Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplemental Materials

An assessment of nanosponges for intravenous and oral drug delivery of BCS class IV drugs: Drug delivery kinetics and solubilization.

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Materials

Meta-chloroperoxybenzoic acid was purchased from Sigma Aldrich and recrystallized in methanol before use. Paclitaxel was obtained from LC Laboratories. Phosphate buffered saline was obtained from Gibco by Life Technologies and pH was adjusted to 7.4 and supplemented with Tween-80 (0.1% v/v). Simulated gastric fluid was prepared by dissolving 2.0 grams sodium chloride in 7.0 mL concentrated HCl, diluting to 1.0 L with water, adjusting pH to 1.2, and supplemented with Tween-80 (0.1% v/v). Spectra/Por® Dialysis membrane was purchased from Spectrum Laboratories Inc. All other materials were obtained from Sigma Aldrich and used as received.

Characterization

Samples for transmission electron microscopy (TEM) imaging were prepared by dissolving 0.5 mg nanoparticles in 1 mL filtered, deionized water. The samples were sonicated for 30 min and stained with 6 drops of 3% phosphotungstic acid. The carbon grids were prepared by slowly dipping an Ultrathin Carbon Type-A 400 Mesh Copper Grid (Ted Pella, Inc., Redding, CA) into the particle solution three times and air drying the grid at room temperature. A Philips CM20T transmission electron microscope operating at 200 kV in bright-field mode was used to obtain TEM micrographs of the polymeric nanoparticles. ^{1}H NMR spectra were obtained from a Bruker AC400 Fourier Transform Spectrometer with $CDCl_3$/TMS as solvent. High-performance liquid chromatography (HPLC) was carried out using a Waters chromatograph equipped with a Waters 2996 variable wavelength photodiode array detector, a Waters 1525 binary HPLC pump, and a reverse phase column (100 x 4.6 mm i.d., pore size 5 μm, Thermo Scientific). All runs were performed using an isocratic gradient of water and acetonitrile (1:1 v/v) at a flow rate of 1

mL/min. Calibration curves of paclitaxel were plotted using different concentrations of paclitaxel in acetonitrile/water (1:1, v/v) or PBS (pH 7.4, 0.1% Tween-80 v/v) and detecting between 30-6,000 ng with correlation coefficients of $R^2 = 0.9998$ and 0.9991, respectively. X-ray diffraction analysis was performed using a Scintag X1 θ/θ automated powder X-ray diffractometer with a Cu target, a Peltier cooled solid-state detector, and a zero-background Si(510) sample support. Scans were collected at a rate of 1°/min in the 2ϑ range from 5 to 65°. Differential scanning calorimetry was conducted using a TA Instruments Differential Scanning Calorimeter (DSC) Model 2920. Samples were added to aluminum pans and non-hermetically sealed. All samples scanned between 25 and 250°C at a rate of 10°C/min after equilibrating at 25 °C.

Polyester nanoparticle synthesis (NP)

Poly(α-allyl-valerolactone, valerolactone)[1] containing 4, 7, or 10% allyl functionality was epoxidized by stirring meta-chloroperoxybenzoic acid (mcpba, 1.2 eq per alkene) in CH_2Cl_2 (0.065 M alkene) for 48 hours and washing with saturated sodium bicarbonate. Resulting polymer was dried under reduced pressure to yield poly(evl-vl) (yield = 75%). In all cases, 100% conversion of allyl to epoxide was achieved as indicated by the complete reduction of the alkene proton shifts. ^{1}H NMR (400 MHz, $CDCl_3$/TMS, ppm) δ: 4.08 (m, –OC*H*$_2$), 2.96-2.91 (m, -CHCH-C*H*$_2$), 2.75, (m, -CHC*H*-CH$_2$), 2.47 (m, -CHCH-C*H*$_2$), 2.34 (m, evl and vl, –O(O)C*H*$_2$CH$_2$), 1.68 (m, evl and vl, –CHC*H*$_2$C*H*$_2$; evl -C*H*CH-CH$_2$), 0.93–0.91 (d, 3-methyl-1-butanol, –OCH$_2$CH$_2$CH(C*H*$_3$)$_2$).

