AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Design and *In-Vitro* evaluation of human serum albumin loaded Paclitaxel nanosuspension

AmarnathReddy Ganji[1,*], Kiran K Jadhav[1], Sanjay P Umachigi[1], Pradeep Shivakumar[1], Palleti SainathReddy[1], Yedla Anilchowdary[2]

1. Gautham College of Pharmacy, Department of Pharmaceutics, Bangalore, India.

2. Lamar University, Department of Chemistry, Beaumont, Texas, U.S.A.

ABSTRACT

At current 40% of the drugs were poorly soluble and were also called as “brick dust”. And the drugs which were belonging to the BCS CLASS II & IV were most eligible for this Nanosuspension technology. Here by using different methods we are reducing the particle size so the surface area will be increases ultimately it leads to increase in the bio availability. This instance was observed mostly in the case of anti-cancer drugs. Paclitaxel with 25% of Human Serum Albumin (HSA) showed very good results in terms of drug content, particle size, zeta potential and %CDR.

Key words: Nanosuspension, Paclitaxel, Human Serum Albumin (HSA), High pressure homogenization.

*Corresponding Author Email: amarnathreddy.ganji@gmail.com

Received 7 February 2012, Accepted 21 February 2012

Please cite this article in press as: Ganji AR *et al.*, Design and *In-vitro* Evaluation of Human Serum Albumin Loaded Paclitaxel Nanosuspension. American Journal of PharmTech Research 2012.

INTRODUCTION:

Cancer is a major disease. About one in four people will get it in some form during their lifetime, and at the present time about one in five of all deaths are due to cancer. Currently there are three major ways of treating cancer. Those are

- ✓ Radiation therapy,
- ✓ Surgery and
- ✓ Cytotoxic drugs.

All of these have significant limitations, but drugs offer the only approach to treat cases where the cancer has spread (metastasised) through the body. Other less well established options include drugs that can stimulate the immune system to assist the body itself to fight the disease, and non-cytotoxic drugs that can prevent cancer cells frommultiplying.This focuses on the development of drugs to combat cancer. Over the last fifty years about 500000 natural and synthetic chemical compounds have been tested for anticancer activity, but only about 25 of these are in wide use today. This gives an indication of the difficulty of this problem. Currently drugs are available that significantly reduce the mortality rates for some cancers (e.g. leukemia and testicular and ovarian cancer), and give longer overall patient survival times. However, there is a long way to go before truly curative drugs are available for most cancers. The reason for this is simple: cancer cells are not foreign to the body but are simply subtly mutated forms of normal human cells, and it is very different to synthesize drugs that can tell the difference. The majority of drugs used for the treatment of cancer today are cytotoxic (cell-killing) drugs that work by interfering in some way with the operation of the cell's DNA. Cytotoxic drugs have the potential to be very harmful to the body unless they are very specific to cancer cells-something difficult to achieve because the modifications that change a healthy cell into a cancerous on ear every subtle. A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects .Initially the specificity of drugs was worked out simply by testing on animals, but no wits possible to use our knowledge of cancer cell biology to actively design drugs to be more specific. However, animal tests still need to be carried out at some point[1].

Nanosuspensions are colloidal dispersals of nanosized drug particles steadied by surfactants. They are also be defined as a biphasic system consisting of pure drug particles discrete in an aqueous vehicle in which the diameter of the adjourned particle is less than 1µm in size[2-4].

Poorly soluble substances were called as brick dust [5]. Drugs which are poorly soluble are not suitable for oral dosage forms. But 40% of the currently available drugs are belongs to this

poorly soluble category[6]. Drugs with high doses are not suitable for IV, IM injections because of large volumes at site. So by using suspension technology we can overcome this problem.

In nanotechnology the particle size was reduced from micron size to nano range. So that small particles having larger surface area and high dissolution velocity so ultimately high bioavailability will occur.Drugs which are insoluble in aqueous media or insoluble in both organic and aqueous media were eligible for this Nanosuspension approach. Drugs belong to the BCSCLASSII & IV is eligible for this approach[7-10].Paclitaxel belongs to the BCS class IV means it has poor solubility as well as poor permeability. So that it is eligible for Nanosuspension approach.

