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EFFECT OF PROCESS PARAMETERS ON FORMULATION OF SOLID LIPID NANOPARTICLES OF PROTEASE INHIBITOR, ATAZANAVIR

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ABSTRACT

AIDS is one of the greatest threats to human health. Millions of people have been infected with HIV and the syndromes remain among the top five fatal diseases. The aim of the present study was to investigate the effect of process parameters on formulation of solid lipid nanoparticles (SLNs) of a protease inhibitor (PI), Atazanavir. Atazanavir is an azapeptide protease inhibitor (PI) of HIV-1 protease. Solid lipid nanoparticles loaded with Atazanavir sulfate, were formulated by hot homogenization followed by ultrasonication method. Compritol 888 ATO and Palmitic Acid were used as lipids and Tween 80 and Poloxamer 188 were used as surfactants. It is observed that Compritol 888 ATO have highest partition coefficient and Palmitic Acid have lower than Compritol 888 ATO but higher than Stearic acid. It shows that homogenization speed plays an important role in the reduction of particle size as compared to the homogenization time and ultrasonication time. Three process parameters - homogenization speed, homogenization time and ultrasonication time were optimized for the formulation of SLNs.

Key Words: Protease Inhibitors, Solid Lipid Nanoparticles (SLNs), Partitioning Behavior, Homogenization, Sonication and Surfactants.

INTRODUCTION:

AIDS is one of the greatest infectious threats to human health. Millions of people have been infected with HIV and the syndromes remain among the top five fatal diseases. *[1]* The causative agent for the disease, the HIV, which is a retrovirus has two subtype; the most common is HIV-1 which occurs world over, and HIV-2 found mainly in Africa. *[2]* AIDS has resulted in millions of deaths and WHO estimates that approximately 40.3 million peoples were living with HIV as of December 2005.

Protease Inhibitors are the new class of anti-

HIV agent. The poor brain permeability of Protease Inhibitors can be attributed to certain key physicochemical properties. For most PIs, the oil/water partition coefficient is much higher than the optimum established oil/water partition coefficient of brain drug permeability. This implies their Blood Brain Barrier Permeability as the extremely high liposolubility lowers the diffusion of the drug from lipid layer into the extracellular fluid of the brain. Thus low brain permeability by Protease Inhibitors can lead to the CNS being a protected viral reservoir.

Nanoparticle drug delivery system of therapeutic agent is critical for successful drug therapy; several delivery systems have been developed to deliver pharmacologically active agent directly to the site of action. It is due to their extreme small size range, has been proposed as potential brain targeting systems. Several studies have been conducted to access the ability of Nanoparticles to our Blood Brain Barrier.

Materials and Methods:

Atazanavir (ATZ) was obtained as a gift sample from Ranbaxy Research Laboratory, Gurgaon, India. Compritol 888 ATO was obtained as a gift sample from Gattefosse, Mumbai, India**.** Palmitic Acid was obtained as a gift sample from Gattefosse, Mumbai, India. Polysorbate 80 (P80) (Tween 80) was obtained from Merck, India. Poloxamer 188 was obtained from HiMedia, Mumbai, India.

Preparation of Solid Lipid Nanoparticles

Many methods have been reported for the preparation of SLNs such as Microemulsion precursor technique *[3]*, membrane contractor technique *[4],* high pressure homogenization technique *[5],* hot homogenization technique *[6],* Cold homogenization *[7],* solvent emulsification technique *[8]* and solvent diffusion technique *[9].* Hot homogenization followed by ultrasonication technique was used for the preparation of SLN because of its simplicity and cheapness *[10].* Atazanavir is a thermo stable drug, not affected by its high temperature process parameters. In this method the lipid was melted at 10°C above melting point of the lipid i.e. 80°C for Compritol 888 ATO and 66°C for Palmitic Acid. It was followed by addition of weighed drug in the melted lipid, which was then added to the hot aqueous phase having surfactant followed by homogenization. Ultrasonicate this emulsion to get nanoemulsion and exposed to cooling to solidify nanoglobules to get nanoparticles.

