Colloids and Surfaces B: Biointerfaces 135 (2015) 133–142

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

# 'One-component' ultrathin multilayer films based on poly(vinyl alcohol) as stabilizing coating for phenytoin-loaded liposomes

Katarzyna Zasada, Magdalena Łukasiewicz-Atanasov, Katarzyna Kłysik, Joanna Lewandowska-Łańcucka, Barbara Gzyl-Malcher, Agnieszka Puciul-Malinowska, Anna Karewicz*, Maria Nowakowska

*Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30- 060 Kraków, Poland*

ARTICLE INFO

*Article history:*
Received 13 April 2015
Received in revised form 19 June 2015
Accepted 13 July 2015
Available online 23 July 2015

*Keywords:*
Poly(vinyl alcohol) derivative
Multilayer film
Phenytoin
Liposome
Coating

ABSTRACT

Ultrathin "one-component" multilayer polymeric films for potential biomedical applications were designed based on polyvinyl alcohol,—a non-toxic, fully degradable synthetic polymer. Good uniformity of the obtained film and adequate adsorption properties of the polymeric layers were achieved by functional modification of the polymer, which involved synthesis of cationic and anionic derivatives. Synthesized polymers were characterized by FTIR, NMR spectroscopy, dynamic light scattering measurements and elemental analysis. The layer by layer assembly technique was used to build up a multilayer film and this process was followed using UV–Vis spectroscopy and ellipsometry. The morphology and thickness of the obtained multilayered film material was evaluated by atomic force microscopy (AFM). Preliminary studies on the application of the obtained multilayer film for coating of liposomal nanocarriers containing phenytoin, an antiarrhythmic drug, were performed. The coating effectively stabilizes liposomes and the effect increases with an increasing number of deposited layers until the polymeric film reaches the optimal thickness. The obtained release profiles suggest that bilayer-coated liposomes release phenytoin less rapidly than uncoated ones. The cytotoxicity studies performed for all obtained nanocarriers confirmed that none of them has negative effect on cell viability. All of the performed experiments suggest that liposomes coated with ultrathin film obtained from PVA derivatives can be attractive drug nanocarriers.



## 1. Introduction

Nanostructural materials have recently gained a considerable attention, mostly due to their potential applications as biomaterials for tissue engineering, drug delivery and regenerative medicine [1–3]. The surface properties of the implant, or any other device which stays in prolonged contact with human tissues, affect such important biological phenomena as protein adsorption, cell adhesion and inflammatory response. Coating of these objects with polymeric thin multilayer films is frequently used to control these processes by providing desirable surface properties [4,5]. Due to the non-toxicity and biocompatibility requirements, natural polymers, such as polysaccharides and proteins, are the materials of choice as surface coatings of various biomaterials [6] . There are, however, some drawbacks, including unsatisfactory reproducibility of such systems due to the significant variation in the exact properties between different batches of the same natural polymer. In some cases an unwanted immunological response may also appear, especially in the case of proteins. The molecular weight and degree of branching of the naturally occurring polymers cannot be controlled unless some additional procedures to modify these properties are applied. Synthetically obtained polymers, on the other hand, allow for control in this regard. Many of them, however, show some toxicity and lack of biocompatibility and therefore are poorly tolerated by human body.

Synthetically obtained poly(vinyl alcohol) (PVA) is commonly used in medical devices [7] due to its low protein adsorption, high biocompatibility and water solubility, as well as chemical resistance [8]. Some of the most common medical uses of PVA are in soft contact lenses, eye drops (artificial tears), as embolization agent, tissue adhesion barriers, and in the cartilage and orthopedic applications. PVA widely used as a coating [9] was, however, hardly

* Corresponding author. Fax: +48 12 634 05 15.
*E-mail address:* karewicz@chemia.uj.edu.pl (A. Karewicz).

http://dx.doi.org/10.1016/j.colsurfb.2015.07.033
0927-7765/

studied as a component of the nanostructural materials. Here we propose a new multilayer, nanostructural material, based on ionic derivatives of PVA and obtained using the Layer-by-Layer (LbL) deposition technique, a procedure which is especially suitable for fabrication of materials for biomedical applications [10,11]. Application of polyelectrolytes with the same main chains increases the probability of their interpenetration and entangling, what results in higher stability of such "one- component" films formed [12].

It is expected that these materials can be particularly suitable for stabilizing coating of liposomal drug carriers. According to the previous studies [13] PVA-coated liposomes were found more stable compared to uncoated ones and the encapsulation efficiency increased with increasing polymer concentration. Takeuchi et al. [14] have also shown in their in vivo studies in rat models that the circulation and distribution of the liposomes coated with alkyl derivative of PVA (PVA-R circulated in animal blood) are significantly longer than those of PVA-coated or non-coated ones, independently of liposomal formulation tested (regardless of the lipid composition, e.g. various cholesterol content or lipid charge). The possible explanation may be that the alkyl chain plays a role of an "anchor" providing a more stable coating than unmodified PVA. We believe that the system can be improved by using the PVA derivatives containing ionic group substituents providing better solubility in water and allowing for strong electrostatic interactions with charged liposomal vesicles. The liposomes with such coating are also expected to interact strongly with cell membrane and to be more resistant against aggregation, which may be difficult to avoid in the system containing only pending alkyl chains. The possibility to modify the thickness of the liposome coating by adding more or less surface layers is a strong advantage, as it allows controlling the surface charge and release rates for liposomal drug carriers. Here we describe the coating of liposomal drug carriers with synthesized ionic derivatives of PVA, their surface properties, stability and interactions with lipid monolayer. Biological studied on their cytotoxicity were also performed. Phenytoin, highly hydrophobic compound used as an effective antiepileptic and antiarrhythmic drug, which is known to have limited stability in aqueous media and to induce severe side effects to the patient (when applied both, orally or parenterally), was used as a model biologically active agent.

