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Physico-chemical characterization of polymeric micelles loaded with platinum derivatives by capillary electrophoresis and related methods

Farid Oukacine [a], Stephane Bernard [b], Iulian Bobe [c], Hervé Cottet [a,*]

[a] Institut des Biomolécules Max Mousseron (IBMM, UMR 5247 CNRS–Université de Montpellier1–Université de Montpellier 2), Place Eugène Bataillon, 34095 Montpellier Cedex 5, France
[b] Debiopharm International SA, Espace «après–demain», EPFL, Innovation Park, Lausanne 1015, Switzerland
[c] Nanocarrier Co. Ltd., Yaesu Yamagata Bldg., 3-2-Nihonbashi, Japan

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ABSTRACT

(1,2-diamino-cyclohexane)Platinum(II) ((DACH)Pt) loaded polymeric micelles of poly(ethylene glycol-b-sodium glutamate) (PEG-b-PGlu) are currently studied as a potential candidate to replace oxaliplatin in the treatment of cancers with the aim to reduce side effects like cumulative peripheral distal neurotoxicity and acute dysesthesias. As for all synthetic polymeric drug delivery systems, the characterization of the (co)polymer precursors and of the final drug delivery system (polymeric micelles) is crucial to control the repeatability of the different batches and to get correlation between physico-chemical structure and biological activity. In this work, the use of capillary electrophoresis (CE) and related methods for the characterization of (DACH)Pt-loaded polymeric micelles and their precursor (PEG-b-PGlu copolymer) has been investigated in detail. The separation and quantification of residual PGlu homopolymer in the PEG-b-PGlu sample were performed by free solution capillary zone electrophoresis mode. This mode brought also information on the PEG-b-PGlu copolymer composition and polydispersity. It also permitted to monitor the decomposition of polymeric micelles in the presence of NaCl at room temperature. Interactions between PEG-b-PGlu unimers, on one hand, and polymeric micelles or surfactants, on the other hand, were studied by using the Micellar Electrokinetic Chromatography and Frontal Analysis Capillary Electrophoresis modes. Finally, weight-average hydrodynamic radii of the loaded polymeric micelles and of the PEG-b-PGlu unimers were determined by Taylor Dispersion Analysis (an absolute size determination method that can be easily implemented on CE apparatus).

1. Introduction

Numerous Pt-containing compounds have been synthesized to cure different types of cancer in humans [1]. Among them, cisplatin (cis-diaminedichloro-platinum(II)) and oxaliplatin ([(1R,2R)-cyclohexane-1,2-diamine](ethanedioato-O,O′)platinum(II)) are widely used alone and in combination with other molecules for treatment of various cancers [2]. The use of oxaliplatin for treatment of colorectal cancer has prolonged the lives of many people diagnosed in later stages of the disease [3]. The most recent statistics indicate a 5-year relative survival rate for colorectal cancer of 64.3% [4]. However, their use has been limited due to dose-limiting toxicities [5]. The use of controlled drug release systems has certain advantages compared to conventional form of dosages, as they can minimize side effects, and also prolong the efficacy of the drug [6]. Consequently, enormous effort has been dedicated to develop drug delivery systems that increase the blood residence time of the chemotherapeutic agent [7]. Nanoparticules based on polymeric micelle have also been described, which are interesting with regard to adjust the drug release to physiological needs of the body [8–10]. The release of active drug is controlled by the rate of drug diffusion in the micellar core or break up of the polymeric micelles into single polymeric chains [11]. Fig. 1 shows the schematic representation of (1,2-diamino-cyclohexane)platinum(II)-loaded polymeric micelles based on polyethylene glycol-b-polyglutamic acid (PEG-b-PGlu) copolymers.

