Electrochimica Acta 212 (2016) 694–700

Contents lists available at ScienceDirect

Electrochimica Acta

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

EQCM verification of the concept of drug immobilization and release from conducting polymer matrix

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ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form 7 July 2016
Accepted 11 July 2016
Available online 12 July 2016

Keywords:
drug delivery
conducting polymers
electrochemical quartz crystal microbalance
betulin
PEDOT

ABSTRACT

Local drug delivery based on conducting polymer carriers is an innovative approach of medical treatment joining the concept of regional release of biomolecules with ion-exchange properties of conjugated polymers. In this study, we have applied electrochemical quartz crystal microbalance (EQCM) to monitor the process of three-step immobilization and release of anti-cancer drug, disuccinyl derivative of betulin, in PEDOT matrix. Each step of this process has been carefully investigated, i.e. electrochemical polymerization of monomer in the absence of drug, removal of primary dopant during the process of matrix reduction and drug incorporation during the process of matrix oxidation. The release of drug from PEDOT matrix has been performed via three paths, i.e. spontaneous release with no application of external potential, active release under potentiostatic conditions and active release under potentiodynamic conditions. EDS elemental analysis, scanning electron microscopy, IR and Raman spectroscopies, have been used to analyze structural and surface properties of drug-loaded PEDOT matrices.

1. Introduction

Local drug delivery is an innovative approach of medical treatment used to decrease its toxic effects against healthy tissues by introducing therapeutic levels of drugs for prolonged period of time [1]. This is of a significant importance when patients are treated with potent drugs, especially antibiotics and anti-cancer agents. The concept of localized drug delivery may be realized by means of conjugated polymers, which are materials that have found numerous applications in biomedical engineering [2] and can be successfully applied as actuators [3], materials for tissue engineering [4] as well as drug delivery systems [5]. This is due to the fact that they not only exhibit high electrical conductivities, but also biocompatibility and ion exchange properties [6]. Conducting polymers are able to undergo reversible redox reactions [7] allowing highly controlled, reversible electrostatic immobilization of a wide range of biologically active compounds, e.g. anti-inflammatory drugs [8–10], anti-cancer agents [11,12], antibiotics [13,14], growth factors [15] and proteins [16]. Conducting polymers used as drug nano-reservoirs allow to release precise amounts of biomolecules as a result of electrical stimulation [17]. The application of conducting polymers is limited by their non-biodegradability, however some literature reports have shown that by using chemical modification it is possible to obtain the material cleavable by enzymes found in vivo [18].

As we showed earlier [19], there are two main electrochemical methods of drug immobilization in conducting polymer matrices. One of them is the one-step immobilization, in which the process of drug incorporation proceeds simultaneously with the process of matrix formation during the electrochemical polymerization. Although it is an easy method, the presence of drug molecules in the solution of monomer can lead to the suppression of electrochemical polymerization process resulting in the formation of thin and poorly conducting organic coating with low drug capacitance [8]. This method can be, however, improved if the polymer backbone is modified with cation exchangers [20] or carboxylated gold nanoparticles [21]. During the three-step modification of conducting matrix, the polymer film is synthesized from bare electrolyte solution of monomer in the absence of biomolecules. Drug immobilization is a result of the ion-exchange processes comprising removal of primary dopant during the

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http://dx.doi.org/10.1016/j.electacta.2016.07.055
0013-4686/

process of matrix reduction and drug incorporation during the process of matrix oxidation. This approach allows to separate the processes of electropolymerization and drug immobilization, preventing the interference of biomolecules with the growth of polymer matrix [19–22]. Due to the fact that drug molecules are immobilized in the surface regions of polymer matrix, the drug capacity of such systems is limited. The final amount of incorporated drug is dependent on several factors, such as the doping level reached during the electropolymerization process, the efficiency of removal of a primary dopant as well as the conditions of oxidation in the presence of drug molecules.

Numerous literature reports have proven that conjugated polymers are advantageous materials to be used as matrices for controlled delivery of both positively [20,23] and negatively [19,24] charged biomolecules This study presents the investigations of the process of drug immobilization and release by means of electrochemical quartz crystal microbalance (EQCM), a superior technique allowing to study the mass change of the conducting matrix resulting from the incorporation and release of ions [25,26]. As a model drug we have chosen betulin, which is naturally abundant triterpene displaying antitumor properties against various types of cancer cell lines, including breast (MCF-7), lung (A549) and prostate (PC3) cancer cell lines [27,28]. Due to the high stability of conductivity [29] and excellent stability against biological reducing agents [30], poly(3,4-ethylenedioxythiophene) (PEDOT) is used to serve as a nano-reservoir of disuccinyl derivative of betulin (DS-Bet). The process of drug incorporation is realized with the use of the three-step modification of conducting polymer matrix. The release of DS-Bet from drug-loaded PEDOT matrix is performed via three paths, i.e. passive release when there is no application of external potential, active release under potentiostatic conditions and active release under potentiodynamic conditions, as the result of cyclic voltammetry. Together with electrochemical and EQCM data, the elemental composition as well as surface parameters of bare and drug-loaded polymer matrices are characterized and compared.

2. Experimental

2.1. Materials

Betulin (Natchem), pyridine (Chempur, analytical grade), succinic anhydride 99% (Sigma-Aldrich), hydrochloric acid (Chempur, analytical grade), ethanol (Chempur, analytical grade), sodium hydroxide (POCH, 1 M standard solution), 3,4-ethylenedioxythiophene (Sigma Aldrich, 97%), potassium chloride (Sigma Aldrich, BioReagent, ≥99.0%) were used as received. Grade 1 (R > 10 MΩ cm^{-1}) deionized water was employed as solvent for all prepared solutions.

