Research Article

Preparation and evaluation of salbutamol liposomal suspension using chloroform film method

Nidhal Khazaal Marie *PhD*, ***Athmar Dhahir Habeeb*** *MSc*

Department of Pharmaceutics, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq.

Date Submitted :17.2.2012

Date Accepted: 27.5.2012

Address for Correspondence:
Dr. Nidhal Khazaal Marie

Abstract

Background: Liposomes are new drug delivery systems consist of lipid bilayer membrane that encapsulate water and lipid soluble drugs.

Materials and methods: Liposomal suspension was prepared using chloroform film method. Different amount of cholesterol and lecithin (100, 200, 300, 400 and 600 mg) were used and their effect on particle size, encapsulation efficiency and drug release was studied. Cholesterol used in different ratios such as (1:1, 1:2 and 2:1) for Cholesterol: Lecithin respectively to evaluate its effect on particle size, encapsulation efficiency and drug release. The effect of increasing salbutamol HCl amount from 10mg to 20mg on encapsulation efficiency was also studied.

Results: The morphology of the prepared formulae showed uniform unilamellar liposomes enclosing the drug in the internal aqueous phase. The observed results of our work suggest that chloroform film method is a good alternative method for the preparation of liposomes for water soluble drugs because of its simplicity, low cost, time saving and few processing steps.

Conclusions: The work suggests a formula that may improve the permeability of salbutamol HCl through mucosal membrane.

Keywords: Salbutamol liposomal suspension, Cholesterol, Lecithin

INTRODUCTION

Liposomes are a new form of drug delivery systems.[1, 2] They are lipid microparticles formed spontaneously when phospholipids are dispersed in water. They consist of lipid bilayer membrane enclosed an aqueous internal phase.[3] Liposomes are loaded with different drug molecules ranging from small molecules to proteins.[4, 5] The drug is either bound to the lipid bilayer (lipid soluble drugs) or solubilized in the internal aqueous phase (water soluble drugs).[6] They are widely used as tools in biology, biochemistry, cosmetics and medicine. Their use is versatile in medicine such as ocular, anticancer, topical, inhalation, injection and vaccination.[7-11]

Liposomes are present in different sizes and shapes depending on their method of preparation.[12, 13] Liposomes were first prepared in 1961 by Bangham and his method is still used nowadays. However this method provides low encapsulation efficiency and large size liposomes.[14] Over the years different methods have been developed from Bangham such as reverse phase evaporation method,[15] freeze thaw extrusion method,[16] ether injection method,[17] lipid hydration method, sonication method, French pressure cell method, calcium induced fusion method, microfluidization method, dehydration rehydration method[18-22] and chloroform film method.[23] These methods ranging from simple ones, using simple equipment with few steps, to

complicated methods that use sophisticated equipment. The method we chose for our work is chloroform film method.[23] The advantages of this method are simplicity, require few processing steps, few equipment and no sonication which may provide stable and small particle size liposomes.

The drug we chose for our work is salbutamol HCl. Salbutamol HCl is beta 2 adrenergic bronchodilator used for the treatment of various diseases such as asthma. It is freely soluble in water and has low solubility in organic solvents. 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.

The aim of our work is to explore chloroform film method as a simple, effective and easily applicable for the formulation of salbutamol liposomes with proper size, encapsulation efficiency and release. Also this method offers promising way to prepare liposome dry powder for formulation of salbutamol inhaler.

MATERIALS AND METHODS

Materials

Lecithin and cholesterol were a gift from the College of Pharmacy Baghdad University. Salbutamol HCl (from Samarra Drug Industry-Samarra Iraq).

Equipment

Rotary evaporator, pH meter, Optical microscope, Scanning electron microscope, UV-165-OPC spectrophotometer (Shimadzu, Japan) Dissolution Tester USP XXII, cooling centrifuge (Sigma, Germany).

