ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Development and Evaluation of Felbamate Loaded Solid Lipid Nanoparticles

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Abstract

Solid lipid nanoparticles (SLN's) or lipid nano particles is typically sphere-shaped, with an average diameter of 1nm to 1000nm. They are alternative carrier systems to tradition colloidal carriers, such as, emulsions, liposomes, and polymeric micro and nanoparticles. The main aim of the current research work is to prepare and optimize the Felbamate loaded solid lipid nanoparticles using by placket and burman design of experiments. SLN's were prepared by microemulsion technique. Based on preliminary experiments and on literature, the influence of independent variable parameters selected were lipid (X1), surfactant (X2), and co-surfactant concentration(X3), aqueous phase volume (X4), magnetic stirrer rate (X5), probe sonication duration (X6), volume of beaker used for sonication (X7), volume of cold aqueous phase (X8) on the dependent variable such as average particle size (Y1) was studies. Other parameters, i.e., magnetic stirrer rate and probe sonication duration were not having a significant impact on particle size and their levels were kept constant for all the experiments. Magnetic stirrer rate has an impact on particle size and was included in the design. It was concluded from the study that the composition prepared with lipid concentration of 50 mg, surfactant concentration of 75 mg, Co-surfactant concentration of 0.75 ml, aqueous phase volume of 5 ml, magnetic stirring speed of 400 rpm, probe sonication duration 30 minutess, volume of beaker used for sonication 500 ml and volume of cold aqueous phase 30 ml has shown the smallest particle size of 165 nm.

Key words: *Solid Lipid, Nano Particles, Particle Size, Sonication, Felbamate.*

Introduction

Epilepsy is chronic neurological disease confirmed by pattern of repeated seizure. The term "Epilepsy" created from Greek word "epilepsia", that means "seizure". These seizures are electrical impulses fired by nerve cells of brain at a rate of maximum 4 times higher than normal rate which leads a type of strong electrical impulse in the brain. Feasible causes of seizures include injuries of head, brain tumor, poor development of the brain cell, genetic and infectious disorder but in most of the cases, no causes are recognized.

Proper medication helps to control seizures for most of the patients [1, 3]. About 50,000,000 people in the earth having epilepsy & nearly 90% of the population suffering from epilepsy are discovered in growing countries. 70% of time, Epilepsy responds to its treatment and about 3/4 of the affected people in growing countries do not have the proper treatment.

Rising of new affected patients come out most commonly in child & elderly patients. Another cause is a co senescence of brain surgery, epileptic/ convulsant seizures can observed in improving patients [1, 3]. Epilepsy/convulsion is mainly controlled with drug, it can't be completely cured. Whereas, more than thirty percent of population posses epilepsy can't have seizure control with the best suitable therapy. Surgical process required in complicated cases.

Epilepsy is not only a single disease but also instead it is symptomatic with a variety of diversified symptoms concerned in episodic abnormal electrical activity in the brain. Antiepileptic drugs are separate group of drugs used in the treatment of epileptic seizures.

Main role of anti-epileptic is to reduce the successive firing of the neuron that starts seizures [1, 3].Anti-epileptic drugs are also known as anti-seizure or anti convulsant drugs. The major targets for the available marketed anti-epileptic products are voltage gated Na+ (sodium) channel & parts of GABA receptor (GABAA receptor, GAT-1- GABA transporter). Other considerable targets are voltage gated Ca+ (calcium) channels, SV2A & α2d [1, 3]. Therapeutic efficacy of any drug depends mainly on four fundamental pharmacokinetic pathways of drug transportation and modification within the body such as its absorption, distribution, metabolism and excretion.

Failure in drug therapy includes insufficient drug concentration due to poor absorption, rapid metabolism and elimination, poor drug solubility, and high fluctuation of plasma levels due to unpredictable bioavailability. A promising strategy to overcome these problems involves the development of a suitable drug colloidal carrier system. Among the colloidal carrier systems solid lipid nanoparticles have many advantages and limited disadvantages as compared to other colloidal carrier systems.

