Asian Journal of Chemistry; Vol. 25, Supplementary Issue (2013), S333-S336

ASIAN JOURNAL OF CHEMISTRY

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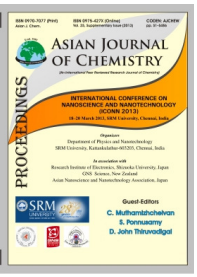

Development and Characterization of Poly-ε-Caprolactone Based Microspheres-A Sustained Drug Delivery System for Treating Rheumatoid Arthritis†

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AJC-12868

Rheumatoid arthritis is an autoimmune disorder with a characteristic feature of chronic inflammation of the joints. This disease can also cause inflammation and injury in other organs in the body, but principally attacks flexible (synovial) joints. The commonly prescribed medication for this disorder includes steroids which can cause side effects like bruising, cataracts, muscle weakness, osteoporosis, infections and blocking of other immune response. Hence a polymeric drug carrier has been designed to provide a stealth characteristic to the delivery system and prolonged blood circulation. In the present study we have developed two different poly-ε-caprolactone based delivery systems with dexamethasone and prednisolone. Efficient encapsulation of dexamethasone and prednisolone in poly-ε-caprolactone microspheres were carried out using oil-water single emulsion solvent evaporation method. The microspheres formed were characterized for encapsulation efficiency, particle size analysis, morphological characteristics and the drug release profiles. The particle size distribution of the poly-ε-caprolactone spheres was narrow in the range 5-20 μm. The characterization processes carried out by SEM, particle size analyzer, AFM and FTIR showed efficient encapsulation and the *in vitro* release studies highlighted them as potential sustained drug delivery systems. The specificity of the system might be granted by conjugating the polymeric microcarrier with the respective ligand so that it functions effectively.

Key Words: Poly-ε-Caparolactone, Rheumatoid arthritis, Sustained drug delivery systems.

INTRODUCTION

Rheumatoid arthritis (RA) is a common rheumatic disease, affecting *ca.* 1.3 million people in the United States[1], according to current census data. The disease is three times more common in women as in men. It is believed that the tendency to develop rheumatoid arthritis may be genetically inherited (hereditary). Certain genes have been identified that increase the risk for rheumatoid arthritis. It is also suspected that certain infections or factors in the environment might trigger the activation of the immune system in susceptible individuals. This misdirected immune system then attacks the body's own tissues. This leads to inflammation in the joints and sometimes in various organs of the body, such as the lungs or eyes.

Various treatments are available for rheumatoid arthritis. Non-pharmacological treatment like physical therapy, orthoses, nutritional therapy and occupational therapy do not stop the progression of joint destruction. Disease-modifying antirheumatic drugs (DMARDs) are necessary to restrain or stop the underlying immune process and put a stop to long-term damage. Whereas, analgesia and antiinflammatory drugs as well as steroids are utilized to repress the symptoms. In recent times, the newer group of biologics has increased treatment options[2]. Cortisone therapy has offered relief in the past, but its long-term effects have been deemed undesirable[3].

Dexamethasone is a glucocorticoid that acts as an anti-inflammatory and immunosuppressant. The advantageous clinical effects of both low dose and high dose glucocorticosteroid treatment are well known. Treatment of treatment resistant rheumatoid arthritis with intravenous high dose dexamethasone pulse therapy is typically effective in repressing the symptoms of rigorous inflammation[4]. Prednisolone is a synthetic corticosteroid drug that is particularly effective as an immunosuppressant drug. Prednisolone which is the active drug and also a steroid, is derived from the prodrug prednisone and this conversion takes place in the liver. Used for many different indications including: asthma, COPD, rheumatic disorders, allergic disorders *etc*. In 1950, the Noble Prize in Medicine and Physiology was awarded to Kendall, Reichstein, who independently isolated and synthesized cortisol and to

†International Conference on Nanoscience & Nanotechnology, (ICONN 2013), 18-20 March 2013, SRM University, Kattankulathur, Chennai, India

