Original Paper

Formulation and Characterization of Ciprofloxacin loaded PLGA Microspheres for Applications in Orthopedic Infections

ANDREEA-GABRIELA MOCANU[1], IONELA BELU[2], O. CROITORU[3], MARIA VIORICA CIOCÎLTEU[3], C.V. MANDA[3], J. NEAMTU[3]

[1]University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Doctoral School

[2]University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Department II of Pharmacy

[3]University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Department I of Pharmacy

ABSTRACT: Purpose-Osteomyelitis is a bone infection that appears as a complication after a fracture or orthopedic surgery. Ciprofloxacin is a broad spectrum antibiotic that can be used in local drug delivery systems for the treatment of bone related infections due to its bactericidal activity against both Gram-negative and Gram-positive bacteria. The purpose of the present study was to include ciprofloxacin in poly (lactic-co-glycolic acid) (PLGA) microspheres. Material and methods-Microspheres were prepared by both water/oil/water (w/o/w) solvent evaporation method and solid/oil/water (s/o/w) dispersion solvent evaporation method. The obtained microspheres were characterized by Fourier Transform Infrared Spectroscopy. High performance liquid chromatography method was deployed to determine the encapsulation ratio. Results-The solvent evaporation method chosen for this experiment resulted in microspheres with good entrapment efficiency. Furthermore the microspheres obtained by the s/o/w method displayed better entrapment efficiency. Conclusion-The particles obtained through the s/o/w technique should be further investigated in order to develop a local drug delivery system.

KEYWORDS: *ciprofloxacin-PLGA microspheres, solvent evaporation, orthopedic infections, osteomyelitis, local drug delivery*

Introduction

Orthopedic infections are still a major concern in orthopedic surgery. Furthermore they have a significant relapse rate [1,2]. Osteomyelitis is the medical term used when referring to a bone infection. Osteomyelitis may occur as a complication after orthopedic surgery or a traumatism (e.g. fracture) or through the bloodstream. Moreover chronic osteomyelitis is characterized by a poor vascular perfusion and necrosis of the bone which makes it difficult to treat [2-5].

Staphylococcus aureus is the most prevalent species and is accounted for 20-30% of the orthopedic infections. Furthermore antibiotic resistant bacteria are a major concern regarding the treatment of orthopedic infections. Methicillin-resistant *Staphylococcus aureus* orthopedic infections are considered to have a higher mortality due to the limited treatment possibilities [2,4,6-8].

Biofilms are formed by different microorganisms on the surface of orthopedic devices. Bacteria found in biofilms are less susceptive to antibiotics due to a series of factors such as low growth rate and antibiotic resistant bacterial subpopulations. Therefore considerably higher concentrations of antibiotics are required to eliminate the bacteria found in biofilms [4,9].

The current approach for the treatment of orthopedic infections implies debridement of the bone and surrounding area followed by surgical revision treatment and prolonged systemic antibiotic therapy (minimum 4 to 6 weeks). The prolonged systemic treatment may determine adverse systemic reactions and affect vital organs [1,9-11]. Moreover the concentration of the antibiotic at the infection site may not be sufficient to eradicate the biofilm. This approach implies high costs and the risk of the patient getting a functional impairment [2].

Local delivery of the antibiotic at the infection site eliminates the systemic treatment adverse reactions and disadvantages [10]. Local drug delivery systems ensure local antibiotic concentrations that exceed the minimum inhibitory concentration (MIC) [12].

Antibiotic poly (methyl methacrylate) (PMMA) beads can be used for the treatment of osteomyelitis. The major disadvantage is that because PMMA is a non-biodegradable material, a second surgery is required for its removal. Furthermore there is a possibility for the biofilm to adhere and develop on the surface of the PMMA beads [10].

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Microspheres are drug delivery systems that are specifically formulated to ensure a prolonged and controlled delivery of the drug. This improves bioavailability and facilitates release of the drug with a predetermined rate at a specific site [13-15].

For this study the method used for ciprofloxacin encapsulation considering its hydrophilic character was solvent evaporation technique with two variations: water/oil/water (w/o/w) double emulsion solvent evaporation and solid/oil/water (s/o/w) dispersion solvent evaporation [16,17].

