Sys Rev Pharm 2020; 11(4): 629 – 639
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Preparation of Inhalable Salbutamol Proliposome Using Different Core Carriers and Investigate the Impact of Simulated Lung Fluid Use in Evaluation

Basma Yahya Al-Najjar[1*], Mowafaq M. Ghareeb[2]
[*1]Alrafidain university college, Department of pharmaceutics, Iraq
[2]University of Baghdad, College of Pharmacy, Department of Pharmaceutics, Iraq
E-mail: alnajjarbasma@yahoo.com

Article History: *Submitted: 02.02.2020* *Revised: 10.04.2020* *Accepted: 03.05.2020*

ABSTRACT

Objective: Pro-liposomes are free flowing precursor of liposomes. Liposome low stability can overcome by preparation of Pro-liposomes as high stability precursor of liposome produced upon hydration. Salbutamol sulphate is bronchodilator widely used for the treatment of asthma. Because of its high water solubility its penetration through lipid barriers is low. The preparation of water soluble drugs as liposomes improves their solubility across biological membranes.

Method: Microparticulate sucrose, mannitol, trehalose, and lactose separately as core carriers were prepared using mini spray dryer. Ethanolic lipid (soya phosphatidylcholine) solution having salbutamol sulphate and micro particulate core carriers at 1:5 w/w phospholipid to carrier ratio was spray dry to find proliposomes. The prepared liposomes were evaluated for particle size distribution, polydispersity, Z.P., entrapment capacity, and morphology. This investigation was undertaken to compare the evaluation results of the produced liposomes that hydrated by simulated lung fluids that mimic human interstitial lung fluid and deionized water.

Results: Good proliposomes were obtained with different properties using spray drying. The results show that carrier type has significant effect on the properties of prepared liposome. In addition, there are obvious differences in results between liposomes generated in simulated lung fluid and deionized water. Overall, simulated lung fluid had significant effect on liposomes size, polydispersity, zetapotential, and entrapment efficiency but had minimal effect of liposome morphology.

Conclusion: It can be concluded that use of simulated lung fluid is preferable than deionized water in evaluation of prepared liposomes.

Key word: Simulated lung fluid, Proliposome, Salbutamol, liposomes

Correspondence:
Basma Yahya Al – Najjar
Alrafidain University College, Department of Pharmaceutics, Iraq
E-mail: alnajjarbasma@yahoo.com

DOI: 10.31838/srp.2020.4.94

INTRODUCTION

Liposomes are microscopic spherical vesicles of a size series among around 25 nm, 20 μm (Bangham et al. 1965). Liposomes consist of a bilayer of phospholipids separated by aqueous buffer or water compartments (Weiner et al. 1989).Liposomes are designed in such a way that materials can be entrapped between its bilayers. Encapsulation of agents in liposomes may effectively protect them from *in vivo* metabolic deactivation or clearance and provide a targeted or sustained drug delivery system (Mathiowitz 1999). Unfortunately, liposomes are unstable to storage as aqueous dispersions. This is due to that phospholipids are liable to oxidation and hydrolysis, resulting in liposomes aggregation and subsequent leakage of encapsulated material (Hunt and Tsang 1981; Hernández-Caselles et al. 1990; Kensil and Dennis 1981; Grit et al. 1989; Elhissi et al. 2012).

Particulate-based proliposomes technologies have been investigated in many studies to provide stable carrier particles e.g. carbohydrates coated for phospho-lipids to produce liposomes upon dispersion in aqueous phase (Payne et al. 1986). The carbohydrates on which phospholipid is coated could be mannitol, lactose, glucose, or sorbitol (Payne et al. 1986). Proliposomes has been studied for oral delivery of nonsteroidal anti-inflammatory drugs (N.S.A.IDs) to provide better gastric protection in model animals (Katare et al. 1990). Also, proliposomes have been investigated for transdermal administration of nicotine, nasal delivery of propranolol hydrochloride and nicotine, pulmonary delivery and intravenous delivery of methotrexate, doxorubicin ciclosporin and NSAIDs (Hwang et al. 1997; Ahn et al. 1995; Jung et al. 2000; Elhissi and Taylor 2005; Park et al. 1994; Lee et al. 1995; Lee et al. 1996; Lee. et al. 1999). Particulate-based proliposomes can be manufactured on a large scale using fluid-energy milling, fluidized-bed coating and spray through (Desai et al. 2002; Kumar et al. 2001; Kim 2001; Alves and Santana 2004).

In this study spray drying has been employed to control stability of liposomes. Spray drying is a frequently used technique to prepare a variety of inhalable powders such as antibiotics, peptides, biodegradable carrier particles and vaccines (Arpagaus and Meuri 2010; Arpagaus and Schafroth 2009). The main advantages of spray drying are: it is a one step process, the possibility to control important characteristics of the particles and the opportunity to spray dry heat sensitive macromolecules with insignificant degradation (Ståhl et al. 2002). The release of the entrapped drug and the biological distribution of liposomes are greatly reliant on liposome morphology, size, charge, and bilayer fluidity (Mathiowitz 1999).

The application of a simulated lung fluid system in place of deionized water to produce liposome could provide extra accurate results regarding size, charge and entrapment efficiency of liposomes. Simulated lung solution is known as Gamble's solution which is a combination of water and inorganic salts such as carbonate, phosphates and chlorides (Drysdale et al. 2012). Takaya *et al*. (2006) has been modified Gamble's solution to include proteins and other organic compounds to mimic human interstitial lung fluid. This paper seeks the affects of prolieposome hydration media on size, charge, and entrapment efficiency of liposomes produced from particulate-based liposomes in simulated lung fluids (Takaya etal. 2006).The present study is focus on the priority of simulated lung fluid over deionized water in revealing the real evaluation parameters of the produced liposomes.

MATERIALS AND METHODS

2.1. Materials

Lactose monohydrate (LMH) was purchased from (VWR, UK). Sucrose, D-Manitol, Trehalose dihydrate was all (SIGMA-ALDRICH, UK). Soya phosphatidylcholine (SPC) and cholesterol were a gift from lipoid, Switzerland. Salbutamol sulphate (SS) was supplied by Alfa-Aesar, UK. Chitosan was SIGMA-ALDRICH. Also, the simulated lung fluid constituents: $CaCl_2.2H2O$, Na_2SO_4, Na_2HPO_4, Glycine, Sodium lactate, Sodium pyruvate were all (SIGMA-ALDRICH, UK). $MgCl_2.6H2O$ was AnalaR, NaCl and Sodium tartrate were (Fisher scientific, UK), $NaHCO_3$ Specified Laboratory Reagent, Trisodium citrate dehydrate is Biochemical grade.

