Research Article

Investigation of Effect of Different Stabilizers on Formulation of Zaltoprofen Nanosuspension

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ABSTRACT

Solubility of a drug is a major concern for its therapeutic action and becomes more prominent when the drug is lipophilic in nature. Zaltoprofen is a novel non–steroidal anti-inflammatory class of drug, but problem associated with is the poor solubility in biological fluids. The present study was performed to investigate the effect of different stabilizers (in single or in combination form) on solubility, particle size, zeta potential, dissolution rate and stability of nano suspension of water insoluble drug Zaltoprofen. Nanosuspension is prepared by using wet mill, high pressure homogenizer, precipitation, and melt emulsification method. In the present work nano suspension was prepared by solvent/anti solvent precipitation method using different steric stabilizers like pluronic F-68, F-127and electrostatic stabilizer like sodium lauryl sulphate. The optimized formulation showed an average particle size 179nm and a zeta potential of -28.4mV. The rate of dissolution of the optimized nano suspension was enhanced (82% in 45min), relative to bulk drug of Zaltoprofen (22% in 45min), mainly due to the formation of nanosized particles. The obtained results showed that nano suspension prepared in combination form of stabilizers like pluronic F-68 and sodium lauryl sulphate has improved dissolution rate. The increase in in-vitro dissolution rate may favourably affect bioavailability and improve safety for the patient by decreasing gastric irritancy. Stability study revealed that nano suspension was more stable at room and refrigerator condition with no significant change in particle size distribution. The results indicate that the combination of stabilizers may contribute in enhancement of the solubility, dissolution rate, and stability.

Keywords: Dissolution rate, Nanosuspensions, Solubility, Zaltoprofen.

INTRODUCTION

Amongst the various routes of administration, the oral route is most commonly used and most convenient for the drug delivery. In pharmaceutical practice these systems has taken more attention because of its simplicity and flexibility in designing the dosage form.[1] Now a day's novel formulations like Nanosuspension having remarkable advantage over conventional formulations which includes enhancement of solubility and bioavailability of drugs.[2]

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.[3, 4] 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.[5, 6]

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.[7] Zaltoprofen acts by inhibiting prostaglandin synthesis through a peripheral mechanism by inhibition of bradykininB_2 receptor mediated responses in primary afferent neurons.

Most commonly used stabilizers to stabilize nanosuspensions are either polymer like (e.g., polyvinyl pyrrolidone (PVP), crystalline cellulose,[8] amphiphilic amino acid,[9] hydroxy propyl cellulose (HPC),[10] hydroxy propyl methyl cellulose (HPMC),[11]and d-α-tocopherol polyethylene glycol 1000 succinate (TPGS 1000),[12,13,14] where as surfactant such as ionic are (e.g., sodium dodecyl sulphate (SDS), sodium lauryl sulphate (SLS), poly(ethyleneimine)(PEI),[15] chitosan[16] and non-ionic surfactant (e.g., polysorbate (tween 80), block co-polymer like pluronic) and some food protein are also used as stabilizers such as soya bean protein isolate, whey protein isolate and β-lactoglobuline.

There are two methods for preparation of Nanosuspension first is conventional method are called Bottom-up method and second is Top-down method. In the present work Nanosuspension is prepared by bottom-up method and study the effect of different stabilizers on the formulation, when all process parameters are kept constant. Response such as particle size, polydispersity index, zeta potential, dissolution rate, drug content etc. were evaluated in this study.[17]

MATERIALS AND METHODS

Materials

Zaltoprofen JP 2-(10, 11-dihydro-10-oxodibenzo [b, f] thiepin-2-yl) propionic acid was obtained as a gift sample from ToniraPharma Limited, Ankleshwar, (Gujarat), India. Pluronic F68 (Poloaxamer 188) and Pluronic F127

(Poloxamer 407) obtained gift sample from BASF (Germany). Sodium lauryl sulphate was purchased from Loba chemicals, (Mumbai).

