Journal of Nanotechnology & Advanced Materials

http://dx.doi.org/10.12785/jnam/

Transfersomal nanoparticles of keratolytic and antibacterial agents for enhanced transdermal delivery

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Received: 20 Jun. 2015, Revised: 22 Mar. 2015, Accepted: 24 Mar. 2015.
Published online: 1 May 2015.

Abstract: The aim of this study was to prepare transfersomal formulations as transdermal deliveries for drugs of bad solubility. Transfersomes (TRSs) were chosen and prepared containing sulfur and salicylic acid by thin film hydration method. Moreover, the size, zeta potential and morphology were investigated. The formulated TRSs showed a good, small and uniform particle size of 210±0.5, 212±0.5 and 230±0.5nm for plain TRSs, sulfur TRSs and salicylic acid TRSs; respectively. The TRSs had highly negative zeta potentials of -41±0.5mV, -24±0.4mV and -72±0.5mV for plain TRSs, TRSs loaded with sulfur, and TRSs loaded with salicylic acid; respectively. The prepared TRSs were formulated into gel which can be used as topical preparation with an enhanced permeation and penetration compared with conventional gels.

Keywords: Transferosome, Gels, Transdermal delivery, Salicylic acid, Topical preparation, Sulfur.

1 Introduction

Lyposomes, LiPOs, are non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations. LiPOs reduce the toxicity of the encapsulating agent e.g. (amphotericin B, Taxol) (1). Transferosomes (TRSs) are 'ultradeformable' LiPOs with enhanced skin penetrating properties which are used in our study.

TRSs are composed of phospholipid, surfactant, and water for enhanced transdermal delivery of drugs. These are ultradeformable LiPOs with enhanced skin penetrating properties. Transferosome is elastic in nature which can squeeze itself through a pore which is many times smaller than its size owing to its elasticity (2). TRSs were developed in order to take the advantage of phospholipids vesicles as transdermal drug carrier. These self-optimized aggregates, with the ultra-flexible membrane are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency (3). Flexibility of TRSs membrane is achieved by using suitable surface-active components in the proper ratios. The resulting flexibility of TRS membrane minimizes the risk of complete vesicle rupture in the skin and allows TRSs to follow the natural water gradient across the epidermis (4). They act as a carrier for low as well as high molecular weight drugs e.g. analgesic, corticosteroids, sex hormone, anticancer, insulin and albumin. It used for both systemic as well as topical delivery of drug. Highly flexible so have higher flux rate across skin and higher rate of penetration compared to other vesicular systems (5-6). All methods of preparation for TRSs are composed of two steps. First, a thin film is prepared, hydrated then brought to the desired size by sonication. Secondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane (3, 7-8).

Although the intact skin is much less permeable and also associated with low risk, the absorption of nanoparticles is primarily carried out through it. Depending on physicochemical properties of the compound, different pathways of penetration across the skin have been recognized that are intercellular, trans-cellular, and transappendageal through hair follicles and sweat glands (3). Number of factors that influence the dermal absorption of nanoparticles that can be divided into three groups as location and skin conditions at the application site, physicochemical properties of the penetrating molecule, and physicochemical properties of the vehicle dispersing the penetrating molecule. Apart from these factors like lipophilic-hydrophilic gradient, pH gradient and isoelectric point have their vital role which influences dermal absorption of nanoparticles (9-11). The presence of molecules such as solvents, surfactants, enhancers, and

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others may alter or damage stratum corneum by different processes thus causing a potential increase in the absorption of all or selected ingredients of the applied formulation (11).

Sulfur is an essential element for all life, and is widely used in biochemical processes. In metabolic reactions, sulfur compounds serve as both fuels (electron donors) and respiratory (oxygen-alternative) materials (electron acceptors). Sulfur is insoluble in water but soluble in carbon disulfide and, to a lesser extent, in other nonpolar organic solvents, such as benzene and toluene. Sulfur was used, mainly in creams, to alleviate conditions such as scabies, ringworm, psoriasis, eczema, and acne. Sulfur does oxidize slowly to sulfurous acid, which in turn (through the action of sulfite) acts as a mild reducing and antibacterial agent (12-13).

Salicylic acid is also known as 2-hydroxybenzoic acid. It is poorly soluble in water (2 g/L at 20 °C). Salicylic acid is a key ingredient in many skin-care products for the treatment of seborrhoeic dermatitis, acne, psoriasis, calluses, corns, keratosis pilaris, acanthosis nigricans, ichthyosis, and warts (14).

