Advanced Drug Delivery Reviews 142 (2019) 62–74

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Self-emulsifying drug delivery system: Mucus permeation and innovative quantification technologies

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ARTICLE INFO

Article history:
Received 17 January 2019
Received in revised form 3 April 2019
Accepted 5 April 2019
Available online 8 April 2019

Keywords:
SEDDS
Mucus
Oral delivery
Mucus diffusion
Hydrophilic peptides
Mucolytic agent

ABSTRACT

Mucus is a dynamic barrier which covers and protects the underlying mucosal epithelial membrane against bacteria and foreign particles. This protection mechanism extends to include therapeutic macromolecules and nanoparticles (NPs) through trapping of these particles. Mucus is not only a physical barrier that limiting particles movements based on their sizes but it selectively binds with particles through both hydrophilic and lipophilic interactions. Therefore, nano-carriers for mucosal delivery should be designed to eliminate entrapment by the mucus barrier. For this reason, different strategies have been approached for both solid nano-carriers and liquid core nano-carriers to synthesise muco-diffusive nano-carrier. Among these nano-strategies, Self-Emulsifying Drug Delivery System (SEDDS) was recognised as very promising nano-carrier for mucus delivery. The system was introduced to enhance the dissolution and bioavailability of orally administered insoluble drugs. SEDDS has shown high stability against intestinal enzymatic activity and more importantly, relatively rapid permeation characteristics across mucus barrier. The high diffusivity of SEDDS has been tested using various in vitro measurement techniques including both bulk and individual measurement of droplets diffusion within mucus. The selection and processing of an optimum in vitro technique is of great importance to avoid misinterpretation of the diffusivity of SEDDS through mucus barrier. In conclusion, SEDDS is a system with high capacity to diffuse through intestinal mucus even though this system has not been studied to the same extent as solid nano-carriers.

Contents

Abbreviations: BCS, Biopharmaceutics Classification System; MPT, Multiple Particle Tracking; Capmul 907 P, Propylene Glycol Heptanoate.; MSD, Mean Squared Displacement; Capmul MCM EP, glyceryl monocaprylate.; Myrj® 52, polyoxyethylene 40 stearate; Captex 355, Capric Triglyceride.; NAC, *N*-acetyl Cysteine; CF, Cystic Fibrosis; NIKKOL HCO-40, PEG-40 hydrogenated castor oil; Cremophor RH40, PEG-40 hydrogenated castor oil; NIKKOL HCO-60, PEG-60 hydrogenated castor oil; Cremophor EL, PEG-35 hydrogenated castor oil; NP, Nanoparticle; DDAB, Dimethyldioctadecylammonium Bromide; PA, 3-sn-Phosphatidic acid, 1,2-dipalmitoyl sodium; Dermofeel MCT, Glycerol tricaprylate.; PAA, Poly (acrylic acid); FDA, Fluorescein diacetate; PAM, poly (allylamine); FRAP, Fluorescence Recovery after Photo-Bleaching; PEC, Polyelectrolyte; GIT, Gastrointestinal Tract; PEG, Poly (ethylene glycol); GSH, Glutathione; P-gp, P-glycoprotein; HIP, Hydrophobic Ionic Pairing Technique; PGSE-NMR, Pulsed-gradient spin-echo NMR; HLB, Hydrophilic Lipophilic Balance; SANS, Small Angle Neutron Spectroscopy; IAP, Intestinal Alkaline Phosphatase enzyme; SEDDS, Self-Emulsifying Drug Delivery System; Labrafac CC, Capric Triglyceride.; Soluplus®, Derivative of polyethylene glycol graft copolymer.; Labrasol, Caprylocaproyl Polyoxyl-8 glycerides.; TBA-D, Thiobutyl Amidine Dodecylamine; Kolliphor EL, PEG-35 hydrogenated castor oil.; TGA-O, 2-Mercapto-N-octylacetamide; Migylol 840, Propylene Glycol Dicaprylocaprate.; TPGS, d-Alpha-Tocopheryl Poly(ethylene glycol.; M.wt, Molecular Weight; Transcutol, Diethylene glycol monoethyl ether.

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https://doi.org/10.1016/j.addr.2019.04.001
0169-409X/

1. General introduction

Mucus is a dynamic barrier that protects the underneath mucosal epithelial membrane in the body. Mucus barrier has a varied thickness depending on the site of the body which it can be as thin as 0.578 μm in the eye [1] to a thick barrier of 150 μm in the colon [2]. Any therapeutic agent administered through mucosal routes needs to permeate through mucus to reach the underlying epithelial membrane and be further absorbed by the systemic circulation [3]. Although permeation of small molecules through mucus barrier is not restricted process [4], macromolecules like peptides and proteins are highly susceptible to being trapped by mucus or degraded by protease enzymes in the mucus layer [5]. The incorporation of such therapeutic agents into a proper nano-system can improve their diffusion through the mucus barrier and provide protection against enzymes [6].

However, the permeation of these nano-carriers can be highly constrained due to either size exclusion by mucin network or interaction with mucus components where these interactions can be either of electrostatic or lipophilic nature [7,8]. Therefore, an ideal nano-carrier for mucosal delivery should avoid mucus entrapment [9] and shield the loaded drugs from intestinal enzymes [10]. The candidate nano-system should escape mucus clearance through fast diffusion across mucus barrier [11] and stay close to the epithelial surface to ensure a maximum absorption of the released drug close to the epithelial surface [12]. The key requirement for nano-carriers is to be permeable across mucus barrier. This aim was achieved using the following strategies: (i) synthesising of particles with hydrophilic inert surface to eliminate the possibility of interaction with mucus components, or (ii) loading of particles with mucolytic agents to reversibly disrupt the mucus barrier [13,14].

Self-Emulsifying Drug Delivery System (SEDDS) is very auspicious nano-systems. This systems can easily be formed by simply mixing ingredients [15] which makes it highly suitable for industrial purposes since large batches can be prepared without any sophisticated technology. SEDDS was introduced to overcome the solubility issue of lipophilic agents which in turn affects their oral bioavailability [16]. Moreover, this system was found to have longer persistence in the GIT and improved drug cellular uptake [17]. More importantly, recent studies have shown that SEDDS is a very efficient nano-carrier for oral delivery of hydrophilic peptides and proteins through protecting of these peptides from GIT environment and enhance their penetration through intestinal mucus barrier [18].

The purpose of this review is to describe the mucus barrier in general and to explicitly elucidate the mechanisms by which intestinal mucus barrier traps droplets of SEDDS. The reason to focus on the intestinal mucus barrier is due to the fact that SEDDS and most of the solid nano-carriers are mainly delivered orally. We will compare the main nano-strategies to improve the permeation of solid core nanoparticles (NP) and liquid core nano-carriers across the intestinal mucus barrier. SEDDS will be reviewed extensively in relation to how the liquid core nano-carrier can improve the mucus delivery of both lipophilic and hydrophilic agents. In this review, SEDDS will be used to define all types of self-emulsifying systems including the Micro-Emulsifying and Nano-Emulsifying systems (SMEDDS/SNEDDS) since SEDDS has been used to describe these systems more recently [19]. Lastly, technologies to quantify SEDDS permeation across mucus will be outlined.