2.2 Methods

2.2.1 Production of microparticulate core carrier by spray drying

The feed solution was prepared by dispersing sucrose, d-mannitol, trehalose dihydrate and lactose in distilled water (1% w/v). The dispersion was fed into the Bauchi Mini Spray Dryer B-290 equipped by a great performance cyclone with a 0.7 mm nozzle. The produce was collected and alienated via the directed and cyclone into the collecting chamber. Microparticulate sucrose, mannitol, trehalose and lactose were used as core carriers. The mini spray dryer permits the making of particles by a range size of 2 to 25 µm. The spray dryer was performed utilizing inlet temp. of 120 - 130 °C, a feed rate of 17%, aspirator setting of 100 % ($38m^3/h$), nozzle air flow rate of 600 L/h, and outlet temperature of 70°C ± 2. The resultant carbohydrate micro particles was utilized as carry of the preparation of proliepsomes (Rahimpour et al. 2014).

2.2.2 Production of proliposomes via spray drying

proleiposomes were prepared and spray dried utilizing Bauchi Mini Spray Dryer B.-290 (Buch Laboratory-Techniques, Switzerland) to products dried particles. A lipid solution having micro particulate sucrose, trehalose salbutamol sulphate mannitol, and lactose was spray dried to find proleiposomes. proleiposomes were prepared using 1:5 w/w phospholipid to carrier ratio (Sucrose, D-Manitol, Trehalose dihydrate and Lactose). Lipids (100mg) was composed of a mix of S.P.C and CH in the ratio mole of 1:1 about of 96% ethanol by dissolve in 100 ml to find an ethanoic lipid solution. SS (10 mg) by additional and sonicated of one min. to find a pure solution. Micro particulate spray-dried sucrose, trehalose, mannitol and lactose was dispersed in the solution and sonicated of 15 min to deaggeregate the particles carbohydrate before spray drying. The ethanoic suspension was stirred whereas being fed into the spray drier in order to provide homogeneity of the suspension. The spray dryer was performed using inlet temperature of 120 - 130 °C, a feed rate of 11%, aspirator setting of 100 % (38m3/h), nozzle air rate flow of 600 L/h, and outlet temperature of 73°C ± 3. The drying time about 0.1 seconds to few seconds. The proliposomes powder was stored in a desiccator ready of future utilize (Chu et al. 2002).

2.2.3 Preparation of simulated lung fluids

The simulated lung fluid was prepared according to the composition listed in Table 1.

Table 1: Simulated lung fluid composition (modified from Takaya et al. 2006)

Component	Concentration (g/L)
NaCl	6.415
$CaCl_2.2H_2O$	0.255
$MgCl_2.6H_2O$	0.212
$NaHCO_3$	2.703
Na_2HPO_4	0.148
Na_2SO_4	0.079
Sodium tartrate	0.199
Trisodium citrate dihydrate	0.180
Sodium pyruvate	0.172
Sodium lactate	0.175
Glycine	0.118

2.2.4 Laser diffraction size analysis of liposomes

Size analysis was carried out utilizing laser diffraction (Malvarn Mastiersizer 2000, Malvarn Instrument L.td., U.K). The instrument software represents the size and size distribution as volume median diameter (VMD; 50% undersize) and Span at the same order. Span is a unit-less term used via by Malvern instrumants Ltd., UK to represent the polydispersity (size distribution) of the particles. Span = (undersize 90% - undersize 10%)/VMD. The VMD and Span of particles of hydrated prepared proliposomes in simulated lung fluids or water were recorded. Samples were run at least in triplicate.

2.2.5 Laser diffraction charge analysis of liposomes

Zeta potential of prepared liposomes was measured by dynamic light scattering with a Malvern Zetasizer (Malvern Instruments, Malvern, UK) at 25°C. Liposome samples were diluted 1 in 10 with PBS for the zeta potential measurements.

2.2.6 Light Microscopy of liposomes

A drop small of the sample was placed in glass slide clean and enveloped by a glass cover-slip. The light microscope Nikon Eclipse Ti-S microscope (Nikon Instrumants Inc., Melvelle, N.Y) was attached to a computer operation software scanning method and a images and camera, was taken in magnifications 20 x 0.40.

2.2.7 High-performance liquid chromatography analysis

HPLC system with HPLC Spectrophotometer detector (Agilent 1200 series with UV detector; Hewlatt-Packard Co., U.S.A) was employed to measure the entrapment efficiency of salbutamol sulphate loaded liposomes utilizing a Symmetry column C18 (250 x 4.6 mm; synergy 4u fusion-RP 80.A; Waters L.td, U.K). The modified HPLC method was validated for accuracy, precision and reproducibility.

The calibration curve of Salbutamol sulphate was prepared in a concentration range from 5 to 80 µg/ml. The injection volume was 20 µl, rate flow was 1ml/min, temperature column at 25°C, The mobile phase was utilized to rinse the column of 15 minute nextall assay. A mixing of Acetonitrile and HPLC grade water (60: 40) was utilized to prepare the stock solutions of salbutamol (Chitlange et al. 2011).

The equivalent quantities of the spray dried formulations containing 10 mg of salbutamol were dispersed in 50µL deionized water and vortex mix of 2 minute form liposome. This was followed via added of D.W to make up1 ml volume. The dispersion was then left of annealing for approx. one h. Then, the dispersion was dilute to 8 mL with deionized water and they centrifuged at 55,000 rpm at 6 °C for 35 min using ultracentrifuge (Beckman Coulter Instruments) to yield liposomes pellets and supernatant. The percentage of Salbutamol entrapment and un-entrapped drug were measured within liposomes pellets and supernatant respectively. The obtained centrifuged liposomes pellets were solubilized in methanol followed by dilution with water before sample injections. The EE% was calculated as follows: (T-C)/T* 100, where T is the quantity of whole drug and C is the quantity of drug detected only in the supernatant.

2.2.8 Hydration protocol of spray-dried proliposomes

The powders Spray-dried was hydrated via added of aqueous phase (simulated lung fluid or deionized water). Preparations was shaken via vortex mixture at best speed of 2 minute to generate the liposome dispersion. Dispersions was allowable to "anneal" for approx. one h before carry out further characterization.

RESULTS AND DISCUSSION

Proliposomes of different properties were prepared by formulation with four different carriers using spray drying technique. Particle surface morphology, zeta potential, vesicle size, distribution size, and entrapment capacity of salbutamol were studied after hydration of proliposomes powder in simulated lung fluid or in deionized water.

