An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

PREPARATION AND CHARACTERIZATION OF NEVIRAPINE CHITOSAN NANOPARTICLES

Nimmagadda Srinivas[1]*, Anusha Yedire[1], Surampalli Gurunath[2]

[1]Department of Pharmaceutics and Department of Pharmacology[2], Bharat Institute of Technology, Hyderabad, Telangana, India

*Corresponding author E-mail: nsap9042@gmail.com

ARTICLE INFO

Key Words

Nanoparticle, Chitosan, Nevirapine and solvent evaporation method, *In-Vitro* drug Release

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

The aim of the present study is to prepare and characterize nanoparticles containing Nevirapine using Chitosan as the polymer. The Nevirapine loaded nanoparticles were prepared by Solvent evaporation method. Nanoparticles were prepared and characterize for process yield, drug entrapment efficiency, particle size, in vitro drug release, kinetic studies and stability studies. The prepared nanoparticles were spherical in shape. The infra red spectra and scanning electron microscopy showed stable character of Nevirapine in the drug-loaded nanoparticles and revealed the absence of drug polymer interactions. The in vitro release behavior from all the drug loaded batches were found to follow first order and provided sustained release over a period of 8 h. No appreciable difference was observed in the extent of degradation of product during 90 days in which Nanoparticles were stored at various temperatures. The best-fit of release kinetics was achieved with first order Higuchi plot and the formulation F7 was found to be best formulation. The release of Nevirapine was influenced by the drug to polymer ratio and particle size and was found to be diffusion controlled. According to the data obtained, this Chitosan-based nanoparticles opens new and interesting perspectives as drug carriers for treating acquired immunodeficiency syndrome (AIDS).

INTRODUCTION

Pharmaceutical Nanotechnology deals with the formation and development of small structures like atoms, molecules or compounds of size 0.1 to 100 nm into structures which can be further developed into special devices with desired characteristics and properties[1]. 'Chitosan' the natural cationic polymer derived from chitin has received growing attention mainly due to their biocompatible, innocuous nature, mucoadhesive properties besides certain medicinal properties like antimicrobial and antioxidant properties. This enhances its potential in different biomedical applications [2-4]. Hence, chitosan based nanoparticles are for the first time explored for as drug delivery carrier for Nevirapine. Therefore, the main aim of this study is to achieve prolonged release of Nevirapine such that the dosing frequency can be reduced by which we may reduce the side effects and increase the patient compliance. It is used in the treatment of HIV infection [5]. Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site [6].

2. MATERIALS AND METHODS

2.1 Materials: Nevirapine was collected as a gift sample from Hetero Labs, Hyderabad, Synthetic polymers and other excipients were purchased from AR chemicals.

2.2 Methodology

Drug excipient compatibility studies [7]: Fourier transform infra red spectroscopy (FTIR) is utilized for evaluating the integrity and compatibility of the drug in the formulation. Pure drug and optimized formulations were analyzed by Fourier transform infra-red (FTIR) spectroscopy. FTIR spectra of pure drug and its formulations were obtained by a FT-IR Shimadzu 8400S (Japan) spectrophotometer using the KBr pellet method. The samples were scanned from 400 to 4,000 cm^{-1} wave number.

Preparation and Evaluation of Nevirapine Chitosan Nanoparticles

Preparation Procedure [8]: Nanoparticles formulations were prepared by solvent evaporation method. The formulation details of eight batches are shown in Table-1. The various amounts of polymer were dissolved in solvent mixture of methanol (2 ml) and dichloromethane (8 ml) very slowly on a magnetic stirrer and Nevirapine (100mg) was added to it and the contents were allowed to stand at room temperature for 30 to 45 minutes with occasional vortexing to allow complete solubilisation of drug and polymer. This solution was poured into 5 ml of each different concentration aqueous polyvinyl alcohol solution. The resulting solution was homogenized by using high pressure homogenizer for 3 minutes to form o/w emulsion. This emulsion was immediately added drop wise to 125 ml of aqueous PVA solution. The contents were stirred for 6 hours at room temperature with a magnetic stirrer to evaporate organic volatile solvent, allowing the formation of a turbid nano particulate suspension. The suspension, so prepared was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and supernatant was collected. Further the ultracentrifugation (35000 rpm for 1 hour) was carried for supernatants. Following ultracentrifugation, the pellet was washed and collected two times with deionized water to remove adsorbed drug and was suspended in deionized water to prevent clumping on storage.

Evaluation Parameters

Particle size [9]: All the prepared batches of nanoparticles were viewed under microscope to study their size. Size of nano-vesicles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles was determined.

