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RESEARCH PAPER

The Effect of Storage Temperatures on the In Vitro Properties of a Polyanhydride Implant Containing Gentamicin

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ABSTRACT

Septacin® is a biodegradable sustained-release implant containing 20% (w/w) gentamicin sulfate. The matrix of the implant is a polyanhydride copolymer composed of erucic acid dimer (EAD) and sebacic acid (SA) in a one-to-one weight ratio. The effect of storage temperatures (−15°C and 25°C) on the stability of Septacin® was evaluated with respect to gentamicin potency, copolymer molecular weight, and in vitro drug release. The drug in polymer matrix was stable for at least 12 months when stored at 25°C, but the molecular weight of the copolymer declined rapidly at this temperature. At −15°C, there was no change in the molecular weight of the copolymer. However, the placebo (copolymer without gentamicin) exhibited a significant drop in copolymer molecular weight at both temperatures. The drug release profiles showed no change for samples stored at −15°C for the duration of this study, while the release of drug slowed down significantly for samples stored at 25°C for longer than one month. A pronounced difference in the morphology of the −15°C samples and the 25°C samples was observed during the in vitro dissolution test; cracking of the −15°C samples was evident, but the 25°C samples remained intact.

Key Words: *Gentamicin; Polyanhydride; Septacin®; Stability*

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INTRODUCTION

Osteomyelitis is a deep bone infection which is difficult to treat. For years, various drug delivery systems have been studied for the treatment of osteomyelitis. Bohner et al.[1] studied gentamicin-loaded hydraulic calcium phosphate bone cement for this indication. Gentamicin release from polymethylmethacrylate (PMMA) bone cement was also reported by van de Belt et al.[2] and Ethell et al.[3] However, these systems are not biodegradable. The PMMA/gentamicin implants need to be removed after the drug is released.

Polyanhydride is a biodegradable polymer which has been well studied in controlled-release drug delivery systems.[4–10] Polyanhydride has been evaluated for local delivery of antibiotics for the treatment of osteomyelitis.[11–13] Septacin® is a polyanhydride implant product commercially developed for this indication. Septacin® contains 20% (w/w) gentamicin sulfate and a copolymer of erucic acid dimer (EAD) and sebacic acid (SA) in a one-to-one weight ratio.

The stability of the polymer in terms of its molecular weight during long-term storage is an important factor in determining product shelf-life. The stability of some polyanhydrides, such as p(SA) (polysebacic acid), p(CPP:SA) (polycarboxyphenoxy propane), and p(CPH) (polycarboxyphenoxy hexane and sebacic acid) has been studied.[6,14] Results from these studies demonstrated that the molecular weight of these polymers decreased markedly at 21°C. However, the impact of the decrease in the molecular weight of the copolymer on the drug release characteristics of the polyanhydride matrix has not been fully investigated.

The objective of this study is to evaluate the effect of storage temperature and time on the stability of Septacin® including gentamicin potency, molecular weight of the copolymer, and in vitro drug release.

MATERIALS

For the preparation of Septacin®, the polyanhydride copolymer of EAD and SA in a one-to-one weight ratio was first melt-mixed with gentamicin sulfate, which was in a spray-dried powder form. The polymer/drug mixture was subsequently injection molded into strands. One strand consisted of five beads (4 mm in diameter and 10 mm in length) joined by four linkers (1 mm in diameter and 4 mm in length) of the same composition as the beads. Two strands, a total of 10 beads, were packed in a sealed foil pouch flushed with nitrogen to prevent moisture absorption. Septacin® containing 20% (w/w) gentamicin sulfate and Septacin® placebo were manufactured by Abbott Laboratories (North Chicago, IL). Gentamicin sulfate was obtained from Lek Pharmaceuticals (Czechoslovakia).

METHODS

Sample Storage

Septacin® and placebo samples were stored at two temperatures: −15°C and 25°C (40% relative humidity). During storage, the temperature at each stability station was monitored using a chart recorder. Samples were removed for evaluation at predetermined time intervals.

