

Preformulation Parameters Characterization to Design, Development and Formulation of Pregabalin Loaded Nanoparticles

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Abstract

Objective: The purpose of the present study was to systematically investigate some of the important physicochemical properties of pregabalin loaded nanoparticles. Almost all drugs are marketed as tablets, capsules or both. Prior to the development of these major dosage forms, it is essential that certain fundamental physical and chemical properties of the drug molecule and other divided properties of the drug powder are determined.

Method: The physicochemical properties such as solubility, pKa, dissolution, melting point, assay development, stability in Solution, stability in solid state, microscopy, bulk density, flow properties, excipient compatibility, entrapment efficiency and release profile of pregabalin loaded nanoparticles was carried out.

Results: A modified release nanoparticles of pregabalin were prepared by using PLGA as coating material. So before selection of excipients, the Preformulation study of drug pregabalin is completed for successful formulation of modified release nanoparticles. The result of Preformulation studies shows good flow properties, excipient compatibility, entrapment efficiency and release profile of nanoparticles.

Conclusion: From this study we concluded that pregabalin with PLGA can be used to formulate pregabalin nanoparticles for modified release.

Key words: Nanoparticles, entrapment efficiency, Pregabalin, modified release, Preformulation

INTRODUCTION

Pregabalin S-(3)-amino methyl hexanoic acid, is a structural analogue of γ -amino butyric acid (GABA). They constitute an important group of compounds that are used in the treatment of epilepsy and neuropathic pain. It is a white and crystalline solid. It is soluble in water and both acidic and basic aqueous solutions. Pregabalin has been studied for use in variety of disorders, including monotherapy in refractory partial seizures, diabetic neuropathy, surgical dental pain and other pain syndromes, post therapeutic neuralgia and social anxiety disorders. Pregabalins innovator is Pfizer-global and appears worldwide under the brand name Lyrica. The half-life of pregabalin is 5-6.5 hrs.¹ Preformulation commences when a newly synthesized drug shows sufficient pharmacologic promise in animal models to warrant evaluation in man. These studies should focus on those physicochemical properties of the new compound that could affect drug performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rational for formulation design, or support the need for molecular modification.² The aim of this study was to determine some of the physicochemical properties such as solubility, pKa, dissolution, melting point, assay development, bulk density, flow properties, excipient compatibility, entrapment efficiency and release profile of nanoparticles

MATERIALS AND METHODS

Pregabalin (99.79%) donated by M/S Shasun pharmaceuticals, Puducherry and PLGA was procured from Sigma Aldrich, St.Louis Dichloromethane, tween

80, (Tokyo, Japan) model which consisted of a LC10AD and LC10 ADvp solvent delivery module, SPD 20 PDA detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20 μ l loop, and PDA detector (SPD-20). The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1.11SP1) installed on it was used for the assay of Pregabalin. All chemicals used in the study were of analytical grade and used without further purification.

EXPERIMENTAL STUDIES

Determination of solubility

The pregabalin was evaluated for solubility in water, acetone, methanol, diethyl ether chloroform and ethanol in accordance with the British pharmacopoeia specifications.³

Determination of bulk density, bulkiness and compressibility index

The bulk density of pregabalin was determined by the three tap method. 10g of pregabalin powder was carefully introduced into a 100 ml graduated cylinder. The cylinder was dropped onto a hard wood surface 3 times from a height of 1inch at an interval of 2 seconds. The bulk density was obtained by dividing the weight of the sample by volume of the sample contained in the cylinder. Reciprocal of bulk density or the specific bulk volume gave the bulkiness.⁴ The percent compressibility index (I) of the pregabalin was calculated using following formula

$$I = \left(1 - \frac{V}{V_0}\right) \times 100$$

Angle of repose

The static angle of repose, α , was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel.⁵ The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

$$\tan \alpha = 2h/D$$

The data presented here is for triplicate determinations.

