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Full Length Article

Layer-by-layer films containing emodin or emodin encapsulated in liposomes for transdermal applications

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ABSTRACT

Dermal drug release systems are an important area of research because they can be applied to the skin in a non-invasive procedure using a lower concentration of drugs. In this study, we have developed two types of Layer-by-Layer (LbL) films for releasing emodin (EM). In one system, EM was intercalated with poly(ethylenimine) PEI and poly(vinyl sufonate) (PVS) polyelectrolytes, forming $(PEI/PVS)_2(PEI/EM)_7$; in another, EM was incorporated in liposomes obtained by mixing dipalmitoyl phosphatidyl glycerol (DPPG) and palmitoyl oleoyl phosphatidyl glycerol (POPG) lipids, forming $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$. UV–vis and FTIR spectroscopies were used to characterize the LbL films. These showed that the depositions of material by LbL were efficient, with increases in the absorbance of each bilayer evidencing the presence of EM in the film. The $(PEI/PVS)_2(PEI/EM)_7$ and $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$ films released EM in three and five days, respectively. The cyclic voltammetry (CV) assay of the $(PEI/PVS)_2(PEI/EM)_7$ results are in agreement with UV–vis measurements, which suggest that EM was protonated in acid environments, while the CV of $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$ demonstrated distinct protonation behaviour for EM within the inner liposome structure, even in acid solutions. Therefore, this study presents two systems based on LbL films and provides additional details about the release of EM from these films to create a viable alternative for transdermal applications.

1. Introduction

Skin diseases are ubiquitous across cultures, ages and subpopulations [1], and may include atopic eczema, psoriasis, leg ulcers, mycoses, urticarial, and cancers [2]. In addition, the skin can be damaged by shocks, surgeries [3] and diseases such as diabetes which cause dermatological changes. When the skin is injured or burned, the place under it is left unprotected and vulnerable to infections [4]. The study of transdermal systems is therefore essential for both helping solve these problems and preventing the development of skin diseases, which can improve quality of life, shorten hospital stays, and reduce mortality.

Dermal drug delivery is becoming increasingly popular because it may be applied over the integrated skin, is non-invasive, is portable, allows the use of lower drug concentrations, and has targeted action [4,5]. Transdermal systems may be built with the Layer-by-Layer (LbL) self-assembly technique [6–8], which is versatile in terms of the large number of materials that can be employed, simplicity in the fabrication method, and use of small quantities of materials since the films are nanostructured. LbL films can be assembled with different types of interactions, including hydrogen bonds, covalent bonds, charge-shift interactions or electrostatic interactions. Many external stimuli can be used for delivery in LbL films such as light [9], mechanical vibration [10], electricity [11], changes in pH [12–15], ionic strength [16] and temperature [17], for widely used molecules such as diclofenac [18], protein lysozyme [19,20], neurotrophin [21], doxorubicin [22,23], metronidazole [24], thrombin and vancomycin [25] and ibuprofen [26].

LbL films for delivery can be constructed using polymers [27], block copolymer micelles [28], silk fibroin [29], graphene [30], and liposomes [17]. The LbL technique favours the preservation of biomolecules activity [31] because of entrapped water in the film structure. This effect is improved when the system is combined with liposomes, which are colloidal vesicles formed by phospholipids that have the ability to protect molecules encapsulated in their inner structure [32–35]. Liposomes are used in delivery systems since they are biodegradable, biocompatible, non-toxic, and

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non-immunogenic, also allowing for loading of both hydrophilic and hydrophobic drugs [36]. Liposomes can be produced by lipids such as saturated phospholipids (dipalmitoyl phosphatidyl glycerol (DPPG)) and insaturated phospholipids (palmitoyl oleoyl phosphatidyl glycerol (POPG)). The properties of the liposomes as carrier systems are determined by the fluidity of the liposome and in this context a mixture of saturated and insaturated phospholipids may be an alternative to reach predetermined, desired characteristics. For example, the glass transition temperature is 41 °C and −2 °C for DPPG and POPG, respectively, and the combination of these two lipids may allow for tuning liposome fluidity to promote a specific drug release pattern. When applied *in vivo*, liposomes formed by phospholipids are removed from the circulation by the phagocytic cells, rapidly preventing the drug release action [37]. For this reason, the immobilisation of liposome on a surface by LbL requires that it first be released from the film so that it may enter the circulation at a later point, therefore delaying the phagocytosing process.

Emodin, a natural compound also known as 1,3,8-trihydroxy-6-methylanthraquinone, is studied for its uses as an antibacterial, anti-inflammatory, immunosuppressant, vasorelaxant, antiulcerogenic, and antineoplastic agent [38,39]. The low water solubility of emodin complicates its oral and intravenous application [40], which has motivated development of formulations using poloxamers [41,42] and nanoemulsions with capryol, cremophor, and transcutol [43] or loading emodin in microspheres of polylactic acid [44], mesosporous silica [45–47], silk-fibroin-coated liposomes, and electrospinned blended nanofibrous membranes. Wang et al. [29] produced an LbL film with cationic microgels of poly(allylamine hydrochloride) with dextran (PAHD) and sodium carboxymethyl cellulose (CMC) forming PAH/CMC and used this material as a support for loading emodin borate and releasing emodin. In this work, we immobilised emodin directly in LbL films with poly(ethyleneimine) PEI $(PEI/EM)_7$ and incorporated emodin in a liposome formed by DPPG-POPG with PEI intercalated $(PEI/DPPG\text{-}POPG\text{-}EM)_7$. The two films were tested in an *in vitro* release assay and compared. Electrochemical studies were performed to obtain information on the films pH-dependent behaviours and predict their actions in biological systems. This work combines the efficiency of liposomes in drug delivery systems with the simplicity of LbL methods and shows the capacity for applying both types of films in transdermal systems.