3.1 Liposome Morphology

This study set out with the aim of assessing the importance of conducting experimental analysis of liposomes in simulated lung fluids rather than in deionized water. Light microscopy was used to assess morphology below static conditions in simulated lung fluids and in deionized water. It is important to study the morphology of liposomes because lipid has a role in morphogenesis of biological vesicular structures (Hotani 1984).Also, analysis of surface morphology might assistance at prediction of the aerosol performance of liposomes (Pilcar et al. 2012). Spherical particles are extra probable to deposit deeper in the lung. Carrier-based liposomes that are formed from proliposomes in simulated lung fluid were small spherical liposomes, and even when proliposomes were hydrated in deionized water the structure was apparently unaffected (Figure1,2,3 and 4) as small spherical liposomes in deionized water and in simulated lung fluid.

Figure 4 show crystallization formed after hydration of mannitol liposomes in simulated lung fluids and in deionized water, possible because mannitol solubility in simulated lung fluid and in deionized water lower than that of the other carriers.

In general, regardless of carrier type microscope showed that no significant difference between liposomes generated from carrier based proliposomes in simulated lung fluids and in deionized water and they were not affected by the dissolution medium. However, the finding of the current study does not support the previous research which showed that manipulation of the osmotic pressure of the environment surrounding the liposomes could change the morphology of liposomes (Hotani 1984).Thus, future studies are suggested to investigate morphology of liposomes in simulated lung fluids using scanning electron microscopy.

On the other hand, it has been found that the manual dispersion could effect on morphology and aggregation of liposomes generated from carry based proliposomes. Also, it has been reported that morphology liposome was not reliant on lipid composition or lipid to carrier ratio but greatly reliant on carry solubility (Elhissi et al. 2012). Moreover, it was mentioned in the literature review, morphological changes of liposomes can be caused by using specific types of copolymers, or by inclusion specific types of lipid (Momekova et al. 2008; Zarif 2005). These results show that morphology of liposomes depends on carrier type rather than hydration medium.

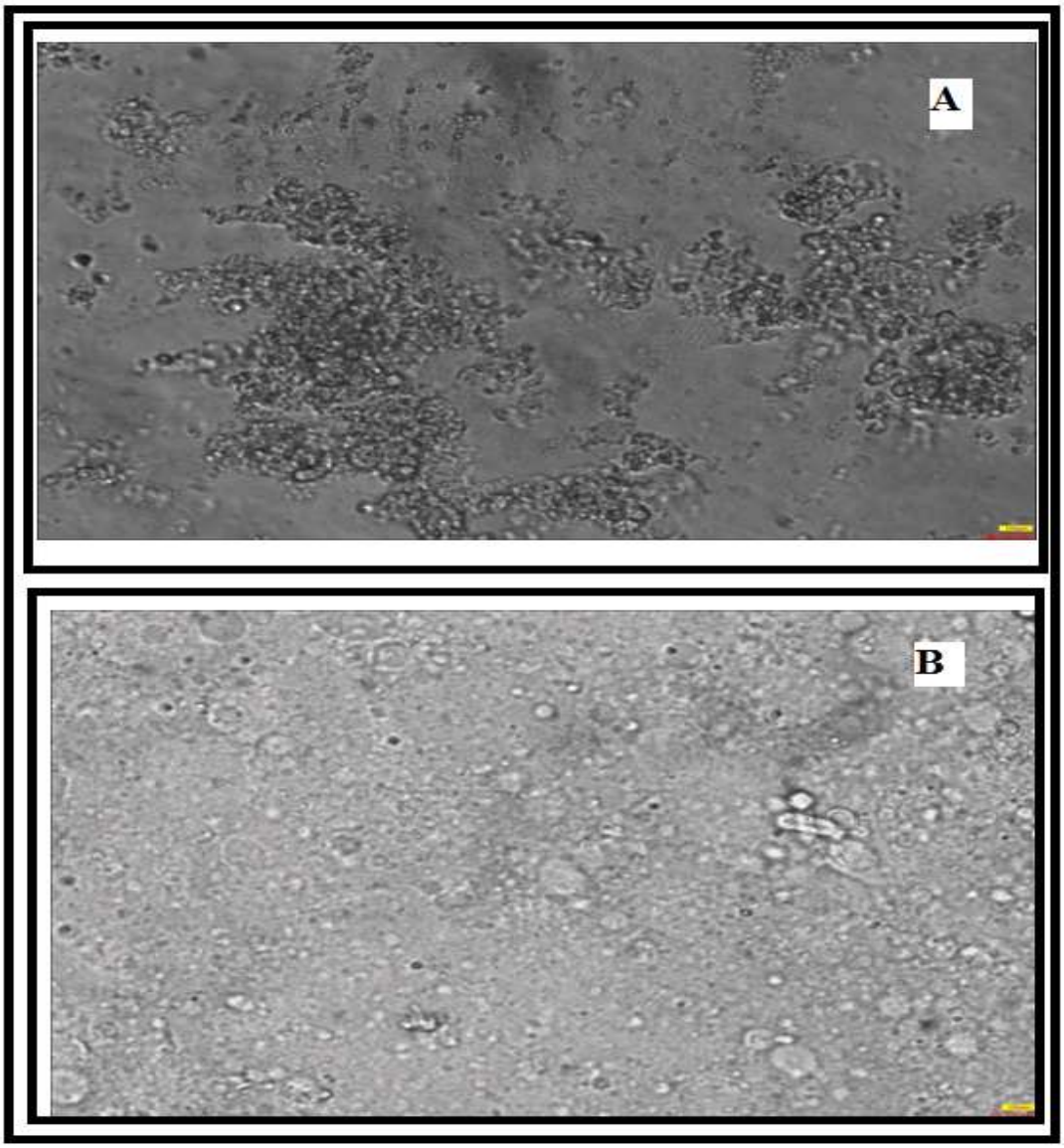

Fig. 1 a: Light microscopy appearing the hydration by simulated lung fluids of lactose based proliposomes (b) appearing the hydration by deionized water of lactose-based proleiposomes

