Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

FORMULATION AND INVITRO EVALUATION OF NELFINAVIR NANOPARTICLES PREPARED BY NANOPRECIPITATION METHOD

M. Purushothaman*[1] and S. Siva Prasad[2]

*[1]Principal, Scient Institute of Pharmacy, Hyderabad, Telangana, India.
[2]Associate Professor, Vasavi Institute of Pharmaceutical Sciences, Kadapa, Andhra Pradesh, India.

ABSTRACT

Nanotechnology refers to the creation and utilization of materials whose constituents exist at the nanoscale; and, by convention, be up to 100 nm in size. Nanotechnology explores electrical, optical, and magnetic activity as well as structural behavior at the molecular and submolecular level. It has the potential to revolutionize a series of medical and biotechnology tools and procedures so that they are portable, cheaper, safer, and easier to administer. Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labelling and treatment of some cancers. due to their exceptional properties including antibacterial activity, high resistance to oxidation and high thermal conductivity, nanoparticles have attracted considerable attention in recent years. Nanoparticles can be synthesized chemically or biologically. Metallic nanoparticles that have immense applications in industries are of different types, namely, Gold, Silver, Alloy, magnetic etc. This study aims to present an overview of nanoparticles, with special reference to their mechanism of biosynthesis and type.

Key words: Nanoparticles, Bactericidal, Thermal Conductivity.

INTRODUCTION

There are various reasons why using nanoparticles for therapeutic and diagnostic agents, as well as advancement of drug delivery, is important and much needed. One of them is that, traditional drugs available now for oral or injectable administration are not always manufactured as the optimal formulation for each product. Products containing proteins or nucleic acids require a more innovative type of carrier system to enhance their efficacy and protect them from unwanted degradation [1]. It is notable that the efficiency of most drug delivery systems is directly related to particle size (excluding intravenous and solution). Due to their small size and large surface area, drug nanoparticles show increase solubility and thus enhanced bioavailability, additional ability to cross the blood brain barrier (BBB), enter the pulmonary system and be absorbed through the tight junctions of endothelial cells of the skin [2]. Specifically, nanoparticles made from natural and synthetic polymers (biodegradable and non-biodegradable) have received more attention because they can be customized for targeted delivery of drugs, improve bioavailability, and provide a controlled release of medication from a single dose; through adaptation the system can prevent endogenous enzymes from degrading the drug [3]. Secondly, the development of new drug delivery systems is providing another advantage for pharmaceutical sales to branch out. Innovative drug delivery is driving the pharmaceutical companies to develop new formulations of existing drugs. While these new formulations will be beneficial to the patients, it will also create a powerful market force, driving the development of even more effective delivery methods [4].

Furthermore, not only will the companies thrive to develop new formulations for their own intellectual property, but will have motivation due to patent expirations [5]. The benefit of pharmaceutical companies taking advantage of this new technology is that nanotechnology gives new life to those drugs those were previously

Corresponding Author :- **M.Purushothaman** Email:- sagiliprasad2003@gmail.com

Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

considered unmarketable due to low solubility and bioavailability, and high toxicity and marked side effects [6]. Finally, we would like to highlight a very recent article from Prof. Robert Langer's group, at the Massachusetts Institute of Technology [7], with an up-to-date survey of the types of polymeric systems used in the drug delivery.

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. The main aim of this study is to achieve prolonged release of Nelfinavir such that the dosing frequency of the drug can be reduced by which we may reduce the side effects and increase the patient compliance. By formulating Nelfinavir as nanoparticles we can directly deliver the drug to the cancer cell and prevent the normal cells from the adverse effects of Nelfinavir. **Nelfinavir** A potent HIV-1 protease inhibitor. It is used in combination with other antiviral drugs in the treatment of HIV in both adults and children.Nelfinavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Nelfinavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs.

Nelfinavir is an inhibitor of the HIV-1 protease. Inhibition of the viral protease prevents cleavage of the gag and gag-pol polyprotein resulting in the production of immature, non-infectious virus.

