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Review article

Polymeric micelles for oral drug delivery

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ABSTRACT

In the case of chronic therapies, the oral route is often the preferred route for drug administration given its acceptability and convenience. However, various factors which limit drug absorption through the gastro-intestinal (GI) mucosa contribute to restricting the bioavailability of the drug, that is, the actual amount which reaches the bloodstream. Among these factors, poor drug permeability through the GI mucosa and/or low aqueous solubility are of central importance. Polymeric micelles, which form upon self-assembly of amphiphilic macromolecules, can act as vehicles for the oral delivery of these drugs. This manuscript summarizes the literature in relation to the design of these micellar systems and their characterization with respect to drug loading and retention properties as well as the ability to withstand dissociation and drug discharge upon oral administration. Also, the role of certain polymers in improving drug absorption through the GI mucosa, either by increasing membrane permeability to the drug and/or carrier or by inhibiting drug efflux transporters in the GI mucosa, is discussed. Finally, this review reports other drug delivery strategies such as using bioadhesive polymers which may lengthen residence time in the GI tract and promote drug permeation, or rendering the polymeric micelles pH-sensitive in order to ensure drug release from the carrier at its site of absorption.

1. The gastro-intestinal tract

The oral route presents several advantages that make it the preferred route of drug administration. From the patient's viewpoint, it allows for painless self-medication and is thus considered the most convenient, especially for chronic therapy. However, formulating a therapeutic agent for oral administration remains an intricate process. Many variables must be taken into consideration as they may affect drug bioavailability, that is to say the extent of absorption. For a drug administered orally, bioavailability represents the fraction of the dose that is absorbed intact and is able to avoid intestinal or hepatic metabolism. For many molecules, the actual portion of a dose that reaches the general circulation remains low as various factors contribute to limit their absorption. These barriers may be either physiological or related to the intrinsic properties of the drug.

The gastro-intestinal (GI) tract comprises an ensemble of organs and glands that work together to extract nutrients from ingested food. The pH in the GI tract varies greatly according to location, with a pH of 1.2 in the empty stomach, 5–7 in the small intestine and 6–7.5 in the colon [1]. Although part of the digestion process occurs in the oral cavity and stomach due to the presence of salivary amylase and gastric protease (pepsin), the small intestine remains the major site for both digestion and absorption [2]. In the small intestine, three structures are responsible for the significant surface area available for absorption. First, the circular folds and villi increase the absorption surface by 3- and 30-fold, respectively. The villi are lined with an epithelium composed of enterocytes which represent the major intestinal cell type [1]. The luminal side of each enterocyte is covered with small (1 μm) protrusions, or microvilli, which further increase (~600-fold) the surface available for digestion and absorption. The microvilli along with the associated glycocalyx (a mixture of mucus, proteins and cholesterol anchored to the membrane) compose what is generally referred to as the brush border. The latter constitutes a major physical and enzymatic barrier to drug absorption.

Aside from its role in the digestion process, the intestine is also involved in the protection against pathogen invasion. As such, its epithelium contains more lymphoid cells and produces more antibodies than any other organ in the body [1]. The immunological function in the intestine is ensured by the presence of the gut-associated lymphoid tissue which comprises intra-epithelial lymphocytes intercalated between adjacent epithelial cells as well as

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organized structures of lymphoid tissue, namely Peyer's patches. The epithelial cells covering the patches may differentiate into specialized M cells that are particularly suited for the transport of particles, antigens or macromolecules [1]. These cells lack the brush border and theoretically constitute a more favorable site for drug or particle internalization. However, M cells represent less than 1% of the intestinal epithelial cells and as such do not constitute a major route of absorption.

2. Drug absorption

While there exists many specific pathways for nutrient absorption, the absorption of orally administered drugs is restricted to either transport through the cell (transcellular pathway, Fig. 1A) or between adjacent cells (paracellular pathway, Fig. 1E). Drugs absorbed by the transcellular pathway are generally low-molecular weight hydrophobic entities which are able to diffuse through the membrane, either on their own or associated with a specific membrane transporter (Fig. 1). In both instances, the rate is determined by the concentration gradient across the intestinal membrane, with the blood acting as a sink [3]. 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 (Fig. 1F). However, once inside the cell, a fraction of these vesicles may fuse with enzyme-rich lysosomes leading to the degradation of the drug. Alternatively, the 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 the degradation of the absorbed substances [1].

Typically, hydrophilic molecules cannot freely diffuse through the intestinal membrane, due to their low affinity for the lipidic constituents [4]. Therefore, in the absence of an appropriate membrane transporter, the paracellular route is the only one available for their absorption (Fig. 1E). In the paracellular space, the presence of junctions restricts the passage of large molecules. These include the tight junction, a protein complex that establishes links between adjacent cells through the intercellular space [3]. Two other protein complexes, namely the adherens junctions and desmosomes further tighten cell association, without fusing cell membranes. Independently of the pathway, it is possible to improve drug absorption by using permeation enhancers. These are generally amphiphilic molecules that are able to increase the fluidity of the membrane or loosen tight junctions. Also, chelating agents and ionic polymers such as chitosan and poly(acrylic acid)s (PAAs) have been shown to act as permeation enhancers. Although quite efficacious, all these agents could be associated with an increased risk of toxicity by permitting the entry of unwanted pathogens through a leakier epithelium. As a consequence, the use of alternate avenues that indirectly improve drug absorption is generally preferred.

3. Physico-chemical issues

The bioavailability of a drug strongly depends on its nature and physico-chemical properties. For instance, proteins and poly(nucleic acids) administered orally are susceptible to both inactivation in the acidic environment of the stomach and degradation by digestive enzymes. Furthermore, their highly hydrophilic character as well as their large size will prevent their diffusion through the intestinal membrane. This in turn contributes to restricting their absorption to the paracellular pathway, which represents less than 1% of the total surface available for absorption [1].

In contrast, for a drug absorbed through the epithelium, the bioavailability is mainly dictated by the aqueous solubility of the drug and its permeability across the cellular membrane. This has been aptly summarized in the biopharmaceutical classification systems (BCS) (Table 1). From this system, it can be seen that, except for class I drugs, all pharmaceuticals will be faced with bioavailability issues due to their poor dissolution rate, their poor permeability or both.

Several strategies have been proposed to overcome these limitations including the reduction of drug particle size [5], salt formation [6] or prodrug synthesis [7]. Another interesting approach relies on the encapsulation of the drug inside nanosized carriers such as polymerized liposomes [8,9] and nanoparticles [10–13].

Table 1
BCS drug classification.