Determination of Partition Coefficient

10 mg drug was added in 50 ml of n-Octanol (pre saturated with water) and it was shaken and then 50 ml of distilled water (pre saturated with n- Octanol) was added and was shaken the mixture by mechanical shaker for 24 hours. After 24 hour both phases are separated. Absorbance was taken of both the phases and calculated the concentration in each phases.⁶

$$\text{Partition Coefficient} = \left(\frac{\text{Drug concentration in Octanol}}{\text{Drug concentration in water}} \right)$$

Percentage of moisture loss

The pregabalin loaded nanoparticles were evaluated for percentage of moisture loss which sharing an idea about its hydrophilic nature. The nanoparticles weighed initially and kept in desiccator containing calcium chloride at 37 °C for 24 hours. When no further change in weight of sample was observed, the final weight was noted down.⁷

$$\% \text{ of moisture loss} = \left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \right) \times 100$$

Determination of Particle Size distribution

5ml of 1% w/v dispersion of pregabalin in glycerine was prepared. A smear of the dispersion was made and examined under microscope. The size of 500 particles was measured using a calibrated eyepiece micrometer.⁸

Preparation of Calibration Curve of Pregabalin in Distilled Water:

A stock solution (100 mg / ml) of Pregabalin prepared and from this different –different concentration solutions were prepared and then the absorbance of dilutions was measured on a UV- Visible spectrometer (UV – 160 IPC, Shimadzu, Japan) at 205.0 nm.^{9,10}

Dissolution Test

In-vitro dissolution studies were carried out using a dissolution apparatus USP (Paddle type) at a paddle speed of 50 rpm. The dissolution medium was 900 ml of phosphate buffer, pH 7.4, which was maintained at 37 ± 0.5 °C. 5 ml of dissolution samples were withdrawn and replaced with equal volume fresh phosphate buffer, pH 7.4 at regular intervals.¹¹ Collected dissolution samples were used for

determination of released pregabalin concentrations by using a UV-VIS spectrophotometer (UV – 160IPC, Shimadzu, Japan) at 205 nm wavelength against a blank.

Preparation of Nanoparticles**Nanoprecipitation method**

Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of surfactant. Polymers, drug, and or lipophilic surfactant are dissolved in a semi polar water miscible solvent such as acetone or ethanol. The solution is then poured or injected into an aqueous solution containing stabilizer under magnetic stirring.¹² Nano particles are formed instantaneously by the rapid solvent diffusion. The solvent is then removed from the suspensions under reduced pressure.¹³ The rates of addition of the organic phase into the aqueous phase affect the particles size. It was observed that a decrease in both particles size and drug entrapment occurs as the rate of mixing of the two phase increases. Nano precipitation method is well suited for most of the poorly soluble drugs. Nanosphere size, drug release and yield were shown to be effectively controlled by adjusting preparation parameters. Adjusting polymer concentration in the organic phase was found to be useful in the production of smaller sized nanospheres through restricted to a limited range of the polymer to drug ratio.¹⁴

Percentage yield

The dried nanoparticles were weighed and percentage yield of the prepared nanoparticles was calculated by using the following formula.¹⁵

$$\text{Percentage yield} = \left(\frac{\text{Weight of nanoparticles}}{\text{Weight of polymer} + \text{drug}} \right) \times 100$$

Particle Size Analysis of Nanoparticles

Average particle diameter and size distribution of nanoparticle were determined by laser diffractometry using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Approximately 10 mg of nanoparticles were dispersed in 2 to 3 ml distilled water containing 0.1% Nonidet P40 for several minutes using an ultrasonic bath. Then, an aliquot of the nanoparticles suspension was added into the small volume recirculation unit, which was subsequently circulated 3500 times per minute. Each sample was measured in triplicate for the analysis.¹⁶

Encapsulation efficiency (EE)

Drug loaded nanoparticles (100 mg) were powdered and suspended in water and then sonicated for about 20 minutes. It was shaken for another 20 minutes for the complete extraction of drug from the nanoparticles. The mixture was filtered through a 0.45 µm membrane filter (MILLIPORE).¹⁷ Drug content was determined by UV- visible spectrophotometer (UV – 160IPC,

Shimadzu, Japan) at 205 nm. The percent entrapment was calculated using the following formula.¹⁸

$$\text{Encapsulation efficiency} = \left(\frac{\text{Actual weight of drug in sample}}{\text{Nanoparticles Sample Weight}} \right) \times 100$$

Invitro drug release

The USP XXIV dissolution rate testing apparatus was employed to study the release of pregabalin using phosphate buffer PH 7.4 as a dissolution medium. 100mg equivalent of pregabalin nanoparticles was filled in hard gelatine capsule and dissolution test was being carried out at 50 rpm maintained at 37°C±0.5°C. 5ml of sample were withdrawn at specific time interval for 12 hours. The sample volume was replaced by an equal volume of fresh medium. The concentration was determined by UV- visible spectrophotometer (UV – 160IPC, Shimadzu, Japan) at 205 nm. The percentage of drug release at various time intervals was calculated and plotted against time.¹⁹

RESULTS AND DISCUSSION

The results of solubility, true density, bulk density, compressibility index, angle of repose, moisture content, pH, Partition Coefficient and melting point was calculated and the results are given in Table 1.

