Formulation and Characterization of Ketorolac Tromethamine Nanoparticle with Eudragit RS-100 and RL-100 by Nano precipitation Method

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ABSTRACT

Nano-carriers with optimized physicochemical and biological properties are taken up by cells more easily than larger molecules, so they can be successfully used as delivery tools for currently available bioactive compounds. Nanoparticles of Ketorolac tromethamine, a potent analgesic and moderate anti-inflammatory activity with eudragit RS-100 and RL-100 for enhance dissolution and bioavailability of the drug by nanoprecipitation method. SEM studies on optimized formulations (F3, F6) were conducted. Partition coefficient (2.2), particle size (0.276 ±0.015 to 1.236 ±0.115nm), zeta potential (+32.5±1.2 to +42.3±2.9 mV), drug content (70.196 ± 8.26 to 95.882 ± 15.33%), drug release kinetic studies best fitted to zero order release. Size range of all the batches was within 500 nm with polydispersity index of 0.4 to 0.6. Additionally, SEM images showed almost spherical particles with smooth surface were obtained. No major drug polymer interaction was detected using FTIR, DSC, PXRD studies done for solid state characterization. Drug encapsulation efficiency was found to be in the range of 75% to 95% which is high due to the solubility of ketorolac. In terms of Encapsulation efficiency, batch F3 and F6 showed relatively higher drug Encapsulation efficiency. The percentage yield of all the formulations ranged between 27-42%. All the batches showed sustained drug release profile. The percentage release for all the formulations ranged from 70-93% for all the formulations after 12 h. Short term stability studies of around two months revealed stable nanoparticle with no significant change in drug content.

Keywords: Ketorolac tromethamine, Nanoprecipitation, transdermal, DSC studies

INTRODUCTION

Oral drug delivery has been known for decades as the most widely used route of administration among all the routes attributed to its ease of administration. Oral controlled drug delivery systems, designed to deliver drugs at predetermined rates for predefined periods of time have been used to overcome the short comings of conventional drug formulations that could revolutionize method of medication and provide a number of therapeutic benefits like single dose, minimized duration of treatment, drug delivery at site of action and minimizing or eliminating side effects [1-3]. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Nanocarriers with optimized physicochemical and biological properties are taken up by cells more easily than larger molecules, so they can be successfully used as delivery tools for currently available bioactive compounds. The gastrointestinal side effects of nonsteroidal anti-inflammatory drugs (NSAIDs) have limited their widely oral use as analgesics in the treatment of local inflammation. This has prompted researchers to investigate the feasibility of alternative dermal and/or transdermal drug delivery systems. Ketorolac is a pyrrolizine carboxylic acid derivative of NSAIDs with potent analgesic and moderate anti-inflammatory activity, a relatively favorable therapeutic agent for the management of moderate to severe pain [4]. Ketorolac tromethamine is administered intramuscularly and orally in divided multiple doses for short-term management of postoperative pain. Its oral bioavailability is 90% with a very low first pass metabolism. However, the drug is reported to cause severe gastrointestinal side effects such as gastrointestinal bleeding, perforation, peptic ulceration, and acute renal failure [5]. Because of the short half-life (4-6 h) of ketorolac, frequent dosing is required to alleviate pain. To avoid intramuscular injection and frequent dosing regimens, dermal and transdermal delivery of ketorolac is an attractive alternative. Additionally, high analgesic activity and low molecular weight of ketorolac make it a good candidate for transdermal delivery. Several transdermal
Archna et al. “Formulation and Characterization of Ketorolac Tromethamine Nanoparticle with Eudragit RS-100 and RL-100 by Nano precipitation Method”

delivery strategies such as use of permeation enhancers [6], proniosomes [7], its prodrugs [8], iontophoresis [9], ultrasound [10], cyclodextrins and liposomes [11], and nanostructured lipid carriers (NLCs) [12] have been developed so far.

The present study aims to prepare and characterize nanoparticle of ketorolac tromethamine with RS-100 and RL-100 as polymers to enhance dissolution and bioavailability of the drug.