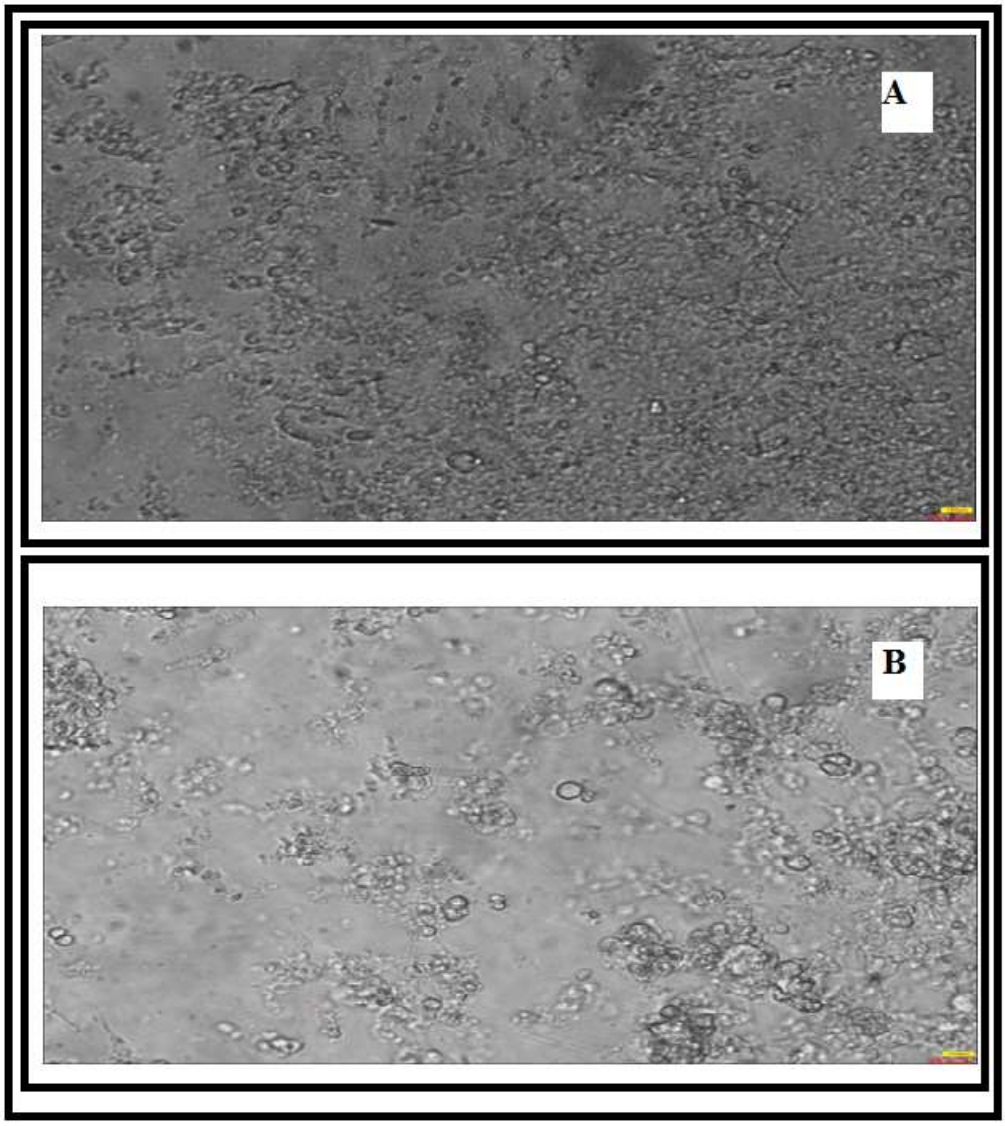

Fig. 2 a: Light microscopy appearing the hydration by simulated lung fluids of sucrose based proliposomes (b) appearing the hydration by deionized water of sucrose-based proleiposomes

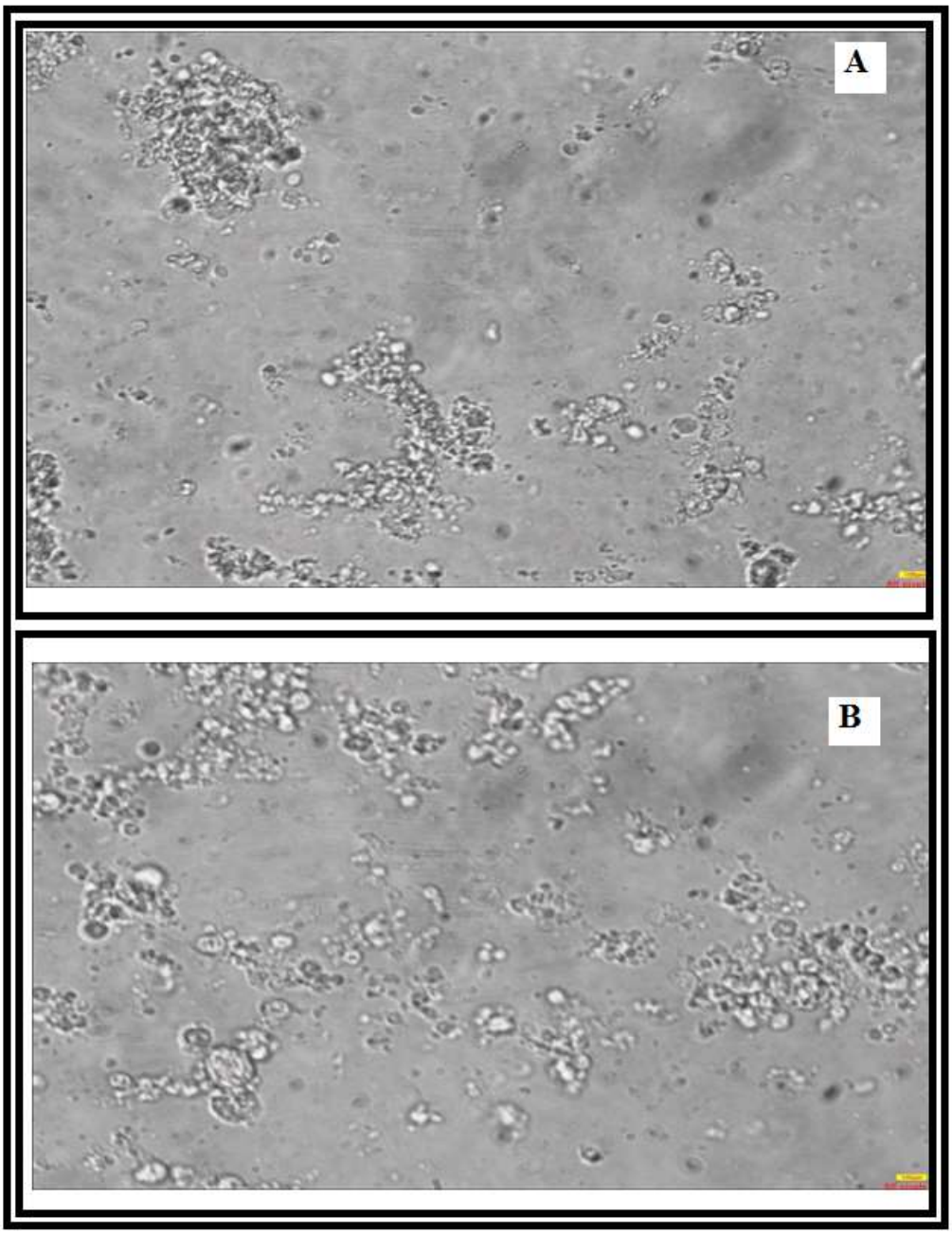

Fig. 3 a: light microscopy appearing the hydration by simulated lung fluids of trehalose based proliposomes (b) appearing the hydration by deionized water of trehalose-based proleiposomes

