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Formulation and Evaluation of Pregabalin Loaded PLGA Nanoparticles

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Abstract : The aim of the present research was formulation and evaluation of Pregabalin loaded PLGA Polymeric naoparticles (PNP1-PNP9) for the controlled release of Pregabalin using different concentrations of PLGAand surfactant. Pregabalin loaded PLGA Polymeric naoparticles (PNP1-PNP9) Nanoparticles were characterized for various physical parameters such as particle size, zeta potential and particle size distribution and chemical parameters such as drug content, entrapment efficiency and *In vitro* drug release studies. The prepared Pregabalin loaded PLGA Polymeric nanoparticles with 150 mg of PLGA and 1.5% of surfactant concentration have shown average particle size 125.7±0.43nm, average zeta potential of --25.4±0.43 mV, average entrapment efficiency 95.35±0.31%, average drug content of 99.82±0.73% and average i*n vitro* drug release 99.85±0.09 % at the end of 24 hrs. DSC and FTIR study concluded that there was no interaction took place between the Pregabalin and other excipients used in the formulation of nanoparticles.
Key words: PLGA, Pregabalin, Zeta potential, Particle size.

Introduction

In recent years, nanoparticles are gaining more attention as these systems deliver the drug controlled release of drug for long period of time to maintain the steady state blood level concentration of drug, therefore providing reduction in the dosing frequency and increasing patient compliance. These systems are designed mainly for the drugs which are required to be taken frequently[1,2].

Pregabalin is a gamma-aminobutyric acid analog anticonvulsant drug used for neuropathic pain and partial seizures. Conventional pregabalin formulations are not very effective as the drug does not reach the target site in effective concentrations. Thus, effective treatment needs an increased dose size, which may lead to unwanted side effects. To overcome these conditions, a controlled release drug delivery approach is employed to bring about controlled release of Pregabalin to brain cells in effective concentration. To achieve this PLGA is selected for the preparation of nanoparticles because of their excellent physicochemical properties and biocompatible nature which are beneficial for biomedical use.

Pregabalin has a shorter biological half life which are required to be taken frequently which leads to poor patient compliance. Hence it is a need to reduce the frequency of administration[1-4].

These systems have been investigated primarily for controlled drug delivery of Pregabalin, and also for the enhancement of dissolution rate/bioavailability of Pregabalin.

So the aim of the present study was to formulate Pregabalin nanoparticles to deliver the controlled release of drug so that its frequency of administration and its bioavailability can be enhanced.

Materials and Methods

Materials

Pregabalin was obtained as a gift sample from Bafna pharmaceuticals chennai. PLGA ,Pluronic F68 and Acetone were purchased from Chemika-Biochemika-Reagents, Mumbai. All other chemicals and reagents used were of Analytical grade.

Methods

Formulation of Pregabalin Loaded PLGA Nanoparticles

Pregabalin loaded PLGA nanoparticles by nanoprecipitation

PLGA in various concentrations and Pregabalin are dissolved in acetone to form the organic phase. The organic phase was added slowly to 10 ml of aqueous phase containing various concentrations of Pluronic F-68 following which the organic solvent was allowed to evaporate for 4 hours with continuous stirring (50 rpm) on magnetic stirrer . The NP suspension was then centrifuged at 13,000 rpm for 1hr at 4º C using high speed centrifuge and the sediment is comprising Pregabalin loaded PLGA nanoparticles was freeze dried for 24 hours using 2% D-mannitol as a cryoprotectant[5-7]. Formula used for the preparation of Pregabalin loaded PLGA nanoparticles were given in table 1.

Table 1. Formula used for the preparation of Pregabalin loaded PLGA nanoparticles

S.No	Formulation	Drug (mg)	Polymer PLGA (mg)	Pluronic F-68 (%)	Acetone (ml)
1.	PNP1	100	100	0.5	25ml
2.	PNP2	100	100	1	25ml
3.	PNP3	100	100	1.5	25ml
4.	PNP4	100	150	0.5	25ml
5.	PNP5	100	150	1	25ml
6.	PNP6	100	150	1.5	25ml
7.	PNP7	100	200	0.5	25ml
8.	PNP8	100	200	1	25ml
9.	PNP9	100	200	1.5	25ml

Evaluation[6,7]

Compatibility studies – By FTIR and DSC

Fourier Transform Infra Red spectroscopy

IR spectra of Pregabalin and other excipients used in the formulation were recorded by using "Perkin-Elmer FTIR." The sample for the IR spectroscopy was prepared by mixing the samples with spectroscopic grade KBr and compressed into transparent pellets, then scanned in the IR range from 500 to 4000 cm^{-1} with a resolution of 4 cm.[1]The results were given in results and discussion section.

Differential Scanning calorimetry (DSC technique)

Differential Scanning Calorimetry studies were carried out using "Schimadzu DSC-60.[1] In this study, Pregabalin was mixed with the excipients used in the formulation and thermal analysis of each sample was

carried out. During the study, the temperature range from 25 to 600º C, heating rate 10ºC/min and flow rate of nitrogen 30 ml/min were maintained. Approximately 5mg of samples was taken in aluminum pan sealed and the thermogram was recorded.

