Materials Science and Engineering C 33 (2013) 1193–1196

Contents lists available at SciVerse ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# Immobilization of aloin encapsulated into liposomes in Layer-by-layer films for transdermal drug delivery

Aline Carla Farrapo Xavier, Marli Leite de Moraes, Marystela Ferreira *

*Universidade Federal de São Carlos, Campus Sorocaba, 18052-780, Sorocaba, SP, Brazil*

ARTICLE INFO

*Article history:*
Received 16 February 2012
Received in revised form 25 October 2012
Accepted 2 December 2012
Available online 9 December 2012

*Keywords:*
Drug delivery
Liposomes
Aloin
Layer-by-layer film
Patches

ABSTRACT

Layer-by-layer (LbL) films have been exploited in drug delivery systems that may be used in the form of patches, but the encapsulation of poor water soluble drugs and their release with a controlled rate are still major challenges to be faced. In this paper, we demonstrate the controlled release of aloin (barbaloin), an important component of the widely used *Aloe vera*, encapsulated into liposomes and immobilized in LbL films with a polyelectrolyte. With a systematic study using fluorescence spectroscopy of aloin release from solutions and from LbL films with different phospholipid liposomes, we inferred that optimized release was achieved with aloin incorporated into palmitoyl oleyl phosphatidyl glycerol (POPG) or dipalmitoyl phosphatidyl glycerol (DPPG) liposomes immobilized in LbL films. Significantly, with this optimized system aloin was almost completely released within 30 h, with a small release rate at the end, which followed a sharp release in the first 5 h. Upon comparing the rates of the distinct systems, we conclude that the main factors controlling the release are the electrostatic interactions involving the negatively charged phospholipids. Because these interactions can be tuned in LbL films, the approach used here opens the way for new drug delivery systems to be developed with fine control of the drug release.



## 1. Introduction

Transdermal drug delivery systems (TDDS) or patches use the skin as an alternative route for the delivery of an effective amount of drugs [1]. These systems are advantageous in comparison to those based on oral administration, because the drug concentration in the plasma varies to a lesser extent, which also minimizes side effects [1,2]. There are only few patches available commercially [3,4], however, owing to the difficulties associated with the low drug solubility and need to find appropriate carriers [3]. New approaches have been proposed to overcome these limitations, including incorporation of drugs into dendrimers, liposomes, carbon nanotubes and polymeric nanospheres [1]. Liposomes, in particular, are widely studied for drug incorporation and controlled release in solution, due to their good protective capacity, fluidity and biocompatibility [5]. Most importantly, liposomes can be used with various types of drugs, regardless of their solubility. Hydrophilic drugs can be entrapped into their internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be coupled to the lipid bilayer by hydrophobic and/or electrostatic interactions. The clinical applications of liposomes include selective delivery of the anticancer agent doxorubicin from polyethylene glycol (PEG) liposomes for treating solid tumors, and delivery of amphotericin B in the treatment of visceral leishmaniasis [4].

For the specific application in patches, liposomes need to be immobilized in a solid film, which should be biocompatible. A suitable method to produce biocompatible films or functionalized surfaces is the layer-by-layer (LbL) technique [6], which allows for molecular-level control of film properties [7]. Furthermore, LbL films are amenable to preserve the activity of biomolecules for considerable periods of time owing to the mild film fabrication conditions and presence of entrained water in the films [8]. LbL films for drug delivery may include linear polymers, nanoparticles, micelles and liposomes, with the release rate being controlled through film degradation and/or changes in parameters such as pH, ionic strength and temperature [9]. For instance, glucose has been used to trigger the release of anticancer drug (Doxorubicin) through pH-triggered degradation of PVA-borate and chitosan films [10]. These triggers provide the dose of drugs on demand with reduced toxicity and increased efficacy, but in real systems the release may be ineffective because these parameters vary very little [11]. Indeed, the difficulties in obtaining the total release of a drug from liposomes immobilized in LbL films have been highlighted by Geraldo et al. [12].

In this study, we investigate the release of aloin (barbaloin) (10-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9-anthracenone) anthraquinone from *Aloe vera* incorporated into liposomes and immobilized in LbL films. In order to mimic the real delivery conditions, in addition to the use of liposomes, the experiments were made at 37 °C, with PBS buffer possessing pH and ionic force close to physiological conditions. Aloin is used to manufacture beverages, food products, cosmetics and medicines. Because it also exhibits anti-inflammatory properties, by

* Corresponding author. Tel.: +55 15 3229 5969; fax: +55 15 3229 6000.
*E-mail address:* marystela@ufscar.br (M. Ferreira).

0928-4931/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2012.12.021

diminishing cytokine production in human T-lymphocytes and endothelial cells, aloin can act on the skin to decrease inflammatory processes [13,14]. Here, aloin was incorporated into dipalmitoyl phosphatidyl glycerol (DPPG) and palmitoyl oleyl phosphatidyl glycerol (POPG) liposomes and deposited in LbL films in conjunction with the polyelectrolyte poly(ethylene imine) (PEI). The incorporation and immobilization of aloin were investigated using UV–vis and fluorescence spectroscopy, while the release was monitored with fluorescence spectroscopy.

## 2. Materials and methods

### 2.1. Materials

Aloin (barbaloin) and the phospholipids dipalmitoyl phosphatidyl glycerol (DPPG) and palmitoyl oleoyl phosphatidyl glycerol (POPG) were purchased from Sigma-Aldrich and Avanti Polar Lipds, respectively, while the polyelectrolytes polyethylene imine (PEI) and poly(vinyl sulfonic acid) (PVS) were obtained from Sigma. Their chemical structures are shown in Fig. 1.

