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# Redox-sensitive Polymeric Nanoparticles for Drug Delivery

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*1. Materials*

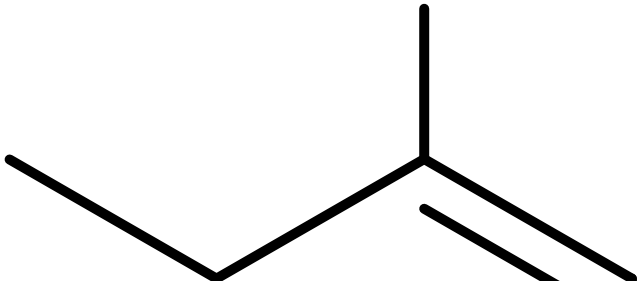
Trimethyl hydroquinone was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. 2-Amino-1,3-propanediol (serinol) was purchased from Alfa Aesar (Ward Hill, MA) and used as received. All other chemicals were obtained from Fisher Scientific (Pittsburgh, PA) and used without further purification, except adipoyl chloride. Adipoyl chloride was distilled under reduced pressure right before use. Paclitaxel was purchased by LC laboratories® (Woburn, MA, USA). All solvents, unless otherwise stated, were used without further purification.

*2. Synthesis of the monomer*

NHS-activated benzoquinone compound **4** was synthesized, as reported previously.[9a] To a mixture of 2-amino-1,3-propanediol (1.639 g, 18 mmol) and triethyl amine (5 mL) in isopropanol (100 mL), the synthesized compound **4** (4.168 g, 12 mmol) in THF (50 mL) was added dropwise at room temperature. After being stirred overnight at room temperature, the mixture was collected and concentrated under reduced pressure. The crude compound was washed with $NaHCO_3$ three times and extracted by ethyl acetate (50 mL×3), then dried over by sodium sulfate. The reductive monomer **1** (yellow crystalline) was purified by recrystallization in ethyl acetate with an isolated yields of 61.3% (2.379 g). NMR spectra were recorded on a Bruker Ultrashield™ 400 PLUS at 400 and 100 MHz for $^1H$ and $^{13}C$, respectively. The residual solvent peaks (δ = 2.50 or 7.24 for DMSO-$d_6$ or $CDCl_3$, respectively) were used as the solvent residual references for $^1H$ NMR spectra, and chemical shifts of the solvent peaks (δ = 39.52 or 77.00 for DMSO-$d_6$ or $CDCl_3$, respectively) were used as the reference for $^{13}C$ NMR spectra.

Monomer **1**, yellow crystal; mp 161–162 °C (from EtOAc) , $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 7.54 (d, *J* = 8.3 Hz, 1H), 4.56 (t, *J* = 5.5 Hz, 2H), 3.60 (dt, *J* = 8.2, 5.6 Hz, 1H),3.32 (m, 4H),

2.71 (s, 2H), 2.01 (s, 3H), 1.90 (s, 3H), 1.87 (s, 3H), 1.32 (s, 6H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ = 190.20, 186.85, 171.31, 154.94, 144.03, 136.07, 135.37, 60.13, 52.70, 47.58, 37.69, 28.06, 13.69, 12.77, 11.70; IR (neat, $cm^{-1}$) 1528, 1600, 1638, 3333; Found: C, 63.20; H, 7.71. Calcd for $C_{17}H_{25}O_5$: C, 63.14; H, 7.79.



