inhalers (MDI) were prepared by suspending SS niosomes equivalent to 20 mg SS in hydrofluoroalkane (HFA). The metered valve was investigated for leakage rate, the total number of puffs/canister, weight/puff, dose uniformity and particle size.

Results: The results showed spherical niosomes with 400-451 nm particles that entrapped 66.19% of SS. 76.54±0.132% SS release from niosomes that showed a controlled release profile for 8h. The leakage test was not exceeding 4 mg/3 d, the number of puffs were up to 200 puffs/canister, the dose delivered/puff was 0.1 mg and 0.64-4.51 µm niosomal aerosol.

Conclusion: The results indicate an encouraging strategy to formulate a controlled drug delivery by entrapping (SS) in niosomes which could be packaged into (MDI) that meet the requirements of (USP) aerosols guidelines which offering a novel approach to respiratory delivery.

Keywords: Pulmonary drug delivery, Metered dose inhalers, Controlled release, Salbutamol sulphate, Niosomes, Mastersizer

INTRODUCTION

The pulmonary delivery route is characterized with rapid onset, non-invasive nature and lack of proteolytic enzymes in lungs compared to gastrointestinal tract. In addition, onset is more rapid in case of localized delivery to lungs by inhaled medications [1-3]. Metered dose inhalers (MDIs) effectively made asthma medications simple for patients to administer to themselves. The combination of the large dispersion area of both aerosolized drug delivery systems and lungs synergistically facilitate the rapid uptake of the drug into systemic circulation offering a controlled method to deliver a known therapeutic amount with each dose [4-5]. Niosome is one of the most promising nano-carriers that can be used in targeted drug delivery systems in a controlled manner [6] showing bioavailability enhancement, continuous therapeutic effect over a longer period of time, preventing the excess drug from pouring into the systemic circulation, protecting drugs from degradation in vivo and therefore, results in reduced side-effects [7-8].

Salbutamol sulphate (SS) was developed and indicated for the symptomatic relief and prevention of bronchospasm due to bronchial asthma, chronic bronchitis and other chronic broncho pulmonary disorders in which bronchospasm is a complicating factor. In addition, it prevents exercise-induced bronchospasm. SS is clinically supplied by The Arab Drug Co., Egypt. Cholesterol (95% stabilized) was purchased from Acros organics, (U. K). Span 60 and chloroform were purchased from Sigma-Aldrich, Germany.

MATERIALS AND METHODS

Experimental

Materials and reagents
Salbutamol sulphate (99.90%), propellant HFA and soya lecithin were kindly supplied by The Arab Drug Co., Egypt. Cholesterol (95% stabilized) was purchased from Acros organics, (U. K). Span 60 and chloroform were purchased from Sigma-Aldrich, Germany.

Instruments
Ultraviolet-visible spectrophotometer (V-630, Jasco, Japan), Digital precise Shaking Water Bath (WSB-18, Daham Scientific Co. Ltd., Korea), Scanning electron microscope (JEOL JSM-5500 LV, Tokyo, Japan). Transmission electron microscope (JEOL JSM-6510 LV, Tokyo, Japan), Rotary evaporator (DSB-2100, N-1200A, Shanghai Eyela Co. Ltd., China), Freeze centrifuge (T-16K, Sigma Laborzentrifugen GmbH, Germany), (PAMASOL 2015, Switzerland) and Malvern Instruments Ltd (Zetasizer Nano-Ze90, MPT-Z, UK) were used.

Preparation of niosomes
Niosomes containing SS were prepared by reversed-phase evaporation method (REV) that representing formula (N). A mixture of Span 60 and cholesterol (1:1 molar ratio) was dissolved in chloroform. Subsequently, 0.5 g SS was dissolved in water then the aqeous phase was added to the lipid phase. The mixture was then emulsified using bath sonicator for 10 min at 10 °C. The emulsion was rotary-evaporated at 40 °C with a rotating speed of 50 rpm for about 15 min to remove the organic solvent; Traces of chloroform were eliminated by employing rotary evaporator for extra 10 min. The suspension then was centrifuged using a refrigerated centrifuge at 4 °C, 10,000 rpm for 1 h, lyophilized and kept at 4 °C for further investigations [12-13].

Characterization of niosomes
Scanning electron microscopy (SEM) of niosomes
Samples were sprinkled on SEM holder with double-sided adhesive tape, coated with a layer of 150 Å gold for two minutes by SPI.
Transmission electron microscopy (TEM) of niosomes

A 10-fold aqueous diluted drop of the niosomal dispersion was subjected to colloid-coated 300 mesh copper grid, left for 5 min, adsorbed using filter paper then a drop of 2% aqueous uranyl acetate was applied for 1 minute, the remaining solution was removed and the samples were air dried and examined at 80 KV [14].