### 2.2. Incorporation of the aloin (barbaloin) into liposomes

The incorporation of Aloin into liposomes was carried out according to the procedure described by Moraes et al. [15]. DPPG or POPG was dissolved in methanol/chloroform (2:8) at a concentration of 1 mmol.l$^{-1}$. Aloin in methanol (0.75 mmol.l$^{-1}$) was mixed in the phospholipid solution, after which the organic solvent was removed using $N_2$ (g) until a film was produced around the tube. This lipid film was hydrated and sonicated for 2 h with a phosphate buffer saline (PBS) at pH 7.4 for the formation of multilamellar vesicles (MLV).

### 2.3. Measurement of size distribution

The size distribution of DPPG and POPG liposomes in PBS solutions, either pure or incorporating aloin, was obtained on a Nanotrac Wave Particle Analyzer, which was controlled by Microtrac Flex V.10.5.0 software. The average diameter of DPPG liposomes increased from 46 nm for the pure phospholipid to 60 nm when aloin was incorporated. For POPG, the size was 68 nm for the pure phospholipid and 33 nm for the aloin-containing liposomes. This decrease in size for POPG liposomes may be attributed to attractive interactions between POPG and aloin.

### 2.4. LbL film fabrication

Layer-by-layer (LbL) films were assembled by immersing a previously hydrophilized [16] quartz substrate in an aqueous solution of PEI (1 mg.ml$^{-1}$) during 3 min, alternated with immersion for 10 min into solutions containing free aloin or aloin encapsulated into liposomes. After deposition of each layer the substrate was washed to remove weakly adsorbed molecules. This procedure was repeated until 10-bilayer films were formed. Film growth at 25 °C was monitored by UV–vis and fluorescence spectroscopies using a Thermo Scientific Spectrophotomer model Genesys 6 and a Hitachi spectrometer model FL 4.500, respectively.

### 2.5. In vitro release of aloin encapsulated into liposomes

The controlled release of aloin from solution and from LbL films was monitored using fluorescence spectroscopy with excitation at 270 nm. The solution containing aloin in liposomes was transferred to a dialysis bag, with molecular weight cut off of 12000–16000 Da. Then the dialysis bag was immersed in a beaker with PBS buffer at pH 7.4 and 37 °C. The sample was removed from the solution out of the dialysis bag after several time intervals (10 min each until 1 h and then after 1, 2, 4, 8, 12, 24 and 48 h) and the spectrum was measured. The PBS buffer in the beaker was renewed after each measurement. For aloin immobilized in LbL films the same procedure was used, however the film was immersed directly in the PSB buffer without the dialysis bag.

## 3. Results and discussion

### 3.1. Characterization of aloin into liposomes

Fig. 2a shows that solutions containing free aloin and incorporated into DPPG and POPG liposomes display three main absorption bands at 269, 296 and 354 nm, where absorption was reduced upon incorporating aloin into the liposomes [12]. The emission spectra with excitation at 270 nm showed two maxima at 300 and 362 nm, in agreement with the literature [3]. The fluorescence intensity increased with incorporation of aloin in the liposomes, probably because the latter offer a more stable environment. Therefore, upon excitation a greater loss of energy occurs as the aloin molecules are far away from each other, thus leading to higher probability of electrons decaying to the ground state and larger fluorescence intensity [3]. This result also confirms the incorporation in the liposomes, inferred from the absorption spectra.

### 3.2. Immobilization of aloin in layer-by-layer films

Aloin free and incorporated into liposomes were immobilized using the LbL method, inspired in earlier work with biomolecules [17,18], with the aim of monitoring their release from the films. Fig. 3 depicts the film growth according to UV–vis absorption data for aloin free, and incorporated into DPPG and POPG liposomes. In all films, the layers containing aloin were alternated with PEI layers. The adsorption time used (10 min.) for aloin was established in an optimization procedure from the adsorption kinetics (results not shown). The almost linear growth pointed to an efficient adsorption of aloin in each deposition step, regardless of the conditions used. Film adsorption in this case was driven by electrostatic interactions

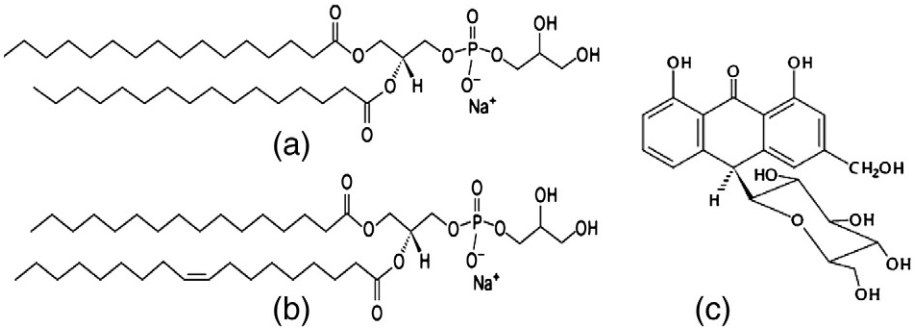


**Fig. 1.** Chemical structures of the phospholipids dipalmitoyl phosphatidyl glycerol (DPPG) (a) and palmitoyl oleoyl phosphatidyl glycerol (POPG) (b) and aloin (c).