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Injectable methotrexate loaded polycaprolactone microspheres: Physicochemical characterization, biocompatibility, and hemocompatibility evaluation

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ABSTRACT

In this study, bare polycaprolactone microspheres (PCL MPs) and methotrexate (MTX) loaded PCL microspheres (MTX-PCL MPs) have been developed by oil-in-water emulsion solvent evaporation method using hydroxypropyl methylcellulose (HPMC) as an emulsifier. Encapsulation efficiency and loading capacity of methotrexate were found to be 51.28% ± 0.52 and 2.8% ± 0.06 respectively. Environmental scanning electron microscopy showed the PCL MPs and MTX-PCL MPs to have a spherical shape and smooth surface morphology. The mean size of microspheres (23 μm) was found within injectability criteria. High-Resolution X-ray diffraction of microspheres revealed that PCL retained its semi-crystalline nature after processing in microspheres, but the drug looses its crystallinity. Fourier transmittance infrared spectroscopy and thermogravimetry analysis of the microspheres indicated that no physicochemical modification occurred. *In vitro*, MTX release study from MTX-PCL MPs in phosphate buffer saline (pH 7.4) showed controlled release profile and only 31% of MTX released in 306 h. The microspheres in lyophilized form are physicochemically stable for 8 months. Furthermore, L929 cells treated with microspheres showed cell viability > 80%. The different concentrations of microspheres found hemocompatible and did not affect the biconcave shape of red blood cells (RBCs). The physiochemical and biological evaluation of microspheres suggests their further use for drug delivery application.

1. Introduction

Over the past few decades, the various types of novel biomaterials have been developed for applications in biomedical engineering such as drug delivery, cell encapsulations and tissue engineering. The various types of active molecules (protein, gene, drugs and several others) of delivery systems have been developed including hydrogels, microparticles, nanoparticles, nanofibers, liposomes and micelles, *etc.* for improving their overall therapeutic efficacy [1–14]. Microspheres prepared from natural as well as synthetic polymers have been widely investigated as a drug delivery carrier [15,16]. Their use has gradually increased in the pharmaceutical field due to its advantages such as controlled release, protection from premature degradations, increased bioavailability, and decreased toxicity, reduced dosage frequency and improved patient compliance [15–17]. Also, they are easily injectable compared to surgical insertion of large implants. Microspheres have been administered through different routes such as intravenous, intramuscular, subcutaneous, and pulmonary, *etc.* [15–18]. Recently, non-biodegradable polymers have been replaced by biodegradable polymers for the development of polymeric microspheres based delivery systems in pharmaceutical technology. Microspheres made from biocompatible and biodegradable polymer produce a non-toxic product from their biodegradation and avoid the surgical removal from injected site [17–20]. Recently, many reports are available on the injectable microspheres for localized delivery of the drug in solid tumor and joints. Because these injectable depots of microspheres are related with site-specific drug release, the drug toxicity at non-targeted tissues is avoided [21–24]. Among synthetic polymers, polylactic acid (PLA), polyglycolic acid (PGA) and poly (lactide-*co*-glycolide) acid (PLGA) have been most widely used for the development of microspheres based drug delivery systems owing to their excellent biocompatibility, biodegradability, and non-immunogenicity [17,18,23,25–30]. However, their high cost and generation of the acidic environment of degradation which can produce inflammation are responsible for their limited use in the microsphere development for biomedical applications. Polycaprolactone (PCL) is a synthetic polymer and is suitable for the development of controlled drug delivery systems due to its high permeability for drug molecules and slow degradation kinetics compared to polylactide and polyglycolide. It is a cost-effective polymer and does not create an acidic environment during its degradation. Also, it is

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biocompatible, biodegradable and non-immunogenic with hydrophobic semi-crystalline nature. It is a Food and Drug Administration-approved polymer for pharmaceutical applications and had been used for a long time for development of drug delivery systems in the pharmaceutical industry. PCL based microspheres have been developed for encapsulation and controlled delivery of hydrophilic and hydrophobic drugs [19,31–34]. Drug-loaded microspheres preparation mainly depends on the solubility of drug and polymer in various solvents. The solvent evaporation is a most widely used simple and reproducible technique for microspheres preparation [21,30,32,35,36].

Methotrexate (MTX) is a potent antimetabolite for folic acid with anti-cancer, anti-inflammatory, anti-rheumatic and disease modifying properties [37–42]. In 1970, MTX was included as disease modifying anti-rheumatic drug (DMARDs) and is currently the most widely used drug for treatment of rheumatoid arthritis. Due to its high effectiveness compared to other drugs for rheumatoid arthritis treatment, it is a popular second line drug among the doctors. The clinical application of MTX has limitations such as poor solubility, large amount excreted by the kidney, short half-life, and rapid diffusion into the body. However, its use is also associated with serious side effects, due to which around 30% patients worldwide leave the treatment of rheumatoid arthritis. The most frequent side effects arising out of MTX dosing includes mouth sores, stomatitis, and a decrease in white blood cells count. A high dose for longer time produces severe hepatotoxicity, nephrotoxicity, and bone marrow suppression. Other side effects caused by MTX include skin rash, itching, dizziness, hair loss, and drowsiness. In the clinical application, regular monitoring of MTX amount in the blood is required to ensure the minimal toxic level [23,39,40,43,44]. To decrease the toxicity the localized intra-articular and intra-tumoral free MTX suspension has been administered, but the efficacy was found low due to rapid clearance from the target site [22,29,38,40,42,45–48]. Therefore, MTX is an ideal drug for microencapsulation into PCL polymer.

