Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(2):70-79

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Formulation and i*n vitro* characterization of Carbamazepine polymeric nanoparticles with enhanced solubility and sustained release for the treatment of Epilepsy

Shashank Tummala*[1], M. N. Satish Kumar[2] and Ashwati Prakash[1]

[1]*Department of Pharmaceutics, J. S. S. College of Pharmacy (Off-campus), J. S. S University, Mysore, India*
[2]*Department of Pharmacology, J. S. S. College of Pharmacy (Off-campus), J. S. S. University, Mysore, India*

ABSTRACT

Carbamazepine is used in the treatment of epilepsy, but it is having limitations such as low solubility leading to lower oral bioavailability. Carbamazepine conventional formulation despite having good anti epileptic activity, its therapeutic activity was limited due to its slow and limited release in gastrointestinal tract. So the major objective was to formulate polymeric nanoparticles, which can increase solubility and drug release along with sustained release property of the drug. In the present study, it was proposed to develop nanotechnology-based systems, for selected poorly water-soluble drug carbamazepine using PLGA as polymer (with different drug: polymer ratios) selected randomly by factorial method designing and was expected to improve dissolution properties that may increase its bioavailability. The polymeric nanoparticles were subjected to particle size evaluation, SEM study, drug content, entrapment efficiency and in vitro release studies. Nanoparticles with drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency of 130±2.1nm, 61.28% and 18.15 % respectively. Optimized batch in drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency of 126.8±0.19m, 34.81±0.01% and 64.28±0.09% respective ly. Contour plots and 3D-scatter plots were drawn for statistical supportive evaluation in optimization using Minitab 17. In vitro drug release studies concluded that carbamazepine nanoparticles released drug in biphasic pattern by initial burst release of 50±0.12% wit h in 4 hours, which was followed by sustained release of 89.92±0. 01% till 24 hours concluding its solubility enhancement.

Keywords: Carbamazepine, Polymeric nanoparticles, Epilepsy, Factorial design, Sustained

---

INTRODUCTION

Epilepsy is a disease characterized by recurrent seizures, which are nothing but episodes of paroxysmal neural discharges [1]. Twice or thrice the mortality was observed in people facing epilepsy when compared to regular population [2]. Most of the newly developed molecules for treatment of epilepsy are suffering from variability in absorption that limits their therapeutic efficacy, which can be attributed to their change in physicochemical properties. Most of the drug delivery to brain has been limited mainly due to the lower solubility. Biopharmaceutical Classification system (BCS class) II indicates low solubility and high permeability for the drug molecules. As the drug is having low solubility, it dissolute very poorly so it delays the absorption that indicates the rate of dissolution is the controlling step for absorption [3-4].

This gives us an indication to follow another approach to enhance its solubility and also sustaining its release.

Nanoparticulate approach has been successfully incorporated to some extent and also been filed for patent which are waiting for successful release into the market (http://www.google.co.in/patents/WO2004078162A1?cl=en). So more research was needed in order to prepare effective nanoparticulate formulations, which can deliver the drug in the intended therapeutic dose and also sustaining its release. Topiramate is the drug, which has been filed for patent for use for epilepsy that was formulated in nano form. Nanoparticulate approach also offers site specificity owing to its sub micron size [5].

Also the nano scale has shown their ability to encapsulate both hydrophilic and hydrophobic drugs. Most of the drugs in nanoparticulate systems shows better CNS targeted drug therapy because of improved penetration of the active pharmaceutical ingredient (API) leading to reduced risk when compared with the conventional formulation [6]. Nanoparticles also can achieve reduced toxicity to peripheral organs.

The idea behind selecting carbamazepine as active pharmaceutical ingredient is that it is an effective anti-epileptic drug, which is characterized by irregular and often slow absorption into the systemic circulation [7]. Despite having good anti epileptic activity, it belongs to BCS class II which indicates low solubility and high permeability leading to oral bioavailability owing to its slow and limited release in gastro intestinal tract [8]. Significant decrease in the elimination half-life was observed when carbamazepine is administered repeatedly which is attributed to auto induction. It shows an elimination half-life of 24 hours when given as single dose and it was lowered to less than 12 hours on chronic dosing [9-10].

Polymeric nanoparticles has gained attraction for drug delivery systems in the last few decades owing to their ability to deliver the drug in a controlled manner at the site of action [11]. Biodegradable nanoparticles were lot more useful for developing controlled/sustained release and can be compliant to patient [12]. Also biodegradable nanoparticles can provide constant rate of degradation, which can be beneficial in term of sustained release approach [13]. Poly (lactide-co-glycolide) (PLGA) can be chosen as the polymer because it is selected widely in preparing various drugs and proteins [14-16] and also used for the marketed products like microparticles, which conforms to its applicability [17-18]. These biodegradable nanoparticles using PLGA that can deliver controlled drug delivery can reduce the serious systemic side effects caused by the drug administration [19-20]. Particle size, surface morphology, size distribution, drug content have a major effect on the controlled drug release from the hydrophobic drug [21].

So this motivates us to formulate carbamazepine polymeric nanoparticles by encapsulating this hydrophobic drug inside PLGA. PLGA polymeric nanoparticles were prepared by solvent evaporation method using different ratios of polymer, which gives best particle size, surface morphology, drug content etc. The prepared polymeric nanoparticles has to improve the solubility and achieve sustained release property of carbamazepine which may help the drug to overcome its poor oral bioavailability that may benefit it therapeutic activity, which has been previously lowered because of its poor pharmacokinetics.