There are many diseases affecting the skin, sulfur (S) and salicylic acid (SA) are known to be effective topical delivery depending on their anti-inflammatory and comedolytic properties (15). The main aim of this study is to formulate, sulfur as an antibacterial as well as salicylic acid as a keratolytic agent as TRS in order to improve the penetrating properties of both S and SA through the skin.

2 Subjects and Methods

The transferosomes were prepared by thin film hydration method. The phospholipid (phosphatidyl choline) and the surfactant (span 80) were dissolved at 9:2 molar ratios into mixture of organic solvents (methanol and choloroform) at ratio of 1:2 v/v. After dissolving the mixture, the organic solvent was evaporated using rotary evaporator till the thin film layer is formed. The film was hydrated by PBS, and then put the film in Sonicator to reduce the size of the formed vesicles. The residual traces of organic solvents were removed by keeping the preparation into desicator overnight. For preparation of sulfur, salicylic acid loaded nanoparticles; the same previous steps were used but differ in encapsulation of the drugs into nanoparticles. Lipophilic drugs as sulfur added into organic solvents (methanol and chloroform), while hydrophilic drugs as salicylic acid was added to aqueous medium. The prepared suspension is characterized by particle size analysis, zeta potential and Encapsulating efficiency.

2.1. Size and zeta potential measurements by dynamic light scattering

To determine the size, count rate and zeta potential of TRSs (plain and loaded), the samples were adjusted to 25oC and laser light scattering analysis was performed with an incident laser beam of 633 nm at a scattering angle of 90o using the Malvern zetasizer nano 6.01 (Malvern Instruments GmbH, Herrenberg, Germany) (*16-18*).

2.2. Determination of encapsulating efficiency (EE %) determination

Encapsulation efficiency of drug in transfersomal formulations was performed by Freeze thawing/centrifugation method. 1 mL of each sample of TRSs was centrifuged at 14,000rpm for 60 min at 4°C. The precipitated TRSs were washed by PBS (PH 7.4) twice. The supernatant was separated each time from TRSs and assayed of free drug. The drug content was determined spectrophotometrically at 203nm using PBS (pH 7.4) (*19*).

EE% = Total drug concentration – Free drug concentration / Total drug concentration X 100

2.3. Gel preparation and characterization of rheological properties

For the preparation of gel, containing 2% W/V of carbapol 934, 2gm of carbapol 934 were dispersed in a half amount of water and stirred for 24 hours (mix 1). On the other hand, 0.4% W/V of sodium hydroxide were dissolved and stirred overnight. The dissolved NaOH was added on the (Mix 1) and stirred for around 6 hours. TRSs containing S and SA were mixed with the plain carbopol gel.

The gel samples were characterized by placing onto the bottom plate of the rheometer; the upper plate was then lowered to a gap size of 1000 µm. Storage (G') and loss modulus (G") were recorded at 25 °C and 1 Hz oscillatory frequency as a function of the applied stress. Gel properties and rheological analyses were studied in triplicate using a stress programmable rheometer (DV.Ultra, RVDV-111 U), Book field, USA. Be equipped with a cone-plate geometry (4/40) operating in the oscillation mode. The gap was 150cm. The following tests then were carried out.

3 Results

3.1. Particle size and zeta potential

Regarding the size, the results showed that good, small and uniform TRS nanoparticles were obtained as determined by DLS Malvern, Worcestershire, UK. The plain TRSs have uniform particle size of 210±0.5 nm. DLS recorded one peak, indicating that the nanoparticles of only one size. The sulfur containing TRSs have particle size of 212±0.5 nm. The particles of only one size and in uniform one. The particle sizes of salicylic acid containing TRSs were 230±0.5 nm, and showed another peak which could be due to some aggregations was recorded. In addition, the count rate and PDI were monitored. The recorded high-count rate value (185 kpcs) indicated that the concentration of nanoparticles was high enough for measurements. Also, the values of PDI were very small (0.16), indicating that the TRSs containing S and SA were uniform and monodisperse, This result is in accordance with the previously reported data as shown in figure (1) (*20-22*).

