Surfactant effects on lipid-based vesicles properties: a review

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Abstract:

Understanding the effect of surfactant properties is critical when designing vesicular delivery systems. This review evaluates previous studies to explain the influence of surfactant properties on the behaviour of lipid vesicular systems, specifically their size, charge, stability, entrapment efficiency (EE), pharmacokinetics, and pharmacodynamics. Generally, the size of vesicles decreases by increasing the surfactant concentration, carbon chain length, the hydrophilicity of the surfactant head group, and the hydrophilic-lipophilic balance (HLB). Increasing surfactant concentration can also lead to an increase in charge, which in turn reduces vesicle aggregation and enhances the stability of the system. The vesicles entrapment efficiency not only depends on the surfactant properties but also on the encapsulated drug. For example, the encapsulation of a lipophilic drug could be enhanced by using a surfactant with a low HLB value. Moreover, the membrane permeability of vesicles depends on the surfactant's carbon chain length and transition temperature (Tc). Additionally, surfactants have a clear influence on pharmacokinetics and pharmacodynamics such as sustaining drug release, enhancing the circulation time of vesicles, improving targeting and cellular uptake.

Keywords: lipid based vesicles, Surfactant, Liposome, Transfersome, Hydrophilic- lipophilic balance, Entrapment efficiency, Zeta potential, and Stability.

1. Introduction:

In recent years, the development of better delivery systems for drugs with some undesirable properties has gained much interest. Improving patient compliance is one of the main drivers for research through achieving a good therapeutic profile and reducing unwanted side effects. Substantial attempts have been made to alter pharmacokinetic and pharmacodynamic properties such as solubility, permeability, release profile and targeting [1,2]. They are generally achieved by designing delivery systems such as particulates, polymeric micro/nano spheres, and vesicular systems [1,3,4]. Vesicular systems usually consist of amphipathic lipids which are self-assembled in aqueous media to form one or multi bilayers enclosing a hydrophilic core [5]. They are employed as a carrier for both hydrophilic and lipophilic active pharmaceutical ingredients (APIs). Hydrophilic drugs are usually encapsulated in the aqueous core or adsorbed on the surface of polar head part of the lipid, whereas the lipophilic drugs are encapsulated between the concentric bilayer (lipid tails form a suitable lipophilic environment) [6,7] (Figure 1, A). Lipid based vesicular systems may be classified depending on many factors such as their constituents, size and number of bilayers, which accumulatively affect the final vesicle properties in several ways [8,9]. Table 1 summarizes the main vesicular systems that are employed for drug delivery.

Surfactants are found in many therapeutic and cosmetic compounds and were usually considered as penetration enhancers [10]. However, surfactants are used in the pharmaceutical industry for their ability to solubilise poorly soluble drugs, to improve the elasticity of the delivery system and to aid drug delivery across biological membranes such as the skin or blood brain barrier[11-14]. Many researchers have also tried to modify delivery systems (e.g. polymeric-nanoparticles) using several surfactants to achieve better properties such as enhanced cellular uptake [13]. The effect of surfactant molecular structure on their aggregation, adsorption at interfaces and cellular uptake have been extensively reported in the literature [13,15-17]. In order to enhance lipid-based vesicular system properties, a new generation of liposomes were developed to be flexible and ultra-deformable (Figure 1,B). Alterations to the conventional liposome composition were reported, since surfactants were incorporated into their structure as well as the lipid component (Table 1). Niosomes, as an example of the mew generation of liposomes, were developed using non-ionic surfactants in combination with cholesterol and, although they still bear the same characteristics as liposomes, were proven to be more permeable and stable [18,19]. As another example transfersomes were considered a new generation of liposome with a high deformability, which allows them to squeeze easily through biological barriers [20]. The presence of surfactants in the composition of lipid-based vesicular systems has been stated as the reason for the improvement in many properties such as the entrapment efficiency (EE), stability and permeability [21,22]. However, the consequence of surfactant presence in the vesicles composition may vary as the properties of the surfactant itself change. The aim of this review is to highlight the numerous studies carried out to date and to summarize our current understanding of how surfactant properties influence the behaviour of vesicle drug delivery systems.

Table 1 summary of the main lipid based vesicular systems, with their main constituents, distinctive properties and disadvantages

2. Surfactants:

Surfactants, also referred to as surface-active agents or edge activators, are amphipathic molecules and composed mainly of two main moieties; the polar hydrophilic part, which is attached to the non-polar lipophilic part [38,39]. The lipophilic part is usually a straight or branched hydrocarbon chain (tail) consists of eight to eighteen carbon atoms (Figure 1, C and D) [40]. At low concentrations surfactants exist as monomers, and usually in aqueous medium they adsorb on the interfacial surfaces (solution- air interface) and consequently they displace some surface molecules and reduce the intermolecular forces, thus lowering the surface tension [41,42]. However, above a certain concentration (critical micelle concentration (CMC)) they aggregate and form micelles (Figure 1 E and F). The CMC value for each surfactant may vary as it depends on the method of determination and other factors such as surface tension, viscosity, temperature and conductivity [43,44] . Additionally, many studies reported that CMC is not a precise value, but represents a range of concentrations over which the self-assembly of surfactant molecules to form micelles are induced [45]. It was also reported that increasing the temperature of the system could cause a reduction in the CMC value, which was explained by the destruction of the hydrogen bonds that usually form between the hydrophilic groups of surfactants and the water molecules [46-48]. However, surfactants form micelles due to the hydrophobic effect, and they could adopt several arrangements [49,50]. In aqueous medium, the hydrophilic heads face the aqueous surroundings and the lipophilic tails directed toward the non-aqueous medium. However, in a non-polar medium, they work similarly but the micelles form in an opposite arrangements where the polar groups face each other and the tails project out towards the non-aqueous medium [42] (Figure 1 E and F).

