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A novel method of studying polymer biodegradation

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A novel combination of laser light scattering (LLS) and the micronization of a water-insoluble polymer into narrowly distributed nanoparticles stable in water has provided not only an accurate, reliable and microscopic method to study polymer biodegradation, but also a novel and fast way to evaluate the biodegradability of a given polymer. Using poly(ε-caprolactone) (PCL) as a typical example, we have shown that its biodegradation time can be shortened by a factor of more than 10^3 times in comparison with the time required to biodegrade a thin film ($10 \times 10 \times 0.1$ mm^3). Moreover, the biodegradation kinetics can be *in-situ* monitored in terms of the decrease of the time-average scattering intensity and the particle number. A comparison of static and dynamic LLS results revealed that the enzyme, Lipase Pseudomonas, "eats" the PCL nanoparticles in an one-by-one manner and the enzymatic biodegradation of PCL follows a zero-order kinetics. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: PCL nanoparticles; enzymatic biodegradation; laser light scattering)

Introduction

Some biocompatible, biodegradable and non-toxic synthetic aliphatic polyesters, such as poly(ε-caprolactone), polylactide and poly(glycolic acid), are completely biodegradable inside body after its interaction with body fluid, enzyme and cells and the resultant low molar mass molecules in the biodegradation can be absorbed by body or/and removed by metabolism[1], so that they are very useful in biomedical applications. The biodegradability and stability of these polyesters have been extensively studied[2–10]. Most of the methods used, such as the total weight loss and oxygen consumption, are conventional and time-consuming, only leading to macroscopic and rough results.

For biomedical applications, both *in vivo* and *in vitro* biodegradation studies are important. Special research interests have recently been paid to the *in vitro* enzymatic biodegradation[11–13]. Chemistry of the enzymatic biodegradation of polyester primarily involves the hydrolysis of the polymer chain backbone, much depending on both microscopic and macroscopic properties, such as chemical structure, molar mass, morphology, size and shape of a given sample. Our previous study already revealed that Lipase Psuedomonas (PS) was able to accelerate the biodegradation of poly(ε-caprolactone) (PCL)[14]. It has also been indicated that the enzymatic biodegradation happens mainly on the surface because it is difficult for a hydrophilic enzyme to diffuse into a hydrophobic polymer[15].

Recently, we developed several novel methods to prepare polymer nanoparticles[16,17]. It is known that the micronization of a polymer sample can greatly increase its total surface area. Therefore, a combination of the micronization and enzymatic biodegradation can provide a quick method to study polymer biodegradation. On the other hand, laser light scattering (LLS), as a nonintrusive, sensitive and widely used analytical method in the characterization of polymers and colloids in solution[18–20], will be most suitable for *in-situ* monitoring the enzymatic biodegradation of polymer nanoparticles in terms of the changes of the scattering intensity and the hydrodynamic size distribution of polymer nanoparticles.

Figure 1 shows the biodegradation time dependence of $[R_{VV}(q)]/[R_{VV}(q)]_0$ of the PCL nanoparticles in aqueous solution at 25°C, where $[R_{VV}(q)]_0$ represents the initial Rayleigh ratio before the enzymatic biodegradation and the enzyme/polymer ratio is 0.86. In static LLS, the Rayleigh ratio $R_{VV}(q)$ is proportional to the excess absolute time-average scattered intensity which is approximately proportional to both the concentration (C) and the weight-average molar mass M_W of the nanoparticles (not the polymer chains) for a very dilute solution at a very small scattering angle (θ)[21]. Therefore,

$$R_{VV}(q) \approx KCM_W \tag{1}$$

where K is a constant for a given polymer solution. Thus, $[R_{VV}(q)]/[R_{VV}(q)]_0 = [CM_W]/[CM_W]_0$ and the decrease of $[R_{VV}(q)]/[R_{VV}(q)]_0$ could be related to the decrease of C or/and M_W where the subscript "0" represents the initial state before the biodegradation.

The insert in *Figure 1* shows two normalized hydrodynamic radius distributions before and after the biodegradation, where each $f(R_h)$ was calculated from the corresponding intensity–intensity time correlation function $G^{(2)}(q,t)$ measured in dynamic LLS. According to dynamic LLS theory[20,22], $G^{(2)}(t,q)$ is related the normalized electric field ($E(t)$) time correlation function $g^{(1)}(t,q)$. For a polydisperse sample, $g^{(1)}(t,q)$ is related to the line-width distribution $G(\Gamma)$ by[22]

$$g^{(1)}(t,q) = \langle E(t)E^*(0)\rangle = \int G(\Gamma)\exp(-\Gamma t)\,d\Gamma \tag{2}$$

