Polyketal Nanoparticles: A New pH-Sensitive Biodegradable Drug Delivery Vehicle

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In this report, we present an acid-sensitive drug delivery vehicle, termed polyketal nanoparticles, which are designed to target therapeutics to the acidic environments of tumors, inflammatory tissues, and phagosomes. The polyketal nanoparticles are formulated from poly(1,4-phenyleneacetone dimethylene ketal) (PPADK), a new hydrophobic polymer which contains ketal linkages in its backbone. The polyketal nanoparticles undergo acid-catalyzed hydrolysis into low molecular weight hydrophilic compounds and should therefore release encapsulated therapeutics at an accelerated rate in acidic environments. Importantly, the polyketal nanoparticles do not generate acidic degradation products after hydrolysis, as with polyester-based biomaterials. Dexamethasone-loaded nanoparticles, 200−600 nm in diameter, were fabricated with PPADK via an emulsion procedure using chloroform and water. The hydrolysis half-life of PPADK was measured to be 102 h at pH 7.4 and 35 h at pH 5.0. PPADK was synthesized by a new polymerization strategy based on the acetal exchange reaction. This new delivery system should find numerous applications in the field of drug delivery because of its ease of synthesis and excellent degradation properties.

Drug delivery vehicles based on polyesters and polyanhydrides have been widely used for the sustained release of therapeutics because of their excellent biocompatibility profiles and slow hydrolysis rates (*1*−*4*). However, numerous medical applications, such as targeting the acidic environment of lysosomes and tumors, require drug delivery systems that undergo rapid, pH-sensitive degradation (*5*, *6*). The majority of degradable polymers used for drug delivery cannot fulfill this requirement because they are composed of ester linkages, which degrade by base-catalyzed hydrolysis at physiological pH values. Recently, pH-sensitive hydrophobic microparticles based on poly(ortho esters) and poly(β-amino esters) have been successfully used for intracellular drug delivery and tumor targeting, thus demonstrating the potential of acid-sensitive biomaterials for drug delivery (*7*−*10*). Consequently, there is great interest in developing new strategies for the synthesis of pH-sensitive biodegradable polymers.

In this communication, we present an acid-sensitive hydrophobic nanoparticle based on a new polymer, poly(1,4-phenyleneacetone dimethylene ketal) (PPADK), which complements existing biodegradable nanoparticle technologies. This polymer has ketal linkages in its backbone and degrades via acid-catalyzed hydrolysis into low molecular weight compounds that can be easily excreted. PPADK forms micro- and nanoparticles, via an emulsion procedure, and can be used for the delivery of hydrophobic drugs and potentially proteins. In contrast to polyester-based biomaterials (*11*), the hydrolysis of polyketal nanoparticles does not generate acidic degradation products. Also, PPADK decomposes on a time scale that is faster than PLGA, but slower than poly(ortho esters) and poly(β-amino esters), and therefore provides the drug delivery community with a greater ability to tailor drug release kinetics to a particular application.

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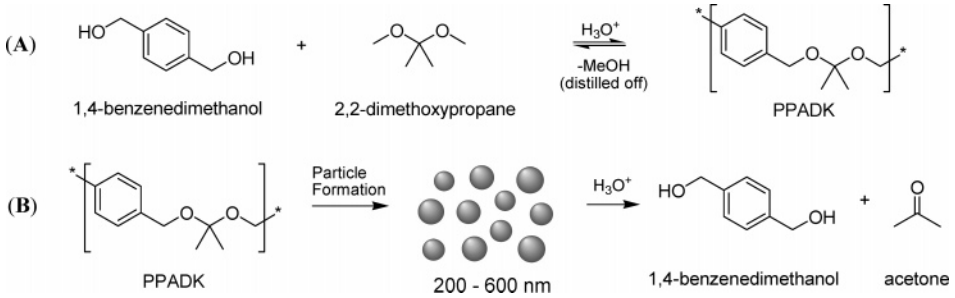
PPADK was synthesized via a new polymerization strategy based on the acetal exchange reaction. The acetal exchange reaction (*12*) is generally used to introduce protecting groups onto low molecular weight alcohols (*13*). However, we demonstrate here that the acetal exchange reaction can be used to generate acid labile polymers through the step growth polymerization of 1,4-benzenedimethanol and 2,2-dimethoxypropane (Scheme 1). This polymerization begins with an equilibrium reaction between 1,4-benzenedimethanol and 2,2-dimethoxypropane, generating dimers and trimers, which can further combine to form polymers. The formation of polymers is favored by distilling off the methanol byproduct formed during the polymerization reaction.

