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The effect of pore formers on the controlled release of cefadroxil from a polyurethane matrix

Ji-Eon Kim [a,1], Seung-Ryul Kim [a], Sun-Hee Lee [a], Chi-Ho Lee [a], Dae-Duk Kim [a,*]

[a] *College of Pharmacy, Pusan National University, Pusan 609-735, South Korea*

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Abstract

The effect of various pore formers on the controlled release of an antibacterial agent from a polymeric device was examined in order to develop a novel biomaterial that prevents bacterial adhesion and growth on its surface. Cefadroxil was chosen as the model antibiotic and was incorporated into a polyurethane matrix by the solvent-casting method. Polyethylene glycol (PEG) 1450, D-mannitol, or bovine serum albumin (BSA) was used as a pore former. The amount of cefadroxil released from various formulations at 37°C was measured by HPLC. The morphological change of matrices before and after release studies was investigated by scanning electron microscopy (SEM). The duration of antimicrobial activities of matrices against *Escherichia coli* and *Bacillus subtilis* was evaluated by measuring the diameters of the inhibition zone. Changing the weight fraction and particle size of the pore formers/drug mixtures could control the release of cefadroxil from the matrix. The release rate of cefadroxil increased as the loading dose of the pore former increased ($15 < 20 < 25\%$). Cefadroxil released from these devices exhibited antibacterial activity for 5–6 days. These results imply that an antibiotic-loaded polymeric device that could prevent bacterial infection on its surface can be formulated using appropriate pore formers.

Keywords: Cefadroxil; Pore former; Polyurethane; Polymeric matrix; Controlled release

1. Introduction

Bacterial adhesion, colonization onto biomaterial surfaces and subsequent infectious complications are common reasons for the failure of many medical devices and implants such as, cardiovascular implants, catheters and urinary tract access (Ander, 1993). Initial adhesion of bacteria to the biomaterial surfaces is the critical event in the pathogenesis of foreign body infection (Sugarman and Young, 1984; Gristina et al., 1987, 1991; Gebelein et al., 1988). A variety of methods have been utilized in an attempt to reduce the incidence of infections. Prevention of bacterial adhesion or

* Corresponding author. Tel.: +82-51-5102800; fax: +82-51-5136754.

E-mail address: ddkim@hyowon.pusan.ac.kr (D.-D. Kim)

[1] Present address: Biotech Research Institute II, LG Chemical Ltd., Taejon 305-380, South Korea.

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at least significant lowering of the number of viable bacteria seems to be the effective way to minimize foreign body infections. Thus, controlled release of anti-infectious substances over a long period of time is obviously needed for the efficacious prevention of foreign-body infections (Golomb and Shipigelman 1991; Sampath et al., 1995; Schierholz et al., 1997).

Sustained-release preparations have frequently been prepared by dispersing a drug in the polymer matrices where the polymer is to act as a rate-controlling barrier. Use of such preparations may result in a number of benefits, such as, reduction of the frequency of dosing, lowered adverse reactions and improved patient compliance (Uchida and Goto, 1988). Polyurethane is widely used in prosthetics and catheters due to its favorable physicochemical and biocompatible properties (Lelah and Cooper, 1986; Levy et al., 1989; Golomb and Shipigelman 1991; Szycher, 1991).

Early studies reported that drug release from the polymers are affected by the particle size and loading dose of drugs in the polymers (Sanders et al., 1986; Kim et al., 1998; Sung et al., 1998). However, a recent study also reported that the diffusion-controlled release of antibiotics from the polyurethane matrices was dependent on the solubility of antibiotics and loading concentration in the matrix (Golomb and Shipigelman, 1991; Schierholz et al., 1997). Thus, the purpose of this study is to investigate the feasibility of controlling the release rate of antibiotics from the polymers by using 'pore formers' incorporated into a polyurethane matrix. Pore formers are biologically inactive and water-soluble compounds that can help the release of drugs by forming channels in the polymer matrix, thereby leaching out the drug regardless of the drug solubility. D-mannitol, PEG1450, and BSA were chosen as pore formers for their high aqueous solubility and low biological activity and toxicity. The effect of the particle size and loading dose of the pore formers was systematically investigated. The duration of antibacterial activity of polymer matrix was also determined by measuring the inhibition zone.

