



Research Paper

Design Prepare and characterization of Pregabalin loaded solid lipid Nanoparticles

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ABSTRACT

Targeted drug delivery systems are one of the most challenging study topics in the pharmaceutical sciences. New obstacles have emerged in the quest to perfect medication delivery as a result of the development of colloidal delivery methods such as liposomes, micelles, and nanoparticles. In order to increase the bioavailability of pregabalin, this research aimed to quantify the impact of lipids during the manufacture of pregabalin-loaded SLN. The FT-IR study showed that the chosen lipid, medication, surfactant, and combination had no negative interactions with one another. Using lipid GMS and surfactants Tween80, Polaxamer 188, and Span20, SLNs were produced through heat homogenization followed by ultrasonication. It was found that the best formulation would have the desired drug release profile if the dispersity index, nanoparticle size, zeta potential, and entrapment efficiency were all consistent across the board. Thepoloxamer188 had significant influence onthedrugreleaseofPregabalin. Therefore, SLNs are a potentially useful delivery strategy for improving the bioavailability of the poorly soluble medication Pregabalin.

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1. INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained¹. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as polyethylene glycol (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes².

Nanoparticles are transport carrier compartments for drugs other than non-liposomal character in the nanometer size range (10nm-1µm).The advantage of nanoparticles as drug delivery systems they are biodegradable, nontoxic and stored being up to one year .

Main objective in designing nanoparticles as a drug delivery system is to control particle size, surface properties and release of pharmacologically active agents (i.e,drug) . In order to achieve the site-specific action of the drug at the therapeutically optimal rate and regimen³⁻⁴.

Drug profile

Pregabalin is short-acting (elimination half life [t_{1/2}] 5-7 hrs) analogue of gabapentin used as an adjunct treatment for focal epilepsies, neuropathic pain, fibromyalgia, and anxiety disorder in adult patients. Initial approval was granted by the FDA and EMA in 2004. Although pregabalin is

patent protected in the USA until 2018, a generic version is currently available in the UK and Canada. Pregabalin has been investigated for use in cancer induced bone pain and as a premedication to provide postoperative analgesia. Pregabalin can be an effective treatment for peripheral neuropathic pain provided adverse reactions are carefully monitored and dosages are correctly determined⁵.

It demonstrates good bioavailability ($\approx 98\%$), minimal drug interactions, and low protein binding. The poor binding of pregabalin means it can be effectively cleared by hemodialysis techniques. A major portion of the drug is excreted unchanged in the urine, with a clearance of equal to the glomerular filtration rate. Thus, the dosage regimen should be adjusted in geriatric patients as well as those with renal impairment. TDM of pregabalin is restricted to dosage adjustment and to assess compliance. Although no specific reference concentration range has been established, a comparative value of 3-8mg/l has been suggested⁶.

Pregabalin trough concentration are subject to high inter subject variability. Significant adverse drug reactions are sedation, obesity, cerebellar symptoms, and peripheral edema. Enzyme-inducing AEDs can moderately lower pregabalin concentrations. An increased risk of major birth defects after first-trimester exposure to pregabalin has been recently documented. An initial clinical study has demonstrated the use of pregabalin as an adjuvant to labor pain relief associated with late termination of pregabalin⁷.

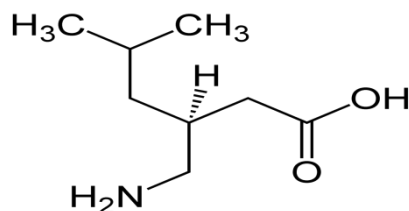


Figure no. 1 Structure of Pregabalin

Pregabalin binds presynaptically to the $\alpha 2$ -delta subunit of the voltage-gated calcium channels in CNS tissue located in the brain and spinal cord. The mechanism of action has not been fully elucidated but studies suggest that pregabalin produces a disruption of calcium channel trafficking or a reduction of calcium currents. The inhibition of subunits of voltage-gated calcium channels release which in order inhibits the release of several neurotransmitter. Studies also suggest that the descending noradrenergic and serotonergic pathways originating from the brainstem may be involved with the mechanism of pregabalin. Interestingly, although pregabalin is a structural derivative of inhibitory neurotransmitter gamma-aminobutyric acid (GABA), it does not bind directly to GABA or benzodiazepine receptors⁸.

