Heparin Release from Hydrophobic Polymers :(I) *In Vitro* Studies

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Abstract □ The release of heparin from monolithic devices composed of different ratios of polyethylene oxide (PEO MW 20,000) and hydrophobic polydimethylsiloxane or polyurethane was investigated. Water soluble PEO blended into the polymers provided a controlled release of heparin. The release rate of heparin could be controlled by varying the content of PEO. The heparin release rate from the devices increased as the content of PEO in the devices increased. The release mechanism may be associated with the creation of pore or domain through the devices following the swelling and the change in the physical structure of the polymer network. Hydrophobic polydimethylsiloxanes and polyurethanes containing PEO can provide an antithrombogenic material for prolonged release of heparin from a heparin blended system.

Keywords □ Heparin release, Nonthrombogenic polymers, Polyurethanes, Silicone rubber.

Polydimethylsiloxans (silicone rubber) and polyurethanes have long been used for biomedical applications such as catheters, artificial hearts and heart and assist pumps. The advantages of silicone rubber and polyurethanes for long term drug delivery systems have been recognized because of their good biocompatibility. Until recently, the release of drugs from hydrophobic silicone rubber and polyurethanes were limited to nonpolar and liphophilic compounds.

The release of heparin from silicone rubber and polyurethanes have not been successful. Thus the potential for use of these materials combined with heparin as nonthrombogenic polymers has been limited. We have attempted an alternative approach in development of nonthrombogenic polymers by incorporating pharmacologically active agents into existing compatible polymers via bulk dispersion or surface immobilization (1).

Nonthrombogenic action of ionically bound heparin to polymer was first studied by Gott *et al.* (2). Grode *et al.* studied silicone rubber treated with tridodecyl methyl ammonium chloride (TDMAC), a cationic surfactant, and placed in a heparin solution. The heparin was ionically bound to the quarternary amines (3). The ionic bonding of heparin to quarternary amines could be applied to polyurethanes and polypropylenes.

Tanzawa *et al.* reported on a heparinized hydrophilic polymers containing quarternary ammonium groups in a copolymer that has prolonged nonthrombogenic action (4). Catheters, polymer films, and tubing, etc. could be coated with 2% to 3% of the above polymer solution by solvent casting. The *in vivo* and *in vitro* evaluation of these heparinized materials showed minimum heparin release rate of 4×10^{-2} $\mu g/cm^2/min$ as a critical parameter for nonthrombogenicity. This release rate was maintained for about two weeks *in vivo*. The critical nonthrombogenicity could be maintained for longer periods *in vivo* using polymers containing 15% by weight heparin, with a hydrophilic:hydrophobic ratio that provided 30% w/w equilibrium swelling in water (5, 6).

Holland *et al.* have reported new methods for ionically binding heparin to a polymer surface (7). Another method of making heparinized polymer is to blend heparin throughout the polymer matrix (8, 9). Kim *et al.* have reported that low molecular weight heparin was released at a higher rate than regular heparin from polyurethane and poly (vinylchloride) polymer matrices (1). Catheters coated with a 9% heparin dispersion in the polyurethane system provided improvement in nonthrombogenic action (10). However, the application of this sys-

tem for a period longer than 3 hours *in vivo* seemed unsuccessful due to a lack of a high enough dose of heparin release (11).

Preparation of the heparin blended polymer which release the drug by diffusion can be accomplished by considering the effective dose level of released heparin and the desired duration for use in a blood contacting device. In order to improve the release rate of hydrophilic drugs through hydrophobic polymers, water soluble carriers functioning as swelling agents, such as polyethylene glycol (PEO), glycerol, ethylene glycol, sodium chloride, and sodium alginate were applied to silicone rubber to increase the release rate of the drugs (12-14).

PEO has been selected for two reasons. PEO is a hydrophilic polymer that is soluble in an aqueous environment. PEO covalently bonded surfaces have been shown to have decreased platelet adhesion on its surfaces. It is our hypothesis that the PEO, aside from increasing the water content of the hydrophobic matrices, will slowly diffuse with the heparin to the surface. This localized concentration of heparin and PEO will actively prevent thrombogenesis at the blood-polymer interface but not affect systemic hemostasis. Thus, PEO has a dual role of increasing hydration of the matrices and decreasing platelet adhesion to prevent thrombus formation at the blood-polymer interface.

