WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 5, 2227-2240. Research Article ISSN 2277– 7105

FORMULATION AND CHARACTERIZATION OF SAQUINAVIR NANOPARTICLES PREPARED BY NANOPRECIPITATION METHOD

S. Sivaprasad*, [1]P. Subhash Chandra Bose, [2]Ch. Sadakvali, [3]G. Ravi and [4]V. Swathi

[*]Associate Professor, Department of Pharmaceutics, MNR College of Pharmacy, Fasalwadi (V), Sangareddy (Dist), Telangana.

[1]Professor, Department of Pharmaceutics, MNR College of Pharmacy, Fasalwadi (V), Sangareddy (Dist), Telangana.

[2]Associate Professor, Department of Pharmaceutics, Avanthi College of Pharmacy, Hyderabad, Telangana.

[3]Associate Professor, Department of Pharmaceutics, MNR College of Pharmacy, Fasalwadi (V), Sangareddy (Dist), Telangana.

[4]Assistant Professor, Department of Pharmaceutics, MNR College of Pharmacy, Fasalwadi (V), Sangareddy (Dist), Telangana.

Article Received on
17 March 2020,
Revised on 07 April 2020,
Accepted on 28 April 2020
DOI: 10.20959/wjpr20205-17476

***Corresponding Author**
S. Sivaprasad
Associate Professor, Department of Pharmaceutics, MNR College of Pharmacy, Fasalwadi (V), Sangareddy (Dist), Telangana.

ABSTRACT

The development of new delivery systems for the controlled release of drugs is one of the most promising fields of research in pharmaceutical sciences. Nanoparticles were specially designed to release the drug at the desired target sites. The main objective of this work was to prepare and characterize Saquinavir nanoparticles as potential drug delivery system for anti-HIV chemotherapy. The particle size and the surface morphology results revealed that Saquinavir nanoparticles were smooth with a size ranging from 248 nm-412 nm. The drug entrapment efficiency was found to be nearly 98%. *In vitro* release studies revealed that the rate of drug release from F5 was 98.97% in 16 hours. Release of drug follows first order and show controlled release behavior. Koresmeyer- peppas model shows that the drug follow non-Fickian transport as the value of n>0.5. The results suggest that TPGS polymer based nanoparticulate formulations are potential means to achieve release of saquinavir for the prolonged period of time for effective therapy.

KEYWORDS: Saquinavir, TPGS, Control release, HIV treatment.

INTRODUCTION[1-4]

Nanoparticles are one of the most promising novel delivery systems used for prolonged and/or controlled drug delivery, improved bioavailability, stability, and targeted drug delivery to specific sites. nanoparticles can also offer advantages such as limiting fluctuation within a therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance.

In spite of these advantages nanoparticles do have limitations like,

1. Altered physical properties which lead to particle – particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms due to smaller size and larger surface area.
2. Smaller the particles size greater the surface area and this property makes nanoparticles very reactive in the cellular environment.
3. Small particles size results in limited drug loading and burst release. These practical problems have to be sorted out before nanoparticles can be used clinically or made commercially available.

Nanoparticles are formulated to target the drug to the specific organ site and to control the rate of release of drug. By encapsulating a drug into nano structures, the existence of the drug in the systemic circulation can be prolonged and thus enhance penetration into target tissue and reduce the toxicity.

PLGA (poly **(lactic-*co*-glycolic acid)**) has been used as a successful biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These two monomers under normal physiological conditions, were by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there was minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications.

D-α-tocopheryl polyethylene glycol succinate (Vitamin E TPGS or TPGS) has been approved by FDA as a safe adjuvant and widely used in drug delivery systems. The biological and physicochemical properties of TPGS provide multiple advantages for its applications in drug delivery like high biocompatibility, enhancement of drug solubility, improvement of drug permeation.

Saquinavir is an antiretroviral protease inhibitor that is used in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). Conventional dosage forms of Saquinavir suffers with poor bioavailability and permeation problems of crossing blood brain barrier (BBB). To overcome these problems a novel dosage forms nanoparticles were prepared by using nanoprecipitation method.

MATERIALS AND METHODS

Saquinavir was purchased from Scion Pharma, Taiwan. PLGA was purchased from Durect corporation Birmingham, UK, TPGS was obtained as a gift sample form Eastman company, UK, Dialysis membrane was purchased from HiMedia Laboratories Pvt. Ltd, Mumbai. All other chemicals used were of analytical grade.