2. MATERIALS AND METHOD

10mg Pregabalin was precisely gauged and moved into 10ml volumetric carafe. It was broken down and weakened to volume with $\text{CH}_3)_2\text{CO}$ to give stock arrangement containing 1000 $\mu\text{g/ml}$. The standard stock arrangement was then sequentially taken 1ml of arrangement from first stock arrangement weakened with $\text{CH}_3)_2\text{CO}$ to get 1 to 100 $\mu\text{g/ml}$ or auxiliary stock arrangement. From optional stock arrangement take 1ml

of the answer for get 10 $\mu\text{g/ml}$ or tertiary stock arrangement. The absorbances of the arrangement were estimated against $\text{CH}_3)_2\text{CO}$ as clear at 210 nm utilizing UV spectrophotometer. The absorbance esteems were plotted against fixation ($\mu\text{g/ml}$) to acquire the standard adjustment bend⁹.

The exact measurement of 10mg Pregabalin was carried out and transferred to a 10ml volumetric container. It was broken down and weakened to volume with ethanol to give the stock arrangement containing 1000 $\mu\text{g/ml}$. The standard stock arrangement was then sequentially taken 1ml of arrangement from the first arrangement weakened with $\text{CH}_3)_2\text{CO}$ to get 1 to 100 $\mu\text{g/ml}$ or auxiliary stock arrangement. From optional stock arrangement take 1ml of the answer to get 10 $\mu\text{g/ml}$ or tertiary stock arrangement. The absorbances of the arrangement were estimated against $\text{CH}_3)_2\text{CO}$ as clear at 210 nm utilizing UV spectrophotometer. The absorbance esteems were plotted against fixation ($\mu\text{g/ml}$) to acquire the standard adjustment bend.

Pregabalin Nanoparticles by Nanoprecipitation Method.

Pregabalin-containing nanoparticles were produced by using nanoprecipitation method. Nanoparticles were set up by utilizing distinctive medication to polymer proportion. Dissolving the medication in 3 mL of refined water was followed by the addition of cosolvent ($\text{CH}_3)_2\text{CO}$ (1 mL) to this mixture. To improve the homogeneity of the inward stage, a cosolvent was necessary. At that point, the polymer was dispersed in 4 ml of chloroform, and this mixture was included in the medication solution for structure scattering. At that point polymer were broken up in 4 ml of chloroform, and this arrangement was added to the medication answer for structure scattering. The scattering was added to 10 ml of fluid ethanol arrangement (70%). Following 5 minutes of blending, the natural solvents were expelled by vanishing at 35°C under ordinary tension, nanoparticles were isolated by utilizing cooling axis (10000 rpm for 20 min), supernatant were evacuated and nanoparticles washed with water and dried at room temperature in a desicator¹⁰.

S. no	Formulation code	Pregabalin (mg)	chloroform	acetone	Tween 80 (mg)	Poloxamer 188(mg)	Span 20 (mg)
1	F1	100	4ml	1ml	25	-	-
2	F2	100	4ml	1ml	50	-	-
3	F3	100	4ml	1ml	75	-	-
4	F4	100	4ml	1ml	-	25	-
5	F5	100	4ml	1ml	-	50	-
6	F6	100	4ml	1ml	-	75	-
7	F7	100	4ml	1ml	-	-	25
8	F8	100	4ml	1ml	-	-	50
9	F9	100	4ml	1ml	-	-	75

Table.no 1 Formulation of Nanoparticles

Evaluation of nanoparticles

1) Particle size and surface morphology

In order to examine the particle surface morphology and shape, scanning electron microscopy (SEM) was used. A Pregabalin nanoparticles solution was spread over a slab and dried under vacuum. All the samples such as 1) Pregabalin+ Tween 80, 2) Pregabalin+ Span 20 nanoparticles, 3) Pregabalin +Poloxamer 188. nanoparticles were shadowed in a cathodic evaporator with gold layer 20nm thick photograph were taken using a JSM-5200 SEM (Tokyo, Japan) operated at 20Kv¹¹.

2) Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a thermo analytical method used to study thermal transitions involving energy or heat capacity changes.

Thermal properties of the powder samples were investigated with differential scanning calorimetry. Approximately 10mg of samples was analyzed in an open aluminum pan and heated at scanning rate of 10°C/min between 0°C and 400°C. Magnesia was used as the standard reference materials. The thermograms were obtained for the samples 1) Pregabalin+ Tween 80, 2) Pregabalin+ Span 20 nanoparticles, 3) Pregabalin +Poloxamer 188. Nanoparticles.

3) Zeta potential

The zeta potential of nanoparticles is commonly used to characterize the surface charge property of nanoparticles. Zeta potential is also an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems. The surface charge on the microscope particle produced a difference in the electric potential in millivolts between the surface of each particle and bulk of the suspending liquid. That difference is called as zeta potential¹².

It is easily measured because the charge of the potential will move as the nanoparticles is placed between the two electrodes that have D.C. voltage across them and the velocity will be proportional to the zeta potential of the particle. The technical term for this is electrophoresis. Thumb rule describes the relation between zeta potential determination responses of the suspension being tested, particularly hydrophobic colloids. The prepared nanoparticles suspensions were characterized with respect to zeta potential by using zeta potential analyzer (Malvern Zeta sizer).

The electrophoretic mobility and zeta potential were measured using a zeta potentiometer (Malvern Zeta sizer). To determine the zeta potential, nanoparticles sample were diluted with KCl (0.1 mm) and placed in the electrophoretic cell where an electric field of 15.2 V / cm was applied. Each sample was analyzed in triplicate.

4) FT-IR

FT-IR is used for the drug-excipient compatibility samples of about 5mg was mixed thoroughly with 100mg KBr IR powder and compacted under vacuum at a pressure of about 12psi, for 3min. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and

the IR spectrum was recorded from 4000cm⁻¹ to 400cm⁻¹ in a scan time of 12min. these studies were done for 1) Pregabalin+ Tween 80, 2) Pregabalin+ Span 20 nanoparticles, 3) Pregabalin +Poloxamer 188.

5) Drug entrapment efficiency

Drug entrapment efficiency was determined by the dialysis method.

Drug entrapment efficiency was calculated for the samples 1) Pregabalin+ Tween 80, 2) Pregabalin+ Span 20 nanoparticles, 3) Pregabalin +Poloxamer 188. nanoparticles Take a beaker containing 100ml of phosphate buffer of pH 7.4 and keep them on the magnetic stirrer for stirring.

10mg of samples was weighed and taken in a broken test tube containing 10ml of phosphate buffer of pH 7.4.

Bottom end of the test tube was packed with the diffusion membrane.

Place the test tube inside the beaker and adjust it such that the test tube is submerged in the beaker containing phosphate buffer of pH 7.4 Now collect the samples i.e. 10ml from the beaker for every 1hr till 10hrs and replace it with the fresh medium of buffer.

6) In-vitro drug release profile:-

In-vitro drug release experiments were conducted in the release medium used was phosphate buffer of pH 7.4 which contains ascorbic acid and butylated hydroxytoluene at a concentration of 1% and 0.1% respectively to prevent degradation.

3. RESULT AND DISCUSSION

Determination of λ_{max} & preparation of calibration curve:

Pregabalin has a maximum absorbance (λ_{max}) at 288 nm. Figure 13 depicts the Pregabalin calibration standard curve. Good linearity ($r^2=0.998$) can be seen in the reference calibration curve.

