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Formulation and In Vitro Evaluation of Bisphosphonate Loaded Microspheres for Implantation in Osteolysis

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ABSTRACT Chitosan and poly(lactide-co-glycolide) acid (PLGA) microspheres loaded with alendronate sodium (AS) were prepared for orthopedic as well as dental applications. In orthopedics the aim was to make the total joint prostheses stay in the body for a long time without causing bone tissue loss, while in dentistry it was aimed to treat the alveolar bone resorption caused by periodontitis and also to make the dental treatment using implants easier by reducing the bone loss in patients with osteoporosis. Solvent evaporation method was used to prepare AS loaded PLGA microspheres and emulsion polimerization method was used to prepare AS loaded chitosan microspheres. Particle size, loading efficacy, surface characteristics, and in vitro release characteristics were examined on prepared formulations. After the examination of the scanning electron microscopy photographs of microspheres, chitosan microspheres were observed to have spherical structure and smooth surface characteristics while PLGA microspheres were observed to have spherical porous surface structure. Loading efficacy was found to be 3.30% for chitosan microspheres and 7.70% for PLGA microspheres. It was observed that 85% of AS had been released at the end of the third day from chitosan microspheres whereas 58% was released at the end of the fifth day from PLGA microspheres. It was found that chitosan microspheres gave first order release while PLGA microspheres gave zero order release.

KEYWORDS Alendronate sodium, Osteolysis, Implants, PLGA, Chitosan, Microspheres

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INTRODUCTION

Alendronate sodium (AS), an aminobisphosphonate, is a potent inhibitor of osteoclast-mediated bone resorption and is used for the treatment of bone disorders, osteoporosis, and Paget's disease of the bone (Lin et al., 1994). It may also be useful in the treatment of bone-related diseases including periodontal disease, malignant hypercalcemia, and orthopedic reconstruction

(Sharpe et al., 2001). Utilization of AS can facilitate reduction of bone loss associated with periodontal disease while preventing the bone loss and erosions in rheumatoid arthritis and loosening of joint prostheses. A third generation bisphosphonate, AS has been approved by the Food and Drug Administration (FDA) for the treatment of diseases characterized by osteoclastic bone resorption (Rosen & Kessenich, 1996). Alendronate sodium (AS) has two disadvantages, firstly its poor absorption (less than 1%) from the gastrointestinal tract because of its poor lipophilicity and large molecular size, and secondly, its association with adverse gastrointestinal effects in humans (Lin et al., 1994).

In parenteral routes of administration, it has been mentioned that the sodium salt caused serious pain and tissue necrosis at the site of injection; other methods of delivery are limited due to the relatively high dose required and local side effects. Therefore, local implantation of bisphosphonates can prevent local irritation and tissue necrosis associated with intramuscular injection of bisphosphonates. The implantation studies on bisphosphonates and their controlled release formulations have been mentioned in the literature (Ezra & Golomb, 2000; Patashnik et al., 1997). The controlled release of bisphosphonates as novel anticalcification agents from a biodegradable implant (chitosan matrices) as well as formulation and evaluation of a controlled release drug delivery system have been reviewed (Golomb et al., 1992). Patashnik et al. (1997) described the preparation and evaluation of chitosan microspheres to be used locally as an implant for the treatment of bone disorders. Also in literature, Perugini et al. (2001) described the formulation of a biodegradable microparticulate drug delivery system containing clodronate, a bisphosphonate intended for the treatment of bone diseases.

Aseptic loosening of implant components is a major complication of total joint replacements (Kim et al., 1993). Polymethylmethacrylate bone cement and other biomaterial wear particles are thought to contribute to the osteolysis of aseptic loosening (Sabokbar et al., 1998). Since implant-related bone loss appears to be the result of osteoclastic resorption, it has been thought that osteolysis might be controlled by the administration of bisphosphonates. It was mentioned in the literature that AS may also be administered in the treatment of osteolysis induced by particles of orthopedic wear debris (Mochida et al., 2002).

