

# LIDOCAINE HYDROCHLORIDE CHITOSOMES: PARETO CHARTS TO DETERMINE THE MAIN EFFECTS AFFECTING FORMULATION

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## Abstract

Lidocaine hydrochloride (Lid) chitosomes were prepared by coating multilamellar liposomes with chitosan (CH). A technique based on a  $2^{k-p}$  fractional design was applied for the optimization of multicomponent liposomal formulations. The CH concentration, the dropping rate into the liposomal dispersion, the stirring rate of this preparation, time period until the coating process has been done and the Lid amount enclosed into the liposomes, comprised the five factors of the system. The encapsulation efficiency (EE), the coating efficiency (CE) and the zeta potential were the three response variables of the system to be optimized. Data analysis revealed that Lid amount was the predominant factor increasing the entrapment capacity. The CE variable was mainly affected by the CH concentration and the dropwise rate of the CH solution into the liposomal dispersion. Also, this later factor had a certain influence on zeta potential values, as well as the low stirring rate, increasing the positive charge on the liposome surface.

## Introduction

Many attempts have been made to provide an efficient anaesthesia of the skin or mucosal tissues in the case of pain itching and buening associated with cutaneous inflammatory response to different agents and with minor surgical operations. However, several studies indicated the limited efficiency on intact skin of available anaesthetic preparations and the need of prolonged applications and high drug concentrations (1, 2).

Lid is a tertiary amine base that is administered as water soluble hydrochloride. In their percutaneous or dental applications, the drug should remain in the skin surface as its uncharged, lipophilic form for a substantial period of time, so that it penetrates the stratum corneum and desensitize the underlying pain receptors within the skin (3).

Therefore, the great interest in Lid delivery systems prompted us to evaluate the possibility to load it in liposomes, already studied by other authors (4, 5). Liposomes incorporating local anaesthetics (LA) showed greater effectiveness making shorter application periods possible as well as decrease of side effects (6).

Cationic polysaccharides, such as chitosan, are large-scale commercial products that have many useful characteristics, such as hydrophilicity, biocompatibility and antibacterial properties. This group of polymers can interact effectively with water, causing the medium to thicken. In addition, owing to their amino/ammonium groups, cationic polysaccharides show a natural attraction for some components of the skin and the hair. On the basis of this property, several authors have used CH as a liposome coating agent, to increase the stability towards drug release (7, 8) and for targeting purposes (9).

Several authors (10) showed that the CH-coated liposomes were formed via ionic interaction between the positively charged CH and negatively charged phosphatidylcholine on the surface of the liposomes. The number of factors that can influence a polymer/colloid system such as chitosan/liposome ratio is large and small

change in one factor may seriously alter the stability as well as other characteristics of the system.

The purpose of the present work was to investigate the effect of some formulation factors on the charge, encapsulation efficiency and coating efficiency from the CH-liposome complex.

## Materials and Methods

**Materials.** L  $\alpha$ -phosphatidylcholine, PC (60 % w/w purity) and cholesterol, CHOL (99 % w/w purity), were purchased from Sigma (Barcelona, Spain). Lid (Genox Farma, Barcelona) was used as drug. CH samples (viscosity: 50 cp) were kindly provided by Padetec Interprice (Brazil). Chloroform (Panreac Chemistry, Barcelona) and all other chemicals were at least reagent grade and used as received.

**Preparation of CH-coated liposomes.** Multilamellar liposomes were prepared by the hydration method. In a typical procedure, 78.94  $\mu$ mol PC, 103.46  $\mu$ mol CHOL and 2.32  $\mu$ mol  $\alpha$ -tocopherol, were dissolved in a small amount of chloroform. The solution was placed in a rotary evaporator at 58 °C until a thin lipid film was obtained. This was then hydrated with 4 mL of Sorensen phosphate buffer pH 5.0 by vortexing six cycles, followed by incubation for 30 minutes. For polymer coating of liposomes, appropriate amounts of CH were dissolved in a solution containing 0.5 % v/v of glacial acetic acid. CH solution (containing 0.1 % w/v or 1 % w/v) was added dropwise into the respective liposomal suspension under controlled stirring at room temperature, followed by incubation at 10 °C for 1 h. The dropping and stirring rates were varied from 0.17 to 0.67 mL/min and 1 to 6, respectively. The final concentrations of the lipids and CH were half of the original solutions.