Gentamicin Potency Determination

Gentamicin potency in Septacin® was determined using the USP ⟨24⟩ bioassay method. Septacin® samples were dissolved in methylene chloride and gentamicin was extracted using a 0.05 M sulfuric acid solution. The drug in the aqueous phase was determined following the USP turbidimetric method. Gentamicin potency was recorded as micrograms of activity in terms of USP reference standard.

Molecular Weight Determination

Molecular weight of EAD : SA (1:1) copolymer was determined using a gel-permeation chromatography (GPC) method. Samples were dissolved in methylene chloride and solutions were filtered through a glass fiber filter prior to analysis. A Waters Ultrastyragel "Linear" column was used. The mobile phase was methylene chloride and a refractive index detector (Shodex Model RI-71) was used for detection. Monodisperse polystyrene standards were used to calibrate the GPC column. The average molecular weight (M_w) was calculated relative to the polystyrene standards.

In Vitro Dissolution

The drug-release profiles of Septacin® samples were determined using the method previously described by Stephens et al.[15] One bead with the linker was placed in a glass vial containing 100 mL of water for injection as the dissolution medium. The vial was stoppered and placed in a 37°C water bath with reciprocal shaking at 100 rpm. At each sampling interval, the entire volume of dissolution medium was replaced with 100 mL of fresh medium. The amount of gentamicin in the dissolution medium was determined using a spectrofluorometric method. The samples were derivatized with *o*-phthaldehyde reagent (Fluoraldehyde™, Pierce Chemical, Rockford, Illinois, USA). The fluorescence of the samples was subsequently measured using a flow injection system consisting of an HPLC pump (Thermo Separation Products P4000), an HPLC autosampler (Thermo Separation Products AS3000), and a fluorescence detector (Thermo Separation Products FL2000). Fluorescence was monitored using an excitation wavelength (λ_{ex}) of 340 nm and an emission wavelength (λ_{em}) of 456 nm. The sample response was quantitated against a linear calibration curve that was generated using gentamicin standard solutions. Samples were prepared in triplicate. The cumulative percent of drug release was plotted against time in days and the error bars represent +/− one standard deviation.

Morphological Evaluation

The morphology change of Septacin® beads during the dissolution test was monitored. At a predetermined time interval, Septacin® beads were removed from the dissolution medium and photomicrographs were taken using a Sony DXC-97MD color video camera.

RESULTS AND DISCUSSION

Effect of Room Temperature Storage on Gentamicin Stability

The stability of gentamicin sulfate in Septacin® stored at room temperature was determined. Table 1 shows the potency results of gentamicin sulfate in Septacin® samples that were stored at room temperature for 12 months. There was no significant change in gentamicin potency.

Table 1

Effect of Room Storage Temperature and Time on Gentamicin Potency of Septacin® Samples

Interval (Months)	Gentamicin Potency (%)	RSD (%) ($n = 10$)
Initial	105.4	±1.0
1	104.6	±1.6
3	105.3	±0.6
6	105.8	±0.5
12	105.7	±1.0

Effect of Storage Temperatures on Copolymer Molecular Weight

The changes in the copolymer molecular weight as a function of storage temperature and time are shown in Table 2. There was no significant change in the copolymer molecular weight for samples stored at −15°C for 18 months. However, a marked decrease in copolymer molecular weight was seen in samples stored at 25°C; more than 50% reduction in molecular weight was shown for samples stored at this temperature for one month. The molecular weight decline continued at 25°C, but the percentage change was less dramatic.

Table 3 shows the molecular weight of placebo samples stored at 25°C and −15°C. A significant decrease in molecular weight was shown for samples stored at both temperatures. After three months, the molecular weights decreased 39% for samples stored at −15°C and 81% for those stored at room temperature. These results clearly indicate that the copolymer with gentamicin incorporated is more stable than the placebo copolymer at the same storage temperature. It has been reported that the main mechanism responsible for depolymerization of a polyanhydride copolymer is the exchange of anhydride bonds between the copolymer chains.[14] Therefore, the stabilization effect of gentamicin sulfate can be attributed to its function as an inert filler that reduces the degree of contact between copolymer chains and thus retards the anhydride linkage exchange and the resultant decrease in molecular weight.[16]

Effect of Storage Temperature on In Vitro Drug Release

The drug-release profiles for Septacin® samples stored at −15°C are displayed in Fig. 1.