Table 1: physicochemical properties of Pregabalin

Parameters	Results
Description	Pregabalin occurs as white to off white powder.
Solubility	For pregabalin, it is pH-dependent, going from 0.1 normal hydrochloric acid (HCl) to 0.1 normal sodium hydroxide, with the solubility being lowest in water and the phosphate buffer, pH 7.4.
Tab density (gm/cc)	0.46 ± 0.53
Bulk density (gm/cc)	0.69 ± 0.03
Compressibility Index (%)	13.17 ± 0.22
Angle of repose (°)	26.51 ± 0.82
Moisture content (%)	8.52 ± 0.45
Partition Coefficient	1.35 ± 0.13
Melting Point (°C)	188-190

Particle size distribution of drug has influence on many bulk properties of pharmaceutical interest such as flow properties, packing, packing densities, compressibility segregation characteristics etc. Hence, it must be the aim of pharmaceutical technologist to study the particle size distribution. The size distribution of the pregabalin particles was estimated. The results are given in Table 2 and shown in Figure 1.

Table 2: Particle size distribution of pregabalin powder

Size range(µm)	Number of particles
0-30	20
30-60	114
60-90	212
90-120	129
>120	25

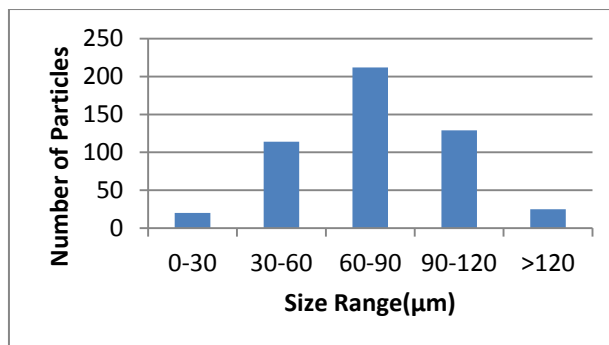


Figure 1: Particle size distribution of Pregabalin

Calibration Curve of pregabalin

The absorbance data are given in the Table 5 and the calibration curve is plotted is shown in Figure 2 a,b and 2c.

Table 3: Absorbance Data for Calibration Curve of pregabalin at 205.0 nm

S.No	Concentration (µg/ml)	Absorbance		
		pH 1.2	pH 7.4	pH 6.8
1	5	0.026	0.028	0.042
2	10	0.053	0.054	0.085
3	15	0.074	0.083	0.126
4	20	0.102	0.107	0.173
5	25	0.126	0.129	0.213
6	30	0.154	0.160	0.258
7	35	0.177	0.180	0.301
8	40	0.205	0.209	0.336
9	45	0.224	0.229	0.381
10	50	0.256	0.260	0.429