Methods

Preparation of the liposome suspension

Different liposomal suspensions were prepared using the chloroform film method. Cholesterol and lecithin (in different proportions) were mixed and dissolved in 9 ml organic mixture of chloroform: methanol (2:1) respectively. The organic solvent was evaporated using rotary evaporator and a thin film of lipids deposited inside the round bottom flask of the devise. Sixty ml of 0.2 M phosphate saline buffer pH 7.4 containing 10 mg of salbutamol HCl and 1 mM EDTA was added to the deposited film with vigorous shaking and liposome suspension immediately formed.[23]

Evaluation of liposome suspension

Effect of total lipid content

Different amount of lecithin and cholesterol were used to evaluate the effect of their total amount on morphology, particle size, encapsulation efficiency and drug release. The total amounts of lipids used were 100, 200, 300, 400 and 600 mg in 1:1 ratio.

Effect of cholesterol amount

Different ratio of cholesterol (1:1, 1:2 and 2:1 of cholesterol: lecithin) were used to evaluate the effect of its amount on morphology, particle size, encapsulation efficiency and drug release.

Effect of drug amount

Different amount of salbutamol HCl were used to evaluate its effect on morphology, particle size, encapsulation efficiency and drug release.

Morphology

An optical microscope and a scanning electron microscope (SEM) were used to study liposome morphology. For light microscope one drop of the liposomal suspension for each formula was placed on a glass slide and covered with plastic cover and examined. For SEM one drop of the suspension was placed on a stub covered with clean glass and a polaron E5100 sputter-coater was used to sputter coat the sample with gold and the sample was examined under the SEM.

Particle size

Particle size was measured using a modified optical microscope that contained a calibrated ocular and stage micrometer. The size of 100 vesicles were measured under 10, 40, 100x magnification.

Encapsulation efficiency

Liposomal suspension was centrifuged using a cooling centrifuge for 1 hour at 4000 rpm. The supernatant was isolated and analyzed by UV spectrophotometer for drug amount. The encapsulation efficiency was calculated according to the following equation:

$$\text{Encapsulation efficiency \%} = (\text{Total amount of drug} - \text{Free drug}) / \text{Total amount of drug} \times 100\%$$

Drug release

A paddle dissolution apparatus was used for this test. A tube with 2.5 cm in diameter was used. The tube was filled with 10 ml liposomal suspension and the open end of the tube covered with filter paper and the tube turned upside down and immersed to about 10 cm in the 400 ml saline phosphate buffer pH 7.4 (collecting media).The

system was maintained at 37^0C and dissolution apparatus set on 150 rpm for 3 hours. Samples were withdrawn from the collecting media every 30 minutes and replaced with fresh buffer. The samples were analyzed for their drug content at wave length 275nm.

RESULTS

Different liposomal formulae were prepared as shown in table 1. The effect of total lipid amount was studied and it was found that increasing the total amount of lipids as in F1 to F5 lead to increased mean particle size and encapsulation efficiency of the produced liposomes. The encapsulation efficiency increased from 8.4%, 28.4%, 30.88%, 35.54% and 39.77% respectively. The particle size increased from 0.43, 0.47, 0.562, 0.59 and 0.67 micrometer respectively.

Table 1. Various liposomal composition, encapsulation efficiency and mean size of liposomal suspension of salbutamol HCl.

Formula code	Lecithin in mg	Cholesterol in mg	Mean particle size in micrometer +I SD	Encapsulation efficiency
F1	50	50	0.43 ± 0.23	8.4%
F2	100	100	0.472 ± 0.2	28.4%
F3	150	150	0.562 ± 0.2	30.88%
F4	200	200	0.59 ± 0.15	35.54%
F5	300	300	0.67 ± 0.099	39.77%
F6	150	300	0.61 ± 0.17	34.48%
F7	300	150	0.469 ± 0.21	26.89%
F8	300	300	0.65 + 0.1	46.48%

The effect of using different ratios of cholesterol was studied. Cholesterol: Lecithin in ratios 1:1, 1:2 and 2:1 used as in F5, F6 and F7 respectively. The encapsulation efficiency was 39.77%, 34.48% and 26.89% respectively. The morphology of liposome particles is shown in figure 1 using optical microscope and in figure 2 using SEM is a typical image for a liposome.

