Polymer 54 (2013) 4538–4544

Contents lists available at SciVerse ScienceDirect

Polymer

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

Encapsulation of Nile Red in polypyrrole microvessels

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ARTICLE INFO

Article history:
Received 24 March 2013
Received in revised form
11 June 2013
Accepted 12 June 2013
Available online 19 June 2013

Keywords:
Polymer hollow structures
Microvessels
Rotational dynamics in confined volumes

ABSTRACT

Polymeric hollow structures are a popular topic in modern chemistry and materials science because of their potential importance in biomedical applications such as smart drug delivery systems. We report on the preparation of polypyrrole microvessels that are capable of encapsulating Nile Red, a solvatochromic dye that serves as a model of lipophilic drugs. Using a range of modern physicochemical methods including electron and optical microscopy, vibrational and fluorescence spectroscopy, and zeta potential measurements, we show that the dye can be encapsulated effectively through chemical polymerization of pyrrole onto *p*-xylene droplets that contain Nile Red. The fluorophore resides within the organic core of the microvessels but interacts with the polymer wall material (or pyrrole oligomers formed during polymerization) which is manifested as slower rotational dynamics and shorter fluorescence lifetime of the dye in comparison to bulk solvent. We also demonstrate release of the dye to the surrounding solution and show that this process is governed primarily by the miscibility of the organic core (*p*-xylene) with the external phase (solvent).

1. Introduction

Conjugated polymers have attracted considerable attention due to their unique properties, many of which make this class of materials promising for technological applications [1–4]. Recently, the use of conjugated polymers in biomedical research has emerged; for biologically related applications polypyrrole is especially promising because it is non-toxic and biocompatible [5–8]. Redox doping of polypyrrole (PPy) results in generation of positive charges in polymer chains (polarons and bipolarons) which is accompanied by incorporation of anionic counter ions to maintain electroneutrality. Employing this capability, PPy films have been used to incorporate and then to release dexamethasone, an anti-inflammatory drug, from polypyrrole-coated electrodes under *in vitro* conditions [9]. The controlled release of salicylate, naproxen, nicoside and neurotrophin was also demonstrated in several papers [10,11]. PPy is also used as a cell-growth substrate. For example, PPy-coated indium tin oxide was shown to be a better cell growth support than bare ITO for chromaffin cells [12].

Recently, the idea of using hollow structures formed from PPy as potential vehicles for controlled drug delivery has emerged [13]. For such applications, the polymer is deposited onto the surface of a colloidal particle, forming a thin spherical coating [14–18]. Once the PPy shell is formed, the core can be removed by dissolution or etching, leaving an empty PPy vessel [19]. This empty vessel can be loaded with the pharmaceutical compound of interest. Loading the PPy hollow structures with guest molecules can be accomplished either before deposition of the polymer coating onto the support surface or after the formation of the PPy shell. The first approach is typically used when liquid droplets are used as the templating structures. In such cases the compound to be encapsulated is dissolved in a solvent which is dispersed into microdroplets which are subsequently coated with PPy. In the second approach, the already-fabricated polymer vessels are typically conditioned in a concentrated solution of the compound, which permeates into the vessel's core. Depending on the nature of the vessel's core material, either hydrophilic or hydrophobic compounds can be encapsulated.

Incorporation of various hydrophilic species into polypyrrole vessels has been demonstrated by several authors. These approaches are based on loading the vessels with aqueous solutions of the compound to be encapsulated. Bajpai et al. loaded hollow PPy structures with fluorescein cadaverine [20]. Mohwald et al. reported on the electrochemical preparation of PPy vessels and demonstrated post-formation loading with Rhodamine 6G (R6G) [21]. A similar approach for photopolymerized vessels was reported by our group recently [22,23]. We have also reported on the preparation of PPy vessels loaded with aqueous doxorubicin solution; doxorubicin is a potent chemotherapeutic agent [24]. The encapsulation of lipophilic compounds requires polymer vessels

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http://dx.doi.org/10.1016/j.polymer.2013.06.029

with a hydrophobic core. We have recently demonstrated encapsulation of lipophilic fluorophores such as pyrene and perylene in PPy vessels filled with toluene [25,26].

In this paper we focus on the encapsulation of Nile Red (NR) as a model for lipophilic drugs. The main advantage of Nile Red is that it is solvatochromic, providing spectroscopic information on its immediate microenvironment. The fluorescence spectra of microvessels containing Nile Red provide direct information on the location of the dye within the polymer structure. We demonstrate in this work the encapsulation of the fluorophore in PPy microvessels and its subsequent release to the surrounding solution based on simple solubility phenomena. We also show that the rotational dynamics of the encapsulated probe is considerably slower in comparison to bulk solvent.

2. Materials and methods

2.1. Chemicals

All chemicals were of the highest quality commercially available and were used as received: *p*-xylene (Aldrich, Chromasolv, >99%), pyrrole (Aldrich, 98%), Nile Red (Sigma), iron(III) chloride (Aldrich, 97%, anhydrous), methanol (POCh, reagent grade). Aqueous solutions were prepared from high purity water (Milli-Q Plus).

2.2. Instrumentation

Scanning electron microscopy was performed with a Zeiss Merlin field emission SEM while transmission electron microscopy data were collected using a Zeiss Libra 120 EFTEM. An energy-dispersive X-ray microanalysis system, Zeiss Merlin FE-SEM, was used to analyze the composition of the microvessels.

