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Fabrication and characterization of solid lipid microparticles of ketoprofen

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ABSTRACT

Solid lipid microparticles (SLMs) loaded with ketoprofen were prepared by single emulsion-solvent evaporation method, in which glyceryl monostearate and Tween 80 were employed. The particle size was found to be 99.80±2.1µm. Microparticles observed by scanning electron microscope (SEM) showed spherical shape. The entrapment efficiency (EE %) and drug loading capacity (DL %) were found to be 72.60±1.6 % and 17.98±0.7% respectively. Results of stability evaluation showed relatively long term stability after storage at 4˚C for 3 months. The in-vivo study revealed slightly better per cent inhibition of pain i.e. 74% in comparison with 68% produced by plain drug.

KEY WORDS: Solid lipid microparticles, ketoprofen, solvent evaporation method.

RESUMEN

Las micropartículas lipídicas sólidas (MLS) cargadas con ketoprofeno se han preparado a través del método de evaporación del disolvente en emulsión simple, en el que se ha utilizado monoestearato de glicerilo y Tween 80. El tamaño de la partícula ha resultado ser de 99,80±2,1 µm. Las micropartículas observadas a través del microscopio electrónico de barrido (MEB) han mostrado una forma esférica. La eficacia de compresión (EC %) y la capacidad de concentración (CC %) del fármaco han resultado ser de 72,60±1,6% y 17,98±0,7% respectivamente. Los resultados de la evaluación de estabilidad han mostrado una estabilidad relativa a largo plazo después de una conservación a 4˚C durante 3 meses. El estudio in vivo ha revelado un ligero mejor porcentaje de inhibición del dolor, es decir, un 74% en comparación con un 68% producido por un fármaco corriente.

PALABRAS CLAVE: Aceclofenaco, profármaco polimérico, dextrano, índice de úlcera, histología patológica.

INTRODUCTION

Solid lipid microparticles are particles of micron size made from lipids that remain in a solid state at room temperature and body temperature and stabilized by a surfactant[1]. Except for their size, they are very similar to solid lipid nanoparticles[2]. Ketoprofen is a widely preferred therapeutic agent in the treatment of varied pains i.e. osteoarthritis, rheumatoid arthritis and postoperative pain[3]. However, the clinical application of ketoprofen is limited due to its multiple unwanted effects on gastrointestinal tract[4]. Moreover, the unfavorable physicochemical characteristics of ketoprofen viz. poor aqueous solubility and photo degradation are difficult to be dealt by conventional dosage forms in practice[5].

In the current study, lipid based microparticles (SLMs) have been conceived to target ketoprofen, while providing a conducive microenvironment in favour of hydrophobic and photo labile ketoprofen molecule. Glyceryl monostearate has been used as a matrix ingredient for a biodegradable, implantable, controlled release dosage form[6,7]. It is enlisted Generally Recommended As Safe (GRAS) and included in Food and Drug Administration (FDA) inactive ingredients guide.

MATERIALS AND METHODS

Materials

Ketoprofen was received as gift sample from BEC Chemicals Pvt. Ltd., India. All other ingredients were procured from SD Fine Chemicals Limited, India.

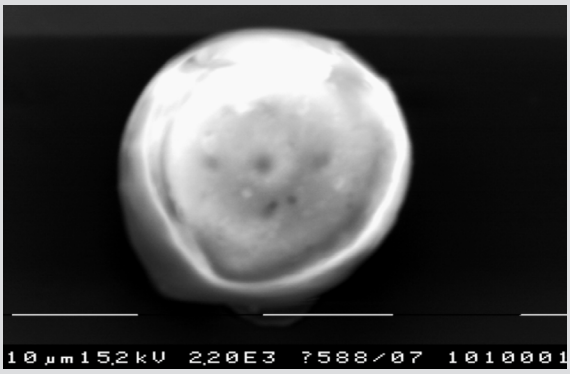
Preparation of solid lipid microparticles:

The SLMs were prepared by a single emulsion solvent evaporation method.[8] Ketoprofen and glyceryl monostearate in a ratio of 1:3 were stirred in a solvent mixture of ethanol and chloroform (1:2 v/v) with the aid of magnetic bead, till a uniform dispersion resulted. The drug polymer dispersion was added slowly in 150 ml of liquid paraffin (light) containing 0.4% v/v of tween-80. The resultant mixture was stirred using a mechanical stirrer at a speed of 1000 rpm at room temperature for a period of 4 hours. Ketoprofen loaded microparticles were then filtered, washed with acetone and freeze-dried. The preparation method was optimized for effect of drug: lipid ratio, stirring speed, amount of crosslinking agent and concentration of emulsifier.

