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Effect of solvents on thedevelopment of biodegradable Polymeric nanoparticles of Nevirapine

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Abstract : The present research work was carried out with an objective of preparing Polycaprolactone nanoparticles for the brain targeted delivery of Nevirapine(an antiretroviral drug). The Nevirapine loaded Polycaprolactone nanoparticles were prepared by Emulsion solvent evaporation technique in three organic solvents (Ethyl acetate, Dichloromethane and Chloroform). In this aspect, the effect of organic solvents on the physicochemical characterisations such as Particle size, Zeta potential, Percentage drug entrapment efficiency (%EE), Percentage drug loading (%DL), Scanning electron microscopy(SEM) , Transmission electron microscopy(TEM)and *in vitro* release study of polymeric nanoparticles were carried out.On the basis of physicochemical characterisations, the appropriate solvent was chosen for preparing Polycaprolactone nanoparticles for Brain targeted delivery of Nevirapine. The percentage drug entrapment efficiency (%EE) was 39.4±0.48% for Ethyl acetate, 32.7±0.4%forDichloromethane and 19.8±0.16% for Chloroform. The SEM and TEM photomicrographs obtained showed smaller size below 50 nm in Ethyl acetate while between 50 nm to 100 nm in Dichloromethane and Chloroform. The result showed that the %EE, %DL and the Particle size distribution was directly proportional to the aqueous solubility of the organic solvents. Polycaprolactone nanoparticles prepared using Ethyl acetate showed smaller particle size (125.57±7.66) nm, having Polydispersity index (PDI) of 0.295±0.005 with an optimum zeta potential (-72.1mv) as compared with Dichloromethane and Chloroform.The nanoparticles prepared in Ethyl acetate showed the highest release of 84.8% than that of Dichloromethane and Chloroform. It can be concluded that the choice of solvents plays an important role in the physicochemical characterisation of Polymeric nanoparticles.

Keywords : Nevirapine, Nanoparticles, Polycaprolactone, Ethyl acetate, Brain delivery, Neurocognitive disorder.

Introduction

The treatment of Neurocognitive disorders such as Neurocognitive impairments (NCIS) associated with HIV infectionis a concerned field of research due toinappropriate availability of treatmentcausing the death of millions of people in the world.The essential features of NCIS are confusion,difficulty in finding words, difficulty with fine motor skills, difficulty in coordination and concentration—for example, difficulty with a task like handwriting and needing to concentrate to write legibly, loss of short and long-term memory—for example, trouble remembering appointments and names, diminished capacity for planning, processing information and problem-solving, apathy and lack of motivation, personality changes, difficulty with multi-tasking. Initially, it remains asymptomatic but as the disease becomes progressive, it leads to AIDS dementia (in adult) and HIV encephalopathy (in children)[1]. Currently availableHighly Active Antiretroviral Therapy

(HAART)are the cornerstone in treating Neurocognitive disorders but treatment fails as they suffer from several limitations such as liver toxicity, requirement of lifelong treatment, patient incompliance causing development of viral resistance, chance of drug interactionsetc. Moreover, most of the antiretroviral drugs in HAART failto cross the Blood Brain Barrier (BBB) due to poor lipophilicity, high molecular weight. Ultimately, there is a poor bioavailability of these drugs in the brain[2]. Till now, several strategies are available, which include invasive strategies (Hyperosmolar BBB disruption, intracerebral implants) and Pharmacological based strategies (Prodrug, Nanoparticles,liposomes)[3].Pharmacological based strategy isknown to be a better option for brain targeted delivery as it could overcome the limitations associated with HAART therapy. So,based on this strategy, measurable remedies were taken by researchers for targeting Nevirapine to the brain by incorporating colloidal carriers such as Liposomes, Polymeric nanoparticles and Lipid nanoparticles. In this regard, Ramana et al, reported on utilising Liposomes (prepared from egg phospholipid) as colloidal carrier for Brain targeted delivery of Nevirapine[4]. While Kuo et al,reported on targetingNevirapine across the Human Brain Microvascular Endothelial Cells(HBMECs) incorporating Solid Lipid Nanoparticles(SLNs) and Nanostructured Lipid Carriers(NLCs) as colloidal carriers[5].Liposomes suffers from the drawback of unstability, easy phagocytosis by Reticuloendothelial Cells (RES),sterilisation problem as phospholipid are thermolabile,poor encapsulation etc[6] whilst Lipid nanoparticles have suffered from poor drug loading, drug expulsion after polymeric transition during storage,low capacity to load hydrophilic drugs,particle growth,unpredictable gelation tendency, relatively higher water content of the dispersion (70 – 99.9%)[7]. In recent years, considerable interests have been shown in utilising Polymeric nanoparticles as colloidal carrier. Polymeric nanoparticles are colloidal particles consisting of polymers, which are biocompatible, biodegradable, inert, nontoxic, small sized(<100 nm), small molecular weight, capable of incorporating both hydrophilic and hydrophobic drugs[8]. The several advantages of polymeric nanoparticles[9] over conventional treatment of HAART and other colloidal carriers (such as Lipid nanoparticles, Liposomes)have attracted lots of researchers to undergo research in Brain targeted delivery of drugs using Polymeric nanoparticles as carrier. In this connection, current research is being undertaken with an aim of targeting an anti-HIV drugNevirapine to the Brain using Polymeric nanoparticles as the carrier. Nevirapine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), is a highly permeable,low soluble drug[10].Currently it is one of the drug in HAART therapy which is administered orally in the form of capsules, tablets and suspensions.The orally administered Nevirapine belonging to Biopharmaceutical Classification System (BCS) class II (low solubility/high permeability)have poorbioavailability in brain due to its poor aqueous solubility,hepatotoxicity, patient incomplianceon frequent dosing etc[11]. These drawbacks associated with orally administered Nevirapine could be overcome by biodegradable Polymeric nanoparticles which are to be administered parenterally[12].Kuo et al reported on successful targetingof Nevirapine across Human Brain Microvascular Endothelial Cells usingPoly (lactide) co glycolide (PLGA) nanoparticles grafted with Transferrin (Tf)[12]. PLGA is one of the extensively used polymersin the Brain targeted delivery of nanoparticlesdue to its biocompatibility and biodegradability. PLGA suffers from the drawback of coverting the medium into acidic pH upon degradation. It could be overcome by Polycaprolactone which is known for its slower degradation and less-acidic pH upon degradation[13].Thus, using Polycaprolactone as the biodegradable polymer, Nevirapine Polycaprolactone nanoparticles were prepared by Emulsion Solvent Evaporation technique in different solvents. The present study was undergone with an objective of investigating the impact of different solvent systems in the physicochemical characterisations and *invitro* release study of Nevirapine loaded Polycaprolactone nanoparticles. Three solvent systems viz. Chloroform, Dichloromethane andEthyl acetatewere used to prepare the Polymeric nanoparticles. The solvents were chosen according to their solubility in water (Ethyl Acetate 8.7 % w/w, Dichloromethane1.6%w/wandChloroform0.815%w/w[14] as well as Dielectric constant of 6.02, 8.9 and 4.8 respectively) [15].

