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Safety of surfactant excipients in oral drug formulations

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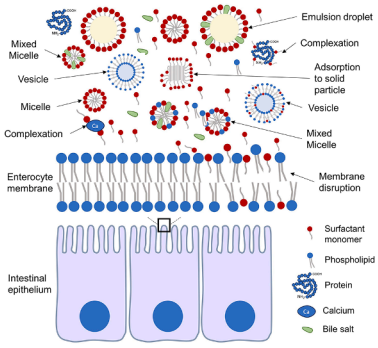
GRAPHICAL ABSTRACT



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ABSTRACT

Surfactants are a diverse group of compounds that share the capacity to adsorb at the boundary between distinct phases of matter. They are used as pharmaceutical excipients, food additives, emulsifiers in cosmetics, and as household/industrial detergents. This review outlines the interaction of surfactant-type excipients present in oral pharmaceutical dosage forms with the intestinal epithelium of the gastrointestinal (GI) tract. Many surfactants permitted for human consumption in oral products reduce intestinal epithelial cell viability in vitro and alter barrier integrity in epithelial cell monolayers, isolated GI tissue mucosae, and in animal models. This suggests a degree of mis-match for predicting safety issues in humans from such models. Recent controversial preclinical research also infers that some widely used emulsifiers used in oral products may be linked to ulcerative colitis, some metabolic disorders, and cancers. We review a wide range of surfactant excipients in oral dosage forms regarding their interactions with the GI tract. Safety data is reviewed across in vitro, ex vivo, pre-clinical animal, and human studies. The factors that may mitigate against some of the potentially abrasive effects of surfactants on GI epithelia observed in pre-clinical studies are summarised. We conclude with a perspective on the overall safety of surfactants in oral pharmaceutical dosage forms, which has relevance for delivery system development.

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1. Introduction

Surfactants are amphiphilic molecules that accumulate at the boundary between distinct forms of matter where they lower surface tension. This feature makes amphiphiles useful as pharmaceutical excipients, food additives, and as components of cosmetics. In oral pharmaceutical dosage forms, surfactants are included as wetting agents, dispersants, emulsifiers, foaming agents, solubilizers, glidants, lubricants, and preservatives.

Surfactants used in oral pharmaceutical dosage forms are considered safe for human consumption by regulators when used within acceptable dose limits. Although surfactants are synonymous with detergency and solubilization, the orally ingested ones cause mild effects compared to household and industrial detergents, which cause extensive GI toxicity even at low concentrations [1]. Still, many soluble surfactants used as oral formulation excipients can disrupt biological membranes [2 3 4], causing an alteration to intestinal epithelial permeability in in vitro models [5]. It is unclear, however, if the quantities used in oral formulations can reach and sustain the threshold concentrations at the intestinal mucosa to cause epithelial barrier reduction in vivo. There is also controversy over whether they may contribute to the development of autoimmune disease as a result of barrier dysregulation, microbiome changes, and changes in mucus overlying the epithelium [6 7]. Recent publications based on data in mouse models have presented concerns that commonly used surfactants might be implicated in ulcerative colitis [8], colon cancer [9], and metabolic disorders [8].

Increasing evidence suggests that synthetic surfactants that increase GI permeability might play a role in the increasing rates of allergic and autoimmune diseases [10 11]. This article reviews the safety data for surfactants used as excipients in oral drug formulations. Included is a summary of the structure and function of surfactants to detail how some surfactants damage biological membranes while others have no observable effect. As the safety data on surfactants is derived largely from pre-clinical models, (with perhaps limited prediction for human exposure), a section is outlined on the factors that may give rise to differences in the effects observed in humans compared to those models. Five case studies are included to provide a focal point for important themes related to surfactant safety. The cases highlight oral formulations with high surfactant concentrations and also contextualise surfactant safety. The article concludes with an overall perspective on the safety of surfactants used in oral pharmaceutical dosage forms in the GI tract.

2. Surfactant classification

Surfactants are most commonly categorized based on structure and/or function. The capacity of a surfactant to adsorb at interfaces is due to distinct hydrophilic and lipophilic regions in the molecule. Surfactants can be defined by their hydrophilic moiety (non-ionic or ionizable) and occasionally by their lipophilic moiety (aliphatic or aromatic). Ionizable hydrophilic moieties are in turn sub-categorized as cationic (weak or strong bases), anionic (weak or strong acids), or amphoteric (zwitterions or non-zwitterions). Sub-categorization of non-ionic surfactants is based on the hydrophilic functional group (e.g., maltosides, sucrose esters, ethoxylates, polysorbates, and macrogol glycerides). For the hydrophobic moiety, most excipient surfactants display aliphatic groups, whereas those with polycyclic aromatic groups include bile acids, which function as natural emulsifiers in the small intestine. Aliphatic surfactants are sub-categorised as short, medium, or long hydrocarbon chains. They may be saturated or unsaturated, branched or unbranched, and there may be more than one hydrocarbon chain in the structure. Additional categories may include further sub-division as natural, semi-synthetic or synthetic surfactants, and as small molecules or polymers (Table 1).

Surfactants are also grouped into soluble and insoluble categories to permit a description of their interfacial properties. Insoluble surfactants

Table 1
Selected surfactants used in oral dosage forms of active pharmaceutical ingredients approved in the US and EU. The maximum human dose (mg) was taken from the FDA IID, if cited.

Category (sub-category)	Excipient	Max potency per unit dose (mg)	Example Applications
Non-ionic (Block co-polymer)	Poloxamer 124	0.09	Emulsifier Solubilizer
	Poloxamer 188	–	Emulsifier Solubilizer
	Poloxamer 331	1000	Emulsifier Solubilizer
	Poloxamer 407	106.7	Emulsifier Solubilizer
Non-ionic (Fatty acids ethoxylates)	PEG-5 oleate	300	Emulsifier Solubilizer
	PEG-8 stearate	25	Emulsifier Solubilizer
	Polyoxyl 40 stearate	8.48	Emulsifier Solubilizer
	Polyoxyl 15 hydroxystearate	–	Emulsifier Solubilizer
Non-ionic (Fatty alcohol ethoxylates)	Steareth 40	–	Emulsifier Solubilizer
Non-ionic (Ethoxylated sorbitan ester)	PEG-40 sorbitan diisostearate	100	Emulsifier Solubilizer
	Polysorbate 20	4.2	Emulsifier Solubilizer
	Polysorbate 40	0.05	Emulsifier Solubilizer
	Polysorbate 60	25	Emulsifier Solubilizer
	Polysorbate 80	418.37	Emulsifier Solubilizer
Non-ionic (Fatty acid ester)	Ascorbyl Palmitate	12	Antioxidant
	Beeswax / white beeswax	–	Viscosity adjustor Coating agent
	Polyglyceryl 3-oleate	310	Vehicle Co-emulsifier
	Propylene glycol monocaprylate	–	Co-emulsifier Vehicle
	Propylene glycol monolaurate	–	Co-emulsifier Vehicle
Non-ionic (Fatty alcohol)	Cetostearyl alcohol	62	Viscosity adjustor Matrix former
	Cetyl Alcohol	59	Viscosity adjustor Matrix former
	Myristic alcohol	2	Viscosity adjustor Matrix former
	Stearyl alcohol	244	Lubricant Matrix former
Non-ionic (Glyceride)	Castor oil	32.1	Vehicle
	Coconut oil	–	Vehicle
	Corn glycerides	318.85	Vehicle Co-emulsifier
	Corn oil	918	Vehicle
	Corn oil mono- & di-glycerides, C_{18}	86	Co-emulsifier Vehicle
	Diacetylated monoglycerides	240	Co-emulsifier
	Fatty acid glycerides	–	Vehicle Co-emulsifier
	Glyceryl 1-stearate	–	Co-emulsifier Lubricant
	Glyceryl dibehenate	5.6	Viscosity enhancer
	Glyceryl distearate	39.2	Co-emulsifier
	Glyceryl mono & dicaprylocaprate	765	Vehicle Co-emulsifier

(continued on next page)

Table 1 (*continued*)

Category (sub-category)	Excipient	Max potency per unit dose (mg)	Example Applications
	Glyceryl mono & dipalmitostearate	2.02	Co-emulsifier
	Glyceryl monocaprylate	400	Vehicle Co-emulsifier
	Glyceryl monocaprylocaprate	–	Vehicle Co-emulsifier
	Glyceryl monooleate	–	Co-emulsifier
	Glyceryl monostearate	264.3	Co-emulsifier
	Glyceryl palmitostearate	150	Co-emulsifier
	Glyceryl ricinoleate	11.25	Co-emulsifier
	Glyceryl Stearate/PEG-100 stearate	1.8	Emulsifier Solubilizer
	Glyceryl tristearate	225	Vehicle
	Hard fat	–	Vehicle
	Hydrogenated castor oil	410.82	Vehicle
	Hydrogenated soybean oil	48	Vehicle
	Medium-chain triglycerides	3390	Vehicle
	Olive oil	425	Vehicle
	Peanut oil	313.8	Vehicle
	Sesame oil	162.5	Vehicle
	Soybean oil	841	Vehicle
	Vegetable oil	2	Vehicle
	Vegetable oil glyceride, hydrogenated	35	Vehicle
	Vegetable oil, hydrogenated	40	Vehicle
Non-ionic (Macrogol glycerides)	Caprylocaproyl polyoxylglycerides 8 (e.g., Labrasol®)	61.2	Emulsifier Vehicle
	Lauroyl PEG-32 glycerides (e.g., Gelucire® 44/14)	0.15	Emulsifier Solubilizer
	Lauroyl polyoxylglycerides	218	Emulsifier Solubilizer
	Linoleoyl polyoxyl-6 glycerides (e.g., Labrafil® M2125)	–	Vehicle Emulsifier
	PEG-7 linoleoyl glycerides	300	Emulsifier Solubilizer
	Stearoyl polyoxyl glycerides (e.g. Gelucire® 50/13)	960	Emulsifier
Non-ionic (PEGylated triglycerides)	Polyoxyl 35 castor oil	515	Emulsifier Solubilizer
	Polyoxyl 40 hydrogenated castor oil	450	Emulsifier Solubilizer
	PEG-40 castor oil	–	Emulsifier Solubilizer
Non-ionic (PEG ester of Vitamin E)	Vitamin E polyethylene glycol succinate	300	Antioxidant Emulsifier
Non-ionic (Sugar ester)	Sorbitan monolaurate	2.5	Co-emulsifier
	Sorbitan monooleate	153.9	Co-emulsifier
	Sorbitan monostearate	62.5	Co-emulsifier
	Sorbitan trioleate	1.5	Co-emulsifier
	Sucrose palmitate	1.25	Co-emulsifier Dispersant
	Sucrose stearate	44.57	Co-emulsifier Lubricant
Amphoteric (Phospholipid)	Lecithin	167	Emulsifier Co-emulsifier
Anionic (Fatty acids/ salts)	Calcium stearate	91.9	Lubricant
	Magnesium stearate	256.4	Glidant Lubricant
	Sodium caprylate	–	PE
	Myristic acid	–	Co-emulsifier Lubricant
	Oleic acid	0.72	Co-emulsifier Vehicle
	Palmitic acid	6	Co-emulsifier Lubricant
	Polyoxyl glyceryl stearate	23.3	–
	Stearic acid	1203	Lubricant Co-emulsifier
Anionic (Sulphate)	Sodium lauryl sulphate	705	Wetting
Anionic (Sulphate, ethoxylate)	Sodium laureth-3 sulphate	3.5	–
Anionic (Sulphonate)	Dioctyl sodium sulphosuccinate (DSS)	8.2	Wetting
Cationic (QAC)	Benzalkonium chloride	–	Preservative Solubilizer
	Cetylpyridinium chloride	1.5	Preservative

do not appreciably dissolve in water and do not form stable micelles, whereas soluble surfactants dissolve and, above a threshold concentration, spontaneously form micelles. Soluble surfactants are used as detergents, dispersants, foaming agents, wetting agents, preservatives, solubilizers, and oil-in-water (o/w) emulsifiers. There is a further subdivision of soluble surfactants based on structures formed at high concentrations. Sub-group 1 can form lyotropic liquid crystalline mesophases that include cubic, lamellar, or hexagonal liquid crystals. Sub-group-2 does not exhibit such properties. Surfactants in sub-group 1 are linear aliphatic structures (e.g., polysorbate 20, fatty acids) whereas those in sub-group 2 are aromatic (e.g., bile salts). Insoluble surfactants can be used as antifoams, water in oil (w/o) emulsifiers, and co-surfactants and vehicles in lipid-based formulations (LBFs). The insoluble type can be sub-divided into non-swelling amphiphiles that do not form structures in water (e.g., diglycerides and long chain fatty acids), and swelling amphiphiles which may exist as lamellar liquid crystals (e.g., monoglycerides and phospholipids) [12].

Surfactants are typically assigned a hydrophilic-lipophilic balance (HLB) number, an indicator of the net contribution from the hydrophilic and lipophilic functional groups. HLB numbers assist in emulsifier selection, although values are used as aids for other applications [13]. The higher the HLB value the greater the hydrophilicity, hence surfactants with values between 10 and 20 comprise o/w emulsifiers, detergents, and solubilizers. The HLB scale also provides information about dispersion characteristics: HLB values of 1–4 do not disperse; HLB 3–6 disperse with difficulty; HLB 6–10 form course emulsions; HLB 10–13 form cloudy-to-clear dispersions, while HLB 13–20 form micellar systems [14].

The concentration above which soluble surfactants form micelles, the critical micelle concentration (CMC), is another attribute that characterizes surfactants. It is a measure of the concentration above which surfactants form micelles and is a measure of the solubility of the monomeric form of the surfactant, the form responsible for its detergent properties (detergency). Ionizable surfactants have higher CMC values than non-ionic surfactants due to greater electrical work required to bring charged hydrophilic head groups into proximity during the formation of micelles. This is why anionic and cationic surfactants are effective detergents, while non-ionic surfactants are better suited for micellar solubilization.

3. Summary of the interaction of surfactants with GI epithelial cells

The disruption of biological membranes with associated solubilization relates to detergency, the process of removing foreign matter from a

solid. The interaction of surfactant detergents with cells causes insertion of surfactant monomers into the plasma membrane of cells resulting in membrane destabilization and removal and solubilization of membrane fragments. Although the processes are different, the most effective detergents will separately perturb plasma membranes to cause solubilization, in which the cell is dissolved into a mixed micelle solution (Fig. 1).

Surfactants that interact with mammalian cells in a detergent-like manner cause lysis by compromising the integrity of the plasma membrane, which precedes solubilization. The implications for safety in humans depend on the formulation type, exposure levels over time, and the route of administration. Examples include infusion site reactions (e.g., thrombophlebitis induced by polysorbate 80 [15]), irritation at mucosal surfaces (e.g., nasal mucosae exposed to benzalkonium chloride [16]), and perturbation of the skin's stratum corneum (e.g., by sodium lauryl sulfate (SLS) [17]). In GI epithelial models, lysis of enterocytes can compromise the integrity of the intestinal epithelial barrier. However, it is unclear if orally-ingested surfactants cause physiologically-relevant damage to the intestinal mucosa in humans in vivo. This will depend on the concentration that reaches the intestinal epithelial surface after dilution, spreading, and interaction with constituents of luminal fluid of the small intestine (Section 8). Even if they do cause damage in vivo, is it of physiological relevance if the GI epithelium can repair itself so quickly?

The information on the interaction of surfactant detergents with plasma membranes and membrane proteins is mostly derived from biochemical and biophysical methods, where the focus is on cell solubilization and extraction of membrane proteins [2 3 4]. Detergent monomers adsorb to and penetrate phospholipid bilayers causing an increase in surface pressure associated with tighter packing [5]. As the concentration of surfactant further increases, there is sharp decrease in surface pressure, indicating that membrane packing has been compromised. This may be due to removal of individual phospholipids with surfactants that desorb from the membrane or due to buckling of the membrane. These released fragments can form mixed micelles with detergent monomers or micelles [18]. Although both detergent monomers and (mixed) micelles are required for cell solubilization, it is the monomers that compromise the plasma membrane at concentrations below the CMC. Additionally, mixed micelles containing detergent monomers and membrane phospholipids can form at concentrations below the CMC.

The effect of surfactant concentration on membrane perturbation is not straightforward. This is because surfactants exist in two principal forms in the dispersion: free monomers and micelles. As it is the monomers that interact with the plasma membrane, increasing the concentration of the surfactant above the CMC may not lead to a concentration-dependent increase in perturbation. This is because increasing the concentration above the CMC does not affect monomer concentration (the form responsible for membrane damage). However, in many cases, perturbation can still increase above the CMC. This may be because (a) the process of solubilization is more efficient when there are detergent micelles to solubilize phospholipids and membrane

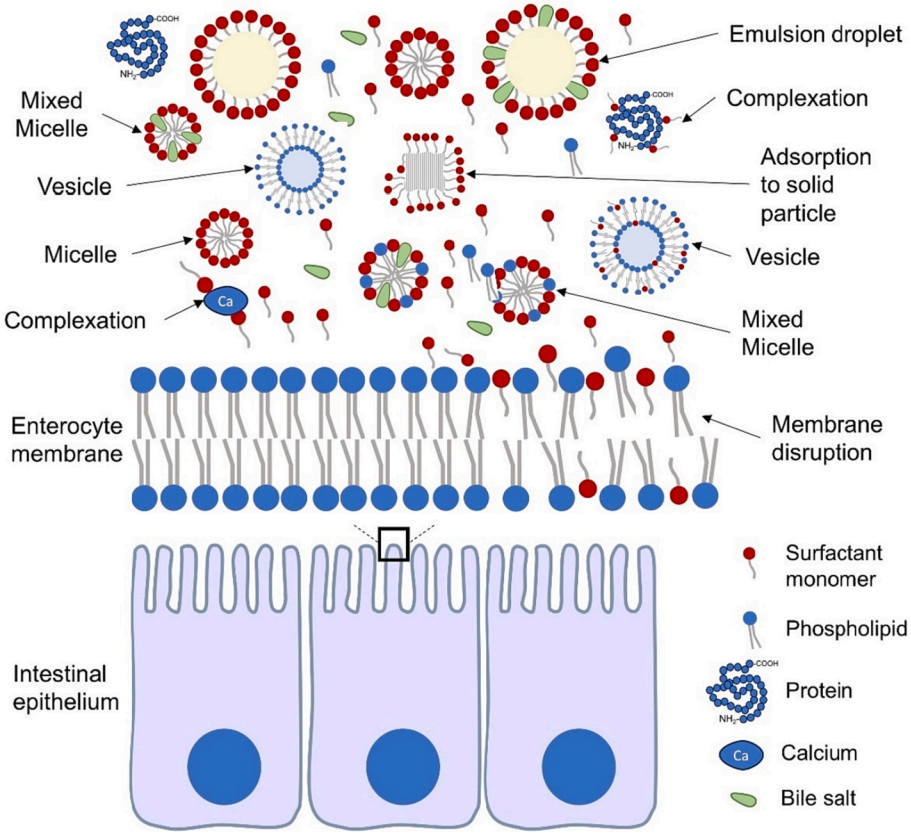

Fig. 1. Interaction of surfactants with constituents of luminal fluid and the epithelial surface. The type of dispersion formed in the GI lumen and the nature of the interaction with the membrane depends on the physicochemical properties of the surfactant, its concentration, and the composition of luminal fluid. This figure illustrates some of the structures that may form when surfactants interact with constituents of luminal fluid. The surfactant may adsorb to undigested solid particles, partition in and emulsify dietary lipids, and form colloidal structures with endogenous surfactants (including vesicles and mixed micelles). They may also bind to dissolved species in luminal fluid such as proteins and divalent cations. These interactions reduce the amount of monomer available to interact with intestinal epithelial membranes. However, If a sufficiently high concentration of surfactant is present, then monomers may interact with the epithelium at a concentration where membrane alterations are observed. Detergent-like effects involve insertion into the lipid bilayer resulting in membrane destabilization, and removal of phospholipids and membrane fragments into mixed micelles, followed by loss of cell integrity.

fragments, or (b) because the micelles act as reservoirs to replenish the monomers that leave the solution to interact with the plasma membrane [19]. Alternatively, a threshold concentration of surfactant may be achieved in the membrane before it is disrupted. The consequence is that the concentration at the CMC is insufficient to achieve membrane disruption and therefore more monomers sourced from micelles are required. If the CMC is very low, then the concentration of monomers will also be low and may never reach a threshold in the membrane to cause perturbation. This is because further increases in the concentration will only increase the number of micelles present in the dispersion.

4. Surfactant properties that favour interaction with biological membranes

Some surfactants lyse cells at very low concentrations (e.g., medium-chain alkyl sulfates), while others have little effect on the plasma membrane (e.g., long-chain fatty acids). The capacity of a surfactant to disrupt cell membranes depends on the balance between its hydrophilic and lipophilic groups. A polar hydrophilic moiety is required for surfactant monomers to dissolve in water and there will be low membrane perturbation if the solubility is low. Surfactants with low CMC values form micelles above that concentration resulting in few monomers available to perturb the membrane. If these monomers do not efficiently penetrate and perturb biological membranes, the surfactant has a higher chance of qualifying as an excipient in formulations.There is, however, no strict correlation between monomer solubility (reflected in the CMC) and membrane perturbation for a homologous series of surfactants. For example, sodium caprylate (C_8) (CMC: ~300 mM) has higher monomer solubility than sodium laurate (C_{12}) (CMC: >3 mM) [20], yet C_{12} is the more potent perturbant [21 22]. For C_8, it is less energetically favorable for its hydrocarbon region to form micelles, and it is therefore weak at penetrating the plasma membrane even at low concentrations. Hence, the hydrocarbon tail of C_8 has insufficient lipophilicity for membrane insertion and disruption. For efficient perturbation, there is a balance required between monomer solubility (hydrophilicity) and membrane insertion (lipophilicity).

The HLB values assigned to surfactants do not entirely predict whether there will be mucosal perturbation. In general, micelle-forming surfactants with HLB values of > 10 are more likely to cause mucosal perturbation than insoluble surfactants with values < 10. However, there is no linear correlation between HLB and perturbation, even within a homologous surfactant series. This is because surfactants in a related series with very high HLB values can be inefficient at penetrating and disrupting membrane structure (e.g., sodium hexanoate, C_6), whereas surfactants in the series with low HLB values (e.g., sodium stearate C_{18}) have low monomer solubility. The HLB where there is greatest mucosal perturbation is observed for such a homologous series will be the one where monomer solubility and membrane insertion are optimal (i.e., C_{10} or C_{12} for fatty acids). The optimal HLB and hydrocarbon chain length will be different for dissimilar hydrophilic head groups.

Ionizable surfactants are more widely used as detergents than non-ionic surfactants. The former group has high CMC values and high free monomer solubility even at long hydrocarbon chain lengths. The existence of the monomeric form in solution is favored over micelle formation because there is repulsion between charged head groups that prevents close interaction. It is only at high concentrations that it is more energetically favorable for micelles to form. There is, therefore, a large free monomer concentration available to interact with the membrane and, because the hydrocarbon chain length is longer, they efficiently penetrate the membrane. Insertion of ionic surfactants into neutral zwitterionic phospholipids bilayers will be more favorable than the formation of ionic micelles, so while micelles may not efficiently form, there can be membrane perturbation at concentrations below the CMC.

Overall, membrane perturbation is more widely observed for ionic surfactants than non-ionic ones. Ionic surfactants are typically included in formulations at low concentrations (e.g., wetting agents or preservatives) and, in some cases, their use is restricted to certain routes of administration (e.g., topical, mucosal). While non-ionic surfactants are considered overall to be milder perturbants than ionic surfactants, there are still some efficient perturbants in the former category, where examples used as cell lysis and protein extract buffers include alcohol ethoxylates ($C_{12}E_9$, Brij® 35), ethers (octyl glucoside, Nonident P40, Triton™ X-100) and esters (e.g., polysorbates). For such applications, non-ionic surfactants may be called mild detergents because they do not denature membrane proteins during extraction, and in some cases have a less pronounced interaction with zwitterionic phospholipid bilayers. However, the term "mild" does not equate with safety as both surfactant categories nonetheless contain some efficient membrane perturbants and solubilizers. Examples of non-ionic surfactant excipients that cause cell perturbation include polysorbate 20, medium chain macrogol-8 glycerides, nonoxynol-9, D-α-tocopherol polyethylene glycol succinate (TPGS), macrogol 15 hydroxystearate (HS15), macrogol 35 castor oil, and sucrose laurate. We discuss these in more detail below. Additionally, a select group of non-ionic surfactants are shown to modulate the activity of the intestinal transporter, P-glycoprotein ((P-GP), including polysorbate 80, PEGylated triglycerides, and TPGS (discussed below) [23].

5. Relationship between membrane damage and overall safety

New candidate excipients are approved by pharmaceutical regulators only as components of a drug formulation, so their safety data in humans is based on overall assessment in an oral dosage form of an active pharmaceutical ingredient (API). Clinical trials report side effects as they impact upon subjects, but alteration to GI barrier integrity or the microbiome may be asymptomatic, for example with microscopic colitis [24]. At the same time, there may be GI side effects in humans that may still be acceptable as part of an overall risk–benefit analysis for a given formulation. Mild-to-moderate GI side effects such as nausea, abdominal pain, and diarrhea are rarely directly attributable to excipients. Even in the unlikely event that biopsy samples could be taken to assess GI safety in humans, the tissue sample would neither be taken at the site where the mucosa is most exposed to the highest concentration of the dosage form constituents before dilution in the lumen, nor before commencement of epithelial repair.

Membrane damage caused by surfactants has been tested in artificial membranes [2 4], brush border membrane vesicles [25], cell cultures [26 27], isolated tissues [99], and animal models [28]. Relating such data to safety in humans requires caution, because the concentration of the surfactant present in the GI tract of humans may be below the threshold for perturbation and, in addition, the exposed tissues in vivo may be less sensitive than in those models. The effect of a dosage form on the GI barrier can be similar in humans and large animals, but identifying the intestinal region where a formulation dissolves and the mucosa is exposed is also difficult when oral dosage forms are given to animals. An additional consideration is the rapid repair of the barrier in vivo before examination of the epithelium, thus likely underestimating initial mucosal perturbation.