EXPERIMENTAL SECTION

Materials

Carbamazepine, used as active pharmaceutical ingredient was obtained from Sigma Aldrich, Mumbai. PLGA and Acetonitrile were obtained from Sigma Aldrich, Mumbai that was used as polymer and solvent respectively. Polyvinyl alcohol was obtained from BDH Laboratories. All other chemicals were of analytical grade. Doubly distilled water was used throughout the study.

Preparation of carbamazepine-PLGA nanoparticles

The method used for the preparation of PLGA nanoparticles containing carbamazepine is solvent emulsification evaporation technique. Different ratios of drug: polymer (1:1, 1:2, 1:3, 1:4, 1:5) was selected in order to optimize the best one and also to observe the effect of polymer on the formulation. Acetonitrile was used as organic solvent and PVA as surfactant in a fixed concentration of 0.5% w/v. Drug was dissolved in ethanol with varying polymer ratios (1:1,1:2,1:3,1:4,1:5). Then it was followed by addition of aqueous surfactant polyvinyl alcohol using high-speed emulsifier and was stirred continuously for 3 hours. Then the emulsion was subjected to centrifugation (SIGMA, Germany) for 30 minutes at 12000 rpm. Supernatant was removed and washed repeatedly three times and subjected to lyophilisation using 5% mannitol as cryoprotectant (Christ Alpha 2-4 LD, Freeze Drying Solutions, UK).

The selected ratios for the methodology were selected based on the factorial design using design of experimentation

(DOE) by Minitab 17 software. The effect of the variables has been studied thoroughly and the method was optimized properly. The effect of the lipid on the entrapment efficiency was screened using DOE.

Characterization of Nanoparticles

Differential scanning calorimetry (DSC) [22]

DSC analysis was performed using DSC Q200(TA instruments, Mumbai, India). DSC analysis was performed for PLGA, carbamazepine, physical dispersion of carbamazepine and PLGA, carbamazepine polymeric nanoparticles. The samples were kept in aluminium pans and heated at a rate of 10° C per/min in a 30 to 300° C temperature under nitrogen flow of 40 mL/min.

X-ray diffraction studies (XRD) [23]

Molecular arrangements of drug carbamazepine in nanoparticulate formulations were performed on an X-ray diffractometer (PANalytical X'pert PRO; Lelyweg, Almelo, The Netherlands) using CuKα radiation. The data were collected over an angular range from 3 degrees to 50 degrees 2θ in continuous mode using a step size of 0.02 degree 2θ and step time of 5 seconds. XRD analysis was performed for PLGA, Carbamazepine, physical dispersion of carbamazepine and PLGA, Carbamazepine polymeric nanoparticles.

Particle size and zeta potential [24]

The average particle size and zeta potential of the carbamazepine-PLGA nanoparticles were determined by particle Size Analyzer (Zetasizer Ver System;Malvern Instruments Ltd, Malvern, UK). Nano suspension was diluted and filtered (0.22µm) with ultra pure water to analyze particle size.

Drug content and Entrapment efficiency [25]

Freeze dried nanoparticles were collected and evaluated for drug content and entrapment efficiency. Nanoparticles need to be added to the solvent for removal of the coat. Nanoparticles (20mg) suspension was subjected to evaporation for further removal of the solvent prior to filtration. Then the residue was washed and diluted appropriately with phosphate buffer of pH 7.4 to determine drug content and entrapment efficiency. Samples were measured at an absorbance of 285 nm in Double beam U.V Spectrophotometer. The effect of the lipid and particle size on the entrapment efficiency was performed using DOE by Minitab 17 software. They were shown as 3D-surface plots and contour plots in order to determine the best lipid and particle size range. Drug content loading and entrapment efficiency of gliclazide in nanoparticles were determined by the following equations

$$\text{Drug loading content (\% w/w)} = \frac{\text{Weight of drug in nanoparticle}}{\text{Weight of nanoparticle recovered}} \times 100$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of the drug in nanoparticle}}{\text{Weight of the drug fed initially}} \times 100$$

Statistical analysis

Statistical analysis was performed for different formulae by applying factorial design using DOE by Minitab 17. The effect of the lipid and particle size of different formulations on the entrapment efficiency was found out to optimize the best formulation for further studies. 3D surface plots and contour plots were drawn for supporting the selected ratios and selected formulations.

Scanning electron microscopy (SEM) [26]

Scanning electron microscopy (SEM) was used to verify uniformity of particle shape and size. Freeze-dried nanoparticles were resuspended in distilled water and were later dropped onto a silicon grid and dried under room temperature. The nanoparticle suspension was vacuum coated with gold for 3min. The surface morphology of the samples was observed under a scanning electron microscope (JEOL–JAPAN) operated at 15-keV pulse at different resolutions.