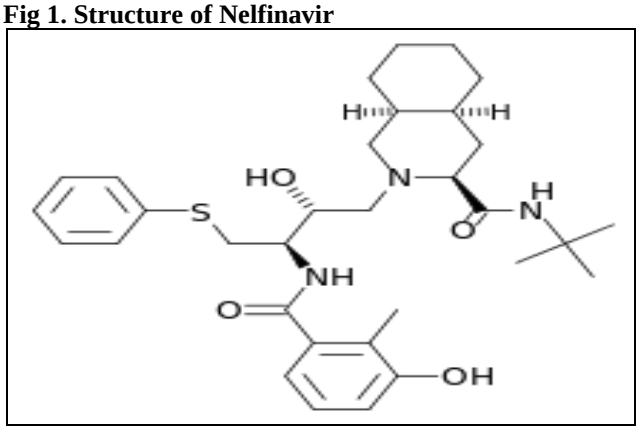
Antiviral Activity in Cell Culture:The antiviral activity of nelfinavir has been demonstrated in both acute and/or chronic HIV infections in lymphoblastoid cell lines, peripheral blood lymphocytes, and monocytes/ macrophages. Nelfinavir was found to be active against several laboratory strains and clinical isolates of HIV-1, and the HIV-2 strain ROD. The EC_{95} (95% effective concentration) of nelfinavir ranged from 7 to 196 nM. Drug combination studies with other HIV-1 protease inhibitors showed nelfinavir had antagonistic interactions with indinavir, additive interactions with ritonavir or saquinavir, and synergistic interactions with amprenavir and lopinavir. Minimal to no cellular cytotoxicity was observed with any of these protease inhibitors alone or in combination with nelfinavir. In combination with reverse transcriptase inhibitors, nelfinavir demonstrated additive (didanosine or stavudine) to synergistic (abacavir, delavirdine, efavirenz, emtricitabine, lamivudine, nevirapine, tenofovir, zalcitabine, or zidovudine) antiviral activity without enhanced cytotoxicity. Nelfinavir's anti-HIV activity was not antagonized by the anti-HCV drug ribavirin.

Review of Literature

K. Vanitha Prakash A reverse phase HPLC method is described for the determination of Nelfinavir Mesylate in tablet dosage form [8]

D. Gowrisankar A simple, rapid and reproducible high performance reversed phase liquid chromatographic method has been developed for the estimation of nelfinavir in bulk drug sample and pharmaceutical dosage forms [9]

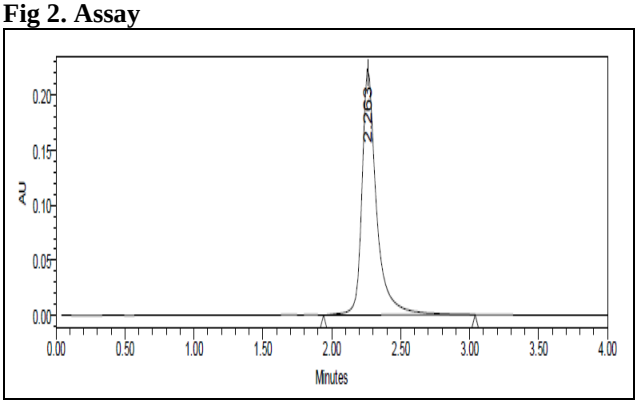
Fig 1. Structure of Nelfinavir



Optimized Chromatographic conditions

Column : Symmetry C18 (4.6 X 150 mm ;5µm Waters).
Column temperature : 250C
Flow rate : 1 ml/min.
Injection volume : 20 µl.
Wavelength : 238 nm.
Run time : 10 min.
Diluent : mobile phase.
Mobile phase composition : methanol: water (60:40%v/v).
Injector : Rheodyne.
Stationary phase : C18 (4.6 X 150 mm; 5µm Waters)
Operating temperature : Room temperature.

Fig 2. Assay



Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

Accuracy

Table 1. Accuracy

Solvent Name	Retention Time	Avg. Area	Resolution	Tailing Factor	Theoretical Plates
Nelfinavir	2.262	789546	0	1.5	2804

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	823686.2	5.0	5.0	100.1%	99.5 %
100%	1634793	10	9.93	99.3%	
150%	2451939	15.0	14.9	99.3%	

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3353.0	1.5
2	1	2804.8	1.5
3	1.2	2384.0	1.4