2. Barrier properties of mucus

2.1. Compositions of intestinal mucus and mucin structure

Generally, regardless of site in the body, mucus is mainly composed of macromolecular glycoprotein units called mucin, high water content (90–95%) and other ingredients including DNA, lipids, electrolytes, bacteria and sloughed epithelial membrane [20,21]. The ratios of these ingredients vary depending on the site of the body. Measurement of the percentages of components in dried intestinal mucus revealed the presence of 5% mucin and 6% DNA [22]. The same study showed that lipids and proteins are existed in high percentages (40%) within the intestinal dried mucus. The high lipid content in the intestinal mucus is associated with breakdown of food rich with lipids while the high DNA percentage is related to the regularly shedding of intestinal epithelial membrane [23]. At average concentration of 30 mg/ml, mucin units connect to each other via hydrogen bonding, hydrophobic and electrostatic interactions to form a network having the properties of hydrogel system [23]. This mucin network swells upon absorption of water leading to formation of gel like structure of mucus [24,25]. Structurally, mucin is a macromolecular glycoprotein having large molecular weight (M.wt) (2000–10,000 kDa) [26,27]. Purified mucin units are curvilinear fibres with an average diameter of about 5–7 nm and a length of approximately 200 to 4000 nm [28,29]. Each macromolecular unit consists of 3 to 4 subunits with average M.wt of 4×10^5 Da [30]. Mucin subunits are large polypeptide chains that composed of glycosylated and non-glycosylated domains in a sequential manner where polypeptide chains in the glycosylated domain are densely covered with polysaccharide glycosylated side chains while non-glycosylated domains are cysteine-rich domains that connecting glycosylated regions by intramolecular disulphide bonds [31]. Functionally, the main roles of mucus are lubrication, hydration and protection of the underlying epithelial membrane against mechanical stress, foreign particles and pathogens [32,33]. Hence, mucus in the GIT is relatively thick with high mechanical strength to protect against the high content of pathogens and microorganisms as well as the highly acidic environment [34,35]. Physically, intestinal mucus can be described as a semipermeable membrane

Fig. 1. Mucin macromolecular conformation.

from which only particles as small as nutrients and drug molecules can permeate [36]. If these molecules are stable against pH and enzymes in the GIT environment, they can be absorbed through the intestinal epithelial layer. On the other hand, crossing the gastrointestinal mucus barrier is a challenge to enzymatically labile therapeutic macromolecules like peptides due to their propensity to hydrolyse and degrade in the GIT. Hence, these peptides must be protected from the GIT environment by encapsulation within a proper nanocarrier [13]. However, as was described above, these particles get trapped by the intestinal mucus [37]. The trapping of these NPs is due to the unique physiochemical properties of mucus [38–40].

2.2. Physicochemical properties of mucus

Mucin units, as the main building entities of the mucus, are mainly responsible for the physicochemical properties of mucus, specifically, through the unique chemical nature of the glycosylated protein domains and the non-glycosylated cysteine-rich protein regions. Thus, the negatively charged nature of mucus is associated with the glycosylated protein domains which are rich with sialic acid and also contains to less extent galactose sulphate ester units [41]. With a pKa of approximately 2, Sialic acid units are totally charged at the physiological pH which results in the negatively charged nature of mucus in the intestine [42]. Besides that, the negatively charged nature of mucin is also obtained from its polypeptide backbone chain which conatins both aspartic acid and glutamic acid units [43]. On the other hand, the lipophilicity of mucus is mainly due to the high lipophilicity of the cysteine-rich circular domains of mucin (Fig. 1) [44]. Furthermore, the polysaccharide side chain exerts some lipophilicity within the mucin through the methyl groups of the polysaccharide fucose units [45].

Accordingly, the viscoelastic nature of mucus is directly related to the gel network of mucin which is formed through the disulphide bridges between mucin units [46]. The mechanical strength of this network is directly related to the extent of interdigitating among glycosidic side chains which results in a stable three-dimensional mucin network with higher mechanical strength [47]. The lengths of these polysaccharide side chains are responsible for the interdigitating process where long polysaccharide chains are responsible for forming of hard gel mucus while small chains are associated with forming of mucus with weak viscous properties [48].

Mucus has different mechanical and viscoelastic properties depending on where it is situated in the body. In the intestine where SEDDS and most of the solid and liquid core nano-carriers are delivered, there are two layers of mucus with different mechanical properties [49]. These are: the outer layer which is a loose structure that colonised with bacteria and inner layer which is a dense structure that is resistant to bacterial penetration [50,51]. The loose mucus layer is characterised by a shear thinning rheological behaviour which has protective lubricating effect against bacteria and foreign particles [52].

2.3. How physicochemical properties affect barrier properties of intestinal mucus against NPs permeation

The mechanism of trapping of orally given NPs and SEDDS depends on the physicochemical properties of mucus and includes two processes which occur simultaneously upon the passage of any foreign particles. Firstly, a gluey cage of mucus (Fig. 2) is formed in which droplets/particles are efficiently trapped by mucus components through formation of numerous dynamic interactions of both lipophilic and hydrophilic nature [53]. Concurrently, the passage of particles/droplets under peristalsis movement exerts a shear thinning effect on the mucus layers which leads to sliding of these layers over each other and dropping of the viscosity [54]. As a result, a lubricated pathway of mucus layers with low viscosity is formed through which droplets/particles trapped in cages are moved. As a result, macromolecular NPs/SEDDS in extracellular mucus are highly trapped as compared with particles at molecular level. Ideally, NPs/SEDDS should permeate quickly through the loose intestinal mucus layer to avoid being rapidly eliminated with the loose mucus clearance [10]. This allows enough time for the complete release of drug molecules adjacent to the epithelial membrane.

3. Strategies in nanotechnologies to improve mucus permeation

Nanotechnologies that have been explored for mucus permeation include solid nano-carriers and lipid based liquid nano-carriers like liposomes and SMEDDS. Solid NPs provide the opportunity for surface modifications to form inert or electrostatically neutral surfaces which can minimise their mucus interaction and consequentially allow permeation [55,56]. On the other hand, lipid based liquid nano-carriers can offer enhanced leverage towards mucus permeation by virtue of their flexible structure and ability to squeeze through the mucus networks [18]. Both nano-systems offer the ability for tuning of their particle sizes and the capacity to be loaded with various hydrophilic and lipophilic agents [57,58].

3.1. Solid nano-carrier

A variety of strategies were examined to improve the mucus diffusivity of solid nano-carriers. These strategies have been classified into three fundamental categories: (i) formation of slippery surface nano-carriers by modulating of particles' surface properties to enhance their diffusion through the mucus barrier (ii) formation of mucolytic nano-carriers to reversibly disrupt the mucus barrier (iii) a combination of slippery surface and mucolytic particles where a mucolytic agent is loaded onto a surface modified nanocarrier to allow synergistic permeation.

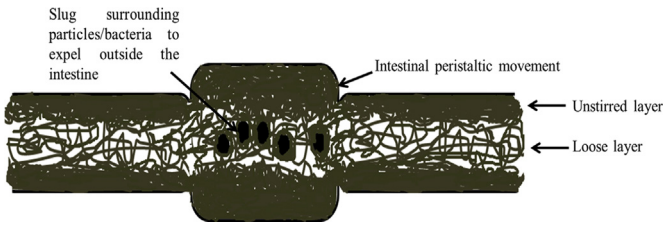

Fig. 2. Trapping mechanism of intestinal mucus towards orally administered particles/droplets.

3.1.1. Slippery surface NPs

This strategy was adopted from certain viruses with inert neutral surfaces like poliovirus and human papilloma which were found to diffuse freely across mucus barrier [59]. These mucus diffusive viruses are covered with densely-charged but neutral capsid shell (without lipophilic membrane) [60] to avoid any electrostatic/H-Bond interactions with mucus [61]. Similarly, inert surface NP is a NP in which the neutral hydrophilic surface totally covers the internal lipophilic core [62] to avoid lipophilic interaction and an inert neutral surface to avoid any electrostatic/H-bond interaction with the mucus components [63].