A representative polymerization of 2,2-dimethoxypropane and 1,4-benzenedimethanol gave PPADK with a 48% yield.[1] Figure 1A shows the GPC trace from one batch in which M_w = 4000 was obtained, corresponding to a degree of polymerization of 22.5 repeating units, with a polydispersity index of 1.54. The ^{1}H NMR spectrum

[1] The polymerization was carried out in a 25 mL two-necked flask connected to a short-path distilling head. 1,4-Benzenedimethanol (1.0 g, 7.3 mmol, Aldrich) dissolved in 10 mL of warm ethyl acetate was added to 10 mL of distilled benzene kept at 100 °C. Recrystallized *p*-toluenesulfonic acid (5.5 mg, 0.029 mmol, Aldrich) dissolved in 550 μL of ethyl acetate was then added. After allowing the ethyl acetate to distill off, distilled 2,2-dimethoxypropane (900 μL, 7.4 mmol, Aldrich) was added to initiate the reaction. Five additional doses of 2,2-dimethoxypropane were added via a metering funnel, with each dose consisting of 2 mL of benzene plus 300 to 500 μL of 2,2-dimethoxypropane. Each dose was added over a 30 to 40 min period with a 30 min interval in between. The total duration of the reaction was 7 h. The reaction was stopped with the addition of 100 μL of triethylamine and was precipitated in cold hexanes. The crude product was vacuum filtered, rinsed with ether and hexanes, and vacuum-dried to yield 600 mg of white solid product (48% yield). The recovered polymer was analyzed by GPC and ^{1}H NMR.

10.1021/bc050176w CCC: $30.25
Published on Web 10/05/2005

Scheme 1. Synthesis of the Polyketal, Poly(1,4-phenyleneacetone dimethylene ketal) (PPADK), via the Acetal Exchange Reaction[a]



[a] (A) Stepwise polymerization based on the acetal exchange reaction between 1,4-benzenedimethanol and 2,2-dimethoxypropane to produce PPADK. (B) Formation of drug-loaded nanoparticles by the solvent evaporation method. Particles exhibit pH-sensitive degradation into low molecular weight excretable compounds.

