*J Vet Clin* 22(3) : 175-180(2005)

# Controlled Release of Cefadroxil from Chitosan Beads in Dogs

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**Abstract:** The purpose of this study is to investigate the effects of formulation variables on the release of cefadroxil form chitosan beads, to optimize the preparation of chitosan beads loaded with the drug for controlled release, and to evaluate the drug release form chitosan beads in dogs. Chitosan beads were prepared with tripolyphosphate (TPP) by ionic cross-linking and those sizes were less than 1 mm in diameter. The release behaviour of cefadroxil was affected various factors. As pH of TPP solutions decreased, the entrapment efficiency of cefadroxil increased, whereas the release of cefadroxil decreased. The release rate of cefadroxil form chitosan beads decreaed with the increased TPP solution concentration. When cross-linking time increased, the release of the drug from chitosan beads decreased. The cefadroxil loaded beads were implanted to 4 mixed breed dogs. The concentration of cefadroxil in sera due to chitosan beads implanted with 50 mg/kg body weight of beads was sustained more than 1 ug/ml for the whole 7 days period. Therefore, the cefadroxil loaded beads can be used successfully in pyoderma of dogs. These results indicate that chitosan beads may become a potential delivery system to control the release of drug.

***Key words*** : chitosan, cefadroxil, dog.

## Introduction

Pyoderma is one of the most common skin disease encountered in dogs. *Staphylococcus intermedius* is the most common bacteria associated with canine pyoderma, but other coagulase-positive *Staphylococcus* spp. has been identified[1]. The treatment for pyoderma usually requires proper antibiotic agents for more than 3 weeks at least. It is a boredom task for both owner and dog. Thus, the agent had controlled release capacity is necessary.

Chitosan beads have been investigated as a controlled drug releasing agent. Chitin is the most abundant natural amino polysaccharide from crabs or other sources. Chitosan[poly(β-(14)-2-amino-2-deoxy-D-glucose)] is a natural cationic polysaccharide derived from chitin, which is copolymer, a glucosamine and an N-acetyl glucosamine units, combined together[2,3]. Chitin and chitosan are recommended as suitable functional materials, because these natural polymers have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption and properties[2].

A wide variety of medical applications for chitin and chitin derivatives have been reported[2,4]. Malettas *et al.* studied the effect of treatment with chitosan and saline solution on healing and fibroplasia of wounds made by scalpel insertions in skin and subcutaneous tissue in the abdominal surface of dogs[11]. Kweon *et al.* studied preparation of water-soluble chitosan/heparin complex and its application as wound healing accelerator[5]. Minami *et al.* found that subcutaneous injection of chitosan induces systemic activation of both PMN cell and complement component 3 (C3) in dogs[10]. Yusof *et al.* proposed chitin beads as a wound dressing material[12]. Numerous studies have demonstrated that chitosan is an effective and safe vehicle for the sustained release of drugs[3,6-9].

In this study, a drug loaded on chitosan bead for bacterial pyodermatitis is cefadroxil. Cefadroxil is a first-generation cephalosporin. The first-generation cephalosporins are effective against most gram-positive bacteria, but have limited activity against gram-negative organisms. Cefadroxil is used in treating susceptible infections of the skin, soft tissue, and genitourinary tract in dogs and cats[13,20,25].

Drug release from chitosan microparticles can be controlled by the matrix density, which is affected by various factors, such as concentration of cross-linking agent and cross-linking time. Therefore, the purpose of this study is to investigate the effects of formulation variables on the release of drug from chitosan beads, to optimize the preparation of chitosan beads loaded with drug for controlled release, and to evaluate the drug release from chitosan beads in dogs.

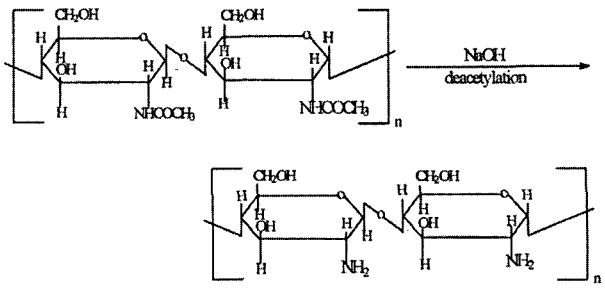


**Fig 1.** Structures of Chitin and Chitosan

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## Materials and Methods

### Materials

The following chemicals were obtained from Sigma Chemical Company (St. Louis, MO): chitosan, tripolyphosphate, and cefadroxil. The chitosan was deacetylated in minimum 85%. UV-Spectrophotometer (UK INON922, Kontron Ins., Italy) was used to measure the concentration of cefadroxil *in vitro*. Microcaliper (DIGIMATC CALIPER®, Mitutoyo, Japan) and 6 mm blank paper discs (Bector, Dickinson) were used to measure the concentration of cefadroxil *in vivo*. Four healthy mixed breed dogs were used.