Particle size, polydispersity index and zeta-potential of niosomes

Nanoparticle size distribution, polydispersity index and zeta-potential were determined using photon correlation spectroscopy (Zeta Potential Analyzer; Malvern Zetasizer Nano-zs90, Malvern Instruments, Malvern, UK). The size distribution analysis was performed at a scattering angle of 90 degrees and at a temperature of 25 °C using samples appropriately diluted with dispersant water. For each sample, the mean diameter±standard deviation of 10 determinations was calculated applying multimodal analysis [15].

Entrapment efficiency (EE %) of SS in niosomes

Two mg of SS niosomes was mixed with 10 ml of absolute alcohol; 0.1 ml of the resultant SS niosomal dispersion was diluted with absolute alcohol and sonicated for ten minutes to obtain a clear solution. The concentration of entrapped SS was determined spectrophotometrically at 276 nm using UV spectrophotometer against the sample withdrawn from empty niosomal dispersion treated in a similar manner. The entrapment efficiency was determined relative to the original drug concentration as [EE % = ED/TD *100, Equation (1)] Where EE % is the entrapment efficiency percent, ED is the entrapped drug concentration and TD is the theoretical drug concentration [16].

Characterization of niosomal MDI according to US pharma-copeia, 2004

The particle size of niosomal aerosol was measured by laser diffraction Malvern Mastersizer as a suitable alternative to impaction methods [22]. Particle size analysis of each sample was performed with reference particle refractive index (1.5295, 0.1000) and reference dispersant refractive index (1.3300). The particle size of niosomal aerosol is converted into a volume based size according to a reported method [23]. The residual volume was 1.820%. The preparation was appropriately diluted with purified water inside the Malvern sample dispersion unit for analysis using the 300 RF lens as recommended by the manufacturer, and laser radiation unit class 3B with a measurement beam lens of 2.40 mm and a range lens of 45 mm for each measurement attached to a measuring cell. The obscuration level was kept between 40-55% at a stable count rate. The average particle size was determined from triplicate of each sample.

Leakage rate was carried out using ten aerosol containers. Each container was weighed (W1) then allowed to stand in an upright position at a temperature of 25±2°C for 3 d. Each container was weighed again (W2) then the h (T) during which the containers under test were recorded. The leakage rate (mg/year) of each container was calculated by the formula of 365*(24/T)*(W1-W2).

To calculate weight per puff, the valve of each container was actuated for 10 puffs, and then; each container was weighed again (W2). The total weight loss from each canister was calculated (W1-W2). The total number of puffs was determined by counting the number of priming discharges until the container was empty using 10 cans.

In vitro release of SS from niosomes

The release of SS from niosomes was determined using the membrane diffusion technique [17-18]. One ml phosphate buffer (pH 7.4) was used to suspend SS; equivalent to 10 mg. The suspension was transferred to a glass tube with a soaked cellulose membrane that enclosed its lower end. Then, the glass tube was placed in a beaker containing 50 ml phosphate buffer (pH 7.4) that was maintained at a temperature of 37°C [19]. Using shaker water bath; the beaker was kept under mild agitation (50 rpm). Aliquots were withdrawn at predetermined time intervals for 8h, then the drug concentration was determined at 276 nm. The experiment was run in triplicates. The obtained data were kinetically analyzed to determine the pattern of the drug release. For better characterization of the drug release behaviour for the systems studied and to understand the corresponding mechanism, Korsmeyer-Peppas semi-empirical model was applied [20-21].

Preparation of MDI

Two formulae were used. MDI (F1), containing 20 mg SS (0.01% of the whole canister) in propellant HFA and considered the control batch, while MDI (F2) containing the REV SS niosomes (table 1). The prescribed amount of the selected niosomes (20 mg SS) was suspended in the propellant HFA that was used as a solvent applying soya lecithin as a dispersing agent and then quantitatively placed in the aerosol container, the valve assembly was inserted and crimped into place, and pressure was introduced into the container equal to propellant vapor pressure from a pressure burette. When the pressure in the container equals that in the burette, the process was stopped. The desired pressure was obtained by increasing the pressure in the filling apparatus through the use of compressed air, using the typical pamasol packaging line that was used to fill the metered dose aerosol inhalers.