***In vitro* drug release study [24]**

In vitro release of carbamazepine from the polymeric nanoparticles was evaluated by performing *in vitro* studies using USP type II (TDT 08T, Electro-lab, Mumbai, Maharashtra, India) dissolution test apparatus. Dissolution test

was conducted in phosphate buffer p^H 7.4, which was maintained at 37.5 ^{0}C, and paddle rotation speed was maintained at 100 rpm. The main rationale behind selecting phosphate buffer(PB) of p^H 7.4 as carbamazepine is a hydrophobic drug it was found to be appreciably more soluble (2-4 times) in phosphate buffer of p^H 7.4 as solvent. Nanoparticles were suspended in 900 ml of PB with continuous stirring at 100 rpm. Samples were with drawn from the dissolution medium at particular time intervals and replenished with fresh buffer after each sampling .The sample solutions were filtered and diluted up to 10 ml and the absorbance was measured at 285 nm using double Beam UV/VIS spectrophotometer. The study was done in triplicate, which suggest each data point in the *in vitro* release graph represents an average of three measurements. The drug release was followed till 24 hours with samples at 0.15,0.30, 1, 2, 4, 6,8,10,12 and 24 hours in order to conclude its sustained release property.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry (DSC)

Thermogram of carbamazepine, PLGA, physical dispersion of PLGA and carbamazepine, carbamazepine-PLGA polymeric nanoparticles was illustrated in **figure number 1**. The DSC curve of carbamazepine exhibited corresponding peak at peak temperature of 186.8^{0}C corresponding to its melting point. Polymer PLGA had shown peak at a temperature of 62.89^{0}C. Carbamazepine PLGA polymeric nanoparticles have shown a minor peak at 62^{0}C and peak position of active pharmaceutical ingredient was found to be vanished, which can be attributed to the fact that drug can be molecularly dispersed into the polymer matrix. Physical dispersion has shown peaks at 62.2^{0}C and 181.2^{0}C, which corresponds to the polymer and active pharmaceutical ingredient. This shows the distinction from the polymeric nanoparticles where the peak was found to be vanished indicating its chances of entrapment.

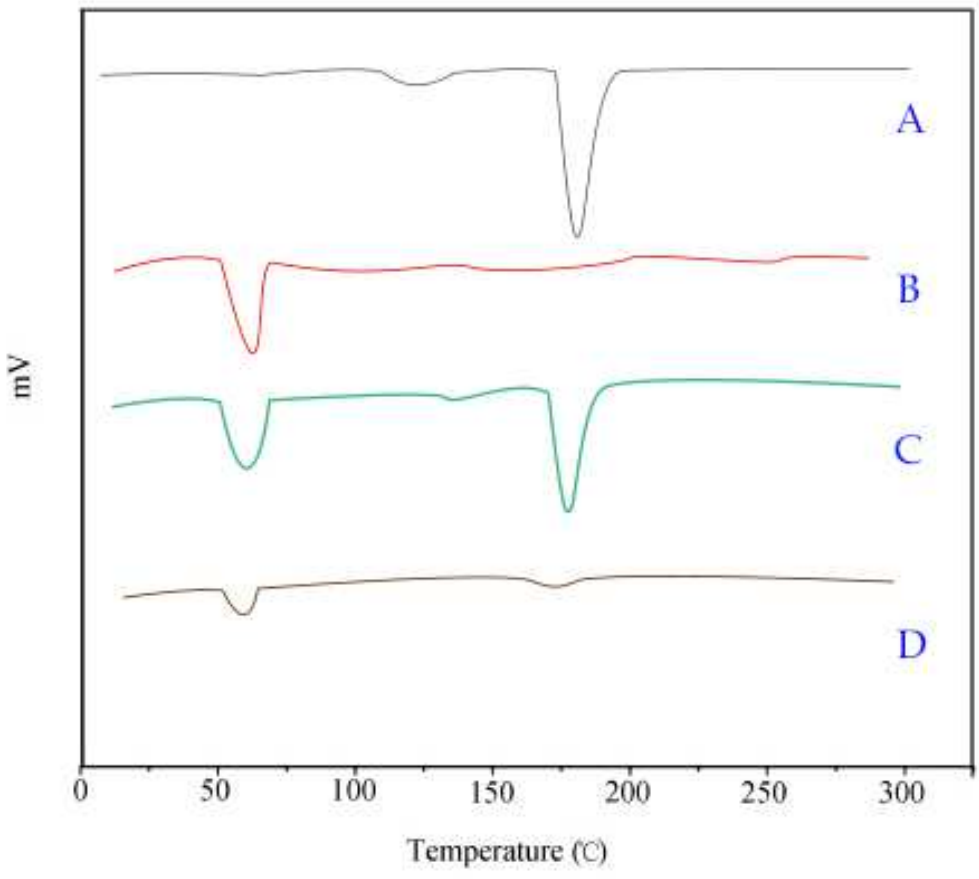

Figure Number 1 Overlaid DSC Thermogram of (A) Carbamazepine (B) PLGA (C) physical mixture of carbamazepine and PLGA(D) Carbamazepine polymeric nanoparticles

Polymeric nanoparticles have exhibited reduction in the height and thickness of the peaks that can be attributed to the presence of the polymer as the drug alone has shown peak that was of more height and thickness. These studies further strengthen the evidence that there is compatibility between the drug and the polymers and also the chance of entrapment of drug inside the polymer in the polymeric nanoparticles. The DSC studies supports our rationale, as stability is the primary concern, which can effect the formulation in many ways. To achieve stability, compatibility between the drug and the polymer must be ensured which can be confirmed by the DSC and further by XRD studies.

XRD studies

An XRD peak mainly depends on the crystal size as they indicate the crystalline nature at particular value at 2θ range. In this study, pure drug carbamazepine had shown a sharp single peak and the highest one at 2θ equals 16.1 ^{0} that indicates its crystalline nature and also minor peaks were observed at 11^0. Polymer PLGA diffractogram had shown peaks at 21.4^0, which can be seen in Figure number 2.