The results were given in results and discussion section.

Particle size and zeta potential

The prepared nanoparticles were evaluated for their particle size and zeta potential by photon correlation spectroscopy (PCS) using Zetasizer. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was carried out at 25°C with an angle of detection of 90°.

Drug content[6,7]

Standard preparation

Weighed accurately 25mg of Pregabalin and transferred in to a 25 ml standard flask. The sample was dissolved with 5 ml of pH 6.8 phosphate buffer and diluted to 25 ml with buffer. 1ml of this solution was diluted to 25ml with buffer solution.

Sample preparation

Weighed accurately 1gm of Pregabalin nanoparticles and transferred in to a 25 ml standard flask. The sample was dissolved with 5 ml of pH 6.8 phosphate buffer and diluted to 25 ml with pH 6.8 phosphate buffer. 1ml of this solution was diluted to 25ml with buffer solution.

Then the standard and sample absorbancewere measured at 210 nm using a UV-Visible spectrophotometer. From the absorbance values the percentage of drug content was calculated.

Entrapment efficiency

Separation of unentrapped Pregabalin from the prepared Pregabalin nanoparticles were carried out by centrifugation method. Pregabalin nanoparticles formulations were centrifuged at 15000 rpm for 30min. The supernatant solution was separated. 1ml of this supernatant was diluted with water and the absorbance was measured at 210 nm using water as blank. The amount of Pregabalin unentrapped in the supernatant was calculated. The amount of Pregabalin entrapped was determined by subtracting amount of free unentrapped Pregabalin from total amount of Pregabalin taken for the preparation[8,9].

The formula used to calculate encapsulation efficiency was given below

$$\text{Encapsulation efficiency} = \frac{\text{Entrapped drug (mg)}}{\text{Total amount of drug added (mg)}} \times 100$$

In vitro drug release studies

In vitro release studies were performed using dialysis membrane method. The prepared Pregabalin nanoparticles formulation was placed inside a dialysis membrane immersed in pH 6.8 phosphate buffer. At predetermined time intervals the sample was withdrawn and the amount of Pregabalin released was determined by measuring the absorbance at 210 nm using a UV-Visible spectrophotometer. From the absorbance values the cumulative percentage drug release was calculated [6,7,9,10].

Results and Discussion

Compatibility study using IR and DSC

- In the IR spectrum of Pregabalin standard consists of characteristics band values at 3834.20 cm^{-1}(C-H-bending), 2846.72 cm^{-1}(C-H-stretching) and 1550.65cm^{-1} (N-H-stretching). These characteristic band values were observed in all the recorded IR spectra.
- DSC of Pregabalin showed a sharp endothermic peak at 204.92^{0}C (melting point). The physical mixture of Pregabalin and other excipients also showed the same thermal behavior as the individual component.
- DSC results also revealed that the physical mixture of Pregabalin with excipients showed superimposition of the thermograms. There was no significant change observed in melting endotherm of physical mixture of Pregabalin and excipients.
- From the IR and DSC studies, it was found that there were no interaction took place between Pregabalin and the other ingredients used in the formulation of Pregabalin nanoparticles. The IR spectra and DSC images were shown from **Fig.1 to Fig. 5.**