Figure 1. A) The dispersion of drug molecules within the lipid based vesicles, B) comparison between conventional liposome on right half and transfersome (elastic liposome) left side, C) surfactant monomer with one tail, D) surfactant monomer with two hydrocarbon tail, E) micelle (surfactant assembly) in aqueous medium, F) micelles in non-aqueous medium.

2.1. Classification

Surfactants may be classified depending on either their molecular weight or their hydrophilic-lipophilic balance (HLB) [51] (Figure 2). Moreover, they are further categorised into several sub-groups based on the properties such as charge of the hydrophilic head group [51].

Figure 2. Illustrated diagram of surfactant classification based on molecular weight and hydrophilic lipophilic balance.

2.1.1. Classification based on Molecular weight

A. Low molecular weight surfactants:

There are four major types of low molecular weight surfactants where the classification depends on the nature of the hydrophilic parts. **Anionic surfactants have negatively charged** hydrophilic parts. They are widely used due to

their low cost. Generally, they could be carboxylates ($C_nH_{2n+1}COO^{-x}$), sulphates (C_nH_{2n+1} OSO_3^{-X}), sulphonates (C_nH_{2n+1} SO_3^{-x}), or phosphates ($C_nH_{2n+1}OPO$ (OH) O^{-x})), where n is the number of carbon atoms (i.e. n= 8-18) [39,51]. **Cationic surfactants have positively charged** hydrophilic parts and often a natural fatty acid. Quaternary ammonium compounds are the most commonly used cationic surfactants such as alkyl dimethyl benzyl ammonium chloride (benzalkonium chloride), which is widely used as a preservative in pharmaceutical formulation (i.e. bactericidal) [39,52]. **Amphoteric surfactants (zwitterionic)** contain both cationic and anionic groups and their behaviour is dictated by the pH of the medium in which they are dissolved. They act as anionic surfactants in alkaline pH due to their acquisition of a negative charge, whereas in acidic medium they gain a positive charge and behave like cations. They show good water solubility, improved stability, and better compatibility with other surfactants and within different mediums in comparison with the cationic and anionic surfactants. **Non-ionic surfactants** are characterised by the presence of uncharged hydrophilic groups that do not dissociate in aqueous solution such as alcohol, ether, ester or amide groups. They contain wide range of classes such as alcohol ethoxylate, sorbitan esters ethoxylate, and fatty acid ethoxylates. Additionally, there are multihydroxy products such as glycol esters, glycerol esters, glucosides and sucrose esters [39,51,52] (Table 2).

B. Polymeric surfactants:

Polymeric surfactants have been developed in the last two decades and can assembled into one or several macromolecular structures that have hydrophilic and lipophilic character. They are now commonly employed due to their wide application as stabilizers in emulsion and suspension formulation. Several modifications have been carried out on these surfactants to improve their properties and get molecules that are effective in several pH conditions, temperature, and media[39,51]. The number of the hydrophilic and lipophilic groups as well as their distribution along the carbon chain is considered to be a distinctive property of the polymeric surfactants. The high structural complexity of the polymeric surfactants exhibit several behavioural differences in comparison with low molecular weight surfactants [53]. Depending on the distribution of the hydrophilic and lipophilic moieties, these polymeric surfactants are usually sub-categorised into two main classes; polysoaps and macrosurfactants [54].

2.1.2. Classification depends on HLB

The hydrophilic-lipophilic balance classification system was first developed by Griffin in the last century, and it is a scale that represents the percentage of hydrophilic to lipophilic groups in surfactant molecules [55]. HLB is subdivided into several categories based on the range of HLB value, each represents a group of surfactants with similar behaviour [39,51]. Surfactants with HLB values of 3-6 show more lipophilicity and they tend to form water in oil (W/O) emulsion, and micelles/vesicles that are more soluble in non-aqueous media. While HLB values of 8-18 represent oil in water (O/W) emulsifiers or solubilisers, which are more hydrophilic and water-soluble. However, surfactants with HLB values between 7-9 are considered as wetting agents and therefore exhibit both properties. Sometimes it is possible to use two or more emulsifying agents (surfactants) at once to achieve the desired solubilisation effect. For example, mixing tween 80 (i.e. polysorbate with a HLB value of 15) with Span 80 (sorbitane monooleate, which has a HLB value of 4.3) in different proportions may cover a range of HLB values in order to choose better composition to achieve the desired properties [39,52]. Therefore, the optimum use of the HLB value is to enable the selection of the surfactant composition.

Table 2. Chemical structures of the most commonly used non-ionic surfactants.

3. Surfactants in lipid- based vesicles:

The uses of surfactants in lipid-based vesicles has progressed over the last few decades [56]. Some studies have focused on using surfactants from one group e.g. studying the non- ionic surfactants in case of niosomes [57], whereas others have investigated the effects of using several surfactants with different characteristics on vesicle properties. Additionally, most studies have aimed to maximise the effect of the chosen surfactant in order to optimise the formation of the lipid vesicles to achieve the desired size, drug loading and physiochemical properties.