Noticeable change was found in the diffractograms of pure polymer and drug loaded polymeric nanoparticles. There was change in intensity of the peak, which can be observed in the figure number 2 that can be attributed to dispersion of drug in molecular level leading to lower level of detection. Moreover the slight disappearance of the carbamazepine peak indicates the entrapment of drug inside the polymer and also indicates the amorphous state of the encapsulated drug.

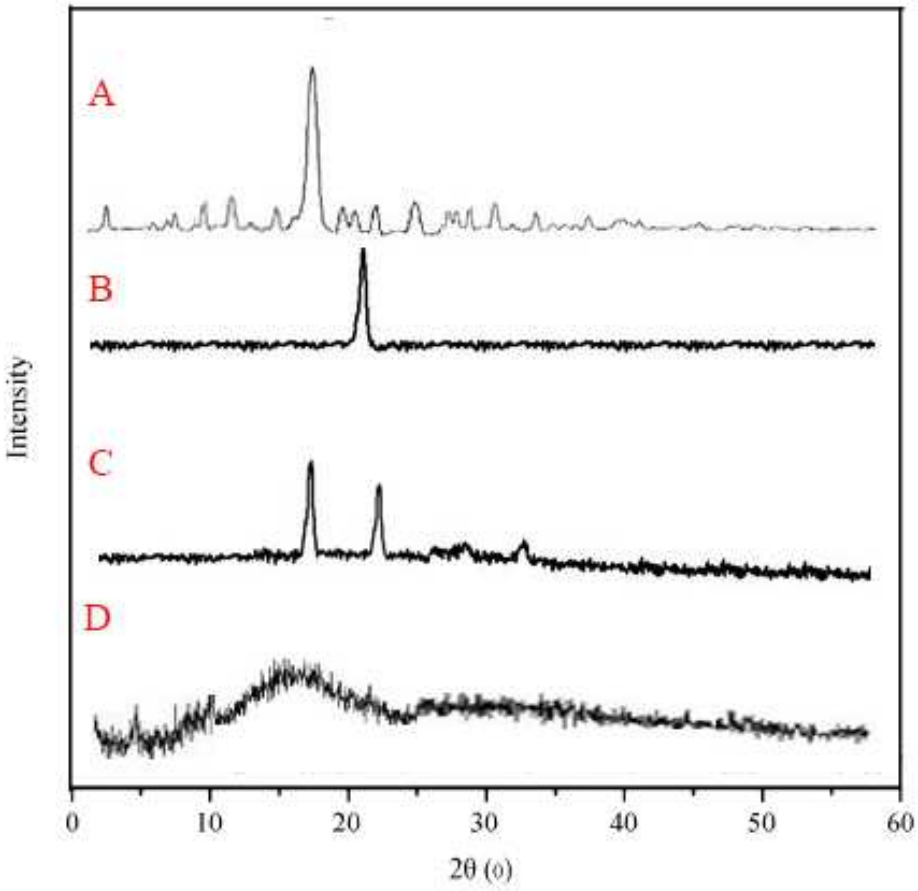

Figure Number 2 Overlaid XRD pattern of (A) Carbamazepine (B) PLGA (C) Physical mixture of PLGA and carbamazepine (D) Carbamazepine polymeric nanoparticles

Particle size and zeta potential analysis

The mean particle size of carbamazepine nanoparticles was found to be smaller than 130 nm. Particle size plays an important role in the drug delivery to the brain as nanoparticles with smaller size can easily cross the barrier comparable to the particles with size more than 500nm. Also decrease in the particle size leads to increase in the

surface area particularly effective surface area, which leads to increase in solubility of the hydrophobic drug. So the nanoparticles size range was found to be satisfactory and was according to the specifications. This was performed in replicate of three times (n=3) in order to ensure reproducibility to minimize the error. PDI values were found to be lesser than 0.2, which indicates that the system has a relatively narrow distribution. Zeta potential was found to be in the limit and it further proves the stability of the prepared polymeric nanoparticles, which justifies the rationale of preparing stable nanoparticles, as stable nanoparticles can be easily dispersed which enhances its solubility. The results evaluated were shown for different ratios of drug: polymer in Table number 1. The particle size intensity for drug: polymer ratio of 1:1 was 126.8± 0.19nm, which can be seen in Figure Number 3. This ratio was found to be satisfactory and can be optimized among the all by evaluating the entrapment efficiency, which was confirmed later.

Table Number 1 Particle Size, Entrapment efficiencies and Drug loading (%) of Carbamazepine polymeric nanoparticles

Drug: Polymer ratio	Particle size (nm)	Entrapment efficiency (%)	Drug loading (%)
1:1	126.8± 0.19	64.28±0.09	34.81±0.01
1:2	131.7±0.17	58.12±0.10	33.21±0.14
1:3	147.1±1.01	47.28±1.07	28.14±0.19
1:4	181.2± 0.12	41.28±0.05	22.81±0.23
1:5	242.2±0.09	31.12±0.07	15.91±1.25