Solid lipid nanoparticles (SLN) have gained attention as carriers for the preparation of a wide variety of poorly water soluble drugs due to their biodegradable and biocompatible properties and low toxicity. The main aim of the current research work is to develop solid lipid Nanoparticles of felbamte (antiepileptic drug) using placket and burman design of experiments [4, 10]. Felbamate is a PEGylated phenylcarbamate derivative that acts as an antagonist of NMDA receptors. It is used as an anticonvulsant, primarily for the treatment of seizures in severe refractory epilepsy. It is slightly soluble in water with the logP value of 0.56 [11, 16].

Materials and Methods

Materials

Felbamate was received as gift sample from Aurobindo Pharma as gift sample. Stearic acid (Arjun Industries, India), Poloxamer 407 (Signet, Mumbai), Polysorbate 80 (Sisco Research Laboratories, Chennai), Chloroform and Methanol (Rankem, Chennai), Dialysis Membrane 50-LA 387 (Hi media, Mumbai) were purchased from the local market. All the reagents used were of analytical grade.

Methods

Optimization of Formulation by Plackett-Burman Experimental Design

The development of Felbamate slid lipid nano particle process includes, many preparation variables appear to have a noticeable influence on the formulation characteristics (average particle size, span, surface area and poly dispersity index). The formulation can be optimized with proper optimized preparation variables. Evaluating the effect of many preparation variables usually requires many experiments, which are often expensive and time consuming.

Consequently, prudent to minimize the total number of experiments done in the optimization process, without sacrificing the quality of prepared Felbamate solid lipid nano particles. The process and formulation can be understood by using numerous statistical designs of experiments. However, we have preferred Plackett-Burman design (PBD), which has been frequently used for screening the large number of factors and identifying the critical one in a minimal number of runs with good degree of accuracy. In PBD, the main effect of each variable was calculated as:

$$Exi = 2\,(\Sigma Hxi - \Sigma Lxi) / N$$

Where, Exi is the particular variable main effect, ΣHxi is the summation of response value at the higher level, ΣLxi is the summation of response value at the lower level and N is the number of trials. A main effect figure with a negative sign indicates an inverse relationship between that particular variable, while a positive sign indicates the effect that favors the optimization. The linear equation of Plackett-Burman design is as follows.

$$Y = b0 + b1\,X1 + b2\,X2 + b3\,X3 + b4\,X4 + b5\,X5 + \cdots + bnXn$$

Where, Y is the response, b0 is the constant and b1, b2...bn are the coefficient of variables X1, X2...Xn (representing the effect of each variable ordered within −1, +1).

Optimization and Statistical Analysis

Based on preliminary experiments and on literature, the influence of independent variable parameters selected were lipid (X_1), surfactant (X_2), and co-surfactant

concentration(X_3), aqueous phase volume (X_4),magnetic stirrer rate (X_5), probe sonication duration (X_6), volume of beaker used for sonication (X_7), volume of cold aqueous phase (X_8) on the dependent variables such as average particle size (Y_1) and poly dispersity index (Y2) of the formulated Felbamate loaded solid lipid nano particles (Table 1) was studied. Other parameters, i.e., magnetic stirrer rate and probe sonicator duration were not having a significant impact on particle size and their levels were kept constant for all the experiments. Magnetic stirrer rate has an impact on particle size and was included in the design.

Table 1: Optimization process parameters at lower and higher levels

Factor	Process Parameter	Levels	
		Lower	Lower
X_1	Lipid concentration (mg)	50	150
X_2	Surfactant Concentration (mg)	25	75
X_3	Co-surfactant Concentration (mL)	0.25	0.75
X_4	Volume of aqueous phase (mL)	5	15
X_5	Magnetic Stirrer rate (rpm)	200	400
X_6	Probe sonicator duration (min)	10	30
X_7	Volume of beaker used probe sonication (mL)	125	250
X_8	Volume of clod aqueous phase (mL)	30	50

Twelve experimental runs (Table 2) involving 10 process parameters at higher and lower levels were generated using Design-Expert® (Version 7.1.5; Stat-Ease, Inc. USA).

