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AEROSOLIZED LIPOSOMES OF CROMOLYN SODIUM FOR ALVEOLAR TARGETING; PART II: FORMULATION AND CHARACTERIZATION

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ABSTRACT

This is second part of a three article series on aerosolized liposome based lung targeting of cromolyn sodium (CS) focusing on formulation and characterization aspects of the system. In total eleven products were formulated out of which one was designed to contain pre-formed liposomes of CS, one to contain CS in proliposome form and nine to contain cetrimide-cromolyn conjugates (3C) dissolved in propellant- phosphalipid solution. The pressure packs were characterized for aerosol parameters as well as liposome parameters i.e. appearance, leak test, internal pressure, discharge rate, spray pattern, airways penetration efficiency and liposomal size.

Keywords: Sodium Cromolyn, Aerosolized Liposomes, Cetrimide-Cromolyn Conjugate, Liposome, Aerosol Characterization.

INTRODUCTION

As described earlier [1-4] aerosolized liposomes are most suited drug delivery system for pulmonary administration of respiratory drugs having short alveolar half-life. Here, aerosol helps the drug reach to the alveolar region while liposomes provide controlled release [5-7]. Moreover, as the liposomes are formed in-situ, the system does not pose any stability problem.

Having a proper mix of active pharmaceutical ingredients, propellants, phosphalipids, stabilizers and solvents constitutes aerosolized liposome system. Perhaps, it is very vital that all ingredients, when mixed together, result in a true solution state as it is only in the molecular dispersion of phosphalipids and drugs that the in-situ liposome formation would occur. Since CS was not soluble in the propellants or in the desired solvent mixtures, a prodrug, cetrimide-cromolyn conjugate (3C) was synthesized, which fortunately showed optimum solubility and compatibility profile [8].

The critical factors determining the region of drug deposition in respiratory tract (RT) are propellant and valve assembly as they determine the spray pattern as well as particle size of the generated plume. It is only 1 to 5µ particles that reach to the alveolies; particles larger than this tend to settle in the upper portion of the RT, while smaller are breathed-out to the atmosphere without being settled [9-11]. Usually, inclusion of dichlorodifluoromethane (P_{12}) is essential to get the desired results. This propellant, because of low boiling point and high vapors pressure, is either filled through pressure burette in previously sealed containers or by cold filling method.

Characterization of aerosolized liposomal system calls upon for inclusion of parameters to depict the both i.e. aerosol as well as liposome profile [12] of the system. Aerosol parameters of importance are appearance, leak-test, discharge rate, spray-pattern and particle size or penetration efficiency for reaching the alveolies. Liposomal characterization parameters of concern are vesicle size, charge, entrapment efficiency, in-vitro drug release profile etc.

This article, the second of its series deals with formulation and characterization aspects of the system. The release kinetics, which was studied using a specially designed instrument ex-vivo, is presented in the third and final part of the article series.

MATERIALS AND METHODS

Materials

Phosphalipids i.e. phosphatidylcholine (soybean, 21%) (SPC), phosphatidylcholine (egg, 60%) (EPC), ysophosphatidylecholine (egg yolk, 99%) (LPC), phosphatidylserine (bovine brain, 98%) (PS) and sphingomyelin (chicken egg yolk, 99%) (SM) were purchased

from Sigma. Cholesterol was purchased from Loba Chemie and propellants i.e. trichloromonoflouromethane (P_{11}) and dichlorodiflouromethane (P_{12}) and aerosol containers along with valve assemblies were procured from local sources. Other chemicals used were of extra pure laboratory grade.

Preformed Liposome Preparation

Preformed liposomes were prepared by ether injection method of Deamer [13]. EPC (100 mg), cholesterol (100 mg) and 3C (50 mg) were dissolved in 2 ml ether. The resultant solution was injected using a specially designed needle into 5 ml aqueous phase maintained at 55^{0}C at a rate of 0.2 ml per minute in the form of a very fine stream and the aqueous phase was kept under negative pressure.

Aerosolized Liposome Formulation

Aerosolized liposome formulations were prepared using method described by Farr et al [1]. Accurate amounts of phosphalipids, cholesterol and CS or 3C were added to aerosol containers (glass, 10 ml capacity) and P_{11} was added in excess. Evaporation of P_{11} was permitted until the required weight was achieved, which served to evacuate the air from bottles. The bottles were then fitted with valve assembly and crimped to seal. Finally the required quantities of P_{12} were added with the help of pressure burette. The exact formulas are shown in Table 1.

Characterization

The formulations were characterized for various aerosol as well as liposomal parameters i.e. Appearance, Leak test, Internal pressure, Discharge rate, Spray pattern, Penetration efficiency and Liposomal particle size.

Appearance

Pressure packs were observed visually through naked eyes and rated for the number of phases present as well as the homogeneity of the phases. Two-phase homogeneous systems were kept for further evaluation and the rest were discarded.

Leak test

Pressure packs were passed completely immersed through a hot water bath maintained at 50^{0}C and examined for leakage.