3.1. Surfactant effects on the size and polydispersity index (PDI):

The presence of surfactants in lipid-based vesicle systems has a noticeable effect on their size. In 2016, Singh et al studied the role of surfactant in the formulation of elastic liposomes for the transdermal delivery of the opioid analgesic tramadol. The effect of several surfactant (i.e. span 80, tween 80, and sodium deoxycholate) was investigated in liposome formulations, where an indirect relationship was observed between liposomes vesicle size and surfactant concentration [58]. It was suggested that the higher surfactant concentration covered the surfaces of the liposomes and therefore prevent them from aggregation [58,59]. A small polydispersity index was also reported with the higher surfactant concentration and the consistent size distribution was thought to be an important factor in reducing interfacial tension and producing a homogeneous emulsion [60]. The same three surfactants were also used by Jain et al to prepare transfersomes and no significant differences in vesicle size was expected, as a result of the homogenization method (through polycarbonated membrane) used during the preparation of the formulations [29,61]. However, a reduction in vesicle size was noted when higher surfactant concentrations were used, it might be attributed to the fact that surfactant with more than 15% induce micelle formation rather than vesicle formation [29]. A similar study of the influence of several surfactants on elastic liposome properties was carried out by Barbosa et al. [62]. They investigated the incorporation of the non-ionic surfactants that have either one hydrophobic chain (such as octaethylene glycol laurate (PEG8L), polyoxyethylene glycol-4-laurate (PEG4L), and pentaethylene glycol monododecyl ether (C12E5)) or two hydrophobic chains (such as polyoxyethylene glycol-8-dilaurate (PEG8DL), and polyoxyethylene glycol-4-dilaurate (PEG4DL)). The study revealed similar results, exhibiting higher surfactant concentration lead to the formation of smaller vesicles [62]. Additionally, surfactants with two hydrophobic chains exhibited better and homogeneous PDI in comparison to those with one carbon chain, which could be explained by their better capability to anchor within the lipid bilayer [62-64]. However, not only does the number of the hydrophobic chains affect the vesicle size, but also the length of the carbon chain of the surfactant. Duangjit et al. studied the effect of carbon chain length and content of the surfactant on meloxicam loaded liposomes [65]. The size of the obtained liposomes decreased as the length of carbon chain of the surfactant increased from C4 to C16 [65]. This was attributed to the rise of the surfactant

hydrophobicity as its carbon chain length increase, which in turn led to improve the solubility of the surfactant molecules within the lipid bilayer [65,66].

Moreover, a reduction in vesicle size was also reported to be influenced by the hydrophilicity of the head group of the surfactant, which was thought to be due to the shortness of the hydrophobic backbone in comparison to the hydrophilic head group, which was asparagine grafts in that case [65,67]. Similar results were achieved during niosomes loading with a β-carotene as a model of lipophilic moiety, with the more hydrophilic surfactant (higher HLB value) producing smaller vesicles [33]. In contrast, the size of elastic transfersomes optimized with several surfactants for the transdermal delivery of pentoxifylline increased as the HLB of the surfactants increased. The surfactants were ranked as they formed larger transfersomes in the following order Span 80 < Span 20 < Tween 21 < Tween 20 [61]. Similar ranking of several Spans on the niosomes size were obtained, since the size of the vesicles increased as the HLB progressively increased, Span 20 (HLB= 8.6) showed larger niosomes size, after that the size gradually decreased with Span 40 (HLB= 6.7), Span 60 (4.7) and Span 80 (HLB= 4.3) [68]. This could be due the effect of the surface free energy, which might decrease as the hydrophobicity increases [69].

Some researchers have investigated the effect of the lipid type on vesicle size. The inclusion of some lipids, for example, an anionic lipid such as dicetylphosphate (DCP) with Span 20-based niosomes reduced vesicle size. The reduction was explained by the increased the curvature of the bilayer caused by the electrostatic repulsion between the ionized head group of both the lipid (DCP) and the surfactant [70].

It has been also suggested that the lipid to surfactant ratio can effect vesicle size. Using cholesterol at higher concentrations than usually specified was observed to increase niosomes size [71]. Parallel results have been obtained by many studies, where the incorporation of cholesterol in niosomes or liposomes at higher concentration than the surfactant lead to increase the vesicle size. It was suggested that the competition between cholesterol and surfactant to keep their place in the lipid bilayer may increase the size of vesicle [70-73].

In summary, the inclusion of surfactants within lipid based vesicles has an obvious effect on vesicle size and many factors need to be considered when a surfactant is incorporated into the formulation. Parameters such as surfactant concentration, number of carbon chains, carbon chain length, and the hydrophilicity of the head groups have an inverse effect on vesicle size. While the competition of other moieties with the surfactant molecules during the arrangement of the lipid bilayer clearly showed an increase in lipid vesicle size.

3.2. Surfactant effects on entrapment efficiency:

Achieving a good EE is considered to be the main goal during the development of any vesicular delivery system. Many researches have tried to incorporate surfactants into lipid- based vesicles in order to improve the encapsulation of both hydrophobic and hydrophilic drugs as well as reduce drug leakage in liposome formulations [33,66,67]. Although many attempts have been carried out to investigate surfactants effects on improving the EE, there is still no definitive proof that specific surfactant properties could lead to certain entrapment. It may entail many surfactant properties, such as the type and concentration, that could have an effect on the EE of a certain drug within a certain lipid composition [71]. General trends could be observed from a set of surfactant properties on

a hydrophilic drug entrapment, but that effect could be totally different when a hydrophobic drug is encapsulated. The effect of various parameters on the EE are discussed in more details in the following sections.

