# Role of Surface Active Agents in Transferosome for Systemic Drug Delivery

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**Abstract:**

The recent utilization of transferosomes (a type of vesicular drug-carrier system) has shown promising results in enhancing the transport of drugs through the skin when administered topically. Phospholipids and surface active agents constitute the primary components of these entities. Surface active agents play a crucial role in enhancing the permeability and flexibility of lipid bilayers. The present research critically examines previous studies to gain insights into the influence of surface active agents on the characteristics and performance of transferosomes. Specifically, it focuses on the effects of these agents on transferosome size, entrapment efficiency (EE), zeta potential, stability, and transdermal flux. The type of surface active agent generally exerts a notable influence on the size of vesicles, encapsulation efficiency (EE), and zeta potential. This phenomenon could potentially be attributed to variations in the hydrophilic-lipophilic balance (HLB), the hydrophilicity of the surface active agent, and the length of the carbon chain. Therefore, it is imperative to investigate the influence of surface active agent properties in the development of transferosomes. The evaluation of the cytocompatibility of surface active compounds was also incorporated into the study.

**Key words:** lipid based vesicles, surface active agent, surfactant, transferosome.

# دور العوامل النشطة السطحية في الترانسفيروسوم لتوصيل الدواء الجهازي

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**الخلاصة:**

أظهر الاستخدام الحديث للاجسام الناقلة (نوع من النظام الحويصلي الناقل للأدوية) نتائج واعدة في تعزيز نقل الأدوية عبر الجلد عند تناولها موضعيًا. تشكل الدهون الفوسفاتية والعوامل النشطة السطحية المكونات الأساسية لهذه الحويصلات. تلعب العوامل النشطة السطحية دورًا حاسمًا في تعزيز نفاذية ومرونة طبقات الدهون الثنائية للحويصلات. يدرس البحث الحالي بشكل نقدي الدراسات السابقة لاكتساب نظرة ثاقبة حول تأثير العوامل النشطة السطحية على خصائص وأداء الاجسام الناقلة. على وجه التحديد، فإنه يركز على آثار هذه العوامل على حجم النقل، وكفاءة الانحباس، والشحنة السطحية، والاستقرار، والتدفق عبر الجلد. عادةً ما يكون لنوع العامل النشط السطحي تأثير ملحوظ على حجم الحويصلات وكفاءة الانحباس والشحنة السطحية. يمكن أن تعزى هذه الظاهرة إلى الاختلافات في التوازن المحب للماء والدهون، وذوبانية العامل النشط السطحي للماء، وطول سلسلة

الكربون. لذلك، لا بد من دراسة تأثير خصائص العامل النشط السطحي في تطور الاجسام الناقلة. تم أيضًا دمج تقييم التوافق الخلوي للعوامل النشطة السطحية في الدراسة.