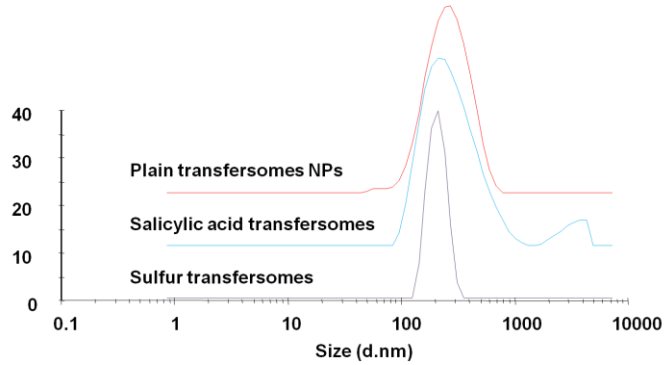

Figure 1; DLS of Particle size distribution of transfersomes nanoparticles

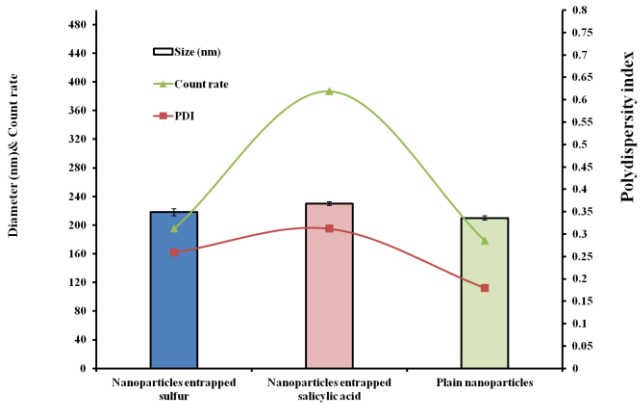

Figure 2; Particle size, PDI and count rate for plain, and formulated TRSs

3.2. Zeta potential results:

The zeta potential for all kinds of TRSs was measured in PBS buffer at pH 7.4. DLS measured zeta potentials highly negative surface charge of -41±0.5mV, -24±0.4mV, -72±0.5mV and -40±0.5mV for plain TRSs, TRSs loaded with sulfur and TRSs loaded with salicylic acid respectively. The highly negative zeta potential indicates that the particles are electrically stabilized to resist aggregation (*23*). This result is in accordance with the previously reported data (*23*).

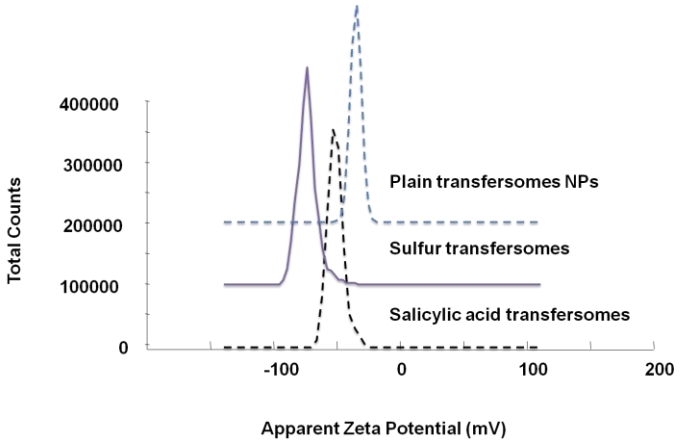

Figure 3; Zeta potential for TRSs nanoparticles as measured by DLS

3.3. Encapsulation effeciency :

The encapsulation efficiency was defined as the percentage ratio of the entrapped drug concentration to the total drug concentration. After centrifugation of 1 ml sample, samples were measured spectrophotometrically at wavelength 400nm and 205nm for S and SA respectively. The PC for S and SA was multiplied by absorbance then concentration was calculated indicating that the E.E of TRSs loaded with S and SA were 78.8%±0.95 and 93.3%±0.8 respectively.

3.4. Evaluation of TRSs gels

3.4.1. Visual appearance

Gel containing sulfur and salicylic acid was found to be uniform in consistency. Gel was evaluated microscopically for the presence of particulate matter, which showed no appreciable particulate under the microscope. Hence, the gel formulations fulfilled the requirement of freedom from particulate matter and grittiness. As desired for any topical preparation pH determination, the pH of each gel was noted and the results were taken as a mean of three determinations. The viscosity was 483×103±20 as determined by viscometer, indicating that the gel was formed in high and good gel formation.

The results obtained by dynamic light scattering (DLS) indicated a successful formulation of transfersomes, where the average diameters of all TRSs were in nano-size. All of the DLS results yielded a reasonable polydispersity index (PDI), all below 0.7. PDI is an index which indicates stability, since it represents the size distribution range in colloidal solution. High PDI indicates the heterogeneity of the particle size in suspension, while smaller PDI values indicate the homogeneity of the particle size in suspension. Polydispersity indices lower than 0.7 are ideal, as it indicates that the particle size distribution falls within a narrow range of sizes (*20-21, 24-26*). The average PDI results of plain, sulfur and salicylic acid containing TRSs were 0.311, 0.20 and 0.321 which show that all of nanoparticles are in ideal range below 0.7 and homogenous formulations. The high-count rate indicates that the concentration of nanoparticles were high enough for measurements. the average count rate for plain 321 , sulfur 217 and salicylic acid 378(*23*). The surface charge also plays an important role in the stability of the TRSs, and the magnitude of zeta potential is indicative of the colloidal stability of the system (*27*). The highly negative zeta potential indicates that the particles are electrically stabilized to resist aggregation (*23*). All results show that the value of z-potentail indicates a good stable TRSs. The increased E.E. % of S and SA within the transfersomal vesicles was resulted in a high concentration of drugs entrapped into the vesicles. The high EE % lead to high therapeutic effect (*19*).