3.2.1. Surfactant concentration

Many researchers have studied the effect of surfactant concentration on vesicle's EE and it has been commonly reported that higher surfactant concentration reduce the EE. This effect has been explained by the possible formation of micelles when the surfactant concentration in the bilayer exceeds a critical lamellar/micellar transition temperature [29,58,59,74]. Furthermore, the permeability of the vesicles membrane might increase due to the arrangement of surfactant molecules within the lipid bilayer structure, which could introduce pores within the membrane and increase its fluidity [75]. Overall, this will prompt entrapped drug leakage [58,76,77]. Additionally, it is thought that the optimum amount of surfactant depends on the packing density of the phospholipid used and the surfactant-phospholipid interaction. When the surfactant concentration increases and it is known to have a high tendency to interact with the lipid, this leads to a reduction in entrapment due to competition on the loading within the bilayer [72,76,78]. For example, transfersomes were prepared and loaded with dexamethasone as a model lipophilic drug to evaluate sodium deoxycholate (SDC), Tween 80 and Span 80 as edge activators at five different lipid-surfactant ratios (95:5, 90:10, 85:15, 80:20, 75:25) [29]. The study revealed that encapsulation efficiency decreased as the concentration of the surfactant increased. Transfersomes prepared with SDC showed the lowest encapsulation of dexamethasone, as both surfactant and drug possess similar steroidal structure and therefore competing each other for their entrapment [29]. Similarly, Patel et al. studied the effect of surfactant concentration on the entrapment of a lipophilic drug (i.e. curcumin) in lipid-based vesicles and demonstrated that higher surfactant concentration lowered the entrapment [76].

Conversely, other researchers have reported that increasing surfactant concentration will increase the number of vesicles formed, which consequently leads to a higher volume of the hydrophobic bilayer domain available to house a hydrophobic drug [68,79,80]. The impact of surfactant concentration was similar when a low concentration was used to prepare Span-based niosomes, a small number of niosomes was obtained, and it was recommended that a higher surfactant concentration may improve drug entrapment [77].

3.2.2. Surfactant structure (carbon chain length, saturation, hydrophilic head group) and transition temperature (Tc)

Generally it is suggested that by increasing the carbon chain length of the surfactant, the solubility of a lipophilic drug in the lipid bilayer should increase and consequently the entrapment efficiency will increase [81,82]. On the other hand, a point not to forget is that a surfactant with a long carbon chain might compete with a lipophilic drug as they assemble themselves within the lipid bilayer, excluding the drug and thus reducing its entrapment [58,83]. Similar results were observed when niosomes were formulated with different types of Span. All Span surfactants have similar head groups and only differ by their hydrophobic chain. Niosomes that were prepared with Span 60 showed the highest entrapment as it has the longest carbon chain [68,77]. In contrast, Span 80 resulted in the lowest entrapment efficiency, which was suggested to be related to the unsaturated double bond in its alkyl carbon chain. The presence of the double bond within the carbon chain might make it bend and thus would make the niosomes bilayer to be more permeable as the packing of the adjacent molecules may not be tight [68,77]. Comparable

outcomes were observed by El-Laithy et al., when they prepared proniosomes by using several non-ionic surfactants such as Tween (80 and 20) , Span (80 and 20) and sugar esters (such as, sucrose stearate, sucrose palmitate, sucrose myristate, and sucrose laurate) [79]. Although the Tween-based proniosomes showed the lowest entrapment efficiency, Tween 80 revealed better encapsulation due to its long carbon chain. Moreover, all sugar ester surfactants showed good encapsulation due to their long carbon chains in spite of their high HLB values [79,84].

Additionally, it was proposed that not only the properties of tail but also the head group of the surfactant might influence drug entrapment within vesicles (table 2). The physiochemical properties of Span 60/Tween 60 niosomes with ellagic acid as a drug were evaluated [85]. The study revealed that entrapment efficiency increased with Tween 60 niosomes, possibly due the nature of the surfactant head group. The head group of Tween 60 (polyoxyethylene groups) is larger than the head group of Span 60, which in turn could help solubilize more ellagic acid [85]. In addition, the formation of hydrogen bonds may be possible between the head group of Tween and the phenolic groups and lactone moiety of the ellagic acid [85,86].

Furthermore, the phase transition temperature (Tc) of the surfactant could be an important factor in explaining surfactant effects on EE of lipid-based vesicles. It was reported that the higher the surfactant transition temperature, the better their ability to form a more ordered gel structure and a less leaky bilayer, which may further improve the entrapment efficiency [79,82,87]. While surfactants with a lower Tc could be more liquid in form, leading to irregular structural formation and increased fluidity of the vesicles bilayer, that in turn reduces the drug entrapment [69,77,88,89]. For example, Gupta et al. showed that Span 80 gave the lowest entrapment as it has the lowest transition temperature (Tc= -12^{o}C) in comparison to Span 60, 40, and 20 since their transition temperature are 53^{o}C, 42^{o}C and16^{o}C respectively [68]. These results were consistent with several other studies where the highest entrapment of drug was obtained from vesicles prepared using Span with the highest transition temperature [77,90,91].

3.2.3. Surfactant HLB value and surfactant physical state

Evaluating surfactant effects on vesicle entrapment efficiency not only depends on its chemical structure, but also requires an understanding of the influence of the hydrophilic-lipophilic balance (HLB). However, the effect of the surfactant HLB value on the entrapment still depends on the drug lipophilicity [29,92]. Literature suggests that the maximum entrapment of a lipophilic drug could be achieved by using a surfactant with a low HLB value [71,93,94]. For example, Tween 60 was reported to give better encapsulation of β-carotene (a model lipophilic drug) when compared to Tween 20 since their HLB values are 14.9 and 16.7 respectively [33]. Niosomes showed a lower tendency to entrap the lipophilic carvedilol, as the HLB value of the surfactant used increased [71,95]. Chaudhary et al., obtained a higher encapsulation of curcumin from transfersomes prepared using Span 80 (HLB value 4.3) as an edge activator compared with Tween 80 (HLB value 15) [58]. Similar findings were also exhibited when dexamethasone loaded transfersomes were prepared, with both Span 85 and Span 80, with HLB values of 1.8 and 4.3 respectively, showed higher encapsulation than Tween 80 (HLB 15) and sodium deoxycholate (HLB 16) [29].