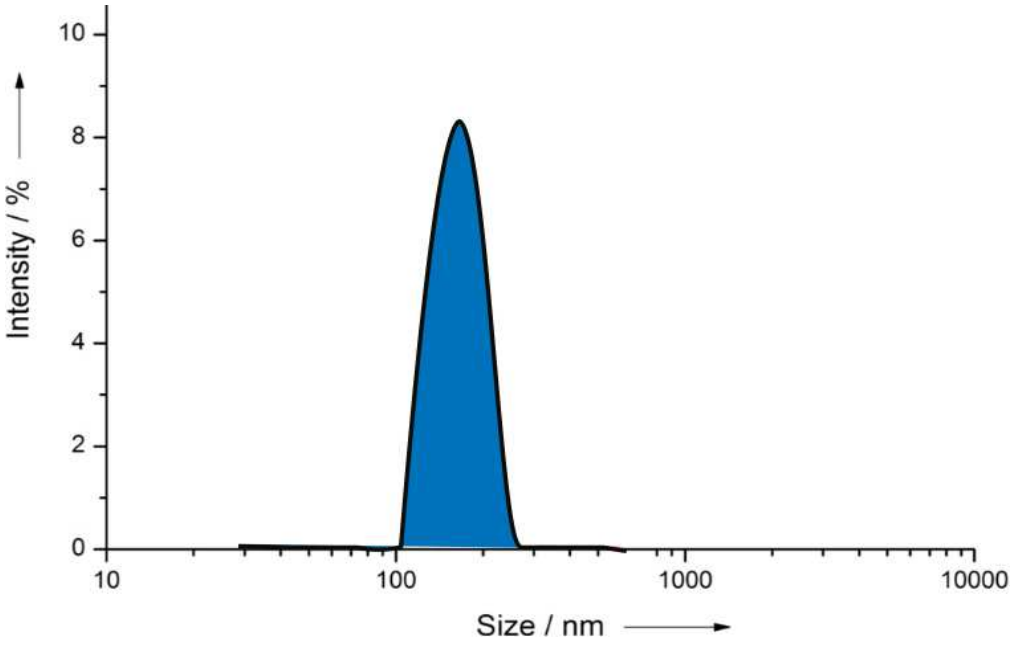

Figure number 3 Average particle size of carbamazepine PLGA nanoparticles

Drug Loading and Entrapment efficiency studies

The reason behind selecting polymeric nanoparticles over solid lipid nanoparticles is their ability to get drug incorporated and also exhibit more encapsulation efficiency. Entrapment efficiency is considered as an important parameter as improper entrapment leads to the initial burst release of the drug, which hinders its sustained release property. Also intended therapeutic dose has to be available for the formulation to achieve required therapeutic effect. Entrapment efficiency of all the polymer nanoparticles made of different polymer ratios was shown in Table Number 1.

Based on the evaluation parameters of entrapment efficiency and drug content, 5-FU polymeric nanoparticles of ratio 1:1 (126.8± 0.19nm) was optimized. The polymeric nanoparticles of drug: polymer ratio of 1:1 has shown an entrapment efficiency of 64.28±0.09%, which was found to be good for hydrophobic drug. As the polymer concentration increased, there was a noticeable change in the entrapment efficiency as it keeps on decreasing, which may be due to the formation of more compact polymer coat, which hinders the proper entrapment of the drug. This was observed as contrary to the some researches where the polymeric concentration increases, it caused increase in

the entrapment efficiency. Carbamazepine nanoparticles with drug: polymer ratio of 1:5 has shown entrapment efficiency of 31% which was very low considering the fact that improper entrapment of intended drug will lead to the decreased therapeutic effect.

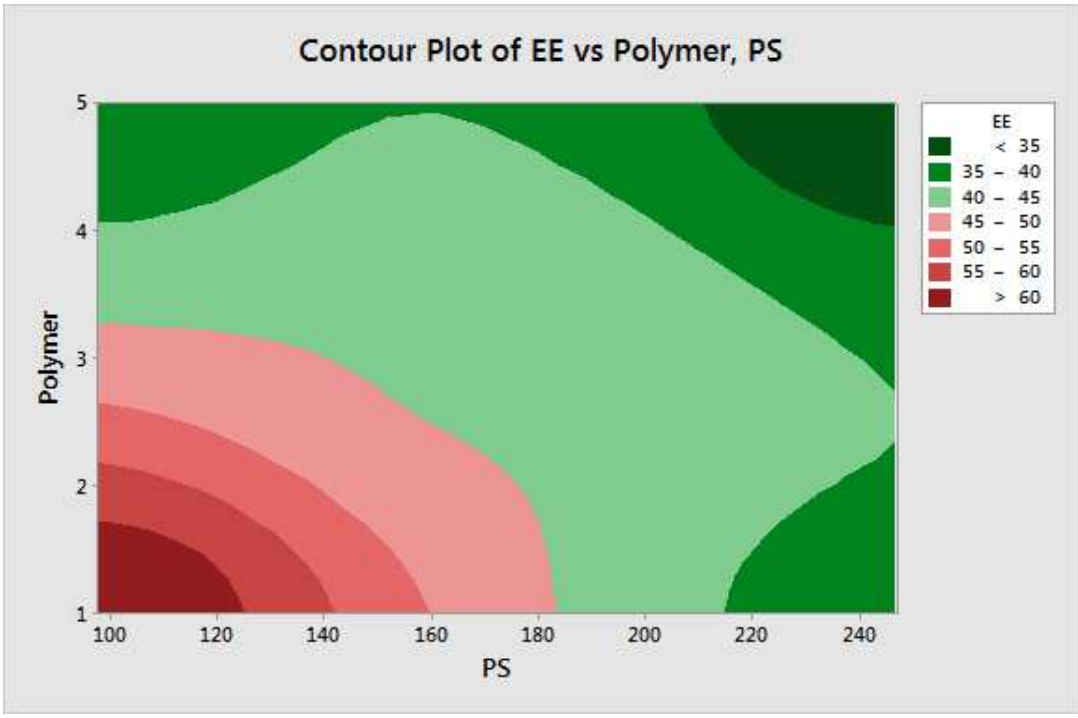

Figure number 4 Contour plot of Entrapment efficiency versus Lipid and Particle size

