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Ultrasound-enhanced polymer degradation and release of incorporated substances

(controlled release/drug delivery systems)

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ABSTRACT **The effect of ultrasound on the degradation of polymers and the release rate of incorporated molecules within those polymers was examined. Up to 5-fold reversible increases in degradation rate and up to 20-fold reversible increases in release rate of incorporated molecules were observed with biodegradable polyanhydrides, polyglycolides, and polylactides. Up to 10-fold reversible increases in release rate of incorporated molecules within nonerodible ethylene/vinyl acetate copolymer were also observed. The release rate increased in proportion to the intensity of ultrasound. Temperature and mixing were relatively unimportant in effecting enhanced polymer degradation, whereas cavitation appeared to play a significant role. Increased release rates were also observed when ultrasound was applied to biodegradable polymers implanted in rats. Histological examination revealed no differences between normal rat skin and rat skin that had been exposed to ultrasonic radiation for 1 hr. With further study, ultrasound may prove useful as a way of externally regulating release rates from polymers in a variety of situations where on-demand release is required.**

We report here a way to enhance the degradation of solid polymers and the transport of incorporated substances within polymers. This method involves the exposure of the solid polymer to ultrasound. We also report experiments conducted to elucidate the mechanism of this phenomenon and to explore the therapeutic potential of this method for delivering incorporated substances *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials. All chemicals were reagent grade. *p*-Nitroaniline (PNA) and *p*-aminohippuric acid (PAH) were from Aldrich. Methoxyflurane was from Pitman–Moore (Washington Crossing, NJ). The aquasonic gel used for skin application was therasonic coupling medium from EM Science (Cherry Hill, NJ). Poly(lactic acid) and poly(glycolic acid) were from Polysciences (nos. 6529 and 6525, respectively). Polyanhydrides were synthesized as described (1). Ethylene/vinyl acetate copolymer (EVAc) was obtained from DuPont (Elvax 40P) and washed with ethanol and water prior to incorporation of substances within the polymer (2). A temperature probe (747 digital thermister thermometer) was from Omega Engineering (Stamford, CT). For shaking experiments a 250-rpm Junior Orbit shaker (Lab-Line Instruments) was used. An RAI Research (Hauppauge, Long Island, NY) model 250 ultrasonic bath was used for *in vitro* release studies (75 kHz). For *in vivo* experiments, a Vibra Cell 250 (20 kHz; Sonics and Materials, Danbury, CT) was used. The animal hair clipper was from Oster (model A2) (Milwaukee).

***In Vitro* Experiments.** The polylactides and polyglycolides were film-cast at room temperature with PNA in chloroform and 1,1,1,3,3,3-hexafluoro-2-propanol, respectively. The PNA-loaded films were then ground, sieved (90–150 μm), and compression-molded into circular disks in a Carver test cylinder (Menomonee Fall, WI) at 30,000 psi (1 psi = 6.89 kPa) and room temperature for 10 min (3). The polyanhydrides, ground and sieved into a particle size range of 90–150 μm, were mixed manually with PNA sieved to the same size range. The mixture was compression-molded into circular disks (14 mm in diameter, 1 mm thick) in a Carver test cylinder at 30,000 psi and 100°C, for poly[bis(*p*-carboxyphenoxy)methane] (PCPM), and room temperature, for copolymers of bis(*p*-carboxyphenoxy)propane with sebacic acid (PCPP/SA). In all cases, the polymer disks were loaded with 10% (wt/wt) PNA. The polymer erosion and drug release kinetics were followed by measuring the UV absorbance of the periodically changed buffer solutions in a Perkin–Elmer 553 spectrophotometer. The optical densities at 381 nm (absorption maximum for PNA) and 250 nm (for degradation products) were measured to determine the respective concentrations.

Bovine serum albumin (BSA) was incorporated (30%, wt/wt) into EVAc by dissolving the polymer in methylene chloride, adding powdered BSA to the solution, and film-casting the mixture at −80°C as described (4). The temperature increase of the specimens while exposed to ultrasound was recorded by placing a temperature probe on the surface of the specimen. To determine the effect of temperature on release rates, the samples were placed in jacketed vials filled with 10 ml of 0.1 M phosphate buffer (pH 7.4); the samples were then exposed to alternating periods at 37°C and 41.3°C. For degassing experiments, degassing of the buffer was achieved by boiling it for 15 min under vacuum.

