ISSN: 2347-4742

Research Article

Journal of Pharmaceutical and Biomedical Analysis Letters

www.pharmaresearchlibrary.com/jpbmal

Formulation and evaluation of effect of different stabilizer at nanosuspension of satranidazole

Vijay kumar singh, Preeti singh, Dinesh Chandra, Saundarya kumar*, Ushers Rai, Praveen singh

Kamala Nehru Institute of Technology & Management, Uttar Pradesh Technical University, Faridipur, Sultanpur, Uttar Pradesh, India

Abstract

The solubility and dissolution properties of drugs play an important role in the process of formulation development. Among all newly discovered chemical entities most of the drugs are lipophilic and fail to reach market due to their poor water solubility. Literature survey reveals that, amoebiasis is the second leading cause of death from parasitic disease worldwide. Satranidazole was selected as the drug of choice because it is most potent nitroimidazole derivative and clinically useful against common protozoa; it is twice as effective as other nitroimidazole against amoebiasis. The aim of the present investigation was to find out the effect of different stabilizer on the formulation of satranidazole nanosuspension. The prepared nanosuspensions were evaluated for Particle size, Polydispersity Index , Zeta potential analysis, SEM, solubility, %yield, drug content, %EE, invito drug release studies and DSC curves obtained confirms the transfer of drug crystalline form to amorphous form. Solubility studies and *in-vitro* drug release studies shows that the prepared nanosuspension has increased solubility and dissolution rate compared to pure drug.

Keywords: Satranidazole, Nanosuspension, Nanoprecipitation method, solubility enhancement

Contents

Corresponding author
Saundarya kumar
E-mail: saundarya.kumar07@gmail.com
MS. ID: PRL2014-JPBMAL1962

Copyright © 2014, JPBMAL All Rights Reserved

PAPER-QR CODE

1. Introduction

Nearly 40% of the new chemical entities currently being discovered are poorly water soluble drugs.The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility. The formulation of nano-sized particles can be implemented to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility and hence partition into gastrointestinal barrier. Several techniques have been developed concerning the optimization of the dissolution rate of these drugs. Such methods include particle

size reduction, solubilization, salt formation and preparation of solid dispersion systems. Nanosizing refers to the reduction of the active pharmaceutical ingredient (API) particle size down to the sub-micron range, with the final particle size typically being 100–1000 nm. The reduction of particle size leads to a significant increase in the dissolution rate of the API, which in turn can lead to substantial increases in bioavailability.

In the present research work an attempt was made to improve the solubility and dissolution rate of model drug Satranidazole (SAT), is a novel nitroimidazole derivative. Chemically, it is 1-methyl sulfonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone1. It is used as antiprotozoal and anitibacterial agent in the treatment of amoebiasis. It posses a C-N linkage at C2 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. In this present study, nanoprecipitation technique is used where a drug solution in a water miscible organic solvent is mixed with an aqueous solution containing a surfactant(s). Upon mixing, the supersaturated solution leads to nucleation and growth of drug particles, which may be stabilized by surfactants. Stabilizer are used to improve the stability of poloxamer, SLS, PVA, Tween 80, are used as stabilizer.

2. Experimental

Materials and Method

Pure drug Satranidazole was obtained from Alkem pharmaceuticals, as a gift sample. and the SLS, poly(ε-caprolactone) (MW 40 000) was supplied by Aldrich (Milwaukee, WI, USA), PVA, Tween 80, poloxamer, was supplied by S.D Fine Chemicals, Ltd, Mumbai,India. The solvents used to prepare nanoparticles ie. Acetone used of analytical grade.