**الكلمات المفتاحية:** حويصلات دهنية، العامل النشط السطحي، خافض التوتر السطحي، الاجسام الناقلة.

## Introduction

Pharmaceutical dosage forms are referred to as topical preparations that are administered to the epithelium covering a body surface, such as the skin, the eye cornea, the nasal, vaginal, or rectal mucosa. Generally speaking, topical medicines are utilized for the delivery of localized effects at the application site by penetrating the drug into the skin's or mucous membranes' deeper layers. Even though some inadvertent systemic medication absorption may happen, it often happens in negligible amounts and is therefore of little significance. However, some topical formulations, such as the transdermal drug delivery system, are intended for the systemic absorption of medicinal compounds in therapeutic levels[(1)]. Transferosomes, a vesicular drug-carrier system, have recently been demonstrated to improve transdermal drug delivery when applied topically without occlusive contact. Transferosomes are synthetic vesicles that are far more deformable than typical liposomes. Utilizing the right ratio of surface active agents will increase the deformability of liposomes for enhanced drug molecule penetration into the skin. Surface active agents provide deformability to transferosome and help them to squeeze through stratum corneum pores that are fve times smaller than their diameter. Upon topical application, the subsequent enhancement of transferosome membrane flexibility decreases the likelihood of complete vesicle rupture and allows transferosomes to traverse the epidermis in accordance with the inherent water gradient[(2).] Because transferosomes are made of both synthetic and natural phospholipids and edge activators, they are both biocompatible and biodegradable in the body. Transferosomes are widely used by researchers as carriers for protein molecules, anti-cancer medications, non-steroidal anti-inflammatory medicines (NSAID), anaesthetics, insulin, and corticosteroids for transdermal and cutaneous drug administration because of their ultradeformability[(3)] , as shown in Figure (1). They are able to transport medications with a high molecular weight through intact skin because they can quickly and easily change their size and form in response to external stressors, allowing them to pass through pores considerably smaller than their own. Transferosomes are an alternative to subcutaneous insulin administration because it's high molecular weight makes it difficult for them to reach the bloodstream and the skin's deepest layers[(4)].

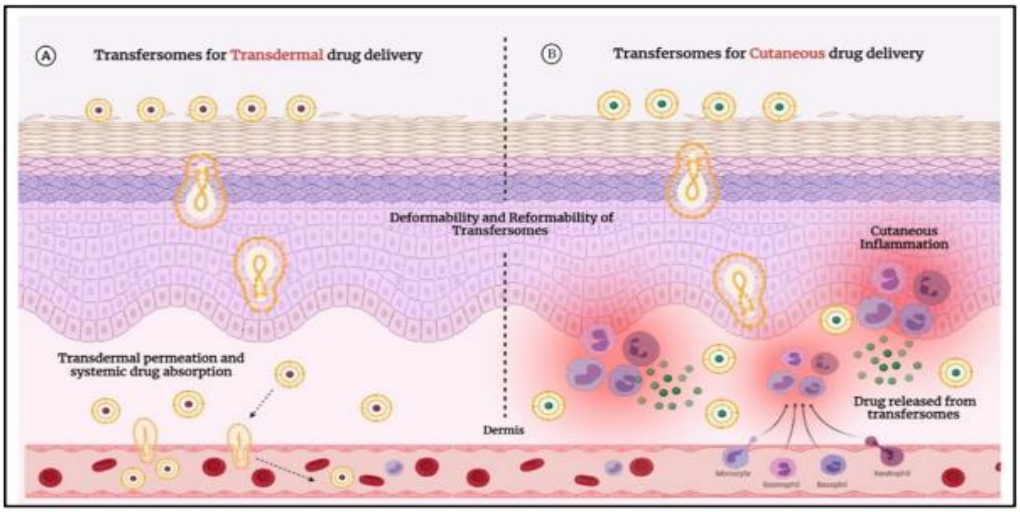


**Figure (1): ( A) Transdermal drug delivery via transferosomes (B) Cutaneous drug delivery via transferosomes[2]**

## Transferosome composition

There are two primary components that make up a transferosome: First, phosphatidylcholine, an amphipathic component in which the aqueous solvents self-assemble form a lipid bilayer that closes into a straightforward lipid vesicle and the second is the surface active agent that boosts the permeability and flexibility of lipid bilayers [5], Figure (2).