Two strategies have been utilized to form slippery NPs with inert surface properties. The first is through coating of NP with a neutral hydrophilic polymer such as PEG polymer. The mucus permeability of densely PEGylated NPs has been deeply explored in term of the effect of degree of surface PEGylation on their diffusion [55]. Studies on different mucus sources revealed that densely PEGylated NPs have high diffusion across vaginal mucus [64], cystic fibrosis (CF) mucus [65] and respiratory mucus [66]. Accordingly, other hydrophilic surfactants were examined for formation of inert surface NPs and their permeation through mucus [67]. For example, hydrophilic pluronic surfactant with low M.wt was used to coat PLGA NPs where coated particles showed high diffusion across chronic rhinosinusitis mucus that is retarded by only 20 times in comparison with its free diffusion in water [68]. The second strategy used to form inert surface NP involves mixing of polymers with opposite charges as such alginate (−ve) and chitosan (+ve) where electrostatic interaction leads to precipitation of polyelectrolyte (PEC) polymer in nanoscale range to form PEC NPs with a densely neutrally charged surface [69]. For example, self-assembled polyelectrolyte NPs of poly(acrylic acid) (PAA) and poly(allylamine) (PAM) show a diffusion coefficient of 2.5 times higher than the positively charged NPs and around two times the diffusion of (−ve) PAA NPs [70]. Similarly, condensation of the negatively charged DNA with the positively charged dimethyldioctadecylammonium bromide (DDAB) on the surface of PLGA NP particles show a tenfold higher diffusion coefficient to that of the same size lipophilic NP [71]. Other coating mixtures such as dextran–protamine (near neutrality charge) was reported to increase mucus permeability of solid lipid NPs [72].

3.1.2. Mucolytic NPs

Mucolytic agents can vastly enhance the NPs diffusion across mucosal barrier through reversible destruction of the mucin network [73] where loading into and releasing of these agents from NPs reduce the resistance of mucus towards the permeation of NPs [73]. Three types of mucolytic agents have been utilized to improve NPs permeation across mucus [74]. Firstly are disulfide breaking agents which cleave disulfide bonds within mucin network. These agents are exemplified by *N*-acetyl cysteine (NAC), dithiotreitol and glutathione. Secondly are proteolytic agents which break mucin's peptide bonds (bromelain, trypsin and papain). Thirdly are DNA hydrolysing agents which split DNA tangles that entangled within the mucin network [75,76].

Muller el al. showed that polyacrylic acid (PAA) NP loaded with the papain proteolytic agent has three times faster diffusion through intestinal porcine mucus than the PAA NPs containing no papain [77]. In vivo examination of PAA-papain NPs in the Sprague Dawley rats showed an extended residence time in the jejunum which indicates high permeability through the mucus loose layer [78]. On the other hand, disulfide breaking agent represented by NAC significantly increased the permeation of 3.2 μm polystyrene micro-particles through rat intestinal mucus [79].

Moreover, a mucolytic agent can be used as adjuvant factor in combination with modified surface NPs to boost the mucus diffusion of inert surface NPs where this combination can have a synergistic effect. For instance, prior treatment of CF mucus with disulfide breaking agent (NAC) showed a synergistic effect on the diffusion coefficient of surface-modified particles with PEG where the diffusion of particles with combined strategies approached its free diffusion in water. Conversely each of the strategies applied separately had limited improvement on mucus permeation [80].

3.2. Mucus permeation of lipid based nano-carrier with liquid core

Liposomes and SEDDS are the main lipid based nano-carriers with liquid core being investigated for oral delivery of peptides [81,82]. While SEDDS has been studied in more depth, liposomes have been reported in a few publications for their ability to permeate through the mucus barrier. This drug delivery system consists of an aqueous core covered by multiple or singular bilayers made of biocompatible lipids of natural or synthetic origin. This unique form of Liposomes provides an opportunity to load both water soluble and lipophilic agents in their aqueous cores or lipid bilayer respectively [83].

Modification of liposomes' surfaces was utilized to enhance oral delivery of proteins with the intent to have high mucus permeation [84]. Specifically, slippery liposome coated with an inert PEG polymer was the main strategy to enable muco-diffusive liposomes. For example, work by Sanders et al. where lipoplex densely covered with inert PEG (5000) was used for gene transfection [85]. This study showed that the PEGylated lipoplexes had better gene transfection efficiency by virtue of minimal interaction with CF mucus as compared to cationic lipoplexes. In a similar study for oral delivery of cyclosporine, liposome was coated with inert an hydrophilic polymer (Pluronic F-127) in comparison with chitosan coat [86]. The study showed that liposome coated Pluronic F-127 expressed higher diffusion through intestinal mucus, higher stability in the GIT environment and double the bioavailability of the liposomes coated with chitosan.

Another study showed that PEGylated liposomes permeated 10% slower through vaginal mucus compared to its movement in water with the diffusion being proportional to PEG density on the surface of liposomes [87]. The PEGylated Liposomal system was also found to be effective in the delivery of oligonucleotides based antibiotics against *H. pylori* at the surface of gastric epithelia. PEGylated liposomes improved the mucus permeation of these oligonucleotides agents which on their own were found to be highly trapped due to the macromolecular nature of these antibiotics [88].

4. Mucus permeation of self-emulsifying drug delivery system

4.1. SEDDS for delivery of hydrophilic/lipophilic agents

This system is an isotropic mixture of oils, aqueous phase and emulsifiers in which oil phase and emulsifiers can be simultaneously transformed into nano-droplets upon contacting any aqueous solvents such as intestinal fluids [89]. Oil and surfactants combinations can be diluted in the excess GIT fluid to form these systems [90]. This unique formation mechanism makes these systems highly suitable for oral delivery. A further convincing reason for suitability of SEDDS for oral nano-delivery is that the preparation of these systems do not require sluggish size reduction techniques associated with other nanosystems [91]. For oral administration of SEDDS, the combination of oils and surfactants are generally administered via a gastro-resistant capsular system so that the acidic barrier of the stomach can be avoided. The capsule consequently gets into intimate contact with intestinal fluid once reaching the intestine; SEDDS is readily formed with nano sized droplets. The emulsification process is spontaneous due to the usage of more than one surfactant and/or co-surfactant for the formation of SEDDS which can minimise the interfacial tension between the two phases when they come in contact [92,93]. This combination usually comprises short or medium chain triglyceride oils along with surfactant/co-surfactant mixtures consisting of the derivatives of glycerides and non-ionic surfactants with high HLB value [94,95].

SEDDS is a very efficient system to deliver lipophilic drugs from Class 2 and 4 in the Biopharmaceutics Classification System (BCS) since SEDDS can improve their solubility by dissolving them in the oil phase

and preventing precipitation in the GI tract [93]. The choice of oil phase is mainly based on the solubility of the lipophilic agent in it. The selected oil should improve the loading capacity and sustain the release profile to avoid drug precipitation. Indeed, SEDSS can eliminate the factors that reduce the bioavailability of BCS4 represented by low solubilisation, enzymatic degradation, gut wall efflux and low permeability [96]. Besides improving the solubilisation of BCS4 drugs, SEDD systems are characterised by high concentrations of surfactants/co-surfactants which can reversibly disrupt intestinal epithelial membrane and enhance the intestinal permeability of these agents [97]. SEDDS can also inhibit the gut wall efflux through the inhibition of P-glycoprotein (P-gp) [98]. i.e., P-gp is a transporter protein served as efflux pump so substrate of these transporters will permeate higher if P-gp is inhibited.