MATERIALS AND METHODS

Materials

Paclitxel and Human serum albumin was purchased from sigma Aldrich, Bangalore, India. Chloroform and ethanol was purchased from the S.D Fine Chem.

Method of preparation

Paclitaxel Nanosuspension was prepared by using ultra turrax high speed homogenization followed by high pressure homogenization. Firstly required quantity of chemicals was weighed accordingly. In the first step Paclitaxel was dissolved in required quantity of Chloroform and ethanol 3:1 ratio. The clear solution was added to Human Serum Albumin (HSA) under the ultra turrax high speed homogenizer by using de ionizer water with 4,000 rpm. In the next step the pre suspension was transferred to high pressure homogenization[11]. Thereby uniform Nanoparticles containing suspension will form. Later it was freeze dried by using organic solvents and stored for further use.

Evaluations

Particle size

For Detection of Particle Size Diameter Photon Correlation Spectroscopy (PCS) is used by this we can measure upto 3nm to 3 µm[12-16]. And the SEM images were mentioned in below Figure 1.

Particle charge / Zeta potential

By using zeta potentiometer we can detect particle charge/ zeta potential. This charge will give the physical stability of the prepared suspension. For an electrostatically stabilized Nanosuspension should have a minimum of ±30mV.For combines electrostatic and steric stabilization a minimum of ±20mV is required[17]. By using Zeta seizer the zeta potential was measured and the reports were mentioned below Figure 2.

Crystalline state and morphology

Crystalline state and particle morphology was detected by using DSC[18-20]. These DSC were performed and the graph was mentioned in the below Figure 3.

Drug content:

10mL of prepared nanosuspension was taken and it was diluted with Saline Phosphate buffer pH 7.4. and dilutions were made up to 10 µg/mL. And drug content was estimated spectrophotometrically by using UV-Visible spectrophotometer at 230nm. The drug content results were mentioned in the below Figure 4.

***In vitro* dissolution study**

This study was performed by using modified Franz diffusion cell. Firstly required amount of cellophane membrane was soaked in a Saline Phosphate Buffer pH 7.4 for 24 hrs. Then the soaked cellophane membrane was cut accordingly and it was tied to the diffusion cell and 10ml of Paclitaxel Nanosuspension was measured and poured into the above diffusion cell it acts like a donor compartment. Now in a 250 ml beaker 200 ml of Saline Phosphate Buffer pH 7.4 was taken and it was placed on a magnetic stirrer and now it acts like a donor compartment. Now the donor compartment was place just above to the receptor compartment and 200 rpm/min and 37±0.5 ºC was maintained throughout the experiment. 2ml sample was withdrawnat 1 to 24 hrs. And the same amount was replaced with fresh Saline Phosphate Buffer pH 7.4 every time. And the concentration was checked by using UV-Visible Spectrophotometer at 230nm[21-24]. And the values were tabled and figured in the below Table 1 and Figure 5.

FT-IR studies

The compatibility studies were performed and observed there was no interaction or any change in Paclitaxel FT-IR spectrum. And spectrum images was mentioned in the below Figure 6,7 & 8.

RESULTS AND DISCUSSION

The formulation which was prepared by using Paclitaxel with Human Serum Albumin by High Pressure Homogenization methods was shown very good results in terms of Particle size, Shape, Zetapotential, DSC and Invitro drug release studies. The Scanning Electron Microscopy shown in the below Figure 1. The zeta potential for the prepared nanosuspension was mentioned in the below Figure 2. The average particle size was observed 252±26.7 nm, the Zeta potential was observed -13.8mV. DSC graphs for the Pure drug and the Formulation was cited in the below Figure 3 There is no melting point change in the Paclitaxel after formulation it was observed by DSC study.

