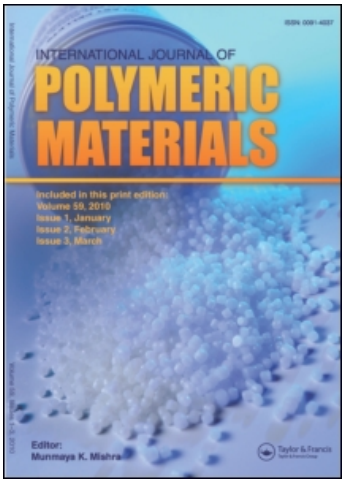

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THE EFFECT OF TEMPERATURE ON CONTROLLED RELEASE OF HEPARIN FROM POLYURETHANE AND ETHYLENE VINYL ACETATE COPOLYMER

Jan-Chan Huang[a]; Ellen M. Jennings[a]
[a] Plastics Engineering Department, Lowell, MA, USA

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THE EFFECT OF TEMPERATURE ON CONTROLLED RELEASE OF HEPARIN FROM POLYURETHANE AND ETHYLENE VINYL ACETATE COPOLYMER

Jan-Chan Huang
Ellen M. Jennings
Plastics Engineering Department, University of Massachusetts, Lowell, MA, USA

The objectives of this study were to examine the effect of elevated temperatures on the release kinetics of heparin from polyurethane (PU) and ethylene vinyl acetate copolymer (EVA), and on the biological activity of heparin released from the polymer matrix. Monolithic samples, prepared by dispersing heparin in solutions of PU and EVA, were fabricated and evaluated for in vitro release rates. Comparisons were made on the release rate of heated and non-heated samples. Heating at 120°C had little effect on the release kinetics from PU and an increase on release from EVA. Bioactivity of pure heparin and heparin released from PU was also evaluated and were found to remain stable up to 210°C.

Keywords: controlled release, heparin, polyurethane, Factor Xa, EVA, temperature effect

INTRODUCTION

Thrombogenicity is one of the most important concerns when developing implantable medical devices such as catheters, artificial organs, and joint replacements. When blood interacts with a foreign surface, thrombosis occurs. A popular technique for increasing thromboresistance to biomaterials has been the incorporation of heparin. Heparin, a naturally occurring blood component, is an effective anticoagulant drug. It is present in mammalian tissues and is usually obtained from the intestinal mucous of mammals. It is a linear anionic

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Address correspondence to Jan-Chan Huang, Plastics Engineering Department, University of Massachusetts, Lowell, MA 01854, USA. E-mail: Jan_Huang@uml.edu

polyelectrolyte, composed of alternating derivatives of D-glycosamine and uronic acid joined by glycosidic linkages [1]. The molecular weight of heparin varies from 6,000 to 20,000 depending on the source and method of determination. It increases negative surface charge [2], thus preventing thrombosis. It has the property of prolonging the clotting time of blood, mainly through the formation of a complex with a plasma protein, antithrombin, to inactivate thrombin, and to inhibit other coagulation proteases such as activated Factor Xa in the clotting sequence [1].

Work has been done by many researchers [2–12] in attempts to improve the antithrombogenicity of biomaterials. Common approaches to this problem have been surface modification methods and pharmaceutical methods [3]. In surface modification the polymers are modified or new polymers synthesized with surface properties considered to be blood compatible. The pharmaceutical approach incorporates heparin into polymers, followed by controlled release at the blood/surface interface. The methods of incorporation include: (1) ionic binding of heparin to polymers, (2) covalent binding of heparin to polymers, and (3) blending of heparin with polymers [4].

In ion-exchange methods the polymer surface is modified to allow heparin adsorption, and the length of protection is controlled by the ion-exchange process. One of the early studies in this approach was the use of a cationic surfactant, Zephiran chloride, to treat a graphite modified surface, followed by immersion into a heparin solution. The heparin was strongly attached even after 100 rinses [5]. Grode et al. [6] examined a procedure for ionic binding of heparin to several polymers. The polymer was swollen in a mixture of toluene and petroleum ether containing 1 to 5% tridodecylmethyl-ammonium chloride (TDMAC). The TDMAC-treated polymer was then placed in a heparin solution and heparin was ionically bound to the quaternary amine. The technique was applicable to materials such as PU, polypropylene, polycarbonate, and poly (vinvyl chloride). Lagergren and Eriksson [7] prepared a crosslinked heparin monolayer surface after ionic binding. First, a cationic surfactant was absorbed onto a polypropylene surface; cationic surface groups bind heparin similarly to other ionically bound systems. The heparin molecules ionically bound to the cationic surface sites were cross-linked with glutaraldehyde to provide a covalently stabilized heparin monolayer.