Preparation of satranidazole nanosuspension

The Satranidazole nanosuspension was prepared by using nanoprecipitation technique coupled with ultrasonication method. . PCL was dissolve in acetone at 45^0C , volume changes due to evaporation of acetone was adjusted at room temperature.and then satranidazole was dissolved in acetone and added to polymer solution and dissolved. This organic solution was injected in distilled water containing different amount of stabilizer. The stabilizer was accurately weighed and dissolved in 2 ml of nanopure water with continuous stirring with magnetic stirrer. The solution of different stabilizer added simultaneously under stirring to a beaker containing 12 ml of nanopure water and then organic solution was added drop by drop by using syringe to the solution containing stabilizer . Stirred the mixture at 2500 rpm for 15 minutes using magnetic stirrer ,then the beaker containing the mixture was sonicated (Vibro cell VCX-750, M/s Sonics and Materials Inc., USA) to produce satranidazole nanosuspension. The formulated nanosuspension was preserved in an air tight container for further characterization. All formulations were evaluated for particle size, Polydispersity index , and zeta potential and shape, swellability and *In-vitro* dissolution studies from nanosuspension. The particle size was examined by digital photomicroscope.

Scanning Electron Microscopy

The shape and surface morphology of 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 dd H2O, and values are represented as *z*-average diameter. Determination of zeta potential was performed by laser anemometry, using the Malvern Zeta Master (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 3.

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer. The FTIR analysis was performed separately for drug and polymer and drug with polymer to confirm the identity of drug and chemical interaction between drug and polymer. The FTIR spectrum was performed by using a Perkin Elmer 1600 spectrophotometer with a resolution of 2 cm^{-1}. The samples were scanned in the spectral region between 4000 and 400 cm^{-1} by taking an average of 8 scans per sample.

Determination of Nanosuspension Process Yield

The nanosuspension production yield was calculated by gravimetry. Fixed volumes of nanoparticles suspension were centrifuged (15,000×g, 30 min, 15ºC) and sediments were dried.

The percentage process yield (% P.Y.) was calculated as follows:

$$\% \text{ P.Y.} = \frac{\text{Nanoparticle weight}}{\text{---------- x100}}$$

Table.3 Particle diameter, Polydispersity index, Zeta potential of nanosuspension of satranidazole

Total solids weight

Determination of % Entrapment Efficiency

The Nanosuspension with known amount of drug incorporated was centrifuged at done at speed of 15000 rpm for 30 minutes.The supernatant solution was separated. and the free drug present in the supernatant was analyzed by UV-Visible spectrophotometer at 318 nm using a caliberation curve.

The amount of drug unentrapped in the supernatant was calculated. The amount of drug entrapped and percentage entrapment was determined from drug unentrapped. Standard deviation was determined for 3 trials. The entrapment efficiency was calculated using the following equation:

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

In-vitro dissolution studies

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 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 9 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.

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. And the observation was take for the physical appearance. Loose, thin layer of sediment can be observed when nanosuspension was stored at room temperature for 1 month. If these sediment were disappeared with slight hand shaking then the good stability of the formulation was achieved. If these 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.

Table. 1 Formulation of Satranidazole nanosuspension using different stabilizer

Ingredients	N_1	N_2	N_3	N_4	N_5
Satranidazole(mg)	10	10	10	10	10
SLS (mg)	5				
PVA (mg)		10			
Tween 80 (mg)			2		
Poloxamer 188				15	
Poloxamer 127					25
Acetone (ml)	2	2	2	2	2
Water (ml)	30	30	30	30	30

Table.2 Time to dissolve 50% drug (t50%) from pure satranidazole and its nanosuspensions

Batch code	N_1	N_2	N_3	N_4	N_5	Pure drug
t 50%	15	19	18	23	25	>>7hr

Table.3 Particle diameter, Polydispersity index, Zeta potential of nanosuspension of satranidazole

Batch No.	**Particle diameter (nm)**	**Polydispersity index (PI)**	**Zeta potential (mv)**
N_{blank}	531.7	0.481	-7.49
N_1	362.3	0.222	-17.3
N_2	427.1	0.382	-11.7
N_3	517.2	0..383	-13.5
N_4	427.3	0.521	-9.25
N_5	628.3	0.471	-8.29

Table.4 Percentage drug content, Drug entrapment efficiency of Nanosuspension

Batch No.	% Drug content	Entrapment efficiency
N_1	**95.21**	**79.37**
N_2	**93.52**	**75.63**
N_3	**83.69**	**69.12**
N_4	**91.20**	**72.06**
N_5	**89.35**	**71.20**