MATERIAL AND METHODS

Materials

Ketorolac Tromethamine (KT) was obtained from Venkatasai Lifesciences, Hyderabad. Eudragit RL 100 and Eudragit RS 100 were purchased from VikramThermo India, Gujrat. Potassium dihydrogen Phosphate and n-Octanol were purchased from SD Fine Chemical Limited, Mumbai. Tween 80 was obtained from Thomas Baker, Mumbai. Methanol and HCl were purchased from Merck Specialities Pvt. Ltd., New Delhi, India.

Method

Preparation Methodology of Nanoparticles

NP were prepared by the nanoprecipitation method with slight modification [13-15]. A modified nanoprecipitation method utilizes use of a co-solvent to either increase the entrapment efficiency of the drug in nanoparticles or to reduce the mean particle size of the nanoparticles. Different amount of Eudragit RS 100 or RL 100 and 200 mg of KT were dissolved in 10 ml methanol. The mixture was then added to 100 ml of 0.1 N HCl solution and then added 0.25 ml of Tween 80.

Table1. Formulation design for preparation of ketorolac nanoparticles

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Formulation Code</th>
<th>Drug (mg)</th>
<th>Polymer</th>
<th>Drug: polymer ratio</th>
<th>0.1N HCl (ml)</th>
<th>Tween 80 (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>200</td>
<td>Eudragit RS 100</td>
<td>1:2</td>
<td>100</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>200</td>
<td>Eudragit RS 100</td>
<td>1:3</td>
<td>100</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>200</td>
<td>Eudragit RS 100</td>
<td>1:5</td>
<td>100</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>200</td>
<td>Eudragit RL 100</td>
<td>1:2</td>
<td>100</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>200</td>
<td>Eudragit RL 100</td>
<td>1:3</td>
<td>100</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>200</td>
<td>Eudragit RL 100</td>
<td>1:5</td>
<td>100</td>
<td>0.25</td>
</tr>
</tbody>
</table>

The mixture obtained was stirred using a mechanical stirrer for 1 h. The resulting particle suspension was separated from unincorporated drug by centrifugation. The supernatant discarded and the NP precipitate washed with distilled water. The process was repeated till the washing was completely drug free (n=3). After that the nanoparticles were lyophilized (−40°C) the sample. Powder form obtained as nanoparticle.

Fourier Transform Infrared Spectroscopy (FTIR)

Before formulation development, FT-IR spectra of physical mixtures of ketorolac with polymers were compared with the standard FT-IR spectrum of the pure drug by finely grounding and dispersing in KBr using FTIR spectroscopy was performed on a Spectrum 65 (Perkin Elmer). Spectra were recorded between 4000 and 500 cm⁻¹ range[16].

Powder X-ray Diffractometric (PXRD) Study

The powder X-ray diffraction pattern of the obtained sample of the ketorolac was determined to confirm the crystalline structure of drug. XRD patterns were obtained with a Seifert Germany ISO debyeflex 2002 apparatus (Japan) using Cu-Kα radiation (λ = 1.541 nm), a voltage of 40 kV and a 100 mA current. Samples were scanned from 0-60° 2θ for qualitative studies and the scanning rate was 4°/min.

Differential Scanning Calorimetric (DSC) Study

DSC study was done on pure Ketorolac. Samples (3-4 mg) were placed in aluminum pan and heated at a rate of 10°C/min, in an atmosphere of nitrogen to a temperature of 300°C, using indium in the reference pan [16].

Scanning Electron Microscopy (SEM) Study

These studies were performed using scanning electron microscope (Zeiss EVO 60 with Oxford EDS detector) at 250X magnification to determine the surface morphology of the drug. The studies were carried out using the optimized formulation F3 and F6.
Archna et al. “Formulation and Characterization of Ketorolac Tromethamine Nanoparticle with Eudragit RS-100 and RL-100 by Nano precipitation Method”

**Partition Co-Efficient Determination**

The Partition coefficient between n-octanol/water was determined at room temperature (30°C). 10 ml of n-octanol and 10 ml of distilled water was taken in a glass stoppers graduated tube and 5 mg of accurately weighted drug was added, the mixture was then shaken with the help of mechanical shaker for 24 h at room temperature the mixture was then transferred to a separating funnel and allowed to equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane and drug content in aqueous phase was analyzed by UV-Visible spectrophotometer.

**Particle Size Determination [17]**

Particle diameter was determined using the particle size analyzer (Malvern Mastersizer, Malvern Instruments Ltd., Malvern, UK). To analyze particle size, nano-suspensions were diluted five times with distilled water. Each value was measured in triplicate determination.