Internal pressure

This was measured at 24^{0}C with a pressure-gauze having a suitable adaptor to fit the valve orifice. The pressure packs were inverted and pressed to clear the diptube, and then, the gauze was applied to the valve orifice of the inverted pack.

Discharge rate

The containers were immersed in water bath maintained at 24^{0}C to attain the temperature, then discharged for about 2 seconds to clear the valve. Now the containers were wiped, dried and weighed and in each case valve was pressed down for 5 seconds and the containers reweighed. Three determinants were made and the discharge rate was calculated in mg/sec.

Spray pattern

Shape and cross sectional area of plume (at 15 cm from origin) was determined using the photographic variant method of Benzamine et al [14]. The photographs were taken using Pentax-900 camera loaded with a black and white film at a shutter speed of 1 sec.

Airways penetration efficiency (Particle size)

This was determined by an empirical method described by Kirk [15]. The aerosols were fired for 5 seconds into the airways penetration apparatus resembling the RT and the amount of drug released from the pressure pack and the amount deposited on the filter fitted in the apparatus were determined. The penetration efficiency was calculated as the fraction of drug reaching to the filter.

Liposome characterization

Studying the spontaneous formation of liposomes did liposome characterization of the formulations. Method of Farr et al [1] was adopted for this purpose. Each aerosol unit was fired for two seconds into Single Stage Liquid Impinger (SLI) and the samples were withdrawn at a particular time intervals using a syringe. Photomicrographs of the samples were taken and then these were scrutinized for presence of liposomes as well as their size profiles.

RESULTS AND DISCUSSION

Formulation of Pressure Packs

The formulation matrix was so designed as to result in an adequately comprehensive study. Two products were formulated as controls using SC and remaining all contained 3C. In SC control products, one was designed to have preformed liposomes while the other, proliposomes. These controls served as baselines for comparing preformed-liposome versus pro-liposome and also SC versus 3C. In the SC proliposome product, SC was dissolved with the help of ethylene glycol. How-

ever, ethylene glycol layer did not become miscible with propellant layer, hence, the system was three-phase, and unfit to result into in-situ liposome formation.

Main phosphalipid selected for the study was EPC. Products containing EPC were made in three concentrations so as to find the effect of concentration and also the optima. SPC was used to workout the economy of the system, as it was of lesser purity, hence, of much lesser cost. PS and SM were included to make the charged liposomes, however, as they were exorbitantly expensive only small amounts were used. Cholesterol is known to provide stability to the vesicles hence this was also included in the study, and in addition to a product containing EPC and cholesterol, one was made with cholesterol alone to serve as control.

Characterization

Characterization of aerosolized liposomal products was a relatively tough task as the parameters are quite complex and devices required to measure them are hardly available commercially. This called for in-house construction of many devices. Complete account of aerosol characteristics of pressure packs is given in table 2. Some of the formulations were dropped from the study after testing first two or three parameters. Spray pattern and the airways penetration efficiency were finer rudiments in describing the aerosol performance. Spray-pattern in most of the products was found to be satisfactory with conical plumes. A typical plume is shown in figure 1.

Airways penetration efficiency

The influence of formulation variable on aerosol particle size [10, 11] and airways penetration efficiency is already widely studied and optimized conditions are reported in literature with respect to required propellant mix. Hence, in this study, the point of main focus was the effect of lipid concentration. Highest efficiency was found at the lowest lipid concentration (Table 2). High lipid concentration could have resisted the aerosol disruption by virtue of relatively delayed propellant evaporation. Henceforth, it could be concluded that if lipid concentration is increased, the faction of P_{12} should also be increased.

Table 1: Pressure Pack Compositions

Formulation Code	Ingredients										
	CS (mg)	3C (mg)	SPC (mg)	EPC (mg)	LPC (mg)	PS (mg)	SM (mg)	CHOL (mg)	Ethylene Glycol (ml)	P11 (g)	P12 (g)
SPC		100	500							2.0	8.0
EPC-CS	50	-		500					1.0	2.0	8.0
EPC-200		100		200						2.0	8.0
EPC-400		100		400						2.0	8.0
EPC-600		100		600						2.0	8.0
EPC-CHOL		100		200				200		2.0	8.0
CHOL		100						500		2.0	8.0
LPC		50			100					1.0	4.0
PS		10				5		5		0.4	1.6
SM		20					10	10		0.4	1.6

CS: Cromolyn Sodium; 3C: Cetrimide-Cromolyn Conjugates; SPC: Phosphatidylcholine (Soybean); EPC: Phosphatidylcholine (Egg); LPC: Lysophosphatidylecholine; PS: Phosphatidylserine; SM: Sphingomyelin; CHOL: Cholesterol; P_{11}: Trichloromonoflouromethne, P_{12}: Dichlorodiflouromethne.