The purpose of this work was to examine the heparin release from the silicone rubber and polyurethane as a function of PEO content for the effective release does and also promotion of long term release of heparin.

EXPERIMENTAL METHODS

Materials

Polydimethylsiloxane (382 Medical Grade Silastic Elastomer, Dow Corning) and stannous octoate (Dow Corning), polyurethane (Biomer) (Ethicon, Inc.), polyethylene oxide (PEO MW 20, 000, Sigma Chemical Company), sodium heparin (Diosynth, Inc.), dimethylacetamide (DMAC, Fisher Chemical Company), sodium chloride (Fisher Chemical Company), and chloroform (Fisher Chemical Company) were used as received. Heparin was passed through a 200 mesh screen prior to use.

Methods

Monolithic matrices of heparin/PEO/silicone rubber were prepared on polyethylene plates by vulcanization. PEO was dissolved in 3 m*l* of chloroform. This solution was added to the silicone rubber and stirred to obtain a homogenous mixture. After evaporation of the chloroform, heparin was physically dispersed homogenously into the PEO/silicone rubber mixture. Stannous octoate was then dispersed into the sample to catalyze the vulcanization process.

The heparin/PEO/silicone rubber blend was applied onto polyethylene plates and then degassed under high vacuum (20 μmHg). After vulcanization at room temperature for 24 hours, the matrices were removed from the polyethylene plates and vacuum dried at room temperature for 24 hours. Circular devices were cut having dimensions of 0.29 cm radius and 0.06 cm thickness.

The matrices of heparin/PEO/polyurethane were casted onto polyethylene plates from a solution of 15 m*l* dimethylacetamide (DMAC) with 3 m*l* chloroform containing a blend of heparin, PEO, and polyurethane. The solutions were allowed to dry at room temperature for 24 hours. The matrices were then removed from the polyethylene plates and dried in vacuum at 24℃ for 24 hours. Circular matrices were cut having the dimen-

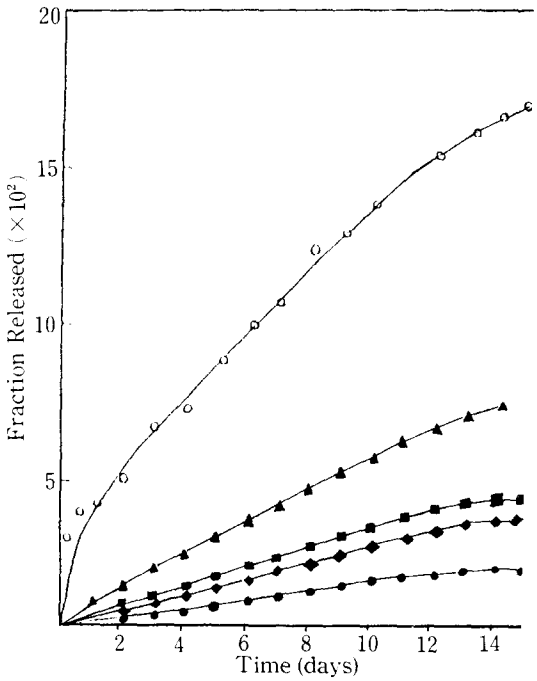

Fig.1. Fractional release of heparin from silicone rubber matrices in PBS containing 5% heparin and different PEO loadings

○ = 20% PEO/silicone rubber
▲ = 15% PEO/silicone rubber
■ = 10% PEO/silicone rubber
◆ = 5% PEO/silicone rubber
● = 0% PEO/silicone rubber

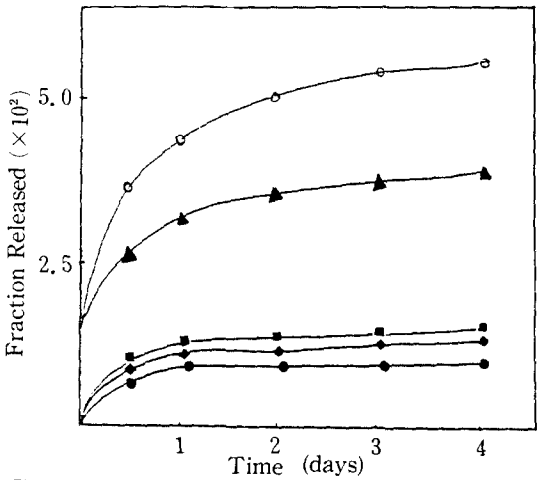

Fig.2. Fractional release of heparin from polyurethane matrices in PBS containing 5% heparin and different PEO loadings.