Experimental

Materials and reagents

Polycaprolactone (Sigma-Aldrich,Kolkata),Pluronic F68 (BASF, Mumbai), Ethyl acetate (Nice Chemicals,Kerala), Dichloromethane (QualigenFine Chemicals, Mumbai) and Chloroform (Rankem,Kolkata) were used.

Preparation of Nevirapine loaded Polycaprolactone nanoparticles:

Using the composition as shown in Table 1, Nevirapine Polycaprolactone nanoparticles were prepared (in triplicate) in three solvents namely Ethyl Acetate, Dichloromethane and Chloroform respectively by Emulsion solvent evaporation technique[16].Briefly in Emulsion solvent evaporation technique, the organic phase was prepared by dissolving Polycaprolactone in organic solvents into which Nevirapine was dissolved. An aqueous solution of Pluronic F68 (1% w/v) was prepared. The required quantity of aqueous solution was placed on magnetic stirring to which the required quantity of organic phase was added dropwise using syringe with 22 gauze needles. The formed emulsion was stirred for 3-5 minutes using magnetic stirrer. After that, the emulsion was homogeinized using High speed Homogeinizer(IKA T25,Karnataka, India) at 20,000 rpm for 20 minutesfollowed by Ultrasonication(Spectralab UCB320,Mumbai, India)for 20 minutes. After that, an 8 -12 hours magnetic stirring was done at 200 -300 rpm for the complete evaporation of organic solvent to form nanosuspension.

Table 1:Composition of the formulation.

Organic Phase: Aqueous Phase ratio	Percentage of Pluronic F 68 (%w/v)	Drug : Polymer ratio
3:10	1	1:4

Determination of Particle size and Polydispersity index (PDI):

The Particle size and Polydispersity Index (PDI) of Nevirapine loaded Polycaprolactone nanoparticles in three solvents was characterized by Dynamic laser scattering technique using particle size analyzer (Brookhaven 90 plus,New York, USA) using distilled water as medium.The measurements of particle size were made in triplicate.

Determination of Drug entrapment efficiency and Drug loading:

The %EE and %DL was determined by Centrifugation method[17].The prepared nanosuspension was centrifuged at 20,000 rpm for 15 minutes at 25 ˚C to separate the unentrapped drug in the supernatant. Concentration of unentrappedNevirapine in the supernatant was determined using UV visible spectrophotometer (Shimadzu UV 1800,Japan) at 214nm after suitable dilution.The residue was pre-freezed at -20^{o}C overnight and followed by lyophilization in a freeze drier (Labconco, 4.5 Plus freezone, USA) for 24-36 hours. The % EE and % DL was determined using the following relationship[18]:

$$\%EE = \frac{\text{Practical Drug content x100\%}}{\text{Theoritical Drug content}}$$

$$\%DL = \frac{\text{Practical Drug content x 100\%}}{\text{Mass of nanoparticles recovered}}$$

Determination of Zeta potential:

The Zeta potential of Nevirapine loaded Polycaprolactone nanoparticles in three solvents was characterised by Laser Doppler Micro-electrophoresis technique using Zetasizer (Malvern ZetasizerNanoseries, ZS90,and UK) after dilution(1:100) with distilled water[7].

Drug Polymer Compatibility Studies

Determination of FT-IR Spectroscopy:

The FT-IR spectra of the drug(Nevirapine),Polycaprolactone, Pluronic F68, Physical mixture of Nevirapine and Polycaprolactone; Nevirapine and Pluronic F68;Polycaprolactone and PluronicF68;Physical mixture of Nevirapine, Pluronic F68 and Polycaprolactone;Blank nanoparticles and Nevirapine loaded Polycaprolactone nanoparticles were obtained using Bruker Alpha IR spectrometer in the mid IR region (wavenumber from 200 to 4000 cm^{-1}), to study the drug and polymer compatibility[19].

Determination of Differential Scanning Calorimetry (DSC):

Possible drug and polymer interactions were detected in the DSC thermograms of the drug (Nevirapine), Polycaprolactone, Pluronic F68, physical mixture of Nevirapine, Polycaprolactone, Pluronic F68;Blank and Nevirapine loaded nanoparticles using a JADE DSC System (Perkin Elmer, UK) with the N_2 purge gas flow rate of 20 mL min^{-1} and heat flow rate at 10 °C min^{-120}.

Determination of Scanning electron microscopy (SEM):

SEM JSM-6360 (JEOL, Japan) was used to examine the shape and surface morphology of NevirapinePolycaprolactone nanoparticles. The nanoparticles were previously coated with a thin layer of gold under vacuum so as to make them electrically conductive. Then surface morphology was examined by photomicrographs at an excitation voltage of 20 kV under different magnification.

Determination of Transmission electron microscopy (TEM):

The shape of NevirapinePolycaprolactone nanoparticles were examined using a TEMJEM 2100 (JEOL, Japan).Initially the sample preparation was undertaken by the deposition of diluted nanoparticles on a copper gridcoated with thin carbon film. Then the nanoparticles were examined by photomicrographs taken at magnification of 60000X, 40000X[21].