Methods

Nanosuspensions were prepared according to nano precipitation method. Zaltoprofen was dissolved in acetone at 40^{0}C to form uniform organic solution. The prepared organic solution was then injected slowly drop wise with the help of a syringe into an aqueous phase (20ml) containing different concentrations of stabilizers (F68, F127, SLS etc.) under high speed mechanical agitation of 8000 rpm to get desired nanodispersion. Prepared Nanosuspension was then stirred magnetically at 500rpm at room temperature for 12h 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.[18] The batches were prepared according to the formulation design in Table 1.

Table 1: Zaltoprofen nanosuspensions prepared with individual and combination of stabilizers

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zaltoprofen (mg/ml)	80	80	80	80	80	80	80	80	80
Pluronic F-68(mg/ml)	40	-	-	-	-	-	20	-	20
Pluronic F-68(mg/ml)	-	8	-	-	-	-	-	-	-
Pluronic F-127(mg/ml)	-	-	40	-	-	-	-	20	-
Pluronic F-127(mg/ml)	-	-	-	8	-	-	-	-	20
Sodium Lauryl Sulphate(mg/ml)	-	-	-	-	40	-	20		-
Sodium Lauryl sulphate(mg/ml)	-	-	-	-	-	8	-	20	-
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^{o}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 Nanosuspensions

Particle size analysis

The mean particle size and the polydispersity index (PDI) were determined by Malvern Zetasizer ZS (Nano series ZS 90 UK). The particle size should be less than 1000nm in Nanosuspension. Prior to the measurement, the sample were diluted with double distilled water to a suitable scattering intensity and re-dispersed by hand shaking.

Zeta potential measurement

The zeta potential was determined by a Malvern Zetasizer ZS (Nano series ZS 90 UK). It is measure of electric charge at the surface of particles indicating the physical stability of colloidal systems. The samples were diluted with de-ionized water and conductivity is adjusted by addition of sodium chloride before measurement. Each sample was measured at least three times.

Physically stable nanosuspensions solely stabilized by electrostatic repulsion, a zeta potential of ±30mV is required as a minimum. Combined with the steric stabilization, the absolute value of zeta potential about ±20mV is sufficient to fully stabilize the nanosuspensions system.[19]

Total Drug Content

An aliquot (0.5ml) was evaporated to dryness. The residue was then dissolved in acetone and filtered with 0.45 µm filter paper. The samples were analysed using UV spectrophotometer (UV-1600, Shimadzu, Japan) at λmax of 227 nm. Total drug content (TDC) and %TDC were calculated Equations 1 and 2.

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

$$\%\ TDC = \frac{TDC}{TAD} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where, Vol. Total/ Vol. Aliquot is the ratio of total Nanosuspension volume to the volume of aliquot taken and the total amount of drug taken for the formulation of nanosuspension.[20]

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and stabilizer. The FTIR (FT-IR 8400, Shimadzu, Japan) spectrum was performed by using a spectrophotometer. The samples were scanned in the spectral region between 4000-400cm^{-1}. Solid powder samples were oven dried at around 300^{o}C. Finely crushed, mixed with potassium bromide (1:10 ratioby weight) and pressed at 15000psig to make disc and then scanned it.[21]

Differential Scanning Calorimetry

DSC of the powered samples of Zaltoprofen and optimized formulation were recorded using DSC-Shimadzu 60 (Shimadzu Co., Kyoto, Japan) with TDA trend line software. All samples were weighed (8-10mg) and heated at a scanning rate of 20°C/min under dry air flow (100ml/min) between 50°C-275°C. Aluminium pans and lids were used for all samples.