In this study, chitosan and poly(lactide-co-glycolide) acid (PLGA) microspheres loaded with AS for implantation were prepared and particle size, drug loading, surface characteristics, and in vitro release characteristics were examined on prepared formulations. The aim of this study was to formulate and evaluate the AS-loaded microspheres for implantation in the treatment of osteolysis in orthopedics (by providing high local drug levels while avoiding the systemic therapy) and also in the treatment of bone loss in the area of dental prostheses.

MATERIALS AND METHODS

Materials and Reagents

Alendronate sodium trihydrate was obtained from Dabur India Limited (Ghaziabad, India) (Batch No.: MAT-2/F04/01). Chitosan medium molecular weight (m.w.) (m.w.: 272.000 g/mol, degree of deacetylation: 84%) was supplied from Fluka (Buchs, Switzerland). Poly (lactide-coglycolide) (PLGA) 50:50 (m.w.: 34.000) was supplied from Medisorb, Dupont Merck (Wilmington, DE, USA). Diethylether (extra pure 99.5% >), glacial acetic acid (99–100%), glutaraldehyde solution 25%, n-hexane (min. 95%), Tween 80, dichloromethane [high performance liquid chromatography (HPLC) grade], citric acid anhydrous, and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Liquid paraffin was obtained from Aromas Food & Chemical Industry Ltd. (Izmir, Turkey). Sodium oleate (SO) and polyvinyl alcohol (PVA) (Av. m.w.: 30.000–70.000) were supplied from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of Chitosan Microspheres

The chitosan microspheres containing AS were prepared by an emulsion polymerization technique (Arica et al., 2002a; Patashnik et al., 1997). For CH-MS-1 formulation, 2% chitosan solution was prepared in 2% acetic acid solution into which AS was added. Thirteen milliliters of this solution was dispersed in 50 mL of liquid paraffin containing Tween 80 [2% (w/v)]. It was then stirred using a mechanic mixer (Heidolph RZR, Germany) at 500 rpm for 2 min. Twenty five percentage glutaraldehyde solution (12 mL) was then added to this medium and the stirring continued for 30 min, following which an additional 25%

TABLE 1 The Experimental Conditions for the Preparation of Chitosan and PLGA Microspheres

Formulation code	Polymer type	Polymer concentration (%)	Amount of cross-linking agent (ml)	pH of dispersion medium	Theoretical drug loading (%)
CH-MS-1	Chitosan	2	24	—	10
CH-MS-3	Chitosan	2	2	—	10
PLGA-MS-1*	PLGA	3	—	—	10
PLGA-MS-2*	PLGA	3	—	2	10
PLGA-MS-3*	PLGA	3	—	6.8	10
PLGA-MS-4*	PLGA	3	—	12	10
PLGA-MS-5**	PLGA	1.5	—	—	10

*Microspheres prepared using o/w single emulsion-solvent evaporation method.
**Microspheres prepared using w/o/w double emulsion-solvent evaporation method.

glutaraldehyde solution (12 mL) was added. The stirring process continued for 3 h until microspheres were obtained. They were then gathered by centrifugation, washed with n-hexane once and with ether three times, and dried at room temperature. For CH-MS-3 formulation, 6 mL of chitosan solution that contained AS was dispersed in liquid paraffin and 2 mL glutaraldehyde solution was added totally. The only difference between chitosan formulations (CH-MS-1 and CH-MS-3) was the amount of glutaraldehyde solution included in prepared formulations. Blank microspheres were prepared similarly for use as a control in the characterization studies. The experimental conditions and preparation details of chitosan microspheres are summarized in Table 1.

Preparation of Poly(lactide-co-glycolide) Microspheres

The Poly(lactide-co-glycolide) (PLGA) microspheres were prepared using o/w (oil/water) single emulsion–solvent evaporation method (Arica et al., 2002b; Yenice et al., 2003), and w/o/w (water/oil/water) double emulsion–solvent evaporation method (Bozdag et al., 2005). Blank microspheres were prepared similarly for use as a control in the characterization studies. The experimental conditions and preparation details of PLGA microspheres are summarized in Table 1.

