World Journal of Pharmaceutical research

Volume 3, Issue 1, 1460-1472. Research Article ISSN 2277 – 7105

PREPARATION AND CHARACTERIZATION OF POLY (Ɛ-CAPROLACTONE) NANOSUSPENSION CONTAINING SATRANIDAZOLE

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Article Received on
20 October2013
Revised on 24 November 2013,
Accepted on 30 December 2013

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ABSTRACT

The main aim of this research is to design a new system to incorporate the drug into the body ie. nanosuspension of satranidazole. Poly(ε-caprolactone) nanosuspension were prepared by nanoprecipitation method and were characterized in terms of particle size, zeta potential, polydispersity index, in vitro drug release,and drug entrapment efficiency,stability studies. Nanosuspension of different drug and polymer ratio were formulated for the purpose of optimization. The nanoparticles have a particle diameter as 241.6 nm and zeta potential as -21.3 mv. There was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. Formulation NS_{1a} was considered as optimum batch as it delivered 67.90 % of drug into colon. No appreciable difference was observed in the drug content of product during 60 days in which nanoparticles were stored at 4˚C and room temperature. According to the data obtained, this delivery system opens new and interesting perspectives way to formulate the nanosuspension of satranidazole..

Keywords : Nanoparticulate suspension; Satranidazole; PCL, Bioavailability.

INTRODUCTION

Litrature survey reveals that amoebiasis is the second leading cause of death from parasitic disease worldwide Satranidazole was selected as a drug of choice because it is very potent and useful against common protozoans. Chemically drug Satranidazole (MF: $C_8H_{11}N_5O_5S$; MW: 289) is 1-methyl sulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone and is soluble in acetone. It is a nitroimidazole derivative possessing a C-N linkage at C_2 of the imidazole ring. It is more active towards anaerobes than many other nitroimidazoles. It shows activity against, common protozoa like *E.* hystolytica, T.vaginalis and giardia, and also acts as antibacterial agent in the treatment of amoebiasis.

The synthetic polymers most commonly used in nanoparticle-based drug delivery systems are biodegradable. Among the FDA-approved biodegradable polymers, poly(lactide) (PLA), poly(L,L-lactide-co-glycolide) (PLGA) and poly(caprolactone) (PCL) have been used most often according to the drug delivery system literature. PCL occupies a unique position: it is at the same time both biodegradable and miscible with a variety of polymers, and it crystallizes very readily. In addition, it lacks toxicity and has great permeability. In this research, SLS was incorporated into PCL as a surfacting agent and enhancer of drug release.

Nanosuspensions consist of the pure poorly water-soluble drug without any matrix material suspended in dispersion. The reduction of drug particles into the sub-micron range leads to a significant increase in the dissolution rate and therefore enhances bioavailability. Satranidazole is poorly water soluble drug. One of the major problem is associated with them is low bioavailability due to less absorption. Formulation of drug in the form of nanosuspension can over come this problem. Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose.

2.MATERIALS AND METHOD

2.1. Materials

Drug Satranidazole was obtained as a gift sample from Alkem Laboratories Mumbai,India. poly(ε-caprolactone) (MW 40 000) was supplied by Aldrich (Milwaukee, WI, USA).` Sodium laury sulphate was supplied by S.D Fine Chemicals, Ltd, Mumbai,India. The solvents used to prepare nanoparticles were:Acetone used of analytical grade. Demineralized water filtered on 0.22 mm membranes (MilliQ water).

For quantitative analysis, different reagents were used ie : methanol (sigma), sodium hydroxide in solution (NaOH) 1 mol/1±0.5% (Sigma), hydrochloric acid (HCl) 36% (Sigma), phosphate-buffered saline (PBS) 10 mM at pH 7.4 (Sigma).