**Scheme 1**. Synthetic reactions for a trimethyl-lock quinone-based redox-sensitive monomer.

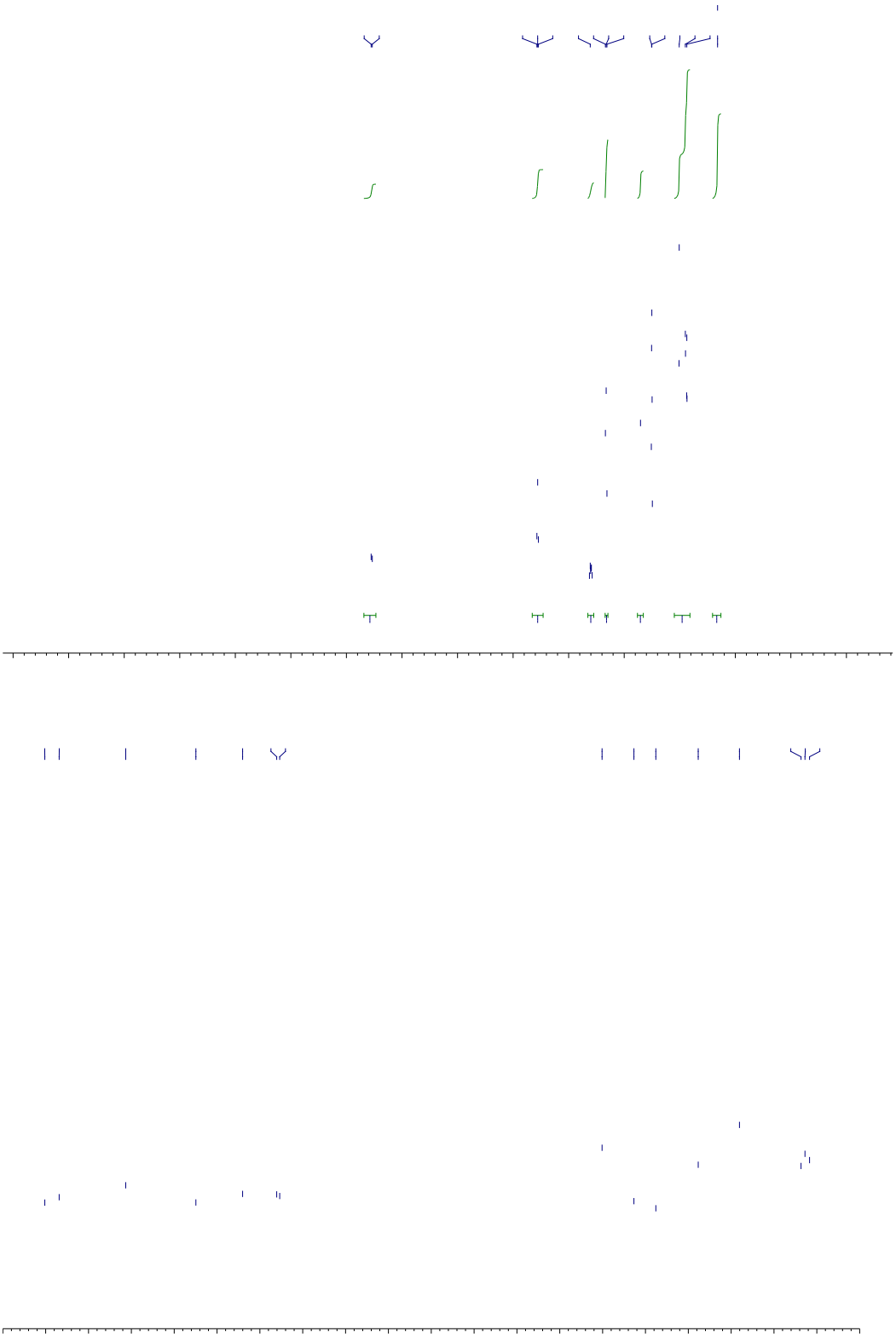


**Figure 1.** $^{1}H$ and $^{13}C$ NMR spectra of compound **1** in DMSO-$d_6$.

*3. Polymerization with adipoyl chloride*

To a solution of the synthesized monomer (1.3 g, 4 mmol) in pyridine (3.2 mL), adipoyl chloride (0.732 g, 4 mmol) in dichloromethane (25 mL) was added dropwise over 10 minutes at room temperature. After being stirred overnight, the mixture was collected and washed with water three times. The collected organic layer was dried with sodium sulfate and concentrated under reduced pressure. The polymer was precipitated in cold ethyl ether (20 mL), yielding yellow polymer with an isolated yield of 84.7% (1.6 g). Molecular weight and PDI of the synthesized polymer was determined to be 9800 Da ($M_n$) with 1.51, respectively, by GPC measurements using polystyrene standards.