Hench, who first applied it and described its dramatic efficacy on patients with developed RA in 1949[5,6]. Since then and despite their long-term unfavorable side effects, corticosteroids are still used extensively and successfully in treatment of many inflammatory conditions, including RA, either alone or co-administered with other drugs[7]. Recent reports highlighted the effectiveness of corticosteroids in reducing inflammation and slowing joint damage, especially when administered at an early stage of disease propagation[8]. In addition, it was also reported that prednisolone given for an extended period is capable of ameliorating joint damage in both collagen- and antigen-induced murine arthritis[9]. So, a sustained-release biocompatible and biodegradable drug delivery system, containing a steroid, such as prednisolone and dexamethasone, are believed to be beneficial in management of RA, especially at its early stages. Efforts need to be made to seek therapeutic agents that can be used for long-time administration. Recent literatures have reviewed the mostly applied natural or synthetic polymers, such as poly(d,l-lactic acid) (PLA), poly-ε-caprolactone) (PCL) and poly(lactic-coglycolic acid) (PLGA) containing several drugs (anaesthetics, antibiotics, antitumoural drug, proteins, *etc*.)[10]. Of all the drug carrying polymers, PCL microspheres are suitable for long-term drug release system[11].

Poly-ε-caprolactone (PCL) is a biodegradable, biocompatible and semicrystalline polymer having a very low glass transition temperature. Due to its slow degradation, PCL is ideally suitable for long-term delivery extending over a period of more than one year. This has led to its application in the preparation of different delivery systems in the form of microspheres, nanospheres and implants. Various categories of drugs have been encapsulated in PCL for targeted drug delivery and for controlled drug release. Microspheres of PCL either alone or of PCL copolymers have been prepared to obtain the drug release characteristics[11].

The present study is about development and characterization of drug delivery system based on dexamethasone and prednisolone encapsulated within PCL microsphere.

EXPERIMENTAL

Prednisolone (P6004) was purchased from Sigma Aldrich, Bangalore, dexamethasone was obtained as gift samples from Cipla Ltd. Mumbai Central, India. Poly(ε-caprolactone) (PCL) (Mn 14,000) was purchased from Sigma Aldrich, Banglore India. Dichloromethane (SISCO Research Lab Pvt. Ltd.), poly vinyl alcohol (PVA) (SDFCL Chemicals, Mumbai, molecular weight 85000-124000), phosphate buffered saline (PBS), dialysis memberane (HIMEDIA, India. Avg Flat width 24.26 mm, Avg Diameter 14.3 mm), Double distilled water. All other materials and reagents were of analytical grade of purity.

Preparation of polycaprolactone (PCL) microspheres

Free PCL microspheres: Microspheres of PCL were synthesized by using the single emulsion technique. 200 mg PCL, 20 mL DCM were mixed by stirring in a beaker for 2 min at 3000 rpm using a mechanical stirrer. 20 mL PCL stock solution was thus obtained. This solution constituted the first emulsion. Then, 20 mL PCL stock solution and 60 mL of PVA (polyvinylalcohol) were mixed at 3500 rpm for 0.5 h at 30 ºC using mechanical stirrer.oil-water emulsion was thus obtained. The emulsion was magnetically stirred for *ca.* 3 h. This was followed by centrifugation for 0.5 h at 3000 rpm. The pellets thus obtained were washed 3 times with distilled water and kept for drying.

Drug loaded PCL microsphere: Prednisolone and dexamethasone(individually) loaded poly(ε-caprolactone) (PCL) microspheres were prepared with water in oil in water (w/o/w) solvent extraction/evaporation method. 200 mg PCL, 20 mL DCM and 0.66 mg drug were mixed by stirring in a beaker for 2 min at 6000 rpm using a mechanical stirrer. The polymer is to drug ratio was kept 3:1. 20 mL PCL stock solution is thus obtained. The organic phase (20 mL PCL stock solution) was added slowly into the external aqueous phase containing polyvinyl alcohol (60 mL) and stirred at 4500 rpm for 0.5 h using a mechanical stirrer. Water-oil-water emulsion was obtained. The resulting emulsion was magnetically stirred for about 3 h till DCM evaporated. This was followed by centrifugation for 0.5 h at 3000 rpm. Pellets carrying the microspheres were formed. These were filtered and washed 3 times with distilled water to remove contaminants. The product was kept for air- drying in a laminar flow hood.

