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Design and characterization of zaltoprofen nanosuspension by precipitation method

Amruta Papdiwal*, Vishal Pande and Kishor Sagar

Sanjivani Rural Education Society's. Sanjivani College of Pharmaceutical Education and Research, Kopergaon, India

ABSTRACT

Poor water solubility and slow dissolution rate are issues for the majority of upcoming and existing biologically active compounds. Zaltoprofen is a novel non-steroidal anti-inflammatory class of drug, but problem associated with is the poor solubility in biological fluids. Zaltoprofen is BCS Class-II having low solubility and high permeability. The purpose of the present investigation was to increase the solubility and dissolution rate of Zaltoprofen by the preparation of nanosuspension by nanoprecipitation technique. Prepared nanosuspension was evaluated for its particle size and in-vitro dissolution study and characterized by differential scanning calorimetry and scanning electron microscopy. The optimized formulation showed an average particle size (237 nm) and zeta potential (-21.5 mV). The rate of dissolution of the optimized nanosuspension was enhanced (89 % in 50 min) relative to micronized suspension of Zaltoprofen (30 % in 50 min), mainly due to the formulation of nanosized particles. Stability study revealed that nanosuspension was more stable at room and refrigerator condition with no significant change in particle size distribution. These results indicate that the Zaltoprofen loaded nanosuspension significantly improved in-vitro dissolution rate and thus possibly enhance fast onset of therapeutic drug effect.

Keywords: Zaltoprofen, Nanosuspension, Nanoprecipitation, Solubility, Dissolution rate

INTRODUCTION

Amongst the various routes of administration the oral route is the one commonly used and most convenient for the drug delivery. Oral drug delivery system has received more attention in the pharmaceutical field, because of its more flexibility in designing the dosage form than other drug delivery system [1]. More than 40% of the new chemical entities being generated through drug discovery programmes are faced the problem for aqueous solubility and become a hurdle for the formulation [2]. Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement [3]. Nanosuspensions are basically suspension where the particle size of the suspended material is within the range of 10-1000 nm [4, 5].

Nanosuspension platform is an efficient and intelligent drug delivery system for water insoluble drugs, as the saturation solubility and the surface area available for dissolution increased [6, 7]. Generally, the biopharmaceutical advantages of water insoluble drugs formulated as nanosuspensions including improvement in formulation performance, such as high drug loading, reproducibility of oral absorption, improved dose-bioavailability proportionality, reduced toxicity and side effects and increased patient compliance via reduction of number of oral units to be taken [8, 9].

Zaltoprofen is model drug; it is a novel non-steroidal anti-inflammatory class of drug acts as a potent and superior analgesic for the treatment of chronic Rheumatoid arthritis, post trauma, chronic inflammation, and acute respiratory

infections. Zaltoprofen acts by inhibiting prostaglandin synthesis through a peripheral mechanism by inhibition of bradykininB₂ receptor mediated responses in primary afferent neurons [10, 11].

Most commonly used stabilizers to stabilize nanosuspension are either polymer like (e.g., polyvinyl pyrrolidone (PVP), crystalline cellulose [12], amphiphilic amino acid [13], hydroxypropyl cellulose (HPC) [14], hydroxypropyl methyl cellulose (HPMC) [15], and d- α -tocopherol polyethylene glycol 1000 succinate (TPGS 1000) [16, 17, 18] whereas surfactant such as ionic are (e.g., sodium dodecyl sulphate (SDS), sodium lauryl sulphate (SLS), poly(ethyleneimine) (PEI) [19], chitosan [20] and non-ionic surfactant (e.g., polysorbate (tween 80), block copolymer like pluronic) and some food protein are also used as stabilizers such as soyabean protein isolate, whey protein isolate and β -lactoglobuline.

Nanosuspension are prepared by two methods first is Bottom-up and second is Top-down method. In the present work nanosuspension is prepared by bottom up method in which drug is dissolved in a solvent, which is then added to non-solvent that cause precipitation of the fine drug particle and the system is stabilize by polymer and or surfactant to prevent them from aggregation or agglomeration [21]. The objective of this work is to formulate Zaltoprofen nanosuspension by nanoprecipitation method. The response such as particle size, polydispersity index, zeta potential, drug content, dissolution rate etc were evaluated in this study.