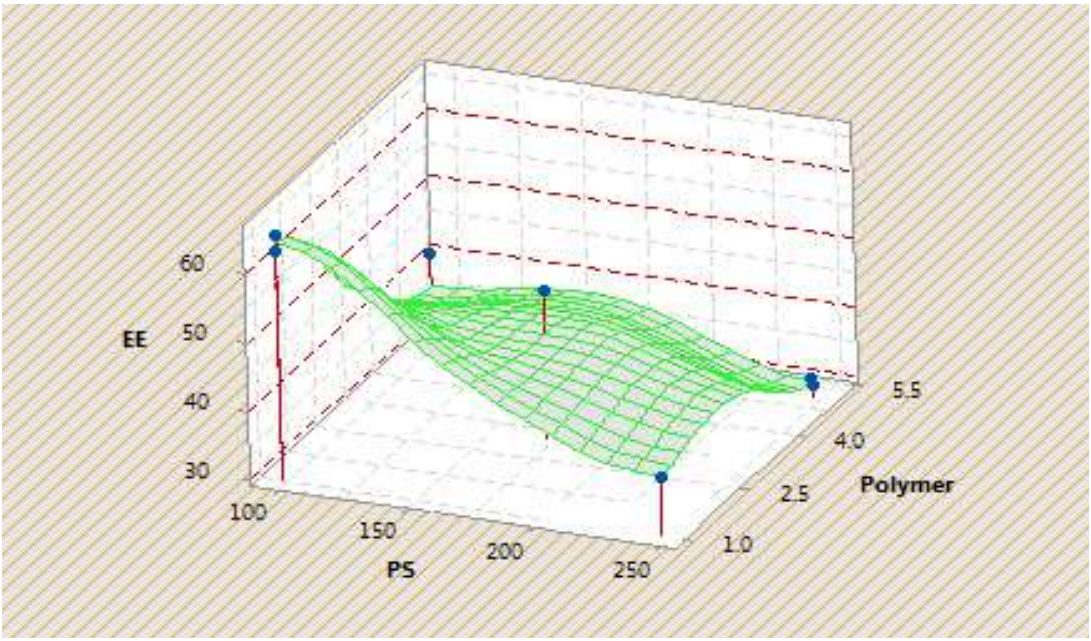

Figure number 5 3D-Scatter plot for Entrapment Efficiency

The optimized ratio of 1:1 ratio of carbamazepine nanoparticles was given primary importance for further studies. Also the particle size of the optimized ratios was found satisfactory which enables us to consider it for further *in vitro* release studies.

Further the effect of the particle size and PLGA was thoroughly proven by using full factorial design. Contour plot and 3D-scatter plots were generated using Minitab 17, which was shown in Figure number 4 and 5 respectively. The scatter plot has shown the influence of the different ratios of the polymer and particle size on the entrapment efficiency. The best was selected based on the statistical data supportive evaluation. In the graph it can be seen that the polymer ratio of 1 has correlated with the highest entrapment efficiency and also the lowest particle size. Contour plot further strengthens the evidence that indicates the various colored regions with different entrapment efficiency.

Scanning electron microscopy

Scanning electron microscopy was performed for polymeric nanoparticles with drug: polymer ratio of 1:1 to obtain more information on the particle size and morphology. The photos of polymeric nanoparticles had shown that the formulated carbamazepine polymeric nanoparticles of PLGA polymer were of spherical shape with size range from 112 to 139 nm, which were shown in Figure Number 6.

The other major aspect where the polymeric nanoparticles were considered advantageous was because of their smooth surface. The presence of smooth surface contributes to the drug release in sustained manner contrary to the rough surface. So the nanoparticles sustained property can be evaluated in further studies as sustained release of the drug have major benefits to the patient reducing the frequency of the administration.