Table.5 In vitro Drug release studies of all batches

S.No.	Time (hr)	% Drug release					
		N_1	N_2	N_3	N_4	N_5	Pure drug
1	0	**0**	**0**	**0**	**0**	**0**	**0**
2	1	22.31	18.5	15.25	16.09	14.37	13.70
3	2	34.52	32.26	31.4	27.36	22.79	16.3
4	3	43.28	39.35	35.70	34.43	33.91	17.39
5	4	58.04	52.62	45.82	38.71	35.27	18.92
6	5	65.8	55.84	53.72	48.52	41.72	19.37
7	6	69.37	62.23	56.09	52.21	45.82	20.57
8	7	78.10	69.75	65.37	59.26	49.35	22.61
9	8	84.21	74.09	71.43	68.38	59.28	25.33
10	9	93.73	81.27	79.1	73.9	60.79	28.53

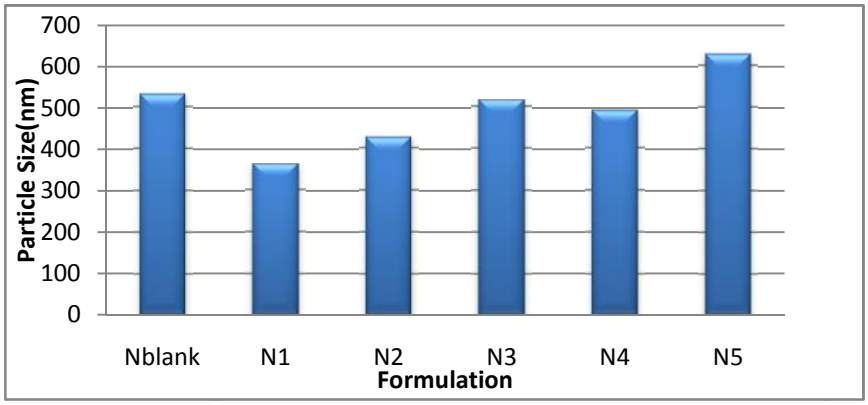

Figure. 1 Comparision of particle size with different stabilizer.