Fig 3. Linearity

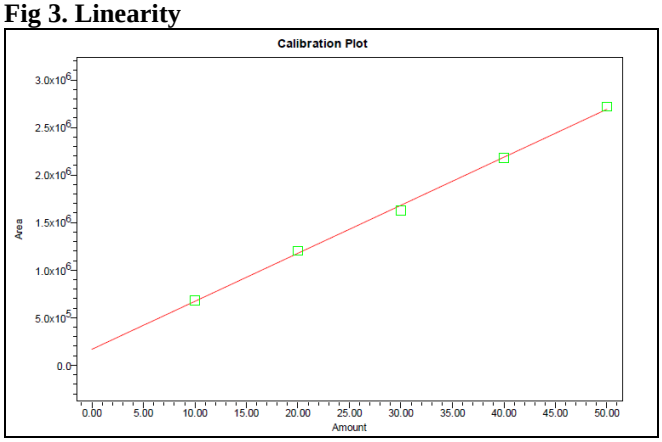
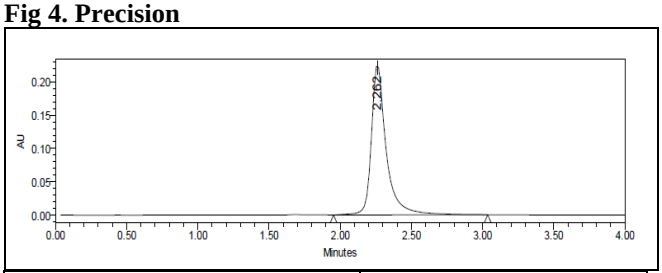


Fig 4. Precision



Injection	Area
Injection-1	1631295
Injection-2	1630511
Injection-3	1636464
Injection-4	1628557
Injection-5	1635684
Average	1632502.2
Standard Deviation	3420.4
%RSD	0.2

ROBUSTNESS

Table 2. Organic Composition in the Mobile Phase

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2396.0	1.3
2	*Actual	2804.8	1.5
3	10% more	2218.0	1.4

METHODOLOGY

IR spectroscopic study of Nelfinavir

Compatibility study (IR spectroscopy)

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

METHOD OF PREPARATION OF NELFINAVIR LOADED NANOPARTICLES

Solvent dispersion (Nanoprecipitation)

The nanoparticles are prepared by dissolving the drug 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.

Note: In above all formulations (F1 to F8) 250mg of the drug was added. The above formulations were prepared and the entrapment efficiency was determined for choosing best formulation.

EVALUATION OF NELFINAVIR LOADED NANOPARTICLES

1. Particle size
2. Zeta potential
3. Entrapment efficiency
4. In vitro drug release

Particle Size: Particle size was determined by using MALVERN instrument.

Zeta Potential: Zeta potential was determined by using MALVERN instrument UK.

Lyophilization: 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.

Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

Drug Encapsulation Efficiency: 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 Nelfinavir in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Nelfinavir PLGA nanoparticles was expressed as loading capacity.

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

In-Vitro Nelfinavir Release

10 mg drug equivalent freeze-dried Nelfinavir 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.

Mathematical Modeling of The Drug Release

The mechanism of drug release from the formulations during the diffusion in pH 7.4 phosphate buffer was determined using the Zero order, First order, Higuchi equation and Korsmeyer-Peppas plot.

Stability studies

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 250C/60% RH analyzed every month for period of three months.
2. 300C/75% RH analyzed every month for period of three months.
3. 400C/75% RH analyzed every month for period of three months

RESULTS AND DISCUSSION

The present study was aimed to developing Nnano particles of Nelfinavir using various polymers. All the formulations were evaluated for physicochemical properties and invitro drug release studies.

Evaluation Parameters

Optimised formulations: Based on the entrapment efficiency, a set of formulations (F6, F7 and F8) were considered as optimized compositions which can be taken up further studies and evaluated for the diffusion studies.

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 18 hours. Initially the release of drug from all the three batches was found to be about 25-35% in 3 hours. This was due to the release of adsorbed drug from the surface of Nanoparticles. Later on a constant and slow drug release was observed for 18hrs.

The drug diffusion for F6, F7 and F8 formulations was found to be approximately same i.e., 97.63%, 95.22% and 92.90% respectively. Therefore the F6 formulation which had drug polymer ratio of 1:30 was decided to be the optimized formulation.

Diffusion study profile for F6, F7 and F8 formulations

The drug release from the Nanoparticles was found to follow first order release based on the "r" value obtained for first order (0.989)) for F8 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.991 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "n" value of 0.907 obtained for Peppa's equation.

FTIR studies

The IR spectra of the nelfinavir, polymer mixture were portrayed in above fig. The IR spectra of nelfinavir exhibited distinctive peaks at 2900 cm−1 due to NH stretching of the secondary amine, 1572.66 cm−1 owing to –C=O stretching of the carboxyl ion and at 745.35 cm−1 because of C-Cl stretching. The FTIR spectra of polymer mix displayed characteristic peaks at 2981.41 due to CH aliphatic stretching and at 1724.05 due to –C=O stretching. In the IR spectra of the polymer mix the peak due to the drug carboxyl group was shifted to 1577.49 cm−1 whereas the signal resulting from the polymer carboxyl appeared at 1734.66 cm−1 ruling out the possibility of any chemical interaction between the drug and polymers. A weak electrostatic interaction between the carboxyl group of the drug and the ammonium group of the polymers during co-dissolution and solvent evaporation could not be ruled out.