On the other hand, surfactants with high HLB values are thought to give better encapsulation of hydrophilic drugs [96,97]. This was proved by Shaji et al., who prepared piroxicam loaded-transfersomes as sodium deoxycholate based

transfersomes, highest encapsulation was obtained in comparison to Tween 80, Span 80 and Span 65 [96]. Surprisingly, contrasting results were obtained when the hydrophilic drug diclofenac sodium was loaded within transfersomes using different types of surfactant [92]. The surfactants used were ranked according to their ability to give the highest encapsulation as: Span 85 > Span 80 > sodium cholate > sodium deoxycholate > Tween 80 [92]. Although Tween 80 did not show higher encapsulation than sodium cholate or sodium deoxycholate, Span based transfersomes showed the highest EE regardless of their low HLB values [92].

Moreover, the physical state of the surfactant could have an effect on vesicles EE [70]. Surfactants could be solid, such as sodium deoxycholate, gel form such as Span 60 and 40 or a liquid such as Span 80. Gel-type surfactants are likely to produce less permeable vesicles than liquid surfactants [70]. Several types of surfactant were used in the preparation of insulin-loaded niosomes and the presence of the gel-type surfactants such as Span 60 and Span 40 were found to improve drug entrapment. Whereas niosomes prepared using liquid surfactants such as Span 20 and Span 80 were thought to be more permeable and showed lower entrapment efficiency [70].

3.3. Surfactant effects on pharmacokinetics and pharmacodynamics:

There are many claims that the presence of surfactant could have an effect on the pharmacokinetic and pharmacodynamics properties of lipid-based delivery systems, such as enhancing drug release, permeability through the route of administration, circulation time and cellular uptake. For example, in surfactant-based liposomes, increasing surfactant concentration enhanced the release of the encapsulated drug (ciprofloxacin) from the delivery system. However, the amount of ciprofloxacin released was dependant on the type of surfactant used and using Tween 80 significantly enhanced release [83]. Similar results were obtained in other studies, where the use of Tween 80 in liposomal formulations enhanced the flux of drug through the skin [98,99]. Although Tween 80 enhanced the skin permeation of celecoxib-loaded liposomes, it did not show any enhancement of celecoxib cellular uptake in comparison to conventional celecoxib liposomes [99]. Niosomes were prepared with several types of Span for the delivery of the antitumor agent 5-flurouracil (5-FU) and the release rate from formulations prepared using Span 40 and Span 60 was slower than those prepared using Span 20 and Span 80 [100]. This trend could be due to the difference in the rigidity and permeability of the formed bilayer, since both Span 40 and Span 60 have a high Tc and form less permeable bilayer than Span 20 and Span 80. Additionally, in comparison with free drug solution, all surfactant-based vesicles of 5-FU showed higher concentrations of drug in several organs for a longer duration that could enhance the possibility of the preferential phagocytic uptake and reduce its cytotoxicity[100]. Also, the pharmacokinetic studies of the 5-FU vesicles reported an increase in the half-life and a decrease in the clearance, which in turn maintained a sustained action of 5-FU [100]. Similarly, paclitaxel was also formulated in niosomes with several surfactants for oral delivery to slow release rate and reduce the toxic side effects [93]. Moreover, Span 40-based niosomes were more efficient in protecting paclitaxel from the degradation by the gastrointestinal enzymes [93]. Span 60- based vesicles were also prepared to deliver doxorubicin, showed prolonged release, doubled its therapeutic effect (tumoricidal effect) and reduced the drug clearance [101,102]. Similar results were achieved when several Spans were used to prepare ketoprofen loaded vesicles and Span 60 was reported to be superior in maintaining the anti-inflammatory effect of ketoprofen for a longer time with

slower release rate in comparison to Span 40 [103]. Confalonieri et al. carried out a comparison between surfactant-based vesicles and non-niosomal formulations for the delivery of flurbiprufen [104]. After IV administration to dairy cattle, there was no immunogenical reaction. Furthermore, the surfactant based niosomes showed longer circulation time in the vascular space, which could improve the flurbiprufen distribution into the required organs/ tissue as well as its short half-life [104]. In addition, a high permeation through the skin with an improved pharmacological activity and reduced side effects were reported after applying meloxicam-loaded niosomes during *in vivo* animal studies, which were prepared using both Span 60 and ethanol as surfactants [105]. A significant enhancement in the cellular uptake of gene-loaded niosomes were reported in comparison to a conventional liposomal delivery [106]. Span 60 and Span 40 were more effective in mediating the cellular uptake of the gene (antisense oligonucleotides) during the *in vitro* study using a COS-7 cell line [106]. In summary, surfactants have a clear influence on the pharmacokinetic and pharmacodynamic properties of lipid delivery systems, such as achieving sustained release, enhancing circulation time, improving targeting and cellular uptake. Nevertheless, the impact is not always constant and could alter depending on the properties of the surfactant.