Preparation of nanoparticles[6-7]

Nanoparticles containing Saquinavir were prepared by using nanoprecipitation method. The nanoparticles are prepared by dissolving the Saquinavir in organic phase along with the polymer (PLGA) and added to the aqueous solution containing TPGS which acts as an emulsifier. The solution of organic phase was added in drop wise into aqueous phase under homogenization at 11,000 rpm. The dispersion was kept under magnetic stirring for 4hrs at room temperature. The solution is kept under reduced pressure for about 2-3min. This process forms nanoparticles loaded with drug. In all the formulations (F1 to F8) 500mg of the drug was added. The first part of the plan of work was to optimize the concentration of surfactant to be used in the formulation of nanoparticles. To achieve this, the first three formulations (F1, F2 & F3) were planned with TPGS concentrations 15, 30, 60 respectively. Since F2 formulation was having the least particle size the same concentration (30mg) is used for F4, F5 & F6 formulations. The next part of the plan of work was to optimize the drug polymer ratio. For this, 5 batches were planned (F4 to F8) using the drug polymer ratios of 1:4, 1:5, 1:6, 1:7 and 1:8 respectively. The optimum drug polymer ratio was selected on the basis of entrapment efficiency of the polymer. The entrapment efficiency was found to be very low for 1:4(94%).

The composition of the Saquinavir nanoparticles and drug polymers ratios were given in Table1&2 respectively. Nanoparticles were separated by using cooling centrifuge (10000 rpm for 20 min), supernatant were removed and nanoparticles washed with water and dried at room temperature in a desicator. The obtained centrifuged samples were lyophilized and stored at 2-8^{0}C. The samples are lyophilized to attain stability. The obtained lyophilized

powder is utilized for determination of entrapment efficiency and *in-vitro* drug release parameters.

Table no. 1: Composition of the Saquinavir Nanoparticles.

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
PLGA (50:50)(mg)	500	1000	1500	2000	2500	3000	3500	4000
TPGS (mg/ml)	15	30	60	30	30	30	30	30
Saquinavir (mg)	500	500	500	500	500	500	500	500
Acetone (ml)	30	30	30	30	30	30	30	30
Water (ml)	100	100	100	100	100	100	100	100

Table no. 2: Formulations used for entrapment efficiency, drug loading capacity & *in vitro* diffusion studies.

Ingredients (mg)	F5	F6	F7	F8
PLGA (50:50)	2500	3000	3500	4000
TPGS%(g/ml)	30	30	30	30
Saquinavir (mg)	500	500	500	500
Acetone (ml)	30	30	30	30
Water (ml)	100	100	100	100

METHODS

Drug polymer compatibility studies[8]

Compatibility of drug with excipients was determined by carrying out FTIR studies. Infrared spectrum of Saquinavir, PLGA, TPGS and physical mixture of drug and polymer was determined on Perkin Elmer FT-IR Spectrophotometer, series 1600 which was calibrated with polystyrene using KBr dispersion method in the region between 400-4000 cm-1.

Differential scanning calorimetry (DSC)[10]

The thermal analysis was performed using a Mettler Toledo star system 822e differential scanning calorimeter, equipped with liquid nitrogen cooling system. The machine was calibrated with pure indium (M.P.1550C) and zinc (M.P.419.5 0C) as standard calibrators prior to experiments. Samples, API and drug excipient mixed powders (5-8mg) were crimped in aluminium pans with lids and tested under dry evaporation of any water or residual solvent existing in the sample. The test was conducted with scanning rate of 10^{o}C /min over the temperature range of 50^{o}C-250^{o}Cfor drugs/ excipients/ mixtures. An empty aluminium pan was used as a reference.

Physico chemical Evaluation[5]

To evaluate particle size and zeta potential, Freeze-dried nanoparticles were reconstituted in distilled water. The size of the nanoparticles were determined by Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) based on dynamic light scattering technique. The mean particle size of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Each sample was diluted with phosphate buffer (pH 7.4) and the surface charge (zeta potential) of the NPs determined by measuring their electrophoretic mobility of the NPs by the zeta sizer (Malvern Instruments, UK).

Particle size analysis and zeta potential measurement[5]

Freeze dried NPs were reconstituted in distilled water. The size of the NPs was determined by Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) based on dynamic light scattering technique. The mean particle size of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Each sample was diluted with phosphate buffer (pH 7.4) and the surface charge (zeta potential) of the NPs determined by measuring their electrophoretic mobility of the NPs by the zeta sizer (Malvern Instruments, UK).

Surface Morphology

The Saquinavir loaded nanoparticles were subjected to Scanning Electron Microscope (SEM) for determining its size and shape. The nanoparticles size and shape were to be characterized.