2.2. Method

Nanosuspension of Satranidazole was prepared by nanoprecipitation method developed by Fessi et al.1992. PCL and drug were dissolved in acetone at 45^0C. The organic solution was injected dropwise into distilled water containing SLS under magnetic stirring at room temperature. Acetone was removed under vacuum using a rotavapor (Buchi, Switzerland). Different batches of nanosuspensions varying Satranidazole and polymer ratios were shown in Table 1

Figure 1. Image of prepared nanosuspension

2.2.1. Size reduction of Particles

2.2.1.1. Ultrasonication:

Nanosuspension of Satranidazole was transferred to a 10 mL beaker and placed in bath sonicator for 15-20 minutes in order to get smaller particle size of the prepared nanosuspension. The sample was left to cool down and placed in the fridge at 4 °C for 1 day prior to further test e.g. size analysis and centrifugation.

All formulations were evaluated for particle size and shape, swellability and percentage drug entrapment. The particle size was examined by digital photomicroscope.

Scanning Electron Microscopy

The shape and surface morphology of PCL Nanoparticle were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then

coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

Particle size , polydispersity index , and zeta potential

The average size of nanoparticles was determined by photon correlation spectroscopy (PCS) (Malvern Instruments 1000, Malvern Instruments, Malvern, UK). The nanoparticle suspension was dispersed in 1ml of 0.2 _m Wltered ddH2O, and values are represented as *z*-average diameter. Determination of zeta potential was performed by laser anemometry, using the Malvern ZetaMaster (Malvern Instruments, UK) following dilution of the nanoparticles samples in 0.001M KCl solution. Nanoparticles were characterised by a mean *z*-average diameter and. Polydispersity index, zeta potential, And Results presented in Table 2 .

Drug entrapment efficiency

To determine drug entrapment efficiency after preparing the fresh nanosuspension, it was centrifuged and the free drug present in the supernatant was analyzed by UV-Visible spectrophotometer . The amount free drug can be calculated by using caliberation curve.

For separation of nanoparticle from unentraped active ingredient, a portion of the nanosuspension was transferred to 3 mL thick wall polycarbonate centrifuge tubes. The samples were then centrifuged. Centrifugation was done by using BECKMAN COULTER centrifuge. The centrifugation was done at speed of 15000 rpm for 30 minutes.

The entrapment efficiency was calculated using the following equation:

$$\text{Entrapment efficiency} = \frac{\text{Total Drug content} - \text{Free dissolved Drug}}{\text{Drug amount used}} \times 100$$

***In vitro* study**

The *in-vitro* drug release studies of Satranidazole nanosuspension was performed by dialysis method in an open end tube sealed with dialysis membrane (Himedia laboratories Pvt. Ltd., Mumbai, India. pore diameter 2.4 nm) was fitted in an USP dissolution apparatus containing 1000 ml of buffer solution as dissolution medium at pH 1.2 and pH 7.0 with stirring at 60 rpm at 37 ^{0} C.

Satranidazole nanosuspension (5ml) was added into the dialysis tube and samples of buffer (1ml) were withdrawn at predetermined time intervals from the external release medium for a period of 6 hours and replaced by same volume of fresh buffer to maintain sink condition. Absorbances of withdrawn samples were measured using a double beam UV-visible spectrophotometer at 318 nm. The amount of drug present in each aliquot was determined from standard calibration curve.

The *in-vitro* drug release study was performed for all optimized formulation and compared the observed data to obtain the better bioavailability of drug.

Short term stability study of nanosuspension

Stability study was performed for Physical appearance of the nanosuspension.Samples were stored at 4^0C for 1 month. The observation was done to check the physical appearance. Loose, thin layer of sediment can be observed when nanosuspension was stored at room temperature for 1 month. If sediment were disappeared with slight hand shaking then the good stability of the formulation was achieved. If sediment did not disappeared with slight hand shaking then the poor stability of the formulation was achieved. And that formulation batch was not considered as optimized batch. The optimization of the suitable batch on the basis of stability parameter can be done also by evaluating the average particle diameters when samples were stored at room temperature and 4^0C respectively. The particle size for the batches was evaluated before performing stability study and after performing stability study (Samples were stored at 4^0C for 1 month). Then the observed data was compared to show the stability of formulation after the duration of 1 month.

