

Note

Biodegradable implantable teicoplanin beads for the treatment of bone infections

İ. Yenice^a, S. Çalış^{a,*}, H.S. Kaş^a, M. Özalp^b, M. Ekizoğlu^b, A.A. Hıncal^a

^a Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, 06100 Ankara, Turkey

^b Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, 06100 Ankara, Turkey

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Abstract

Parenteral antibiotic therapy for acute bone infections, soft tissue infections and osteomyelitis may result in high serum concentrations, associated with nephrotoxic, ototoxic and allergic complications. After taking these above mentioned disadvantages into consideration, recent investigations have explored the use of antibiotic-loaded biodegradable implants, incorporating antibiotics for potential use in the treatment of bone infections. In this study, biodegradable implants containing teicoplanin for the prevention or the treatment of bone infections were designed by using sodium alginate as the polymer material. Therefore, teicoplanin, a glycopeptide antibiotic, active against gram-positive bacteria was incorporated in a natural polymer in order to prepare bead formulation for implantation purpose in bone for the localized treatment of osteomyelitis. In vitro characterization was realized by determining particle size, surface characteristics, loading capacity and in vitro release characteristics of the beads. © 2002 Elsevier Science B.V. All rights reserved.

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Osteomyelitis is still the cause of many problems in orthopaedics in terms of therapy and repeated infections. 4–6 week systemic antibiotic therapy is necessary along with bone and soft tissue debridement in the therapy of chronic osteomyelitis (Schmidt et al., 1995; Liu et al., 1999; Yenice, 2001b). Prolonged-release local antibiotic therapy has been taken into consideration due to

side effects encountered in long-term high-dose antibiotic use and the duration of hospitalization of the patients. Although local antibiotic therapy has been achieved by bone cement, a second surgical operation is needed for the removal of the system (Schmidt et al., 1995; Iannuccelli et al., 1996a,b; Liu et al., 1999; Yenice et al., 2001a). On the other hand, the use of heat in the preparation of bone cement limits the use of heat-sensitive active ingredients. Therefore, more recently there are a number of studies about bioabsorbable materials such as poly-lactic acid and ceramics to be

* Corresponding author. Tel.: +90-312-310-15-24; fax: +90-312-311-47-77

E-mail address: sucalis@tr.net (S. Çalış).

used as filling materials for osteomyelitis treatment (Schmidt et al., 1995; Iannuccelli et al., 1996b; Liu et al., 1999; Yenice, 2001b). However, poly-lactic acid devices could contain residual organic solvents used in the preparation, whereas ceramic materials are generally very brittle to handle (Schmidt et al., 1995; Iannuccelli et al., 1996b). Thus, it is suggested that natural polymers such as calcium alginate could be an alternative polymeric carrier for biodegradable, implantable antibiotic beads for the treatment of bone infections. Teicoplanin was selected as the drug because it is a new investigational glycopeptide antibiotic with a broad spectrum of activity covering most gram-positive aerobic and anaerobic organisms, including methicillin-resistant *Staphylococcus aureus*, which is the most frequent osteomyelitis-inducing microorganism. Teicoplanin does not seem to be ototoxic and appears less nephrotoxic and reported to be well tolerated in humans and animals (Shea et al., 1995).

In this study, teicoplanin, a glycopeptide antibiotic, active on gram-positive bacteria was incorporated in a natural biodegradable alginate polymer in order to prepare bead formulations for implantation purpose in bone for the localized treatment of osteomyelitis.

Teicoplanin was a gift from Hoechst Marion Roussel (Italy) sodium alginate was supplied by Pronova Biopolymers (Norway) and calcium chloride from Merck (Darmstadt, Germany), and Mueller Hinton agar (MHA) was obtained from Difco Laboratories (Detroit, MI, USA).

Alginate beads were prepared by physical cross-linking of calcium ions to sodium alginate polymer. For this purpose, sodium alginate was dissolved in distilled water at a concentration of 4 w/v%. To a 25 ml of alginate solution, 100 mg of teicoplanin (10%) was added and dispersed thoroughly by stirring. The dispersion was added dropwise by an injector to 0.1 M calcium chloride solution.

Characterization of teicoplanin–alginate beads was realized by determining particle size, surface characteristics, loading capacity, differential scanning calorimetry (DSC) analysis and in vitro release characteristics.