Cefadroxil was chosen as a model drug in this study for its short biological half-life (1.5 h) (Uchida and Goto, 1988). It is a semi-synthetic cephalosporin antibiotic intended for oral administration. The antimicrobial spectrum of cefadroxil includes important gram-positive and gram-negative pathogens usually associated with infections of the urinary and respiratory tracts (Buck and Price, 1977; Hartstein et al., 1977; Tenrisaver and Santella, 1986). Due to its rapid elimination from the body, frequent administration is essential. A sustained-release preparation of cefadroxil, therefore, seemed advantageous (Uchida et al., 1992).

2. Materials and methods

2.1. Materials

Cefadroxil was kindly provided by Cho-A Pharmaceutical Co. (Pusan, South Korea). Polyurethane resin (Lot No. 6193) was received from Dong-Sung Chemical Co. (Suwon, South Korea). D-mannitol, bovine serum albumin (BSA), polyethylene glycol (PEG) 1450, and tetrahydrofuran (THF) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of special reagent grade and used as purchased. *Escherichia coli* (NIHJ) and *Bacillus subtilis* (ATCC 6633) were obtained from the microbiology laboratory of Pusan National University (Pusan, South Korea). Nutrient agar was purchased from DIFCO Laboratories (Detroit, USA) and nutrient broth was purchased from Fisher Scientific (Pittsburgh, USA).

2.2. Preparation of polyurethane matrices

Matrix discs were fabricated by the solvent-casting method (Rhine et al., 1980). Briefly, 2 g of cefadroxil and 2 g of pore former (D-mannitol, BSA, or PEG1450) (1:1weight ratio) were completely dissolved in 150 ml of double-distilled water. The mixed aqueous solution was lyophilized and sieved to fractionate into various particle sizes (<62, 62–88, 88–125, 125–177 μm). In order to make a control matrix without a pore former, various particle sizes of cefadroxil was also prepared by the same procedure with 4 g of cefadroxil dissolved in 150 ml of double-distilled water.

Polyurethane resin (1.4, 1.2, and 1.0 g) was dissolved in 20 g of THF (resulting mixture ranged from 5 to 7%, w/w) with constant stirring for 3 h at 60°C, and then mixed with various weight fractions of the lyophilized drug/pore former powder (0.6, 0.8, and 1.0 g) of specific particle sizes to make 30, 40 and 50% (w/w) powder in polymer matrices (equivalent to 15, 20 and 25% pore former, respectively). This mixture was degassed with an aspirator, and poured into a Teflon mold. The solvent was evaporated overnight at ambient temperature followed by vacuum drying for another 48 h. Final matrices with thickness of 0.4–0.5 mm were then trimmed to 1.0 cm^2, and stored at 4°C until used.

2.3. Cefadroxil release study

The matrices were placed in vials containing 20 ml of isotonic phosphate buffer (IPB) solution (pH 7.4) and placed in a shaking water bath at 37°C. Aliquots (10 ml) of medium were taken at predetermined time intervals for 12 h, and replaced with the same amount of fresh medium. High-performance liquid chromatography (HPLC) was used for the quantitative determination of cefadroxil in the samples. The amount of cefadroxil released was expressed as the percent of the starting amount in the matrix. Each release study was performed in triplicates, i.e. three samples taken from one polyurethane matrix.

2.4. HPLC analysis

Cefadroxil concentration was determined using a HPLC with a binary pump system (Gilson Model 305 and 306) and automatic injector (Gilson Model 234). A Merck C_{18} LiChroCART® column (5 μm particle size, 125 × 4 mm, Merck, Darmstadt, Germany) was used as an analytical column at ambient temperature. The mobile phase was 5% (v/v) methanol in 10 mM acetate buffer (pH 4.8) at a flow rate of 1.0 ml/min. The variable wavelength ultraviolet detector (Gilson Model 118) was set at 240 nm. Injections of 20 μl were made for all solutions to be analyzed. The retention time of cefadroxil was about 5.4 min.