$G(\Gamma)$ can be calculated from the Laplace inversion of $G^{(2)}(t,q)$. For a pure diffusive relaxation, Γ is related to

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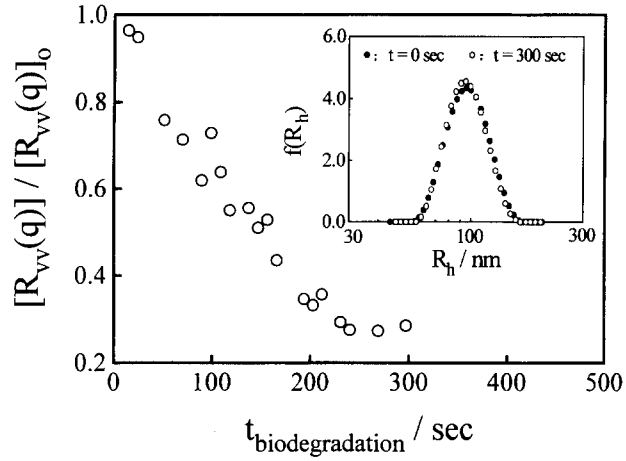

Figure 1 Typical biodegradation time dependence of the relative Rayleigh ratio $[R_{VV}(q)]/[R_{VV}(q)]_0$ of the PCL nanoparticles in water, where $[R_{VV}(q)]_0$ is the initial Rayleigh ratio before the biodegradation, $\theta = 15°$ and $T = 25°C$, $C_{0,PCL} = 6.25 \times 10^{-6}$ g mL^{-1} and $C_{0,LipasePS} = 5.39 \times 10^{-6}$ g mL^{-1}. The insert shows the corresponding hydrodynamic radius distributions $f(R_h)$ of the PCL nanoparticles before and after the enzymatic biodegradation

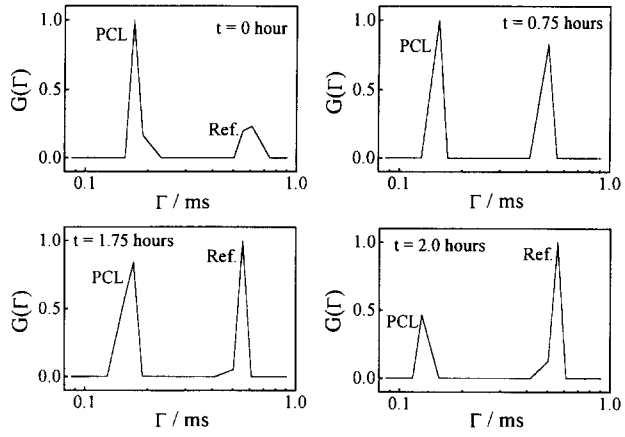

Figure 2 Biodegradation time dependence of the hydrodynamic radius distribution $f(R_h)$ of the PCL nanopartieles, where the initial concentrations of PCL and Lipase PS were 3.13×10^{-6} g mL^{-1} and 2.69×10^{-8} g mL^{-1}, respectively

the translational diffusion coefficient D by $\Gamma/q^2 = D$ at $C \rightarrow 0$ and $q \rightarrow 0$. In this case, $G(\Gamma)$ can be directly converted to a translational diffusion coefficient distribution $G(D)$ or a hydrodynamic radius distribution $f(R_h)$ by using the Stocks–Einstein equation: $R_h = k_B T/(6\pi\eta D)$ with k_B, T and η being the Boltzman constant, the absolute temperature and the solvent viscosity, respectively.

The insert shows that there is no change in $f(R_h)$, i.e. no change in the molar mass distribution of the nanoparticles. It should be noted that in LLS only those remaining PCL nanoparticles can be "seen" because they scatter much, much more light than those small molecules, such as $HO(CH_2)_5COOH$ and $HO(CH_2)_4COOH$, resulted from the biodegradation. Therefore, the decrease of $[R_{VV}(q)]/[R_{VV}(q)]_0$ in *Figure 1* actually reflects the decrease of the number of the nanoparticles due to the biodegradation, or more precisely, the decrease of the relative concentration (C/C_0). This concentration decrease can be more clearly viewed in *Figure 2* from the relative decrease of the peak area of $G(\Gamma)$ related to the PCL nanoparticies, where the reference peak was from the added inert poly(*N*-isopropylacrylamide) spherical microgel particles and the peak area