The type and quantity of surfactant used in medicinal products depends on the route of administration, which can be grouped into topical, mucosal, and injectable. The skin is more resistant to the effects of detergents like sodium lauryl sulfate (SLS) (or its alternative name, sodium dodecyl sulfate (SDS)), cetrimide, benzalkonium chloride, and soaps than other sites in the body. SLS can be present at high concentrations in shampoos, creams, and gels, but is present in only low quantities in oral dosage forms, while it is not used in pulmonary or ophthalmic formulations. Oral dosage forms containing surfactants including polysorbate 20, SLS, macrogol glycerides, TPGS, and poloxamers are deemed safe for human consumption by regulators, despite the capacity to lyse mammalian cells in vitro and (under certain conditions) to alter epithelial barrier integrity in animal models. The widespread use of

surfactants in food and pharmaceutical products suggests that any GI adverse effects are mild and reversible. Controversial evidence that prolonged exposure of mice to selected surfactants could be linked to intestinal inflammation, colitis, and disruption to the microbiome has raised concerns about their chronic use, especially in ultra-processed foods (see Section 6.1.1). Given the established use of surfactants and other emulsifiers in humans and the absence of clinical data indicating GI dysfunction, there is uncertainty as to whether the findings in pre-clinical animals will be observed in humans. Studies are underway to assess whether reducing ingestion of foods containing selected emulsifiers has any deleterious effect on patients with inflammatory bowel disease (IBD) (see Case 5).

6. Surfactants in oral dosage forms

A list of surfactant categories used in oral pharmaceutical dosage forms in the USA and the EU is shown in Table 1. This is not a conclusive inventory as not all of the excipients found in FDA-approved products are listed in the FDA Inactive Ingredients Database (IID). Furthermore, an equivalent inventory is not available for authorized products in the EU, making it difficult to identify all surfactants permitted for use there. For example, there are technical documents [29] and reference compendia [30] supporting the use of polyoxyl 15-hydroxystearate (Kolliphor® HS15, BASF, Ludwigshafen, Germany) in oral products, yet even though it is present in FDA-approved parenteral and ophthalmic products [31], it is unclear if this surfactant is present in any marketed oral products. Naming convention is another problem that makes it difficult to identify if an excipient is listed on the FDA IID. For example, the United Stated Pharmacopoeia National Formulary (USP-NF) and the European Pharmacopoeia (EP) use the name caprylocaproyl polyoxyl-8 glycerides, but this name is not listed in the FDA IID. Users of the inventory are required to use the FDA Preferred Substance Name when searching for ingredients, in this case, caprylocaproyl polyoxylglycerides 8. There may be further difficulty in identifying publications that report safety information with excipients that are solely referred to by trade names, for example, Labrasol® ALF (Gattefosse, St. Priest, France) or Acconon MC8-2 (Abitec Corp. Ohio, USA).

The surfactants in Table 1 are categorized according to charge and sub-categorized based on chemical structure. Sections 6.1 and 6.2 provide an outline of the actions of prominent surfactants within the GI tract. A case study is included for lipid-based formulations (LBFs) due to the prevalence of surfactants in these dosage forms (Case 1). Surfactants are also used as intestinal permeation enhancers (PEs) for macromolecules. These excipients are intentionally added to oral formulations in particular concentrations to alter epithelial barrier integrity leading to improved intestinal permeability. A case study on PEs is included to show that a degree of intestinal barrier alteration induced by excipients can be acceptable for selected products (see Case 2).

6.1. Non-ionic surfactants

Non-ionic surfactants are the largest category of amphiphiles present in oral dosage forms, where they aid dissolution and improve solubility (Table 1). They are also key constituents of LBFs, functioning as non-aqueous vehicles, co-emulsifiers, and emulsifiers (Table 2). There is no clear relationship between the physicochemical properties (e.g., CMC, HLB, ethoxylate chain length) of non-ionic surfactants and the capacity to cause mucosal perturbation. A relatively low level of mucosal perturbation was seen for TPGS and polyoxyl 35 hydrogenated castor oil (Cremophor® EL), whereas a higher degree was evident for glycerides, macrogol glycerides, and polysorbates. Strong non-ionic surfactants primarily used for cell solubilization are not used in oral dosage forms (e.g., $C_{12}E_8$, Triton™ X-100, Nonident™ P40, Brij®35). Some of the non-ionic surfactants listed in Table 1 are hydrolyzed into ionizable forms, but it is unclear which structures are responsible for mucosal perturbation. For example, digestion of insoluble glycerides liberates

Table 2
Licensed products formulated in LBFs.

Product (API, manufacturer)	Surfactant	Undesirable GI effects†
Norvir® soft capsules Ritonavir (Abbott Laboratories, USA)	Polyoxyl 35 castor oil Oleic acid	Nausea, vomiting, diarrhea, abdominal pain
Norvir tablets Ritonavir (AbbVie, USA)	Sorbitan laurate	Stomach ache, vomiting, nausea, diarrhea
Aptivus® soft capsules Tipranavir (Boehringer Ingelheim, Germany)	Polyoxyl 35 castor oil Mono- and di- glycerides of C_8 and C_{10}	Diarrhea, nausea, abdominal pain
Aptivus® oral solution Tipranavir (Boehringer Ingelheim, Germany)	TPGS Mono- and di- glycerides of C_8 and C_{10}	Diarrhea, nausea, vomiting, abdominal pain
Kaletra® oral solution (EU) Lopinavir and Ritonavir (AbbVie, USA)	Polyoxyl 40 hydrogenated castor oil	Nausea, vomiting, abdominal pain, diarrhea
Kaletra® oral solution (USA) Lopinavir and Ritonavir (AbbVie, USA)	Oleic acid Polyoxyl 35 castor oil	Nausea, diarrhoea, abdominal, pain, vomiting
Kaletra® tablets Lopinavir and Ritonavir (AbbVie, USA)	Sorbitan laurate	Abdominal pain, diarrhea, nausea, inflammation
Agenerase® soft capsules Amprenavir GlaxoSmithKline, UK	TPGS	Nausea, vomiting, diarrhea
Agenerase® oral solution Amprenavir GlaxoSmithKline, UK	TPGS	Nausea, vomiting, diarrhea
Dutasteride soft capsules Dutasteride (RivoPharm UK Ltd)	Propylene glycol monocaprylate	None listed
Avodart® soft capsules Dutasteride GlaxoSmithKline, UK	Mono- and di glycerides of C_8 and C_{10}	None listed
Sandimmun® soft capsules Cyclosporin (Novartis, Switzerland)	Maize oil	Nausea, vomiting, abdominal discomfort, diarrhea
Neoral® soft capsules Cyclosporin (Novartis, Switzerland)	Polyoxyl 40 hydrogenated castor oil Corn oil mono-, di- and tri-glycerides	nausea, vomiting, abdominal discomfort, diarrhea
Roaccutane® soft capsules Isotretinoin (Roche Pharm AG, Germany)	Soybean oil Beeswax	Abdominal pain, bloody diarrhea (rare), vomiting
Absorica® Isotretinoin (Sun Pharm, India)	Soybean oil Stearoyl macrogolglycerides Sorbitan monooleate	Nausea, constipation, abdominal pain, diarrhea, vomiting
Altavita® D3 soft capsules Cholecalciferol (SMB Technol. SA, Belgium)	Medium chain triglycerides	None listed
Deximune® soft capsules	Polysorbate 20 Sorbitan oleate	Vomiting, nausea, diarrhea, abdominal pain

(continued on next page)

Table 2 (*continued*)

Product (API, manufacturer)	Surfactant	Undesirable GI effects†
Cyclosporin (Dexcel Pharma Ltd, UK)	Lecithin Polyoxyl 40 hydrogenated castor oil	
Imodium® soft capsules Loperamide HCl (Johnson and Johnson, USA)	Propylene glycol monocaprylate	Nausea, constipation, vomiting
Omacor® soft capsules Omega 3 fatty acids (BASF, Germany)	Medium chain triglycerides Lecithin	Abdominal distension, diarrhea, constipation, nausea, abdominal pain, vomiting
One-Alpha® soft capsules Alfacalcidol (Leo Laboratories, Denmark)	Sesame oil	Nausea, vomiting, constipation, diarrhea, abdominal pain
Peppermint soft capsules Peppermint oil (Sanofi, France)	Medium chain triglycerides Lecithin	Nausea, vomiting
Solferol® soft capsules Cholecalciferol (Windzor Pharma, UK)	Medium chain triglycerides	Altered bowel movements, nausea, abdominal pain
Vesanoid® soft capsules Tretenoin (Arzneimittel, Germany)	Soybean oil Hydrogenated vegetable oil Beeswax Soy wax	Abdominal pain, nausea
Abidec® oral drops solution Multivitamin (Polpharma, Poland)	Arachis oil Polysorbate 60	None listed
Rocaltrol® soft capsules Calcitriol (Atnahs Pharma, UK)	Medium chain triglycerides	Abdominal pain, nausea, vomiting, constipation
Depakene® soft capsules Valproic acid (AbbVie, USA)	Corn oil	Abdominal pain, constipation, diarrhea, nausea, vomiting
Marinol® soft capsules Dronabinol (AbbVie, USA)	Sesame oil	Nausea, vomiting, abdominal pain, diarrhea
Fortovase® soft capsules Saquinavir (Roche, Switzerland)	Medium chain mono- and di- glycerides	Diarrhea, nausea, abdominal discomfort, ulceration,
Prometrium® soft capsules Progesterone (AbbVie, USA)	Peanut oil Lecithin	Abdominal cramps, nausea, vomiting
Targretin® soft capsules Bexarotene (Eisai Co., Ltd, Japan)	Polysorbate	Nausea, diarrhea, vomiting, constipation, abdominal pain
Zemplar® soft capsules Paricalcitol (AbbVie, USA)	Medium chain triglycerides	Stomach discomfort, constipation, diarrhea, nausea, vomiting
Hectorol® soft capsules Doxercalciferol (Sanofi-Genzyme, USA)	Fractionated coconut oil triglycerides	Nausea, vomiting
Rapamune® oral solution Sirolimus (Pfizer, USA)	Soya oil Polysorbate 80 Phosphatidyl choline Mono- and diglycerides Ascorbyl palmitate	Stomach pain, diarrhea, constipation, nausea
Gengraf® capsules Cyclosporin (AbbVie, USA)	polyoxyl 35 castor oil polysorbate 80 sorbitan monooleate	Stomach pain and discomfort, vomiting, diarrhea

Table 2 (*continued*)

Product (API, manufacturer)	Surfactant	Undesirable GI effects†
Lovaza® soft capsules Omega 3 fatty acid ethyl esters (GSK, UK)	Soybean oil	Upset stomach
Lipofen® hard capsules Fenofibrate (Kowa Pharma, USA)	Gelucire 44/14 (lauroyl macrogol glyceride type 1500)	Nausea, stomach pain, vomiting, diarrhea
Hycamtin® hard capsules Topotecan (Novartis, Switzerland)	Hydrogenated vegetable oil Glyceryl monostearate	Diarrhea, abdominal pain, intestinal inflammation
Claritin® Liqui-Gel capsules Loratadine	Caprylic/capric glycerides Polysorbate 80	None listed
Xtandi® capsules Enzalutamide Astellas, Japan	Caprylocaproyl macrogol-8 glycerides	Upset stomach, nausea, vomiting, diarrhea
Ofev® soft capsules Nintedanib Boehringer Ingelheim, Germany)	Medium chain triglycerides Hard fat Soya Lecithin	Diarrhea, nausea, vomiting, abdominal pain, inflammation
Vargatef® soft capsules Nintedanib Boehringer Ingelheim, Germany)	Medium chain triglycerides Hard fat Soya Lecithin	Diarrhea, nausea, vomiting, abdominal pain, inflammation
Rayaldee® soft capsules Calcifediol (CLS Vifor, Switzerland)	Paraffin Hard paraffin Glycerol monostearate Lauroyl macrogolglycerides	Constipation, nausea, diarrhea, abdominal discomfort, vomiting
Pravafenix® hard capsules Pravastatin, fenofibrate (Laboratoires SMB, Belgium)	Ascorbyl palmitate Lauroyl macrogolglycerides	Abdominal pain, nausea, vomiting, diarrhea, constipation
Lynparza® hard capsules Olaparib (AstraZeneca, UK)	Lauroyl macrogol-32 glycerides	Nausea, vomiting, diarrhea
Rydapt® soft capsules Midostaurin (Novartis, Switzerland)	Macrogol glycerol hydroxystearate Maize oil mono-di-triglycerides	Nausea, vomiting, constipation, abdominal pain, diarrhea
Lamprene® capsules Clofazimine (Novartis, Switzerland)	Beeswax	Diarrhea, nausea, vomiting, GI intolerance
Drisdol® capsules Ergocalciferol (Validus Pharma, USA)	Soybean oil	Nausea, vomiting, constipation
Claravis® capsules Isotretinoin (Barr Pharma, USA)	Hydrogenated vegetable oil Soybean oil White wax Polysorbate 80	Nausea, vomiting, diarrhea, bloody stools
Amitaza® soft capsules Lubiprostone (Takeda, Japan)	Medium chain triglycerides	Diarrhea, nausea, vomiting, abdominal distension, pain and discomfort
Ibuprofen soft capsules Ibuprofen (GSK, UK)	Soya lecithin Medium chain triglycerides Glyceryl stearate Oleic acid Ascorbyl palmitate	Abdominal pain, distension, nausea, vomiting, diarrhea, gastritis, ulceration, inflammation

(*continued on next page*)

Table 2 (*continued*)

Product (API, manufacturer)	Surfactant	Undesirable GI effects†
Ibuprofen soft capsules Ibuprofen (Brill Pharma Ltd, Ireland)	Polysorbate 80	Abdominal pain, distension, nausea, vomiting, diarrhea, gastritis, ulceration, inflammation
Nurofen® 400 mg liquid capsules Ibuprofen (PCO Ltd, Ireland)	Medium chain triglycerides Lecithin	Abdominal pain, distension, nausea, vomiting, diarrhea, gastritis, ulceration, inflammation, perforation

† Taken from the summary of product characteristics and/or patient information leaflet. These effects may be common or less common depending on the specific products.
#Discontinued.

free medium-chain fatty acids that are known to cause mucosal perturbation [32]. Intestinal lipases can also hydrolyze medium chain macrogol-8 glycerides (Labrasol®, Gattefosse, St. Priest, France) into free fatty acids, although a recent study found that mucosal perturbation was more likely caused by the parent non-ionic surfactant rather than by free fatty acids liberated during enzymatic degradation [33].

6.1.1. Polysorbates

Polysorbates are fatty acid esters of sorbitan that are commonly ethoxylated with 20 ethylene oxide sub-units. The polyoxyethylene sorbitan is the hydrophilic moiety and the fatty acid ester is the hydrophobic moiety. For common polysorbates, the numbers 20, 40, 60, or 80 refer to the fatty acid chain lengths of C_{12}, C_{16}, $C_{16:1}$, and C_{18}, respectively. There are, however, cases where this number refers to the subunits of ethylene oxide, for example, polysorbate 21 (polyoxyethylene 4 sorbitan monolaurate). Aside from the monoesters, there are tristearate and trioleate forms named polysorbate 65 and 85, respectively. The most common polysorbates used as excipients are polysorbate 20 (polyoxyethylene sorbitan monolaurate) and polysorbate 80 (polyoxyethylene sorbitan monooleate). The HLB of polysorbate 20 is 16.7 and for polysorbate 80 it is 15, which puts them in the category of soluble surfactants that principally function as o/w emulsifiers and solubilizers. The low CMC values for polysorbate 20 (60 mg/L) and 80 (14 mg/L) indicate that micelles form at low concentrations, so there is low solubility of the monomeric form compared to anionic surfactants like SDS with its higher CMC value (CMC: 2.45 g/L).

Commercially available polysorbates are complex blends where the primary chemical structure often accounts for only a minor fraction. This is due to structural variation in the number and position of ethylene oxide sub-units in the hydrophilic moiety and to variation in the hydrocarbon chain length, where 50–60% of polysorbate 20 is monolaurate ester and the remainder comprises a range of C_8 to C_{18} esters [34]. For polysorbate 80, the monooleate ester accounts for 58–85%, with C_{14} to C_{18} esters accounting for the remaining fraction. Further variability may also arise from the susceptibility of polysorbates to hydrolysis and oxidation [35]. Polysorbates are widely used excipients in topical, mucosal, oral, and injectable formulations. In Germany, they are present in over 3000 licensed medicines [36]. Oral products containing polysorbates on the FDA IID include tablets, suspensions, and solutions [37]. They are also present in several LBFs (Table 2). Based on acceptance levels in food and pharmaceutical products (Section 5), an oral dosage form is allowed to contain over 50 mg, whereas food may contain much higher quantities. The EMA has outlined the effects of polysorbates on membranes and different cell populations [36].

The effect of polysorbates on intestinal barrier integrity has been reviewed alongside 250 other compounds [5]. Although polysorbates can cause cell membrane perturbation (e.g., hemolysis [38]) and alter the GI barrier [32], they are not regarded among the leading intestinal PEs. Polysorbate 80 is a constituent of the Transient Permeation Enhancer® technology (TPE®) of Chiesi Farmaceutici S.p.A (Parma, Italy) (originally from Chiasma, Jerusalem, Israel), which facilitates oral administration of octreotide (Mycapssa®, Chiesi) [39]. However, it is likely to play a minor role in inducing permeability compared to other constituents of the TPE® formulation (see Case 2).

In filter-grown Caco-2 monolayers, polysorbate 80 (3.8 mM or 0.5 % w/v) increased the apparent permeability coefficient (P_{app}) of ^{14}C mannitol by a modest 4-fold, which was 10 to 100 times lower than other surfactants including SDS (2 mM, 140-fold), sodium taurocholate (50 mM, 400-fold), dioctyl sulfosuccinate (1.6 mM, 90-fold), and sodium taurodeoxycholate (5 mM, 200-fold) [26]. In another study, mannitol permeability across monolayers was not altered by 0.01 to 1 % w/v polysorbate 80 and was similar to the lack of effect of Cremophor® EL [40]. This result was in contrast to the non-ionic detergents, Nonident™ P-40 and Triton™ X-100, which caused a 40-fold increase in mannitol permeation at concentrations as low as 0.05% w/v. These data emphasize that polysorbates have a milder effect on epithelial monolayers than detergents used in cell solubilization buffers. The same Caco-2 study showed that low concentrations of polysorbate 80 (0.0001–1 % w/v) had an inhibitory effect on P-GP efflux [40]. Others have also shown that polysorbates can inhibit P-GP in vitro [41]. With polysorbate 20, there was a concentration-dependent increase in the transport of metformin across Caco-2 monolayers between 1 and 5 % w/v and a 20-fold increase in permeation at the higher 5% concentration [42]. No increases in metformin flux were detected for polysorbate 60 or 85. Increased metformin flux coincided with a loss of viability, suggesting that transport was due to transcellular perturbation of the epithelial barrier and not necessarily due to inhibition of P-GP.

In rectal perfusion of rats, there was a small increase in plasma AUC of sulfanilic acid when presented in PBS containing 5% w/v polysorbate 80 (1.4-fold increase), 60 (1.5-fold), 40 (2-fold), or 20 (3.4-fold) [43]. A 10-fold increase in AUC was also detected when each polysorbate was perfused in Miglyol® or olive oil, emphasizing the behavior change that can occur in lipid-rich environments. In contrast, the presence of Ca^{2+} in perfusion fluid counteracted sequestration and attenuated the barrier alteration by polysorbates in rat colonic perfusions [44], an example of the influence of luminal fluid composition on excipient interaction with the mucosa. In rat small intestinal perfusions, 1 % w/v polysorbate 80 slightly increased the percentage absorption of the marker, sulfaguanidine, to 10.8% versus 7.4% in controls [45]. The effect was much lower than the ionisable surfactants, SDS (29%) and cetyltrimethylammonium bromide (CTAB) (26%). Perfusion of 1% w/v polysorbate 60 and 80 into rat ileal segments caused epithelial release of the intracellular lysosomal enzyme, N-acetyl-β-glucosaminidase 15 min after instillation [46], which was accompanied by a modest increase in permeation of fluorescein. The authors speculated that these surfactants might increase permeability to harmful compounds in the gut. The effects of polysorbate 80 and Triton™ X-100 on intestinal epithelial cell integrity were assessed via lactate dehydrogenase (LDH) release from rat jejunal and colonic sacs [47]. There was a two-fold increase in LDH release from rat jejunum with 1% w/v polysorbate 80 versus control compared to a 7-fold increase for 1% w/v Triton™ X-100. Increases in mucus turnover were also recorded for each surfactant. Both LDH release and mucus turnover were reversed over 4 h when perfusion of polysorbate was stopped, but they remained elevated with Triton™ X-100. Additionally, Triton™ X-100 caused histological damage compared to minimal effects with polysorbate 80.

Polysorbates permeabilize and solubilize bilayer vesicles [48 49 50], but are slower to perturb membranes than detergents like Triton™ X-100 and octyl β-D-glucopyranoside. This may be due to slower partitioning in the membrane and a lower free monomer concentration that gives rise to a slower build-up of polysorbates in the membrane from micelles [50]. Given the short residence time of oral formulations at any particular focal point in the GI tract, the graduated effects of polysorbates may limit membrane damage to formulations containing high

concentrations. This counters the argument that modest alterations to the permeability of Caco-2 monolayers induced by polysorbates translate to mucosal perturbation from oral dosage forms containing low quantities of polysorbates in vivo. The integrity of the GI barrier of rats was assessed by administering the transmucosal marker, phenol red 15 h after gastric instillation of 200 mg in 2 mL of polysorbate 80, SLS, or Triton™ X-100 via intubation [51]. There was comparable urinary recovery of phenol red for polysorbate 80 (2.5%) versus control (2.3%) indicating that the barrier was intact 15 h after administration of a high dose of polysorbate 80. On the other hand, there was a higher recovery of phenol red in the urine of rats treated with SLS (5.7%) and Triton® X-100 (6.8%), evidence that high doses of these detergents cause prolonged alteration to GI barrier integrity.

Toxicology studies indicate that polysorbates are well tolerated in animals following repeated oral administration [52 36]. In one example, body weight, food consumption, and histopathology in mice, rats, dogs, and monkeys receiving 10 mg/kg daily for 3 months was comparable to control [53]. The animals received 5 mL/kg of polysorbate 80 (0.2 % w/v), a dilute concentration that does not represent GI exposure to higher concentrations that may be observed in the GI lumen with some oral formulations listed on the FDA IID [53]. On the other hand, repeated daily administration of higher concentrations of polysorbate 80 (2.5 to 5% w/v) in food for two years resulted in hyperplasia, inflammation, and ulcers of the fore-stomach of mice [54]. Another two-year study in mice recorded diarrhea at high doses (10 to 20% w/v) [55], but this was due to an osmotic laxative effect caused by the presence of the unabsorbed polyoxyethylene sorbitan moiety [54].

Polysorbate 80 also increased oral absorption of the plasticizer, diethyl hexyl phthalate (DEHP) [56], and its metabolite mono ethyl hexyl phthalate (MEHP) [57]. However, the 25 mg/kg dose was higher than those permitted by the EMA for medicinal products. These studies on plasticizers also assessed the interaction of polysorbate 80 with intestinal epithelial cells, finding a concentration-dependent reduction in the viability of Caco-2 cells between 0.25 and 0.5% w/v [56]. At sublethal concentrations (0.05 to 0.125% w/v), there was disruption to the mitochondrial membrane potential (MMP), which may lead to inhibition of P-GP by lowering intracellular ATP [56]. Other dietary surfactants have also been shown to disrupt MMP before loss of cell integrity (e.g., fatty acids [58]). The researchers subsequently showed a reduction in mucin-2 (muc-2) in the GI of rats and mice dosed with 25 mg/kg polysorbate 80 [57]. Decreases in muc-2 levels were also seen in HT29 cells exposed to 0.025% to 0.3% w/v [57], confirming previous findings of altered mucus turnover [47]. Rodents that received water containing 1% w/v polysorbate 80 for 2 weeks had a reduced luminal mucus layer, which exacerbated mucosal perturbation and histological damage induced by 5-aminosalicylic acid (5-ASA) [59]. The effect could be reversed with rebamipide, a compound that promotes mucus secretion in goblet cells. Changes to mucus levels were accompanied by reduced levels of the tight junction (TJ) proteins claudin and occludin in mice and rats dosed at 25 mg/kg [57]. Similar reductions of these TJ proteins were also seen in Caco-2 cells at concentrations of 0.025% – 0.3% w/v [57]. While alteration to the expression of TJ proteins suggests alteration of intestinal barrier integrity occurs via a paracellular mechanism, this may be a consequence of transcellular perturbation because loss of cell viability was observed in the same concentration range in Caco-2 cells [56] and there was increased intracellular accumulation of MEHP-fluorescein in Caco-2 and HT29 cells [57].

Pre-treatment of isolated human Peyer's patch follicle associated epithelium (FAE) and villous epithelium (VE) mounted in Ussing chambers with 0.1% w/v polysorbate 80 for 30 min caused a 2-fold increase in translocation of *E. coli* over 4 h [60]. The same study also showed translocation across co-culture-based cell models of intestinal M−cells. There was no reduction in transepithelial electrical resistance (TEER). The authors suggest penetration of the surfactant into the cell membrane could alter bacterial adhesion and translocation. The authors speculated that food emulsifiers might have a potential involvement in Crohn's pathogenesis.

Low concentrations of polysorbate 80 induced low-grade inflammation and obesity/metabolic syndrome in mice, and colitis in predisposed mice [61]. These effects were attributed to changes to the composition of the microbiota and disruption to host–guest interactions (detailed below). The authors concluded that the widespread use of emulsifiers could be a contributing factor in the increased incidence of chronic inflammatory diseases.