In vitrodrug release study:

The *invitro* release study of various formulations prepared in different solvents was carried out by Dialysis bag diffusion technique[22]. 80 mg of dried nanoparticles(equivalent to 3 mg of drug) (as explained in the determination of %EE and %DL) were redispersed in 5 ml of Phosphate buffer (pH 7.4) and put in a Dialysis membrane bag which was tied to one of the paddle of USP dissolution apparatus II (Electrolab Dissolution Tester,TDT-08L,Mumbai,India) containing 900ml of Phosphate buffer, pH 7.4 in the presence of a cosolvent(50 % v/v PEG 4000)maintained at 100 rpm and at a temperature of 37± 0.5^{o}C. At preset time interval, 10 ml of sample was withdrawn and was replaced with fresh phosphate buffer pH 7.4, so as to maintain the sink condition. The withdrawn samples were filtered through a membrane filter (0.45μm) and were analysed for drug content spectrophotometrically at 214 nm using a UV-Visible spectrophotometer(Analytica Zena, Specord 50 Plus, India) after suitable dilution with water (1:20).The concentration of drug was calculated from the standard calibration plot of Nevirapine($y=0.015x+0.008$, $R^2 = 0.994$).The *invitro* release study graph of the formulations were plotted by taking time along the X axis and the cumulative percent drug release(%CDR) along the Y axis.

Modelling and comparison of drug release profiles:

The *in vitro* release data were inserted in various Kinetic equations to find out the drug release mechanism of the prepared nanoparticles. The Kinetic models used were the Zero order equation, the First order equation and the Higuchi and the Korsmeyer-Peppas models[12].

Result and Discussion

Determination of particle size:

It was observed that particle size of nanoparticles depend on the solubility of organic solvents in water and nanoparticles particle sizedecreases as solubility of the solvent in water increases. The solubility of three solvents in water follows as Ethyl acetate (8.7 % w/w), Dichloromethane (1.6 % w/w) and Chloroform (0.815 % w/w)[14]. Ethyl Acetate has partial water solubility due to its low interfacial tension between the organic phase and aqueous phase,while Dichloromethane and Chloroform have poor water solubilitydue to their high interfacial tension between the organic phase and aqueous phase.There immiscibility may lead to significant aggregation causing larger particle size.Hence the particle size of nanoparticles follows the decreasing order of the solvent solubility in water as shown in Table 2:

Chloroform> Dichloromethane> Ethyl Acetate.

Table 2: Particle size distribution in different solvents.

Solvents	Formulation code	Mean Particle size (nm) ±*S.D.* *(n=3)*	PDI±*S.D.* *(n=3)*
Ethyl acetate	F1	125.57±7.66	0.295±0.005
Dichloromethane	F2	269.48±8.03	0.288±0.013
Chloroform	F3	664.8± 33.34	0.263±0.18

The Polydispersity Index (PDI) of particles ranges from 0 to 1. PDI close to zero indicates homogenous distribution. Dichloromethane and Ethyl Acetate show relatively homogenous distributionfollowed by Chloroform.

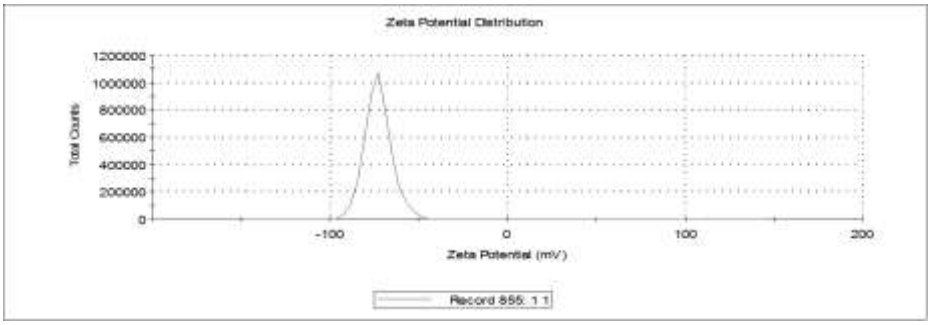

Fig. 1: **Zeta Potential of Nanoparticles prepared in Ethyl acetate**

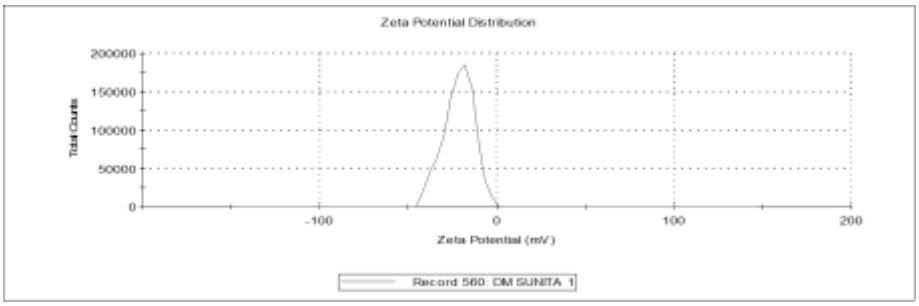

Fig. 2:Zeta Potential of Nanoparticles prepared in Dichloromethane

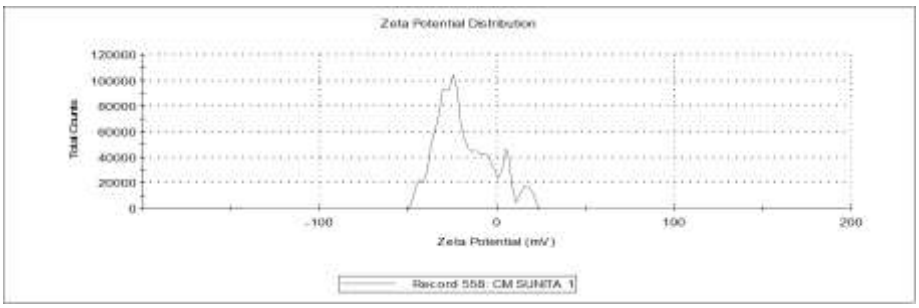

Fig.3: Zeta Potential of Nanoparticles prepared in Chloroform

The physical stability of the nanosuspensionwas characterised by Zeta potential i.e. surface charge on the particles could control the particles stability of the nanoparticulate formulation through strong electrostatic repulsion of particles with each other[23].A negative Zeta potential was observed for all three formulations in three solvents i.e.-72.1 mv, -21.4 mv and -18.5 mv (Ethyl acetate, Dichloromethane and Chloroform respectively) as shown in Fig.1, Fig. 2 and Fig.3 respectively. A higher negative charge indicates electrostatic repulsion between similarly charged nanoparticles which overcome the attractive forces between nanoparticles preventing the chance of aggregation or flocculation. Thus the nanosuspension remains stable on prolonged

storage.A sharp uniform peak signifies homogenous distribution in Dichloromethane and Ethyl Acetate. While uneven peak signifies nonhomogenous distribution in Chloroform due to high PDI.