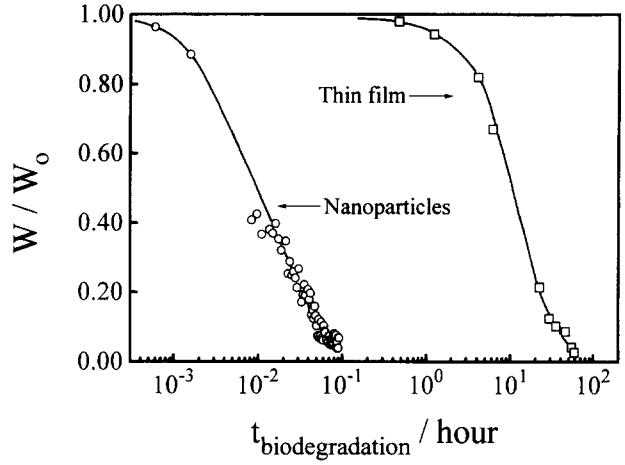

Figure 3 Comparison of the weight loss of the PCL nanoparticles (~100 nm in diameter) and a thin PCL film ($10 \times 10 \times 0.1$ mm^3) in the biodegradation, where $T = 37°C$ and the initial concentrations of PCL and Lipase PS were 6.67×10^{-4} g mL^{-1} and 5.00×10^{-4} g mL^{-1}, respectively

in $G(\Gamma)$ is directly proportional to the scattering intensity[23]. It should be noted that in *Figure 2* the enzyme/polymer ratio has been greatly reduced to slow down the biodegradation so that it can be followed in dynamic LLS.

It should be noted that for a given solution (C/C_0) equals the relative weight (W/W_0), a more commonly used parameter in the field of biodegradation. The line in *Figure 1* represents a least square fitting of $[R_{VV}(q)]/[R_{VV}(q)]_0 = 1 - 3.05 \times 10^{-3} t_{biodegradation}$, revealing that the enzymatic biodegradation follows a zero-order kinetics. *Figure 3* shows that for the same enzyme/polymer ratio the decrease of W/W_0 (the weight loss) of the PCL nanoparticles is more than $\sim 10^3$ times faster than that of a thin PCL film, where the results of the nanoparticles and the thin film were obtained by laser light scattering and a conventional weighting method, respectively, and the thin film was casted from solution. Besides possible difference in crystallinity, the difference in the biodegradation rate can be mainly attributed to the huge surface area of the nanoparticles. Therefore, a combination of LLS and micronization has provided not only an accurate and reliable microscopic method to study the enzymatic biodegradation kinetics, but also a novel and fast way to evaluate the biodegradability of a given polymer.

Methods

Materials. Poly(ε-caprolactone) (PCL) was synthesized by ring-opening polymerization of ε-caprolactone using yttrium trifluoroacetate and triisobutylaluminum $Y(CF_3COO)_3/Al(i\text{-}Bu)_3$ as catalyst. The average molar mass of PCL used in this study is 1.43×10^5 g mol^{-1}. Hexadecyltrimethylammonium bromide from Eastman Kodak was used as stabilizers in the micronization of PCL without further purification. Lipase PS from *Pseudomonas cepacia* (Courtesy of Amano Pharmaceutical Co. Ltd, Nagoya, Japan) was further purified by freeze-drying.

Micronization of PCL. The PCL nanoparticles were prepared by adding dropwise a dilute PCL acetone solution (2.5×10^{-3} g mL^{-1}) into a large amount of aqueous solution containing HTAB. The acetone and aqueous solutions were constantly mixed by a magnetic stirring during the addition process. When the PCL acetone solution was

added into the aqueous solution, acetone quickly diffused into the water phase so that the hydrophobic PCL chains started to aggregate with each other in water to form nanoparticles which were stabilized by HTAB absorbed on the particle surface. Finally, acetone together with a portion of water were removed under a reduced pressure until the mixture reached a desired concentration.

Laser light scattering (lls). A modified commercial LLS spectrometer (ALV/SP-125) equipped with an ALV-5000 multi-τ digital time correlator and a solid state laser (ADLAS DPY42511, out power is approximately 400 mV at $\lambda = 532$ nm) was used. This spectrometer is capable of measuring both static and dynamic LLS continuously from 6° to 154°. The accessible small angle range is particularly useful for the study of large particles. The PCL nanoparticle suspension and the Lipase PS aqueous solution used in LLS were respectively clarified by 0.8 and 0.5 μm Millipore filters. In a typical enzymatic biodegradation experiment, a proper amount of the dust-free Lipase PS aqueous solution was *in situ* added into 2 mL of the dust-free PCL nanoparticle suspension. Both $R_{VV}(q)$ and $G^{(2)}(t,q)$ were simultaneously measured in the course of the enzymatic biodegradation. All the biodegradation experiments were *in situ* conducted inside the LLS cuvette at $T = 25$°C and $\theta = 15$°, except otherwise stated.

Acknowledgements

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