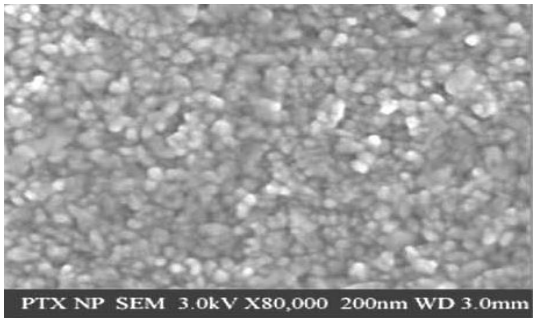

Figure 1: SEM Image of Human Serum Albumin Loaded Paclitaxel Nanosuspension.

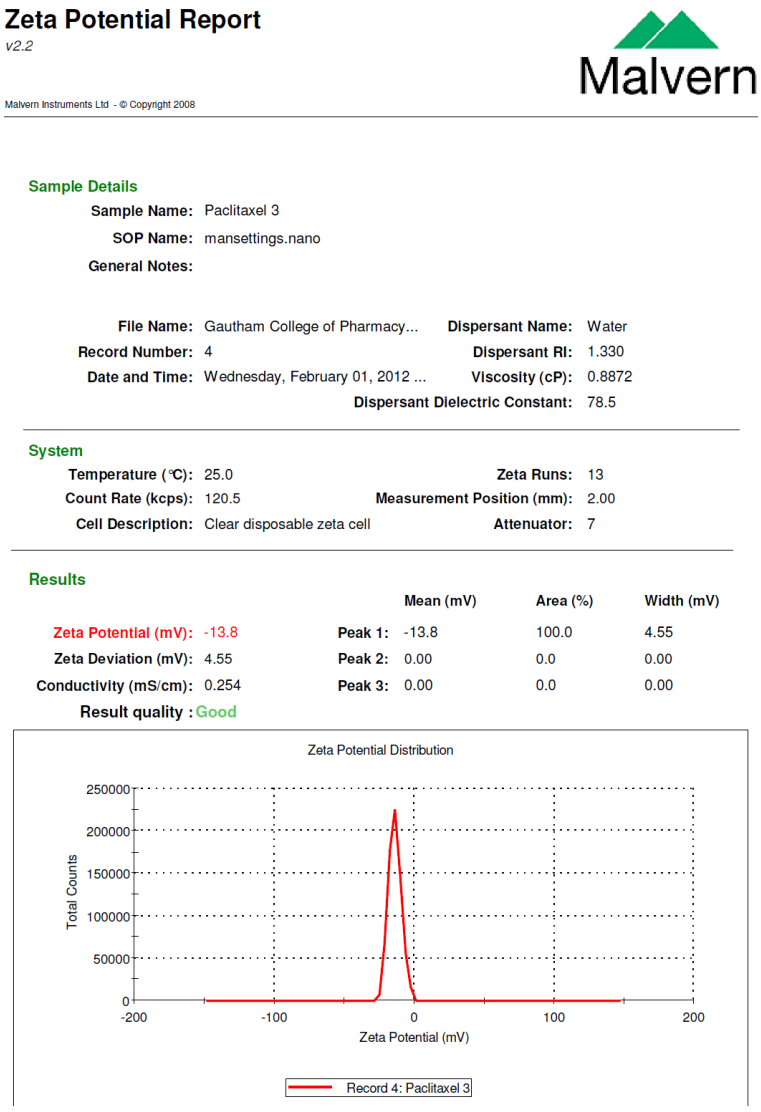

Figure 2: Zeta potential for the prepared Human Serum Albumin Loaded Paclitaxel Nanosuspension.

Drug content for the prepared nanosuspension was mentioned in the below Figure 4, The *Invitro* drug release profile for the prepared formulation up to 24 hrs was mentioned in the below Table 1 & Figure 5.

