P-081 **Nonwoven gauzes functionalization with liposomes entrapping piroxicam**

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INTRODUCTION AND OBJECTIVES

Non-steroidal anti-inflammatory drugs (NSAIDs) could suppress the imbalance of chronic inflammation, which characterizes chronic wounds (Nizamutdinova 2009), leading to wound healing. On the other hand, from the ancient times, a suitable material had to be used to cover the wound in order to prevent any infection. In fact, the use of textile materials in wounds presents various advantages (Gouveia 2011). In addition, prolonged contact time of a drug with a body tissue, through the use of gauzes, for example, can significantly improve the performance of many drugs.

In this work, it was used liposomes to entrap piroxicam and further their attachment onto nonwoven gauzes it was performed. It was used two types of liposomes: multilamellar liposomes (MLVs) and large unilamellar liposomes (LUVs). LUVs were produced, from MLVs of egg-yolk phosphatidylcholine (EPC), using ultrasound. Besides liposomes type, it was also investigated the influence of EPC concentration (1500 and 3000 μM) in the piroxicam encapsulation efficiency. The liposomes production was followed by their characterization, which included the determination of size, polydispersity index (PDI), zeta-potential, morphology and piroxicam encapsulation efficiency. After production optimization and characterization of the liposomes, they were attached onto nonwoven gauzes in order to produce a functionalized biomaterial with the ability to deliver the pharmaceutical agent in a control manner. The quantification of piroxicam released from liposomes attached onto gauzes surface it was evaluated by UV spectrophotometry.

MATERIALS AND METHODS

Reagents

The chemical reagents were purchased from Sigma-Aldrich and all were used as supplied. The nonwoven gauzes were commercial available in a local pharmacy.

Liposomes preparation and drug incorporation

Liposomes were prepared by the thin film hydration method. Briefly, a known amount of EPC dissolved in chloroform was mixed or not with a piroxicam solution in chloroform/methanol (1:1, v/v). Piroxicam:EPC ratio used was 1:3. The resulting dried lipid film, obtained after removal of organic solvents, was dispersed by the addition of phosphate buffered saline solution (PBS; 0.01 M, pH 7.4) and the resultant mixture was vortexed, at room temperature, to yield MLVs. The sonication of this suspension was carried out with a total treatment of 21 min monitored in 3 min increments, at 25 ± 1 ºC. A pulsed duty cycle of 8 s on, 2 s off was used for all the experiments with indicated power delivery of 40%. After LUVs preparation, the suspension was submitted a one centrifugation (2500 g, 15 min) to remove titanium particles. Liposomes were separated from non-entrapped drug by size exclusion chromatography.

Determination of size, PDI, zeta-potential and morphology of liposomes

Size distribution and zeta-potential values of EPC liposomes, with and without incorporated drug, were monitored at pH 7.4 (PBS), at 25.0 ± 0.1 ºC, in a Malvern Zetasizer Nano Series. Liposomes morphology was determined using scanning electron microscopy (SEM). Samples were dried, at the room temperature, and further coated with gold, in vacuum. This technique was also used to evaluate the attachment of liposomes onto the nonwoven gauzes.

Quantification of piroxicam encapsulation into liposomes

Piroxicam concentration was determined by UV spectrophotometry, at 353 nm. The encapsulation efficiency of drug into liposomes was evaluated by:

$$\text{Encapsulation efficiency}(\%) = \frac{[\text{NSAID}]_i - [\text{NSAID}]_f}{[\text{NSAID}]_i} \times 100$$

where $[NSAID]_i$ and $[NSAID]_f$ is the initial and final NSAID concentration in supernatant, respectively, after separation of the liposomes.

Chemical activation of gauzes

The cationization was performed in sealed, stainless steel pots of 120 cm^3 capacity in a laboratory scale dyeing machine (AHIBA Spectradye). The treatment was carried out using a material:liquor ratio of 1:20, 10% (on weight of the fabric) of poly(diallyldimethylammonium chloride) and 5 $g.L^{-1}$ of sodium hydroxide, over 60 min at 50 ºC.

Attachment of liposomes containing piroxicam onto nonwoven gauzes and drug release

The attachment of liposomes was achieved by incubating MLVs or LUVs with the gauzes in a water shaking bath, at 25 ºC, over 24 h, under constant shacking (50 rpm). After incubation, the samples were washed with deionized water. The presence of liposomes onto gauzes samples was evaluated by staining the samples and controls (nonwoven gauzes, cationized and non-cationized, without liposomes) with Comassie brilliant blue G250 and Reactive red 66. The staining was executed in AHIBA

machine, using 0.25 and 1 g.L^{-1} of the dyes, at 60 ºC for 60 min. The colour measurements were determined using a Spectraflash spectrophotometer (illuminant D_{65} at 600 nm for Comassie dye and 540 nm for Reactive red 66). The colour strength was evaluated as K/S (K is absorption coefficient and S is scattering coefficient).