These nanoparticles have many advantages. They reduce the cumulative toxicity by the micellar self-dissociation into unimers who have a molar mass lower than that of the threshold of glomerular excretion. They allow deeper tumor penetration due to the sub-100 nm size [8]. The outer poly(ethyleneglycol) (PEG) shell of micelles may inhibit the surface adsorption of biological components. The adsorption of proteins to the surface of a drug delivery vehicle can cause damage and lysis of the vehicle, including leakage of the drug entrapped within the carrier. This adsorption makes it nearly impossible to predict the pharmacokinetics of a drug incorporated in such system. Also, a multimodal population distribution of the micelles may cause differences in blood circulation times and in biodistributions in the body. For these reasons, it is important to well characterize the polymeric micelles and their precursors in terms of purity, molar mass, size, polydispersity, stability and physicochemical properties.

* Corresponding author. Tel.: +33 4 6714 3427; fax: +33 4 6763 1046.
E-mail address: hcottet@univ-montp2.fr (H. Cottet).

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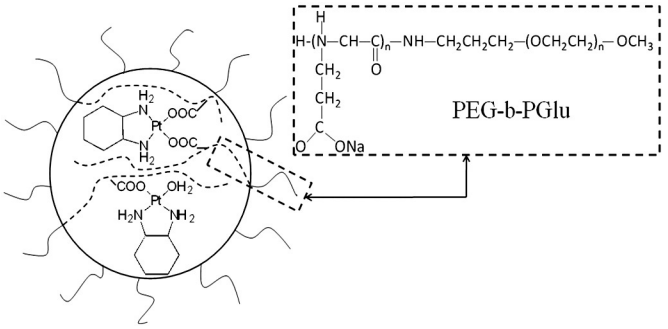

Fig. 1. Schematic representation of (DACH)Pt-loaded polymeric micelles based on PEG-b-PGlu diblock copolymers.

Several methods are used in the literature for the characterization of polymeric micelles used for drug delivery. Their size and their morphology are characterized by atomic force microscopy (AFM), dynamic light scattering (DLS) and transmission electron microscopy (TEM) [8,9,12]. The molar mass distribution of PEG-b-PGlu is generally characterized by size-exclusion chromatography [8,9] and the average polymerization degree of PGlu is obtained by ^{1}H NMR [8,9,13].

The ICP–MS is preferably used today, in laboratory and clinical studies, for the determination of the total concentrations of Pt in Pt-based drugs in different biological tissues [14–16]. As well as ICP–MS, coupling of liquid chromatography–tandem mass spectrometry (LC–MS–MS) can be advantageously applied [17].

Capillary electrophoresis (CE) is a powerful technique for the characterization of charged synthetic or natural polymers and nanoparticles. Due to different separation mechanisms, CE techniques are complementary to chromatographic methods. Advantages of CE-related techniques include low sample consumption, no need of sample filtration, no stationary phase (no unwanted interactions), low running costs, and straightforward and relatively fast analysis. The aim of this work is to evaluate the potential of CE and related methods for the characterization of drug-loaded polymeric micelles and their precursors. Different electrophoretic modes (free solution Capillary Zone Electrophoresis (CZE), Micellar Electrokinetic Chromatography (MEKC), Frontal Analysis Continuous Capillary Electrophoresis (FACCE) and Taylor Dispersion Analysis (TDA)) were implemented for the characterization of PEG-b-PGlu unimers and the subsequent polymeric micelles loaded with (DACH)Pt.

2. Materials and methods

2.1. Chemicals

Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium dodecyl sulfate ($C_{12}H_{25}NaO_4S$), pthtalic acid ($C_6H_4(COOH)_2$), and poly-L-glutamic acid sodium salt (PGlu, M_w = 6 600 g mol^{-1}, DP = 45) were purchased from Aldrich (Steinheim, Germany). Sodium hydroxide (NaOH) was from VWR (Leuven, Belgium). Deionized water was further purified with a Milli-Q system from Millipore (Molsheim, France).

