International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

FORMULATION AND *INVITRO* EVALLUATION OF NANOSUSPENSION OF GLIMEPIRIDE

Ethiraj T*, Sujitha R, Ganesan V

Dept. of Pharmaceutics, The Erode College of Pharmacy, Perundurai Main Road, Vallipurathanpalayam (po), Veppampalayam, ERODE – 638112, Tamilnadu, INDIA.

***Corresponding author e-mail:** ethiraj1974@yahoo.co.in, sssujitharaavi@gmail.com

ABSTRACT

The present research work is an attempt to develop and evaluate Nanosuspension of Glimepiride in order to improve the solubility and bioavailability of poorly water soluble drugs. Nanosuspensions of Glimepiride were developed with different ratios of Urea and PVP combinations by nanoprecipitation technique. Nanoprecipitation method being simple and less sophisticated was optimized for the preparation of nanosuspension. The Fourier transform infrared (FTIR) spectroscopy was used to confirm compatibility and to rule out any possible interactions between drug and carriers. Four formulations (F1, F2, F3 and F4) consisting PVP and urea in the ratios of 1:3 and 1:6 respectively were prepared. All formulations were prepared using poloxamer as stabilizer, mixture of drug and acetone as organic phase and distilled water containing carriers as aqueous phase. . Physicochemical characteristics of nanosuspension in terms of size, zeta potential, entrapment efficiency (% EE) and *in vitro* drug release were found within their acceptable ranges. Differential scanning calorimetry studies provided evidence that enhancement in solubility of drug resulted due to change in crystallinity of drug within the formulation. Data of in vitro release from nanosuspensions were fit in to different equations and kinetic models to explain release kinetics.

Key words: Urea; PVP; In vitro; Glimepiride; nanoprecipitation technique

INTRODUCTION

Solubility [1] is the property of a solid, liquid, or gaseous chemical substance called *solute* to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent. More than 40% NCEs [3] (new chemical entities) developed in pharmaceutical industry are practically insoluble in water. These poorly water soluble drugs having slow drug absorption leads to inadequate and variable bioavailability and gastrointestinal mucosal toxicity. For orally administered drugs solubility is the most important one rate limiting parameter to achieve their desired concentration in systemic circulation for pharmacological response. Problem of solubility is a major challenge for formulation scientist. The poor solubility and low dissolution rate of poorly water soluble drugs in the aqueous gastrointestinal fluids often cause insufficient bioavailability. It is a common problem for those drugs belonging to the biopharmaceutical classification system (BCS) classes II and IV. As for BCS class II drugs rate limiting step is drug release from the dosage form and solubility in the gastric fluid and not the absorption, so increasing the solubility in turn increases the bioavailability for BCS class II drugs. For increasing solubility of poorly water soluble drugs various techniques are employed. Solubility improvement techniques can be categorized in to physical modification, chemical modifications of the drug substance, and other techniques. Physical Modifications[1] [3]**:** Particle size reduction like micronization and nanonisation, modification of the crystal habit like polymorphs, amorphous form and cocrystallization, drug dispersion in carriers like eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques. Chemical Modifications [1] [3]: Change of P^H, use of buffer, derivatization, complexation, and salt formation. Miscellaneous Methods[1][3]*:* Supercritical fluid process, use of adjuvant like surfactant, solubilizers, cosolvency, hydrotrophy, and novel

excipients. Other techniques like liposomes, emulsions, microemulsions, solid-dispersions and inclusion complexes using Cyclodextrins show reasonable success but they lack in universal applicability to all drugs. These techniques are not applicable to the drugs, which are not soluble in both aqueous and organic medias. Hence there is need of some different and simple approach to tackle the formulation problems to improve their efficacy and to optimize the therapy with respect to pharmacoeconomics. Nanotechnology can be used to resolve the problems associated with these conventional approaches for solubility and bioavailability enhancement.

NANOSUSPENSION [2]**:** A Nanosuspension is a submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid Biphasic, dispersed, solid drug particles in an aqeous vehicle, size below 1µm, without any matrix material, stabilized by surfactants and polymers, prepared by suitable methods for Drug Delivery applications, through various routes of administration like oral, topical, parenteral, ocular and pulmanary routes. A nanosuspension not only solves the problem of poor solubility and bioavailability but also alters the pharmacokinetics of drug and that improves drug safety and efficacy. . In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability.