Drug Entrapment Efficiency[11]

Lyophilized nanoparticles 3mg were dissolved in 1ml of diluents and the drug amount was determined by HPLC analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Saquinavir in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Saquinavir PLGA nanoparticles was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

***In vitro* release studies**[12]

In vitro release studies were carried out by using dialysis membrane with an artificial membrane. 10 mg drug equivalent freeze dried Saquinavir loaded nanoparticles were

dispersed in 3 ml pH 7.4 phosphate buffer solution which is transferred in dialysis bag and suspended in 100 ml of isotonic pH 7.4 Phosphate buffer solution (PBS). The bag was placed under magnetic stirring in a water bath maintained at 37 ± 0.5° C. At fixed time intervals 5ml of samples were taken out and fresh buffer was replaced. The obtained solution was analyzed by HPLC to determine the drug content.

Kinetic modeling

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equations like zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), Peppas model (log % drug release vs. log time). R^2, K, n values were calculated for the linear curve obtained by regression analysis of the above plots.

Stability studies

The stability of Saquinavir nanoparticles was performed as per (ICH) QIA (R2) guidelines to assess the physical appearance, drug content and drug release studies. Saquinavir loaded nanoparticles were packed separately in screw capped HDPE bottles sealed with aluminium seals and were stored at 40°C/75% relative humidity (RH) in the stability chamber for 6 months. Samples were taken at 0, 1, 3 and 6 months during the testing period and these samples were tested for physical appearance, drug content and drug release studies.

Statistical analysis

All experiments were repeated at least three times. Data are expressed as mean ± standard deviations (SD, n = 3).

RESULTS AND DISCUSSION

From the FTIR results, we examined the emission spectra of pure Saquinavir (Fig.1) and optimized formulation nanoparticles (Fig.2) were found to be identical. The FTIR spectrum of the pure showed the characteristic peaks for Saquinavir at 3307.37 cm^{-1} due to amide N-H stretch and hydroxyl stretch and 1664.05 cm^{-1} due to the amide carbonyl, and 1506.96 cm^{-1} due to N-H bend and 889 cm^{-1} due to out of plane aromatic bend. The FTIR spectrum of the optimized formulation also shows distinct band at 3332 cm^{-1} due to amide N-H stretch and hydroxyl stretch and 1669.32 cm^{-1} due to the amide carbonyl, and 1547 cm^{-1} due to N-H bend and 866.71 cm^{-1} due to out of plane aromatic bend. The FTIR spectra obtained

indicated that no chemical interaction occurred between the pure drug and optimized formulation. But, a slight shift in absorption peaks position was noticed which indicated that physical interaction might have occurred between drug and the polymer/ excipients used.

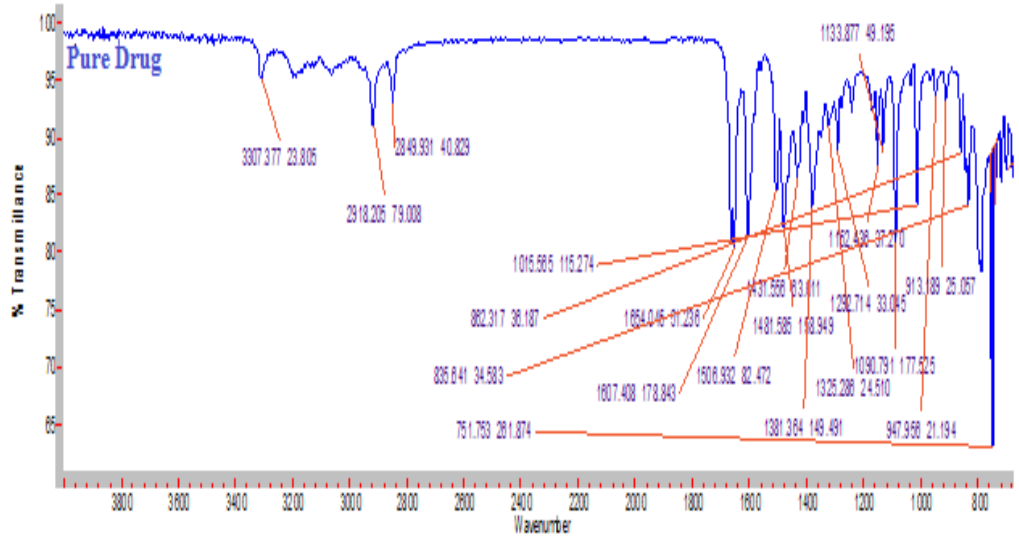

Fig. 1: I.R spectra of Saquinavir.

