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Relevance to oral drug absorption

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Review

Nonionic surfactants modulate the transport activity of ATP-binding cassette (ABC) transporters and solute carriers (SLC): Relevance to oral drug absorption

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Nonionic surfactants modulate the transport activity of ATP-binding cassette (ABC) transporters and solute carriers (SLC): Relevance to oral drug absorption

Running title: Nonionic surfactants modulate membrane transport proteins

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Abstract:

Recently, it has become evident that pharmaceutical excipients may interfere with the activity of ATP-binding cassette (ABC) transporters and solute carriers (SLC). The present review aims to provide an overview of surfactants shown to modulate substrate transport via SLCs and ABCs, and to discuss the relevance for oral drug absorption. *In vitro,* more than hundred surfactants have been suggested to decrease the efflux activity of P-glycoprotein (P-gp, ABCB1), and many of these surfactants also inhibit the breast cancer resistance protein (BCPR, ABCG2), while conflicting results have been reported for multidrug resistance-associated protein 2 (MRP2, ABCC2). In animals, surfactants such as pluronic® P85 and polysorbate 20 have been shown to enhance the oral absorption of P-gp and BCRP substrates. Many surfactants, including cremophor® EL and Solutol® HS 15 inhibiting ABC transporters, were also found to inhibit SLCs in cell cultures. These carriers were SLC16A1, SLC21A3, SLC21A9, SLC15A1-2, and SLC22A1-3. This overlap in specificity of surfactants that inhibit both transporters and carriers might influence the oral absorption of various drug substances, nutrients, and vitamins. Such biopharmaceutical elements may be relevant for future drug formulation design.

Key words: Nonionic surfactant, Co-surfactant, ATP-binding cassette transporters, Solute carriers, Oral absorption, Lipid-based formulations.

1. Introduction:

Today, many drug substances approved for oral and parenteral use are prepared in surfactant containing formulations such as lipid-based formulations, suspensions and solid dosage forms (Savla et al., 2017). In these formulations, nonionic surfactants are used as solubilizes, stabilizers, wetting agents etc. In ~~the last~~recent years, it has become evident that some surfactants may affect the function of ~~biological~~ membrane transport proteins by altering drug substance uptake (Engel et al., 2012; Rege et al., 2002) and/or cellular efflux (Batrakova et al., 2003a; Rege et al., 2002; Yamagata et al., 2007b). Membrane transport proteins relevant to drug transport are from two major families, i.e. the ATP-binding cassette (ABC) family of efflux transporters and the solute carrier (SLC) family of cellular influx and efflux carriers. In brief, members of the ABC family (hereafter termed "transporter") depend directly on the use of cellular ATP to complete their transport cycle, which in mammals result in cellular efflux. Members of the SLC family do not directly depend on using cellular ATP, but are driven by substrate concentrations and in many cases the concentration gradient of other substrates such as ions. A SLC facilitates either cellular influx or efflux, and are hereafter termed "carrier". Currently, the ABC and SLC families consist of 51 and 417 members (HUGO Gene Nomenclature Committee, 2019), respectively. The first indication that pharmaceutical excipients could alter the transport function of transporters and carriers came from observations that nonionic surfactants such as cremophor® EL (Woodcock et al., 1990), Solutol® HS 15 (Coon et al., 1991), and polysorbate 80 (Woodcock et al., 1992) reversed multidrug resistance in cancer cells. In cell cultures, nonionic surfactants inhibited different members of the ABC family such as P-glycoprotein (P-gp, MDR1, ABCB1) (Lo, 2003; Rege et al., 2002), breast cancer resistance protein (BCRP, ABCG2) (Yamagata et al., 2007a, b), and multidrug resistance-associated protein 2 (MRP2, ABCC2), although conflicting results have been

observed for MRP2 (Hanke et al., 2010; Li et al., 2013a, 2014). *In vivo*, in wild type animals, nonionic surfactants have been shown to enhance the intestinal absorption and bioavailability of P-gp substrates such as digoxin (Cornaire et al., 2004; Nielsen et al., 2016; Zhang et al., 2003), etoposide (Akhtar et al., 2017; Al-Ali et al., 2018a), and paclitaxel (Varma and Panchagnula, 2005), and the BCRP substrate topotecan (Yamagata et al., 2007b). Interestingly, corresponding control experiments in transporter deficient animals showed that co-administration of nonionic surfactants with digoxin (Nielsen et al., 2016) or etoposide (Al-Ali et al., 2018a) in *mdr1a* deficient rats, or topotecan (Yamagata et al., 2007b) in *abcg2* deficient mice did not alter the oral absorption and bioavailability of these substrates. This indeed indicates that surfactants increase intestinal absorption through P-gp or BCRP inhibition, and for the drug substances in question, not through unspecific effects related to solubilizing of the drug substances or through permeation enhancing effects.

Recently, *in vitro* studies have also shown that nonionic surfactants such as polysorbate 20 and cremophor® EL inhibit the transport via several SLCs expressed ion the apical membrane of enterocytes such as the organic anion transporting polypeptide 1A2 (OATP1A2, SLC21A3) (Engel et al., 2012) and organic cation transporters (OCT1-3, SLC22A1-3) (Otter et al., 2017; Soodvilai et al., 2017). These observations indicate that biopharmaceutical considerations need to be an important part of new formulation development when formulations contain pharmaceutical excipients such as surfactants and co-surfactants because these excipients may have different impacts on transporters and carriers. Therefore, addition of surfactants to obtain an enabling formulation may potentially influence the oral absorption (positively or negatively) of a co-administered drug substance if this is a substrate for a carrier and/or transporter. On the other hand, enabling formulations provide the formulation scientist with the possibility to adjust drug absorption to become more consistent by selecting

appropriate excipients for drug substances that are ABC and/or SLC substrates. To do so, it becomes important to understand: 1) the different impacts of nonionic surfactants on carriers and/or transporters, 2) the mechanism behind surfactant-protein interactions, and 3) whether such impacts of surfactants on substrate-protein interactions observed in cell cultures may affect the pharmacokinetics parameters of the substrates *in vivo*. Currently, the translational aspects of how excipients affect carriers and transporters *in vivo* and how this may be exploited for formulation design are largely unexplored.

This review aims to provide an overview of surfactants shown to modulate substrate transport via transporters and carriers, and to discuss the relevance for oral drug absorption, and when possible the mechanism behind the interaction. In this paper, essential data is presented in tables, whereas more comprehensive overviews are provided in a supplementary table~~s~~ in order to enhance the readability of the review.

2. Modulation of intestinal transporters and carriers

In the 1980s, modulation of membrane transport proteins was originally proposed as a strategy for chemo-sensitizing cancer cells and to increase the oral bioavailability of drug substances that were substrates for the efflux transporter P-gp in humans. Therefore, inhibitors of efflux transporters such as P-gp and BCRP were ~~then~~ identified and these inhibitors, for example verapamil (Tsuruo et al., 1981), dexverapamil (Gramatté and Oertel, 1999), valspodar (vanAsperen et al., 1997), and GF120918 (Hyafil et al., 1993), showed promising results in inhibiting P-gp (GF120918 also inhibited BCRP) (Maliepaard et al., 2001a), in pre-clinical studies. However, this strategy failed to produce safe and effective treatment in human clinical trials (Dalton et al., 1995; Greenberg et al., 2004; Lehnert et al.,

1998; Mross et al., 1999; Planting et al., 2005; Ries and Dicato, 1991; Sparreboom et al., 1999; Warner et al., 1998). Subsequently, other strategies based on natural products (Appendino et al., 2003; Yoshida et al., 2005) and pharmaceutical excipients (Lo, 2003; Rege et al., 2002; Regev et al., 1999; Zhang et al., 2003) were suggested. In the latter group of compounds, nonionic surfactants gained quite some attention since many surfactants were found to enhance the intracellular accumulation of anticancer drugs including daunorubicin, vinblastine, and etoposide in cancer cells (Buckingham et al., 1995; Woodcock et al., 1990), and to influence the translocation activity of several transporters (Batrakova et al., 2001; Lo, 2003; Rege et al., 2002) and carriers (Rege et al., 2002). In terms of intestinal absorption, it has been reported that nonionic surfactants such as cremophor® EL (Rege et al., 2002), polysorbate 80 (Lo, 2003), and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS 1000) (Bogman et al., 2005) increased the absorptive permeability and decreased the secretory permeability of the P-gp substrates drug substances rhodamine 123, epirubicin, and talinolol, respectively, *in vitro* using the Caco-2 cell monolayers model. Likewise, in intestinal segments of rats, surfactants such as TPGS 1000 (Varma and Panchagnula, 2005) and polysorbate 40 (Zhu et al., 2009) enhanced mucosal to serosal (M-S) permeability and decreased S-M permeability of P-gp substrates paclitaxel and rhodamine 123, respectively. Moreover, *in vivo*, it has been shown that Solutol® HS 15 (Bittner et al., 2002), polysorbate 80 (Zhang et al., 2003), and pluronic® P85 (Föger et al., 2006) enhanced the oral absorption and exposure of P-gp substrates colchicine, digoxin, and rhodamine 123, respectively. Consequently, it was evident that nonionic surfactants could be potential alternatives to conventional P-gp inhibitors.

Since the majority of research performed until now, has been focused on investigating the influence of nonionic surfactants on P-gp, BCRP, and MRP2 transporters, and on carriers including monocarboxylic

127 acid transporter (MCT), OATP1A2, OATP2B1, OCT1-3 and peptide transporters 1 and 2 (PEPT1 and
128 2), this review will therefore summarize and discuss the impact of nonionic surfactants on these
129 transporters and carriers *in vitro* and on the intestinal absorption of substrate drug substances *in vivo*.

130

131 **3. Expression of selected transporters and carriers along the human intestine**

132 The intestinal expression of transporters and carriers have been studied for years using various mRNA-
133 based techniques, such as northern blotting and RT-PCR, as well as protein quantification methods, e.g.
134 western blotting. As an example, Broberg et al. studied the expression of the proton-coupled amino
135 acid transporter PAT1 along the length of the rat intestine taking samples from each 5 cm and
136 measured the *pat1* mRNA expression in each segment (Broberg et al., 2012). Recently, the emerging of
137 high-resolution MS/MS-based techniques, membrane proteomics has received attention as a tool to
138 describe the absolute transporter or carrier abundance in enterocytes. Knowing transporter and carrier
139 abundancies in the intestine is important for understanding the absorption windows of drug substances
140 (Oswald et al., 2006) and drug-drug interactions (Giacomini et al., 2010). Furthermore, absolute
141 transporter and carrier abundance in different segments of the intestine is necessary for PBPK
142 modelling (Harwood et al., 2013). The present section will briefly review the transporter and carrier
143 abundance in the human intestine and Caco-2 cells of the selected transporter and carriers discussed in
144 the present review.

145 Table 1 shows protein concentrations of selected transporters and carriers in Caco-2 cells and in
146 different regions of the human intestine. Naturally, cell differentiation affects transport and carrier
147 protein expression, which ~~is~~ was also shown by Uchida et al. (Uchida et al., 2015). Therefore, we only

compare expression levels in cells with a similar degree of differentiation, and all three studies have ~~shown~~ investigated the expression in Caco-2 cells cultured for three weeks. Expression levels in cells obtained from different cell banks may vary to a high degree as illustrated for the glucose carrier SGLT1 by Steffansen and co-workers (Steffansen et al., 2017). For this reason, we have compared studies of Caco-2 cells from different cell banks (DSMZ, ECACC, and ATCC). The most extensive and systematic work in regional transporter and carrier expression in the intestine has been performed by Drozdzik and co-workers (Drozdzik et al., 2019; Drozdzik et al., 2014)

Regarding transporters, the P-gp expression rangeds from low to high (Akazawa et al., 2018; Drozdzik et al., 2019; Drozdzik et al., 2014; Gröer et al., 2013; Harwood et al., 2015; Lloret-Linares et al., 2016), and the expression increaseds from the proximal small intestine towards the distal small intestine (Akazawa et al., 2018; Drozdzik et al., 2014; Gröer et al., 2013) and ~~drops~~ decreased in the colon to levels similar to those in the proximal small intestine (Table 1) (Drozdzik et al., 2019; Drozdzik et al., 2014). Caco-2 cells expressed slightly elevated levels of P-gp, compared to the small intestine (Brück et al., 2017; Uchida et al., 2015; Ölander et al., 2016). BCRP expression varieds between the studies from low (Drozdzik et al., 2014) to very high (Akazawa et al., 2018) expression in the small intestine with an increasing expression from the proximal to the distal part of small intestine (Drozdzik et al., 2019; Drozdzik et al., 2014). In the colon, very low to intermediate expression of BCRP has been reported (Drozdzik et al., 2019; Drozdzik et al., 2014). Similarly, reported expression of BCRP in Caco-2 cells varieds greatly. MRP2 and MRP3 ~~generally seem~~were intermediately to highly expressed along the entire intestine (Akazawa et al., 2018; Drozdzik et al., 2019; Drozdzik et al., 2014; Gröer et al., 2013; Harwood et al., 2015) with a slight tendency of elevated expression in the colon (Drozdzik et al., 2019; Drozdzik et al., 2014). The expression of MRP2 in Caco-2 cells ~~is~~ was similar to the

expression in the small intestine (Brück et al., 2017; Uchida et al., 2015) with one exception, where lower expression was reported (Ölander et al., 2016). Ölander and co-workers quantified MRP3 in Caco-2 cells, and the expression of this protein was similar to the expression in the small intestine (Ölander et al., 2016). Brück et. al. and Uchida et. al. could not quantify MRP3 in Caco-2 cells (Brück et al., 2017; Uchida et al., 2015).

Regarding carriers, PEPT1 exhibiteds high to very high expression in the small intestine (Akazawa et al., 2018; Drozdzik et al., 2019; Drozdzik et al., 2014; Gröer et al., 2013; Miyauchi et al., 2016), and increasing expression in the proximal to distal direction in the small intestine (Drozdzik et al., 2019; Drozdzik et al., 2014), however, the expression in the colon is was highly reduced (approx. 10- to 30-fold) (Drozdzik et al., 2019; Drozdzik et al., 2014), (see Table 1). In Caco-2 cells, PEPT1 showeds different levels of expression from colon-like levels to small intestine-like levels (Brück et al., 2017; Uchida et al., 2015; Ölander et al., 2016) (Table 1).

Absolute amounts of transporters and carriers in the human gastrointestinal tract highly depend on the site of sampling for the determination. Therefore, it is important to note that the site of sampling from anatomical structures varies between studies in the field (Drozdzik et al., 2019; Drozdzik et al., 2014; Lloret-Linares et al., 2016; Miyauchi et al., 2016). Additionally, tissue samples are occasionally only defined as ‘jejunal’, ‘ileal’, ‘distal jejunum’, or ‘distal ileum’ with no further definition (Akazawa et al., 2018; Gröer et al., 2013; Harwood et al., 2015). Likewise, great inter-individual variation of intestinal transporter and carrier protein expression is likely, and we have left out all statistical deviation parameters in (Table 1) to enhance the overview. Moreover, protein expression is affected by external factors, for example certain drug compounds (Lin and Yamazaki, 2003) and dietary elements (Erickson

et al., 1995) along with general health condition and diseases (Englund et al., 2007; Wojtal et al., 2009).

Finally, inter-laboratory variation is a well-documented factor, and we refer to the excellent cross-laboratory study by Wegler and co-workers (Wegler et al., 2017). Herein, the authors have shown large variabilities, depending on the methods applied for quantification, especially when they compare whole-lysate and membrane fractionation techniques in the sample preparation. Depending on the drug formulation, pharmaceutical excipients, such as nonionic surfactants, are likely to be present at different concentrations in different segments of the intestine. To firmly understand how the excipients will affect drug absorption influenced by transporters or carriers, it is crucial to obtain more PBPK modelling knowledge regarding transporter and carrier expression patterns.