Table 2

Effect of Storage Temperature and Time on Copolymer Molecular Weight of Septacin® Samples (Standard Deviations in Parentheses)

	Storage Time Interval (Months)					
Storage Conditions	0	1	3	6	12	18
25°C, RH[a] 40%	53,835 (988.5)	19,754 (404.0)	10,167 (1,261.2)	5,494 (319.7)	7,649 (234.9)	NT[a]
−15°C	53,835 (988.5)	55,081 (1,117.9)	51,004 (907.9)	56,802* (907.2)	59,685 (1,137.0)	59,273 (1,414.9)

[a]RH = relative humidity; NT = no testing.
*Tested at seven months.

Table 3

Effect of Storage Temperature and Time on Copolymer Molecular Weight of Septacin® Placebo Samples (Standard Deviations in Parentheses)

	Storage Time Interval (Months)				
Storage Conditions	0	1	3	6	12
25°C, RH[a] 40%	42,609 (808.5)	8,637 (108.6)	8,064 (201.3)	5,385 (591.3)	7,381 (512.8)
−15°C	42,609 (808.5)	NT[a]	27,320 (576.4)	25,862* (701.0)	19,959 (781.3)

[a]RH = relative humidity; NT = no testing.
*Tested at seven months.

The drug-release profile did not change as a function of storage time at this temperature. Figure 2 shows the release profiles for the samples stored at 25°C for up to 12 months. Progressively slower drug-release profiles were exhibited by Septacin® stored at room temperature (25°C) for a prolonged time. After 30 days of in vitro dissolution, the extent of drug release was 90% for the initial and one-month samples, 60% for the three-month samples, 45% for the six-month samples, and less than 40% for the 12-month samples. In addition to the significantly lower extent of drug release, there was a relatively higher burst release of gentamicin within the first week of dissolution for the samples stored at 25°C for three months or longer.

After reviewing the effect of storage temperature on the molecular weight of the implants (Table 2) and their in vitro drug-release profiles (Figs. 1 and 2), a decline in the copolymer molecular weight clearly shows a pronounced retarding effect on the drug release from implants. The controlled-release mechanism for gentamicin sulfate from Septacin® in water was previously investigated by Stephens et al.[15] They monitored the degradation/erosion of the matrix by measuring the release of sebacic acid from the implant in water. Their results showed that the release of sebacic acid was much slower than that of the drug. This led them to propose that the release of gentamicin from the polyanhydride matrix was not controlled by the surface erosion of copolymer. They further proposed that because of the relatively high percent (20%) of gentamicin sulfate in the implant, the dissolution and release of the drug would result in water-filled channels in the matrix through which subsequent release of drug could occur via diffusion.

The polyanhydride matrix of Septacin® stored at room temperature for longer than three months exhibited extensive depolymerization (Table 2). The release of sebacic acid from the surface of a highly depolymerized matrix (low molecular weight) was likely to be faster, and thus resulted in a higher

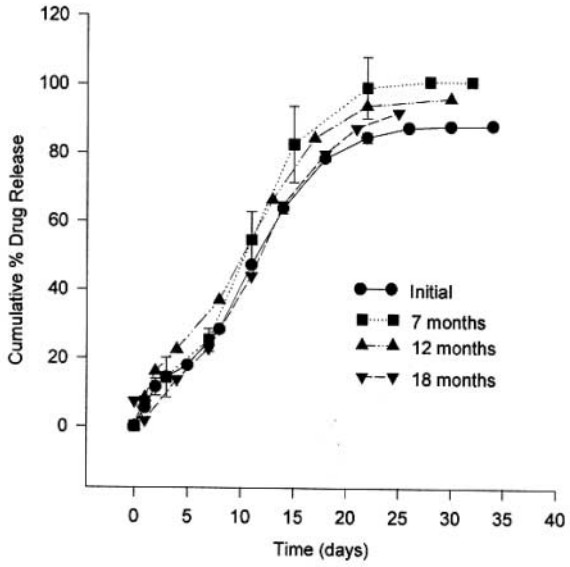

Figure 1. Release profiles of gentamicin from Septacin® samples stored at −15°C ($n = 3$).