2.5. In vitro antibacterial activity study

Antibacterial activities of cefadroxil released from the matrices against gram negative (*E. coli*) and gram positive (*B. subtilis*) bacteria were determined using a modified ditch-plate method (Kennedy et al., 1974). Briefly, into culture plates (90-mm diameter and 15-mm height), 25 ml of nutrient agar inoculated with 25 μl of bacterial broth was placed. Ditch plates were prepared by placing three stainless steel cylinders (I.D., 9 mm; O.D., 10 mm) before solidifying and agar was allowed to harden. After solidifying, the three steel cylinders and the contents were removed to give three cylindrical ditches, to each of which the disc (1.0 cm^2) was planted and wetted with a few drops of agar solution. These plates were incubated for 24 h at 37°C, and the diameters of the inhibition zone were determined. To investigate the duration of the antibacterial activity of each matrix, they were transferred to a freshly prepared plate after 24 h of incubation, and incubated for another 24 h at 37°C. This procedure was continuously repeated until the inhibition of bacterial growth was no longer noticed. The duration of the antibacterial activity of the matrices was investigated using a plain matrix without cefadroxil as a control in each study. Each study was performed in triplicates, i.e. three samples taken from one polyurethane matrix.

2.6. Morphological analysis of matrices

Scanning electron microscopy (SEM) was performed on Au sputter-coated samples before and after release studies using a Hitachi S-4200 SEM (Tokyo, Japan). Cross-section of matrices was carefully examined to study the effect of pore formers on the formation of pores/channels in the matrices.

3. Results

3.1. The effect of pore formers on the release of cefadroxil

Fig. 1 shows the release profiles of cefadroxil

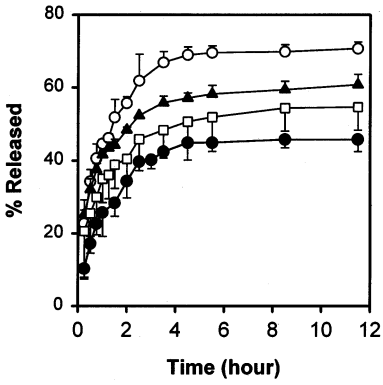

Fig. 1. The effect of different pore formers on the release of cefadroxil from the polyurethane matrix, ○, BSA; ▲, D-mannitol; □, PEG1450; ■, no pore former. Matrix contains 50% lyophilized powder (particle size, 62–88 μm; pore former:cefadroxil, 1:1), equivalent to 25% (w/w) pore former. The bars represent S.D., $n = 3$.

from the polyurethane matrices containing 25% (w/w) of various pore formers, which is equivalent to 50% powder (25% pore former and 25% cefadroxil) in matrices. Compared with the matrix containing 50% (w/w) cefadroxil powder without the pore former, the release rate and the amount of cefadroxil release significantly increased in the order of PEG1450 < D-mannitol < BSA. Fig. 2 shows the effect of particle size of pore formers on the release of cefadroxil from the polyurethane matrix. Increase in the particle-size of pore former enhanced the rate and the total amount of cefadroxil, released from the matrix containing 15% (w/w) pore former (equivalent to 30% powder). The release rate and the total amount of cefadroxil release also increased as the weight fraction of pore former increased from 15 to 25% (Fig. 3). It is interesting to note that the amount and the rate of release were always in the increasing order of PEG1450, D-mannitol, and BSA. The release rate of cefadroxil was always the highest, when BSA was incorporated as a pore former.

3.2. In vitro antibacterial activity studies

Fig. 4 shows the antibacterial activity of polyurethane matrices against *E. coli* and *B. subtilis*. All polyurethane matrices containing 25% (w/w) pore formers (particle size, 62–88 μm) produced inhibition zone for at least 5–6 days, while the control polymer matrix without cefadroxil showed no zones of inhibition (data not shown). The diameter of the inhibition zone was about 7 cm on the first day of incubation, and then gradually decreased for 5–6 days, which means that all the matrices released significant amount of cefadroxil and inhibited the growth of both model gram negative and positive bacteria.