In vitro release of salbutamol HCl from liposomal suspension was studied and the results are shown in Table 2. The effect of total lipid amount on the release of salbutamol HCl from liposomal suspension was shown in figure 3. The % of drug released within the first 3 hours from F3, F4 and F5 are 60%, 32% and 29% respectively. The effect of using different ratios of cholesterol on drug release was studied and the results shown in figure 4. The % of drug released within the first 3 hours from F5, F6 and F7 are 29%, 30% and 70% respectively.

Table 2. The correlation coefficient of the in-vitro release of salbutamol HCl from liposomal suspension.

Formula code	Zero order R^2	First order R^2	Higushi R^2
F3	0.996	0.779	0.915
F4	0.98	0.92	0.966
F5	0.986	0.919	0.956
F6	0.978	0.937	0.972
F7	0.992	0.787	0.932

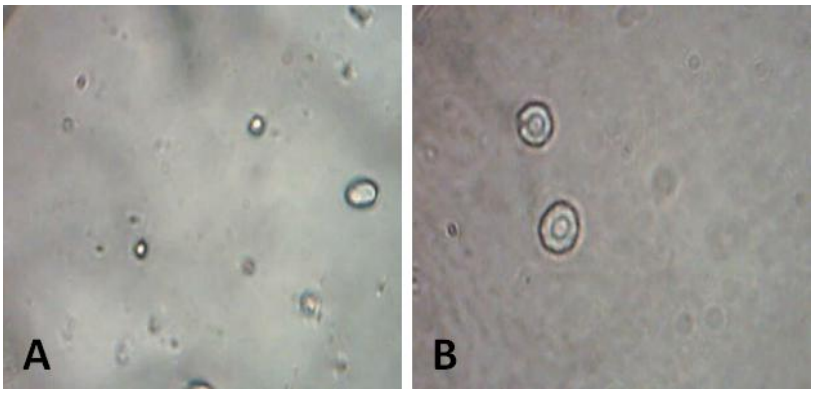

Figure 1. Liposomal suspension of salbutamol HCl (F5) under optical microscope. (**A)** Under optical microscope magnification x40. (**B**) Under optical microscope magnification x100.

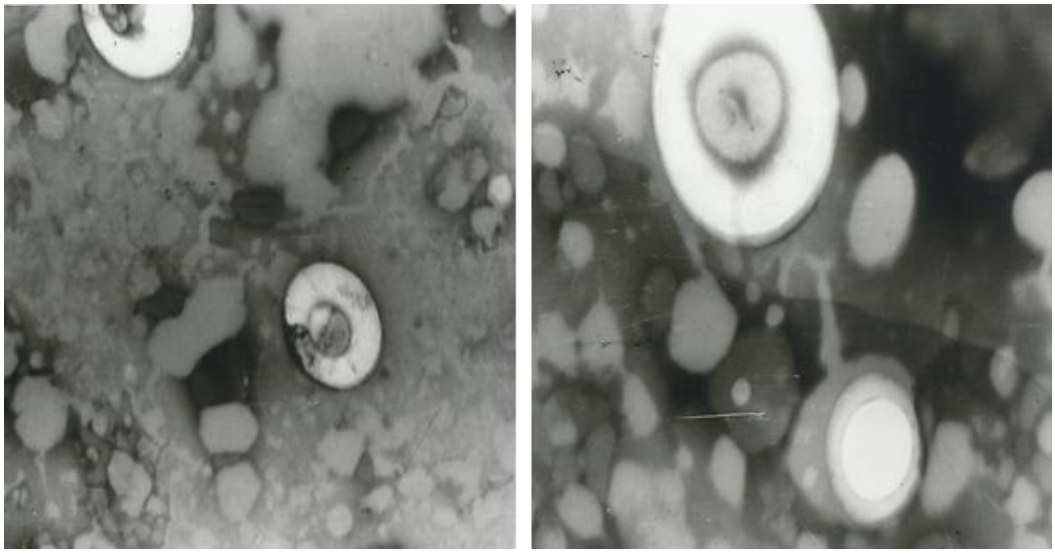

Figure 2. Liposomal suspension of salbutamol HCl (F5) under SEM x 36000 magnification shows unilamellar vesicles.