Sterilization of SLNs

SLNs can be sterilized by autoclave at 121°C for 15 minutes, maintaining an almost spherical shape, without any significant increase in size or nanoparticles distribution. The stability of sterilized SLN over time appears depends on the lipid matrix, but the

dispersing media may also play an important role. *[11]*

Lyophillisation of SLNs

SLNs can be lyophilised by alpha 1-2 LD plus freeze dryer. Normally we add some cryoprotectant in the SLNs suspension before lyophillisation, which helps in preventing the agglomeration of particles. Type and concentration of cryoprotectant plays an important role in maintaining the particle size of the formulation. *[11]*

Partitioning behavior of Drug in lipids

Three volumetric flasks (capacity 5ml) were taken thoroughly rinsed with distilled water, first labeled for Compritol, second was labeled for Palmitic Acid and third was labeled for Stearic acid.

Table 1: Composition of different flasks for partitioning behavior of Atazanavir sulfate

Flask No.	Atazanavir sulfate (mg)	Lipid	Lipid (gm)	Water (ml)
I	10	Compritol 888 ATO	1	1
II	10	Palmitic Acid	1	1
III	10	Stearic acid	1	1

1gm of Compritol 888 ATO, Palmitic Acid and Stearic acid were weighed accurately and transferred to their respective flasks. Flask I and III were subjected to 80°C (Approximately 10°C above their melting points) in a water bath and flask II was subjected to 66°C in another water bath. After melting of the lipids 1 ml of distilled water (heated to respective temperature) was added in the respective flasks and 10 mg of drug was added in each flask. These flasks were shaken in water bath at 100 rpm at the same temperature for 30 minutes. After 30 minutes the emulsions were transferred to centrifuge tubes and these were refrigerated centrifuged at 3000 rpm for 5 minutes. After this the supernatant aqueous phase were taken out with the help of syringe and filtered through separate 0.1 µm membrane syringe filters. The filtrate was analyzed for drug content by HPLC. [10]

Table 2: Observation of the partitioning behaviour of Atazanavir sulfate

Flask No.	Lipid	Drug in water (mg)	Partition coefficient
I	Compritol 888 ATO	0.083	119.19
II	Palmitic Acid	0.107	92.59
III	Stearic acid	0.147	66.94

It was observed that Flask III have highest amount of Atazanavir sulfate left in water as compared to Compritol 888 ATO and Palmitic Acid. It is observed that Compritol 888 ATO have highest partition coefficient and Palmitic Acid have lower than Compritol 888 ATO but higher than Stearic acid.

Preparation of Solid Lipid Nanoparticles (SLNs)

The blank SLNs were first optimized (without drug loading). Compritol 888 ATO and Palmitic Acid were selected for the preparation of SLNs as these two shown the sufficient partitioning of drug.

Preparation of blank Solid Lipid Nanoparticles

Venkateshwarlu V. et al developed hot homogenization followed by ultrasonication method for the preparation of Solid Lipid Nanoparticles. [*10*] In this method the lipid is melted at 10°C above melting point of the lipid, which is then added to the hot aqueous phase having surfactant followed by homogenization. Ultrasonicate this emulsion to get nanoemulsion which is exposed to cooling to solidify nanoglobules to get nanoparticles.

Effect of process parameters

There were three process parameters (two were of homogenization and one was of ultrasonication) which were optimized for the formulation of SLNs. In homogenization parameters, first parameter was homogenization speed and second was homogenization time. In ultrasonication, ultrasonication time was the parameter to be optimized. The concentration of compritol 888 ATO, Tween 80 and Poloxamer 188 was taken 1% for each with 97 % of water for each group.

It was observed that formulation coding PP SLN 2, 4, 6, and 8 showed satisfactory particle size. It shows that homogenization speed plays an important role in the reduction of particle size as compared to the homogenization time and ultrasonication time. In both the formulation, homogenization speed was 8000. 5 minutes homogenization time is sufficient for the production of SLNs, there is not so much difference found in the particle size when homogenization time was increased to 10 minutes. Ultrasonication time optimized was 30 minutes. So, we selected the process parameters of PP SLN 6 for the further process.

Effect of formulation parameters

Next step we adopted was, optimization of lipid: surfactant concentration ratio which is varied from 1:2, 1:2.5, 1:3, 1:3.5 and 1:4 with different concentration of lipid.