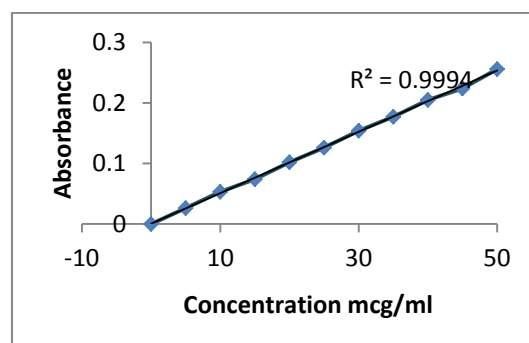


Figure 2(a): Calibration Curve of Pregabalin in pH 1.2 at 205. nm

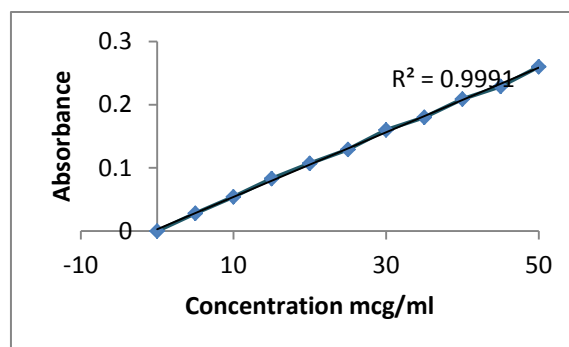


Figure 2(b): Calibration Curve of Pregabalin in pH 7.4 at 205. nm

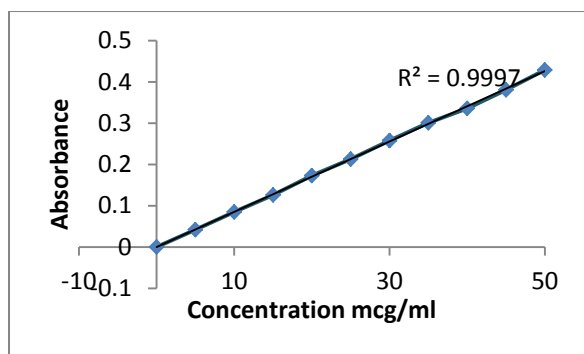


Figure 2(c): Calibration Curve of Pregabalin in pH 6.8 at 205. nm

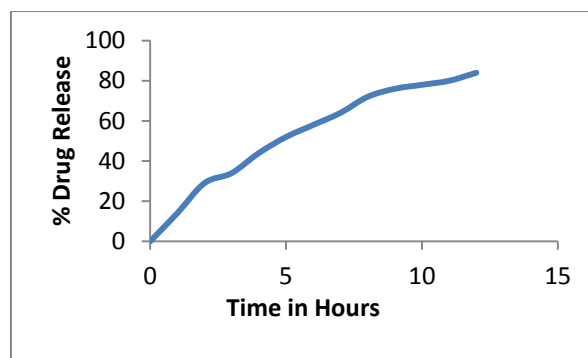


Figure 5: Drug Release Profile of formulated nanoparticles

The dissolution rate of pregabalin of tablets was over 90% after 40 min (Figure 3), which conformed to the pregabalin standards written in the official monograph.

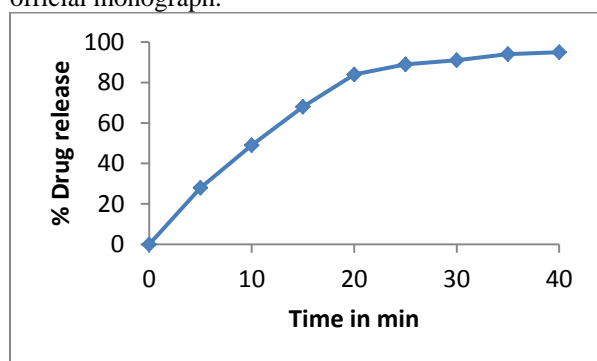


Figure 3: Dissolution rate of Pregabalin

Nanoparticles Evaluation

The formulated nanoparticles are evaluated for various properties like particle size, yield, entrapment efficiency and in vitro drug release from the nanoparticles. The formulated nanoparticles had good results of percentage yield, particle size, entrapment efficiency and in-vitro drug release at various time intervals of formulated nanoparticles are given in table 4. and shown in Figure 4 and Figure 5.

Table 4: pregabalin nanoparticles evaluation

Formulation	Particle Size	% yield	% Entrapment efficiency	% Drug release
F1	236.50	89.64	86.57	82.07

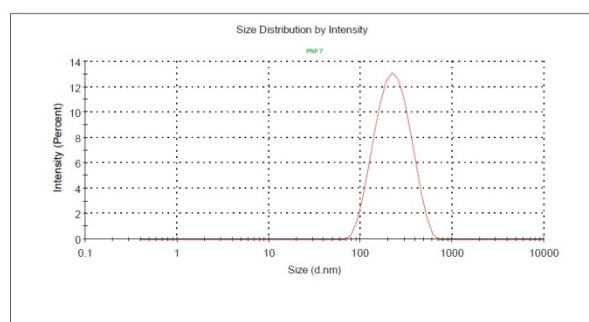


Figure 4: Particle size of pregabalin loaded nanoparticles

CONCLUSION

The preformulation phase is a critical phase in establishing the properties of CDs that will allow suitable risk assessment for development. Typically it begins during the lead optimization phase, continues through prenomination, and on into the early phases of development. Decisions made on the information generated during this phase can have a profound effect on the subsequent development of those compounds. Therefore, it is imperative that preformulation should be performed as carefully as possible to enable rational decisions to be made. The quantity and quality of the drugs can affect the data generated as well as the equipment available and the expertise of the personnel conducting the investigations. In this study we successfully completed the physicochemical characterization of pregabalin properties like morphology, size, solubility, pH, Partition coefficient, Surface area flow property, drug content and release study. This knowledge can be useful in developing modified release formulations mainly formulation of pregabalin nanoparticles.