O/W Emulsion Solvent Evaporation Method

After dissolving PLGA (50:50) (300 mg) in dichloromethane, AS (30 mg) was dispersed in this polymer solution. This dispersion was then added to 100 mL of aqueous phase containing 0.4% (w/v) of PVA and 0.1% (w/v) of SO, and stirred (750 rpm) at room temperature for 2 h until the evaporation of dichloromethane was completed. PLGA-MS-1 formulation was prepared without changing the pH of the aqueous continuous phase. For PLGA-MS-2, PLGA-MS-3, and PLGA-MS-4 formulations, the pH of the aqueous continuous phase was adjusted to 2, 6.8, and 12, respectively, using 0.2 N HCl solution and 0.2 N NaOH solution. Microspheres formed at the end of this process were collected by centrifugation, washed with distilled water, and dried at room temperature.

W/O/W Multiple Emulsion Method

To prepare w/o/w microspheres, AS (15 mg) was dissolved in distilled water and this solution was dispersed into a solution of PLGA (150 mg) in 1.8 mL of dichloromethane. It was vortexed for 2 min to form primary w/o emulsion. This primary emulsion was then added dropwise into the PVA solution (6%) in distilled water and it was stirred (300 rpm) using a mixer at 15°C to form w/o/w multiple emulsion. In order to extract dichloromethane, this solution was added to the aqueous solution (35%) of PVA and stirred (300 rpm) at room temperature for 1 h. Microspheres obtained after this process were washed with distilled water and dried at room temperature.

Characterization of the Formulations

Instrumental Parameters and Chromatographic Conditions

Chromatographic analyses were performed using a high performance liquid chromatography (HPLC)

system (Agilent Technologies HPLC 1100) in which a reverse phase (PRP-1) column (10 μm particle size, 250 × 4.1 mm, i.d., Hamilton, NV, USA) was used. The mobile phase, which was a mixture of 0.05 M sodium citrate and sodium phosphate buffer (pH 8.0) – acetonitrile – methanol (75:20:5, v/v/v), was delivered at a flow rate of 1.0 mL/min at room temperature. Detection of the 9-fluorenyl chloroformate derivative was carried out using spectrophotometric detector (DAD) at 266 nm. Derivatization was required to realize the quantitative analysis of AS (De Marco et al., 1989). As previously described, derivatization procedure was followed for determination of AS amount in the microspheres and of the amount released from the microspheres to the medium (Samdancioglu et al., 2003).

Determination of the Amount of Alendronate Sodium Loaded in Microspheres

For each formulation of PLGA and chitosan microspheres, the amount of AS loaded in the microspheres was determined by HPLC at 266 nm as previously described in the literature (Samdancioglu et al., 2003).

Particle Size Distribution of Microspheres

The particle size distribution of both chitosan and PLGA microspheres was measured using HELOS Laser Diffraction Particle Size Analyzer (Sympa, Germany). Before measuring the particle size of samples, they were dispersed in distilled water containing 1% Tween 80.

Surface Morphology of Microspheres

A scanning electron microscope (SEM) (Jeol-SEM 6400, Japan) was used to evaluate the surface characteristics of both chitosan and PLGA microsphere formulations. Samples were mounted on metal stubs with conductive silver paint and then splattered with a 150 Å thick layer of gold in a Bio-Rad apparatus, and SEM photographs of the samples were taken.

In Vitro Release Studies

In vitro release of AS from PLGA and chitosan microspheres was determined at pH 7.4 in 0.1 M sodium citrate solution. The in vitro release profiles of samples were investigated by placing microspheres (10 mg for CH-MS-3 and 100 mg for PLGA-MS-1) in 5 mL of 0.1 M sodium citrate solution (pH 7.4) in vials placed in a horizontally shaken water bath at 37 ± 0.5 °C(rotated at 50 rpm). Aliquots (1.5 mL) were withdrawn at predetermined times and replaced with fresh medium. Aliquots were filtered through a membrane filter with a pore size of 0.22 μm and this solution was then transferred to polypropylene tubes for derivatization and analyzed using HPLC. The results were taken as the average of six readings. The same procedure was followed in the in vitro release study of AS powder to compare the release profiles. Ten milligram of AS powder was placed in a vial containing 50 mL of 0.1 M sodium citrate solution (pH 7.4) and aliquots were analyzed using HPLC.