Table 3: Various process parameters and effects of these parameters on particle size

Batch number coding	Homogenization speed coding	Homogenization time coding	Sonication time coding	Particle size range (nm)
PP SLN 1	-1	-1	-1	902-1245
PP SLN 2	+1	+1	+1	220-530
PP SLN 3	-1	-1	+1	835-1229
PP SLN 4	+1	+1	-1	246-552
PP SLN 5	-1	+1	-1	768-1039
PP SLN 6	+1	-1	+1	212-425
PP SLN 7	-1	+1	+1	748-943
PP SLN 8	+1	-1	-1	224-530

Homogenization speed coding: -1= 4000, +1= 8000; Homogenization time coding: -1= 5 minutes, +1= 10 minutes; Ultrasonication time coding: -1= 20 minutes, +1= 30 minutes; PP SLN= Process Parameters Solid Lipid Nanoparticle

Table 4: Formulation parameters at different lipid and lipid: surfactant ratio.

Batch number coding	Compritol 888 ATO (%)	Poloxamer 188 (%)	Tween 80: Poloxamer 188 (1:1) (%)	Water (%)
BC SLN 1	1	-	2.0	98.0
BC SLN 2	1	-	2.5	97.5
BC SLN 3	1	-	3.0	97.0
BC SLN 4	1	-	3.5	96.5
BC SLN 5	1	-	4.0	96.0
BP SLN 1	-	1	2.0	98.0
BP SLN 2	-	1	2.5	97.5
BP SLN 3	-	1	3.0	97.0
BP SLN 4	-	1	3.5	96.5
BP SLN 5	-	1	4.0	96.0
BC SLN 6	2	-	4.0	94.0
BC SLN 7	2	-	5.0	93.0
BC SLN 8	2	-	6.0	92.0
BC SLN 9	2	-	7.0	91.0
BC SLN 10	2	-	8.0	90.0
BP SLN 6	-	2	4.0	94.0
BP SLN 7	-	2	5.0	93.0
BP SLN 8	-	2	6.0	92.0
BP SLN 9	-	2	7.0	91.0
BP SLN 10	-	2	8.0	90.0
BC SLN 11	3	-	6.0	91.0
BC SLN 12	3	-	7.5	89.5
BC SLN 13	3	-	9.0	88.0
BC SLN 14	3	-	10.5	86.5
BC SLN 15	3	-	12.0	85.0
BP SLN 11	-	3	6.0	91.0
BP SLN 12	-	3	7.5	89.5
BP SLN 13	-	3	9.0	88.0
BP SLN 14	-	3	10.5	86.5
BP SLN 15	-	3	12.0	85.0

BC SLN: Blank Compritol 888 ATO Solid Lipid Nanoparticle; BP SLN: Blank Palmitic Acid Solid Lipid Nanoparticle

Table 5: Particle size difference at different lipid and lipid: surfactant concentration ratio.

Batch number coding	Particle size range (nm)
BC SLN 1	403-50345
BC SLN 2	372-6287
BC SLN 3	218-3278
BC SLN 4	195-1062
BC SLN 5	190-663
BP SLN 1	397-50024
BP SLN 2	353-5999
BP SLN 3	206-3109
BP SLN 4	187-984
BP SLN 5	185-583
BC SLN 6	289-51378
BC SLN 7	170-7178
BC SLN 8	127-238
BC SLN 9	115-230
BC SLN 10	112-229
BP SLN 6	276-49245
BP SLN 7	166-6846
BP SLN 8	124-235
BP SLN 9	114-226
BP SLN 10	114-228
BC SLN 11	428-55382
BC SLN 12	400-32187
BC SLN 13	327-6186
BC SLN 14	302-964
BC SLN 15	299-927
BP SLN 11	419-52984
BP SLN 12	401-32298
BP SLN 13	373-5839
BP SLN 14	316-852
BP SLN 15	287-839

Particle size of BC SLN 8, 9, 10 and BP SLN 8, 9, 10 were found to be in narrow size distribution range which is required for nanoparticles point of view. We only, go for formulation BC SLN 9 and 10 and BP SLN 9 and 10 for drug loading.