Determination of Percent Drug Entrapment Efficiency(%EE) and Percent Drug loading(%DL):

The %EE of Nevirapine was found to be39.4±0.48% for Ethyl acetate, 32.7±0.4% for Dichloromethane and 19.8±0.16% for Chloroformrespectively. The solubility of solvents in water influences the %EE. Polycaprolactone got an excellent solubilityin Chloroformbut as Chloroformis having low solubility in water(0.815 % w/w water solubility), so, Polycaprolactone remains only in the dispersed phase (Chloroform)causing a high dispersed phase to continuous phase (aqueous phase) ratio. It led to high volume of dispersed phase. As the dispersed phase volume is high, so, more time was required for the complete removal of Chloroform causing slow precipitation of the Polycaprolactone. Hence, the %EE reduces. While Ethyl acetateis partially soluble in water(8.7 % w/w water solubility), so it gets easily diffused into the aqueous phase. Diffusion of Ethyl acetate into the aqueous phase leads to reduction in the dispersed phase volume. The low volume of the dispersed phase in turn makes an easy removal of Ethyl acetate upon stirring of prepared nanosuspension. This phenomenon leads to a fast precipitation of Polycaprolactoneleading to reduction in the particle size which in turn increases the%EEof Nevirapine[24]. The %DL in Ethyl Acetate, Dichloromethane and Chloroform are (14.83±0.14)%,(10.94±0.17)% and(6.65±0.13)%respectively. The low Drug loading efficiency of the nanoparticles was a result of further transition of the drug to the water phase because of the better miscibility of water and organic solvents. Thus,an increase in the solubility of organic solvent increases in water enhances the %DL of nanoparticles. The %DL follows the following order:

Ethyl acetate>Dichloromethane> Chloroform[25].

The %EE and %DL in Ethyl acetate, Dichloromethane and Chloroform are given in Table 3.

Table 3: The Percentage Drug entrapment efficiency and Percentage Drug loading in different solvent systems.

Solvents	Formulation code	Mean (%EE) ±*S.D. n=3*	Mean (%DL) ±*S.D. n=3*
Ethyl acetate	F1	39.4±0.48	14.83±0.14
Dichloromethane	F2	32.7±0.4	10.94±0.17
Chloroform	F3	19.8±0.16	6.65±0.13

Drug Polymer Compatibility Studies

Determination of FT-IR Spectroscopy:

The FTIR spectra of Nevirapine, physical mixture (Nevirapine, Polycaprolactone) and NevirapinePolycaprolactone nanoparticles show that there were no changes in the position of absorption peaks.The pure Nevirapine (Fig.4) showed the main peaks contributed by the functional groups of molecule such as carbonyl –C=O stretching (1642.26 cm^{-1}), –OH stretching (3183.27 cm^{-1}), N-H stretching (3184.76 cm^{-1}), c-c stretching,aromatic(1486.30 cm^{-1}). The physical mixture of Polycaprolactone, Nevirapine (Fig.5) showed similar absorption peaks at the same position as that of Nevirapine.NevirapineloadedPolycaprolactone nanoparticles prepared by Emulsion solvent evaporation technique in three solvents – Ethyl acetate (Fig.6), Dichloromethane(Fig.7) and Chloroform (Fig.8) showed peaks resulting from simple superposition of their separated components in the infrared spectra. Fig. 6, Fig.7 and Fig.8 showed peaks at 3315.80 cm^{-1}(–OH stretching), 1635.87 cm^{-1}(carbonyl –C=O stretching) and 3298.81 cm^{-1}(–OH stretching), 1636.01 cm^{-1}(carbonyl –C=O stretching) as well as 3293.54 cm^{-1}(–OH stretching), 1636 cm^{-1}(carbonyl –C=O stretching) respectively.Spectral analysis indicated that the specific functional groups of polymeric material in the nanoparticles surface have almost the same chemical characteristics of the pure polymer. The study suggests that molecular interactions that could alter the chemical structure of the drug did not occur. Therefore, no chemical interaction between functional group of Nevirapine and Polycaprolactone exist[20].