The inhibitory mechanism is related to certain types of surfactants used in SEDDS which can influence the efflux role of P-gp through modifying the structure of lipids within the epithelial membrane [99]. Surfactants like PEG based surfactants, TPGS (d-Alpha-Tocopheryl Poly (ethylene glycol), polysorbate 80 [100], polyoxyethylene 40 stearate (Myrj® 52) [101] and cremophor EL [102] were reported to inhibit P-gp efflux and improve the bioavailability of P-gp substrate agents. In addition to the effect on P-gp, surfactants like Polysorbate 80, TPGS, sucrose laurate, Cremophor RH 40 and Cremophor EL (PEG-40 and PEG-35 hydrogenated castor oil) can supress the enzymatic activity of cytochrome P450 3A4-mediated and hence it can influence the pharmacokinetics of these enzymes substrate agents [103]. SEDDS containing Cremophor RH40 or Tween 80 have shown inhibitory effect on cytochrome P450 3A in murine hepatocytes model [104].

Beside the main use of SEDDS for the delivery of hydrophobic agents, recently, this system was explored as a carrier for orally administered hydrophilic macromolecules which are prone to intestinal enzymatic degradation. To be loaded into the oil phase, these hydrophilic agents are turned into hydrophobic agents through a technique known as the Hydrophobic Ionic Paring technique (HIP) [105]. This HIP technique includes the pairing of peptides with a macromolecular hydrophobic counter ion which turns the peptides into hydrophobic agents with high oil solubility. Designing this technology enables the loading of peptide/proteins into SEDDS to provide a high protection against enzymatic degradation.

4.2. SEDDS as a muco-diffusive system: role of glycols at the surface of oil droplets

Originally, the main purpose of employing SEDDS for oral delivery of hydrophobic agents is to boost their bioavailabilities by improving both their solubility and loading capacity [106]. In other words, the mucus barrier is not the limiting step for the permeation of hydrophobic agents. SEDDS has not been utilized to enhance the diffusion of lipophilic agents across the intestinal mucosal barrier. Therefore, studying the diffusion of a lipophilic drug loaded into SEDDS through the mucus barrier has rarely been reported. For example, Sunazuka et al. used SEDDS to load a class 2 agent (Fenofibrate) then studied the permeation of the system through the mucus barrier consisting of 3% (*w*/w) porcine gastric mucin layer using the Transwell Membrane technique [107]. This study showed that SEDDS containing a surfactant with a lower M.wt PEG NIKKOL HCO-40 (PEG-40 hydrogenated castor oil) exhibited higher mucus permeation compared to the system containing PEG with higher M.wt NIKKOL HCO-60 (PEG-60 hydrogenated castor oil).

The mucus barrier and mucosal enzymes are the main reasons that limit the oral delivery of peptides [108]. Hence, more recently, utilizing of SEDDS as a nano-carrier to improve their mucus permeation has been extensively studied. There are a number of reasons for this. Firstly, SEDDS can provide effective protection against enzymatic degradation due to their efficient encapsulation and sustained release capability which renders a free peptide unavailable which in turn extremely minimise their hydrolysis by the enzymatic activity in the intestine [109,110]. The HIP technique, as was described above, has been used as the main pathway to improve loading of peptides into SEDDS systems. The HIP of peptides was widely studied to explore the best pathways to improve the loading of peptides into SEDD systems. For example, Griesser et al. investigated a variety of ion pairing surfactants and their complexation efficiency with different peptides like leuprorelin, insulin and desmopressin [111]. Sodium docusate emerged as the most efficient ion paring agent irrespective of the peptides, which translated into efficient loading capacity into a SEDDS model. The second reason to make SEDDS an excellent nano-carrier of peptide through intestinal mucus barrier is the high content of surfactants within the SEDDS which can highly reduce the intestinal enzymatic activity against loaded peptides [112].

However, high permeation across the intestinal mucosal barrier is the main reason to utilise SEDDS as a nano-carrier for peptides mucus delivery. SEDDS can improve the mucus permeation of macromolecular peptides due to the flexible nature of the fluidic droplet and the high hydrophilic nature of the droplets' surface [113]. Interestingly, most of the published work on SEDDS with high diffusivity across intestinal mucus barrier exhibited a mutual factor. This factor is the presence of a glycol moiety within the surfactants/co-surfactants compositions of the SEDDS. The mucus-diffusive SEDDS that is reported in the literature is mainly composed of surfactant/*co*-surfactant of polyglycol derivatives of glycerides. Thus, it seems that these glycol moieties at the surface of oil droplets form a muco-inert hydrophilic surface that avoids the interaction of oil droplets with mucus components in a similar mechanism of PEGylated solid NPs and PEGylated liposomes.

For this reason, the majority of the published work on the improvement of mucus permeation of peptides employing SEDDS involve use of the HIP technique, with loading of the ion paired lipophilic peptide into a SEDDS with glycol moiety at the surface. For example, Zupančič et al. demonstrated that complexation of daptomycin peptide with a dodecylamine hydrochloride improved its lipophilicity and loading capacity into the SEDDS system comprising an oil phase consisting of Dermofeel MCT (Glycerol tricaprylate) and Capmul MCM EP (glyceryl monocaprylate) and PEG based nonionic surfactants consisting of Cremophor RH40 and Cremophor EL [114]. This SEDD system improved the permeation of daptomycin twofold through pig intestinal mucus. The same researcher demonstrated a similar advantage of HIP and loading into SEDDS on the oral delivery of enoxaparin peptide. Incorporation of this peptide into a SEDDS with medium chain length lipids in which the surfactant mixture is compsed of 30% Cremophor EL (PEG ethers of hydrogenated castor oil) and 10% propylene glycol improved the mucus permeation twofold and improved its oral bioavailability [115].

Hintzen et al. also reported the protective effect of incorporating leuprolide (ion paired with sodium oleate) into a SMEDDS system composed of Capmul MCM and captex 355 as oils and a surfactant mixture of Cremophor EL (PEG ethers) and 10% propylene glycol [116]. The enzymatic degradation in a trypsin solution of leuprolide oleate loaded into the SEDDS was compared with a loading of the free leuprolide acetate and the leuprolide acetate. Fig. 3 shows that SEDDS significantly enhanced the stability of leuprolide oleate against the enzymatic degradation while both free leuprolide acetate and leuprolide acetate loaded into SEDDS expressed a fast degradation. In vivo studies showed an improved oral bioavailability of leuprorelin in rat models.

SEDDS is one of the widely exploited nano-systems to enhance the bioavailability and mucus diffusivity of insulin [117]. Karamanidou et al. explored the hydrophobic ion pairing of insulin with dimyristoyl phosphatidylglycerol to improve its hydrophobicity and loading capacity into an SEDDS containing a derivative of PEG emulsifier (Cremophor EL) [118]. The stability of insulin in a solution of common intestinal enzymes was highly improved indicated efficient shielding by the system and up to 40% permeation after six hours across purified porcine intestinal mucus in transwell inserts.

