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Fabrication and characterization of sildenafil citrate loaded transfersomes as a carrier for transdermal drug delivery

Abstract

The aim of this study was to enhance the transdermal delivery of Sildenafil Citrate by encapsulation in nano sized ultra deformable vesicles, transfersomes. The thin film hydration and sonication technique was utilized in the preparation of Sildenafil Citrate loaded Transfersomes and both non ionic and ionic edge activators (anionic and cationic) were used in varying ratios to study their effects on the physicochemical characteristics and the release properties of the prepared formulations. Results revealed that the mean size of the prepared Sildenafil Citrate loaded nano vesicles ranged from 69.08nm to 265.80nm, the encapsulation efficiencies ranged from 59.90% to 92.49% and the *in vitro* drug release through synthetic membranes ranged from 40.21% to 99.99%. All formulations were subjected to rank order based on their physicochemical characteristics and *in vitro* release profiles. Transmission Electron Microscope imaging of the chosen formulations revealed the general images of transfersomes formulations and the predicted differences between the formulations. Results for the *ex vivo* release of the chosen formulations through isolated skin of rats ranged from 76.29% to 99.90%. Stability studies for the chosen formulations showed that formulations were generally stable for at least 3 years.

Keywords: sildenafil citrate, transfersomes, transdermal, anionic, cationic, edge activators

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Abbreviations: SC, sildenafil citrate; SLS, sodium lauryl sulfate; PS, particle size; PDI, poly dispersity index; ZP, zeta potential; EE%, entrapment efficiency; SC-aTS, sc loaded anionic transfersomes; SC-cTS, sc loaded cationic transfersomes; SLS, sodium lauryl sulphate; T80, tween 80; S80, span 80

Introduction

Transdermal drug delivery has acquired an increasing interest in the recent decades as it overcomes the disadvantages of conventional delivery systems. The major barrier for transdermal drug delivery is the *stratum corneum* which is the outermost layer of the skin being composed of many layers of dead dehydrated corneocytes embedded in a lipid matrix.[1] Utilization of ultra flexible drug delivery system, Transfersomes, which can penetrate through the deep layers of the skin, was the aim of this work. These ultra flexible nano sized vesicles have extra ordinary flexibility that they can squeeze themselves to penetrate through pores smaller than their own diameter by 5-10 folds. Having both hydrophilic and lipophilic moieties, Transfersomes can accommodate a varying range of biogenic molecules of varying solubilities and molecular weights.[2] Sildenafil citrate (SC), the drug model utilized, has been utilized to treat erectile dysfunction, a major sexual problem.[3,4] In addition, it was utilized to increase feto placental perfusion in pregnancy, to aid in vitro fertilization, to prevent preeclampsia[5] to treat pulmonary arterial hypertension[6] and in cellulite and skin aging formulae.[7] However, oral administration of SC encounters numerous obstacles. The drug is vulnerable to considerable intestinal and hepatic first-pass metabolism (approximately 71% of oral dose). The drug is metabolized in the liver by the enzyme cytochrome P3A4, with an oral bioavailability of 40%.[8] In addition, relatively delayed onset (30-45 minutes) and short duration of action have been demonstrated for SC, with a half-life of 4hours, thus repeated doses are required to sustain drug plasma levels.[9] Moreover, numerous side effects such as blood pressure reduction, headaches, flushing, and nasal congestion are concomitant with oral administration. Encapsulation of SC in these nano sized ultra deformable Transfersomes can enhance the transdermal delivery of SC through a local tissue area and this could be an alternative to the oral route, in order to avoid these adverse side effects, to shorten onset time, and to sustain the effect for longer periods. The characteristics of Transfersomes are dependant to a great extent on the type and concentration of the used edge activator. In this work, the use of predetermined concentrations of edge activators of varying characteristics such as Tween 80(T 80), Span 80(S 80), Sodium Lauryl Sulfate (SLS) and Cetremide (Cet) was studied to take advantage of their varying effects on the formulated Transfersomes particle size (PS), Poly Dispersity Index (PDI), Zeta potential (ZP), entrapment efficiency (EE%) and both the in vitro and the ex vivo drug release characteristics. Furthermore, release kinetics, permeation studies and stability studies of the chosen formulations at 25°C and at 4°C were performed.

Materials and methods

Materials

Sildenafil citrate (SC) was a free sample from Nile drug company, Cairo, Egypt. The sample was of 100gm from batch number 284072009SC with purity of 99.31%, Phospholipid, Phospholipon 90 G (PL), Soybean lecithin was a generous gift from Lipoid Phospholipid GmbH, Nattermannallee 1, Germany, Methanol Analar SD company, Mumbai, India., Chloroform from Honeywell speciality chem.,

Germany, Cetremide (Cet) from Winlab Chemicals, U.K, Sodium Lauryl Sulphate (SLS) and Tween 80(T80) were purchased from ADWIC Company, Cairo, Egypt, Span 80 (S80) was supplied from Sigma Aldrich, USA, Sodium hydroxide and potassium dihydrogen phosphate were purchased from ADWIC Company, Cairo, Egypt. All reagents used were of analytical grade.

Preparation and characterization of the formulations

Preparation of SC loaded nonionic Transfersomes (SC-TS), SC loaded anionic Transfersomes (SC-aTS) and SC loaded cationic Transfersomes (SC-cTS)

Transfersomes are prepared by either two methods:

a. Rotary evaporation and sonication method.

b. Vortexing and sonication method.