***In Vivo* Experiments.** Rats (Sprague–Dawley, 200–250 g) were anesthetized with methoxyflurane. Their abdominal and cervical fur was shaved with an electric animal hair clipper and the shaved area was cleaned with Betadine solution. In the cervical region a 1- to 2-cm incision was made in the skin with a no. 10 scalpel blade. A pair of round-edged scissors was inserted subcutaneously through the incision in the closed position and opened to form a pocket. The implant (0.05–0.08 g) was then placed (with forceps) in the far end of the pocket, and the wound was closed by 5-0 nylon (Ethicon) suture. For the catheterization a 2-cm midline abdominal incision was made through the abdominal musculature. As the bladder was exposed, a 26-gauge needle (Becton Dickinson) was inserted to withdraw the urine. A catheter (Intra-

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Abbreviations: BSA, bovine serum albumin; EVAc, ethylene/vinyl acetate copolymer; PAH, *p*-aminohippuric acid; PCPM, poly[bis(*p*-carboxyphenoxy)methane]; PCPP/SA, copolymer of bis(*p*-carboxyphenoxy)propane and sebacic acid; PNA, *p*-nitroaniline.

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medic PE-50, 7411) was then inserted through an 18-gauge needle and secured to the bladder by a 4-0 polyglycolic (Dexon) suture. After urine flow was verified, the abdominal musculature as well as the skin incision were sutured with 4-0 Dexon and 5-0 Ethicon, respectively. After implantation the rats were placed in rat restrainers. Urine was collected every 30 min, and the concentration of PAH in the urine was evaluated by HPLC (Hypersil C_{18}, 5 μm, 25-cm column; 1 ml/min flow rate; UV detection at 278 nm). Urine samples were diluted to 2 ml with distilled water and filtered through a 0.22-μm Millipore membrane. The sample injection size was 20 μl. The ultrasound probe was applied to a shaven site above an aquasonic gel, which was applied to the treated area, and the rats were exposed for 20 min to ultrasound (pulsed mode, 50% duty cycle, 5 W/cm^2). For histological evaluation, rats exposed to ultrasound were killed by asphyxiation with carbon dioxide. Sections were fixed in 10% neutral buffered formalin and evaluated by hematoxylin and eosin staining.

RESULTS AND DISCUSSION

Experiments were first performed by subjecting polymer matrices containing incorporated substances to ultrasound *in vitro*. Bioerodible (biodegradable) polymers evaluated included polyglycolides, polylactides, poly[bis(*p*-carboxyphenoxy)alkane anhydrides], and copolymers of these monomeric anhydrides with sebacic acid. Nonerodible EVAc copolymer was also examined.

The pronounced effects of ultrasound on the degradation of PCPM matrices and release rates of incorporated PNA are shown in Fig. 1 *a* and *b*. The polyanhydride matrices were exposed repeatedly to ultrasound. The duration of exposure to ultrasound was 15 min. The intervals between the exposures were 15 min to 1.5 hr. Similar experiments were performed on the other erodible and nonerodible polymers.

Fig. 1*c* displays the rates of PNA release from poly(lactic acid) and poly(glycolic acid) as modulation vs. time. Modulation is defined as the ratio of the release rate during ultrasound exposure to the mean of the rates during the time intervals before and after that exposure, in which the sample was not exposed to ultrasound. PNA was used as a marker because it absorbs light in a region different than the polymer degradation products. The response of release rate increase to the ultrasonic triggering was rapid. On-line analysis showed that the lag time to response was <2 min in turning on the ultrasound and <1 min in turning it off (data not shown). The enhancement increased in proportion to the intensity of the ultrasound (Fig. 2).

While the effect of ultrasound on polymers in solutions has been studied (5), there has been almost no previous examination of the effect of ultrasound on solid polymers. The phenomenon observed may be due to a number of factors that affect diffusion and matrix decomposition, such as temperature, mixing, and cavitation. Experiments were therefore conducted to elucidate the mechanism.

The temperature increase of the specimens while exposed to ultrasound was recorded by placing a temperature probe on the surface of the specimen and found to be <2.5°C (Fig. 3*a*). A separate release experiment done at 41.3°C instead of 37°C, however, showed that the rate increase was <20% (Fig. 3*b*). This suggests that the enhancement cannot be attributed only to temperature. To determine whether ultrasound could affect a diffusion boundary layer, release experiments performed under vigorous mixing were compared to those under stagnant conditions (Fig. 3*c*). The difference (<20%) was insignificant compared to the effect of ultrasound. Again, the effect of eliminating a boundary layer alone cannot be held responsible for a 10- to 20-fold increase in release rate (Fig. 1). (Other experiments, conducted without ultrasound application, showed that the vigorous mixing was in the range where increases in mixing speed do not affect the release rate, suggesting that in this range of mixing the boundary layer is eliminated.)