Table 1: Composition of SS alone and SS niosomes for aerosol studies

| Formula | Amount of ingredients in (g) | Molar ratio | Amount of SS in the whole canister |
|---------|-----------------------------|-------------|-----------------------------------|
|         | SS | Span60: cholesterol | Span60: cholesterol |
| F1      | 0.02 | - | - |
| F2      | 1.5 | 6:4:5:6 | 1:1 |
|         | 20 | 0.01 | 20 |
|         | 0.01 |      | 0.01 |

Note: Niosomes in formula 2 (F2) are entrapping 20 mg SS (SS): Salbutamol Sulphate

Characterization of niosomal vesicles

Entrapment efficiency (EE %)

The EE% of SS in niosomes was found to be 66.19±0.48. This result was in accordance with [25] who reported that REV method is the most efficient method used to entrap water-soluble drug. Furthermore, [26] reported that REV method used to prepare Hydroxychloroquine in niosomes achieved the highest entrapment efficiency (86.4%). Reverse-
Phase evaporation method has these unique advantages for encapsulating water-soluble materials such as SS as the organic solvent is simply removed from the inverted micelles resulting in vesicles with larger aqueous space to lipid ratio and consequently higher EE%.

Particle size, polydispersity index and zeta-potential of niosomes
A particle size ranged from 400-450 nm (fig. 1. a) with a dispersant RI 1.330 and viscosity (cP) equals 0.8872 was considered optimum for pulmonary application. Polydispersity index (PdI) value was 0.354 that indicates the homogenous distribution of formed niosomes (fig. 1. a). Zetapotential of RE niosomes was -46.5 which reflects the stability of formed niosomes (fig. 1. b). [27].

Scanning electron microscopy (SEM)
SEM illustrated surface characteristics of REV SS-loaded niosomes (fig. 1. c) which appeared spherical with some discontinuities in the membrane. This may be explained as the acyl-chain structure of span 60 could affect cholesterol interactions causing variations in cholesterol distribution. The polar head group of non-ionic surfactant must cover the non-polar portion of cholesterol; this coverage is essential to avoid the unfavourable free energy of cholesterol that when contacts with water decrease the repulsion between cholesterol molecules [28].

Transmission electron microscopy (TEM)
The morphology of the selected formula of REV SS-loaded niosomes (fig. 1. d), revealed the presence of well identified spherical multi-lamellar vesicles existing in disperse pattern.

The results illustrated in (fig. 2. a) showed that SS release percent after eight hours from REV SS niosomes was 76.54±0.132. Furthermore, the slow release rate depends on both polymer breakdown and diffusion out of the matrix. Correlation of release data revealed that the release profile followed Higuchi model with regression parameters of $R^2=0.992$, for REV SS niosomes (fig. 2. b). This kinetic pattern indicated that SS release is dominated by diffusion model which normally depends on drug concentration gradient between nano-vesicles and dissolution media with penetration of this media through a porous wall which accompanied by matrix disruption [29].
In this light, release can be changed by varying system surface area and wettability; determined by size and uniformity. Meanwhile, loading percentage directly affects the drug concentration gradient and release rate [30]. Further kinetic studies were carried out and the release pattern was non-fickian with $n = 0.6929$ for N (table 2).

| Formula | Higuchi diffusion | Korsmeyer-peppas | Mechanism of drug release |
|---------|-------------------|-------------------|---------------------------|
|         | $R^2$             | $K$               | $n$                       |                          |
| N       | 0.992             | 0.999             | 0.006                     | 0.6929 Non Fickian       |

Note: formula (N): Niosomes containing salbutamol sulphate (SS) that prepared by reversed-phase evaporation method (REV), $(R^2)$: linear regression, $(K)$: a constant incorporating characteristics of the particle system, $(n)$: the diffusion exponent

Characterization of niosomal MDI according to USP

Characterization of niosomal MDI was performed. Statistical analysis using one way ANOVA ($p<0.05$) was carried out by graphpad prism6® showing that the results complies with pharmacopeial specifications according to USP 2004. Fig. (3) illustrates the particle size of aerosolized REV SS niosomes which ranged from 0.64 to 4.51 μm. The results also indicate that niosomal SS particle size measured by (TEM) and (SEM) were in good agreement with malvern masterizer results, which was almost below 5μm. Particles larger than 5μm are rapidly removed from the lung by coughing or swallowing while particles smaller than 0.5 μm may escape impaction in the upper airways, thus only aerosols 3-5 μm in diameter show efficient penetration into the lungs [31]. All characterization parameters were studies according to US Pharmacopoeia, 2004 [32].