The proximity of bacteria to the intestinal epithelium was reduced by > 50% from 25 μm in control to 10 μm in mice that were given 1% w/v polysorbate 80 in drinking water for 12 weeks [61]. There was a two-fold increase in bacteria adhered to the colon of mice given polysorbate 80. This encroachment correlated with reduced mucus thickness. Changes to the composition of the microbiota included reduced levels of bacteria associated with gut health (*Bacteroidales*) and increased levels of mucus-degrading bacteria such as *Ruminococcus gnavus*. Both of these populations appear to increase during the onset of symptoms in patients with Crohn's disease. There was also a reduction in microbial diversity and increases in proinflammatory proteobacteria as well as increased levels of proinflammatory LPS and flagellin in faecal samples. Polysorbate 80 promoted colitis in two engineered strains of mice that are likely to develop shifts in microbiota and inflammation (namely IL-10$^{-/-}$ and TLR5$^{-/-}$). The surfactant did not induce overt colitis-like symptoms in wild-type mice, although there was evidence of low-grade inflammation. As there are links between low-grade inflammation and metabolic syndrome, the authors then assessed weight and fat mass in wild-type mice, revealing increases in both. Dysglycaemia was also seen in fasted blood glucose measurements and tolerance testing. The effects on metabolic syndrome persisted for 6 weeks after ceasing consumption. The low-grade inflammation and metabolic syndrome did not occur in germ-free mice, suggesting an interplay between mucosa, polysorbate 80, and the microbiota rather than a direct interaction between the surfactant and the epithelium. There were no changes to levels of short-chain fatty acid, bile acids, mucus thickness or penetration of 0.5 mm beads into mucus in germ-free mice. Additionally, transferring the microbiota from mice treated with polysorbate 80 to germ-free mice led to the same GI problems observed in mice given the surfactant, including encroachment, low-grade inflammation, and metabolic syndrome. In a subsequent study, mice fed polysorbate 80 for 12 weeks had shortened colons at the end of the study period, a surrogate for intestinal inflammation [62]. There was greater food intake in animals fed polysorbate 80, accompanied by an increase in body weight in the form of fat deposits.

Mice that were fed 1% w/v polysorbate 80 in drinking water for 1 month also had elevated plasma levels of LPS, as well as higher TNF-α expression in muscle tissues compared to control [63]. There were higher plasma levels of insulin in the polysorbate-fed mice, and both groups had similar levels of blood glucose, giving rise to impaired insulin sensitivity due to a higher homeostasis model assessment ratio (HOMA-R). After running exercises, there were reductions in muscular pH, mitochondrial cytochrome oxidase activity, and glycogen content versus mice not given polysorbate, data that suggests impaired skeletal muscle metabolism.

The incorporation of polysorbate 80 into mouse chow mimicked the pro-inflammatory effects and metabolic changes observed in drinking water, suggesting adverse events in fasted- and fed state conditions [61]. This latter example suggests that the deleterious effects of polysorbate 80 do not relate to detergent-like perturbation of the epithelial surface.

To determine if low-grade inflammation and metabolic syndrome resulted from direct changes to the microbiota or if there was involvement from the host, an in vitro microbiota model, M−SHIME (mucosal simulator of the human intestinal microbial ecosystem), was used [64]. Polysorbate 80 directly altered the composition of the microbiota and increased inflammatory mediators such as flagellin. The transfer of the polysorbate-treated M−SHIME microbiota to germ-free mice led to low-grade inflammation in the host and evidence of metabolic syndrome.

These results suggest that the direct interaction of polysorbate 80 with the microbiome is responsible for these inflammatory disorders in mice.

It is difficult to estimate how constituents of food or the formulation might impact surfactant-induced alteration to the intestinal microflora because the mechanism by which changes occur is not clear. It is tempting to suggest that changes occur due to the direct effects on the colonic microflora by unabsorbed surfactants like polysorbate 80. However, it is plausible that changes to the microflora are initiated by interaction between bacteria and ingested surfactants in the upper GI tract as the concentration of polysorbate administered to mice in drinking water is higher than in aqueous dispersions shown to alter bacterial growth rate [65] and cause loss of viability [66]. Since microbiota changes have also been observed in mice receiving polysorbate 80 in chow, there is also a possibility that these effects are observed when the surfactant is administered both in food and pharmaceutical dosage forms.

The same authors assessed the potential for low-grade inflammation to promote carcinogenesis in the colon [9]. Mice that consumed 1% w/v polysorbate 80 in drinking water for 90 days had larger tumors in a subsequent colitis-associated cancer model (dextran sodium sulfate) and azoxymethane) and there was greater immune cell infiltration. Changes to the microbiota were associated with the development of a pro-inflammatory environment in the small intestine, which predisposed to subsequent tumorigenesis. Consumption of polysorbate 80 was associated with proliferation of colonic epithelial cells and increased apoptosis both before and after DDS/azoxymethane treatment, indicative of increased cell turnover. The unbalanced proliferation and apoptosis caused by polysorbate 80 did not occur in germ-free mice, again indicating an interplay between the mucosa, polysorbate, and microbiota.

The dose of polysorbate in mice studies [61] is above the ADI of 25 mg/kg/day set by EFSA and above the estimated average consumption in the US [67] and selected EU countries [52]. . Study investigators acknowledged that the dose administered to mice is higher than the estimated average daily consumption of polysorbate 80 in the UK, which is somewhat offset by differences in the exposure duration [8]. . Studies are underway to determine if ingestion of dietary emulsifiers are implicated in intestinal inflammation in IBD patients [68].

Although intestinal inflammation was attributed to actions of polysorbate 80 on intestinal microbiota, there is also evidence of direct action on epithelial cells and the mucus gel layer. Treatment of isolated porcine mucus with 1% polysorbate 80 for 2 h slightly decreased the median pore size from 109 nm to 88 nm [69]. The mucin pore sizes suggest that monomers and even polysorbate micelles (with an approximate diameter of 8 nm) are free to diffuse through mucin pores [69]. However, a study of purified pig gastric mucin showed that polysorbate 80 monomers freely diffuse, whereas diffusion of micelles was moderately obstructed by mucin [70]. There was a greater obstruction to the diffusion of micelles in native mucus versus purified mucin, which was due to interaction with other constituents in mucus, in particular lipid depots [70]. Such interaction is more a function of the amphiphilic properties of polysorbate 80 molecules rather than the polyoxyethylene chains, which generally do not interact with mucus [71]. At a time-scale of 3 s, there was reduced diffusivity of anionic and neutral nanoparticles into mucus treated with the surfactant [69]. This may be due to disruption to hydrophobic interactions in the mucus structure and/or increased viscosity. The effect of polysorbate 80 on the mucus gel layer of a Caco-2 HT29-MTX co-culture was assessed at 0.5% w/v polysorbate 80 [69]. There was partial removal of the mucus gel layer from the co-culture after 1 h exposure. When 1% polysorbate 80 was injected into rat intestinal loops for 30 min the mucus layer was more loosely attached to the mucosa and there was a greater amount of mucus on the villus tips and in the lumen compared to control. Exposure of rat intestinal tissue mounted in Ussing chambers to 0.5% or 1% polysorbate 80 increased the concentration of dissolved mucus sloughed into the bathing solution.

The significance of findings in pre-clinical animals and cell-based models remains unclear. In a safety evaluation in humans, the ingestion of 3 g (6 × 500 mg capsules) polysorbate 60 twice daily for 28 days did not cause clinically recognizable adverse effects in human subjects [72]. There were isolated instances of diarrhea in some subjects, although investigators did not attribute these to the surfactant. A re-evaluation of polysorbates by the EFSA concluded that further studies are required before any alteration to the ADI for polysorbates [52].

The documented effects of surfactants on intestinal barrier integrity raise concerns that they could inadvertently increase the absorption of drugs that are not completely absorbed in humans [73]. However, there was no change in the absorption of valacyclovir (500 mg tablets, Mylan Pharma, PA, USA), enalaprilat (2.5 mg/2mL solution, West-Ward Pharma, NJ, USA) or chenodeoxycholate (250 mg Chenodal, Travere Therapeutics, CA, USA) when healthy volunteers were given these drugs before or after a six-day pre-treatment with polysorbate 80 capsules (400 mg, twice daily) [74]. As each volunteer received one 400 mg polysorbate 80 capsule at the same time as drug administration, this study shows no acute alteration to permeability at the time of administration and shows an absence of progressive barrier alteration caused by pre-treatment for six days. Study investigators concluded that polysorbate 80 is unlikely to cause bioinequivalence.

6.1.2. Poloxamers

Poloxamers are synthetic tri-block co-polymers used widely in oral, topical, mucosal, and injectable formulations. These macromolecule surfactants have the general formula $E_mP_nE_m$ or less commonly $P_nE_mP_n$, where E is a hydrophilic polyoxyethylene chain and P is a hydrophobic polyoxypropylene chain. A catalogue of poloxamers is available and although they are collectively viewed as safe, there are concerns relating to individual ones. For example, Poloxamer 407 is associated with renal toxicity and is therefore not used in injectable formulations, although it is used in oral products [75]. Poloxamer 188 ($E_{80}P_{30}E_{80}$) (P188) is listed in several formulations of the FDA IID. This surfactant has a molecular weight (MW) of 8750 Da, a HLB value of ~ 29, and a CMC of 0.48 mM. Renal toxicity in early clinical trials with P188 was greatly reduced when low MW constituents (e.g., glycols) were removed from the commercial grade [76]. P188 has low hemolytic potential [77] and did not alter the viability or barrier integrity of Caco-2 monolayers up to a concentration of 50 mg/mL [78]. While membrane perturbation is common for many small molecule surfactants, P188 has been assessed for its capacity to repair mammalian membranes [79]. Protective effects have also been seen with other poloxamers. For example, P407 attenuated the hemolytic action of the amphiphilic drug, miltefosine [80]. P188 is also used as a stool softener. In one example, an oral suspension contains 1 g per 5 mL (Co-Danthramer, Pinewood, Ireland), a high dose that suggests it is not a potent mucosal perturbant.

6.1.3. Fatty acid ethoxylates

Fatty acid ethoxylates are non-ionic surfactants formed by the ethoxylation of fatty acids. The simplest forms are monoesters of fatty acid and PEG. Examples include PEG-5 oleate, PEG-8 stearate, and polyoxyl 40 stearate. Long-chain fatty acid ethoxylates are considered mild surfactants. For example, polyoxyl 40 stearate (Myrj™ 52) did not affect permeability or histology in isolated rabbit gastric mucosa, unlike a panel of Brij® surfactants [81]. Structurally-related excipients (e.g. PEG-32 stearate, Gelucire® 48/16, Gattefosse) are under investigation as solubility enhancers [82]. The fatty acid ethoxylates are similar to macrogol glycerides, which are blends containing fatty acid monoesters and diesters of PEG, and a proportion of glycerides (mono- di- and tri-esters). Macrogol glycerides are discussed in Section 6.1.8.

6.1.4. Fatty alcohol ethoxylates

Fatty alcohol ethoxylates are non-ionic surfactants formed by ethoxylation of fatty alcohols. They are structurally similar to fatty acid ethoxylates, the difference being that fatty alcohols are bound to the

PEG moiety by a monoether whereas the fatty acid link is a monoester. An extensive number of surfactants are in this category, but few are used in oral products. Strong detergents in this group are present in cleaning products and personal care products for external use. Just one fatty alcohol ethoxylate, steareth-40 ($C_{18}E_{40}$), has a listing on the FDA IID, and this entry does not list a maximum daily dose. Increased permeability across isolated rabbit gastric mucosa and membrane solubilization was evident with homologs of medium hydrocarbon chain length (C_7 to C_{10}) and ethylene oxide chains between E_{10} to E_{20}, [81]. A related homolog of Steareth-40 (Steareth-30) caused less damage to the oral mucosa compared to SLS in toothpaste formulations given to healthy volunteers [83].

6.1.5. Fatty acid esters

A group of fatty acid esters form a miscellaneous group of non-ionic insoluble surfactants (Table 1). Examples include mono/di esters of medium/long chain fatty acids with propylene glycol, polyglycerol, or ascorbate. Although these hydrophilic head groups are not structurally related, they are all non-ionic surfactants with HLB values of < 10 (e.g., propylene glycol monocaprylate (HLB: 6.7), polyglyceryl-3 oleate (HLB: 6.2)). Excipients in this group function as non-aqueous vehicles and co-emulsifiers, the exception being ascorbyl palmitate which is a fat-soluble form of ascorbic acid [84]. Surfactants with HLB values in this range do not form micelles and possess low monomer solubility, so there is a low potential for detergent-like membrane perturbation compared to ones with more soluble hydrophilic head groups. However, there may be improved solubility and greater membrane perturbation when used in formulations containing soluble surfactants, co-solvents, and/or oils [85].

Propylene glycol monocaprylate is available in two dispersions, type I (40–75% monoester) and type II (>75% monoesters). Exposure of Caco-2 monolayers to 0.05–0.25% v/v propylene glycol monocaprylate (Type I, Caproyl® 90, Gattefosse) caused a gradual reduction in epithelial barrier integrity over 6 h [86]. High concentrations (5–15% v/v) in rat intestinal loops increased barrier permeability to insulin [86] and fluorescent dextrans (4–70 kDa) [25], evidence that selected co-surfactants and vehicles from this category can alter GI barrier integrity. The same concentration range of Capryol® 90 also caused perturbation of rat brush border membrane vesicles [25]. There were reversible changes to TJ proteins in rat intestinal loops after 4 h treatment with 10% v/v Caproyl® 90, an effect that could be reversed after a 4 h washout period.

Propylene glycol monolaurate (HLB: 3) is more lipophilic than the caprylate ester, so a lower quantity of monomers is available to perturb the membrane, which is reflected in a lower capacity for barrier alteration for Lauroglycol™ 90 (Gattefosse) compared to Caproyl® 90 [86]. Overall, these co-surfactants are unlikely to cause appreciable disturbances to GI health due to perturbed membranes.

6.1.6. Fatty alcohols

Fatty alcohols are insoluble surfactants commonly used in topical formulations as co-emulsifiers. Examples listed in the FDA IID include myristyl, cetyl, and stearyl alcohol (Table 1), but they are not common constituents of LBFs (Table 2). The examples in Table 1 are considered non-toxic at permitted levels [87]. There is an inverse association between chain length and irritation, where C_6-C_{11} alcohols are irritants, C_{12}-C_{16} alcohols are mild irritants, and long-chain alcohols (>C_{18}) are non-irritant [88]. A long-chain fatty alcohol may comprise a large portion of an oral or rectal dosage form if included as a matrix agent. However, as there is likely to be gradual release from such dosage forms, the GI epithelium is unlikely to access high enough concentrations to elicit mucosal perturbation.

6.1.7. Glycerides

Glycerides are lipids consisting of mono-, di-, or tri-esters of fatty acids and glycerol. They are subdivided into short-, medium-, and long-chain triglycerides, diglycerides, and monoglycerides (Table 1). Native triglycerides are not amphiphiles, although they are digested into monoglycerides, diglycerides, and free fatty acids. The medium-chain triglyceride, trilaurin, had no effect on barrier integrity to a marker molecule in washed rat jejunal loops, yet when it was free to mix with luminal constituents in unwashed jejunal loops or administered with lipase and bile salts, there was barrier alteration [89].

Mono- and di- glycerides are insoluble non-ionic surfactants that alone do not form micelles or stable emulsions, rather they function as vehicles or co-emulsifiers in LBFs (Table 2). As major constituents of edible oils and fats, there are no safety concerns for the small quantities used as excipients in oral formulations. Indeed, some show a protective effect on the GI barrier [90]. Other glycerides and their degradation products are considered less favorable for gut health [91], and little is known about the role played by excessive consumption in chronic disease. Additionally, many LBFs combine glycerides with soluble surfactants (see Table 2). These combinations may together cause greater intestinal perturbation than the individual constituents [33].

Of the excipients listed in Tables 1 and 2, medium-chain monoglycerides (MCM) have the highest level of monomer solubility of glycerides used in oral formulations, hence these are more likely to freely interact with biological membranes to alter barrier integrity [92]. Alteration to rat and dog intestinal barrier integrity to a marker molecule followed the order monocaprylate > dicaprylate > tricaprylate [92 93]. In one study, GI barrier alteration in rats followed the rank order monocaprylin > monocaprin > monolaurin [93]. In another, the optimal hydrocarbon chain length required for barrier alteration was 10 carbons [92]. The effects of MCMs on barrier integrity are low compared to free fatty acids and their soluble sodium salts [89 94]. Nevertheless, monocaprylin is a constituent PE alongside C_8 and polysorbate 80 in the TPE® technology, although it is unclear if it contributes to barrier integrity alteration (see Case 2).

MCMs and free fatty acids also have antimicrobial activity [95] so may alter the intestinal microflora. There was decreased expression of three inflammatory mediators, toll-like receptor-2 (TLR-2), tumor necrosis factor (TNF), and monocyte chemotactic protein-1 (MCP-1) in mice fed high doses of monocaprylin for 22 weeks [96]. This monoglyceride also contributed to a healthy gut microbiota by increasing microbiome diversity. Changes to the composition included an increase in bacteria that are beneficial to the host (e.g., *Ruminococcus, Lactobacillus*) and a decrease in those that are not (e.g., *Bacteroidetes*).

Alterations to the microbiota by MCMs are not limited to monocaprylin. While monolaurin is not listed on the FDA IID, it is present in some of the listed oils (e.g., coconut oil). Dietary levels (150 mg/kg) of monolaurin promoted metabolic syndrome, alterations to the gut microbiota, and systemic inflammation in mice fed a low-fat diet [97]. The same authors showed that, at higher doses (450 mg/kg), monolaurin altered the intestinal microflora and modulated lipid metabolism to improve metabolic syndrome in mice on a high-fat diet [98]. At a dose of 1600 mg/kg, there was an alteration to the gut microbiota and decreased chronic systemic inflammation in mice fed a low-fat diet [99], along with reduced obesity in mice fed a high-fat diet [100].

6.1.8. Macrogol glycerides

Macrogol glycerides are mono- or diesters formed between a PEG chain and one or two fatty acids liberated from glycerides. They may also contain a small fraction of glycerides and free PEG. The primary constituents are mono- or diesters of free fatty acid and PEG, but despite the naming convention, the structure does not contain glycerol as is the case with PEGylated triglycerides such as polyoxyl 35 castor oil (Kolliphor® EL, Basf, Germany). Examples from Gattefosse are caprylocaproyl polyoxylglycerides 8 (e.g., Labrasol® ALF), stearoyl polyoxyl-32 glycerides (e.g., Gelucire 50/13,), lauroyl PEG-32 glycerides (e.g., Gelucire® 44/14) and linoleoyl polyoxyl-6 glycerides (e.g., Labrafil® M2125). These dispersions are not pure surfactants; they are preconcentrates that spontaneously form emulsions or microemulsions when diluted in

water. Labrasol® contains a blend of high HLB surfactants (PEG-8 monoesters of C_8 and C_{10}), a low HLB surfactant (PEG-8 diesters of C_8 and C_{10}), oil (glyceride), and a solvent (PEG-8). It is the mono and diester fraction of Labrasol® that is principally responsible for the alteration to intestinal barrier integrity [33]. Macrogol glycerides are constituents of LBFs where they may be used alone or in combination with oil, solvent, or emulsifiers to prepare dosage forms for poorly soluble drugs.

Caprylocaproyl polyoxylglycerides 8 (Labrasol®) is mainly used as a solubilizer in oral drug applications. It is the vehicle used in the LBF of enzalutamide (Xtandi® soft gelatin capsules, Astellas, Tokyo, Japan). Labrasol® reduces the intestinal epithelial barrier to small molecules and macromolecules [33]. The barrier was evaluated in isolated rat colonic mucosae, where exposures up to 10 mg/mL Labrasol® for 2 h increased permeation of ^{14}C mannitol by 3- to 5- fold [101]. No histological perturbation was observed for 2 mg/mL Labrasol®, but there was mucosal aberration at higher concentrations consistent with surfactant-like perturbation. Labrasol® had lower cytotoxicity in Caco-2 cells (MTT IC_{50}: 0.021%v/v) compared to polysorbate 20 (MTT IC_{50}: 0.004% v/v) and polysorbate 80 (0.005 % v/v) [102]. For each surfactant, loss of viability was accompanied by loss of membrane integrity as measured by LDH release [102]. The cytotoxicity and alteration to the integrity of Caco-2 monolayers by Labrasol® were greater than Cremophor® EL, a common constituent of licensed LBFs [103] (Table 2). A concentration of 0.5 μL/mL Labrasol® caused 50% hemolysis of human erythrocytes and, while this was a lower value than that induced by SLS (0.04 mg/mL), no hemolysis was observed for Cremophor® EL, Miglyol® 812, and polysorbate 80 at concentrations up to 80 μL/mL [104]. Rat intestinal instillations of 8 mg/mL Labrasol® did not lead to histological damage [33]. This concentration was an estimate of luminal concentration based on complete dissolution of soft gelatin capsules containing 500 mg in ~ 60 mL gastric or intestinal fluid. However, local high concentrations have the potential to cause GI damage. The oral LD_{50} for Labrasol® in rats was 22 g/kg [105]. Adverse events and mortality were observed at 10 mL/kg [106]. Labrasol® had a NOAEL of 3000 mg/kg/day following oral administration to rats or dogs [105].

Gelucire® 44/14 is another medium-chain macrogol glyceride used in commercial LBF products (Table 2). The major constituent is a blend of PEG-32 mono and diesters of C_{12}, with a small fraction of C_{12} glycerides and PEG-32. The ethylene oxide chain length is outside of the range where the greatest level of membrane perturbation was observed for the related alcohol ethoxylates (E_{10}-to-E_{20}) [107]. There was a greater alteration to intestinal barrier integrity with Labrasol® compared to Gelucire® 44/14 in rat duodenal instillations [108]. Here, Gelucire® 44/14 had no effect on absorption of low MW heparin whereas Labrasol® increased absorption by 4-fold over 2 h. The alteration to barrier integrity by Labrasol® was still mild compared to C_{10} (24-fold) and sodium laurate (C_{12}) (25-fold) [108]. Gelucire® 44/14 had lower cytotoxicity in Caco-2 cells (MTS IC50: 0.05 to 0.1% v/v [109]) compared to Labrasol® (MTT IC_{50}: 0.021% v/v). Loss of viability was accompanied by loss of cell membrane integrity. Gelucire® 44/14 had a NOAEL value of > 2500 mg/mL in dogs and a NOAEL value in rats of 2400 mg/kg/day [105].

6.1.9. Pegylated triglycerides

Polyoxyl castor oil derivatives are PEGylated glycerides that form soluble non-ionic surfactants. The glycerol backbone forms mono- di or tri-ethers with the ethylene glycol moiety of ethoxylated fatty acid esters of ricinoleate. Commercial preparations may also contain a small fraction of free ricinoleate and PEG esters (e.g., Kolliphor® EL, BASF). The most common examples are polyoxyl 35 castor oil (Cremophor® EL or Kolliphor® EL), and polyoxyl 40 hydrogenated castor oil (Cremophor RH40 or Kolliphor RH40). The approximate MW of Cremophor® EL is 2.5 kDa, so it is larger than many other non-ionic surfactants. Similarly, the presence of a hydroxyl group in the hydrophobic ricinoleate moiety may contribute to less efficient micelle formation and hence a higher CMC (200 mg/L) compared to other non-ionic surfactants (e.g., Labrasol (CMC: 42 mg/L), polysorbate 80 (CMC: 14 mg/L) and polysorbate 20 (CMC: 60 mg/L). However, while there may be a higher free concentration of Cremophor® EL in the molecular form that is free to interact with mammalian membranes, the presence of the hydrophilic group in the hydrocarbon tail may also contribute to inefficient penetration into lipid bilayers, thus limiting the potential for perturbation.

Cremophor® EL did not cause hemolysis of human erythrocytes at concentrations ranging between 1 and 8 % v/v [104]. There was no alteration to the integrity of Caco-2 monolayers treated with 10% w/v Cremophor® EL, whereas extensive alteration was noted with 0.01 to 1% w/v with 3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate (CHAPS), Nonident™ P-40 and Triton™ X-100 [40]. Similarly, there was no loss of cell viability or alteration to cell membrane integrity in Caco-2 cells treated with 5% w/v Cremophor EL for 1 h, although there was a near complete loss of viability and extensive membrane perturbation after 24 h incubation [110]. Other studies show a modest alteration to viability [111] and permeability [112] in Caco-2 cells and monolayers at concentrations up to 1.25% w/v. The oral LD_{50} for Cremophor EL in mice ranged between 100 mg/kg [105] to 6500 mg/kg [113], which is lower than Labrasol® (22 g/kg) [105], polysorbate 80 (25 g/kg) [114], and SLS (1.29 g/kg) [30].

While Cremophor® EL causes mild cell perturbation on par with other non-ionic surfactants, it is associated with side effects that do not appear to relate to membrane perturbation. These include hypersensitivity to injectable formulations that contain Cremophor® EL (e.g., paclitaxel [115 116], multivitamin [116], and cyclosporin [117]). This was initially attributed to type I hypersensitivity reactions based on clinical symptoms [118]. In vitro evidence suggests that it may be due to activation of the complement system [119 120].

The capacity of Cremophor® EL to inhibit intestinal P-GP is also reported, although its relevance to humans is not clear as any net alteration in absorption may be due to an interplay of several mechanisms [121]. This could be why the absorption of some P-GP substrates is inhibited and others are not. For example, low concentrations of Cremophor EL (0.1 mg/mL) did not affect the absorption of the P-GP substrates, atenolol, and rhodamine 123, in rat intestinal perfusions [122], whereas it caused a 2.3-fold increase in norverapamil uptake into the systemic circulation in the same model [123]. In human volunteers, an oral formulation containing 720 mg Cremophor EL decreased the $AUC_{0\text{-}24}$ of the P-GP substrate saquinavir by ~ 30% [124]. However, in vitro testing led authors to suggest that this was due to reduced availability for epithelial uptake due to the partitioning of the drug in micelles created by the surfactant. In the same study, 1440 mg Cremophor EL increased $AUC_{0\text{-}24}$ of another P-GP inhibitor, fexofenadine, by ~ 30% whereas no effect was observed at the lower Cremophor dose of 720 mg. There was also an increase in saquinavir absorption in volunteers given doses of 100 mg, 1000 mg or 5000 mg Cremophor EL, but it was unclear if the increases were due to membrane perturbation, inhibition of PGP, or of cytochrome *P*-450 3A [125].