2. Experimental section

2.1. Materials

Emodin (molar mass 270.24 g mol^{-1}) was obtained from plant frangula, poly(ethyleneimine) (PEI, branched) and poly(vinyl sufonate) (PVS) were purchased from Sigma-Aldrich. The phospholipids dipalmitoyl phosphatidyl glycerol (DPPG) and palmitoyl oleoyl phosphatidyl glycerol (POPG), with molar masses of 744.96 g mol^{-1} and 770.99 g mol^{-1}, respectively, were purchased from Avanti Polar Lipids.

2.2. Substrate

LbL films were assembled onto quartz slides for UV–vis spectroscopy and delivery tests, onto silicon substrates for FTIR spectroscopy and on ITO-coated glass substrates (Indium-Tin Oxide, one side coated on glass by Delta Technologies) for electrochemical measurements. The quartz plates were cleaned with $NH_4OH/H_2O_2/H_2O$ (1:1:5, v:v:v) and $HCl/H_2O_2/H_2O$ (1:1:6, v:v:v) hydrophilization solutions at 75 °C for 10 min. ITO and silicon were cleaned with chloroform and isopropanol in a sonicator for 30 min and rinsed with ultrapure water (Sartorius system).

2.3. Polymeric solutions

PEI and PVS solutions were prepared at concentrations of 1 mg mL^{-1} and 4 μL mL^{-1} respectively, in water for the $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$ film and in sodium phosphate buffer at pH 8 for the $(PEI/PVS)_2(PEI/EM)_7$ film. Emodin was prepared at 8.8 mmol L^{-1} in an ethanol stock solution and diluted to 1/10 in ethanol/buffer.

2.4. Liposomes preparation

The method used for incorporating emodin in liposomes was based on a procedure described by Moraes et al. [34]. DPPG and POPG at concentrations of 0.95 mmol L^{-1} and 0.05 mmol L^{-1}, respectively, were dissolved in methanol/chloroform (1:8). Emodin was previously dissolved in methanol at 24 mmol L^{-1}. The organic solvent was dried in a rotary evaporator. The lipid film was hydrated with ultrapure water and sonicated for 2 h. The liposomes were evaluated using a Nanoparticle Tracking Analysis (NTA) apparatus manufactured by Malvern Instruments (UK) with a NanoSigth LM 10 cell green laser of wavelength 532 nm and a camera CMOS (*Complementary Metal Oxide Semiconductor) with the software*NanoSigth (version 2.3). The liposomes were diluted 50 times, and each sample was measured five times. This technique was used to determine the size distribution and concentration of the liposomes.

2.5. LbL assembly

The LbL films were created with two bilayers of polyelectrolytes as a cushion to reduce the influence of the substrate morphology on film growth [48]. The $(PEI/PVS)_2(PEI/EM)_7$ film was assembled by immersing the substrate in PEI and PVS for 3 min each and PEI and EM (7 min), also intercalating with a washing procedure. This was repeated for the $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$ sample, which was dipped in DPPG-POPG-EM solution for 10 min. This procedure was repeated until seven bilayers had been formed. Film growth was monitored by measuring the absorbance spectrum after each bilayer was deposited using a Thermo Scientific Genesys 6 UV–vis spectrophotometer at room temperature.

2.6. Film characterization

The films $(PEI/PVS)_2(PEI/EM)_7$, $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$, $(PEI/PVS)_{10}$, emodin solution, and DPPG/POPG solution in cast films were characterized by FTIR using an Agilent Technologies Cary 630 spectrometer with a baseline taken using silicon. All measurements were carried out in the transmission mode. Cyclic voltammetry measurements were performed under N_2 flow at potentials between −0.9 and 0.5 V versus a saturated calomel electrode (SCE) at a scan rate of 0.1 V s^{-1} in Autolab PGSTAT 30 using a conventional electrochemical cell containing a sodium phosphate buffer at pH 5.5, 6.7 or 8.0, and an auxiliary electrode made of platinum foil (1 cm^2); the SCE and the ITO working electrodes were modified with LbL films of $(PEI/PVS)_2(PEI/EM)_7$ or $(PEI/PVS)_2(PEI/DPPG\text{-}POPG\text{-}EM)_7$.

2.7. In vitro *release of emodin*

The release profile of emodin from the films was analysed using an adapted support of a falcon tube cut to a height of 4 cm with gaps of 2 by 1.5 cm. A dialysis membrane with a molecular weight cut off of 12000–16000 Da covered the support outside, and the films on quartz were placed inside. The support was filled with PBS buffer pH 7.4 inside and outside and kept at 37 °C in a water bath. The solution outside the support was replaced at regular intervals (from 15 to 7200 min) and the concentration of emodin was measured in