Laser scanning confocal microscopy fluorescence imaging was performed using a Leica TCS SP2 system. Wide field optical microscopy in fluorescence or reflection mode was performed with a Nikon Eclipse LV 100 optical microscope.

Raman spectra were recorded with a LabRAM HR spectrometer (Horiba Jobin Yvon) coupled to an Olympus BX41 confocal microscope. An Excelsior-CDRH diode-pumped laser (Spectra Physics) operating at 532 nm was used as the excitation source. The same instrument was used to record the fluorescence spectra of Nile Red released from PPy vessels into the surrounding solution. Other fluorescence measurements were performed using a Fluorolog FL3-2-IHR320 spectrometer (Horiba Jobin Yvon).

Infrared measurements in transmission mode in KBr pellets were acquired with a Nicolet 8700 spectrometer (Thermo Electron Corporation).

The zeta potential of the PPy microvessels suspended in water was determined with a Zetasizer NanoZS instrument (Malvern).

Fluorescence lifetime and anisotropy decay measurements were performed with a time-correlated single photon counting (TCSPC) instrument. This instrument has been described in detail elsewhere and we provide a brief description here [27]. The light source was a CW mode-locked Nd:YVO_4 laser (Spectra Physics Vanguard) that produces 2.5 W average power at both 355 nm and 532 nm, at 80 MHz repetition rate with 13 ps pulses. The UV output of the Nd:YVO_4 laser is used to excite a cavity-dumped dye laser (Coherent 702) operating at 525 nm with Coumarin 500 dye (Exciton) and producing 5 ps pulses at a repetition rate of 4 MHz (Gooch and Housego cavity dumping electronics). Pulses from the dye laser are divided into excitation and reference arms, with the reference channel being detected by a photodiode (Becker & Hickl PHD-400-N), and the excitation arm directed to the sample. Emission is collected using a 40× reflecting microscope objective (Ealing). The collected emission is separated into components polarized parallel (0°) and perpendicular (90°) with respect to the vertically polarized excitation pulse with a polarizing cube beam splitter. The parallel and perpendicular polarized signal components are detected simultaneously using two microchannel plate photomultipliers (MCP-PMT, Hamamatsu R3809U-50), each equipped with a subtractive double monochromator (Spectral Products CM-112). The detection electronics (Becker & Hickl SPC-132) produce a *ca.* 30 ps response function for each detection channel. Data acquisition, detector bias, and collection wavelength are all controlled using a LabVIEW® program (National Instruments) written in-house. Samples were measured at 23 ± 0.1 °C using a temperature controlled brass block sample holder.

2.3. Preparation of the vessels

The Nile Red containing *p*-xylene/water emulsion was prepared by mixing 3 ml of Milli-Q water with 100 μl of Nile Red solution in *p*-xylene (10^{-4} M). Then, the polymerization solution prepared by adding 2 ml of aqueous pyrrole (0.21 M) to 2 ml of aqueous ferric chloride (0.1 M) was poured to the emulsion and allowed to react for 30 min. The resulting polypyrrole microvessels were separated by centrifugation. For preparation of microvessels not loaded with Nile Red the neat *p*-xylene instead of NR solution was used to prepare the emulsion.

3. Results and discussion

3.1. Fabrication of microvessels

The microvessels were prepared by deposition of polypyrrole onto the surface of *p*-xylene microdroplets dispersed in aqueous medium. The synthesis starts with preparation of a *p*-xylene-in-water emulsion followed by the *in situ* oxidative polymerization of pyrrole onto the surfaces of the droplets (*vide infra*). The optical microscopy image of the *p*-xylene-in-water emulsion is shown in Fig. S1 (Supplementary data). The average diameter of the *p*-xylene droplets is *ca.* 2.5 μm. These droplets were used to template the growth of PPy to form hollow structures. The polymer was deposited by the addition of monomer and oxidant to the emulsion.

The resulting polymer structures were studied with transmission electron microscopy (TEM). Shown in Fig. 1 is a typical TEM image of an individual polymer microvessel. The vessel is collapsed, likely due to evaporation of the *p*-xylene. The thickness of the microvessel wall is estimated to be ~50 nm based on the TEM image. The TEM data clearly show that vessel walls are highly flexible.

Even though the vessels are fragile and collapse under vacuum conditions, when they are suspended in aqueous solution they can be stored for extended periods of time. To study the interactions between individual vessels in the aqueous phase we performed zeta potential measurements.

Shown in Fig. 2 is dependence of zeta potential of PPy microvessels dispersed in water as a function of pH. The value of the zeta potential decreases with increase of pH. The isoelectric point is at *ca.* 2.7. These data can be explained as follows. At low pH the polypyrrole is protonated which contributes to positive value of the zeta potential. The pK_a of the polypyrrole protonation process is *ca.* 3 [28], which coincides with the isoelectric point determined from the zeta potential measurements. With increase of pH the zeta potential gradually decreases reaching the value of *ca.* −42 mV at pH 11. This potential decrease is likely due to deprotonation of polypyrrole. Another explanation for the negative value of the zeta potential at higher pH would be incorporation of $FeCl_4^-$ as doping ions. However, we do not observe any traces of iron in the sample as