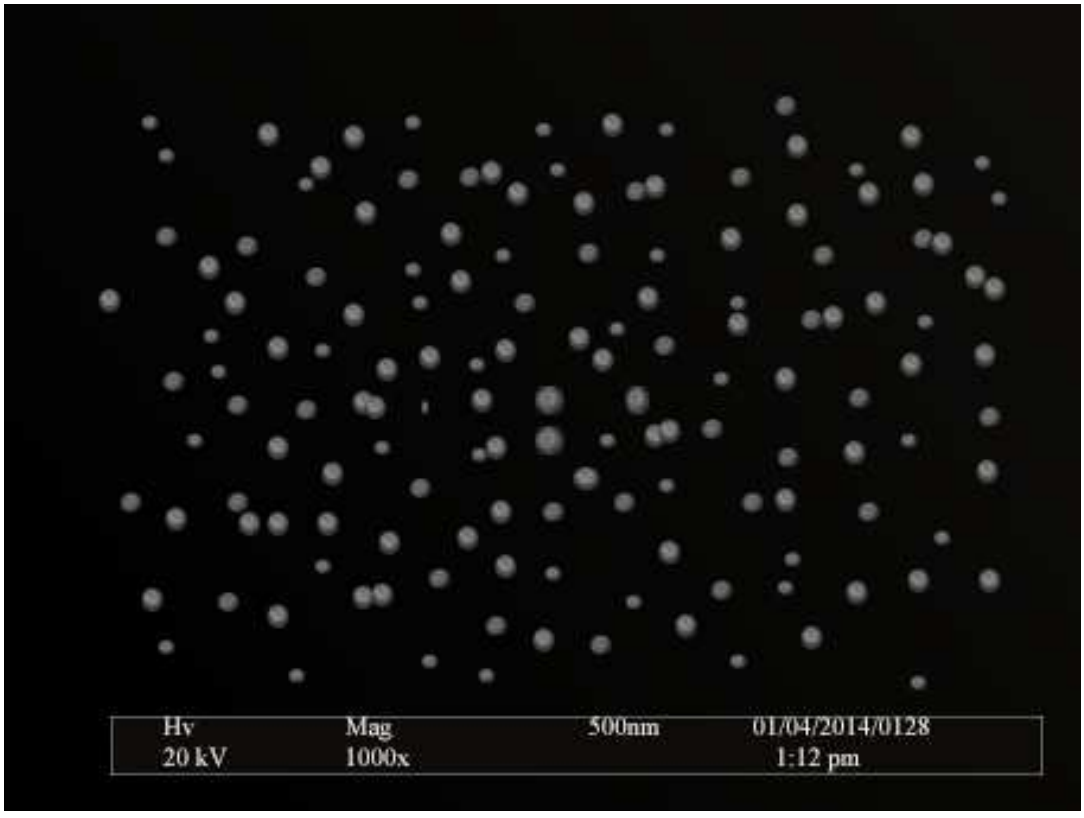

Figure number 6 SEM image of Carbamazepine PLGA nanoparticles (Drug:polymer ratio of 1:1)

***In vitro* drug release studies**

The cumulative percentage drug release from carbamazepine polymeric nanoparticles with drug: polymer ratio of 1:1 was observed by using dissolution test apparatus in buffer p^H 7.4. This was shown in figure number 7.

Carbamazepine nanoparticles released 50 percentage of the drug with in 4 hours, which correlates with the onset of action. Later the nanoparticles has shown significant sustained release effect by prolonging the drug release at 89.92±0.01% till 24 hours. The use of the polymer PLGA has a significant effect on the drug sustained release over a prolonged time, but the initial drug release more than 50±0.12% with in 4 hours can be attributed to the part of unencapsulated drug inside the polymer. This suggests *in vitro* drug release exhibited biphasic pattern by initial burst release followed by sustained release. Pure drug was also used in order to the compare the effectiveness of the carbamazepine polymeric nanoparticles. Pure drug has initially shown an increased release of the drug when compared to the nanoparticles till one hour, there after 90 minutes it has reached 49.21± 0.02%. The cumulative drug release was observed constant for the pure drug, which did not increase with increase in the time, which elevates the importance of sustained release property of the polymeric nanoparticles

So the main rationale behind opting for *in vitro* studies is to find the drug release at particular intervals, which gives us an indication about the solubility of the drug. Increased release of the drug correlates with the increased solubility which can be attributed to the fact that lower particle size of the nanoparticles had caused an increase in the effective surface area which in turn increases the solubility. This supports our rationale of increasing solubility, which may improve the drug oral bioavailability due to the polymeric nanoparticles with sustained release property. So this formulation can be used as an alternative to the oral conventional formulation, which suffers poor oral bioavailability to the low solubility of the drug.

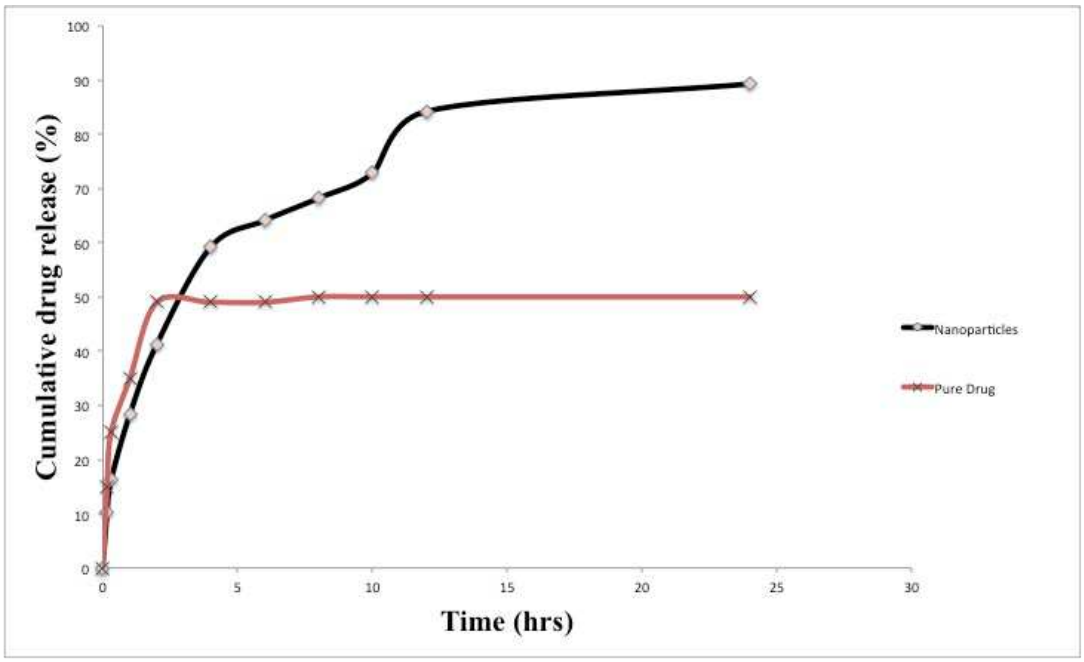

Figure number 7 *In vitro* drug release profile of Carbamazepine nanoparticles
Results were presented as mean ±SD, n=3