In this study, bare polycaprolactone microspheres (PCL MPs) and methotrexate loaded PCL microspheres (MTX-PCL MPs) have been developed from single O/W emulsion solvent evaporation method. Here, hydroxypropyl methylcellulose (HPMC) has been used as an emulsifier for the development of PCL MPs and MTX-PCL MPs. The developed microspheres size, shape, and morphology were characterized by Environmental Scanning Electron Microscopy (ESEM), and Fourier Transform Infrared Spectroscopy did physiochemical characterization (FTIR), Thermogravimetric Analysis (TGA) and High-Resolution X-ray Diffractometry (HR-X-RD). The stability study for lyophilized PCL microspheres powder sample was performed up to 8 months. The biocompatibility was performed on the L929 fibroblast cells for 24 h and hemocompatibility was performed on the human blood. This system will pave the way for further advancement in microsphere-based injectable drug delivery system for treatment of localized tumor and joint-rheumatoid arthritis.

2. Materials and methods

2.1. Materials

PCL (molecular weight 14,000 Da) was purchased from Sigma-Aldrich (Mumbai, India), and hydroxypropyl methylcellulose (HPMC, K15M) procured from Otto Chemie Ltd. (Mumbai, India). Methotrexate was kindly gifted by Sun Pharma Ltd. (Gujarat, India). Organic solvent like dichloromethane (DCM) HPLC grade and dimethyl sulfoxide (DMSO) analytical grade were obtained from Merck Ltd. (Mumbai, India). Chemicals for the preparation of Phosphate Buffer Saline (used for drug release studies) disodium hydrogen phosphate, sodium chloride, and potassium dihydrogen phosphate was purchased from Merck Ltd. (Mumbai, India) and potassium chloride purchased from Sigma-Aldrich (Mumbai, India). Cell culture media (DMEM), antibiotics (Penicillin and streptomycin) and PBS were purchased from HiMedia (Mumbai, India). The Triton X-100 was purchased from Fisher Scientific Ltd. (Mumbai, India), and glutaraldehyde (38%) supplied by Merck Ltd. (Mumbai, India).

2.2. Preparation of microspheres

The PCL MPs and MXT-PCL MPs were prepared by using O/W emulsion solvent evaporation method. Briefly, MTX was dissolved into 500uL DMSO and mixed into 2 mL DCM containing 100 mg of dissolved PCL polymer. After polymer and drug containing organic phase was added by using syringe pump with the speed 1 mL/min into 30 mL of water phase containing HPMC (0.2%w/v) as an emulsifier. Simultaneously the resulting emulsion was emulsified by homogenizer with the speed 3000 rpm for 5 min and then the emulsion was kept on a magnetic stirrer at 800 rpm for evaporation of organic phase for 6 h. After complete evaporation of organic phase, the MTX-PCL MPs were collected by centrifuging at 10000 rpm for 15 min and microspheres dried by lyophilisation. PCL MPs were also obtained by the same method but without the addition of MTX. MTX loaded PCL microspheres were prepared in dark condition due to light-sensitive nature of MTX.

2.3. Morphology and size analysis of microspheres

The shape and surface morphology of PCL MPs and MTX-PCL MPs were characterized by environmental scanning electron microscope (ESEM) (FEI-ESEM, Quanta 200, Netherlands). The dried samples of microspheres were stacked on double-sided carbon tape, and samples were coated with platinum for 300 s with 10 mA (JFC-1600, Fine Coater, Japan). The microspheres were observed under different magnifications with accelerated voltage from 5 to 20 kV. Average size and size distribution of PCL MPs and MTX-PCL MPs were from ESEM images calculated by using Image J online software.

2.4. Production yield (% PY) of microspheres

The total amount of PCL MPs and MTX-PCL MPs were estimated by weighing the obtained lyophilized microspheres powder on a weighing machine. The production yield of PCL MPs and MTX-PCL MPs were calculated by using Eq. (1).

$$\text{Production yield (\%)} = (Wa/Wb) \times 100 \quad (1)$$

The microparticles production yield was calculated by weight of microspheres produce (Wa) and weight of compounds used in the formulation for microspheres preparation (Wb), PCL or PCL plus MTX.

2.5. Drug encapsulation efficiency and loading capacity

To calculate the encapsulated amount of MTX, the 5 mg of MTX-PCL MPs were dissolved in 2 mL DCM, and then 1 mL DMSO was added to solubilize the drug. After the mixture was kept for bath sonication of 1 min, and then added into 10 mL phosphate buffer saline (PBS, pH 7.4) and stirred until complete evaporation of DCM. The obtained solution was centrifuged at 10000 rpm for 15mins, and collected supernatant was analyzed using UV-spectrometer at 304 nm wavelength. PCL MPs were also analyzed using the same procedure and used as a control. The encapsulation efficiency (%EE) and loading capacity (%LC) was calculated by using the following formulas (2) and (3).

$$\%EE = \frac{\text{Mass MTX in microspheres}}{\text{Total mass of MTX used in formulation}} \times 100 \quad (2)$$

$$\%LC = \frac{\text{Mass of MTX loaded microspheres}}{\text{Total mass of MTX used in formulation}} \times 100 \quad (3)$$