4. Nonionic surfactants modulate the transport activity of several ABC transporters

4.1 Impact of nonionic surfactants on P-glycoprotein

The first discovered member of the ABC family, P-glycoprotein (Juliano and Ling, 1976), has until now been the most investigated transporter. ~~Since P-gp was found to mediate the cellular efflux of drug substances belonging to different drug classes, e.g. anticancer drugs and antibiotics, extensive research has been performed to modulate the transport activity of the transporter *in vitro* and *in vivo*. The next sections will focus on P-gp molecular characterization and substrate transport via P-gp, and cellular expression and tissue distribution of P-gp. In the subsequent sections, the effects of surfactants and co-surfactants on P-gp activity *in vitro* and on the intestinal absorption of P-gp substrates *in vivo* will be discussed.~~

~~P-glycoprotein molecular characterization and substrate transport~~

P-glycoprotein is a 170 kDa efflux transporter that requires energy from ATP hydrolysis to pump substrates out or across cellular membranes. It has been estimated that P-gp requires two ATP molecules to transport one substrate (Ambudkar et al., 1997; Sauna and Ambudkar, 2001). Due to the direct use of ATP, the transporter is able to transport its substrates against the concentration gradient, and therefore ~~P-gp can~~ limit the cellular accumulation and retention of certain drug substances. In human, P-gp is expressed in the cell membrane in different tissues such as the luminal membrane in enterocytes, the canalicular membrane in hepatocytes, in the luminal membrane in proximal tubular cell, in the luminal membrane of endothelial cells of the central nervous system and testes, bronchial cells of lungs, and placenta (Cordon-Cardo et al., 1990), hence affecting the ADMET properties of its substrates. P-gp substrates include a wide range of hydrophobic and amphipathic substrates such as drug substances and toxins with diverse molecular weight ranging from approximately 300-4000 Da (Fromm, 2004; Rao et al., 1999; Su et al., 2009). Hundreds of drug substances are P-gp substrates (Drugbank, 2019) including anti-cancer drugs, antibiotics, cardiac drugs, immuno-suppressants, lipid-lowering agents, HIV drugs, and hormones (Chan et al., 2004; Fromm, 2004; Seelig, 1998).

~~It has previously been suggested that P-gp substrates might diffuse through the membrane bilayer and reach the cytoplasmic leaflet, where the substrate gets access to the protein (Raviv et al., 1990). P-gp will then according to this mechanistic proposal act as a hydrophobic "vacuum cleaner" that pumps the substrate to the extracellular environment (Raviv et al., 1990). Another model referred as "flippase model" assumed that the P-gp substrate first partition into the lipid bilayer and reach the inner leaflet, where the substrate has access to P-gp. At this stage, the substrate will be pumped directly to the extracellular environment by P-gp or flipped by the protein to the outer leaflet of the lipid bilayer~~

~~(Higgins and Gottesman, 1992). In both cases, the movement of the substrate would be driven by either the equilibrium between the concentration of the substrate in the extracellular environment and in the outer leaflet, or between the concentration of the substrate in the inner leaflet and in the cytoplasm (Higgins and Gottesman, 1992). There is accumulating evidence supporting the flippase model for P-gp mediated substrate transport across the membrane bilayer (Abulrob and Gumbleton, 1999; Eckford and Sharom, 2005; Romsicki and Sharom, 2001; van Helvoort et al., 1996).~~

It was proposed that the transmembrane (TM) organization of P-gp consists of two TM domains (Chen et al., 1986). ~~, which e~~Each domain has six TM helices and one nucleotide-binding domain (NBD) located in the cytoplasm ~~(Fig. 1a)~~ (Chen et al., 1986) (Fig. 1a). Recently, the inward-facing conformation of mouse P-gp proposed that the TM helices are arranged to form an internal cavity of approximately 6000 Å^3, which is integrated in the lipid bilayer (Aller et al., 2009). ~~It has been suggested that P-gp might have portals open to the cytoplasmic region and to the inner leaflet of the lipid bilayer (Aller et al., 2009). The P-gp substrates may therefore via these portals get access to the binding sites in the internal cavity of the P-gp, where two P-gp substrates could simultaneously be accommodated (Aller et al., 2009).~~ By using cryo-electron microscopy at 3.4 Å resolution, recent research has also shown the outward-facing conformation of human P-gp (Kim and Chen, 2018). In brief, a P-gp substrate may bind to the internal cavity of the inward-facing conformation of P-gp, as it has been suggested by Aller and co-workers (Aller et al., 2009), and this initiates ATP binding to the NBDs of the protein (Kim and Chen, 2018). In the process of reaching the outward-facing confirmation, the NBDs ~~may~~ dimerize resulting in the NBD's becoming closer, while the TM helices re-arrange toward the extracellular space and compress, preventing the binding of the substrate to P-gp (Kim and Chen, 2018) (Fig. 1a). In its outward-facing confirmation, the extracellular part of TM helices seems to be flexible for the substrate release and transport to the extracellular environment.

ATP hydrolysis will then reset the P-gp to the inward-facing conformation (Kim and Chen, 2018). ~~as it has been illustrated for the mouse P-gp (Aller et al., 2009). The inward- and outward-facing conformations of P-gp which were proposed to occur during the substrates translocation may support that the substrates transport across the membrane bilayer most likely occur by the flippase model.~~

~~4.1.1 Nonionic surfactants inhibit P-glycoprotein *in vitro* via different mechanisms~~

The most investigated nonionic surfactants that inhibit P-gp transport *in vitro* are cremophor® EL, Solutol® HS 15, TPGS 1000, polysorbate 20, polysorbate 80 and pluronic® P85, (~~see~~ Table 2). Several *in vitro* assays have been utilized to investigate the P-gp inhibitory properties of these surfactants including: 1) measuring the impact of surfactants on substrate absorptive and/or secretory transport using cell-based systems or intestinal segments excised from animals, 2) measuring the ATPase activity ~~of P-gp~~ using membrane vesicles from cells overexpressing P-gp, and 3) fluorescence-based (e.g. calcein-AM) efflux assay using cells ~~highly~~ overexpressinged ~~with~~ P-gp. In bi-directional transport assays, nonionic surfactants were shown to enhance the absorptive permeability of ~~model~~ P-gp substrates such as digoxin (Al-Ali et al., 2018b; Batrakova et al., 2001; Collnot et al., 2010; Nielsen et al., 2016) and rhodamine 123 (Collnot et al., 2010; Guan et al., 2011; Kiss et al., 2014; Rege et al., 2002; Sachs-Barrable et al., 2007; Zhao et al., 2016), and to decrease the secretory permeability ~~of these substrates~~ across cell monolayers. Consequently, the data presented in Table 2 strongly support that these surfactants inhibit the efflux activity of P-gp, thus provide a promising approach to inhibit P-gp-mediated efflux of drug substances. In addition, Table S1 provides a comprehensive overview of many other nonionic surfactants and co-surfactants that have shown different abilities to inhibit P-gp-

mediated transport *in vitro*. ~~The results provided in this table may assist providing an overview of likely interactions.~~

Different mechanisms have been proposed to explain the mechanisms behind how nonionic surfactants increase drug absorption by inhibiting P-gp-mediated cellular efflux (~~see~~ Fig. 2). Work from Seelig and co-worker has suggested that the inhibition may occur through partitioning of the hydrophobic tail of the surfactant into the cell membrane, while the hydrogen bond acceptor groups in the hydrophilic moiety of the surfactant form hydrogen bonds with the hydrogen bond donor groups in the TM domain of the protein (Li-Blatter et al., 2009; Seelig and Gerebtzoff, 2006), (Fig. 2). These hydrogen bonds between the surfactant and the TM domain in P-gp may explain the higher affinity of surfactants with large number of hydrogen bond acceptor groups such as n-octyl-β-D-maltopyranoside (C_8-malt) and 3-cyclohexyl-1-propyl-β-D-maltopyranoside (Cymal-3), than surfactants with fewer hydrogen bond acceptor groups e.g. n-heptyl-β-D-glucopyranoside (C_7-gluc). Thus C_8-malt and Cymal-3 with a similar number of hydrogen bond acceptor groups exhibit almost the same affinity to the membrane as C_7-gluc, but a higher affinity to the P-gp protein, due to the duplication of the sugar moiety in maltopyranoside based-surfactants compared to the single sugar moiety in glucopyranoside (Li-Blatter et al., 2012; Li-Blatter et al., 2009; Li-Blatter and Seelig, 2010; Xu et al., 2015). This observation may be supported by the results from a recent study (Al-Ali et al., 2018b), which reported that polysorbate 20 elicited higher affinity to P-gp in the calcein-AM efflux assay than the mono-saccharide based surfactants, e.g. lauroyl methyl glucamide, and the di-saccharides based surfactants, e.g. lauryl-β-D-maltoside, since the former surfactant had a higher number of hydrogen acceptor groups when retaining the laurate side chain in the surfactants (Al-Ali et al., 2018b). The latter study also suggested that extending the alkyl side chain to more than laurate e.g. stearate in polysorbate 60 and oleate in polysorbate 80, or attaching multiple

alkyl groups such as tri-stearate in polysorbate 65, while retaining the hydrophilic group in these surfactants, may decrease the affinity of the surfactant to P-gp (Al-Ali et al., 2018b). Therefore, the study concluded that *in vitro* both the hydrophobic and hydrophilic moieties in nonionic surfactant may contribute to the surfactant mediated P-gp inhibition.

The second proposed mechanism of P-gp inhibition by nonionic surfactants relates to the alteration of membrane bilayer fluidity induced by surfactants, an alteration that might indirectly inhibit the ATPase activity (Fig. 2). It was shown that polysorbate 20, Nonidet™ P-40 and Triton™ X-100, which all increase the fluidity of artificial membranes, inhibited P-gp ATPase activity in membrane vesicles prepared from Chinese hamster ovary AA8 cells (Regev et al., 1999), (Table 2). In addition, it was reported that polysorbate 80 and cremophor® EL, which increased membrane fluidity significantly, also inhibited P-gp. The inhibition resulted in a significant increase in the absorptive permeability across Caco-2 cells of the model P-gp substrate rhodamine 123 and a significant decrease in the secretory permeability (Rege et al., 2002). In a subsequent study, the surfactant N-octyl glucoside did not modulate membrane bilayer fluidity and did not change the absorptive and secretory permeability of rhodamine 123 (Rege et al., 2002).

Wei and co-workers proposed that the intracellular depletion of ATP was the main mechanism of P-gp inhibition by pluronic-based surfactants such as pluronic® P123 suggesting a third mechanism of P-gp inhibition (Wei et al., 2010; Wei et al., 2013), (see Fig. 2). This third suggested mechanism of P-gp inhibition is supported by previous studies which reported that pluronic® P85 and pluronic® L64 enhanced the intracellular accumulation of the P-gp substrate rhodamine 123 and decreased intracellular ATP *in vitro* (Batrakova et al., 2003a), (Table 2).

The fourth proposed mechanism of surfactant mediated P-gp inhibition suggested a combined effect of depleted intracellular ATP and alteration in cellular membrane fluidity (Batrakova et al., 2003a; Batrakova et al., 2003b), (~~see~~ Fig. 2). In support of this, it was reported that pluronic® P85 and pluronic® L81, which enhanced membrane fluidity, were able to deplete ~~the~~ intracellular ATP, and significantly enhanced the intracellular accumulation of rhodamine 123 in bovine brain microvessel endothelial cells (Batrakova et al., 2003b; Batrakova et al., 2004), (Table S1).

Consequently, the mechanisms of P-gp inhibition by surfactants seem complex. It could be that one or more mechanisms ~~or more~~ are involved ~~in such inhibition~~. However, further research focused on ~~further~~ characterizing the underlying mechanism(s) of surfactant-mediated P-gp inhibition is needed, which might assist in choosing the appropriate surfactant(s) or developing new surfactant(s) that could be more potent than the ones available and perhaps transporter specific.

~~4.1.2~~4.1.1 Surfactants used in preparing lipid-based formulations may inhibit P-glycoprotein *in vitro*

Surfactants can be used in pharmaceutical formulations such as lipid-based formulations (LBF) (Pouton, 2006). Currently, many drug substances available in the market are incorporated into LBFs such as tipranavir (Aptivus®), bexarotene (Targretin®), and sirolimus (Rapamune®), where the surfactants function as solubilizing agents and emulsifiers (Savla et al., 2017). For formulations of P-gp substrates, inclusion of surfactants that have P-gp inhibition properties might be advantageous with respect to enhancement of substrate transport across biological membranes. Therefore, studies have reported the use of the LBFs to enhance the oral absorption of different P-gp substrates (Akhtar et al., 2015; Zhao et al., 2013). The self-micro-emulsifying drug delivery systems (SMEDDS) containing

cremophor® RH 40, cremophor® EL, or polysorbate 80 (Zhao et al., 2013), and the self-nano-emulsifying drug delivery systems (SNEDDS) containing cremophor® RH40 and Transcutol® P (Akhtar et al., 2015) were shown to enhance etoposide permeability across intestinal tissues and cell monolayers partly due to the inhibition of P-gp by these surfactants. In human, several studies showed enhanced oral absorption of the P-gp substrate cyclosporine A when formulated in LBFs compared to the oral absorption of cyclosporine A from conventional oral dosage forms (Bekerman et al., 2004; Drewe et al., 1992; Postolache et al., 2002). Cyclosporine A is an immunosuppressant used in prophylaxis and treatment of graft rejection in organ transplantations, and in treatment of autoimmune diseases e.g. rheumatoid arthritis, aplastic anemia, and myasthenia gravis (Italia et al., 2006). Cyclosporine A has low aqueous solubility (0.04 mg mL^{-1}) (O'Leary et al., 1986), low permeability in cell cultures (Augustijns et al., 1993; Fricker et al., 1996), and high variations in oral bioavailability among patients (Czogalla, 2009; Lown, 1997). In the studies where cyclosporine A was prepared in LBFs (Bekerman et al., 2004; Drewe et al., 1992; Postolache et al., 2002), the possible P-gp inhibition effect of excipients, e.g. nonionic surfactants, was however not mentioned. Interestingly, the excipients used to prepare LBFs-containing cyclosporine A were polysorbate 80, cremophor RH 40 (Bekerman et al., 2004), sucrose monolaurate, hydrogenated castor oil, and polyethylene glycol (Drewe et al., 1992), which were later shown to possess P-gp inhibitory properties *in vitro* (Al-Ali et al., 2018a; Al-Saraf et al., 2016; Ashiru-Oredope et al., 2011; Chiu et al., 2003; Cornaire et al., 2004; Gurjar et al., 2018; Hanke et al., 2010; Hodaei et al., 2015; Hugger et al., 2002; Johnson et al., 2002; Kiss et al., 2014; Rege et al., 2002; Shono et al., 2004) (Table 2 and S1), and/or *in vivo* (Shimomura et al., 2016; Zhang et al., 2003; Zhao et al., 2013) (Table 3).

Furthermore, previous research have reported that several surfactants such as lauroyl methyl glucamide, lauryl-β-D-maltoside, and trehalose 6-laurate that inhibited the efflux of P-gp substrate calcein-AM in MDCKII MDR1 cells, (Table S1), might also possess paraceullar and/or transcellular permeation enhancing effects in cell cultures (Al-Ali et al., 2018b; Eley and Triumalashetty, 2001; Petersen et al., 2012). Such effects might be advantageous when designing LBFs to enhance the oral absorption of P-gp substrate drug substances with limited oral bioavailability induced by intestinal P-gp.