The release of piroxicam by liposomes attached onto gauzes (≈ 0.07 g) was assessed by incubation of the textile samples in deionized water (3.5 mL). At determined time points, aliquots were taken, and the piroxicam release was monitored by absorbance measurements at 356 nm.

RESULTS AND DISCUSSION

Characterization of liposomes containing piroxicam

The values of size and PDI obtained for LUVs were 70.6 ± 1.10 nm and 0.30 ± 0.04, respectively, and for MLVs the size obtained was equal to 1730 ± 10.25 and the PDI presented a value of 1.00. As expected, the MLVs population presents a higher size and PDI, due to their heterogeneity. On the other hand, there was no significant reduction of the zeta-potential values between LUVs and MLVs with piroxicam (≈ -1.3 mV), when compared with liposomes without drug (≈ -0.91 mV). This is in agreement with the presence of zwitterionic forms of piroxicam, at pH 7.4. SEM analysis demonstrates that LUVs and MLVs, with or without piroxicam, present a similarly spherical shape. Finally, the values obtained for encapsulation efficiency of piroxicam were greater when it was used an higher phospholipid concentration, but, for the same concentration, were similar for MLVs and LUVs (≈ 52% and 56% for 1500 μM and 3000 μM of EPC, respectively). These results can be related with the hydrophobicity of piroxicam that will allow its location mainly between phospholipids in the lipid bilayer.

Attachment of liposomes containing piroxicam onto nonwoven gauzes

The presence of liposomes onto nonwoven gauzes led to a decrease of the colour strength comparatively to the control. Additionally, a superior decrease of K/S values was obtained for the cationized samples, suggesting an higher attachment of the liposomes onto positively surfaces charge of the fibres. SEM analysis of the fibres also corroborates the results obtained for K/S.

Comparing piroxicam concentrations released by the cationized and non-cationized gauzes (Figure 1), it is possible to conclude that the cationized gauzes, for the same EPC concentration, led to higher concentrations of the NSAID in the aqueous environment. As referred before, the cationization process would favour the attachment of the liposomes, which presents a negative surface charge, onto the structure of the gauzes material. In Figure 1, it is also possible to observe that the NSAID is released, mainly, in the first hours. Furthermore, the use of higher concentration of phospholipids (3000 μM), promotes an increase of the NSAID concentration in the aqueous phase. In fact, the greater the number of MLVs or LUVs in suspension, the greater is the amount of NSAID encapsulated. Moreover, comparing piroxicam release by MLVs and LUVs, an higher concentration of this drug in the aqueous phase was obtained when the gauzes had attached MLVs, which can be related with the higher decrease of K/S values.

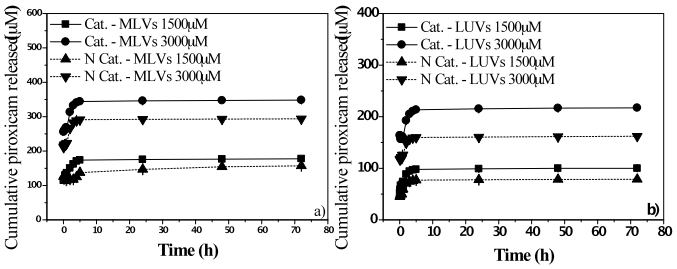

Figure 1: Release profile of piroxicam encapsulated on MLVs (a) and LUVs (b) of EPC (1500 and 3000 μM) from cationized and non-cationized gauzes.

CONCLUSIONS

The chemical activation of gauzes improved the content of liposomes at the fibres surfaces' and, consequently, a higher concentration of piroxicam is released. Additionally, the highest concentration of piroxicam released can be reached when it is used MLVs prepared with an higher EPC concentration. The results achieved indicate that these functionalized gauze bandages may potentially be used as powerful bioactive carriers to control and to heal inflammatory conditions, as in wound healing.

REFERENCES

- Nizamutdinova I. T. et al (2009) *Anthocyanins from black soybean seed coats stimulate wound healing in fibroblasts and keratinocytes and prevent inflammation in endothelial cells*. Food and Chemical Toxicology 47 (11) 2806–2812.
- Gouveia I. C. (2011) *Synthesis and characterization of a microsphere-based coating for textiles with potential as an in situ bioactive delivery system*. Polymers for Advanced Technologies: DOI: 10.1002/pat.1877.

ACKNOWLEDGEMENT

Helena Ferreira thanks POPH/FSE for co-financing and FCT for fellowship SFRH/BPD/38939/2007.