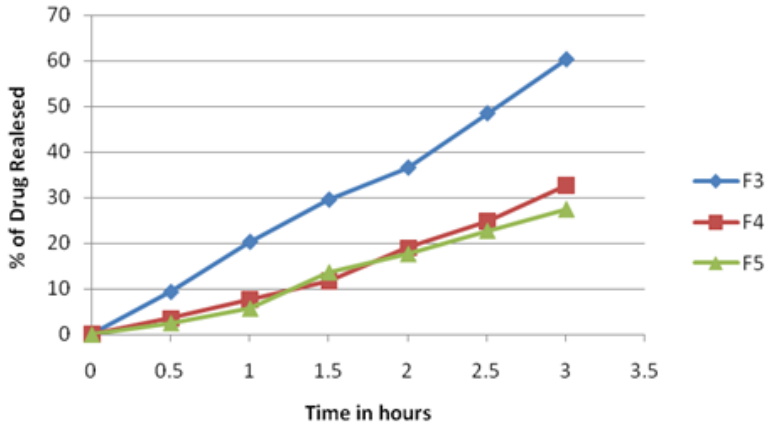

Figure 3. Effect of total amount of lipids on in-vitro salbutamol HCl release from liposomal suspension.

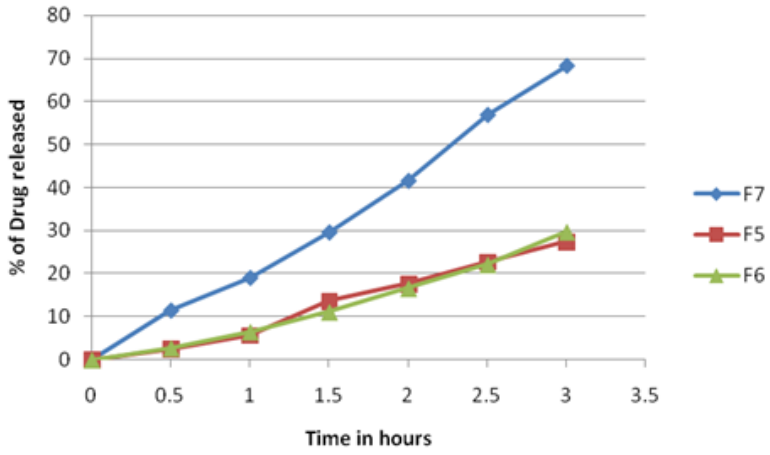

Figure 4. Effect of amount of cholesterol on in-vitro salbutamol HCl release from liposomal suspension.

DISCUSSION

The effect of total lipid amount on liposome characteristics was studied. It was found that increasing the total amount of lipids from 100, 200, 300, 400 to 600 mg as in F1 to F5 lead to an increase in the mean particle size and encapsulation efficiency of the produced liposomes. The encapsulation efficiency increased from 8.4%, 28.4%, 30.88%, 35.54% and 39.77% respectively. The particle size increased from 0.43, 0.47, 0.562, 0.59 and 0.67 micrometer respectively. This is probably because increasing the total amount of lipids lead to the formation of rigid, uniform spherical liposomal suspension due to the formation of more stable liposomes.[23-26] It was noticed that encapsulation efficiency was increased with increasing particle size and this was probably because Salbutamol HCl is a water soluble drug and dissolved in the aqueous buffer encapsulated inside the liposomes, the larger the particle size, the greater the encapsulated fluid and the greater the encapsulation efficiency.[25]