Table 2: Aerosol Characterization of Pressure Packs

Formulation Code	Appearance	Leak Test	Internal Pressure (psi)	Discharge Rate (n=3,±sd)	Penetration Efficiency	Spray Pattern	
						Shape of Plume	Area at 15 cm (cm^2)
SPC	Two phase-homogeneous	Passed	32	150±45	30.9%	Conical	17.5
EPC-CS	Three Phase	Passed	-	- -	-	-	
EPC-200	Two phase-homogeneous	Passed	35	142±23	46.2%	Conical	16.8
EPC-400	Two phase-homogeneous	Passed	31	136±31	34.8%	Conical	17.8
EPC-600	Two phase-homogeneous	Passed	33	161±18	25.3%	Conical	16.5
EPC-CHOL	Two phase-homogeneous	Passed	38	152±27	33.7%	Conical	15.8
CHOL	Semisolid mass	Failed	-	-	-	- -	
LPC	Suspension	Passed	-	-	-	- -	
PS	Two phase-homogeneous	Passed	33	-	65.8%	Conical	16.1
SM	Turbid	Passed	-	-	-	-	-

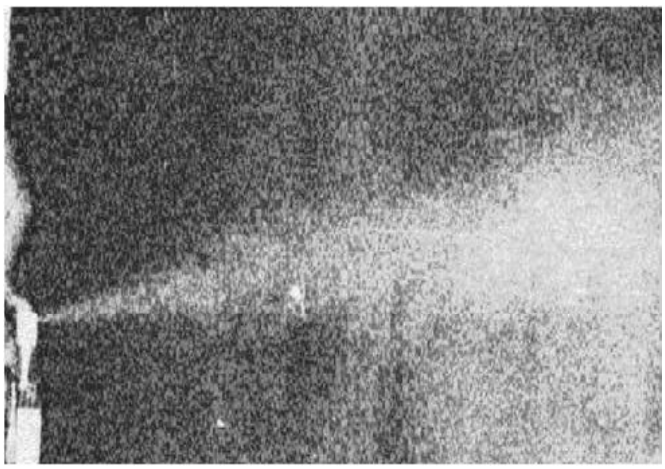

Figure 1: A typical aerosol plume (EPC-400)

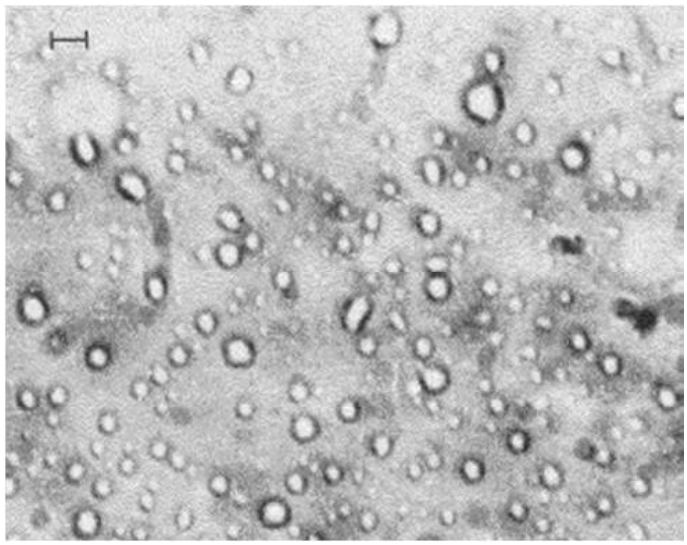

Figure 2: In-situ formed liposomes (EPC-600). Bar represents 5 microns.

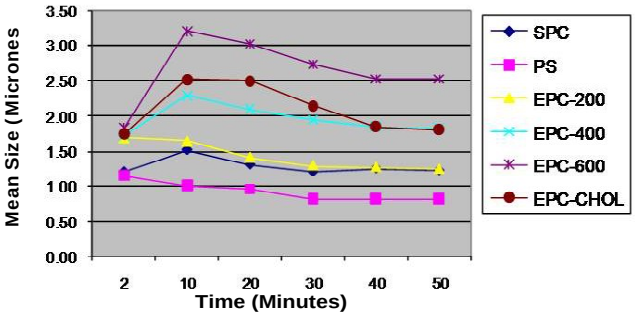

Figure 3: Particle size profile of in-situ formed liposomes

Particle size of in-situ formed liposomes

Photomicrographs (Figure 2) confirmed the in-situ liposome formation on firing the aerosol onto wet surface. Figure 3 shows mean size-time profile of selected pressure pack formulations on deposition in SLI. A rapid change is noticeable in most of the cases until equilibrium attained in 20-40 minutes. The particle size profile of higher lipid containing systems was found to be comparatively different then that of lower lipid containing systems, as these show higher initial size increase before reaching to the stable equilibrium.

Soluble non-volatile components are known to retard the flashing and evaporation of aerosol propellants. Particularly, phosphalipids by virtue of being amphiphilic in nature are likely to show formation of a coherent resistance or barrier at the droplet-air interface, slowing down the evaporation of propellant. Initial size increase of the vesicles could be accounted for internal vaporization followed by slow diffusion of small amounts of residual propellant trapped in hydrating phosphalipid interfaces.

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