Tanzawa et al. [4] chemically modified a graft copolymer of vinyl chloride, ethylene, and vinyl acetate by a hydrophilic ammonium group followed by ionic binding of heparin. This ionically heparinized copolymer provided heparin release over a long period of time. Heparin was initially released by ion-exchange and then diffused

through the swelling polymer. A minimum heparin release rate of 4×10^{-8}g/cm^2/min was established as a critical parameter for nonthrombogenicity. However, there were adverse conditions associated-with ionic binding. Heparin was released by an ion-exchange process, leaving the treated surface with positive charges, which had undesirable platelet adhesion and activation [8]. For example, using a solution of a PU with poly(ethylene oxide) (PEO) soft segment and heparin to coat a polyester-PU tube, Lin et al. showed that the minimum critical release rate for nonthrombogenesis was 4×10^{-9}g/cm^2/min [9]. The difference was attributed to the effect of charges on the polymer surface, which increased the heparin requirement in the earlier study.

Heyman et al. [3] developed a method of interposing a spacer arm between the surface and the affixed heparin. This method provided greater quantities of heparin immobilized onto the spacer arm and also increased the ability of the immobilized heparin to bind antithrombin (AT III). Comparisons were made between catheters coated with a heparin dispersion in PU (controlled release system) and heparin-immobilized PU. Heyman et al. found, via *in vitro* and *in vivo* bioactivity testing, that immobilized heparin retained its ability to bind antithrombin III; the controlled release system proved to be superior in short term experiments. However, in the long term (months and years), which would be the case for artificial organs, the immobilized heparin systems would probably be the choice. Park et al. [10] studied PEO spacers of different length on Polyurethane-urea (Biomer) surface and concluded that minimal platelet adhesion occurred at PEO molecular weight of 1000. More recently, Marconi et al. [11] modified the surface of a commercial ethylene vinyl alcohol (EVAL) by covalently bonded heparin using adipoyl chloride and hexamethylene diisocyanate. Heparin was also bonded ionically to EVAL functionalized by introduction of quaternary ammonium groups bonded covalently to the hydroxyl groups of the polymer. The results showed that the covalent bonding of heparin provided better haemocompatibility than the ionic system.

Controlled release of heparin from a polymer matrix was studied by Ebert et al. [12]. Heparin and prostaglandin, which prevents platelet aggregation, were both dispersed in poly(2-hydroxyethyl methacrylate), (HEMA). By incorporating the two agents, both antithrombogenicity and adverse platelet interaction were controlled. A common use of controlled-release systems with the incorporation of heparin is as coatings on catheters. This method was also used by Heyman et al. [3]. The polymer is dissolved in solution and a known amount of heparin is dispersed in the solution. Catheters are then dip-coated with the polymer/heparin solution and allowed to dry. Once placed in

the body, in contact with environmental fluids, the heparin will release.

In all the work that has been done with heparin and polymer systems, the effect of elevated temperatures had not been addressed. This is due to the fact that the methods of preparation of these devices have not involved high temperature plastic processing techniques such as extrusion, injection molding or compression molding. Previous work used solution casting, surface modification and dip coating methods. Therefore, the effect of heat was not a parameter that needed to be taken into account.

The objective of this study has two parts: one, to determine the effect of elevated temperature on the bioactivity of heparin, which was released from a polymer matrix; two, to determine if elevated temperature affected the release of heparin from polymer matrices. The results can help to determine whether heparin can withstand the temperatures associated with plastics processing techniques. PU and EVA were chosen because of their known biocompatibility and ease of processing. Pellethane PU was especially prepared for medical use. The biocompatibility of EVA copolymer has been demonstrated by Langer et al. [14].