The effect of using different ratios of cholesterol was studied. Cholesterol: Lecithin in ratios 1:1, 2:1 and 1:2 were used as in F5, F6 and F7 respectively. The encapsulation efficiency was 39.77%, 34.48% and 26.89% respectively. It was found that reducing the amount of cholesterol lead to reduction in encapsulation efficiency. This is probably because cholesterol imparts rigidity and stability to the liposome wall and reducing its amount causes lyses and fusion of the liposomes which result in low encapsulation efficiency.[25] Formula 5 was chosen to study the effect of increasing the amount of drug loaded because it shows higher encapsulation efficiency and uniform particle size. The amount of salbutamol HCl increased from 10 mg in F5 to 20 mg in F8. It was observed that the encapsulation efficiency increased from 39.77% in F5 to 46.48% in F8.

The morphology of liposome particles shown in figure 1 using optical microscope is a typical image for a liposome. The images revealed that the suspension contains unilamellar vesicles with mean particle size of (0.67 micrometer $\pm$ 0.099). To confirm the formation of liposomes SEM was also used. The image in figure2 confirms the presence of typical unilamellar liposomes enclosing internal aqueous phase in which the drug is soluble.

From the above results F5 was chosen as the best formula for the preparation of salbutamol HCl liposome suspension because it shows the higher encapsulation efficiency and the best uniform vesicles with acceptable particle size.

The release of the drug from F3-F7 was studied as described before. The mechanism of release kinetics was evaluated by fitting the data to zero, first order and Higushi equation models. All the formulae show zero order Kinetics according to R^2 values as shown in Table 2. The effect of total lipid amount on the release of salbutamol HCl from liposomal suspension is shown in Figure 3 where 60% of the drug was released within the first 3 hours when using 300 mg of total lipids (F3) while only 32% of the drug was released when using 400 mg total lipids (F4) and 29% of the drug released when using 600 mg of total lipids as in (F5). This is because increasing lipid amount lead to the formation of a stable rigid bilayer wall that gives better controlled release liposomes. It was noticed that there is no significant difference in the release of the drug upon using 600 mg total lipids (as in F5) in comparison to F4.This may indicate that increasing lipid content above 400 mg may lead to reducing drug release since more rigid vesicles are produced.

When using Cholesterol: lecithin in 2:1 and 1:2 ratios as in F6 and F7 respectively only 30 % of the drug was released within the first 3 hours in F6. It was noticed that increasing the amount of cholesterol from 33% (as in F7, 1:2 ratio) to 50% (as in F5, 1:1 ratio) showed a dramatic decrease in drug release profile from 70% to 29% respectively within the first 3 hours. This indicates that cholesterol gives more rigid and stable liposomes enclosing the drug inside the internal aqueous phase which will take a longer time to be released.[24] Increasing the amount of cholesterol to 67% (as in F6, 2:1 ratio) shows non-significant difference in drug release (30%) in comparison to F5 (29%). This indicates that using 50% cholesterol (F5) gives maximum stability, better encapsulation efficiency and more uniform liposomes. Therefore any further increase in the amount of cholesterol (as in F6) will have no significant effect on drug released and gives less encapsulation efficiency.

Conclusion

From the above results it was found that salbutamol HCl liposomal suspension prepared using 600mg total lipids and 1:1 cholesterol to lecithin ratio had the best encapsulation efficiency (39.77%) and 29% of the drug released within the first 3 hours compared to the other formulae. The prepared liposomal suspension shows uniform unilamellar vesicles under the microscope. This proves that chloroform film method is a good replacement for the complicated methods for the formulation of liposomes where it gives liposomes with better proper size, morphology, encapsulation efficiency and drug release that will improve the mucosal permeability of water soluble drugs.