RESULTS AND DISCUSSION

Nanosuspension of Satranidazole were prepared by nanoprecipitation method. The prepared Nanosuspension were eualuated for particle mean diameter, Polydispersity index (PI) and the zeta potential, Drug intrapment efficiency, In vitro study, and Stability study. The nanosuspension prepared with PCL was creamy white in colour. In the nanoscale, which is normally seen as going from 100 nm to 1000 nanometers range , is suitable for the nanoparticle suspension formulation. In this nano formulation the particle mean diameter was found to be in range of 241.6 nm to 999.5. The usual range of PDI values is; 0-0.05 (monodisperse standard), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid range polydispersity), > 0.7 (very polydisperse). In this nano formulation the PDI was found to be in range of 0.133 toa 0.845. In order to obtain a nanosuspension exhibiting good stability, for

an electrostatically stabilized nanosuspension a minimum zeta potential of ± 30 mv is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desired. In this nano formulation the zeta potential was found to be in range of -7.9 to -21.3.Batch Ns_{1a} was called as optimized formulation because the particle size, PDI and zeta potential of Ns_{1a} batch was found under the suitable range of nanoparticle suspension. Particle size of this batch was 241.6 nm, PDI was 0.133 and zeta potential was -21.3 mv. Then Ns_{1b} had particle size of 440.7 nm and PDI was 0.453 and zeta potential was -13.6 mv. And the batch Ns_{2a} had exibited particle size of 377 nm and PDI was 0.433 and zeta potential was -18.0 mv. DEE of the satranidazole loaded nanosuspension was found to be 72.61 %, 55.34 %, 34.11 %,67.42 % ,42.25 %, for the selected batches on the basis of particle size,poly disparity index, zeta potential. The low DEE values indicate relatively low affinity of the drug with the polymer matrix. Another explanation for poor entrapment is probably solubility and ionization of the drug. Satranidazole is less soluble in water. The SLS (surfactant) is present in the aquous phase. Therefore, when the organic phase is added drop wise into the aqueous surfactant solution, part of the drug is ionized and escapes from the nanoparticles during diffusion of the acetone into the aqueous phase. Batch Ns_{1a} was found to be DEE in range of 72.61 % .Where the polymer and surfactant conc. was used as 50 mg and 5 mg respectively. Three strategies were used to enhance DEE of the batches such as effect of changing polymer content, and addition of SLS in a quantity in the range of 5 to10 mg. Changing the content of polymer in the formulation $Ns_{1a,}$ Ns_{2a} did not improve the DEE of nanosuspension but changing in conc. of surfactant the drug entrapment efficiency was increased. The SEM picture of NS_{1a} , nanoparticles were found to be spherical with a smooth surface and less aggregate. According to all kind of information which are mentioned above regarding to particle size, zeta potential and PDI, and SEM image, the NS_{1a} preparation was proved as a best formulation among all batches. In vitro drug release from the nanosuspension in SIF pH 7.0 and SGF pH 1.2 was performed by the dialysis experiment using the static Franz diffusion cell. The calibration curve of the drug was constructed to determine concentration from the absorbance at 318 nm with SIF pH 7.0 and SGF pH 1.2. The in vitro drug release profiles obtained from the dialysis experiment was shown in Figure 6**.** The amount of stabilizer incorporation in the formulation and drug entrapment efficiency have a direct effect on the drug release profile. As the content of the drug entrapment efficiency (DEE) in the formulation increased, the release rate also increased. The drug release (phosphate buffer, pH 7.0) and drug entrapment efficiency (DEE) were found as 67.9 %, 72.61 %, respectively, with a smaller average particle size (241.66 nm). Batch NS_{1b} had a

DEE of 55.34 % with a larger average particle size (440.7 nm), gave a prolonged drug release profile with only about 48.5 % drug release after 6 hours. A similar tendency was observed for Batch NS_{2a} (DEE ,67.42 % and particle size, 377 nm) which released about 53.8 % of the drug after 6 hours. Thus, a correlation between drug release from the nanosuspensions with mean particle size was observed. Thus, it can be inferred that larger particles have a small initial burst release and a longer sustained release than smaller particles.