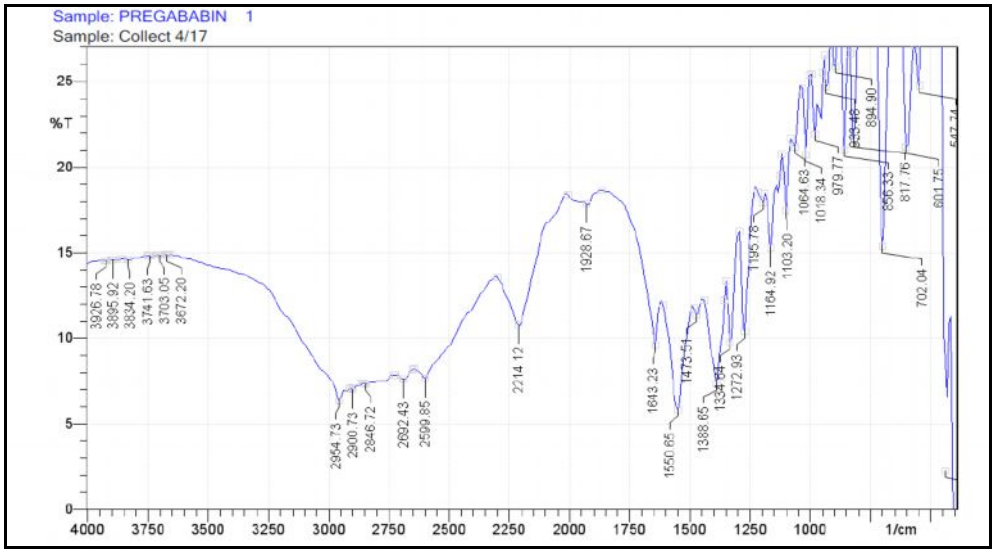

Fig.1. IR spectrum of Pregabalin

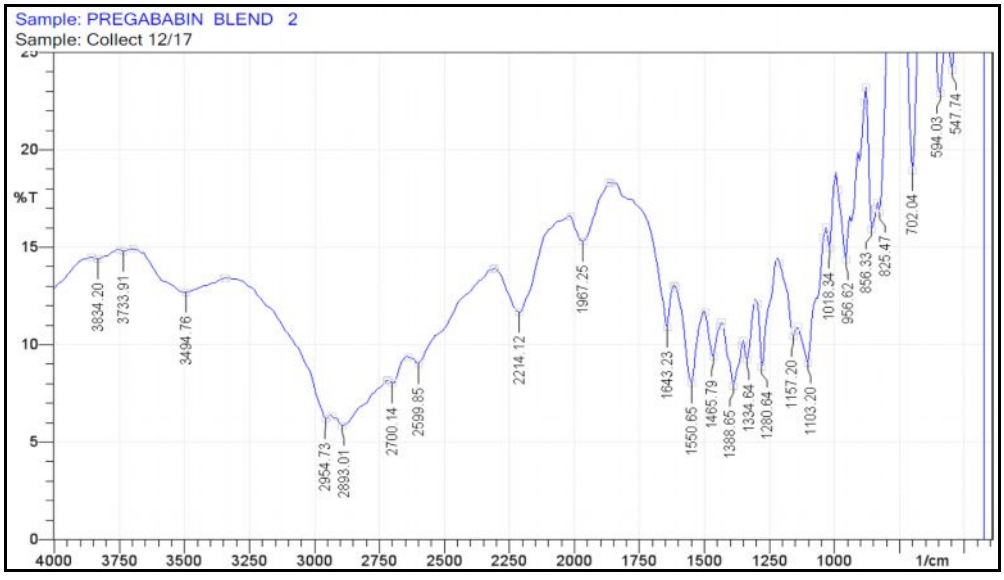

Fig.2. IR spectrum of blend of Pregabalin nanoparticles

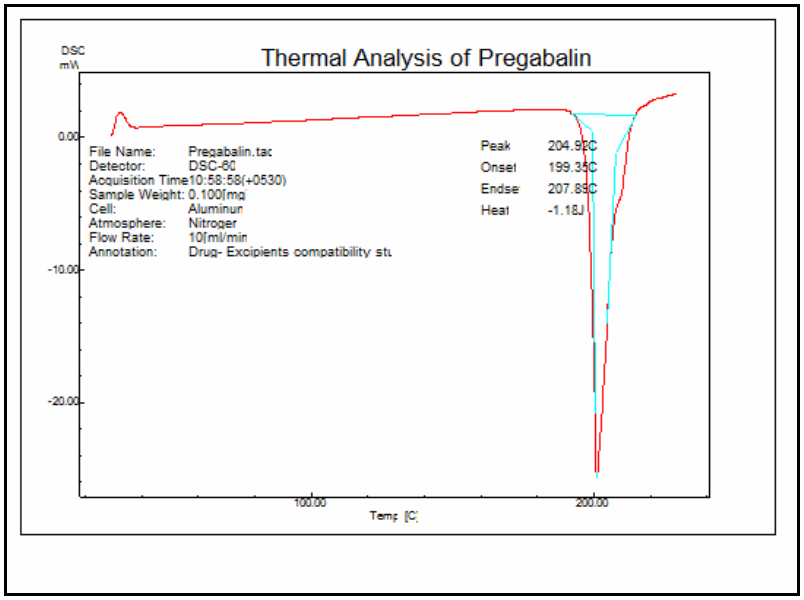

Fig.3. DSC of Pregabalin

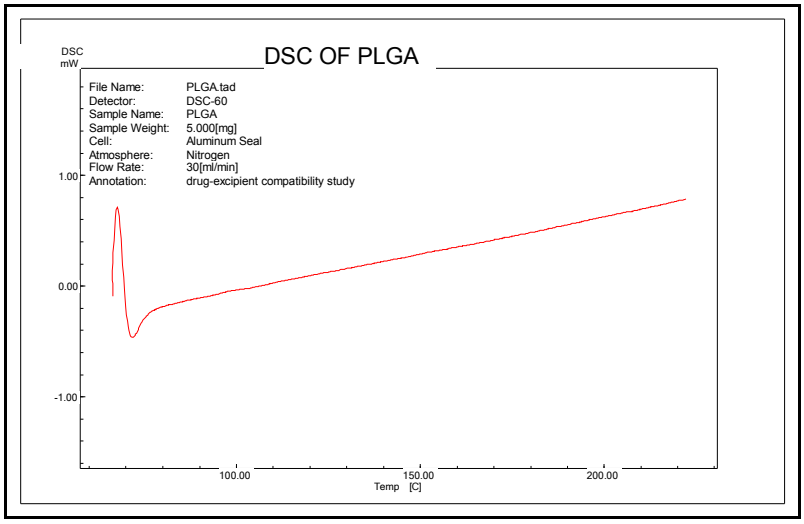

Fig.4. DSC thermograms of PLGA

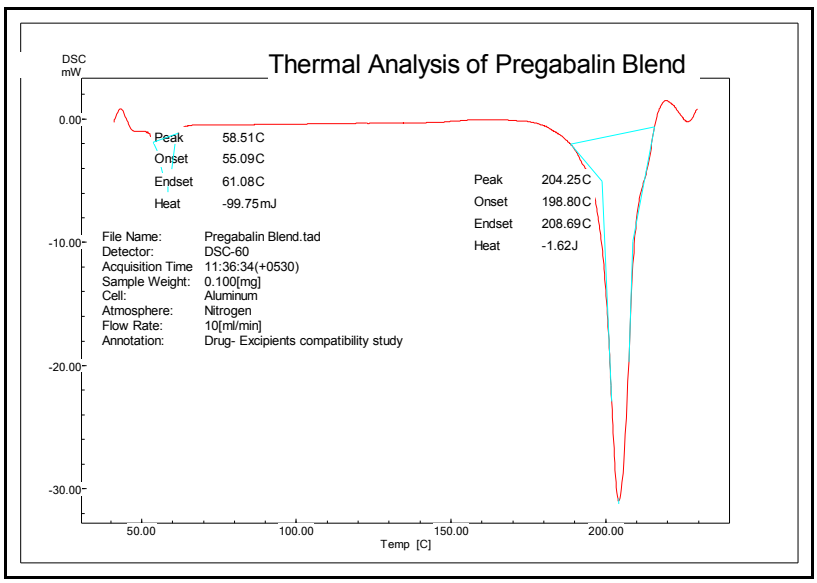

Fig.5. DSC of Pregabalin-PLGA nanoparticles

Particle size , Zeta potential, Entrapment efficiency and drug content[11-16]

Table 2. Zeta potential, Particle size and Entrapment efficiency of PNP1-PNP9

Trials	Particle size(nm)	Zeta potential (mV)	Entrapment Efficiency (%)	Drug content(%)
PNP1	85.7±0.24	-26.8±0.35	45.81±0.34	99.34±0.11
PNP2	80.3±0.17	-25.3±0.46	53.73±0.18	99.56±0.22
PNP3	75.7±0.43	-24.9±0.81	60.84±0.62	99.62±0.55
PNP4	155.8±0.29	-28.7±0.65	72.96±0.09	99.39±0.87
PNP5	125.7±0.43	-26.6±0.25	86.83±0.19	99.65±0.16
PNP6	95.5±0.28	-25.4±0.43	95.35±0.31	99.82±0.73
PNP7	300.8±0.65	-29.7±0.37	95.47±0.72	99.29±0.18
PNP8	245.3±0.57	-27.3±0.58	95±.610.52	99.53±0.38
PNP9	178.3±0.87	-25.2±0.29	95.84±0.56	99.78±0.27

mean±S.D, n=3

- The mean particle size of Pregabalin loaded PLGA nanoparticles is shown in Table …. .. The average particle sizes of the formulations were range from 75.7±0.43 nm to 300.8±0.65 nm (PNP1-PNP9)
- The smallest particle size of the developed formulation was found in trial PNP1 (75.7±0.43nm) which contains 100 mg of PLGA and 1.5% of surfactant concentrations
- The largest particle size was found in trial PNP7 (300.8±0.65 nm) which contains 200 mg of PLGA and 0.5% of surfactant concentrations.