Additionally, the mixed micelles formulations such as pluronic® 105/pluronic® F-127, pluronic® P123/pPluronic® F127, and polysorbate 80/pluronic® F-127 were shown to inhibit the P-gp-mediated efflux of docetaxel (Chen et al., 2013), paclitaxel (Wei et al., 2010), and morin (Choi et al., 2015), respectively, in cells highly over expressing P-gp (Table S1). Despite the fact, that pluronic® F-127 was used in the latter formulations and proposed to inhibit P-gp in another study (Guan et al., 2011), several studies reported that pluronic® F-127 did not inhibit P-gp mediated efflux of several P-gp substrates e.g.such as rhodamine 123 (Batrakova et al., 2003b; Wei et al., 2013), nelfinavir (Shaik et al., 2008), etoposide (Al-Ali et al., 2018a), and digoxin (Gurjar et al., 2018). In these types of formulations, one limitation could be that the drug substances might also be adsorbed to the core of surfactant micelles, which may decrease the free fraction of unbound substrate in the formulation, thus affecting the subsequent oral absorption and bioavailability of the substrate *in vivo*. Therefore, investigating the release of drug substances from micelles is important and should be performed *in vitro* in order to avoid or understand such impacts of surfactants *in vivo*. The LBFs containing nonionic surfactants that have P-gp inhibitory properties seem, however, as a promising approach to improve P-gp substrates permeability across cellular membranes.

~~4.1.3~~4.1.2 Nonionic surfactants inhibited P-glycoprotein *in vitro* at below and above critical micelle concentrations

In several studies performed in cell cultures, it was observed that the inhibition of P-gp transport activity by nonionic surfactants decreased at concentrations at or above the surfactants critical micelles concentration (CMC) compared to concentration below CMC. It was reported that pluronic® P85 (Batrakova et al., 2003b; Batrakova et al., 2004), cremophor® EL (Shono et al., 2004), and polysorbate 40 (Zhu et al., 2009) were effective in inhibiting P-gp transport activity at concentrations lower than their CMCs *in vitro*; however, this inhibitory effect decreased at or above the surfactants CMC values (Batrakova et al., 2003a; Batrakova et al., 2003b; Batrakova et al., 2004; Shono et al., 2004; Zhu et al., 2009) (Table 2 and S1). A possible explanation to this observation could be that most P-gp substrates are lipophilic substances, which after incorporation into the hydrophobic core of micelles, lead to a decrease in the unbound fraction of the substrate available for the transcellular transport, and hence the observed reduction in substrate transport, termed the solubility/permeability interplay (Beig et al., 2017; Beig et al., 2015; Dahan et al., 2010; Miller et al., 2011).

In addition, using the parallel artificial membrane permeability assay (PAMPA), the passive permeability of the P-gp substrate paclitaxel across artificial membranes have been shown to decrease significantly when the TPGS 1000 concentration in the donor chamber was above the CMC (Varma and Panchagnula, 2005). Furthermore, using PAMPA, increasing the concentration of the surfactants such as sodium lauryl sulfate above the CMC values decreased the passive permeability of etoposide across artificial membranes (Beig et al., 2015). The PAMPA studies may support the hypothesis that the decreased permeability of the substrates was related to the incorporation of paclitaxel and etoposide

in the micelles, this may support the effect of surfactant on the thermodynamic activity of the substrate rather than the decreased inhibitory effect of surfactant above the CMC.

Moreover, many studies have reported that nonionic surfactants, used at concentrations above their CMCs, inhibited the efflux of P-gp substrates *in vitro* such as (Surfactant: P-gp substrate/s): (cremophor® RH 40: rhodamine 123) (Kiss et al., 2014), (Brij® 58: digoxin and rhodamine 123) (Gurjar et al., 2018; Zhao et al., 2016), (cremophor® EL: etoposide and digoxin) (Al-Ali et al., 2018a; Gurjar et al., 2018), (Labrasol®: rhodamine 123) (Lin et al., 2007), and (polysorbate 20: etoposide, doxorubicin, digoxin, and epirubicin) (Al-Ali et al., 2018b; Al-Saraf et al., 2016; Gurjar et al., 2018; Lo, 2003). Consequently, it seems that nonionic surfactants at concentration higher than their CMC values are able to inhibit P-gp *in vitro*; however, care should be taken in the interpretation due to the potential influence ~~for~~ of the solubility/permeability interplay.

~~4.1.4~~4.1.3 Polyethylene glycol (PEG) derivatives inhibited P-glycoprotein *in vitro*

In addition to nonionic surfactants, co-surfactants such as polyethylene glycol (PEG) derivatives are used in a broad spectrum of drug delivery systems where these excipients are used as solubilizers, stabilizers, release-modifiers, and bioavailability enhancers (D'Souza and Shegokar, 2016). From Table 2 and S1, it can be noticed that several PEG derivatives with different molecular weight including PEG 300, PEG 400, PEG 2000, PEG 6000, and PEG 20000 were reported to decrease the efflux of several P-gp substrates in Caco-2 cells and in rat intestinal segments. *In vitro*, it has been shown that PEG 400 at concentration of 0.1-20% (w/v, or v/v) may decrease the efflux of several P-gp substrates e.g. digoxin (Johnson et al., 2002), ranitidine (Ashiru-Oredope et al., 2011), and rhodamine 123 (Hodaei et

al., 2015; Shen et al., 2006). Furthermore, recent studies have reported that PEG 400 may decrease the P-gp expression in Caco-2 cells (Hodaei et al., 2015) and increase the P-gp ATPase activity (Ashiru-Oredope et al., 2011), suggesting two different mechanism of P-gp inhibition. The latter effect might refer to the direct interaction of PEG 400 with P-gp, thus competitively inhibited the protein (Ashiru-Oredope et al., 2011). Moreover, PEG 300 was reported to inhibit P-gp through altering Caco-2 membrane fluidity (Hugger et al., 2002), suggesting a third mechanism of PEG derivatives mediated P-gp inhibition *in vitro*.

~~4.1.5~~4.1.4 Nonionic surfactants increased the oral absorption of P-glycoprotein substrates *in vivo*

The impact of nonionic surfactants on the oral absorption of P-gp substrates have mainly been investigated in wild type rats~~,~~ (Table 3). Several surfactants such as polysorbate 20 and 80 have been shown to increase the oral absorption of different P-gp substrates ~~such as polysorbate 80, which has been shown to enhance the oral absorption of~~ such as digoxin (Zhang et al., 2003), etoposide (Zhao et al., 2013), and rifampicin (Shimomura et al., 2016). The concentrations of surfactants that increased the oral absorption of different P-gp substrates range from 1-25% (w/v)~~,~~ (~~see~~ Table 3). Recently, wild type and *mdr1a* deficient rats have been used to investigate the role of intestinal P-gp for the oral absorption of digoxin (Nielsen et al., 2016) and etoposide (Al-Ali et al., 2018a). In these studies, there was approximately 2- and 8-fold increase in the AUC of digoxin and etoposide, respectively, in *mdr1a* deficient rats compared to wild type rats. When 5% and 10-25 % (v/v) polysorbate 20 was co-administered with etoposide and digoxin, respectively, in wild type rats, the oral bioavailability was enhanced significantly. However, in *mdr1a* deficient rats, the presence or absence of similar doses of the surfactant did not influence the bioavailability indicating that the enhanced oral absorption in the

wild type rats was most likely related to P-gp inhibition effects mediated by polysorbate 20 rather than enhancement of the substrate solubility by the surfactant.

In relation to scaling between *in vitro* and *in vivo* studies of the surfactants, it has been shown that a concentration of 20-500 µM polysorbate 20 decreased the efflux ratio of digoxin and etoposide in cell cultures (Al-Ali et al., 2018a; Nielsen et al., 2016), whereas in pre-clinical studies in wild type rats, the minimum doses of polysorbate 20 required to increase the oral bioavailability of digoxin (Nielsen et al., 2016) and etoposide (Al-Ali et al., 2018a) were 10% (v/v, 90 mM) and 5% (v/v, 45 mM), respectively. However, in *mdr1a* deficient rats, it was noticed that the oral absorption and bioavailability of etoposide decreased when co-administered with 25% (v/v) polysorbate 20 compared to the oral absorption with 0 or 5% (v/v) polysorbate 20 (Al-Ali et al., 2018a). *In vitro* dialysis studies demonstrated that etoposide release from a 25% polysorbate 20 containing formulation was minimal, most likely due to the incorporation of etoposide into the micelles (Al-Ali et al., 2018a). Furthermore, it has been reported that 5% labrasol® increased the oral bioavailability of etoposide more than 10% labrasol® (Akhtar et al., 2017), indicating that etoposide release from the micelles was concentration dependent (Akhtar et al., 2017), (Table 3).

TPGS 1000 enhanced the oral bioavailability of paclitaxel in wild type rats with a factor of six relative to the bioavailability when administered without the surfactant (control) (Varma and Panchagnula, 2005), (Table 3). Verapamil was further demonstrated to enhance the oral bioavailability of similar doses of paclitaxel four times compared to control (Varma and Panchagnula, 2005). With respect to the use of surfactants *in vivo*, it should be noted that some of these surfactants undergo digestion in the intestinal tract (Christiansen et al., 2010; Cuiné et al., 2008; Devraj et al., 2013; Mohsin, 2012),

and might thus be less efficient ~~the use of these relative to~~than small-molecular P-gp inhibitors like verapamil~~, should be considered with respect to the hypothesis of the studies~~.

From Table 2, 3 and S1, it is evident that research is still needed to establish how inhibition observed *in vitro* translates into increased absorption *in vivo*, since many nonionic surfactants such as Brij® 78, pluronic® P123, and polysorbate 40, have not yet been investigated for their abilities to inhibit intestinal P-gp *in vivo*. Moreover, for some surfactants that were extensively investigated *in vitro* such as cremophor® EL, pluronic® 85 and Solutol® HS 15, (see also Table 2), only few *in vivo* studies have reported the effect ~~on PK~~ of these surfactants on PK in animals (Bittner et al., 2002; Föger et al., 2006; Zhao et al., 2013), (Table 3). Consequently, further *in vivo* studies are needed to advance the knowledge about the effect of ~~these~~ surfactants on the oral absorption of different P-gp substrates, which may assist in designing and performing clinical studies in humans.

4.2 Impact of nonionic surfactants on breast cancer resistance protein

The ABC transporter BCRP is as a monomeric protein of 72 kDa (Doyle and Ross, 2003; Mao, 2005) consisting of one TM domain of six TM helices, and one NBD located in the cytoplasm (Chen et al., 2015; Mao and Unadkat, 2015; Wang et al., 2008). Two BCRP monomers dimerize to form a functional BCRP transporter (Fig. 1b) (Rosenberg et al., 2010; Rosenberg et al., 2015). The helices are arranged to form a cavity, where BCRP substrates bind, while TM helices one and six are attached to amino and carboxyl termini in the cytoplasm, respectively (Wang et al., 2008). BCRP substrates belong to different therapeutic classes such as anticancer drugs, HIV drugs, antihistamines, and anti-hyperlipidemia drugs (Mao and Unadkat, 2015). BCRP shares many substrates with P-gp e.g.

topotecan (Jonker et al., 2000; Maliepaard et al., 1999), doxorubicin (Allen et al., 1999; Mechetner et al., 1998), irinotecan (Gupta et al., 1996; Maliepaard et al., 1999), and etoposide (Allen et al., 2003; Keller et al., 1992). In humans, BCRP is highly expressed in normal tissues such as the apical membrane of small intestinal and colonic enterocytes, canalicular membranes in the liver, endothelial cells of brain microvessels (Mao, 2005), veins and capillaries, and in cancer cells (Doyle and Ross, 2003; Maliepaard et al., 2001b). Since BCRP is expressed in different tissues, its modulation in humans may influence the ADMET properties of its substrates. In the past, BCRP inhibitors were developed to overcome the multidrug resistance phenomenon, as well as to enhance the oral absorption of the substrates (Gupta et al., 2006; Gupta et al., 2004; Houghton et al., 2004; Matsson et al., 2009). BCRP inhibitors may act as competitive inhibitors at the substrate binding sites, as allosteric inhibitors by binding to the protein at a site different from the substrate binding site ~~in the BCRP cavity~~, or by inhibiting ATPase activity (Mao and Unadkat, 2015). Of the BCRP inhibitors identified; some of these also inhibit P-gp e.g. GF120918, and the tyrosine kinase inhibitors imatinib, and the antifungal drug substance itraconazole (Mao and Unadkat, 2015; Matsson et al., 2009).

Until now, few s~~S~~tudies ~~were performed by Yamagata and co-workers to~~ have investigated~~ing~~ the effect of nonionic surfactants on BCRP transport activity *in vitro* and *in vivo* (Sawangrat et al., 2018a; Sawangrat et al., 2018b; Xiao et al., 2016; Xu et al., 2015; Yamagata et al., 2007a, b; Yamagata et al., 2009). In MDCKII BCRP cells, nonionic surfactants such as cremophor® EL, polysorbate 20, span 20, pluronic® P85, and Brij® 30 increased the uptake of the BCRP substrate mitoxantrone (Yamagata et al., 2007a) (Table 4). Yamagata and coworkers were also able to enhance the uptake of mitoxantrone in MDCKII MDR1 by the use of the same surfactants indicating the ability of these surfactants to modulate both BCRP and P-gp (Yamagata et al., 2007a), effects that w~~a~~sere demonstrated both *in vitro*

(Al-Ali et al., 2018a; Al-Ali et al., 2018b; Al-Saraf et al., 2016; Gurjar et al., 2018; Li-Blatter and Seelig, 2010; Lo, 2003; Nielsen et al., 2016; Rege et al., 2002; Shaik et al., 2008), and *in vivo* (Al-Ali et al., 2018a; Föger et al., 2006; Nielsen et al., 2016; Zhao et al., 2013), for further details see ~~(see~~ Table 2, 3 and S1~~)~~. With respect to the effect of nonionic surfactants on BCRP, ~~it~~ another study ~~was~~ reported that pluronic® P85 and polysorbate 20 enhanced the ~~mucosal-to-serosal~~M-S transport of BCRP substrate topotecan across ileum everted sacs derived from wild type mice (~~see~~ Table 4) (Yamagata et al., 2007b). Interestingly, in everted intestinal sacs derived from *Abcg2* deficient mice, topotecan absorption rate was significantly enhanced in comparison to the absorption rate in everted intestinal sacs from wild type mice (Yamagata et al., 2007b). However, the presence of surfactants did not further improve the absorption rate of topotecan in *Abcg2* deficient everted intestinal sacs, demonstrating the surfactants' impact~~s~~ in mediating ~~the~~ Bcrp inhibition in the wild type animals (Yamagata et al., 2007b). *In vivo*, pluronic® P85 and polysorbate 20 administered orally 15 min before oral administration of topotecan to wild type mice increased the AUC of topotecan significantly, when compared to the administration of similar doses of topotecan without the surfactant (Yamagata et al., 2007b). It was later noticed that the interaction of pluronic® P85 and polysorbate 20 with Bcrp was reversible and transient upon removal of these surfactants (Yamagata et al., 2009). It is worth noticing that these surfactants were also able to inhibit P-gp *in vitro* (Al-Ali et al., 2018a; Al-Ali et al., 2018b; Al-Saraf et al., 2016; Batrakova et al., 2003a; Batrakova et al., 2004; Gurjar et al., 2018; Nielsen et al., 2016; Shaik et al., 2008) (Table 2, S1) and *in vivo* (Al-Ali et al., 2018a; Föger et al., 2006; Nielsen et al., 2016) (Table 3).