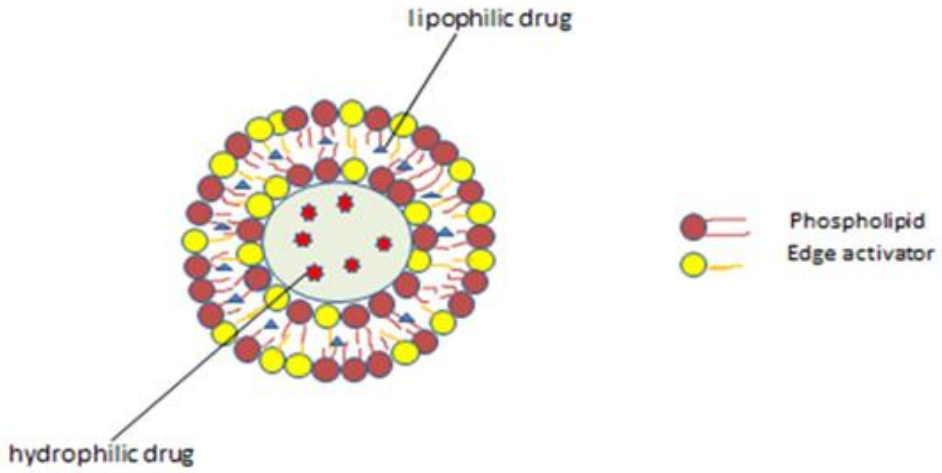


**Figure (2): Structure of transfersomes**

## Surface active agents (SAAs)

Surface active agents are amphipathic molecules that are primarily made of two primary moieties, a non polar lipophilic half that is connected to a polar hydrophilic component[6]. The tail of the lipophilic component, which typically has a branching or straight hydrocarbon chain with eight to

eighteen carbon atoms. Surface active agents often adsorb on the interfacial surfaces in aqueous media and exist as monomers at low concentrations. As a result, they displace certain molecules of the surface and weaken inter-molecular interactions, which lowers surface tension[7]. However, they aggregate and form micelles above a specific concentration, known as the critical micelle concentration (CMC). The technique of measurement, as well as additional variables like surface tension, viscosity, temperature, and conductivity, might affect the CMC value for each surface active agent[8, 9]. The hydrophilic heads and tails of an aqueous molecule face the aqueous environment and the non-aqueous environment, respectively. In a non-polar medium, micelles exhibit a similar functionality; however, they adopt an inverted arrangement wherein the polar groups orient towards one other and the tails extend outward into the non-aqueous medium[10].

## Surface active agents classifications

### Classification depends on molecular weight

### Low molecular weight surface active agents

Low molecular weight surface active agents can be divided into four main categories based on how their hydrophilic components behave.

Firstly, the hydrophilic components of anionic surface active agents are negatively charged. They are used widely because they are inexpensive. They could, in general, be phosphates, sulphonates, carboxylates or sulphates[11].

Socondly, Positive hydrophilic components and frequently a natural fatty acid are features of cationic surface active agents. Quaternary ammonium compounds, namely alkyl dimethyl benzyl ammonium chloride, are widely utilized as cationic surface active agents. These compounds find frequent application as pharmaceutical preservatives in various formulation[12].

Thirdly, Zwitterionic (amphoteric surface active agents) include both anionic and cationic groups, and the pH of the liquid in which they are dissolved affects how they behave. In an alkaline pH environment, anionic surface active agents are able to accept a negative charge, but in an acidic pH environment, they are capable of accepting a positive charge and exhibit cationic behavior. Cationic and anionic surface active agents are found to have lower water solubility, reduced stability, and limited compatibility with other surface active agents and various media, in contrast to the observed characteristics of the mentioned agents[13].

Fourthly, Non-ionic surface active substances are differentiated from cations by the presence of uncharged hydrophilic groups such as alcohol, ether, ester, or amide groups, which exhibit limited solubility in aqueous solutions. Fatty acid ethoxylates, alcohol ethoxylates, and sorbitan esters ethoxylates represent a limited selection of the diverse range of compounds that fall within this category. Glycerol esters, sucrose esters, glycol esters, and glucosides are other examples of polyhydroxy compounds[14].