The results illustrated in (table 3) showed that, weight varies from 22.65-23.69 gm with no significant differences between the 20 cans ($p<0.05$) as (23.42±0.24) for SS and (20.65±0.33) for niosomal SS. Weight per puff (mg) of 10 canisters of SS and niosomal SS were found to be (83±0.31) and (80±0.27) mg respectively which is not less than 71 mg and not more than 83 mg/puff. Leakage test (mg/year) showed (425±1.89) for SS and (472±0.56) for niosomal SS. Number of puffs per canister (delivery/canister) for 10 containers showed (190±0.53) and (215±0.61) (del/can) for SS and niosomal SS respectively. Regarding dose uniformity per puff, the results showed (115%±0.19) for SS and (95%±0.55) for niosomal SS, this in accordance with the recorded values in USP that ranged from 85% to 115% using 10 canisters. Moreover, the results of dose uniformity per the whole canister showed that (99%±0.45) and (105%±0.11) for SS and niosomal SS, respectively which meet the compendia requirements that ranged from 85% to 115% using 10 canisters.

![Fig. 3: Malvern mastersizer report of the particle size of REV SS niosomes in MDIs](image-url)

Note: D(4,N), D(3,N), D(2,N), D(1,N) are the equivalent derived diameter at 90, 80, 50 and 10% cumulative volume.
niosomes can be formulated as MDI with physical properties met the USP requirements, and so it could be delivered to lung as targeting to minimize its clearance, that guarantees controlled drug delivery with reduced number of dosing which helps to reduce the side effects. It also

**CONCLUSION**

Finally, all authors wrote, reviewed and approved the manuscript. Fonte P, Soares S, Sousa F, Costa A, Seabra V, Reis S, et al. Stability study perspective of the effect of freeze-drying using cryoprotectants on the structure of insulin-loaded into PLGA nanoparticles. Biomacromolecules 2014;15:3753-65.

**REFERENCES**

The authors are grateful for Dr. Abd-El Sabour Ahmed head of the Aerosol Department, Arab drug company, Cairo 11813, Egypt for the MDIs packaging.

**CONFLICT OF INTERESTS**

The authors declare no competing interests. We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

**ACKNOWLEDGMENT**

Note: All values were represented as mean±SD (n=3), P<0.05

| Test                         | Salbutamol sulphate aerosol | Niosomal salbutamol sulphate aerosol | Pharmacopeial specifications |
|------------------------------|-----------------------------|--------------------------------------|------------------------------|
| Leakage test (mg/year)       | 425±1.89                    | 472±0.56                             | Comply                       |
| Weight variations (g)        | 23.42±0.24                  | 20.65±0.33                           | Comply                       |
| Weight per puff (mg)         | 83±0.31                     | 80±0.27                              | Comply                       |
| Number of puffs/can (del/can)| 190±0.53                    | 215±0.61                             | Comply                       |
| Dose uniformity/puff%        | 115±0.19                    | 95±0.55                              | Comply                       |
| Dose uniformity/can%         | 99±0.45                     | 105±0.55                             | Comply                       |