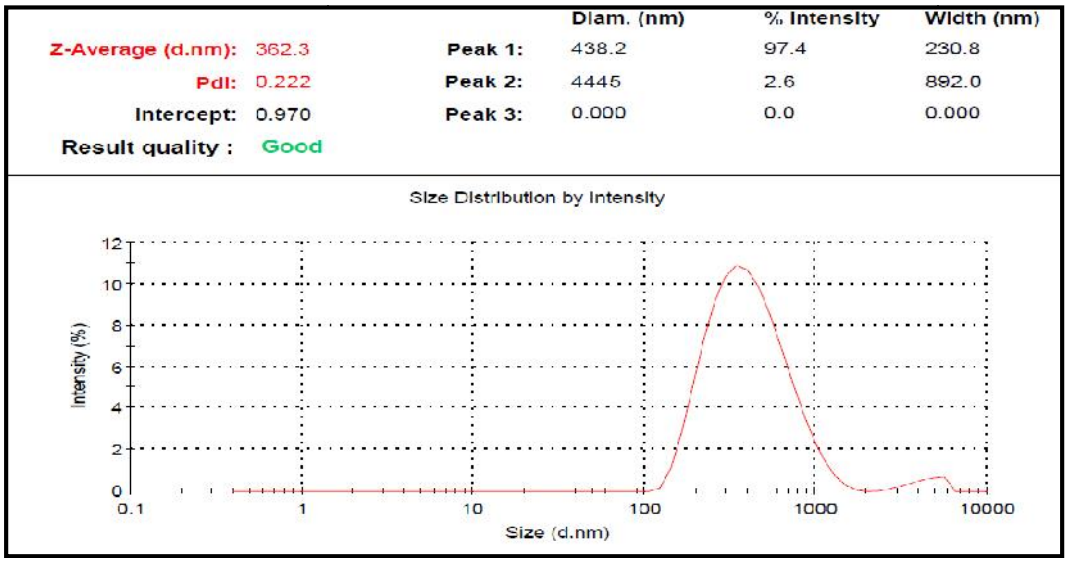

Figure .2 Particle size of formulation N_1

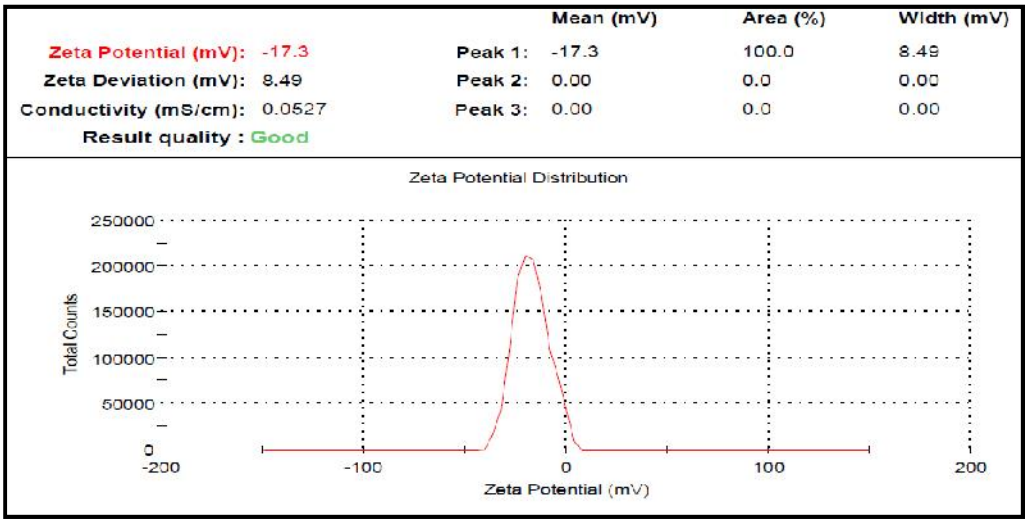

Figure.3 Zeta potential of formulation N_1

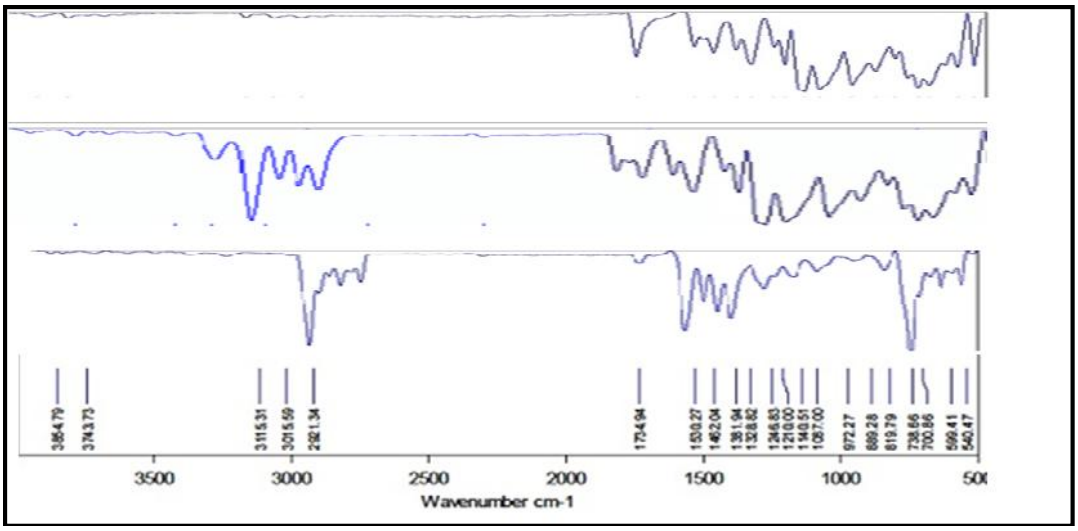

Figure.4 FTIR spectra of (A)- spectra of pure drug satranidazole, (B)- polymer PCL, (C)- Drug + polymer(mixture)