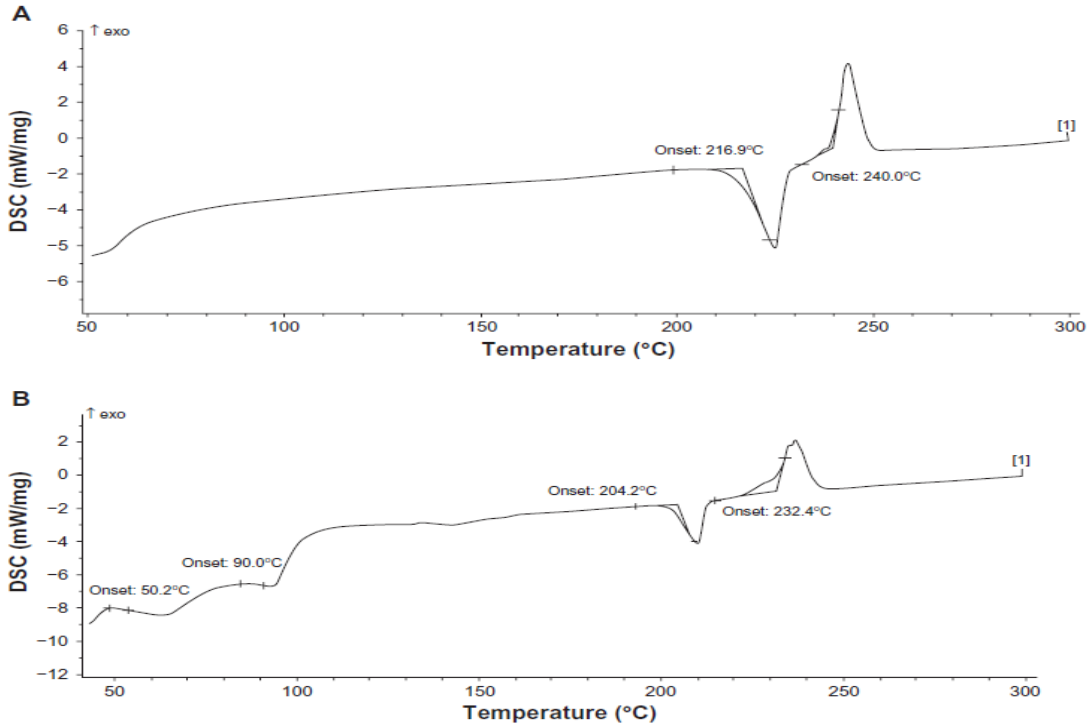

Figure 3: DSC graphs of A: Raw material of Paclitaxel, B: Human Serum Albumin Loaded Paclitaxel Nanosuspension.

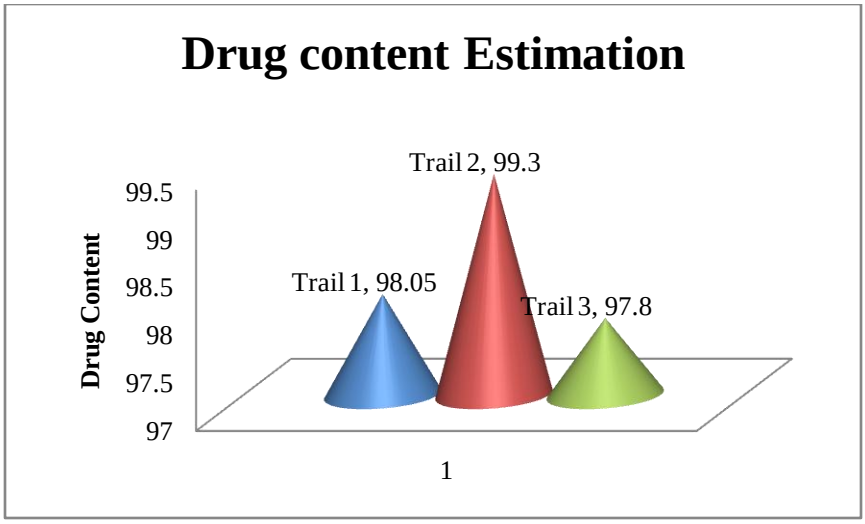

Figure 4: Dug content for the prepared Human Serum Albumin Loaded Paclitaxel Nanosuspension

The FTIR spectrums for the Paclitaxel, Human serum Albumin and the Physical mixture was mentioned in the below Figures 6, 7 and 8 respectively and there was no interaction between Paclitaxel and Human Serum Albumin was observed by FTIR data observations. The *In-vitro* drug release was observed 97.89% after 24 hrs. The drug content for the prepared Nanosuspension was found to be 98.3833±0.8036.