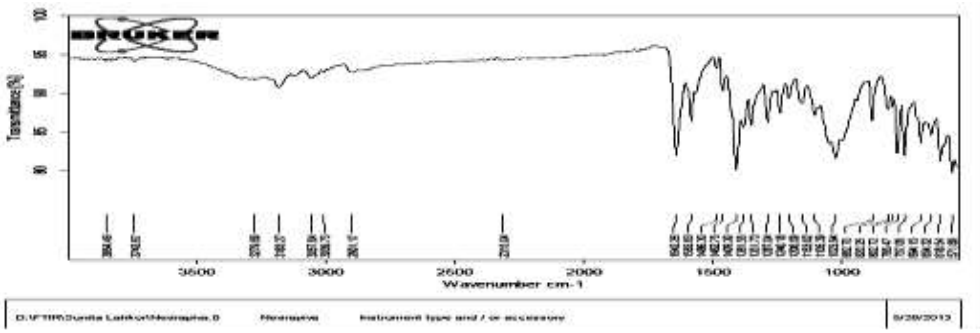

Fig. 4: FTIR of Nevirapine

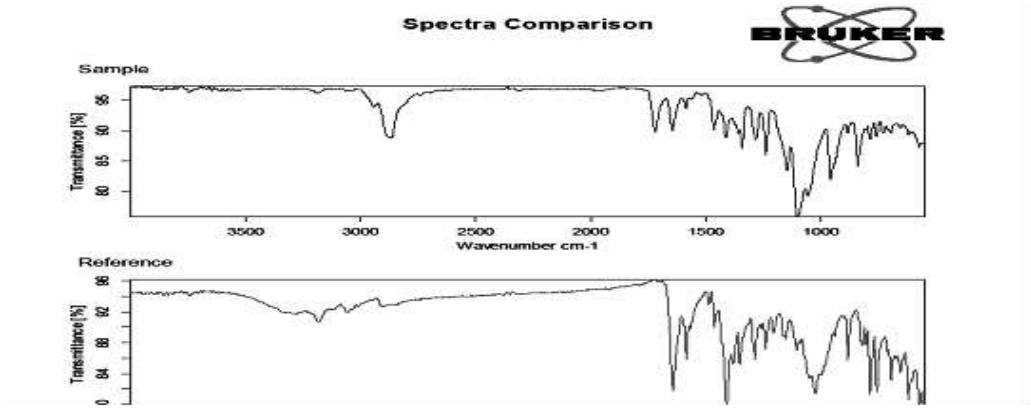

Fig. 5: FTIR of physical mixture

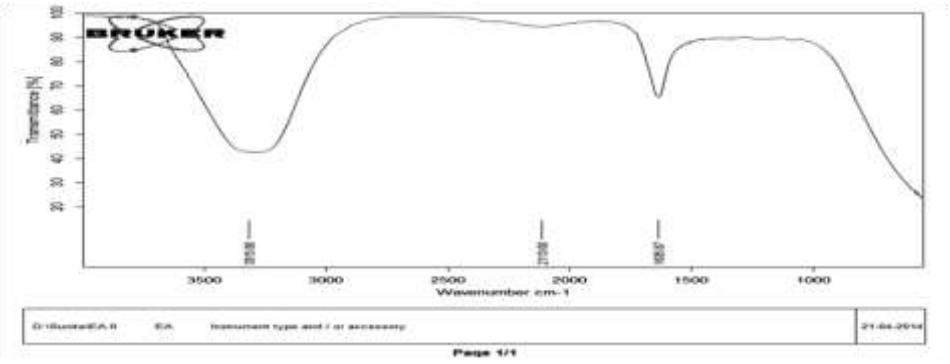

Fig. 6: FTIR of nanoparticles prepared in Ethyl acetate as the solvent.

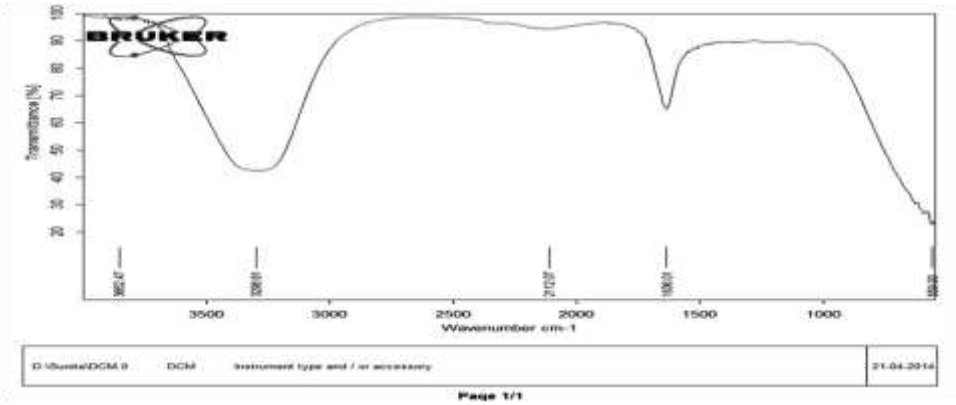

Fig. 7: FTIR of nanoparticles prepared in Dichloromethane as solvent.

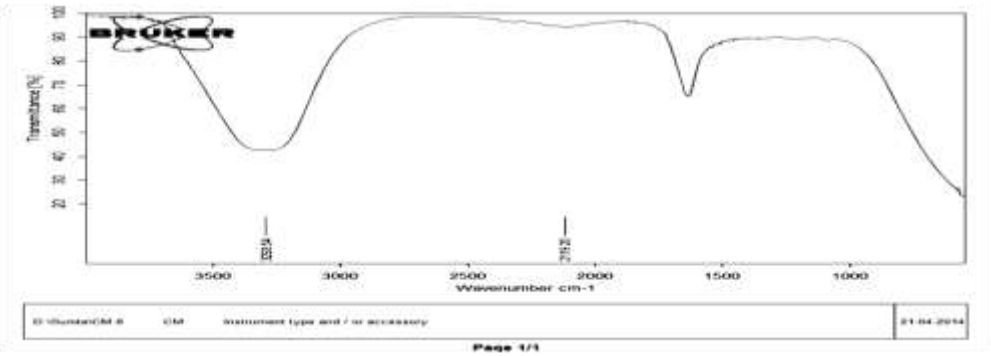

Fig. 8: FTIR of nanoparticles prepared in Chloroform as solvent.

Determination of Differential Scanning Calorimetry (DSC):

The DSC of Nevirapine showed sharp endothermic peak at 250.57 ^{0}C corresponding to its melting point which indicate its crystalline nature (Fig. 9). The DSC of Polycaprolactone, NevirapinePolycaprolactone and Pluronic F68 physical mixture showed sharp endothermic peaks at, 68.76 ^{0}C(Fig.10) and 69.51 ^{0}C(Fig.11). Less intense endothermic peaks with an absence of Nevirapine peaks were obtained for NevirapinePolycaprolactone nanoparticles due to the encapsulation of Nevirapine in the Polycaprolactone nanoparticles (prepared in Ethyl acetate,Dichloromethane and Chloroform) in the amorphous state as shown in Fig.12, Fig.13 and Fig.14respectively[26].There is a depression in melting point towards lower temperature for Nevirapine, Polycaprolactone in polymeric nanoparticles when compared to individual DSC peaks for Nevirapine, Polycaprolactone. The small particle size corresponds to high surface area which requiresless energy than the perfect crystalline substance, which needs to overcome lattice force. The less ordered lattice arrangement in the amorphous state of polymeric nanoparticles entraps the drug[27].