4 Conclusion

The TRSs nanoparticles were prepared using dry thin film layer. They were characterized for particle size and zeta potential and encapsulation efficiency. The results of particle size and zeta potential showed that good, small and uniform TRSs were obtained using thin film hydration method. The formulated TRSs were of a uniform particle size of 210 ± 0.5 nm for plain TRSs, 212 ± 0.5 nm for those containing sulfur and 230± 0.5 for salicylic acid. From our study it can be concluded that the encapsulation efficiency of both S and SA in to TRSs showed good therapeutic efficiency.

Acknowledgements

I would like to thanks Ms Zobida Hassan, Rehab Saeed and Nada Elsaid for supporting us with materials and zetasizer measurements.

References

1. C. Yucel, Z. Degim, S. Yilmaz, *Biomed Pharmacother* **67**, 459 (Jul, 2013).
2. T. Rattanapak, K. Young, T. Rades, S. Hook, *J Pharm Pharmacol* **64**, 1560 (Nov, 2012).
3. I. A. Alvi *et al.*, *Anticancer Drugs* **22**, 774 (Sep, 2011).
4. S. Duangjit *et al.*, *Biol Pharm Bull* **37**, 239 (2014).
5. S. Duangjit, P. Opanasopit, T. Rojanarata, T. Ngawhirunpat, *J Drug Deliv* **2011**, 418316 (2011).
6. A. Schatzlein, G. Cevc, *Br J Dermatol* **138**, 583 (Apr, 1998).
7. T. A. Ahmed, *J Liposome Res* **25**, 1 (Mar, 2015).
8. W. S. Zheng, X. Q. Fang, L. L. Wang, Y. J. Zhang, *Int J Pharm* **436**, 291 (Oct 15, 2012).
9. S. Konda, S. R. Meier-Davis, B. Cayme, J. Shudo, H. I. Maibach, *Skin Therapy Lett* **17**, 1 (May, 2012).
10. K. Higaki *et al.*, *Int J Pharm* **239**, 129 (Jun 4, 2002).
11. V. Shahi, J. L. Zatz, *J Pharm Sci* **67**, 789 (Jun, 1978).
12. A. K. Gupta, K. Nicol, *J Drugs Dermatol* **3**, 427 (Jul-Aug, 2004).
13. F. Grossi, *Clin Ter* **131**, 413 (Dec 31, 1989).
14. R. K. Madan, J. Levitt, *J Am Acad Dermatol* **70**, 788 (Apr, 2014).
15. S. Akarsu, E. Fetil, F. Yucel, E. Gul, A. T. Gunes, *J Dermatol* **39**, 433 (May, 2012).
16. F. Destremaut, J. B. Salmon, L. Qi, J. P. Chapel, *Lab Chip* **9**, 3289 (Nov 21, 2009).
17. A. B. Leung, K. I. Suh, R. R. Ansari, *Appl Opt* **45**, 2186 (Apr 1, 2006).
18. Ahmed A. H. Abdellatif. J. Pharm. Sci. & Res., **7(1)**, 14-20, (Jan, 2015).
19. N. Berger, A. Sachse, J. Bender, R. Schubert, M. Brandl, *Int J Pharm* **223**, 55 (Jul 31, 2001).
20. J. Vieville, M. Tanty, M. A. Delsuc, *J Magn Reson* **212**, 169 (Sep, 2011).
21. M. Aghajani, A. R. Shahverdi, A. Amani, *AAPS PharmSciTech*, (Sep 21, 2012).
22. X. Liu, H. Xu, H. Xia, D. Wang, *Langmuir* **28**, 13720 (Sep 25, 2012).
23. R. Greenwood, K. Kendall, *Journal of the European Ceramic Society* **19**, 479 (1999).
24. G. Lewis, Y. Li, *J Mech Behav Biomed Mater* **3**, 94 (Jan, 2010).
25. J. Pereira-Lachataignerais *et al.*, *Chem Phys Lipids* **140**, 88 (Apr, 2006).
26. C. Rosenfeld, C. Serra, C. Brochon, G. Hadziioannou, *Lab Chip* **8**, 1682 (Oct, 2008).
27. E. D. Kaufman *et al.*, *Langmuir* **23**, 6053 (May 22, 2007).