○ = 15% PEO/polyurethane
▲ = 10% PEO/polyurethane
■ = 5% PEO/polyurethane
◆ = 1% PEO/polyurethane
● = 0% PEO/polyurethane

sions of ≈ 2.05 cm radius × 0.024 cm thickness.

All devices were washed with deionized water to remove surface contaminants. The heparin/PEO/silicone rubber devices were immersed in 70 m*l* of saline solution or deionized water at room temperature (24±1℃). The heparin/PEO/polyurethane devices were immersed in 3 m*l* of deionized water at room temperature (24±1℃). To minimize the boundary layer effect, the releasing media were stirred continuously by constant speed syncronous motors or a magnetic stirrer. The releasing media were withdrawn at timed intervals and replaced with fresh solvent.

The concentration of released heparin was assayed by spectrophotometry at 655 nm according to Hurst (15). The experiments were performed in duplicate and the mean results were reported. The water content of these devices was determined by measuring the weight of wet and dry devices.

RESULTS

Polymer matrices of silicone rubber and polyurethane containing a constant heparin load (5%) and different PEO contents were prepared. The PEO dependent release rate from these monolithic matrices are shown in Fig.1 and 2. The release of heparin from the matrices in the absence of PEO was very low. As the PEO content in the matrices increased, significant increases in the amount of heparin released resulted.

The silicone rubber matrices seem to have a constant, zero order, release rate. The release rates increased with increased PEO loadings. No burst effect was evident until the matrices had 20% PEO loading. The burst effect was followed by a period of constant release.

All polyurethane matrices had a burst effect period. A period of relatively constant release rates followed the burst period. The fraction of heparin load released from the matrices increased with the PEO loadings. Furthermore, the rate of release increased with the PEO loadings.

Considerations of Table I shows the equilibrium water content for the polymer matrices. The fraction of heparin released are for 14 days in saline in the case of silicone rubbers and for 4 days in distilled water in the case of polyurethanes. It is evident that the water content of the matrices increased with the PEO loadings, although heparin

Table I. Effect of PEO Loadings on Silicone Rubber and Polyurethane Matrices with 5% Heparin Loading.

PEO loading (%)	Silicone Rubber water content (%)*	Heparin Fraction released (%)+	Polyurethane water content (%)*	Heparin Fraction released (%)++
0	6.3	2.7	5.1	1.0
5	10.6	3.4	12.4	1.6
10	14.6	4.0	22.2	4.0
15	19.4	7.1	31.7	5.7
20	22.9	16.7	–	–

* water content = $\frac{\text{(wet weight)} - \text{(dry weight)}}{\text{(wet weight)}} \times 100$

+ Silicone rubber matrices released in saline for 14 days

++ Polyurethane matrices released in distilled water for 14 days

Table II. Effect of Heparin and PEO Loadings on Silicone Rubber and Polyurethane Matrices.

Heparin loading (%)	PEO loading (%)	Silicone Rubber water content (%)*	Heparin Fraction released (%)*	Polyurethane water content (%)	Heparin Fraction released (%)*
5	20	31.4	25.6	44.5	35.7
10	15	18.9	9.7	39.3	25.4
10	20	47.1	57.7	15.1	31.4
10	25	–	–	27.2	59.6
15	25	–	–	25.2	46.8

* All matrices were released in distilled water for 14 days

loading does contribute to increased water content. For a given PEO loading, the polyurethane matrices had a higher water content than silicone rubber matrices.

The effects of heparin loadings on heparin release rate was studied. It is well known that the release rate of drugs from monolithic matrices depends on the initial drug load. The total amount of heparin released from the prepared matrices are shown in Figs.3 and 4. The release rates for these same matrices are shown in Figs.5 and 6. Table II shows the equilibrium water content and the fraction of heparin released into distilled water for 14 days.

