ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Saravanakumar et al /J. Pharm. Sci. & Res. Vol. 12(4), 2020, 488-491

Formulation Development and Characterization of Meloxicam Cationic Nanoparticles

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Abstract

Meloxicam (MLX), a potent nonsteroidal anti-inflammatory drug, is prescribed to relieve postoperative and/or chronic joint pain. Its frequent oral administration often results in serious in duodenal ulceration and GIT disturbances. Thus, novel cationic nanoparticles (NPs) was formulated to minimize the gastric and/or systemic exposure and increase the retention time of MLX in the joint after intra-articular (IA) injection, due to formation of micronized electrostatic clusters with endogenous hyaluronic acid (HA) in the synovial cavity. The prepared formulations were subjected to physicochemical characterization includes particle size, drug loading in NPs and *in vitro* drug release studies. MLX-loaded NPs consisting of poly [lactic-co-glycolic acid] (PLGA) and Eudragit RL are prepared by solvent evaporation method. The F3-NPs (PLGA: Eudragit RL ratio 7:3) with a particle size of 212.0 ± 0.8 nm with drug loading of 4.50 ± 0.12 % w/w is selected as an optimized one. *In vitro* dissolution studies using the dialysis method indicates sustained release pattern of MLX in conventional, cationic F3-NPs and cationic F3-NPs.

Keywords: Meloxicam (MLX), Nanoparticles (NPs), Intra-articular (IA) injection, Solvent evaporation technique.

INTRODUCTION

In the treatment of osteoarthritis, rheumatoid arthritis and joint pain intra-articular (IA) administration of drug(s) has an advantage to selectively deliver drug(s) to the site of action. IA delivery of drugs reduces systemic exposure and undesirable adverse effects. Direct IA delivery of active compounds to affected tissues offers the chance to boost therapeutic outcomes with lower doses [1, 2]. Currently various non steroidal anti inflammatory drugs (NSAIDs), methotrexate and corticosteroids are been common using for IA therapy [2, 3]. Due to leakage and redistribution into systemic circulation, their therapeutic action is not completely achieved. The outer synovial membrane consists of a discontinuous layer of synoviocytes with loose intercellular gaps ranging from 0.1–5.0 µm [4]. It was reported that IA administration of paracetamol, indomethacin, methyl salicylate and diclofenac showed a short elimination half-life of 1.1, 2.8, 2.4, 5.2 h respectively in patients [5, 6]. Hence developing nano particles (NPs) as drug carriers for increased localized drug action after IA injection is more advantageous [7, 8]. Poly (lactic-co-glycolic acid) (PLGA) has been widely used in parenteral NPs, because of its biodegradability and sustained drug release characteristics [9,10]. PLGA based NPs could prolong anti-inflammatory action of betamethasone, a corticosteroid by delaying its clearance rate from the synovial cavity [11]. Frequent IA injection administration should be done with NPs smaller than 250 nm in size due to their quick escape from the joint cavity [12]. Cationic NPs are a better option to acquire prolonged drug retention in the synovial joint [9]. The electrostatic interaction between the hyaluronic acid (HA) in synovial fluid and the cationic surface-charged NPs forms micro-sized clusters which restricts the efflux and increases the retention time in the synovial cavity after IA injection. Meloxicam (LRX), a member of the oxicam group of NSAIDs, is often administered to reduce postoperative and/or chronic pain in joints, due to its strong and long-lasting analgesic and anti-inflammatory effects. Adverse effects like GIT ulceration, bleeding, and renal failure will occur after its repeated oral administration [13-15]. To reduce the gastrointestinal damages of oral therapy, IA administration has been attempted [16]. Therefore, the objective of this study was to formulate a cationic NPs of MLX to reduce its systemic exposure and simultaneously prolong its retention in the synovial cavity after IA injection. MLX cationic NPs were prepared by emulsification and solvent evaporation technique and were characterized in terms of particle size, surface charge, loading amount of drug, and *in vitro* release profile.

MATERIALS AND METHODS

Materials: Meloxicam (purity >99.0% w/w) was obtained as a gift sample from A to Z Pharmaceuticals Pvt. Ltd. Chennai, India. PLGA, polyvinyl alcohol, phosphate-buffered saline (PBS) tablets, Sodium hyaluronate, dichloromethane and Eudragid RL were obtained from S.D. Fine Chemicals Pvt. Ltd. All the chemicals and reagents were used for the study are of analytical grades.

Methods

Drug-excipient compatibility studies

Fourier Transform Infrared (FTIR) studies

Were performed on MLX and (1:1 ratio) physical mixtures of MLX with polymers (PLGA and Eudragit RL) by an IR spectrophotometer (Bruker FTIR, ALPHA II), in the region between 400 and 4000 cm^{-1} by the direct sampling method [17-18].