SEM analysis [10]: The morphology of nanoparticles was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the nanoparticles was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.

Drug encapsulation efficiency [11]: Lyophilized nanoparticles weighing 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Nevirapine in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Nevirapine nanoparticles was expressed as loading capacity.

Entrapment Efficiency (%) = Amount entrapped /Total drug loadedX100

***In-vitro* drug release studies [12]:** The release studies were carried out by Franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped Nevirapine dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of

aliquots was withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium. Percentage of drug release was determined using the following fo rmula. % drug release = concentration × no.of dilutions × volume of dissolution fluid / 1000

Percentage of drug release = Da/Dt X 100

Where, Dt = Total amount of the drug in the patch, Da = The amount of drug released .

Release kinetics [13]: Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile. Mathematical models are used to evaluate the kinetics and mechanism of drug release from the tablets. The model that best fits the release data was selected based on the correlation coefficient(R) value in various models. The models that have shown high 'R' value were considered as the best fit on the release data. Various mathematical models are: Zero order release model, First order release model**,** Higuchi release model, Korsmeyer – Peppas release model**.** Zero Order Release Equation: The equation for zero order release is

Where , $Q_t = Q_o + K_o t$

Q_o = Initial amount of drug, Q_t = Cumulative amount of drug release at time "t", K_o= Zero order release constant, T= Time in hours

The zero-order kinetics describes the systems in which the drug release rate is independent of its concentration of the dissolved substance. A graph was plotted between the time taken on x-axis and the cumulative percentage of drug release on y-axis.

2. First Order Release Equation: The first order release equation is

$$\text{Log } Q_t = \text{Log } Q_o + K_t / 2.303$$

Where, Q_o = Initial amount of drug, Q_t = Cumulative amount of drug release at time "t", K= First order release constant, T= Time in hours. Here, the drug release rate depends on its concentration .The first order kinetics describes the systems in which the drug release rate is concentration dependent.

Higuchi Release Equation: The Higuchi release equation is

$$\mathbf{Q_t = K_H\sqrt{t}}$$

Where , Q = Cumulative amount of drug release at time "t", K_H = Higuchi constant, T = Time in hrs

Higuchi described the release of drug from an insoluble matrix as square root of time dependent process. The Higuchi square root model also gives the drug release from a planar surface of an insoluble heterogeneous matrix by diffusion through the intra granular openings created by porosity of the formulation. A graph is plotted between the square root of time taken on x-axis and the cumulative percentage of drug release on y-axis.

4. **Korsmeyer -Peppas Release Equation:**

Korsmeyer –Peppas equation is

Where, $\mathbf{F=M_t / M = K_m t^n}$

F = fraction of drug released at time't', M_t = amount of drug released at time 't' , M = total amount of drug in dosage form **,** K_m= kinetic constant, n = diffusion or release exponent, t = time in hrs, 'n' = Linear regression of log (M_t / M) versus log t

Stability studies: Selected Formulation (F7) was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^0C/60% RH analyzed every month for period of 3months.
2. 30^0C/75% RH analyzed every month for period of 3 months.
3. 40^0C/75% RH analyzed every month for period of 3 months.

3. RESULTS AND DISCUSSION

FT-IR Spectrum of Nevirapine: FT-IR Spectra of Nevirapine and F7 formulation were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between Nevirapine and polymer. It also confirmed that the stability of drug during process.

Table-:1 Formulation development of the Nanoparticles

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F6	F6
Chitosan	100	150	200	250	300	350	400	450
PVA	150ml	150ml	150ml	150ml	150ml	150ml	150ml	150ml
Methanol	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
Nevirapine (mg)	100	100	100	100	100	100	100	100