Characterization of prepared solid lipid microparticles:

Surface morphology and Shape: The morphological study of microparticles was carried out by scanning electron microscopy. Samples were prepared from dilutions in distilled water of particle suspensions and dropped on to stubs. After drying in air, particles were coated with a thin layer of gold and then examined by SEM. The SEM image of SLMs is shown in Figure 1.

Figure 1. SEM image of ketoprofen solid lipid microparticles



Particle size: Average particle size of prepared microparticles was determined by laser diffraction using a Malvern Mastersizer 2000. The average particle size of optimized batch was found to be 99.80±2.1µm.

Drug loading, encapsulation efficiency and process yield:

Microparticles were separated from aqueous medium by centrifugation and kept in 4% HCl solution for 24 hours. The amount of drug present in the microparticles was determined as the difference between the total amount of drug used to prepare the microparticles and the amount of drug present in aqueous medium, utilizing following formulae:

$$DE\ (\%w/w) = \frac{(MTD - MFD)}{MTD} x100$$

$$DL\ (\%w/w) = \frac{(MTD - MFD)}{MTM} x100$$

$$\text{Process yield } (\%) = \frac{(\text{mass of microparticles}) \times 100}{\text{Total mass of drug + lipid}}$$

DE: Drug entrapment;
MTD: Mass of total drug;
MFD: Mass of free drug;
MTM: Mass of total microparticles

The results of these determinations are given in Table 1.

In-vitro drug release studies:

The optimized microparticle formulation of ketoprofen was further evaluated for in-vitro drug release kinetics and in-vivo sustained action. The drug release studies were

carried out by rotating basket method. Weighed amount of microparticles containing 100 mg equivalent of ketoprofen were placed in the basket of dissolution rate apparatus USP XIX. 900 ml of 0.1 N HCl was used as dissolution fluid. The basket was rotated at 100 rpm and temperature was maintained at 37±1°C. 1 ml samples were withdrawn for analysis at regular intervals and immediately replaced with dissolution fluid to maintain sink conditions. The withdrawn samples were suitably diluted and analyzed spectrophotometrically at a wavelength of 254 nm.

Stability studies:

Prepared formulations were stored in screw capped, small glass bottles at 4˚C, 25˚C, and 50˚C. Samples were analyzed for residual drug content after a period of 15,30,45,60 and 90 days.

In vivo studies:

Rat tail flick method was used for assessment of analgesic activity of prepared SLMs. The results were compared with a control group and a group treated with plain drug. Cross over study design was employed. Following formula was used to calculate % inhibition of pain.

% inhibition = (Vc-Vt)/Vc × 100

where

Vc = mean time of tail flick in control group

Vt = mean time of tail flick in test and standard group

RESULT AND DISCUSSION

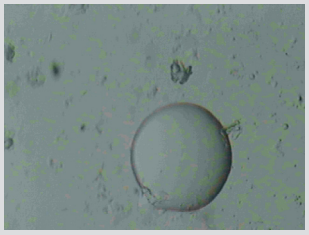
The effects of variables such as polymer concentration, stirring rate, and emulsifier concentrations on the particle size of the microparticles were studied. The mean diameter of the lipid microparticles on increasing glyceryl monostearate concentrations (i.e., at drug-glyceryl monostearate ratios from 1:1 to 1:5) increased from 87.60µm to 108.30µm. This increase in particle size of the microparticles could be attributed to an increase in viscosity with increasing polymer concentrations, which resulted in larger emulsion droplets and finally in greater microparticle size. The mean diameters of microparticles prepared at various agitation speeds (i.e., 500, 1000, and 1500 rpm) were 101.0, 99.20, and 68.50µm, respectively. The dispersion of a drug and glyceryl monostearate into the droplets in the oil phase was dependent on the agitation speed of the system. As agitation speed increased, the size of microparticles reduced, but beyond a certain agitation speed it led to increased size of microparticles and loss of spherical shape. This could be due to agglomeration of particles and generation of surface charge. The mean diameter of the microparticles prepared using various