PREPARATION OF NANOSUSPENSION [2] [16]

For the preparation of nanosuspensions, mostly two methods namely "Bottom up technology" and "Top down technology" are used. Bottom up technology is an assembling method to form nanoparticles like precipitation, microemulsion, melt emulsification method and top down technology involves the disintegration of larger particles into nanoparticles, examples of which are high-pressure homogenization and milling methods.

Materials: Glimepiride (Micro Labs, Banglore), Poly vinyl pyrrolidine (Shreeji chemicals, Mumbai),Urea (Shreeji chemicals, Mumbai), Poloxamer (S.D. Fine Chem. Ltd., Mumbai), Sodium Lauryl Sulphate(SLS) (S.D. Fine Chem. Ltd., Mumbai), Acetone (S.D. Fine Chem. Ltd., Mumbai), Tween 80 (S.D. Fine Chem. Ltd., Mumbai).

METHODOLOGY

Preformulation Studies: Before formulation of drug substances into a dosage form, it is essential that the drug and polymer should be chemically and physically characterized. Preformulation studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipient in the fabrication of a dosage form. The perform- ulation studies like determination of melting point, determination of λ_{max} of Glimepride were carried out.

Preparation Of Glimepiride Nanosuspension By Nanoprecipitation: The nanosuspension was obtained by the precipitation process. The drug Glimepiride was initially dissolved in 3ml of acetone. This is organic phase. The carriers Urea, PVP and surfactants SLS, Poloxamer and Tween 80 were added to 10 ml of distilled water (antisolvent). This is aqueous phase. The organic phase was slowly added drop wise with syringe into the aqueous phase which is kept at room temperature and stirred with a speed of 900-1000rpm for 1hr using Magnetic stirrers. After 1hr the solution is sonicated for 1hr to remove excess acetone.

EVALUATION OF GLIMIPRIDE NANOSUSPENSION

Fourier Transform Infra Red Spectroscopy (FTIR) [7] [8]**:** Compatibility study of Glimepride with the carriers Urea and PVP and mixture of urea and pvp used to formulate nanosuspension was determined by FTIR Spectroscopy using Perkin Elmer RX1. Spectral analysis of Glimepiride, Urea and PVP and combination was carried out to investigate the changes in chemical composition of the drug after combining it with excipients. The compatibility study on FTIR was carried by JASCO FT/IR 4100, MD, USA in the frequency range 4000-400cm^{-1}

Scanning Electron Microscopy (SEM) [7] [8]**:** Surface morphology of the specimen will be determined by using a scanning electron microscope (SEM), Model JSM 84 0A, JEOI, Japan. The samples are dried thoroughly in vaccum desicator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10 with plasma voltage about 20mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current about 80mA.The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and working distance WD=39mm.

Zeta potential measurement [7] [8]**:** Zeta potential of the suspension is measured by malveren zetasizer. The zeta sizer mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the center of the sample cell, and the scattered light at an angle of about 130 is detected. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential is calculated.

Thermal analysis by differential scanning calorimetry (DSC) [7] [8]**:** DSC scans of the prepared lyophilized powdered drug sample and pure drug samples were recorded using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 10°C/min under dry nitrogen flow (100 ml/min) between 50 and 300° C. Aluminium pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

Drug entrapment efficiency (DEE) [7]**:** The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5 ^{0}C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 363 nm using UV spectrophotometer against blank/control Nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated [53].

Saturation Solubility Studies[9]: Saturation solubility measurements were assayed through ultraviolet absorbance determination at 221 nm using shimadzu UV-Visible spectrophotometer. The saturation solubility studies were carried out for both the unprocessed pure drug and different batches of lyophilized nanosuspensions. 10 mg of unprocessed pure drug and nanosuspensions equivalent to 10 mg of Glimepride were weighed and separately introduced into 25 ml stoppered conical flask containing 10 ml distilled water. The flasks were sealed and placed in rotary shaker for 24 hrs at 37°C and equilibrated for 2 days. The samples were collected after the specified time interval, and it is filtered and analyzed. The diluted samples were analyzed using UV spectrophotometer at 221nm.