### Preparation of cefadroxil-loaded chitosan beads

The chitosan beads containing cefadroxil were prepared by the method of Bodmeier *et al.*[14] Cefadroxil (1% w/w) was dissolved in a solution of chitosan (2% w/w) in diluted acetic acid (1% v/v). The beads were formed by dropping the bubble-free solution or dispersion (10 ml) through a disposable syringe onto a gently agitated tripolyphosphate (TPP) solution (100 ml). The chitosan beads were separated after one hour and rinsed five times with deionized water. The gel beads were air-dried for 48 hours at 25°C.

The cefadroxil-loaded beads were made in three groups. Beads in the group I were prepared in 5% TPP solutions of pH 6, 8, and 10 and cross-linking time was 1 hour. Beads in the group II were prepared in 3%, 5%, and 7% TPP solutions (pH 8), and cross-linking time was 1 hour. Beads in the group III were prepared into 5% TPP solutions (pH 8), and each cross-linking times were 15 minutes, 30 minutes, 1 hour, and 2 hour, respectively. Finally, drug-free beads were a 5% TPP solution (pH 8), and cross-linking time was 1 hour. The morphology of beads was examined by using a microscope and the size of them also was measured.

### Drug leakage into TPP solutions *in vitro*

The beads were prepared in 5% TPP solutions of pH 6, 8, and 10, and then the leakage amount from chitosan beads was determined by a spectrophotometer at 0.5, 1, 1.5, 2, and 3 hour. Calibration curves were obtained by assaying the standard solutions of cefadroxil of 10, 20, 30, 40 μg/ml concentration at 265 nm.

For cefadroxil in 5% TPP solution;

$$ABS = (0.0228 \times conc.) + 0.1405$$

$$R^2 = 0.9999$$

### Drug release in phosphate buffer saline *in vitro*

Fifty mg of chitosan beads was immersed in a separated test tube containing phosphate buffer saline (pH 7.4) as a releasing medium. The sealed tubes were placed in a shaking incubator at 37°C and agitated at 50 rpm. The supernatant was withdrawn at predetermined time intervals over one week and replenished again. The concentration of cefadroxil in the medium was determined spectrophotometrically at 229 nm. Calibration curves were obtained by assaying the standard solutions of cefadroxil of 10, 20, 30, 40 ß¹/ml concentration at 229 nm.

For cefadroxil in PBS;

$$ABS = (0.0421 \times conc.) + 0.2704$$

$$R^2 = 0.9968$$

### Drug release *in vivo*

Cefadroxil-loaded chitosan beads were administrated to four healthy dogs. The beads (50 mg/kg) were implanted in subcutaneous tissue by stab incision. The beads were made in the condition of 7% TPP solution at pH 8 and the cross-linking time of 15 minutes.

Blood samples were collected from jugular vein of each dog in 12 hour, 1, 2, 4, and 7 day after implantation. The sera were separated with centrifugation and stored at -20°C until being assayed.

*Staphylococcus intermidius* was used in microbiological assay. Serum cefadroxil concentrations were determined by agar plate diffusion technique[15,16,17,25]. Standard solutions were obtained from the serum of healthy dog.

Calibration curves were obtained by assaying the standard solutions of cefadroxil of 10, 20, 30, 40 ß¹/ml.

For cefadroxil in sera;

$$\text{Inhibitor zone} = (2 \times conc.) + 5.4283$$

$$R^2 = 0.9962$$

## Results

### Morphology of chitosan beads

Solutions of chitosan in 1% acetic acid were dropped onto TPP solutions and gel like spheres formed instantly by ionotropic gelation. The color of chitosan beads was white initially, however, got yellowish as dried.

One percent chitosan solution was crushed after dried. But 2% chitosan solution didn't lost their sphere shape. Therefore, 2% chitosan solution was used in this study. Fig 2. showed the shape and size of these chitosan beads. The mean diameter of beads was less than 1 mm approximately.

### The leakage of cefadroxil from chitosan beads

It was determined that the leakage amount of cefadroxil not entrapped into chitosan beads on the hypothesis that pH of TPP solution might affect the entrapment of drug[3,14,18,19]. The time profiles of cefadroxil leaked out of the chitosan gel into the 5% TPP solution were shown (Fig 3). The leakage amount was increased according to increase of the pH of TPP solution.