- The ideal particle size (95.5±0.28 nm) and drug entrapment efficiency (95.35±0.31) was obtained with trial PNP6 which contains 150 mg of PLGA and 1.5% of surfactant concentrations. The presence of suitable concentration of surfactant are effective in lowering the interfacial tension resulting in smaller particle size of 95.5±0.28 nm.
- The results suggested that the particle size and drug entrapment efficiency of prepared nanoparticles, dependant on PLGA and surfactant concentrations.
- Based on the results it was found that an increase in PLGA concentration increases the particle size and entrapment efficiency significantly. The concentration of surfactant plays a major role in the reduction of particle size and zeta potential and in the enhancement of drug entrapment efficiency.
- Particle size and entrapment efficiency of the Pregabalin Nanoparticles (PNP1-PNP6) were increased with increasing the PLGA concentration up to 150 mg. This may be due to high amount of availability of PLGA to encapsulate the drug, upon increasing the PLGA concentration, number of layers of coated drug was increased, and this resulted in increased particle size and entrapment efficiency.
- Further increase in the PLGA concentration to 200 mg as in (PNP7 and PNP9), there is no much increase in the entrapment efficiency due to the availability of the drug to be incorporated is low which is not enough for further encapsulation of drug by PLGA.
- There was no significant changes in the drug content of all the formulations. The results of particle size and zeta potential were given in fig.6 to fig.14.