Preparation of Solid Lipid Nanoparticles loaded with Atazanavir sulfate

Venkateshwarlu V. et al [*10*] developed hot homogenization followed by ultrasonication method for the preparation of Solid Lipid Nanoparticles. Twelve formulations of SLNs were prepared by varying the concentration of drug i.e. Atazanavir sulfate. Table 6 shows the composition of various formulations of ATZ-sulfate-SLNs. 400 mg of Compritol 888 ATO or Palmitic Acid (according to formula given in table) was weighed and transferred to a clean and freshly rinsed 50 ml capacity borosilicate glass beaker. Lipid was heated at 10°C above melting point of lipid in water bath. ATZ-sulfate was added to hot melt of lipid.

To solubilize drug completely in lipid, 0.2 ml of PG was added in the mixture of hot melt lipid and drug. It was shaken manually and vortex at the same temperature until a clear solution formed. An aqueous phase was prepared by dissolving required amount of surfactants (Tween 80: Poloxamer 188) in double distilled water, maintained at the same temperature of the molten lipid phase. Hot solution of drug and lipid was transferred to the aqueous phase and stirred for 2 minutes at magnetic stirrer at same temperature, and resultant was homogenized at 8000 rpm at temperature 10°C above melting point of lipid with the help of RQT-127A homogenizer for 5 minutes. Coarse hot oil o/w emulsion so obtained was ultrasonicated for 30 minutes.

SLNs were obtained by allowing hot nanoemulsions to cool to room temperature.

Table 6: Composition of various formulations of ATZ-sulfate-SLNs.

Batch number coding	Atazanavir sulfate coding	Compritol 888 ATO (%)	Palmitic Acid (%)	Tween 80:Poloxamer 188 (1:1)	Propylene glycol (%)	Water (%)
ATZ-sulfate SLNs A	-1	-	2	7.0	1.0	91.0
ATZ-sulfate SLNs B	0	-	2	7.0	1.0	91.0
ATZ-sulfate SLNs C	+1	-	2	7.0	1.0	91.0
ATZ-sulfate SLNs D	-1	2	-	7.0	1.0	90.0
ATZ-sulfate SLNs E	0	2	-	7.0	1.0	90.0
ATZ-sulfate SLNs F	+1	2	-	7.0	1.0	90.0
ATZ-sulfate SLNs G	-1	-	2	8.0	1.0	91.0
ATZ-sulfate SLNs H	0	-	2	8.0	1.0	91.0
ATZ-sulfate SLNs I	+1	-	2	8.0	1.0	91.0
ATZ-sulfate SLNs J	-1	2	-	8.0	1.0	90.0
ATZ-sulfate SLNs K	0	2	-	8.0	1.0	90.0
ATZ-sulfate SLNs L	+1	2	-	8.0	1.0	90.0

-1: 0.15%, 0: 0.30%, +1: 0.45%

Sterilization of Atazanavir sulfate-SLN

ATZ-sulfate-SLNs formulation D were prepared as described previously and sterilized by steam sterilization (Autoclaving) at 121°C for 15 minutes in aseptic environment. 5 ml sterilized ATZ-sulfate-SLNs suspensions was kept at various temperature (20°C, 25°C, and 30°C) for 7, 14, and 30 days to evaluate, whether it shows any microbial growth or not. Sample was evaluated visually as well as microscopically, for bacterial and fungal growth. No microbial growth was observed.

Lyophillization of ATZ-sulfate-SLNs

The ATZ-sulfate-SLNs formulation D were prepared as described previously and lyophilized using Alpha 1-2 LD plus Freeze Dryer. 2% Sucrose was added as cryoprotectant before freezing. Slow freezing was carried out at -30°C and subsequently lyophilized for 24 hr. Lyophilized SLNs were kept for stability study.

Results and Discussion:

Partitioning behaviour of Drug in Lipids

In order to select a suitable lipid, partitioning behavior of the drug was studied in three lipids (Compritol 888 ATO, Palmitic Acid, Stearic acid). Partition coefficient is the ratio of amount of Atazanavir sulfate in lipid to the amount in aqueous phase. It depends upon the structure of the lipid as well as on the structure of drug also. Figure 1 shows the partition of Atazanavir sulfate in three lipids. Partition coefficient of Atazanavir sulfate obtained maximum in Compritol 888 ATO than in Palmitic Acid and then lowest in Stearic acid. Because of very much low partitioning of Atazanavir sulfate in Stearic acid as compared to other lipids, the approach to prepare Stearic acid SLNs were dropped at the same time and we didn't made Stearic acid SLNs.