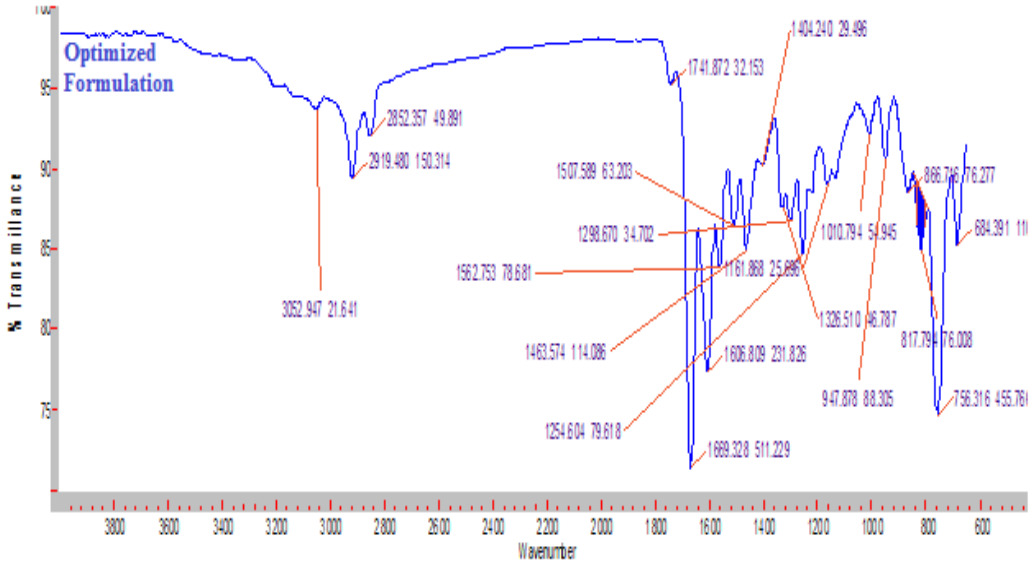

Fig.2: I.R spectra of Saquinavir optimized formulation.

DSC characterization

DSC thermo grams for drug excipients compatibility studies are shown in Fig.3&Fig.4 and the changes in melting endotherm are summarized. In all the thermo grams The melting onset was in the range of 222.50 ^{0}C to 225.42 ^{0}C and the melting peak was in the range of 192.32^{0}C to 276.8^{0}C for Saquinavir with all the excipients selected. From the above studies, it may be concluded that there is no incompatibility between the drugs and excipients.

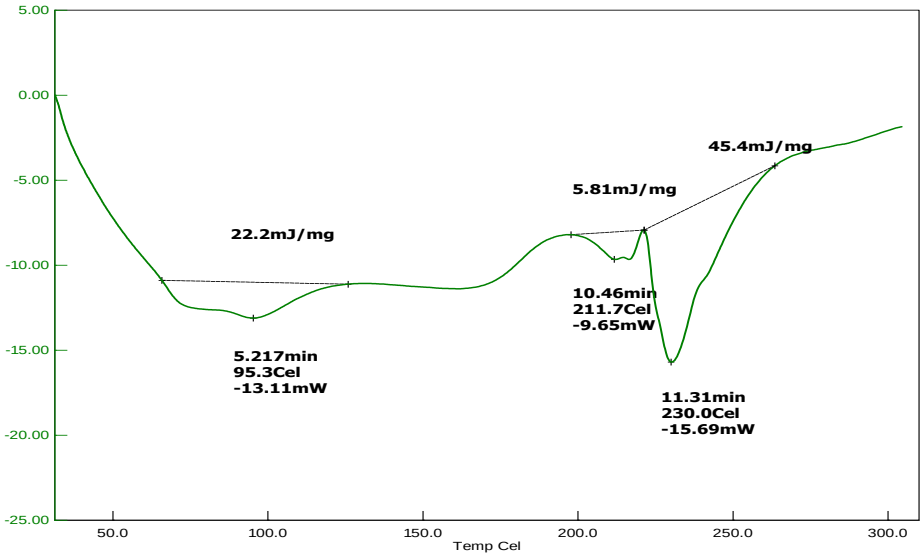

Fig.3: D.S.C Thermogram of Saquinavir.