3.4. Surfactant effects on charge and stability:

Measuring the electrostatic charge of lipid vesicles is important factor in order to evaluate their surface properties, as it might play a crucial role in their stability by either creating repulsive forces or agglomeration [61,96]. The net charge on the vesicle surfaces was thought to be the combination of both lipid and surfactant charge. However, it was reported that the type of surfactant could greatly affect the zeta potential. For example; between several types of surfactant-based transfersomes, cholate-based transfersomes exhibited the highest negative zeta potential value [96]. Additionally, as the concentration of the surfactant increased, the net charge of the transfersomes increased as well [96]. This high negative charge was considered to be advantageous as the research aimed to prepare transfersomes for transdermal drug delivery [61]. Since it was thought to enhance the transfersomes permeability and stability due to the repulsive forces between the charge of the vesicles and skin surface [61,78]. On the other hand, the same research revealed that all Tween-based transfersomes showed positive charge, and the greater the hydrophilicity of the Tween (i.e. HLB value) the larger the positive charge on the vesicle surfaces [61]. Similarly, many studies have reported that as the surfactant concentration increases, the vesicles hold a larger zeta potential. Generally, the high charge could improve vesicles stability by reducing aggregation due to the electrostatic repulsions that could occur between them when they bear similar charge on their surfaces [59,60,87,107]. On the other hand, several studies suggested that increasing the surfactant concentration may reduce the physical stability by forming several types of aggregates. These could be elongated vesicles or tubules due to the fusion of the spherical vesicles [108]. That could be related not only to the surfactant concentration but also to the medium pH, since it was reported that at high pH surfactant monomers might have looser packing and form more elongated and aggregated vesicles [108].

Additionally, the surfactant transition temperature was also reported to have an effect on the vesicular system stability. It was thought that the surfactant with higher transition temperature could be useful to prepare more stable vesicles [68,109,110]. Moreover, it was claimed that a surfactant molecular structure such as polyoxyethylene alkyl ether could enhance the stability of liposomes. This is because its large hydrophilic group induced a steric

hindrance which impaired liposome aggregation and improved the stability [111]. Similar findings were reported when a weak acid type anionic surfactant sodium 3,6,9,12,15-pentaoxaheptacosanoate (AEC4-Na) was used to form vesicles. Inhibition of aggregation and the stability were enhanced because of the steric repulsion which was induced by the hydrophilic oxyethylene unit of AEC4-Na [112].

4. Conclusion:

This review has summarised previous studies from the literature on the influence of surfactants on vesicular system properties. Parameters such as surfactant concentration, number of carbon chains, carbon chain length, the hydrophilicity of the head groups, the competition of other moieties with the surfactant molecules during the arrangement of the lipid bilayer and the HLB values of the surfactant clearly demonstrate an effect on vesicle properties such as size, charge and drug entrapment. Apparently, the size of the prepared vesicles decreases by increasing the surfactant concentration, its carbon chain length and number, the hydrophilicity of the surfactant head group, and the hydrophilic- lipophilic balance (HLB) value. However, the competition that could be raised between a surfactant and the lipid could increase the vesicle size. Moreover, the effect of surfactant on the EE not only depends on the surfactants properties but also on the nature of encapsulated drug. The encapsulation of a lipophilic drug could be enhanced when surfactants with low HLB values are used, whereas surfactants with high HLB values enhance the encapsulation of hydrophilic drug. Generally, it has been reported that increasing surfactants carbon chain length and using gel like surfactants or surfactants of high transition temperature (Tc) could improve the EE. Moreover, unsaturated bonds (in their carbon chain) or using more liquid form surfactants may enhance the permeability of the vesicles across membrane; however, it is associated with drug leakage and therefore reduced drug entrapment. Higher concentration of surfactant sometimes improves EE due to increasing the number of vesicles formed, but conversely other studies showed a reduced drug entrapment due to improved membrane permeability. It was also reported that higher surfactant concentration could lead to an increase in the charge, which in turn reduces vesicles aggregation and enhances the system stability. Surfactants have a significant bearing on the pharmacokinetic and pharmacodynamic properties of lipid-based delivery systems. Mainly, surfactants maintain a desirable sustained release, enhance circulation time, targeting and cellular uptake of vesicles.

