DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 4, pp. 341–346, 2004

RESEARCH PAPER

Effect of Postmolding Heat Treatment on In Vitro Properties of a Polyanhydride Implant Containing Gentamicin Sulfate

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ABSTRACT

A polyanhydride implant containing gentamicin sulfate was fabricated using a laboratory-scale injection-molding machine. After injection molding, the implants were subject to heat treatment at 60°C for various time periods with or without nitrogen protection. The impact of this heat treatment on the in vitro properties of the implants including copolymer molecular weights, mechanical properties, and in vitro drug-release profiles was investigated. This heat treatment caused a drastic drop in the molecular weight of the copolymer. Heating without nitrogen protection resulted in the hardening of the implant, but heating in the presence of nitrogen rendered the implant less rigid. It was also found that a faster in vitro drug release profile was shown by implants heated without nitrogen protection and a pronounced slowing down in drug release was exhibited by implants heated with nitrogen protection.

Key Words: Poly(EAD:SA 1:1); Gentamicin sulfate; Implant; Injection-molding; Heat treatment; In vitro properties; Copolymer molecular weights; Mechanical properties; Drug-release profiles.

INTRODUCTION

Septacin™ is a biodegradable implant composed of a polyanhydride copolymer and gentamicin sulfate. Septacin was injection molded into cylindrical beads with linkers having the same composition as the beads.[1,2] This product was developed for the treatment of osteomyelitis. When the beads are implanted in a patient, the sustained release of gentamicin can provide a constant therapeutic level of the drug at the

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DOI: 10.1081/DDC-120030928
0363-9045 (Print); 1520-5762 (Online)
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site of implantation for an extended period of time without exposing the patient to high toxic systemic drug levels. Polyanhydrides are degradable in vivo through the hydrolysis of the anhydride bonds,[3–8] therefore, no implant removal is necessary upon completion of treatment.

When Septacin was stored at room temperature, the drug exhibited long-term stability, but the copolymer degraded extensively and the in vitro drug release rate decreased as a function of storage time.[9] This result has led to the possibility that a postmolding heat treatment of the implant at an elevated temperature can be used to produce implants with significantly slower drug release characteristics. In this study, 60°C was used as the accelerated temperature for the postmolding heat treatment of Septacin. The impact of this heat treatment with or without nitrogen protection on the in vitro properties of Septacin was determined.

MATERIALS

The synthesis and characterization of polyanhydrides have been reported.[10–12] Poly(EAD:SA 1:1) is a copolymer of erucic acid dimer (EAD) and sebacic acid (SA) in a one-to-one weight ratio. The two monomers were copolymerized in a molten state using a melt-copolymerization method. In this process, a batch-type planetary mixer was used as a reactor as well as a polymer-drug compounder. The copolymerization took place under vacuum to remove the residual acetic anhydride and acetic acid produced as the reaction by-products. A copolymer lot with a weight-average molecular weight about 50,000 daltons was used in this study. Figure 1 shows the molecular structures of sebacic acid, erucic acid dimer, and poly(EAD:SA 1:1).

Gentamicin sulfate bulk drug was a spray-dried white powder purchased from Lek Pharmaceutical and Chemical Company, Ljubljana, Slovenia.

PREPARATION PROCEDURES AND EQUIPMENT

Preparation of the Copolymer-Drug Mixture

Upon the completion of copolymerization of the polymer in the reactor, dried gentamicin sulfate powder was added and mixed with the molten copolymer for 15 minutes at 125°C. The molten polymer-drug mixture was poured onto a Teflon® coated foil and allowed to resolidify under ambient conditions. The solidified material was manually cut into small pieces, which were later used for injection molding.