Particle size of Pregabalin nanoparticles (PNP1-PNP9)

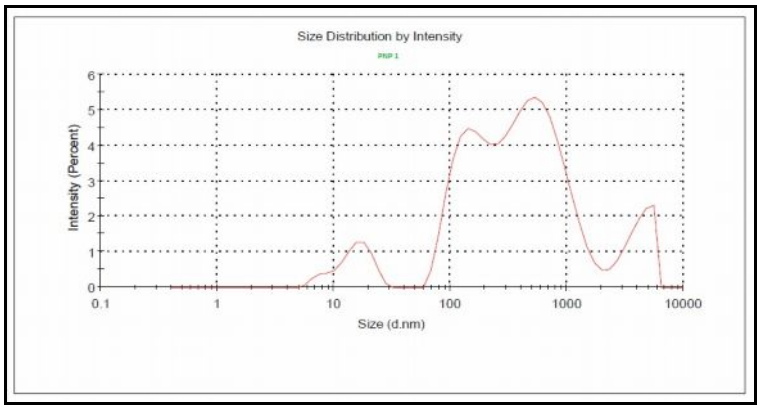

Fig.6. Pregabalin nanoparticles PNP1

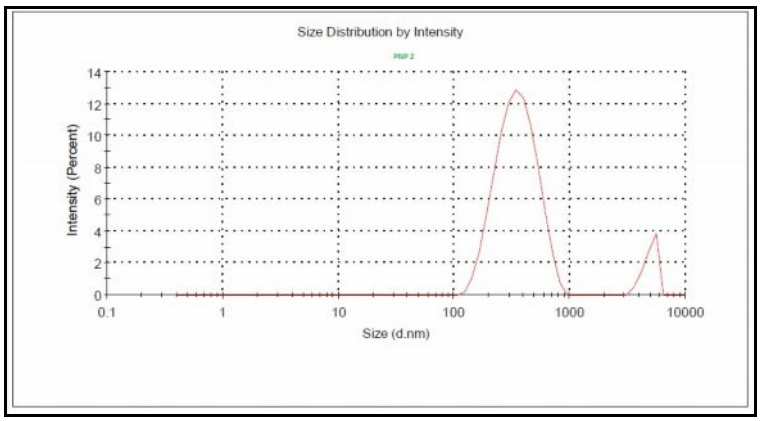

Fig.7. Pregabalin nanoparticles PNP2

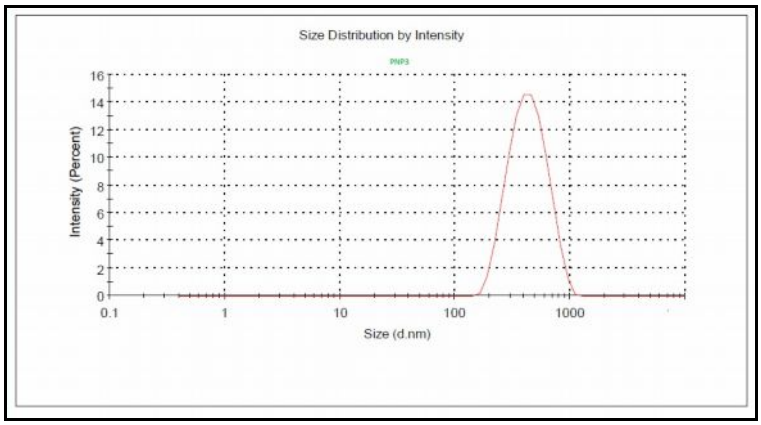

Fig.8. Pregabalin nanoparticles PNP3

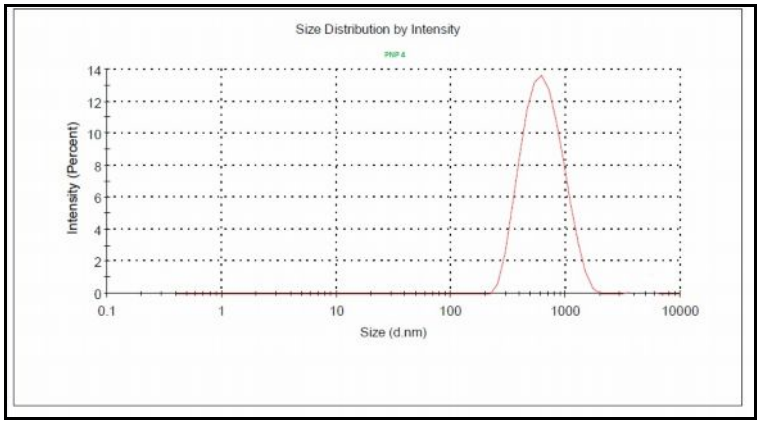

Fig.9. Pregabalin nanoparticles PNP4

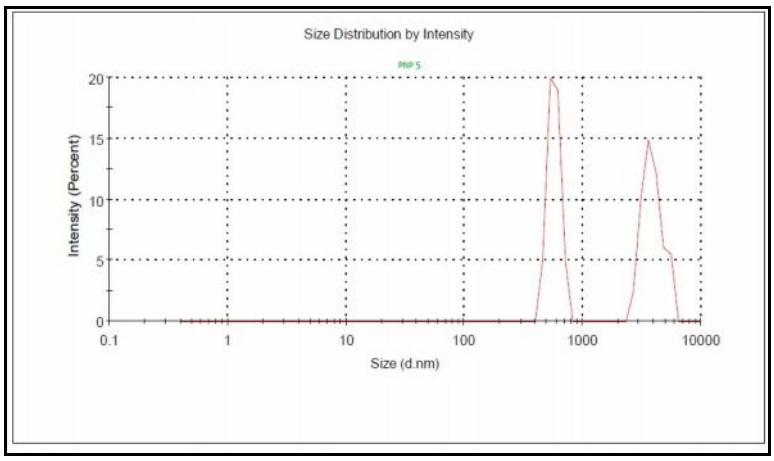

Fig.10. Pregabalin nanoparticles PNP5

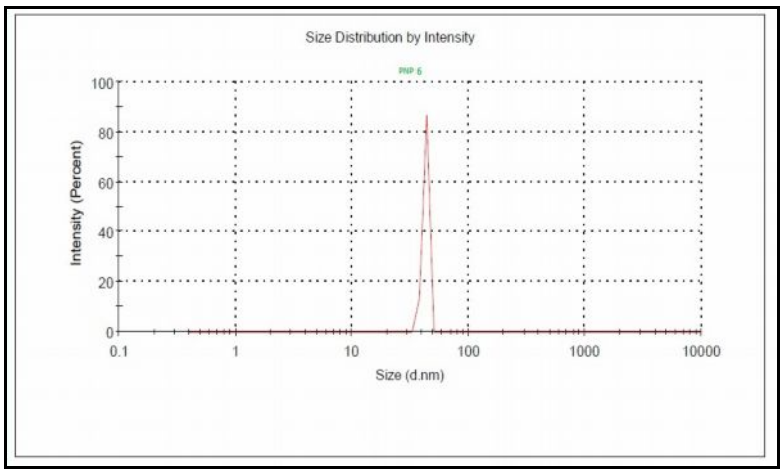

Fig.11. Pregabalin nanoparticles PNP6

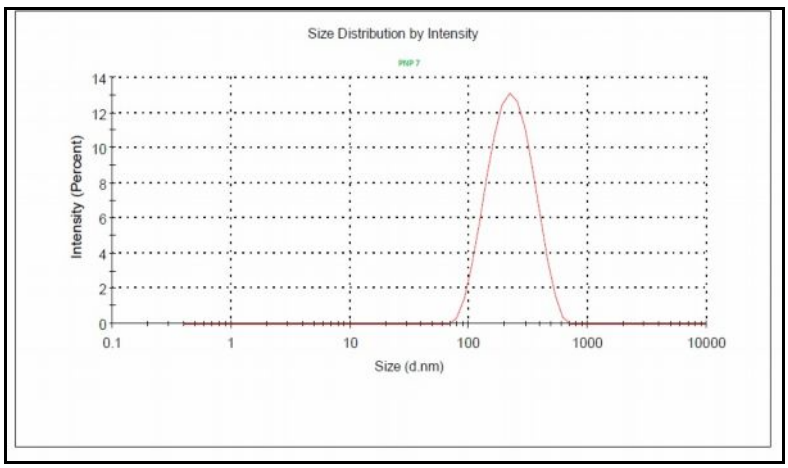