The drug release of nanosuspension in SGF pH 1.2 were found as 28.4%,17.7%,20.5%. respectively for $NS_{1a,}$ $NS_{1b,}$ NS_{2a} batches. In this way it was found that the nanosuspensions of drug satranidazole show less % of drug release in SGF pH 1.2 than the SIF pH 7.0.

Physical appearance of the NS_{1a} nanosuspension did not change when samples were stored at 4^0C for 1 month. A loose, thin layer of sediment was observed when nanosuspension was stored at room temperature for 1 month. However, the sediment disappeared with slight hand shaking. The average particle diameters were found to be 294.7 nm and 231.5 nm when samples stored at room temperature and 4^0C respectively. The particle size for the batch NS_{1a} was 241.6 nm before performing stability study. It can be inferred from the observed data that the prepared nanosuspension NS_{1a} was stable after 1 month of storage at room temperature and 4^0C.

Table : 1. Composition of different batches of Satranidazole nanosuspension

Batch No.	Drug (mg) satranidazole	Polymer PCL (mg)	Surfactant SLS (mg)	Acetone (ml)	Water (ml)
Ns_0	10	50	_	25	50
Ns_{1a}	10	50	5	25	50
Ns_{1b}	10	50	10	25	50
Ns_{1c}	10	50	15	25	50
Ns_{2a}	10	100	5	25	50
Ns_{2b}	10	100	10	25	50
Ns_{2c}	10	100	15	25	50
Ns_{3a}	10	150	5	25	50
Ns_{3b}	10	150	10	25	50
Ns_{3c}	10	150	15	25	50

Table: 2. Particle diameter, Polydispersity index, Zeta potential of nanosuspension of satranidazole

Batch No.	Particle diameter (nm)	Polydispersity index (PI)	Zeta potential (mv)
Ns_{blank}	481.7	0.530	-8.31
Ns_{1a}	241.6	0.133	-21.3
Ns_{1b}	440.7	0.453	-13.6
Ns_{1c}	517.2	0..383	-16.4
Ns_{2a}	377.0	0.433	-18.0
Ns_{2b}	605.0	0.446	-8.4
Ns_{2c}	464.2	0.395	-12.0
Ns_{3a}	841.5	0.845	--14.7
Ns_{3b}	999.5	0.816	-8.6
Ns_{3c}	725.2	0.583	-7.9

Table: 3. Selected batch on the basis of Particle size polydispersity index, and zeta potential of nanosuspension of satranidazole

Batch No.	D: P ratio (mg)	Name of Surfactant	Quantity of Surfactant (mg)	Stirring speed (rpm)
Ns_{1a}	1:5	SLS	5 mg	1500
Ns_{1b}	1:5	SLS	10 mg	1500
Ns_{1c}	1;5	SLS	15 mg	1500
Ns_{2a}	1:10	SLS	5 mg	1500
Ns_{2c}	1;10	SLS	15 mg	1500

Table :4. Physical properties of Satranidazole nanosuspension of optimized formulation.

S No.	Batch No.	Shape
1	Ns_{1a}	Spherical and no aggregation
2	Ns_{1b}	Spherical and less aggregation
3	Ns_{1c}	Spherical and less aggregation
4	Ns_{2a}	Spherical and aggregation
5	Ns_{2c}	Spherical and aggregation

Table : 5. % Drug Entrapment efficiency of the nanosuspension of satranidazole

Batch No.	Total amount of Drug taken(mg)	Amount of Drug in supernatant (mg)	% Drug Entrapment efficiency
Ns_{1a}	10 mg	3.26	72.61
Ns_{1b}	10 mg	5.53	55.34
Ns_{1c}	10 mg	7.41	34.11
Ns_{2a}	10 mg	4.74	67.42
Ns_{2c}	10 mg	6.25	42.25