Encapsulation efficiency: Encapsulation efficiency was determined for each batch of microspheres by analyzing the filtrate of the double emulsion once the microspheres were removed from it after centrifugation at 5000 rpm for 0.5 h. The amount of drug present in the filtrate was calculated from calibration curves of concentration *vs*. absorbance already prepared with known standards of the drug. The amount of the drug encapsulated and the percent encapsulation in the microspheres is given by:

Drug (encapsulated) = Drug (total) - Drug (filtrate)

$$\text{Encapsulation (\%)} = \left[\frac{\text{Drug (encapsulated)}}{\text{Drug (total)}}\right] \times 100$$

Surface morphology: The surface morphology of the microspheres was investigated using scanning electron microscopy. The dry samples of microspheres were mounted on metal stubs using double sided adhesive tapes. They were then, sputter coated with gold particles under reduced pressure conditions and observed under the scanning electron microscope. The accelerating voltage was kept constant at 4 kV.

Particle size distribution: Particle size distribution was studied using particle size analyser. A specific quantity of the microspheres was dispersed in water in an ultrasonic disperser for 1 min to bring about disaggregation. Particle size of the microspheres was then measured by laser diffractometry using laser particle size analyzer zeta potential.

FTIR spectra: FTIR spectra were recorded to understand the intermolecular interactions in the polymer drug formulation. FT-IR analysis was performed between 4000 and 450 cm^{-1} averaging 10 scans for the polymer drug formulation.

Drug release studies: Drug release profiles of prednisolone and dexamethasone was determined by placing 10 mg microspheres, from each batch, in 25 mL of release medium (PBS buffer, pH 7.4). The samples were kept in an orbital shaker maintained at 37 ± 5 ºC stirring at 50 rpm. At specified time intervals, 1 mL aliquot of the release medium was removed and replaced with fresh media. These samples were then centrifuged at 3000 rpm at room temperature. The

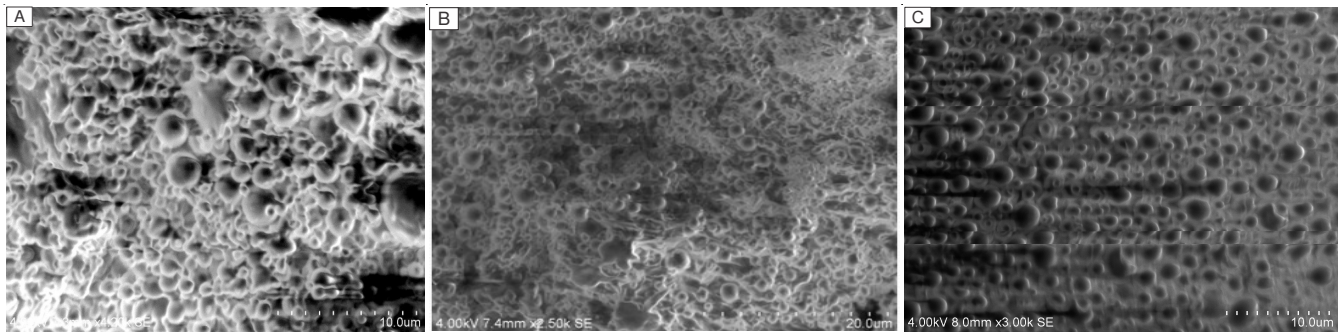

Fig. 1. Scanning Electron Microscopy images of A = free PCL microsphere, B = Prednisolone-loaded PCL microspheres, C = dexamethasone loaded PCL microsphere

supernatant was then analyzed for drug content by UV-visible spectrophotometry.