Differential Scanning Calorimetric (DSC) studies

Thermal analysis was conducted through DSC (Sirius DSC, 3500) in order to detect the comparability between the drug and polymers (PLGA and Eudragit RL). The DSC thermograms were recorded by sealing 10 mg samples in flat bottomed aluminium pans and heated in the nitrogen flow rate of 100 mL/min, with the heating rate of 10 °C/min in a temperature range of 20-300 °C [19].

Preparation of MLX-loaded NPs

MLX-loaded NPs were prepared by oil-in-water emulsification and solvent evaporation method [20]. A total of 125 mg of the polymers (PLGA and Eudragit RL) and 25 mg of MLX were dissolved in 8 mL of dichloromethane. NPs prepared with PLGA and Eudragit RL with the weight ratios of 10:0, 9:1, 8:2, and 7:3 were named as conventional NPs, F1-NPs, F2-NPs, and F3-NPs, respectively, which are represented in (Table1). The above solution was poured into 20 ml of 0.1%; w/v PVA solution and ultrasonicated for 7 min. The organic phase was evaporated by stirring the o/w emulsion at 400 rpm for 4 hr, and the suspension of NPs was centrifuged at 20,000×*g* for 30 min. Finally, the supernatant was discarded, and the distilled water was used for re-dispersion of NPs.

Table1: Formulation table of MLX-NPs

Formulations Code	PLGA: Eudragit RL wt. ratio
Conventional NPs	10:0
F1-NPs	9:1
F2-NPs	8:2
F3-NPs	7:3

Characterization of Nanoparticles

The prepared nanoparticles [21] were characterized by following parameters includes,

Morphology and particle size

Dispersion of NPs in water was dropped onto a cover glass and air dried under reduced pressure. The dried, sample-loaded cover glass was placed onto a copper grid using double-sided tape and coated with platinum for 2 min under vacuum. Prepared samples were viewed with field-emission scanning electron microscope (FE-SEM) [Zeiss Sigma, Germany] is at a voltage of 3 kV for to study their morphology and particle size.

Drug loading

Powder of NPs equivalent to 5 mg of MLX was added to few mL of methanol and the volume was made up to 5 mL with pH 7.4 PBS and mixed for 30 min. Then, the resultant solution was filtered through 0.45 µm poly tetra flour ethylene (PTFE) filter disc, suitably diluted if necessary and its absorbance was measured by UV-Visible spectrophotometer at 346 nm. The loading amount of MLX in NPs was calculated by the following equation:

$$\% \text{ Loading amount} = \frac{\text{Wt.of the encapsulated LRX}}{\text{Total Wt.of the NPs}} \text{x100}$$

***In vitro* drug release studies**

In vitro release profiles of MLX from conventional NPs and optimized formulation based on characterization studies: F3-NPs aggregates were assessed using the dialysis method [22]. Each formulation containing the equivalent amount of MLX (2 mg) was sealed in a dialysis bag. Drug-containing bags were then immersed into 200 mL of pH 7.4 PBS and rotated at 50 rpm. 500 µL of the medium was collected, filtered through 0.45 µm PTFE filter disc, suitably diluted if necessary and its absorbance was measured by UV-Visible spectrophotometer at 346 nm. The constant medium volume was maintained by the addition of the same volume of fresh pH 7.4 PBS.

Statistical analysis

All the values are expressed as mean ± SD and results were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

The standard calibration curve of MLX in pH 7.4 PBS

The λ_{max} was determined as 346 nm. A straight line with an equation, y = 0.048 x + 0.006 and a regression coefficient (R^2) of 0.999 was obtained, which indicates the curve follows the Beers-Lambert law in the conc. range of 0-10 µg/mL. Standard calibration curve of MLX in pH 7.4 PBS at 346 nm was shown in (Fig.1).