6.1.10. TPGS

TPGS is a non-ionic surfactant formed through the esterification of vitamin E succinate and PEG1000. Commercial blends contain 80% PEG monoesters, 12% PEG diesters (where two vitamin E monomers are bonded to each end of the PEG1000), free tocopherol succinate, and free PEG1000 [126]. TPGS may be used as a soluble source of vitamin E (e.g., Vedrop®, Recordati, Milan, Italy), as an excipient to improve drug solubility, and as an antioxidant. The FDA IID lists a maximum dose of 300 mg per unit and the permitted dose of TPGS in pediatric Vedrop® for a 15 kg child is 250 mg. An initial application of TPGS was as a solubilizer in Agenerase® capsules (GSK, London, UK) containing 50 mg/150 mg amprenavir and 247 mg/740 mg TPGS. As patients received a daily dose of 600 mg to 2400 mg of amprenavir, the total daily intake of TPGS ranged between 3 and 12 g [127]. TPGS has self-affirmed generally recognized as safe (GRAS) status for use in food products. It was also

viewed as a suitable food for special medical purposes by the EFSA scientific panel on food additives, flavorings, processing aids, and materials in contact with food [126]. The EFSA report noted a NOAEL of 1000 mg per kg body weight in rats. There are no reports of mucosal perturbation caused by TPGS.

TPGS, like Cremophor® EL, has also been shown to inhibit P-GP [128]. In Caco-2 monolayers, low concentrations of TPGS (0.13 mM) increased membrane rigidity and reduced apical-to-basolateral transport of the P-GP substrate, rhodamine 123 [129]. TPGS did however increase the secretory transport of rhodamine 123 [129] and other studies have shown an increase in apical-to-basolateral transport of rhodamine 123 induced by it in Caco-2 monolayers [130]. In the presence of P-GP substrates, micromolar concentrations of TPGS caused a concentration-dependent reduction in the ATPase activity of the efflux pump leading to energy depletion [131]. This inhibitory action occurred without causing membrane leakage of LDH [130]; this suggests discrimination between efflux pump inhibition and alteration to plasma membrane integrity. At higher concentrations of TPGS (3.3 mM), there was increased membrane fluidity in Caco-2 cells [131], which is consistent with leakage of cellular LDH [130]. In a similar concentration range (2.5 to 6.5 mM), TPGS had no effect on barrier integrity in isolated rat colonic mucosae and there was no histological damage or disruption to electrogenic chloride secretion [101]. The effect of TPGS on histology was mild compared to several other surfactants including Labrasol®, sucrose laurate, ox bile extract, and the medium chain fatty acids, C_{10}, and C_{12} [101].

Low concentrations of TPGS (0.07 to 0.14 mM) increased apical-to-basolateral flux of paclitaxel across isolated rat ileal mucosae mounted in Ussing chambers [132]. There was also an increase in the effective permeability (P_{eff}) when paclitaxel was co-perfused with 0.07 mM TPGS in rat ileal perfusions. Improved permeation at concentrations below the CMC (0.13 mM [133]) was attributed to inhibition of P-GP and not micellar solubilization or membrane perturbation [132]. Membrane perturbation by TPGS was also discounted because increased paclitaxel flux did not also occur in the secretory direction across polarised models [132]. There was also an absence of perturbation in artificial membranes [132], no effect on the integrity of Caco-2 monolayers to mannitol [133], and polarised transport of another P-GP substrate (amprenavir) in Caco-2 [133]. In rat jejunal everted sacs, TPGS also increased permeation of another P-GP substrate, etoposide, at concentrations below the CMC [134]. There was, however, no alteration to permeation of digoxin in rat jejunal mucosa mounted in Ussing chambers when treated with a similar concentration of TPGS [135]. TPGS at 2.5% v/v had no effect on the solubility of berberine, nor was there histological damage to rat ileum 5 h after oral gavage of the test formulation, which led the authors to suggest that improvement to berberine absorption in rats over 36 h was due to inhibition of P-GP [136]. TPGS (50 mg/kg) also increased absorption of paclitaxel following bolus administration to rats [132], although it was not possible to uncouple the effects of micellar solubilization, changes in membrane fluidity, and P-GP inhibition at concentrations that are above the CMC; in this example, improved solubility caused by TPGS is likely to play a major role in the increased flux.

6.1.11. Sugar esters

Sugar esters are non-ionic surfactants formed by esterification of medium- or long- chain fatty acids with mono or disaccharide polyols. The HLB values of sugar esters are higher for disaccharides versus those made with monosaccharides. Blends of sugar esters may also contain more lipophilic polyesters compared to single conjugates if more than one fatty acid is reacted with each sugar.

Sorbitan is a hexahydric alcohol derived from the dehydration of sorbitol. Fatty acid esters of sorbitan are often referred to as Span®. Sorbitan laurate and oleate are constituents in some LBFs (Table 2). There are few reports on the interaction of sorbitan esters with the intestinal mucosa. The HLB value of sorbitan laurate is 8.6 and for the longer chain sorbitan oleate, the HLB is 4.3, suggesting poor dispersion characteristics in water and relatively low monomer solubility for the latter. Of the sugar esters listed in Table 1, sorbitan monolaurate has the highest monomer solubility, the form responsible for membrane insertion. This is reflected by increasing hemolysis capacity in the order: sorbitan oleate (HLB: 4.3, % hemolysis: 7%), stearate (HLB: 5, % hemolysis: 9%), palmitate (HLB: 7, % hemolysis: 9%), and laurate (HLB: 8.6, % hemolysis: 17%) [137]. A test concentration of 1 mM (~0.35 mg/mL) sorbitan laurate caused a lower level of hemolysis compared to SDS, CHAPS, Nonident™ P-40, and Triton™ X-100 [40], but higher than selected ethoxylated surfactants (e.g., polysorbate 80, and Cremophor™ EL [104]). Span® 20 increased permeation of ranitidine across rat jejunal mucosa by 20% and it increased oral BA by 24% in male rats and by 31% in female rats [138]. This effect was accompanied by reduced expression of P-GP at the mRNA and protein level. A separate study showed that the IC_{50} for inhibition of P-GP was 7.7 mM for Span® 20, which was 100 to 1000 folder higher than other non-ionic surfactants including polysorbate 80 (IC50: 12.5 μM) [139], suggesting that it is not physiologically-relevant.

In acute toxicity testing, sorbitan laurate had a very high LD50 of 34 g/kg when orally administered to fasted rats [140]. In sub-chronic toxicity testing in rats, incorporation of sorbitan laurate into food had no effect between 1 and 10 % w/v, but there were toxicity markers detected at 15 to 25 % w/v [140]. Oral administration of 2 g daily to rhesus monkeys for 6 weeks had no effect on growth rate or blood measurements, although there was some evidence of kidney damage [141]. An ADI of 10 mg/kg sorbitan (e.g., 21 mg/kg sorbitan monolaurate) was recommended for fatty acid esters of sorbitan by EFSA's scientific panel on food additives and nutrients added to food [141]. The FDA IID lists a maximum safe daily exposure of 800 mg in a tablet dosage form, though the maximum quantity per unit dose is not listed.

6.2. Ionisable surfactants

There are fewer ionizable surfactants present in oral formulations compared to non-ionic ones (Table 1), but those that are allowed are widely used in pharmaceutical products and some can also be used in foods with limits on consumption (e.g., SDS, and stearic acid). In general, anionic, and cationic surfactant excipients cause more damage to cells at lower concentrations compared to non-ionic surfactants. In a head-to-head comparison, the damage caused to cells by low concentrations of SDS was greater than high concentrations of polysorbate 80 [142]. Ionizable anionic and cationic surfactants are sub-divided based on whether the hydrophilic head group is a weak or strong acid. A small number of quaternary ammonium compounds (QACs) are used at low concentrations for their antimicrobial action. They are not used as emulsifiers and solubilizers due to the potential for detergent-like perturbation at high concentrations. Similarly, anionic surfactants with hydrophilic head groups that are derived from strong acids, including sulfonic acid (e.g., dioctyl sulphosuccinate (DSS)) and sulfuric acid (e.g., SDS), are used in low quantities as wetting agents. DSS or docusate contains two branched hydrocarbon tails that create a bulky structure that is ideally suited to wetting because micelle formation is less favored [143]. Docusate is one of a select group of excipients that also function as active pharmaceutical ingredients (as a stool softener) [144]. If the hydrophilic head group is a weak acid or weak base, there will be changes to both the solubility of the molecular form and the ability to form micelles depending on the pH in the GI lumen. Consequently, there may be differences in the ability of the surfactant to cause mucosal perturbation in different GI regions. Fatty acids are prominent examples in this sub-category. Medium and long-chain fatty acids have negligible solubility in gastric fluid compared to small intestinal fluid, where millimolar concentrations exist in molecular and micellar forms. Some ionizable surfactants have zwitterionic head groups. The most relevant example for oral dosage forms is lecithin (Table 2). The simultaneous presence of the quaternary ammonium group and a phosphate confers a net neutral charge over a wide pH range on

phosphatidylcholine, thus it is an insoluble surfactant with little capacity for detergent like perturbation of biological membranes.

6.2.1. Sodium lauryl sulfate (SLS)

SLS is an alkyl sulfate consisting of a saturated C_{12} hydrocarbon tail and a hydrophilic sulfate head group. To avoid confusion with SDS, we use "SLS" throughout this Section. The compendial monograph for SLS states that it is a mixture that contains > 85% of the lauryl ester, a low percentage of C_{14} and C_{16} esters, and < 8% sodium chloride/sodium sulfate [30]. The pKa value for the anionic sulfate group in SLS is 1.9, so it is ionized at most pH values in the GI tract and there will not be major pH-dependent changes to its solubility and surface-active properties in gastric fluid unless the pH is at or below 2. The anionic sulfate group imparts high aqueous solubility and a HLB value of 40, which lies outside the scale of 1 to 20 initially developed for ethylene oxide-based surfactants. The soluble anionic sulfate group in SLS confers a relatively high CMC (8 mM), hence there is a higher dissolved monomer concentration compared to many other surfactants with a 12-carbon hydrophobic tail. By comparison, the CMC of polysorbate 20 is 0.05 mM, dodecyl maltoside is 0.15 mM, and sucrose laurate is 0.3 mM.

SLS is used mostly in products for external applications, typically as a detergent in medicated shampoos and as a co-emulsifier with cetostearyl alcohol in aqueous creams. In the UK, 2.4% of marketed topical products contain SLS [145]. It can also be present in oral dosage forms at relatively low concentrations as a wetting agent, a tablet lubricant, and as a dispersant [30] with the overall aim of increasing solubility. It is not used in parenteral products.

Although SLS is an allowed excipient, it is contained in fewer oral products than non-ionic surfactants and it is not permitted for use as a food additive in the EU [145]. In the US, SLS is a GRAS food additive, but there are limits on consumption ranging from 10 to 1000 ppm depending on the food matrix [146]. In oral medicinal products, SLS is more commonly used in solid dosage forms rather than in liquids. It is generally used at concentrations ranging between 0.2 and 2 % w/w [30], so 500 mg tablets or capsules may contain up to 10 mg SLS. The majority of examples listed on the FDA IID quote quantities of less than 10 mg, but there are examples where the maximum potency per unit dose is higher than 10 mg and the maximum daily exposure is > 100 mg [37]. The EMA and FDA do not stipulate a maximum permitted oral dose of SDS in medicinal products. A NOAEL of 100 mg/kg/day was measured in rats and 400 mg/kg/day in dogs [147]. The safety of orally administered SLS has been assessed in acute, sub-chronic, and chronic toxicity testing in animals [148]. Chronic oral administration of 0.2 to 1% w/w SLS to rats for 2 years did not lead to gross or microscopic abnormalities [149 150]. Similarly, dogs receiving up to 2% SLS in their diet had no anatomical abnormalities. The estimated lethal oral dose of SLS in humans is 0.5 to 5 g/kg [30].

Several studies have evaluated the interaction of SLS with the intestinal epithelium [5]. Low mM concentrations of SLS (0.4 mM) caused rapid disruption to Caco-2 monolayers [27]. The barrier recovered following exposure to 0.4 mM SLS for 20 min, but not for 2 h [27]. Monolayers that were exposed to 3.5 mM SLS for 20 min, however, did not recover after 24 h [151]. In an in vitro evaluation of several excipients used in drug solubilization, SLS compromised Caco-2 monolayer integrity to a greater extent than polysorbate 80, Pluronic™ F68, Gelucire® 44/14, Transcutol® P, and PEG-40 hydrogenated castor oil (HCO-40) [142]. SLS caused truncation of microvilli, disbandment of actin, and disruption of TJs of Caco-2 monolayers [27]. Truncation of microvilli is associated with mucosal perturbation, whereas disbandment or actin and disruption of TJs is usually attributed to a paracellular mode of action; although both of the latter parameters can also be disrupted by mucosal perturbation [152]. The mechanism through which a compound alters barrier integrity is typically concentration-dependent, where low concentrations are associated with small changes in permeability via the paracellular route and high concentrations tend to result in gross mucosal perturbation. A mechanistic model in Caco-2 monolayers assigned K values to estimate the relative contribution of the transcellular and paracellular routes on a scale of 0 to 1 (where 0 represents a predominant transcellular action and 1 represents a predominant paracellular effect) [153]. Here, dilute concentrations of SLS (0.035 mM) had a K value of 0.31, suggesting some degree of separation between barrier alteration and cell perturbation. However, for 0.35 to 3.5 mM SLS, the K values were zero, indicating that transcellular perturbation dominates at higher concentrations. Indeed, SLS (0.1 % w/v, 3.5 mM) is often used as a positive control in cytotoxicity studies in Caco-2 monolayers as a consequence of its capacity to solubilize cells [151 154].

SLS caused a concentration-dependent decrease in Caco-2 cell viability between 0.1 and 1 mg/mL as measured in the MTT and LDH assays after 1 h exposure [155]. In the same study, there was a concurrent loss of barrier integrity with SLS as measured by increased permeability of [^{14}C]-mannitol over 1 h [155]. At low concentrations (64 mcg/mL and 80 mcg/mL), SLS also increased mannitol permeability in Caco-2 monolayers, but without causing cytotoxicity. This suggests there may be disruption to TJs before loss of cell integrity and that these events can be dissociated. Additionally, barrier integrity at these low concentrations fully recovered when SLS was removed. It remains unclear if this relates to membrane action as electron micrographs of monolayers showed that in addition to disruption to TJs, there was also intracellular perturbation and shortening of microvilli at these low concentrations. Additionally, the concentration of SLS required to induce 50% haemolysis of human erythrocytes was ~ 30 mcg/mL (0.1 mM) [156], therefore interaction with membranes can still occur at concentrations where there is an absence of LDH release or loss of mitochondrial function [155].

Exposure of isolated intestinal mucosa to SLS causes alteration to barrier integrity that was associated with markers of perturbation. In rat everted sacs, a panel of alkyl sulfates (including SLS) altered intestinal permeability to acetylsalicylate and this was accompanied by protein release [157]. SLS also caused a 5-fold increase in LDH release from rat colonic mucosae incubated with 20 mM for 3 h [158]. There was a concentration-dependent release of protein and phospholipid from rat everted sacs incubated with SLS (0.05 to 50 mM) for 60 min [159]. Perfusion of 1% w/v SLS and phenol red in rat small intestine for 1 h increased the absorption rate constant (Ka) by 8-fold, which was higher than several other surfactants including polysorbate 80 [160]. The alteration to barrier integrity by SLS in rat intestinal perfusions was accompanied by a 10-fold increase in luminal phospholipids, which was confirmed histologically as severe mucosal erosion [160].

SLS has been assessed as an intestinal PE, where it was grouped as an agent that induces strong permeability enhancement and slow recovery of barrier integrity [161]. For example, there was greater absorption of cefoxitin when it was co-perfused into rat small intestine with SLS (50 mM) compared to C_{10}. When SLS perfusion was stopped after 1 h however, absorption of cefoxitin remained elevated for a further 3 h, whereas the effect of C_{10} reversed within 1 h [162].

Repair of barrier integrity was also assessed in a rat rectal perfusion model with the permeability marker, sulfanilic acid. The absorption of sulfanilic acid when co-perfused with 5 mM SLS was reduced by 50-to-90% when perfusion of the marker was staggered by 0–2 h after cessation of the surfactant [163]. The permeability changes were accompanied by perturbation in gross histology. There may be the expectation that repeat mucosal perturbation causes progressively more GI damage, as can be observed with repeated topical exposure to mild detergents [164]. On the contrary, when repeated co-perfusion of sulfanilic acid and SLS was performed 2 or 24 h after the first treatment, there was a 40% reduction in the amount of sulfanilic acid absorbed compared to the former [163].

Intragastric administration of phenol red (6 mg/2mL) to rats with 0, 1, 1.5, or 2% w/v SLS increased absorption ($AUC_{0\text{-}300\ min}$) from 108 μg/mL·min to 294, 436, or 665 μg/mL·min, respectively [28]. When phenol red was administered 0.25, 0.5, 1, or 3 h after the 1% SLS solution, there

was a progressive recovery of the barrier such that the $AUC_{0\text{-}300\ min}$ decreased from 294 μg/mL·min to 242, 168, 111 or 131 μg/mL·min, respectively. There was also near complete recovery of the barrier to the increased in phenol red absorption 3 h after administration of 1.5% w/v SLS, whereas only partial recovery was observed with 2% w/v SLS. Histology of the rat duodenum 10 or 15 min after intragastric administration of 2 mL SLS (1% w/v) revealed swollen villi and sloughing of epithelial cells leading to partial denudation. After 30 min, light micrographs showed a continuous layer of epithelial cells covering the mucosa. Electron micrographs at 30 min still showed extensive loss of microvilli and disruption to junctional complexes. The duodenal mucosa appeared normal in light micrographs 1 h after SLS administration. At the same time point, electron micrographs showed reorganization of junctional complexes and the initial stages in regeneration of microvilli. There was no evidence of transcellular perturbation in the jejunum following intra-gastric administration, suggesting dilution of SLS as it moves down the small intestine.

The extensive damage and slow barrier recovery caused to the mucosa by SLS has led researchers seeking candidate excipients to increase permeability of macromolecules to focus on milder surfactants (e.g., C_8 [39] and C_{10} [165]). Although SLS may not be ideal for inclusion as a PE in an oral dosage form, its presence in oral formulations for other reasons (e.g., as a wetting agent or lubricant) may unintentionally increase permeability depending on the concentration. Such excipients are termed absorption-modifying excipients (AMEs) [73] and they have come under scrutiny recently because their presence in pharmaceutical products may contribute to the ADME profile of an innovator product. This could lead to bioinequivalence versus generic formulations that do not contain the same excipients [166].

A series of studies have examined the effect of AMEs including SLS on drug permeability in a rat single-pass intestinal perfusion (SPIP) model. SLS was tested at 0.1 and 0.5% w/v, a concentration range that may be transiently encountered at focal points where a dosage form dissolves in the GI tract. Continuous perfusion of SLS solutions over 75 min led to a concentration-dependent increase in absorption of acyclovir, atenolol, enalaprilat, and phenol red [167]. There was also a 7.5-fold increase in blood to-lumen clearance of ^{51}Cr-EDTA with 0.5% SLS. At the same concentrations, SDS also caused alterations to colonic barrier integrity as measured by increased absorption of atenolol and enalaprilat in rat single colonic perfusion and increased blood-to-lumen flux of ^{51}Cr-EDTA [168].

In dogs, there were no changes to $AUC_{0\text{-}6h}$ of acyclovir, atenolol, ketoprofen, metoprolol, or phenol red following intra-intestinal administration in a 20 mL bolus containing a marker cassette dose with or without SLS (11.4 mg/mL) [169]. There was, however, a 1.8-fold increase in $AUC_{0\text{-}6h}$ of enalaprilat. In contrast, instillation of 0.5 mL of this cassette in rat duodenum increased $AUC_{0\text{-}2h}$ of acyclovir, atenolol, enalaprilat, and phenol red, but not metoprolol or ketoprofen. It is thus difficult to relate the action of SLS in different species and for different drugs. The effect of SLS in rat intestinal instillations was also less efficacious than the continuous exposure seen in perfusions, raising the important issue of exposure time in the GI tract (Section 8). In the rat SPIP model, exposure of the small intestine to 0.5% SLS for either 15 min or 60 min increased absorption of enalaprilat, acyclovir, atenolol, and phenol red [170]. When SLS perfusion was stopped after 15 min, there was still a continued increase in absorption of all markers over the following 30 min [170]. For the 60 min exposure, there was prolonged elevation of plasma levels of all drugs over the following 60 min [170], thereby indicating a relationship between exposure time and recovery of barrier integrity. Other studies in rats have confirmed that marker absorption remains elevated after a pre-treatment SLS exposure period (e.g., sulfadimethoxine [45]). The alteration to barrier integrity caused by perfusion of 0.5% SLS in the rat SPIP model was prevented using a combination of two mucosal protective agents, misoprostol and melatonin, as measured by a decrease in luminal clearance of ^{51}Cr-EDTA [171]. Neither agent alone could prevent SLS-induced barrier alteration, but they did promote recovery following removal of SLS [171].

Several studies have assessed the interaction of SLS with plasma membranes [172 173 174 175 176]. While there are subtle differences in how detergents perturb membranes, most fit within the general model of membrane solubilization (Section 3). However, there are subtle differences in the mechanism. For example, the mechanism that SLS perturbs membranes differs from that of non-ionic surfactants (e.g., Triton™ X-100), as SLS only equilibrates in the outer leaflet of the plasma membrane due to a lower rate of flip-flop into the inner leaflet compared to non-ionic surfactants [174 176]. Accumulation of SLS monomers on intact large unilamellar vesicles led to insertion into the outer leaflet of the membrane [176]. This event caused an initial 25% expansion of the hydrodynamic radius, i.e. vesicle swelling [176]. The SLS monomers in the outer leaflet of the membrane have a high positive spontaneous curvature compared to membrane lipids that have nearly zero spontaneous curvature in both giant [174] and large [176] unilamellar vesicles. This mismatch causes bilayer bending, separation of membrane lipids, and vesicle perturbation [176]. Subsequent accumulation of SLS monomers on the outer leaflet promoted outward bending of the membrane, which leads to the formation of local invaginations and the formation of mixed micelles [176]. This process may lead to the formation of macroscopic pores and fragmentation of the intact vesicle with some fragments detaching and forming mixed micelles with SLS [174 176]. While it is generally accepted that penetration of detergent monomers is principally responsible for membrane perturbation, there is evidence that SLS micelles can directly interact with dimyristoylphosphatidylcholine liposomes [175]. Others have noted that after initial adsorption of SLS to plasma membranes, the time needed to form mixed micelles is inversely dependent on the surfactant concentration [173]. In other words, the time to reach a dynamic equilibrium (between the concentration of mixed micelles and vesicles and mixed micelles alone) is dependent on the concentration of SLS. This has direct relevance in the GI tract where transit and dilution may prevent SLS from reaching the threshold to solubilize intestinal epithelial cells.

There has been attention on the capacity of polysorbate 80 and carboxymethyl cellulose to cause low-level intestinal inflammation in part via changes to the intestinal microbiota (Section 6.1.1). Alterations to GI bacteria have also been reported in preliminary studies with SLS. Oral administration of 0.6% w/v SLS to Drosophila flies for 5 days caused extensive damage to intestinal epithelial cells isolated from the mid-gut, including loss of microvilli and swelling of mitochondria [177]. The mid-gut microflora of control flies primarily consisted of *Wolbachia*, which changed to *Klebsiella* (in particular to *Klebsiella aerogenes*) in the SLS treatment group. A current project funded by the Medical Research Council (MRC) in the UK is investigating the short- and long- term effects of SDS and other emulsifiers on the GI tract of wild-type mice and $IL10^{-/-}$ mice that display colitis-like features [68]. The project aims to assess the impact of emulsifiers on microflora, intestinal inflammation, and alteration to barrier integrity including bacterial translocation.

6.2.1.1. Fatty acids and fatty acid salts. Fatty acids are among the most abundant ionizable surfactants present in the GI tract. They may be present in food or are liberated during digestion of glycerides or from other surfactants including polysorbates, macrogol glycerides, or sugar esters. As fatty acids are major dietary constituents, their use as excipients is viewed as safe. All naturally occurring saturated fatty acids ranging from butyric acid (C_4) to stearic acid (C_{18}) are listed on the FDA Substances Added to Food Database. In their acidic form, fatty acids are insoluble and are unlikely to cause major perturbation of the intestinal epithelium. At pH values in the small intestine (above their respective pKa values), the deprotonated form is a soluble anionic surfactant, which may cause mucosal perturbation depending on hydrocarbon tail length. Soluble salts of fatty acids, or soaps, are widely used as detergents in cleansing products, although some salts ranging between C_4 to C_{18} are also on the FDA Substances Added to Food Database. Fatty

acids that are included in the FDA IID include caprylic acid (and the sodium salt), myristic acid (C_{14}), palmitic acid (C_{16}), oleic acid ($C_{18:1}$), and stearic acid C_{18} (and sodium and magnesium salts). Others such as capric acid (C_{10}) and lauric acid (C_{12}) are allowed as constituents of some vegetable oils listed on the IID (e.g., coconut oil). Despite this, it cannot be assumed that medium-chain fatty acids will be approved in an oral macromolecule dosage form at the high concentrations required to act as PEs.

Long-chain fatty acids (C_{14} to C_{18}) are principally used as lubricants or glidants for tablets/capsules, where only a small quantity (<10 mg) is required. Magnesium stearate is a common lubricant and glidant in oral dosage forms. At typical use concentrations of 0.25 to 5% [30], it is unlikely to cause mucosal perturbation, but higher doses could have a laxative effect or cause mucosal irritation [30]. There are, however, no studies reporting these effects in humans at the low levels used as excipients. Mild diarrhea can occur in a small percentage of adults taking large doses of soluble magnesium salts (360 + mg Mg^{2+}/day) [178]. No evidence of genotoxicity was found in vitro or in vivo [179] and this fatty acid salt has GRAS status and is permitted for use up to 2.5 g/kg per day.