Rotary evaporation and sonication method was found to give more stable Transfersomes.[10] Hence this technique was chosen for formulation development and batch processing. Table 1 shows the composition of the Transfersomal formulae containing SC prepared by thin film hydration technique. Formulations of SC-TS from 1 to 8 were prepared by weight ratios while formulae SC-aTS from 1 to 3 and formulae SC-cTS from 1 to 3 were prepared by molar ratios. The amounts of SLS in formulae SC-aTS were written in mgs which were equivalent to 0.27, 0.29 and 0.31mM of SLS (7.7, 8.0 and 8.9mg). Also, the amounts of Cet in formulae SC-cTS were written in mgs which were equivalent to 0.27, 0.29 and 0.31mM of Cet (9.8, 10.5 and 11. mg). The thin film hydration and sonication method was employed as described by El Zaafarany GM et al.,[11] Jain S.[12] The amount of PL, the edge activators (T 80, S 80, SLS and Cet) and SC as a drug were accurately weighed and dissolved in 1:1 mixture of chloroform and methanol. The mixture was sonicated for one minute with probe sonicator (Qsonica, model Q125, Qsonica LLC., USA) to ensure complete dissolution of all components. The resultant ivory yellow clear solution was transferred to the eggplant shaped round bottom flask of the rotary evaporator which was previously cleaned and oven dried. The temperature of the water bath was adjusted to 45°C which is about 10 degrees above the transition temperature of the phospholipids component (32 °C). The rotor speed was adjusted to 60rpm. Complete removal of the organic solvent was accomplished after 45 minutes resulting in a homogenously distributed thin film on the wall of the flask. The flask was kept under vacuum overnight to ensure complete removal of any residuals of the organic solvent. The thin film was then hydrated with phosphate buffer pH 5.8 and the volume was made up to 20ml, thus the total concentration of SC in all formulae was 500µg/ml. The flask was stirred at 120rpm for 30minutes till complete dissolution of the thin film. The resulting vesicular suspension was kept overnight for annealing. The vesicular suspension was then sonicated using probe sonicator at 4°C for 20minutes. The resulting formulae were kept refrigerated in a clean tightly closed umber colored glass containers.

Table 1 Composition of the formulations

Group	Formula code.	SC(mg)	PL(mg)	T 80(mg)	S 80(mg)	SLS(mg)	Cet(mg)
Group I	SC-TS 1	10	950	50			
	SC-TS 2	10	900	100			
	SC-TS 3	10	850	150			
	SC-TS 4	10	750	250			
Group II	SC-TS 5	10	950		50		
	SC-TS 6	10	900		100		
	SC-TS 7	10	850		150		
	SC-TS 8	10	750		250		
Group III	SC-aTS 1	10	758			7.7	
	SC-aTS 2	10	758			8	
	SC-aTS 3	10	758			8.9	
Group IV	SC-cTS 1	10	758				9.8
	SC-cTS 2	10	758				10.5
	SC-cTS 3	10	758				11.3

SC, sildenafil citrate; PL, Phospholipon G90; T 80, tween 80; S 80, span 80; SLS, sodium lauryl sulphate; Cet, cetyl pyridinium bromide; TS, transfersomes; aTS, anionic transfersomes; cTS, cationic transfersomes. Value for EE% is a mean of 3 observations ±SD

Drug encapsulation efficiency and physicochemical characterization

Drug encapsulation efficiency

Indirect method described by Patel R et al.,[13] was employed to calculate the amount of SC entrapped in the PL in each formula. Samples from the prepared formulations were transferred to eppendorff tubes and refrigerated at -20°C. The frozen samples were centrifuged at 14000 rpm for 30 minutes. The supernatant solutions were assayed for drug content spectrophotometrically after dilution with phosphate buffer pH 5.8.[14] The EE% was calculated from the equation:

$$\frac{\left(Initial\ SC\ concentration\ -\ concentration\ of\ unentraped\ SC\right)}{\left(Initial\ SC\ concentration\right)}\times\left(100\right)$$

Particle size, poly dispersity index and zeta potential determination

Vesicle size, size distribution and Zeta potential were determined by dynamic light scattering (DLS) technique.[15] Samples were prepared in buffer and diluted with distilled water in 1:50 ratio and then the required measurements were done by Malvern Zetasizer, model Nano Z.S., U.K.

In vitro drug release and release kinetics

In vitro SC release

Locally fabricated Franz diffusion cells were employed. The receptor chambers were of a size of 57 ml. The donor chamber was fixed to the receptor chamber by means of adhesive tape. The cells were shacked constantly at 100rpm by means of a thermostatically controlled electric shaker whose temperature was kept at 32±1°C.[16] Mixed Cellulose Ester membrane of average pore size of 0.45µm was used. The membranes employed were soaked in the release media-phosphate buffer-at room temperature over night in order to allow the membrane pores to swell. The sampling port was employed to withdraw samples at predetermined time intervals and to add fresh buffer to the media. Samples were collected at time 0, 0.5, 1, 2, 3, 4, 5 and 6hours. The results were compared to that of pure drug suspension in the same concentration of the formulae (500mcg/ml).

SC release kinetics

The *in vitro* drug release data were fitted to three different kinetic models which are often used to describe the behaviour of the drug release from nano spheres, i.e. zero-order, first-order and Higuchi diffusion models. The proper mode of release was determined based on the correlation coefficient (r) for the linear regression fit of the parameters involved, where the highest correlation coefficient represents the actual mode of the release.

Rank order of the prepared formulations

Based on their physicochemical characteristics and on their *in vitro* release profile, all formulations were subjected to rank order to choose one formula from each group to proceed into further processing.