Moreover, ~~R~~recent research has reported that several surfactants, including 6-tetradecyl-β-D-maltopyranoside, (C6-malt) (Xu et al., 2015), cremophor® EL (Al-Ali et al., 2018a; Al-Saraf et al.,

2016; Gurjar et al., 2018; Rege et al., 2002; Shono et al., 2004), BL-9EX, Brij® 92, Brij® 97 (Zhao et al., 2016), and labrasol® (Akhtar et al., 2017; Cornaire et al., 2004; Lin et al., 2007; Ma et al., 2011), which have been shown to inhibit P-gp (Table 2), ~~have~~ also ~~been able to~~ inhibit BCRP in MDCKII BCRP cells (Xiao et al., 2016), in membrane vesicles containing human BCRP (Xu et al., 2015), and ~~Bcrp~~ in rat intestinal membrane and *in vivo* using the *in situ* closed intestinal loop method (Sawangrat et al., 2018a). The doses of the surfactants used to inhibit BCRP *in vitro* and *in vivo* (Sawangrat et al., 2018a; Xiao et al., 2016; Xu et al., 2015) were comparable to the doses used to inhibit P-gp (Akhtar et al., 2017; Lin et al., 2007; Ma et al., 2011; Zhao et al., 2016) (Table 2, 3, and S1). As similar doses of nonionic surfactants appeared to be able to inhibit P-gp and BCRP, and since many drug substances that are BCRP substrates share substrate specificity with P-gp, drug formulators should thus consider the surfactants used in their formulations, and avoid using the surfactants that may have overlap in inhibiting effect on both transporters in cases where this may have an influence on the biopharmaceutical properties of the ~~compound~~substrate. Cremophor® EL was shown to enhance the absorptive permeability and decrease the secretory permeability of scutellarin in MDCKII BCRP (Xiao et al., 2016). Scutellarin is a flavonoid glucuronide approved in China to treat patients with cerebral infarction and paralysis caused by cerebrovascular diseases (Xiao et al 2016). In wild type rats, cremophor® EL enhanced scutellarin oral absorption, however, this study also reported that the surfactant affected other transporters such as MRP2 and MRP3 (See section 4.3). Another ~~recent~~ study by Sawangrat and co-workers showed that 0.05 % (w/v) cremophor® EL enhanced the absorptive permeability and decreased the secretory permeability of topotecan significantly in Caco-2 cells, and enhanced the intestinal absorption of topotecan in rats using the *in situ* closed-loop method (Sawangrat et al., 2018b). Similar concentration of cremophor® EL did, however, not influence the absorptive or secretory permeability of the BCRP substrate sulfasalazine across rat intestinal segments in diffusion

chambers (Sawangrat et al., 2018a). Furthermore, using the *in situ* closed-loop method, 0.05% (w/v) polysorbate 20 enhanced the intestinal absorption of topotecan significantly in rats (Sawangrat et al., 2018b). In contrast~~; however~~, higher concentration (0.1 and 0.5% w/v) of the surfactant did not enhance the absorption of sulfasalazine in another study (Sawangrat et al., 2018a). The effect of nonionic surfactants on the transport of BCRP substrates across the intestine therefore seems to differ as a function of substrate and/or method used. Further studies are needed to investigate the effects of nonionic surfactants on the transport activity of BCRP *in vitro* and *in vivo*. It is recommended that different BCRP substrates and different methods are used in the prospective investigations.

4.3 Impact of nonionic surfactants on ~~the~~ multidrug resistance-associated protein 2

In human tissues, the efflux transporter multidrug resistance-associated protein 2 (MRP2) is expressed in the hepatocyte canalicular membrane, gallbladder epithelial cells, the proximal tubule of the kidney, duodenum, jejunum, ileum, brain, bronchi, and placenta (Jedlitschky et al., 2006; Kool et al., 1997; Nies and Keppler, 2007). MRP2 is highly expressed in cancer cells, such as non-small cell lung cancer and adeno-lung carcinoma (Kool et al., 1997). MRP2 consists of two TM domains, each has six TM helices, linked intracellularly with two NBDs located in the cytoplasm (Fig. 1c) (Jedlitschky et al., 2006). To the NBDs, ATP molecules bind, which is required for hydrolysis initiating substrate transport (Jedlitschky et al., 2006). In addition, a third TM domain consisting of five helices is attached to the first TM domain via a linker (L0), which is located in the cytoplasm. The third TM domain is extracellularly attached to NH_2 terminus of the first TM domain (~~see~~ Fig. 1c) (Jedlitschky et al., 2006). MRP2 transports different endogenous compounds such as glutathione, leukotrienes, bilirubin

glucuronides and steroids, and drug substances of different classes, e.g. anticancer drugs, HIV drugs, antibiotics, and the metabolites of these substances (Dietrich et al., 2003; Jedlitschky et al., 2006).

The effect of nonionic surfactants on MRP2 has been investigated *in vitro* using different assays, e.g. bi-directional transport, uptake assay, ATP measurements, and phosphate release measurements. It was reported that pluronic® P85 enhanced the intracellular accumulation of the MRP2 substrates vincristine and doxorubicin in MDCKII MRP2 cells (Batrakova et al., 2003a). This was confirmed by decreased ATP levels in MDCKII MRP2 cells and decreased ATPase activity in the membrane vesicles isolated from these cells (Batrakova et al., 2004). Based upon these data, Batrakova and co-workers proposed that the mechanism of MRP2 inhibition could be related to the change in membrane fluidity or binding of the surfactant to the cell membrane, thereby competitively preventing the drug-protein interaction (Batrakova et al., 2004). Beside the effect of pluronic® P85 on MRP2, it was found that similar concentrations of the surfactant inhibited MRP1 and P-gp (Batrakova et al., 2004).

Recent studies have shown that surfactants including cremophor® EL, cremophor® RH 40, pluronic® F68, and pluronic® P-127, and co-surfactants PEG 400, and PEG 2000 decreased the efflux ratio of the MRP2 substrate scutellarin in Caco-2 cells (Li et al 2013, Li et al 2014). ~~Scutellarin is a flavonoid glucuronide approved in China to treat patients with cerebral infarction and paralysis caused by cerebrovascular diseases (Xiao et al 2016).~~ Scutellarin has a poor oral bioavailability, which is partly related to the efflux effect of membrane transporters such as MRP2 and BCRP (~~see~~ Table 4 and 5). In agreement with Li and co-workers (Li et al 2013, Li et al 2014), Chen *et al.* have shown that pluronic® F68, pluronic® F-127, pluronic® P85, and pluronic® P105 increased A-B permeability and decreased B-A permeability of the MRP2 substrate, baicalein, in MDCK MRP2 cells (Chen et al., 2017), (Table 5).

Chen and co-workers have suggested that these observations were due to MRP2 inhibition (Chen et al., 2017).

In contrast, vinblastine transport across MDCKII MRP2 cells was not affected significantly by 0.1 % (w/v) pluronic® L61 (Evers et al., 2000). Likewise, using a 5-chloromethylfluorescein diacetate (CMFDA) based accumulation assay in MDCK-MRP2 cells, Bogman *et al.* (2003) found that the surfactants TPGS 1000, cremophor® EL, polysorbate 80, pluronic® F68, pluronic® L61, and pluronic® L81 were unable to inhibit MRP2-mediated methylfluorescein-sulfoglutathione complex (MF-SG) transport (Bogman et al., 2003).

From Table 4 and 5, it can be noticed that the dose of cremophor® EL needed to inhibit MRP2 or BCRP in cell cultures and in rats are similar (Xiao et al 2016). Xiao and co-workers have also reported that cremophor® EL was able to activate the efflux protein MRP3 (Xiao et al 2016), which was found to be expressed on the basolateral membrane of enterocytes (Kool et al., 1997; Kool et al., 1999). Therefore, effects of cremophor® EL on scutellarin seems to be related to the effect on multiple efflux transporters. Thus, activating the efflux transporters, being located in the basolateral membrane in enterocytes by surfactants could also be a strategy to improve the absorption of substrate drug substances across intestinal membranes; however, further investigations are needed for this to be a robust formulation strategy.

An interesting finding reported was that pluronic® F-127 decreased the efflux ratio of scutellarin and baicalein in Caco-2 cells (Li et al 2013, Li et al 2014) and MDCKII MDRP2 (Chen et al., 2017), respectively, but had no inhibitory effect on the P-gp substrates rhodamine 123 (Batrakova et al., 2003b), nelfinavir (Shaik et al., 2008), etoposide (Al-Ali et al., 2018a), and digoxin (Gurjar et al., 2018). Consequently, it may be that there is limited cross inhibitory effects for surfactants inhibiting

MRP2 towards other efflux membrane transporters and vice versa. The ability of nonionic surfactants to inhibit MRP2-mediated transport seems to be complex and dependent on the model system employed to investigate and understand the influence of the surfactant. Therefore, further studies and specific MRP2 model systems are needed to understand the consequences of MRP2 inhibition by nonionic surfactants.

5. Nonionic surfactants modulate solute carriers *in vitro*

In humans, solute carriers transport endogenous and exogenous (Yu Liang Siqi Li Ligong, 2015), charged, and uncharged substrates (Koepsell et al., 2007), in and/or out of cells in different tissues, e.g. intestine, kidney, liver and brain (Giacomini et al., 2010). The SLC family consists of 62 sub-families (HUGO Gene Nomenclature Committee, 2019). For a protein to be assigned to the SLC family they need to be responsible for membrane solute transport and to have an amino acid identity of > 20% to other members of the family (Hediger et al., 2013). Within the SLC family uniporters, symporters and antiporters are found. The symporters may depend on the driving force of ions such as K^+, Na^+, H^+, or Cl^-, and at a cellular level, they are therefore known as secondary-active transporters (e.g. K^+, Na^+, or Cl^- -dependent carriers) or tertiary-active transporters (H^+-dependent carriers), because the cellular homeostasis of ions eventually will involve transport by the active NA^+/K^+-ATPase enzyme. The use of transporter in this context is a reminiscence of a notion and literature source present prior to the establishment of the SLC system, which was pioneered by Hediger (Hediger, 2004; Hediger et al., 2013).

644 SLC proteins are diverse in their structures, however, the most common predicted folds of this family
645 proteins are the Major Facilitator Superfamily (MFS, LacY) and the Leucine transporter (LeuT) that
646 has folding consisting of 12 and 10 TM helices, respectively. However, some carriers in the SLC
647 family may possess a lower number of helices, e.g. the glucose uniporter that has a unique fold of
648 seven TM helices (Colas et al., 2016). For the LacY like fold (Fig. 1d), the protein is oriented in a V-
649 shape conformation opened to the extracellular side of plasma membrane where the substrate is
650 assumed to bind. The substrate may then move to an intermediate state inside the protein, before it may
651 release from the inverted V-shape conformation of the SLC to the cytoplasm (Fig. 1d) (Colas et al.,
652 2016).

653 The oral absorption of a large variety of important nutrient such as amino acids, sugars, peptides, fatty
654 acids, and vitamins are mediated by carriers, which are important for oral absorption of drug substances
655 that are structurally similar to the nutrients (Steffansen et al., 2004). Despite the large number of
656 carriers expressed in the intestine, the effect of nonionic surfactant on these transport systems is largely
657 uninvestigated. In 2002, Rege and co-workers reported that polysorbate 80 decreased the absorptive
658 permeability of the prototypic PEPT1 substrate glycyl-sarcosine and cremophor® EL decreased the
659 transport of the monocarboxylic acid transporter (MCT) substrate benzoic acid in Caco-2 cells (Rege et
660 al., 2002), (Table 6). Recently, these surfactants have been shown to inhibit other carriers in transfected
661 cells models. Polysorbate 80 and cremophor® EL inhibited OCT1-3 and PEPT2 in MDCKII OCT1-3
662 cells and MDCKII PEPT2 cells, respectively. From Table 6, it can be noticed that polysorbate 80
663 appeared more potent than cremophor® EL with respect to inhibition of OCT1-3 and PEPT2 in cell
664 cultures (Otter et al., 2017; Soodvilai et al., 2017). In addition, it was reported that cremophor® EL
665 inhibited OATP1A2 and OATP2B1 in HEK OATP1A2 and HEK OATP2B1 cells, respectively (Engel

et al., 2012). Furthermore, poloxamer 188 and 407 (Otter et al., 2017), and polysorbate 20 and 60 (Otter et al., 2017; Soodvilai et al., 2017), have shown different abilities to inhibit the organic cation transporters in cell cultures. In MDCKII OCT1 cells, the estimated IC_{50} of poloxamer 407 (pluronic® F-127) was approximately 2600-fold and 900-fold higher than the estimated IC_{50} of polysorbate 80 and polysorbate 20, respectively (Otter et al., 2017).

Surfactants inhibiting carriers (Table 6) were also reported to inhibit transporters (Table 2-5, and S1) in cell cultures as exemplified by the observations that Solutol® HS 15 inhibited OATP1A2, OATP2B1 (Engel et al., 2012), OCT1-3, PEPT2 (Otter et al., 2017), and P-gp (Akhtar et al., 2017; Buckingham et al., 1995; Coon et al., 1991; Cornaire et al., 2004; Gurjar et al., 2018; Lamprecht and Benoit, 2006); polysorbate 80 inhibited OCT1-3, PEPT1-2 (Otter et al., 2017; Rege et al., 2002; Soodvilai et al., 2017), P-gp (Al-Ali et al., 2018b; Al-Saraf et al., 2016; Cornaire et al., 2004; Hanke et al., 2010; Kiss et al., 2014; Lo, 2003; Nerurkar et al., 1996; Nielsen et al., 2016; Shono et al., 2004; Woodcock et al., 1992; Yu et al., 2011), and MRP2 (Hanke et al., 2010); and cremophor® EL inhibited OATP1A2, OATP2B1 (Engel et al., 2012), OCT1-3, PEPT2 (Otter et al., 2017), MCT (Rege et al., 2002), P-gp (Al-Ali et al., 2018a; Al-Saraf et al., 2016; Buckingham et al., 1995; Chiu et al., 2003; Nerurkar et al., 1996; Rege et al., 2002; Shono et al., 2004; Woodcock et al., 1990; Woodcock et al., 1992), BCRP (Sawangrat et al., 2018a; Xiao et al., 2016; Yamagata et al., 2007a), and MRP2 (Hanke et al., 2010; Li et al., 2013a; Xiao et al., 2016). Cremophor® EL seems to be the surfactant with the widest range of inhibition of different carriers and transporters. Importantly, impact of surfactant on transporters and carriers simultaneously may lead to unpredictable drug-transporter interactions. Therefore, further knowledge about the ability of nonionic surfactants to inhibit carriers under relevant *in vivo* conditions

is needed for drug formulators to make enlightened choices on nonionic surfactants as pharmaceutical excipients.

As generally presented in this review, a broader class of surfactants that are often used as pharmaceutical excipients, may have effects on drug absorption through interactions with either transporters, or carriers, or both. While a lot of insights have been generated *in vitro*, less is available *in vivo* from non-clinical trials, and no information is publicly available from human trials systematically investigating the influence of pharmaceutical excipients on transporters or carriers. Drug prescribers and pharmacists should therefore be aware that in treatment of patients with polypharmacy prescriptions of drug compounds that are known substrates to transporters or carriers should be administered separately from drug products containing nonionic surfactants and co-surfactant polymers in order to avoid unexpected interactions of drug-excipient at transporters and/or carriers, as this might lead to unpredicted side effects.