**Encapsulation efficiency.** The EE was determined by an indirect method, by using dialysis bags. 3 mL of the drug-loaded vesicles were placed into the dialysis bag which was then transferred into 150 mL of phosphate buffer solution (PBS) pH 5.0. Samples of 10 mL were withdrawn at fixed time periods from outside of the bag and replaced with equal volumes of PBS solution. They were spectrophotometrically analyzed at 263 nm.

**Coating efficiency.** 4 mL of liposomes and CH solution were centrifuged at 3000 rpm for 45 minutes to separate the fraction of CH adhered and non-adhered to the vesicles. To calculate the amount of free CH in solution, a colorimetric method was applied (11). Protonated amino groups of CH can act as cationic sites for anionic dyes. In this work, Cibacron brilliant red 3B-A (also known as Reactive Red 4), was used.

### Determination of zeta potential

Surface charges of chitosomes were measured on a Malvern Zetasizer 2000 using a dip cell. Measurements were made in triplicate

### Screening study

A two-level  $2^{k-p}$  fractional factorial design was employed in this study. The experimental matrix for this design is shown in table 1.

This specific design, comprising 16 runs, is described as a  $2^{(5-1)}$  design of resolution V (five). The 16 formulations listed in table 2 were evaluated in random order to nullify the effect of extraneous or nuisance variables. After the three responses had been collected, the system was ready for analysis.

**Table 1.** Composition of the different formulations. CH: chitosan concentration (factor A); DR: dropping rate (factor B); SR: stirring rate (factor C); Time: time period from the fabrication of the liposomal dispersion until coating process (factor D); Lid: lidocaine hydrochloride amount added (factor E).

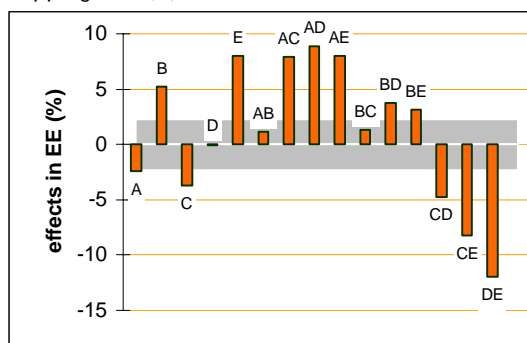
Batch	CH (%)	DR (mL/min)	SR (rpm)	Time (h)	Lid (mg)
1	0,1	0,17	30	0,5	100
2	1	0,17	30	0,5	40
3	0,1	0,67	30	0,5	40
4	1	0,67	30	0,5	100
5	0,1	0,17	100	0,5	40
6	1	0,17	100	0,5	100
7	0,1	0,67	100	0,5	100
8	1	0,67	100	0,5	40
9	0,1	0,17	30	24	40
10	1	0,17	30	24	100
11	0,1	0,67	30	24	100
12	1	0,67	30	24	40
13	0,1	0,17	100	24	100
14	1	0,17	100	24	40
15	0,1	0,67	100	24	40
16	1	0,67	100	24	100

The effects of CH, DR, SR, Time and Lid, on the EE (%), CE (%) and zeta potential of the formulations were analyzed by using the Yate's algorithm and plotted by using the Pareto charts. This graph shows the estimates of the factor main effect plotted against all the factors and their interactions. The factors are ordered from A to E, according to its nomenclature, with the interactions among them. This design allows to separate the factor size and its sign. From these results, two regions (lower and upper bounds) were calculated to indicate the threshold for statistical significance.

## Results and Discussion

### Pareto charts of the effects

In figure 1 the obtained main effects and the interactions among them on the EE have been shown. With respect to the main factors (A to E), a clear positive effect was obtained for the dropping rate (B).