**Surface Charge (Zeta Potential) Studies [18]**

The zeta potential of the NPs was measured using the Zeta-sizer 2000 (Malvern instruments Ltd. Malvern, UK). Measurements were carried out at 25°C in 10^{-3} M NaCl solution to keep the ionic strength constant. For each preparation, three samples were injected in the capillary cell of the Zetasizer and each of them was determined 20 times. Afterwards, the average values of three replicates were calculated.

**Drug Content Determination**

All the formulations were then subjected for determination of drug content. The drug content was determined by diluting 1 ml of the formulation to 100 ml with buffer pH 1.2. Aliquot of 1 ml was withdrawn and further diluted to 10 ml with buffer pH 1.2. Ketorolac concentration was then determined at 319 nm by using UV-Vis spectrophotometer. Afterwards, the average values of three replicates were calculated.

**In Vitro Release Studies**

The *in vitro* release of Ketorolac from all the formulations was studied through dialysis membrane 12,000 MWCO (Spectra por, Sigma, USA) retaining NPs and allowing free drug using a dialysis membrane diffusion technique. The dissolution medium used was buffer pH 1.2. An amount of formulation equivalent to 20mg was accurately kept in dialysis membrane, previously soaked overnight in the dissolution medium to form a pouch. This pouch was immersed in a receiving compartment containing 100 ml buffer pH 1.2. This volume provided complete sink conditions for the drug. The entire system was kept at 37 ± 0.5°C with continuous magnetic stirring at 100 rpm. At pre-determined time intervals samples (2ml) were withdrawn and aliquots volume of fresh medium at the same temperature were diluted with the dissolution medium and analyzed by UV-Vis spectrophotometer at 319 nm.

**Drug Release Kinetics Studies**

Drug release kinetics was studied by curve fitting method to different kinetic models of zero order, first order, Higuchi and Korsmeyer-Peppas models. To find out the mechanism of drug release, 60% drug of release data was first fitted in the Korsmeyer-Peppas model. The model was used to study the drug release mechanism by analyzing ‘n’ as the diffusion exponent. According to this model if ‘n’ is below 0.45, Fickian mechanism governs drug release; if between 0.45 to 0.89, Non-Fickian mechanism governs drug release and if n is 0.89 or greater than 0.89, then release mechanism is governed by case-II transport or super case II transport mechanism respectively[19].

**Stability Studies**

Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. Two randomly selected formulations (F3 and F6) (one from each polymer) were subjected to stability studies conditions at 4°C, room temperature (25°C) with ambient humidity, 37°C with 80% relative humidity and 60°C for a period of two months. The samples were withdrawn after 15, 30 and 60 days and were evaluated for drug content.
RESULT AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR)

Polymers, physical mixtures of ketorolac with polymers shows compatibility of drug and polymer. IR spectra of the pure drug, and optimized formulations F3 and F6 (Figure 1).

![Figure1. FTIR spectra of pure drug (a), F3 (b) and F6 (c)](image)

Appearance of the peaks at the functional group of the drug in the spectra shows the entrapment of the drug. Pure drug exhibits spectra at 3348.35 cm\(^{-1}\) (free OH group) and 3350.46 cm\(^{-1}\) (bonded OH group); whereas in F3 and F6 free OH group disappear indicating the complex formation.

Powder X-ray Diffractometric (PXRD) Study

PXRD patterns of pure drug and optimized formulations F3 and F6 were studied (Figure 2).

![Figure2. P-XRD of pure drug (a), F3 (b) and F6 (c)](image)

The PXRD of ketorolac exhibited characteristic sharp peaks at numerous 2\(\theta\) values of 9.64, 12.72, 14.1, 16.46, 18.78, 19.22, 20.3, 22.1, 22.9 and 29.3 indicating its crystalline nature. Although most characteristic peaks of ketorolac were disappeared in the F3 and F6, some portions of such small and sharp peaks still existed in the curve exhibiting the crystallinity of the formulations.