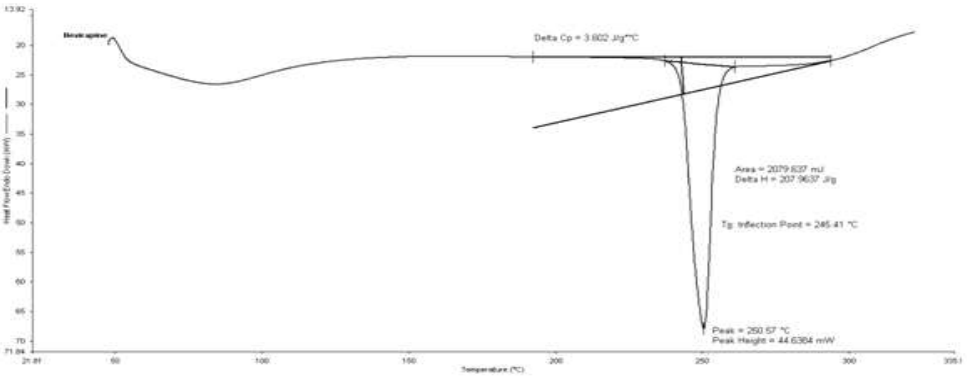

Fig. 9: DSC of Nevirapine

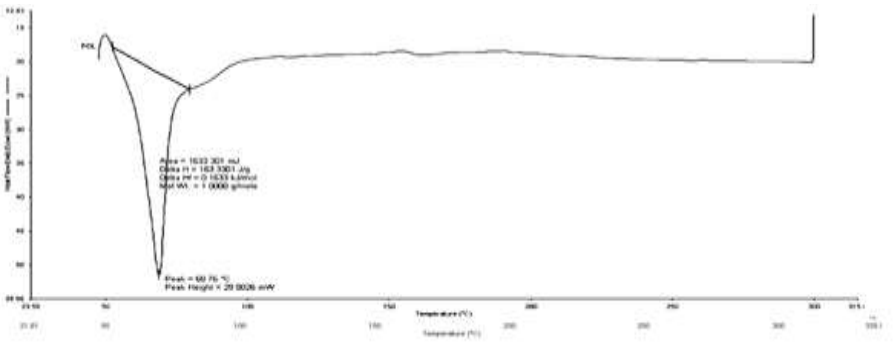

Fig. 10: DSC of Polycaprolactone.

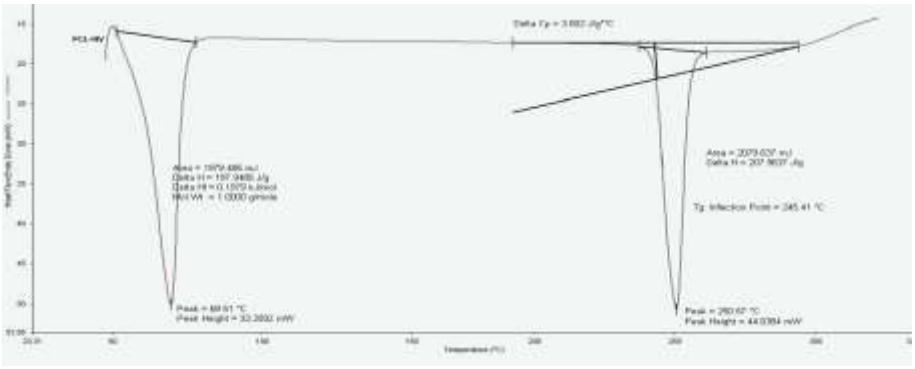

Fig. 11: DSC of Nevirapine Polycaprolactone physical mixture.

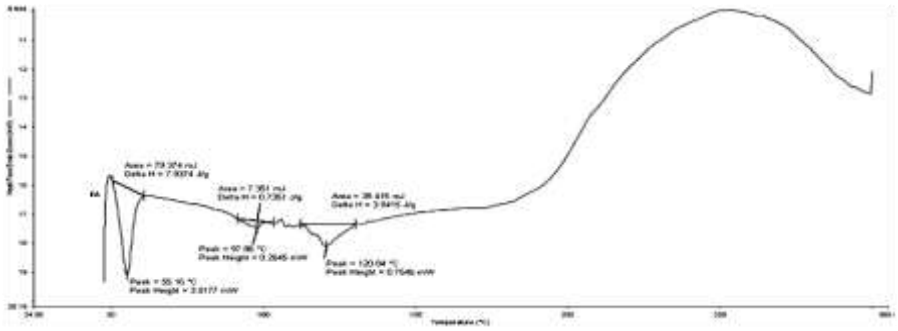

Fig. 12: DSC of nanoparticles prepared in Ethyl acetate

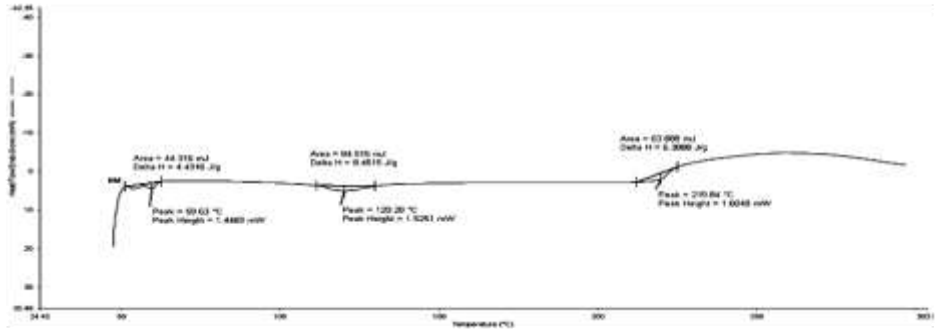

Fig. 13: DSC of nanoparticles prepared in Dichloromethane

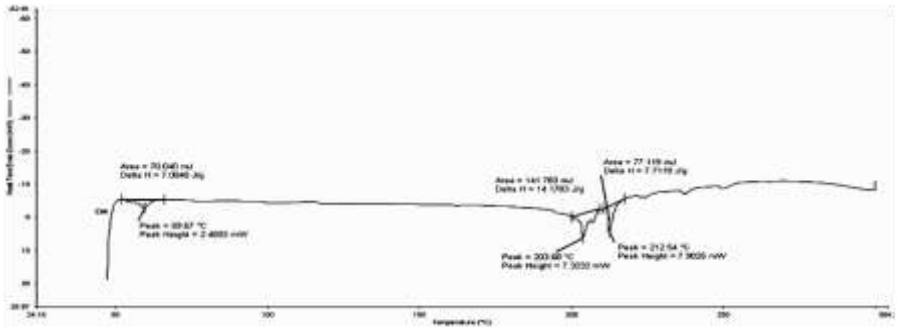

Fig. 14: DSC of nanoparticles prepared in Chloroform

Determination of Scanning Electron Microscopy (SEM):