The intestinal mucus permeation of another peptide used for diabetes mellitus treatment (exenatide) was studied from SEDDS, prepared using Cremophor EL (PEG) as surfactant and Propylene glycol as cosolvent. Hydrophobic ion pairing of exenatide with sodium docusate

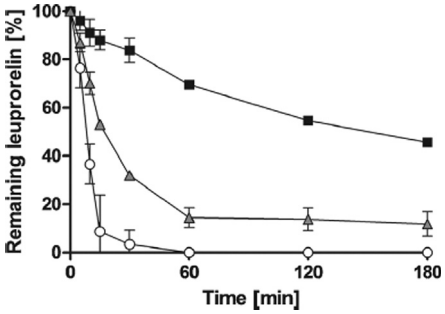

Fig. 3. Degradation profile in a trypsin solution of free leuprolide acetate (0), leuprolide acetate loaded into SEDDS (Δ) and leuprolide oleate loaded into SEDDS. Values are expressed as mean ± SD ($n = 3$). Adopted from Hintzen et al. [116].

improved the hydrophobicity of the peptide (logP 2.1) and the loading capacity [119]. Mucus permeation of exenatide loaded into SEDDS was enhanced by 2.7 times and oral bioavailability of 14.62% was achieved compared to the subcutaneous application. The SEDDS system was reported to improve the intestinal mucus permeation of another peptidic drug (octreotide) where this peptide was ion paired and loaded into a SEDDS comprising BrijO10, octyldodecanol, propylene glycol and ethanol [113]. This SEDD system showed a very high diffusion coefficient through pig intestinal mucus, high stability against intestinal lipase enzymes and significant increase in bioavailability. The same group showed the same outcome of improving the mucus permeation of desmopressin peptide by using SEDDS with glycol surfactants [120].

Ijaz et al. [121] employed a SEDDS strategy to protect a model peptide (lanreotide) which is susceptible to sulphide-thiol exchange owing to a disulphide linkage present in its molecule. These disulphide groups can interact with the intrinsic reduced glutathione (GSH) and reduce them into thiols and thus degrade the peptides. Lanreotide was efficiently paired with sodium deoxycholate then incorporated within a system composed of Capmul MCM (oil) and a surfactant mixture of Kolliphor EL(PEG-35 hydrogenated castor oil) and Migylol 840 (Propylene Glycol Dicaprylocaprate). The formulation exhibited a significant protection against GSH and enriched casein peptone within the first three hours with 50% of lanreotide remaining intact. A similar protection against Glutathione was reported for Desmopressin where an ion paired with Sodium docusate was loaded onto a SEDDS composed of Capmul 907 P (Propylene Glycol Heptanoate) as oil and Cremphor RH40 and Transcutol (Diethylene glycol monoethyl ether) as surfactants [122].

4.3. Other strategies to improve mucus permeation of SEDDS: mucolytic SEDDS

unhealthy mucus in CF and other pathological conditions is characterised by atypical viscosity and water content where mucolytic agents can be highly effective to eliminate the entrapment efficiency of mucus [123]. A rational approach to achieve better muco-penetration can involve localised micro-mucolysis around the droplets to weaken mucus viscosity and thus improve droplets permeation [73,77]. The aim is to load the SEDDS with a mucolytic agent with the ability to slowly release the agent during the transit of droplets through the mucus in order to reduce the mucus resistance to the moving droplets without inducing a massive destruction to the whole mucus barrier. This strategy has shown a great improvement in the mucus permeation of SEDDS. Leichner et al. investigated the potential of a mucolytic protease enzyme (Papain ion paired with Sodium deoxycholate by HIP) to disrupt intestinal mucus barrier [124]. In this study, SEDDS system loaded with papain- deoxycholate exhibited up to twofold higher diffusion across intestinal mucus barrier compared with the unloaded SEDDS.

A more exhaustive study to understand the effect of mucolytic agents like bromelain, papain and trypsin was conducted by Efiana et al. [125]. The enzymes were ion paired with a hydrophobic surfactant (palmitoyl chloride) with a maximum conjugation of up to 47.8% for papain compared with other peptides. Mucus permeation of papain-Palmitate loaded into a SEDDS with a derivative of PEG surfactant was found to be around 5 times higher than unloaded SEDDS when measured using the Transwell Method. In another study, Rohrer et al. exploited the capacity of thiol groups to break the disulfide linkages of the mucus network using two novel thiomers, thio butyl amidine dodecyl amine (TBA-D) and 2-mercapto-N-octylacetamide (TGA-O) [126]. The incorporation of TBA-D and TGA-O incorporated into a SEDDS formulation (propylene glycol 10%) resulted in a reduction of the dynamic viscosity. Multiple Particle Tracking (MPT) studies on these systems diffusion revealed a high difference in the diffusion of the SEDDS loaded with TBA-D (66 folds) compared with thiol free SEDDS.

4.4. Other strategies to partially improve mucus permeation of SEDDS

4.4.1. Zeta potential inverting SEDDS

The surface charge of oil droplets is an important parameter that can affect the diffusivity of the SEDDS in a similar manner to solid NPs [68]. The surface of an oil droplet should be neutral or slightly negatively charged to avoid any electrostatic or ionic interaction with the mucus components having sialic and sulfonic acid residues [127]. However, a negatively charged droplet would be significantly impeded for endocytosis mediated absorption at the intestinal epithelial interface [128]. The mutually opposite requirement of surface charge at the two subsequent interfaces can be achieved using a system capable of reversing its zeta potential in response to certain variables at these interfaces and thereby achieve efficient mucus permeation as well as cellular absorption.

Suchaoin et al. demonstrated that when formulating a SEDDS containing 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid sodium (PA), it formed a negatively charged system which favoured efficient mucus permeation. However, because this PA is a synthetic substrate of intestinal alkaline phosphatase enzyme (IAP), rapid cleavage of anionic phosphates from PA took place when the SEDDS reached the intestinal epithelia, where IAP are overexpressed. This inverted the overall surface charge of SEDDS from −ve to +ve providing improved permeability across the intestinal epithelial membrane. The enzymatic cleavage and subsequent release of anionic phosphate groups was ascertained using caco-2 monolayer in vitro studies expressing IAPs and using male SD rat intestine fixed in an Ussing-type chamber (ex vivo). In both cases, the released phosphate was measured using malachite green assay. The in vitro studies showed a 12.3% release of the total phosphate, the ex vivo experiment (rat intestine) suggested a fast release of 23.1% [129]. Further work reported by Griesser showed that SEDDS, comprising phosphorylated polysaccharides (hydroxypropyl starch phosphate and maize starch phosphate), achieved higher mucus permeation as compared to control groups due to the net negative charge provided by the phosphate groups [130]. Subsequently, these phosphate groups were cleaved down by IAP at the intestinal epithelia which inverted the surface charge from negative to positive and thus could facilitate intestinal absorption in vivo.

Recently, a conjugate of phosphorylated tyrosine with octadecylamine was reported to be a flip-flop agent possessing both negative and positive charge groups within the same molecule [131]. This agent when incorporated into a SEDDS would initially impose a negative charge on the surface due to phosphate groups and provide efficient mucus permeation of the nanodroplets. The surface charge would alter significantly after exposure to IAP and subsequent cleavage of phosphate groups would leave the amine groups to populate onto the surface and provide a positive charge to the nano-droplets which in turn would cause intestinal absorption and inhibit back diffusion.

4.4.2. Supersaturated SEDDS

The use of considerably large amounts of surfactants in these systems pose the danger of GI side effects [132,133]. A novel class of SEDDS has recently emerged where SEDDS is composed of lower

concentrations of surfactants and a precipitation inhibitor to achieve a supersaturated state of a drug in the intestinal fluid. This strategy can be achieved by utilizing a hydrophilic polymeric system along with the surfactants which will inhibit the crystallization of the loaded drug. Thus this strategy enables the poorly water soluble drugs to reach their supersaturated state within the SEDDS and still not-precipitated [134].