Dissolution study[9]**:** *Invitro* drug release studies were performed in USP apparatus-Type II using paddle method at rotation speed of 50 rpm. Dissolution was carried out in pH 1.2 buffer as a dissolution medium. The volume and temperature of the dissolution medium were 900 ml and 37.0 + 0.5oC. 5 ml of sample was withdrawn periodically (after 5minutes) and replaced with an equal volume of fresh P^H 1.2 buffer up to 60min. Samples were suitably diluted and filtered through a filter paper (0.22 µm, Whatman Inc., USA). The filtrate was then subject to the UV analysis against the blank (distilled water). Percent cumulative release was calculated based on the standard UV calibration curve at 221nm (Systronic 2203, Japan).

RESULTS

Preformulation study: Determination of melting point: Melting point of Glimepiride was found to be in the range of 206-207^{0}c as reported in the literature, thus indicating purity of sample. The melting point of the obtained drug was found to be 207^{0}C, hence the obtained drug was found to be pure without any impurities.

Solubility: Glimepiride is insoluble in water. Soluble in acetone, dichloromethane and methanol. Sparingly soluble in ethylacetate.

Drug - Excipient Compatibility Studies

Drug - Excipient compatibility is confirmed by FTIR Spectroscopy for which, FTIR spectra of Glimepiride alone compared with FTIR spectrum of best formulation. The spectrum of Glimepiride showed a characteristic peaks at 1706 cm^{-1} (C=O), 1671 cm-^{1} (C=O Carbonyl), 1347.32 cm^{-1} (N-O) Nitro), 615.55 cm^{-1} C-Cl (Alkyl halide) indicating purity of the drug. The characteristic peaks of Glimepiride were prominently observed in FTIR spectra of best formulation with slight shift in their positions.

Calibration Of Curve Of Lornoxicam

The absorbances of the resultant solution was recorded at 221nm and values are reported in table no.3.

Particle Size Analysis: The particle size distribution studies showed that the optimised formulation particle size was in the range 446 nm and where as unprocessed drug shows 50µm sizes. All the formulations having a particle size in the nanometre range and showing ideal surface morphology.

Saturation Solubility Studies: The saturation solubility studies indicating that nanosuspensions

showing maximum solubility compared to unprocessed drug which is due to the crystalline nature of puredrug. Results of solubility and drug entrapment efficiency of Formulations were tabulated in table no:9.

Differential scanning colorimetry: The physical state of raw Glimepiride and lyophilized drug nanoparticles was examined by DSC. The DSC of Glimepiride shows an endothermic curve at its melting point 208.9°C (ΔH = -15.80 J/g) and the precipitated formulation shows an endothermic peak at 224.0°C (ΔH =6.50 J/g). This observation can be explained by the presence of poloxamer on the surface of nanoparticles, as it exhibits a melting peak at 224.0°C.

Shape and surface morphology: Nanoparticle surface morphology and shape were visualized using SEM(JSM-T330A, JEOL). SEM surface studies showed elongated nanoparticles with porous surface. The size of pure drug particles Glimepiride was found to be 50µm.The size of optimised formulation(F12) was found to be 446nm.

ZETA POTENTIAL STUDY: Zeta potential is a term related to the stability of samples for molecules and particles that are small enough, high zeta potential will confer stability i.e.it resist aggregation .Here zeta potential of the prepared nanosuspension was found to be -34.4.

INVITRO DRUG RELEASE STUDIES

The results were recorded in table no.4.

KINETIC MODELING OF DRUG RELEASE

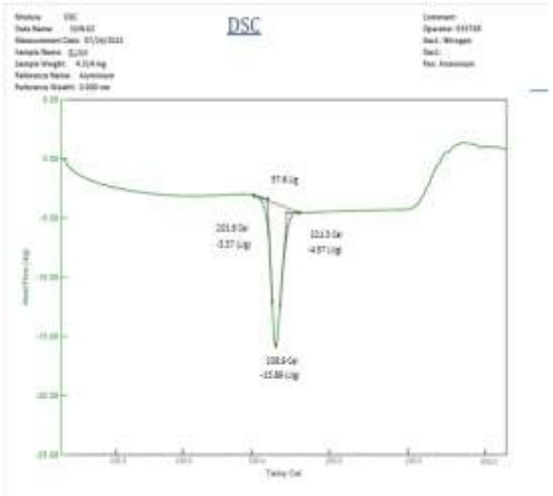
The data from the in vitro study was fitted to various kinetic models to determine the kinetics of drug release. The main models are Zero order, First order, Higuchi and Korsmeyer to understand the drug release from the nanosuspension. The coefficients of regression and release constant values were computed. However drug release was found to be very close to first order kinetics(Table no:5).The R^2 values were found in the range of 0.804-0.996.The corresponding plot(cumulative percent drug release vs time) for first order equation indicated good linearity. The plot of Higuchi's model was found to be linear. The R2 values were found in the range of 0.802-0.995.The prepared formulation follows Higuchi model.