RESULTS AND DISCUSSION

Chitosan Microspheres

Chitosan microspheres seem highly suitable for use in implantable drug delivery systems for treating bone diseases since they are osteoconductive. In the literature, chitosan microspheres containing pamidronate, which is an important member of the bisphosphonates, were prepared by a w/o emulsion method (Patashnik et al., 1997). In our studies, chitosan microspheres that included AS were prepared by an emulsion polymerization method using glutaraldehyde as the cross-linking agent (Arica et al., 2002a). In pre-formulation studies, microspheres were prepared using different brands of chitosan having different molecular weights. Amount of chitosan, surfactant type, and stirring rate were the optimized parameters. Following the preformulation studies, 2% (w/v) chitosan solution was prepared in 2% acetic acid solution to form microspheres (Arica et al., 2002a; Patashnik et al., 1997) and Tween 80 was used as emulsifying agent. During these studies, the stirring rate was kept constant at 500 rpm, whereas the amount of chitosan solution that was dispersed in oil phase (12 mL for CH-MS-1 and 6 mL for CH-MS-3) and the amount of cross-linking agent (24 mL for CH-MS-1 and 2 mL for CH-MS-3) were changed.

PLGA Microspheres

In therapeutic use, implantation of PLGA microspheres may improve the treatments by possible localization of the drug at the site of action and by

prolonged release of drugs (Ghaderi et al., 1996). It has been reported in literature that the physical state of the drug in the polymer solution has an important role in the encapsulation efficiency and other properties of microspheres (O'Donnell & McGinity, 1997). Alendronate sodium (AS) is very soluble in water, but insoluble in organic solvents. Therefore, in the preparation of AS-loaded PLGA microspheres, AS did not dissolve in organic solvent or co-solvent, but it was dispersed into the polymer solution (PLGA-MS-1). For a water-soluble active substance, the entrapment efficiency might be low due to the partitioning of the drug to the external aqueous phase during the evaporation process (Ghaderi et al., 1996). The encapsulation of water-soluble active substances by the o/w solvent evaporation method will generally result in a rapid partitioning of the drug into the aqueous phase from the organic phase (Watts et al., 1990). Because of the loss of the drug in the aqueous phase, the solvent evaporation method is limited to water-insoluble drugs or to drugs for which the solubility in the external aqueous phase could be minimized by pH adjustment (Alex & Bodmeier, 1990). Moreover, the drug will diffuse out into the external aqueous phase, resulting in low loading of the drug in the microspheres. It was reported in the literature that reducing the solubility of a small peptide (with seven amino-acids) in the external phase resulted in a better encapsulation; the same strategy has also been applied to a protein by adjusting the pH to values close to the pH at which the protein is characterized by a low water solubility (Leo et al., 1998). For drugs with pH-sensitive solubility, it may be possible to reduce partitioning by adjustment of the aqueous phase pH (Watts et al., 1990). Buffering the internal and/or external aqueous phase could also affect the ionization of the drug and polymer (Herrmann & Bodmeier, 1995). In order to encapsulate AS in microspheres and to increase the loading efficacy, microsphere formulations were also prepared by changing the pH value of the external phase. The pH of the external phase was adjusted to 2 (PLGA-MS-2) and 6.8 (PLGA-MS-3) by using 0.2 N HCl solution and to 12 (PLGA-MS-4) by using 0.2 N NaOH solution. Since AS has 5 pKa values (Lin et al., 1994), the solubility did not change by changing the pH value, and the loading efficacy was found to be low. During the preparation of microspheres using o/w single emulsion-solvent evaporation method, PVA and SO were used as polymeric stabilizers to stabilize the polymer droplets to prevent aggregation of microspheres in a 4:1 ratio mixture and dichloromethane, which is the most widely used solvent for producing microspheres by the emulsion–solvent evaporation method was used (Watts et al., 1990). It was reported in the literature (Tuncay et al., 2000) that during the preparation process, 2 h stirring time would be adequate for the complete evaporation of dichloromethane from the medium (Watts et al., 1990). The double emulsion/solvent evaporation method (w/o/w) is one of the most useful approaches for entrapping water-soluble compounds (Leo et al., 1998; Ghaderi et al., 1996). In literature, PLGA microspheres containing clodronate, a member of bisphosphonates, were prepared with PLGA copolymers of various molecular weights and molar compositions by a w/o/w double emulsion solvent evaporation process because of its high water solubility (Perugini et al., 2001). In our study, when preparing the microspheres, the w/o/w double emulsion–solvent evaporation method was used to increase the loading efficacy in PLGA microspheres.