A Waters gel permeation chromatography (GPC) system (Waters, Milford, MA) equipped with a binary pump (Waters 1525), a refractive index detector (Waters 2414), and a Styragel HR4E column (300 × 7.8 mm I.D., 5$\mu$m particle size) were used for the molecular weight measurements. THF of HPLC grade was eluted at a flow rate of 1mL/min at 25°C. Polystyrene standards (1,000-50,000Da) were also run to obtain a calibration curve.

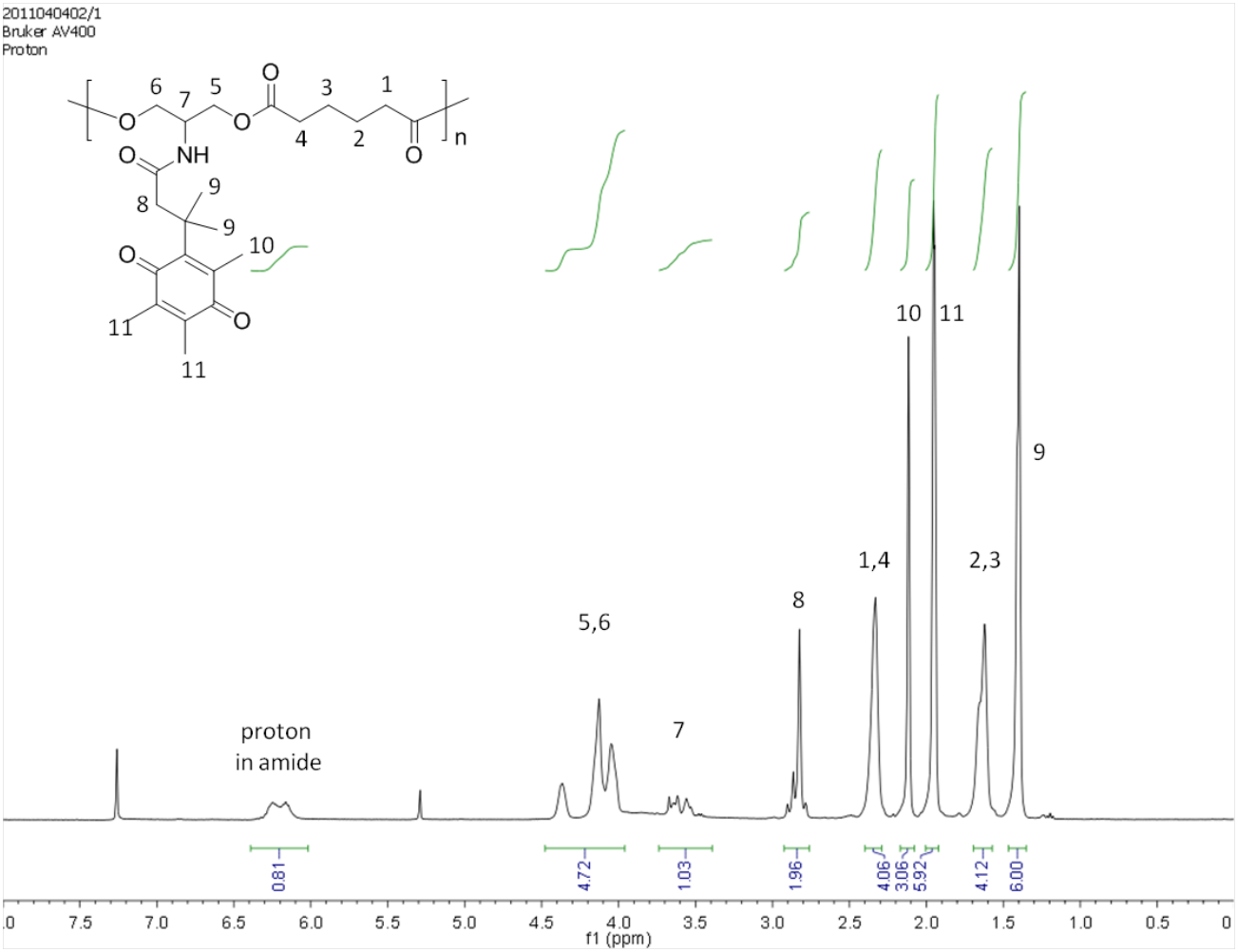



**Figure 2.** $^1$H NMR spectrum of the synthesized polymer.

*4. Preparation of nanoparticles by single emulsion method*

For the preparation of blank redox-sensitive nanoparticles, a synthesized polymer solution (25mg) in 0.5 mL dichloromethane was added to 9.4 mL of PBS containing 0.1 mL of tween 80 as a surfactant, while stirring at room temperature. The mixture was stirred at 1000 rpm for another 10 mins and, then, emulsified by sonication for 1 min in an ice-bath with a probe sonicator. After magnetically stirring the mixture overnight at ambient temperature, the hardened nanoparticles were filtered through a 0.45 μm filter to remove large particles and rinsed with PBS three times to remove surfactant via centrifugation. The nanoparticles were lyophilized and collected as a yellow fluffy powder (17 mg, 68%). For the preparation of paclitaxel-loaded nanoparticles, 2.5 mg of paclitaxel