REFERENCES

1. Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: an update review. ***Curr Drug Deliv*** 2007;4:297-305.
2. Boyd BJ. Past and future evolution in colloidal drug delivery systems. ***Expert Opin Drug Deliv*** 2008;5:69-85.
3. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. ***Adv Drug Deliv Rev*** 2002;54:S131-55.
4. Panico A, Pignatello R, Pulglisi G. Effects of liposome composition on Erythromycin encapsulation and invitro antibacterial activity. ***Inter J pharm Adv*** 1996;1:443-58.
5. Betageri GV, Vutla NB, Banga AK. Liposomal formulation and characterization of the opiod peptide leucine enkephalin. ***Pharmacy and Pharmacology Communications*** 1997;3:587-91.
6. Cullis PR, Mayer LD, Bally MB, Madden TD, Hope MJ. Generating and loading of liposomal systems for drug delivery applications. ***Adv Drug Deliv Rev*** 1989;3:267-82.
7. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. ***Adv Drug Deliv Rev*** 2007;59:522-30.
8. Wong HL, Bendayan R, Rauth AM, Li Y, Wu XY. Chemotherapy with anticancer drugs encapsulated in solid lipid nanoparticles. ***Adv Drug Deliv Rev*** 2007;59:491-504.
9. Couvreur P, Fattal E, Andremont A. Liposomes and nanoparticles in the treatment of intracellular bacterial infections. ***Pharm Res*** 1991;8:1079-86.
10. Wissing SA, Müller RH. Cosmetic applications for solid lipid nanoparticles (SLN). ***Int J Pharm*** 2003;254:65-8.
11. Hathout RM, Mansour S, Mortada ND, Guinedi AS. Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. ***AAPS PharmSciTech*** 2007;8:1.
12. Yokoyama S, Inagaki A, Tsuchiya K, Sakai H, Imura T, Ohkubo T, et al. Stearylamine changes the liposomal shape from MLVs to LUVs. ***Journal of OLEO Science*** 2005;54:251-4.
13. Szoka F, Papahadjopoulos D. Comparative Properties and methods of preparation of lipid vesicles (Liposomes). ***Annu Rev of Biophys Bioeng*** 1980;9:467-508.
14. Riaz M. Liposomes preparation methods. ***Pak J Pharm Sci*** 1996;19:65-77.
15. Szoka F Jr, Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. ***Proc Natl Acad Sci U S A*** 1978;75:4194-8.
16. Mayer L.D, Hope MJ, Cullis PR. Vesicles of variable sizes produced by a rapid extrusion procedure. ***Biochim Biophys Acta*** 1986;858:161-8.
17. Song W, Wall Z. Ether injection Azithromycin liposomes were prepared. ***Chinese pharmaceutical journal*** 2005;6:876.
18. Mozafari MR. Liposomes: an overview of manufacturing techniques. ***Cell Mol Biol Lett*** 2005;10:711-9.
19. Yan B, Qin X, Bai J, Zhang YH. Preparation of cefazolin sodium liposomes by supercritical carbon dioxide method. ***Wuli Huaxue Xuebao*** 2006;22:226-9.
20. Kshirsagar NA, Gokhale PC, Pandya SK. Liposomes as drug delivery system in Leishmaniasis. ***J Assoc Physicians India*** 1995;43:46-8.
21. Kshirsagar NA. Drug Delivery Systems. ***Indian J Pharmacol*** 2000;32:S54-S61.
22. Arrowsmith M, Hadgraft J, Kellaway IW. The in vitro release of steroids from liposomes. ***Int J Pharm*** 1983;14:191-208.
23. Changsan N, Chan HK, Separovic F, Srichana T. Physicochemical characterization and stability of rifampicin liposome dry powder formulations for inhalation. ***J Pharm Sci*** 2009;98:628-39.
24. López-Pinto JM, González-Rodríguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. ***Int J Pharm*** 2005;298:1-12.
25. Socaciu C, Jessel R, Diehl HA. Competitive carotenoid and cholesterol incorporation into liposomes: effects on membrane phase transition, fluidity, polarity and anisotropy. ***Chem Phys Lipids*** 2000;106:79-88.
26. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. ***Eur J Pharm Biopharm*** 2007;67:398-405.