Saturated Solubility

Saturation solubility of bulk Zaltoprofen powder 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 RemiLab India) for 24hrs. After 24hr., solutions were analysed using UV spectrophotometer at 227 nm, which was the absorption maxima determined earlier and drug concentrations were calculated.[22-24]

In-vitro Drug Release

An *in-vitro* dissolution test was conducted in a of USP type II paddle type of dissolution apparatus (Electro lab Dissolution Tester USP TDT-08L). The temperature was maintained at 37±0.5°C, and the stirring rate was at 100rpm. Accurately weighed bulk drug and nanosuspensions (all equivalent to 80 mg of Zaltoprofen) were dispersed in 900ml of dissolution medium. Five millilitre samples were drawn and the same volume of fresh dissolution medium was added at5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60min respectively. Then, the samples were filtered through a 0.1 µm syringe filter immediately before dilution, when necessary. Drug content was determined with a UV spectrophotometer at 227 nm.[25]

Stability Study

Prepared Nanosuspension (batch F7) was chosen to perform short term stability study of the Nanosuspension. Samples were stored in glass vials for 3 months at room temperature (25°C) and refrigerator (4°C). After 3month, samples were visually observed for any sedimentation. The particle size was performed using zeta sizer and also determines the drug content after 3 months storage.

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 nanosuspensions of Zaltoprofen. The ratio of solvent to antisolvent was kept constant i.e. 1:20 and stirring speed 8000 rpm and stirring time 9hr. 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

Three stabilizers (Pluronic F-68, F-127 &SLS) and in combination of stabilizers were tested for their stabilization potential. The important function of stabiliser is that they can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual nanoparticles. Also, stabilizer type and concentration play an important role in creating a stable formulation. It must be capable of wetting the surface of the drug particles and providing a steric or ionic barrier. Too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Ostwald's ripening. When drug-to-stabilizer ratio was kept 1:0.5, it markedly improved stabilization of Nanosuspension. The mechanism of the adsorption is likely by the formation of steric barrier. Steric barrier are produced when the adsorbed stabilizer extends its chain to the water phase, which helps in maintaining the distance between closely approaching solid particles. The obtained results showed that Nanosuspension prepared in combination form of stabilizer of Pluronic F-68 and SLS (F-7) had reduced in particle size as compared to individual stabilizer.

Polydispersity index gives degree of particle size distribution. It ranged from 0.273 to 0.603 depending on formulation variables (Table- I). Higher value of polydispersity index indicates broad particle size distribution. A narrow size distribution is essential to prevent particle growth due to Ostwald ripening and maintaining stability of Nanosuspension. Batches with lower polydispersity values showed long- term stability and were preferred for studies. The particle size of optimized batch (F7) was found to be 179nm shown in (Figure 1).

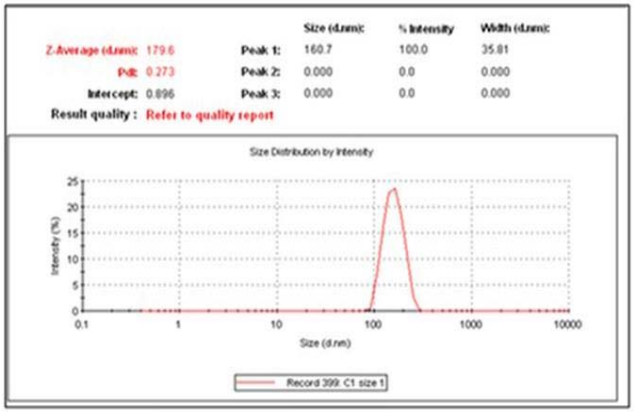

Figure 1: Particle size graph of optimized Zaltoprofen Nanosuspension (F7)

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 -28.4 mV. Zeta potential of Zaltoprofen Nanosuspension (F-7) was found to be -

28.4mV (Figure 2).Thus, it was concluded that the system had sufficient stability.