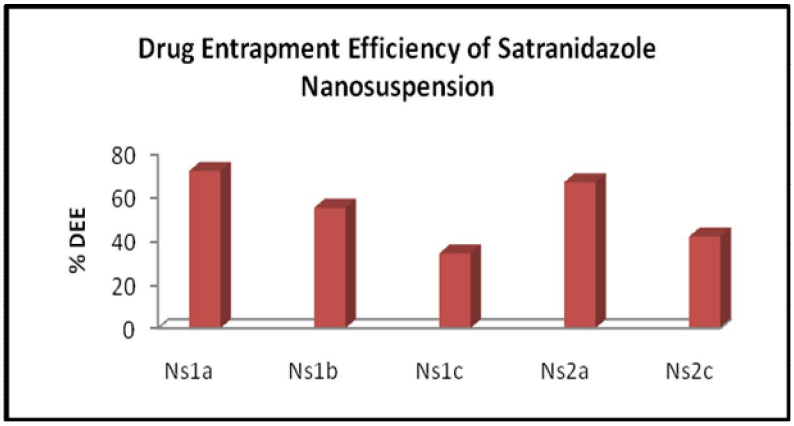

Figure : 2. Entrapment efficiency of the nanosuspension of satranidazole

Graph showing the comparative study of % DEE, Ns_{1a} shows the highest peak than the other formulation.The decreasing order of % DEE was found as $Ns_{1a.} > Ns_{2a} > Ns_{1b} > Ns_{1c} > Ns_{2c.}$