Stability Studies

There were no significant changes in physical and chemical properties of capsule of formulation F-6 after 2 months. Parameters quantified at various time intervals were shown

Table 3. Materials used

Sino	Materials	Manufacturer / Supplier
1.	Nelfinavir	Scion Pharma, Taiwan
2.	PLGA	Lactel, Durect corporation Birmingham Division
3.	TPGS	Eastman company, UK
4.	Acetone	SRL
5.	Dialysis membrane	Himedia

Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

Table 4. Equipments used

S. No.	Instrument	Manufacturer / Supplier
1.	Homogenizer	Kinematica AG(Poly tron PT2100)
2.	Rotary evaporator	Super fit
3.	Analytical balance	ShincoDeshi .,Ltd, Japan
4.	pH meter	Polmon, LP-139S
5.	Microscope	Olympus, CH20
6.	HPLC	Shimadzu
7.	Sonicator	Enertech electronic Pvt. Ltd
8.	Lyophilizer	Lyophilisation systems India PVT LTD
9.	Particle size analyzer	MALVERN
10.	Zeta potential analyzer	MALVERN
11.	Magnetic stirrer	Rimek

Table 5. Composition of the Nanoparticles

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
PLGA (50:50)(mg)	1300	1300	1300	2500	5000	7500	10000	12500
TPGS(%g/ml)	3	4	5	6	7	8	9	10
Nelfinavir (mg)	250	250	250	250	250	250	250	250
Acetone (ml)	30	30	30	30	30	30	30	30
Water (ml)	100	100	100	100	100	100	100	100

Table 6. Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size, Zeta Potential and Drug Loading

Batch No	Particle size (nm)	Zeta potential (mV)	DrugLoaded (mg)	EntrapmentEfficiency (%)
F1	152.3	-25.34	124.2	49.88
F2	164.21	-24.16	153.4	61.36
F3	174.25	-23.41	189.54	75.816
F4	253.1	-22.18	193.48	77.39
F5	100.3	-21.34	202.14	80.856
F6	124.8	-12.14	222.1	88.84
F7	121.5	-6.47	236.47	94.58
F8	152.4	-16.48	241.1	96.44

Table 7. Formulations used for in vitro diffusion study

Ingredients (mg)	F6	F7	F8
PLGA (50:50)	7500	10000	12500
TPGS%(g/ml)	8	9	10
Nelfinavir (mg)	250	250	250
Acetone (ml)	30	30	30
Water (ml)	100	100	100

Table 8. Results of stability studies of optimised formulation F6:

Formulation code	Parameters	Initial	1st Month	2nd Month	Limits as per specifications
F8	25˚C/60%RH % Release	97.62	96.85	96.14	Not less than 85%
F8	30˚C/75%RH % Release	97.54	97.14	96.91	Not less than 85%
F8	40˚C/75%RH % Release	96.92	96.12	95.17	Not less than 85%
F8	25˚C/60%RH Assay value	98.15	98.02	97.99	Not less than 90% Not more than 110%
F8	30˚C/75%RH Assay value	98.12	98.10	98.05	Not less than 90%

Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

					Not more than 110%
F8	40˚C/75%RH Assay value	97.95	96.54	96.34	Not less than 90% Not more than 110%

Fig 5. Diffusion study profile Cumulative % release Vs Time (hrs)