**Figure 1.** Pareto chart for the factors main effect on the EE (%)

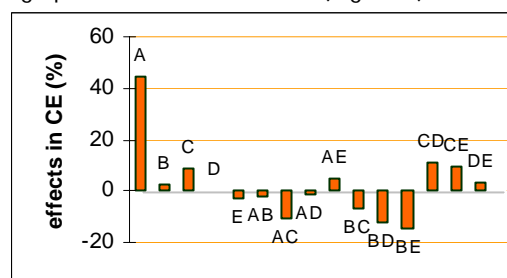
So, because the effect is located in the upper site of the graph, the pass from the lower level (0.17 mL/min) to the higher level (0.67 mL/min) implies an increase in the EE values. Therefore, we should fix the addition rate of the CH solution to the liposome dispersion at 0.67 mL/min.

The factor D had not a significant relevance individually; however, its interaction with all other factors, had a great importance in the whole system. We can also observe in figure 1 how the A and C effects have been multiplied when the interaction between them is produced and when the factor D has been considered. So, we can conclude that the whole coating mechanism could affect the drug encapsulation. CH has

strong affinity to the phospholipid due to its positive charge. The adhesion of a polymer layer on the liposome surface tends to stabilize the system.

Also, on the basis of obtained results, we can conclude that higher Lid concentrations (factor E) can be used to formulate chitosomes.

With respect to the second response (CE), it was observed that the CH concentration (A) exerts a high positive effect on the CE (Figure 2).



**Figure 2.** Pareto chart for the factors main effect on the CE (%)

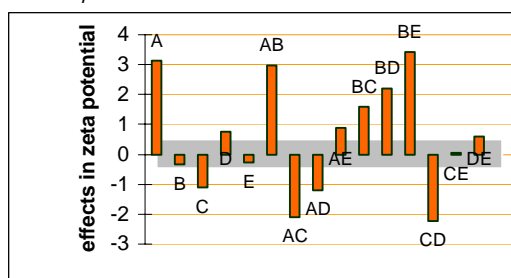
Therefore we can conclude that the coating process was favoured by passing from the lower CH concentration to the higher CH concentration used. The factor C (SR) also affects significantly the response in a positive sense. An electrostatic interaction between positively charged CH and the opposite charge on liposomes exists. The CH covers the surface of the liposomes by forming the ion-complex with PC in the liposomal formulation. Since all formulations possessed high positive charge, they have a resistance to flocculation and coagulation (12).

With respect to zeta potential, the Pareto charts show that the coating mechanism significantly affects the surface charge of the vesicles (Figure 3): the increase in the CH concentration up to 1 % affects positively to the zeta potential. This response must be maximized, since a good adhesive reaction between the chitosomes and the skin may be produced.

The most accepted theory with respect to liposome-chitosan interaction is that CH covers the surface of the liposomal formulation (12, 13). In this work, the positive values of the resultant liposomes increased on increasing the amount of CH in the liposome formulations.

On the other hand, an inverse relationship between the stirring rate and the zeta potential has been found.

**Figure 3.** Pareto chart for the factors main effect on the zeta potential



Therefore, this factor must be fixed in the lower level. Also, when liposomes were coated 24 h after production, it was observed an increase in the zeta potential, as a consequence of the better coating process. The fifth factor studied (E), that is, the Lid amount, has not a statistical significance on this response, meaning that the drug molecules remain in the aqueous liposome compartment.

As a conclusion, the use of this screening design diminished considerably the number of experiments and gave us a very useful information about the main effects of the examined factors.

If a high Lid EE is desired, a high drug amount should be used, in combination with a high dropping rate. If a high CE was required, the CH concentration must be high and being added at a high dropping rate. If an increase in the positive charge on the liposome surface is desired, a high CH concentration should be added, at a low stirring rate.

The optimization process of the formulation combining all the factors affecting significantly these responses is currently in progress. Several optimization methods are being applied and they will be the purpose of next papers.

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