded poly(anhydride) nanoparticles.

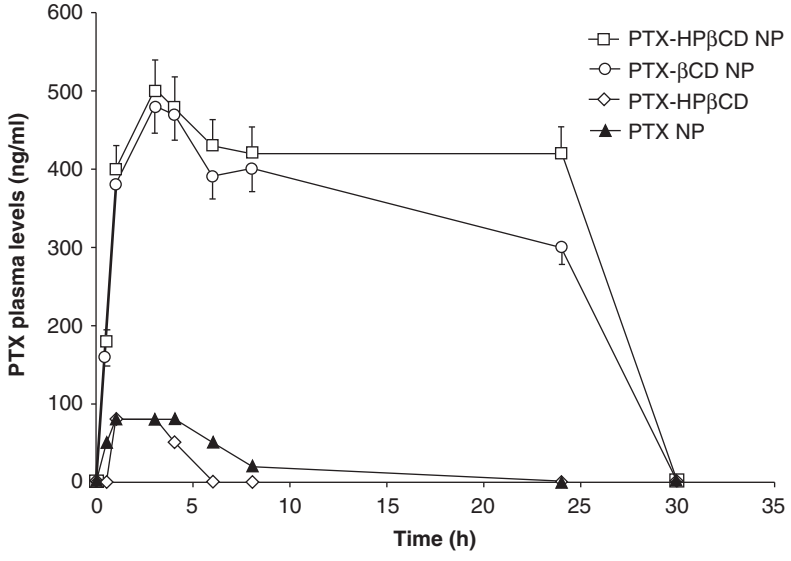

Figure 4. Paclitaxel plasma levels after oral administration of a single dose of 10 mg/kg in mice. Data expressed as mean ± s.d. (n = 6).

PTX-βCD NP: Paclitaxel-βCD complexes loaded in nanoparticles; PTX-HPβCD NP: Paclitaxel-HPβCD complexes loaded in nanoparticles; PTX NP: Paclitaxel-loaded nanoparticles.

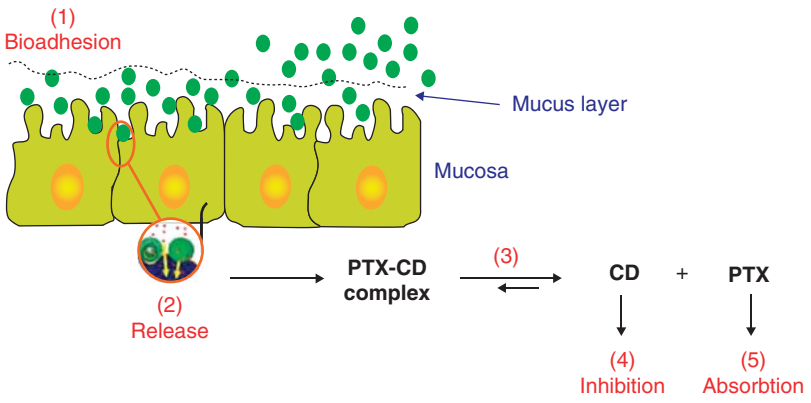

Figure 5. Representation of the mechanism by which the combination between cyclodextrins and poly(anhydride) nanoparticles would improve the absorption of PTX. (1) Bioadhesive interactions between nanoparticles and components of the mucosa; (2) cargo release; (3) dissociation of the PTX-cyclodextrin inclusion complex; (4) inhibition of P-glycoprotein and cytochrome P450 by free cyclodextrin molecules; (5) PTX absorption.
CD: Cyclodextrin; PTX: Paclitaxel.

or physically mixed with empty nanoparticles. In all cases, those levels were always above the therapeutic threshold (85.3 ng/ml) [86]. Similar results were obtained when animals were treated with either PTX NP or Taxol.

Concerning the relative oral bioavailability of paclitaxel in rats, it was calculated to be ~ 80% for both PTX-βCD NP and PTX-HPβCD NP. For PTX-NHβCD NP the oral bioavailability was found to be 18%, which would be related to a rapid release of an important fraction of the loaded drug in the stomach [19,76]. In mice, the relative oral bioavailabilities would be slightly lower than in rats (~ 60% for PTX-HPβCD NP and PTX-βCD NP).