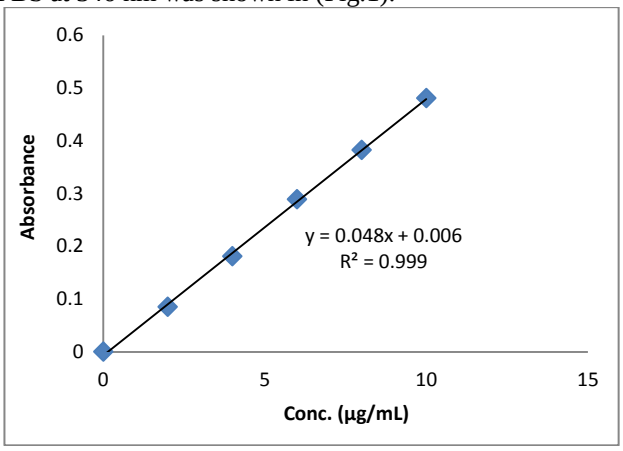

Fig.1. Standard calibration curve of Meloxicam in pH 7.4 PBS at 346 nm

Drug-excipient compatibility studies

FTIR studies

The FTIR spectra of MLX, MLX+PLGA and MLX+ Eudragit RL (1:1 ratio) shows the presence of characteristic peaks of MLX at 3287 cm^{-1} for NH_2 stretching, 2997 cm^{-1} for C-H aromatic-stretch, 2922 cm^{-1} for C-H aliphatic-stretch, 1618 cm^{-1} for NH_2 scissoring, 1549 cm^{-1} for C≡N stretch and 1180 cm^{-1} for S=O stretching respectively, indicating that there is no interaction between the drug and polymers used in the study. FTIR spectra of MLX (pure drug) and MLX: polymers **(**1:1 ratio) were shown in (Fig.2)

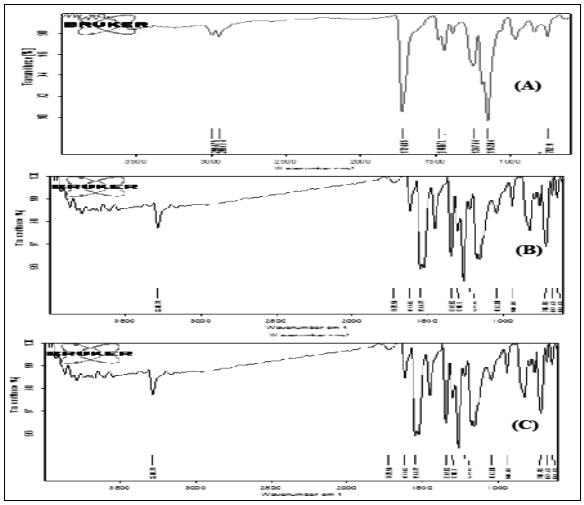

Fig.2. FTIR spectra: A. Meloxicam (MLX), B. MLX+ PLGA & C. MLX+ Eudragit RL

DSC studies

The DSC thermographs of MLX, MLX+ PLGA and MLX+ Eudragit RL were showed in (Fig.3). MEL endothermic peak was sharp at 259.61 °C corresponding to its melting point, while in PMM+ PLGA and MLX+ Eudragit RL; MEL endothermic peak appeared reduction in intensity but still near the range of its melting point i.e. 248.32C˚, and 247.33C˚respectively. The polymers endothermic peaks appeared also within the range of their melting points (55-65) C˚. This indicated that there was no well observed interaction between MEL and polymers used in the study.

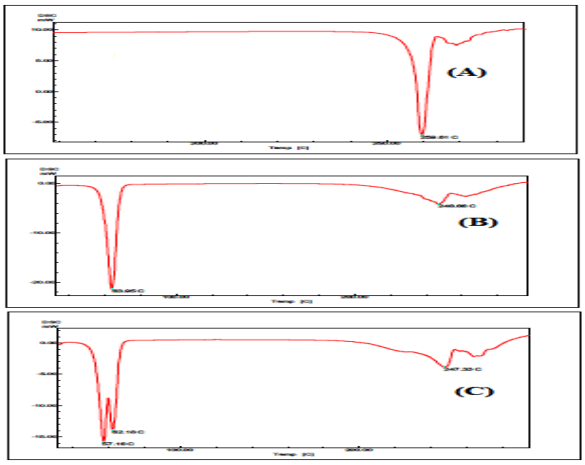

Fig.3. DSC thermographs: A. Meloxicam (MLX), B. MLX+ PLGA & C. MLX+ Eudragit RL

Characterization studies on Nanoparticles

Morphology and particle size

Studies by FE-SEM reveals both MLX loaded conventional and cationic NPs were uniform and spherical (Fig.1). The particle size (198.3 ± 0.9 to 229.4 ± 0.6 nm) of all NPs as observed by FE-SEM (Table 2). Hence, there was no significant change in particle size, regardless of the ratio of PLGA to Eudragit RL. Previous studies concluded that PLGA and hydrophobic group of Eudragit RL form the inner core structure of the NPs, while the positively charged portion (quaternary ammonium group) of Eudragit RL was on the outer surface of the NPs [23-25]. Hydrophobic MLX was predominantly entrapped inside the core compartment of the cationic NPs. The dispersion of the NPs without precipitation and/or aggregation in aqueous medium is aided by the small quantity of PVA adsorbed on their surface [26].

Drug loading

Percent drug loaded decreased from F1-NPs to F3-NPs may be due to high viscosity because of the increase of the conc. of cationic polymer Eudragit RL (Table 2).