In a study reported by Lee et al. Soluplus® (derivative of polyethylene glycol graft copolymer) was employed to prevent precipitation due to high drugs concentration and thus forming a supersaturated SEDDS for the delivery of dutasteride [135]. The system achieved a 1.3-fold higher bioavailability for dutasteride in rats in comparison with pristine SEDDS and a 2-fold improvement in maximum plasma drug concentrations.

Thomas et al. developed a supersaturated SEDDS system through freeze-thawing approach to stabilise Simvastatin (a poorly water-soluble drug) at supersaturating concentrations. In vivo studies showed significant improvements in oral bioavailability and terminal half-life compared with the control SEDDS [136]. Similarly, oral bioavailability of Silybin was found to be improved as much as 3-fold compared to conventional SEDDS when HPMC (Hydroxy Propyl Methyl Cellulose) was employed as a precipitation inhibitor in a SEDD system composed of SLB, Labrafac CC (Capric Triglyceride), Cremophor RH40, Labrasol (Caprylocaproyl Polyoxyl-8 glycerides) [137].

5. Techniques to assess the diffusion of SEDDS through the mucus barrier

The quantification of diffusion of NPs or SEDDS through mucus barrier posed great difficulties to researchers [138]. Mucus barrier properties can be highly affected during the experiment, for example, atmospheric factors can increase the humidity or dryness of mucus sample in the in vitro testing which in turn can affect the diffusion data of particles/droplets through mucus [139]. Designing of the In vitro test should be carried carefully to avoid equivocal results leading to misjudgement of the diffusivity of particles/droplets through the mucus laden barrier. This review will describe only the techniques that have been reported in previous work to quantify the permeability of SEDDS in mucus. Other sophisticated techniques like Pulsed-Gradient Spin-Echo NMR (PGSE-NMR) [140] that widely utilized to quantify the diffusion of NPs in mucus but not for SEDDS will not be reported in this review. Similarly, less frequently used techniques with SEDDS like Small Angle Neutron Spectroscopy (SANS) will not be described in this review [141].

5.1. Transwell chamber techniques

The method is considered to be the most frequently utilized technique to measure the permeation of SEDDS across a static layer of mucus. The technique is essentially composed of a parallel or vertical arrangement of donor and acceptor chambers which are separated through a mucus barrier loaded between two layers of membranes. The quantification of permeated SEDDS is associated with the quantity of drug in the receptor media indicating the crossing of drug and SEDDS through the mucus barrier. The receptor compartment is filled with a suitable buffer in which the drug/dye is highly soluble [142,143].

This technique is simple, cost effective and flexible in terms of changing the parameters during the experiments. For example, with this technique, it is possible to use a small receptor compartment which enables testing the permeation at low drug doses. Also the Transwell Chamber Technique allows to change the mucus constituents throughout the course of the experiment to resemble some pathological circumstances [144]. In this regard, Boegh et al. (2015) utilized alternative method in which caco-2 cells were grown first then porcine intestinal mucus was added to form mucosal layer inserted between the two vertical compartments [3,145]. This technique, however, measures the bulk permeation only of the loaded drug/dye but it does not measure the behavioral movement of individual particles/droplets in the mucus [144]. Also, this method takes considerably long time to allow drug movement through the layer of mucus. This delay might suffice the chances of mucus enzymatic degradation or diluting the mucus sample which in turn cause leaking of mucus into receptor chamber [146].

The method has been widely used to test the mucus permeation of SEEDS. Friedl et al. set a Transwell Technique to study the factors affecting the permeation of SEDDS through a pig intestinal mucus layer where the crude mucus was centrifuged at high speed to yield a robust packed mucus layer [147]. The study showed that SEDDS with smaller particle size (12 nm) expressed about nine times higher diffusion than the SEDDS with particle sizes of 455 nm. Accordingly, Zupančič et al. assessed the permeation of a peptide (daptomycin ion-paired with dodecylamine hydrochloride) loaded into an SEDDS versus the free permeation of the SEDDS that dissolved in buffer and was added into the receptor compartment [114]. In this study, Transwell inserts having a pore size of 3 μm were employed to minimise the membrane effect and 50 mg of mucus was added onto the membrane to form a layer of 100–150 μm thickness to mimic the mucus intestinal barrier. The study suggested that the permeation of daptomycin loaded into the SEDDS was significantly higher than that of free daptomycin (Fig. 4).

Mucus diffusion of Desmopressin-docusate loaded into a variety of SEDDS formulations (with varying particle size and zeta potential) was determined utilizing a Transwell Method analogous to Friedl et al. [58]. The comparison was also conducted with other nano-carriers including liposomes and Polyacrylic acid-based NP as reference. The Transwell studies indicated an unequivocal improvement in the mucus permeation capabilities of SEDDS as compared to reference nano-carriers. This study also showed that SEDDS with a smaller particle size (25 nm) and most negatively charged (zeta potential −25 mV) is the most effective muco-diffusive compared with other SEDDS [58]. The Transwell method was utilized to understand the influence of papain on the mucus diffusion of a SEDDS [124]; to understand the mucus penetration profiles of phosphorylated zeta potential changing SEDDS [130]; and for the determination of the permeation potential of curcumin loaded into SEDDS [148].

5.2. Rotating silicone tube

This technique is similar to other traditional techniques where it involves the measurement of the bulk movement of particles, dyes or drugs in a mucus sample confined in a tube [56]. The process is simple; mucus is added to a tube (usually silicon based tubing) being closed from one end with a cap. The length of the tube can be up to 5 cm and the diameter up to 0.4 cm. Then SEDDS (loaded with a dye or drug) is added through the open end, which is then locked using a separate cap and the tube is kept rotating horizontally at 37 °C for a predetermined time (8–24 h) [149]. The tubes, after that, are frozen at −80 °C for about 1 h and then cut into 2 mm pieces starting from the end where particles were added and finally the quantity of drug/dye is measured within each piece.

The technique enables measurement of how deep particles can diffuse through a mucus sample and also quantifies the permeation of these particles which can be described in relation to the rate of diffusion of the tested SEDDS. Moreover, this cost-effective technique enables variation of the content of mucus within the tube where mucus with different properties can be filled within each tube segment [150]. However, the quantification process involved reflects the amount of loaded drug/dye that is diffused through the mucus and not the quantity of the SEDDS where the loaded cargo can be released in the mucus during the time of experiment.

The Bernkop-Schnürch group have widely the rotating silicone tube method to assess SEDDS permeation in mucus. For example, Suchaoin et al. tested the diffusion of a zeta potential changing SEDDS loaded with Lumogen red through a pig intestinal mucus sample using the rotary tube technique where the crude mucus was washed with 0.1 M sodium

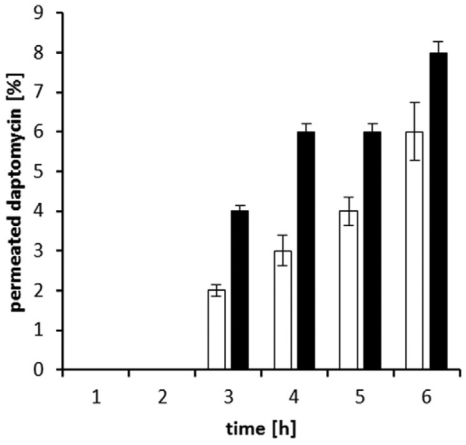

Fig. 4. Free daptomycin diffusion in phosphate buffer pH 6.8 (as white bars) compared to the diffusion of daptomycin loaded into SEDDS (black bars) through the pig intestinal mucus barrier utilizing Transwell technique. All data are reported as mean ± SD (n = 3). Adopted from Zupančič et al. [114].

chloride then centrifuged at 9000 rpm prior to the experiment [129]. Quantifying the Lumogen red in subsequent segments of the tube enabled the study of the effect of shifting the surface charge on the diffusion of the SEDDS. Negatively charged SEDDS permeated efficiently at the early stage of the experiment whereas, within time, converting the SEDDS into a positively charged system led to trapping in the mucus.