6. Conclusion

From the present review, it is quite evident that pharmaceutical excipients are not just compounds required for processing drug formulations, but they also possess the ability to alter drug transport across biological barriers by interacting with transporters and carriers. Pharmaceutical excipients frequently used in enabling formulations, notably nonionic surfactants, alter the function of carriers and/or transporters, thereby affecting drug transport. The main body of evidence for this is based on *in vitro* experiments using cell culture models or excised tissue, whereas pre-clinical studies available in the literature are limited. Few studies have investigated if surfactants reduce transporter-mediated transport

and thereby increase the oral bioavailability; while to the best of our knowledge no *in vivo* study has investigated if excipients inhibit carriers *in vivo*, and hence could decrease oral bioavailability. Therefore, more pre-clinical studies are needed to investigate if surfactants at concentration likely to be reached in the intestinal lumen may alter the exposure of substrates of transporters and carriers. It seems likely that inhibition of transporters by surfactants could be incorporated into a formulation approach, while a potential inhibition of solute carriers should be avoided as this would decrease oral absorption. The key points missing are what the scaling between *in vitro* and *in vivo* effects is and if enabling formulations containing surfactants are safe i.e. without toxic effects. Such biopharmaceutical insight may assist in the active development of formulations where excipients are bioactive components included for inhibition of intestinal efflux transport. Interestingly, some surfactants, e.g. cremophor® EL, Solutol® HS 15 and polysorbate 20, have been shown to share inhibiting effects on several transporters and carriers *in vitro*. In addition, the concentration of surfactants that inhibit the efflux transporters P-gp, BCRP, and MRP2 were comparable in cell cultures. Hence, when drug substances such as doxorubicin and etoposide are substrates for multiple efflux transporters, co-administration of these with surfactants can generate a complex absorption mechanism.

As more and more discovered compounds have limited aqueous solubility, the need for enabling formulations, that may include surfactants, are increasing. Given that surfactants can have multiple physico-chemical as well as biopharmaceutical properties, drug formulators may need to bring this perspective into consideration when defining the formulations of the future.

Declaration of interest

729 The authors do not have any conflict of interest to report.

730

731 **Author contribution**

732 Writing - original draft: AAAA, CUN, and RBN. Writing - review & Editing: AAAA, RBN, BS, RH
733 and CUN. Final approval of the version submitted: AAAA, RBN, BS, RH and CUN.

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Figures Legend:

Figure 1: Structures of membrane transport proteins in the absence and presence of a substrate.

Cartoons illustrate: **a)** Inward-facing P-glycoprotein (P-gp) (mouse Abcb1, left) and the outward-facing P-gp (human ABCB1, right, substrate release), transmembrane domain 1 (TMD1) (1-6 transmembrane helices (TMH)), TMD2 (7-12 TMH), extracted with modifications from (Aller et al., 2009; Kim and Chen, 2018); **b)** Two monomers of breast cancer resistance protein (BCRP), BCRP monomer-1 (1-6 TMH, white) and -2 (1-6 TMH, dark gray), substrate-free state (left) using MsbA from *Escherichia coli* as a template, substrate-bound state (right) using mouse Abcb1 as the template, extracted with modifications from (Rosenberg et al., 2010; Rosenberg et al., 2015); **c)** Multidrug resistance-associated protein 2 (MRP2), TM0 (1-5 TMH), TMD1 (6-11 TMH), TMD3 (12-17 TMH), Lasso motif (L_0), left is when substrate-free state, and right when substrate-bound state, the molecular structure is determined using bovine Mrp1 as a template, extracted with modifications from (Dallas et al., 2006; Johnson and Chen, 2017); **d)** Lactose permease of *Escherichia coli* (LacY) representing a solute carrier (SLC) member with 12 TMHs, LacY consists of two segments, each containing two repeat units of three TMHs (1-3, 4-6, 7-9 and 10-12) as dark gray, black, white and light gray rods, respectively , outward-open conformation (V-shape, substrate-free state, left) and inward-open conformation (inverted V-shape, substrate-released state, right) facing the extracellular and cytoplasmic side of the cellular membrane, respectively, extracted with modifications from (Colas et al., 2016; Kumar et al., 2018; Kumar et al., 2014; Radestock and Forrest, 2011). Nucleotide binding domain (NBD), adenosine tri-phosphate (ATP), TMHs are depicted as rods, straight-dashed arrow represents the direction of substrate movement, curved arrow represents the direction of helices movement during conformational changes, post-translational modifications are not shown in the sub-figures, and black circle is a substrate.

Figure 2: Proposed mechanisms of P-glycoprotein inhibition by nonionic surfactants.

Cartoon shows: I) Surfactant-P-gp interaction via hydrogen bonding, the hydrophobic moiety of the surfactant partitions into the cell membrane, while the hydrogen bond acceptor groups in the hydrophilic moiety of the surfactant form hydrogen bonds with the hydrogen bond donor groups in P-gp, II) Alteration of membrane fluidity and/or (III) depleted intracellular ATP. Transmembrane helices are depicted as rods. Nucleotide binding domain (NBD), adenosine tri-phosphate (ATP), adenosine diphosphate (ADP), extracellular (Ex.), intracellular (In.), black triangle (P-gp substrate), red circle attached to a tail (nonionic surfactant), and black dashed lines (hydrogen bonds).

Table 1: Expression of selected transporters and carriers along the human intestine and in Caco-2 cells.

Logarithmic 10-step color scale and annotation of expression levels (very low-very high) have *arbitrarily* been defined for overview in the range 0-15 pmol/mg total protein and 0-450 fmol/mg total tissue[d]:

To be inserted as a footnote under Table 1: LC-MS/MS-determined protein concentrations (pmol/mg total protein) of selected transporters and carriers in Caco-2 cells and segments of the human gastrointestinal tract. Protein concentrations from Caco-2 cells were obtained three weeks after seeding. Caco-2 cells were from three different sources: American Type Culture Collection (ATCC)[a], The European Collection of Authenticated Cell Cultures (ECACC)[b], and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)[c]. The average values are depicted without statistical deviation parameters. For (Akazawa et al., 2018), the average were obtained from two reported values from two humans, and if one of the two values was below the lower limit of quantification (LLOQ), the other value is depicted. Intestinal segments were adapted from (Drozdzik et al., 2014): Duodenum (D),

jejunum (J1-2), ileum (I1-2), and colon (C1-4). BLQ = below the LLOQ. ~~Logarithmic 10-step color scale and annotation of expression level (very low-very high) have *arbitrarily* been defined for overview in the range 0-15 pmol/mg total protein and 0-450 fmol/mg total tissue[d].~~

Table 2: *In vitro* ~~I~~impact of selected nonionic surfactants and polyethylene glycol (PEG) derivatives on P-glycoprotein ~~*in vitro*~~.

To be inserted as a footnote under Table 2: Accumulation (accum.), Approximately (approx.), Respectively (resp.), Surfactant (surf.), Concentration (conc.), Dependent (dep.), Not specified in the study (ns), Apical to basolateral (A-B), Mucosal to serosal (M-S), Permeability (P_{app}), Any increase or decrease described in the table means significant $P<0.05$, Resistance Modification Index (RMI), Mouse embryo fibroblasts transfected with MDR1 (NIH-MDR1-G185), Mouse embryo fibroblasts transfected with MDR1 (NIH-MDR1-G185), P-gp overexpressing human melanoma cell line (MDA-MB-435/LCC6MDR1), P-gp overexpressing human ovarian carcinoma cell line (NCI/ADR-RES), Adriamycin-resistant of murine leukaemia P388 cells (P388/ADR), P-gp variant of human epithelial cells KB 3-1 (KB 8-5-11 cells), In Vitro Diffusion Chamber Method (In vitro DCM), ATPlite 1step Assay kit was from PerkinElmer, P-gp containing membranes of Chinese hamster lung fibroblasts (DC-3F/ADX cells), MDR cell subline of Chinese hamster ovary cells Aux-B1(CH^{r}C5), Bovine brain microvessel endothelial cells (BBMEC), Vinblastine-resistant derivative of Human Caucasian acute lymphoblastic leukaemia CCRF-CEM cells (R100 cells), Human lung adenocarcinoma cell line A549 treated with paclitaxel (A549/Taxol), Porcine kidney epithelial cell line (LLC-PK1-MDR1), LLC-PK1 stably expressing MDR1 (LLC-MDR1), P-gp overexpressing human oral epidermal carcinoma (KBv), For cremophor® EL and Solutol® HS 15, RMI was measured at 10 µg/mL, P-gp overexpressing human melanoma cell line (MDA-MB-435/LCC6MDR1), P-gp overexpressing human ovarian carcinoma cell

line NCI/ADR-RES, MDR cell subline of human breast carcinoma MCF-7 cells (MCF7/ADR), Resistance reversion index (Log (IC_{50}.0/IC_{50})) was determined as a ratio of IC_{50} of Doxorubicin in the assay buffer and surfactant solution, Vincristine-resistant derivative of K562 (7962 cells), Human lung cancer cells (A549), Human P-gp overexpressing membranes obtained from baculovirus-infected insect cells (High Five, BTI-TN5B1-4), Clonal isolate derived from the Spodoptera frugiperda cell line IPLB-Sf-21-AE (Sf9), The disappearance of the drug in perfusate (P_{lumen}) as well as the appearance of the drug in mesenteric vein blood (apparent permeability coefficient, P_{blood}), P-gp overexpressing of Chinese hamster ovary AA8 cells (Emt^{R1}). For an overview of the effects of more surfactants on P-gp, (see Table S1).

Table 43: Impact of nonionic surfactants on intestinal P-glycoprotein in rats.

In vivo pre-clinical studies were performed in male [a]: Sprague-Dawley rats, [b]: Wistar albino rats.

Synonyms of surfactants are available in Table 2.

Table ~~34~~: *In vitro* and *in vivo* ~~I~~impact of nonionic surfactants on breast cancer resistance protein, BCRP~~, *in vitro*~~.

To be inserted as a footnote under Table 4: Concentration (conc.), Approximately (approx.), Respectively (resp.), Plasma membrane vesicle of cells containing human ABCG2 (Membrane vesicles BCRP), Clonal isolate derived from the Spodoptera frugiperda cell line IPLB-Sf-21-AE (Sf9 insect cells), *In Vitro* Diffusion Chamber Method (*In vitro* DCM), Serosal to mucosal (S-M), *In situ* closed-

loop method (*In situ* CLM), Wild type (WT), Sprague-Dawley (SD), Synonyms of surfactants are available in Table 2.

~~**Table 4:** Impact of nonionic surfactants on intestinal P-glycoprotein in rats.~~

~~*In vivo* pre-clinical studies were performed in male [a]: Sprague-Dawley rats, [b]: Wistar albino rats.~~

~~Synonyms of surfactants are available in Table 2.~~

Table 5: *In vitro and in vivo* Iimpact of nonionic surfactants and co-surfactants on multidrug resistance-associated protein 2, MRP2~~, *in vitro*~~.

To be inserted as a footnote under Table 5: Concentration (Conc.), Respectively (resp.), Membrane vesicles prepared from Spodoptera frugiperda (Sf9) insect cells over-expressing human MRP2 (Membrane vesicles of Sf9 MRP2), ATP measurements were performed using ATP luciferin/Luciferase assay, Wild type (WT), Synonyms of surfactants are available in Table 2 and 3.

Table 6: Nonionic surfactants inhibit~~ed~~ solute carriers (SLCs) *in vitro*.

To be inserted as a footnote under Table 6: IC_{50} were estimated from upatke transport assay. For Regev et al. 2002, imact of surfactant on bi-directional transport assay was shown. 1-methyl-4-phenylpyridinium acetate (MPP^+), Monocarboxylic acid transporter (MCT, SLC16A1), Organic cation transporter 1 (OCT1, SLC22A1), (OCT2, SLC22A2), (OCT3, SLC22A3), Peptide transporter 1 (PEPT1, SLC15A1), (PEPT2, SLC15A2), Organic anion transporting polypeptide 1A2 (OATP1A2, SLC21A3), (OATP2B1, SLC21A9). Human embryonic kidney cells stably transfected with OATP1A2

(HEK OATP1A2), or with OATP2B1 (HEK OATP2B1), Chinese hamster ovary cells stably transfected with rbOCT1(CHO-K1 rbOCT1), Madin-Darby canine kidney cells stably transfected with OCT1-3 (MDCKII OCT1-3), or with PEP2 (MDCKII PEPT2). Synonyms of surfactants available in Table 2 and 5.

Table S1: *In vitro* ~~I~~impact of nonionic surfactants and polyethylene glycol (PEG) derivatives on P-glycoprotein ~~*in vitro*~~.

Accumulation (accum.), Approximately (approx.), Respectively (resp.), Surfactant (surf.), Concentration (conc.), Dependent (dep.), Not specified in the study (ns), Apical to basolateral (A-B), Mucosal to serosal (M-S), Permeability (P_{app}), Any increase or decrease described in the table means significant $P<0.05$, Resistance Modification Index (RMI), Mouse embryo fibroblasts transfected with MDR1 (NIH-MDR1-G185), Mouse embryo fibroblasts transfected with MDR1 (NIH-MDR1-G185), P-gp overexpressing human melanoma cell line (MDA-MB-435/LCC6MDR1), P-gp overexpressing human ovarian carcinoma cell line (NCI/ADR-RES), Adriamycin-resistant of murine leukemia P388 cells (P388/ADR), P-gp variant of human epithelial cells KB 3-1 (KB 8-5-11 cells), In Vitro Diffusion Chamber Method (In vitro DCM), ATPlite 1step Assay kit was from PerkinElmer, P-gp containing membranes of Chinese hamster lung fibroblasts (DC-3F/ADX cells), Concentration of half-maximum activation (K_1), Concentration of half-maximum inhibition (K_2), MDR cell subline of Chinese hamster ovary cells Aux-B1(CH^{r}C5), Bovine brain microvessel endothelial cells (BBMEC), Vinblastine-resistant derivative of Human Caucasian acute lymphoblastic leukemia CCRF-CEM cells (R100 cells), Human lung adenocarcinoma cell line A549 treated with paclitaxel (A549/Taxol), Porcine kidney epithelial cell line (LLC-PK1-MDR1), LLC-PK1 stably expressing MDR1 (LLC-MDR1), P-gp overexpressing human oral epidermal carcinoma (KBv), For cremophor® EL and Solutol® HS 15, RMI

was measured at 10 µg/mL, P-gp overexpressing human melanoma cell line (MDA-MB-435/LCC6MDR1), P-gp overexpressing human ovarian carcinoma cell line NCI/ADR-RES, MDR cell subline of human breast carcinoma MCF-7 cells (MCF7/ADR), Resistance reversion index (Log (IC_{50}.0/IC_{50})) was determined as a ratio of IC_{50} of Doxorubicin in the assay buffer and surfactant solution, Vincristine-resistant derivative of K562 (7962 cells), Human lung cancer cells (A549), Human P-gp overexpressing membranes obtained from baculovirus-infected insect cells (High Five, BTI-TN5B1-4), Clonal isolate derived from the Spodoptera frugiperda cell line IPLB-Sf-21-AE (Sf9), The disappearance of the drug in perfusate (P_{lumen}) as well as the appearance of the drug in mesenteric vein blood (apparent permeability coefficient, P_{blood}), P-gp overexpressing of Chinese hamster ovary AA8 cells (Emt^{R1}).