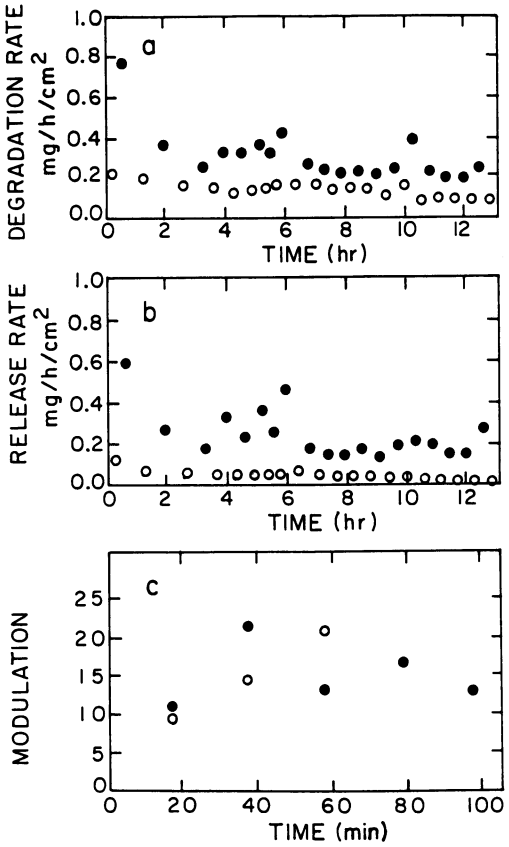

FIG. 1. Effect of ultrasound (75 kHz, 16 W into 500-cm^3 water tank) on *in vitro* polymer degradation and drug release. The repeated ultrasound exposure durations were 15 min for the "on" period (●). The intervals between these exposures in which the samples were not exposed to ultrasound were 15 min to 1.5 hr (○). (*a*) Degradation rate of PCPM vs. time. (*b*) Rate of PNA release from PCPM loaded with 10% PNA vs. time. (*c*) Modulation vs. time of PNA release from poly(lactic acid) loaded with 10% PNA (●) and from poly(glycolic acid) loaded with 10% PNA (○). (Modulation is defined as the ratio of degradation or release rates during ultrasound exposure to the mean of the rates during the time intervals before and after that exposure, in which the sample was not exposed to ultrasound.)

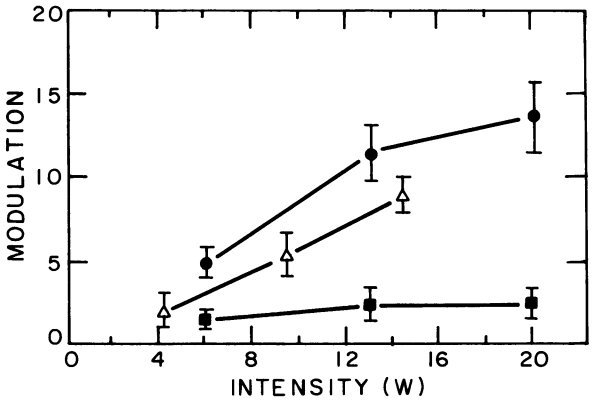

FIG. 2. Modulation vs. ultrasound intensity *in vitro* for PNA release from PCPM (●), PCPM degradation (■), and BSA release from EVAc loaded with 30% BSA (△). Mean and standard deviation of at least seven data points in which the sample was exposed repeatedly to ultrasound are displayed.