A high quantity of fatty acids may be present in LBFs. For example, Norvir® soft gelatin capsules (AbbVie, Illinois, USA) contain ritonavir dissolved in a LBF containing oleic acid, ethanol, and polyoxyl 35 castor oil (Cremophor® EL). Fatty acids may also be constituents of several vegetable oils used as vehicles in LBFs including soybean, olive, peanut, corn, sesame, and coconut. For example, soybean oil [containing C_{16} (10%), C_{18} (4%), $C_{18:1}$ (18%) linoleic ($C_{18:2}$) (55%) and linolenic ($C_{18:3}$) (13%) [180]] is a major constituent of soft gelatin capsules containing isotretinoin (Roaccutane®, Roche Pharma AG, Germany).

Vegetable oils used in LBFs may also have a high proportion of medium-chain fatty acids. Coconut oil for example contains C_8 (8%), C_{10} (7%), C_{12} (49%) C_{14} (8%) C_{16} (8%), C_{18} (2%), $C_{18:1}$ (6%), and $C_{18:2}$ (2%) [181]. The quantities present in LBFs are low in comparison to dietary consumption. There is also no evidence that the ionized soluble form of each fatty acid formed at the pH in the small intestine reaches and sustains a high enough concentration to cause damage to the intestinal mucosa at concentrations present in LBFs [5]. The selected medium chain length can provide an optimal balance between solubility and capacity for membrane insertion and membrane disruption. This is why soluble salts of medium-chain fatty acids are widely tested for their capacity to transiently alter intestinal barrier integrity to enable absorption of therapeutic peptides [5].

There is concern that intentional alteration to intestinal barrier integrity may lead to uptake of bacteria and toxins, but there is little evidence to support this argument in humans to date. On the contrary, there is typically low and variable uptake of the macromolecule that is co-delivered with the PE. Extensive clinical testing of oral peptide dosage forms containing C_8 [39] and C_{10} [165] has not raised major safety concerns about this possibility to date. While cytotoxicity in isolated cell cultures and histological damage in isolated and intact GI tissues has been demonstrated for MCFAs presented in mM concentrations, this does not seem to translate to in vivo preclinical models. C_8 is the main surfactant present alongside polysorbate 80 and monocaprylin in TPE™ (Mycapssa®) (Case 2). Several publications show that it is the major component that alters intestinal epithelial barrier integrity in TPE™ [39]. It is noteworthy that at low concentrations, below a threshold for membrane disruption, C_8 is also used at lower concentrations in injectable products to stabilize albumin (e.g., Pazenir®, Teva, Israel) [182]. The key role of TPE™ in oral octreotide (Mycapssa®) shows that modulation of intestinal barrier integrity by mild surfactants can be acceptable.

6.2.1.2. Sodium laureth-3 sulfate. Although sodium laureth-3 sulfate is on the FDA IID as an excipient used via the oral route, it is more commonly associated with external applications. Internal use of laureth-3 is only allowed at low concentrations (<5mg). The hydrophilic head group of sodium lauryl ether sulfates (SLESs) contains an ethoxylate chain of varying length (3-mer for laureth-3). Ion dipole interactions between the sulfate and these PEG chains contribute to a lower CMC [183], leading to a lower free monomer concentration available to perturb epithelia compared to SLS. The CMC of SLES is 3 mM [184] compared to 8 mM for SLS [20]. Hence, SLESs are mild detergents, which is reflected in low levels of contact irritation [185]. There is a paucity of studies reporting the effect of laureth-3 on intestinal barrier integrity, but 5% laureth-9 caused a 2-fold increase in rectal absorption of insulin, similar to that seen with EDTA but slightly lower than sodium salicylate [186].

6.2.1.3. Dioctyl sulphosuccinate. DSS or sodium docusate is an alkyl sulfate consisting of two short-branched 8-carbon hydrophobic moieties and a sulphosuccinate hydrophilic moiety. This irregular shape is more favorable for wetting as steric effects impede micelle formation [143]. DSS is also used therapeutically as a stool softener and as an earwax softener. The concentration range used in wetting (0.01 – 1% w/v) is well below the therapeutic dose as a stool softener (120 mg), but comparable to the concentration in eardrops (e.g., Waxsol® 0.5% w/v eardrop solution, Mylan, India). The effect of 1.6 mM DSS (88-fold) on epithelial flux of mannitol across Caco-2 monolayers was less than for 2 mM SLS (>138 fold) but higher than for 3.8 mM polysorbate 80 (4-fold). In rat intestinal loops, there was a 50-fold increase in absorption of the barrier integrity marker, phenolsulfonphthalein (PSP), with 1% DSS. Oral administration of a solution containing 500 mg DSS to volunteers caused a 2-fold increase in permeability of PSP, suggesting alteration to barrier integrity [187]. However, there was no elevation to systemic PSP when it was administered one day after 6 consecutive days of treatment with 500 mg DSS, suggesting that its effects are temporary and reversible. As docusate is recommended for short durations, it is therefore unlikely to cause prolonged changes to GI health. Moreover, there is no evidence of long-term effects in patients who take docusate more regularly.

6.2.1.4. Quaternary ammonium compounds (QACs). QACs are cationic surfactants with good solubility across a wide pH range due to the presence of a quaternary ammonium group. Benzalkonium chloride (BAC) is a common example in pharmaceutical products, where it is used as an antimicrobial preservative in solutions for topical or mucosal routes. It may also assist with wetting, solubilization, and permeation enhancement across skin and other epithelia. The preservative action is associated with topical and mucosal irritancy [16]. Relatively few oral dosage forms contain BAC and there is limited safety information for this route. As low concentrations of BAC are required for preservation, patients will ingest relatively low doses of this surfactant. For example, patients receiving an oral dose of 5 or 10 mL of sodium oxybate 500 mg/mL oral solution (Neuraxpharm, Lagenfeld, Germany) will ingest 0.25 or 0.5 mg BAC. As BAC exhibits potent antimicrobial activity, it has the potential to alter the intestinal microflora, especially as it is not well absorbed from -the upper GI tract. In addition to potent antimicrobial action, BAC also displays potent hemolytic action and induces cytotoxicity in intestinal epithelial cells, which was associated with loss of membrane integrity [188]. Treatment of mice with 80 ppm BAC for 21 days exacerbated inflammation and tumorigenesis in mice treated with dextran sulphate for seven days [189]. Overall, it is unclear if dilution of formulations containing BAC in the GI tract reduces the concentration below the threshold for antimicrobial activity and also for barrier integrity changes in the GI tract. The oral LD_{50} for BAC ranged between 400 and 525 mg/kg in rats [190], which is lower than for SLS (1.29 g/kg) [30]. Toxicity of 500 mg/kg/day BAC in mice was attributed to local effects in the GI tract following oral administration [191]. The NOAEL was 190 mg/kg/day in a sub-chronic mouse study and 14 mg/kg/day in a chronic dog study [191].

6.2.1.5. Lecithin. Lecithin is a natural lipid blend principally composed of phospholipids. It is a common food emulsifier and is used in several LBFs (Table 2). Although lecithin increased permeation of FD10 across Caco-2 monolayers by over 1000-fold [192], there was no evidence of intestinal toxicity in humans despite exposure to high quantities in food and endogenous bile secretions. Phosphatidylcholine (PC) is one of the main constituents of lecithin. This zwitterionic insoluble surfactant is a common constituent of the colloidal structures in the GI tract. As PC membranes are present in the mucus gel layer, it may have protective effects [193]. Lecithin is one of several emulsifiers under assessment for a potential contribution to altered bowel and metabolic health [68]. Toxicity data suggests that lecithin is safer than many other widely used ionizable surfactants with an oral LD50 in rats of $>$ 5 g/kg [194]. The NOAEL based on a 12-week study in rats was $>$ 3.75 g/kg, the highest dose tested [195].

Native lecithin has low aqueous solubility due to the presence of two long-chain hydrocarbon tails and a neutral zwitterionic head group (HLB value: 3–5 [196]). Thus, there are fewer dissolved monomers available to interact with and perturb biological membranes. Pancreatic phospholipase A2 can hydrolyze native lecithin to lysolecithin, a more soluble variant containing only one hydrocarbon tail. The HLB value of palmitoyl lysophosphatidyl choline is 13 [197], and unlike native phosphatidylcholine, it forms micelles (CMC: 0.0025% w/v [198]). These degradation products therefore have greater potential to interact with biological membranes due to higher solubility. For example, the IC_{50} value for palmitoyl lysophosphatidyl choline was 0.07% w/v in Caco-2 cells compared to $>$ 0.5% for dipalmitoyl lysophosphatidyl choline [198]. Lysophosphatidyl choline also had a more pronounced reduction of the epithelial barrier of Caco-2 monolayers to macromolecules compared to phosphatidyl choline [198] and it caused a reduction in ileal barrier integrity in rats [199 200]. Older publications debate the effects of lysolecithin on mucosal barrier integrity [201]. Although lysolecithin is not on the FDA IID, it may form when native lecithin is digested. A study in humans found higher levels of lysophosphatidyl choline in patients with gastric or duodenal ulcers versus normal controls [202]. More recently, lysophosphatidyl choline exacerbated dextran sodium sulphate-induced colitis in mice. There was also increased production of this surfactant by the microbiota of mice devoid of fucosyltransferase 2, a gene identified as an IBD risk locus. Recent studies have emphasized that phospholipids are under-exploited as pharmaceutical excipients [203]. It is possible that irritation to the gastric mucosa caused by aqueous micellar dispersions of lysolecithins is of less consequence because they are present in emulsified systems where the surfactant is adsorbed at the oil–water interface and is not available to perturb membranes [203]. This is partly why enzyme-modified lecithin has GRAS status for use as an emulsifier.

Case 1. ***Lipid-based formulations.***

Surfactants are present at high concentrations in many LBFs (Table 2). In these oral formulations, they function as solubilizers, vehicles, and emulsifiers. The simplest LBFs contain only glycerides and rely on endogenous lipid digestion pathways to undergo dispersion in the small intestine. Other categories detailed in the Lipid Formulation Classification Scheme (LFCS) [204] contain soluble and insoluble surfactants, and some contain polar co-solvents. The presence of these surfactants promotes spontaneous emulsification into coarse emulsions, nanoemulsions, or microemulsions. The majority of individual excipients in LBFs have been tested in intestinal models. However, there is limited information on how marketed formulations containing combinations of surfactants, co-surfactants, oils, and co-solvents affect the intestinal barrier.

The majority of excipients used in LBFs are non-ionic surfactants with a history of safe use in humans as food additives and excipients. Some of the surfactants used in LBFs have been shown to cause alteration to intestinal permeability in cells and tissues modeling the human GI tract (e.g., polysorbates, macrogol glycerides) [205]. There is no evidence that LBFs used in marketed products cause GI aberration in humans. There has been a focus on evaluating the mechanism by which some surfactants in LBFs inhibit drug efflux pumps (e.g., TPGS, Cremophor EL), but it remains unclear if inhibition observed in vitro is relevant in vivo in humans [121].

Fatty acids are the only ionizable surfactant category used in LBFs; they may be constituents of the formulation or liberated during enzymatic degradation. The quantities of fatty acids present in LBFs are well below those ingested in food and although medium-chain fatty acids are known to cause mucosal perturbation [5], they cause only focal transient alteration to intestinal permeability when delivered directly to the intestinal mucosa as soluble salts.

The GI side effects for marketed LBFs are consistent with common GI side effects for all medicines (Table 2). It is noteworthy that the side effects reported in Table 2 relate to the marketed product, rather than the API alone. There are occasions where mechanistic studies indicate that the API is the cause of GI side effects (e.g., ischemic colitis caused by lubiprostone [206]). There is a tendency for safety data to be attributed to the API rather than to a combination of the API with the formulation excipients. Indeed, the side effects listed in the patient information leaflet and summary of product characteristics of generic products granted regulatory biowaivers, are taken from the patient information leaflet (PiL) and the summary of product characteristics (SmPC) for the innovator product, which often contain different excipients. Accordingly, the side effects in these documents relate to the branded formulation (API and excipients) rather than the generic formulation.

Case 2. *Surfactants as intestinal permeation enhancers (PEs).*

Intestinal PEs are excipients that are intentionally added to a formulation to improve permeation of poorly absorbed drugs across the intestinal epithelium. Surfactants are among the most widely tested PEs in clinical trials (reviewed in [207]). There is one surfactant-based PE formulation of a peptide licensed for use (Mycapssa®, Chiesi, Parma, Italy [39]) and one non-surfactant-based peptide product (Rybelsus®, Novo Nordisk, Denmark). Leading ionizable surfactant PEs are C_{10} [165], C_8 [39], lauroylcarnitine chloride [208], and bile salts [209]. Their interaction with the intestinal mucosa has come under scrutiny due to concerns relating to intentional GI barrier alterations. While barrier alteration is a concern for any surfactant excipient, the probability is higher for PE formulations designed to repeatedly alter epithelial integrity when administered in daily tablets or capsules. Surfactant PEs are therefore prime candidates to examine the effect of mucosal aberration on GI function.

In the 1990s, the medium-chain fatty acid salt, C_{10}, was used as a PE in rectal ampicillin suppositories (Doktacillin™, Meda, Sweden). This formulation improved rectal BA from 13% in the suppository base alone to 23% with C_{10} [210]. When rectal biopsies were taken after 25 min in humans, the suppository containing hard fat (950 mg) and C_{10} (25 mg) had a significantly higher mean histology score compared to patients receiving a suppository containing only hard fat [210]. C_{10} was also the main constituent of the GastroIntestinal Permeation Enhancement Technology (GIPET™) developed by Merrion Pharmaceuticals (Dublin, Ireland) [211]. This enteric coated tablet contains high quantities of C_{10} (at least 500 mg). GIPET™ was licensed to Novo Nordisk who showed in an 8-week phase II trial of type 2 diabetics that an oral long-acting stable basal insulin formulated with GIPET™ caused a comparable drop in fasting plasma glucose compared to a low dose of subcutaneous insulin glargine, which was achieved with an estimated 2% oral bioavailability [212]. No severe adverse events were reported for the oral formulations over 8 weeks of administration. Although 12% of patients in the oral group experienced diarrhea, this was not directly attributed to the formulation as the same percentage of patients had this adverse reaction in the injectable cohort. In a Phase 2b trial, the number of adverse events reported was similar for oral formulations containing the macrocyclic peptide, MK-0616, with 180 mg C_{10} and the placebo control group that did not contain the active or the PE [213]. Side effects reported were

diarrhea and nausea. Several other clinical trials of oral formulations containing C_{10} report mild gastrointestinal disturbances such as diarrhea, abdominal pain, and constipation. The cause of these mild and infrequent disturbances has not been determined.

Several pre-clinical studies show that mM concentrations of C_{10} cause mucosal perturbation (reviewed in [165]). Incubation of Caco-2 cells with C_{10} causes a concentration-dependent loss of membrane integrity and cell viability [58]. C_{10} also caused histological damage to isolated rat and human intestinal tissues when incubated for 2 h at low mM concentrations [214]. There was also histological damage following rat intestinal instillations with high mM concentrations after 2 h, although barrier integrity quickly recovered to near control levels [215] (Fig. 2). Mode of action studies performed by high content image analysis in Caco-2 cells show that barrier alteration is closely associated with transcellular perturbation and loss of cell viability [58]. The reported alteration to barrier integrity by surfactants can be fast for some surfactants (e.g., C_{10} [215]) and relatively slow for others (e.g., SLS [28]), although this is often concentration-dependent.

Mycapssa® is an enteric coated capsule containing octreotide and the TPE® formulation: an oily suspension of octreotide, C_8, and polyvinyl pyrrolidone (PVP) dispersed in an oily blend of polysorbate 80, glyceryl monocaprylate, and glycerol tricaprylate [39 216]. TPE® causes a reversible alteration to barrier integrity, which enables improved permeation of octreotide and this has been ascribed largely to C_8 [217], but there may be minor contributions from polysorbate 80 [217 43], glycerol monocaprylate [218] and other constituents.

The transient aspect of TPE® highlights that the alteration to barrier integrity is quickly reversed. For example, in rat intestinal instillations there was a reduction in absorption of FD4 when it was administered 10, 30, or 60 min after intestinal instillation of TPE® [217]. Although TPE® is believed to act via opening TJs to increase paracellular permeability [217], excipients like C_8 are known to cause transcellular perturbation at higher concentrations [219]. Depending on the local mucosal concentration, alteration to barrier integrity may occur via a combination of TJ opening or membrane perturbation [39].

Preclinical safety studies reported no morbidity or mortality following single or multiple dosing of TPE® to nearly 300 rats [217]. Daily administration of oral octreotide in TPE® to cynomolgus monkeys for 9 months did not affect body weight and there was no hematological or clinical pathology. There were also no abnormalities in macroscopic and histopathological evaluation of target organs. Participants of a Phase I trial experienced mild GI-related adverse events including abdominal pain, diarrhea, and nausea [220]. However, similar side effects were also experienced in patients receiving the injectable formulation of octreotide. In a Phase III trial, patients experienced mild-to-moderate GI-related adverse events including nausea (30%), diarrhea (20%), and abdominal pain (10%) [221]. These mostly occurred during the first two months of treatment and resolved as the treatment progressed. The GI side effects were consistent with the injectable somatostatin receptor ligand (SRLs) drug class. A similar observation was noted in a second Phase III trial [222].

7. Relating the safety data from pre-clinical models to humans

The data from cells, isolated tissues, and animal models shows the potential for some surfactants to non-specifically perturb biological membranes causing alteration to epithelial barrier integrity along with cell death. Some surfactants can inhibit efflux transporters while there is emerging evidence that induced changes to the microbiota may be involved in inflammation and metabolic disorders in murine models. How these effects relate to humans is difficult to predict because it is not clear whether a surfactant reaches and sustains a concentration at the mucosal surface for long enough to cause these effects.

Exposure to concentrations that have potential to cause toxicity may be limited by dilution in GI fluid, spreading and transit, interaction with constituents of luminal fluid, and the barrier presented by the mucus gel layer covering the epithelial surface (Section 8). The luminal concentration may also be reduced by absorption of the surfactant itself if it is permeable. A very small quantity of SLS is required as a wetting agent in solid dosage forms, so studies assessing the interaction of mM SLS concentrations with isolated enterocytes do not represent in vivo conditions in the small intestine.

The nature of the dosage form may limit potential exposure to the mucosal surface. A surfactant may have a high free luminal concentration from an aqueous micellar solution, but there will be a lower available concentration if the surfactant is stabilizing an emulsion droplet and is not free to desorb from the oil–water interface. If the surfactant gradually dissolves from a solid dosage form moving along the GI tract, the luminal concentration at any focal point will remain low. Formulations containing high surfactant concentrations, such as LBFs (Case 1) or PE-based dosage forms (Case 2), will lead to a high luminal surfactant concentration, although it is difficult to determine the concentration of free surfactant that diffuses through the mucus gel layer and for how long the epithelium is exposed to it.

Cell viability and barrier integrity assessments performed in cell cultures and isolated tissues directly show the potential for membrane perturbation, but relating it to the concentration, treatment duration, and fluid composition to the human GI tract is not possible. Oral administration of dosage forms to animals allows an assessment of the surfactant in conditions that account for dilution, transit, interaction with luminal constituents, and barrier recovery. However, interspecies differences in transit, fluid volume, and physiology are known to play a role in drug disposition and the action of surfactants to improve permeation of poorly absorbed molecules (Case 2). Dosage forms that are designed for humans can be administered to pigs and dogs, which allows a comparison of exact excipient quantities. Attempts to use miniaturized formulations for rodents do not allow assessment of comparable concentrations from different formulations in different species. A 20 mg size 9 minitablet administered to 200 g rats is comparable to a formulation weighing 7 g in an average human weighing 70 kg. If the minitablet contains 1 % w/w surfactant (0.2 mg) (1 mg/kg), this does not represent the amount of surfactant ingested by a human taking a 500 mg tablet with 1% w/w surfactant (5 mg) (0.07 mg/kg). While the luminal diameter and fluid volume are smaller in rats, it is not possible to predict the difference in luminal concentration of PE across species

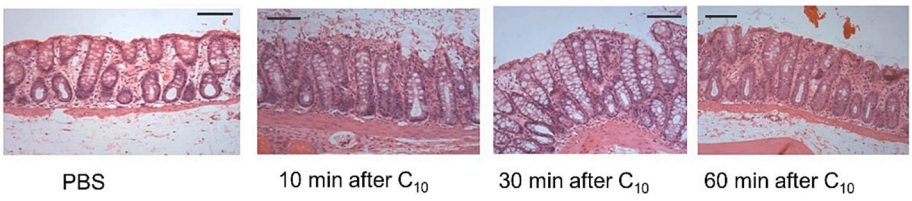

Fig. 2. Representative light micrographs illustrating the effect of 100 mM C_{10} on surface morphology of the intact rat colonic epithelium at different timepoints following instillations. Horizontal bars = 250 μm. Absorption of co-administered FD4 was highest over 120 min when co-administered with C_{10}, but it tailed off if C_{10} was added as a pretreatment followed by FD4 instillation at sequential time points. This reflects both the rapid absorption of C_{10} and the recovery of the epithelium from surfactant effects. Edited with permission from Wang, X., et al (2010). *Therapeutic Delivery*. 1 (1): 65–72. https://doi.org/10.4155/tde.10.5.

and hence it is difficult to predict toxicological effects in humans from studies in rodents. There may be the expectation that instillation of a known concentration into the GI lumen of rats or mice will compare with the effects observed in humans, but there is often a disparity in the effects observed.

8. Factors affecting the interaction of surfactants with the intestinal mucosa

This section outlines some of the factors that may limit the concentration of surfactant available at the intestinal mucosal surface and offset potential damage associated with perturbation in vivo. Additional information on the factors that affect the luminal excipient concentration is found elsewhere [73 216 223].

8.1. Effect of GI fluid volume on mucosal damage by surfactants

The concentration of surfactant present in the GI lumen depends on the quantity in the dosage form in the available volume of intestinal fluid. If there is a high luminal volume, the surfactant will be diluted below the threshold level where perturbation of the epithelium may have been observed in pre-clinical models. If a dosage form dissolves in a low fluid volume, the concentration of surfactant may reach that threshold. However, the potential for mucosal perturbation also depends on other factors.

In the fasted state, the mean gastric fluid volume is 45 mL (range 13 – 72 mL) [224]. In another study, the gastric fluid volume in fasted human volunteers increased from 35 mL to 242 mL after ingestion of 240 mL of water, the volume recommended for bioequivalence testing [225]. This volume of gastric fluid decreased by 50% after 13 min and returned to baseline after 45 min [225]. Fasted state human small intestinal fluid volume was measured as either 43 mL (range: 5 – 159 mL) [225] or 105 mL (45 – 319 mL) [224]. Fluid in the small intestine is not a continuous liquid reservoir, rather, a collection of fluid-filled pockets averaging 13 mL in the fasted state and 4 mL after a meal [224]. There was an increase from 43 mL in the fasted state to 94 mL ~ 10 min after ingestion of 240 mL water [225]. After 12 min, there was also an increase in the number of fluid-filled pockets from 8 × 4 mL to 15 × 6 mL [225]. After 45 min, the fluid volume was 77 mL (16 × 5 mL). In addition to the large fluid pockets in the small intestine, there are many small fluid pockets (<2.5 mL).

The impact of GI fluid on surfactant concentration depends on whether the dosage form is solid or liquid and the type of release profile. An immediate release (IR) tablet may dissolve in 50 to 250 mL of fluid depending on the amount of ingested water, so the concentration of surfactant may range by several orders of magnitude depending on the amount in the tablet. For example, a dosage form containing 10 mg SLS may be present at a concentration of 0.02% w/v if it dissolves in 50 mL of gastric fluid or 0.004 % w/v if it dissolves in 250 mL. A dosage form containing a higher quantity of surfactant (e.g., 0.5 g) may be present at a concentration ranging between 0.2 and 1% w/v. It is difficult to estimate the volume of water that an enteric-coated dosage form will be exposed to in the small intestine and hence difficult to estimate the concentration of surfactant at the epithelium. An enteric formulation encountering a fluid pocket of 10 mL could reach concentrations up to 20 times higher than those measured in gastric fluid.

Studies that assess the effect of intestinal conditions on the interaction of surfactants with the intestinal mucosa have been carried out with PEs or AMEs. Such studies can determine how intestinal conditions influence the interaction of PEs with the epithelium to improve drug flux. Other studies can also determine if there are unintentional barrier alterations with AMEs contained in the formulation for some other purpose [167]. A rat SPIP experiment assessed the interaction of 0.1 or 0.5 w/v SLS on barrier integrity assuming 0.2–1 g doses of the excipient dissolved in a 250 mL volume of water [170]. The estimated luminal fluid is close to the measured gastric fluid volume if the formulation is administered with a full glass of water [225]. However, these quantities of SLS were higher than the amounts allowed for it on the FDA IID. Perfusion of 0.1 or 0.5% w/v SDS altered epithelial permeability to selected compounds [170]. The magnitude of effect depended on both the concentration and the exposure time. Surfactants that transiently alter intestinal barrier integrity are less efficacious when diluted in larger fluid volumes. Thus, there was a sequential decrease in effective permeability of salmon calcitonin (sCT) across isolated rat small intestinal mucosa when formulations containing sCT, citric acid, and the surfactants lauroylcarnitine chloride or sodium taurocholate were diluted in 10 mL, 50 mL or 150 mL fluid prior to tissue exposure [226].

