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QUANTITATIVE ANALYSIS OF THE MONOMER PRODUCTS FORMED ON THE HYDROLYSIS OF POLY(ESTERS) AND POLY(ANHYDRIDES)

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BACKGROUND

Biodegradable polymers have a variety of potential biomedical applications in addition to their current use as suture materials [1]. Among the most important of these potential applications is their use in the controlled release of therapeutic agents [2]. The biodegradable polymers used in controlled release applications are primarily insoluble polymers which undergo chemical hydrolysis to soluble monomer or oligomer units [2]. These polymers have both advantages and disadvantages when compared to non-degradable polymers used for controlled delivery. The major advantage is that biodegradable polymers do not require removal after they have delivered their dose. In addition, adverse tissue reactions from implanted polymer are ameliorated as the polymer degrades [3]. However, because these polymers degrade with time, their removal is often difficult, as when a change in therapy is required before an entire dose is delivered. Also, because of their degradation, toxicology must be performed on both the polymer and all its decomposition products as well as their metabolites. The future utility of biodegradable for controlled delivery applications depend on developing accurate methods for the measurement of their biodegradation products. Ideally, these methods should be useful in measuring concentrations of each soluble monomeric and oligomeric product and should be sensitive while tolerating interfering substances.

This research focuses on two classes of biodegradable polymers, poly(esters) and poly(anhydrides) as potential carriers of recombinant proteins for vaccine and drug use. Poly(esters) used medically as suture material [1] were the first type synthetic biodegradable polymer used in a drug delivery application, the release of narcotic antagonists from poly(lactate) [4]. Poly(anhydrides) were developed by the textile industry because of their fiber-forming properties but interest waned when it was determined that their hydrolytic lability prevented their application for such purposes. Initial experiments demonstrated the usefulness of these polymers in the controlled release applications [5] and further work has resulted in the successful application of poly(anhydrides) in preliminary clinical trials examining the delivery of an anticancer agent within the brain [6]. The results presented in this manuscript examine various methods to measure the biodegradation products formed during the hydrolysis of poly(esters) and poly(anhydrides).

MATERIALS AND METHODS

Poly(anhydride), purified bis(p-carboxyphenoxy) propane sebacic acid copolymer (PCPP-SA 20:80) of Mw 30,000 [7] was a gift from Drs. R. Langer and E. Ron of the Department of Chemical Engineering, MIT. Poly(esters) including poly(L-lactate) Mw 100,000, poly(D,L-lactate-co-glycolate, 80:20), poly(3(-)hydroxybutyrate) MW 50,000, and poly(3-)hydroxybutyrate-co-hydroxyvalerate, 80:20) were from Polysciences Inc.

Polymers were used either as powders or were spray-dried from a 1-10(w/v)% solution in methylene chloride at 25°C using a Yamoto Pulvis Min-Spray GA-32 spray-drier. The particles formed by spray drying were measured by scanning electron microscopy.

A 100 mg sample of poly(anhydride) or poly(ester) sample was suspended with stirring in 50 mL of distilled water and adjusted to pH 7.0 and 60°C. Titrant (0.1M sodium hydroxide) was added in quantities sufficient to maintain the pH at 7.0 using a 665 Dosimat (Metrohm) titrator. Both time and volume of titrant was recorded.

A 100 mg sample of poly(anhydride) was suspended with stirring in 50 mL of phosphate buffered saline (150 mM sodium phosphate at pH 7.2 containing 0.9(w/v)% sodium chloride, pbs) at 60°C. Periodically aliquots were removed, filtered of particles and the absorbance of the solution was measured at 250 nm.

A 100 mg sample of poly(anhydride) or poly(ester) was suspended with stirring in 50 mL of pbs at 85°C. Periodically aliquots were removed, filtered of particles and analyzed using a Dionex QIC ion-chromatography system on a HPICE-AS1 column eluted with 2mM octane sulfonic acid in 2(w/v)% 2-propanol at a flow rate of 0.75 mL/min at 25°C. A post column AMMS-ICE membrane supressor run with 10mM tetra-n-butylammonium hydroxide at a flow rate of 1 mL/min was used prior to ion detection by conductivity at 100 uS full scale.

RESULTS AND DISCUSSION

The hydrolysis of a powdered sample of insoluble poly(anhydride) was measured using absorbance, titrametric and ion-chromatography/conductivity methods. The hydrolysis rate of PCCP-SA 20:80 has been previously determined by measuring absorbance at 250 nm as 160 ugcm^{-2}h^{-1} in pbs at 37°C [7]. The hydrolysis rate of this polymer was sufficiently fast at 60°C to obtain an initial rate of 0.1 mmol/h over the course of several hours. This method of determining the hydrolysis rate measures the formation of soluble absorbance in the form of PCCP monomer or oligomer containing PCCP. Since SA has little measurable absorbance at 250 nm this hydrolysis product as well as soluble oligomers solely containing SA remain undetected by this method. Titrametric analysis was examined in an effort to develop a method capable of measuring all the products formed from hydrolysis of this poly(anhydride). Titrimetric determination measures the moles of both soluble and insoluble acid formed during the hydrolysis of the polymer's anhydride backbone. Using this method of measurement, an initial hydrolysis rate of 0.066 mmol/h was determined over the course of several hours. Although they measure different hydrolysis products, both absorbance and titrimetric methods fail to distinguish between individual products, giving instead the concentration of an aggregate of products (ie, absorbance measures all UV active products). The examination of poly(esters) by these methods failed to give the desired hydrolysis rate data. Even poly(esters) with a 6 micron average particle size failed to hydrolyze at sufficient rates at 60°C to give measurable rates by titration over a period of hours. The failure of aliphatic hydroxyacids to have sufficient UV absorbance prevented the measurement of hydrolysis rate by absorbance. Ion-chromatography using conductance detection has been applied to the determination of organic acids, diacids, and hydroxyacids [8]. When used in conjunction with a supressor column this method has been demonstrated to have high sensitivity. The coupling of a high resolution separation step to this high sensitivity detection method should permit the determination of the concentration of a wide variety of soluble hydrolysis products including both monomers and oligomers. The expected monomer products of poly(ester) and poly(anhydride) hydrolysis could be separated and detected by this method. Initial studies on the poly(anhydrides) and poly(esters) have demonstrated that it is possible to determine the rate of formation of monomers and various as yet unidentified oligomers. Work is in progress to identify the structure of these soluble oligomeric products and to construct a quantitative model of product formation during both poly(anhydride) and poly (ester) hydrolysis.

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REFERENCES

1. Anon. Chem. Ind., 905 (1970).
2. Linhardt, R.J., Biodegradable Polymers for the Controlled Release of Drugs in Perspectives in Controlled Release, M. Rosoff, ed., Verlag Chemie, Critical Review Series, Chapter 2, (1989).

3. Yoles, S. and Sartori, M.F., Drug Delivery Systems, 84 (1980).

4. Yoles, S., Eldridge, J.E., and Woodland, J.H.R., Polymer News, 1, 9 (1971).

5. Rosen, H.B., Chang, J., Wnek, G.E., Linhardt, R.J., and Langer, R., Biomaterials 4, 131 (1983).

6. Chasin, M., Lewis, D., and Langer, R., BioPharm Manufacturing, 1, 33 (1988).

7. Leong, K., Simonte, V., and Langer, R. Macromolecules, 20, 705 (1987).

8. Rocklin, R.D., Slingsby, R.W., and Pohl, C.A., J. Liq. Chromat., 9, 757 (1986).