Table 2: Physicochemical characterization of F-1 to F-9 batches

Batch Code	Particle size (nm)	Polydispersity index	Zeta potential (mV)	% Total Drug Content
F1	207	0.457	-21.9	87
F2	263	0.373	-18.5	89
F3	237	0.415	-18.2	92
F4	363	0.603	-16.2	80
F5	556	0.257	-8	86
F6	320	0.543	-8.3	89
F7	**179**	**0.273**	**-28.4**	**94**
F8	258	0.472	-21.5	90
F9	307	0.560	-18.3	89

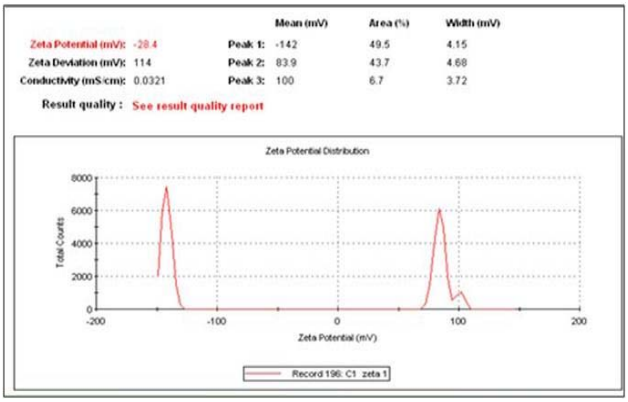

Figure 2: Zeta potential graph of optimized Zaltoprofen Nanosuspension (F7)

Total Drug Content (TDC)

Table 2 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.

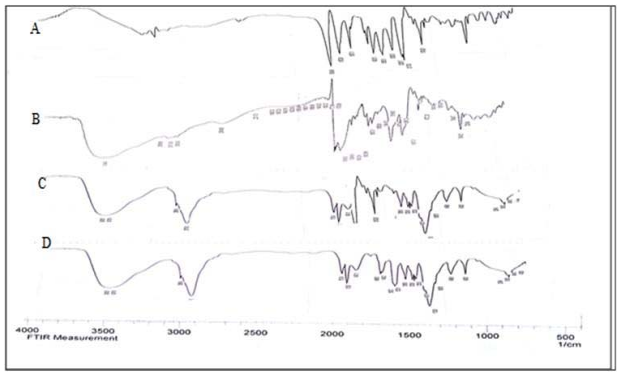

Figure 3: FT-IR of a) Zaltoprofen b) Sodium lauryl sulfate c) Pluronic F-68 d) Formulation (F7)

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 F127, sodium lauryl sulphate and the Zaltoprofen Nanosuspension of optimized formulation are shown in Fig. IV. FTIR spectra showed characteristic peaks such as C-H stretch at 3058cm^{-1}, aryl C-H stretch at 2992cm^{-1}, carbonyl C=O stretch of ketone and acid at 1703 and 1694cm^{-1} respectively, O-H stretch at 2533cm^{-1}, C=C stretch at 1670cm^{-1}, C-O stretch at 1279cm^{-1} and C-S stretch at 1418cm^{-1}.[21] 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^{0}C at a heating rate of 20^{0}C/min. From thermogram, it can be concluded that the drug and the surfactant do not interact with each other. The data was represented in Figure 4.

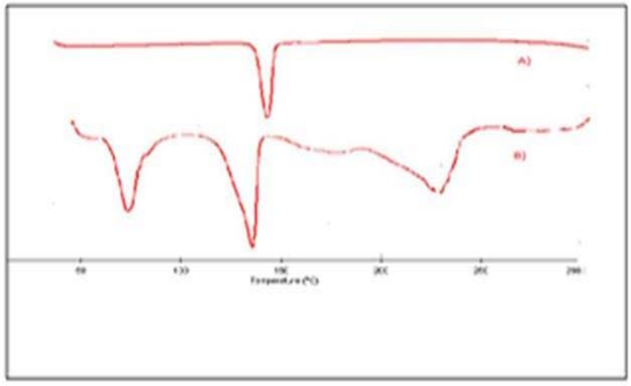

Figure 4: DSC thermogram of A) Zaltoprofen B) Lyophilized Nanosuspension of optimized formulation without cryoprotectant

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 (F7) was shown in figure 5.