RESULTS AND DISCUSSION

Encapsulation efficiency: The encapsulation efficiency of the drug loaded PCL microsphere was found to be moderate (dexamethasone-75.2 %, prednisolone- 79.32 %). In the microparticles prepared by the w/o/w double emulsion technique, there is the presence of an internal aqueous phase that is enveloped by the polymer PCL. The major percentage of drug tends to get inside this internal aqueous phase during the formulation of the emulsions and therefore more drug gets encapsulated in the microparticles. However, leakage of the drug is sure to happen from the inner aqueous phase of the microspheres since there is the high volume of aqueous phase present outside and this is the major reason for a non-ideal encapsulation of the drug.

Encapsulation efficiency of prednisolone:

Drug (encapsulated) = 0.133 mg – 0.02756 mg

= 0.1055 mg

$$\text{Efficiency (\%)} = \left(\frac{0.1055}{0.133}\right)\times 100$$

= 79.32 %

Encapsulation efficiency of dexamethasone:

Drug (encapsulated) = 0.133 mg – 0.0336 mg

= 0.1 mg

$$\text{Efficiency (\%)} = \left(\frac{0.1}{0.133}\right)\times 100$$

= 75.2 %

Surface morphology: The shape and morphology of the microspheres was observed using SEM. The surface of both the empty and the drug loaded microspheres prepared by the w/o/w method revealed that the particles were spherical in shape with diameter ranging from 10-100 μm as shown in Fig. 1.

The PCL microspheres, both free and drug loaded ,presented a smooth surface without apparent porosity. Moreover, microspheres were of good morphological characteristics, spherical, with smooth surface, no drug crystals were observed on the surface.

Particle size distribution: The particle size analysis showed the range in which the microspheres were formed and it was to be a very narrow range which indicated the formation of microspheres was almost uniform indicating a uniform size distribution of the polymeric microspheres.

FTIR spectra: Typical spectra of free PCL microspheres, prednisolone-loaded microspheres, dexamethasone loaded microspheres and dual-encapsulated microspheres were obtained by FTIR analysis. The FTIR peaks of PCL displays a characteristic absorption band at strong bands such as the carbonyl stretching mode around 1727 cm^{-1} ν(C=O), asymmetric stretching 2949 cm^{-1} ν(CH_2) symmetric stretching 2865 cm^{-1} ν(CH_2)[20]. The spectrum of prednisolone loaded PCL microspheres shows an additional peak at 1712 cm^{-1} indicating carbonyl stretching and OH stretching at 3350 cm^{-1}. The spectrum of dexamethasone loaded PCL microsphere shows additional absorption bands at 3300 cm^{-1} (O-H bond) and 1300 cm^{-1} (C-F bond) which can be seen in dexamethasone spectrum (Figs. 2-4).