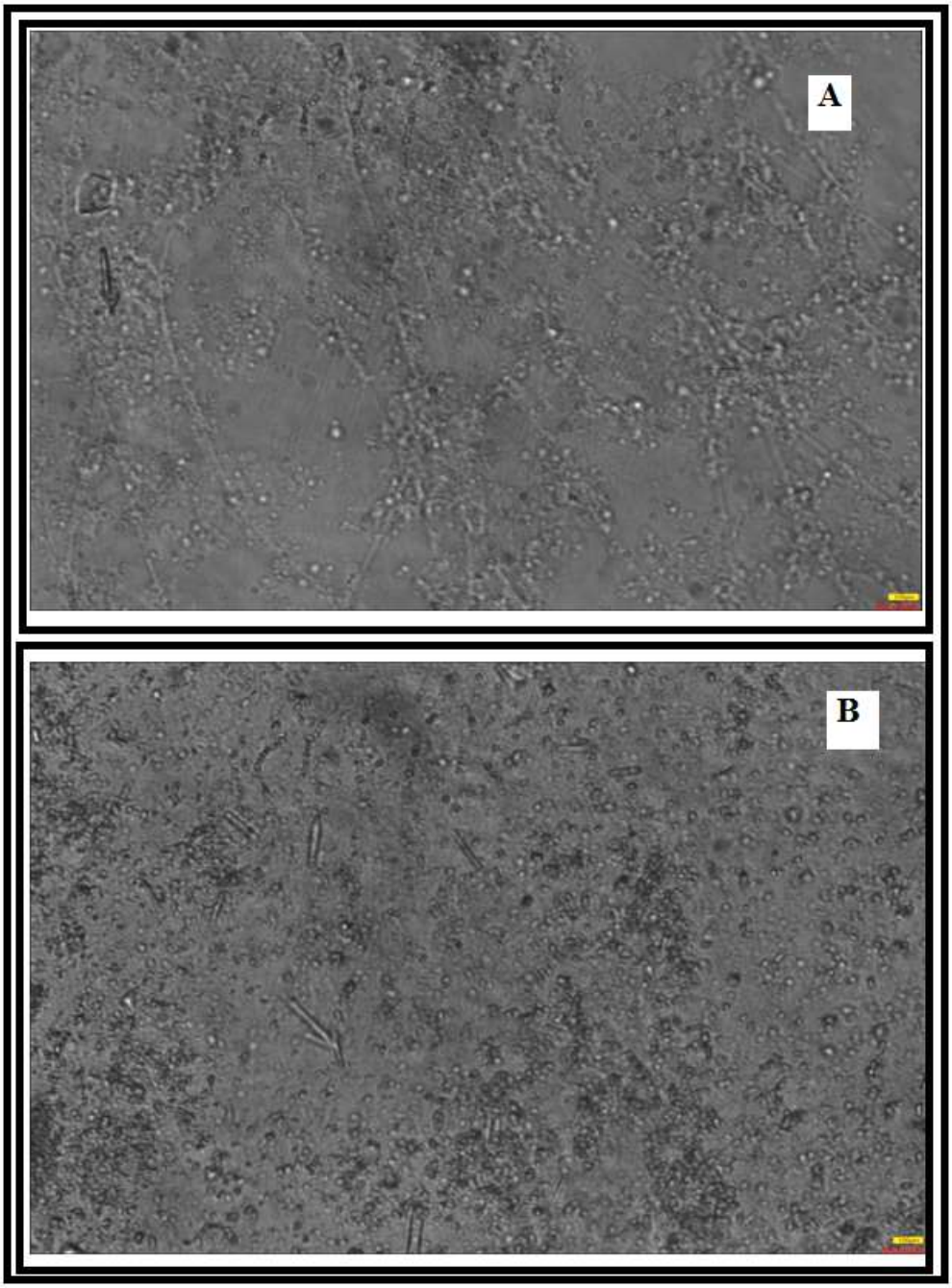

Fig. 4 a: Light microscopy appearing the hydration by simulated lung fluids of mannitol based proliposomes (b) appearing the hydration by deionized water of mannitol-based proleiposomes

3.2 Size Analysis of Liposomes Generated from proliposomes

To assess the size and polydispersity of particulate based liposomes, the analysis was conducted through recording the V.M.D and span respectively to most of size and polydispersity of particulate based liposomes. Liposomes may be produced via a series of sizes that influences drug encapsulation efficiency, the retaining time in the lung and the sustained released possessions (Hupfeld et al. 2006). Larger liposomes by multiple bilayers might increase the capability of vesicles to encapsulate proportions drug and prolong the duration of drug action in the lung. Liposomes increase drug retaining time and decrease drugs toxic next administration (Pilcer and Amighi 2010).In order to assess accurate results, repeated-measures were used. The average results of VMD and span in simulated lung fluids and in deionized water were compared in order to investigate the influence of simulated lung fluids on size and span of liposomes. The results, as shown in figure 5 (a), indicate that liposomes generated from lactose, trehalose and sucrose-based proliposomes in simulated lung fluid had smaller size than in deionized water for similar carry kind and phospholipid to carry ratio. This might be attributed to the water utilized in this study D.W data in the accumulation of liposomes because they are no force data in deaggeregation of liposomes. However; liposomes generated from mannitol

based proliposomes in simulated lung fluids had larger size than in water and this is might be due to crystallization of mannitol during spray drying. This is probably justified via the liposomes of the morphology (Figure 3). The affect of carry kind on the size of particlute-based liposomes in simulated lung fluids was slight with generally similar size for liposomes generated from lactose, trehalose, mannitol and sucrose. However, the effect of carry kind on the size of particulate-based liposomes in deionized water was significant for liposomes generated from lactose, trehalose, mannitol and sucrose.

Span values which are utilized to study the size distribution of liposomes were influenced by hydration medium. On average, span values of liposomes generated from the particulate-based proliposomes in simulated lung fluids was shown to have smaller values than that in deionized water suggesting that less liposomes aggregates were formed in simulated lung fluids than in deionized water. This is due to the water utilized in this study was water deionized data in the accumuletion of liposomes because no force result in deaggreigation of liposomes. However, in case of liposomes generated in simulated lung fluid there is a force result in deaggreigation of liposomes.