EXPERIMENTAL PROCEDURE

Polyurethane (Pellethane 2363-80 AE from Dow Chemical) was dissolved in tetrahydrofurane to give a 10% solution (w/w). EVA (EY 90125 from USI) was dissolved in methylene chloride to give a 10% solution. A weighed amount of polymer was added to the solvent and placed in an incubator (37°C) until fully dissolved. A weighed amount of heparin was added to a weighed amount of solution in a glass vial, to give a 40% heparin (w/w) loading of total solid. The mixture was vortexed to yield a uniform suspension. The mixture was then poured on a Teflon sheet and cast with a Gardner Blade, setting up a gap of 0.05 inches. The film was allowed to dry and a second coat (gap setting of 0.075 inches) was cast on top of the first. Film was allowed to dry for twenty-four hours to assure that all solvents had evaporated. The thickness of the samples was 16 and 28 mil for PU and EVA, respectively. Using a copper tube and hammer, small disks, 1.4 cm in diameter, were punched out of the polymer film.

Heating of samples was performed in a convection oven at various temperatures. Five disks were heated at each temperature. Each disk was placed on a glass slide, put into an oven and exposed to heat for ten minutes. The following temperatures were examined: 100, 130,

160, and 210°C. Disks not subjected to heat were used as controls. After the disks were heated, they were placed in vials containing 20 ml of phosphate buffer solution (PBS) (pH 7.4). Test tubes were placed in an incubator at 37°C for time periods from one to 165 hours, allowing heparin to release from the matrix. The samples were removed from the test tubes and the solutions refrigerated for later testing. Weighed amounts of heparin were placed in the oven and exposed to the above temperature for ten minutes. Untreated heparin at room temperature was also used as the control. It was noted that after heating the weight loss of heparin was 10–25 wt%.

Two methods were used to determine the amount of heparin: Azure A Assay and Factor Xa (FXa) Assay methods. The Azure A method determines the mass of heparin in the solution, while the FXa method determines the bioactivity of the heparin. The Azure A result supplements the result from the FXa assay. A comparison of both results on samples from the same source can give an indication of the effect of temperature on the activity of heparin. Azure A method uses spectrometry to determine the unknown concentration of heparin released from samples. Azure A dye was used in order to obtain absorption in the visible range. Standard solutions of untreated heparin were prepared for comparison. The absorbance at 631 nm was measured. This method measured the total concentration of heparin in solution, including both active and inactive form.

Active units of heparin per unit mass may be determined by comparison of clotting time of sample solutions with the heparin material vs. the clotting time obtained using heparin solutions of known concentrations. This study used the Coatest Heparin Test method to determine heparin bioactivity. This assay analyzed heparin activity in phosphate buffer solution (PBS) by determining its effect on antithrombin (AT III) in the inhibition of activated FXa.

The heparin formed a complex consisting of heparin and antithrombin III (AT III). The concentration of this complex depended on the availability of AT III. The diluted solution was incubated with an excess of coagulation Factor Xa. FXa was neutralized in proportion to the amount of heparin, which determined the amount of [Heparin* AT III] complex. The remaining amount of FXa catalyzed the splitting of p-nitroaniline (pNA) from a synthetic peptide substrate Bz-Ile-Glu-Gly-Arg-pNA (S-2222) [13]. The amount of pNA was measured photometrically at 405 nm after stopping the reaction with acetic acid. The principle of the procedure is as follows [13]:

$$\text{Heparin} + \text{AT III (excess)} \rightarrow [\text{Heparin}^* \text{ AT III}]$$

$$[\text{Heparin}^* \text{ AT III}] + \text{Xa (excess)} \rightarrow [\text{Heparin}^* \text{ AT III}^*\text{Xa}] + \text{Xa (remaining)}$$

$$\text{Substrate} \xrightarrow{\text{Xa}} \text{Peptide} + \text{pNA(measured spectrophotometrically)}$$

Chemicals for bioactive test were obtained from Sigma in a Coatest Heparin - Bioactivity Test kit (FXa), which contained lyophilized proteins that could be reconstituted into reagent solutions.