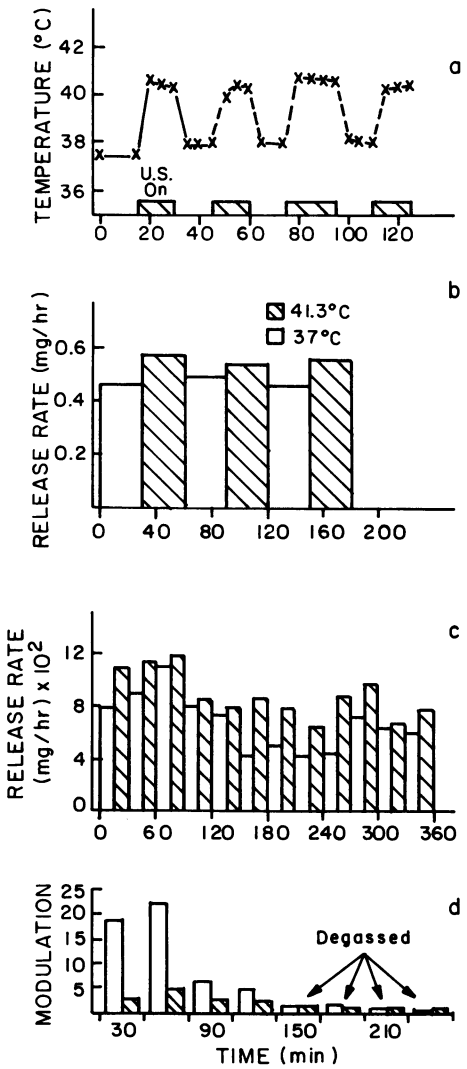

FIG. 3. (*a*) *In vitro* temperature profiles of samples exposed and not exposed to ultrasound (U.S.). (*b*) Rate of BSA release from EVAc copolymer matrices at 37°C and at 41.3°C. (*c*) Effect of shaking (hatched bars), and not shaking (open bars) on rate of BSA release from EVAc samples loaded with 30% BSA. (*d*) Effect of nondegassed and degassed buffer on modulation of PNA release *in vitro* from PCPM matrices loaded with 10% PNA (open bars) and on PCPM degradation (hatched bars).

To evaluate the importance of cavitation (6), experiments were conducted in exhaustively degassed buffer. The enhancement of degradation and release rates in the degassed buffer, where cavitation was minimized, was greatly reduced (Fig. 3*d*). When the samples were transferred back to undegassed buffer, release and degradation rates returned to the high levels observed before the exposure to nondegassed buffer. We conclude therefore that cavitation induced by the ultrasonic waves is a major cause for the enhanced release rates. In solutions of polymers exposed to ultrasound, main-chain rupture is thought to be induced by shock waves created during cavitation, which are assumed to cause a rapid compression with subsequent expansion of the liquid. On a molecular level, this implies a rapid motion of solvent molecules to which the macromolecules embedded in the solvent cannot adjust. Thus, friction is generated that causes strain and eventually bond rupture in the macromolecules (5).

Apart from the action of shock waves, the collapse of cavitation bubbles could create pronounced perturbations in the surrounding liquid. We speculate that such a perturbation might increase the penetration of water species into the polymer, thereby promoting hydrolytic degradation. The enhanced release was also observed in nonerodible polymeric systems exposed to ultrasound (Fig. 2), in which the release is normally diffusion-dependent (7), suggesting that in addition to matrix degradation, ultrasound affects the transport of the dissolved molecules. The effect of ultrasound on transport phenomena can also be seen when release and degradation rates are compared (Fig. 1 *a* and *b*). In general the increase in release rate is more pronounced than the increase in polymer degradation rate, as the release is due to erosion and diffusion. Ultrasound seems to affect both.

To examine the effect of ultrasound on model releasing agents, 20-ml solutions of PNA, insulin, BSA, and PAH were exposed to ultrasound for up to 2 hr at various frequencies (20 kHz, 75 kHz, and 1 MHz, at 10 W). No difference in the chemical integrity of the molecules was detected due to the ultrasound exposure as analyzed by HPLC or UV spectroscopy (data not shown).

Next we examined the ultrasound-induced drug release in rats. The implant used was a matrix of PCPP/SA, 20:80, loaded with 10% (wt/wt) PAH. As a urine function marker, PAH is not metabolized and is excreted unchanged in the urine (8). The PAH-loaded disk was implanted subcutaneously (9) into the upper back of rats whose bladders were catheterized (10) for continuous urine collection. To direct the ultrasonic source to the implant, an ultrasonic unit with an applicator head was used. This probe had been tested under *in vitro* conditions to yield augmented degradation and release rates comparable to those described in Fig. 1. In the *in vivo* experiments an aquasonic gel was placed on top of a shaven implant site and then the ultrasonic applicator was applied. The rats were then exposed for 20 min to ultrasound at 20 kHz in a pulsed mode of 50% duty cycle at 5 W/cm^2.