Particle size distribution of the beads was determined by sieve analysis (Endocott Ltd., London, UK) procedure.

Scanning electron microscopy (SEM) evaluation of the alginate beads was carried out to examine surface morphology. Beads were mounted on metal stubs with conductive silver paint and then sputted with a 150 Å thick layer of gold in a Bio-Rad apparatus. A scanning electron microscope (JOEL-SEM ASID-10 device in 80 kV) was used to evaluate surface characteristics. DSC was carried out by using Dupont DSC 910 instrument. The samples were heated in hermetically sealed aluminum pans at a rate of 10 °C/min from 25 to 200 °C in nitrogen atmosphere.

Thirty ml of phosphate buffer pH 7.4 was added to 250 mg of teicoplanin-incorporated alginate beads and kept in ultrasonic bath for 5 min. Then, this solution was stirred for 24 h at room temperature. After filtration with 0.22 µm Millipore filters, teicoplanin amount in filtered samples was measured by microbiological assay (Yenice, 2001b).

Fifty mg of alginate beads was weighed in 50 ml capacity of glass vials, and 25 ml of phosphate buffer pH 7.4 was added. Glass vials were placed in thermostated bath at 40 rpm at 37 °C. Samples were collected at various time-points and replaced by an equal volume of dissolution medium. Subsequently, released teicoplanin amount was determined by zone-inhibition measurements (Yenice, 2001b).

The particle size of alginate beads was measured as 1200 ± 400 µm, while the amount of loaded teicoplanin was determined as 6.6%. The particle size of the beads was affected by factors such as preparation technique, polymer concentration, needle size and stirring time (Takka et al., 1998; Fundueanu et al., 1999). SEM photographs revealed nearly spherical shaped beads which have a tight and rough surface, while they swell and became looser after in vitro release (Fig. 1a, b). Fig. 2 showed that in DSC thermograms teicoplanin did not display a distinct melting endotherm. For alginate beads, a peak at 200 °C was observed which might be interpreted as a deformation. However, the DSC thermograms do not provide sufficient data about the physico-chemical state of the drug within the micropartic-

ulate system due to the lack of melting or deformation peaks of both the drug and the polymer. The *in vitro* release profiles of teicoplanin beads revealed that 60% of the antibiotic was released at the end of 3 h, while 100% release was completed at the end of 8 h (Fig. 3). The antibiotic release of the teicoplanin from our alginate beads was faster than the sodium alginate-kind, containing a high proportion of mannuronic sequences capable of forming a complex with the drug (Iannuccelli et al., 1996a). It could be stated that preparation technique, dissolution medium, polymer concentration, bead size and material as polymer types,

and molecular weight of polymer were the parameters which might affect the *in vitro* release of alginate beads (Bodmeier et al., 1993; Takka et al., 1998; Fundueanu et al., 1999).

Although this period of release should be extended for the treatment of osteomyelitis, beads prepared from natural polymers offer the advantage of convenient implantation to bone due to their appropriate particle size. It might be concluded that beads prepared using a natural polymer, calcium alginate, might be a right choice for the treatment of osteomyelitis; however, further investigation is required.