From these findings it may be hypothesized that nanoparticles would transport the drug complexes to the surface of the mucosa where these carriers would remain immobilized in intimate contact with the absorptive membrane. Then, nanoparticles would progressively release their contents to the environment, yielding, after dissociation, the free drug and the oligosaccharide molecules. The anticancer drug would be rapidly absorbed, whereas the cyclodextrins would interact with lipophilic components of the membrane, disturbing the activity of the efflux pump and cytochrome P450 (**Figure 5**). From the authors' point of view, this is the key point because efficient P-gp/CYP3A4 co-modulation is necessary to inhibit paclitaxel absorption [86].

5. Expert opinion

Oral delivery remains the preferred route for drug administration thanks to its patient convenience and compliance. However, in some cases the oral bioavailability is limited by factors such as rapid degradation, low solubility, poor membrane permeability or extensive presystemic metabolism.

It is well known that the drug, before absorption through the epithelial cells, has to be sufficiently and rapidly dissolved in the aqueous fluids of the gastrointestinal tract. Poor dissolution properties within the lumen, as observed for lipophilic molecules, may, importantly, limit the fraction of drug absorbed. On the other hand, the permeability of a molecule may be negatively affected when the drug is characterized by the presence of hydrophilic or ionizable groups in its chemical structure. Similarly, this permeability is also low when the drug is a substrate of the efflux transport systems that are found on the surface of enterocytes. Another important factor that can negatively affect the bioavailability of a molecule is its sensitivity to the harsh conditions of the gastrointestinal tract or its degradation by luminal enzymes or enzymatic complex located in either the enterocytes (presystemic metabolism) or the liver (first-pass metabolism). In any case, overcoming these hurdles is a major challenge for the development of oral therapies.

To solve these drawbacks several strategies have been developed. Thus, for oral delivery of lipophilic compounds, the synthesis of more soluble structural analogues, micronization, solid dispersions, or the development of self-microemulsifying drug delivery system (SMEEDS) formulations have been proposed. Similarly, several nanoparticle-based formulations developed for oral delivery applications have succeeded in protecting the encapsulated drugs (including peptides and proteins), promoting their absorption through the gastrointestinal mucosa and enhancing their oral bioavailability.

Oral administration of drugs belonging to Class IV of the BCS, however, still represents a major challenge. In general, these drugs display poor aqueous solubility and specific permeability characteristics. In fact, most of these compounds are substrates for the biological transporters

(including P-glycoprotein) and for metabolism by means of cytochrome P450 enzymes, resulting in a significant presystemic metabolism.

This group of drugs, characterized by unfavorable physicochemical properties in tandem with an important sensitivity to the physiological mechanisms of detoxification, includes several antineoplastics. These facts limit the possibilities for developing oral formulations and treatments. However, oral chemotherapy is attractive because it improves patients' comfort. It is also especially appropriate where prolonged drug exposure is desirable. At present, many current anticancer therapies are cytostatic in nature and thus are optimally effective when given chronically for continuous tumor exposure [87,88]. This mechanism of action virtually requires oral daily and prolonged therapies [89]. On the other hand, from the economic standpoint, oral administration is more attractive because it reduces the cost of hospitalization and infusion equipment supplies [90], which makes it a 'dominant strategy' in pharmacoeconomic terms [91].

In this context, one possible strategy may be a combination between bioadhesive nanoparticles and 'soft' P-gp inhibitors, without pharmacological activity. It has been reported that some pharmaceutical excipients could inhibit the function of intestinal P-gp [92]. These excipients offer several advantages, including their well-known use in the formulation of parenteral and enteral medicines, safety, and regulatory acceptance. Among others, surfactants (i.e., Pluronic derivatives) [93,94], co-solvents (i.e., polyethyleneglycols) [95] and solubilizing agents (i.e., cyclodextrins) [47,52] have been proposed.