MATERIALS AND METHODS

Materials

Zaltoprofen JP 2-(10, 11-dihydro-10-oxodibenzo [b, f] thiepin-2-yl) propionic acid was purchased from ZCL chemicals Limited, Gujarat, India. Lutrol F68 (Poloaxamer 188) gift sample from BASF (Germany). Hydroxypropyl methyl cellulose 5 cps (HPMC) was purchased from Samsung fine chemical Co., LTD., Korea. All other solvents and chemicals used were of analytical grade and obtained from S.D Fine Chemicals (Mumbai, India).

Method

Nanosuspensions were prepared according to nanoprecipitation method. Pure drug Zaltoprofen and HPMC (5cps) was dissolved in (1ml) acetone at 40°C to form uniform organic solution. The prepared organic solution was then injected slowly dropwise with the help of a syringe into an aqueous phase (20 ml) containing stabilizers (pluronicF68) under high speed mechanical agitation of 8000 rpm to get desired nanodispersion. Prepared nanosuspension was then stirred magnetically at 500 rpm at room temperature for 12 h to evaporate organic solvent. Complete evaporation of acetone was determined by spectrophotometric method. The volume was then adjusted with the addition of triple distilled water to recover loss in keeping other parameters constant [22]. The batches were prepared according to the formulation design in Table no. I

Table no. I: Formulation of Zaltoprofen nanosuspensions

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg/ml)	80	80	80	80	80	80	80	80	80
Pluronic F-68(mg/ml)	3	6	30	3	6	30	3	6	30
HPMC (5 cps) (mg/ml)	6	6	6	18	18	18	30	30	30
Acetone(ml)	1	1	1	1	1	1	1	1	1
Water(ml)	20	20	20	20	20	20	20	20	20

Lyophilisation and Redispersibility of Nanosuspensions

Zaltoprofen nanosuspension were frozen and lyophilized using lyophilizer (Decibel digital, India) for 24h (-40°C). The freeze-dried samples were diluted to original volume with triple distilled water and redispersibility was observed. Freeze-dried samples were further used for solid state characterization.

Characterization of Nanosuspension

a. Particle Size Analysis

Average particle size and polydispersibility index of formulations were determined by Malvern Zetasizer ZS (Nano series ZS 90 UK) using water as dispersion medium. The sample was scanned 100 times for determination of particle size.

b. Zeta Potential

Zeta potential of formulation was measured using Malvern Zetasizer ZS (Nano series ZS 90 UK). The samples were diluted 10 times with solvent before analysis. Physically stable nanosuspensions solely stabilized by electrostatic repulsion, a zeta potential of ± 30 mV is required as a minimum. Combined with the steric stabilization, the absolute value of zeta potential about ± 20 mV is sufficient to fully stabilize the nanosuspensions system [23].

c. Total Drug Content

An aliquot (0.5ml) was evaporated to dryness. The residue was then dissolved in acetone and filtered with 0.45µm filter paper. The samples were analyzed using UV spectrophotometer at λ_{max} of 337 nm. Total drug content (TDC) and %TDC were calculated Eqs. 1 and 2.

$$\text{TDC} = \frac{\text{Vol. total}}{\text{Vol. Aliquot}} \times \text{Drug amount in aliquot} \times 100 \dots \dots \dots 1$$

$$\% \text{ TDC} = \frac{\text{TDC}}{\text{TAD}} \times 100 \dots \dots \dots 2$$

Where, Vol. Total/ Vol. Aliquot are the ratio of total nanosuspension volume to the volume of aliquot taken and the total amount of drug taken for the formulation of nanosuspension [24].

d. Scanning Electron Microscopy

The morphological features of Zaltoprofen nanosuspension are observed by scanning electron microscope at different Magnifications.

e. Fourier Transform Infrared Spectroscopy

The FT-IR analysis was conducted to check any interaction of chemical bonds between drug and excipients. FT-IR spectrum was performed by using a Shimadzu 8400 spectrophotometer. The samples were scanned in the region between 4000 and 400cm⁻¹. Solid powder sample were oven dried at around 300⁰C, finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15000psig (using a Carver Laboratory Press, Model C, Fred S. carver Inc., WIS 53051) to make disc and then scanned it [25].