Concentration(mcg/ml)	Absorbance(nm)
0	0
3	0.142
6	0.272
9	0.424
12	0.567
15	0.689

Table No2 :Standard calibration curve of Pregabalin

Compatibility studies

The infrared spectra of medication and lipid excipients, respectively. The spectrum was examined from 400 to 4000 centimeters per second. The spectral evidence strongly suggested that the chosen lipids, drugs, and mixes did not interact with one another. To conclude, the chosen lipid was found to be compatible in entrapping the chosen medication with carriers without any mutual interactions.

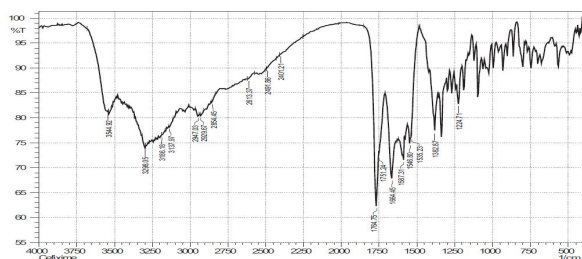


Figure no.2 FT-IR spectra of Pregabalin

Determination of entrapment efficiency

Table displays the results of entrapment efficiency tests performed on formulated SLNs. The entrapment efficiency of formulations F1 through F3 (Tween 80) at varying concentrations (1.5%, 2%, and 1%, respectively) is 73.830.83, 77.830.35, and 80.60.70, respectively. Formulation F4-F6 (Poloxamer 188) entrapment efficiency at various ratios (1:1, 1.5:1) shows 81.30.74, 83.50.45, and 84.80.51. Results reveal that when surfactant concentration is increased, so too does the entrapment efficiency of the formulations, with values of 69.30.60, 73.20.4, and 75.10.2 for formulations F7-F9 (Span 20) at 1%, 1.5%, and 2%, respectively. That's because there'd be more lipid to ensnare the drugs in if the lipid concentration was higher. The surfactant poloxamer188 exhibits the greatest drugentrapment among those used.

S.No	Formulationcode	Entrapmentefficiency(%)	
1	F1	Tween80	73.83±0.83
2	F2		77.83±0.35
3	F3		80.6±0.70
4	F4	Poloxamer188	81.3±0.74
5	F5		83.5±0.45
6	F6		84.8±0.51
7	F7	Span20	69.3±0.60
8	F8		73.2±0.4
9	F9		75.1±0.2

Table No.3: Entrapment efficiency of Pregabalin loaded SLN

Effect of surfactants on the particle size of Pregabalin SLN

There was a significant difference in particle size between SLN dispersions made with polyoxamer 1882.0% as a stabilizer (F4, F5, and F6) and those prepared with the other surfactants.

S.No	Formulation code	Surfactant	Meandiameter	PDI	Zetapotential
1	F1	Tween80	381.1	0.056	-14.2
2	F2		151.5	0.647	-19.1
3	F3		69.51	1.000	-12.3
4	F4	Poloxamer 188	33.58	0.354	-1.10
5	F5		22.03	0.281	-1.20
6	F6		5.229	0.273	-3.65
7	F7	Span20	421	0.071	-17.3
8	F8		908.8	0.054	-14.267
9	F9		478.0	0.315	-11.07

Table No.4: Particle size, PDI, Zetapotential

The increased molecular weight and HLB value of poloxamer 188 compared to tween 80 and span 20 may account for this finding. Particle size of different SLN stabilized with different surfactants rose as follows: Poloxamer 188 > Tween 80 > Span20.