	High permeability	Poor permeability
High solubility	Class I	Class III
Poor solubility	Class II	Class IV

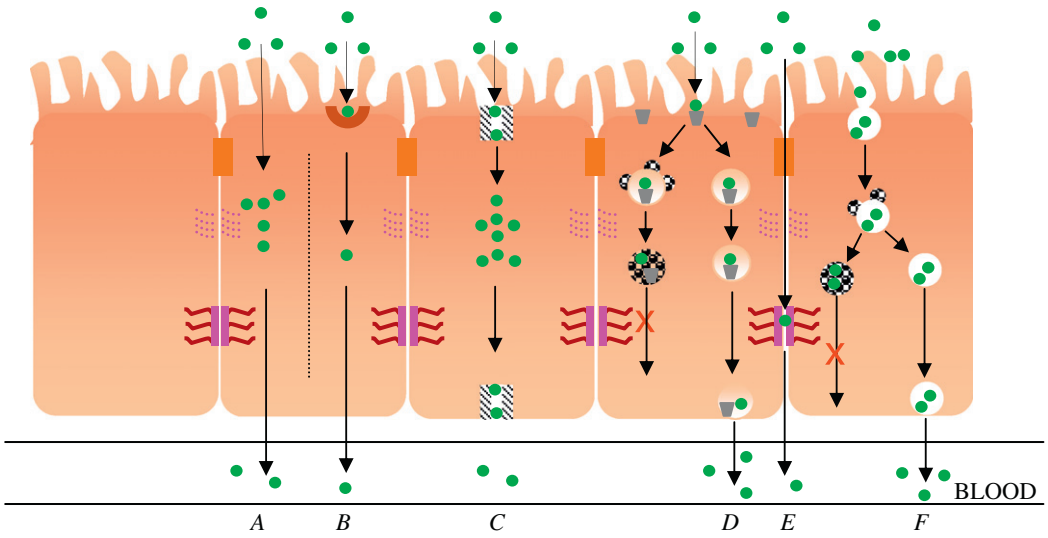

Fig. 1. Schematic representation of the mechanisms involved in the absorption of xenobiotics in the small intestine. (A) Transcellular transport; (B) active transport; (C) facilitated diffusion; (D) receptor-mediated endocytosis; (E) paracellular transport; (F) pinocytosis.

Over the past decade, polymeric micelles (PMs) have been investigated as potential delivery systems for such therapeutic compounds [14–17]. These vectors, as classically defined, possess a hydrophobic core which acts as a reservoir for lipophilic molecules, surrounded by a hydrophilic corona exposed to the aqueous environment. The corona confers aqueous solubility and steric stability to the ensemble. Depending on the physico-chemical properties of the drug as well as those of the polymer chains forming the micellar structure, the core of PMs is capable of solubilizing considerable amounts of guest molecules which otherwise would precipitate in the aqueous fluids of the GI tract. This review will focus mainly on the application of PMs for the oral delivery of class II therapeutic compounds. Furthermore, only micelles based on macromolecules of molecular weight greater than 1000 g/mol which have been developed for the oral route will be discussed herein.

4. Improvement of drug solubility

As previously mentioned, class II therapeutic agents are characterized by poor aqueous solubility and high permeability. In order for these compounds to be absorbed through the GI mucosa and reach the blood circulation at concentrations above the therapeutic threshold, they must first be fully dissolved. However, the time required for the drug molecules to dissolve may surpass the transit time in the GI tract to the intended site of absorption. Hence, the dissolution step becomes the rate-limiting step which determines the overall oral bioavailability of the drug. Herewith lies a major challenge for the successful delivery of highly efficacious yet poorly water-soluble therapeutics *via* the oral route [18].

Various polymeric micellar systems have been investigated as a means to improve the oral delivery of hydrophobic compounds (Table 2). The group of Winnik studied the solubilization of cyclosporin A (CyA), a poorly water-soluble immunosuppressant (solubility in water at 20 °C of 23 μg/mL), by PMs [19–22]. They reported a net increase in the aqueous solubility of the drug once incorporated into hydrophobically modified polysaccharide-based micelles. Here, the core of the structure consisted of long alkyl chains (C16 or C18) grafted onto a hydrated dextran or hydroxypropylcellulose (HPC) backbone which formed the micelle corona (dextran-*g*-PEO-C_n and HPC-*g*-PEO-C_n). Drug-loading levels for CyA in such vehicles were found to range from 5.5% to 8.5% (w/w), with a direct correlation between the alkyl chain grafting density and final drug loading. Also, for the same grafting density, hexadecyl (C16) chains offered greater solubilization capacity than octadecyl (C18) chains, indicating that the micropolarity of the core played an important role in its ability to host a hydrophobic cargo.

Elsewhere, paclitaxel, an anticancer agent with poor water solubility (<1 μg/mL), was loaded into 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy(poly(ethylene glycol)/D-α-tocopheryl polyethylene glycol succinate (PEG-DSPE/TPGS, 1:1 mol/mol) micelles [23]. TPGS is basically a water-soluble vitamin E-PEG conjugate. When added to PEG-DSPE, TPGS brought about an increase in the drug loading capacity from 1.2% to 3.5% (w/w), indicating that the nature of the core components can directly impact cargo incorporation. Overall, the aqueous solubility of paclitaxel was enhanced up to 5000 times (~5 mg/mL) upon its incorporation into the hydrophobic core of these mixed micelles. However, perhaps the most impressive example of solubility improvement by PMs was that reported by Park and coworkers. Nicotinamide derivatives, *i.e. N,N*-diethylnicotinamide and *N*-picolylnicotinamide, were shown to be powerful hydrotropes for paclitaxel [31]. Copolymers featuring a segment containing a nicotinamide derivative could yield PMs exhibiting hydrotropic properties toward paclitaxel. As such, it was reported that micelles composed of PEG-*b*-poly(vinylbenzyloxy)-*N,N*-diethylnicotinamide) (PEG-*b*-PVBODENA) could accept up to 37.4% (w/w) paclitaxel [24], thereby raising the aqueous solubility of the drug to 38.9 mg/mL. The loading capacity increased proportionally with the length of the hydrotropic DENA segment. In comparison, plain

Table 2
PMs employed for the oral delivery of hydrophobic compounds.

Polymer	CMC (mg/L)	Incorporated drug (% w/w)	Reference
PEG-DSPE/TPGS[a]	45.9–62.6	Paclitaxel [1.2 without TPGS] [3.5 with TPGS]	[23]
PEG-*b*-P(VBODENA)[b]	36–70	Paclitaxel [18.4–37.4]	[24]
PEG-*b*-PLA	70–90	Griseofulvin [4–6.5]	[25]
PEG-*b*-P(CL-*co*-TMC)[c]	30	Risperidone [<3.8] Ketoconazole [<2.2] Indomethacine [<4] Hydrocortisone [<3.9]	[26]
PEG/MOG/SA[d]	340–1000	Risperidone [13.3] Ketoconazole [1.7] Indomethacine [9.5] Hydrocortisone [3.8] Cyclosporin [1.4]	[27]
PEG-*b*-PLA/PLA-COOH	NA	Itraconazole [>8]	[28]
PEO-*b*-PPO-*b*-PEO	NA	Genistein [11.2]	[29]
HPC-*g*-PEO-C_n C_n: C16 or C18	15–135	Cyclosporin A [1.7–6.7]	[22]
(1) PEO-*b*-PPO-*b*-PEO (2) PEG-DSPE	(1) 1.6×10^{-4} to 6.9×10^{-5} (2) 1.0×10^{-5} to 1.4×10^{-5}	Camptothecin [<0.065] Meso-tetraphenyl porphine [<8.7] Octaethylporphine [<2]	[30]

NA: non-applicable.
[a] PEG-DSPE/TPGS: 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy(poly(ethylene glycol)/D-α-tocopheryl polyethylene glycol succinate (PEG-DSPE/TPGS, 1:1 mol/mol).
[b] PEG-*b*-P(VBODENA): PEG-*b*-poly(vinylbenzyloxy)-*N,N*-diethylnicotinamide).
[c] PEG-*b*-P(CL-*co*-TMC): PEG-*b*-poly(caprolactone-*co*-trimethylcarbonate).
[d] PEG/MOG/SA: random copolymers composed of PEG, monooleylglyceride and succinic acid.