Table 1: *In-vitro* drug release study for the prepared Human Serum Albumin Loaded Paclitaxel Nano suspension

S.No	Time in hrs.	% CDR
1	0	0
2	0.3	0.124
3	1	0.198
4	2	0.599
5	3	0.813
6	4	1.44
7	5	1.988
8	6	2.442
9	7	3.065
1	8	3.495
11	9	4.448
12	10	9.479
13	11	14.71
14	12	20.68
15	13	22.54
16	14	26.36
17	15	34.69
18	16	42.88
19	17	46.37
20	18	52.20
21	19	58.53
22	20	66.09
23	21	79.02
24	22	84.72
25	23	93.02
26	24	97.89

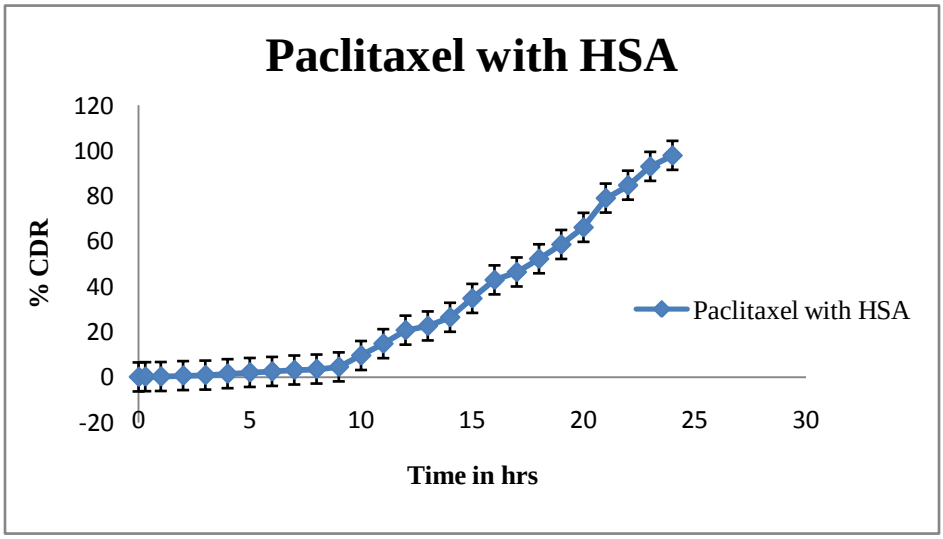

Figure 5: *In-vitro* drug release study for the prepared Human Serum Albumin Loaded Paclitaxel Nanosuspension