Characterization of the chosen SC loaded transfersomal formulations

Morphology of the prepared nano transfersomes

The selected formulae were visualized using TEM microscope at Al- Azhar University Center for Fungi Research to determine the morphological characteristics of the resultant vesicles.

Ex vivo skin permeation studies

The abdominal skin of four male Sprague Dawley (SD) rats (200±25g) was removed after shaving by means of an electric shaver and animal clipper. The underlying superficial fascia and the attached blood vessels and fats were removed by means of forceps, surgical scissors and cotton pads impregnated with alcohol. The isolated skin was washed thoroughly by fresh buffer, treated with sodium azide in a concentration of 0.1% in order to stop all metabolic processes. The dermal part was inspected by means of a magnifier to ensure integrity of the stratum corneum. The skin was cut into pieces of 3x3cm and kept in nylon bags at -20°C for further processing.[17] Skin pieces were mounted between the donor and acceptor chambers with the dermal part facing the donor part to which samples of 0.5ml were added to assess the skin permeation characteristic of the selected formulae. The results were compared to that of pure drug suspension in the same concentration of the formulae. The ability of the prepared formulae to enhance the flux of the drug through isolated animal skin was assessed compared to the drug suspension. Fick's law was applied to the obtained results to determine the flux rate and lag times for the selected formulae and SC suspension.

Stability studies

Samples from the chosen formulae were kept in tightly closed clean umber coloured glass containers and stored at 4°C and 25°C. To assess the stability of the chosen formulae, we studied the effect of storage on their.

Physical stability

By studying the effect of storage on the prepared vesicles PS, PDI and ZP

Chemical stability

The amount of SC entrapped in each formula was assayed spectrophotometrically at one month intervals for threemonths by taking samples of the formulae stored at 4°C and at 25°C.[9] Results of the drug content measurements were subjected to kinetics study to determine the degradation rate constants of SC at the two storage temperatures. Arrhenius equation was applied to the obtained degradation rate constants at 4°C and 25°C. By the knowledge of SC degradation rates constants at these tempratures, the degradation rate constant at 20°C.and the corresponding activation energy (Ea) could be determined.The aforementioned parameters were used to determine the time required to 50% of the original amount of SC to be degraded (t50) and the time required for 10% of the original amount of SC to be degraded (t90). The latter represents the expiry date of each of the selected formulae

Results and discussion

Drug encapsulation efficiency and physicochemical characterization

Results for SC EE%, vesicles particle size, PDI, zeta potential and cumulative in vitro release are listed in Table 2.

Effect of the edge activator type on EE%

Values for the EE% measurements were greatly varied depending on the nature of the used edge activator. In the case of Tween 80 formulae which include SC-TS 1, SC-TS 2, SC-TS 3 and SC-TS 4, the lipid competes with the surfactant for the drug. The high HLB of the surfactant explains that at low surfactant concentration, the lipid which has higher concentration has higher chance to accommodate more fraction of the drug. Decreasing the lipid ratio decreases the amount of the drug entrapped in the lipid bilayer from 81.08% to 62.2% by increasing the surfactant ratio from 5% for SC-TS 1 to 25% for SC-TS 4. Results are demonstrated in Figure 1.

Table 2 Results for EE%, PS, PDI, ZP and IVR% for the prepared formulations

Group	Formula	EE%±SD	PS	PDI	ZP	IVR%
I	SC-TS 1	81.08±0.01	102.5	0.202	0.213	99.98%
	SC-TS 2	78.45±0.04	95.31	0.204	0.722	80.57%
	SC-TS 3	65.52±0.48	77.62	0.238	-10.3	99.99%
	SC-TS 4	62.20±0.02	80.84	0.235	-3.22	99.99%
II	SC-TS 5	52.51±0.03	237.6	0.574	0.708	85.35%
	SC-TS 6	63.83±0.04	138.7	0.41	0.613	67.49%
	SC-TS 7	87.65±0.02	69.08	0.343	0.653	93.62%
	SC-TS 8	80.04±0.03	118.3	0.134	-0.646	71.99%
III	SC-aTS 1	92.49±0.09	265.8	0.368	-1.3	57.52%
	SC-aTS 2	82.43±0.08	122	0.377	-24.9	69.03%
	SC-aTS 3	93.85±0.05	131.4	0.415	-2.19	40.21%
IV	SC-cTS 1	97.23±0.10	98.92	0.343	9.37	76.51%
	SC-cTS 2	59.90±1.09	69.08	0.343	0.653	74.78%
	SC-cTS 3	67.67±0.07	81.75	0.388	15.4	71.91%

N.B. EE% (encapsulation efficiency), PS (particle size), PDI (poly Dispersity index), ZP (zeta potential), IVR% (cumulative release percent after 6 hours of in vitro release)

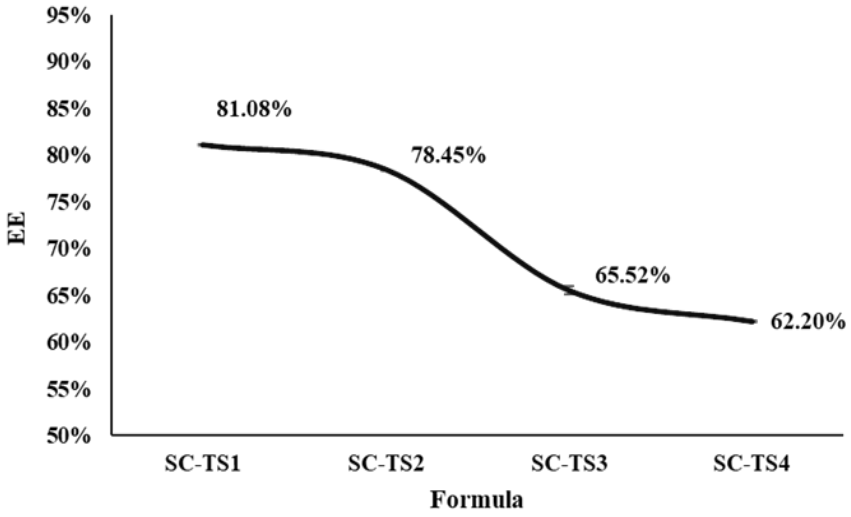

Figure 1 EE% of SC in T80 formulae.

