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Thermoresponsive coacervate formation of random poly(phosphonate) terpolymers

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ABSTRACT

Coacervates are partially hydrated, colloidal polymer droplets in water held together by hydrophobic interactions and are considered promising candidates for drug delivery applications. We present the first coacervates made from temperature-induced phase separation of aqueous poly(phosphoester) terpolymer solutions. Such coacervates are interesting for drug carrier applications as they are non-toxic, fully biodegradable and form spontaneously upon heating above a threshold temperature (lower critical solution temperature, LCST). The investigated poly(ethylene alkyl phosphonate) terpolymers are synthesized via the organocatalytic anionic ring-opening polymerization of cyclic phosphonate monomers. This way polymers with high control over molecular weight, terpolymer composition (and hence physical and chemical properties) and rather narrow molecular weight distributions ($Đ < 1.30$) are produced. The terpolymers bear functional pendant groups for further modifications and have a finely tunable balance of hydrophilic and hydrophobic side-chains randomly distributed over the whole chain, as proven by ^{31}P NMR polymerization kinetics. These functional terpolymers spontaneously phase separate into a polymer rich coacervate phase in water upon heating above the LCST, providing an elegant method to prepare degradable and non-toxic carrier system.

1. Introduction

The precise synthesis of polymers for the preparation of nanometer- or micrometer-sized carriers is an important field of current research. The entrapment of reactive or labile drugs inside a polymeric carrier reduces adverse or systemic reactions and prevents their premature exclusion and degradation in the body [1–4]. One of the many systems discussed for these applications are coacervates, liquid-liquid phase separated polymer solutions which form spontaneously upon certain stimuli and are known to have exceptionally high loading-capacities [5–8]. One such stimulus can be the temperature induced desolvatisation and subsequent phase separation into a polymer-poor aqueous phase and a polymer-rich coacervate phase. Polymers showing such phase separation behavior are generally called LCST (lowest critical solution temperature) polymers and are extensively studied in literature for many applications [9–13]. LCST-induced coacervate formation, however, is far less often observed in synthetic polymers [6,14–17].

Some of the main requirements to be fulfilled for polymers to be used as carrier systems are biocompatibility, low toxicity, solubility in water, the potential to induce a release mechanism and degradability. Today, even though there are a growing number of polymer-drug formulations being approved by the FDA, poly(ethylene glycol) (PEG) is still considered as *the* gold standard for water-soluble, biocompatible polymers [18–21]. Despite its many assets, however, PEG suffers from several drawbacks: the polyether backbone is non-degradable under biological conditions which might lead to accumulation of high molecular weight PEG. Due to its

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abundance in many formulations, a significant percentage of people have developed “anti-PEG” antibodies over the past decades. This reduces the efficacy of PEG-based drug formulations in many cases [21–25]. Finally, chemical modification of PEG is usually only possible on the α- and ω- end of the polymer, restraining the adjustment of PEG properties towards specific needs. To overcome some of these shortcomings of PEG, several research groups have suggested various synthetic or natural polymers as PEG-alternatives [20,26–29]. One class receiving growing attention in recent years are poly(phosphoester)s (PPEs) going back to the pioneering works of Penczek et al. and Carraher et al. in the 1950s as they mimic the natural poly(phosphoester)s found ubiquitous in nature: DNA and RNA [30–33].

Today, the majority of these PPEs are produced via the organocatalytic anionic ring-opening polymerization (AROP) of cyclic phosphoester. This provides excellent control over molecular weight and narrow molecular weight distributions. Furthermore, several functional groups have been introduced to the pendant ester to date [28,34–46]. However, if well-defined PPEs are demanded, the polymerization needs to be terminated at moderate conversions (50–70%) or special catalysts need to be added to prevent excessive transesterification reactions that would broaden the molecular weight distribution. Another strategy to well-defined PPEs was recently presented by our group: the preparation of poly(alkylene alkyl phosphonate)s (PPn)s, aliphatic PPEs with a hydrolytically stable P−C bond in the side chain. We found that this small structural change successfully prevents transesterification even at high conversions (> 95%). The AROP of 2-alkyl-2-oxo-1,3,2-dioxaphospholanes, the respective cyclic monomers, was already performed with methyl-, ethyl- and isopropyl-side-chains resulting in non-toxic and still fully degradable polymers with excellent control over molecular weight and narrow molecular weight distributions [41,43]. Copolymers of 2-alkyl-2-oxo-1,3,2-dioxaphospholanes bearing an isopropyl and a cyclohexyl group showed a linear correlation between the glass transition temperature (T_g) and the copolymer composition [44].