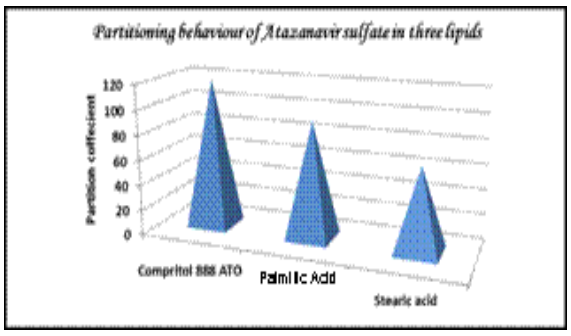

Figure 1: Partition coffecient of Atzanavir sulfate in Compritol 888 ATO , Palmitic Acid and Stearic acid

In Compritol 888 ATO and Palmitic Acid, the first one exhibited higher partitioning but we will go for both the lipids because we assumed that there may be increase in EE% and DL%, as of surfactants.

The lipid crystalline structure related to the chemical nature of the lipid is a key factor to determine whether a drug would be expelled or firmly incorporated into the carrier system. Lipid forming highly crystalline state with a perfect lattice would lead to drug expulsion. On the other hand imperfection (lattice defects) of the lipid structure could offer space to accommodate the drugs *[12].*

Preparation of Solid Lipid Nanoparticles

SLN have been prepared by various researchers using various methods. *[13, 5, 14, 9, 15].* In the present study we adopted an economical, simple, and reproducible method for the preparation of solid lipid nanoparticles i.e. hot homogenization followed by ultrasonication at above the melting point of the lipid. This method was described by Venkateshwarlu V. et al. [10] to prepare SLNs loaded with clozapine and obtained a particle size range of 60-380 nm.

First we prepare blank SLNs to determine the process and formulation parameters. Before determining the formulation parameters we determined the process parameters.

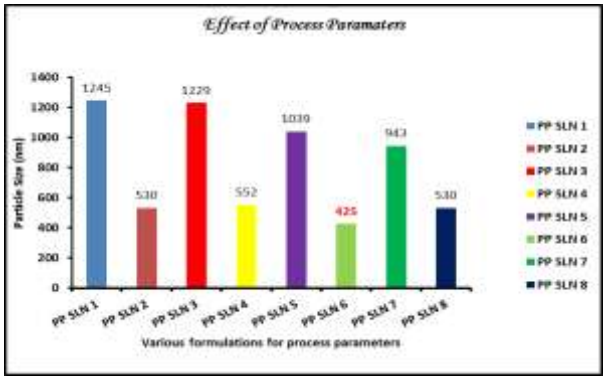

Figure 2: Effect of homogenization speed, homogenization time and sonication time.

Effect of process parameters

We studied three process parameters, in which two were of homogenization and one was of ultrasonication. Figure 2 shows the effect of homogenization speed, homogenization time and ultrasonication time. Formulation PP SLN 6 was optimized for process parameters and its process parameters were used for further work.

Effect of Formulation Parameters

Figure 3 showed maximum particle size (nm) for various formulations of blank Compritol 888 ATO Solid Lipid nanoparticles. The concentration of lipid was varied at 3 levels i.e. -1, 0, +1 and lipid : surfactant ratio was also varied i.e. 1:2, 1:2.5, 1: 3, 1:3.5 and 1:4 at each level of lipid. Three formulations showed good results i.e. BC SLN 8, 9, and 10. Maximum particle size of these three formulations is below 250 nm. In these formulations, lipid concentration is at 0 levels. Formulation 9 and 10 have lipid: surfactant concentration ratio 1:3.5 and 1:4 respectively.

The same was observed with various Blank Palmitic Acid Solid Lipid Nanoparticles, with 1: 3.5 and 1:4 of lipid: surfactant ratio produced good results which are acceptable for nanoparticles point of view (Figure 4).