RESULTS AND DISCUSSION

The results of *in vitro* testing are shown in Figures 1 and 2. Figure 1 shows the results of heparin release from PU and EVA at room tem-

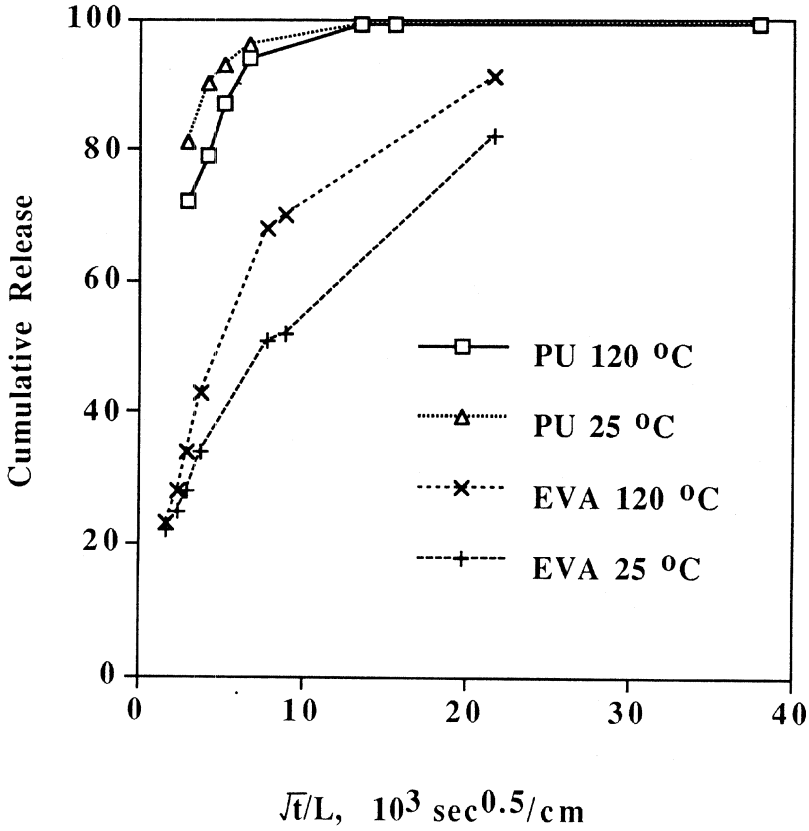

FIGURE 1 Heparin release from PU and EVA.

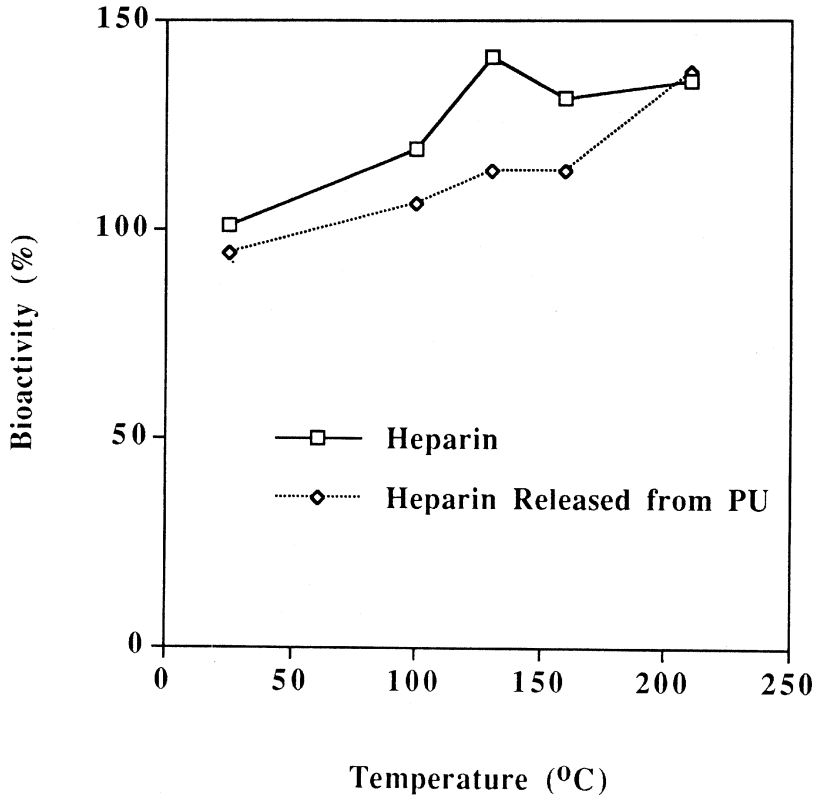

FIGURE 2 Bioactivity of pure and released heparin.