Differential Scanning Calorimetric (DSC) Study

DSC studies were performed using to obtain the endotherms of the drug and polymer respectively. These studies further confirmed the entrapment of the drug and also helped in understanding the behavior of the complex. DSC thermograms of pure drug, optimized formulations (F3 and F6) were obtained. The unique melting point of pure ketorolac is 169.04°C. But incorporation of higher concentration of polymers Eudragit RS 100 and RL 100 (1:5) exhibits different melting points (Figure 3).

![Figure3. DSC of pure drug (a), F3 (b) and F6 (c)](image)

The sharp peak of F3 indicates the crystalline nature of the formulation and broad peak of F6 indicates the amorphous nature of formulation.
Scanning Electron Microscopy (SEM) Study

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. The optimized formulation F3 and F6 were chosen for these studies and the images revealed the rectangular size and shape of them (Figure 4).

Figure 4. SEM of pure drug (a), F3 (b) and F6 (c)

Partition Coefficient Determination

Partition coefficient for the ketorolac in octanol/water was found to be 2.2 indicating that the drug is lipophilic in nature.

Particle Size Determination

The mean particle size of F2 (0.276 ±0.015nm) was very small but the size of F1 is bit higher size (1.236 ±0.115nm). Both the optimized formulations F3 and F6 were found satisfactory. Optimal particle size should be needed for a drug delivery, so that it will not easily leak out of the capillaries and up taken by the macrophages. It will help in the active targeted delivery of nanoparticle into the site leads to faster accumulation and release of drug to that particular site (Table 2).

Table 2. Particle size, zeta potential and percentage of drug content values for formulations

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Particle Size ±S.D. (nm)</th>
<th>Zeta Potential ± S.D. (mV)</th>
<th>Drug Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.236 ±0.115</td>
<td>(+36.4±2.2</td>
<td>83.529 ± 10.25</td>
</tr>
<tr>
<td>F2</td>
<td>0.276 ±0.015</td>
<td>(+34.4±1.5</td>
<td>89.411 ± 11.18</td>
</tr>
<tr>
<td>F3</td>
<td>0.462 ±0.019</td>
<td>(+40.2±3.2</td>
<td>93.725 ± 14.03</td>
</tr>
<tr>
<td>F4</td>
<td>0.898 ±0.025</td>
<td>(+36.3±1.3</td>
<td>70.196 ± 8.26</td>
</tr>
<tr>
<td>F5</td>
<td>0.362 ±0.012</td>
<td>(+32.5±1.2</td>
<td>85.098 ± 10.94</td>
</tr>
<tr>
<td>F6</td>
<td>0.462 ±0.019</td>
<td>(+42.3±2.9</td>
<td>95.882 ± 15.33</td>
</tr>
</tbody>
</table>

Surface Charge (Zeta Potential) Studies

F3 and F6 formulations were found satisfactory because of their higher (40.2±3.2 and 42.3±2.9 mV) zeta potential values as mentioned in classification of colloidal system according to zeta potential distribution. Greater the zeta potential, greater the stability. Zeta potential was a reliable indicator in the prediction of stability of particles in liquid medium and the possible interactions with other materials.

Drug Content Determination

The drug content of all formulations was around 98%. HPLC data confirmed that there was no drug degradation during preparation of SLNs. The entrapment efficiencies of all formulations (F1-F6) were 96-97%. This confirmed that the drug dissolved in lipid matrix remained associated with the matrix and there was no drug diffusion.

In Vitro Release Studies

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its in vivo behavior. It was found that cumulative percent drug release was 83.52%, 89.41%, 93.72%, 70.19%, 85.09% and 86.22% for formulation F1, F2, F3, F4, F5 and F6 respectively after 12 h. The rapid initial release involved the diffusion of the bound or adsorbed drug at the surface of the nanoparticles. The prolonged release in the later stage can be attributed to the slow diffusion of the drug through polymer matrix (Figure 5).
Archna et al. “Formulation and Characterization of Ketorolac Tromethamine Nanoparticle with Eudragit RS-100 and RL-100 by Nano precipitation Method”

22 International Journal of Research in Pharmacy and Biosciences V4 ● I1 ● January 2017

**Figure 5.** In Vitro drug release studies of F1-F6 formulation

Drug Release Kinetics Studies

Plots of Zero order, First order, Higuchi matrix, Korsmayer Pappas and Hixson Crowell for the formulations were plotted and the regression coefficient ($r^2$) values and the ‘n’ values for Korsmayerpappas(Table 3).