104

1. **Patton JS, Bukar J, Nagarajan S. Inhaled insulin. Adv Drug Delivery Rev 1999;35:235-47.**
2. **Kaparisissides C, Alexandrioud S, Kotti K, Chatidiou S. Recent advances in novel drug delivery systems. J Nanotechnol 2006;2:1-11.**
3. **Wall DA. Pulmonary absorption of peptides and proteins. Drug Delivery 1995;2:1-20.**
4. **Anselmo AC, Mitragotri S. An overview of the clinical and commercial impact of drug delivery systems. J Controlled Release 2014;190:15-28.**
5. **Taburet AM, Schmit B. Pharmacokinetic optimization of asthma treatment. Clin Pharmacokinetics 1994;26:396-418.**
6. **El-najjar BY, Hussain SA. Chitosan microspheres for the delivery of chemotherapeutic agents: paclitaxel as a model. Asian J Pharm Clin Res 2017;10:1-5.**
7. **Mariancic C, Di Marzio L, Rinaldi F, Celia C, Paolino D, Alhajque F, et al. Niosomes from 80s to present: the state of the art. Adv Colloid Interface Sci 2014;205:187-206.**
8. **Duarah S, Pujari K, Durai RD, Narayanan VHB. Nanotechnology-based cosmeceuticals: a review. J Appl Pharm 2016;8:8-12.**
9. **Morgan D, Paul J, Richmond B, Wilson-Evered E, Ziccone S. Pharmacokinetics of intravenous and oral salbutamol and its sulphate conjugate. Br J Clin Pharmacol 1986;22:587-93.**
10. **Moham J, Costello JF. Textbook of medicine. ed. Souhami RL, Moham J. 506 (Chapter 15). (3rd ed., Churchill Livingstone, London, 1997); 1997.**
11. **Hutchings M, Paul J, Wilson-Evered E, Morgan D. Pharmacokinetics and metabolism of salbutamol in premature labour. Br J Clin Pharmacol 1987;24:69-75.**
12. **Hua W, Liu T. Preparation and properties of highly stable innocuous niosome in Span 80/PEG 400/H2O system. Colloids Surf A 2007;302:377-82.**
13. **Sozka JF, Papadopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc Natl Acad Sci USA 1987;75:4194-8.**
14. **Abd-Elbary A, El-laihthy HM, Tadros MI. Sucrose stearate-based proniosome-derived niosomes for the nebulisable delivery of cromynol sodium. Int J Pharm 2008;357:189-98.**
15. **Fonte P, Soares S, Sousa F, Costa A, Seabra V, Reis S, et al. Stability study perspective of the effect of freeze-drying using cryoprotectants on the structure of insulin-loaded into PLGA nanoparticles. Biomacromolecules 2014;15:3753-65.**
16. **Arafa MG, Ayoub BM. DOE optimization of the nano-based carrier of pregabalin as hydrogel: new therapeutic and chemometric approach for controlled drug delivery systems. Sci Rep 2017;7:41503.**
17. **Devaraj GN, Parakh SR, Devraj R, Apte SS, Rao BR, Rambhau D. Release studies on niosomes containing fatty alcohols as bilayer stabilizers instead of cholesterol. J Colloid Interface Sci 2002;251:360-5.**
18. **Gavras-Dodov M, Goracinova K, Madenovska K, Fredro-Kumbharadzi E. Release profile of lidocaine HCl from topical liposomal gel formulation. Int J Pharm 2002;242:138-41.**
19. **Gursoyum A, Kut E, Ozkirimli S. Co-encapsulation of isoniazid and rifampicin in liposomes and characterization of the liposome by derivative spectroscopy. Int J Pharm 2004;271:115-23.**
20. **Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 1983;15:25-35.**
21. **Kulkarni A, Mandhare T, Aloorkar N. Application of novel natural polymer for controlling the release of fenoverine from controlled release matrix tablets. Int J Appl Pharm 2017;9:1-9.**
22. **De-Boer AH, Le Brun PPH, Van Der Woude HG, Hagedoorn P, Heijerman HGM, Frijlink HW. Dry powder inhalation of antibiotics in cystic fibrosis therapy, part I: Development of a powder formulation with colistin sulfate for a special test inhaler with an air classifier as a de-agglomeration principle. Eur J Pharm Biopharm 2002;54:17-24.**
23. **Jones SA, Martin GP, Brown M B. High-pressure aerosol suspensions-a novel laser direct fractionation particle sizing system for hydrofluoroalkane pressurized metered dose inhalers. Int J Pharm 2005;302:15-65.**
24. **Marie PF, Pierre L, Anne G. The influence of carrier roughness on adhesion, content uniformity and the in vitro deposition of terbutaline sulphate from dry powder inhalers. Int J Pharm 2004;275:201-9.**
25. **Mohgangami S, Hadjiyadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. J Controlled Release 2014;185:22-36.**
26. Bendas ER, Abdullah H, El-Komy MHM, Kassem MAA. Hydroxychloroquine niosomes: a new trend in topical management of oral lichen planus. Int J Pharm 2013;458:287-95.
27. Jassem NA, Rajab NA. Formulation and invitro evaluation of azilsartan medoxomil nano suspension. Int J Pharm Pharm Sci 2017;9:110-9.
28. Parker A, Miles K, Cheng K, Huang J. Lateral distribution of cholesterol in dioleoylphosphatidylcholine lipid bilayers: cholesterol-phospholipid interactions at high cholesterol limit. Biophys J 2004;86:1532-44.
29. Davies NM, Feddah MR. A novel method for assessing dissolution of aerosol inhaler products. Int J Pharm 2003;255:175-87.
30. Hanif SN, Garcia-Contreras L. Pharmaceutical aerosols for the treatment and prevention of tuberculosis. Front Cell Infect Microbiol 2012;2:118-24.
31. Glyn T, Ian K. Pulmonary drug delivery, chapter 10 in drug delivery and targeting. Anya MH, Andrew WL, James S. ed. Taylor and Francis, Inc, New York; 2001.
32. US Pharmacopeial Convention 27, Rockville, MD; 2004. p. 2253-8.