The SEM photomicrographs were taken at 20 kV under different magnification. It was observed that using Ethyl acetate (Fig.15) as the solvent, spherical shaped, discrete, regular shaped nanoparticles of sizes below 100 nm could be obtained. While Dichloromethane (Fig.16) when used as solvent, showed comparatively slightly bigger sized nanoparticles and Chloroform(Fig. 17) as a solvent, showed the biggest sized particles

among the three. The nanoparticles prepared using Ethyl acetate could be observed only under higher magnification of 55000 kV, while Chloroform prepared nanoparticles was observed at low magnification of 27,000 kV.

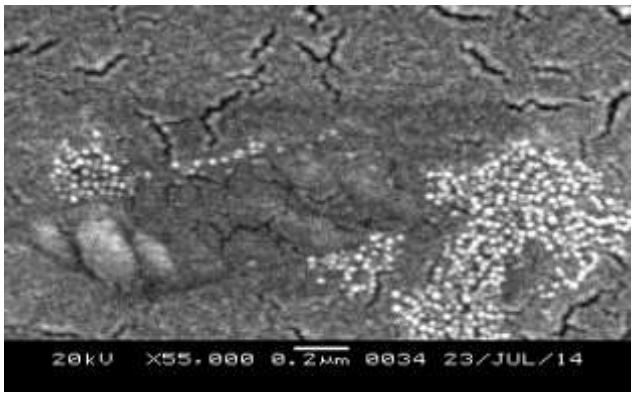

Fig. 15: SEM of nanoparticles prepared in Ethyl acetate

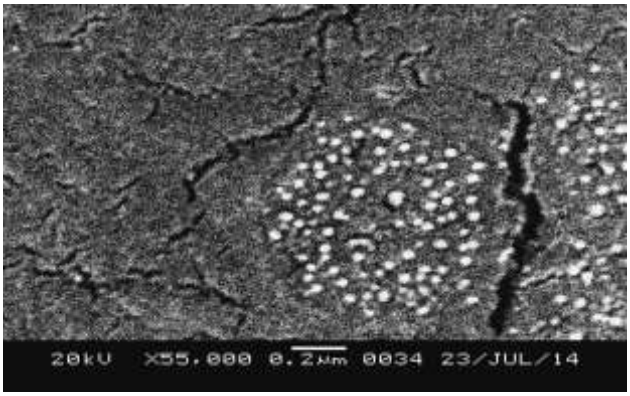

Fig. 16: SEM of nanoparticles prepared in Dichloromethane

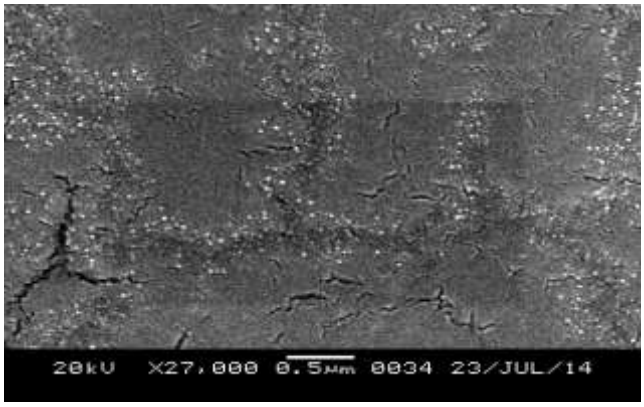

Fig. 17: SEM of nanoparticles prepared in Chloroform

Determination of Transmission Electron Microscopy (TEM):

The TEM microphotographs of NevirapinePolycaprolactone nanoparticles prepared in different solvents Ethyl acetate(Fig.18), Dichloromethane(Fig.19) and Chloroform(Fig.20) showed small sized, smooth surfaced,uniform, regular, spherical shaped, discrete particles of size 50nm to 100 nm. A higher magnification of 60,000 X was required for observing the nanoparticles prepared in Ethyl acetate while the nanoparticles prepared in Chloroform, Dichloromethane required a magnification of 40,000 X. The nanoparticles prepared in Ethyl acetate was found to have size below 50 nm while the nanoparticles prepared in Chloroform and Dichloromethane was found to have size between 50 nm to 100 nm.