Table 1: Expression of selected transporters and carriers along the human intestine and in Caco-2 cells. ~~LC-MS/MS-determined protein concentrations (pmol/mg total protein) of selected transporters and carriers in Caco-2 cells and segments of the human gastrointestinal tract. Protein concentrations from Caco-2 cells were obtained three weeks after seeding. Caco-2 cells were from three different sources: American Type Culture Collection (ATCC)[a], The European Collection of Authenticated Cell Cultures (ECACC)[b], and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)[c]. The average values are depicted without statistical deviation parameters. For (Akazawa et al., 2018), the average were obtained from two reported values from two humans, and if one of the two values was below the lower limit of quantification (LLOQ), the other value is depicted. Intestinal segments were adapted from (Drozdzik et al., 2014): Duodenum (D), jejunum (J1-2), ileum (I1-2), and colon (C1-4). BLQ = below the LLOQ.~~ Logarithmic 10-step color scale and annotation of expression level (very low-very

high) have *arbitrarily* been defined for overview in the range 0-15 pmol/mg total protein and 0-450 fmol/mg total tissue[d]:

	Very low expression		*Low expression*		*Intermediate expressio*	
pmol protein/mg total protein	0-0.0099	0.0100-0.0248	0.0249-0.0621	0.0622-0.154	0.155-0.386	0.387-0.9
fmol protein/mg total tissue [d]	0-0.299	0.300-0.747	0.748-1.86	1.87-4.65	4.66-11.5	11.6-28

Transporter or carrier	Caco-2	Reference	Intestinal segment									Reference
			D	J1	J2	I1	I2	C1	C2	C3	C4	
P-gp	2.06[a]	(Ölander et al., 2016)	7.67[d]	33.02[d]	47.41[d]		70.78[d]		9.98[d]			(Drozdzik et al., 2019)
	4.1[b]	(Uchida et al., 2015)	0.290	0.408	0.475	0.711	1.06	0.145	0.304	0.228	0.368	(Drozdzik et al., 2014)
	1.0[c]	(Brück et al., 2017)		1.22								(Lloret-Linares et al., 2016)
				0.614		0.656						(Gröer et al., 2013)
					1.89		0.20					(Harwood et al., 2015)
				2.43		4.93						(Akazawa et al., 2018)
BCRP	0.0117[a]	(Ölander et al., 2016)	5.51[d]	19.58[d]	26.97[d]		30.47[d]		5.13[d]			(Drozdzik et al., 2019)
	1.79[b]	(Uchida et al., 2015)	0.190	0.277	0.356	0.405	0.359	0.150	0.0438	0.153	0.160	(Drozdzik et al., 2014)
	0.5[c]	(Brück et al., 2017)			1.25							(Miyauchi et al., 2016)
				0.574		0.241						(Gröer et al., 2013)
					2.56		1.60					(Harwood et al.,

					4.65		8.07						2015) (Akazawa et al., 2018)
MRP2	0.134[a]	(Ölander et al., 2016) (Uchida et al., 2015) (Brück et al., 2017)	11.88[d]	22.37[d]	22.52[d]		19.84[d]		16.69[d]				(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Lloret-Linares et al., 2016) (Gröer et al., 2013) (Harwood et al., 2015) (Akazawa et al., 2018)
	0.649[b]		0.758	1.03	0.946	0.764	0.808	1.41	1.77	1.13	0.951		
	0.8[c]			0.116									
				1.07		0.350							
					0.59		BLQ						
				0.835		1.16							
MRP3	0.423[a]	(Ölander et al., 2016) (Uchida et al., 2015) (Brück et al., 2017)	17.28[d]	30.47[d]	31.25[d]		22.58[d]		28.79[d]				(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Lloret-Linares et al., 2016) (Gröer et al., 2013) (Akazawa et al., 2018)
	BLQ[b]		0.850	0.639	0.506	0.552	0.696	1.53	2.10	2.11	1.72		
	BLQ[c]			1.91									
				0.309		0.686							
				0.501		0.303							
PEPT1	0.342[a]	(Ölander et al., 2016) (Uchida et al., 2015) (Brück et al., 2017)	25.61[d]	84.17[d]	109.6[d]		107.3[d]		3.27[d]				(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Miyauchi et al., 2016) (Gröer et al., 2013) (Akazawa et al., 2018)
	1.48[b]		2.63	3.49	4.23	4.62	4.89	0.298	0.210	0.188	0.310		
	5.2[c]				1.60								
				2.45		4.73							
				8.34		10.7							
OATP2B1	2.66[a]	(Ölander et al., 2016) (Uchida et al., 2015) (Brück et al., 2017)	5.30[d]	7.21[d]	8.02[d]		8.06[d]		8.00[d]				(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Miyauchi et al., 2016) (Gröer et al., 2013) (Akazawa et al.,
	0.771[b]		0.428	0.556	0.486	0.464	0.482	0.478	0.731	0.638	0.591		
	3.3[c]				0.540								
				0.299		0.267							
				BLQ		BLQ							

													2018)
OATP1A2	BLQ[b] BLQ[c]	(Uchida et al., 2015) (Brück et al., 2017)	BLQ BLQ	BLQ BLQ	BLQ BLQ BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	BLQ BLQ	(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Miyauchi et al., 2016) (Gröer et al., 2013) (Akazawa et al., 2018)
OCT1	BLQ[a] BLQ[b] BLQ[c]	(Ölander et al., 2016) (Uchida et al., 2015) (Brück et al., 2017)	1.61[d]	4.22[d]	6.02[d]		5.12[d]		2.79[d]				(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Miyauchi et al., 2016) (Gröer et al., 2013)
			0.665	0.647	0.566	0.802	0.842	0.469	0.695	0.725	0.632		
					BLQ								
					BLQ	0.480							
OCT3	BLQ[b] BLQ[c]	(Uchida et al., 2015) (Brück et al., 2017)	BLQ 0.06 70	BLQ 0.0 564	BLQ 0.06 29	BLQ 0.0 531	BLQ 0.0 687	BLQ 0.1 26	BLQ 0.10 7	BLQ 0.1 16	BLQ 0.1 35		(Drozdzik et al., 2019) (Drozdzik et al., 2014) (Miyauchi et al., 2016) (Gröer et al., 2013) (Akazawa et al., 2018)
					BLQ								
					BLQ	0.077							
				0.551		BLQ							
MCT	1.72[a] 0.871[b]	(Ölander et al., 2016) (Uchida et al., 2015)	61.13[d]	78.81[d]	75.14[d]		43.65[d]		112.6[d]				(Drozdzik et al., 2019) (Miyauchi et al., 2016) (Akazawa et al., 2018)
					1.85								
				1.54		2.41							

Table 2: *In vitro* Iimpact of selected nonionic surfactants and polyethylene glycol (PEG) derivatives on P-glycoprotein ~~*in vitro*~~.

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vein blood (apparent permeability coefficient, P_{blood}), P-gp overexpressing of Chinese hamster ovary AA8 cells (Emt^{R1}). For an overview of the effects of more surfactants on P-gp, (see Table S1).

	Conc.	Substrate	Cell line/Tissue	Assay	Impact	Refer
EL,	(1:1000)	Daunorubicin	R100 cells	Uptake transport	Increased intracellular accum.	(Wood 1990)
L, **astor**	(1:1000)	Daunorubicin	7962 cells	Uptake transport	Increased intracellular accum.	(Wood 1990)
astor **5**	(1:1000)	Daunorubicin	R100 cells	Uptake transport	Increased intracellular accum.	(Wood 1992)
	(1:1000)	Daunorubicin	P388/ADR	Uptake transport	Increased intracellular accum.	(Wood 1992)
	0.0001-0.1% (w/v)	Acf(N-Mef)$_2$NH$_2$	Caco-2 Cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Neru 1996)
	100 µg/mL	Rh 123	KB 8-5-11 cells	Uptake transport	Enhanced the fluorescence of Rh 123 by 3-fold.	(Buck 1995)
	3-20 µg/mL	Doxorubicin	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI =1.5 ± 0.0	(Buck 1995)
	3-20 µg/mL	Vinblastine	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI =1.1 ± 0.1	(Buck 1995)
	3-20 µg/mL	Colchicine	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI =1.3 ± 0.1	(Buck 1995)
	3-20 µg/mL	Etoposide	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI =1.2 ± 0.3	(Buck 1995)
	3-20 µg/mL,	Actinomycin D	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI =1 ± 0.2	(Buck 1995)
	0.01-1 mM	Rh 123	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app} in a conc. dependent manner.	(Rege
	0.02-2% (w/v)	Cyclosporine A	Caco-2 cell monolayers	Bi-directional transport	Decreased B-A P_{app}.	(Chiu
	0.005-0.5% (w/v)	Rh 123	Rat intestinal membrane	Bi-directional transport (In vitro DCM)	Increased S-M and decreased M-S $P_{app.}$	(Shon
	400 µM	Doxorubicin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B $P_{app.}$	(Al-Sa 2016)
	300 µM	Etoposide	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Al-A
	1% SMEDDS containing 50% (w/w) of surf.	Etoposide (SMEDDS)	Intestinal segments from rats´ ileum	In situ single-pass perfusion experiments	Increased intestinal $P_{app.}$ ~~i~~Increased P_{Blood} and $P_{Lumen.}$	(Zhao
	0.3-1000 µM	Digoxin	MDCKII MDR1	Uptake transport	Increased intracellular accum. in a conc. dependent manner. IC_{50} = 12 µM	(Gurja
glycol	2.5-20% (w/v)	Paclitaxel	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app} in a conc. dependent manner.	(Hugg

20% (w/v)	Paclitaxel	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app} in a conc. dependent manner.	(Hugg
20% (v/v)	Ranitidine	Caco-2 cell monolayers	Bi-directional transport	Decreased ranitidine ER.	(Ashir al., 20
300 µM			PREDEASY ATPase Kit	Increased P-gp ATPase activity.	(Ashir al., 20
1, 5 and 20% (w/v)	Digoxin	Rat jejunal membrane	Bi-directional transport (In vitro DCM)	Decreased S-M flux by 47, 57 and 64%, resp., compared to control.	(Johns 2002)
0.1-20% (v/v)	Rh l23	Rat intestinal membrane	Bi-directional transport (In vitro DCM)	Decreased S-M P_{app} in a conc. dep. manner.	(Shen
20% (v/v)	Ranitidine	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Ashir al., 20
0.5 and 1% (v/v)	Ranitidine	Caco-2 cell monolayers	Bi-directional transport	Enhanced A-B and decreased B-A P_{app}.	(Ashir al., 20
300 µM			PREDEASY ATPase Kit	Increased P-gp ATPase activity.	(Ashir al., 20
1 and 2% (w/v)	Rh 123	Caco-2 cells	Uptake transport	Enhanced Rh 123 intracellular accum.	(Hoda
1 and 2% (w/v)		Caco-2 cells	Western blotting	Decreased P-gp expression.	(Hoda
	Doxorubicin	KBv		Log ($IC_{50.0}/IC_{50}$) = 0.7	(Batra 1999)
	Doxorubicin	MCF7/ADR		Log ($IC_{50.0}/IC_{50}$) = 0.8	(Batra 1999)
	Doxorubicin	$CH^{r}C5$		Log ($IC_{50.0}/IC_{50}$) = 2	(Batra 1999)
Log M = -5	Rh 123	KBv	Uptake transport	Enhanced Rh 123 accum. by approx. 6.5-fold.	(Batra 1999)
0.001-1%	Rh 123	LLC-PK1-MDR1	Uptake transport	Increased Rh 123 accum.	(Batra 2001)
0.001-1%	Digoxin	LLC-PK1-MDR1	Uptake transport	Increased digoxin accum.	(Batra 2001)
0.01-1%	Digoxin	BBMEC	A-B transport	Increased A-B transport.	(Batra 2001)
0.01%	Digoxin	BBMEC	A-B transport	Increased A-B and decreased transport.	(Batra 2001)
0.01 and 0.1% (w/v)	Digoxin	Rat jejunal membrane	Bi-directional transport (In vitro DCM)	Decreased S-M flux.	(Johns 2002)
0.01% (w/v)	Rh 123	BBMEC	Uptake transport	Enhanced Rh 123 accum. by approx. 2-fold. Depleted intracellular ATP content. Decreased the P-gp ATPase	(Batra 2003)

				activity.	
	0.01% (w/v)	Rh 123	BBMEC	ATP luciferin/ luciferase	Depleted intracellular ATP content.	(Batra 2003)
	0.01% (w/v)	Rh 123	KBv	Pgp ATPase activity	Decreased the P-gp ATPase activity.	(Batra 2003)
	0.1% w/v		P-gp membranes from Gentest Co.	P-gp ATPase Assay	Decreased V_{max} and increased K_m significantly.	(Batra 2004)
	0.01 and 0.1% w/v	Vincristine	P-gp membranes from Gentest Co.	P-gp ATPase Assay	Decreased V_{max} and increased K_m significantly.	(Batra 2004)
	0.5 % (w/v)	Rh 123	Rats´ jejunal segments	M-S transport. (Ussing chamber)	Increased M-S P_{app} by1.9-fold.	(Föger
	0.1% w/w		P-gp membranes (High Five, BTI-TN5B1-4)	P-gp ATPase Assay	Abolished P-gp ATPase activity completely.	(Shaik
	0.01% w/w	Verapamil	P-gp membranes (High Five, BTI-TN5B1-4)	P-gp ATPase Assay	Inhibited verapamil-stimulated P-gp ATPase activity.	(Shaik
	0.01% w/w	Nelfinavir	P-gp membranes (High Five, BTI-TN5B1-4)	P-gp ATPase Assay	Abolished the nelfinavir stimulated P-gp ATPase activity.	(Shaik
	0.01% w/w	Nelfinavir	MDCKII MDR1	Uptake transport	Enhanced nelfinavir accum.	(Shaik
	0.01% w/w	Saquinavir	MDCKII MDR1	Uptake transport	Increased saquinavir accum. by 2-fold.	(Shaik
	0.01% w/w	Saquinavir	LLC-PK1-MDR1	Uptake transport	Increased saquinavir accum. by 5-fold.	(Shaik
0,	0-600 ng/mL		Membrane vesicles of Emt^{R1} cells	Phosphate release measurements	Reduced P-gp ATPase activity in a conc. dep. manner.	(Reger
ne	0-300 ng/mL	Doxirubicin	Large unilamellar vesicles (LUV)	Trans-bilayer movement	Decreased Flip-Flop Life-Time of doxorubicin in a conc. dep. manner.	(Reger
	30-100 ng/mL	Clacein-AM	Emt^{R1} cells	Calcein-AM efflux	Enhanced calcein-AM uptake in a conc. dep. manner.	(Reger
	0.5% (w/v)	Digoxin	Rat everted gut sac model	Uptake transport	Enhanced digoxin accum.	(Corna 2004)
	200 µM	Epirubicin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Lo, 2
	20-200 µM	Epirubicin	Caco-2 cells	Uptake transport	Enhanced fluorescent epirubicin accum. in a conc. dep. manner.	(Lo, 2
	200 µM	Epirubicin	Everted sacs of rat´s jejunum or ileum	M-S transport	Increased M-S $P_{app.}$	(Lo, 2
	200 µM	Doxorubicin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B $P_{app.}$	(Al-Sa 2016)