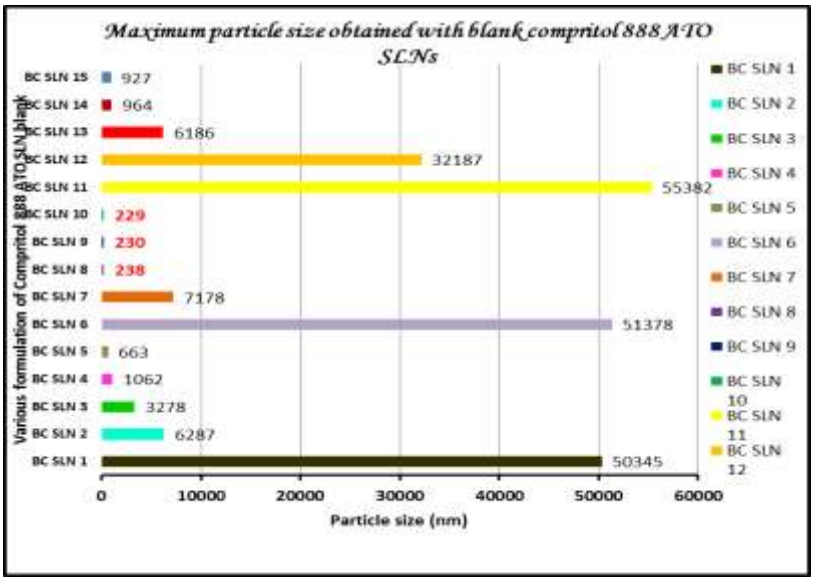

Figure 3: Effect of lipid, lipid : surfactant concantration ratio on particle size of blank compritol 888 ATO SLNs

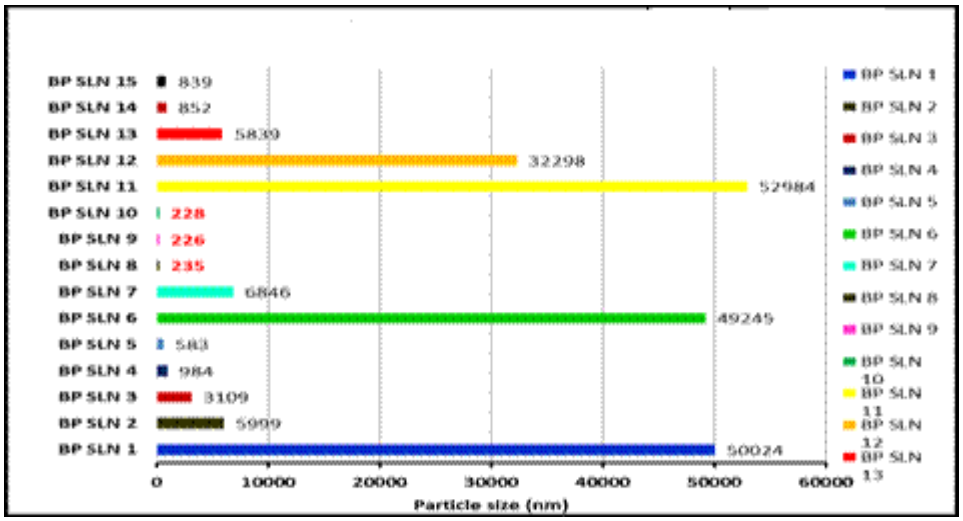

Figure 4: Effect of lipid, lipid : surfactant concantration ratio on particle size of blank Palmitic Acid SLNs

We observed that particle size of compritol 888 ATO is greater as compared to Palmitic Acid at the same formula. Figure 5 show the comparative data for both type of SLN i.e BC SLN 1 to 15 and BP SLN 1 to 15 (BC SLN: Blank Compritol 888 ATO Solid Lipid Nanoparticle, BP SLN: Blank Palmitic Acid Solid Lipid Nanoparticle).