### Cefadroxil release *in vitro* from chitosan beads

Chitosan beads were prepared by the pH 6, 8, and 10 of TPP solutions, respectively. The release profiles of cefadroxil from chitosan for various pH of TPP solutions were shown (Fig 4). The beads prepared in pH 8 of TPP solution had the

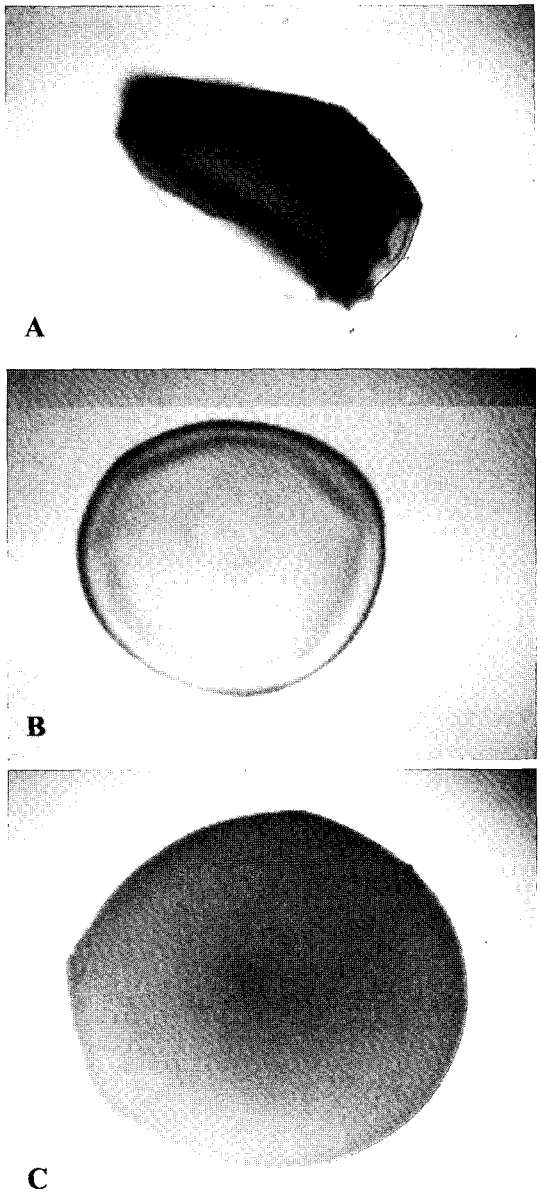


**Fig 2.** Morphology of chitosan beads. 1% chitosan bead (A), 2% chitosan bead that cefadroxil unloaded (B), 2% chitosan bead that cefadroxil loaded. Mean diameter was less than 1 mm.

highest ability in releasing the drug, whereas the beads in pH 10 of TPP solution had the lowest release.

### Effects of TPP concentration

Cefadroxil concentration from chitosan beads for various TPP concentrations was shown in Fig 5. The release rate of cefadroxil from chitosan beads decreased with the increased TPP solution concentration.

### Effects of cross-linking time

Chitosan beads were prepared by different cross-linking time of 15, 30, 60, and 120 minutes to determine the proper

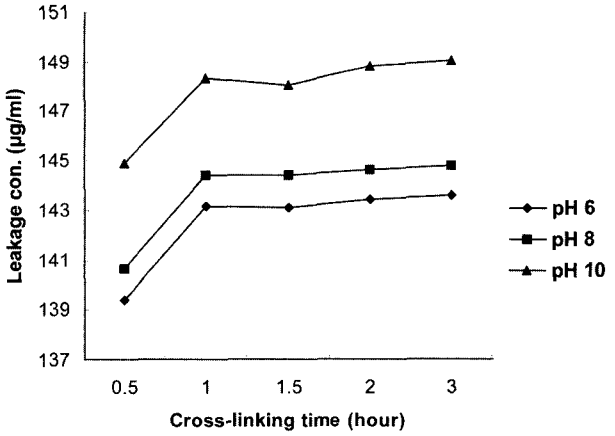


**Fig 3.** Leakage amount of cefadroxil from chitosan beads into TPP solutions of each pH 6, 8, and 10. TPP concentration was 5%.