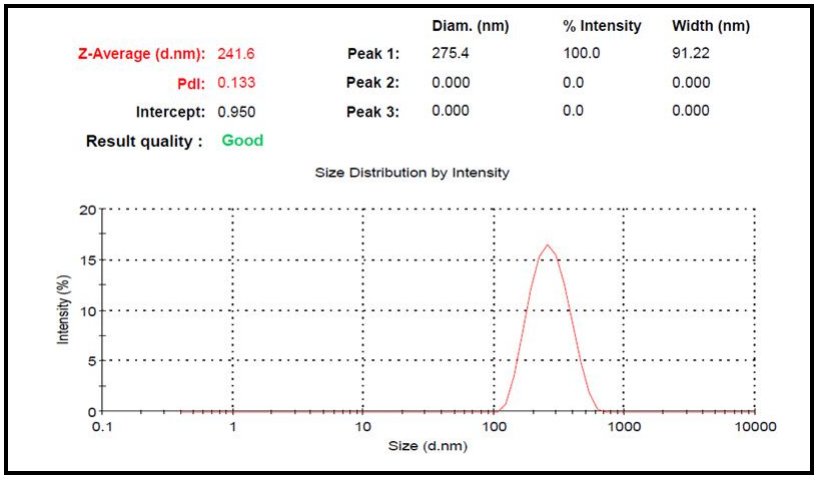

Figure : 3. Particle size of sample Ns_{1a}

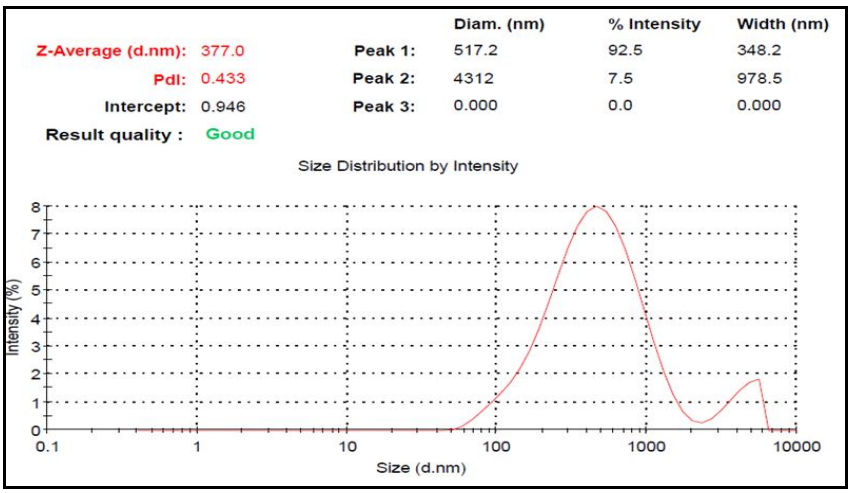

Figure: 4. zeta potential of Ns_{1a}

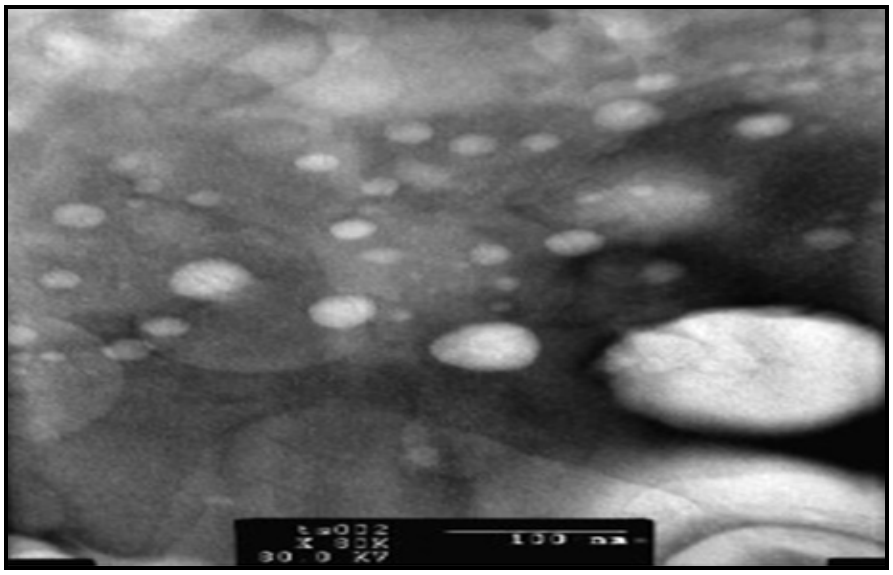

Figure : 5. show the SEM picture of NS_{1a}

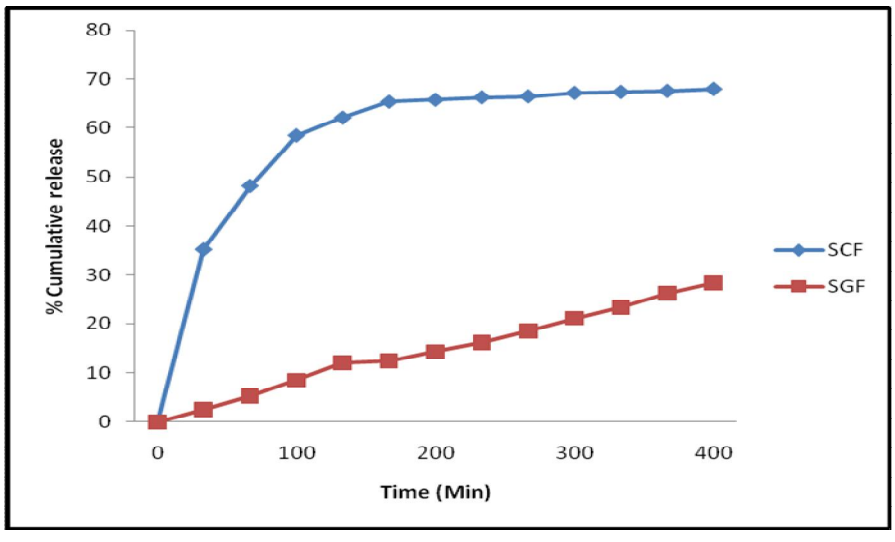

Figure: 6. *In vitro* evaluation of batch NS_{1a}

CONCLUSION

From the results of present study, it can be concluded that PCL based satranidazole nanosuspension provides better bioavailability of the drug, high degree of drug entrapment efficiency and deliver most of the drug load in the colon and allow drug release at the desired site. Hence PCL based satranidazole nanosuspension are a potential system for colon delivery in case of amoebic infection.

ACKNOWLEDGEMENT

The authors acknowledge the kind help received from Alkem Laboratories Mumbai, India, for gift samples of satranidazole. The authors gratefully acknowledge to the Kamla Nehru Institute of Technology & Management, Faridipur, Sultanpur, Uttar Pradesh Technical University, India. for providing facilities to carry out this research.

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