Also, span of liposomes in simulated lung fluid was independent of carrier type, with similar values for each carrier type. However, the affect of carry kind on the span of liposomes in deionized water was statistically important by commonly higher span for liposomes of lactose and mannitol-based prolieposomes (Figure 5 (b)).

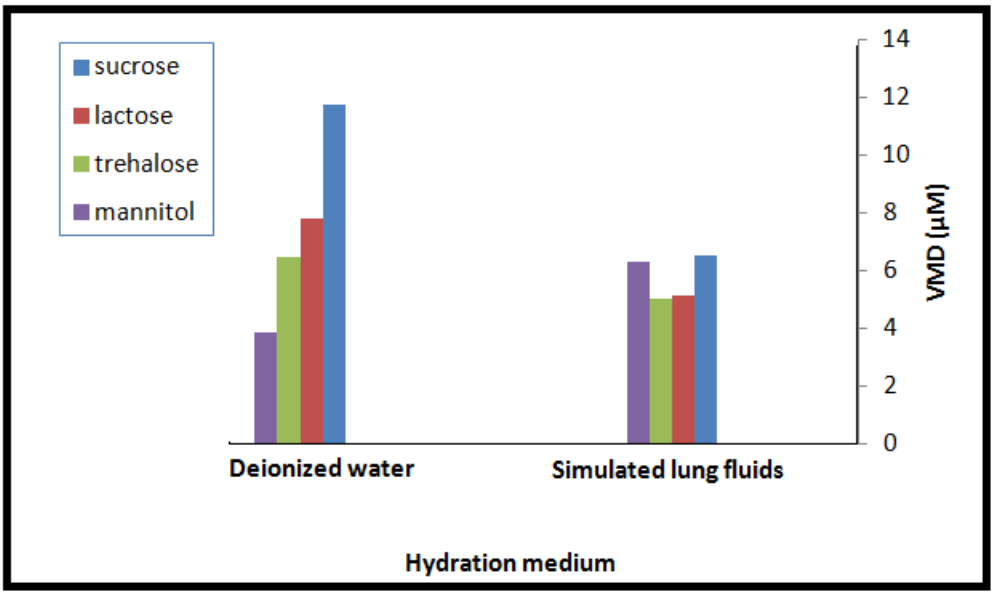

Fig. 5 a: Size of liposomes in simulated lung fluids and deionized water

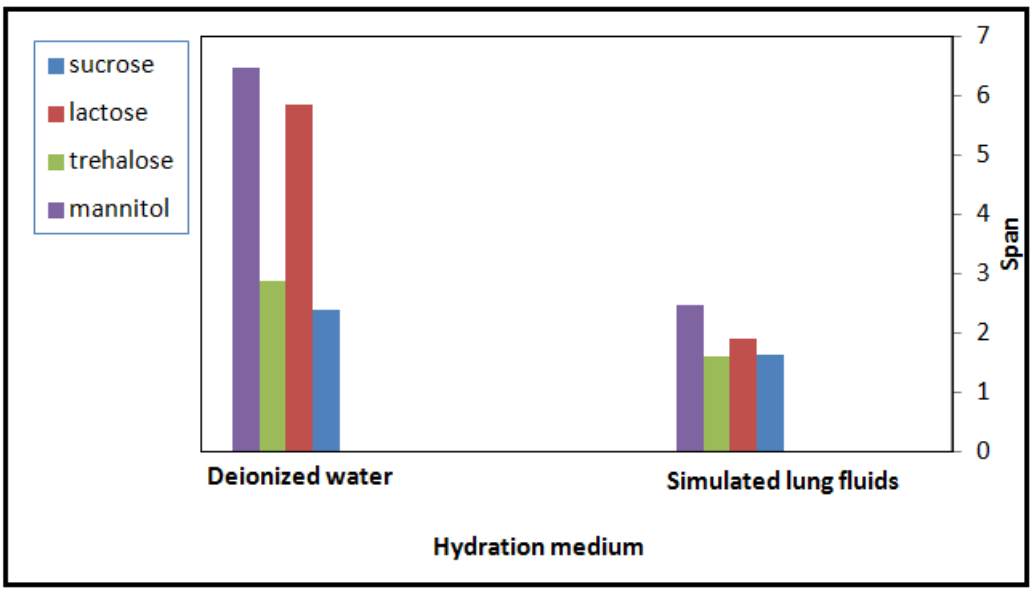

Fig.5 b: Polydispersity of liposomes in simulated lung fluids and deionized water

3.3 Zetapotential of Liposomes Generated from proliposomes in simulated lung fluids and water

Zeta potential values are used to study charge of liposomes and predict the stability of colloidal systems *in vitro*. Thus, the charge surface density effects the bio distribution of liposomes *in vivo*. Z.P. of liposomes was most influenced by the hydration medium. The results of zeta potential of liposomes in simulated lung fluid in comparison to those in deionized water are interesting, it can be seen that the zeta potential values of the particulate-based liposomes in simulated lung fluid were found to be negative in charge, while those in deionized water were positive as shown in figure 5 (c). The slightly negative Z.P. may be attributed to a negative charge of the simulated lung fluid. Also, zeta potential was independent of carrier type, with comparable values for each carrier type. The density and nature of charge on the liposome surfaces are essential factor can affect the mechanism and extent of liposome cell interaction (Sharma et al. 2010). Negative surface charge might raise intracellular uptake of liposomes (Gabizon et al. 1990).