The effect of fluid volume on surfactant alteration to barrier integrity was also assessed in rats following bolus intestinal administration of C_{10} in volumes of fasted state simulated intestinal fluid (FaSSIF) [227]. Dilution of 16 mg C_{10} led to less pronounced absorption of the epithelial barrier integrity marker, FD4, in the order 0.27 mL (BA: 22%) > 0.8 mL (BA: 21%) > 1.6 mL (BA: 14%). The comparable level of FD4 flux at 0.27 mL and 0.8 mL suggests that the concentration remains above a threshold level required to induce flux at low dilution volumes. There may therefore be focal alteration in barrier integrity when dosage forms dissolve in small fluid volumes in the small intestine, although this will depend on the type of surfactant, its concentration in the dosage form, and GI transit.

While surfactants may initially be diluted in a large fluid volume, absorption of water from the GI tract may concentrate poorly absorbed molecules in GI fluid [228]. The intestinal luminal concentration of TPGS in rats was 2-fold more concentrated than that from the initial oral dose, whereas the concentration of DMSO decreased because it was absorbed [228]. The concentration of TPGS was also monitored in the duodenum and jejunum of humans who were given a 1200 mg dose of amprenavir as eight Agenerase® soft gelatin capsules (each containing 150 mg of the drug and 280 mg of TPGS) [229]. TPGS levels in the duodenum increased to ~ 8 mM after 40 min, then decreased to less than 2 mM after 105 min. The concentration in jejunal fluids gradually increased to 4 mM after 105 min, thereafter, decreasing to less than 1 mM. At no time was the average TPGS concentration above the theoretical concentration of 2240 mg dissolved in 180 mL of water. Nevertheless, the ratio of TPGS to amprenavir increased in the jejunum, suggesting slow absorption of TPGS.

8.2. Effect of GI transit time on mucosal damage by surfactants

GI transit contributes to limiting the exposure of the intestinal mucosa to high concentrations of surfactant excipients for prolonged periods. The effect of GI transit depends on the release properties from the pharmaceutical dosage form. Surfactants present in suspensions, solutions, or IR dosage forms may be dispersed in gastric fluid where they may either interact with the gastric mucosa or exit the stomach in liquid that moves freely into the small intestine. The half-emptying time from the stomach was 16 min after administering 300 mL water [230], 13 min after 240 mL [225], and 4 min after 150 mL [231]. In these scenarios there may be dilution and only a short exposure time at epithelia in the stomach or duodenum. Tablets and capsules that are slow to dissolve may rest against the wall of the gastric mucosa prior to gastric emptying. Gradual dissolution from these dosage forms in the vicinity of the gastric mucosa may prolong exposure of the epithelium to constituents of the formulation. This is part of the mechanism by which mucosal perturbation is caused by acetylsalicylate and why dispersible forms cause less gastric damage compared to IR tablets [232] (see Case 4). Gradual co-release of semaglutide and the (non-surfactant) PE, sodium salcaprozate, over 30–50 min is also part of the mechanism by which the flux of semaglutide occurs in the stomach [233]. There are no examples of surfactant-induced perturbation of the gastric mucosa from licensed products.

A study in minipigs assessed how dosage form release characteristics and spreading affect the local surfactant concentration and barrier

integrity [234]. Upon intraduodenal administration capsules containing 250 mg C_{10} and 12.5 mg cyanine-5 labelled peptide ruptured after ~ 11 min. The regional C_{10} concentration measured 10 to 15 min after administration was over 150 mM at the capsule release site and dropped to ~ 10 mM at 3 cm from the capsule shell. C_{10} caused perturbation of the epithelial surface, cell sloughing, and increased mucus secretion at the capsule release site, whereas there was no mucosal damage 10 cm from this location.

Simulations of intestinal peristalsis were used to determine how peristaltic speed, contraction levels, fluid pocket size, release location, and occlusion ratio influence the regional concentrations of C_{10} and insulin [235]. There was a 4-fold increase in the maximum concentration of C_{10} at the epithelial surface when the peristaltic wave speed was reduced from 1.5 to 0.5 cm/s. Thus, a slow more contracted peristaltic wave was more efficient at transporting dissolved C_{10} to the intestinal wall. However, when partially occluded pockets became more open, (i.e., the GI segment took the form of a more open cylindrical tube rather than a set of fluid pockets), the concentration at the epithelial surface became negligible. The C_{10} concentration at the epithelial surface was also lower when wave propagation occurred in a GI segment containing 7×10 mL fluid pockets compared to 12×2 mL fluid pockets. Here, the C_{10} was distributed over several small fluid pockets or one large fluid pocket. There was a higher concentration of C_{10} at the epithelial surface when it was released at the partially occluded points between fluid pockets compared to the center of a pocket. When there was an increase in the extent of occlusion, C_{10} became less spatially dispersed and there was a greater tendency for vortex mixing which led to a higher concentration at the epithelial surface. Together, this study suggests that conditions in the GI tract play a part in restricting sustained exposure to high concentrations at the epithelial surface.

The mean small intestinal transit time of pharmaceutical dosage forms was estimated as 3 ± 1 h based on a *meta*-analysis [236]. There is also wide variability in the small intestinal residence time [237]. The migrating motor complex (MMC) moves undigested materials toward the large intestine and facilitates mixing, digestion, and absorption. Movement is therefore non-uniform and discontinuous [238]. This makes it challenging to predict the time that any focal point in the small intestine will be exposed to any ingested surfactant. It is estimated that chyme moves through the small intestine at a rate of 1–4 cm/min [239]. An estimated velocity for dosage forms with a 3 h small intestinal transit time is 2 cm/min if the average length of the small intestine is 356 cm [240]. The average fasted flow rate measured in humans was 0.73 mL/min in the jejunum and 0.33 mL/min in the ileum, values that increased to > 2 mL/min in the fed state [241]. When a 3 mL solution of citric acid and Optiray^{TM} 320 were instilled into the duodenum of intubated dogs, a drop in pH was recorded 15 cm past the instillation port after only 1 min [242]. Spreading and dilution at the site where liquids are instilled into the small intestine therefore limits barrier integrity alterations caused by surfactant excipients. For example, instillation of palmitoyl carnitine with cefoxitin into a ligated segment of rat small intestine led to a 4-fold higher transmucosal permeation of cefoxitin compared to the same mixture instilled an open segment [243]. The time therefore that any segment of the intestinal mucosa is exposed to an excipient in an oral dosage form ranges from seconds to minutes, potentially limiting perturbation of the epithelium compared to Caco-2 cell monolayers, intestinal sacs, and tissues mounted in Ussing chambers. Recent articles note that a short residence time in the small intestinal lumen is a major factor that limits the efficacy of PEs in formulations [216 207].

8.3. *Effect of luminal fluid composition on mucosal damage by surfactants*

Studies evaluating the effect of surfactants on in vitro and ex vivo models are often performed in culture media or tissue bathing buffer solutions. While these controlled physiological environments may preserve cell viability and permit determination of fluxes following surfactant exposure, they do not represent conditions in the intestine. Human gastric and intestinal fluids are complex heterogeneous fluids containing endogenous secretions and dietary substances. These include carbohydrates, lipids, proteins, amino acids, micronutrients, minerals, enzymes, bile acids, mucin, phospholipids, solid particles or liquid globules, and lysates of sloughed epithelial cells and microorganisms. There is therefore a cocktail of dissolved molecules, colloidal structures, and coarse particles that can influence the free concentration of surfactant in the GI tract (Fig. 1).

Some surfactants are sensitive to chemical or enzymatic degradation in the small intestine. There may be degradation of surfactants into absorbable nutrients, for example, when hydrophilic and hydrophobic moieties are bonded via hydrolyzable esters (e.g., sorbitan fatty acid esters). Enzymatic degradation of insoluble surfactants and some lipid vehicles can release soluble surfactants that can cause enhanced perturbation of the intestinal mucosa. For example, release of monoglycerides and free fatty acids from the enzymatic degradation of medium-chain glycerides accentuated the barrier integrity alteration associated with an increased absorption of the epithelial permeability marker cefoxitin in rats [89]. The alteration to rat intestinal barrier integrity to insulin by macrogol-8 glycerides (Labrasol®, Gattefosse) was somewhat attenuated in the presence of the lipase inhibitor, orlistat®, suggesting that metabolites of Labrasol® contribute partially to its role in barrier perturbation [33]. In the digestive tract of rats, there was 100% hydrolysis of polysorbate 80 following oral gavage, 98% for polysorbate 60, and 84% for polysorbate 65 [244]. Free fatty acids are then absorbed in the GI tract while the hydrophilic polyoxyethylene sorbitan moiety is largely excreted in feces [244 245]. As GI degradation of polysorbates removes the hydrophobic moiety and diminishes the surface action of the native compound, the potential for adverse events related to the intact surfactant is reduced.

The availability of surfactant monomers to interact with the intestinal epithelium is also influenced by physical interaction with constituents of luminal fluid and dietary substances. As surfactant monomers play a prominent role in detergent action, luminal conditions that alter the CMC will change the concentration of free surfactant and will impact their interaction with the intestinal mucosa. The formation of ionic micelles is sensitive to the ionic strength of the vehicle because increasing the concentration of counterions lowers the repulsive forces between similarly charged hydrophilic head groups and favors micellization. Hence, the CMC for ionizable surfactants is lower at higher ionic strength. For example, the CMC of SLS is 8.1 mM in water and 0.7 mM in 0.3% NaCl [246]. The CMC for SLS was also reduced in water containing two undisclosed compounds and the values were further reduced in FeSSIF and simulated gastric fluid containing the same compounds [247]. The ionic strength of luminal fluid may therefore reduce the maximum concentration of surfactant monomers by over 10-fold and can reduce the concentrations to below the threshold required for inducing membrane perturbation and plasma membrane solubilization. In high ionic strength conditions, there is also an increase in the number of surfactant monomers per micelle, and an increase in the hydrodynamic radius [248]. As an increase in micelle size is associated with a decrease in particle surface area exposed to the solvent, there is potential for slower desorption from the larger particles to replenish the free monomers used up during perturbation. The capacity of SLS to alter barrier integrity to some hydrophilic drugs was accentuated in hypotonic conditions (0.45% NaCl versus 0.9% NaCl) in a rat *in situ* single pass intestinal perfusion [249]. When the free surfactant interacts with cells or other interfaces in the GI lumen, it is effectively removed from solution and, although monomers are replenished from micelles, the concentration will not exceed the CMC. As fluid is moving through the GI tract in vivo, there is therefore a low possibility of cumulative perturbation.

Ionizable surfactants that are weak acids are also sensitive to pH changes in the GI tract. At pH values 1 to 2 units below their pKa, the molecular acid will exist as an insoluble surfactant, displaying negligible

monomer solubility and the absence of micelles. Consequently, insoluble forms of medium-chain fatty acids do not cause comparable barrier integrity alterations. Solubility of ionizable surfactants can also be reduced in the presence of divalent counterions. For example, the anionic carboxylate ion of medium-chain fatty acids reacted with Ca^{2+} to form insoluble calcium soaps with reduced capacity to alter barrier integrity in Caco-2 monolayers [250].

There may also be complexation of ionizable surfactants with drugs or excipients containing an opposite charge, leading to precipitation of an insoluble complex. In the rat SPIP model, SDS and C_{10} increased the permeation of enalaprilat, but had a negligible effect on permeation of hexarelin, perhaps because of interaction between the cationic hexarelin and the surfactants [251]. There is interest in the use of surfactants as hydrophobic ion pairing (HIP) agents to increase the lipophilicity of poorly permeable peptides [246]. Complexation between the anionic surfactant, sodium docusate, and a cationic peptide caused precipitation of the complex due to charge neutralization and the incorporation of one or more hydrocarbon moieties into the peptide structure. While drug delivery researchers are attempting to create peptide salts with greater lipophilic properties, the findings highlight the potential for ionizable surfactants to interact with oppositely charged species in the intestinal milieu.

Surfactants may also interact with large proteins or other polymers to reduce the free concentration available to cause membrane perturbation. For example, SDS displayed a reduced hemolytic action in the presence of bovine serum albumin (BSA) [252]. The authors suggest that the stabilizing effect is due to binding of BSA to SDS. A similar effect is possible with dietary proteins as SDS has been shown to bind and precipitate ovalbumin, lysozyme, and conalbumin [253]. SDS also forms a precipitate with gelatin, leading to a slow dissolution of hard gelatin capsules in acidic dissolution media (pH $<$ 5) [254]. The interaction between SDS and gelatin raises concerns about dissolution of capsules containing poorly soluble drugs [254]. The study indirectly shows that other excipients present in oral dosage forms can reduce surfactant presentation at the mucosal surface.

Luminal fluid contains micro heterogeneous colloidal structures and coarse liquid/solid particles (Fig. 1). Surface active agents preferentially adsorb at the interface between these distinct phases, removing monomers from solution. This also has potential to reduce the availability of monomers at the GI epithelium. Colloidal structures present in the stomach and small intestine include micelles, mixed micelles, vesicles, and nanoemulsions (Fig. 1). These consist of mixtures of fatty acids, mono- and di- glycerides, phospholipids, bile salts, proteins, and amphiphilic food additives. The extent to which excipients in pharmaceutical dosage forms partition in these structures is unclear. If the concentration of the surfactant is above the CMC, then any monomers that are depleted by adsorption to luminal particles will be replenished by monomers desorbing from micelles. The capacity of alkyl maltosides to promote flux of FD4 across Caco-2 monolayers was attenuated in the presence of mixed micelles (taurocholate, lecithin) by causing a decrease in the amount of free monomer in the buffer [19]. There was also a similar reduction in the amount of FD4 absorbed from the small intestine of rats when dodecyl maltoside was instilled in fasted state simulated intestinal media (BA: 5%) compared to transport media (BA: 20%) [19].

The extent of barrier integrity alteration to Caco-2 monolayers caused by the zwitterionic surfactants, hexadecylphosphocholine (HPC) and palmitoyl carnitine chloride (PCC), was also attenuated in FaSSIF, which the authors suggested was due to incorporation of taurocholate and phosphatidyl choline into mixed micelles [255]. In the same study, the presence of FaSSIF had no effect on the barrier alteration caused by the C_{10}, emphasizing that partitioning effects are not universal. Reasons suggested for this include a lower propensity for medium hydrocarbon chains to partition in micelles and that the much higher concentration of C_{10} required for barrier alteration saturates the mixed micelles in the FaSSIF [255]. More recently, rat intestinal instillation of 50 or 300 mM C_{10} in buffer had a similar effect on barrier integrity to FD4 in either FaSSIF or FeSSIF emphasizing that colloidal structures may not impede permeation enhancement when high enough surfactant concentrations are presented. In coarse grain molecular dynamic (CG-MD) simulations, the free monomer concentrations of C_8 and C_{10} were higher in FaSSIF than FeSSIF due to reduced incorporation into colloidal structures in the former [256 257]. A reduced free monomer concentration in FeSSIF was associated with an approximately 3-fold decreased penetration into phosphatidyl choline-based artificial membranes compared to FaSSIF. The composition of the micelles also influenced the number of C_{10} monomers moving in and out of the micelle, which affected insertion into the membrane.

In a rat SPIP perfusion of 0.5% w/v SLS in FaSSIF caused a 7.6-fold increase in absorptive flux of atenolol, indirect evidence of alteration to intestinal barrier integrity. The barrier-altering effect of SLS was decreased to 5.6-fold when FaSSIF was replaced by FeSSIF, which the authors suggest could be due to an abundance of colloidal structures in the latter. While the interaction of surfactant monomers with constituents of luminal fluid can partially prevent alteration of intestinal barrier integrity in selected examples, this effect depends on the type of surfactant, the luminal concentration, and the nature of the interaction with the intestinal mucosa. On the contrary, it is also possible that epithelial effects of some surfactants can be accentuated. For example, in the presence of PEG-based co-solvents [85], or when fatty acids were mixed with macrogol glycerides [258].

Fatty acids may form anionic micelles at high pH values above their pKa or insoluble oils below their pKa, but at pH values close to the pKa the presence of both the acid and conjugate base can lead to the formation of vesicles, nanoemulsions, and coarse emulsions. These depend on the concentration of surfactant and the fraction of surfactant in the acid and conjugate base forms. The pKa of C_{10} monomers is approximately 4.0, whereas surfactant monomers in micelles have a lower tendency to donate their proton and have a higher pKa of 7.0 [259]. At pH 8.5 where over 90% of C_{10} exists as the conjugate base and less than 10% exists as the insoluble acid, micelles (<5 nm) were observed by cryo-TEM [227] (Fig. 3). In addition to micelles forming at high pH values, *in silico* studies by CG-MD confirm that the proportion of free C_{10} monomers is also higher due to the greater solubility of the carboxylate anion [257]. At pH 6.5, approximately 50–90% of C_{10} exists as the insoluble acid form and only 10–50% exists as the soluble conjugate base; this is why vesicles and other larger structures (50–200 nm) were observed in cryo-TEM [227]. Bolus intestinal administration of 50 mM C_{10} in pH 6.5 buffer (forming vesicular structures) to rats had a greater effect on promoting FD4 absorption compared to C_{10} micelles formed at pH 8.5. This pH effect was not observed at higher concentrations (100–300 mM). As C_{10} in the micellar form (pH 8.5) is more quickly absorbed than the vesicular form (pH 6.5), the greater effect on barrier integrity for the vesicular form compared to micelles may be due in part to longer retention at the instillation site where a higher concentration of C_{10} at the epithelium occurs. The greater effect on barrier integrity in buffers of lower pH values was somewhat unexpected as the CMC of MCFAs increases at higher pH values [260]. Additionally, CG-MD simulations show different interactions at the membrane for capric acid and the caprate anion [261]. Here, a greater proportion of neutral capric acid undergoes flip-flop events across lipid bilayers and there is reduced expulsion into the vehicle compared to caprate. In sum, there are additional factors to the availability of free monomers that may contribute to barrier perturbation.

Studies in rats have shown that the free concentration of bile acid surfactants in luminal fluid is impacted by dietary fibre [262]. These luminal effects are said to play a role in reabsorption in the ileum. Mechanisms proposed include binding/complexation from solutions, physical entrapment in intestinal fluid that has been rendered more viscous by the presence of soluble fiber, and/or reinforcement of the unstirred water layer to restrict micelle diffusion to the surface of epithelial cells [262 263 264]. For insoluble fiber, adsorption to

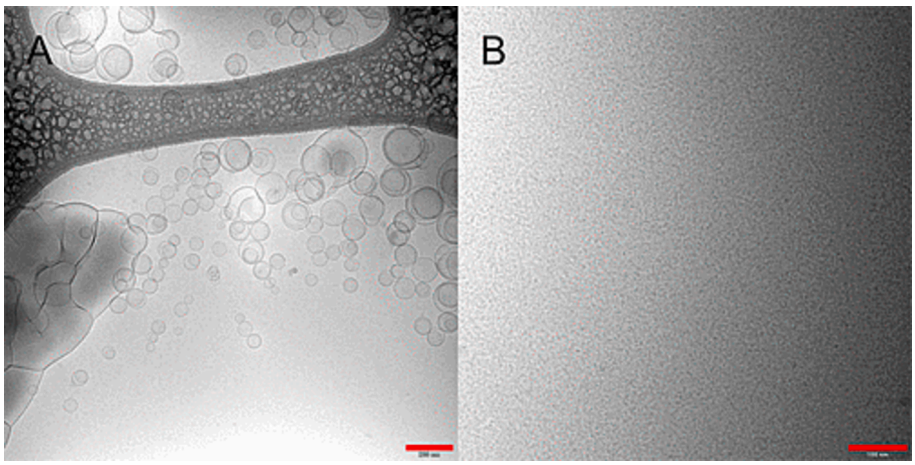

Fig. 3. Cryo-TEM images of C_{10} solutions in FaSSIF. (A) 100 mM C_{10} @ pH 6.5. Vesicles varying in size from 50 to 200 nm are present with larger aggregated structures. (B) 300 mM C_{10} @ pH 8.5. A homogenous sample of spherical micelles smaller than 5 nm. Scale bar: 200 nm in (A) and 100 nm in (B). Reproduced from Berg, S et al. (2022) Mol. Pharm.; 19(1): 200–212. https://doi.org/10.1021/acs.molpharmaceut.1c00724 with permission under a CC-BY 4.0 licence.

particulates can also alter the free bile acid concentration, which is another proposed mechanism by which fiber affects reabsorption in the ileum [264].

8.4. *Effect of the mucus gel layer on mucosal damage by surfactants*

Mucus is a viscoelastic gel that lubricates and protects the intestinal epithelium from luminal debris, enzymes, and micro-organisms. There is a tightly adhered layer of mucus close to the epithelial surface and a more mobile diffuse layer in the lumen. The extrinsic barrier formed by mucus restricts diffusion of large particles to the epithelial surface and also slows diffusion of smaller particles, macromolecules, and small molecules [265]. Mucus secretion is part of the initial GI protective response to surfactant perturbants [266]. Bile acids and phospholipids in FaSSIF and FeSSIF cause perturbation of enterocyte monolayers that do not produce an extrinsic mucus gel layer [267 268]. The application of a layer of biosimilar mucus to Caco-2 monolayers partially countered reductions in cell viability and barrier integrity caused by exposure to FeSSIF and salts of medium-chain fatty acids [269]. The protective effect was nullified at high concentrations of free fatty acids, so there are limits to the protection afforded by biosimilar mucus in static cell culture models.

Damage to Caco-2 monolayers induced by oleic acid was reduced by overlaying the cells with porcine gastric mucin [270]. The production of mucin induced by instillation of low concentrations of oleic acid to rat jejunum in vivo helped to protect against damage induced at higher concentrations [270]. Perfusion of epidermal growth factor into the rat GI lumen stimulated goblet cell mucus secretion, which attenuated the barrier integrity alterations caused by oleic acid [271]. In the same study, inhibition of mucus production by atropine accentuated the epithelial damage caused by oleic acid and also abolished the protective effects observed with epidermal growth factor.

The protective role of mucus is apparent in intestinal diseases that cause goblet cell pathology and reduction in secretion. In Crohn's disease, there is an increase in *muc-2*, which alters the viscoelastic properties of mucus gel and its barrier properties [272]. In contrast, a thinning of the mucus gel layer is observed in ulcerative colitis, which is correlated to decreased expression of *muc-2* [272]. Although alteration to GI mucus is just one feature of IBD, such alterations can lead to greater exposure of the epithelial surface to the intestinal milieu. Polysorbate 80 interferes with the structure and function of mucus in cell culture models [69], rat intestinal loops [69], and following oral administration to mice pre-disposed to colitis-like symptoms [61] (Section 6.1.1). The consequences of changes in GI mucus thickness in humans remains unclear. Studies demonstrating alteration to mucus thickness in mice were based on 12-week daily exposure to surfactants in drinking water. There are no equivalent studies demonstrating such effects following daily administration of oral dosage forms. Researchers have also used surfactants to reduce the mucus gel layer to facilitate better penetration of nanoparticles. In rat intestinal instillations, there was therefore greater mucus penetration of poly(lactic-co-glycolic acid) (PLGA) nanoparticles following a 30 min pre-treatment of SDS, polysorbate 80, or poloxamer 407 [273].

Instillation of 0.5–10 mM deoxycholate into rat colon caused a concentration-dependent increase in mucus secretion [274]. There were also partial increases in mucus secretion seen with hyodeoxycholate and chenodeoxycholate, but no effect with 10 mM polysorbate 20, cholate, or ursodeoxycholate [274]. A concentration-dependent increase in mucus secretion was also shown in rabbit colonic loops perfused with chenodeoxycholate, an event that preceded mucosal perturbation [275]. These data suggest that detergent-like perturbation plays a role in bile acid-induced secretion and depletion of mucus, rather than a secretagogue effect as observed with cholinomimetics.

Although surfactant-induced disruption to the mucus gel layer in preclinical models raises potential concerns about potential increased exposure of the epithelium to xenobiotics and micro-organisms, to date there is no evidence that bile acids or ingested surfactants alter the GI mucus gel layer in humans in vivo. Further investigations in humans are warranted as a clinical evaluation showed that structural weakening of colonic mucus could be associated with the pathogenesis in a sub-group of ulcerative colitis (UC) patients [276]. There was a reduction in expression of *muc-2* in GI mucus from these patients, which was linked to greater epithelial passage of microorganisms.

8.5. *Effect of dosage form properties on mucosal damage by surfactants*

The effects of excipient-excipient and drug-excipient interactions on oral BA is poorly understood, as is the effect of these interactions on the luminal excipient concentration and epithelial barrier integrity. The concentration of surfactant used in oral dosage forms can range from low mg quantities (when used to aid manufacturing) to high mg quantities (when used to stabilize emulsions/suspensions, to assist dissolution, or to improve intestinal permeation). Although the concentration is a key factor impacting free monomer concentration, the characteristics of the dosage form also play a part in determining the level of monomer that is available to interact with the intestinal epithelium. For example, the amphoteric surfactant, palmitoyl carnitine, had a greater effect on absorption of the permeability marker, cefoxitin, in the small intestine of

rats when it was instilled as an aqueous dispersion versus a minitablet [243]. Therefore, data showing the effects of aqueous solutions administered via intestinal instillation, oral gavage, or in drinking water to rats do not represent the behavior of a surfactant administered in an oral dosage form. This is one of the reasons why performance of intestinal PEs can fail to translate from rodent models to humans [216]. Surfactants may be present in tablets, solid/liquid-filled capsules, or oral liquids including solutions, suspensions, and emulsions. The formulation type can influence the rate of release of the active molecule and the surfactant and can contain other excipients that influence the availability of monomers.

There is little emphasis in the literature on the dissolution rate of excipients from conventional tablets and capsules, although release kinetics play a role in barrier integrity alteration by surfactants [216]. Dissolution of soluble surfactant salts, such as SLS, will proceed quickly from fast-disintegrating IR tablets. This results in a spike in the regional concentration that will eventually decrease due to a combination of spreading, dilution in luminal fluid, and SLS absorption. A more gradual release can arise due to slower dissolution of the surfactant or because there is modified release from the dosage form. The consequences of slower dissolution depend on the conditions in the GI tract and are difficult to predict. If the dosage form is continuously moving along the GI tract, there will be a lower concentration of surfactant released at any focal point compared to an IR formulation. For example, the effect of C_{10} on absorption of sulpiride in rats was two-fold higher for IR tablets (90–100% release over 10–20 min) compared to a sustained release (SR) formulation, where both agents were released over 4 h [277].