### Polymeric surface active agents

In the past two decades, there has been significant progress in the development of polymeric surface active agents. These agents possess hydrophilic and lipophilic properties and can be integrated into various macromolecular architectures. They are currently widely utilised because of their significant usage as stabilisers in the creation of emulsions and suspensions. To improve their qualities and produce molecules that function well in a range of pH, temperature, and medium conditions, these surface active agents have undergone a number of alterations[15]. One distinguishing feature of polymeric surface active agents is thought to

be the quantity and distribution of hydrophilic and lipophilic groups along the carbon chain. The enormous structural complexity of polymeric surface active molecules demonstrates a variety of behavioural anomalies when compared to low molecular weight surface active agents[16]. These polymeric surface active agents are often divided into two primary types, polysoaps and macrosurface active agents, depending on how the hydrophilic and lipophilic moieties are distributed[17].

### Classification depends on the hydrophilic-lipophilic balance (HLB)

The hydrophilic-lipophilic balancing classification system was created by Griffin throughout the 20th century. The scale in question is a quantitative representation of the relative abundance of hydrophilic and lipophilic groups within surface active substances[18]. The classification of HLB is based on the spectrum of HLB values, wherein each group encompasses a set of surface active agents that exhibit similar characteristics. HLB values between 3-6 increase a surface active agent's lipophilicity and ability to form micelles or vesicles that are more soluble in non-aqueous fluids and W/O emulsions. Hydrophilic and water-soluble oil in water (O/W) emulsifiers or solubilizers have HLB values of 8 to 18. Surface active compounds with HLB values between 7-9 are considered wetting agents and have both properties.Sometimes two or more emulsifying agents (surface active agents) can be used together to solubilize. Mixing Span 80 (sorbitane monooleate, which has an HLB value of 4.3) and tween 80 (polysorbate, which has an HLB value of 15) in varied ratios may span a range of HLB values to find the best composition for desired qualities[19].

### Surface active agents in trasnferosomes

Over the past few decades, surface active agents have increasingly been used in lipid-based vesicles[20]. Some studies have concentrated on employing certain surface active agents, such as studying non-ionic surface active agents in the case of niosomes[21]. While other studies have investigated the effects of using several surface active agents with different attributes on vesicle properties. The bulk of studies have also tried to improve lipid vesicle synthesis in order to get the right size, drug loading, and physiochemical characteristics. By maximising the effects of the selected surface active agent, this is accomplished[22].

## Surface active agent effects on the vesicles size

### Effect of surface active agent type on the vesicles size

The size of transferosome is significantly affected by the presence of surface active agents. For example; in a study of cox-2 inhibitors, transfersomes made with tween 80 typically had smaller vesicles, followed by transfersomes made with sodium lauryl sulfate (SLS), and then transfersomes made with cetrimide[25]. The observed conclusion can be attributed to the physicochemical characteristics of the surface active chemicals under investigation. Specifically, the enhanced solubility of these agents in the lipid bilayer can be attributed to the elongated hydrocarbon chain present in tween 80. This improved solubility ultimately leads to a reduction in the size of the vesicles. Tween 80 possesses the ability to establish hydrogen bonding with the hydrophilic polar head of the lipid, resulting in the formation of more flexible transfersomes. These transfersomes are notably influenced by sonication and exhibit reduced vesicle size[26]. Previous research stated that the produced vesicles reduced surface free energy caused the size of the vesicles to shrink when the HLB dropped[27]. However, due to the repulsive force inside the bilayers of the vesicles, transfersomes made with ionic surface active

agents (cetrimide and SLS) showed increased particle size[28]. Generally speaking, a surface active agent with a lower HLB value reduces the particle size[23].