## 2. Experimental part

### 2.1. Materials

Polyvinyl alcohol, $M_p \approx 14{,}000$ g/mol and $M_p \approx 25{,}000$ g/mol; GTMAC, glicydyl trimethylammonium chloride, ≥90% (FLUKA); sodium monochloroacetate, p.a. (Sigma–Aldrich); potassium dihydrogenphosphate (V), p.a. (POCH S.A.); disodium hydrogenphosphate (V) 12 hydrate, p.a. (POCH S.A); potassium hydroxide p.a. (POCH S.A); sodium chloride p.a. (POCH S.A); sodium hydroxide p.a. (POCH S.A); buthyl acetate p.a. (Sigma–Aldrich); deuterated water p.a. (Aldrich); poly(allylamine hydrochloride) PAH, p.a (Sigma–Aldrich); poly(sodium styrene sulfonate), PSS, p.a (Aldrich Chemical Company); L-α-phosphatidylcholine (type XIII-E, SIGMA)—100 mg/ml solution in ethanol; phenytoin (5,5-diphenylimidazolidine-2,4-dione) (p.a. SIGMA), dihexadecyl phosphate (DHP, SIGMA); cholesterol (ALDRICH); Triton X-100 (p.a. POCH Gliwice, Poland) methanol p.a. (POCH Gliwice); acetone p.a. (POCH Gliwice), Primary Dermal Fibroblasts; Normal, Human, Adult, Organism: Homo sapiens, human/tissue: skin (ATCC® PCS-201-012™), Minimum Essential Medium (MEM) Eagle With Earle's salts, L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture (Sigma), Sodium pyruvate solution 100 mM, sterile-filtered, BioReagent, suitable for cell culture (Sigma), MEM Non-essential Amino Acid Solution (100×) without L-glutamine, liquid, sterile-filtered, BioReagent, suitable for cell culture (Sigma), Research Grade Fetal Bovine Serum (FBS), South-American origin, Triple 0.1 μm Sterile Filtered, HyClone (Thermo Scientific), Penicilin–Streptomycin Solution 10.00 units/ml, Penicilin/10.000 μg/ml, Streptomycin 0.1 μm Sterile Filtered, In Vitro Toxicology Assay Kit, XTT based (Sigma) were used as received.

### 2.2. Synthesis of the poly(vinyl alcohol) modified with cationic groups (C-PVA)

2 g of PVA (100 % hydrolyzed, MW ≈ 14,000 Da) were dissolved in 22.5 ml of distilled water and 12.2 ml of undiluted GTMAC, previously dissolved in 3 ml of water, was then added. The mixture was heated to 80 °C and continuously mixed using magnetic stirrer under reflux. Then 1 ml of 5 M NaOH was added and the reaction mixture was further stirred at 80 °C for one hour. Next the mixture was cooled down to the room temperature, neutralized with 1 M HCl and dialyzed against water. When the conductivity of water outside the dialysis tube dropped down below 15 μS, the dialyzed solution was freeze-dried and the resulting solid deposit was further dried under vacuum at 60 °C.

### 2.3. Synthesis of the poly(vinyl alcohol) modified with anionic groups (PVA-COOH)

1.0 g of PVA (88 mol% hydrolysis, $M_p \approx 25{,}000$ Da) was dissolved in 50 ml of distilled water at 70 °C. 50 g of sodium monochloroacetate were then added and, after its complete dissolution, the reaction mixture was cooled down and kept at 4 °C for 24 h. Next, 42 ml of the saturated (50%) aqueous sodium hydroxide solution was added slowly to the mixture, which was next stirred for another 24 h at room temperature. Finally the mixture was neutralized with 6 M HCl and dialyzed against water. When conductivity of the water dropped down below 15 μS, dialyzed solution was freeze-dried and obtained solid deposit was dried under vacuum at 60 °C.

### 2.4. Preparation of the multilayer polyelectrolyte films

#### 2.4.1. Spectroscopic detection

The polyelectrolyte multilayer films were prepared by electrostatic self-assembly (LbL technique) using quartz plates and 0.25 mg/ml solutions of polymers in 0.1 M sodium chloride. Quartz plates were cleaned prior to polymer adsorption by immersion in freshly prepared *piranha* solution (1:3 v/v mixture of 30% $H_2O_2$ and 95% $H_2SO_4$) followed by washing with deionized water. Cleaned plates were then stored in water before use. In the first step, plates were flushed with deionized water, dried under stream of nitrogen and immersed in the polycation solution for 25 min at room temperature. In the next step they were again flushed with water, dried and placed in the polyanion solution for another 25 min under the same experimental conditions. Procedure was repeated to obtain desirable number of layers. The outer layer was always coated with polyanion. After the deposition of each bilayer the sample was measured spectrophotometrically in the range of 200 – 400 nm. Measurements were performed using Varian Cary 50 Conc. UV–Vis spectrophotometer with a single cell Peltier accessory. All spectra were collected at room temperature.

#### 2.4.2. AFM studies

The polyelectrolyte multilayer films were prepared by the same procedure as described above but silicon plates support and 1 mg/ml polymer solutions in 0.1 M NaCl were used. Atomic force microscopy (AFM) measurements were performed using Nanoscope IV multimode microscope (Digital Instruments, Veeco) working in the tapping mode. Standard silicon cantilevers (Veeco)