Fig.12. Pregabalin nanoparticles PNP7

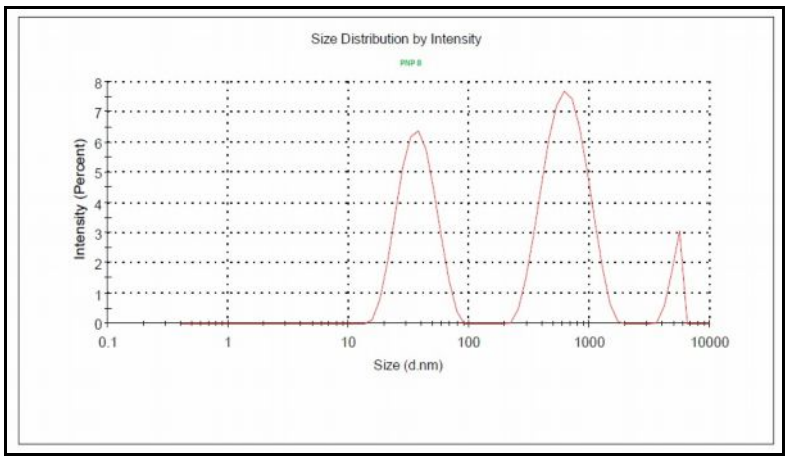

Fig.13. Pregabalin nanoparticles PNP8

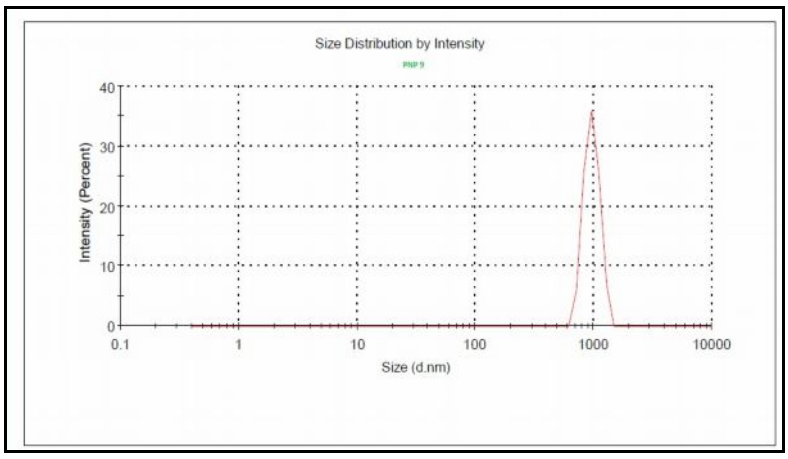

Fig.14. Pregabalin nanoparticles PNP9

In vitro drug release studies

The results of in vitro release were given in table 3

Table 3. Percentage *In vitro* drug release of PNP1-MNP3

Trials/ Time (h)	PNP1	PNP2	PNP3
0	**0**	**0**	**0**
1	6.25±0.17	7.04±0.05	8.18±0.14
2	12.48±0.32	14.35±0.16	15.86±0.07
4	24.96±0.69	26.36±0.56	28.45±0.53
6	37.41±0.73	39.35±0.83	41.62±0.45
8	49.91±0.56	51.46±0.29	53.41±0.36
