

STUDIES CONCERNING THE DEVELOPMENT OF LIPID NANOSTRUCTURES IN BIOPRODUCTS ENCAPSULATION

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Abstract

In recent years, a considerable effort was dedicated to researching methylxantine derivatives (MX), because of their effect on the hemorheology, increasing deformability, decreasing the aggregation trend of the red blood cells and the fibrinogen concentration. All of these properties turned MX into a drug eligible to be in peripheral and cerebral vascular disorders. At the same time, the pharmacological profile of MX and their short half-life make it a good candidate for encapsulation of drugs.

Lipid nanostructures are a new technology for the encapsulation and delivery of bioactive agents. Because of their biocompatibility and biodegradability, along with their nanosizes, they have potential applications in a vast range of fields. They are able to improve the solubility, bioavailability and stability of bioactive agents, to provide protection of drugs and as well to prevent their unwanted interactions with other molecules, to ensure cell-specific targeting, to minimize adverse effects on healthy cells and tissues.

This study aimed at developing the encapsulation of MX into biodegradable, biocompatible and non-toxic carriers. Lipid vesicles were prepared through a physical dispersion method using different ratios of lipids. We studied the changes that occurred in the entrapment efficiency, the particle size and the drug stability when different formulation parameters were modified. The physicochemical properties of the vesicles were significantly affected by the formulation parameters.

Key words: liposomes, methylxantine derivatives, physicochemical characterization

INTRODUCTION

MX derivatives are used both in humans to treat cerebrovascular and peripheral vascular diseases, and in dogs to improve microcirculation and as a consequence, to diminish inflammation and enhance healing of many kinds of skin lesions including: ulcerative dermatosis of Collies and Shelties; dermatomyositis; ear margin seborrhea; atopic disease; and other skin diseases with underlying vasculitis and in horses to treat endotoxemia, laminitis and navicular disease. Because of their pharmacokinetic

properties, they are recommended as good candidates to from the modified release, in order to improve our bioavailability and compliance (Banderas et al., 1997; Hardman et al., 1992; Grigoleit and Leonhardt,1997).

This study was aimed at the development of a method for encapsulating MX derivatives in order to improve their bioavailability and to achieve a controlled drug release profile. Also, the evaluation of the encapsulated forms was studied.

Current research show the preoccupation for the production of vectors ensuring a selective targeting and a controlled release of the drug at the targeted organ or cell (Varshosaz et al., 2010; Lian and Ho, 2001). This approach involves modifying the pharmacokinetic profil of various therapeutic classes of bioactive compounds through their incorporation in colloidal nanoparticulate carriers in the submicron size range such as liposomes or nanoparticles (Lamprecht A., 2009).

Lipid nanostructures are colloidal carriers, usually with a 0.05-5 μm diameter, which form spontaneously when certain lipids are dispersed in aqueous media. They have been reported to improve the solubility, the stability, the bioavailability and the pharmacokinetic properties of the encapsulated bioproducts. Also, they reduce the associated side effects and improve *in vitro* and *in vivo* activity of encapsulated bioproducts (Achim et al., 2009). One of the great benefits of lipid nanostructures is that they can incorporate both hydrophilic and hydrophobic bioproducts. They generally have a large carrying capacity, but usually not large enough to ferry large molecules (such as proteins). Hydrophilic drugs can be readily entrapped within the aqueous interior of the vesicles, while neutral and hydrophobic molecules may be carried within the hydrophobic bilayers of the vesicles (Popovici and Lupuliasa, 2004).

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine (PC) (Sigma–Aldrich)

Dipalmitoil-phosphatidylcholine (DPPC) (Sigma–Aldrich)

Cholesterol (C) (Fluka)

Pentoxifylline (P) and xantinol nicotinate (XN), which were offered as samples from Terapia.

All the other chemicals, reagents and solvents (potassium chloride, potassium dihydrogen phosphate, acetone, chloroform, methanol) used were of analytical or

HPLC purity degree and were purchased from Merck, Germany. All other materials were of analytical grade or equivalent.

Methods

a) Preparation of designed liposomes

From the various lipid nanostructures preparation methods, the hydration of a lipid film was considered as being the most suitable for the encapsulation of MX derivatives. Four formulas for both derivatives were prepared in order to select an optimum formula (with an adequate size, yield and stability).