Zupanc̆ic̆ et al. utilized this technique to study the permeability of SEDDS loaded with Fluorescein diacetate (FDA) in a system formulated with varied chain length lipids (long to small chain lipid versus no lipid). The study showed the diffusion of SEDDS with no lipids was higher than the diffusion of other tested systems [151]. Similarly, the same group used the rotary tube technique to understand the influence of different mucolytic agents on the relative movement of a SEDDS consisting of Captex 355 (Capric Triglyceride), Kolliphor EL and propylene glycol [152]. This study showed that the SEDDS loaded with papain had a higher diffusion through mucus compared with the same system loaded with other mucolytic agents.

5.3. Fluorescence recovery after photo-bleaching (FRAP)

FRAP is a broadly employed technique to quantify the bulk movement of fluorescently labelled particles/droplets in mucus and any viscous solution or hydrogel biopolymer [153].

This technique involves inoculation of a SEDDS loaded with fluorescent dye into the biopolymer sample on a microscopic slide sealed with a coverslip and placed under fluorescence microscopy. The sample is left for 15 min for equilibration then a confined zone of the mucus sample is exposed to a high intensity laser beam for few seconds resulting in bleaching of the fluorescently tagged droplets. The fresh (unbleached) fluorescent droplets tend to relocate to already bleached section within the biopolymer sample leading to regain the strength of the fluorescence signal at that section. The bulk diffusion is calculated based on the time difference between the loss and regaining of fluorescent signal [154].

In the last decade, this technique was used to measure the diffusion of viruses and peptides. For examples, the bulk diffusions of a huge number of different sizes fluorescent peptides as well as viruses were quantified by FRAP to understand the effect size and surface properties on their diffusions in mucus [61]. Saltzman et al. [155] utilized this method to understand the factors affecting the movement of antibodies in cervical mucus. Accordingly, Afdhal [156] expanded the use of FRAP to study the effect of mucin concentration on the tendency of cholesterol particles to aggregate where it was observed that the size of cholesterol vesicles would be influenced by the relative interaction with mucin resulting in aggregation of vesicles into larger particle sizes. More importantly, in accordance with SEDDS, FRAP was used to measure the diffusion of oil nano-droplets through mucin solution which showed no effect of the droplet size compared to a significant impact of the size of the lipid phase (medium or long chain lipid) on the bulk diffusion of SEDDS [157].

5.4. Multiple particle tracking technique

MPT is a microscopy based technique pioneered by Hanes group to examine the motion of fluorescent particles in soft materials as a function of time [158]. The same group developed this technique to study the diffusion of fluorescent nano-systems across biological fluid and mainly through the mucus barrier [159]. This technique enables researchers to quantify the diffusivity of each individual particles at nanoscale level across a biopolymer system like mucus [160] and also understanding the structural and micro-rheological properties of that biopolymer system [161]. While most in vitro techniques provide the measurement of the bulk diffusion of particles, droplets or loaded drugs through mucus [66], MPT can simultaneously visualize, track and detect the individual diffusion coefficients of hundreds fluorescently labelled particles in a mucus samples [162].

MPT technique includes the use of either epifluorescence or confocal microscopy supplied with a high speed camera to record videos for the movements of fluorescently labelled particles/droplets in mucus [163]. These movements are ordinarily captured in X-Y dimensions within a single plane in the Z direction since mucus is an isotropic system and movements are equal in X, Y and Z dimensions [144]. Auto-fluorescence from mucus should be considered before the selection of fluorescent dye for particles' labelling since it can interfere with tracking [164]. Another factor that needs to be considered is the efficiency of dye loading into the particles/droplets where these loaded dyes should not be heavily leaked outside the particles before/during the experiment to avoid background noise. In this regard, SEDDS should be a very suitable system for the MPT analysis since such systems with an oil core allows the efficient incorporation of lipophilic fluorescent dyes that provides robust MPT studies in mucus [165]. Table 1 shows some of the published work in which SEDDS were efficiently labelled with fluorescent dyes and the method used to analyse the in vitro diffusion of SEDDS through mucus.

Video recording is followed by post-acquisition analysis using tracking software such as ImageJ to simultaneously track the movements of each individual droplet and to convert these movements into trajectories of hundreds of individual droplets [169]. These trajectories are firstly expressed in pixels then converted into the metric system based on the setting of microscopy, i.e., the trajectories are converted into metric distance to calculate the displacements of each particle. The 2-dimentional displacements of any droplets at certain time intervals are calculated as the mean squared displacement (MSD) per time interval $MSD_{(n)} = (X_{\Delta t})^2 + (Y_{\Delta t})^2$). For each SEDDS species, the MSD of hundreds of particles are calculated then the geometric mean of these MSDs is calculated to represent the ensemble MSD <MSD> of that particulate species. Ensemble diffusion coefficient < Deff> of any particle species at certain time interval is calculated by dividing the <MSD> by the frame rate multiplied by 4 since 4 represented the 2 dimentional displacement in X-Y direction and the frame rate is the time scale at which MSD was calculated [65].

MPT technique can reveal not only the diffusion of droplets in the mucus but the behavioral movements of these droplets. Fig. 5A shows the trajectories of oil droplets (SEDDS) in mucus samples where some droplets appeared trapped by the mucus and some appeared diffusive with pearl on string behavioral movement through the mucus (image captured by Gumbleton group) [170]. Fig. 5B represents the measurement of <MSD> through transferring the trajectories into metric displacements in X-Y dimensions then calculating <MSD>. Fig. 5C clarifies the different modes of behavioral movements of particles in which pearl on string movement is associated with the consecutive binding and unbinding of certain particles to the mucin. Random movement suggests the lack of interaction between a particle and its

Table 1
Compositions of SEDD systems which are efficiently labelled with fluorescent dyes for in vitro testing of droplets permeation through mucus barrier.

Composition	Dye employed	Technique	Reference
Brij™O10 as surfactant and octyldodecanol and paraffin as oil	Lumogen red Fluorescence labelled Dextran	Multiple particle tracking Ex vivo permeation	[113]
Capmul MCM (30%), Captex 355(30%), Cremophor EL (30%) and propylene glycol (10%).	Lumogen red fluorescein diacetate (FDA)	Multiple particle tracking Rotating tube method	[166]
Different combinations of oil including Capmul MCM EP and oleic acid. Different combination of surfactants including Capmul PG8, and Cremophor EL.	Lumogen red	Single particle tracking	[167]
Pluronic F-127 coated liposomes	Coumarin 6	CLSM studies on intestinal segments	[86]
Ethyl oleate and Captex as oil with different combinations of Cremophor, transcutol and triacetin as surfactants	Fluorescien diacetate	Standardized Transwell diffusion plates	[168]

environment and immobilised movement is related to a totally trapped particle. In other words, these modes of particles' movements in the mucus actually reflect the mechanism of particles' interactions with mucus components [171]. These particles-mucus interactions can be further analysed to give a clear description of the structural, mechanical and micro-rheological properties of the mucus samples [172].