	200 μM	Digoxin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app}.	(Niels
	0.2-500 μM	Digoxin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app} in a conc. dep. manner.	(Niels
	0.2-500 μM	Digoxin	MDCKII MDR1 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app} in a conc. dep. manner.	(Niels
	200-500 μM	Etoposide	Caco-2 cell monolayers	Bi-directional transport	Increased A-B $P_{app.}$	(Al-A
	0.2-500 μM	Etoposide	Caco-2 cell monolayers	Bi-directional transport	Decreased B-A $P_{app.}$	(Al-A
	20-500 μM	Etoposide	MDCKII MDR1 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Al-A
		Calcein-AM	MDCKII MDR1	Calcein-AM efflux	Increased calcein fluorescence in a conc. dep. manner. IC_{50} = 11 μM.	(Al-A 2018b
	200 μM	Digoxin	MDCKII MDR1	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$ Increased intracellular accum. of digoxin from the apical side.	(Al-A 2018b
	0.3-1000 μM	Digoxin	MDCKII MDR1	Uptake transport	Increased intracellular accum. in a conc. dependent manner. IC_{50} = 74 μM.	(Gurja
0, **ne** **oleate**	200 μM	Epirubicin	Everted sacs of jejunum or ileum of rats	M-S transport	Increased M-S P_{app}	(Lo, 2
	20-200 μM	Epirubicin	Caco-2 cells	Uptake transport	Enhanced intracellular accum. of fluorescent epirubicin in a conc. dep. manner.	(Lo, 2
	200 μM	Epirubicin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Lo, 2
	0.5 w/v	Digoxin	Rat everted gut sac model	Uptake transport	Enhanced digoxin uptake.	(Corna 2004)
	0.01-1 mM	Rh 123	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A P_{app} in a conc. dep. manner.	(Rege
		Rh 123	Caco-2	Uptake transport	Increased Rh 123 accum.	(Kiss
		Rh 123	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Kiss
		Calcein-AM	Caco-2 cells	Uptake transport	Increased calcein accum.	(Kiss
	(1:10000)	Daunorubicin	R100 cells	Uptake transport	Increased intracellular daunorubicin accum.	(Wood 1992)
	0.0001-1 % (w/v)	Acf(N-Mef)$_2$NH$_2$	Caco-2 Cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Neru 1996)
	0.1% (w/v)	Rh 123	Rat intestinal membrane	Bi-directional transport	Reduced S-M/M-S ratio.	(Shon

				(In vitro DCM)		
	0.06-0.66 µM	Verapamil	Membrane vesicles of NIH-MDR1-G185	Phosphate release measurements	Inhibition of verapamil-induced P-gp ATPase activity.	(Li-Bl 2009)
	0.001-0.05 w/v	Clacein-AM	MDCKII MDR1	Calcein-AM efflux	Enhanced calcein fluorescence by approx. 2-fold.	(Hank
	150 µM	Bis(12)-hupyridone	Caco-2 cell monolayers	Bi-directional transport	Increased A-B P_{app} and decreased B-A $P_{app.}$	(Yu et
	10 µM	Bis(12)-hupyridone	Caco-2 cell monolayers	Bi-directional transport	Decreased B-A $P_{app.}$	(Yu et
	200 µM	Doxorubicin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B $P_{app.}$	(Al-Sa 2016)
	200 µM	Digoxin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A flux.	(Niels
	300 µM	Etoposide	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Al-A
	1% SMEDDS containing 50% (w/w) of surf.	Etoposide (SMEDDS)	Intestinal segments from rats´ ileum	In situ single-pass perfusion experiments	Increased intestinal $P_{app.}$ Increased P_{Blood} and $P_{Lumen.}$	(Zhao
		Calcein-AM	MDCKII MDR1	Calcein-AM efflux	Increased calcein fluorescence in a conc. dep. manner. IC_{50}= 69 µM	(Al-A 2018b
	200 µM	Digoxin	MDCKII MDR1	Bi-directional transport	Decreased B-A $P_{app.}$ Increased intracellular accum. of digoxin from the apical side.	(Al-A 2018b
	0.3-100 µM	Digoxin	MDCKII MDR1	Uptake transport	Increased intracellular accum. in a conc. dep. manner. IC_{50} = 45 µM	(Gurja
5, S 15, glycol	0.05-0.5 % (w/v)	Digoxin	Rat everted gut sac model	Uptake transport	Enhanced digoxin accum.	(Corna 2004)
	(1:10000)	Daunorubicin	R100 cells	Uptake transport	Increased intracellular daunorubicin.	(Wood 1992)
ate, ted earic		Etoposide	C6 glioma cells	MTT	Decreased IC_{50} by 10-fold.	(Lamp Benoi
		Etoposide	F98 glioma cells	MTT	Decreased IC_{50} by 3-fold.	(Lamp Benoi
		Etoposide	9L glioma cells	MTT	Decreased IC_{50} by 8-fold.	(Lamp Benoi
ate	35-39% Lipid nanoparticles		P-gp exhibiting membrane vesicles	ATPase kit (SPIbio®, Massy, France)	Decreased ATPase activity.	(Lamp Benoi
	5-100 µg/mL	Rh 123	KB 8-5-11 cells	Uptake transport	Enhanced fluorescence of Rh 123 in a conc. dep. manner.	(Buck 1995)
	3-20 µg/mL	Doxorubicin	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI = 6 ± 3.2	(Buck 1995)

	3-20 µg/mL	Vinblastine	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI = 2 ± 1	(Buck 1995)
	3-20 µg/mL	Colchicine	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI = 4.2 ± 0.7	(Buck 1995)
	3-20 µg/mL	Etoposide	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI = 2.7 ± 0.7	(Buck 1995)
	3-20 µg/mL	Actinomycin D	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI = 2.3 ± 0.9	(Buck 1995)
	3-20 µg/mL	Paclitaxel	KB 8-5-11 cells	MTT	Decreased IC_{50} in a conc. dep. manner. RMI=10 ± 1.2	(Buck 1995)
	0.1 – 100 µM	Colchicine	KB 8-5-11 cells	Colorimetric (Crystal violet)	Decreased IC_{50} in a conc. dep. manner. RMI = 34.5 ± 2.5	(Coon
	0.1 – 100 µM	Vinblastine	KB 8-5-11 cells	Colorimetric (Crystal violet)	Decreased IC_{50} in a conc. dep. manner. RMI = 27.7 ± 2.3	(Coon
	0.1 – 100 µM	Doxorubicin	KB 8-5-11 cells	Colorimetric (Crystal violet)	Decreased IC_{50} in a conc. dep. manner. RMI = 41.7 ± 3	(Coon
	70 µM	Rh 123	KB 8-5-11 cells	Uptake transport	Increased accum. by 50-fold.	(Coon
	0.1-1 % (w/v)	Etoposide	Everted sacs of ileum of rats	M-S and S-M transport	Increased A-B P_{app}	(Akht
	0.3-1000 µM	Digoxin	MDCKII MDR1	Uptake transport	Increased intracellular accum. in a conc. dep. manner. IC_{50} = 180 µM	(Gurja
l	0.05-0.5% w/v	Digoxin	Rat everted gut sac model	Uptake transport	Enhanced digoxin accum.	(Corna 2004)
glycol e, PGS, glycol	0.05 and 0.5 w/v	Celiprolol	Rat everted gut sac model	Uptake transport	Enhanced celiprolol accum.	(Corna 2004)
	0.002-1 mg/mL	Paclitaxel	Intestinal segments from rats´ ileum	Bi-directional transport (Ussing chamber)	Decreased B-A P_{app} in a conc. dep. manner. Increased A-B P_{app}.	(Varm Panch
	0.1 and 1 mg/mL	Paclitaxel	Intestinal segments from rats´ ileum	In situ single-pass perfusion experiments	Increased intestinal $P_{app.}$	(Varm Panch
		Verapamil	P-gp membranes from Sf9	ATPase	Inhibited substrate induced ATPase activity. IC_{50} (µM) = 3.18 ± 1.97	(Colln
		Quinidine	P-gp membranes from Sf9	ATPase	Inhibited substrate induced ATPase activity. IC_{50} (µM) = 0.82 ± 0.47	(Colln
		Progesterone	P-gp membranes from Sf9	ATPase	Inhibited substrate induced ATPase activity. IC_{50} (µM) = 3.25 ± 1.29	(Colln
		Nicardipine	P-gp membranes from Sf9	ATPase	Inhibited substrate induced ATPase activity. IC_{50} (µM) = 0.40 ± 0.17	(Colln
	33.0 µM	Rh 123	Caco-2 monolayers	Bi-directional transport	Increase A-B and decrease B-A $P_{app.}$	(Colln
	33.0 µM	Digoxin	Caco-2 monolayers	Bi-directional transport	Increase A-B and decrease B-A $P_{app.}$	(Colln

	Calcein-AM	NCI/ADR-RES	Calcein-AM efflux	Dose-dependent increase in calcein fluorescence.	(Dong
	Calcein-AM	MDA-MB-435/LCC6MDR1	Calcein-AM efflux	Dose-dependent increase in calcein fluorescence.	(Dong
0.005%	Talinolol	Caco-2 Cell	Bi-directional	Increased A-B $P_{app.}$	(Bogn

	Dose of surfactant	Substrate (Dose)	Impact	Referenc
® EL	1% SMEDDS containing 50% (w/w) surfactant	Etoposide [a] (12 mg/kg)	Increased AUC, C_{max}, and F by 1.7-, 1.3-, and 1.7-fold, respectively.	(Zhao et a
® RH 40 RH 40, tor oil	1% SMEDDS containing 43% (w/w) surfactant	Etoposide [a] (12 mg/kg)	Increased AUC, C_{max}, and F by 1.4-, 1.3-, and 1.4-fold, respectively.	(Zhao et a
	240 mg/kg	Rifampicin [b]	Increased AUC by 1.5-fold, prolonged $t_{½}$ by 25%, and decreased CL to 60%	(Ma et al.
royl lycerides,	1% (w/v)	Etoposide [b] (4.5 mg/kg)	Increased AUC, C_{max}, and F by 1.8-, 4.7-, and 1.8-fold, respectively.	(Akhtar e
ric	5% (w/v)	Etoposide [b] (4.5 mg/kg)	Increased AUC, C_{max}, and F by 3-, 7-, and 3-fold, respectively.	(Akhtar e
	10% (w/v)	Etoposide [b] (4.5 mg/kg)	Increased AUC, C_{max}, and F by 1.6-, 6-, and 1.6-fold, respectively.	(Akhtar e
lene (40)	8.5 mg/table	Rh 123 [a] (1.5 mg/tablet)	Increased AUC by 3.4-fold.	(Föger et

Table 43: Impact of nonionic surfactants on intestinal P-glycoprotein in rats.

In vivo pre-clinical studies were performed in male [a]: Sprague-Dawley rats, [b]: Wistar albino rats.

Synonyms of surfactants are available in Table 2.

68	240 mg/kg	Rifampicin [b]	Increased AUC by 1.5-fold, prolonged $t_{½}$ by 38%, and decreased CL to 60%.	(Ma et al.
35	8.5 mg/table	Rh123 [a] (1.5 mg/tablet)	Increased AUC by 1.6-fold.	(Föger et
20	10-25% (v/v)	Digoxin [a] (0.2 mg/kg)	Increased AUC by 1.4-fold, increased C_{max} by 1.4-1.8-fold, increased k_e by 1.4-1.6-fold, and increased F by approx.1.5-fold.	(Nielsen e
	5 and 25% (v/v)	Etoposide [a] (20 mg/kg)	Increased AUC by 1.8-fold, increased C_{max} by 1.5-2.1-fold, CL decreased by half, and increased F by 1.7-fold.	(Al-Ali et
80	1 and 10%(v/v)	Digoxin [a] (0.2 mg/kg)	Increased AUC by 1.3-1.6-fold and increased C_{max} by 2.5-fold	(Zhang et
	10%	Rifampicin [b] (30 mg/kg)	Increased AUC by 1.7-fold and decreased $t_{½}$ to 36%.	(Shimomu 2016)
	1% SMEDDS containing 50% (w/w) surfactant	Etoposide [a] (12 mg/kg)	Increased C_{max} by 3.5-fold, increased F and AUC by 2.5-fold.	(Zhao et a
7, prylic / rides	1 mg/kg	Digoxin [a] (0.25 mg/kg)	Increased AUC by 1.4-fold and decreased t_{max} by 4.5-fold.	(Cornaire
15	10%	Colchicine (5mg/kg)	Increased AUC by 4-fold.	(Bittner et
	10%	Colchicine (5mg/kg)	Increased AUC by 2-fold.	(Bittner et
	50 mg/kg	Paclitaxel [a] (25 mg/kg)	Increased AUC, C_{max}, and F by 6.3-, 3.1-, and 6.4-fold, respectively.	(Varma a Panchagn

Table 34: *In vitro* and *in vivo* ~~i~~Impact of nonionic surfactants on breast cancer resistance protein, BCRP~~, *in vitro* and *in vivo*~~.

~~Concentration (conc.), Approximately (approx.), Respectively (resp.), Plasma membrane vesicle of cells containing human ABCG2 (Membrane vesicles BCRP), Clonal isolate derived from the Spodoptera frugiperda cell line IPLB-Sf-21-AE (Sf9 insect cells), *In Vitro* Diffusion Chamber Method~~

factants	Conc.	Substrate	Cells/Tissue/ Animal	Assay	Impact of surfactant	Ref
β-D-side, (C_6-malt)			Membrane vesicles BCRP	Phosphate release measurements	Reduced Pgp ATPase activity. $K_2 = 4.6\ 10^3$ μM	(Xu
glycol,	0.05% and 0.075%	Sulfasalazine	Rat intestinal membrane	In vitro DCM	Decreased S-M B-A transport.	(Sav 2018
ene (9) lauryl 09	0.05 %	Sulfasalazine	WT male Wistar rat	*In situ* CLM	Increased AUC and C_{max} by 1.45 and 1.4- folds, resp.	(Sav 2018
	0.1 %	Sulfasalazine	WT male Wistar rat	*In situ* CLM	Increased AUC and C_{max} by 2.2 and 2.1-folds, resp.	(Sav 2018
ene (4) lauryl $_4$, Brij® L4	50 and 100 μM	Mitoxantrone	MDCKII BCRP	Uptake transport	Increased the uptake by approx. 1.7-fold.	(Yar 200

(*In vitro* DCM), *In situ* closed-loop method (*In situ* CLM), Wild type (WT), Sprague-Dawley (SD),

Synonyms of surfactants are available in Table 2.

olyoxyethylene er	0.01% and 0.05%	Sulfasalazine	Rat intestinal membrane	*In vitro* DCM	Decreased S-M B-A transport.	(Sav 2018
	0.1 %	Sulfasalazine	WT male Wistar rat	*In situ* CLM	Increased AUC and C_{max} by 1.8 and 2.3-fold, resp.	(Sav 2018
EL	50 µM	Mitoxantrone	MDCKII BCRP	Uptake transport	Increased the uptake by approx. 1.4-fold.	(Yar 200
	6.25-100 nM	Scutellarin	Membrane vesicles of Sf9 BCRP	Uptake transport	Increased the uptake in a conc. dependent manner.	(Xia 201
	1 and 5 µg/mL	Scutellarin	MDCKII BCRP	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Xia 201
	5 µg/mL	Scutellarin	WT Male SD rats		Increased AUC and C_{max2} by 1.6 and 1.9-folds, resp.	(Xia 201
	0.025 and 0.05% (w/v)	Topotecan	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and/or decreased B-A $P_{app.}$	(Sav 2018
	0.05 % (w/v)	Topotecan	WT male Wistar rat	*In situ* CLM	Increased AUC 2.3-folds.	(Sav 2018
nethyl-*β*-D-side, (Cymal-1)			Membrane vesicles BCRP	Phosphate release measurements	Reduced P-gp ATPase activity. K_2 = 1.51 10^4 µM	(Xu
	0.075%	Sulfasalazine	Rat intestinal membrane	In vitro DCM	Decreased S-M B-A transport.	(Sav 2018
	0.025 and 0.05% (w/v)	Topotecan	Caco-2 cell monolayers	Bi-directional transport	Increased A-B $P_{app.}$	(Sav 2018
8	0.025 and 0.05% (w/v)	Topotecan	Caco-2 cell monolayers	Bi-directional transport	Decreased B-A $P_{app.}$	(Sav 2018
5	20 µM	Mitoxantrone	MDCKII BCRP	Uptake transport	Increased the uptake by approx. 1.8-fold.	(Yar 200
	250 mg kg^{-1} (Oral)	Topotecan (Oral)	WT mice		Increased the AUC by 2-folds.	(Yar 200
	20 µM	Topotecan	Everted sacs from WT mice ileum	Transport	Increased the intestinal absorption rate of topotecan.	(Yar 200
	20 µM	Mitoxantrone	MDCKII BCRP	Uptake transport	Increased the uptake.	(Yar 200
ene (8) lauryl yloctaglyol,			Membrane vesicles BCRP	Phosphate release measurements	Reduced Pgp ATPase activity. K_2 = 6.93 µM	(Xu
20	100 and 250 µM	Mitoxantrone	MDCKII BCRP	Uptake transport	Increased the uptake by approx. 1.6-fold.	(Yar 200
	100 mg kg^{-1}	Topotecan	WT mice		Increased the AUC by 2-	(Yar

	(Oral) 250 µM	(Oral) Topotecan	Everted	Transport	fold. Increased the intestinal	200 (Yar
	Conc.	**Substrate**	**Cells or animal**	**Assay**	**Impact of surfactant**	**Refere**
EL	0.005-0.05% (v/v)	Calcein-AM	MDCKII MRP2	Bi-directional transport	Decreased B-A $P_{app.}$	(Hanke
	100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake.	(Li et a
	0.1%		membrane		transport.	201
	0.025 and 0.05% (w/v)	Topotecan	Caco-2 cell monolayers	Bi-directional transport	Increased A-B and decreased B-A $P_{app.}$	(Sav 2018
	0.05 % (w/v)	Topotecan	WT male Wistar rat	*In situ* CLM	Increased AUC 2.5-folds.	(Sav 2018
nolaurate	100 µM	Mitoxantrone	MDCKII BCRP	Uptake transport	Increased the uptake by approx. 1.4-fold.	(Yar 200

Table 5: *In vitro and in vivo* impact of nonionic surfactants and co-surfactants on multidrug resistance-associated protein 2 MRP2.