For S80 formulae which include SC-TS5, SC-TS 6, SC-TS 7, and SC-TS 8, as illustrated in Figure 2. Increasing the concentration of the more lipophilic surfactant caused an increase in the entrapment efficiency from 52.51% for SC-TS 5 in which the surfactant concentration is 5% to 87.65% for SC-TS 7 in which the surfactant concentration is 15%. For both T80 and S80 formulae, when edge activator concentration exceeded 15%, mixed micelles coexisted with the Transfersomes, with the consequence of lower drug entrapment due to the rigidity and smaller size of mixed micelles as reported by El Zaafarany GM et al.[11] These findings obey the work done by Maghraby GME[17] who found that the displacement between the drug and the surfactant involves competition between species and, thus, becomes noticeable at high concentrations of the more lipophilic surfactant (S80 in this study).

As for anionic and cationic Transfersomes, as shown in Figure 3, it was found that charged surfactants can increase the EE% of the vesicles. Anionic surfactant could increase the repulsive force between intrabilayer of Transfersomes. It also could increase their elasticity by destabilizing the bilayer. Moreover, it may act as solubilizing agents in bilayer, thus anionic Transfersomes showed an increase in the EE% of formulae SC-aTS 1, SC-aTS 2 and SC-aTS 3 than those of cationic Transfersomes in formulae SC-cTS 1, SC-cTS 2 and SC-cTS 3. Cationic surfactants can also increase the EE% of

cationic Transfersomes by the same way. Cet was found to increase the drug content by acting as a solubilizing agent and by destabilizing the bilayer. It should be kept in mind that the lipophilicity of the drug and the high HLB of charged edge activators play a crucial role in explaining the fluctuations in the EE% by changing the concentration of the edge activators as discussed before. These findings obey the findings of[18] in their studies on meloxicam as a drug model.

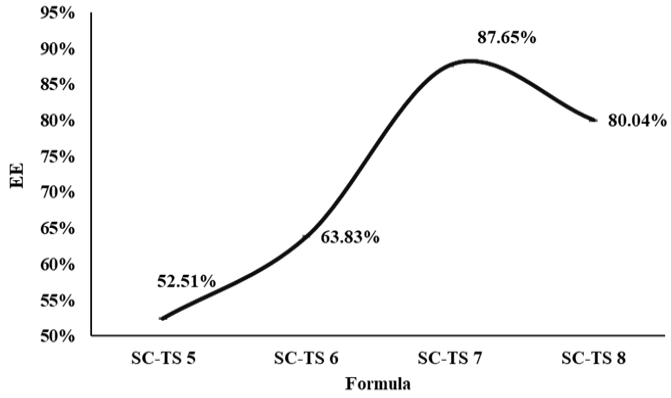

Figure 2 EE% of SC in S80 formulae**.**

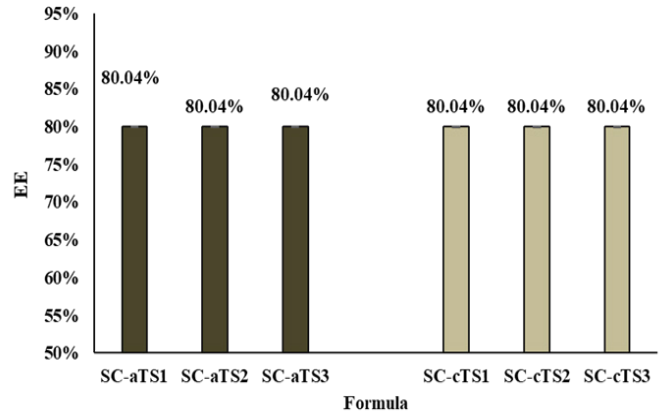

Figure 3 Average PS of TS prepared employing non-ionic surfactants.