To confirm this strategy, paclitaxel as inclusion complex with cyclodextrins was encapsulated in poly(anhydride) nanoparticles characterized by their ability to develop adhesive interactions with components of the gut mucosa. The idea was that the bioadhesive nanoparticles would transport the drug-cyclodextrin complexes to the surface of the mucosa where they would be released. Then, the inhibitory effect of the cyclodextrins on the activity of the P-gp and cytochrome P450 would facilitate oral absorption of paclitaxel. The resulting nanoparticles were orally administered in laboratory animals. The paclitaxel plasma levels (in both rats and mice) were characterized by a plateau close to the C_{max} spanning from T_{max} to 24 h post-administration. Interestingly, these sustained profiles were found in parallel with high bioavailability values. Thus, the relative oral bioavailability of paclitaxel was calculated to be ~ 60% in mice and close to 80% in rats.

Although further studies are necessary, it is clear that effective oral delivery of compounds with poor characteristics of solubility and permeability is highly feasible.

Declaration of interest

The authors declare no conflict of interest. This work was supported by the Ministry of Science and Innovation (project SAF2008-02538) and Foundation 'Caja Navarra: Tú eliges, tú decides' (Nanotecnología y Medicamentos, number 10828) in Spain. M Augüeros is sponsored by University of Navarra, and the Regional Government of Navarra. J Irache is sponsored by the Ministry of Research (Spain) and the Foundation Caja Navarra. S Espuelas is sponsored by the Foundation Caja Navarra, and the University of Navarra.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Washington C, Washington CG, Wilson CG. Physiological Pharmaceutics. Barriers to Drug Absorption. Taylor and Francis, London; 2001
2. Dahan A, Miller JM, Amidon GL. Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. AAPS J 2009;11:740-6
3. Daugherty AL, Mrsny RJ. Transcellular uptake mechanisms of the intestinal epithelial barrier. Part one. Pharm Sci Technol Today 1999;8:144-51
4. Daugherty AL, Mrsny RJ. Regulation of the intestinal epithelial paracellular barrier. Pharm Sci Technol Today 1999;2:281-87
5. Corr SC, Gahan CC, Hill C. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol Med Microbiol 2008;52:2-12

• **Interesting work about the functions of intestinal M cells.**

6. Thummel KE. Gut instincts: CYP3A4 and intestinal drug metabolism. J Clin Invest 2007;117:3173-6

• **Thorough review of cytochrome P450.**

7. Wacher VJ, Salphati L, Benet LZ. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv Drug Deliv Rev 2001;46:89-102

•• **Excellent review about the effects of P-gp.**

8. Bansal T, Akhtar N, Jaggi M, et al. Novel formulation approaches for optimising delivery of anticancer drugs based on P-glycoprotein modulation. Drug Discov Today 2009;14:1067-74

•• **Excellent review about strategies to avoid the inconveniences provoked by P-gp efflux.**

9. Stella VJ, Nti-Addae KW. Prodrug strategies to overcome poor water solubility. Adv Drug Deliv Rev 2007;59:677-94
10. Cai Z, Wang Y, Zhu LJ, Liu ZQ. Nanocarriers: a general strategy for enhancement of oral bioavailability of poorly absorbed or pre-systemically metabolized drugs. Curr Drug Metab 2010;11:197-207
11. Ponchel G, Irache J. Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract. Adv Drug Deliv Rev 1998;34:191-219

• **Interesting review about the interactions between nanoparticles and the mucosa components.**

12. Arun R, Ashok Kumar CK, Sravanthi VVNSS. Cyclodextrins as drug carrier molecule: a review. Scientia Pharm 2008;76:567-98
13. Endo T, Nagase H, Ueda H, et al. Isolation, purification and characterization of cyclomaltooctadecaose (nu-cyclodextrin), cyclomaltononadecaose (xi-cyclodextrin), cyclomaltoeicosaose (o-cyclodextrin) and cyclomaltoheneicosaose (pi-cyclodextrin). Chem Pharm Bull 1998;46:1840-43
14. Miyazawa I, Ueda H, Nagase H, et al. Physicochemical properties and inclusion complex-formation of delta-cyclodextrin. Eur J Pharm Sci 1995;3:153-62
15. McCormack B, Gregoriadis G. Drugs-in-cyclodextrins-in-liposomes: a novel concept in drug delivery. Int J Pharm 1994;112:249-58
16. McCormack B, Gregoriadis G. Entrapment of cyclodextrin-drug complexes into liposomes: potential advantages in drug delivery. J Drug Target 1994;2:449-54
17. Silveira AM, Ponchel G, Puisieux F, Duchene D. Combined poly (isobutylcyanoacrylate) and cyclodextrins nanoparticles for enhancing the encapsulation of lipophilic drugs. Pharm Res 1998;15:1051-55
18. Cirpanli Y, Bilensoy E, Lale Dogan A, Calis S. Comparative evaluation of polymeric and amphiphilic cyclodextrin nanoparticles for effective camptothecin delivery. Eur J Pharm Biopharm 2009;73:82-9
19. Agueros M, Zabaleta V, Espuelas S, et al. Increased oral bioavailability of paclitaxel by its encapsulation through complex formation with cyclodextrins in poly (anhydride) nanoparticles. J Control Release 2010;145:2-8