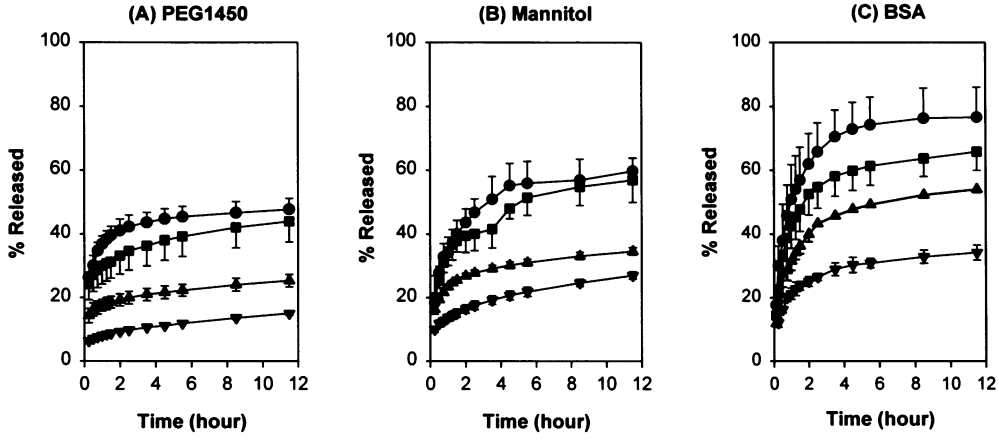

Fig. 2. The effect of particle sizes of pore former (15%, w/w) on the release of cefadroxil from the polyurethane matrix, ●, 125–177 μm; ■, 88–125 μm; ▲, 62–88 μm; ▼, <62 μm. (A) PEG1450; (B) D-mannitol; or (C) BSA was used as a pore former (pore former:cefadroxil, 1:1). The bars represent S.D., $n = 3$.

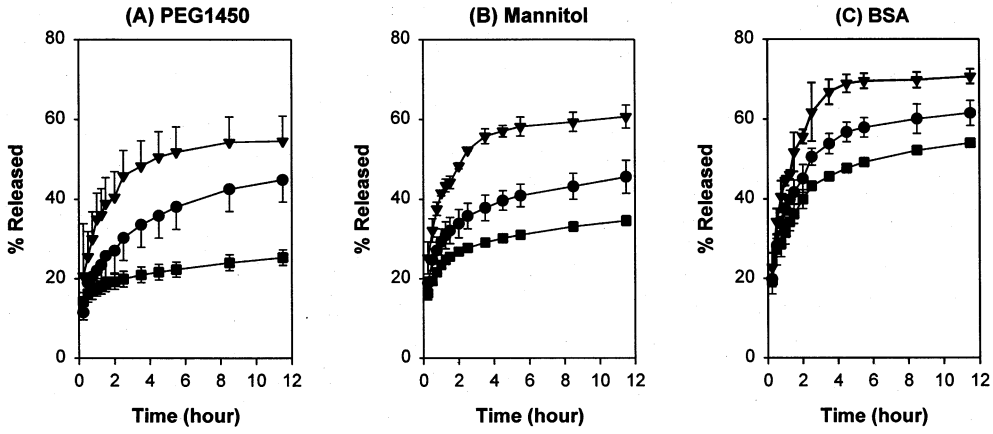

Fig. 3. The effect of loading doses of pore former (particle size, 62–88 μm) on the release of cefadroxil from the polyurethane matrix, ▼, 25%; ●, 20%; ■, 15%. (A) PEG1450; (B) D-mannitol; (C) BSA was used as a pore former (pore former:cefadroxil, 1:1). The bars represent S.D., $n = 3$.

3.3. SEM study

SEM of the cross-section of matrix showed the typical channel formation after 12 h of release study (Fig. 5), which suggested that the pore former leached out together with the cefadroxil during the release study. Moreover, it is clear that the matrix with 25% of the pore former resulted in bigger channels and pores in the matrix than that of 20%, which are well correlated with the in vitro release studies.