Ciprofloxacin (CIP) is a broad spectrum fluoroquinolone antibiotic that has a bactericidal activity against both Gram-negative and Gram-positive pathogens thus having a high therapeutic efficiency. Furthermore, ciprofloxacin has tolerable side effects [16,18,19].

Poly (lactic-co-glycolic) acid (PLGA) is a synthetic polymer that combines poly (L-lactic acid) and polyglycolide and is both biocompatible and biodegradable. This means that its degradation byproducts are nontoxic. PLGA has a series of advantages such as adaptability for different formulation techniques, surface modification to ensure targeted drug delivery that make it fit for microsphere preparation [20-23].

Considering these facts and the constant growing number of antibiotic-resistant bacteria, it is clear that orthopedic infections will continue to be an impediment.

The aim of the study is to formulate ciprofloxacin-PLGA microspheres with a high incorporation rate for the later development of an orthopedic local drug delivery system.

Material and methods

Materials

PLGA (50:50) was purchased from Sigma-Aldrich, polyvinyl alcohol (PVA) and ciprofloxacin were purchased from Merck.

All the other chemicals used were of analytical grade

Microencapsulation

PLGA microparticles were prepared by two methods:

-The w/o/w double emulsion solvent evaporation technique where 20mg CIP were dissolved in 0.5ml water. The oil phase was obtained by dissolving 0.5g of PLGA into 9g of methylene chloride with the addition of the w/o emulsifier (span 80). The water phase was added to the oil phase and mixed at 30000rpm resulting in the water/oil (w/o) primary emulsion. A 0.1% polyvinyl alcohol solution was prepared. The w/o emulsion was mixed with polyvinyl alcohol solution at 30000 rates per minute (rpm) to obtain the w/o/w emulsion which was the poured into the rest of the polyvinyl alcohol solution and stirred at 1500rpm for 4 hour to evaporate the solvent. Then the microparticles were separated and dried.

-The s/o/w dispersion solvent evaporation technique in which 20mg CIP were dispersed in the oil phase. The oil phase was obtained by dissolving 0.5g of PLGA into 9g of methylene chloride. A 0.1% polyvinyl alcohol solution was prepared at $90C^{o}$ and the chilled. Then the oil phase was mixed with polyvinyl alcohol solution to obtain the s/o/w emulsion. The final emulsion was poured into the rest of the APV solution and stirred at 1500rpm for 4 hours to evaporate the solvent. The obtained microspheres were separated and dried.

FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) spectra were recorded for PLGA, ciprofloxacin, PLGA/CIP (w/o/w) and PLGA/CIP (s/o/w) in KBr pellets on an Avatar Nicolet spectrophotometer. The range for this analysis was between 500-4000cm^{-1}.

Encapsulation rate

The drug encapsulation efficiency (EE) was measured after ciprofloxacin extraction from microspheres following the same method for both samples. 10mg of microspheres of both w/o/w sample and s/o/w sample were weighed and dissolved in 3ml methylene chloride.

The 2ml of water was added to this phase and the solution pH was set to 11 with ammonia hydroxide. Furthermore the samples were ultrasonicated for 20 minutes and then centrifuged at 10000rpm for 10min.

The supernatant was withdrawn and completed with mobile phase up to 5ml. Furthermore the samples were analyzed by high-performance liquid chromatography (HPLC) using a Thermo Finnigan Surveyor HPLC System with a diode array detector on a C18 reverse phase column Hypersil Gold.

The mobile phase was composed of a 20mM citrate solution (sodium citrate dihydrate 3.3mM and citric acid hydrate 16.7mM) and acetonitrile (40:60). All experiments were done at room temperature at 280nm.

The ciprofloxacin encapsulation efficiency was determined with the following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{actual\ drug\ encapsulated}{theoretical\ drug\ encapsulated} \times 100$$

Results

FTIR analysis

The peak at 1750cm^{-1} corresponding to the carbonyl (C=O) stretching is present in both PLGA spectrum and microspheres spectra (Fig.1).