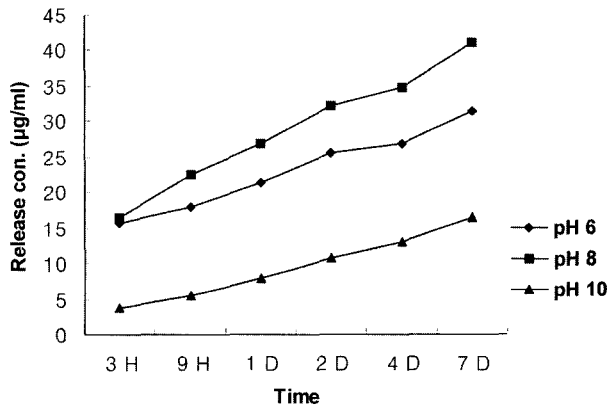


**Fig 4.** Release concentrations of cefadroxil from chitosan beads prepared at TPP solutions of each pH 6, 8, and 10 into PBS. TPP concentration was 5% and cross-linking time was 1 hour. H=hour; D=day.

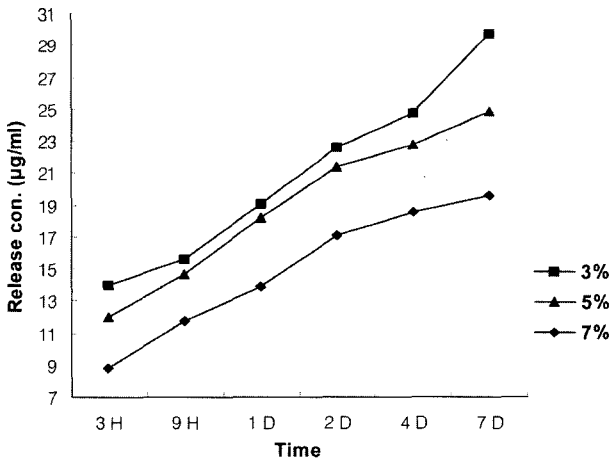


**Fig 5.** Release concentrations of cefadroxil from chitosan beads prepared at 3, 5, and 7% TPP solutions into PBS. The pH of TPP solutions was 8 and cross-linking time was 1 hour. H= hour; D=day.

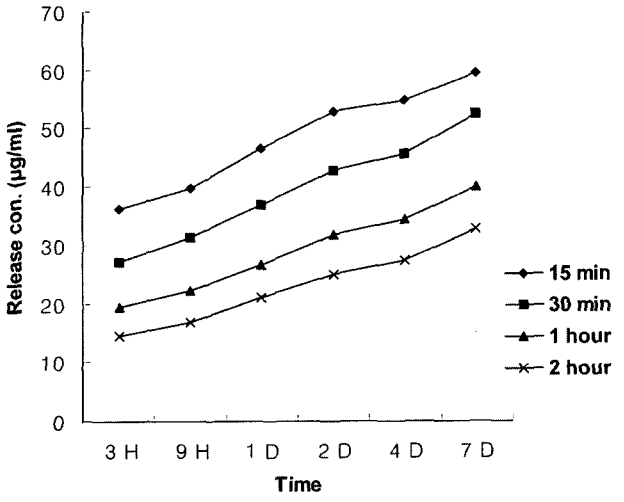


**Fig 6.** Release concentrations of cefadroxil from chitosan beads into PBS cross-linked for 15 and 30 minutes, 1 and 2 hours. The pH of TPP solutions was 8 and the concentration of it was 5%. H=hour; D=day.

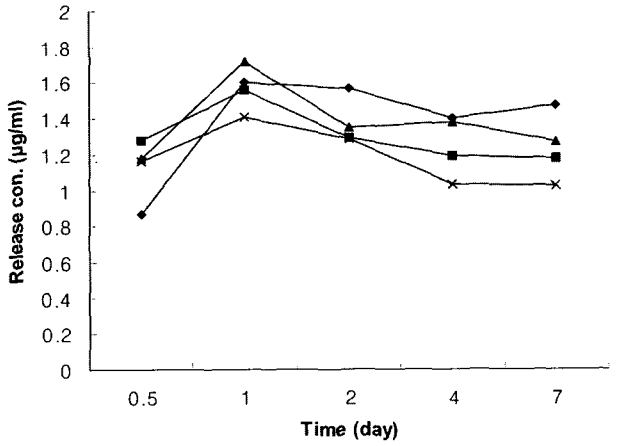


**Fig 7.** Serum concentration of cefadroxil in four dogs after implantation of cefadroxil loaded chitosan beads at a dose of 50 mg/kg body weight. The pH of TPP solutions was 8 and the concentration was 7% and cross-linking time was 15 minutes.

cross-linking time. The longer cross-linking time was, the less releasing drug was (Fig 6).

### Cefadroxil release *in vivo* from chitosan beads

Cefadroxil was released from chitosan beads gradually. The concentration in sera reached the peak at 24 hour and sustained more than 1 μg/ml except the serum of a dog at 12 hours. The drug release in sera of four dogs (Fig 7).