PEG-*b*-poly(D,L-lactide) (PEG-*b*-PLA) micelles could incorporate a maximum of 27.6% (w/w) of the same drug. With such high drug-loading levels in the PMs, the oral administration of a therapeutic drug dose becomes readily achievable. In other instances, however, loading levels may be significantly lower. Indeed, griseofulvin, a poorly water-soluble antifungal agent, was incorporated into PEG-*b*-PLA micelles at levels of approximately 0.45–0.65% (w/w) [25]. As highlighted by the authors, prohibitively large amounts of copolymer would need to be administered to achieve a daily therapeutic dosage (330–375 mg for adults) *via* the oral route. Polymer–drug compatibility is an important element which can impact drug-loading levels into PMs. Indeed, the group of Allen [32] highlighted the connection existing between polymer-drug compatibility, inferred by their respective solubility parameter, and the amount of drug solubilized in different PMs.

5. Micelle preparation

Many approaches have been investigated for micelle preparation. Two main strategies may be envisaged to form adequately sized drug-loaded PMs [16]. The first one, direct dissolution, involves dissolving both the polymer and drug in an aqueous solvent. This method is preferred when the polymeric materials are only moderately hydrophobic in nature, as in the case of several poloxamers (poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide), PEO-*b*-PPO-*b*-PEO) currently investigated [33]. The second strategy, implying the use of organic solvents, is relevant when both the copolymer and the drug are not readily soluble in water. In this case, the copolymer and the drug are first dissolved in a common solvent which is then removed by one of several methods. If the solvent is water-miscible, dialysis may be employed to slowly replace the organic solvent by an aqueous one, thereby triggering micellization. According to literature, dialysis against water is the most widespread method for the preparation of drug-loaded PMs studied for the oral route, although it may not be readily transposable to the industrial scale [19,25,34]. When the solvent is not miscible with water, drug entrapment may be achieved through an oil-in-water emulsion method [35]. Alternatively, a solution casting method has also been reported for micelle formation. Here, both polymer and drug are dissolved in an organic solvent which is then evaporated, yielding a polymeric film in which the drug molecules are uniformly dispersed. Upon hydration of the film with a heated aqueous solvent, micelle formation occurs spontaneously. For example, paclitaxel was loaded into PEG-DSPE/TPGS mixed micelles by this method [23]. Different amounts of paclitaxel and PEG-DSPE/TPGS mixtures were first dissolved in chloroform. The latter was evaporated to form a thin film which was then hydrated with a pH 7.4 buffer. The mixture was incubated at 50 °C for 20 min to induce micelle formation.

For reasons of practicality and convenience, dry dosage forms are generally preferred for drug administration *via* the oral route. They also confer extended shelf-life to the product. Such formulations could be obtained by spray drying drug-loaded micelles or physically mixing the polymeric surfactant with the drug. As such, sustained-release tablets obtained by direct compression of poloxamer covalently conjugated to PAA were investigated for the oral delivery of various drugs (see Section 7.6), including class II nitrofurantoin [36]. Upon disintegration in the GI tract, the polymeric matrices are expected to generate a gel layer from which drug-loaded micellar aggregates can form and be released in the GI media.

6. Micelle stability

To ensure delivery of the carried drug to its site of absorption, the micellar carrier must be able to resist rapid dissociation upon dilution and exposure to the harsh conditions of the GI tract. The critical micelle concentration (CMC) refers to the concentration of copolymer above which micelle formation is favored. Typically, PMs exhibit significantly lower CMCs compared to their counterparts derived from low-molecular weight surfactants, thus offering greater resistance toward dissociation upon dilution. Taking into account the fluid volumes of the GI tract (stomach and small intestine) in the fed (740 mL) and fasted (130 mL) states [37], polymer concentrations may vary anywhere from 135 to 7700 mg/L following the oral administration of a micelle dose of 100–1000 mg. Thus, as an indicative guide, a CMC of less than 135 mg/L should be sufficient to resist rapid dissociation upon oral administration. Lower CMC values denote greater stability and are generally conferred by the presence of highly hydrophobic domains in the micelle core [38]. Therefore, at a constant corona-forming chain length, an increase in the core-forming chain length should bring about a decrease in CMC value. Indeed, higher PEO-C16 or C18 grafting densities yielded an increase in the CMC of dex-*g*-PEO-C_n or HPC-*g*-PEO-C_n micelles [22,38]. Lee et al. [24] found that, given its hydrophilic nature, DENA in the core-forming segment of the PEG-*b*-P(VBODENA) copolymer raised the CMC value compared to a control PEG-*b*-PLA micelle formulation which possessed a more hydrophobic core. Conversely, but to a lesser extent, when the core-forming segments are kept constant, increasing the length of the hydrophilic chains of the corona will lead to an increase in the value of the CMC. For example, when the PEG content exceeded 70 mol%, PEG-*b*-PLA micelles exhibited poor stability, given that the attractive forces between the relatively short PLA chains could not balance out the repulsive forces existing between the large PEG chains in the outer shell of the micelles [25]. Yet, when the PEG content was below this threshold, the micelles showed proper stability with CMC values ranging from 0.07 to 0.09 mg/mL.

Following oral administration, micelles are exposed to pH variations, bile salts and digestive enzymes in the GI tract fluids. Investigating drug release from the micelles upon exposure to both simulated gastric and intestinal fluids (SGF and SIF) may help predict their behavior *in vivo*. Francis et al. [20] studied the release of CyA from dex-*g*-PEO-C_n and HPC-*g*-PEO-C_n micelles in SGF (pH 1.2) and SIF (pH 6.8). In both cases, the amount of drug released from the micelles reached a plateau of <12% of the loaded CyA within ~4 h, indicating that the micelles maintained their integrity and drug-retention properties throughout the experiment. In this work and as in most other *in vitro* studies carried out with PMs, the SIF did not contain the digestive enzymes nor the bile salts normally encountered in the lower part of the GI tract. Moreover, many of these release studies should be interpreted with caution since they were conducted using the dialysis method, *e.g.* a synthetic membrane separates the drug-loaded micelles from the free drug molecules, and true sink conditions are rarely ensured. Thus, the *in vitro* results obtained with respect to drug-release kinetics and micelle stability may not accurately reflect the actual amount of drug which would eventually be released under *in vivo* conditions. Elsewhere, the release of griseofulvin from PEG-*b*-PLA micelles was investigated in phosphate-buffered saline (PBS) (pH 7.4) as well as in SGF (pH 1.2) and SIF (pH 7.5) [25]. In addition, the specific viscosity of the copolymer was monitored in parallel as a means to follow its degradation upon incubation. All three drug-release profiles were similar, with less than 50% of griseofulvin being released within a 6-day period. The PBS incubation was prolonged and revealed a rapid release phase from day 15 to day 30. In all media, slow polymer degradation was recorded during the first 10–15 days of the experiment, followed by rapid polymer degradation. The authors therefore suggested that the release of griseofulvin from the PEG-*b*-PLA micelles was erosion controlled. However, given that polyesters such as PLA are particularly susceptible to degradation in both acidic and basic aqueous media, it is surprising

that the rates of both polymer degradation and drug release were practically identical for all three incubation media investigated. In this case, the fact that the PLA segments were hidden in the micelle core may have protected them against premature hydrolysis. Such slow drug release from the micelles, *e.g.* less than 50% released in 6 days, may not be adequate for oral delivery purposes. Again, no bile salts or enzymes were added to the SIF employed here. Yet, it has been suggested that esterases contained in pancreatin-rich SIF were mainly responsible for the rapid degradation of PLA nanoparticles [39]. Another group investigated the stability of PEG-DSPE/TPGS mixed micelles in SGF and SIF, this time in the presence and absence of bile salts [23]. Overall, the data indicated that the mixed micelles resisted destabilization over a 12-h period as evidenced by a stable paclitaxel content and size distribution. In the presence of bile salts, however, a decrease in micelle size was detected. This partial destabilization was to be expected since bile salts, acting as surfactants for fatty foods in the gut, may have brought about the formation of smaller mixed micelles. Adequate drug retention and micelle stability are without a doubt prerequisites to the drug reaching its absorption site in the GI tract in the solubilized state. However, in most cases, drug release from the micelles is in fact needed for its absorption through the GI mucosa.