was dissolved in the organic phase. Drug loading in the nanoparticles was determined by direct measurements of loaded paclitaxel using a HPLC system after dissolving the dried paclitaxel-loaded nanoparticles in acetonitrile. The drug loading efficiency in the polymeric nanoparticle was determined to be 77.9%. The HPLC system consisted of a binary pump (Waters 1525), a UV detector (Water 2487), and an autosampler (Water 717). Analytical column was Waters $C_{18}$ Symmetry column (150 mm×3.9 mm I.D., 5 μm particle size) and a mixture of acetonitrile and water (55/45, v/v) was used as the eluent solvent. The flow rate was set at 1.0 mL/min with 20 μL of injection volume, and the paclitaxel was detected at an absorption wavelength of 227 nm.

*5. Nanoparticle characterization*

The size distribution of blank and paclitaxel-loaded nanoparticles was measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The polymeric nanoparticles were also characterized by transmission electron microscopy (TEM) using a Tecnai T12 microscope (FEI, Hillsboro, OR) operated at 80 kV. Each sample was sonicated for 5 mins before being mounted on a carbon coated Formvar cooper grid (400 mesh) and dried for 3 minutes. After wicking away excess solution and air drying for 1 minute, samples were then negatively stained with uranyl acetate (2% w/v) for 30 seconds prior to TEM imaging.