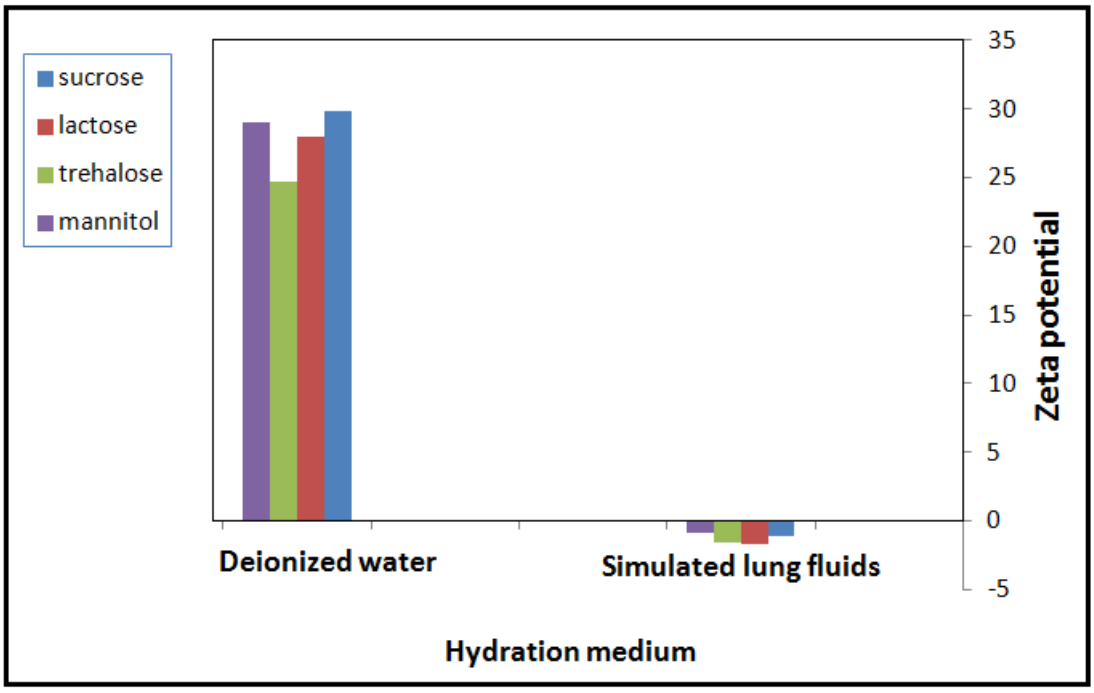

Fig. 5 c: Zeta potential of liposomes in simulated lung fluids and in deionized water

3.4 Entrapment efficiency

To compare the entrapment efficiency of salbutamol in liposomes after hydration by simulated lung fluid or deionized water, HPLC analysis was used. As can be seen from the figure 6, entrapment efficiencies of particulate-based liposomes in simulated lung fluids lower than in deionized water. In reviewing the literature, no data was found on the association between the influence of simulated lung fluid and entrapment efficiency of salbutamol loaded liposomes. A possible explanation might be due to the seinsveral solubility of the carrier based liposomes upon hydration in simulated lung fluid and in deionized water. Hence, it could conceivably be hypothesized that conducting tests using simulated lung fluid is more accurate than in deionized water. This founding have significant implications of developing liposomes as drug delivery system. The test was successful as it was capable to identify the difference in results between conducting tests in simulated lung fluids and in deionized water. In future studies it may be probable to utilize simulated lung fluid instead of deionized water.

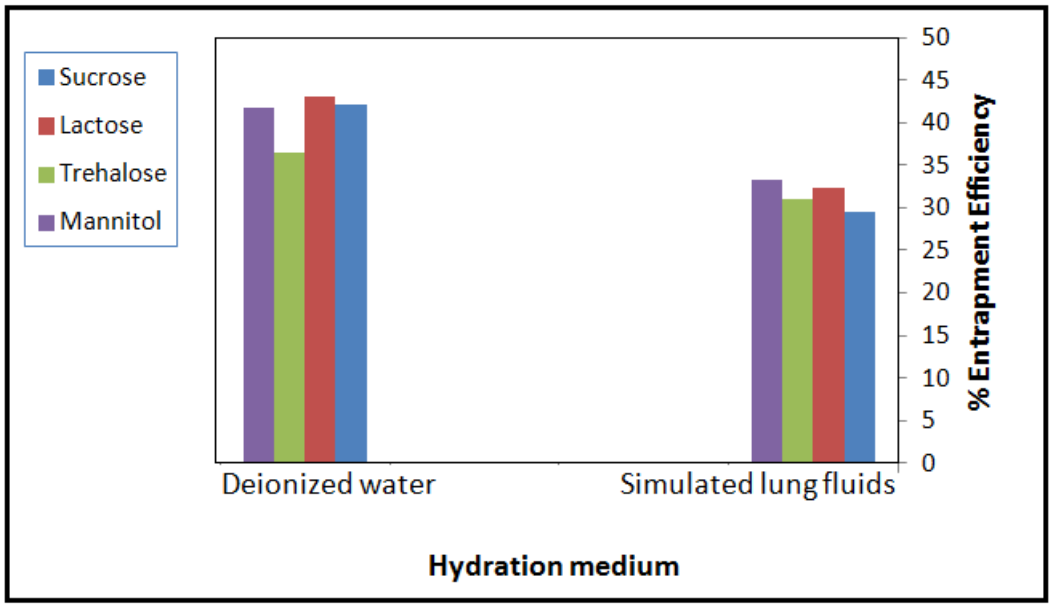

Fig.6: Entrapment efficiency of salbutamol in liposomes in simulated lung fluids and deionized water

CONCLUSION

This study set out to determine the effect of using simulated lung fluid instead of deionized water. This study has shown that VMD, span and entrapment efficiency values of liposomes loaded salbutamol in simulated lung fluids are smaller than in deionized water. The research has also shown that the zeta potential of the particulate-based liposomes in simulated lung fluids was found to be negative in charge, while the zeta potential of liposomes in water was positive. Simulated lung fluid made no significant difference to morphology of liposomes. This study has found that generally there are differences in results between liposomes produced by hydration of simulated lung fluids or deionized water. The current data highlight the importance of using simulated lung fluid instead of deionized water to carry out tests of liposomes.