Fig-1: FTIR Studies of Nevirapine

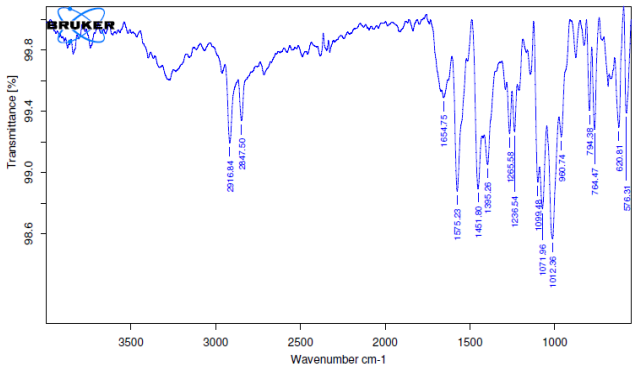

Fig-: 2 FT-IR Sample for Optimized Formulation

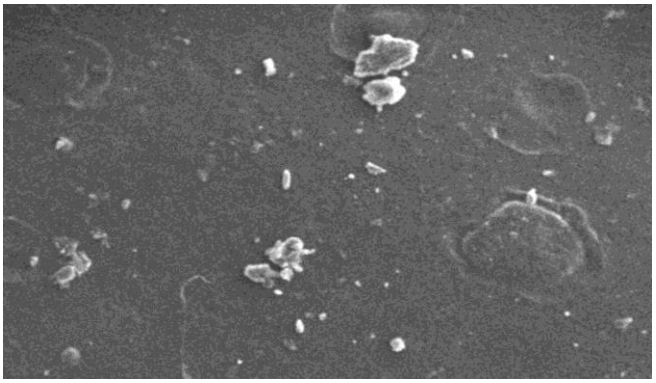

Fig-: SEM analysis of Optimized Nanoparticles

Table: Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	242	65.42
F2	246	69.11
F3	302	71.94
F4	338	72.83
F5	348	75.26
F6	328	76.24
F7	346	78.12
F8	356	81.26

Table-: Diffusion study profiles for all formulations

Time (hrs)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
0	0	0	0	0	0	0	0	0
1	20.12	23.55	22.24	26.55	26.55	23.24	29.55	26.55
2	31.55	33.60	35.43	35.60	31.60	36.43	38.50	35.60
3	44.57	40.55	46.12	44.55	42.55	47.12	48.12	44.55
4	53.85	52.55	51.65	52.55	55.55	50.65	55.65	52.55
5	62.59	65.88	65.58	63.58	65.58	63.51	65.55	63.58
6	72.54	72.10	73.03	70.88	72.90	75.20	77.20	70.88
7	81.42	83.24	82.85	85.15	83.52	83.85	83.85	85.15
8	93.54	91.62	92.19	93.20	91.20	92.55	95.59	93.20