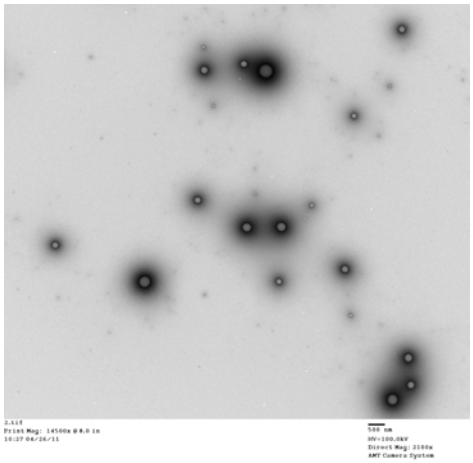


**Figure 3.** The TEM image of paclitaxel incorporated polymeric nanoparticles.

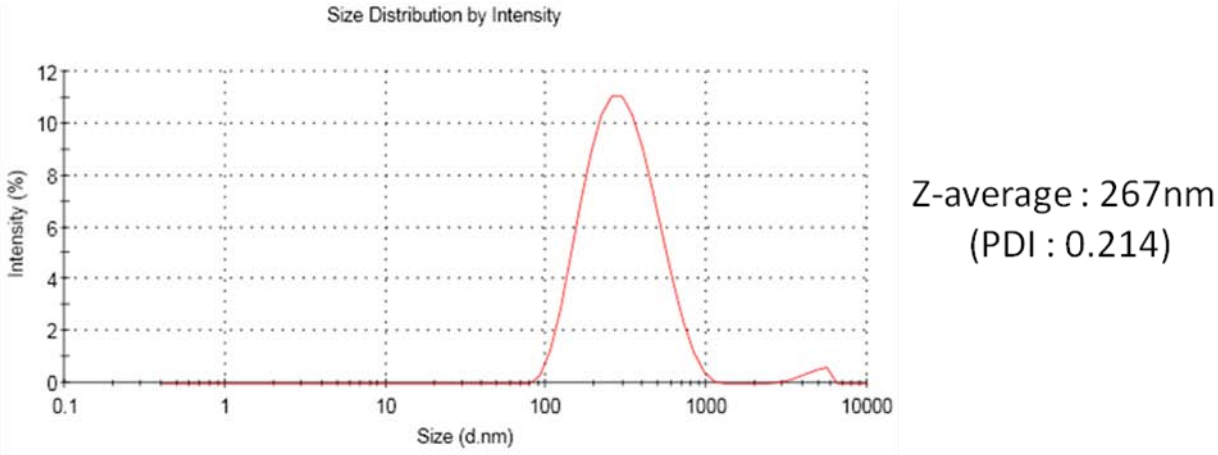



**Figure 4.** Size distribution and z-average diameter of the paclitaxel loaded nanoparticles

*6. Paclitaxel and lactone release by chemical reduction with sodium dithionite ($Na_2S_2O_4$)*

Paclitaxel release from the polymeric nanoparticles was investigated through chemical reduction by sodium dithionite *in vitro*. Drug encapsulated nanoparticles (230 $\mu$g) were suspended in PBS buffer with pH 7.4 containing sodium salicylic acid (0.8 M). After an addition of 200-fold molar excess of sodium dithionite to the benzoquinone moieties, aliquots (150 $\mu$L) were collected for the analysis with assistance of HPLC over 24 hours at appropriate time intervals, and the same volume of blank buffer media was added to maintain the total volume (3 mL). The triggered release experiment was achieved in the same manner, except the release media was incubated without sodium dithionite up to 48 hr. The 200-fold molar excess of sodium dithionite was added at 48 hrs time point, and the sample for HPLC analysis was collected essentially same manner as described in the section 4. A control solution of the nanoparticles was prepared with the same buffer solution without sodium dithionite. Since the retention times of released lactone and paclitaxel are different each other, the released lactone upon the chemical reduction was also monitored by HPLC at the same time (Figure 5). Synthesized lactone which was used for the calibration curve to determine the amounts of released lactone, compound 2 in the scheme 1, showed the same retention time as the lactone release from the polymer after reduction.

The effects of the chemical reduction on polymer structure were further examined by the analysis of resulting compounds after polymer reduction. Ten milligrams of the redox-sensitive polymer was dissolved in 1 mL of 50% v/v acetonitrile in deionized distilled water containing sodium dithionite at a 200-fold molar excess to the benzoquinone. After vigorously vortexing the mixture, the mixture was concentrated by the removal of acetonitrile under a continuous flow of nitrogen gas. After freeze drying, the concentrated mixture was extracted with acetonitrile to obtain the organic solvent-soluble products. Remaining mixture was further extracted with water to obtain water-soluble reduction products. The extracts were characterized by NMR after being dissolved either in $CDCl_3$ or $D_2O$. As shown in Figure 6 (a), the organic solvent-soluble portion,

represented by the top spectrum, was turned out to be the benzoquinone lactone of which spectrum is shown at the bottom. On the other hand, NMR spectrum of the water-soluble component indicated that the main water-soluble reduction product was poly(serinol adipate) as shown in Figure 1-(b). Characteristic methylene proton peaks in adipate appeared at 1.7 and 2.6 ppm while the proton peaks from serinol appeared at 4.1 and 4.5 ppm. This result also indicated that poly(serinol adipate) with free amines, resulting product after reduction of the TMBQ-based redox-sensitive polymer, is water soluble.