12	74.84±0.48	76.32±0.12	78.92±0.82
16	99.76±0.37	99.84±0.58	99.82±0.64
24	99.83±0.45	99.84±0.81	99.83±0.27

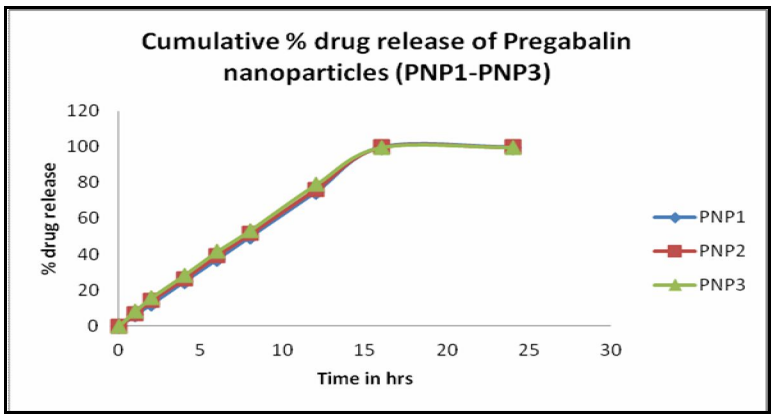

Fig.15. Cumulative percentage drug release of PNP1-PNP3

Table 4. Percentage *In vitro* drug release of PNP4-PNP6

Trials/Time (h)	PNP4	PNP5	PNP6
0	0	0	0
1	4.08±0.35	4.11±0.29	4.14±0.12
2	8.19±0.18	8.28±0.36	8.33±0.45
4	16.35±0.46	16.53±0.16	16.64±0.31
6	24.54±0.78	24.84±0.47	24.96±0.53
8	32.73±0.27	33.10±0.73	33.79±0.34
12	49.08±0.86	49.27±0.08	49.93±0.78
16	65.44±0.19	66.18±0.29	66.57±0.23
24	98.15±0.65	99.28±0.13	99.85±0.09