4. Discussion

The first implantable biomaterial systems providing for a sustained release of antibiotics (gentamicin) and antibacterial agent (iodine) were fabricated from silicone. However, polyurethane (PU) was chosen as the matrix in this study due to its favorable physicochemical and biocompatibility properties. It has been reported that drug-release from the polymers is influenced by the physicochemical properties of both the polymer

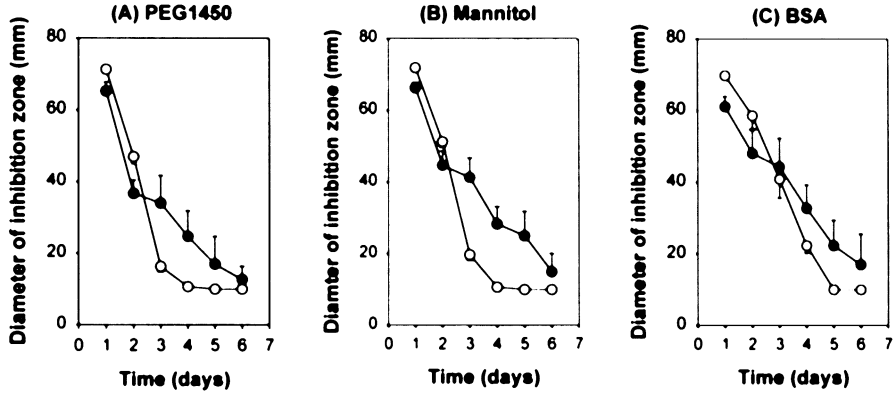

Fig. 4. Antibacterial activity of cefadroxil released from the polyurethane matrix against ●, *E. coli*; and ○ *B. subtilis*. Matrices contain 25% (w/w) of (A) PEG1450 or (B) D-mannitol or (C) BSA as a pore former (particle size, 62–88 μm, pore former:cefadroxil, 1:1). The bars represent S.D., $n = 3$.

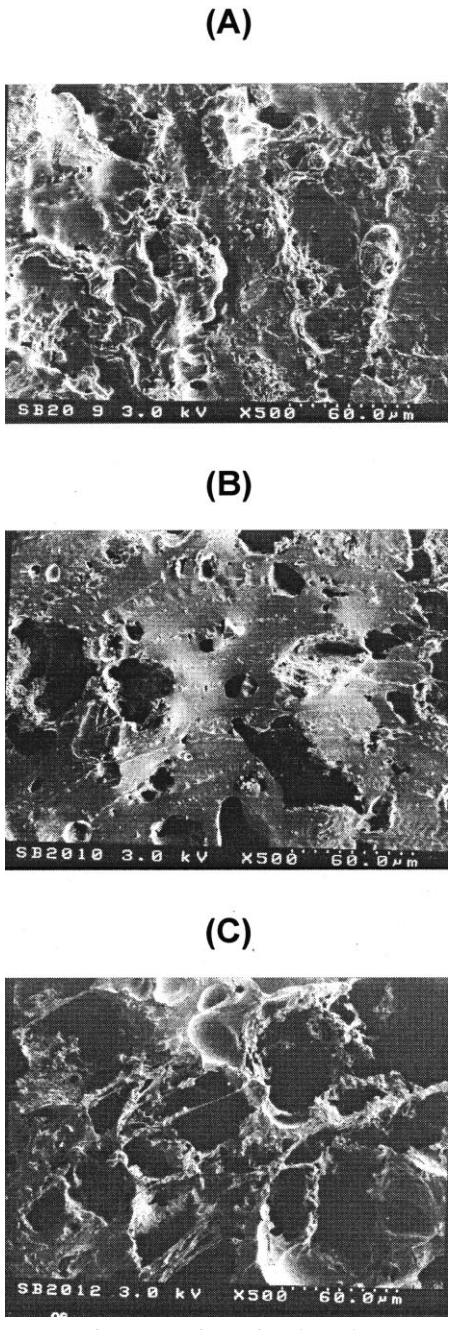

Fig. 5. SEM of cross-section of polyurethane matrix (A) before and (B and C) after cefadroxil release study for 12 h. Matrices contain (A and B) 20% (w/w) or (C) 25% (w/w) D-mannitol as a pore former (particle size, 62–88 μm, pore former:cefadroxil, 1:1).

and the drug, which include the polymer molecular weight, drug loading percentage and drug solubility as well as the fabrication method (Sanders et al., 1986; Sung et al., 1998). An early study demonstrated that the release of macromolecules from the polymer matrix increased as the particle size and loading dose increased (Rhine et al., 1980). The authors suggested that the enhanced release rate by increasing particle size and loading dose resulted from the formation of larger channels or pores in the polymer matrix, which facilitated the movement of water into, and drug out of, the matrix by providing simpler pathways (lower tortuosity) and greater porosity for diffusion (Rhine et al., 1980).