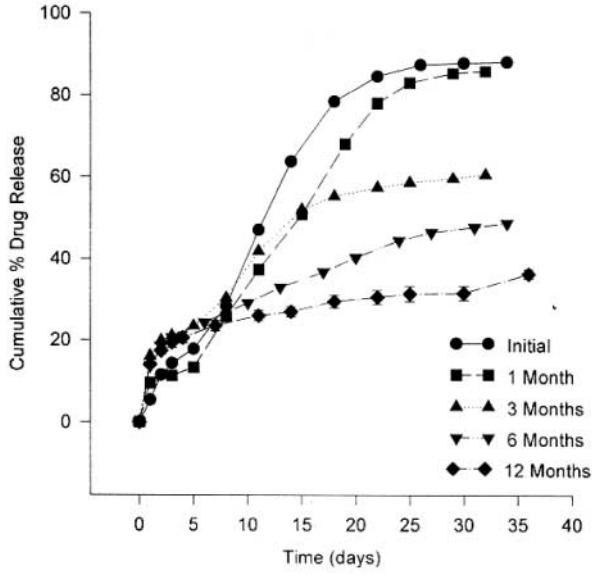

Figure 2. Release profiles of gentamicin from Septacin® samples stored at 25°C ($n = 3$)

burst release of gentamicin. However, the drop in copolymer molecular weight of the matrix fails to provide a plausible explanation for the retarded drug release subsequent to the burst drug release for implants with a copolymer molecular weight significantly less than 20,000.

Morphological Changes

Stephens et al.[15] reported that cracking of Septacin® beads was seen starting on the fifth day after the implant was immersed in water. Since no cracking was seen for placebo beads in water, they concluded that the cracking of the beads was likely attributed to the osmotic pressure build-up by gentamicin dissolved in the water which penetrated into the copolymer matrix. They later pointed out that the cracks formed in the beads could produce an additional new surface area, and thus allowed faster and greater extent of drug release. The morphological changes of Septacin® beads during in vitro dissolution were also visually monitored in this study. Photomicrographs were taken of Septacin® beads that were removed from the dissolution medium at predetermined time intervals. The Septacin® samples stored at −15°C, regardless of time of storage, showed signs of cracking after five days of dissolution testing. The cracking became more severe and finally reached the point where the structural integrity of the bead could no longer be maintained. This sequence of changes is illustrated by Fig. 3a, which shows photomicrographs of Septacin® samples (stored at −15°C for 12 months) during the course of an in vitro dissolution test.

During dissolution, Septacin® samples that were previously stored at 25°C for one month exhibited a cracking pattern of the matrix similar to that observed for beads stored at −15°C. However, samples that were stored at 25°C for three months or longer did not show cracking of the matrix during the course of dissolution tests (Fig. 3b). The absence of crack formation in the matrix of these samples may be attributed to the slow release of gentamicin, because the surface area of an intact matrix exposed to the dissolution medium was significantly less than that of a cracked matrix.

The decrease in copolymer molecular weight in samples stored at 25°C over three months may also contribute to the slow release of gentamicin. When the molecular weight of Septacin® samples decreased from 50,000 D to 5000 D in six months, this may result in the formation of small fragments of poly(EAD:SA). Since EAD is larger in molecular weight and more hydrophobic, SA would dissolve faster and leave behind the waxy EAD in the implant.