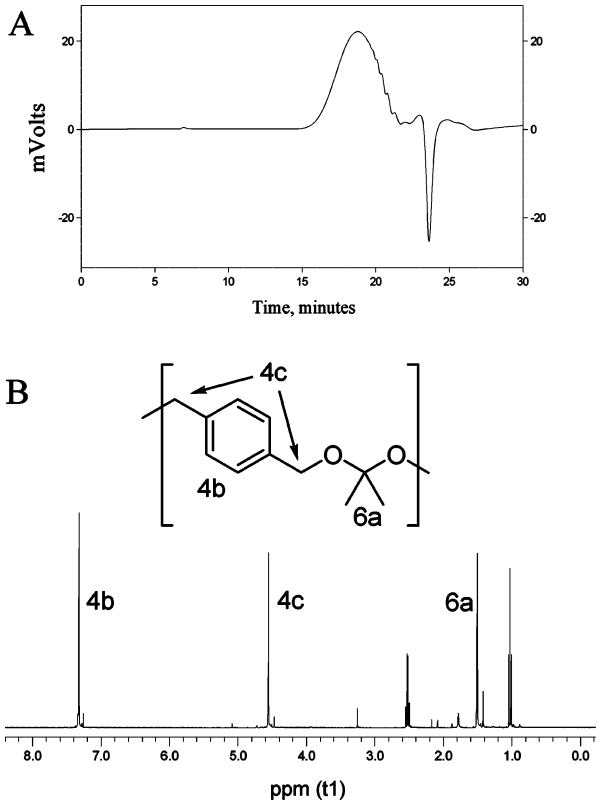


Figure 1. (A) GPC trace of PPADK in THF (Shimadzu SCL-10A). $M_w = 4000$, $M_w/M_n = 1.54$ based on a polystyrene standard (Polymer Laboratories, Inc.) (1060 M_w, 20.8 min; 2970 M_w, 19.0 min; 10,680 M_w, 16.5 min). *Y*-axis indicates relative absorbance at 262 nm. (B) ^{1}H NMR spectrum of PPADK in $CDCl_3$ (Varian Mercury Vx 400); repeating unit peaks at 7.3 ppm (4b), 4.5 ppm (4c), and 1.5 ppm (6a). Peaks at 2.5 and 1.0 are due to triethylamine added to prevent ketal hydrolysis.

(Figure 1B) confirms that the repeating unit of PPADK contains a ketal group ('6a'). Together, the GPC and ^{1}H NMR data provide evidence for the successful synthesis of PPADK**.**

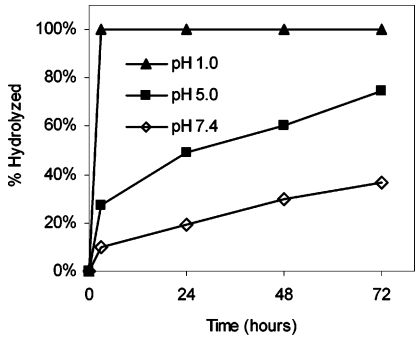


Figure 2. Hydrolysis kinetics of PPADK (finely ground powder) at pH 1.0, 5.0, and 7.4 (37 °C). Exponential decay half-lives are 102 h (pH 7.4) and 35 h (pH 5.0). The pH 1.0 control batch was completely hydrolyzed before the first time point.

The hydrolysis kinetics of PPADK was measured at pH values corresponding to lysosomes (pH 5.0) and the bloodstream (pH 7.4). The hydrolysis rates were measured by grinding PPADK into a fine powder and adding it to deuterated solutions at pH 7.4 (phosphate buffer), pH 5.0 (acetate buffer), and pH 1.0 (DCl).[2] Exponential decay half-lives were calculated to be 102 h at pH 7.4 and 35 h at pH 5.0, representing a 3-fold rate increase from pH 7.4 to 5.0 (Figure 2). The pH sensitivity of PPADK is significantly less than the 250-fold increase from pH 7.4 to 5.0 reported by Kwon et al. (*14*) for a water-soluble ketal. We hypothesize that the lower pH sensitivity of PPADK is due to its water insolubility, which limits the diffusion of water and creates another rate-limiting step that is pH-independent.

[2] The suspensions were stirred at 37 °C, and data points were taken at 3, 24, 48, and 72 h. Each suspension was centrifuged for 4 min at 1800*g*, and the supernatant was analyzed by ^{1}H NMR. The spectra contained peaks for 1,4-benzenedimethanol (7.24 and 4.47 ppm) and acetone (2.05 ppm). The average of the two 1,4-benzenedimethanol peak integrals was used to determine the relative degree of hydrolysis. The percent hydrolysis was calculated as the 1,4-benzenedimethanol peak average of the pH 7.4 or 5.0 sample divided by the 1,4-benzenedimethanol peak average of the pH 1.0 control batch.

[3] DLS samples were prepared by diluting the particle suspension in 10 mL of pH 9 buffer and allowing the larger particles to settle out. An aliquot from the liquid portion of each vial was then diluted for DLS particle sizing (Brookhaven 90Plus particle sizer). An SEM sample was made with the 0.2:1 ratio of PVA: PPADK by centrifuging the nanoparticle suspension for 10 min (5000*g*, 4 °C), washing with distilled water, and lyophilizing the recovered pellet.