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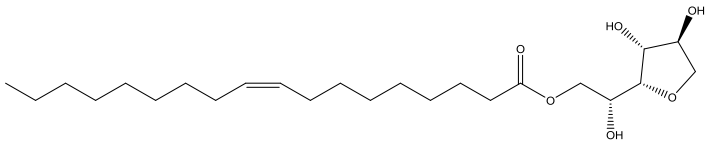
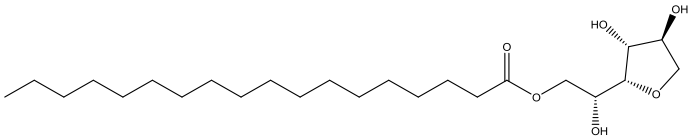
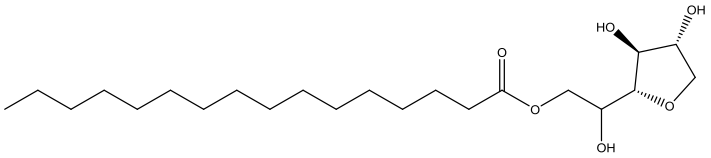
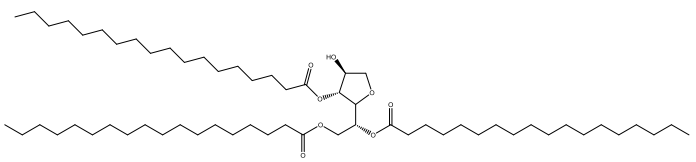
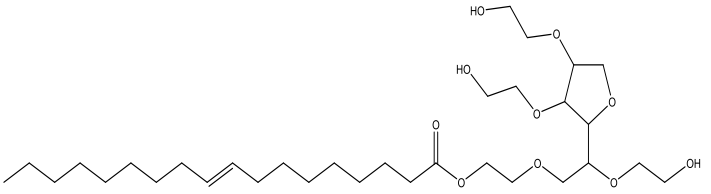
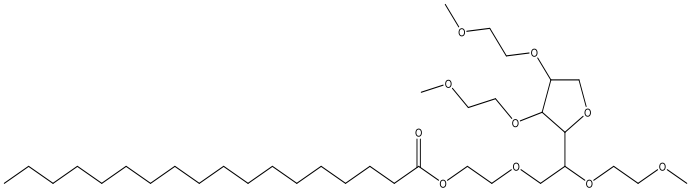
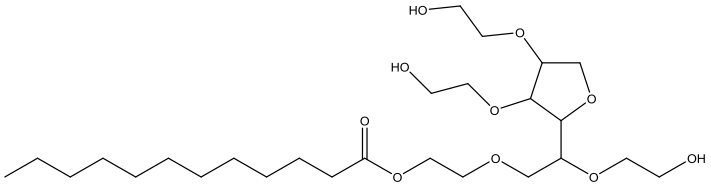
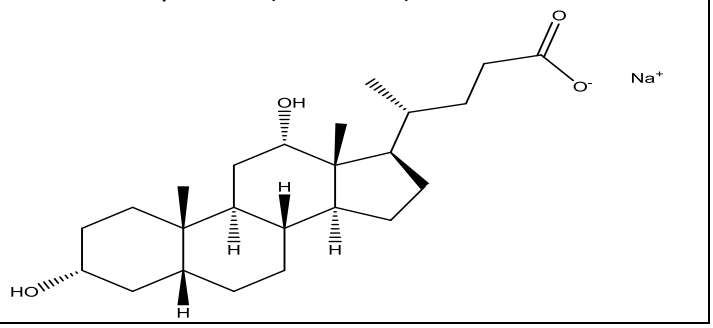
Table 3 summary of the main lipid based vesicular systems, with their main constituents, distinctive properties and disadvantages

Vesicular system	Main constituents	Distinctive properties	References
Liposomes (Figure 1, B)	• Natural or synthetic Phospholipids (neutral or charged). • Cholesterol.	• Sizes vary between 25-2500 nm • Suitable for both hydrophilic, lipophilic, small molecular weight and macromolecular drugs. • Reduced toxicity. • Targeted drug delivery could be achieved. **Disadvantages** • Drug leakage. • Expensive. • Low stability. • Low encapsulation of hydrophilic drugs. **Classified as** • Multilamellar vesicles (MLV): Onion structure, multiple bilayers enclosing many hydrophilic compartments. • Large unilamellar vesicles (LUV). • Small unilamellar vesicles (SUV).	6-8
Niosomes	• Non-ionic surfactant (uncharged single-chain surfactant). • With/or without cholesterol.	• Microscopic lamellar vesicles. • More stable than liposome. • Osmotically active. • Suitable for loading drugs with wide range of solubility. • Relatively less expensive than liposome. **Disadvantages** • Aggregation. • Fusion. • Drug leakage.	9,10
Transfersomes/ Deformable or Ultra-deformable liposome (Figure 1, B)	• Edge activator (surfactant). • Natural or synthetic Phospholipids (neutral or charged).	• High deformability. • Show very high encapsulation for lipophilic drugs. • More stability. **Disadvantages** • Difficulty of loading lipophilic drug without compromising their deformability. • Expensive to formulate. • Chemically unstable as they are more prone to oxidation.	11-13
Ethosomes/Elastic vesicles	• Ethanol (20-45%) as permeation enhancer. • Phospholipid.	• Increase cell membrane lipid fluidity due to the presence of ethanol. • Enhanced permeation profile, especially for dermal application. • Low risk profile or toxicity. • Relatively simple to manufacture.	14-18

		Disadvantages • Poor encapsulation/yield. • Possibility of vesicles disruption.	
Pro-vesicular system (Proliposomes/proniosomes)	• Water-soluble porous carrier (solid particles). • In addition to the same ingredients of the liposome or niosome respectively.	• Mainly to overcome the disadvantages of liposome /niosome. • Free flowing dry form that enhance the stability.	[19,20]
Other: **e.g. Herbosomes/Sphingosomes/Genosomes**	• Similar to liposomes, where lipid charge, type, or nature determine the type of the vesicles.	• Improved stability, e.g. herbosomes as they have phytochemical water-soluble particles that form stronger bonds with phospholipids in comparison with liposomes. • Provide selective passive targeting, e.g. sphingosmoes as they contain sphingolipid which improves targeting. • Suitable for delivering specific substances such as gene, e.g. genosomes.	[1,6,21]

Table 4. Chemical structures of the most commonly used surfactants.

Span 80 ($C_{24}H_{44}O_6$) 	Span 60 ($C_{24}H_{46}O_6$) 
Span 40 ($C_{22}H_{42}O_6$) 	Span 65 ($C_{60}H_{114}O_8$) 
Tween 80 ($C_{32}H_{60}O_{10}$) 	Tween 60 ($C_{35}H_{68}O_{10}$) 
Tween 20 ($C_{26}H_{50}O_{10}$) 	Sodium deoxycholate ($C_{24}H_{39}NaO_4$) 