A pronounced effect of the ultrasound was observed 15–30 min after ultrasound application, where peak PAH concentrations appeared in the urine (Fig. 4). This delay can presumably be attributed to the time for PAH to be released from the polymer matrix, equilibrated with plasma, and removed by the kidney. When control animals were treated by the same procedure, with the power level of the ultrasonic unit at zero, no effect on release rates was detected. In addition, histopathological examination revealed no differences between normal skin and the skin that has been

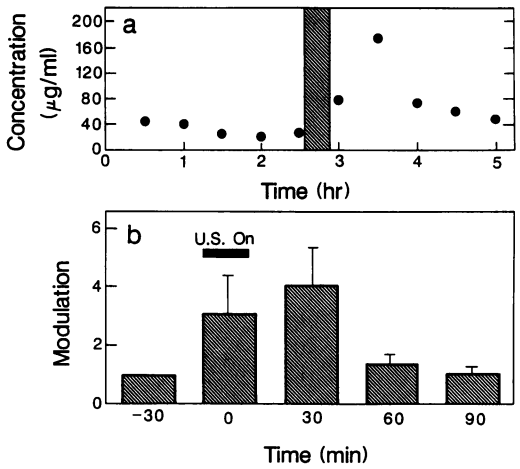

FIG. 4. (*a*) PAH concentration in the urine of Sprague–Dawley rats as a function of time before, during, and after a 20-min exposure to ultrasound (hatched area). (*b*) Modulation vs. time expressed as a mean and standard deviation of four experimental rats. [Modulation was defined as the ratio of PAH concentration during and after the ultrasound (U.S.) exposure to the mean of the PAH concentration before the exposure.] The implants were PCPP/SA copolymers (20:80) loaded with 10% PAH.

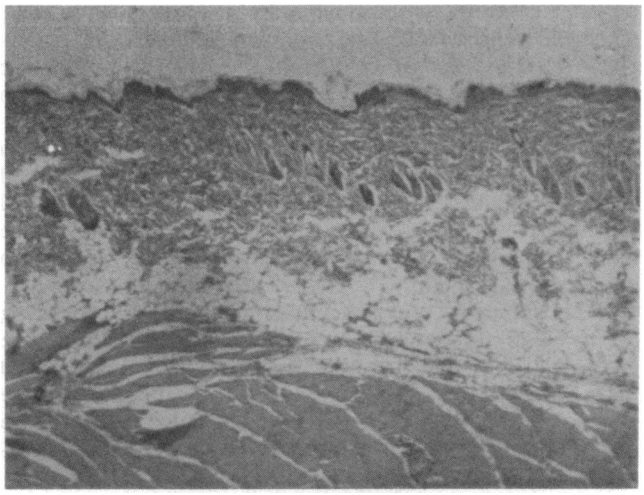

FIG. 5. Hematoxylin- and eosin-stained section (5 mm) of rat's skin exposed for 1 hr to ultrasound (5 W/cm^2). (×75.)

exposed to ultrasonic irradiation as described above for 1 hr (Fig. 5).

While the above experiments suggest the feasibility of ultrasonically augmenting polymer erosion or drug release, considerable future experimentation will be required to enable this to become a practical approach. Among the aims of such research will be to achieve a still greater understanding of the mechanism controlling degradation and release, to study both polymer-related (e.g., composition, molecular weight) and ultrasound-related (e.g., frequency) factors of this phenomenon, and to examine the physiological effects in animals of long-term exposure to ultrasound.

Ultrasonic-responsive delivery systems might be useful in situations where augmented delivery on demand is beneficial (e.g., insulin for diabetes). A number of stimuli including magnetism (11), temperature (12, 13), pH (14), light (15), electricity (16), and specific trigger molecules (17–20) have been shown in experimental systems to provide increased delivery. Each of these stimuli-triggered systems currently requires either a nondegradable polymer or the incorporation of an additional substance (e.g., magnets, enzymes) within a polymer matrix. At present, the only systems in clinical experimentation that can be externally regulated are large implantable pumps that can be controlled by approaches such as telemetry. The ultrasound-based system used in conjunction with biodegradable polymers may, with further study, be an attractive method of externally augmenting drug release rates. The use of a biodegradable polymer-based system minimizes the removal, complexity, and size of the potential implant, because the matrix is bioerodible and has no moving parts or external additives and because the releasing substance is stored in solid form rather than in solution. The polymer-based system contains >100 times more drug per unit volume than a standard "Infusaid" or Siemens pump (21, 22). One might envision that a patient could someday wear a portable triggering device (e.g., like a wristwatch) that could be used to augment release on demand. The possibility of utilizing ultrasound to affect degradation or molecular transport in polymers may also be useful in other areas such as separation science.

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