The bands that show up between 1300 and 1150cm-1 in the PLGA and both microspheres spectra are due to the asymmetric and symmetric C-C(=O)-O vibrations (Fig.1). The bands between 3500 and 3450 are characteristic to the OH groups.

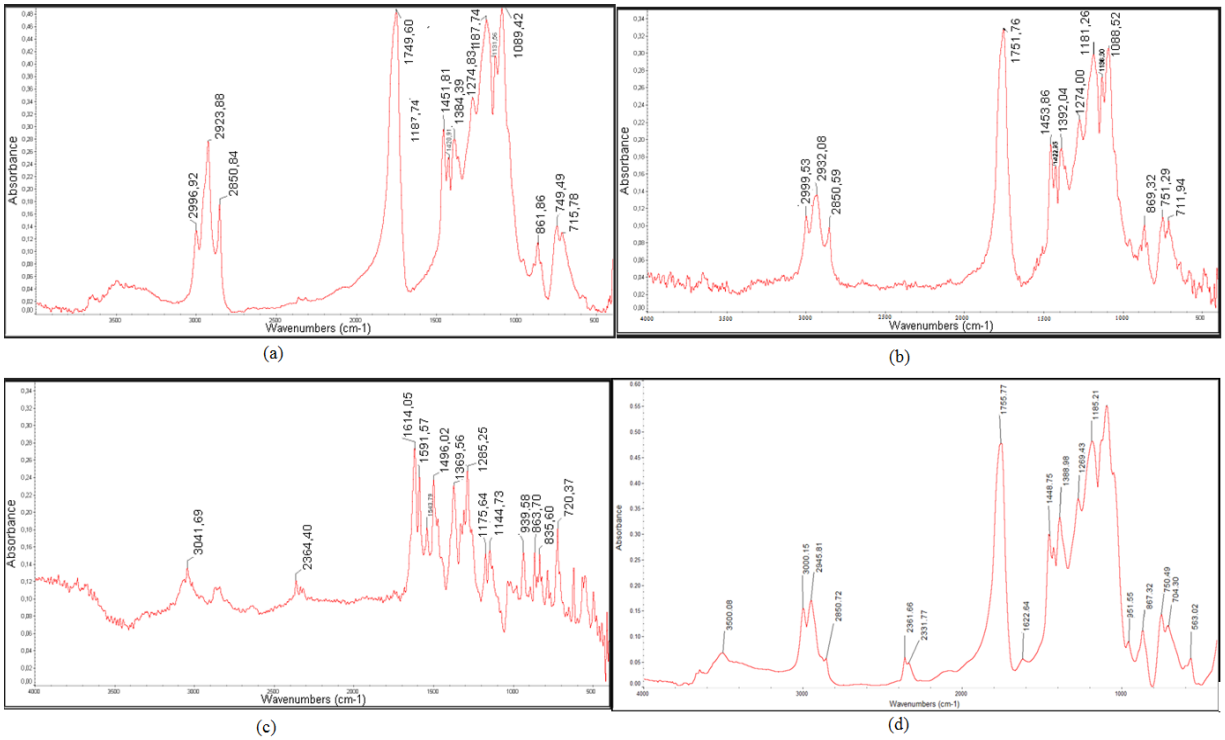

Fig.1. FTIR spectra of PLGA-CIP microspheres (w/o/w) (a), PLGA-CIP microspheres (s/o/w) (b), ciprofloxacin (c) and PLGA (d)

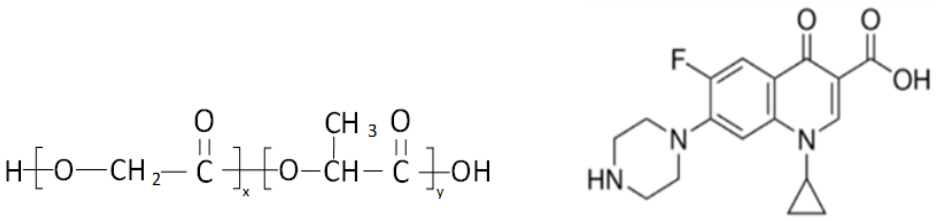

Fig.2. Chemical structures of PLGA and ciprofloxacin

Drug encapsulation

The encapsulation efficiency was calculated after HPLC determination of the samples (Fig.3). For the w/o/w sample the encapsulation was determined to be 9.32% whereas for the s/o/w sample the encapsulation efficiency was 27.78%