Lipid solutions were prepared by dissolving accurate amounts of PC or DPPC and C in chloroform. The solution was introduced in a 100 ml round-bottomed flask and the solvent was evaporated in a rot evaporator Heidolph Laborota 4200, at 35°-40°C, under vacuum. Quantities determined in MX derivatives (P or XN) were dissolved in 10 ml phosphate buffer pH 7,4 (0.25 M) solution and then used for hydrating the dry lipid films formed in bottom glass flask. The lipids were mechanically dispersed in the aqueous medium by stirring for 2 hours. The hydration process was performed 40°C. The liposomes were separated from the unincorporated drug by centrifugation over 30 min. at 12000 rpm and at 5°C. The supernatant was removed and the liposomes were reconstituted in 5 ml aqueous medium phosphate buffer (pH 7.4). This suspension was allowed to hydrate for 2 h in order to anneal any structural defects.

Four formulations of liposome (Tables 1 and 2) were prepared in order to select an optimum formula (with an adequate size, yield and stability).

b) Size measurement

The determination of the diameter was performed by a Mastersizer 2000 Malvern apparatus.

c) Determination of drug content

The content in drug was analyzed spectrophotometrically.

The percentage of encapsulated MX was calculated using the formula:

$$\% P_e = \frac{MX_s}{MX_t} \times 100$$

in which: MX_e = amount of drug in sediment; MX_t = total amount of drug/ml liposomal suspension (Shivhare et al., 2009).

Also, the influence of the lipid composition and of the temperature during the hydration process on the yield was studied.

RESULTS AND CONCLUSION

a) Preparation of designed liposomes

In the design of the experimental plan for the preparation of the MX-loaded liposomes, we varied the ratio between PC, DPPC, C and P or XN (Tables 1, 2).

Table 1: The composition of the experimental liposomes loaded with P (mg)

Sample	PC	DPPC	C	P
L1P	3	3	3	2
L2P	3	3	2	2
L3P	4	4	1	2
L4P	9	9	1	2

Table 2: The composition of the experimental liposomes loaded with XN (mg)

Sample	PC	DPPC	C	XN
L1XN	3	3	3	1
L2XN	3	3	2	1
L3XN	4	4	1	1
L4XN	9	9	1	1

b) Size measurement

The results show that the vesicles are polydisperse, with an average size ranging from 200 to 570 nm, the majority sizing between 300 and 380 nm (Table 3).

Results of particle size analysis showed that with decreasing ratio of phosphatidylcholine and cholesterol occurs and a decrease in the average size of the designed liposomes.

Table 3: Evaluation of the size for experimental liposomes loaded with MX derivatives (nm)

Type of liposomes	Mean particle size (nm± SD)	Type of liposomes	Mean particle size (nm± SD)
L1P	380 ± 4.81	L1XN	320 ± 5.07
L2P	365 ± 3.84	L2XN	290 ± 4.32
L3P	310 ± 2.72	L3XN	220 ± 2.91
L4P	260 ± 1.71	L4XN	180 ± 1.82

(Mean ± SD, n=3)

c) Determination of drug content

The analysis the UV spectra of MX, both in the presence and in the absence of phosphatidylcholines has shown that this method of assay is adequate, as the lipids and any MX derivatives have peak absorptions at different wavelengths.

Table 4: The influence of the lipid composition on the encapsulation degree

Type of liposomes	Degree of encapsulation (%)	Type of liposomes	Degree of encapsulation (%)
L1P	31.73 ± 0.87	L1XN	28.13 ± 0.66
L2P	35.28 ± 0.67	L2XN	31.93 ± 0.73
L3P	42.92 ± 0.46	L3XN	36.22 ± 0.86
L4P	46.32 ± 0.32	L4XN	38.27 ± 0.48

(Mean ± SD, n=3)

The data shows that an increase in the cholesterol percentage results in a decrease of the degree of encapsulation from 46 % to 32 % for P, and that the increase of phosphatidylcholine proportion leads to an increase of the degree of encapsulation, as described throughout the literature (Popovici et al., 1998; Gregoriadis G., 2007). The same observations are valid and for XN. It seems that the degree of encapsulation is lower in each case for P comparative with XN due to its higher solubility.

CONCLUSION

Lipid film hydration is an appropriate method for obtaining liposomes loaded with MX derivatives.

Using this method we obtained a liposomal dispersion type MLV with heterogeneous size and entrapment efficiency according to the composition. An increase in the cholesterol percentage results in a decrease of the degree of encapsulation.

An increase in PC proportion leads to an increase of the entrapment efficiency.

From results of MX derivatives entrapped it was observed that as the amount of cholesterol increased there was subsequent increase in the

stability and rigidity of liposomes but at the same time entrapment efficiency reduced due to reduction in PC and DPPC.

We have determined the optimal parameters for the preparation of MX-loaded vesicles, which ensure the reproductibility of size, content and stability.

In conclusion, these results regarding preparation and characterization of some liposomal formulations could be developed in some sustained or controlled release dosage forms.

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