D-mannitol, PEG 1450, and BSA were chosen as pore formers, and then were incorporated in the polyurethane matrix together with cefadroxil by the solvent-casting method. Since cefadroxil is poorly soluble in THF, it disperses and forms channels or pores in the polyurethane matrix. The addition of pore formers enhanced the release of cefadroxil from the matrix (Fig. 1), which implied that pore formers facilitated the movement of water into the matrix increasing the solubilization of cefadroxil, thereby increasing the movement of drug out of the matrix. Increase in the particle size of the drug/pore former powder enhanced the rate and the total amount of cefadroxil released from the matrix (Fig. 2). The release rate and the total amount of cefadroxil release also increased as the loading dose of the powder increased (Fig. 3). SEM study confirmed that pore formers leached out of the polymer matrix together with cefadroxil by the formation of channels (Fig. 5). Increasing the particle size and loading dose seemed to facilitate the release of cefadroxil by the formation of larger channels or pores in the polymer matrix. These results are consistent with our previous study to develop a controlled release polyurethane device of hirudin using D-mannitol and BSA as pore formers (Kim et al., 1998). Thus, it was possible to control the release rate of cefadroxil from the polyurethane matrix by changing the particle size and loading dose of the drug/pore former powder. This method could be applied to enhance the release rate of non-soluble compounds from the polymer device by incorporating water-soluble pore formers. However, it is necessary to improve the incomplete release of drug from the matrices, which are probably due

to the 'isolation' of drug particles from the channels or the degradation of drug during the fabrication procedures.

It is interesting to note that the release rate of cefadroxil was highest when BSA was used as a pore former, and was the lowest with PEG1450. Since PEG1450 is fully soluble in THF while BSA is less soluble in THF than PEG450, incomplete formation of channels or pores seemed to cause the slower release. Since it is also known that the release rate of drug from the drug-dispersed matrices is controlled by the drug solubility in the matrix (Schierholz et al., 1997), the effect of the solubility of pore formers on the release rate needs further investigation.

Additionally, the duration of antibacterial activity of polyurethane matrix was successfully determined by transferring the matrix to a fresh culture plate after 24 h of incubation. The diameter of inhibition zone gradually decreased for 5–6 days (Fig. 4), which implies the burst release of cefadroxil from the matrix in the beginning and is consistent with in vitro release studies. The release of cefadroxil was completed within 8 h of the in vitro release studies (Figs. 1–3), but the antibacterial activity of polyurethane matrix maintained for 5–6 days, probably due to the slower diffusion of cefadroxil through the medium in the antibacterial activity study than in the release study. However, since it was possible to investigate the duration of antibacterial activity of the matrix, further study is under way in this laboratory to determine the effect of the composition of the matrix on the duration of antibacterial activity.

5. Conclusion

The release rate and the total amount of cefadroxil released from the polyurethane matrix could be controlled by changing the weight fraction and particle size of drug/pore former powders incorporated. When BSA was incorporated as a pore former, the highest release rate of cefadroxil was observed, compared with PEG1450 and D-mannitol. This might be attributed to the different aqueous solubility of the pore formers although further investigation is necessary. SEM of the cross-section of the matrix showed the typical channel formation after 12 h release study and the channel size increased with higher loading dose of the pore former. Antibacterial activity of polyurethane matrices continued for 5–6 days when the diameter of the inhibition zone was measured by repeatedly transferring the matrix to a fresh culture plate after 24 h of incubation. Thus, an antibiotic-loaded polymeric device could prevent bacterial infection on its surface, which could bring about an enhancement in biocompatibility of biomaterials. This device would also be helpful in the local and systemic controlled delivery antibiotics for treating various infectious diseases, such as, chronic osteomyelitis and periodontitis, or for preventing infection by controlled release from suture.

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