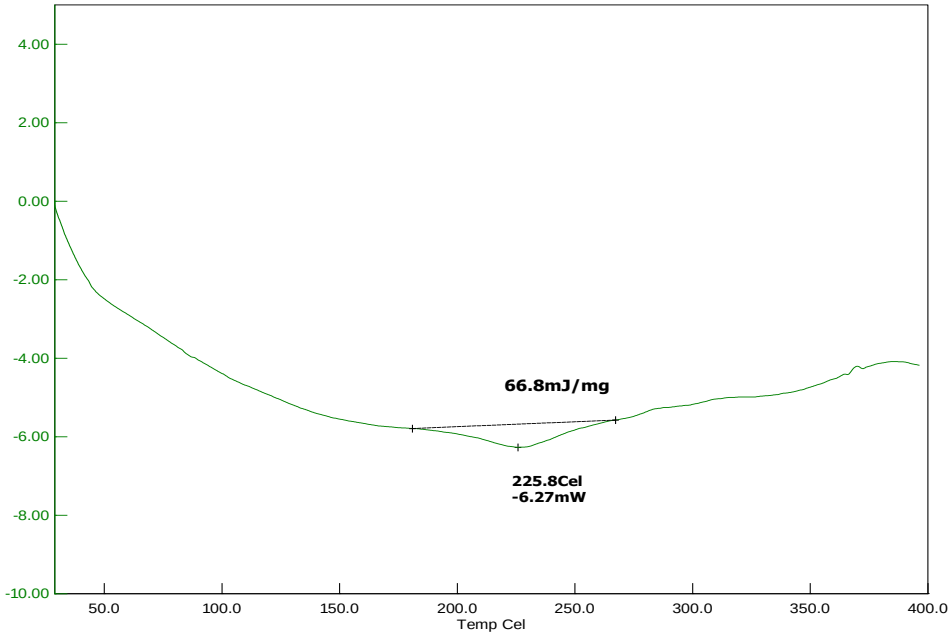

Fig. 4: D.S.C Thermogram of Saquinavir optimized formulation.

HPLC Method[13]

Samples collected in diffusion studies were analyzed by HPLC technique. For this purpose a standard plot was plotted in HPLC by using reference standard of Saquinavir.

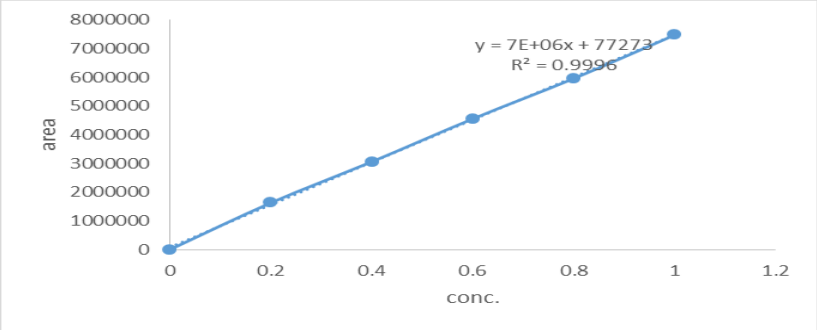

Fig. 3: Standard graph for Saquinavir nanoparticles.

EVALUATION OF SAQUINAVIR NANOPARTICLES

Measurement of Particle size, zeta potential and Drug loading capacity

All the prepared formulations were analyzed in order to determine their particle size distribution, zeta potential and drug entrapment efficiency. The size of the all the formulations was ranging from 237.1 nm to 412.2 nm (Table no. 3). The drug entrapment efficiency of different formulations ranges from 82% to 98.27%. Zeta potential values of Saquinavir formulations were found to be -22.47 mV to -28.59 mV (Table no.3) Thus there was a steady increase in the entrapment efficiency and decrease in particle size on increasing the polymer concentration in the formulation. Among the above formulations F5, F6, F7 and F8 were considered for the *in vitro* release studies based on the entrapment efficiency. SEM photographs (Fig.4) indicate that NPs were spherical in shape and in the nanosize range with discrete spherical outline.

Table no. 3: Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size, Zeta Potential and Drug Loading.

Batch No	Particle size (nm)	Zeta potential (mV)	Drug Loaded (mg)	Entrapment Efficiency (%)
F1	354.2	-12.48	412.21	82.44
F2	251.1	-16.75	431.75	86.35
F3	274.3	-23.17	462.14	92.4
F4	346.8	-17.84	471.24	94.24
F5	412.2	-26.74	493.16	98.632
F6	248.1	-28.59	492.17	98.434
F7	237.1	-26.41	491.38	98.276
F8	302.1	-22.47	491.33	98.266

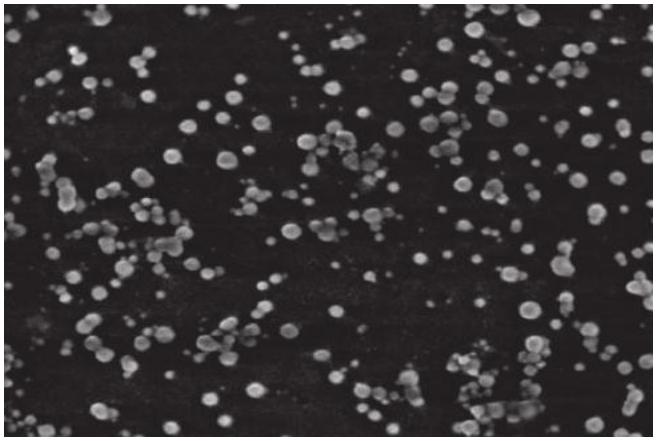

Fig.4: SEM image of optimised formulation Saquinavir.