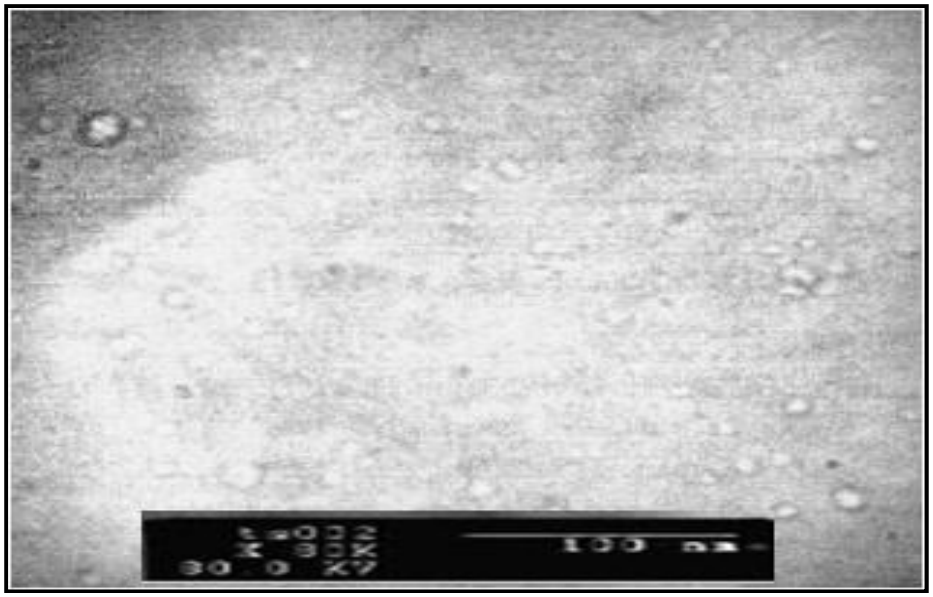

Figure .5 SEM image of batch of N_1

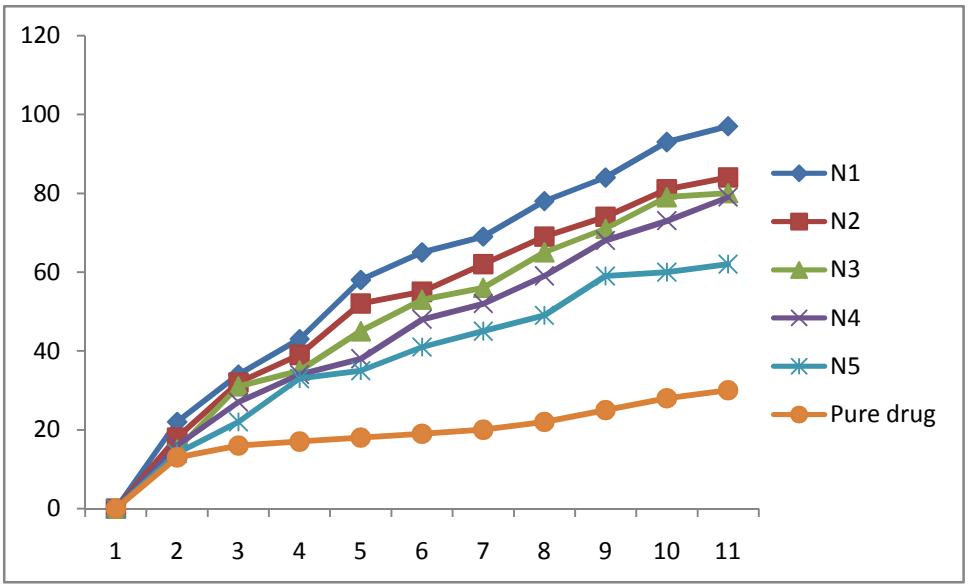

Figure .6 Cumulative % Drug release from all Formulation

3. Result and Discussion

Nanoprecipitation has been employed to produce nanosuspension of satranidazole. The types of different stabilizer were contributing much towards the change in particle size in nanosuspension preparation. Nanosuspension of satranidazole was prepared by as formulation shown in table 1. N1–N_5 formulations were containing different concentration of different stabilizer. Amount of water and acetone was kept constant for all batches. Curdy white nanosuspension was successfully prepared which was compared with distilled water.