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. When the dissolution profile for nanosuspensions with different stabilizers (F-68, F-127 and SLS) was compared with pure drug Zaltoprofen. From that study it was found that formulation of F7 batch gave faster release behaviour compared to other shown (fig. VI). This may be attributed to the fact that due to reduction in particle size there was enhancement in accessible surface area by dissolution medium. Moreover

the surface active coat of ionic and non-ionic stabilizers might also have a significant effect on increase of dissolution rate. The drug release of F7batch was found to be 82.5% within 45min. So, Nanosuspension enhanced rate of dissolution of Zaltoprofen to a great extent.

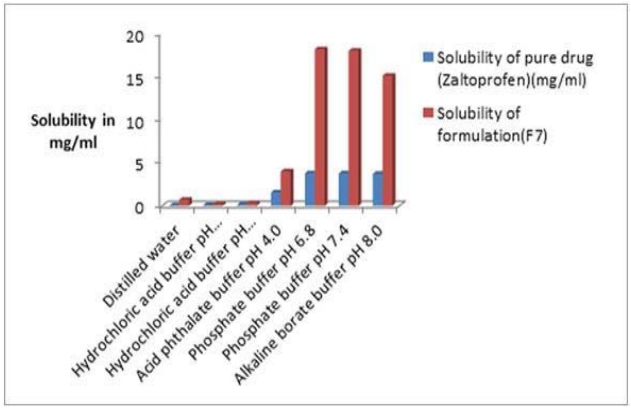

Figure 5: Solubility graph of pure drug Zaltoprofen and F-7 formulation in different pH buffer.

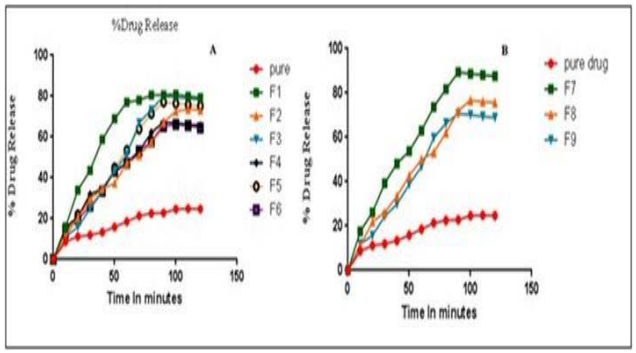

Figure 6: In-vitro drug release study in Phosphate buffer pH 6.8A) F1-F6 batches (single stabilizer) B) F7-F9 batches (combination of stabilizer).

Stability Study

Physical appearance of the batch of F7 nanosuspension does not change when samples were stored at 4^0C for 3months. A loose, thin layer of sediment was observed when nanosuspension was stored at room temperature for 3 months. However, the sediment disappeared with slight hand shaking. The average particle diameters were 181 nm and 180 nm when samples stored at room temperature (25^0C) and refrigerator (4^0C) and respectively. The particle size for the F7 was 179nm before performing stability study. Drug content was found to be 93 % and 94% when samples store at 25^0C and 4^0C respectively for 3 months. It can be inferred from the observed data that the prepared nanosuspension F7 was stable after 3months of storage at different temperature condition.

CONCLUSION

A Solvent/ Antisolvent precipitation method was successfully employed to produce stable Zaltoprofen Nanosuspension which can enhance the solubility and dissolution rate. From the above investigation, it is concluded that the combined effect of non-ionic (Pluronic F-68) and ionic stabilizer (sodium lauryl sulphate) showed a pronounced effect on particle size reduction. According to optimized batch (F7) the mean particle size and zeta potential was found to be 179nm and -28.4mV respectively and stable at various conditions. The rate of dissolution of the optimized Nanosuspension was enhanced (82% in 45min), relative to bulk drug of Zaltoprofen (22% in 45min), mainly due to the 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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