**Effect of surface active agent: phospholipid ratio on vesicles size**

There was no link between the ratio of surface active agent to phospholipid and vesicle diameters in a study of risperidone transferosomes made with tween surface active agent; nonetheless, the presence of surface active agent is essential for consistently tiny and small sizes[26]. A study found that surface active agents are essential for lowering interfacial tension, preventing vesicles from aggregating, and thus reducing their size in lipid-based vesicles[10]. No interaction was observed between the effects of the drug:phospholipid ratio or the surface active agent:phospholipid ratio on particle size in transfersomes produced using either tween 80 or cetrimide.The ratio between surface active agents and phospholipids, as well as the ratio of phospholipids, are important factors to consider in this study[24]. An additional investigation revealed that altering the ratio of surface active agents to phospholipids leads to a notable decrease in vesicle size. This reduction can be attributed to the diminished interfacial tension resulting from a higher concentration of surface active agents. Furthermore, the negative charge of the prepared transferosomes contributes to steric stabilization and electrostatic repulsion, further influencing the reduction in vesicle size[25]. As a conclusion, there is no general effect can be considered in this investigation and the effect of surface active agent: phospholipid ratio on vesicles size may depends on other factors like the type of drug used, type of surface active agent and method of preparation.

**Effect of amount of surface active agent on vesicles size**

Results showed that an optimal dosage of 2% w/v of surface active agent for total transferosomal dispersions, produces particles of the ideal size in the creation of piroxicam transferosomes. Increasing or decreasing this amount causes changes in particle size. In terms of comparison, it was observed that transferosomes formulated with higher concentrations of surface active agents exhibited smaller sizes in comparison to those formulated with lower concentrations of surface active agents until a certain limit depending on the type of surfactants, beyond this limit the vesicle siz will increase[26]. There was a prior finding which suggested that the inclusion of substantial amount of SAA inside the lipid bilayer might augment its diameter, hence resulting in an increase in transferosomal size[27].

**Surfactant effect on entrapment efficiency**

**Effect of surface active agent type on entrapment efficiency percent (EE%)**

Any vesicular delivery system's primary objective during development is thought to be achieving a good EE.Several studies have attempted to add surface active agents to lipid-based vesicles to enhance drug entrapment of hydrophilic and hydrophobic compounds and lessen drug leakage from the vesicles[27]. There is currently no concrete evidence that certain surface active agent qualities could cause a particular entrapment, despite numerous attempts to study surface active agents' influence on enhancing the EE. It could include a variety of surface active agent characteristics, such kind and concentration, that could impact a drug's EE inside a certain lipid composition[28].The entrapment of Ivabradine HCl within the produced transfersomes was found to be significantly influenced by surface active agent type ($P < 0.05$). Transfersomes prepared with SLS exhibited EE% higher

than those prepared with tween 80 and the lowest one are those prepared by cetrimide[24]. For the formation of piroxicam transferosomes, four distinct surface active agents under experimental conditions were used. The major criterion for selecting an surface active agent was entrapment efficiency. In contrast to sodium deoxycholate, which created nice and clear dispersions with higher entrapment efficiency, tween 80 produced good dispersions but with poorer entrapment[26]. Additionally, it was shown that as the ratio of sodium deoxycholate to tween 80 increase the EE% in risperidone transferosomes increase[29]. The sodium cholate, pluronic F-127, and sodium deoxycholate exhibited HLB values of 22, 18, and 16, respectively. The molecular weights associated with these compounds were 12,600 Da, 430.6 Da, and 416.6 Da, correspondingly. During the centrifugation process for the separation of transfersomes, it has been observed that surface active chemicals with higher molecular weight possess the capability to disrupt the bilayer membrane of the vesicle. This disruption hinders the entrance of drugs into the surrounding aqueous medium. Although both sodium cholate and sodium deoxycholate possess anionic properties, the differential trapping efficacy can be attributed to the presence of an extra hydroxyl group in the structure of sodium cholate[22, 30]. In a nother recent study, report that the entrapment efficiency of ondansetron HCL largely increased upon the addition of a sodium deoxycholate[31].