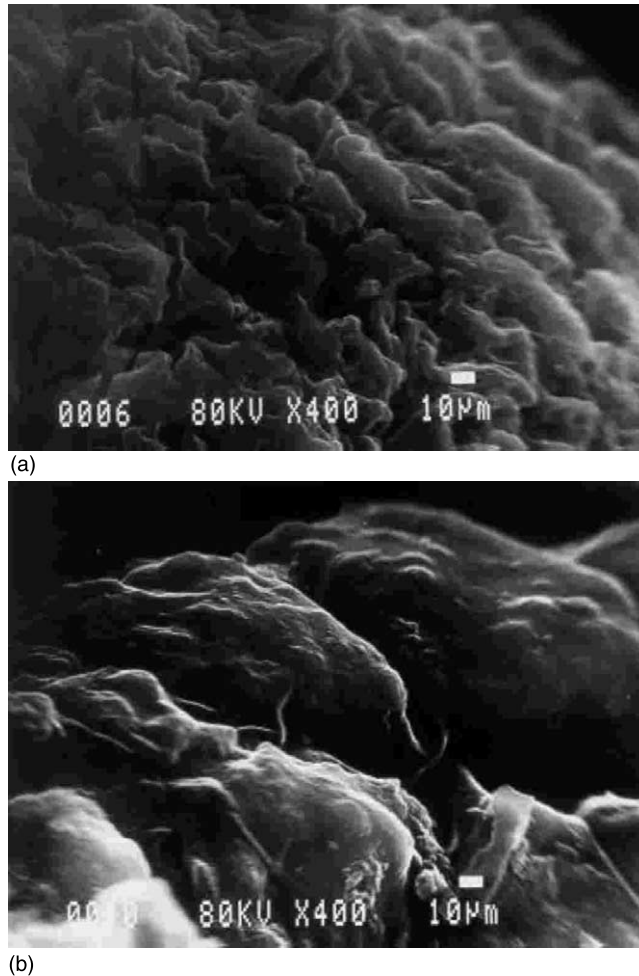


Fig. 1. SEM photographs of alginate beads (a) before release and (b) after release.

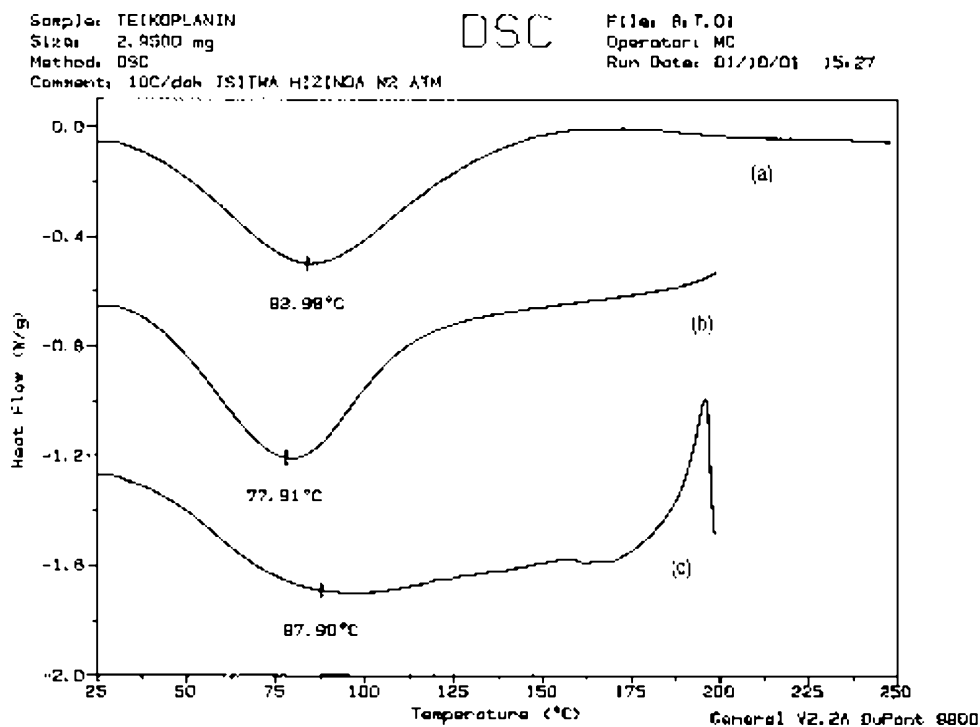


Fig. 2. DSC thermograms of (a) teicoplanin, (b) sodium alginate and (c) teicoplanin-loaded alginate beads.

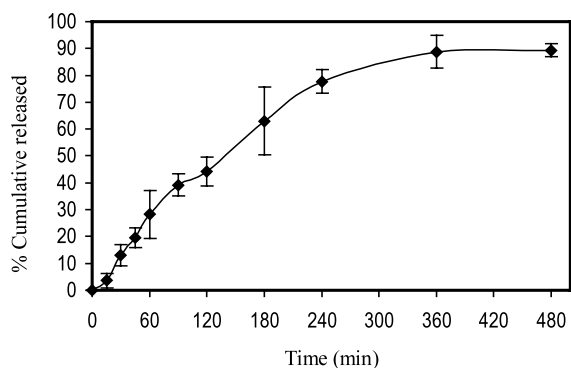


Fig. 3. In vitro release profile of teicoplanin-loaded alginate beads.

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