~~Concentration (Conc.), Respectively (resp.), Membrane vesicles prepared from Spodoptera frugiperda (Sf9) insect cells over-expressing human MRP2 (Membrane vesicles of Sf9 MRP2), ATP measurements were performed using ATP luciferin/Luciferase assay, Wild type (WT), Synonyms of surfactants are available in Table 2 and 3.~~

	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decrease ER.	(Li et a
	0.1-100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER in a conc. dependent manner.	(Li et a
	0.1-100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Li et a
	6.25-100 nM	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Xiao e
	1 and 5 µg/mL	Scutellarin	MDCKII MRP2	Bi-directional transport	Decreased B-A $P_{app.}$	(Xiao e
	5 µg/mL	Scutellarin	WT Male Sprague-Dawley rats		Increased AUC and C_{max2} by 1.6 and 1.9-folds, resp.	(Xiao e
EL + 27	100 µg/ml + 100 µg/ml	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake.	(Li et a
EL +	100 µg/ml + 100 µg/ml	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake.	(Li et a
RH 40	0.02-0.04% (v/v)	Calcein-AM	MDCKII MRP2	Bidirectional transport	Decreased B-A $P_{app.}$	(Hanke
	100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake.	(Li et a
	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	0.1-100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER in a conc. dependent manner.	(Li et a
	0.1- 100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Li et a
	0.1-100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Membrane vesicles transport assay	Increased the uptake.	(Li et a
	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	0.1-100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Li et a
	0.1-10 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased B-A $P_{app.}$	(Li et a
	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Increased A-B $P_{app.}$	(Li et a
	100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake.	(Li et a
	100 µg/mL	Scutellarin	Caco-2 cell	Bi-directional	Decreased ER.	(Li et a

			monolayers	transport		
	0.1-100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	0.1-100 µg/mL	Scutellarin	Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Li et a
	100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake	(Li et a
	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	0.1-100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	10 µg/mL	Baicalcein	MDCKII MRP2	Bi-directional transport	Increased A-B P_{app} and decreased B-A $P_{app.}$	(Chen
7,	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
07,						
7	0.1-100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER in a conc. dependent manner.	(Li et a
	0.1-100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Li et a
	10 µg/mL	Baicalcein	MDCKII MRP2	Bi-directional transport	Decreased B-A $P_{app.}$	(Chen
	0.00005-0.005% (w/w)		MDCKII MRP2	ATP measurements	Decreased ATP levels.	(Batral 2003)
	0.01-0.5% (w/w)	Vincristine	MDCKII MRP2	Uptake transport	Increased intracellular accum. in a conc. dependent manner. Decreased IC_{50} value by 6.6 times.	(Batral 2003)
	0.01-0.5% (w/w)	Doxorubicin	MDCKII MRP2	Uptake transport	Increased intracellular accum. in a conc. dependent manner. Decreased IC_{50} value by 125 times.	(Batral 2003)
	0.1 % (w/v)		Plasma membranes of MDCKII MRP2	Phosphate release measurements	Decreased V_{max}.	(Batral 2004)
	0.1 % (w/v)	Vincristine	Plasma membranes of MDCKII MRP2	Phosphate release measurements	Decreased V_{max} and increased K_m.	(Batral 2004)
	10 µg/mL	Baicalcein	MDCKII MRP2	Bi-directional transport	Increased A-B P_{app} and decreased B-A $P_{app.}$	(Chen
+	10 µg/mL	Baicalcein	MDCKII MRP2	Bi-directional transport	Increased A-B P_{app} and decreased B-A $P_{app.}$	(Chen
+	10 µg/mL	Baicalcein	MDCKII MRP2	Bi-directional transport	Increased A-B P_{app} and decreased B-A $P_{app.}$	(Chen
7						
+	10 µg/mL	Baicalcein	MDCKII MRP2	Bi-directional	Increased A-B P_{app} and	(Chen

5				transport	decreased B-A $P_{app.}$	
5	10 µg/mL	Baicalein	MDCKII MRP2	Bi-directional transport	Increased A-B P_{app} and decreased B-A $P_{app.}$	(Chen
0	0.05% (v/v)	Calcein-AM	MDCKII MRP2	Bi-directional transport	Decreased B-A $P_{app.}$	(Hanke
	0.01-0.05% (v/v)	Calcein-AM	MDCKII MRP2	Bi-directional transport	Decreased B-A $P_{app.}$	(Hanke
	100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake.	(Li et a
rcol						
ier	100 µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decreased ER.	(Li et a
	0.1 and 1µg/mL	Scutellarin	Caco-2 cell monolayers	Bi-directional transport	Decrease ER.	(Li et a
	0.1 and 100 µg/mL	Scutellarin	Membrane vesicles of Sf9 MRP2	Uptake transport	Increased the uptake in a conc. dependent manner.	(Li et a

Table 6: Nonionic surfactants inhibited solute carriers (SLCs) *in vitro*.

~~IC_{50} were estimated from upatke transport assay. For Regev et al. 2002, imact of surfactant on bi-directional transport assay was shown. 1-methyl-4-phenylpyridinium acetate (MPP^{+}), Monocarboxylic acid transporter (MCT, SLC16A1), Organic cation transporter 1 (OCT1, SLC22A1), (OCT2, SLC22A2), (OCT3, SLC22A3), Peptide transporter 1 (PEPT1, SLC15A1), (PEPT2, SLC15A2), Organic anion transporting polypeptide 1A2 (OATP1A2, SLC21A3), (OATP2B1, SLC21A9). Human embryonic kidney cells stably transfected with OATP1A2 (HEK-OATP1A2), or with OATP2B1 (HEK-OATP2B1), Chinese hamster ovary cells stably transfected with rbOCT1(CHO-K1 rbOCT1), Madin-Darby canine kidney cells stably transfected with OCT1-3 (MDCKII OCT1-3), or with PEP2 (MDCKII PEPT2). Synonyms of surfactants available in Table 2 and 5.~~

Nonionic surfactant	~~Transporter~~SLC	Substrate	Cells	IC_{50} Impact of surfactant
Solutol® HS 15	OATP1A2	Estrone-3-sulfate	HEK OATP1A2	0.0074%
	OATP1A2	Taurocholate	HEK OATP1A2	0.0041%
	OATP2B1	Estrone-3-sulfate	HEK OATP2B1	0.011%
	OATP2B1	Bromosulfophthalein	HEK OATP2B1	0.00095%
	OCT1	MPP^+	MDCKII OCT1	0.008%
	OCT2	MPP^+	MDCKII OCT2	0.046%
	OCT3	MPP^+	MDCKII OCT3	0.019%
	PEPT2	Glycyl sarcosine	MDCKII PEPT2	0.014%
Cremophor® EL	OATP1A2	Estrone-3-sulfate	HEK OATP1A2	0.00054%
	OATP1A2	Taurocholate	HEK OATP1A2	0.00034%
	OATP2B1	Estrone-3-sulfate	HEK OATP2B1	0.0011%

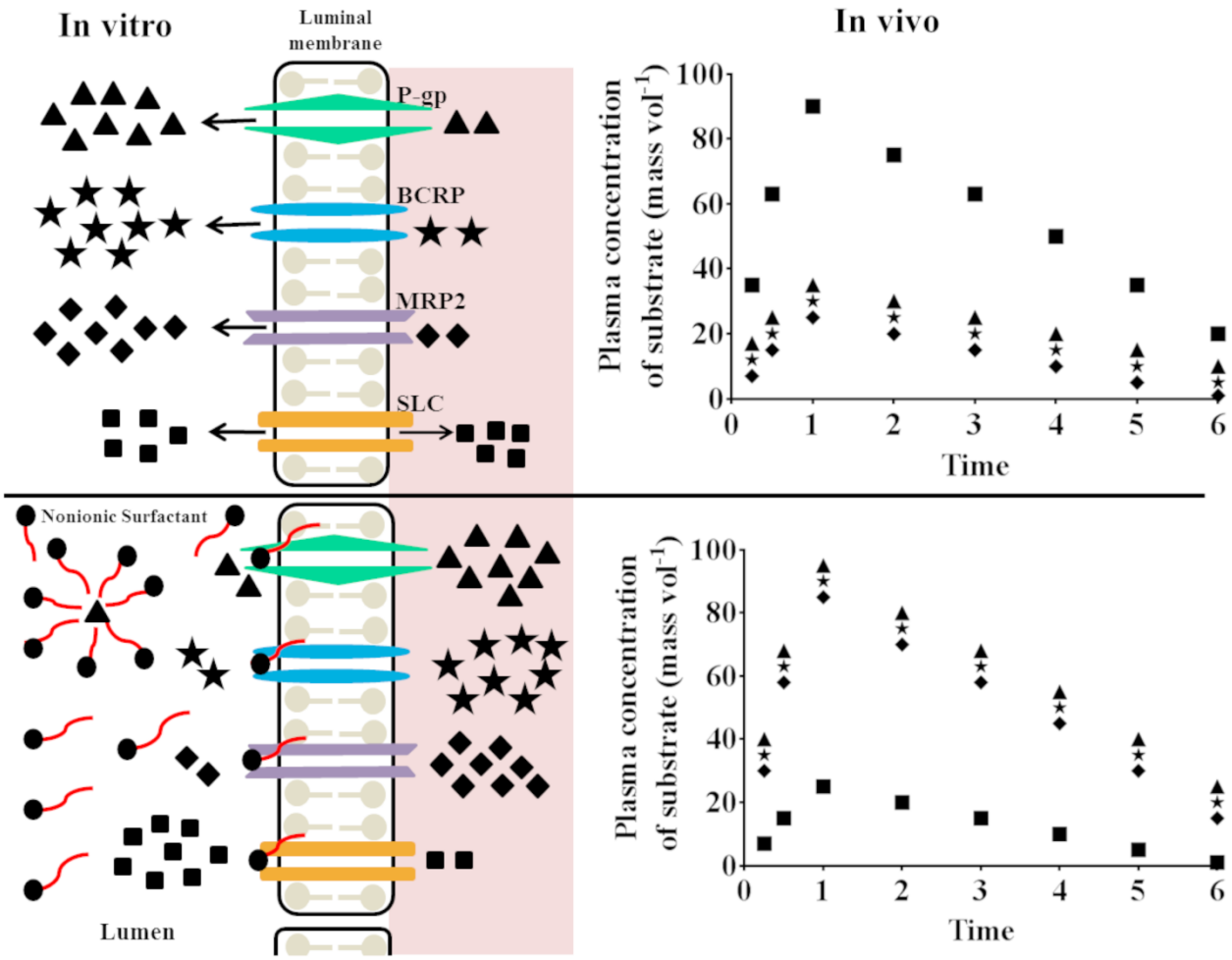
	OATP2B1	Bromosulfophthalein	HEK OATP2B1	0.0098%
	OCT1	MPP^+	MDCKII OCT1	0.019%
	OCT2	MPP^+	MDCKII OCT2	0.46%
	OCT3	MPP^+	MDCKII OCT3	9.77%
	PEPT2	Glycyl sarcosine	MDCKII-PEPT2	0.16%
	MCT	Benzoic acid	Caco-2 cells	Decreased A-B P_{app} of the substrate in a concentration dependent manner.
Kolliphor® P 188, Poloxamer 188	OCT3	MPP^+	MDCKII OCT3	0.024%
Kolliphor® P407	OCT1	MPP^+	MDCKII OCT1	1.85 %
Polysorbate 20	OCT1	MPP^+	MDCKII OCT1	0.002%
	OCT1	MPP^+	CHO-K1 rbOCT1	85 ± 1.12 µg/ml
	OCT2	MPP^+	MDCKII OCT2	0.033%
	OCT2	MPP^+	CHO-K1 rbOCT2	295 ± 1.48 µg/ml
	OCT3	MPP^+	MDCKII OCT3	0.011%
	PEPT2	Glycyl sarcosine	MDCKII-PEPT2	0.005%
Polysorbate 60, Tween® 60, Polyoxyethylene (20) sorbitan stearate	OCT1	MPP^+	CHO-K1 rbOCT1	50 ± 1.26 µg/ml
	OCT2	MPP^+	CHO-K1 rbOCT2	42 ± 1.15 µg/ml
Polysorbate 80	OCT1	MPP^+	MDCKII OCT1	0.0007%
	OCT1	MPP^+	CHO-K1 rbOCT1	106 ± 1.20 µg/ml
	OCT2	MPP^+	MDCKII OCT2	0.039%
	OCT2	MPP^+	CHO-K1 rbOCT2	185 ± 1.20 µg/ml
	OCT3	MPP^+	MDCKII OCT3	0.011%
	PEPT2	Glycyl sarcosine	MDCKII PEPT2	0.037%
	PEPT1	Glycyl sarcosine	Caco-2 cells	Decreased A-B P_{app} of the substrate in a concentration dependent manner.

Declaration of interest

The authors do not have any conflict of interest to report.

Author contribution

Writing - original draft: AAAA, CUN, and RBN. Writing - review & Editing: AAAA, RBN, BS, RH and CUN. Final approval of the version submitted: AAAA, RBN, BS, RH and CUN.


In vitro
Luminal membrane
P-gp
BCRP
MRP2
SLC
Nonionic Surfactant
Lumen
In vivo
Plasma concentration of substrate (mass vol^{-1})
Time
Plasma concentration of substrate (mass vol^{-1})
Time

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