The Characterization of Microspheres

Data Concerning Loaded Alendronate Sodium in Chitosan Microspheres

Since AS does not demonstrate any intrinsic UV properties, derivatization was required to realize the quantitative analysis of AS by HPLC. In order to determine the amount of AS loaded in chitosan microspheres, those microspheres were first degraded in 5 mL 1% citric acid solution. Since the pH value of the obtained eluent was acidic, 0.1 M sodium citrate solution was added to make the solution suitable for derivatization. Drug loading efficacy was found to be 1.10% and 3.30%, respectively, for CH-MS-1 and CH-MS-3 formulations. It was observed that in both CH-MS-1 and CH-MS-3 formulations, the amount of loaded drug had decreased in contrast to the increased amount of glutaraldehyde, and was found to be lower than in PLGA microspheres in both formulations. The amount of the cross-linking agent affected the drug loading capacity of microspheres. Drug loading efficacies are shown in Table 2. Chitosan was cross-linked through Schiff's salt formation between aldehyde groups of glutaraldehyde and amine groups of chitosan by adding a glutaraldehyde solution (Thanoo et al., 1992; Hennink & Nostrum, 2002). A reaction, called self-linking in the

TABLE 2 The Characteristics of Chitosan and PLGA Microspheres

Formulation	Mean particle size* (μm)	Loading efficacy (%)	Yield value (%)
CH-MS-1	111.28 ± 0.17	1.10	65
CH-MS-3	109.28 ± 0.63	3.30	70
PLGA-MS-1	39.13 ± 0.30	7.70	77
PLGA-MS-2	74.36 ± 0.47	2.20	50
PLGA-MS-3	36.81 ± 1.66	1.65	62
PLGA-MS-4	54.65 ± 0.89	7.15	56
PLGA-MS-5	33.98 ± 0.52	7.26	46

n = 3 (samples assayed in triplicate), mean.
*Mean ± SD.

literature, was observed between the amine group of doxorubicin and glutaraldehyde. Glutaraldehyde reduced the free doxorubicin concentration in the implants probably due to the cross-linking with the drug (Fan & Dash, 2001). Since 5-fluorouracil (5-FU) derivatives used in the formulations also contained a terminal amine, glutaraldehyde addition indiscriminately bound the active agent to the polymer as it did between chitosan chains, causing drug immobilization rather than encapsulation (Ravi Kumar, 2000). Thus, during the release studies, it was reported that the amount of 5-FU was decreased by immobilization in the delivery system. In our study, it was considered that a reaction between aldehyde groups of glutaraldehyde and amine groups of AS to form Schiff's salt might have taken place, and this might have caused the decrease in the amount of loaded AS in microspheres.

Data Concerning Loaded Alendronate Sodium in PLGA Microspheres

Drug loading efficacies of AS in PLGA microspheres for PLGA-MS-1, PLGA-MS-2, PLGA-MS-3, and PLGA-MS-4 were found to be 7.70%, 2.20%, 1.65%, and 7.15%, respectively. The solvent evaporation method, in which the drug was dissolved in the organic polymer solution followed by emulsification into an external aqueous phase to form an o/w emulsion, did not result in higher encapsulation efficiencies. Because of its high water solubility, the drug could be partitioned into the external phase during microsphere preparation, and this caused a low drug loading in microspheres. After examination of the PLGA microspheres, a higher yield was observed on microspheres prepared with PLGA-MS-1. Drug loading efficacy of AS in PLGA-MS-5 microspheres prepared by w/o/w emulsion–solvent evaporation method was found to be 7.26%. Using a w/o/w double emulsion–solvent evaporation method also did not increase the drug loading in PLGA microspheres. pH adjustment during the aqueous phase was another attempt to increase the drug loading of samples; however, no significant increase was observed. We believe that the five different pKa values of AS might have had an effect on this result.