CONCLUSION

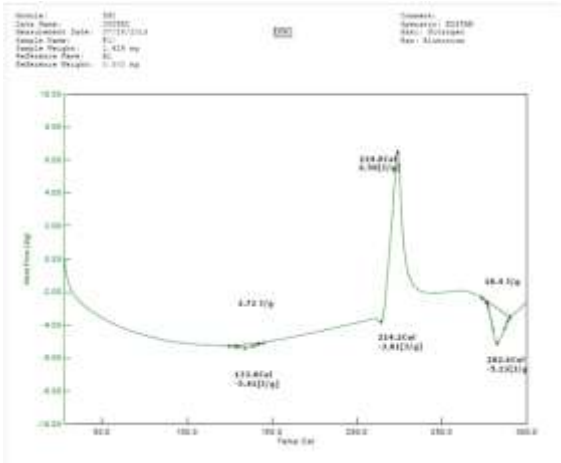
A Nanosuspension is a submicron colloidal dispersion of drug particles. The nanosuspension delivery has gained importance in recent years. The nanosuspension delivery system has potential advantages like improved bioavailability and patient compliance over other drug delivery systems. In the present study nanosuspension of Glimepiride was prepared using urea and PVP as carriers and SLS and poloxamer as stabilizers. The evaluation results confirms that prepared formulation exhibited satisfactory results. Release study of Glimepiride nanosuspension indicated that the drug release from the formulation varies with the different compositions of carriers and stabilizers. Among all the prepared formulations, formulation containing urea as carrier and poloxamer as stabilizer showed better drug release of 99.63% after 20min. By reviewing the results obtained, on the basis of the *in vitro* characterization it was concluded that Glimepiride can be formulated as nanosuspension in our laboratory. Further the therapeutic utility of this system to be estabilished by pharmacokinetics and pharmacodynamic studies on human beings. Thus Nanoprecipitation technique can be employed to produce nanosuspension of Glimepiride, a poorly water-soluble drug, for the improvement of solubility and dissolution rate. In this process, the particle size of Glimepiride can be obtained in the micron and nano-size ranges, by adjusting the operation parameters, such as the stabilizer concentration and the organic to aqueous solvent ratio. The best nanosuspension of Glimepride can be obtained by using Glimepiride 10mg, Urea 30mg as carrier and poloxamer 5mg as stabilizer using Nanoprecipitation technique at laboratory scale. The dissolution of nanosized Glimepiride is significantly enhanced compare with the pure Glimepiride. Nanoprecipitation technique can thus be a simple and effective approach to produce nanoparticles particles of poorly water-soluble drugs. Formulation F_3 holds promise for further *in-vivo* studies.

ACKNOWLEDGEMENTS

I express my gratitude to Management Erode College Of Pharmacy And Research Institute, Erode, for their support and encouragement. I was also thankful to Spectrum Pharma Research Solutions, Hyderabad for providing their help in fullfillment of this work.



Graph No 1: Thermograph Of Glimepiride



Graph No 2:Thermograph Of Best Formulation(F12)