Evaluation of pregabalin loaded solid lipid nanoparticle

Invitro drug release

TIME(MINS)	Tween80			Poloxamer188			Span20		
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
15	6.77±0.21	7.67±0.21	8.12±0.85	9.78±0.63	13.85±0.85	16.71±0.63	5.56±0.21	6.92±0.42	8.38±0.21
30	18.97±0.42	17.63±0.63	18.68±0.42	20.79±0.43	25.01±0.84	28.03±0.42	14.46±0.43	14.76±0.85	20.04±0.21
60	23.98±0.21	24.88±0.64	25.04±0.43	29.71±0.21	38.02±0.42	41.19±0.64	21.12±0.40	23.67±0.21	29.11±0.22
120	39.84±1.2	37.44±2.1	39.69±0.21	43.92±1.4	55.1±0.63	51.81±0.62	35.61±0.43	36.37±0.64	43.92±1.49
180	49.71±0.21	56.78±0.43	57.39±0.43	59.82±0.43	71.18±1.1	70.29±0.63	44.57±0.64	42.32±0.43	53.80±0.43
240	58.40±1.7	65.94±0.44	70.31±1.92	75.01±0.42	83.38±0.2	85.65±0.41	53.69±0.21	58.29±0.53	65.96±0.42
300	63.19±0.2	75.11±0.25	80.40±0.64	83.1±0.01	89.73±0.42	94.71±0.53	60.88±0.42	67.07±0.21	70.47±1.92

Table no5 :In-vitro drugrelease of pregabalin

Scanning electron microscopy

Scanning electron microscopy was used to look at the dispersion of Pregabalin-loaded SLN and see how it looked. For SEM analyses, the optimal formulation, F6 (containing 2% poloxamer), was selected. Figure displayed the SEM image. As can be seen in Figure, the SLN dispersion indicates that the particle size is 500 nm, with a rather smooth surface.

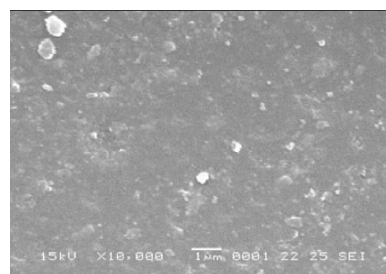


Figure no.3 Image of Pregabalin loaded SLN by Scanning Electron Microscopy

Release kinetics

Code	Zeroorder		Firstorder		HiguchiModel		Korsmeyerpeppas	
	k	r²	k(hr⁻¹)	r²	k(hr⁻¹)	r²	n	r²
F6	1.878	0.8685	0.039	0.9977	11.948	0.9451	0.633	0.9867

Table No.6 : Release kinetics

4.CONCLUSION

This research was conducted to learn how including lipids into the SLN formulation might affect the bioavailability of pregabalin. The FT-IR analysis showed that the tested lipids, medicines, surfactants, and combinations did not react with one another.

Using lipid GMS and surfactants Tween80, Polaxamer 188, and Span20, SLNs were produced through heat homogenization followed by

ultrasonication. According to the findings, the heat homogenization and ultrasonication procedures were a secure and workable approach to produce pregabalin-loaded solid lipid nanoparticles¹³.

The particle size, morphology, entrapment efficiency, release studies, Fourier transform infrared spectroscopy, in vitro kinetics, scanning electron microscopy, and polydispersity index of SLN formulations were calculated. Studies on particle size found that increasing the concentration of surfactant from 1% to 2% reduced the average particle size of the formulation.

In contrast to the other surfactants tested, the particle size of the SLN dispersion generated with polyoxamer 188 (2.0%) as a stabilizer (F4, F5, and F6) was much smaller than that of the other lipids tested. Pregabalin's release from the SLN formulation is governed by a first-order kinetics model. The particle size of formulations with best entrapment was found out in the range of 5.229 nm¹⁴.

Our research shows that using hot homogenization and the ultrasound dispersion method, the poorly bioavailable drug Pregabalin can be successfully incorporated into solid lipid nanoparticles. It was found that the best formulation would have the desired drug release profile if the dispersity index, nanoparticle size, zeta potential, and entrapment efficiency were all consistent across the board. To improve the bioavailability of the poorly soluble medication Pregabalin, SLNs hold promise as a delivery strategy due to the strong impact of the polymer.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

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