• **This paper shows that encapsualtion of paclitaxel-cyclodextrin complexes in poly(anhydride) nanoparticles improves the bioavailability of this anticancer drug.**

20. Pecchio M. Development and characterization of cyclosporine A-loaded Gantrez AN 119 nanoparticles for oral delivery. In vivo behaviour. Ph.D. Thesis University of Navarra, Pamplona, Spain; 2010
21. Calvo J. Nanoparticles as vehicles for the oral delivery of antimalarial drugs. Ph.D. Thesis University of Navarra, Pamplona, Spain; 2010
22. Martin del Valle EM. Cyclodextrins and their uses: a revies. Process Biochem 2004;39:1033-46

•• **Extensive review about the properties and potencial applications of cyclodextrins.**

23. Terada Y, Yanase M, Takata H, et al. Cyclodextrins are not the major cyclic alpha-1,4-glucans produced by the initial action of cyclodextrin glucanotransferase on amylose. J Biol Chem 1997;272:15729-33
24. Takaha T, Yanase M, Takata H, et al. Potato D-enzyme catalyzes the cyclization of amylose to produce cycloamylose, a novel cyclic glucan. J Biol Chem 1996;271:2902-08
25. Astray G, Gonzalez-Barreiro C, Mejuto JC, et al. A review on the use of cyclodextrins in foods. Food Hydrocol 2009;23:1631-40
26. Loftsson T, Brewster ME, Masson M. Role of cyclodextrins in improving oral drug delivery. Am J Drug Del 2004;2:1-15

•• **Excellent review about the benefits of using cyclodextrins for oral drug delivery.**

27. Shimada K, Kawano K, Ishii J, Nakamura T. Structure of inclusion complexes of cyclodextrins with triglyceride at vegetable oil-water interface. J Food Sci 1992;57:655-56
28. Szejkli J. Past, present, and future of cyclodextrin research. Pure Appl Chem 2004;76:1825-45
29. Singh M, Sharma R, Banerjee UC. Biotechnological applications of cyclodextrins. Biotechnol Adv 2002;20:341-59
30. Uekama K, Hirayama F, Irie T. Cyclodextrin drug carrier systems. Chem Rev 1998;98:2045-76
31. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product

development. J Pharm Pharmacol 2010;62:1607-21

32. Prabaharan M, Mano JF. Hydroxypropylchitosan bearing beta-cyclodextrin cavities: synthesis and slow release of its inclusion complex with a model hydrophobic drug. Macromol Biosci 2005;5:965-73

33. Bellanger N, Perly B. NMR investigations of the conformation of new cyclodextrin-based amphiphilic transporters for hydrophobic drugs - Molecular Lollipops. J Mol Struct 1992;273:215-26

34. Bilensoy E, Atilla HA. Recent advances and future directions in amphiphilic cyclodextrin nanoparticles. Exp Op Drug Del 2009;6:1161-73

35. Liu FY, Kildsig DO, Mitra AK. Complexation of 6-acyl-O-beta-cyclodextrin derivatives with steroids - Effects of chain-length and substitution degree. Drug Dev Ind Pharm 1992;18:1599-612

36. Roux M, Perly B, Djedaini-Pilard F. Self-assemblies of amphiphilic cyclodextrins. Eur Biophys J 2007;36:861-7

37. Zhang P, Ling CC, Coleman AW, et al. Formation of amphiphilic cyclodextrins via hydrophobic esterification at the secondary hydroxyl face. Tetrahedron Lett 1991;32:2769-70