7. Interaction of micelles with the intestinal mucosa

7.1. In vitro assessment of membrane permeability

Different methods can be employed to study the interaction of a carrier with the intestinal membrane. While the use of artificial liposomal membranes [40] or even isolated gut sacs [41] has been proposed, Caco-2 cellular monolayers remain the gold standard. PMs are not known to interact extensively with cellular membranes, probably due to the steric hindrance provided by the hydrated shell. As a consequence, indicators of paracellular permeability such as transepithelial electrical resistance (TEER) are not normally altered in the presence of PMs [20,42,43]. Sezgin et al. [41] have reported a decrease in TEER when Caco-2 cells were incubated 4 h with meso-tetraphenyl porphine (mTPP)-loaded PEG-DSPE but not with poloxamer PMs. The authors attributed this occurrence to an opening of the tight junctions as neither polymer was shown to be cytotoxic. Nevertheless, the permeability of the encapsulated drug was not improved, confirming the limited contribution of paracellular transport to the absorption of poorly water-soluble drugs.

In most cases, *in vitro* studies involving PMs are performed to assess the effect of micelle solubilization on drug permeability. However, the conditions which bring about micelle dissociation and drug release upon dilution *in vivo* cannot be readily simulated *in vitro*, leading to a biased interpretation of drug permeability. In theory, it is expected that the encapsulation of a class II drug would result in improved drug absorption. However, cell studies have yielded contradictory data. For example, the loading of CyA in either hydrophobically modified dextran or HPC micelles increased its permeability by 1.5- and 3-fold, respectively [20]. In contrast, the loading of risperidone in PEG-*b*-P(CL-*co*-TMC) micelles did not improve its permeability [26]. This could be attributed to the reduced thermodynamic activity of the drug in solution given its micellar solubilization and retention within the carrier.

7.2. Micelle absorption

In its normal state, the intestinal membrane is relatively impermeable to particles in the nanometer size range. However, it has been shown that drug carriers can be absorbed intact either by the enterocytes or the M cells through an endocytotic pathway [44]. In the absence of a targeting moiety, the internalization of a carrier can be triggered by non-specific interactions (hydrogen bonding, hydrophobic or van der Waal interactions) between the carrier and the intestinal cells [44]. Alternatively, the carrier can be absorbed by fluid-phase pinocytosis which also results in the invagination of the membrane and vesicle formation. Both processes are energy dependent and saturable (Fig. 1).

The extent and rate of particle absorption are mainly dictated by the size and surface properties of the carrier [45]. In the case of PMs, while their size should favor their diffusion through the mucus layer, the hydrophilicity of the shell may limit their interaction with the intestinal membrane [46]. Nonetheless, studies performed on various cell models have shown that PMs could be internalized, with fluid-phase pinocytosis appearing as a major route [47,48]. A similar process could be responsible for the absorption of PMs across the intestinal epithelium. Studies performed by Mathot et al. [49] provided some insight as to the fate of PMs. Following oral administration of radiolabeled PMs to rats, radioactivity was detected in the blood, spleen, liver and kidneys. The authors calculated an oral bioavailability of 40% for PMs which is much larger than the 0.01–5% typically reported for large particles (Fig. 2). In this case, this value could be attributed to the presence of long-circulating unimers or polymeric degradation products rather than intact micelles. Indeed, it has been demonstrated that aliphatic polyesters will undergo substantial gastro-intestinal degradation following oral administration [50]. In a subsequent study, the authors confirmed that intact micelles could be absorbed through an energy-dependent process, most likely fluid-phase pinocytosis [51]. However, a large proportion of unimers also passed through the cell monolayer. The exact mechanism governing micelle permeation could not be determined with certainty since the addition of endocytosis inhibitors as well as varying the temperature yielded contradictory results. Despite the relatively high bioavailability of PEG-*b*-P(CL-*co*-TMC) [49], the extent of absorption of risperidone was not altered [52], demonstrating that micelle absorption is not a predominant mechanism to improve drug availability. A recent study by Van Hasselt et al. [53] suggests that the gastro-intestinal absorption of highly lipophilic compounds from polymeric micelles is mediated by free bile and that the uptake of intact micelles through pinocytosis is in fact a minor route of drug absorption. Indeed, in rats which have undergone bile duct ligation, vitamin K

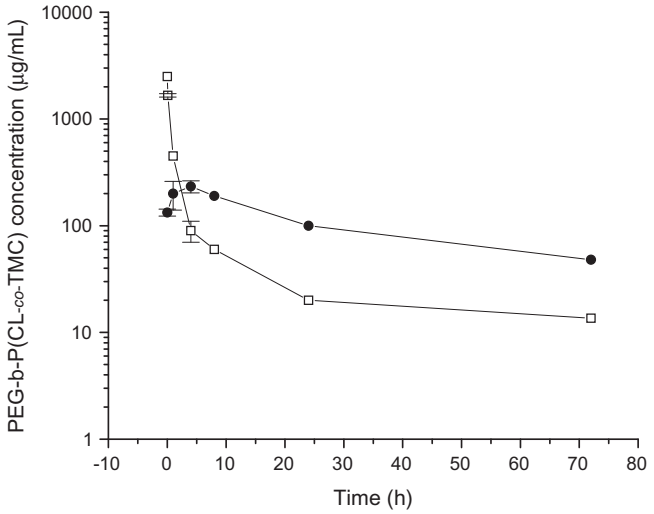

Fig. 2. Plasma profiles of total PEG-*b*-P(CL-*co*-TMC)-related radioactivity after a single oral (circles) or intravenous (squares) administration of 3 mg/kg risperidone solubilized in 10% [^{14}C]-PEG-*b*-(CL-*co*-TMC) solution in male rats (mean ± SD, n = 3). Adapted from Ref. [49].

bioavailability was restored only when the vitamin K-loaded polymeric micelles were administered in the duodenum together with bile acids.

7.3. Receptor-mediated uptake

In order to improve the oral absorption of an encapsulated drug, receptor-mediated endocytosis may prove to be beneficial [54,55]. Ligand-mediated targeting has been widely studied in parenteral drug delivery as a means to improve cellular uptake as well as target specificity. Indeed, colloidal vectors such as nanoparticles and micelles have been fitted with various surface-bound probes including monoclonal antibodies, carbohydrates, folate and transferrin [56–58]. Conversely, research in the field of targeted oral drug delivery is sparse. Yet, a number of ligands, including bacterial toxins, immunoglobulins and vitamin B12 (VB12), have been investigated with respect to their role in enhancing the receptor-mediated endocytosis of various therapeutic entities [55,59]. The role of receptor-enhanced endocytosis in the permeability of Caco-2 cell membranes to CyA was investigated by Francis et al. [19]. Here, dextran-*g*-PEO-C16 micelles were decorated with VB12 moieties. When VB12 is absorbed, it conjugates to the intrinsic factor (IF), a protein produced in the stomach. This VB12-IF complex then binds to IF receptors on the surface of the enterocytes in the small intestine and is transported through the mucosa *via* receptor-mediated endocytosis. Results showed that the apical to basolateral transport of CyA was twice as high when incorporated into VB12-decorated micelles in comparison with naked micelles. Indeed, the permeation coefficient of the drug transported by VB12-tagged micelles was 3.3 vs. 1.4 cm/s for bare micelles.