Table 2: Scheme for the fabrication of Felbamate solid lipid nanoparticles according to Plackett-Burman experimental design

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
FSN01	150.00	75.00	0.25	5.00	200.00	30.00	125.00	50.00
FSN02	50.00	75.00	0.25	15.00	400.00	10.00	250.00	50.00
FSN03	50.00	75.00	0.75	15.00	200.00	10.00	125.00	50.00
FSN04	50.00	25.00	0.25	15.00	200.00	30.00	250.00	30.00
FSN05	150.00	25.00	0.25	5.00	400.00	10.00	250.00	50.00
FSN06	150.00	75.00	0.75	5.00	200.00	10.00	250.00	30.00
FSN07	150.00	25.00	0.75	15.00	200.00	30.00	250.00	50.00
FSN08	50.00	25.00	0.25	5.00	200.00	10.00	125.00	30.00
FSN09	150.00	25.00	0.75	15.00	400.00	10.00	125.00	30.00
FSN10	150.00	75.00	0.25	15.00	400.00	30.00	125.00	30.00
FSN11	50.00	75.00	0.75	5.00	400.00	30.00	250.00	30.00
FSN12	50.00	25.00	0.75	5.00	400.00	30.00	125.00	50.00

Fabrication of Felbamate Solid Lipid Nano particle

The nano scale solid lipid particles which contain the drug Felbamate was fabricated from oil-in-water micro emulsion technique. In this method, lipid phase containing stearic acid was melted at 69-70°C and the drug was dissolved in the melted lipid. Then the lipid phase was added drop wise into the aqueous

phase containing Polaxamer-407 as surfactant and Polysorbate-80 as a co-surfactant, which heated at the same temperature. A transparent, thermodynamically stable o/w micro emulsion was obtained under magnetic stirrer at 400 rpm for 15 min.

This resulting o/w micro emulsion was dispersed into cold aqueous medium under probe sonicator for 30 min to solidify the nano particles in a volume ratio of 1:1 hot micro emulsion to cold water. The fabricated Felbamate loaded nano scale solid lipid particles were freeze dried on a lyophilizer at -40°C temperature and operating pressure 0.4 bar. The dried powder was stored in desiccators till the analysis.

Determination of Particle Size and Poly Dispersity Index

Prepared solid lipid nano particles were maintained at room temperature for 30 days, which were characterized for particle size and poly dispersity index using Malvern Zetasizer Nano ZS (Malvern, UK). About 1 ml of prepared solid lipid nano particles were diluted appropriately using distilled water, which was then taken individually in a zeta cell and measured the average particle size and poly dispersity index. The experiments were performed in triplicate.

Results & Discussion

Development of Solid Lipid Nano particulate Drug Delivery System using Sonication Approach

Felbamate solid lipid nano particles were prepared using micro-emulsion method. During preparation, addition of organic phase containing polymer in to the aqueous phase results in rapid miscibility of organic solvent in to aqueous phase leading to increase in the polarity of organic solvent, which in turn decreases the solubility of polymer and initiate nucleation of polymer. However, sonication process inhibits the nucleation of polymer at the initial stage. The cationic nature of polymer provides higher zeta potential to the formed nano particles and develops an electrostatic force and keeps the nano particles in Brownian motion, which inhibits the further growth of polymeric nano particles resulting in the formation colloidal nano formulation.

Brownian motion of nano particles overcomes the Van der Waals force of attraction and gravitational force resulting in the prevention of aggregation and sedimentation of nano particles. Prepared nano particles were characterized for distribution width, mean particle size, surface area, span, and uniformity using laser particle size analyser.

However, these characterization parameters depends on process parameters such as organic solvent, polymer concentration, percentage of organic solvent, volume of aqueous phase, concentration, temperature generated during sonication process, sonication duration and drug concentration.

Hence, a step-by-step optimization was carried out to evaluate the effect of these process parameters on prepared polymeric nano particles and the particle size spectrum of optimization batch (FSN01 to FSN12).

The experiments were performed in triplicate and characterization results were expressed as mean ± standard deviation and student t test (Graph Pad Prism software; version 6.0) was used to evaluate the significance of difference. The differences were considered significant if P value <0.05 and non-significant if P value >0.05.