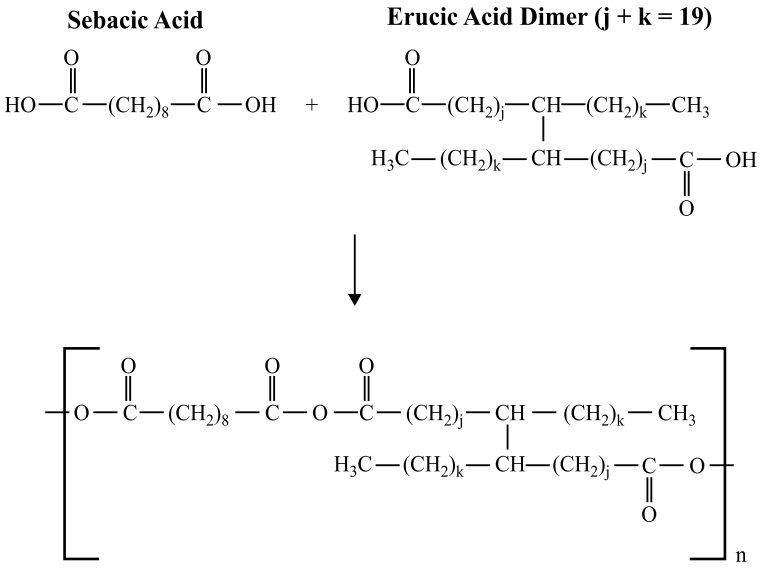

Figure 1. Molecular structures of sebacic acid, erucic acid dimer, and the EAD:SA copolymer.

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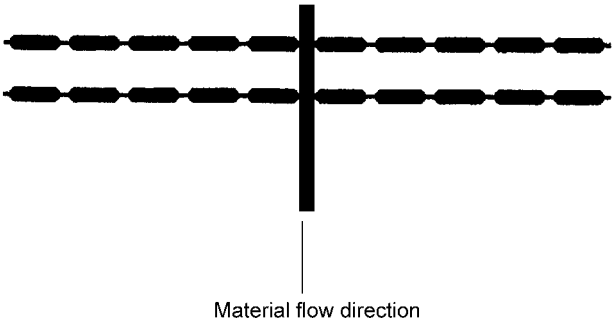

Figure 2. Injection mold configuration.

Injection Molding of Septacin Beads

A laboratory-scale injection-molding machine, Model P20 manufactured by Gluco Inc. (Jenison, MI), was used in this study. It is a plunger-type molder that operates with a predetermined shot size (volume). For each injection-molding cycle, approximately 4.25 g of small pieces of polymer-drug mixture were manually loaded into the heating chamber. The material was melted in the chamber at a controlled temperature for a predetermined time prior to injection. After the molten material was forced into the mold and cooled for a predetermined time, the mold was opened and the product was removed from the cavity. The mold features four identical strands with five beads per strand (Fig. 2).

Heat Treatment of Septacin Beads

Two strands of Septacin beads (a total of 10 beads) were placed into a plastic blister sealed with a Tyvek lid using a heat-seal platen. The blister with the beads was placed into an aluminum foil pouch and flushed with nitrogen prior to final heat-sealing. For samples packaged without nitrogen protection, three beads were placed in a 4-dram scintillation vial under ambient conditions and sealed with the cap. Septacin beads packaged with or without nitrogen flushing were heated at 60°C for 2 weeks in a convection oven.

EVALUATION METHODS

Determination of Molecular Weight

The molecular weight of poly(EAD:SA 1:1) was determined by gel permeation chromatography (GPC) using a Waters Styragel HR-5E column and a Shimadzu RID-6A RI detector. The mobile phase was methylene chloride, the pump rate was set at 1.0 mL/min, and the temperature of the system was maintained at 30°C.

Determination of Stiffness

The effect of heat treatment on the hardness/stiffness of Septacin was investigated using a Dynamic Mechanical Thermal Analyzer (DMTA), Model Mark III, Rheometric Scientific, Piscataway, NJ. Septacin beads were tested using the stress-strain mode of the DMTA under an isothermal condition at 37°C for 2 minutes. This temperature was chosen to reflect the mechanical properties of Septacin at body temperature (37°C). The bead was placed between two parallel discs and was compressed at a constant force rate of 0.043 Newton/sec. The displacement of the compression was recorded as a function of time. The stiffness (N/m) of the bead was calculated as the ratio of the total applied force (5.16 Newton) to the total displacement (ΔH) over the 2-minute test time.

$$\text{Stiffness (N/m)} = [5.16\ (\text{Newton})/\Delta H\ (\mu\text{m})] \times 10^5\ (\mu\text{m/m})$$

The stiffness value is an indicator of the degree of compliancy possessed by a substance. A lower stiffness value means greater compliance and more resistant to stressed related failure of a substance.