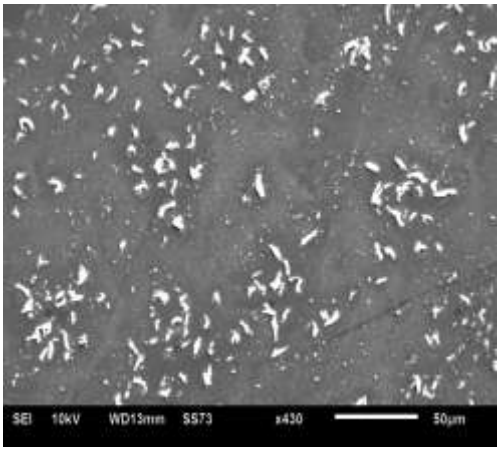


Fig No 2: SEM report of Pure Glimepiride

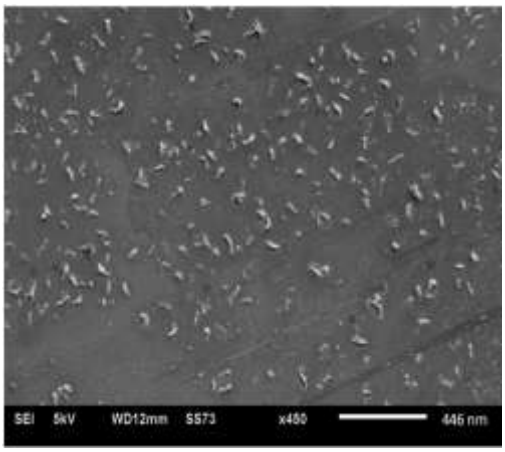
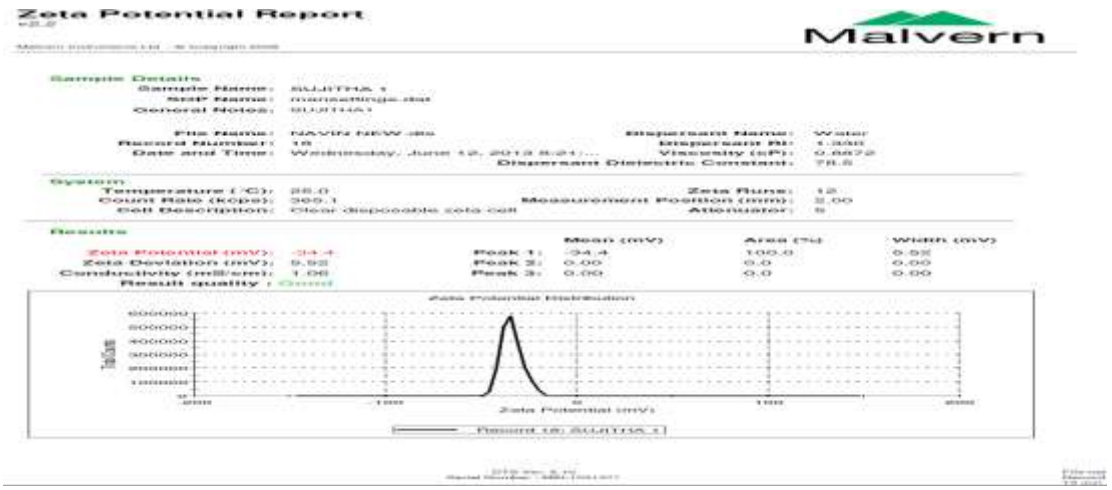


Fig No 3: SEM report Of F_3



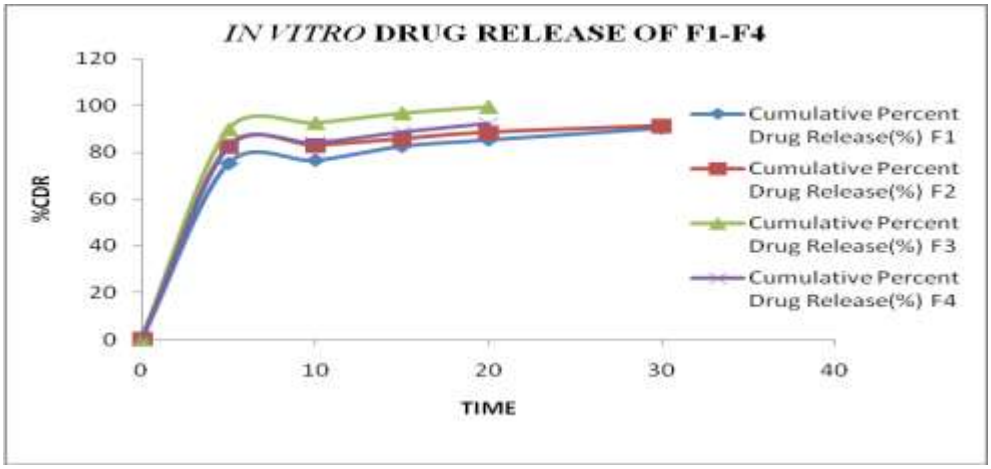


Table 1: List of ingredients taken for formulation

Ingredients	F1	F2	F3	F4
GLIMEPRIDE (mg)	10	10	10	10
PVPK30 (mg)	30	30	_	_
UREA (mg)	_	_	30	30
SLS (mg)	_	_	_	_
TWEEN 80 (mg)	_	_	_	_
POLOXAMER (mg)	5	10	5	10
ACETONE (ml)	3	3	3	3
WATER (ml)	10	10	10	10