Particle Size Distribution of Microspheres

The mean particle sizes of the formulated AS microspheres are shown in Table 2. Samples with a large particle size can be suitable for local implantation. In order to avoid the inconvenient surgical insertion of large implants, injectable biodegradable and biocompatible polymeric particles could be employed for controlled release dosage form (Jain et al., 1998). Microparticles of size less than 250 μm, and ideally less than 125 μm, are suitable for this purpose (Jain, 2000). Following the pre-formulation studies, 500 rpm was selected as the appropriate stirring rate to obtain particles with spherical structure and size to be applied locally as an implant. In CH-MS-1 and CH-MS-3 formulations, results for particle size were found to be 111.28 μm and 109.28 μm, respectively. As can be seen from the results, the particle sizes of chitosan microspheres seem to be suitable for local implantation. In all PLGA formulations, particle sizes were found to be 39.11, 74.36, 36.81, and 54.65 μm for PLGA-MS-1, PLGA-MS-2, PLGA-MS-3, and PLGA-MS-4 microspheres, respectively. Particle size of PLGA-MS-5 microspheres was found to be 33.98 μm. During the preparation of o/w single emulsion-solvent evaporation method, the stirring rate and the amount of polymer and drug were kept constant; only the pH value of external aqueous phase was changed. Therefore, pH value of external aqueous phase might have had an effect on the particle size of PLGA-MS-1, PLGA-MS-2, PLGA-MS-3, and PLGA-MS-4 formulations.

Surface Morphology of Microspheres

Scanning electron microscope (SEM) photographs were informative about the morphology and surface

properties of the microspheres. The Scanning electron microscope (SEM) photographs of chitosan microspheres (CH-MS-3) are shown in Fig. 1. In both CH-MS-1 and CH-MS-3 formulations, microspheres appeared to have spherical structure and smooth surface. During the preparation of chitosan microspheres, 25% glutaraldehyde solution was used as a cross-linking agent. When the amount of glutaraldehyde solution was increased, the cross-linking was also increased, resulting in the formation of a rigid structure. Therefore, a smooth surface could be an effect of the glutaraldehyde solution. The scanning electron microscope (SEM) photographs of PLGA microspheres (PLGA-MS-1) can be seen in Fig. 2. When the SEM photographs of PLGA-MS-1 formulation used in the release studies were examined, it was observed that microspheres had spherical and porous surface structures. These results were in accordance with the results of Arica et al. (2002b). Spherical structure was

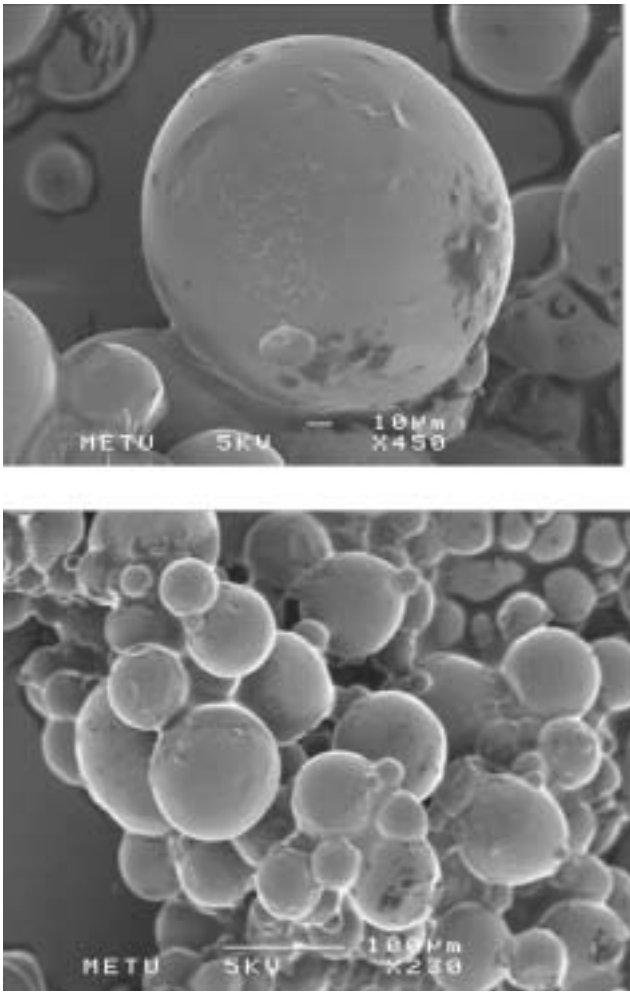

FIGURE 1 The SEM Photographs of AS-loaded CH-MS-3 Microspheres.