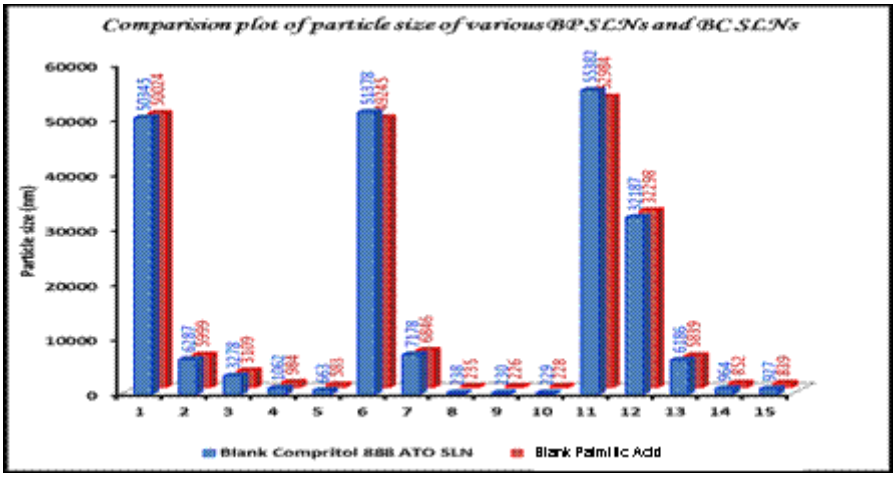

Figure 5: Comparision of particle size between Compritol 888 ATO & Palmitic Acid

This is assumed that it is because of higher melting point of compritol 888 ATO than Palmitic Acid. Average particle size has been shown to increase with increasing lipid melting temperature for both high pressure homogenization and high shear homogenization techniques. *[16, 17]* Factors such as velocity of lipid crystallization, lipid hydrophobicity, and influence of self-assembling properties of the lipid on the

shape of the lipid crystals (and hence the surface area) were found to effect the final size of the SLNs dispersion *[18].* But here the concentration of lipid and other factors are kept same for both the lipids formulation, so it is assumed that the difference in the particle size can be because of melting point difference.

Preparation of Solid Lipid Nanoparticles loaded with Atazanavir sulfate

This method successfully produced ATZ-sulfate-SLNs. BP SLN 9, 10 and BC SLN 9, 10 were used for the preparation of atazanavir sulfate loading. Atazanavir sulfate independently was not completely soluble in lipids, so in order to solubilize it completely in the lipids, PG was used as a co-solvent. Twelve formulations of the SLNs were prepared (Table 7) at the same parameters out of them six were with Palmitic Acid and six were with Compritol. The concentration of drug was varied at three levels i.e. -1, 0, +1.

Table 7: Composition of various formulations of ATZ-sulfate-SLNs

Batch number coding	Atazanavir sulfate coding	Compritol 888 ATO (%)	Palmitic Acid (%)	Tween 80:Poloxamer 188 (1:1)	Propylene glycol (%)	Water (%)
ATZ-sulfate SLNs A	-1	-	2	7.0	1.0	91.0
ATZ-sulfate SLNs B	0	-	2	7.0	1.0	91.0
ATZ-sulfate SLNs C	+1	-	2	7.0	1.0	91.0
ATZ-sulfate SLNs D	-1	2	-	7.0	1.0	90.0
ATZ-sulfate SLNs E	0	2	-	7.0	1.0	90.0
ATZ-sulfate SLNs F	+1	2	-	7.0	1.0	90.0
ATZ-sulfate SLNs G	-1	-	2	8.0	1.0	91.0
ATZ-sulfate SLNs H	0	-	2	8.0	1.0	91.0
ATZ-sulfate SLNs I	+1	-	2	8.0	1.0	91.0
ATZ-sulfate SLNs J	-1	2	-	8.0	1.0	90.0
ATZ-sulfate SLNs K	0	2	-	8.0	1.0	90.0
ATZ-sulfate SLNs L	+1	2	-	8.0	1.0	90.0

-1: 0.15%, 0: 0.30%, +1: 0.45%

Only homogenization produce microparticles, so to get the nanoparticles, this coarse emulsion was further subjected to ultrasonication for about 30 minutes. Ultrasonication produced the desire particle size of SLNs. All the processes were done at above the melting point of the solid lipid i.e. 10°C above the melting point of the lipid because at below the melting point of the lipid it gets solidified and may result in many defects such as, a very wide range of particle size, no attainment of the nanometric size of particles, no spherical shape, no uniform distribution of drug may be observed.