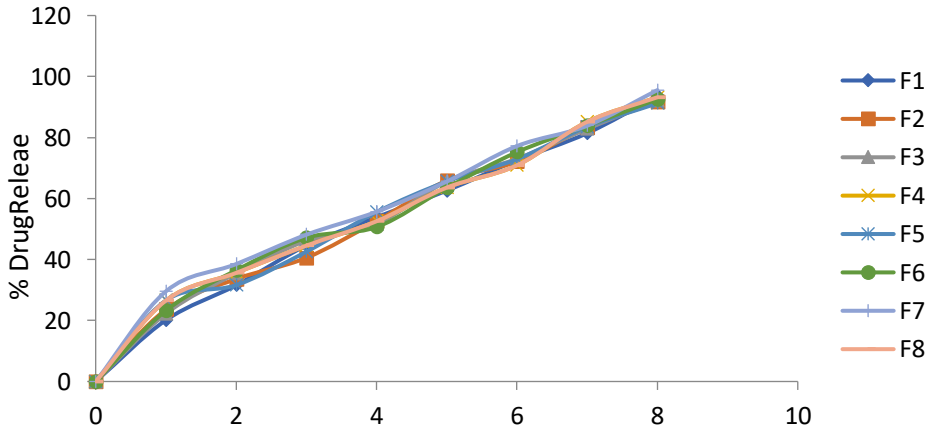

Fig-: *In vitro* drug release studies for all formulations

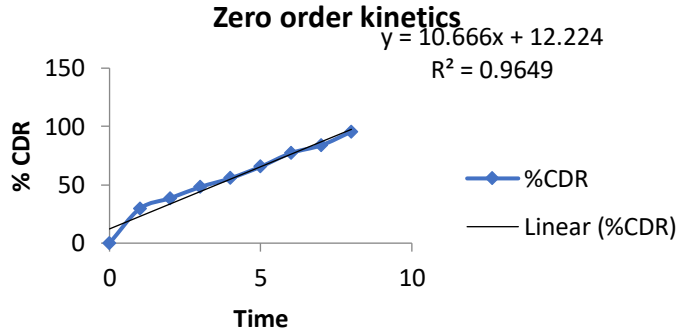

Fig-: Zero order kinetics

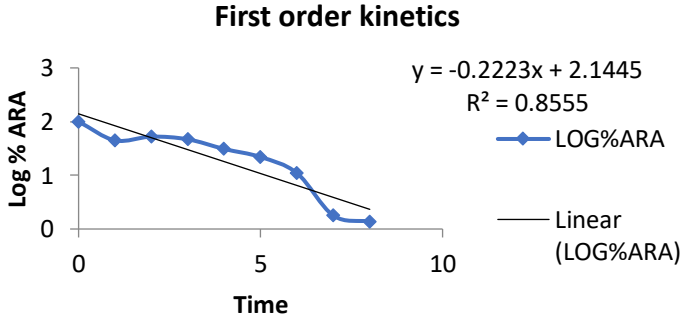

Fig-: First order kinetics

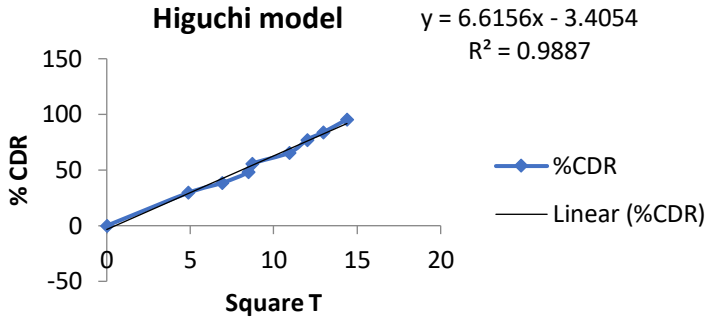

Fig-: Higuchi model

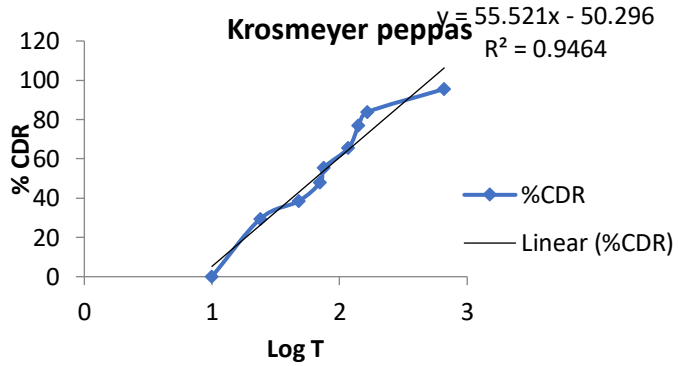

Fig-: krossmayer peppas

Table-: Regression equations of Nevirapine nanoparticles

F. no	*In vitro* release in phosphate buffer P^H 7.4 Regression values			
	Zero order	First order	Higuchi Plot	Kross mayer-peppas
F_7	0.964	0.855	0.988	0.946

Table- : Results of stability studies of optimized formulation F-7

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-7	250C/60%RH % Release	95.59	95.41	95.38	95.34	Not less than 85 %
F-7	300C/75% RH % Release	95.59	95.45	95.37	95.32	Not less than 85 %
F-7	400C/75% RH % Release	95.59	95.50	95.35	95.30	Not less than 85 %

Particle size: The particle size increased with increasing surfactant concentration although the increase was not significant. Entrapment efficiency decreased with increasing PVA concentration. Based on particle size distribution and entrapment efficiency, PVA concentration was selected for further studies.

Surface morphology: Scanning electron microscopy (SEM) SEM revealed that the MTX nanoparticles were smooth and spherical without any aggregation.

Drug entrapment efficiency: The first part of the plan of work was to optimize the concentration of polymers to be used in the formulation of nanoparticles. The optimization of polymer concentration was done on the basis of particle size and entrapment efficiency of nanoparticles obtained.

***In vitro* drug release studies:** Results indicate that the formulation showed initial burst release followed by sustained release of the drug for a prolonged period of time. The rapid initial release may be attributed to the fraction of Nevirapine on the surface of nanoparticles. The in vitro drug release results revealed that the prepared Nevirapine nanoparticles would be able to control drug release for extended period of time. The *in vitro* diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours. Initially the release of drug from all the three batches was found to be about 25-30% in 8 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 8hrs. F7 formulation decided to be the optimized formulation.

Drug release kinetics: All the five formulation of prepared Nevirapine nanoparticles were subjected to in vitro release studies these studies were carried out using Franz diffusion cell apparatus. The dissolution medium consisted of 10 ml of Standard buffer pH 7.4. The results obtaining in vitro release studies were plotted in different model of data treatment as follows: 1. Cumulative percent drug released vs. time (zero order rate kinetics), 2. Log cumulative percent drug retained vs. time (First Order rate Kinetics), Cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation) 4. Log of cumulative % release Vs log time (Peppas Exponential Equation). The values of *in vitro* release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Peppas were respectively. Regression values are higher with Zero order release kinetics. Therefore, the drug release from the Nevirapine nanoparticles followed zero order kinetics. The table indicates that r^2 values are higher for Higuchi's model compared for all the formulation. Hence Nevirapine release from all the nanoparticles followed diffusion rate controlled mechanism

Stability studies: There was no significant change in physical and chemical properties of the tablets of formulation F-7 after 3 months. Parameters quantified at various time intervals were shown.