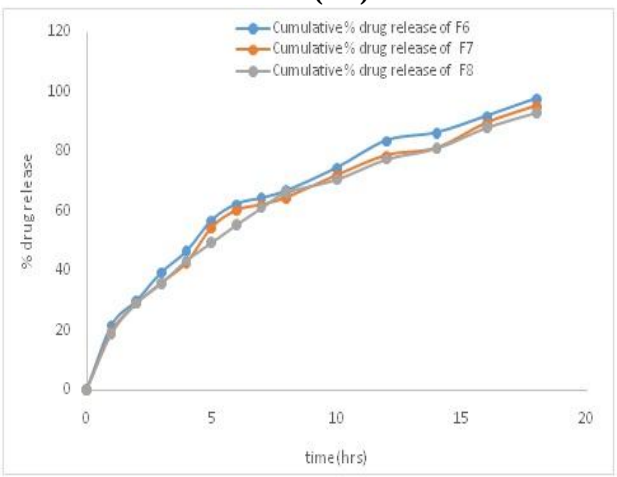
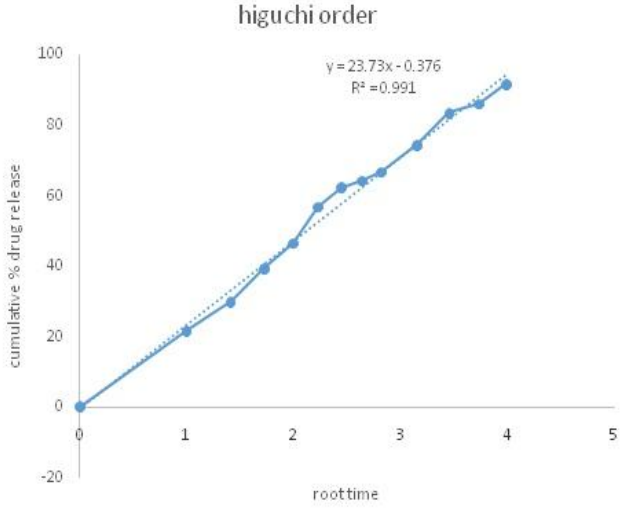


Fig 6. Drug Release Kinetics



DISCUSSION

Assay

The amount of Nelfinavir present in the taken dosage form was found to be 99.6 % respectively.

Accuracy

The percentage mean recovery of Nelfinavir is 99.5% respectively.

System Suitability

The % RSD for the retention times and peak area of Nelfinavir were found to be less than 2%.

Linearity and Range

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Nelfinavir is 0.999.

Precision

Test results for Nelfinavir are showing that the %RSD of Assay results are within limits.

Robustness

The system suitability parameters were within limit at all variable conditions.

Ruggedness

The %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

The validated method is found to be Specific,

Linear, Precise, Accurate, Robust and Rugged for the estimation of Nelfinavir in tablet dosage form. Hence it is concluded that the assay method is found to be valid in terms of reliability, precision, accuracy and specificity for routine analysis as well as for stability analysis

The present research proposed a novel formulation by applying as an emulsifier to fabricate Nanoparticles by solvent dispersion/nanoprecipitation for controlled release of Nelfinavir. Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimized. Our results demonstrated that vitamin E TPGS could be an efficient emulsifier for fabrication of polymeric nanoparticles, which can achieve excellent effects in drug encapsulation efficiency, size and size distribution and in vitro release kinetics of the nanoparticles. In this research, a drug encapsulation efficiency as high as 97% has been achieved. The particle size and size distribution strongly depends on the amount of TPGS added in the fabrication. Drug release kinetics indicated that drug release was best explained by peppas equation, as these plots showed the highest linearity (r^2=0.907) but a close relationship was also noted with first order kinetics (r^2 =0.989)

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No interest

Asian J. Pharm. Res. Vol 8, Issue 2, 32-38, 2018.

REFERENCES

1. Controlled Drug Delivery Fundamentals and Applications, 2nd Edition edited by Robonson JR, Lee VHL, New York, Dekker, 1987, 3.
2. Leon L, Herbert AL, Joseph LK. The Theory & Practice of Industrial Pharmacy, 3rd Edition, Varghese Publishing House, Bombay, 1991, 430-439.
3. Vyas SP and Dixit VK .Pharmaceutical Biotechnology, 1st Edition Edited by, Published by CBS Publishers & Distributors, New Delhi, 1998, 395.
4. Mohanraj VJ and Chen Y. Nanoparticles – A Review**.** *Tropical Journal of Pharmaceutical Research*, 5(1), 2006, 561-573.
5. Singh R, Lillard JW. Experimental and Molecular Pathology 86, 2009, 215–223.
6. Peltonen L, Aitta J, Hyvönen S, Karjalainen M, Hirvonen J. Improved Entrapment Efficiency of Hydrophilic Drug Substance During Nanoprecipitation of Poly(l)lactide Nanoparticles. *AAPS Pharm Sci Tech*, 5(1), 2004,16
7. Musumeci T, Ventura CA, Giannone I, Ruozi B, Montenegro L, Pignatello R and Puglisi G. PLA/PLGA nanoparticles for sustained release of Docetaxel. *International Journal of Pharmaceutics*, 325(1), 2006, 172-179.
8. www3.ntu.edu. UROP Report
9. Mu L and Feng SS. worked on A novel controlled release formulation for the anticancer drug Paclitaxel(taxol);PLGA nanoparticles containing Vit E TPGS. *Journal of controlled Release*, 86, 2003, 33-48.