The hot nanoemulsion was subjected to room temperature for re-crystallization of the lipids which results in solidification of liquid nanoglobules (consisting drug and lipid) into solid nanoparticles. This leads to formation of SLNs loaded with Atazanavir sulfate. Drug loading may increases the particle size as compared to blank formulation.

Sterilization of ATZ-sulfate-SLNs

For intravenous and ocular administration SLNs must be sterile. The high temperature reaches during sterilization by autoclaving presumably cause a hot o/w microemulsion to form in the autoclave, and probably modifies size of the hot nanodroplets may coalescence producing larger SLNs than the initial ones. After sterilization the particle size increases two times but all the nanoparticles were in colloidal range and spherical in shape. It shows that sterilization of SLNs showed an increase in the particle size. In the literature it was shown that autoclaving was possible in case of lecithin stabilized SLNs. However, autoclaving was not suitable for SLNs stabilized with Poloxamers series (sterically stabilized polymers) due to partial collapse of polymer adsorption layer during autoclaving leading to particle aggregation. *[6]*

Type of surfactant, type of lipid plays an important effect on particle size during sterilization. It can be minimized by changing the type and varying the concentration of surfactants as well as lipids. Cavalli R. et al showed the effect of sterilization on various parameters of Diazepam SLNs. Cavalli also showed that SLNs can be sterilized by autoclaving; this is an advantage in comparison with some polymeric NPs. The dispersant media i.e., surfactant plays an effective role in maintaining the particle size of NPs. [*11*]

Particles in colloidal state are preferred for parenteral administration, it means our formulation meet the criteria for parenteral administration, because it's still maintained in colloidal state after sterilization.

No microbial growth was observed visually as well as microscopically, until after 30th days of sterilization at any point of temperature, it shows that sample was effectively sterilized and it doesn't have any microbial growth, which may be harmful during parenteral

administration of formulation. So the ATZ-sulfate-SLNs formulation D was found safe to be administered intravenously.

Lyophillisation

Usually SLNs dispersion shows an increase in particle size in short period of time during the storage. Particles size of prepared SLNs may cross the nanometric range within a couple of weeks or months. Although, lyophillisation has been used widely to improve the chemical and physical stability of SLNs over an extended period of time, it may damage the surfactant film around the nanoparticles due to a freezing out effect and may also cause particle aggregation during the re-solubilization or re-dispersion process. [5] Various cryoprotectants have been used to prevent these problems associated with lyophillization. Typical cryoprotective agents which can be used are carbohydrates including sorbitol, mannose, glucose, fructose, maltose, and trehalose. *[19]*

Sucrose was used as cryoprotectant in the lyophillization of ATZ-sulfate-SLNs. SLNs was re-dispersed in water under mechanical stirring at the dispersion ratio 1:25, after a storage period of 3 months. Size measurement of re-dispersed ATZ-sulfate-SLNs by LD showed an increase in their average particle size. All lipid matrices used formed larger SLNs with a wider size distribution after freeze-drying probably due to the presence of aggregates between NPs.

Conditions of freeze drying process and the removal of water probably promotes aggregation among SLNs; the amount of cryoprotector used during the dispersion was only 2%, probably too low to protect the NPs during this process.

Conclusion:

Three process parameters - homogenization speed, homogenization time and ultrasonication time were optimized for the formulation of SLNs.

It is observed that Compritol 888 ATO have highest partition coefficient.

It was observed that formulation coding PP SLN 2, 4, 6, and 8 showed satisfactory particle size (Table 3). It shows that homogenization speed plays an important role in the reduction of particle size as compared to the homogenization time and ultrasonication time. In both the formulation, homogenization speed was 8000. 5 minutes homogenization time is sufficient for the production of SLNs. Ultrasonication time optimized was 30 minutes. So, we selected the process parameters of PP SLN 6 for the further process.

Particle size of BC SLN 8, 9, 10 and BP SLN 8, 9, 10 were found to be in narrow size distribution range which is required for nanoparticles point of view. We only, go for

formulation BC SLN 9 and 10 and BP SLN 9 and 10 for drug loading (Table 5).

No microbial growth was observed visually as well as microscopically, until after 30th days of sterilization at any point of temperature, so ATZ-sulfate-SLNs formulation D was found safe to be administered intravenously (Table 7).

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