perature and heat treated at 120° C. It can be seen that the rate of heparin release depended strongly on the type of polymer matrix. The effect of heating was more pronounced in EVA samples. The horizontal axis has been adjusted to include thickness, which was different for the two polymers. The initial slopes of these curves are proportional to the apparent diffusivity of heparin through the channels in the polymers. Heparin released more rapidly from PU than EVA. The curve for the PU was incomplete because significant release had taken place when the first measurement was made at one hour. Within the first hour, 72% (non-heated) and 81% (heated) of heparin released from PU samples. Only 23% (non-heated) and 22% (heated) released from EVA samples. After five hours, PU samples were almost completely depleted, whereas less than 50% had been released from the EVA samples. All samples were completely depleted after one week of release. At the beginning stage the cumulative release from EVA samples showed a

linear relationship. This is the characteristic of Fickian diffusion [14–18].

The morphology of EVA and PU had an impact on the release characteristics of the heparin. Because macromolecules are too large to diffuse through a polymer matrix, release occurs via diffusion through channels in the matrix. At high loading heparin particles are apt to touch each other and large clusters of heparin extend from the surface deep into the matrix. These clusters result in connected pore space upon dissolution of heparin particles [15]. Depending on the structure of the polymer, these pores may develop easier in some [15–17], which explains the difference in the release rate from PU and EVA samples. PU is a block copolymer with a melting temperature exceeding 200°C, while EVA is a near random copolymer with a melting temperature below 100°C. During the heating process it is expected that EVA melted and created new pores due to separation of EVA and heparin caused by surface tension difference, leading to a higher release rate.

Langer et al. [17] compared the release of the same protein in different polymers and different proteins in the same polymer. When soybean trypsin inhibitor was loaded into different polymers the release rate decreased in the following sequence: poly(2-hydroxyethyl methacrylate, HEMA), poly(vinyl alcohol), and EVA. The difference in release rate is understandable because different solvents were used for different polymers during sample preparation. Also polyHEMA has a much higher water affinity than EVA and can swell in water. When EVA was used as the matrix for four proteins the release rate followed the sequence: lysozyme > soybean trypsin > alkaline phosphatase > catalase. This sequence was determined by the molecular weight and solubility of the proteins. Recently, the use of a regulatory particle was proposed as a method to control the rate of release in macromolecular systems [18] using the difference in solubility and diffusivity.

The Factor Xa assay was used to determine the bioactivity of the heparin after heating. Bioactivity percentage was defined as the ratio of measured units of active heparin, as measured via Coatest Heparin Assay, to actual units of heparin present in the sample. Theoretically, this percentage would be 100% at 25°C for an untreated sample. It was thought that heparin would begin to degrade in the presence of heat. However, this was not the case in Figure 2, which shows the bioactivity of pure heparin after heating at different temperatures. The reason for this increase in bioactivity is difficult to explain. There is little written on the effect of elevated temperature on heparin's bioactivity or structure. One possible explanation for this phenomenon

is that somehow heat affected the access to binding sites of the heparin molecules. Heparin exerts its main anticoagulant activity by binding to AT III and activating it, thus inhibiting thrombin and FXa. If the heparin's binding ability was increased in some way, then the biological activity would increase. Figure 2 also shows the bioactivity of heparin from PU after two hours of release. There was also a trend of increasing bioactivity when temperature of heat treatment increased. This is similar to pure heparin. Since most of heparin is released in the first two hours for PU samples, this result reflects the increase of heparin activity rather than a change in the amount of heparin released. This result also showed that the use of the PU matrix had no effect on heparin during the heating treatment.

CONCLUSIONS

It can be concluded that heparin can briefly withstand temperatures up to 210°C either in pure state or in PU. It was observed that heat had little effect on the release kinetics. No change in release was seen between 25°C and 120°C for PU. A small increase was observed in EVA after heating at 120°C. However, it was determined that the type of polymer matrix affected the release rate of heparin. Release from PU was quicker than from EVA. Heparin could withstand the high temperatures which would be encountered during plastics processing.

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