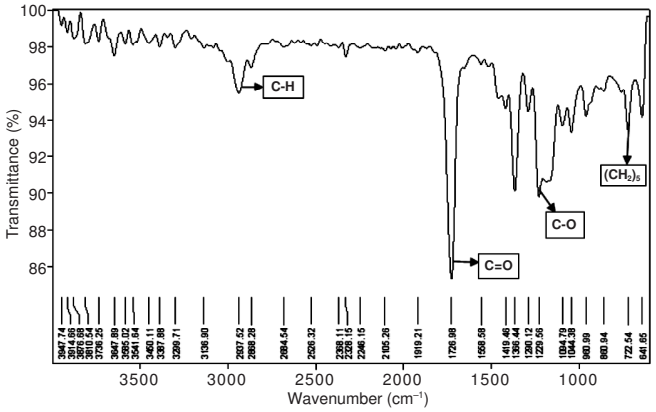

Fig. 2. FTIR Spectra for free PCL microspheres

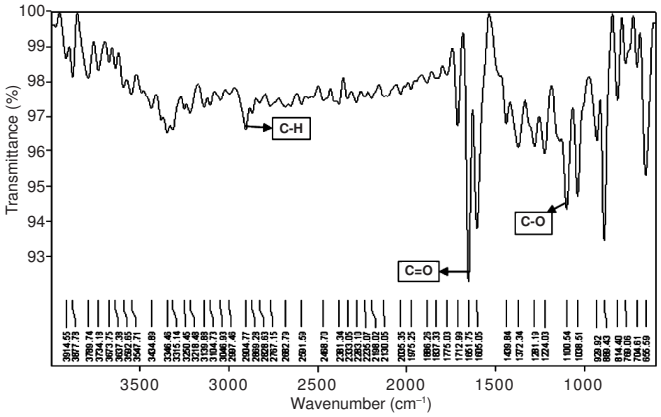

Fig. 3. FTIR spectra for prednisolone loaded microspheres

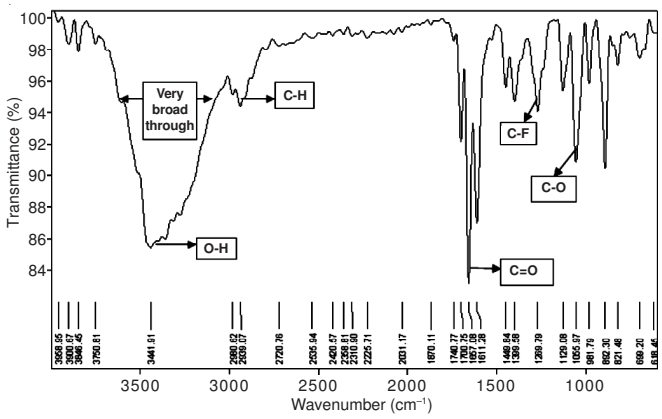

Fig. 4. FTIR spectra for dexamethosone loaded microspheres

Drug release studies: The release profiles of prednisolone and dexamethasone from PCL microparticles prepared by o/w method, in pH 7.4 phosphate buffer at 37 ºC is shown in Fig. 5. It exhibits an initial burst followed by a plateau for over 12 h. The slow release of the drug can also be attributed to hydrophobic interaction taking place between the aromatic rings of drug and the methylene groups of the PCL polymer. Hence, only the drug which are concentrated on the periphery of the microspheres are released immediately and a minor part of the drug is not released thereby explaining the incomplete release of the drug even after 12 h. Further the release of the drug can be enhanced *in vivo* by the activity of various enzymes present in the physiological system. These enzymes will act on the biodegradable PCL polymer and increase the drug release compared to that conducted *in vitro*.

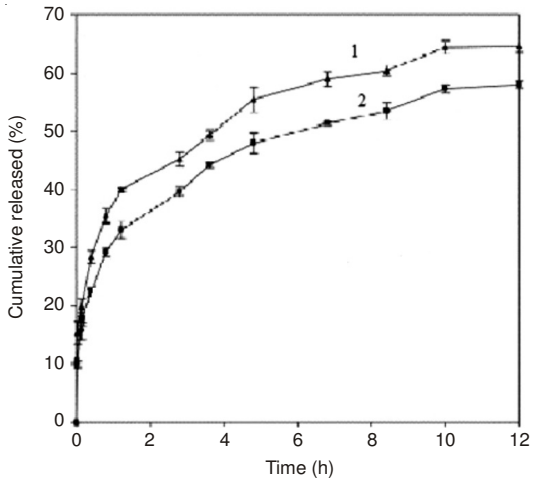

Fig. 5. Drug release study of (1) Prednisolone encapsulated PCL microspheres and (2) Dexamethasone encapsulated PCL microspheres

Conclusion

Poly-ε-caprolactone is one of the extensively studied synthetic biodegradable polymers in various formulations for potential drug delivery and tissue engineering and through this work we have shown that PCL microspheres can encapsulate prednisolone and dexamethasone with agreeable efficiency. These microspheres were fabricated using single o/w emulsion evaporation technique. The encapsulation efficiency of the drug into the spheres was calculated and the characterization was studied by SEM, particle size analysis, FTIR techniques and they indicated positive result for encapsulation of both prednisolone and dexamethasone within the same PCL microsphere. There was a slow and sustained drug release observed by different release behaviours of the two drugs (including a small burst release at the beginning).

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