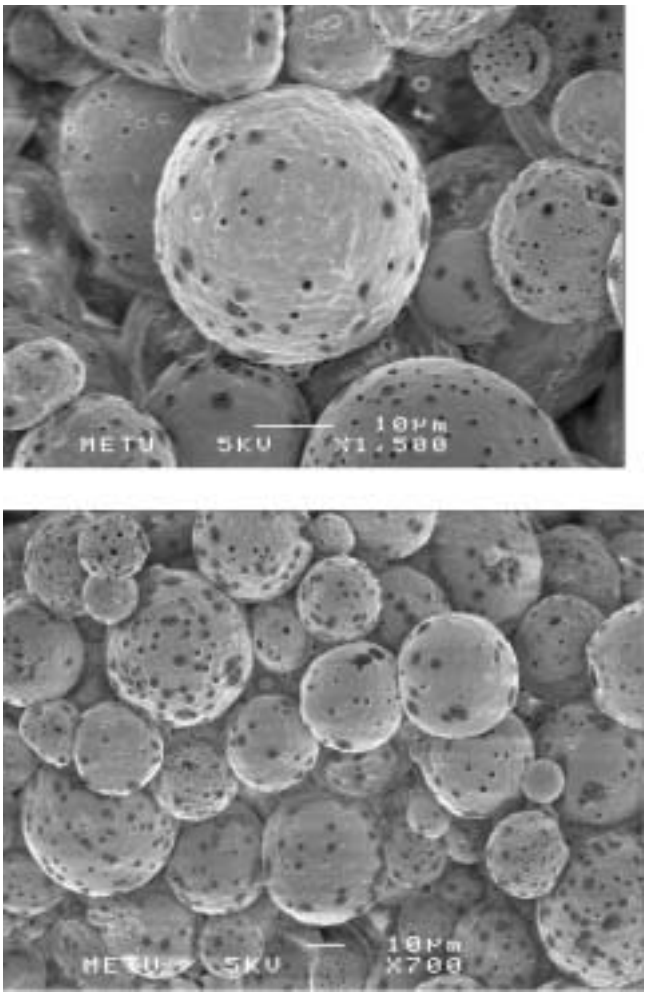

FIGURE 2 The SEM Photographs of AS-loaded PLGA-MS-1 Microspheres.

observed in the SEM photographs of PLGA-MS-2, PLGA-MS-3, and PLGA-MS-4 formulations, which had been prepared at different pH values, and they had less pores on their surface than the PLGA-MS-1 formulation. Poly(lactide-co-glycolide) (PLGA) microspheres (PLGA-MS-5) prepared by the solvent evaporation method using double emulsion were spherical but they had less pores on the surface. Therefore, it was observed that the PLGA-MS-1 formulation had a porous structure, while PLGA-MS-2, PLGA-MS-3, PLGA-MS-4, and PLGA-MS-5 formulations, which had been prepared by applying different pH values, had a less porous structure. Scanning electron microscope (SEM) photographs revealed that PLGA microspheres (50:50 m.w. 34.000) had a characteristic porous structure on the surface, in accordance with the results of Tuncay et al. (2000). For PLGA microspheres, very rapid solvent evaporation may

lead to formation of porous structures on the microsphere (Jain, 2000); addition of buffers or salts to the internal aqueous phase may also lead to formation of porous microspheres (O'Donnell & McGinity, 1997). Scanning electron microscope (SEM) photographs revealed that microspheres were homogeneous and had a spherical surface in all formulations.