f. Differential Scanning Calorimetry

The thermal properties of pure Zaltoprofen, physical mixture were characterized by differential scanning calorimeter (DSC-60, Shimadzu).The samples of about 5 mg were placed in standard aluminium pans, and dry nitrogen was used as effluent gas. All samples were scanned at a temperature ramp speed of 5 min, and the heat flow was set from 0 to 300⁰C. Before the experiment, the DSC was calibrated using pure Indium and heat of fusion (H fusion).

g. X-ray powder diffraction (XRPD)

XRD measurements were carried out with an X'Pert PRO diffractometer. The diffractograms of Zaltoprofen and lyophilized product were obtained for analysis.

h. Saturated Solubility

Saturation solubility of pure drug Zaltoprofen and nanosuspensions formulation was measured in buffer solution having different pH (1.2 to 10) buffers. The solution containing flasks were kept on a rotary shaker (Orbital shaking incubator Remi Lab. India) for 24hrs. After 24hr, solutions were analyzed using UV spectrophotometer at 337 nm, which was the absorption maxima determined earlier and drug concentrations were calculated (Agrawal S et al., 2004; Maheshwari RK et al., 2012; Soni LK et al., 2014).

i. In-vitro Drug Release

In-vitro dissolution test were performed in USP apparatus Type II (Electrolab Dissolution Tester USP TDT-08L) using paddle method at rotation speed of 100 rpm. Dissolution was carried out in 900ml phosphate buffer of pH6.8 as a dissolution medium and maintained temperature 37±0.5⁰C. Accurately weighed bulk drug and nanosuspensions (all equivalent to 80 mg of Zaltoprofen) were dispersed in dissolution medium. 5ml aliquots were removed at predetermined time intervals 0, 5, 10, 15, 25, 30, 35, 40, 45, 60 minute from dissolution medium and replace with same buffer solution for maintain sink condition and the sample were analyzed for the drug release using UV Spectrophotometer at the wavelength of 337 nm.

j. Stability Study

Stability study of optimized Zaltoprofen nosuspension was carried out by placing formulation in glass vials at different temperature condition for 3 months at room temperature (25⁰C), refrigerator (4⁰C). After 3 months samples were visually observed for any sedimentation and changes in particle size and size distribution using zeta sizer.

RESULTS AND DISCUSSION

Zaltoprofen is BCS Class-II drug with low solubility and high permeability. Thus, it was challenging to enhance the solubility and dissolution rate of Zaltoprofen particles in an aqueous solution. Solvent/antisolvent precipitation method was employed to produce nanosuspension of Zaltoprofen. The ratio of solvent to antisolvent was kept constant i.e 1:20 and stirring speed 8000 rpm and stirring time 9 hr was also kept constant. The confirmation of nanosuspension formation of a colloidal nanodispersion can be visualized by the bluish opalescence in appearance

Particle size and Polydispersity Index

The particle size distribution has most important characteristics affecting the in vivo fate of nanosuspension. The average particle size of F1-F9 batches was observed from the ranges of 237 nm to 579 nm as shown in Table II. The largest size 579 nm in F1 batch which could be due to the lower concentration of stabilizer, because too little concentration of stabilizer induces agglomeration or aggregation and too much concentration promotes Ostwald ripening. The optimized formulation (F6) showed an average particle size 237nm as shown in fig.1.

Polydispersity index gives degree of particle size distribution. It ranges from 0.230 to 0.545 depending on formulation variables (Table no. I).The formulation F1 showed lowest PDI (0.231) that indicates good uniformity in particle size distribution.