***In vitro* Release studies:** All the selected formulations of Saquinavir nanoparticles were subjected to release studies and the results were shown in table no.4. Formulations F5, F6, F7 and F8 were prepared with drug: polymer ratio of 1:5, 1:6, 1:7 and 1:8 shown the drug release after 16 hours in pH 7.4 phosphate buffer was found to be 98.97±0.02%, 92.24±0.02, 92.00±0.04 and 93.71±0.04 respectively. The release profiles of different formulations were shown in Fig.5. Among the all the formulations, F5 formulation drug release was higher compared to other formulations. Hence, F5 formulation was considered as optimized formulation. On increasing polymer concentration, the release rate of Saquinavir from nanoparticles decreased drastically. The *in vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism. All the kinetic models are shown in Fig.6 and the results are shown in Table no.5. In the table R^2 value is correlation value, k is rate constant and n is release exponent. On the basis of the R^2 value it is concluded that the optimized formulation F5 of nanoparticles follow the Korsmeyer-Peppas model with release exponent value n= 1.031. The magnitude of the release exponent n indicates the release mechanism is Non Fickian diffusion.

Table no. 4: Diffusion study profiles forF5, F6, F7 & F8.

TIME(hrs)	F5	F6	F7	F8
0	0	0	0	0
1	27.86±0.01	18.87±0.02	28.04±0.05	27.73±0.06
2	41.45±0.02	35.66±0.05	41.65±0.02	42.04±0.01
4	55.25±0.05	51.06±0.02	53.81±0.05	64.33±0.02
6	66.73±0.06	63.63±0.01	64.53±0.02	83.84±0.05
8	76.34±0.02	69.71±0.05	69.43±0.01	84.32±0.08
10	82.52±0.05	79.27±0.02	79.98±0.05	89.44±0.02
12	92.97±0.01	89.02±0.08	85.98±0.02	92.34±0.09
16	98.97±0.02	92.24±0.02	92.00±0.04	93.71±0.04

Cumulative % release Vs Time (hr)

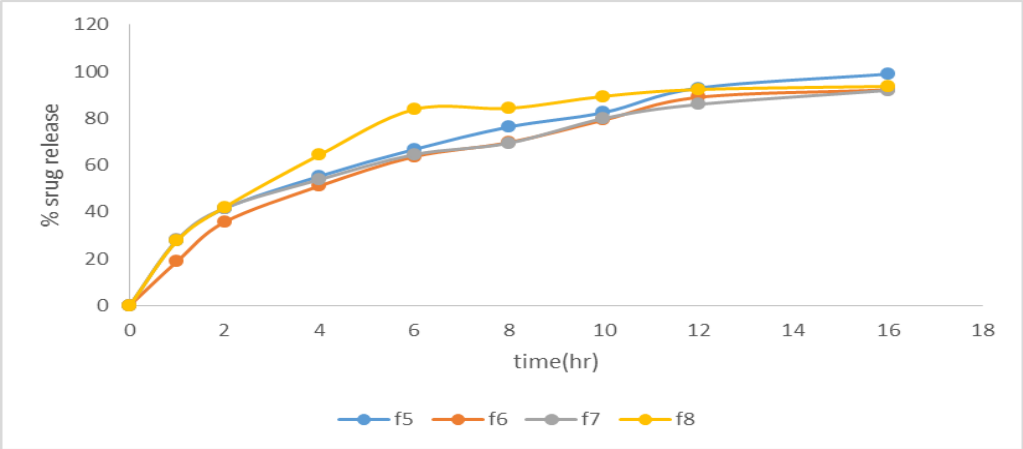

Fig. 5: Diffusion study profile for F5, F6, F7 and F8.