In Vitro Release Studies

The profile of drug release studies from microspheres is shown in Fig. 3. Hundred percent of the AS was dissolved in 1 h. The release studies of chitosan microspheres with CH-MS-3 formulation showed that 85% of AS had been released from chitosan microspheres at the end of the third day. For chitosan microspheres, 50% of loaded AS was released in the first day, and agent AS released from cross-linked chitosan microspheres was characterized by an initial rapid release of AS. Thirty percent of total drug was released from the microspheres within 6 h. It was considered that cross-linking effectively controls drug diffusion from the microspheres. The PLGA-MS-1 formulation was used in the in vitro release studies of PLGA microspheres, and it was observed that 58% of AS had been released from PLGA microspheres at the end of the fifth day. Approximately 25% of total drug was released from microspheres within 75 h. Alendronate sodium (AS) was subjected to goodness of fit analysis. At the end of this analysis, it was found that chitosan microspheres gave first order release, while PLGA microspheres gave zero order release.

The release patterns of AS from microspheres were compared with the dissolution patterns of the AS powder in pH 7.4 0.1 M sodium citrate solution. While the AS powder dissolved completely within 1 h, slow release was obtained from the microspheres, and this may be a promising result for local use in implantation. According to these studies, drug loading of samples and the release studies can be considered inadequate, but the behavior and the release characteristics of polymers like chitosan and PLGA in "in vivo" medium may be different from the "in vitro" medium. As a result, release characteristics may change and prolongation of the drug release may occur.

CONCLUSION

Alendronate sodium (AS) is a potent anti-resorptive agent especially useful for the prevention and treatment of osteoporosis. However, since osteoporosis is a systemic disease, it would be difficult to apply these microspheres locally to treatment regions as implants.

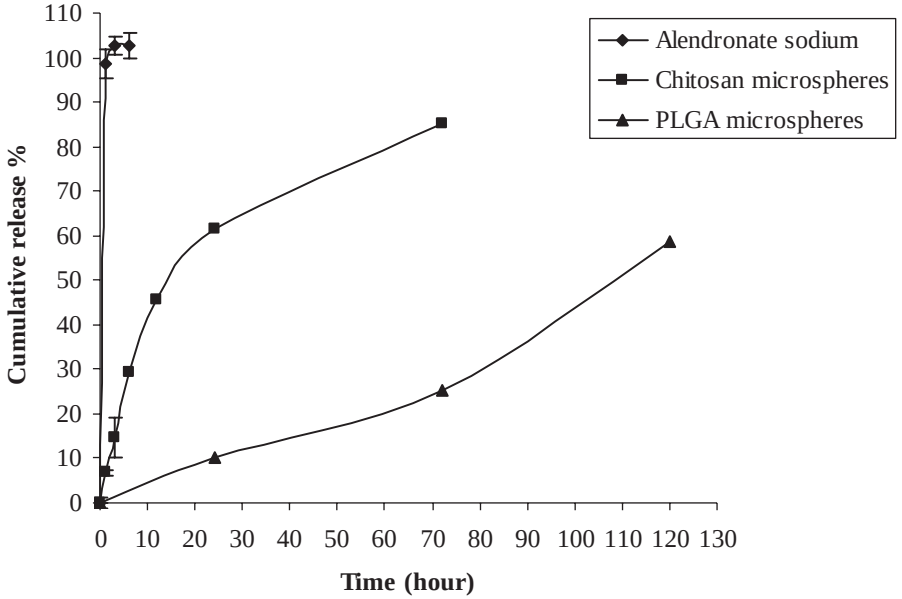

FIGURE 3 The Dissolution Profile of AS Powder (◆), and the Release Profiles of AS from Chitosan Microspheres (■) and from PLGA Microspheres (▲). $n = 6$, mean ± SD.

This study can provide the local delivery of AS to specific regions in dental and orthopedic application. Local application of microspheres as implants may prevent bone resorption during dental and orthopedic procedures. Therefore, AS-loaded microspheres can be used for the treatment of osteolysis in orthopedics. This study has shown that the emulsion polymerization method for chitosan microspheres and the solvent evaporation method for PLGA microspheres can be employed as delivery systems for AS in in vitro studies. It was considered that AS-containing microspheres seem to be promising for local application in osteolysis. Further in vivo studies are in progress.

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