If the tablet or capsule becomes lodged at the gastric mucosa or small intestinal mucosa, gradual release may continuously expose a focal point in the GI tract to a low concentration of surfactant, which, depending on the potency of the surfactant, may cause local barrier alteration. For example, perfusion of 50 mM C_{10} into a segment of rat small intestine for 30 min had a greater effect on barrier integrity than perfusion of 100 mM for 15 min, as determined by absorption of cefoxitin [162]. In contrast, bolus administration of 1 g C_8 had a greater effect on rectal absorption of cefoxitin in humans compared to co-infusion of 0.5 g over 1 h [278]. However, as the latter study was performed in the rectum, there may not be a difference in exposure time between the bolus and infused doses.

The interaction between surfactants and other constituents of the formulation can also alter the free monomeric concentration. Surfactants are commonly included in formulations to adsorb at the interface between distinct phases to alter the interfacial properties. Adsorption of surfactant monomers between oil and water assists in the physical stabilization of emulsions. Surfactants can also be used as dispersants in suspensions, where they adsorb to solid particles in a liquid leading to deflocculation. Adsorption of surfactant monomers at the air–liquid and solid–liquid interfaces is also central to the mechanism of wetting. Surfactant adsorption at a solid–liquid interface is quick, however, the subsequent desorption is slow because the monomers have a more favorable interaction at the solid–liquid interface than in solution [279]. This suggests that once a surfactant adsorbs to a solid particle in the GI tract, it may only slowly desorb as it transits through the GI tract. This also influences the free monomer concentration. The FDA IID provides a maximum potency per unit dose for individual dosage forms, emphasizing that allowed limits relate to specific formulations. In food sciences, the allowed limits of selected surfactants depends on the food matrix (examples include SLS [146] and polysorbates [280]).

There may also be unintentional adsorption of surfactant monomers to insoluble solid particles in the formulation or electrostatic interaction between ionizable head groups and dissolved counterions present in the formulation. These include salts and trace elements (Mg^{2+}, Ca^{2+}), ionizable APIs (e.g., chlorpromazine [281]), and excipients (e.g., gelatin [254]). Divalent counterions in the formulation can precipitate fatty acids to form insoluble salts (e.g., calcium caprate [250]). While monovalent counterions like Na^{+}, Cl^{-}, and K^{+} in the formulation will not precipitate ionizable surfactants at low concentrations, they make micelle formation more favorable and therefore can decrease monomer solubility by decreasing the CMC [250]. The interaction between the surfactant and other excipients in the formulation is similar to interactions with constituents of luminal fluid (see Section 8.3). However, as the additives in the formulation are in close proximity during liberation, there is likely to be a more consistent interaction compared to luminal fluid and food.

Interaction studies of drugs and excipients with super-disintegrants used in oral solid dosage forms found that the free concentration is reduced by electrostatic interactions or lipophilic interactions, depending on the physicochemical properties of the disintegrant and the drug/excipient [282]. The percentage concentration of the cationic surfactant, cetyl pyridinium chloride in aqueous solution, was reduced by the anionic polymers, sodium starch glycolate and croscarmellose sodium [282]. There was no change to the concentration in the presence of the neutral polymer, crospovidone. Adsorption of cetyl pyridinium chloride to Mg^{2+} stearate particles in a tablet-based lozenge reduced the surfactant monomer concentration in solution and reduced its antimicrobial activity [283]. These effects were observed at a concentration range where magnesium stearate is used in oral dosage forms (0.1 to 2% w/w). The effect was not observed for all excipients as there was no adsorption to talc or reduction in antimicrobial activity. On the other hand, adsorption of the cationic amphiphile drug, chlorpromazine, to activated charcoal, talc, and kaolin, reduced diffusion through a dimethyl polysiloxane model membrane whereas lactose and gelatin had no effect [281]. In the same study, there was reduced artificial membrane permeation in the presence of bile salts, with the authors suggesting that this is due to micellar solubilisation and/or complexation. Reduced permeation was also observed for stomach mucin with increased viscosity and/or protein binding suggested as the cause. As surfactant monomers are also responsible for detergent like perturbation of mammalian membranes, these studies suggest interaction with other additives in the formulation may limit interaction of surfactant monomers with the intestinal mucosa.

8.6. *Effect of systemic absorption on mucosal damage induced by surfactants*

The rapid absorption of a surfactant may limit its capacity to damage the intestinal mucosa. Absorption of an intestinal PE can dilute the excipient below a concentration that causes alteration to barrier integrity [216]. Strategies to reduce PE absorption include the selection of polymeric PEs that are poorly absorbed or the synthesis of PE-polymer conjugates that are also retained in the GI lumen.

Few studies have assessed oral absorption kinetics for excipients used in oral dosage forms. Some surfactants have inherently low intestinal permeability and poor oral bioavailability. For example, native polysorbates have low intestinal permeation. They are however hydrolyzed to yield a free fatty acid moiety that is well absorbed and a hydrophilic polyoxyethylene sorbitan moiety which is not [54 244 245]. In a rat SPIP study, absorption of dodecyl sulfate was slow because the fixed negative charge at pH values in the small intestine restricts its epithelial permeability to the paracellular route [284]. The pharmacokinetics of C_{10} was evaluated in humans following oral administration of a tablet containing 550 mg C_{10}, and ~ 50 mg I338 insulin with 100 mL of water [285]. The C_{10} T_{max} was 29 min indicating rapid liberation and absorption once the tablet enters the small intestine. When the formulation was administered with food, the T_{max} of C_{10} was reduced to 23 min and there was a reduction in the amount of insulin absorbed. The authors note that rapid absorption of C_{10} leaves only a small amount of it in the GI lumen to promote further insulin absorption, although they note that other factors may also play a role in how food attenuates barrier integrity alterations. As rapid and complete absorption of C_{10} occurs in the upper small intestine, there is little likelihood of pathology in the large intestine.

8.7. Effect of epithelial repair on mucosal integrity after exposure to surfactants

The capacity of the intestinal epithelium to initiate repair mechanisms that restore barrier integrity contributes positively to the overall safety of surfactants in vivo (Table 3). Exposure of cultured mammalian cells to many soluble surfactants will result in concentration-dependent cell lysis. When intestinal epithelial cells are cultured as monolayers on Transwell™ supports, a concentration-dependent loss of barrier integrity is observed for many surfactants. At high concentrations, these effects are irreversible. However, at carefully selected concentrations and exposure times, recovery of monolayer integrity can be demonstrated upon wash-out (Table 3). Recovery from surfactant exposure is also reported in intestinal tissues mounted in Ussing chambers, but it is easier to demonstrate in intestinal perfusions and instillations *in situ* due to an intact blood supply. Bile salts are commonly used to investigate the physiological response to induced chemical injury in the GI tract (Table 3). The majority of studies in Table 3 assess PE reversibility as a positive feature for their feasibility as excipients in oral macromolecule delivery. Many examples show that barrier integrity alterations by PEs are indeed reversible at set concentrations, highlighting the amazing repair capacity of the GI epithelium [155 286 287 219 288 289 249 290 291].

There are only a few examples assessing damage and repair by surfactants used in LBFs and even fewer studies assessing damage and repair following oral administration of solid dose formulations. Reversibility studies for SDS, Labrasol®, and C_{10} indicate that repair is initiated after exposure to these excipients. However, it is difficult to relate the concentration and exposure time in animal models to exposure in humans. Case 3 provides a summary of a detailed study assessing the effect of repeat administration of surfactants on the GI mucosa.

Case 3. *Repeat oral administration of surfactants to mice*.

A study assessing the long-term safety of two surfactant PEs and one non-surfactant was performed in mice over 1 month [306]. Mice received daily oral gavage (10 μL/g) of 200 mg/kg C_{10} (200 mM) or 390 mg/kg (48 mM) sodium deoxycholate. The doses are generally higher than those that are likely to be administered to humans. A dose of 200 mg/kg C_{10} administered as 10 μL/g in mice translates to a massive dose of 14 g per 700 mL in a 70 kg human. Tablets designed for humans typically contain 500 mg C_{10} [212], and 180 mg has been used in a recent clinical trial [213]. Each week the intestinal barrier integrity was measured by determining systemic levels of FD4, administered three hours after the daily dose of surfactant on days 1, 8, 15, 22, and 30. Microscopy and gene expression analysis were performed on a subgroup of mice on day 30, while a second cohort was allowed to recover for 7 days before barrier integrity of the recovered epithelium was measured and samples were taken for histological analysis and gene expression analysis.

In initial experiments, both C_{10} and deoxycholate caused over a 10-fold increase in plasma FD4 levels versus control. There was partial recovery of barrier integrity after 3 h, where plasma FD4 was 1.7-fold higher for C_{10} versus untreated control and 5-fold higher for deoxycholate. There was no further elevation of plasma FD4 concentration beyond levels seen initially after 3 h on day 1, when mice were given daily oral doses of each surfactant for one month. This suggests that repeat cycles of barrier integrity alteration by these two surfactants do not lead to progressive deterioration of barrier integrity to the macromolecule. Histology of the small intestine on day 30 following chronic daily exposure to either agent showed intact villi and an absence of cell sloughing in all treatment groups, along with no difference in weight gain or changes in a surrogate plasma marker of intestinal permeability, zonulin. While deoxycholate caused a more substantial alteration to barrier integrity, there was no increase in plasma levels of TNF-α after 30 days of oral administration. There was however a progressive decrease in stool quality in the deoxycholate group as determined by a

Table 3
Examples of epithelial repair following exposure to surfactants in preclinical studies.

Surfactant	Model	Recovery	Ref
C_{10} (8.5 mM, 15–60 min)	Caco-2	A reduction in TEER to 15%, 5%, or 0% when exposed to 8.5 mM C_{10} for 15, 30, or 60 min. When the surfactant was removed, integrity of the cell layer recovered to 100% after 2 h, 4 h, or 7 h. Damage observed in electron microscopy recovered after 4 or 24 h and there was progressive recovery of plasma membrane permeability in high content image analysis. Expression of inflammatory markers changed across the recovery period with the most prominent change to IL-8 [292 292].	[294]
Palmityldimethyl ammonio propane sulfonate (PPS) (0.03%, 10–60 min)	Caco-2	TEER reduction to < 20% of control after 10 or 60 min; recovered to over 90% after 24 h	[295]
Palmitoylcarnitine (0.4–1 mM, 30 min)	Caco-2	TEER reduced to 50% of control after 30 min; recovered to > 80% after 22 h. After 22 h, active transport of neutral red into Caco-2 cells was 90% of control monolayers.	[296]
C_{10} (0.1%w/v, 20 min) Deoxycholate (0.1%w/v, 20 min)	Caco-2	C_{10} reduced TEER to ~ 50% of control after 20 min, a value which recovered to 100% between 3 and 6 h. Deoxycholate reduced TEER < 20% of control after 20 min, which recovered to 100% between 6 and 24 h. There was no recovery of TEER following incubation with 0.1% SDS	[151]
Triton™ X-100 (0.06 mM, 5 min)	Ussing (Guinea pig ileum)	Denudation of the epithelium of 86% of villi tips recovered after 2 h. Cells shouldering the injury underwent a conformational change to cover the exposed basement membrane. There was also recovery of potential difference and permeation of barrier integrity markers to control levels after 2 h.	[297]
Deoxycholate (15 mM, 30 min)	Ussing (Porcine colon)	Denuded surface epithelium was re-epithelialized with flattened migrating cells after 8 min and barrier integrity to mannitol was recovered after 40 min.	[298]
Labrasol® (4 mg/mL, 30 min)	Ussing (Rat colon)	TEER reduced to 20% after 30 min recovered to 70% after 2 h	[33]
Deoxycholate (6 mM, 15 min)	Ussing (Porcine, ileum)	There was a gradual recovery of barrier integrity (histology, TEER, mannitol permeability) in the 3 h after replacement of the bile salt with fresh Ringer's solution.	[299]
Deoxycholate (0.5 mM, 10 min)	Ussing (Human colon)	There was recovery from 50% damage of the colonic mucosa after 10 min, partially recovered to 32% after 3 h in recovery fluid. There was no recovery of TEER.	[300]

(*continued on next page*)

Table 3 (*continued*)

Surfactant	Model	Recovery	Ref
Taurocholate and oleic acid (20 mM) (10–40 mM)	Loop Instillation (Rat jejunum)	Damage to villous tips was repaired 50 min after instillation	[32]
Deoxycholate (15 mM, 30 min)	Instillation (Porcine colon)	Cell necrosis and epithelial sloughing led to denudation of the surface epithelium. There was evidence of cell migration and reepithelialisation after 15 min. Morphology and barrier integrity returned to normal after 2 h	[301]
Deoxycholate (5 mM, 60 min)	Instillation (Rat, rectum)	A reduction in the number of enterocytes to 20% of control recovered to over 95% after 2 h. There was more gradual recovery of goblet cells from 0% to 10% after 2 h and only 40% after 24 h.	[163]
SLS (5 mM, 60 min)	Instillation (Rat, rectum)	A reduction in the number of enterocytes to 60% of control recovered to over 100% after 2 h. There was more gradual recovery of goblet cells from 20% to 30% after 2 h and only 50% after 24 h.	[163]
TPE™ (C_8, Polysorbate 80, Glycerides)	Instillation (Rat, jejunum)	FD4 absorption was greatest when 0.3 mL TPE™ was co-instilled together with 1.65 mg FD4. When the FD4 was administered 10, 30, or 60 min after TPE™, there was little FD4 in plasma, indicating repair to barrier integrity.	[217]
Deoxycholate (1.5–100 mM, 30 min)	Intestinal loop (Rat, jejunum)	Different mucosal repair processes were observed via light microscopy depending on the deoxycholate concentration. Moderate to severe damage was repaired over 3 h through spreading of goblet cells to re-epithelialize the mucosa	[302]
Labrasol® (50 mg/kg, 30–60 min)	Instillation (Rat, jejunum)	The alteration to intestinal barrier integrity to LMWH that occurred upon co-presentation with Labrasol® was not observed when Low MW heparin was administered 30 – 60 min after Labrasol®	[303]
Triton™ X-100 (5 mg)	Instillation (Rat, jejunum)	Mucosal damage observed via light microscopy after 20 min was repaired after 1 h	[304]
C_{10} (100 mM)	Instillation (Rat colon)	Surface erosion and sloughing observed after 10 min exposure, recovered between 30 and 60 min. There was also recovery of barrier integrity as determined by a reduction in bioavailability of a macromolecular marker.	[215]
Nonyl phenol polyoxyethylene 10.5 (1% w/v, 60 min)	Perfusion (Rat, jejunum)	Recovery of barrier integrity to phenol red 2 to 4 h after cessation of surfactant perfusion. Histology score increased from 1 in control to 8 following 1 h with the surfactant and then reduced to 1 following a 3 h recovery	[160]
SLS (5 mg/mL)	SPIP (Rat, jejunum)	Increased drug flux caused by 15 min exposure to 5 mg/mL SLS recovered to baseline after 30 min, whereas there was only 50 percent recovery after exposure for 60 min.	[170]

Table 3 (*continued*)

Surfactant	Model	Recovery	Ref
Taurodeoxycholate (1%, 1 h) IgePal® CO-710 (1%, 1 h)	Perfusion (Rat, jejunum)	Barrier integrity to phenol red recovered 3 h after cessation of both surfactants and there was also a reduction to baseline in the cell membrane integrity marker LDH. The elevated histology damage score after 1 h also recovered to control 3 h after cessation of surfactant perfusion.	[160]
Triton™ X-100 (1%, 3 h)	Perfusion (Rat, jejunum)	Elevated levels of luminal LDH and mucous recovered to baseline 3 h after cessation of Triton™ X-100 perfusion.	[47]
SLS (1 % w/v)	Oral gavage (Rat)	Discontinuation and swelling of the duodenal mucosa observed after 10 to 15 min after oral administration of 1% SLS, partially recovered after 30 min and completely recovered after 1 h. There was less extensive damage to the jejunal mucosa after 15 min and complete recovery after 30 min. There was only gradual recovery of barrier integrity over 5 h as determined by measurement of phenol red absorption.	[28]
Doktacillin™ C_{10}, hard fat (25 mg, 950 mg)	Rectal delivery (Human, rectum)	Increase in the average histology score from 0.62 before administration to 1.94 after 25 min After 3 h the histology score recovered to 0.94.	[210]
C_{10} (0.5 g)	Intubation (human)	Baseline human intestinal permeability as measured by the lactulose mannitol urinary excretion ratio (LMER) was 0.02 in 24 healthy volunteers. When the sugars were orally administered 20 min after jejunal instillation of C_{10}, the LMER increased to 0.03, but the value recovered to 0.02 when the recovery period was extended to 40 min.	[305]

quantitative score accounting for solidity, presence of mucus, and blood in stool samples. On day 30, the stool quality on a scale of 0 to 3 was 0.25 for control, 0 for $C_{10,}$ and 1.6 for deoxycholate, suggesting the latter causes GI disturbance. The stool quality score partially recovered to 0.75 following a 7-day recovery period from exposure to deoxycholate.

There were no consistent changes to expression of TJ proteins (junctional adhesion molecule A (JAM-A), claudin 2 and 3) or TJ-associated proteins (zonula occludin 1 (ZO-1)) following oral delivery of both surfactants for 30 days and after the subsequent 7 day washout period. Both surfactants caused an increase in expression of JAM-A versus the control group in the small intestine, but levels returned to baseline after a 7-day washout. There was however a reduction in small intestinal claudin-2 expression in the deoxycholate group after the 7-day washout period. The authors conclude that repeat oral gavage of two surfactant PEs do not cause irreparable damage to the intestinal mucosa of mice. A trend showing increased plasma levels of FD4 by day 30 while not statistically significant, warrants further studies beyond the initial 30 days. It is unclear how relevant this mouse study is in terms of pointers for chronic exposure in humans.

9. Evidence of the effects of surfactants on the GI tract of humans

Some drugs are directly associated with damage to the GI mucosa, effects that include mucosal aberration, gastritis, enteropathy, microscopic colitis, stricture, inflammation, ulceration, or ischemia. There is no evidence that acceptable use levels of surfactants in oral dosage forms cause any of these GI disturbances in humans. It is impractical to assess the real-time effect of formulations at the site of release in the GI tract of humans, so although there is an absence of evidence of mild mucosal perturbations caused by surfactants, this does not mean there are no undesirable interactions at focal points in the GI tract.

A large proportion of oral medicines list GI side effects, but the causes are not reported. Among the most prevalent symptoms from licensed oral products (not necessarily containing surfactants) are nausea, abdominal pain, heartburn, vomiting, constipation, and diarrhea [307 308]. These symptoms may be associated with gastrointestinal perturbation. For example, chemical gastritis was accompanied by nausea, and abdominal pain [307], and microscopic colitis was associated with diarrhea [309]. Harsh surfactants in household detergents and cosmetics can cause common GI side effects if ingested [310]. Common side effects also occur in orally administered pharmaceutical products that contain surfactants (Table 2). The onset of GI disturbance is however not a reliable way to determine if there is damage to the GI tract caused by mild perturbants found in pharmaceutical products. GI side effects do not prove direct interaction with the intestinal mucosa by surfactants because they can also be caused by the API. Nausea and diarrhea are very common side effects of the GLP-1 receptor agonist class and associated vomiting, constipation, and abdominal pain are relatively common [311].

Another reason why GI symptoms are not a reliable predictor of GI pathology is because GI damage can be asymptomatic. Capsule endoscopy has been used to show the extensive macroscopic damage caused by non-steroidal anti-inflammatory drugs (NSAIDS) [312 313 314]. It has been argued that 60–70% of long-term NSAID users have an asymptomatic enteropathy along with increased intestinal permeability and mild inflammation [315]. Even with short-term use, the gastrointestinal lesions in healthy volunteers given diclofenac were asymptomatic [307]. It is not only substances that cause direct damage to the intestinal epithelium that cause side effects. For example, diarrhea, abdominal pain, nausea, and vomiting are some of the adverse events reported for sorbitol, lactose, and saccharin [316].

The consequences of repeat exposure to surfactants in oral dosage forms has not been well studied, with Case 3 being an exception. On one hand, frequent repeated cycles of mild perturbation could strain repair mechanisms, yet there is no evidence of the progressive intestinal damage with surfactants that is observed for some drugs (e.g., NSAIDS). A case study is presented on the interaction of aspirin with the GI tract to highlight the difference in toxicity to surfactants in humans and to show that extensive mucosal injury relates to mechanisms beyond direct mucosal perturbation (see Case 4). The effect of surfactants on patients with IBD is also unknown, but dietary-sourced surfactants may play a role in disease etiology [61, 62].

In food intervention studies assessing the impact of dietary constituents on intestinal inflammation, surfactants are generally grouped with other molecular classes (e.g., polymers, solid particles) and collectively referred to as emulsifiers. There is an important distinction between these types of emulsifiers. Surfactants are amphiphiles that form monomolecular films around oil droplets, whereas many polymers form multimolecular films. Most natural, synthetic, and semi-synthetic polymers can act as emulsifiers, but they may not appreciably lower surface tension, nor do they act as wetting agents, solubilizers, or detergents. Consequently, polymers like gelatin, albumin, casein, acacia gum, carboxymethyl cellulose, carrageenan, and polyvinyl pyrrolidone do not cause cell lysis, nor do they cause perturbation of intestinal epithelia. That is not to say these polymers are not a concern for patients with IBDs (e.g., carboxymethyl cellulose [64] and carrageenan [317]). The 2014 Emerging Risks report by EFSA [318] lists the long-term effects of food emulsifiers on intestinal barriers as a potential cause for concern. Another EFSA report also lists effects of food emulsifiers on the gut microbiome as potentially problematic [319]. Sub-categorization of emulsifiers can distinguish the behavior of surfactants from other types of emulsifiers.

Surfactants have been identified as ingredients that may be a potential cause for concern in IBD (e.g., fatty acids [320]) and there are efforts to exclude them from controlled diets seeking to improve disease management [62 321 322 323 324] (see Case 5). Although these studies show evidence of reduced intestinal inflammation with special diets, the results cannot be directly attributed to surfactants as there is also reduced intake of other dietary constituents within each intervention. Additionally, while it is possible to reduce exposure to artificial surfactant food additives, there is continued exposure of the GI tract to natural surfactant emulsifiers. These include endogenous bile salts, phospholipids liberated from sloughed epithelial cells/ingested animal cells as well as fatty acids, mono- and di- glycerides from enzymatic degradation of dietary lipids [11]. Nevertheless, some of the data emerging from clinical and pre-clinical studies [61] suggests a potential contributory role for emulsifiers in intestinal inflammation. Crohn's disease incidence is also increased in geographical regions where there is higher consumption of emulsifiers and fat [60], although this is just a correlation.

The association between leaky gut and intestinal inflammation has led to concerns that candidate excipients that alter barrier integrity could induce intestinal inflammation [324]. A review of excipients in 98 licensed products for gastroenterology highlighted 11 excipients with potential safety concerns, although surfactants were not among those highlighted [325]. This may be because there was less of a spotlight on emulsifiers like polysorbate 80 when the article was published. Polysorbate 80 is present in some marketed formulations for IBD (e.g., Entocort® CR gastro-resistant capsules (Tillotts Pharma, Rheinfelden, Germany)). Perhaps the low quantities present in oral formulations are not problematic in these patient populations. Surfactants are also present as foaming agents in rectal products. Examples of surfactants in rectal foam products include; polysorbate 60, and cetostearyl alcohol (Salofalk®, Dr Falk Pharma, Freiburg, Germany); cetyl alcohol, emulsifying wax, and polyoxyl 10 stearyl ether (Colifoam®, Mylan, Pennsylvania, USA). The concerns raised for emulsifiers in pre-clinical studies have led some researchers to suggest that foaming agents could adversely affect inflamed mucosa and should be replaced by excipients that do not cause cell perturbation [326]. However, there is no clinical data suggesting foaming agents used in medicated foams exacerbate IBD. On the contrary, foams show no inferiority to liquid enemas that do not contain surfactants and are preferred by most UC patients [327]. In theory, the changes to normal GI physiology in UC patients, such as longer small intestinal residence time [327] and altered mucus thickness [327] could cause greater exposure to perturbants in the GI lumen. Additionally, as repair mechanisms are impaired [328], perturbation may lead to a more pronounced effect in these patients. Nevertheless, there is no reference to excipient restrictions in the FDA or EMA guidance documents on development of drugs for UC or Crohn's disease or in the FDA excipient guidance to industry.

Oral formulations that contain relatively high quantities of surfactant PEs provide insights into the GI symptoms that may be expected when there is transient alteration to intestinal barrier integrity. However, it is difficult to discern if these side effects relate to the API or excipients in the formulation. Indeed, the drug-related side effects that are independent of administration route (e.g., octreotide [39], and insulin [212]) may make it difficult to determine if oral excipients contribute to GI side effects.

Case 4. *Aspirin as a reference point for more extensive mucosal perturbation.*

Aspirin is among the most widely used drugs over the last century despite causing a variety of GI side effects ranging from mild discomfort to life-threatening bleeding. It continues to be prescribed for primary prevention of cardiovascular disease because the benefits outweigh the risk of serious GI side effects.

The effects of aspirin on the GI tract include increased permeability, mucosal erosion, collagenous colitis, anorectal stenosis, ulceration, bleeding, and perforation [329 330 331]. The GI symptoms arising from these effects are widely reported. In one example, prominent upper GI symptoms that were observed in 15% of surveyed patients on low-dose aspirin, included gastroesophageal reflux disease (70%), heartburn (46%), acid regurgitation (45%), bloating (31%), and eructation (30%) [332]. Over 70% of affected patients said these symptoms had a negative effect on their quality of life and 12% reported low adherence to the dosing requirements. Peptic ulcers, bleeding, and GI perforation are more serious adverse events caused by aspirin and other NSAIDs. GI symptoms for peptic ulcers include abdominal pain, bloating, and nausea [333]. These issues can also go undetected until there is clinical presentation of a GI bleed. Perforation is commonly accompanied by abdominal pain, but there are some asymptomatic cases. While some formulations containing surfactants share some of these non-specific GI symptoms (e.g., abdominal discomfort and nausea), no permitted pharmaceutical excipients are the cause of such extensive mucosal perturbation. Moreover, any such excipients would not be approved by current regulatory standards. GI damage caused by aspirin is associated with both regional perturbation and systemic pharmacological actions (both are described below).