Influence of stabilizers on particle size

The stabilizer's characteristics play an important role in creating a stable formulation. It must be capable of wetting the surface of the drug crystals and providing a steric or ionic barrier. Too little stabilizer induces aggregation or agglomeration and too much stabilizer promotes Oswald's ripening. First of all a screening of formulations was designed with different polymeric, anionic and stearic stabilizers. PVA, is a well known efficient polymeric stabilizer forming adsorption layers on drug nanoparticles and SLS was efficient anionic stabilizer, Poloxamer-127 & Poloxamer- 188 it can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual emulsion droplets at their optimum level. It was observed that the particle size (nm) and rate of dissolution has been improved when nanosuspension prepared with the SLS because of the efficient adsorption of the stabilizer on the produced nanoparticles surface and also imparts the stability to the formulation. The rate of dissolution of the optimized nanosuspension (N_1) was enhanced (50% in 15 min), relative to micronized suspension of satranidazole (50 % in >>7hr), mainly due to the formation of nanosized particles.

Particle size, poly dispersivity index, zeta potential

The particle size of different formulation was shown in figure.2, which clearly indicates the batch N_1 had less particle size as compare to other formulation. The batch (N_1) had a Z-average particle size of 362.3 nm with 0.222, poly dispersivity index which indicate the particles are in uniform distribution. And the zeta potential of the N_1 batch was found to be 17.3 which indicate that prepared formulation was stable. The particle size distribution pattern and zeta potential of the nanosuspension prepared with SLS formulation is given in figure.2 and figure.3 respectively.

FTIR studies

IR spectroscopic studies were conducted to determine possible interactions between drug and carrier. IR spectra of pure drug satranidazole and drug with PCL polymer were obtained. Which shows no chemical interaction between drug and excipients? The result of IR study shown in Figure 4.

Scanning electron microscope analysis of batch N_1

The nanoparticles surface appearance and shape were analyzed by scanning electron microscopies (SEM) figure 5 . This was indicating the size and shape of the prepared nanosuspension. And the nanoparticles were found to be spherical with a smooth surface and less aggregate.

Percentage drug content, Drug entrapment efficiency.

In nanosuspension formulation the drug particles were reduced to nano sized. During the formulation process there was not any drug loss step involved, so theoretically the formulation was considered as being 100% drug content. The Percentage drug content, drug entrapment efficiency and percentage yield of all the formulations were calculated and the results were tabulated in table 4. from all the formulations, formulation N_1 gave the highest

percentage drug content with 95.21 % . The drug entrapment efficiency of N_1 was high when compared to other formulations. This indicates that N_1 can be considered as best formulation.

***In-vitro* dissolution studies**

The release rate profiles were drawn as the percentage satranidazole dissolved from the nanosuspension and pure drug versus time. Dissolution studies of pure satranidazole and all other prepared nanosuspension (N1- N_5) were carried out in distilled water. t50% (time to dissolve 50% drug) values calculated from release profile are reported in Table 2. From this data, it was evident that onset of dissolution of pure satranidazole was very low as compare to its nanosuspension.

Stability study

The stability studies of formulation N_1 had been performed. The formulation showed a good stability at 4^0C, at room temperature 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 diameter was found to be 394.2 and 385.3 nm when samples were stored at room temperature and 4^0C respectively.

3. Conclusion

A nanoprecipitation method was developed to prepare satranidazole nanoparticles using SLS as stabilizer. In this process, the particle size of satranidazole can be obtained in the micron and nano-size ranges by selecting proper stabilizer. The best nanosuspension of satranidzole can be obtained by SLS as a stabilizer using nanoprecipitation technique. The dissolution of nanosized satranidazole is significantly enhanced compare with the pure satranidazole suspension. In conclusion, the nanoprecipitation method offers a direct process to obtain drug nanoparticles of desirable size, amenable for continuous and consistent production.