38. Duchene D, Ponchel G, Wouessidjewe D. Cyclodextrins in targeting: application to nanoparticles. Adv Drug Del Rev 1999;36:29-40

•• **Excellent review about the capabilities of cyclodextrins both to improve the drug loading of nanoparticles and to be used as materials for the preparation of nanocapsules or nanospheres.**

39. Wenz G. Synthesis and characterization of some lipophilic per(2,6-di-O-alkyl) cyclomalto-oligosaccharides. Carbohyd Res 1991;214:257-65

40. Wouessidjewe D, Skiba M, Leroy-Lechat F, et al. A new concept in drug delivery based on 'skirt-shaped cyclodextrin aggregates' - Present state and future prospects. STP Pharma Sci 1996;6:21-8

41. Fenyvesi F, Fenyvesi E, Szente L, et al. P-glycoprotein inhibition by membrane cholesterol modulation. Eur J Pharm Sci 2008;34:236-42

42. Yunomae K, Arima H, Hirayama F, Uekama K. Involvement of cholesterol in the inhibitory effect of dimethyl-beta-cyclodextrin on P-glycoprotein and MRP2 function in Caco-2 cells. Febs Lett 2003;536:225-31

43. Bardelmeijer HA, van Tellingen O, Schellens JHM, Beijnen JH. The oral route for the administration of cytotoxic drugs: strategies to increase the efficiency and consistency of drug delivery. Invest New Drug 2000;18:231-41

44. Schellens JHM, Malingre MM, Kruijtzer CMF, et al. Modulation of oral bioavailability of anticancer drugs: from mouse to man. Eur J Pharm Sci 2000;12:103-10

45. Garrigues A, Escargueil AE, Orlowski S. The multidrug transporter, P-glycoprotein, actively mediates cholesterol redistribution in the cell membrane. Proc Natl Acad Sci USA 2002;99:10347-52

46. Carrier RL, Miller LA, Ahmed M. The utility of cyclodextrins for enhancing oral bioavailability. J Control Release 2007;123:78-99

47. Arima H, Yunomae K, Hirayama F, Uekama K. Contribution of P-glycoprotein to the enhancing effects of dimethyl-beta-cyclodextrin on oral bioavailability of tacrolimus. J Pharmacol Exp Ther 2001;297:547-55

• **Study about the inhibition of P-gp by β-cyclodextrin derivatives.**

48. van Waterschoot RAB, Rooswinkel RW, Wagenaar E, et al. Intestinal cytochrome P450 3A plays an important role in the regulation of detoxifying systems in the liver. Faseb J 2009;23(1):224-31

49. Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol 1999;39:1-17

50. Lin JH, Lu AYH. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet 1998;35:361-90

51. van Herwaarden AE, Wagenaar E, van der Kruijssen CM, et al. Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. J Clin Invest 2007;117:3583-92

52. Ishikawa M, Yoshii H, Furuta T. Interaction of modified cyclodextrins with cytochrome P-450. Biosci Biotech Biochem 2005;69:246-8

• **Study about the inhibition of intestinal CYP by cyclodextrin.**

53. McCormack B, Gregoriadis G. Drugs-in-cyclodextrins-in-liposomes: an approach to controlling the fate of water insoluble drugs in vivo. Int J Pharm 1998;162:59-69

54. Duchene D, Ponchel G, Wouessidjewe D. Cyclodextrins and carrier systems. J Control Release 1999;62:263-8

55. Becirevic-Lacan M, Salko N. Hydrocortisone/cyclodextrin complex. Complexation methods and complex incorporation in liposomes. STP Pharma Sci 1997;7:343-47

56. Loukas YL, Jayasekera P, Gregoriadis G. Characterization and photoprotection studies of a model gamma-cyclodextrin-included photolabile drug entrapped in liposomes incorporating light absorbers. J Phys Chem 1995;99:11035-40

57. Loftsson T, Masson M. Cyclodextrins in topical drug formulations: theory and practice. Int J Pharm 2001;225:15-30

58. Fatouros DG, Hatzidimitriou K, Antimisiaris SG. Liposomes encapsulating prednisolone and prednisolone-cyclodextrin complexes: comparison of membrane integrity and drug release. Eur J Pharm Sci 2001;13:287-96