Effect of the edge activator type on the PS of the prepared formulations

It is obvious from the results in Table 2 that Transfersomes prepared using T80 as edge activator were generally smaller in size than those prepared using S80 as edge activator at the same ratio of the surfactants and at the same formulation conditions. This can be explained by the differences in HLBs between the two edge activators which are 15 for T80 and 4.3 for S80. On the one hand, T80 molecule has highly flexible and non bulky hydrocarbon chains in addition to its high hydrophilicity. T80 includes in its structure a large head group (containing about 20 polyoxyethylene units) and an HLB value of 15, making it miscible in water. Thus, T80 is expected to partition between lipid bilayers and aqueous phase. T80 also contains the ethylene oxide and a long hydrocarbon chain imparting both lipophilic and hydrophilic characteristics to the vesicular formulation. It has been reported that T80 may interact with the polar head groups of the lipids and the modification of H-bonding and ionic forces may occur.[19] Thus, Transfersomes prepared using T80 have high elasticity that they can respond to a higher degree to sonication by a more degree of size reduction. T80, by its aforementioned characteristics, causes a higher degree of membrane fluidity by formation of transient hydrophilic pores which also enhance membrane fluidity. On the other hand, the more bulky and more hydrophobic S80 resulted in more rigid membranes which respond to a lesser degree to sonication. This could be a result of its high hydrophobicity, which reduced the formation of transient hydrophilic holes, hence, minimizing the amphiphilic property of the bilayers responsible for membrane fluidity. These findings can be correlated with those obtained by El Zaafarany GM[11] in their studies on Diclofenac Sodium as drug model. The obtained results for aTS and cTS were also affected by the nature of the used edge activator to a great extent. The results show that anionic formulae have greater sizes than cationic formulae prepared at the same edge activator ratio. The addition of anionic edge activator significantly increased vesicle size, and imparts more elasticity to the formulation. Anionic surfactant could increase the repulsive force between interbilayer of the vesicles, thus resulting in an increase the vesicle size. This obeys the findings of.[20] The characteristics of cationic and anionic edge activators were contrast, thus the effect of cationic surfactant on physicochemical characteristics of cationic Transfersomes was different. The addition of cationic surfactant in Transfersomes formulations slightly decreases vesicle size due to a decrease in the repulsive forces between the bilayers. The neutralization of negative charge of SC and positive charge of vesicle may result in a decrease in the repulsive force between the lipidic bilayer, therefore resulting in a decrease in vesicles size. Similar findings were observed by Duangjit S et al.[18]

Effect of the edge activator type on ZP

The results showed that absence of any charge imparting agent caused the potential of nonionic Transfersomes to be about zero. Similar findings obtained by Ahmed TA[21] Mahmood S.[22] The slight negativity of some formulae may be due to the negativity of the lipid component and the drug. The use of charged edge activators affected the net surface charge of Transfersomes prepared at the same molar ratio of the edge activator. The addition of anionic edge activator, SLS, added to the negativity of the formulae. The opposite, as predicted, was observed by the use of the cationic edge activator, Cetremide, which decreased the negativity of the vesicle and the drug switching the net charge to positivity.

In vitro release and release kinetics

In vitro release profiles of SC from the prepared Transfersomal formulations.

The cumulative release percent of all formulations after 6 hours ranged from 40.21% for formula SC-aTS 3 to 99.99% for formulae SC-TS 3 and SC-TS 4. Each formulation was subjected to in vitro drug release studies using a Mixed Cellulose Ester membrane. The cumulative amount of drug release was calculated for each formulation. Results revealed that formula SC-TS 3 (formulation with 15% T80) had the highest cumulative amount of drug release (99.99%) up to 6 hours as compared to other T80 formulae as illustrated in Figure 4. It was also found that formula SC-TS 7 (formulated with 15% S80) had the highest cumulative amount of drug release (93.62%) after 6hours as compared to other S80 formulae as shown in Figure 5. As for anionic and cationic Transfersomal formulae, it was found that

formula SC-aTS 2 (formulated with 0.30 M SLS) had the highest cumulative amount of drug release (69.03%) compared to other SLS formulae. It was also found that formula SC-cTS 1 (formulated with 0.29 MCet) had the highest cumulative amount of in vitro drug release of (76.51%) compared to other Cet formulae. Figures 6 & 7 illustrate the *in vitro* release profiles of anionic and cationic Transfersomal formulae.

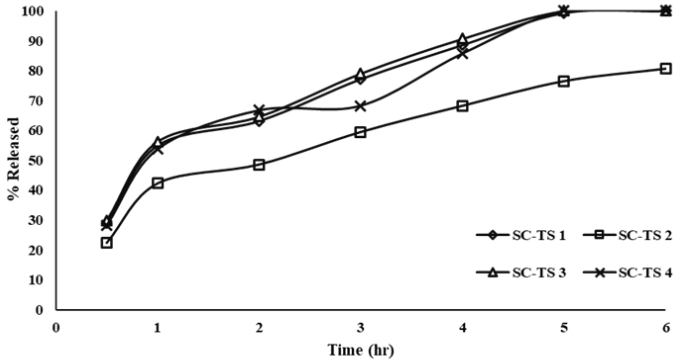

Figure 4 *In vitro* release profiles of T80 formulae.

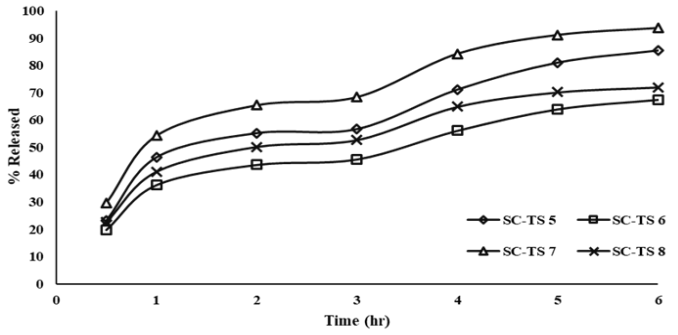

Figure 5 *In vitro* release profiles of S80 formulae.

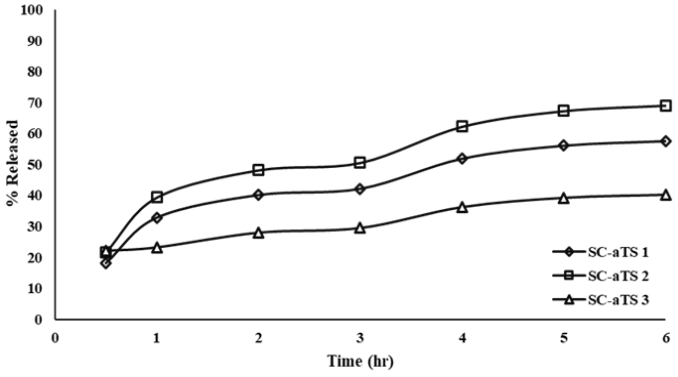

Figure 6 *In vitro* release profiles of SLS formulae.