[4] Control batches were prepared with PPADK/PVA only and Dex only. To measure Dex encapsulation, each particle batch was resuspended in pH 9 buffer, and an aliquot was then further diluted. A portion was filtered through a 0.1 μm Supor membrane Acrodisc syringe filter (Pall Corp.), and the 242 nm absorbance of the filtrate was recorded with a Shimadzu UV-1700 spectrophotometer. The encapsulation efficiency was calculated as $(A_{Dex} - A_{DexPoly})/(A_{Dex} - A_{Poly})$, where A is the absorbance at 242 nm and the subscripts 'Poly', 'Dex', and 'DexPoly' refer to the 'PPADK only', 'Dex only', and 'Dex + PPADK' samples, respectively. These calculations resulted in a Dex encapsulation efficiency of 43% to 53% for various samples.

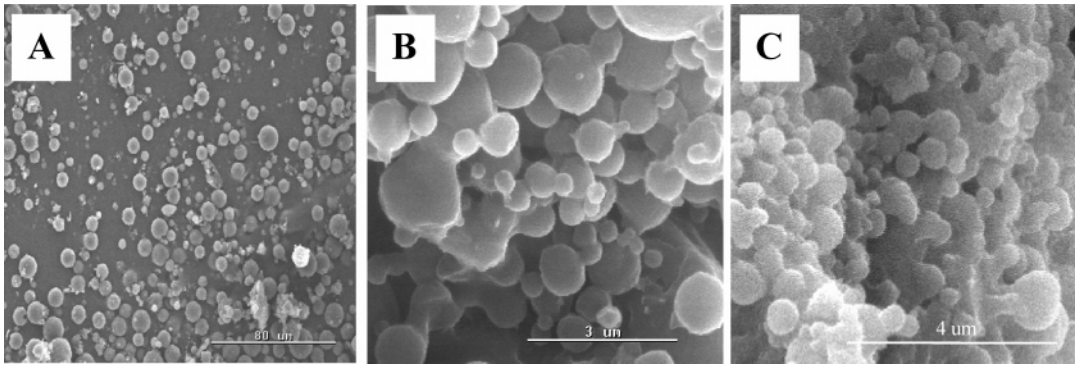


Figure 3. SEM images of particles made with PPADK. (A,B) Microparticles using 0.2:1 ratio of PVA to PPADK. (C) Dexamethasone-loaded nanoparticles made using 1:1 PVA:PPADK. Scale bars are (A) 80 μm, (B) 3 μm, and (C) 4 μm. SEM images were generated with a Hitachi S4100 by Dr. Lisa Detter-Hoskin of the Georgia Tech Research Institute, Atlanta, GA.

Micro- and nanoparticles were synthesized with PPADK using an oil-in-water emulsion method (*15*). Briefly, 50 mg of PPADK dissolved in 1 mL of $CHCl_3$ (with 0.1% triethylamine) was added to 5 mL of pH 9 buffer (10 mM $NaHCO_3$) containing various amounts of poly(vinyl alcohol) (PVA, 31−50 kDa, Aldrich) as the emulsifier. The oil−water mixture was shaken briefly and then sonicated for 2 to 3 min at 40 W (Branson Sonifier 250) to form a fine oil/water emulsion. The emulsion was stirred under N_2 flow for at least 3 h to evaporate the solvent and produce a nanoparticle suspension.

Particle sizes were analyzed by dynamic light scattering (DLS) and SEM.[3] As expected, the particle size was sensitive to the ratio of PVA to PPADK. The DLS particle diameters were 520, 290, and 280 nm for samples containing 0.2:1, 0.8:1, and 2:1 mass ratios of PVA:PPADK, respectively. The SEM images of the 0.2:1 batch (Figures 3A and 3B) confirm that PPADK does form micron-sized particles, with particle size distribution ranging from 0.5 to 30 μm in diameter.