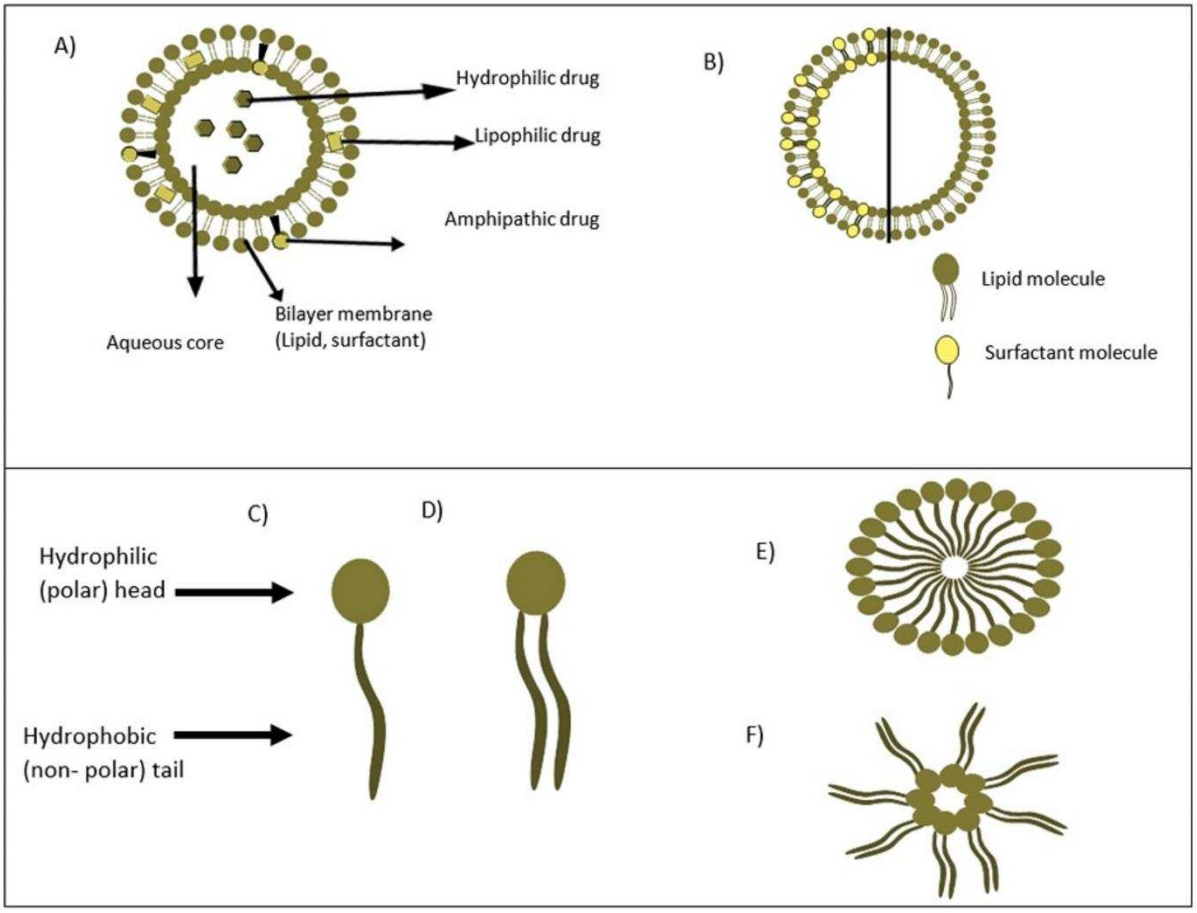


Figure 1. (a) The dispersion of drug molecules within the lipid-based vesicles, (b) comparison between conventional liposome on right half and transfersome (elastic liposome) left side, (c) surfactant monomer with one tail, (d) surfactant monomer with 2 hydrocarbon tails, (e) micelle (surfactant assembly) in aqueous medium, (f) micelle in nonaqueous *medium.*

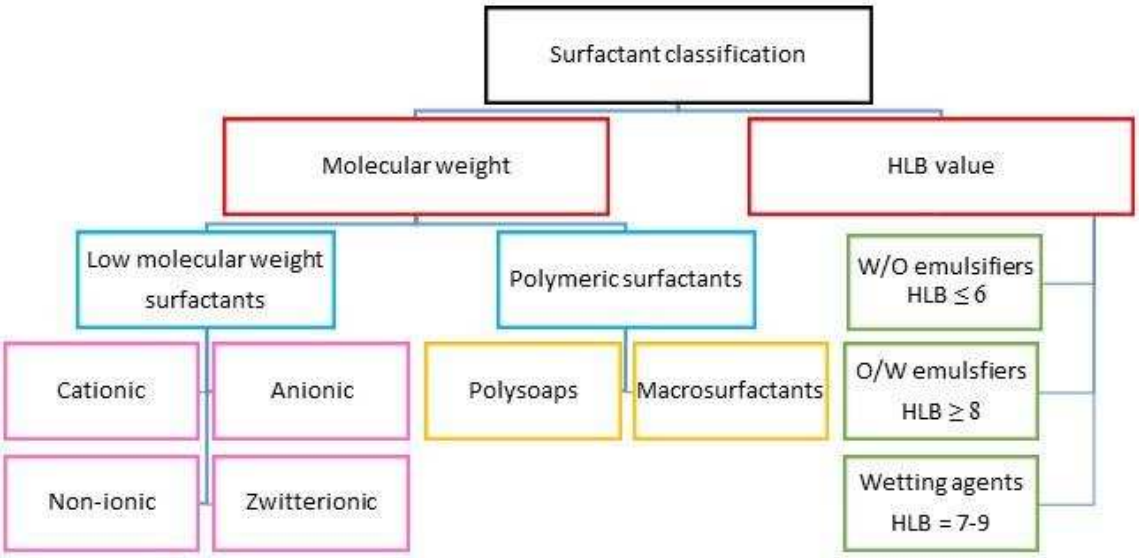


Figure 2. Illustrated diagram of surfactant classification based on molecular weight and hydrophilic lipophilic balance.