REFERENCES

- Pawar RG, Gadhave MV, Jadhac SL, Gaikvad DD. Formulation and evaluation of pregabalin sustained release matrix tablet. International Journal of Pharmaceutical Research and Development. 2011; 4(2): 153-159.
- P.Venkatesan, V. Sree Janardhanan, R.Manavalan, K.Valliappan. Preformulation Parameters Characterization to Design, Development and Formulation of Loxoprofen Loaded Microspheres. International Journal on Pharmaceutical and Biomedical Research (IJPBR). 2011; 2(3): 107-117
- Ohwoavworhua FO, AdelakumTA. Some physical characteristics of microcrystalline cellulose obtained from raw cotton of cochlospermum planchonil. Trop J pharm Res 2005; 4:1-7.
- Martin, A., Bustamante,P. and Chun, A.H.C., Physical Pharmacy, 4th Ed., Indian Reprint,B.I.Waverly Pvt.Ltd., New Delhi, 1994,444.
- Vijay J, Sahadevan JT , Prabhakaran R , Mehra Gilhotra R. Formulation and Evaluation of Cephalexin Extended-release Matrix Tablets Using Hydroxy Propyl Methyl Cellulose as Rate-controlling Polymer. Journal of Young Pharmacists.2012; 4:3-12.
- Venkatesan,P, Manavalan.R and Valliappan.K. Preparation and evaluation of sustained release loxoprofen loaded microspheres. Journal of Basic and Clinical Pharmacy.2011; 2(3): 159-162

07. Ghosh A, Nayak UK and Roy P. Development, Evaluation and Method selection for the Preparation of lamivudine microspheres. *The International. J. Pharmacy* June 2007;9:67-71.
08. M. M. Gupta*, T.R. Saini . Preformulation parameters characterization to design, development And formulation of vancomycin hydrochloride tablets for Pseudomembranous colitis. *IJPRD*, 2009;1:1-7.
09. P.Venkatesan, V. SreeJanardha nan, C. Muralidharan, and K.Valliappan. Improved HPLC Method with the Aid of Chemometric Strategy: Determination of Loxoprofen in Pharmaceutical Formulation. *Acta Chim. Slov.*, 2012; 59: 242-248.
10. KannappanValliappan, Sree Janardhanan Vaithiyathan and Venkatesan Palanivel. Direct Chiral HPLC Method for the Simultaneous Determination of Warfarin Enantiomers and Its Impurities in Raw Material and Pharmaceutical Formulation: Application of Chemometric Protocol. *Chromatographia*, 2013; 76(5): 287-292.
11. A. Arunkumar et al.,: Development and Validation of New Analytical methods for Simultaneous estimation of Epigallocatechin gallate, a component of Green Tea extract and Niacin in a Pharmaceutical dosage form, *J. Pharm. Res.*, 2016; 5(2): 21-24.
12. Venkatesan P, Manavalan R, Valliappan K, et al. Microencapsulation: A Vital Technique In Novel Drug Delivery System. *J Pharm Sci & Res.* 2009;1(4):26-35.
13. Venkatesan P, Muralidharan C, Manavalan R, Valliappan K, et al. Selection of better method for the preparation of microspheres by applying Analytic Hierarchy Process. *J Pharm Sci & Res.* 2009;1(3):64-78.
14. Gohel MC, Parik RK, Amin AF and Surati AK. Preparation and formulation optimization of sugar cross linking gelatin microspheres of diclofenac sodium. *Indian J. Pharm Sci.* 2005;67(8):575-81.
15. T.Sudhamani, K. Noveenkumar reddy, V.R. Ravi Kumar, R.Revathi, V.Ganesan, preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery, *Int.J.Pharma Research and Development.* 2010, 119-125.
16. Zinutti C, Kedzierewicz F, Hoffman M, Maincent P preparation and Characterisation of ethyl cellulose microspheres containing 5- fluorouracil Microencapsule, *1994 Sep- Oct, 11 (5), 555- 563.*
17. R.Sathiya Sundar, A.Murugesan, P.Venkatesan, and R.Manavalan, Formulation Development and Evaluation of Carprofen Microspheres. *Int.J. Pharm Tech Research.* 2010 Vol.2, No.3, 1674-1676.
18. P.Venkatesan, R.Manavalan and K.Valliappan Microencapsulation: A Vital Technique In Novel Drug Delivery System. *J. Pharm. Sci. & Res.* Vol.1 (4), 2009, 26-35.
19. Perumal.D, Microencapsulation of ibuprofen & Eudragit RS 100 by the emulsion solvent diffusion technique, *Int.J.Pharm,* 218, 2001, 1-11.