The antiinflammatory drug dexamethasone (Dex, Sigma) was encapsulated into nanoparticles made with PPADK. Dex-loaded particles were formulated using the same procedure as that described above, except that the oil phase contained a 5 mg/mL concentration of Dex and the mass ratio of PVA:PPADK was 1:1. SEM images of these particles demonstrate that they are 200−600 nm in diameter (Figure 3C). Particle sizing by DLS indicated an effective diameter of 250 nm for the Dex-loaded particle batches. The Dex encapsulation efficiency ranged between 43 and 53%.[4]

In summary, we have developed a new pH-sensitive biodegradable polymer for drug delivery, synthesized via the acetal exchange reaction between 2,2-dimethoxypropane and 1,4-benzenedimethanol. This polymer is unique in that it contains ketal linkages in the polymer backbone, allowing for acid-catalyzed hydrolysis into low molecular weight, water soluble compounds. Dexamethasone-loaded nanoparticles were fabricated in the 200 to 600 nm range, which is suitable for applications involving phagocytosis by macrophages. The relative ease of polymer synthesis and particle formation, as well as its rapid pH-sensitive degradation into low molecular weight excretable compounds, suggest that this new polyketal will find numerous applications in the field of drug delivery.

ACKNOWLEDGMENT

M.J.H. is supported by a National Science Foundation Graduate Research Fellowship. We acknowledge the Georgia Tech/Emory Center for the Engineering of Living Tissues (funded by NSF-EEC-9731643) and NIH UO1 HL80711-01.

LITERATURE CITED

(1) Anderson, J. M., and Shive, M. S. (1997) Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv. Drug Delivery Rev. 28,* 5−24.
(2) Jain, R. A. (2000) The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices. *Biomaterials 21*, 2475−2490.
(3) Mathiowitz, E., Saltzman, W. M., Domb, A., Dor, Ph., and Langer, R. (1988) Polyanhydride microspheres as drug carriers. II. Microencapsulation by solvent removal. *J. Appl. Polym. Sci. 35*, 755−774.
(4) Berkland, C., Kipper, M. J., Narasimhan, B., Kim, K., and Pack, D. W. (2004) Microsphere size, precipitation kinetics and drug distribution control drug release from biodegradable polyanhydride microspheres. *J. Controlled Release 94*, 129−141.
(5) Stubbs, M., McSheehy, P. M. J., Griffiths, J. R., and Bashford, C. L. (2000) Causes and consequences of tumour acidity and implications for treatment. *Mol. Med. Today 6*, 15−19.
(6) Leroux, J.-C. (2004) pH-responsive carriers for enhancing the cytoplasmic delivery of macromolecular drugs. *Adv. Drug Delivery Rev. 56*, 925−926.
(7) Heller, J., and Barr, J. (2004) Poly(ortho esters) − From concept to reality. *Biomacromolecules 5*, 1625−1632.
(8) Heller, J., Barr, J., Ng, S. Y., Abdellauoi, K. S., and Gurny, R. (2002) Poly(ortho esters): synthesis, characterization, properties and uses. *Adv. Drug Delivery Rev. 54*, 1015−1039.
(9) Berry, D., Lynn, D. M., Sasisekharan, R., and Langer, R. (2004) Poly(β-amino ester)s promote cellular uptake of heparin and cancer cell death. *Chem. Biol. 11*, 487−498.
(10) Potineni, A., Lynn, D. M., Langer, R., and Amiji, M. M. (2003) Poly(ethylene oxide)-modified poly(β-amino ester) nanoparticles as a pH-sensitive biodegradable system for paclitaxel delivery. *J. Controlled Release 86*, 223−234.
(11) Fu, K., Pack, D. W., Klibanov, A. M., and Langer, R. (2000) Visual evidence of acidic environment within degrading poly(lactic-*co*-glycolic acid) (PLGA) microspheres. *Pharm. Res. 17*, 100−106.
(12) Lorette, N. B., and Howard, W. L. (1960) Preparation of ketals from 2,2-dimethoxypropane. *J. Org. Chem. 25*, 521−525.
(13) Greene, T. W., and Wuts, P. G. M. (1999) *Protective Groups in Organic Synthesis*, pp 201−230, Wiley-Interscience, New York.
(14) Kwon, Y. J., Standley, S. M., Goodwin, A. P., Gillies, E. R., and Fréchet, J. M. J. (2005) Directed antigen presentation using polymeric microparticulate carriers degradable at lysosomal pH for controlled immune responses. *Mol. Pharm. 2*, 83−91.
(15) Panyam, J., Williams, D., Dash, A., Leslie-Pelecky, D., and Labhasetwar, V. (2004) Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. *J. Pharm. Sci.* 93, 1804−1814.

BC050176W