Table II. Physicochemical characterization of Zaltoprofen nanosuspensions

Batch Code	Particle size (nm)	Polydispersity index	Zeta potential (mV)	% Total Drug Content
F-1	554	0.421	-15.4	84
F-2	451	0.524	-13.5	86
F-3	420	0.356	-17.5	85
F-4	348	0.290	-19.1	88
F-5	254	0.325	-19.5	83
F-6	237	0.230	-21.5	89
F-7	309	0.542	-14.5	90
F-8	285	0.384	-18.1	81
F-9	325	0.356	-18.4	80

Zeta Potential Analysis

Zeta potential analysis was performed to investigate the surface properties of nanosuspension. Zeta potential is an important parameter for prediction of stability of nanosuspension. Zeta potential of formulation of F-1 to F-9 was observed between -8 to -21.5 mV. Zeta potential of Zaltoprofen nanosuspension (F-6) was found to be -21.5 mV (Fig. II). Thus, it was concluded that the system had sufficient stability.

Total Drug Content (TDC)

Table-III shows TDC for the prepared batches. TDC for all batches was satisfactory and was more than 80%, which indicates that loss of drug was lower during preparation process.

Scanning Electron Microscopy

This was carried out to study the surface morphology of particles. It was found that Zaltoprofen nanoparticle revealed a smooth texture (Fig. III). The SEM picture of pure drug particles was abundantly found with larger particle size when compared to F7 formulation. Thus, poloxamer 188 and HPMC produced better surface characteristics. The surface structure of nanosuspension in the SEM of F7 appeared good in shape.

Fourier Transform Infrared Spectroscopy-

FTIR analysis was used to evaluate the possible intermolecular interaction between Zaltoprofen and the excipients. The spectra of pure zaltoprofen, pluronic F68 and HPMC (3cps) and the Zaltoprofen nanosuspension of optimized formulation are shown in Fig. IV. FTIR spectra showed characteristic peaks such as C-H stretch at 3028 cm^{-1} , aryl C-H stretch at 2998 cm^{-1} , carbonyl C=O stretch of ketone and acid at 1703 and 1694 cm^{-1} respectively, O-H stretch at 2534 cm^{-1} , C=C stretch at 1675 cm^{-1} , C-O stretch at 1275 cm^{-1} and C-S stretch at 1420 cm^{-1} (17). These absorption bands all appeared and almost have the same value as the curve of Zaltoprofen nanosuspension.

Differential Scanning Calorimetry

The DSC thermograms of pure drug and optimized nanosuspension formulation were taken between 20-300 $^{\circ}$ C at a heating rate of 20 $^{\circ}$ C/min. From thermogram, it can be concluded that the drug and the polymer do not interact with each other. The data was represented in Fig.V

X-Ray Diffractometry(XRD)

Polymorphic changes in the drug are important since they might affect the dissolution rate and in line bioavailability. Hence it was necessary to study the Polymorphic changes Zaltoprofen in freeze dried nanosuspension product. Fig 6. Shows XRD of (A) pure Zaltoprofen, (B) Freeze dried powder of F-6 batch. X-ray diffraction pattern in fig. demonstrated that pure Zaltoprofen was clearly in crystalline state as it showed sharp distinct peak at 2 θ diffraction angles of 9.5 $^{\circ}$, 15.50 $^{\circ}$, 18.50 $^{\circ}$, 22.50 $^{\circ}$, 32.50 $^{\circ}$, and 48.50 $^{\circ}$. XRPD pattern of formed freeze dried powder showed absence of sharp peak. Such an absence Zaltoprofen constructive reflection (specific sharp peak) in the covered from crystalline to amorphous or solubilized form, such lack of crystallinity in the system.

Saturated Solubility

Saturation solubility enhancement ratio of optimized batch of nanosuspension is 356.07 in phosphate buffer of pH 6.8. This great increase in saturation solubility of zaltoprofen due to particle size reduction and subsequent increase in surface area. This great increase in saturation solubility of zaltoprofen due to particle size reduction can be attributed to enhanced dissolution and justifying the objective of research work. The comparative solubility study of zaltoprofen drug and optimized batch (F6) was shown in fig. VI.

In-vitro Drug Release

The most important feature of nanoparticles is the increase in the dissolution velocity, not only because of increase in surface area but also because of increase in saturation solubility. In-vitro drug release data from the nanosuspension were carried out for 60 min and graphically represented as % drug release v/s time profile (Fig. 7). The percentage drug release curve of formulation F6, coarse suspension of Zaltoprofen and pure drug showed the desired rate in phosphate buffer of pH6.8 up to 60min. From that study it was found that formulation of F6 batch gave faster release behaviour compared to coarse suspension and pure drug. The drug release of optimized batch (F6) was found to be 89% within 50 min. Thus, from the above results it was found that as the particle size is decrease drug release is increased. So, nanosuspension enhanced rate of dissolution of Zaltoprofen to a great extent.