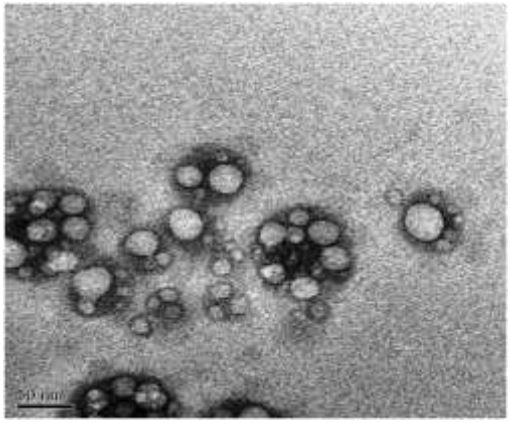

Fig. 18: TEM of nanoparticles prepared in Ethyl acetate

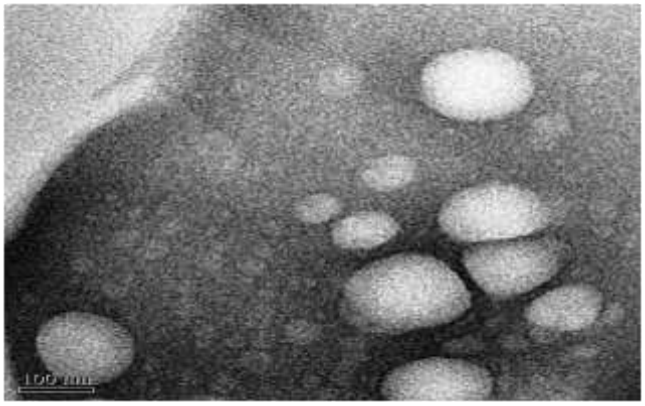

Fig. 19: TEM of nanoparticles prepared in Chloroform

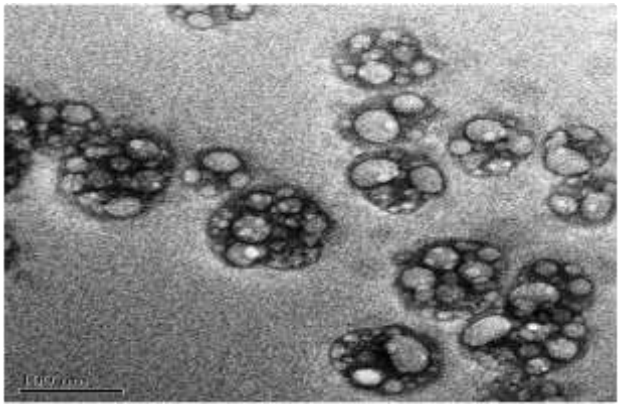

Fig. 20: TEM of nanoparticles prepared in Dichloromethane

In vitro drug release study:

The *invitro* release study of formulations prepared in Ethyl acetate, Dichloromethane and Chloroform are shown in Fig.21. The nanoparticles prepared using Ethyl acetate showed the highest release than nanoparticles prepared using Dichloromethane and Chloroform. It was already observed that the nanoparticles prepared in Ethyl acetate got the smallest particle size than that of Dichloromethane and Chloroform. A small sized nanoparticle exposes more surface area to the dissolution medium. More the surface area, more quantity of drug gets released into the dissolution fluid of pH 7.4,Phosphate buffer.Hence nanoparticles prepared in Ethyl acetate got the highest release of 84.8 % followed by Dichloromethane and Chloroform[28]. The nanoparticles prepared in three solvents of Ethyl acetate, Dichloromethane and Chloroform released the drug for 8 hrs.

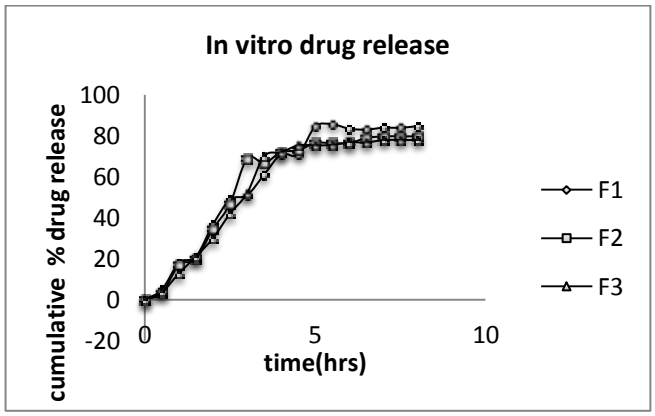

Fig. 21: *invitro* release study of formulation (F1, F2 and F3) prepared in Ethyl acetate, Dichloromethane and Chloroform respectively.

Modelling and comparison of drug release profiles:

The *invitro* release datas of nanoformulations prepared inEthylacetate, Dichloromethane andChloroform was inserted in different kinetic equations such as Zero order, First order, Higuchi model and Korsemeyerpeppas model[29]. The obtained data for correlation coefficients(R^2), rate constants(K) are being shown in Table4.

Table 4: Kinetic model parameters of different formulations in Ethyl acetate.

Formula--tion code	Solvents	Zero order		First order		Higuchi release		Korsemeyer peppas model	
		R^2	K_0	R^2	K_1	R^2	K_H	R^2	n
F1	Ethyl acetate	0.933	0.159	0.856	-0.016	0.979	2.42	0.972	0.986
F2	Dichloro-methane	0.772	0.19	0.749	-0.012	0.88	2.89	0.957	0.753
F3	Chloroform	0.799	0.182	0.673	-0.013	0.893	2.8	0.949	0.748

From the Table4, it was observed that the nanoparticles prepared in Ethyl acetatewere best fitted in Higuchi model with the highest correlation coefficient of (R^2= 0.979 for Ethyl acetate) followed by Korsemeyerpeppas model. The Higuchi model indicated that the drug was released from the nanoparticles by diffusion controlled mechanism. The *n* value obtained from the Korsemeyerpeppas model found to be 0.986 which was more than 0.89 (n > 0.89), indicatinga super case II transport mechanism was followed. When Dichloromethane was used as solvent, the best fitted kinetic model with highest correlation coefficient was found to be 0.957 with Koresemeyer peppas model followed by Higuchi model release. The value of 'n' found to be between 0.753 which was between 0.45 <n< 0.88, showing 'Anomalous' (non fickian) diffusion.Using Chloroformas the solvent, the best fitted model with highest correlation coefficient was obtained to be 0.949 with Korsemeyer peppas model and the value of 'n' found to be 0.748 which was between 0.45 <n<0.88, indicating 'Anomalous' (non fickian) diffusion. Hence, nanoformulations prepared in Ethyl acetate release the drug by diffusion controlled while nanoformulations prepared in Dichloromethane and Chloroformundergoes 'Anomalous' (non fickian) diffusion.

Conclusion

Nevirapine loaded Polycaprolactone nanoparticles were prepared by Emulsion solvent evaporation technique.The aqueous solubility of solvents had significantly influenced thephysiocochemical characterisations and release behaviour of the prepared nanoparticles. The solvent having partial water solubility resulted in high % Drug Entrapment efficiency, smaller particle size, the highest drug release as compared to water immiscible solvents.Hence appropriate selection of solvent is important in preparing Nevirapine loaded Polycaprolactone nanoparticles.Thus for further work, Ethyl acetate is chosen as asuitable solvent for preparing Polycaprolactone nanoparticles for Brain targeted delivery of Nevirapine.

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