In Vitro Dissolution in Water

Three beads were tested for in vitro release of gentamicin in water. Sample bottles containing 100 mL of water were immersed in a shaker water-bath (manufactured by Precision) at 37°C and agitated by reciprocating shaking at 100 rpm. At each time point, a 1-mL sample was drawn from the test bottle, which was then replenished with the same volume of fresh dissolution medium. The assay of gentamicin in the dissolution medium was performed using an high performance liquid chromatography (HPLC) (Model HP 1100) method. The drug release was monitored for 25 days.

Physical Appearance of Bead Remnants

A Navatar zoom macro lens attached to a Sony DXC-970 color video camera purchased from The Fryer Company, Huntley, IL was used to photograph the bead remnants after the in vitro dissolution test.

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Table 1. Copolymer molecular weight and stiffness of Septacin beads with various heat treatments at 60°C.

Sampling treatment	Duration (weeks)	Mw[a] (Dalton)	Stiffness[b] (N/m)
Control	0	48,485	5.87E4 (σ=0.092E4)
Without N_2 flushing	2	7,120	7.00E4 (σ=0.608E4)
With N_2 flushing	2	5,000	3.30E4 (σ=0.283E4)

[a]Each molecular weight result is an average of four values and each value's % difference from the average is no more than 3%.
[b]An average of two measurements with the standard deviation shown in the bracket.

RESULTS AND DISCUSSION

Copolymer Molecular Weight and Mechanical Properties

The results of copolymer molecular weight and stiffness of Septacin beads before and after heating are summarized in Table 1 above.

The copolymer molecular weight of Septacin beads decreased significantly after 2 weeks of heating with or without nitrogen protection. It is noted that beads packaged with nitrogen flushing exhibited a slightly greater reduction in molecular weight compared with those packaged without nitrogen flushing. The stiffness of the beads protected with nitrogen declined significantly after heating, while an increase in stiffness was seen for beads heated without nitrogen protection. The mechanism of the impact of nitrogen flushing on the decrease in copolymer molecular weight and the change in stiffness of the heated implants is not clear. However, the low oxygen and moisture level associated with nitrogen flushing may have contributed to the results shown in Table 1.

In Vitro Drug Release and Physical Integrity in Water

Figure 3 shows the in vitro release profile of gentamicin for unheated Septacin beads and the release profiles for beads heated with or without nitrogen protection. In comparison with the unheated beads, a significantly faster drug release for the first 10 days was shown by the beads heated without nitrogen protection, but a much slower drug release profile with a brief initial burst release was displayed by the beads heated with nitrogen protection. In Fig. 3, the drug release profile of Septacin beads stored at room temperature for 6 months is also included for comparison.[9] It is interesting to note that the 6-month room temperature beads exhibited a drug release profile similar to that for the beads heated with nitrogen protection for 2 weeks. It is noteworthy that the Septacin™ beads used for room temperature stability evaluation were packaged in a foil pouch with nitrogen flushing. This result further confirms the significant impact of the presence of nitrogen on the drug release characteristics of a heat-treated polyanhydride implant.