Stability Study

Physical appearance of the batch of F6 nanosuspension does not change when samples were stored at 4 $^{\circ}$ C and room temperature condition for 3 months. A loose, thin layer of sediment was observed when nanosuspension was stored at room temperature for 3 month. However, the sediment disappeared with slight hand shaking. The average particle diameters were 240 nm and 239 nm when samples stored at room temperature (25 $^{\circ}$ C) and refrigerator (4 $^{\circ}$ C) respectively. The particle size for the F6 was 237 nm before performing stability study. It can be inferred from the observed data that the prepared nanosuspension F6 was stable after 3 months of storage at different temperature condition.

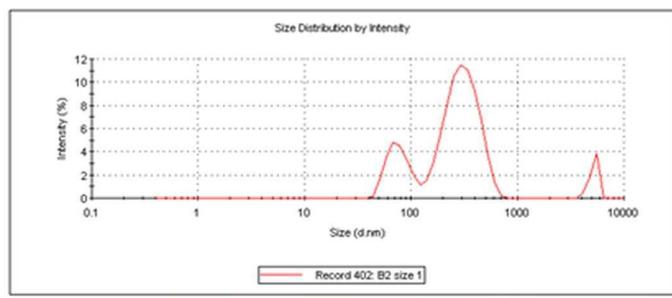


Fig.1. Particle size graph of optimized Zaltoprofen nanosuspension (F6)

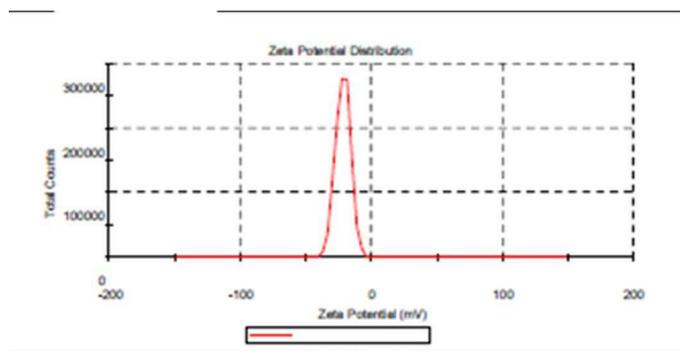


Fig.2. Zeta potential graph of optimized Zaltoprofen nanosuspension (F6)

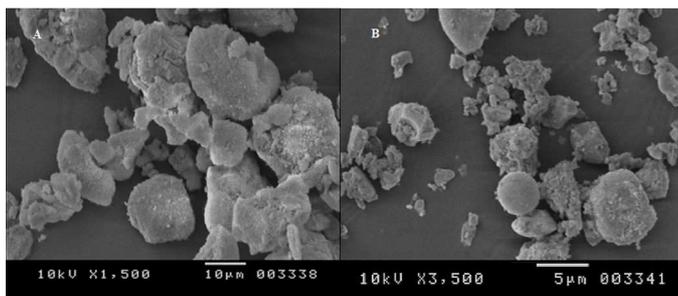


Fig.3. Scanning electron micrographs of A) Pure drug Zaltoprofen B) Optimized Formulation (F6)

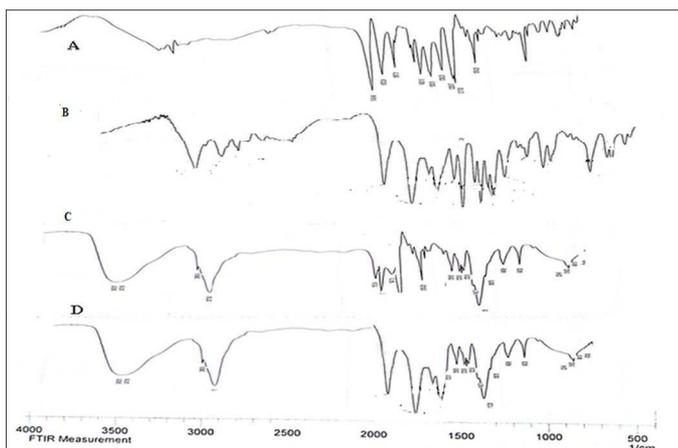


Fig.4. FT-IR of a) Zaltoprofen b) HPMC (5cps) c) Pluronic F68 d) Formulation (F6)