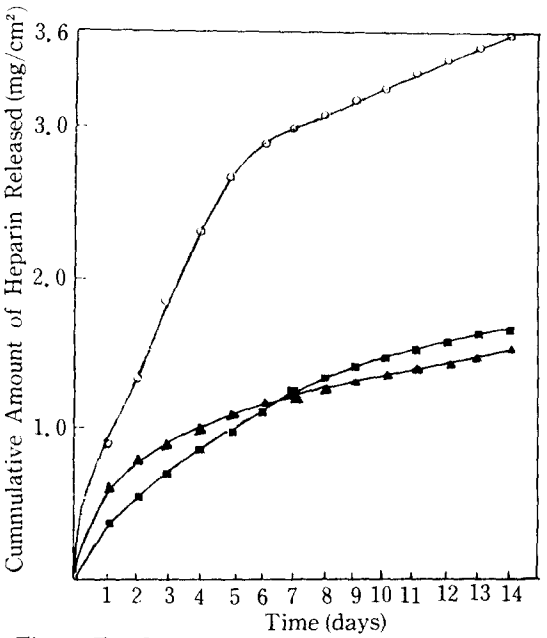

Fig.3. Total amount of heparin released in H_2O from silastic rubber matrices with different heparin and different PEO loadings.

○=10% heparin-20% PEO
▲= 5% heparin-20% PEO
■=10% heparin-15% PEO

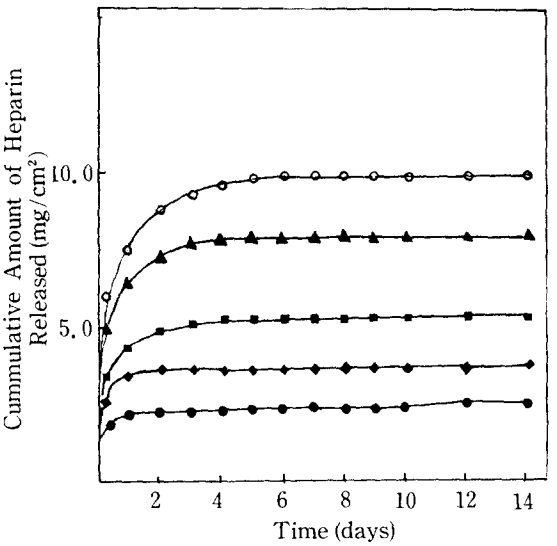

Fig.4. Total amount of heparin in released in H_2O from polyurethane matrices with different heparin and PEO loadings.

○=15% heparin-25% PEO
▲=10% heparin-25% PEO
■=10% heparin-20% PEO
◆=10% heparin-15% PEO
●= 5% heparin-20% PEO

For silicone rubber matrices, the heparin release rate from a 10% heparin/20% PEO loading was about 2 times higher than from a 5% heparin/20% PEO loading (the water content was higher also). The 5% heparin/20% PEO loading and the 10% heparin/15% heparin loading had similar release profile and release rates. Also, under the same loading condition of 5% heparin/20% PEO the water content and the fraction of heparin released in higher in distilled water than in saline (see Tables I and II).

All the polyurethane matrices had similar releas

profiles, these are initial burst effects followed by rapid decline of the release rate (see Fig.6). Significant fractions of heparin remained within the matrices. A series of three polyurethane matrices with 10% heparin and different PEO loadings showed increase amount of heparin released with increasing PEO as with the 5% heparin loadings, but the water content did not increase with the PEO loadings. Triplicate experiments were performed which showed approximately 10% standard deviation in the release rates.

DISCUSSION

In the previous work, the release of heparin from heparin/polyurethane matrices were not sufficient to prevent thrombosis at the blood-polymer interface. A typical release profile showed an initial burst effect followed by a rapid decline to very low release rates. The release rate of heparin from a 10% loading dispersed in polyurethane dropped below the critical release rate of 4×10^{-2} mg/cm^2/min within 40 minutes (11). Furthermore, the fraction of heparin released was less than 10%.

It is hypothesized that the heparin on and close to the polymersolvent interface are released during the initial burst effect period. The minimal hydration of the hydrophobic polyurethane prevents the heparin within the matrix from dissolving and diffusing from the matrix. Thus, the heparin release rate is below the critical level established by Tanzawa, and the heparin remains within the matrix.