CONCLUSION

It can be concluded that carbamazepine, which is normally an poorly water soluble drug having poor oral bioavailability can be improved by formulating it as polymeric nanoparticles. Carbamazepine nanoparticles were prepared using polymer PLGA in drug: polymer ratio of 1:1 using solvent emulsification evaporation method. The optimized batch yielded better entrapment efficiency, drug content and cumulative drug release when compared to others. The optimized batch was selected using statistical software DOE and shown by contour plots and 3D-surface plots. *In vitro* drug release studies concluded that carbamazepine nanoparticles released drug in biphasic pattern by initial burst release, which was followed by sustained release. Formulated polymeric nanoparticles have achieved sustained release over a prolonged period of 24 hours, which can benefit the patient in decreasing the dosing frequency. So we can conclude that nanoparticles prepared by this method using the same polymer with the optimized ratio can represent as potential drug delivery approach for treating epilepsy.

Recommended Future Research
Further *in vivo* studies can be carried to estimate the pharmacokinetic parameters and the sustained release property of the carbamazepine nanoparticles. Oral bioavailability enhancement can be observed clearly in *in vivo* studies.

Acknowledgements
Dr. M. N. Satish Kumar guided the study with his kind help during all stages of my research. Ms. Ashwati Prakash contributed in the preparation of manuscript.

REFERENCES

[1] Banerjee Pn; Filippi D; Allen Hauser W, *Epilepsy research*., **2009** ,85(1), 31-45.
[2] Sharma Nancy; Mishra Neeraj, *Journal of pharmaceutical and scientific innovation*., **2014**,3(3),199-207.
[3] Dressman JB; Amidon GL; Reppas C; Shah VP, *Pharm. Res*., **1998**, 15, 11-22.
[4] Guidance for Industry: Waiver of *in vivo* BA studies for Immediate Release Solid Oral Dosage Forms containing certain Active Moieties/Active Ingredients based on a BCS, US Department of Health, Food and Drug Administration, Center for Drug Evaluation and Research, **January 1999**.
[5] Barratt G, *Cell. Mol. Life Sci*., **2003**, 60,21-37.
[6] L Haibin; D Xuechen, *Nanoscience Reviews*., **2006**, 11, 207-209.
[7] Naima Z; Siro T; Juan-Manuel GD; Chantal C; René C; Jerome D, *Eur J Pharm Sci* .,**2001**,2,395-404.
[8] Vasconcelos T; Sarmento B; Costa P, *Drug Discov Today*.,**2007**, 12,1068-75.
[9] Nahla S; Barakat Mahasen A; Radwan, *Drug Delivery*., **2006**, 13,1–10.
[10] Ramsay R E; McManus D Q; Guterman A; Briggle T H; Vazquez D; Perchalski R, *Therapeut. Drug Monit*.,**1990**, 12,235–241.
[11] McCarron PA; Hall M, *Int J Pharm*., **2008**, 348,115-124.
[12] Shammi Goyal; Jitendra Kumar Rai; R. K. Narang; Rajesh K. S, *Int J Pharmacy Pharm Sci*., **2010**,2(2),1-6.
[13] Pathiraja A; Gunatillake; Raju Adhikari, *European Cells and Materials*., **2003**, 5,1-16.
[14] Lam XM; Duenas ET; Daugherty AL; Levin N; Cleland JL, *J. Control. Release* ., **2000**,67, 281-292.
[15] Cohen S; Yoshioka T; Lucarelli M; Hwang LH; Langer R, *Pharm. Res*.,**1991**, 8,713-720.
[16] Whittlesey KJ; Shea LD, *Exp. Neurol*., **2004**, 190, 1-16.
[17] Woo BH; Jiang G; Yeong W; DeLuca PP, *Pharm. Res*., **2001**, 18,1600-1606.
[18] Dong HN; Youna YS; Leea SD; Sonb MW; Kimb WB; DeLucac PP; leea KC, *J. Control. Release*.,**2003**, 92, 291-299.
[19] Mu L; Feng SS, *J. Control. Release*., **2003**, 86,33-48.
[20] Okada H; Toguchi H, Crit. Rev. *Ther. Drug Carrier Syst*.,**1995**, 12,1-99.
[21] Gabor F; Ertl B; Wirth M; Mallinger R, *J. Microencapsul*., **1999**, 16,1-12.
[22] Shashank T; Satish kumar MN; Ashwati Prakash; Shashank M; Raju KRS, *Indo American Journal of Pharm Research*.,**2014**,4(08), 3579-3587.
[23] Badry M; Fetih G; Fathy M, *Saudi Pharmaceutical Journal*.,**2009**, 17(3), 219-230.
[24] Naik JB; Mokale VJ, *International Journal of Drug Delivery*.,**2013**, 5,300-308.
[25] Avinash B; Steven JS; Karen I, *Int. J. Pharm.*, **2007**, 51,87-92.
[26] Mohideen B; Ezhilmuthu RP, *International Journal of Biological & Pharmaceutical Research*.,**2013**, 4(7),533-540.