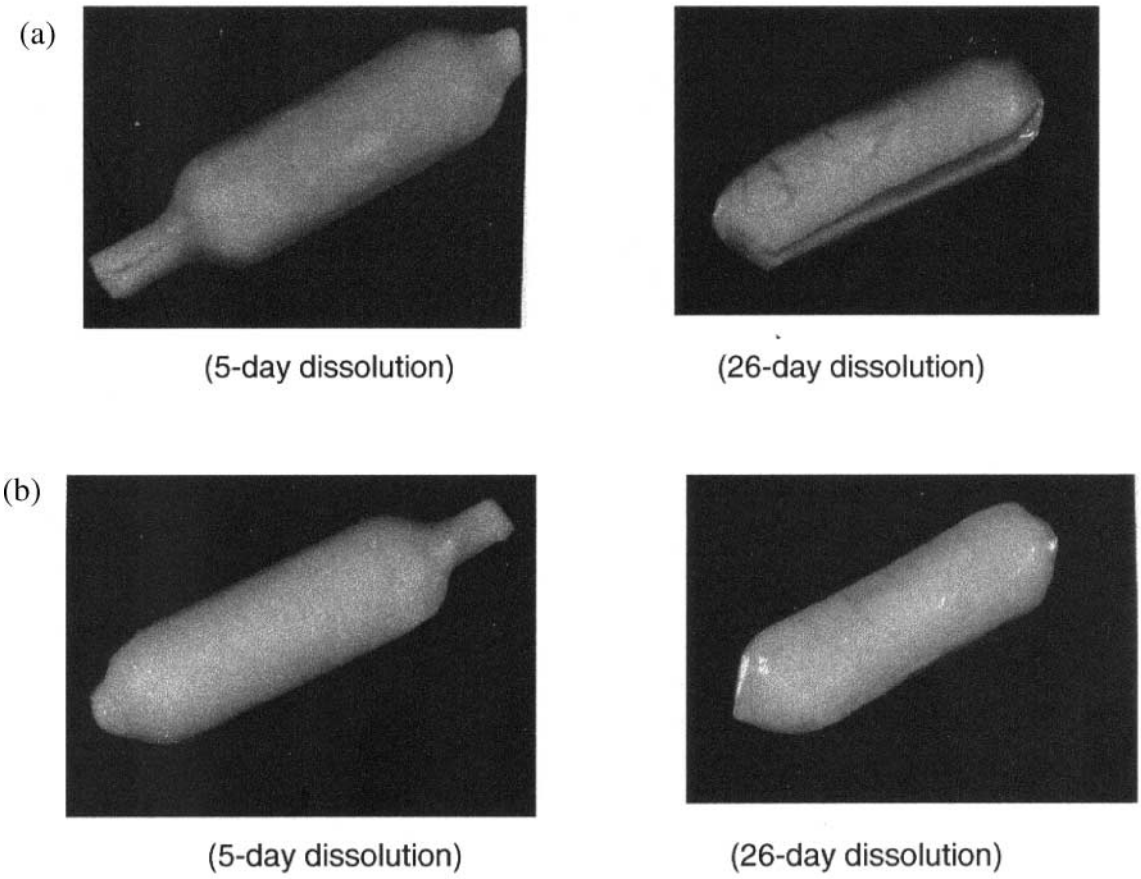

Figure 3. Photomicrographs of Septacin® samples after five days and 26 days of in vitro dissolution test: (a) samples stored at −15°C for 12 months; (b) samples stored at 25°C for 12 months.

Thus, the samples are essentially composed of waxy EAD and some small molecular weight polymers. The hydrophobic EAD is controlling and slowing the release of hydrophilic gentamicin from the degraded polyanhydride matrix upon extended storage at 25°C.

Furthermore, the extent of drug release from the 25°C samples stored for longer than three months (i.e., six and 12 months) decreased gradually as a function of time. However, a discernible difference in the morphology of these beads was not apparent when comparing their photomicrographs (three, six, and 12-month samples). Since the extent of cracking is determined visually and is not quantitative, some micro-cracks may be present within the matrix, which cannot be detected by visual observation. This possibility has led us to speculate that a progressive reduction in these micro-cracks in samples stored for a longer time may contribute to the continuous decrease in the extent of drug release.

The results of this study have strongly suggested that an extensively depolymerized polyanhydride matrix (low molecular weight) is more resistant to cracking in water. It appears that a polyanhydride matrix with a much lower molecular weight is capable of maintaining its structural integrity in water.

CONCLUSION

The stability of a polyanhydride implant containing gentamicin was evaluated. A subzero storage temperature (−15°C) was required to maintain the long-term stability of the copolymer in terms of molecular weight. Samples stored at room

temperature showed a pronounced decrease in copolymer molecular weight and exhibited slower drug release profiles. The copolymer matrix with a significant decrease in molecular weight was able to resist cracking of a matrix during in vitro drug release, leading to a slow drug-release profile.

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