Table 3: Characterization of prepared SLN's

Run	Average Particle size(nm)	Poly dispersity Index
FSN01	723	1.619
FSN02	186	0.199
FSN03	177	0.252
FSN04	225	0.419
FSN05	1175	1.947
FSN06	192	0.428
FSN07	856	1.54
FSN08	388	0.487
FSN09	1025	1.91
FSN10	556	0.849
FSN11	165	0.299
FSN12	196	0.25

Effect of Formulation Variables on Particle Size

Solid lipid nano particles have been extensively investigated in the field of pharmaceutical research and the particle size of SLNs can play an important role as it can instantly influence the physical stability, cellular uptake, bio distribution and the drug release. Be contingent on the desired administration route, the size of the particle should be optimized. The Particle size range 10 to 1000 nm is acceptable for intravenous administration. Following linear model equation can describe the

Y1. [Average particle size =+480.83+283.00 *A-138.33 *B-35.33 *C+23.17*D+69.83 *E-26.67 *F+70.17 *H].

The F-value of the model 560.10 implies that the model is significant and the Values of "Prob > F" less than 0.0500 indicate model terms are significant. The predicted R^2 (0.9633) and adjusted R^2 (0.9980) values implied a good correlation between the obtained and predicted value and those of the fitted models.

The small particle size of 165 nm (FSN11) could be achieved by working the experiment under following experimental conditions, lipid concentration of 50 mg, surfactant concentration of 75 mg, Co-surfactant concentration of 0.75 ml, aqueous phase volume of 5 ml, magnetic stirring speed of 400 rpm, probe soniation duration 30 mins, volume of beaker used for sonication 500 ml and volume of cold aqueous phase 30 ml. Increase in particle size (Y1) was observed to increase in lipid concentration (X1) and as well decrease the probe sonicator duration (X6).

The value of the coefficient of variation (CV) is 3.84% and indicates the precision and reliability of the model. The lipid concentration, volume of the aqueous phase, magnetic stirring rate and volume of cold aqueous phase give a positive effect on the average particle size while surfactant concentration, co-surfactant concentration and magnetic stirrer rate give negative effect. The parameter (X7) volume of beaker used for sonication does not have significant influence on average particle size.

Effect of Formulation Variables on Poly dispersity Index

The fabricated nano particles, size population commonly follows a multimodal distribution. The poly dispersity is a significant parameter, which can provide the information about the homogeneity of the particle size and it should be (<0.3). The below 0.3 of poly dispersity index shows narrow size distribution and it suggest the particles are mono dispersity. Following linear model equation can explain the effect of factors levels Y2, [Poly dispersity index=+ 0.84+0.54 *A-.25* B-0.083 *C+0.060 *E-0.031*F-0.050 *G+0.12 *H].

Where Y2 is the response of poly dispersity index and A, B, C, E, F, G and H are concentration of lipid, concentration of surfactant, concentration of co-surfactant, magnetic stirrer rate, probe sonicator duration, volume of beaker used probe sonication and volume of cold aqueous phase respectively with their coefficient. The value of the coefficient of variation (CV) is 4.27% and indicates the precision and reliability of the model.

The main effect analysis indicates lipid concentration, magnetic stirrer rate and volume of cold aqueous phase positive effect on poly dispersity index while surfactant concentration, co-surfactant concentration, probe sonication duration and volume of beaker used for sonication give negative effect and the volume of aqueous phase parameters does not have significant effect on poly dispersity index.

Conclusion

Solid lipid nano particles were prepared using micro emulsion method by stirring and sonication approach. Plackett-Burman factorial design was used to optimize the process parameters. Nanoparticles prepared were within an average particle size <100 nm, PDI (i.e. uniformity <0.3).

It was concluded from the study that the composition prepared with lipid concentration of 50 mg, surfactant concentration of 75 mg, Co-surfactant concentration of 0.75 ml, aqueous phase volume of 5 ml, magnetic stirring speed of 400 rpm, probe sonication duration 30 minutes, volume of beaker used for sonication 500 ml and volume of cold aqueous phase 30 ml has shown the smallest particle size of 165 nm.

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