7.4. Peyer's patches pathway

As mentioned previously, Peyer's patches are lymphoid follicles made up of highly phagocytic cells called M cells. These cells are believed to actively sample the GI milieu of the small intestine. The internalized material is then processed and excreted on the baso-lateral side of the cells, resulting in a non-specific immune response. Although many authors have investigated the intestinal uptake of nanoparticles through Peyer's patches, their efforts yielded mixed results [55,60]. This is in part explained by the fact that the accessibility of the M cells is restricted by their location at the base of the intestinal villi as well as the presence of glycocalyx at the surface of the intestinal epithelium. To the best of our knowledge, this pathway has yet to be investigated with respect to the oral uptake of PMs.

7.5. Inhibition of efflux transporters

The presence of efflux transporters was first evidenced in cancerous cells, where they are associated with the development of multidrug resistance. Their role is essentially to prevent the accumulation of exogenous drugs considered to be toxic [61]. Of the different efflux transporters, the P-glycoprotein (Pgp) remains the most widely studied. Pgp is a 170-kDa membrane transporter which is part of the ATP-binding cassette [62] and as such its activity requires ATP as an energy source. Due to its ability to expel therapeutics, the presence of intestinal Pgp is associated with a decrease in oral bioavailability. Therefore, modulation of its activity is seen as a potential means to improve drug bioavailability.

The first Pgp inhibitors proposed were themselves substrates that could bind to the protein and inhibit its activity. Several drugs, including CyA and verapamil, have been studied for this purpose [63]. However, these molecules may be associated with toxic side effects, driving the search for safer modulators. Amphiphilic structures were presented as a potential alternative [64]. Different hypotheses have been proposed to rationalize the effect of such molecules on Pgp activity. Mostly, the inhibition of efflux transport appears to be related to a modification of the fluidity of the cellular membrane [65]. This inhibitory effect has been demonstrated with both low-molecular weight and polymeric surfactants, among which TPGS and poloxamers have been the most extensively studied.

The effect of TPGS on the bioavailability of a Pgp substrate was first reported with CyA. It was initially postulated that the improvement in oral availability was due solely to micelle formation and increased drug solubility [66,67]. Subsequently, Chang et al. [68] demonstrated an increased CyA absorption at TPGS concentrations below the CMC. Since CyA is a known Pgp substrate, the authors hinted at a possible mechanism implicating the efflux transporter, a premise which was later confirmed [69,70]. Contrary to other surfactants, TPGS seems to have only a minor effect on membrane fluidity [71], challenging earlier reports [65]. Indeed, it was speculated that the inhibition of Pgp resulted from a decrease in ATPase activity following substrate binding [71]. Further *in vitro* studies using Caco-2 cells aimed at investigating the mechanisms involved in TPGS Pgp inhibition [72]. The data suggest that TPGS does not act as Pgp substrate or competitive inhibitor of Pgp substrate efflux nor does it trigger intracellular ATP depletion. Instead, TPGS is thought to function as an allosteric modulator not involving the Cis(Z)-flupentixol binding site.

In contrast, the interaction of poloxamers with intestinal Pgp has been thoroughly examined mostly though the work of Batrakova and coworkers [73–75]. In this case, most of the knowledge on their inhibitory effect has been acquired through sensitization studies on cancerous cells [76–78]. These studies led to the development of a doxorubicin–poloxamer formulation targeted toward multidrug-resistant tumors [79]. These findings were applied to the oral route, where poloxamers are expected to show a similar behavior on intestinal Pgp. A combination of decreased ATPase activity and ATP depletion due to increased membrane fluidity is thought to be responsible for Pgp inhibition [75]. This effect is maximal at concentrations just below the CMC, where unimer concentration is the most important (Fig. 3). Usually, drug permeability increases consistently with polymer concentration until a maximum is reached near the CMC. If the polymer concentration is further augmented, the apparent permeability significantly decreases [73,76]. The latter observation can be ascribed to an

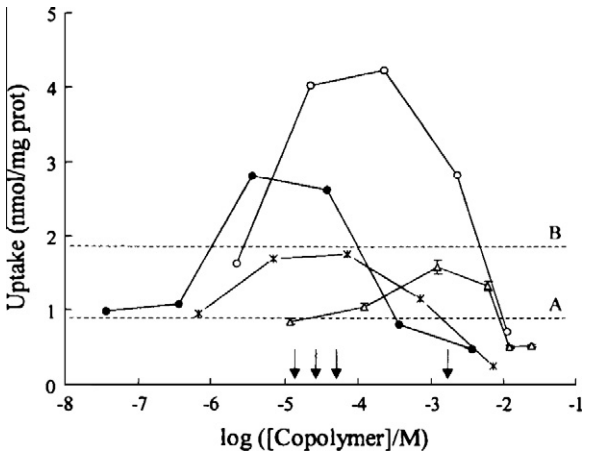

Fig. 3. Effect of poloxamer 231 (filled circles), 235 (open circles), 188 (triangles) and Cremophor EL (crosses) on R-123 accumulation in Caco-2 monolayers (1 h incubation). The dashed lines represent the accumulation of the probe dissolved in assay buffer without (A) or with CyA (4 μg/mL) (B). From left to right, the arrows indicate the CMCs of poloxamer 231, Cremophor EL, poloxamers 235 and 188. Reproduced from Ref. [73].

increased encapsulation of the drug or the probe in the micelles and thus a decrease in the amount of free drug. For both poloxamers and TPGS, the modulation of Pgp activity was highly influenced by polymer composition. In most instances, polymers with intermediate hydrophobicity were the most effective [43,78]. Recently, a study by Zhu et al. [80] demonstrated that PEG stearate could inhibit the Pgp in Caco-2 cells with greater efficacy at concentrations just below the CMC. Mechanistic studies revealed that its potential as Pgp inhibitor was not linked to modulation of intracellular ATP level, basal Pgp ATPase activity, or changes in membrane fluidity. Instead, PEG stearate unimers are thought to interact with activated Pgp, thereby reducing its substrate-stimulated ATPase activity and finally inhibiting the efflux of Pgp substrates.

Zastre et al. [81] performed a comprehensive study of the inhibition of Pgp by PEG-*b*-poly(ε-caprolactone) (PEG-*b*-PCL) micelles of varying compositions. They studied the effect of altering either the hydrophobic (2–10 units) or hydrophilic (17 or 45 units) block length. In all instances, the diblock copolymers were at least as efficient as the controls, verapamil (50 μM) or CyA (4 μM), in inhibiting Pgp activity. The most noticeable effect was observed when PEG_{17} was coupled to either PCL_2 or PCL_5. Both polymers doubled the intracellular accumulation of rhodamine-123 (R-123) compared to the controls. Furthermore, it was found that, contrary to other polymeric structures, PEG-*b*-PCL produced a maximal inhibitory effect at concentrations 8–100 times over their CMC [81]. At this point, less than 20% of the dye was encapsulated inside PMs. Increasing the polymer concentration over 0.5% and 2.5% (w/w) for PEG_{17}-*b*-PCL_2 or PEG_{17}-*b*-PCL_5, respectively, caused a drop in the accumulation of R-123 probably as a result of the increased partitioning of the dye in the micellar phase. The inhibitory effect was found to be due in part to an increase in the rigidity of the membrane, following PEG-*b*-PCL unimer insertion [42,82]. This manifestation has previously been linked to the inhibition of Pgp by some surfactants [82] and high concentrations of low-molecular weight PEG [83,84].