59. Lira MCB, Ferraz MS, Silva DGVCd, et al. Inclusion complex of usnic acid with beta-cyclodextrin: characterization and nanoencapsulation into liposomes. Incl Phenom Macrocycl Chem 2009;64:215-24

60. Maestrelli F, Gonzalez-Rodriguez ML, Rabasco AM, Mura P. Preparation and characterisation of liposomes encapsulating ketoprofen-cyclodextrin complexes for transdermal drug delivery. Int J Pharm 2005;298:55-67

61. Gillet A, Grammenos A, Compere P, et al. Development of a new topical system: drug-in-cyclodextrin-in-deformable liposome. Int J Pharm 2009;380:174-80

62. Maestrelli F, Gonzalez-Rodriguez ML, Rabasco AM, et al. New 'drug-in cyclodextrin-in deformable liposomes' formulations to improve the therapeutic

efficacy of local anaesthetics. Int J Pharm 2010;395:222-31

63. Boudad H, Legrand P, Lebas G, et al. Combined hydroxypropyl-beta-cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir. Int J Pharm 2001;218:113-24

64. Mooguee M, Omidi Y, Davaran S. Synthesis and in vitro release of adriamycin from star-shaped poly(lactide-co-glycolide) nano- and microparticles. J Pharm Sci 2010;99:3389-97

65. Wang T, Zhang C, Liang XJ, et al. Hydroxypropyl-beta-cyclodextrin copolymers and their nanoparticles as doxorubicin delivery system. J Pharm Sci 2010;100:1067-79

66. Jerry N, Anitha Y, Sharma CP, Sony P. In vivo absorption studies of insulin from an oral delivery system. Drug Deliv 2001;8:19-23

67. Irie T, Uekama K. Cyclodextrins in peptide and protein delivery. Adv Drug Deliv Rev 1999;36:101-23

68. Sajeesh S, Sharma CP. Cyclodextrin-insulin complex encapsulated polymethacrylic acid based nanoparticles for oral insulin delivery. Int J Pharm 2006;325:147-54

69. Zhang N, Li J, Jiang W, et al. Effective protection and controlled release of insulin by cationic beta-cyclodextrin polymers from alginate/chitosan nanoparticles. Int J Pharm 2010;393:212-18

70. Trapani A, Garcia-Fuentes M, Alonso MJ. Novel drug nanocarriers combining hydrophilic cyclodextrins and chitosan. Nanotechnology 2008;19:185101

• **Excellent review about the benefits associated with the combination of cyclodextrins and chitosan.**

71. Trapani A, Lopedota A, Franco M, et al. A comparative study of chitosan and chitosan/cyclodextrin nanoparticles as potential carriers for the oral delivery of small peptides. Eur J Pharm Biopharm 2010;75:26-32

72. Arbos P, Wirth M, Arangoa MA, et al. Gantrez AN as a new polymer for the preparation of ligand-nanoparticle conjugates. J Control Release 2002;83:321-30

73. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 6th edition. Pharmaceutical Press; North Yorkshire, UK; 2009

74. Arbos P, Campanero MA, Arangoa MA, et al. Influence of the surface characteristics of PVM/MA nanoparticles on their bioadhesive properties. J Control Release 2003;89:19-30

75. Ojer P, Salman H, Martins RDC, et al. Spray-drying of poly(anhydride) nanoparticles for drug/antigen delivery. J Drug Del Sci Technol 2010;20:353-59

76. Agueros M, Areses P, Campanero MA, et al. Bioadhesive properties and biodistribution of cyclodextrin-poly (anhydride) nanoparticles. Eur J Pharm Sci 2009;37:231-40

77. Arangoa MA, Campanero MA, Renedo MJ, et al. Gliadin nanoparticles as carriers for the oral administration of lipophilic drugs. Relationships between bioadhesion and pharmacokinetics. Pharm Res 2001;18:1521-7

78. Arbos P, Arangoa MA, Campanero MA, Irache JM. Quantification of the bioadhesive properties of protein-coated PVM/MA nanoparticles. Int J Pharm 2002;242:129-36