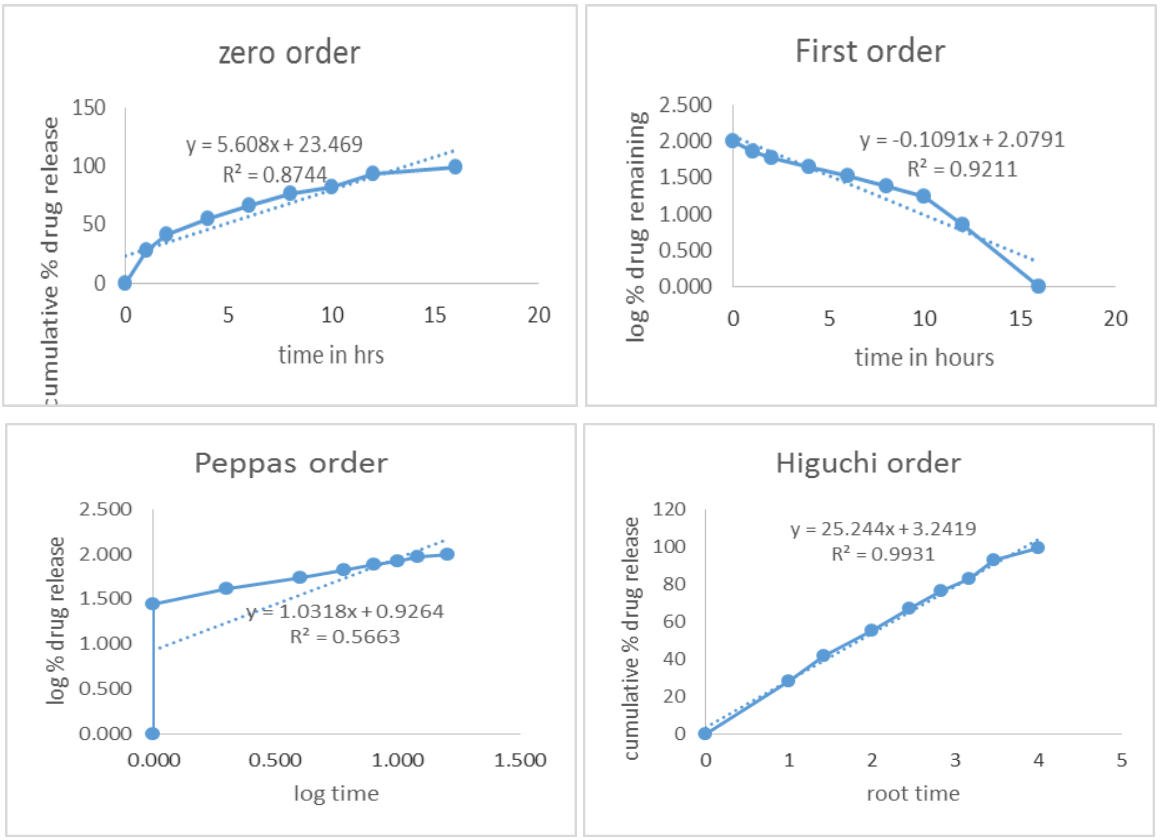

Fig.6: Drug Release kinetic plots for optimized formulation-F5.

Table no. 5: Interpretation of R^2 values and the rate constants of release kinetics of nanoparticles.

Model	R^2	k	n
Zero order	0.874	5.608	-
First order	0.921	-0.109	-
Higuchi	0.993	25.24	-
Korsmeyer-Peppas	0.566		1.031

Stability study

There were no significant changes in physical and chemical properties of formulation F-5 after 6months. However, relatively less change was observed in the assay and entrapment efficiency at different temperatures and different relative humidities. (Table no. 5). The release profiles of optimized formulation F5 during stability studies optimized formulation was mentioned in the Fig.7.

Table no. 5: Results of stability studies of Saquinavir optimised formulation F5 before and after storage during the stability studies.

Formulation code	Parameters	Percentage of Drug content				Limits as per specifications
		Initial	After 1st Month	After 3rd Month	After 6th Month	
F5	25˚C/60%RH % Release	98.97	97.48	96.43	96.12	NLT 85%
F5	30˚C/75%RH % Release	98.45	96.48	96.15	96.24	NLT 85%
F5	40˚C/75%RH % Release	99.15	97.48	97.42	97.14	NLT 85%
F5	25˚C/60%RH Assay value	98.47	98.21	98.20	98.08	NLT 90% NMT 110%
F5	30˚C/75%RH Assay value	97.48	97.34	97.11	96.78	NLT 90% NMT 110%
F5	40˚C/75%RH Assay value	96.87	96.80	96.75	96.16	NLT 90% NMT 110%