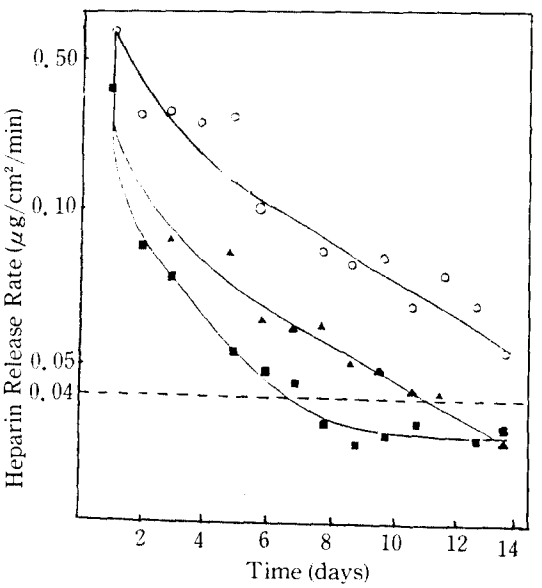

Fig.5. Release rates of heparin released from silastic rubber matrices with different heparin and PEO loadings.

○ = 10% w/w heparin-20% w/w PEO
▲ = 5% w/w heparin-20% w/w PEO
■ = 10% w/w heparin-15% w/w PEO

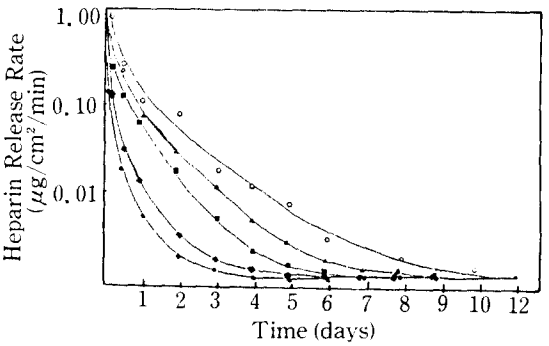

Fig.6. Release rate of heparin released from polyurethane matrices with different heparin and PEO loadings.

○ = 15% heparin-25% PEO
▲ = 10% heparin-25% PEO
■ = 10% heparin-20% PEO
◆ = 10% heparin-15% PEO
● = 5% heparin-20% PEO

PEO, blended into the hydrophobic matrices, is used to increase the water content. The higher water content would allow for dissolution of the heparin within the matrix. Also, the hydration of the matrices is thought to create pores, allowing heparin to diffuse.

According to Graham *et al.* (16) and Polson (17), the interactions between PEO and water increased as PEO concentration increased. These interactions resulted in structural changes such as random coil formation of PEO. The heparin dispersed in PEO may have an ability ot diffuse into the random coil structure of PEO in an aqueous solution. The dissolution of heparin and PEO in the devices, facilitated by water absorption, formed small pores or domains in the devices.

These results show a relationship between the release rate of heparin and the content of PEO. The PEO was found to be a good agent to control heparin release from hydrophobic polymer devices because the release characteristics were related to the water content of polymers (5, 6).

For silicone rubber matrices, the heparin release rate increased with PEO loading. In contrast, the polyurethane matrices had minimal released rates after the initial burst effect periods. It is evident that the silicone rubber matrices were able to sustain a release rate above the critical level of 4×10^{-2} μg/

cm^2/min for up to 14 days with a loading of 10% heparin/20% PEO. Whereas, the polyurethane matrices with 15% heparin and 25% PEO maintained an effective dose level of release for up to 5 days.

It seems necessary to sustain the minimum release rate of heparin of 4×10^{-2} $\mu g/cm^2$/min to maintain antithrombogenic activity on polymer surfaces *in vivo* (3). This rate could produce a minimum surface concentration approaching 0.5 $\mu g/ml$ at the interface, a commonly used therapeutic minidose level (18).

CONCLUSIONS

Polyethylene oxide proved to be an active agent for prolonging heparin release from heparin dispersed hydrophobic systems. The release characteristics of heparin were found to be highly dependent on the PEO content. It will be necessary to examine the heparin release at various loading studies as well as water content. The mechanism controlling heparin release and subsequent improvement of antithrombogenicity of polyurethane and polydimethylsiloxane will be studied further by *in vivo* experiments. Heparin dispersed PEO polymer devices are expected to provide antithrombogenic action in logn-term *in vivo* studies.

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