**Effect of surface active agent: phospholipid ratio on entrapment efficiency**

Surface active agent:phospholipid ratio and EE% had a weakly negative, non-significant connection; nonetheless, as surface active agent concentration rose, EE% dropped[24]. This may be because there is a higher chance that surface active agent will be incorporated into lipids with membranes that are more permeable to vesicles and have a lower EE%[32]. Additionally, as the concentration of the surface active agent increase, mixed micelles form, which have a lower EE% due to their stiffness and smaller size[33]. The transfersomes of raloxifene were prepared using a formulation consisting of transfersomal vesicles composed of a 50:50 mixture of Span 85 and Span 80. The ultimate hydrophilic-lipophilic balance (HLB) value of the combination in the formulation was determined to be 1.769. The combined effect of the mixture on lipids was seen to exhibit synergistic behavior, as evidenced by the greatest recorded entrapment efficiency of 96% at a lipid:surface active agent ratio of 95:5 (w/w). In contrast, a lower entrapment efficiency of 88% was estimated at a ratio of 80:20 (w/w)[34] .

In general, there is no a clear relationship between the the surface active agent to phospholipid ratio and the EE%[24].

**Effect of surface active agent amount on entrapment efficiency**

In the formulation of piroxicam transferosomes when the surface active agent content is 2%, the entrapment efficiency is at its greatest[35]. This phenomenon may arise due to the enhanced medication partitioning, which occurs when the surface active agent molecule interacts with the phospholipid bilayer at a specific concentration. Hence, once the concentration of the surface active agent exceeds 2%, it is likely that the molecules will undergo micellization, leading to the creation of bilayer micelles. This process can cause the formation of pores in the vesicle membranes and ultimately culminate in the complete transformation of the vesicle membranes into mixed micelles[36]. Improved EE was obtained by surface active agent induced vesicle formation and adding fluidity to the membrane bilayer. Additionally, the hole

formation brought about by the high surface active agent concentration caused the vesicles to leak, leading to high drug loss and, consequently, reduced drug entrapment[23]. In another study it was evident that as surface active agent ratios were raised, the entrapment got smaller with respect to the type of surface active agent[37]. In summary, the amount of SAA increases EE until it reaches the mixed micelle concentration, beyond which additional increase causes EE to drop.

**Surface active agent effects on zeta potential and stability**

**Effect of surface active agent type on zeta potential and stability**

The determination of the electrostatic charge of lipid vesicles is crucial for evaluating their surface properties, since it can significantly impact their stability through the induction of aggregation or repulsive forces[38]. The lipid and surface active agent charges were thought to contribute to the vesicle surface charge. Zeta potential was said to be strongly impacted by surface active agent type. Cholate-based transfersomes had the highest negative zeta potential among surface active agent-based transfersomes[26]. Furthermore, the net charge of the transfersomes increased in tandem with the concentration of surface active agent. The substantial negative charge was found to be useful in the context of building transfersomes for transdermal drug delivery[38]. The perception was that the repulsive interactions arising from the charge of the vesicles and the skin surface would enhance the permeability and stability of transfersomes by means of surface active agents[39]. It is clear that the final zeta potential was significantly influenced by the type of surface active agent. SLS-based transfersomes had a lower negative zeta potential than tween80-based transfersomes, but cetrimide-based transfersomes had a positive zeta potential[24]. This outcome was accounted for the different ionic properties of the examined phospholipids that are bad and the surface active agents that are found in soy lecithin[40]. Based on previous research, the presence of both lipid and surface active agent charges leads to the generation of electrostatic charge on the surfaces of vesicles[41].

**Effect of surface active agent: phospholipid ratio on zeta potential and stability**

The ratio of surface active agents to phospholipids has a substantial positive relationship with zeta potential. In a recent study, SLS-based transfersomes demonstrated a significant negative association, whereas tween 80 and cetrimide-based transfersomes demonstrated a non-significant negative association[24]. Similar to this, other investigations revealed that when anionic surface active agent concentration increase, the net charge of transfersomes increase[41].