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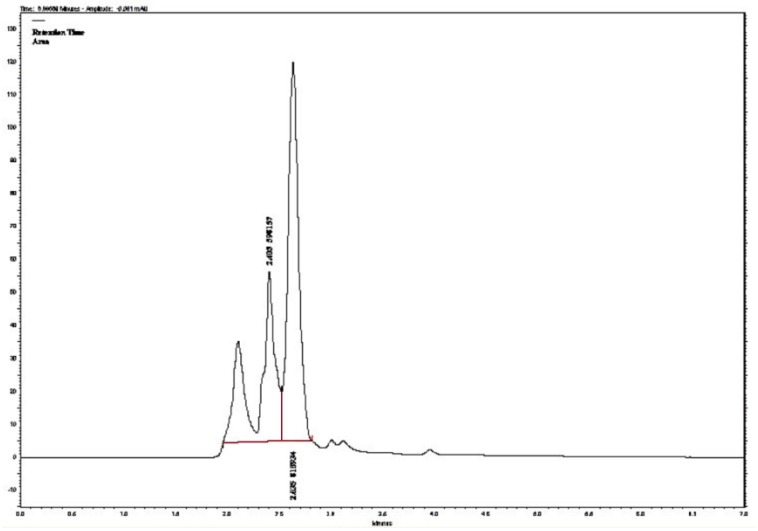

Fig.3. Determination of the entrapment efficacy of ciprofloxacin by HPLC

Discussion

Ciprofloxacin is an antibiotic used in a number of infectious diseases including chronic osteomyelitis. Given that ciprofloxacin is a poorly water-soluble molecule and is not soluble in common organic solvents such as: methylene chloride, chloroform, dimethyl sulfoxide, acetonitrile; two methods were employed to incorporate the drug: the double emulsion solvent evaporation method (w/o/w) and the s/o/w emulsion solvent evaporation [16,24].

Therefore to make the microspheres the w/o/w double emulsion and the s/o/w emulsion, respectively, were broke down and poured into a PVA solution. PLGA with a 50:50 polyglycolide: polylactide composition was chosen for the present study. Furthermore PLGA systems have a controlled drug release rate which could ensure the required amount of drug at the infection site [25].

The bands between 1400 and 1150cm^{-1} are characteristic to esters and are found in PLGA spectrum and in both microspheres (w/o/w; s/o/w) spectra (Fig.1). This suggests that the polymer did not suffer structure modifications during the microencapsulation process (Fig.2). The bands characteristic to ciprofloxacin between 1000cm^{-1} and 1500cm^{-1} overlap the PLGA specific bands. This is due to the small ciprofloxacin: PLGA ratio in the microcapsules. Therefore we continued with the HPLC determination of ciprofloxacin and calculating the entrapment efficiency. We observed that the entrapment efficiency is higher when using the s/o/w method rather than the w/o/w solvent evaporation technique. These findings are consistent with the work of other researchers [24]. This can be explained by various factors including the low ciprofloxacin solubility. Moreover the entrapment efficiency can be optimized by varying synthesis parameters such as agitation rate, viscosity of the dispersed phase, quantity of the drug, temperature and pressure in a new study for the s/o/w evaporation method [17].

Conclusion

In the present study ciprofloxacin loaded PLGA microspheres were fabricated by two variations of the solvent evaporation method. The microspheres showed a better entrapment efficiency when the s/w/o emulsion technique was used.

The local delivery of ciprofloxacin can be an effective method to reduce antibacterial resistance and to target the biofilm bacteria. Therefore further studies must be employed into the development of a local delivery system based on PLGA-ciprofloxacin microspheres.

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Corresponding Author: Andreea-Gabriela Mocanu, University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Petru Rareş St., No. 2. Craiova, 200349, Romania; e-mail: gabriela_deca@yahoo.com