4. Acknoledgement

We are very thankful to Alkem Pharmaceutical Ltd., (Mumbai India.) for generously providing the gift sample of the drug.

5. Reference

1. Avgoustakis. K. “Pegylated poly (lactide) and poly(lactide-coglycolide) nanoparticles: preparation, properties and possible applications in drug delivery”, Current Drug Delivary, October 2004, vol. 1, no. 4, 321–333.
2. Dhaval j Patel, Jayvadan k. Patel, Vikram M .Pandya, Ritu D,Patel, Effect of formulation variables on Nanosuspension containing Famotidine prepared by solvent evaporation technique” IJPST Vol 2.Issue 4.Jan-March 2010.
3. Dillaman R.M., Hart H.V. “X-ray evaluation of SEM technique for determining the crystallography of echinoid skeletons”. Scanning Electron Microscopy, 1981, (Pt 3):313-20.
4. Fessi H., Puisieux F., Devissaguet J.P., Ammoury N. , Benita S. “Nanocapsule formation by interfacial polymer deposition following solvent displacement”, International Journal of Pharmaceutics, October 1989, vol. 55, no. 1, R1–R4.
5. Mainardes R.M, Evangelista R.C. “PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution”. International Journal of Pharmaceutics, February 2005, vol. 290, no. 1-2, 137- 144.
6. Molpeceres J., Guzman M., Aberturas M.R., Chacon M., Berges L. “Application of central composite designs to the preparation of polycaprolactone nanoparticles by solvent displacement ”, Journal of Pharmaceutical Science, February 1996, vol. 85, no. 2, 206–213.
7. Pinto Reis C., Neufeld R.J., Ribeiro A.J., Veiga F. “Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles”, Nanomedicine:Nanotechnology,Biology, and Medicine, March 2006, vol. 2, no. 1, 8-21.
8. Quintanar-Guerrero D, Alle´mann E, Fessi H, Doelker E. “Preparation techniques and mechanism of formation of biodegradable nanoparticles from preformed polymers”, Drug Devlopment and Industrial Pharmacy, December 1998, vol. 24, no. 12, 1113-1128.
9. R.Pignatello , C.Bucolo , G.Spedalieri , A.Maltese , G.Puglisi, Flurbiprofen-loaded acrylate polymer nanosuspensions for ophthalmic application”Dipartimento di Scienze Farmaceutiche, Universit " a degli Studi di Catania, Citt " a Universitaria, viale A. Doria, 6–95125 Catania, Italy ,Bausch & Lomb-Fidia Oftal Pharmaceuticals, Corso Italia 141-95127, Catania, Italy Received 21 May 2001; accepted 28 February 2002
10. Rajalakshmi. R1, Thanda Venkataramudu,Design and characterization of valsartan nanosuspension,Dept. of Pharmaceutics, Sree Vidyanikethan College of Pharmacy, Tirupati, A.P, 517102, India.SV University, Tirupati, A.P, 517102, India. AMR Memorial College of Pharmacy, Narasaraopet, Guntur, A.P, 522601, India,International journal of pharmacotherapy,ISSN 2249 – 7765

11. Selvakumar Kalimuthu, A. V. Yadav,Formulation and Evaluation of Carvedilol loaded Eudragit e 100 Nanoparticles,,Biopharmaceutics Research Group,Department of Biopharmaceutics, Government College of Pharmacy, Karad - 415124, M.S., India, International Journal of PharmTech Research,CODEN(USA): IJPRIF ISSN : 0974-4304ol.1, No.2, pp 179-183 , April-June 2009
12. Vikram M Pandya1 , Jayvadan K Patel2, Dhaval J Patel" Effect of different stabilizer on the formulation of simvastatin nanosuspension prepared by nanoprecipitation technique" Research Journal of Pharmaceutical, Biological and Chemical Sciences" ISSN: 0975-8585.