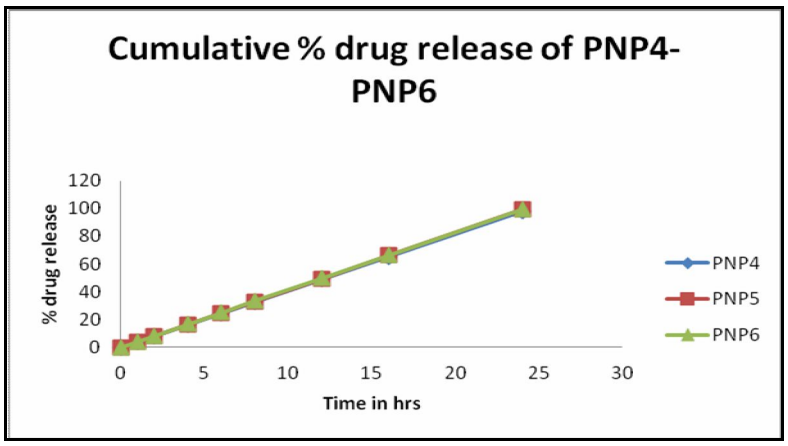

Fig.16. Cumulative percentage drug release of PNP4-PNP6

Table 5. Percentage *In vitro* drug release of PNP7-PNP9

Trials/Time (h)	PNP7	PNP8	PNP9
0	**0**	**0**	**0**
1	4.03±0.27	4.05±0.08	4.06±0.28
2	8.07±0.35	8.11±0.25	8.15±0.55
4	16.13±0.76	16.19±0.11	16.31±0.23
6	24.21±0.87	24.29±0.34	24.46±0.63
8	32.27±0.06	32.38±0.78	32.61±0.39
12	48.41±0.21	48.58±0.92	48.92±0.31
16	64.55±0.47	64.77±0.39	65.21±0.16
24	96.81±0.53	97.15±0.25	97.82±0.05

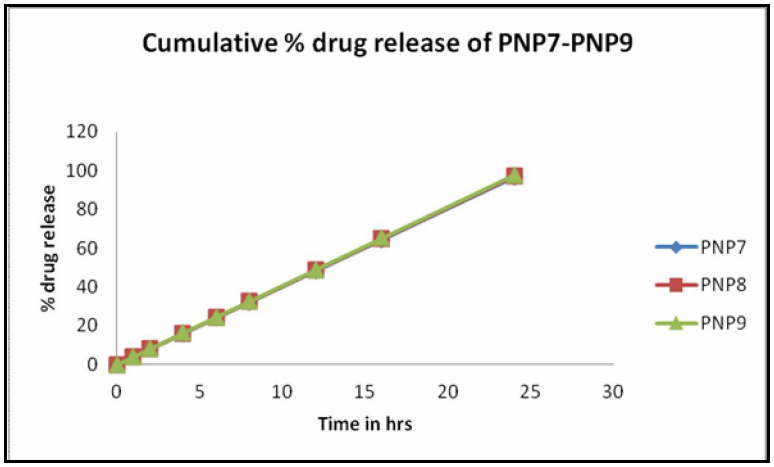

Fig.17. Cumulative percentage drug release of PNP7-PNP9

- The *in vitro* drug release rate of all the trials (PNP1-PNP9) were decreased with increasing the PLGA concentrations and increased with increasing surfactant concentrations.
- The smaller size Pregabalin nanoparticles prepared with lower concentration of PLGA (100mg) as in trials PNP1-PNP3 exhibited maximum drug release within 16hrs(99.76±0.37, 99.84±0.58 and 99.82±0.64 for MNP1,MNP2 and MNP3 respectively) this may be due to the increased surface area of the pregabalin nanoparticles resulting in larger drug fraction exposed to the dissolution medium.
- The *invitro* drug release of PNP1-PNP3 were found to be not desirable as the concentration of PLGA (100mg) and surfactant (0.5%, 1% and 1.5%) were not sufficient to extend the drug release up to 24hrs.
- On the other hand, for the trials (PNP7, MNP8 and PNP9), the maximum percentage of drug release at the end of 24 hrs were found to be 96.81±0.53, 97.15±0.25 and 97.82±0.05 respectively which contains 200 mg of PLGA and surfactant (0.5%, 1% and 1.5%) concentrations that prolongs the Pregabalin release from nanoparticles.
- From the *in vitro* drug release studies results of the Pregabalin nanoparticles (PNP1-PNP9), the maximum percentage drug release (99.85±0.09) at the end of 24h was observed with trial PNP6 which contains 150mg of PLGA and 1.5 % of surfactant concentrations. The controlled release of Pregabalin nanoparticles may be due to the slower diffusion of dissolved pregabalin within the PLGA core of the nanoparticles in to the dissolution medium[11-16].
- From the *in vitro* drug release data for PNP1-PNP9, it was observed that the concentration of PLGA and surfactant plays a major role in the *in vitro* drug release profile of prepared Pregabalin nanoparticles.
- From all the trials PNP6 was selected as optimized formulation for further performance evaluation study, comparative study and stability study because of its ideal particle size, high entrapment efficiency, the desirable and maximum drug release.

Conclusion

In this study an attempt was made to study controlled release pregabalin nanoparticles which can provide controlled drug release for up to 24 hrs. Pregabalin nanoparticles were formulated and evaluated. Pregabalin nanoparticles were prepared with different concentrations of PLGA and surfactants were optimized by conducting various trials. The optimization procedure aided in the preparation of Pregabalin nanoparticles with controlled drug release up to 24 hrs. The *in vitro* dissolution studies revealed that the formulated Pregabalin nanoparticles released the desired concentration of the drug continuously for 24 hrs. Hence it may be concluded that the newly formulated nanoparticulate drug delivery systems of Pregabalin provided an insight into the therapeutic effectiveness of the designed formulation for the treatment of chemotherapy induced neuropathy pain and epilepsy.

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