The extent of mucosal damage by aspirin in humans goes beyond any reported adverse event for surfactants, even though surfactants are more effective perturbants than aspirin in Caco-2 monolayers. For example, 10 mM aspirin causes only a 40% reduction in TEER between 6 and 12 h and only a modest increase in permeation of the macromolecule marker FD4 [334]. By comparison, 10 mM C_{10} causes a 90% reduction in TEER within 5–15 min and there is a greater level of FD4 permeation over a shorter period [214]. This is because direct damage to epithelial cells is only one contributing factor side effects of aspirin in the GI tract. The local effects of aspirin that may play a part in transcellular perturbation include osmotic lysis caused by ion trapping [335], intracellular acidification [336], uncoupling of oxidative phosphorylation [337], and membrane fluidization [338].

Aspirin can be inserted into biological membranes to disrupt membrane integrity (reviewed in [338]). The compound is a weak amphiphile that has weak surface activity, where saturation at the air–water interface is slow and a higher concentration is required for adsorption [339]. Hence, there is likely to be slow and inefficient adsorption and penetration into the cell plasma membrane and inefficient perturbation, especially when consideration is given to the physicochemical properties of the molecule along with the modest perturbation induced in Caco-2 monolayers [334]. Another possible local effect is ion trapping [335]. In this theory, the non-ionized, acidic form of aspirin freely diffuses into gastric cells and becomes deprotonated at the higher intracellular pH. This reduces the intracellular pH and forms the soluble anionic carboxylate ion, which does not freely diffuse across phospholipid bilayers. The ionized form of aspirin accumulates and is trapped inside the epithelial cells to create a hypertonic environment which ultimately leads to osmotic lysis.

Other actions of aspirin do not directly cause cell perturbation, rather, alter protective elements of the barrier and/or attenuate repair mechanisms. Aspirin decreases the surface hydrophobicity of the mucus gel layer through an association with the external layer of adsorbed phospholipids [338 339 340]. This association is believed to make the mucosa less effective at resisting damage caused by constituents of luminal fluid [342].

The inhibition of prostaglandin synthesis is another contributing factor to the epithelial damage caused by NSAIDs. Prostaglandins play a role in defense and repair mechanisms [342]. For example, inhibition of prostaglandin E_2 synthesis by aspirin decreased bicarbonate secretion in guinea pig gastric mucosa and reduced mucin secretion in rats and dogs [343]. This exposes epithelial cells in the stomach and duodenum to acidic gastric fluid and other luminal perturbants including the NSAID itself.

NSAIDs can impair re-epithelialisation of damaged mucosa by reducing epithelial cell responsiveness to Epidermal Growth Factor (EGF) secreted by cells at the margin of the injury [342]. Indomethacin reduced the binding of EGF to the EGF receptor in gastric KATO-III cells and there was a significant reduction in cell proliferation [344]. Aspirin can also block EGF-stimulated cell proliferation [344].

The effect of aspirin on gastric blood flow is dependent on the dose and the route of administration. Increased blood flow is an inflammatory response to direct perturbation of the gastric mucosa by aspirin and other perturbants (e.g., acetic acid, alcohol) [345]. However, unlike other perturbants, there is a subsequent reduction in gastric blood flow caused by inhibition of cyclooxygenase 1 (COX-1) [343]. A reduction in gastric blood flow disrupts control of pH in the sub-mucosa by preventing buffering and dilution of acid that back diffuses from the lumen [346]. The lack of pH control by the microvasculature causes extensive damage to the sub-mucosa.

A reduction in mucosal blood flow also impedes capacity to repair damaged epithelia by disrupting the formation of a protective mucoid cap over denuded mucosa. The mucoid cap forms over the exposed basement membrane when fluid from the vasculature in the sub-mucosa mixes with mucous from damaged epithelial cells [347]. It protects the sensitive basement membrane from further chemical perturbation and allows cells that shoulder the injured mucosal to repair the damage. A reduction in mucosal blood flow by NSAIDs disrupts the formation of this mucoid cap by reducing the volume of fluid that is exuded from the microvasculature. Consequently, there is deterioration of the sensitive basement membrane of denuded mucosa by noxious luminal fluid and then diffusion into the sub-mucosa to yield necrosis and hemorrhage.

The interaction of non-selective NSAIDs with platelets is another contributing factor to gastric bleeding [342]. In normal circumstances, damage to blood vessels in the sub-mucosa stimulates release of thromboxane from platelets, which promotes clotting through vasoconstriction and platelet aggregation. As the synthesis of thromboxane requires the production of prostaglandin H_2 by COX-1, clotting is suppressed by aspirin and other NSAIDs that inhibit COX-1 [348]. There are also COX-independent effects of aspirin that contribute to antiplatelet effects [349].

The most serious injury to the GI tract occurs when there is inhibition of both the protective COX-1 and COX-2 enzymes, an additional mechanism to direct membrane damage. Although aspirin is viewed as a non-selective inhibitor of COX, it was 166-fold more potent in inhibiting COX-1 (IC50: 0.3 μM) than COX-2 (50 μM) in isolated cells [350]. Moreover, aspirin inhibits COX-1 by irreversibly acetylating serine 529 to restrict access of arachidonic acid to the active site of the enzyme. The acetylation of serine 516 in COX-2 causes an incomplete enzyme reaction where 15-hydroxyeicosatetraenoic acid is formed and subsequently converted to 15-epi-(*R*)-lipoxin A_4 (aspirin-triggered lipoxin) by lipoxygenase. The anti-inflammatory agent, lipoxin, limits recruitment of leukocytes to the injured gastric mucosa [351] for example with prostaglandins [352]. Consequently, the mucosa exposed to aspirin retains the ability to generate anti-inflammatory signals and therefore avoid gross haemorrhagic lesions that are observed when both isoforms are inhibited. The inhibition of COX-2 with a selective inhibitor, inhibits the production of prostaglandins and aspirin-triggered lipoxin leading to greater leukocyte recruitment to sites of inflammation, which is implicated in more extensive mucosal injury. There was an increase in duodenal endoscopy score from 0.8 to 5.8 ± 1.8 in healthy volunteers given 100 mg aspirin once daily [351], whereas a higher endoscopy score of 9.9 ± 1.9 was measured in subjects given 100 mg aspirin and 200 mg celecoxib. Hence, the most extensive damage was caused by inhibition of both COX isoforms.

While low doses of aspirin (75 mg) are less likely to cause hemorrhagic lesions, clinical trials show an increased prevalence of GI bleeding induced by it in older persons [353]. There is a good correlation between the dose level and the prevalence of GI disturbances in humans [354]. In animal studies, oral gavage of high-dose aspirin (250 mg/kg) caused extensive hemorrhagic damage to rat gastric mucosa [355]. Interestingly, there was upregulation of COX-2 expression in response to aspirin but not 40% ethanol. The increased expression of inducible COX-2 activity was considered an acute anti-inflammatory defense that may result in increased blood flow, plasma exudation, and inhibition of leukocyte adhesion and infiltration (reviewed in [342]). In sum, extensive mucosal injury occurs through a combination of the aforementioned mechanisms including direct perturbation (e.g., by osmotic lysis, intracellular acidification, uncoupling of oxidative phosphorylation), and other actions resulting from the inhibition of cyclooxygenases.

The widespread use of surfactants at permitted intake levels does not cause the type of extensive perturbation and side effects that are observed with NSAIDs. Aspirin is an example where the risk of GI side effects competes with the pharmacological benefits of the prevention of cardiovascular disease. The same risk–benefit does not apply to surfactants used as excipients, so attempts to justify the use of an excipient because it is less damaging to the GI tract than some ingested drugs is unlikely to be favorably viewed by regulatory agencies. However, there is merit in highlighting that the level of damage caused by some ingested substances (e.g., aspirin) is far greater than the effect of permitted intake levels of surfactants used as excipients (see Cases 1 and 2). Studies in intestinal cell monolayers show that surfactants are stronger direct perturbants than aspirin, but it is the ability of aspirin to impair mucosal defense and repair mechanisms that are especially implicated in mucosal damage in humans. The case presented on aspirin reinforces the role of defense and repair in preventing mucosal perturbation by constituents of diet and endogenous substances. It is noteworthy that acute exposure to a strong perturbant can overcome endogenous defence mechanisms leading to serious injury or death. For example, the accidental ingestion of sulfuric acid causes coagulative necrosis and is often fatal [356].

Case 5. *A low-level emulsifier diet to investigate their role in Crohn's Disease*.

A feasibility study that involved limiting ingestion of emulsifiers was carried out over 14 days in 20 patients with stable Crohn's disease [62]. Patients were required to avoid, 65 food emulsifiers, of which almost 50% were surfactants. These included polysorbates, sucrose esters, phospholipids, fatty acid salts, mono and diglycerides, sorbitan esters, and other fatty acid derivatives. There was 95% adherence to the regimen by patients, defined as a 75% reduction in the frequency of emulsifier intake over 14 days. Also recorded were reductions in consumption of energy, carbohydrates, saturated fat, niacin, Na^{+}, Ca^{2+}, and vitamin B_{12}, although these were not viewed as clinically significant. No significant changes in body weight occurred over the period. There was a reduction in Crohn's disease-related symptoms based on the Patient Reported Outcome-2 (PRO-2) questionnaire and an increase in patient-perceived disease control, IBD-Control-8 (IBD-C-8) questionnaire. This feasibility study did not assess intestinal permeability, inflammatory markers, or changes to the gut microbiome.

10. A perspective on surfactant safety in oral dosage forms

10.1. Interpreting safety data for surfactants

Surfactants are present in a wide range of oral formulations and there is no evidence in humans that, at permitted use limits, they cause overt mucosal injury. On the other hand, surfactants perturb biological membranes leading to cell lysis and loss of viability in a range of pre-clinical models. Studies in Caco-2 monolayers and rat models indicate induced reductions in intestinal barrier integrity, inhibition of intestinal transporters, increased intestinal permeability, and histological damage. A concern is therefore that at least the effects seen in animals could go unnoticed during repeated dietary consumption in humans and might be impossible to ascribe causality. Despite this, the probability of damage occurring in humans with low milligram quantities (<50 mg) of surfactants present for many applications (e.g., wetting, lubricants) appears to be low. There is nonetheless a greater risk of mild mucosal perturbation in humans from oral formulations containing high milligram quantities (>50 mg) such as o/w emulsifiers, vehicles, stool softeners, and PEs. Whether this happens ultimately depends on the properties and dose of the individual surfactant along with whether the dosing regimen is daily and chronic. Even then, the consequences of mild mucosal perturbation remain unclear as the intestinal mucosa is quick to repair any damage.

If data from pre-clinical models are used to infer that ingestion of dietary surfactants poses a safety risk to humans, there is the counter-argument that endogenous bile acids and dietary fatty acids also cause mucosal damage in pre-clinical models (Table 3). It is therefore tempting to conclude that pre-clinical studies in rodents do not represent the conditions in the human GI tract and that some degree of mild mucosal perturbation and repair is part of the normal acceptable function of the GI tract in response to food and drink. The latter is supported by very high cell turnover in the GI tract where there is continuous cell production, steady migration, and sloughing into the GI lumen [357]. The intensity of this process is highlighted by estimates that the small intestinal epithelium is replaced every three days, with approximately half a pound of epithelial cells sloughed into the GI lumen per day [358]. Harsh environments (including air, urine, and GI fluid), and dietary xenobiotics give rise to physiological epithelial regeneration in skin, bladder, and GI tract, whereas non-renewable cells (such as endothelia) that are exposed to blood and extracellular fluid do not undergo such rapid cell turnover [358].

10.2. Safety insights from studies on PEs

Clinical trials where surfactant excipients are used to increase permeation to macromolecules suggest that only small changes to barrier integrity, as reflected in single-digit bioavailability increases, occur from oral dosage forms containing surfactants, even if they cause major changes to barrier alteration in cells, tissues, and rodent models [39 212]. The GI side effects for formulations that cause small increases in oral bioavailability of macromolecules were mild-to-moderate and included nausea, vomiting, and diarrhea. To date, there is no evidence that these side effects relate to PEs in these formulations nor is there evidence that repeated oral administration causes progressive development of side effects. Increases in macromolecule permeability by leading PEs cause mild mucosal perturbation in animal models, which has led to the suggestion that mild, reversible mucosal damage could occur when there is enhancement in humans. There are however no human studies at the microscopic or macroscopic level that assess the upper GI tract during the transport-enhanced state. As altered GI permeability is implicated in intestinal inflammation, there is caution regarding the use of PEs in patients with IBD, especially in Crohn's disease which can affect the small intestine; where most of the PEs mainly act. In some cases, patients with GI disorders have been excluded from clinical trials assessing PEs to improve oral bioavailability of macromolecules [212], although neither Rybelsus® or Mycapssa® are contraindicated in patients with IBD. Overall, there is a lack of information regarding safety of formulations intentionally designed to alter barrier integrity in patients with GI disorders.

10.3. A possible contribution of surfactants to the risk of autoimmune disease

The hypothesis that dietary synthetic surfactants are the cause of intestinal barrier integrity alterations and the increased incidence of

allergic and autoimmune diseases in humans is currently based on findings from pre-clinical cell, tissue, and mouse models [359], and on consumption patterns. Several hundred compounds have been shown to alter intestinal permeability in pre-clinical drug delivery models. Thus, singling out synthetic surfactants as the cause of intestinal diseases is premature. Even the most effective surfactant PEs that have progressed to clinical testing in formulations designed to improve oral peptide absorption have mostly failed to translate due to a lack of sufficient permeability enhancement in humans [207]. This emphasizes that there is only a modest effect on barrier integrity even when high doses are intentionally used to alter barrier integrity. The use of surfactants as excipients and food additives is still widely supported by regulatory authorities in the USA and Europe.

There is an as yet unproven hypothesis that repeated exposure to low levels of dietary substances that alter gut permeability (including emulsifiers and surfactants) plays a role in the initiation or accentuation of autoimmune GI diseases. An increase in intestinal permeability according to the lactulose-mannitol urinary excretion ratio (LMER) preceded relapse in patients with Crohn's disease [360]. While barrier dysfunction is a factor in the etiology of IBD [361], the cause of these complex multifactorial diseases is not fully understood. On the one hand, direct alteration to barrier integrity caused by a local disturbance may trigger the inflammatory response via immune dysregulation [362]. Alternatively, a systemic inflammatory response may lead to an alteration to intestinal permeability [363]. It is therefore not clear if alteration to barrier integrity is a cause or effect of inflammation associated with IBD. Preliminary investigations from patients receiving low emulsifier diets show a decrease in Crohn's disease-related symptoms (see Case 5). Based on evidence to date, it is still premature to implicate surfactants in the onset or maintenance of IBD.

Trends in consumption of emulsifiers versus incidence of intestinal diseases like Crohn's disease present a compelling argument for the role of these additives in intestinal disease, especially when they are considered alongside preclinical data [6 363]. However, emulsifiers are just one additive group associated with barrier integrity alteration. There are also changes to ingestion of other food-derived substances shown to increase permeability in pre-clinical models, some of which are found in high quantities in ultra-processed food: examples include sodium [365], glucose [366], ethanol [367], capsaicin [368], and gliadin [369]. As is the case with emulsifiers, there is no direct evidence that barrier alterations associated with these agents occur in humans. There are also studies associating consumption of some of these substances and incidence of intestinal disorders (e.g., high sucrose intake [370]). As many constituents of a Western diet are implicated in intestinal disorders it is difficult to single out specific food additives or emulsifiers.

10.4. Interpreting studies showing microbiome changes by polysorbate 80

In a controversial study, the non-ionic surfactant, polysorbate 80, has been associated with alteration to the murine microbiome, which in turn seemed to contribute to metabolic disorder and intestinal inflammation in mice [61]. A cautious approach is required when relating studies showing inflammation in mice to humans because the equivalent dose in a 70 kg human would be between 28 and 112 g if a 25 g mouse drinks 1 to 4 mL of water containing 1% w/v polysorbate 80 per day (400–1600 mg/kg/day). These doses are above the ADI of 25 mg/kg/day by EFSA and below the estimated average consumption in the US [67] and selected EU countries [52]. It is improbable that the effects observed in mice at very high daily doses occur in humans at the much lower doses found in oral formulations. Study investigators acknowledge that the dose administered to mice is higher than the estimated average daily consumption of polysorbate 80 in the UK (8.2 mg/kg/day), although they argue that this limitation is somewhat offset by differences in the exposure duration [8]. It is difficult to assert that effects observed in mice fed high doses for 12 weeks will cause similar effects in humans exposed to lower doses for a prolonged period. However, the investigators also note that exposure levels in humans may approach those observed in animal testing when there is excessive consumption of ultra-processed foods. When the discrepancy in daily intake is considered along with differences in diet, species, microbiota it becomes even more difficult to relate these findings to humans. Studies are underway to determine if ingestion of dietary emulsifiers causes intestinal inflammation in IBD patients [68].

The ADI of polysorbates was assigned in humans by the EFSA Panel on Food Additives and Nutrient Sources Added to Food [52]. As part of this evaluation, the panel considered research papers suggesting that the consumption of polysorbate 80 in processed foods may promote Crohn's disease [61 60 363]. The panel noted that barrier integrity alteration by polysorbate 80 in Caco-2 monolayers (Section 6.1.1) did not consider gut lumen physiology (see Section 8) and that in the absence of clinical studies, they could not use these findings as part of the risk assessment. The panel noted that microbiome changes, intestinal inflammation, and metabolic syndrome caused by supplementation of polysorbate 80 to the drinking water of mice warranted further investigation to understand possible implications for human health.

Concerns have been raised that toxicological studies required by EFSA to evaluate or re-evaluate food additives do not require an assessment of barrier integrity alterations, microbiota changes, or tests in genetically modified animals that are pre-disposed to intestinal diseases [371]. This led to the conclusion that the ADI level does not guarantee safety and that specific studies are warranted for surfactants because they alter GI permeability [371]. Even well-designed studies identifying microbiota changes, mild inflammation, colitis, and metabolic syndrome in mice are difficult to relate to effects in humans. This makes it difficult to justify changes to current food regulatory guidelines, which is perhaps partly why consideration of such studies has not led to changes in allowable intake, but rather a call for further studies [52].

While there is a commitment from the EFSA panel to investigate findings that are not systematically included in toxicity studies, it is unclear if subtle adverse effects identified in pre-clinical models could lead to a restriction on the use of an established food additive. Similarly, as diseases such as Crohn's disease, UC, and metabolic syndrome are multifactorial, a diet restricting one ingredient may not provide sufficient evidence to warrant discontinuation. On the other hand, a trial diet restricting structurally or functionally related compounds (e.g., polymers and surfactant emulsifiers) may potentially alleviate intestinal inflammation in patients with IBD. If clinical studies in humans show that surfactant emulsifiers cause microbiome changes that contribute to intestinal inflammation and metabolic disorder, there may eventually be a recommendation for patients suffering from IBD or metabolic syndrome to reduce intake of processed food containing these additives; indeed, this view is supported by many GI clinicians. Other options available to regulators are a reduction in the ADI or withdrawal of food additive status for selected surfactants. While withdrawal of food additive status is rare, there is precedent in selected geographical regions. The food additive status of titanium dioxide was rescinded by EU member states based on a recommendation by EFSA [372]. A 6-month phasing-out period began in 2022 after which a full ban came into effect. The EFSA panel looked at over 11,000 scientific publications and the results from a commissioned toxicity study, which could not rule out concerns regarding genotoxicity. There remains uncertainty about the safety of titanium dioxide in humans and it continues to hold food additive status in the USA and UK.

In cases where a surfactant is permitted for use as both a food additive and pharmaceutical excipient, the quantity of a surfactant in a pharmaceutical product is generally below the amount typically ingested in food. Hence, there would have to be a large reduction in the ADI of a surfactant like polysorbate 80 in food for a re-evaluation of its safety of pharmaceutical products. For example, a 70 kg human may ingest 1.75 g (ADI: 25 mg/kg) of polysorbate 80, well above the current maximum daily exposure listed for an oral solution (432 mg, 6.2 mg/kg) on the

FDA IID [37]. Additionally, lowering the ADI for food products may not change the risk–benefit calculation for a pharmaceutical product. Indeed, some surfactants are permitted for use as excipients, but not as food additives. SLS is used widely as an excipient, though is not permitted for use as a food additive in the EU (It is however used as a food additive in the US) (Section 6.2.1).

10.5. GI physiology can protect against mucosal perturbation by surfactants

In Section 8, a review of the factors that influence interaction with the mucosa shows that dilution in luminal fluid, spreading in transit, interaction with constituents of luminal fluid (including other excipients) and the protective effects of the mucus gel layer collectively help to reduce the concentration at the gut wall. If, however, a sufficiently high surfactant concentration is ingested, these protective barriers may be overcome with damage ranging from mild perturbation of cells at the surface mucosa in the stomach or the apex of the villus to more extensive damage for ingestion of poisons. However, adjacent cells that shoulder the injury spread to cover any denuded mucosa, and then cells from the gastric pits or intestinal crypts migrate to replenish the mucosa.

These events may not occur in experimental models, which is why it is not possible to conclude that surfactants cause epithelial damage in humans at acceptable concentration levels. Exposure of cell cultures or isolated tissues to milligram per mL quantities of SDS, polysorbate, or other surfactants for hours is not representative of dilution and transit in humans nor is apical transport media similar to human intestinal fluid. Additionally, the capacity for repair is limited in such models. These models therefore only represent the potential for barrier integrity alterations by excipients under specific and static conditions. In situ perfusions and instillations in rats more closely represent GI physiology, but the concentrations and exposure times are difficult to relate to oral dosage forms. Additionally, the vehicle used to deliver the surfactant to the lumen of rat models does not closely represent fasted or fed state intestinal fluid.

Oral gavage in rats and mice offers intact GI barriers, but drawbacks include interspecies physiological differences [359], and the reliance on gavage of aqueous liquids rather than intact dosage forms. Studies in dogs and pigs permit the evaluation of tablets and capsules of standard dimensions, which can consider release kinetics and the possibility of localized perturbation of the intestinal mucosa at concentration pockets. Nevertheless, there are still inter-species differences in dogs and pigs in several of the factors that impact exposure of the epithelium to surfactants, including stomach and small intestinal fluid volume, gastric residence time, small intestinal transit time [373], mucus thickness [374], composition of enterocyte membranes [375], fluid composition and pH [376] (reviewed in [152]). For example, the faster small intestinal transit time in dogs versus humans [373] may reduce exposure of the epithelium to noxious luminal substances in the former. Similarly, an increase in the thickness of the mucus gel layer in pigs versus humans [374] may slow diffusion of surfactant to the epithelial surface thereby reducing perturbation. Finally, pigs have a much longer gastric retention time for tablets and particulates, which can confound attempts to correlate absorption from standard oral dosage forms with that of humans [373].

10.6. Does unintentional barrier integrity alteration impact oral drug bioavailability?

Surfactants that alter intestinal barrier integrity have the potential to unintentionally alter the rate and extent of absorption of other constituents in the dosage form (e.g., SLS [145]). Generic formulations that do not contain the surfactant present in the innovator product may have different ADME profiles, that is, they could be bioinequivalent. This is an important consideration for generic products that are granted a biowaiver, waiving the requirement for bioequivalence testing in humans [166]. A generic test formulation of alendronate was not equivalent to the marketed formulation (Fosamax 10 mg tablets) because SLS was deemed responsible for a 5-to-6 fold increase in BA in the generic product [377]. However, it is difficult to solely attribute this change to SLS, especially as the generic manufacturer that performed the study subsequently licensed a generic product containing SLS [166]. Additionally, a bioequivalence trial found that 14 of the top 20 excipients on abbreviated new drug applications (ANDA) submissions (including 25 mg SLS) had no effect on BA of cimetidine or acyclovir [378]. Others have cautioned that these results should not be extrapolated for other drugs. The EMA and FDA will only grant a biowaiver for BCS (Biopharmaceutics Classification System) Class III drugs (those exhibiting high solubility and low permeability) if the excipients do not affect membrane permeation or intestinal transit [169]. In summary, these studies highlight the concerns that excipients, including surfactants like SLS, could contribute to altered intestinal absorption, although it is unclear if such alterations are a result of barrier perturbation.

11. Conclusions

Surfactants are a structurally diverse group of amphiphilic compounds that give rise to many important applications in pharmaceutical sciences. This structural diversity precludes broad generalizations about safety in humans. The in vitro perturbation of cell membranes by many surfactants is less obvious in tissue and animal studies. Surfactants that can cause mucosal injury in animals are used in oral products, but there is no evidence that the local concentration reached at the epithelial surface is sufficient to cause focal damage in humans.

The intestinal epithelium has an amazing capacity for repair and restitution that contributes to reversible perturbation of the intestinal epithelium, although little is known about the long-term consequences of repeated cycles of damage and repair. GI side effects noted in clinical trials of orally administered products containing surfactants range from mild to moderate and include abdominal pain, diarrhea, and constipation, but so far these effects are more associated with the co-administered drug molecule. Even when surfactants are intentionally added to oral formulations to increase macromolecule permeation by altering barrier integrity, there are only relatively small increases in absorption in humans and the GI side effects in these trials are similar to those of many licensed medicines.

It is evident from widespread use in clinical trials and marketed products that surfactants do not cause anywhere near the levels of damage observed with NSAIDs even in the high concentrations required to be presented as PEs. Dietary emulsifiers that cause microbiome changes, low-grade intestinal inflammation in healthy mice, and colitis in pre-disposed mice have nevertheless raised concerns that have led to a re-evaluation of the safety of selected surfactant emulsifiers as food additives. However, effects seen at high doses over a short duration in rodents are not representative of lower doses administered over a long duration in humans. Ongoing studies in animal models and humans aim to determine whether restrictions on use levels are required in food or pharmaceutical products for the general population or in patients predisposed to IBD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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