4. CONCLUSION

In present study nanoparticles of Nevirapine were prepared in eight different drug to polymer ratio using solvent evaporation method. In present study Chitosan polymer is used to achieve the controlled delivery of drug from polymer matrix. The particle size of various formulations varied due to variation in the composition of formulations. The shape of the nanoparticles was found to be spherical by SEM study. Formulations with high polymer content were observed to be fairly spherical and sphericity was decreasing with increasing drug content. Small pores and cavities were present on the surface of nanoparticles. In the FT-IR study all characteristic peaks due to pure Nevirapine were appeared in nanoparticle spectra, without any markable change in their position after successful solvent evaporation method, indicated no chemical interaction and stability of drug during solvent evaporation process. FT-IR was done for pure drug and with different formulation for the identification of drug. It indicates no chemical interaction occurred between drug and polymer and also determined the stability of drug during formulation process. Release of Nevirapine was evaluated in phosphate buffer pH 7.4. Drug release from formulation with high drug content was fast in the medium, drug released within 7 to 8 hours of study. The *in vitro*

release data was applied to various kinetic models like zero order kinetics, Higuchi's plot and Peppa's plot to predict the drug release kinetic mechanism. Results shows that, release best explained by the zero order kinetics. In stability study there was no remarkable change in the drug content of nanoparticles formulation (FN7) during 90 days in which they were stored at various temperatures.

5. REFERENCES

1. T. Musumeci, C.A. Ventura, I. Giannone, B. Ruozi, L. Montenegro, R. Pignatello and G. Puglisi. PLA/PLGA nanoparticles for sustained release of Docetaxel. International Journal of Pharmacy. 2006; 325(1-2): 172-179.
2. Saikia C, Gogoi P, Maji TK. Chitosan: A Promising Biopolymer in Drug Delivery Applications. Journal of Molecular Genetic Medicine. 2015; S4: 01-10.
3. L.Mu and S.S.Feng. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. Journal of controlled Release. 2003; 86(1): 33-48.
4. N.Jawahar, T.Eagappanath, Nagasamy V., Jubie.S, Samanta M.K, Preparation and characterization of PLGA-Nanoparticles containing Anti-hypertensive agents. International Journal of PharmTech Research. 2009; 1(2): 390-393.
5. Vihola H, Laukkanen A, Hirvonen J, Tenhu H. Binding and release of drugs into and from ther-mosensitive poly (N-vinylcaprolactam) nanoparticles. European Journal of Pharmaceutical Sciences. 2002; 16(1-2): 69-74.
6. Dustgani A, Vasheghani Farahani E, Imani M. Preparation of chitosan nanoparticles loaded by dexamethasone sodium phosphate. Iranian J Pharmaceutical Sciences. 2008; 4(2): 111-114.
7. Wilson B, Samanta MK, Santhi K, Sampath Kumar KP, Ramasamy M, Suresh B. Chitosan nanoparticles as a new delivery system for the anti-Alzheimer drug tacrine. Nanomedicine. 2010; 6(1): 144-52.
8. Singh S, Singh M, Gambhir IS. Nanotechnology for Alzheimer's disease detection. Digest Journal of Nanomaterials and Biostructures. 2008; 3(2): 75 – 79.
9. Calvo P, Remunan-Lopez C, Vila Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharmaceutical Research. 1997; 14: 1431-1436.
10. Donga Y, Wai Kiong N, Shen S, Kim S, Tan RBH. Preparation and characterization of spironolactone nanoparticles by antisolvent precipitation. International Journal of Pharmacy. 2009; 375(1-2): 84-88.
11. Papadimitriou S, Bikiaris D, Avgoustakis K, Karavas E, Georgarakis M. Chitosan nanoparticles loaded with dorzolamide and pramipexole. Carbohydrate Polymers. 2008; 73(1): 44-54.
12. Suphiya P., Ranjita M., K. S. Sanjeeb. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomedicine. 2012; 8(2): 147–166.
13. I. Tamai and A. Tsuji. Drug delivery through the blood brain barrier. Advanced Drug Delivery Review. 1996; 19: 401- 424.