2.2. Methods

2.2.1. Synthesis of disuccinyl derivative of betulin, DS-Bet

4.43 g (10 mmol) of betulin and 100 ml of dried pyridine was put into 250 ml flask. The suspension was heated up until dissolution and 10 g (100 mmol) of succinic anhydride was added. Light-brown solution was heated up under condenser and kept at the boiling temperature for 8 hours. After the end of reaction, the solution was cooled and poured into 500 ml of 5% HCl. Light-brown precipitation was filtered under reduced pressure and washed with water until neutral pH of filtrate. The precipitation was dried, dissolved in ethanol and heated with active carbon under condenser for 15 minutes. Hot solution was filtered and poured into water. The white precipitation was formed. The chemical structure of product was confirmed by means of ^{1}H NMR, ^{13}C NMR, DEPT, FT-IR and MS.

^{1}H NMR: 4.68 (1H, d, J = 1.7 Hz, C_{29}-H_a); 4.59 (1H, d, J = 0.2 Hz, C_{28}-H_b); 4.49 (1H, t, J = 7.9 Hz, C_3-Hα); 4.30 (1H, d, J = 11.0 Hz, C_{28}-H_a); 3.80 (1H, d, J = 11.0 Hz, C_{28}-H_b); 2.69–2.62 (8H, m, HOOC-CH_2-CH_2-COO- and −OCO-CH_2-CH_2-COOH); 2.42 (1H, dt, J = 5.7 and 10.3 Hz, C_{19}-H); 1.68 (3H, s, C_{30}-H_3); 1.02; 0.97; 0.84; 0.83 × 2 (15H, singlets, 5 CH_3 groups).

^{13}C NMR: 178.2 (2 x C_q, HOOC-CH_2-CH_2-COO- and −OCO-CH_2-CH_2-COOH); 172.4 (C_q, −OCO-CH_2-CH_2-COOH); 171.8 (C_q, HOOC-CH_2-CH_2-COO-); 150.0 (C_q, C-20); 109.8 (CH_2, C-29); 81.5 (CH, C-3); 63.2 (CH_2, C-28); 48.7 (CH, C-19); 47.7 (C_q, C-18); 47.7 (C_q, C-17); 29.3 and 29.0 (4 x CH_2, HOOC-CH_2-CH_2-COO- and −OCO-CH_2-CH_2-COOH); 19.1 (CH_3, C-30).

DEPT: 6 x CH_3, 16 x CH_2, 6 x CH.

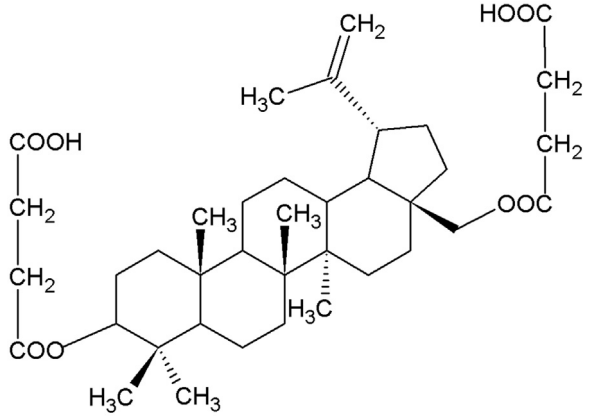
FT-IR spectrum of disuccinyl derivative of betulin (Fig. S1) shows absorption bands at 2943 cm^{-1} and 2872 cm^{-1} corresponding to the stretching of C-H bonds, and at 1450 cm^{-1} and 1360 cm^{-1} associated with the skeletal vibrations [31]. The high intensity signal at 1712 cm^{-1} corresponds to the vibration of carbonyl group (C=O) [31,32], while signals between 1250 cm^{-1} and 1160 cm^{-1} are assigned to the C—O bond [31]. The signals at 1640 cm^{-1}, 976 cm^{-1} and 883 cm^{-1} are attributed to the unsaturated C=C bond stretching and bending, respectively [31].

Mass spectrum (Fig. S2) reveals peaks characteristic for betulin [33,34]; fragmentation pattern for disuccinyl derivative of betulin is shown in Fig. S3.

The presented method was an efficient way to produce DS-Bet (Scheme 1), especially when comparing with the method described in the literature [35]. It was possible to eliminate the need to use the acylation activator, 4-(dimethylamino)pyridine. The efficiency of the process was much higher (94.1%) and because of the high purity of obtained product, the additional purification using a chromatographic column was not required.

2.2.2. Matrix formation and drug immobilization

Electrochemical immobilization was carried out by means of CH Instruments 400c Electrochemical Workstation equipped with time-resolved electrochemical quartz crystal microbalance in a standard three-electrode setup, employing CHI125 gold crystal as a working electrode, Ag/AgCl reference electrode (E = 0.205 V vs. SHE) and a platinum foil counter electrode. The three-step drug immobilization comprised the electrochemical polymerization of EDOT (10 mM) in 0.1 M KCl solution, followed by the dedoping of the polymer layer and oxidative immobilization of drug. Polymerization was realized via 20 potential cycles in the potential range of 0 V ÷ 1.2 V (vs. Ag/AgCl), at a scan rate of 0.1 V s^{-1}. Dedoping of the polymeric deposit was achieved in 0.1 M KCl solution, at a constant working electrode potential of −0.5 V (vs. Ag/AgCl) over 300 s.



Scheme 1. The chemical structure of disuccinyl derivative of betulin, DS-Bet.