**Surface active agent effects on transdermal flux**

Numerous arguments suggest that the transdermal flux of transferosomes via the route of administration may be impacted by the presence of surface active agent. The investigation of raloxifene transfersomes was considered to explore the transderma flux interaction with surface active agent. Transfersomes with Span (80 and 85) demonstrated a consistent pattern of drug release in all formulations. The flow increased dramatically when the surface active agent ratio in the Span 80 formulations increased from 5 to 20%. With the exception of formula 4 of span 85 based transferosomes, all formulations in the span 85 based formulas showed a significant difference when compared to formula 5 of span 85 based transferosomes. Formula 5 of transfeosomes prepared with span 80 + span

85 had a synergistic effect since the flow was higher than in transfeosomes prepared with span 80and transfeosomes prepared with span 85 alone formulations[42]. The different quantities of surface active substances in the formulations can explain the differential transdermal flow of transfersomes formulations. As the concentration of surface active agents in the bilayer of transferosome increases, the vesicle lipid bilayer becomes disordered. Because of the non-occlusive application, the formulations are leaky and allow for increased medicine penetration into the skin[43]. On the other hand, drug is hampered by low surface active agent concentrations, which order the bilayer structure of vesicles. The lipid becomes more soluble and loses its bilayer structure due to the high concentration of surface active agent, making it leakier. Additionally, it causes the development of mixed micelles [44]. Due to their water-sensitive gradient and limited stratum corneum distribution compared to transfersomes, micelles vesicle has lower transdermal potential than transfersome for medication transport[45]. Results of resveratrol transferosome study, showed that the greatest surface active agent ratios, the maximum resveratrol, and the phospholipids ratio to surface active agent were the main determinants of resveratrol permeattion[37]. When the type of surface active agent was taken into account in a research of cinnarizine transfersomes, an inversely proportional relationship between the EE% and the percentage of drug released was discovered[44].

### Cytocompatibility of surface active agents on transferosome

A toxicity experiment was performed on a human retinal epithelial primary cell line (HREP) to examine the safety of using the generated transferosomes in the eye. This cell line was chosen because retinal epithelial cells are extremely sensitive to outside stimuli. 3.5 mg/mL stock dispersions of cyclosporin A (CysA) transferosomes hydrated with 1% v/v surface active agent concentrations were used to compare cell viability to that of the negative control. Tween-based transferosomes had a greater viability percentage than Span-based transferosomes at all dosages tested. HREP cells reacted just as strongly to empty transferosomes as they did to transferosomes containing CysA. The cells' morphology reflects the environment in which they are developing[46]. As a result, the aberrant morphology might represent a stress reaction to the hazardous components in the growing media[47]. The MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) test which is a colorimetric assay for measuring cell metabolic activity, was utilized in another investigation to investigate how different surface active agent-based transferosomes affected the viability of the HepG-2 cell line(a cell line exhibiting epithelial-like morphology that was isolated from a hepatocellular carcinoma). The harmful surface active agent discovered by the viability test was sodium cholate. Tween 80 toxicity increased when combined with sodium cholate or deoxycholate. Even if the exact mechanism is unknown, it is likely that the combination had a synergistic effect on cellular toxicity[48].

## Conclusion

This study provides a summary of prior studies in the literature that have examined the impact of surface active agents on the characteristics of vesicular systems. The influence of various factors on the properties of vesicles, including size, zeta potential, and drug entrapment, can be observed through the modification of parameters such as surface active agent concentration, carbon chains number, carbon chain length and hydrophilicity of the head groups. Effects of surface active agents on transdermal flux was reviewed and found that

increasing surface active agent concentration to a certain limit may lead to increase in the transdermal flux, in addition the use of more than one surface active agent may give synergistic effect to promote the flux. However, substantial increase may lower the transdermal flux of the drug since it may decrease the deformability of transferosomes. In summery, it was reported that certain SAA like tween is more safe than span and both were less toxic than sodium cholate or deoxycholate. In general, the combination of more than one SAA may have more hazardous effect on cell viability.

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