Bead remnants for the unheated beads and beads heated for 2 weeks with and without nitrogen flushing were examined and photographed 25 days after the in vitro dissolution. A marked difference in morphological appearance of these remnants was observed (Fig. 4). The remnant of the bead heated under nitrogen remained intact. While the remnant of both the unheated bead and the bead heated in the absence of nitrogen exhibited cracking, a much less extensive cracking was seen with the remnant of the bead heated under nitrogen. The

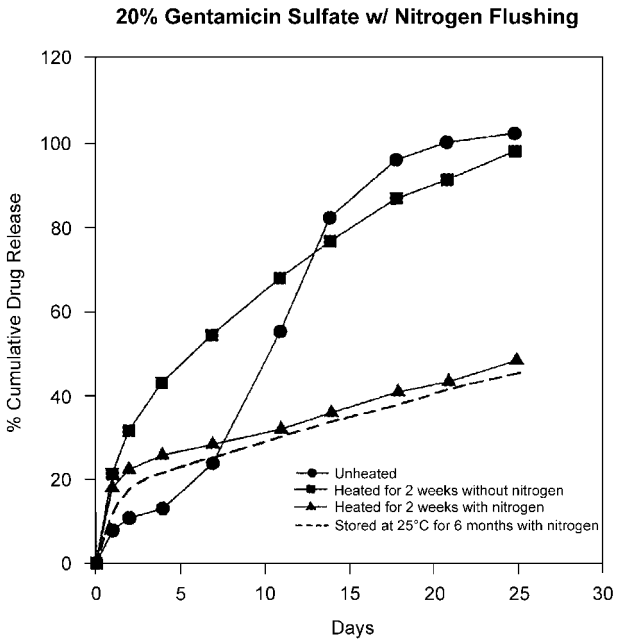

Figure 3. In vitro drug release of unheated and heated Septacin beads.

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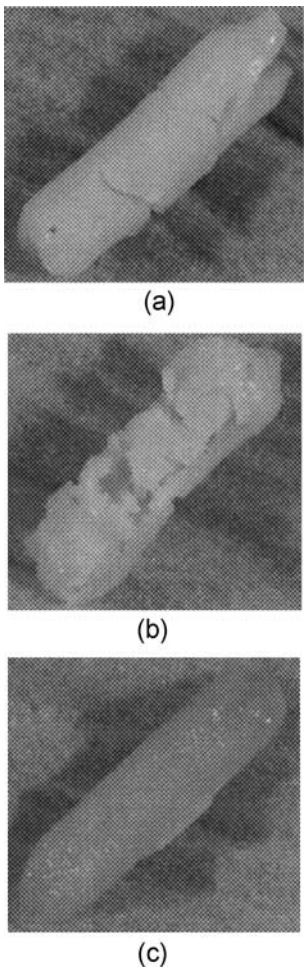

Figure 4. Physical appearance of bead remnants of the (a) control, (b) heated without, and (c) heated with nitrogen flushing.

extent of cracking of the bead remnant appears to correlate well with the rate of drug release: a faster drug release was shown by the bead with more extensive cracking. This is clearly attributed to an increase in the surface area of a cracking bead exposing to the dissolution medium.

The structure failure of a Septacin bead in water was previously shown to be attributed to the osmotic pressure produced by the gentamicin dissolved in the water penetrating into the polymeric matrix. Therefore, the ability of the bead to maintain physical integrity in water is dependent on the mechanical properties of the bead. The stiffness values (Table 1) indicate that the beads heated in the presence of nitrogen were more pliable and likely to resist the cracking caused by the stress generated by the osmotic pressure. The more intact matrix structure of a noncracking bead also resulted in the slower drug release rate because of less surface area exposed to the dissolution medium.

CONCLUSIONS

The effects of various heat treatments on the copolymer molecular weight, mechanical property, and the in vitro drug release of Septacin beads were found to be significant. The copolymer molecular weight of heated beads was reduced as a result of polymer degradation (depolymerization). Noticeable differences in molecular weight reduction were also shown between samples heated with or without nitrogen flushing. The decrease in copolymer molecular weight and stiffness of beads heated with nitrogen flushing was more extensive than those heated without nitrogen flushing. The lower stiffness value indicates a greater resistance of the beads to stress related cracking in water as evidence by the more intact matrix structures of the bead remnants. The slow drug release exhibited by the beads heated under nitrogen was attributed to the less cracking of the beads in water. Results of this study have demonstrated that the drug release of a polyanhydride implant can be further sustained with a postinjection molding heat treatment under nitrogen protection.

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