Nanoparticles were formed by refluxing 2,2'-ethylenedioxy-bis(ethylamine) (1.5 amines per epoxide) with poly(evl-vl) in CH_2Cl_2 (3.24×10^{-3} M epoxide) at 46 °C for 12 hours. For

example, a 4% crosslinked nanoparticle was formed by adding 2,2'-ethylenedioxy-bis(ethylamine) (7.5 μL, 0.51 mmol) to a stirring solution of poly(evl-vl) (0.173 g, M_n = 3,644 Da, 6.83 x 10^{-2} mmol epoxide) containing 4% epoxide dissolved in CH_2Cl_2 (21.1 mL). The refluxing mixture stirred for 12 hours at 46 °C. Residual bisamine was removed by dialyzing with Snakeskin Pleated Dialysis Tubing (MWCO = 10,000 Da) against CH_2Cl_2 to yield nanoparticles (NP, 123.8 mg). ^{1}H NMR (400 MHz, $CDCl_3$/TMS, ppm) δ: The significant change is the disappearance of the epoxide ring protons at 2.96, 2.76, and 2.47 ppm and the emergence of small, broad peaks at 3.50-3.45 ppm from the methylene protons adjacent to the oxygens in the crosslinker and 2.75-2.68 ppm from the methylene protons adjacent to the secondary amine after crosslinking.

Particles with 7% and 10% crosslinking densities were synthesized in a similar manner with poly(evl-vl) containing 7% and 10% epoxide functionality, respectively, and reacting with 2,2'-ethylenedioxy-bis(ethylamine) (1.5 amines per epoxide) in CH_2Cl_2 (3.24 x 10^{-3} M epoxide) for 12 hours at 46 °C.

Paclitaxel encapsulation into nanoparticles (NP-PTX)

A solution of nanoparticle (70.0 mg) and paclitaxel (15.4 mg) in DMSO (0.150 mL) was added drop-wise to a vortexing solution of aqueous 1% d-α-tocopherol polyethyleneglycol (1000) succinate (Vit E-TPGS, 35.0 mL) to form a precipitation, and the purification and analysis of the encapsulated product were modified from a previous study.[2] The resulting precipitation was washed by three cycles of centrifugation at 7830 rpm for 20 minutes and replacing the supernatant each time with fresh deionized water. The precipitation was freeze-dried to yield a white powder. To determine the amount of encapsulated paclitaxel, the product was first

dissolved in acetonitrile before diluting with an equal volume of water and analyzing by HPLC as described in the characterization section. In this way, the encapsulated drug was separated from the nanoparticle and was detected at the same retention time (6.3 minutes) as free paclitaxel using a variable wavelength detector at 227 nm. NP-PTX averaged 13.0% paclitaxel wt/wt.

In vitro drug release

The release of paclitaxel from the nanoparticles was measured in PBS (pH 7.4) or simulated gastric fluid (pH 1.2) containing Tween-80 (0.1% v/v) at 37 °C at a paclitaxel concentration of 0.15 mM. For example, a 4% crosslinked nanoparticle containing 13.0% paclitaxel wt/wt (4.8 mg) was suspended in 5.0 mL PBS (pH 7.4, 0.1% Tween-80 v/v). At each time point, the suspension was centrifuged at 7830 rpm for 15 minutes and the supernatant was collected and replaced with 5.0 mL fresh buffer. For release studies in PBS, the supernatant at each time point was analyzed by HPLC as described in the characterization section. For release studies in simulated gastric fluid, the supernatant was neutralized with sodium bicarbonate to pH 7 and extracted three times with 5 mL CH_2Cl_2. The CH_2Cl_2 was evaporated and the resulting solid was dissolved in acetonitrile/water (1:1 v/v) and analyzed by HPLC. The amount of paclitaxel released at each time point was calculated from paclitaxel calibration curves, and all experiments were performed in triplicate.

1. Stevens, D.; Watson, H.; LeBlanc, M.; Wang, R.; Chou, J.; Bauer, W.; Harth, E., Practical polymerization of functionalized lactones and carbonates with Sn(OTf)(2) in metal catalysed ring-opening polymerization methods. *Polymer Chemistry* **2013,** *4* (8), 2470-2474.
2. Mu, L.; Feng, S., A novel controlled release formulation for the anticancer drug paclitaxel (Taxol (R)): PLGA nanoparticles containing vitamin E TPGS. *Journal of Controlled Release* **2003,** *86* (1), 33-48.