## Discussion

The polycationic polysaccharide, chitosan, forms gels with suitable mutivalent counterions. In this study, chitosan beads were prepared by ion interaction between the positively charged amino group in chitosan and negatively charged counterion, tripolyphosphate (TPP)[14,22,23]. 1% chitosan beads was prepared initially. That were crushed after be dried whereas, 2% chitosan beads was prepared spherically. That result indicates the concentration of chitosan solution affects density of chitosan beads. Bodmeier *et al*[14] reported that the shape and preparation of the beads were critically dependent on the viscosity of the chitosan solution. Because the viscosity of chitosan solution is increased with the concentration of chitosan solution, 2% chitosan beads were not crushed.

Entrapment efficiency is one of the critical parameters to be considered in the preparation of chitosan beads containing the drug. A minimum loss of cefadroxil during preparation would lead to a high loading efficiency of the beads and make the preparation process attractive for future applications in practice. Chitosan is a weak polybase, and the pKa was reported to be 6.3[22]. With the pH of the solution increasing, the ionization of amine groups decreased and hence cross-linking density of beads is decreased with increase of pH in TPP solutions. Thus, the leakage amount into TPP solutions was increased according as the pH of TPP solution was increased.

Charge density is important factor in electrostatic interaction and mainly depends on solution pH. The charge numbers of TPP and chitosan were related to solution pH and the electrostatic cross-linking of anions to chitosan was also influenced by solution pH[6]. Shu and Zhu[22] reported that increasing TPP solution pH led to quicker drug release. By the way, our results were different from that. The release level of cefadroxil from beads formed at pH 6, 8 TPP solutions were agreed with them, but one at pH 10 wasn't agreed. The reason may be considered that the initial loaded dose of beads at pH 10 TPP solution was not so enough that the release level was the lowest of them.

Some studies have been reported the release profile of drug from TPP-chitosan preparations decreased with the increased cross-linking agent concentration[3,26]. As they said, the increased concentration of TPP strengthens the density of chitosan matrix and the release of cefadroxil is decreased.

When chitosan solution was dropped into TPP solution, an ionic interaction occurred between tripolyphosphoric ions ($P_3O_{10}^{5-}$) and the amino group ($NH_3^+$) of chitosan, resulting in cross-linking[24]. Increased cross-linking time would increase the interaction time and lead to denser chitosan matrix. Therefore, increased cross-linking time is associated with the decreased release of drug.

Dogs with *Staphylococcus intermedius* pyoderma were treated successfully with 22-35 mg/kg cefadroxil given q12h for 3-6 weeks[20]. The cefadroxil MIC (minimum inhibitory concentration) for staphylococcal isolates was 1 μg/ml[15]. As shown in Fig 7, the concentration of sera due to chitosan beads implanted with 50 mg/kg body weight of beads was sustained more than 1 μg/ml for the whole 7 day period except the serum of a dog at 12 hours. Therefore, the cefadroxil

loaded beads can be used successfully in pyoderma of dogs.

These results indicate that cefadroxil loaded chitosan beads may become a potential delivery system to control the release of drug in dogs.

## Acknowledgement

This research was supported by Kyungpook National University Research Fund, 2000.

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# 개에서 키토산 비드를 이용한 cefadroxil 방출제어

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**요 약**: 본 연구는 개에서 키토산 비드를 이용한 cefadroxil 방출에 영향을 주는 인자, 약물을 함유한 최적의 키토산 비드의 제조, 그리고 키토산 비드로부터 약물의 방출을 평가하는 것이다. 키토산 비드는 tripolyphosphate (TPP)와 이온결합으로 생성되며 비드의 크기는 1 mm 미만이었다. 비드로부터 cefadroxil 방출은 여러 인자에 영향을 받는다. TPP의 pH가 감소할수록 cefadroxil의 비드내 함유량은 증가하지만, 비드로부터의 방출량은 감소한다. Cefadroxil의 방출속도은 TPP 농도가 증가할수록 감소한다. 결합시간이 길어지면, 방출량이 감소한다. Cefadroxil을 함유한 키토산 비드를 50 mg/kg 용량으로 건강한 개 4두의 피하에 이식한 결과, cefadroxil의 혈청내 농도는 1 μg/ml 이상으로 7일간 유지되었다. 따라서, cefadroxil을 함유한 키토산 비드는 개의 농피증 치료에 유용한 것으로 사료되며 약물방출을 통제할 수 있는 약물수송체가 이용될 수 있다고 사료된다.

**주요어** : chitosan, cephadroxil, dog.