In this work, we extend the scope of poly(ethylene alkyl phosphonate)s with the new side-chain functional monomer 2-allyl-2-oxo-1,3,2-dioxaphopsholane **(3)**. For the first time, we study the terpolymerization behavior of three different dioxaphospholane monomers. The thermoresponsive behavior of the obtained terpolymers in aqueous solution as well as the temperature triggered formation of simple coacervates is presented. In combination with previous studies investigating the hydrolytic and microbial (seawater) degradation of PPn’s, this straightforward preparation of degradable coacervates might be beneficial for future degradable drug delivery or self-healing applications [41,43,47].

2. Materials and methods

2.1. Materials

Solvents and chemicals were purchased from Acros Organics, Sigma Aldrich or Fluka and used as received, unless otherwise stated. All chemicals were purchased in highest purities, dry and stored over molecular sieve (4 Å), if possible. 2-(Benzyloxy)ethanol was purchased from ABCR, distilled from calcium hydride and stored over molecular sieve (4 Å) and under argon prior to use. DBU was purchased from Sigma Aldrich, distilled prior to use and stored over molecular sieve (4 Å) under argon. Deuterated solvents were purchased from Deutero GmbH (Kastellaun, Germany) and used as received.

2.2. Instrumentation and characterization techniques

Size exclusion chromatography (SEC) measurements were performed in DMF (1 g L^{-1} LiBr added) at 60 °C and a flow rate of 1 mL min^{-1} with a PSS SECcurity as an integrated instrument, including a PSS GRAM 100–1000 column and a refractive index (RI) detector. Calibration was carried out using poly(ethylene glycol) standards provided by Polymer Standards Service. The ^{1}H, $^{13}C\{H\}$, and $^{31}P\{H\}$ NMR experiments were acquired on a 500 MHz Bruker AMX system. The temperature was kept at 298.3 K and calibrated with a standard ^{1}H methanol NMR sample using the topspin 3.0 software (Bruker). $^{13}C\{H\}$ NMR spectra were referenced internally to solvent signals. $^{31}P\{H\}$ NMR spectra were referenced externally to phosphoric acid. The $^{13}C\{H\}$ NMR (125 MHz) and $^{31}P\{H\}$ NMR (201 MHz) measurements were obtained with a ^{1}H powergate decoupling method using 30 ° degree flip angle. 2D NMR experiments (1H DOSY (Diffusion ordered spectroscopy)) were measured on a Bruker 500 AMX NMR spectrometer under the same conditions as mentioned above. All spectra were processed with the MestReNova 9.0.1-13254 software. Differential Scanning Calorimetry (DSC) measurements were performed using a Mettler-Toledo DSC823 thermal analysis system in the temperature range from −80 to 50 °C under nitrogen with a heating rate of 10 °C min^{-1}. Cloud points were determined in ultrapure water with a resistivity of 18 MΩ cm^{-1} or dulbecco’s phosphate buffered saline, respectively and detected by optical transmittance of a light beam (λ = 500 nm) through a 1 cm sample cell. The measurements were performed in a Jasco V-630 photo spectrometer with a Jasco ETC-717 Peltier element. The intensity of the transmitted light was recorded versus the temperature of the sample cell. The heating/cooling rate was 1 °C min^{-1} and values were recorded every 0.1 °C. Confocal laser scanning microscopy was performed with a LSM SP5 STED Leica Laser Scanning Confocal Microscope (Leica, Germany), consisting of an inverse fluorescence microscope DMI 6000 CS equipped with a multi-laser combination and five detectors operating in the range of 400–800.

2.3. Experimental

2.3.1. Ethylphosphonic acid dichloride

The compound was synthesized according to literature protocol [43]. Briefly, *O,O*-diethyl ethylphosphonic acid diester (100.0 g, 0.6 mol) and DMF (0.5 mL) was added drop wise to refluxing thionylchloride (180 mL, 1.5 mol). Strong gas evolution of methylene