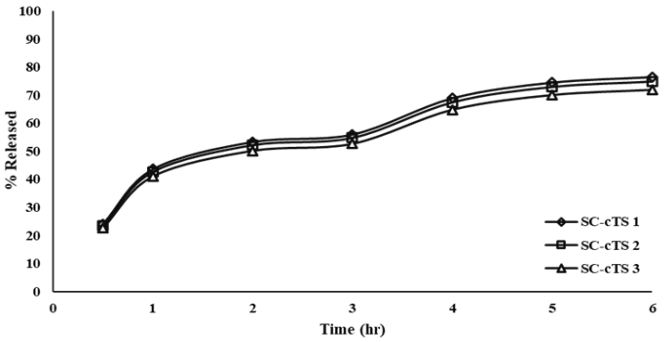

Figure 7 *In vitro* release profile of Cet formulae.

In vitro release kinetics

The study of the *in vitro* release kinetics showed that the release from the prepared formulations was by diffusion mechanism. These findings prove that release occurs mainly from the vesicle structure of the prepared TS, aTS and cTS. The release occurs first from the entrapped drug solution through the vesicle wall into the surrounding solution and then from this solution to the release medium through the membrane filter or the skin in the case of the ex vivo release.

Rank order of the prepared SC loaded transfersomal formulations

All formulations were subjected to rank order based on their physicochemical characteristics and on their in vitro release profiles to choose the best formula from each group. The results showed that formula number SC-TS 3 had the first rank of the formulations prepared using T80 as edge activator, formula number SC-TS 7 had the first rank of the formulations prepared using S80 as edge activator, SC-aTS 2 had the first rank of the formulations prepared using SLS as edge activator and formula number SC-cTS 1 had the first rank of the formulations prepared using Cet as edge activator.

TEM imaging of the chosen formulations

Transmission electron microscopy indicated the general images of Transfersomes vesicular formulations. At 100nm magnification, the particles were found to be unilamellar vesicles. The craters of each vesicle was dense indicating the lipidic bilayers while the core appeared lighter which represented the encapsulated drug solution as seen in Figures 9-11. Morphological studies proved the predicted differences between all formulae. T80 vesicles of formula SC-TS 3 were relatively smaller in size than those of S80 formula SC-TS 7. SLS vesicles of formula SC-aTS 2 were greater in size than those of formula SC-cTS 1 prepared using Cet as edge activator. As discussed earlier, this is owed to the difference in the HLB and surface charge of each surfactant. Having higher HLB, T80 resulted in smaller vesicles with lighter wall due to the formation of hydrophilic pores in the bilayer. SLS causes repulsion in the bilayer due to its negativity resulting in larger vesicles while the positively charged Cet decreased this repulsion resulting in smaller vesicles with more dense wall.

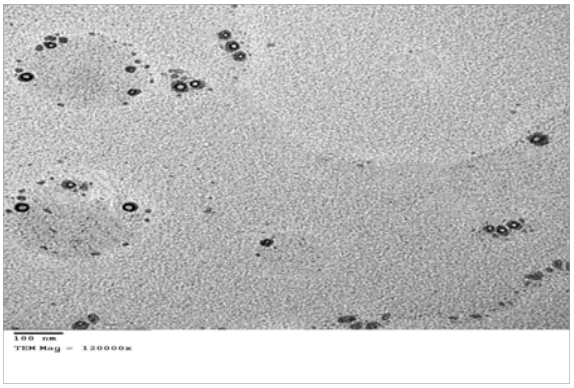

Figure 8 TEM imaging of formula SC-TS 3.

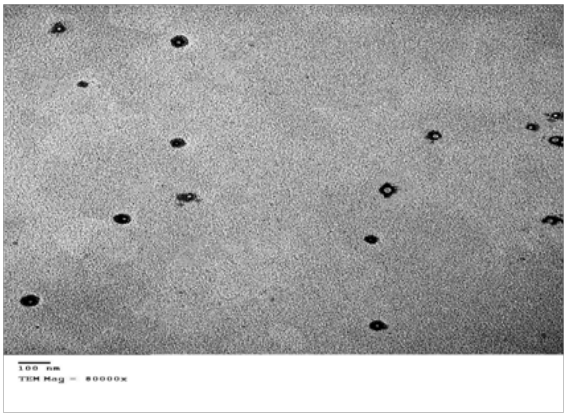

Figure 9 TEM imaging of formula SC-TS7.

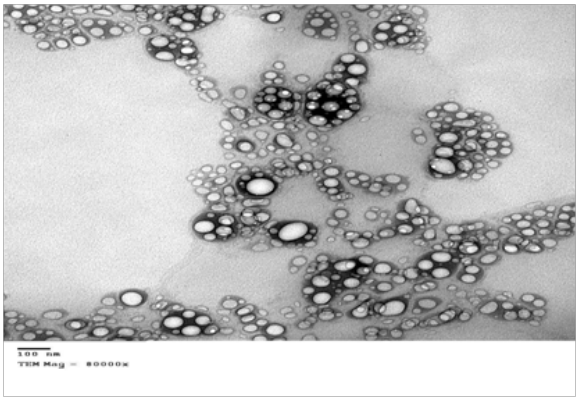

Figure 10 TEM imaging of formula SC- aTS 2.