To this day, what makes a polymer an efficient inhibitor is still unclear. Although it seems that an interaction with the membrane is necessary, some polymers showed no effect on Pgp despite being able to alter the fluidity of the cellular membrane [51]. Until extensive structure–activity studies are performed, the selection of a suitable polymeric inhibitor will remain empirical.

7.6. Bioadhesive PM

Bioadhesive polymers have found applications in various pharmaceutical formulations as a means to enhance drug absorption and/or provide sustained drug levels. By achieving an intimate contact with the mucosa, they reduce pre-systemic drug metabolism, prolong the residence time at the site of drug action or absorption and provide a steep concentration gradient at the absorption membrane. Synthetic bioadhesive polymers currently investigated in pharmaceutical formulations include, among others, PEG, cellulose derivatives such as methylcellulose and HPC, and polyelectrolytes such as PAA and chitosan [85]. These polymers bind to the mucus *via* non-covalent bonds such as hydrogen bonding, electrostatic interactions and van der Waals forces. Mucus interpenetration and chain entanglement may also contribute to the phenomenon of mucoadhesion, particularly with regard to uncharged polymers. For example, it has been shown that PEG-DSPE and poloxamer micelles display some degree of bioadhesion *ex vivo* in the everted rat intestine [41]. However, the significance of this finding in terms of improvement of drug absorption remains to be established. Recently, Chayed and Winnik [86] examined the binding of PMs prepared from hydrophobically modified dextran and HPC to a bovine submaxillary gland mucin layer by impedance quartz crystal microbalance and surface plasmon resonance measurements. They found that grafting of alkyl chains (C16) to HPC and dextran enhanced polymer adsorption on the mucin layer. The thickness of the polymer layer was significantly less than the hydrodynamic radius of the corresponding PMs in solution, indicating a reorganization of the micelles as they came into contact with the mucin. In the case of dextran, it was suggested that hydrophobic interactions in the PMs played a major role in the adsorption process since the unmodified polysaccharide was devoid of bioadhesive properties. Another mucoadhesive system, which has been developed by Bromberg and coworkers, is based on a family of poloxamer-PAA graft polymers [36] (Fig. 4). Depending on concentration and temperature, these polymers form pH-responsive micellar networks [87]. These networks could be chemically cross-linked with degradable or non-degradable cross-linkers to yield microgels exhibiting a pH-dependent swelling behavior [88,89]. Interestingly, cross-linked gels prepared from poloxamer-PAAs demonstrated mucoadhesive properties that could exceed those of PAA gels [90]. Moreover, as in the case of poloxamers, poloxamer–PAA copolymers inhibited the Pgp *in vitro*, allowing increased permeation of doxorubicin in the Caco-2 cell model [91]. In rats, the oral administration of megestrol acetate formulated in poloxamer L92-PAA microgels significantly increased its absorption [92]. Recently, Bromberg reported a 10-fold enhancement in the oral bioavailability of paclitaxel solubilized in Pluronic–PAA micelles upon coadministration with the Pgp inhibitor cyclosporine A in mice [93]. As mentioned previously, an interesting feature of poloxamer–PAA copolymers is that they can be easily compressed in the dry form to obtain sustained-release tablets [36,94].

Although potentially useful, polymers that function only by interacting non-specifically with the mucus through weak bonds may not display optimal mucoadhesive properties. Much stronger bioadhesion can be achieved by functionalizing polymers with targeting ligands (*e.g.* lectins) or reactive groups such as thiols.

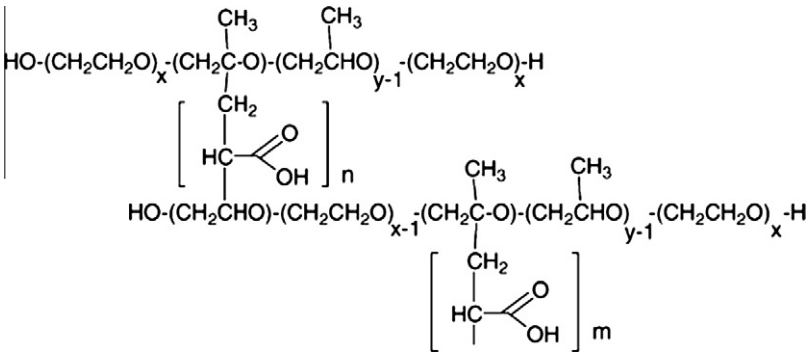

Fig. 4. Structure of the poloxamer-*g*-PAA copolymers. Reproduced from Ref. [36].

Through strong adherence to glycoproteins and glycolipids in the membrane of enterocytes, lectins may prove useful in both prolonging the transit time of a host cargo through the small intestine as well as promoting its uptake *via* receptor-mediated endocytosis [54]. Bernkopf-Schnurch and coworkers [95] have demonstrated that the thiolation of classical bioadhesive delivery systems substantially increases their mucoadhesive properties and therefore further improves the oral absorption of therapeutic proteins. Surface-exposed thiols are thought to form disulfide bonds with cysteine-rich subdomains of mucus glycoproteins. Thiolated polymers also exhibit increased permeation-enhancing effect as well as enzyme inhibitory properties [95]. Thiol-decorated polyion complex micelles (45 nm) prepared through complexation between PEG-*b*-poly(2-(*N,N*-dimethylamino)ethyl methacrylate) and a 20-mer oligonucleotide have been shown to interact with mucin through the formation of disulfide bonds [96]. While these micelles were initially designed to carry nucleic acid drugs, a similar strategy may be applied to deliver hydrophobic drugs through the use of thiol-functionalized PEG-*b*-PLA or PEG-*b*-PCL PMs [97]. A potential limitation of thiolated PMs is their propensity to form intra and intermolecular bonds, requiring their storage under non-oxidizing conditions.

8. *In vivo* data generated with conventional PMs

In vivo data pertaining to orally administered PMs are scarce. Most systems were either studied on cell models or not tested for their efficacy. The most interesting results were obtained with the hydrotropic PMs as carriers for paclitaxel. The formulation was administered intravenously or orally to canulated rats. The oral bioavailability was estimated at 12.4% [24]. While the experiment did not include a control formulation, other studies have reported an oral bioavailability of 6.5% for Taxol® (Cremophor EL micelles) [98]. Interestingly, the PMs injected directly through the portal vein demonstrated a 50% reduction in AUC compared to the i.v. infusion, corroborating the high hepatic metabolism of paclitaxel despite being entrapped in micelles. Elsewhere, a micellar formulation containing itraconazole performed as well as the commercial preparation which uses cyclodextrin as a solubilizing agent [34]. In this case, the pharmacokinetics of the drug was slightly improved, though the difference was not statistically significant. A third study showed that the bioavailability of risperidone was not improved following encapsulation in PMs, thus confirming the *in vitro* data on Caco-2 cells [26]. In all these studies, only a limited number of animals were evaluated, and the formulations were either administered after a fasting period or infused directly into the duodenum. As a consequence, these results do not take into account the effect of stomach acidity or the presence of bile salts or of other constituents of the intestinal fluids that may affect the formulation. Nevertheless, a recent study conducted by Benny et al. [99] yielded promising results with respect to Lodamin, an antiangiogenic drug (TN-470) conjugated to PEG-*b*-PDLLA micelles. Indeed, Lodamin showed significantly greater primary tumor growth inhibition compared to free TN-470. Moreover, Lodamin was able to limit tumor angiogenesis and proliferation as well as liver metastasis development when orally administered to mice (Lewis lung carcinoma and melanoma).