***In vitro* drug release studies**

Compared to the *in vivo* joint environment, the *in vitro* dissolution conditions are assumed to accelerate drug release, because the actual volume of synovial fluid in normal human joints is only 0.5-2.0 mL. Moreover, the movement of synovial fluid in the knee is not fast, with a flow rate of just 0.002 mL/cm^2/ h [26]. Under sink condition, MLX from the drug powder was readily dissolved in the aqueous medium and diffused out of the dialysis membrane, achieving 80% drug release after 120 min. On the other hand, drug release from conventional, cationic F3-NPs and cationic F3-NPs became significantly delayed, exhibiting cumulative release of F1-NPs, F2-NPs F3-NPs were 51%, 46% and 57% after 120 min, respectively (Fig. 5). There were no significant differences in the release profile between conventional and F3-NPs, regardless of the presence of Eudragit RL, in case of cationic F3-NPs.

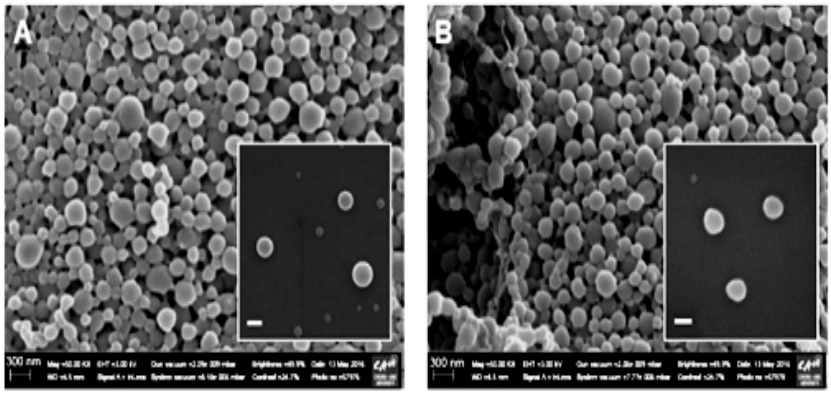

Fig.4. FE-SEM images of (A) Conventional NPs (B) Cationic F3-NPs

Table2. Results of Characterization studies on Nanoparticles

Characteristics	Conventional NPs	F1-NPs	F2-NPs	F3-NPs
Particle size (nm) by FE-SEM	198.3±0.9	229.4±0.6	215.3±0.7	212.0±0.8
Drug loading (% w/w)	5.48±0.12	5.35±0.11	4.79±0.13	4.50±0.12

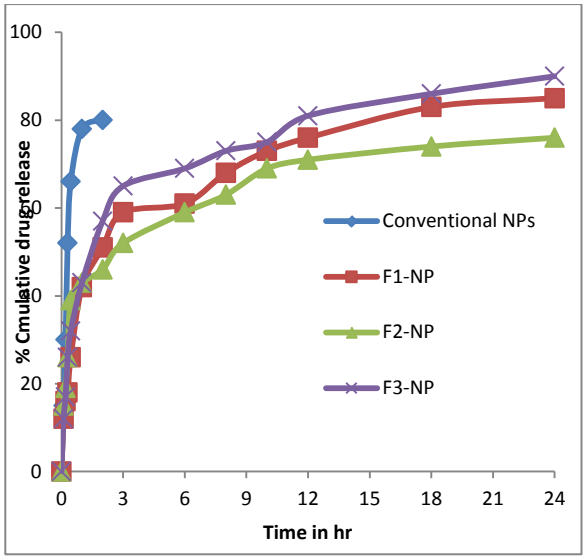

Fig.5. *In vitro* release profiles of MLX from conventional NPs, F1-NPs, F2-NPs F3-NPs aggregates in pH 7.4 PBS solution at 37C.

CONCLUSIONS

In the present study, three cationic NPs of MLX (F1-NPs, F2-NPs, F3-NPs) containing different proportions of PLGA and Eudragit RL were designed to investigate the influence of surface charge on the electrostatic interaction and aggregate-forming behaviour with the anionic polymer which is present in synovial joint, for to diminish systemic exposure and extend retention time of MLX in the joint after IA administration. Drug-excipient compatibility studies by (FTIR & DSC) indicates the MLX and polymers (PLGA and Eudragit RL) used in the study are compatible. Cationic F3-NPs (PLGA: Eudragit RL ratio 7:3) with a particle size of 212.0 ± 0.8 nm; with drug loading of 4.50 ± 0.12 % w/w is selected as an optimized one. *In vitro* dissolution studies indicate that sustained release pattern of MLX was observed in conventional, cationic F3-NPs showing 80% and 90 % drug release after 24 hr respectively. The significant difference in the release profile of cationic F3-NPs is due to the formation of aggregates between positively surface charged NPs with anionic polymer by electrostatic interaction.

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