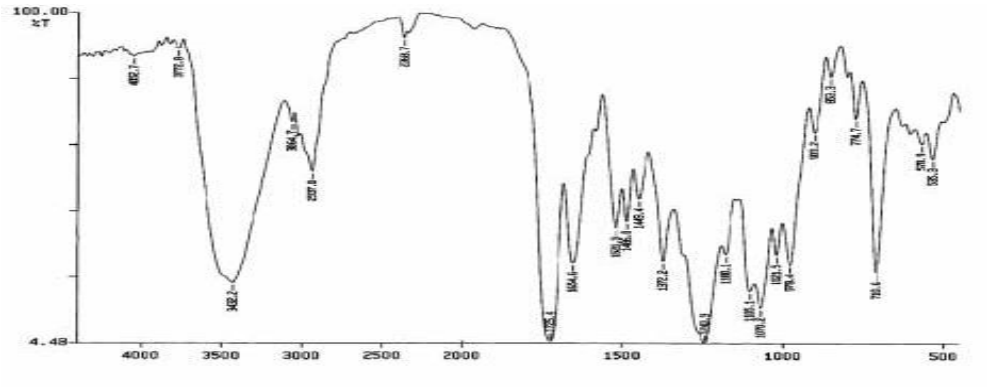

Figure 6: FT-IR spectra of Paclitaxel

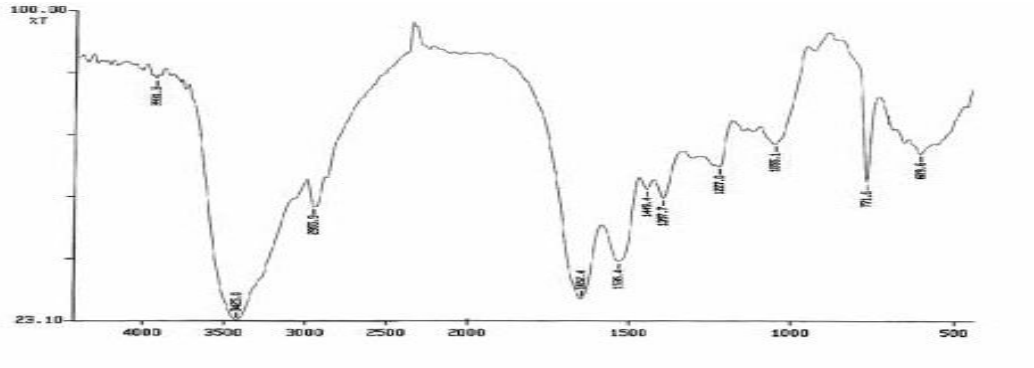

Figure 7: FT-IR Spectra of Human Serum Albumin

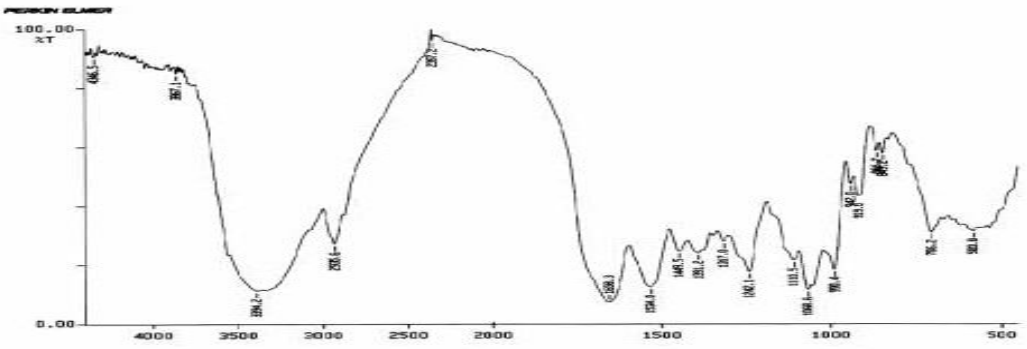

Figure 8: FT-IR Spectra of physical mixture of Paclitaxel with Human Serum Albumin

CONCLUSION

Paclitaxel with 25% of Human Serum Albumin (HSA) shown very good results and its circulation time also increased accordingly up to more than 24 hrs. So that there is a scope to do in-vivo studies.

REFERENCES

1. Bill Denny (Cancer Research Laboratory) and Heather Wansbrough and based on: Denny, William A.; New Directions in Cancer Chemotherapy; Chemistry in New Zealand 1995.