9. pH-sensitive polymeric micelles

Several PM systems designed to increase the oral bioavailability of hydrophobic compounds exhibit release times which largely exceed the transit time in the small intestine [25,26]. This is also true for surfactant micelles which have been found in some cases to impede the absorption of hydrophobic drugs due to excessive retention in the micellar phase [100,101]. Hence, when developing oral PM formulations for class II drugs, it is important to adequately control the release rate in order to avoid either precipitation upon dilution or sequestration within the micellar phase which may lead to incomplete adsorption.

One approach to ensure progressive and complete drug release in the GI tract consists in using PMs that exhibit a pH-dependent ionization/dissociation profile. Such micelles minimize the initial burst release and possible precipitation in the stomach by releasing small amounts of their cargo at acidic pH. At the intestinal pH (pH > 5), they partially or completely ionize, thereby liberating the remaining entrapped drug in a molecularly dispersed form in the small bowel where absorption is maximal. These pH-responsive micelles can be either multimolecular or unimolecular [102,103]. To release their content in a pH-dependent fashion, the inner core of unimolecular PMs contains weakly acidic groups that ionize at the intestinal pH. The polymer deprotonation increases the core's polarity while lowering its affinity for the encapsulated drug [102]. Compared to conventional PMs, unimolecular micelles have the advantage of being intrinsically stable upon dilution and thus do not exhibit a CMC.

Conversely, the self-assembly of pH-responsive multimolecular PMs depends on the pH of the dispersion medium. For instance, PEG-*b*-P(VBODENA) micelles which were rendered pH-sensitive *via* incorporation of acrylic acid moieties ($\leqslant$50 mol%) in the core-forming segment exhibited significantly faster PTX release through micelle destabilization upon incubation in SIF (pH 6.5) compared to their plain hydrotropic counterparts [104]. Alternatively, PEG-*b*-P(alkyl(meth)acrylate-*co*-methacrylic acid)s (PEG-*b*-P(Al(M)A-*co*-MAA)s) are diblock copolymers that display a pH-dependent micellization behavior in aqueous media. The pH-sensitivity is conferred by the pendant carboxylic acid groups of the MAA moieties, whereas the self-association into well-defined core–shell structures is facilitated by the inclusion of hydrophobic non-ionizable Al(M)A units [103,105,106]. In diblock copolymers bearing no Al(M)A unit, the protonation of MAA at acidic pH leads to the formation of large aggregates which are thought to result from extensive hydrogen bonding between the carboxylic acid groups and PEG chains [105]. Particle size and CMC values are dependent on the nature of the Al(M)A units. Small alkyl side groups such as ethyl acrylate (EA) were shown to yield larger particles and to associate at higher concentrations than more hydrophobic larger/bulkier units such as *n*-butyl acrylate (*n*BA), *iso*-butyl acrylate (*iso*-BA) or propyl methacrylate (*Pr*MA). As discussed earlier, the presence of longer aliphatic chains in the Al(M)A units reduces hydrogen bonding between MAA and PEG, thereby preventing the uncontrolled aggregation of polymer chains. PEG-*b*-P(Al(M)A-*co*-MAA)s were found to self-assemble at pHs ranging from 4.5 to 5.5 [35,103,106]. Depending on the polymer composition and molecular weight, micelles of small size (<100 nm) and narrow size distribution could be obtained in SGF (Table 3). The CMC in SGF was low, typically in the order of 10–30 mg/L. Micelles also formed in distilled water but they fully dissociated at near-neutral pH due to the deprotonation of MAA.

PEG-*b*-P(Al(M)A-*co*-MAA) PMs have been investigated for the encapsulation of a number of hydrophobic drugs (*i.e.* indomethacin, fenofibrate, progesterone, candesartan cilexetil (CDN)) by various encapsulation methods (*i.e.* o/w emulsion, dialysis, solvent evaporation). These polymers have been generally associated with high drug loadings (up to ca. 20% w/w in the case of CDN) (Table 3) and pH-dependent release profiles. Fig. 5 shows the *in vitro* cumulative release profiles of CDN, a poorly ionizable drug, from pH-sensitive PEG-*b*-P(*iso*BA-*co*-MAA) and pH-insensitive PEG-*b*-P(*iso*BA-*co*-*tert*-butyl methacrylate) (PEG-*b*-P(*iso*BA-*co*-*t*BMA)) micelles. The PMs were immersed in SGF for 2 h and then exposed to pH 7.2 for an additional 7 h. For all formulations, drug leakage

Table 3
Characteristics of PEG-*b*-P(Al(M)A-*co*-MAA)s and their self-assemblies in aqueous media. Adapted from Ref. [106].

Copolymer	M_n (NMR)	CMC in water (mg/L)	CMC in SGF (mg/L)	d_h in water (nm)	d_h in SGF (nm)	CDN loading (% w/w)	EE[a] (%)
PEG_{115}-*b*-P($n$$BA_{38}$-*co*-$MAA_{43}$)	13,600	63	28	60	64	19.0	95
PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38})	12,800	39	24	56	54	17.8	89
PEG_{115}-*b*-P(*Pr*MA_{36}-*co*-MAA_{36})	12,800	26	12	122	87	17.4	87

[a] Entrapment efficiency of PMs prepared by the solvent evaporation method.

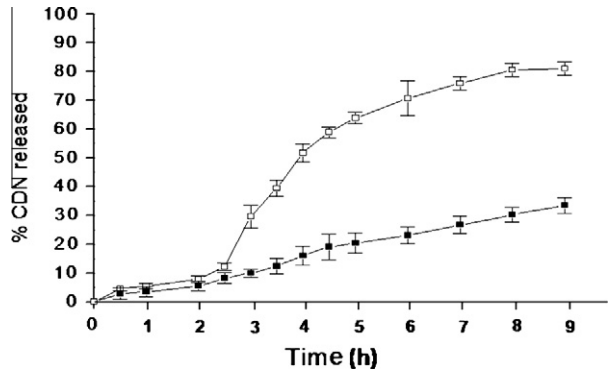

Fig. 5. *In vitro* release profile of CDN from PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38}) (open square) and PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38}) (closed square) PMs prepared by the solvent evaporation method at pH 1.2 for 2 h followed by 7 h at pH 7.2. Mean ± SD (n = 3). Adapted in part from Ref. [106].

was low at acidic pH, with about 10% of CDN released after 2 h. Raising the pH to 7.2 resulted in an abrupt increase in the release rate from PEG-*b*-P(*iso*BA-*co*-MAA) micelles, whereas the release profile from the pH-insensitive formulation was not affected. Dissociation of the pH-responsive PMs allowed the almost complete discharge of CDN within 9 h, while the control micelles retained more than 50% of their content until the end of the assay. In rats, such micelles were found to improve the oral bioavailability of fenofibrate [35] and CDN (Fig. 6). In the case of CDN, the pH-sensitive PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38}) PMs yielded greater drug