*Table 3. Model fitting for the release profile of pH sensitive formulations by using 5 different models*

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi matrix</th>
<th>Korsmeyer-Peppas</th>
<th>Hixson-Crowell</th>
<th>Best fit model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$R^2$</td>
<td>$R^2$</td>
<td>$R^2$</td>
<td>$R^2$</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0.988</td>
<td>0.920</td>
<td>0.927</td>
<td>0.990</td>
<td>0.951</td>
<td>Korsmeyer-Peppas</td>
</tr>
<tr>
<td>F2</td>
<td>0.988</td>
<td>0.935</td>
<td>0.947</td>
<td>0.973</td>
<td>0.964</td>
<td>Zero</td>
</tr>
<tr>
<td>F3</td>
<td>0.979</td>
<td>0.832</td>
<td>0.908</td>
<td>0.971</td>
<td>0.900</td>
<td>Zero</td>
</tr>
<tr>
<td>F4</td>
<td>0.991</td>
<td>0.964</td>
<td>0.942</td>
<td>0.969</td>
<td>0.976</td>
<td>Zero</td>
</tr>
<tr>
<td>F5</td>
<td>0.987</td>
<td>0.964</td>
<td>0.949</td>
<td>0.991</td>
<td>0.980</td>
<td>Korsmeyer-Peppas</td>
</tr>
<tr>
<td>F6</td>
<td>0.990</td>
<td>0.946</td>
<td>0.978</td>
<td>0.978</td>
<td>0.951</td>
<td>Zero</td>
</tr>
</tbody>
</table>

Table shows that the best fit model was Zero order, while ‘n’ exponent value, for Pappas model, for formulations F1 and F5 is greater than 0.45 indicating that formulation is released by Non-Fickian diffusion mechanism. While for formulations F2, F3, F4 and F6 the ‘n’ exponent value for Pappas model is less than 0.45 indicating that formulation is released by Fickian diffusion mechanism.

Stability Studies

Optimized formulations (F3 and F6) were subjected to stability studies at 4°C, room temperature (25°C) with ambient humidity, 37°C with 80% relative humidity and 60°C for a period of two months, and evaluated for percent drug content. Both showed slight decrease in drug content at 25°C and at 37°C after 60 days of storage whereas at 4°C and 60°C showed significant decrease in the drug content. This significant decrease in drug content is due to the precipitation of drug in all three formulations at refrigeration temperature (4°C) and at 60°C temperature. From the stability studies it was confirmed that formulations of ketorolac remained most stable at ambient temperature (25°C) and humidity. After storage at 25°C, there was no significant change ($P > 0.05$) in the mean particle size and in drug content.

CONCLUSION

In this study, the potential of Eudragit RS 100 &Eudragit RL 100 nanoparticle with potential for oral delivery of Ketorolac was investigated. Nanoparticle was prepared by nanoprecipitation technique which is the easiest and reproducible method to prepare nanoparticles without need of any sophisticated instruments. Size range of all the batches was within 500 nm with polydispersity index of 0.4 to 0.6. Additionally, SEM images showed almost spherical particles with smooth surface were obtained. No major drug polymer interaction was detected using FTIR, DSC, PXRD studies done for solid state characterization. Drug encapsulation efficiency was found to be in the range of 75% to 95% which is high due to the solubility of ketorolac. In terms of Encapsulation efficiency, batch F3 and F6 showed relatively higher drug Encapsulation efficiency. The percentage yield of all the formulations ranged between 27-42%. All the batches showed sustained drug release profile. The percentage release for all the formulations ranged from 70-93% for all the formulations after 12 h. Short term stability studies of around two months revealed stable nanoparticle with no significant change in drug content.
Archana et al. “Formulation and Characterization of Ketrorolac Tromethamine Nanoparticle with Eudragit RS-100 and RL-100 by Nano precipitation Method”

Thus the project allowed the development of a novel formulation of ketorolac which would allow sustained release of the drug for more than 12 h and thus would prevent the gastric irritation which is a major setback for the use of Ketrorolac. Besides the sustained action of the formulation would help overcome the issue of short half-life. However there is still a lot of scope for future research especially, in vivo studies which would throw light on the actual bioavailability of the drug as the results obtained in vitro may vary from in vivo results.

REFERENCES