2.2. Synthesis of PEG-b-PGlu diblock copolymer

PEG-b-PGlu diblock copolymers have been provided by Debiopharm. They were synthesized following the previously reported synthetic route of PEG-b-PAsp (PAsp being polyaspartic acid) [18]. Briefly, the N-carboxyanhydride of γ-benzyl-L-glutamate was synthesized by the Fuchs–Farthing method using triphosgene. Then, N-carboxyanhydride of γ-benzyl-L-glutamate was polymerized in N,N-dimethylformamide, initiated by the NH_2 amino group of CH_3O-PEG-NH_2, to obtain PEG-poly(γ-benzyl L-glutamate) (PEG-b-PBLG) block copolymers. PEG-b-PBLG was deprotected by reaction in 0.5 N NaOH at room temperature to obtain PEG-b-PGlu. Molecular weight (*Mw*) was obtained by MALDI-TOF/MS measurement (*Mw* average was found to be 12,400 g mol^{-1}, depending on batches). Polydispersity was 1.02.

2.3. Synthesis of (DACH)Pt-loaded micelles (1,2-diaminocyclohexane) platinum(II)-loaded polymeric micelles

$[Pt(DACH)(OH_2)_2]^{2+}$ was obtained by mixing $Pt(DACH)Cl_2$ and $AgNO_3$ in distilled water with a molar ratio of $[AgNO_3]/[Pt(DACH)Cl_2] = 0.5$. The solution was kept in the dark at 25 °C for 24 h. AgCl precipitates after reaction. Next, the mixture was centrifuged at 3000 rpm for 10 min to eliminate the AgCl precipitate. Afterward, the supernatant was purified by filtration through a 0.22 μm filter. PEG-b-PGlu was added to aqueous solution of $[Pt(DACH)(OH_2)_2]^{2+}$ with a molar ratio of $[Pt(DACH)(OH_2)_2]^{2+}/[PGlu] = 0.5$ and reacted for 72 h to prepare (DACH)Pt-loaded micelles (Fig. 1). The prepared micelles were purified by ultrafiltration (molar mass cutoff (MWCO): 100,000 g mol^{-1}). The polydispersity index measured by DLS, for drug-loaded polymeric micelles, was reported to be 0.16 [10], for a total molar mass of the drug-loaded micelle estimated to ~250,000–300,000 g/mol. Q7

2.4. Capillary electrophoresis and Taylor dispersion analysis

CE and Taylor dispersion analysis (TDA) experiments were carried out with a 3D-CE Agilent technologies system (Waldbronn, Germany) equipped with a diode array UV detector. Separation capillaries prepared from bare silica tubing were purchased from Composite Metal Services (Worcester, UK). Capillary dimensions were 33.5 cm (25 cm to the detector) × 50 μm for all analyses unless otherwise specified. New capillaries were conditioned by performing the following washes: 1 M NaOH for 20 min, 0.1 M NaOH for 15 min and water for 10 min. Between two runs, the capillary was flushed with NaOH 1 M for 2 min, water for 0.5 min and electrolyte for 5 min. The temperature of the capillary cassette was set at 25 °C. Samples were injected hydrodynamically (17 mbars, 3 s). Solutes were monitored by UV absorbance at maximum wavelength absorption (193 nm) unless otherwise specified.

3. Results and discussion

3.1. Determination of PEG-b-PGlu and (DACH)Pt-loaded micelle hydrodynamic radii by Taylor dispersion analysis

Taylor dispersion analysis (TDA) allows to determine the hydrodynamic radii (or diffusion coefficients) of molecules, macromolecules or particles. This absolute method of determination of the hydrodynamic radius is based on the dispersion of a solute plug in a capillary under laminar Poiseuille flow. After injection in the capillary of a narrow band of solute dissolved in the eluent, a pressure is applied at the inlet end of the capillary. Molecules in the band move with different velocities depending on their position in the capillary since the velocity profile is a parabolic function of the radius. The combination of the dispersive velocity profile with the molecular diffusion that redistributes molecules in the capillary cross section leads to a specific mechanism of dispersion described by the Taylor–Aris equation for unretained solutes. TDA does not require any calibration, and it is not necessary to know the injected concentration of the solute [19–23].

The diffusion coefficient *D* of the solute can be determined using Eq. (1):

$$D = \frac{R_c^2}{24\sigma^2} t_R \quad (1)$$

where R_c is the internal radius of the capillary, t_R is the average elution time, and σ^2 is the temporal variance of the elution profile. σ^2 can be obtained by integration of the peak or by a Gaussian curve fitting of