MPT was recently utilized to quantify the diffusion of two discrete SEDDS formulations across freshly excised pig intestinal mucus. In the first MPT study, the mucolytic effect of thiomer loaded into SEDDS was revealed where the SEDDS system loaded with thiol exhibited a significantly higher diffusion coefficient compared to the thiol-free SEDDS [173]. Accordingly, MPT study on the diffusion coefficient of SEDDS showed an inverse relation between the ratio of the oil phase (octyldodecanol) and the diffusion of the system indicating the impact of the lipophilic interaction between the oil phase and the lipophilic components of the mucus [174].

6. Conclusion

SEDDS has shown high tendency to be an effective system for delivery of drug payloads across the mucosa. Literature review showed SEDDS can improve the diffusion of both hydrophilic macromolecule and lipophilic agents across intestinal mucus barrier. In terms of clinical unmet conditions, SEDDS has been extensively used to improve the permeation of loaded drugs, especially agents prone to enzymatic destruction such as peptides. The majority of these SEDD systems which showed high diffusivity across the mucus barrier have one factor in common, which is the presence of PEG derivatives as a co-surfactant. These glycol moieties seem to increase the flexibility of the surface of oil droplets and to diminish any lipophilic bonding between these oil droplets and mucus. In summary, utilizing SEDDS for mucus delivery has the following advantages: (i) high protection of loaded drugs against enzymatic environment; (ii) high permeation through mucus barrier; (iii) high loading capacity; and (iv) ease of preparation compared with solid nano-carriers.

However, even with the promising findings from SEDDS, There have been far fewer studies carried out on mucus diffusion of SEDDS than for the solid nano-systems. This has led to many gaps in knowledge, for example, on how surfactant and co-surfactant properties can change the diffusion of SEDDS through the intestinal mucus barrier. I.e., the published work on how surfactants and co-surfactants can improve mucus permeation of SEDDS is limited on the use of PEG-surfactants derivatives like PEG-35 and PEG-40 Hydrogenated Castor Oil to form a slippery-surface oil droplet that can permeate through mucus barrier. This limitation also applies to the impact of the oil phase where only few studies have been conducted to clarify the influence of the M.wt, size or chain length of the oil/lipid phase on the diffusion of the system.

Moreover, the work on promising SEDD systems like the zeta-changing system is still inadequate with few publications in the last decade. This is similar to the industrial approach where only limited work has been reported even though Sandimmune®, the first SEDDS product was released to the market two decades ago [175]. Therefore, it can be concluded that SEDDS has high capacity to deliver different hydrophilic/lipophilic agents across the intestinal mucus barrier but further studies are required to have a better understanding on the factors affecting the mucus diffusion of this system.

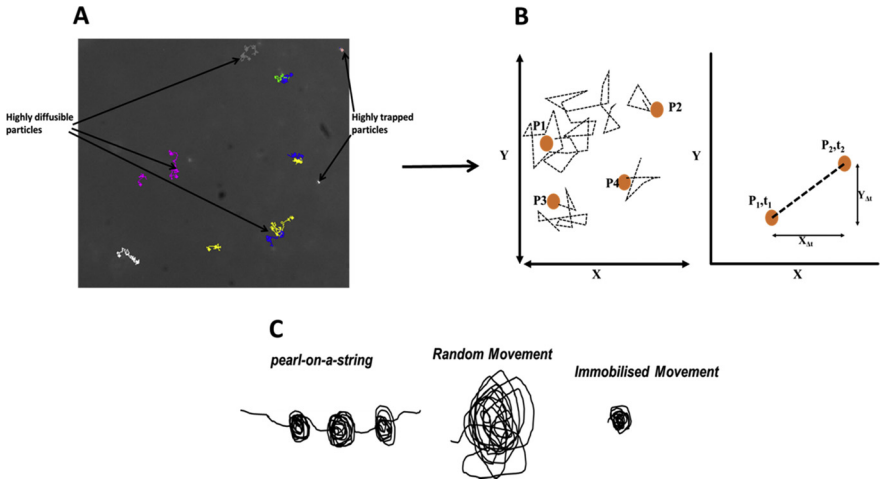

Fig. 5. Tracking of SEDDS oil droplets by the MPT technique using Fiji Image J software (Gumbleton group) [170]. (A) Trajectories of different oil droplets; (B) Transferring on trajectories into metric movement to measure the <MSD> and < Deff> of particles; (C) Modes of particles movements within mucus sample.

7. Progress towards clinical translation

A lot of promise has been put forth by the in vitro success of the mucus permeating SEDDS which is imperative to be translated to patient bedside through exhaustive preclinical and clinical evaluation. Robustness in results in terms of safety, efficacy as well as improvement in the pharmacokinetic performance of candidate drugs is necessary for regulatory approval. There have been a scarce but positive precedence of the in vivo pharmacokinetic performance of mucus permeating SEDDS where significant improvement in oral bioavailability of hydrophilic macromolecules through HIP was reported as discussed above [113,115,116,119]. There are, however, concerns regarding the in vitro-in vivo correlation of SEDDS formulations along with challenges pertaining to stability and manufacturing cost towards the clinical translation of the evident advantages of SEDDS [18]. Another criticism associated with SEDDS is the unpredictable impact of surfactants on the membrane permeability [176].

Notwithstanding the mentioned criticism, SEDDS have found its way into the clinic as a carrier formulation for oral delivery of hydrophobic agents like cyclosporine (Neoral) [177], ritonavir (Norvir) [178], fenofibrate (Lipirex) [179] to mention a few. The potential of SEDDS for improvement of oral absorption of low permeable drugs have been already established and thus must be the carrier of choice of such agents. However, the correlation of the in vitro outcomes such as mucus permeation and controlled release with actual improvement of bioavailability in preclinical as well clinical setting is necessary for the eventual translation of the technology to the commercial products [18]. Further exploration of the quantitative technologies for mucus permeation is necessary to expedite the development of the SEDDS based delivery of hydrophilic macromolecules and hydrophobic agents across intestinal mucus barrier efficiently.

8. Future perspectives

SEDDS is an effective strategy to augment the mucus permeation and enhanced oral bioavailability of hydrophilic peptides/proteins and lipophilic agents. However, the industrial utilisation of SEDDS for mucus delivery is still constrained and requires comprehensive exploration of various aspects and variables to reach the clinic. As is evident from the discussions above, an improvement in the molecular understanding of surfactant/co-surfactant and oil behaviours can be a potential breakthrough in designing SEDDS with enhanced efficiency and in vivo formulation stability which is imperative for clinical translation.

An important aspect which requires attention from the community is the incorporation of stimuli triggers within the SEDDS to pass the various physiological roadblocks present within the path of the mucus barrier. Zeta potential reversing systems exemplify theses innovative stimuli triggers which enable SEDDS to cross mucus barriers into intestinal epithelial absorption sites. In this regard, it can clearly be seen that there is a requisite to execute more studies to determine the fate of SEDDS as a system across the mucus and on the intestinal epithelia. The innovative techniques discussed provide a promising repertoire for precise analysis of SEDDS within the lumen and consequently into the target tissue. However, the selection of the technique which can precisely provide the vital evidence regarding mucus permeation is also a concern considering the cost and time constraints associated with certain techniques. MPT appears to be the right technique to comprehensively explain the parameters affecting mucus permeation of these systems.

Conflict of interest

The authors report no conflict of interest.

Acknowledgment

The authors gratefully acknowledge the financial support from NRN/Life Sciences Research Network Wales.

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