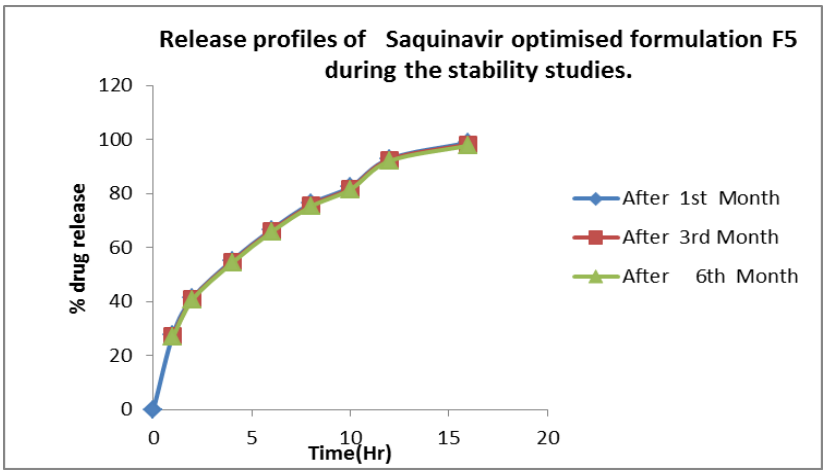

Fig. 7: Release profiles of Saquinavir optimised formulation F5 during the stability studies.

CONCLUSION

In the current work an attempt was made to prepare Saquinavir nanoparticles by using nanoprecipitation method. And positive results were observed it seems that the concentration

of PLGA played a main role in the entrapment and drug loading capability of the formulation. Based on the amount of drug loaded and entrapment efficiency the F5 formulation was considered as optimized formulation and which is considered for *in vitro* studies and stability studies. The compatibility studies also revealed that there was no incompatibility between the pure drug and the optimised formulation. The best formulation (F5) followed first order release kinetics and the release mechanism is non Fickian diffusion.

REFERENCES

1. Robinson JR, Lee VH. Controlled drug delivery. Fundamental and Applications. Marcel Dekker, New York: 2nd edition, 213-216 & 405.
2. Mahajan SD, Roy I, Xu G, Yong KT, Ding H, Aalinkeel R. Enhancing the delivery of anti retroviral drug "Saquinavir" across the blood brain barrier using nanoparticles. Curr HIV Res., Jul, 2010; 8(5): 396–404.
3. Brahmankar D.M, Biopharmaceutics and clinical pharmacokinetics, Vallabh Publications, New Delhi, 145-148.
4. Brewer E, Coleman J, Lowman A. Emerging technologies of polymeric nanoparticles in cancer drug delivery. J Nanomater, 2011; 1–10.
5. Furniss B S, Hannaford A J, Smith P W and Tatchell A R; In: Vogel's Textbook of Practical Organic Chemistry, 5th edition, I R Correlation tables, 1413-1422.
6. S. Schubert, J. T. Delaney Jr, and U. S. Schubert. Nanoprecipitation and nanoformulation of polymers from history to powerful possibilities beyond poly(lactic acid). Soft Matter, 2011; 7(1): 1581–1588.
7. D. Alukda, T. Sturgis, and B. C. Youan. Formulation of tenofovir-loaded functionalized solid lipid nanoparticles intended for HIV prevention. J Pharm Sci., Aug, 2011; 100(8): 3345–3356.
8. Adlin Jino Nesalina J, Anton Smith et al. Preparation and evaluation of Chitosan nanoparticles containing zidovudine. Asian J Pharm Sci., 2012; 7(1): 80-84.
9. Adlin Jino Nesalina J, Anton Smith et al. Preparation and evaluation of Chitosan nanoparticles containing zidovudine. Asian J Pharm Sci., 2012; 7(1): 80-84.
10. Tamizharasi S, Shukla A, Shivkumar T, et al. Formulation and evaluation of lamivudine loaded polymethacrylic acid nanoparticles, Int J Pharma. *Tech Res*., 2009; 1: 411-15.
11. ICH, Stability Testing of New Drug Substances and Products. International Conference on Harmonisation, IFPMA, Geneva, 1993.

12. Monika Baskshi and Saranjit Singh; Stress degradation studies on metronidazole and development of a validated stability indicating HPLC assay method , J Pharm Tecnol.**,** 2003; 148-160.
13. Droste, J. A. H, Verweijvan Wissen, C.P. W. G. M.; Burger, D. M. Simultaneous Determination of the HIV Drugs Indinavir, Amprenavir, Saquinavir, Ritonavir, Lopinavir, Nelfinavir, the Nelfinavir Hydroxymetabolite M8, and Nevirapine in Human Plasma by Reversed Phase High Performance Liquid Chromatography. Therapeutic Drug Monitoring, 2003; 25(3): 393-399.