Table no: 2 Standard Calibration Curve Of Glimepiride

No.	Conc [µg/ml]	Abs (221.00nm)
1	0.0000	0.000
2	1.0000	0.063
3	2.0000	0.095
4	3.0000	0.145
5	4.0000	0.222
6	5.0000	0.307
7	6.0000	0.341

Table no 3: Results of solubility and drug entrapment efficiency of Formulations

Sample	Solubility (µg/ml)	Drug entrapment(%)
F1	440	82
F2	346	82
F3	453	92
F4	423	84

Table no:4 Comparative Cumulative % Drug Release Of Formulations F1-F_4

Time (min)	Cumulative Percent Drug Release (%)			
	F1	F2	F3	F4
0	0	0	0	0
5	75.18	82.56	89.94	82.10
10	76.57	83.02	92.71	83.95
15	82.56	85.79	96.86	88.56
20	85.33	88.56	99.63	92.25
30	90.40	91.33		

Table No. 5: Results of Model fitting for Glimepiride Nanosuspension

Formulation Code	Zero Order	First Order	Higuchi/ Matrix	Peppas
F1	0.654	0.911	0.950	0.891
F2	0.536	0.938	0.967	0.880
F3	0.707	0.965	0.996	0.898
F4	0.532	0.950	0.978	0.882

REFERENCES

1. Ketan T. Savjani, Anuradha K. Gajjar, and Jignasa K. Savjani .2012; 2:1-10.
2.Vishal R. Patel and Y. K. Agrawal. Adv Pharm Technol Res. 2011 Apr-Jun; 2(2): 81–87.
3. Deepshikha Sikarra, Vaibhav Shukla, Ankit Anand Kharia, Chatterjee D. P. Journal of Medical Pharmaceutical and Allied Sciences 2012; 01:1-22
4. Ch.Prabhakar .International Journal of Pharma and Bio Sciences 2011 ; Vol 2/Issue1 Jan-Mar; 546-558.
5. V. B. Patravale, Abhijit A. Date and R. M. Kulkarni.JPP 2004; 56: 827–840.
6. Elaine, M; Liversidge, Gary G and Liversidge, M.Toxicolo0067ic Pathology; 2008:36, 43-48.
7. Sarita Kumari Yadav, Shivani Mishra, and Brahmeshwar Mishra. AAPS PharmSciTech. 2012; 13(4): 1031–1044.

8. R. N. Patel, Umashankar M.S, Mamatha M.C, S. N. Patel, Madhushri M, Mahalingan. K .Am. J. PharmTech 2012; 2(3): 1-19.
9. N.Arunkumar, M.Deecaraman, C.Rani, K.P.Mohanraj, K.Venkates Kumar. International Journal of PharmTech Research CODEN (USA): IJPRIF 2009; Vol.1; 1725-1730.
10. Jacobs C, Muller RH. Pharm Res. 2002; 19(2):189–194.
11.Dandan Liu, Heming Xu, Baocheng Tian, Kun Yuan,Hao Pan, Shilin Ma, Xinggang Yang and Weisan Pan. AAPS PharmSci Tech, March 2012 Vol. 13; No. 1:295-304.
12.Mahendra Nakarani , Priyal Patel, Jayvadan Patel, Pankaj Patel , Rayasa S. R. Murthy , Subhash S. Vaghani. Sci Pharm. 2010; 78: 345–36.
13. Sambit Mishra, Dibya Sundar Panda, Mahitosh Pradhan, Ishaat Hussain. 2011, 2(4):185-189.
14. Nakarani M., Misra A.K., Patel J.K., Vaghani S.S.DARU, 2010; Vol. 18: 84 -90.
15. Meetali Mudgil, Pravin K. Pawar Chitkara. SciPharm, 2013; 81:591–606.
16. Kalpesh S. Wagh, Satish K. Patil, Anup K. Akarte, Dheeraj T. Baviskar. International Journal of Pharmaceutical Sciences Review and Research, 2011; Volume 8:1-5.