2. Barret E .Rabinow. Nanosuspensions in drug delivery. Nat Rev 2004 ;(3): 785-796.
3. Muller RH. Differential opsonization: A new approach for the targeting of colloidal drug carriers. Arch Pharm 1989; 322: 700.
4. Patravale VB, Abhijit A, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmcol 2004; 56: 827-840.
5. Suchika Sharma et al. Novel technologies for oral delivery of poorly soluble drugs: Res J Pharma Biological Che Sci 2010; 1(4): 292-295.
6. Lipinski, C. Poor aqueous solubility – an industry wide problem in drug discovery. Am Pharm Rev 2002; 5: 82–85.
7. Guidance for industry waiver of In-Vivo Bioavailability and Bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. CDER, Aug. 2000.
8. Kasim NA *et al.* Molecular Properties of WHO Drugs and provisional Biopharmaceutical Classification. Molecular Pharmaceutics.
9. Patel HB, Patel HL, Shah ZH, Modasiya MK. Review on Hydrogel Nanoparticles in Drug Delivery. Am J Pharm Tech Res2011; 1(3): 19-38.
10. Bhardwaj A, Veenu L. Colloidal Drug Delivery Systems: A Future Prospective For Treatment of Tuberculosis. Am J Pharm Tech Res 2011; 1(3): 102-123.
11. Yonglu Wang, Xueming Li, Liyao Wang, YuanlongXu, Xiaodan Cheng, Ping Wei. Formulation and pharmacokinetic evaluation of a paclitaxel nanosuspension for intravenous delivery. Int J Nanomedicine. 2011: 6, 1497-1507.
12. Muller BW, Muller RH. Particle size analysis of latex suspensions and microemulsions by Photon Correlation Spectroscopy. J Pharm Sci 1984; 73: 915-918.
13. Montasser H, Fessi AW. Coleman. Atomic force microscopy imaging of novel type of polymeric colloidal nanostructures. Eur J Pharm Biopharm 2002; 54: 281–284.
14. Muller RH, Jacobs C. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm. Res 2002b; 19: 189–194.
15. Acharya M, Patel M, Raval J. Nanoparticulate Drug Delivery System Using Drug Polymer and Aptamer Conjugation. Am J PharmTech Res 2011; 1(4): 88-107.
16. Rao KT, Praneeth VSRS, Suria PK, MuthuPrasanna P. Nano Science in Drug Delivery to Lungs. Am J PharmTech Res 2011; 1(4): 44-59.

17. Sunitha RR, Harika D, Phanikumar A, Suria PK, Muthu Prasanna P. A Review: Nanoparticles as specified carriers in Targeted brain drug delivery system. Am J PharmTech Res 2011; 1(2): 121-134.

18. Muller RH, Bohm BHL. Nanosuspensions. In: Muller, R. H., Benita, S., Bohm, B. H. L. (eds) Emulsions and nanosuspensions for the formulation of poorly soluble drugs. Med pharm Scientific Publishers, Stuttgart: 1998:149–174.

19. Muller RH, Grau MJ. Increase of dissolution velocity and solubility of poorly water soluble drugs as nanosuspension. Proceedings, World Meeting APGI/APV, Paris. 1998; 2:623–624.

20. Shanthakumar TR, Prakash S, Basavraj RM, Ramesh M, Kant R, Venkatesh P, Rao K, Singh S, Srinivas NR. Comparative pharmacokinetic data of DRF-4367 using nanosuspension and HP-_-CD formulation. Proceedings of the International Symposium on Advances in Technology and Business Potential of New Drug Delivery Systems, Mumbai. Vol. 5, B. V. Patel Educational Trust and B. V. Patel PERD Centre 2004:75.

21. Tamizhrasi S, Shukla A, Shivkumar T, RathiV ,Rathi JC. Formulation and evaluation of lamivudine Loaded polymethacrylic acid nanoparticles. Int J PharmTech Res 2009, 1(3): 411-415.

22. Muthu MS, Singh S. Poly (D, L-Lactide) Nanosuspensions of Risperidone for Parenteral Delivery: Formulation and In-Vitro Evaluation. Current Drug Delivery, 2009, 6, 62-68.

23. Dongmei Zhao, Xiuhua Zhao, YuangangZu, Jialei Li, Yu Zhang, Ru Jiang, Zhonghua Zhang. Preparation, characterization, and *in vitro* targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. Int J Nanomedicine 2010:5 669–677.

24. Tao Yang, Fu-De Cui, Min-Koo Choi, Jei-Won Cho, Suk-Jae Chung, Chang-Koo Shim, Dae-Duk Kim. Enhanced solubility and stability of PEGylated liposomal paclitaxel: *In vitro* and *in vivo* evaluation. Int J Pharmaceutics 2007; 338:L317–326.