Fig.5. DSC thermogram of A) Zaltoprofen B) Lyophilized nanosuspension of optimized formulation without cryoprotectant

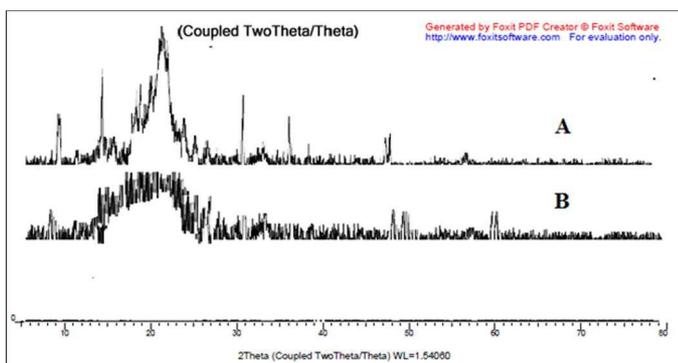


Fig.6. X-RD of pure drug Zaltoprofen and freeze dried nanosuspension powder (F-6)

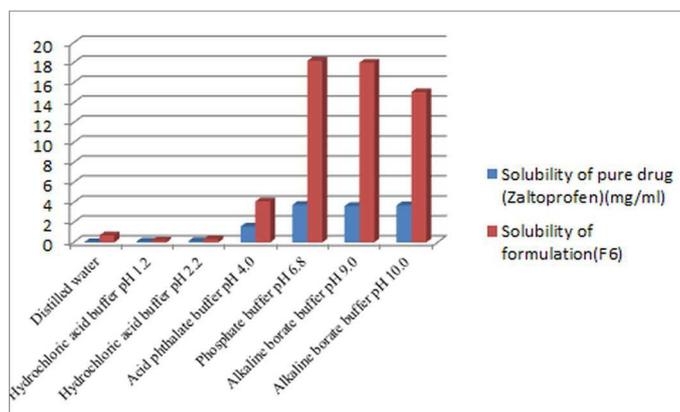


Fig.7. Solubility graph of pure drug Zaltoprofen and F-6 formulation in different pH buffer

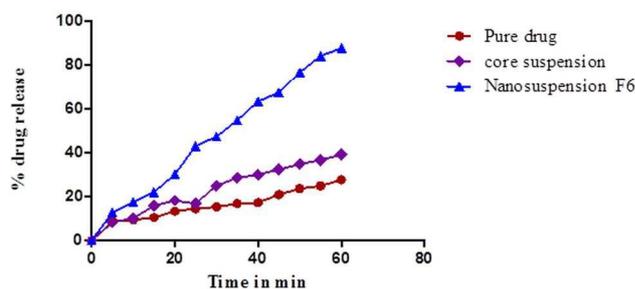


Fig.8. In-vitro drug release study in Phosphate buffer pH 6

CONCLUSION

Nanoprecipitation method was successfully employed to produce stable Zaltoprofen nanosuspension which can enhance the solubility and dissolution rate. In this process, the particle size of Zaltoprofen can be obtained in the nano-size ranges, by adjusting the operation parameters, such as surfactant concentration and polymer concentration and agitation speed (8000 rpm) and time (9hr) was constant. From the above investigation, it is concluded that the drug to stabilizer ratio (1:0.5) and drug to polymer ratio (1:0.3) showed a pronounced effect on particle size reduction. According to optimized batch (F6) the mean particle size and zeta potential was found to be 237 nm and -21.5 mV respectively and stable at various conditions. The rate of dissolution of the optimized nanosuspension was enhanced (89% in 50min), relatively to coarse suspension (30% in 50 min), mainly due to formation of nanosized particles. Thus, the objective of formulated nanosuspension of Zaltoprofen by using nanoprecipitation method has been achieved with success.

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