Table 1: Characterization parameters for ketoprofen SLM

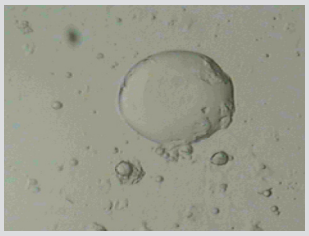
Characterization parameters	Result (mean± SD) n = 3
Drug entrapment (%w/w)	72.6±1.60
Drug loading (%w/w)	17.98±0.70
Process yield (%)	52.23±2.16

SD: Standard Desviation

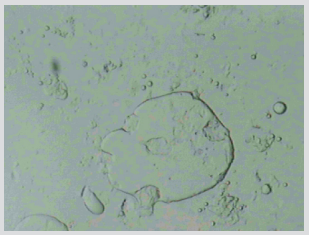
Figure 2. Different stages of dissolution; a) Onset of erosion b) erosion and diffusion c) Advanced stages of erosion and diffusion d) Loss of structural integrity due to diffusion and erosion e) Total Loss of structural integrity and release of drug



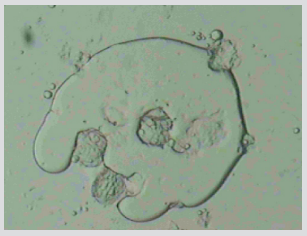
a) After 1 hour of dissolution



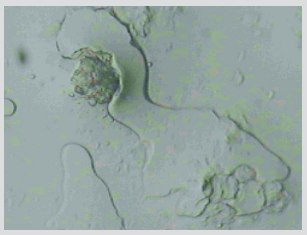
b) After 2 hour of dissolution



c) After 4 hour of dissolution



d) After 6 hour of dissolution



e) After 12 hour of dissolution

concentrations of surfactant (i.e., 0.2, 0.3, 0.4 and 0.5%v/v), were 108.0, 105.0, 99.80, and 85.63µm, respectively. As the surfactant concentration increased, the microparticles become more regular well shaped spheres while the size of the microparticles reduced from 108.0 to 85.63µm. An increase in the level of surfactant may have stabilized a greater interfacial surface area, thus leading to smaller particle size. The turbulence created by the stirrer is better conveyed to every portion of the dispersion medium because of reduced interfacial tension.

These microparticles were spherical in shape and distributed in the size range of 90 to 110 µm, with an average size of 99.80±2.1µm. The particles exhibited a smooth surface, as indicated in SEM (Figure 1). The average entrapment efficiency (EE %) and drug loading capacity (DL %) were found to be 72.60±1.6% and 17.98±0.7% respectively (Table 1). The ketoprofen release from microparticles was found to be 75.5% after 12 hrs. The r^2-value of the microparticles indicated delayed diffusion control with initial burst release (first order kinetics). The value of exponent coefficient (n) for optimized batch (n=0.5593) indicated anomalous type of release. The fate of a microparticle at various stages during dissolution is highlighted in Figure 2a-e.

The results of the stability studies suggest that at 50±1˚C, the microparticles loose their spherical shape indicating their instability at higher temperature which is obvious due to lipoidal nature. There was no significant loss in drug content when the SLMs were stored at 4˚C and 25˚C. For adequate shelf life of microparticles, the ideal storage temperature is a cold place i.e. 4-8˚C. The findings of in-vivo analgesic activity studies of fabricated formulation revealed that it had slightly better per cent inhibition of pain i.e. 74% in comparison with 68% produced by plain drug.

CONCLUSION

Results of present study provided an insight into the significance of lipid microparticles as an oral delivery device for ketoprofen. The investigated system has potential to remain in treated site for a prolonged period and capable of maintaining constant concentration of drug through a longer duration of time due to its sustained action. This shall reduce the frequency of administration and decrease the dose dependent side effect associated with repeated administration of conventional ketoprofen loaded dosage forms, which ultimately improves patient compliance.

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