79. Areses P, Agueros MT, Quincoces G, et al. Molecular imaging techniques to study the biodistribution of orally administered (99m)Tc-labelled naive and ligand-tagged nanoparticles. Mol Imaging Biol 16 Dec 2010 [Epub ahead of print]

80. Agueros M, Ruiz-Gaton L, Vauthier C, et al. Combined hydroxypropyl-beta-cyclodextrin and poly (anhydride) nanoparticles improve the oral permeability of paclitaxel. Eur J Pharm Sci 2009;38:405-13

• **This paper shows the improvement of the permeability of paclitaxel when loaded as a complex with cyclodextrins in poly(anhydride) nanoparticles.**

81. Jain R, Agarwal S, Majumdar S, et al. Evasion of P-gp mediated cellular efflux and permeability enhancement of HIV-protease inhibitor saquinavir by prodrug modification. Int J Pharm 2005;303:8-19

82. Bravo-Osuna I, Vauthier C, Chacun H, Ponchel G. Specific permeability modulation of intestinal paracellular pathway by chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles. Eur J Pharm Biopharm 2008;69:436-44

83. Rowinsky EK, Donehower RC. Paclitaxel (Taxol). N Engl J Med 1995;332:1004-14

84. Kruijtzer CM, Beijnen JH, Schellens JH. Improvement of oral drug treatment by temporary inhibition of drug transporters and/or cytochrome P450 in the gastrointestinal tract and liver: an overview. Oncologist 2002;7:516-30

85. Mardones P, Andrinolo D, Csendes A, Lagos N. Permeability of human jejunal segments to gonyautoxins measured by the Ussing chamber technique. Toxicon 2004;44:521-8

86. Yang S, Gursoy RN, Lambert G, Benita S. Enhanced oral absorption of paclitaxel in a novel self-microemulsifying drug delivery system with or without concomitant use of P-glycoprotein inhibitors. Pharm Res 2004;21:261-70

87. Liu G, Franssen E, Fitch M, Warner E. Patient preference for oral versus intravenous palliative chemotherapy. J Clin Oncol 1997;24:110-15

88. Pasquier E, Kavallaris M, Andre N. Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 2010;7:455-65

89. Weingart SN, Brown E, Bach PB, et al. NCCN task force report: oral chemotherapy. J Natl Compr Canc Netw 2008;6:S1-14

90. Terwogt JM, Schellens JH, Huinink WW, Beijnen JH. Clinical pharmacology of anticancer agents in relation to formulations and administration routes. Cancer Treat Rev 1999;25:83-101

91. Irshad S. Considerations when choosing oral chemotherapy: identifying and responding to patient need. Eur J Cancer Care 2010;19:5-11

92. Buggins TR, Dickinson PA, Taylor G. The effects of pharmaceutical excipients on drug disposition. Adv Drug Deliv Rev 2007;59:1482-503

93. Lo YL. Relationships between the hydrophilic-lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. J Control Release 2003;90:37-48

94. Batrakova EV, Kabanov AV. Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers

to biological response modifiers. J Control Release 2008;130:98-106

95. Hugger ED, Audus KL, Borchardt RT. Effects of poly(ethylene glycol) on efflux transporter activity in Caco-2 cell monolayers. J Pharm Sci 2002;91:1980-90

Affiliation

Maite Agüeros[1], Socorro Espuelas[1], Irene Esparza[1], Patricia Calleja[1], Ivan Peñuelas[2], Gilles Ponchel[3] & Juan M Irache[†4]
[†]Author for correspondence
[1]University of Navarra, School of Pharmacy, Department of Pharmaceutics and Pharmaceutical Technology, Irunlarrea 1, 31080 Pamplona, Spain
[2]Radiopharmacy Unit, Clinica Universidad de Navarra, 31080 Pamplona, Spain
[3]University of Paris South, CNRS UMR 8612, 92296 Chatenay-Malabry, France
[4]Professor, University of Navarra, School of Pharmacy, Department of Pharmaceutics and Pharmaceutical Technology, Irunlarrea 1, 31080 Pamplona, Spain
Tel: +34 948425600; Fax: +34 948425649; E-mail: jmirache@unav.es