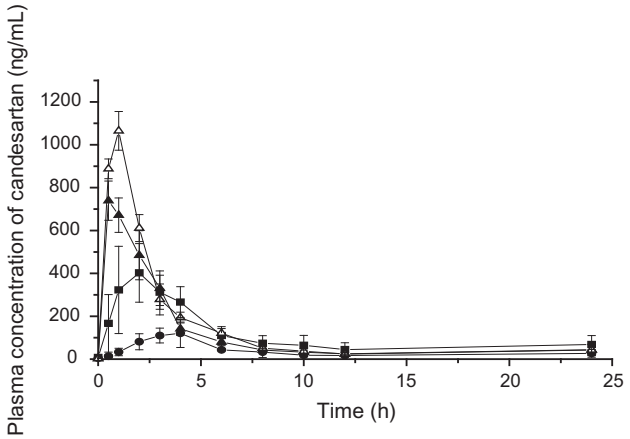

Fig. 6. Plasma concentration vs. time curve of CDN after oral administration of PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38}) (drug loading 18.1% w/w) (closed triangle), PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38}) (drug loading 17.3% w/w) (open triangle), CDN coarse suspension in arabic gum 0.5% (w/v) (circle) and the suspension of commercial tablet formulation in water, Atacand® (square) to fasted Sprague Dawley rats at a dose of 1 mg/kg. Blood samples were collected at regular intervals post-dose *via* the subclavian vein. The content of CDN in plasma was quantified following liquid–liquid extraction by LC-MS/MS using nimodipine as internal standard. Column: Luna C18(2)-HST 50 × 3 mm, 2.5 μm particle size (Phenomenex®), mobile phase: acetonitrile gradient (40–95% over 4 min): flow rate (0.2 mL/min), limit of quantification: 1 ng/mL. Mean ± SD (n = 6). Gaucher et al., unpublished data.

plasma concentrations (AUC 3265 ng·h·mL^{-1}) compared to non-pH-sensitive PEG_{115}-*b*-P(*iso*BA_{35}-*co*-*t*BMA_{38}) PMs (AUC 2649 ng·h·mL^{-1}), the commercial form Atacand® (AUC 2661 ng h mL^{-1}) and a candesartan powder suspension (AUC 872). The positive effect of these PMs on the oral absorption could be solely ascribed to the increased solubilization and favored drug release in the intestine. Indeed, at neutral pH, PEG-*b*-P(Al(M)A-*co*-MAA)s were found to have almost no effect on the activity of Pgp and on transepithelial permeability (Table 4).

10. Administration of hydrophilic compounds

For the most part, the use of PMs as oral delivery agents for hydrophilic drugs has been applied to the transport of nucleic acids. Yet, peptides and proteins are another therapeutic class for which encapsulation in a drug carrier may prove beneficial [107,108]. For all these drugs, the challenge consists in avoiding destabilization upon exposure to acidic pH and degradation by GI enzymes while enhancing permeation through the intestinal membrane. The use of neutral or poly(ethylene imine) grafted poloxamers has been exploited toward that end. Promising results were obtained *in vitro* and *in vivo* in the rat with oligonucleotides [109] and plasmids [110], respectively. In both instances, it was hypothesized that the ability of poloxamers to promote cellular internalization played a determining role. Recently, reverse PMs have been proposed for the oral delivery of therapeutic peptides

Table 4
Effect of PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38}) on Pgp ATPase activity, accumulation of rhodamine-123 (R-123) in Caco-2 cells and mannitol permeability across Caco-2 cell monolayers at pH 7.4. Mean ± SD (n = 3). Gaucher et al., unpublished data.

Medium/ assay	Pgp ATPase activity[b] (nmol/ mg min)	R-123 accumulation[c] (pmol/mg protein)	Mannitol permeability[d] (×10^{-7} cm/s)
Buffer	6.8 ± 0.6	378 ± 58	1.4 ± 1.2
Verapamil[a]			
9.1 μg/mL (20 μM)	26.7 ± 2.1	–	–
22.7 μg/mL (50 μM)	–	548 ± 48	–
PEG_{115}-*b*-P(*iso*BA_{35}-*co*-MAA_{38})			
0.5 mg/mL	4.2 ± 1.1	331 ± 22	–
2 mg/mL	–	–	3.7 ± 0.5

[a] Verapamil is used as a positive control; it stimulates the Pgp ATPase activity and enhances the accumulation of R-123 in Caco-2 cells by inhibiting the efflux pump.
[b] The ATPase assay was carried out using isolated human Pgp membrane suspension. The assay involved monitoring the changes in the liberation of inorganic phosphate from the cleavage of ATP catalyzed by Pgp ATPase.
[c] This assay was used the assess Pgp inhibition. Caco-2 cells were exposed to 5 μM R-123 (Pgp substrate) in either assay buffer (Hank's balanced salt solution + 10 mM HEPES, pH 7.4) or in solutions of copolymer or verapamil. After 90 min of incubation at 37 °C, the cellular accumulation of R-123 was determined by spectrofluorimetry.
[d] The effect of copolymer on tight junctions was investigated by measuring the permeability of [^{14}C]-mannitol across Caco-2 monolayer in Hank's balanced salt solution (pH 7.4, 37 °C).

[111,112]. This delivery system was inspired by the Macrosol™ technology developed by New and Kirby where peptidic drugs were solubilized in oil in the presence of a lyophilized mixture of phospholipids and surfactants [113,114]. Contrary to conventional micelles, reverse PMs are characterized by a hydrophilic interior and a hydrophobic shell. Reverse PMs resulting from the self-assembly of star-shaped alkylated poly(glycerol methacrylate)s were successfully employed for the solubilization of vasopressin, an antidiuretic nonapeptide, in an oily vehicle [112]. When incorporated in the reverse PMs, the affinity of the peptide for ethyl oleate was significantly increased [112]. The presence of oil and the encapsulation of the peptide should both contribute to protect the peptide against degradation in the GI tract [113]. However, preliminary results showed greater *in vivo* antidiuretic efficacy when the vasopressin-loaded reverse micelles were administered *via* the subcutaneous route instead of the oral route [115].

11. Conclusion

For class II drugs, poor solubility in GI fluids remains the main barrier to oral absorption. Drug extrusion by the intestinal Pgp may also play a determining role in restricting oral bioavailability. As a consequence, most drug delivery strategies have been devised with the aim of improving dissolution rate and preventing cellular drug efflux. One such approach lies in the encapsulation of the drug inside nanocarriers such as PMs. For the most part, these polymeric self-assemblies seem to act as potent solubilizers for class II drugs. The use of PMs for oral drug delivery is still fairly new, and few systems have been extensively investigated. Instead, many formulations present optimized properties for parenteral delivery, such as slow drug-release rates which may largely surpass the transit time in the intestine. Moreover, the chosen methodologies can often lead to biased results. As a case in point, *in vitro* release studies are rarely performed under conditions which properly mimic the *in vivo* environment. In addition, much effort is still required in order to gain greater insight into the mechanisms that govern the interaction of a carrier with the intestinal membrane and subsequent drug absorption. These are some of the issues which need to be addressed specifically when designing polymeric micelles intended for per oral drug delivery. Nevertheless, based on the literature discussed herewith, PMs show promise as delivery vehicles for the efficient administration of various therapeutic compounds *via* the oral route.

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