REFERENCES

1. Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. Journal of molecular biology 13(1):238-IN27.
2. Weiner N, Martin F, Riaz M (1989) Liposomes as a drug delivery system. Drug Development and Industrial Pharmacy 15(10):1523-1554.
3. Mathiowitz E, editor (1999) Encyclopedia of controlled drug delivery. Wiley-Interscience, pp 461–492.
4. Hunt CA, Tsang S (1981) α-Tocopherol retards autoxidation and prolongs the shelf-life of liposomes. International Journal of Pharmaceutics 8(2):101-110.
5. Hernández-Caselles T, Villalain J, Gómez-Fernández JC (1990) Stability of liposomes on long term storage. Journal of Pharmacy and Pharmacology 42(6):397-400.
6. Kensil CR, Dennis EA (1981) Alkaline hydrolysis of phospholipids in model membranes and the dependence on their state of aggregation. Biochemistry 20(21):6079-6085.
7. Grit M, de Smidt JH, Struijke A, Crommelin DJ (1989) Hydrolysis of phosphatidylcholine in aqueous liposome dispersions. International journal of pharmaceutics 50(1):1-6.
8. Elhissi AM, Ahmed W, McCarthy D, Taylor KM (2012) A study of size, microscopic morphology, and dispersion mechanism of structures generated on hydration of proliposomes. Journal of dispersion science and technology 33(8):1121-1126.
9. Payne NI, Ambrose CV, Timmins P, Ward MD, Ridgway F (1986) Proliposomes: a novel solution to an old problem. Journal of Pharmaceutical Sciences 75(4):325-329.
10. Payne NI, Browning I, Hynes CA (1986) Characterization of proliposomes. Journal of pharmaceutical sciences 75(4):330-333.
11. Katare OP, Vyas SP, Dixit VK (1990) Effervescent granule based proliposomes of ibuprofen. Journal of microencapsulation 7(4):455-462.
12. Hwang BY, Jung BH, Chung SJ, Lee MH, Shim CK (1997) In vitro skin permeation of nicotine from proliposomes. Journal of controlled release 49(2-3):177-184.
13. Ahn BN, Kim SK, Shim CK (1995) Proliposomes as an intranasal dosage form for the sustained delivery of propranolol. Journal of controlled release 34(3):203-210.
14. Jung BH, Chung BC, Chung SJ, Lee MH, Shim CK (2000). Prolonged delivery of nicotine in rats via nasal administration of proliposomes. Journal of controlled release 66(1):73-79.
15. Elhissi AM, Taylor KM (2005) Delivery of liposomes generated from proliposomes using air-jet, ultrasonic, and vibrating-mesh nebulisers. Journal of drug delivery science and technology 15:261-265.

16. Park JM, Ahn BN, Yoon EJ, Lee MG, Shim CK, Kim CK (1994) The pharmacokinetics of methotrexate after intravenous administration of methotrexate-loaded proliposomes to rats. Biopharmaceutics& drug disposition 15(5):391-407.
17. Lee HJ, Ahn BN, Yoon EJ, Paik WH, Shim CK, Lee MG (1995) Pharmacokinetics and tissue distribution of adriamycin and adriamycinol after intravenous administration of adriamycin-loaded neutral proliposomes to rats. International journal of pharmaceutics 121(1):1-10.
18. Lee HJ, Ahn BN, Paik WH, Shim CK, Lee MG (1996) Inverse targeting of reticuloendothelial system-rich organs after intravenous administration of adriamycin-loaded neutral proliposomes containing poloxamer 407 to rats. International journal of pharmaceutics 131(1):91-96.
19. Lee MK, Choi L, Kim MH, Kim CK (1999) Pharmacokinetics and organ distribution of cyclosporin A incorporated in liposomes and mixed micelles. International journal of pharmaceutics 191(2):87-93.
20. Desai TR, Wong JP, Hancock RE, Finlay WH (2002) A novel approach to the pulmonary delivery of liposomes in dry powder form to eliminate the deleterious effects of milling. Journal of pharmaceutical sciences 91(2):482-491.
21. Kumar R, Gupta RB, Betegeri G (2001) Formulation, characterization, and in vitro release of glyburide from proliposomal beads. Drug Delivery 8(1):25-27.
22. Kim JC (2001) Preparation by spray drying of amphotericin B-phospholipid composite particles and their anticellular activity. Drug delivery 8(3):143-147.
23. Alves GP, Santana MH (2004) Phospholipid dry powders produced by spray drying processing: structural, thermodynamic and physical properties. Powder Technology 145(2):139-148.
24. Arpagaus C, Meuri M (2010) Laboratory scale Spray drying of inhalable particles: a review. In Respiratory Drug Delivery 2:469-476.
25. Arpagaus C, Schafroth N (2009) Laboratory scale spray drying of biodegradable polymers. In Respiratory Drug Delivery 269-274.
26. Ståhl K, Claesson M, Lilliehorn P, Lindén H, Bäckström K (2002) The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation. International journal of pharmaceutics 233(1-2):227-237.
27. Mathiowitz E, editor (1999) Encyclopedia of controlled drug delivery. Wiley-Interscience,pp 461–492
28. Drysdale M, Bjorklund KL, Jamieson HE, Weinstein P, Cook A, Watkins RT (2012) Evaluating the respiratory bioaccessibility of nickel in soil through the use of a simulated lung fluid. Environmental geochemistry and health 34(2):279-288.
29. Takaya M, Shinohara Y, Serita F, Ono-Ogasawara M, Otaki N, Toya T, Takata A, Yoshida K, Kohyama N (2006) Dissolution of functional materials and rare earth oxides into pseudo alveolar fluid. Industrial Health 44(4):639-644.
30. Rahimpour Y, Kouhsoltani M, Hamishehkar H (2014) Alternative carriers in dry powder inhaler formulations. Drug discovery today 19(5):618-626.
31. Chu MQ, Gu HC, Liu GJ (2002) Study on the preparation of tanshinoneproliposomes by spray drying method. CHINESE PHARMACEUTICAL JOURNAL-BEIJING- 37(1):32-35.
32. Chitlange SS, Chaturvedi KK, Wankhede SB (2011) Development and validation of spectrophotometric and HPLC method for the simultaneous estimation of salbutamol sulphate and prednisolone in tablet dosage form. J Anal Bioanal Techniques 2(117):2.
33. Hotani H (1984) Transformation pathways of liposomes. Journal of molecular biology 178(1):113-120.
34. Pilcer G, Wauthoz N, Amighi K (2012) Lactose characteristics and the generation of the aerosol. Advanced drug delivery reviews 64(3):233-256.
35. Momekova D, Rangelov S, Lambov N, Karlsson G, Almgren M (2008) Effects of amphiphilic copolymers bearing short blocks of lipid-mimetic units on the membrane properties and morphology of DSPC liposomes. Journal of Dispersion Science and Technology 29(8):1106-1113.
36. Zarif L (2005) Drug delivery by lipid cochleates. In Methods in enzymology 391:314-329. Academic Press.
37. Hupfeld S, Holsæter AM, Skar M, Frantzen CB, Brandl M (2006) Liposome size analysis by dynamic/static light scattering upon size exclusion-/field flow-fractionation. Journal of nanoscience and nanotechnology 6(9-10):3025-3031.
38. Pilcer G, Amighi K (2010) Formulation strategy and use of excipients in pulmonary drug delivery. International journal of pharmaceutics 392(1-2):1-9.
39. Sharma Vijay K, Mishra D, Sharma A, Srivastava B (2010) Liposomes: present prospective and future challenges. International Journal of Current Pharmaceutical Review & Research 1(2):6-16.
40. Gabizon A, Price DC, Huberty J, Bresalier RS, Papahadjopoulos D (1990) Effect of liposome composition and other factors on the targeting of liposomes to experimental tumors: biodistribution and imaging studies. Cancer research 50(19):6371-6378.