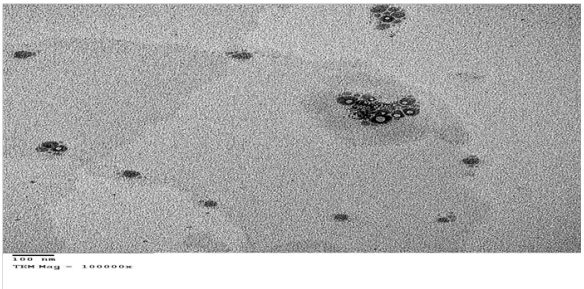

Figure 11 TEM imaging of formula SC-cTS 1.

Ex vivo skin permeation study

The chosen formulae SC-TS 3, SC-TS 7, SC-aTS 2 and SC- cTS 1 were selected for permeation studies through isolated skin of SD rats and compared with SC suspension. Results obtained from the *ex vivo* release of the chosen formulae SC-TS 3, SC-TS 7, SC-aTS 2 and SC-cTS 1 as well as drug suspension (DS) are illustrated in Table 3.

Ex vivo release kinetics

Results for the *ex vivo* release of the chosen formulations exhibited Higuchi model of diffusion which can be correlated to the data obtained from the *in vitro* release studies. The half life of release for the chosen formulations ranged from 3.76 hr for formula SC-TS 3 to 6.34 hr for formula SC-TS 7. The release profiles of SC from TS, aTS and cTS showed a biphasic release process, where initial burst release of the surface-adsorbed drug was observed, followed by slow diffusion from the lipid vesicles. At the initial 3 hours, higher release of SC from TS, aTS and cTS was observed. This could be attributed to more unentrapped drug distributing in the system. Unentrapped drug could pass through release interface earlier compared with drug entrapped in vesicles. Afterward, lipid vesicles played an important role in the release profiles and drug release rate slowed down. The percentages of cumulative drug release from formulae SC-TS 3, SC-TS 7, SC-aTS 2, SC-cTS 1 and DS after 9 hours were 99.09%, 76.29%, 99.90%, 93.14% and 52.33% respectively. As shown in table (3), the%cumulative amount of SC transferred from the chosen SC-TS, SC-aTS and SC-cTS showed a larger amount of drug permeation over time compared to DS. This could be attributed to the fact that TS, aTS and cTS act as a depot, hence, offer a mean for sustained release which can be correlated to the findings of.[15] Two mechanisms have been proposed for the improved skin delivery by deformable vesicles. The first mechanism proposes that vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin, under the influence of the naturally occurring in vivo transcutaneous hydration gradient.[2,23] This mechanism can explain why TS were able to permeate much larger amounts of SC through the skin, compared to DS. The second mechanism proposes that vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify its intercellular lipids, hence, raising its fluidity and weakness. Thus, drugs can further penetrate in higher amounts.[24] Moreover, phospholipids have a high affinity for biological membranes, thus, the mixing of vesicle-phospholipid bilayers with the intercellular lipid layers of the skin may also contribute to permeability enhancement of TS. This mechanism explains the higher skin permeation of Transfersomes over the DS. Both the penetrating enhancing effect and the intact vesicle permeation into the skin played a role in the enhanced skin delivery of SC by TS under non-occlusive conditions. This conclusion complies with the findings of[25,17] who reported that one of the 2 mechanisms might predominate according to the vesicle composition and characteristics, as well as, the physicochemical properties of the drug.

Permeation study

The ability of the chosen formulae to enhance the flux of the drug through isolated animal skin was compared to the drug suspension. Fick's law was applied to the obtained results to determine the flux rate and lag times for the selected formulae and SC suspension. Results for permeation studies are illustrated in Table 4. The values of the transdermal flux for formula SC-TS 3, SC-TS 7, SC-aTS 2

and SC-cTS 1 observed were (2.32, 1.79, 2.27 and 2.18 mcg/cm^2/h) respectively. Compared to the value obtained for drug suspension (1.52 mcg/cm^2/h), the formulated TS, aTS and cTS increased SC transdermal flux by 152.86% for formula SC-TS 3, 117.69% for formula SC-TS 7, 149.39% for formula SC-aTS 2 and by 143.68% for formula SC-cTS 1. The probable reason for the high permeation, diffusivity and partitioning of transfersomal formulations may be the partitioning of vesicles into the stratum corneum, which is an important process as it drives the partitioning of vesicle-bound drug into the skin. It can, therefore, strongly influence the flux and lag times obtained. The transfersomal formulation consists of polar lipids (Phospholipid + Edge activator) that have a tendency to attract water due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. So, when the transfersomal formulation was applied on the skin surface that was partly dehydrated by water loss due to evaporation, the lipid vesicles tried to evade complete drying by moving along the hydration gradient, resulting in the faster penetration of vesicles into the stratum corneum and other deeper layers of the skin.

Effect of storage on the stability of the chosen formulations

Physical stability

The effect of storage on the prepared vesicles PS, PDI and ZP was determined by DLS technique. No sedimentation was found in any vesicle formulation after fresh preparation. After storage at 4°C for 3months, as shown in Table 5, there was no sedimentation, but the average size of the vesicles in all formulations increased in different ratios ranged from about 18% for formula SC-TS 3 to about 10 folds for formula SC-TS 7 which explains the increase in the PDI.