We have also tested the effect of sodium dithionite on paclitaxel at the same molar ratio used for the release study and confirmed that sodium dithionite did not affect paclitaxel analysis even after 24 hrs incubation as shown in Figure 7.

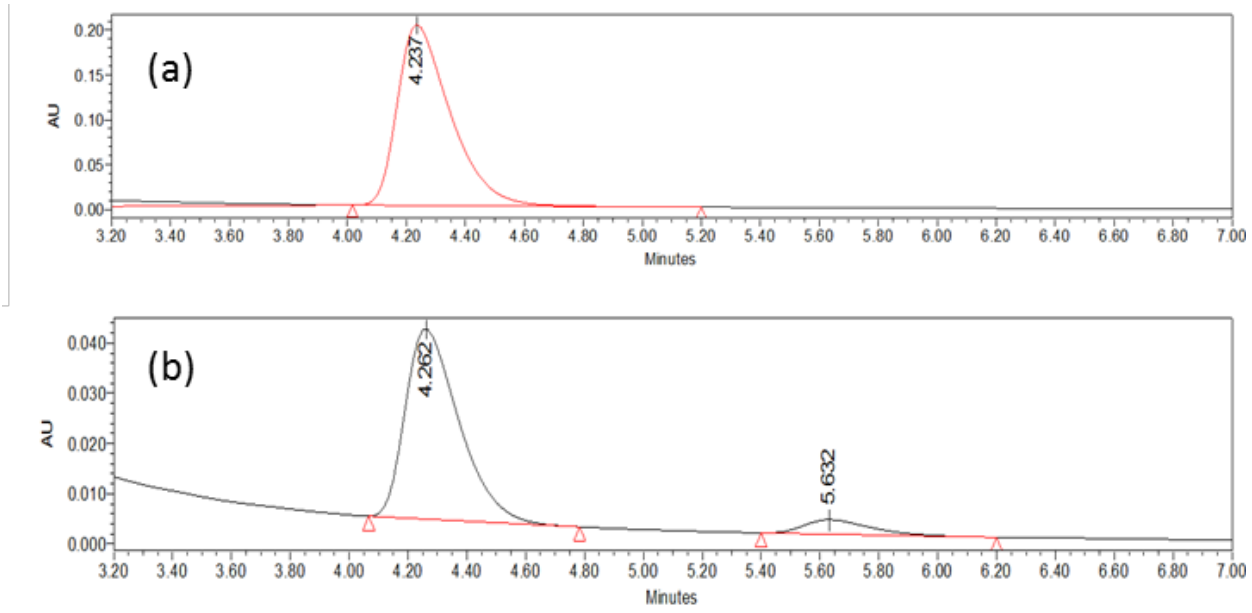



**Figure 5.** HPLC chromatograms of (a) the synthesized lactone and (b) a sample from the in vitro release study.

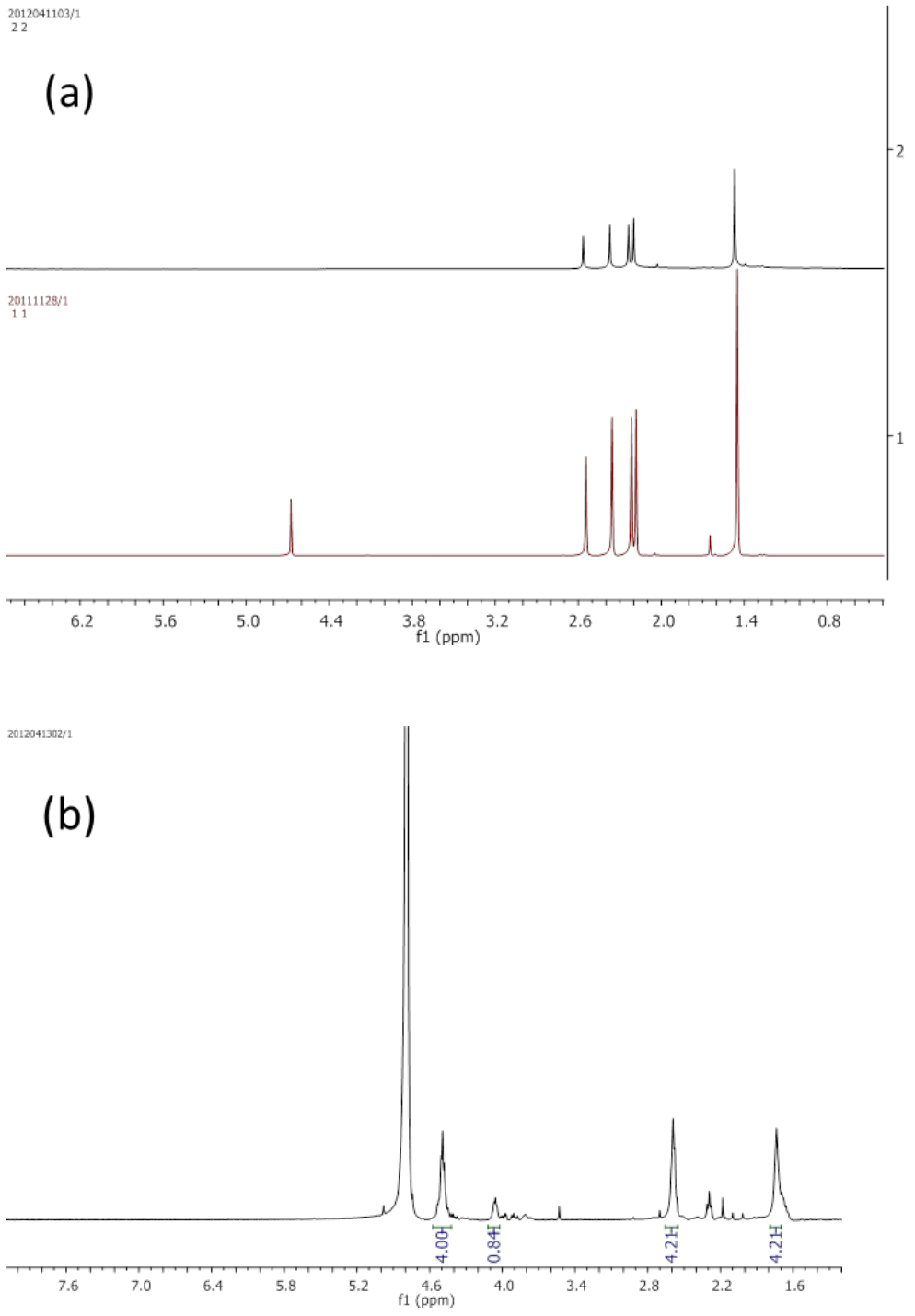



**Figure 6**. NMR spectra of the reduction products after polymer reduction by sodium dithionite. (a) NMR spectra of the organic solvent-soluble components after polymer reduction (top ), and synthesized benzoquinone lactone (bottom) in $CDCl_3$. (b) NMR spectrum of the water-soluble components after polymer reduction in $D_2O$.

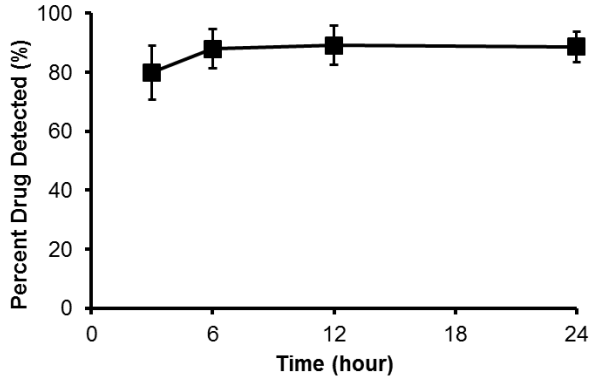



**Figure 7.** The effect of sodium dithionite on the detection of paclitaxel by HPLC.

*7. Statistical analysis*

Student's t-test used for statistical analyses of data. The differences were considered significant for p value of <0.05.