Table 3 *Ex vivo* cumulative release results

Formula	Time in hours										
	0	0.5	1	2	3	4	5	6	7	8	9
SC-TS 3	0.00%	30.66%	44.48%	57.99%	68.87%	74.29%	80.60%	90.60%	95.88%	98.90%	99.09%
SC-TS 7	0.00%	23.60%	34.25%	44.65%	53.03%	57.20%	62.06%	69.76%	73.82%	76.15%	76.29%
SC-aTS 2	0.00%	34.33%	49.82%	64.94%	77.13%	83.20%	90.27%	95.96%	98.90%	99.90%	99.90%
SC-cTS 1	0.00%	28.82%	41.81%	54.51%	64.74%	69.83%	75.76%	85.16%	90.12%	92.96%	93.14%
DS	0.00%	8.07%	10.67%	28.76%	28.76%	33.64%	42.01%	49.71%	51.42%	51.99%	52.33%

Table 4 Permeation parameters values

Formula	*Jss* (mcg/cm^2/h)	LT (h)	P (cm hr-1)	D (cm^{2}hr^{-1})	K
SC-TS 3	2.324951	3.02126	0.00465	2.21E-07	42.1457
SC-TS 7	1.790105	3.0212	0.00358	2.21E-07	32.4496
SC-aTS 2	2.272181	3.71096	0.004544	1.80E-07	50.5918
SC-cTS 1	2.185294	3.02147	0.004371	2.21E-07	39.6168
DS	1.520987	0.94649	0.003042	7.04E-07	8.6376

Table 5 Results for PS measurement on storage at 4 °C

Formula	PS(nm) at 0M	PS(nm) at 1M	PS(nm) at 2M	PS(nm) at 3M
SC-TS 3	77.62	77.59	77.48	92.16
SC-TS 7	42.89	60.42	193.8	465.3
SC-aTS 2	122	131.32	157.4	409.4
SC-cTS 1	98.92	95.44	91.49	167.6

Chemical stability

The effect of storage on the drug content in the selected formulae was studied and hence accelerated stability testing was performed to determine the shelf life for each formula. The amount of SC retained in each formulation was assayed spectrophotometrically at one month intervals for threemonths for samples of the formulae stored at 4°C and at 25°C. Results for the drug content measurements at the predetermined time intervals are illustrated in Table 6. All results are taken in µg/ml.[26–28] Upon evaluating the chemical stability of the formulae, the percentage of SC remaining at 4°C for 3months ranged from 97.49% for formula SC-TS 7 to 95.51% for formula SC-TS 3 as illustrated in Figure 12.

Table 6 Results for rug content measurement at 4°C and at 25°C over 3months of storage

Formula	4°C				25°C			
	M0	M1	M2	M3	M0	M1	M2	M3
SC-TS 3	327.6	326.211	323.086	312.883	327.6	316.134	310.057	267.996
SC-TS 7	438.25	435.344	432.636	427.254	438.25	417.433	404.526	383.618
SC-aTS 2	412.15	407.076	402.814	394.984	412.15	391.625	374.384	342.698
SC-cTS 1	486.15	483.71	480.563	473.372	486.15	468.263	464.149	459.11

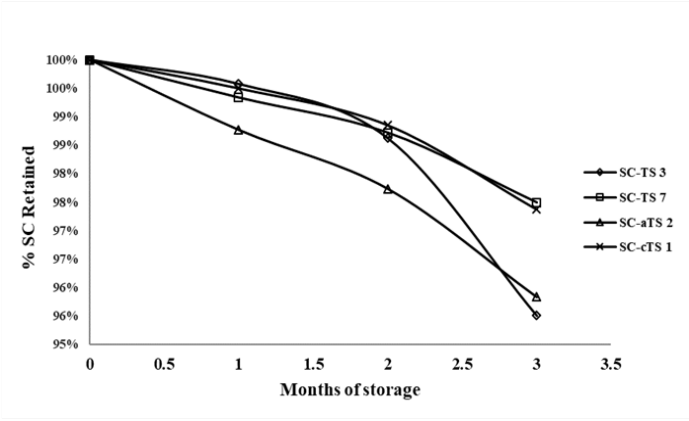

Figure 12 %SC Retained upon storage at 4°C for 3months.

Conclusion

Sildenafil citrate was successfully formulated in Transfersomes which improved its transdermal permeation compared to suspension of the drug. In employing non ionic surfactants in the formulation of Transfersomes, Tween 80 gave better results in the aspects of EE%, PS, PDI, in vitro and ex vivo permeation compared to Span 80. As for anionic and cationic Transfersomes, the use of the cationic surfactant, Cetremide, proved to give better results in the aspect of PS while Sodium Lauryl Sulphate gave better results in the aspects of ZP, EE%, in vitro and ex vivo drug permeation. The difference in the EE%, PS, ZP, and PDI in addition to the in vitro and ex vivo skin permeation may be owed to the physicochemical characteristics of the drug and the edge activator employed. For T80 formulae, formula SC-TS 3 which contained 15% T80 was superior to other T80 formulae giving a PS of 77.62nm, ZP of -10.3 mV, EE% of 65.52% and permeated about 99% of the contained drug over 6 hours of the in vitro and 9 hours of the ex vivo release studies. As for anionic Transfersomes, the anionic formula SC-aTS 2 which contained 0.30 mM of SLS was superior to other anionic formulae giving a PS of 122.0nm, ZP of -24.9 mV, EE% of 82.43% and permeated 69.03% of the contained drug over 6hours of the in vitro and 99.90% of the contained drug during the *ex vivo* permeation studies. According to the permeation studies, formulae SC-TS 3, SC-TS 7, SC-aTS 2 and SC-cTS 1 improved the flux of the drug through hairless SD rat skin by 152.68%, 117.6%, 149.39% and 143.68% compared to the DS respectively. As for the stability of the chosen formulae, both physical and chemical stabilities of the prepared formulae evoked that all formulae were generally stable.

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Conflict of interest

Author declares that there is no conflict of interest.

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