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Preparation and Characterization of Polymeric Nanoparticles used in the Treatment of Epilepsy

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Abstract

The present study was aimed to prepare PLGA nanoparticle with an anti-epileptic drug Tiagabine Hydrochloride. Nanoparticles were prepared by Emulsification Solvent Evaporation Method. Various formulation and process variables which could affect the preparation and properties of nanoparticles. These formulation variables were identified and optimized to get uniform preparation with highest encapsulation efficiency. Formulation variables include amount of drug, polymer concentration and stabilizer concentration and process variables include stirring speed, stirring time and sonication time. The influence of formulation variables on the particle size, zeta potential, drug entrapment efficiency and *in vitro* drug release profile characteristics were investigated. Based on the evaluation results, the formulation which consists of drug/polymer in the ratio of 1:15 was selected as best formulation. Hence the developed formulation was found a promising carrier especially for blood brain barrier as a challenging factor for brain drugs delivery.

Keywords: Epilepsy, Nanoparticles, Formulation, Characterization and PLGA.

INTRODUCTION

Epilepsy is a chronic neurological condition, affecting nervous system, and is characterized by recurrent seizures episodes. Seizures are sudden disturbances in the electrical discharge in the brain. Rapid termination of seizure activity is required; if the episodes of epilepsy remains untreated it may lead to a permanent damage to the brain. The major symptoms of epilepsy involves loss of awareness, vigorous shaking, muscle stiffness, sudden jerking of group of muscles, sudden loss of muscle tone etc. Often seizures arise from a small part of brain and then spread into other regions of the brain. The possible causes of epilepsy involve brain injuries, brain malformation such as cerebral arteriovenous malformation, gene and tuberous sclerosis, tumor, infections such as encephalitis and meningitis.

Systemic drug treatment of epilepsy is a huge challenge due to the unique protective barriers of the Central Nervous System (CNS). The main challenge for efficient drug delivery is to cross, or to bypass, the pervasive tight barrier that is otherwise vital to the homeostasis and normal functioning of the brain. In normal circumstances, the Blood-Brain Barrier (BBB) plays a vital role in protecting the delicate environment of the brain; however, when the introduction of exogenous treatment into the CNS is desired, the BBB prevents 98% of small molecules and an even greater percentage of large molecules from reaching their intended targets. This lack of access to the brain is a major bottle neck for CNS drug development. The BBB prevents the brain uptake of most pharmaceuticals, with the exception of small hydrophilic compounds with a mass lower than 150Da and highly hydrophobic compounds with a mass lower than 400- 600Da that can cross the membrane by passive diffusion [1].

Nanotechnology, which employs engineered materials or devices on a scale between 1 and 100 billionth of a meter (1–100 nm), represents an innovative and promising approach for targeted drug delivery across BBB. Nanotechnology is more evident in diseases related to the Central Nervous System (CNS), mainly due to the highly elaborate structural and functional properties of the CNS. Recently nanotechnology based approaches are widely used in the pharmaceuticals research to treat the neurodegenerative diseases. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-300 nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres, nanocapsules can be obtained. 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. Polymeric nanoparticles are prepared from polymers. These are polymeric particles made of natural or artificial polymers. They are stable and loading of many agents and made control drug delivery and many polymers. The drug is dissolved, entrapped, encapsulated or attached to nanoparticles and depending upon the method of preparation, nanospheres or nanocapsules can be obtained [2].

Antiepileptic drugs can be classified into different categories depending on the mechanism of action; Drugs that affect Gamma-amino-butyric-acid (GABA) activity (Barbiturate, Benzodiazepine, Valproate, Vigabatrin, Gabapentin, Tiagabine) Drugs that affect calcium current (Ethosuximide, Trimethadione, Valproate), Drugs that affect sodium channel (Phenytoin, Carbamazepine, Lamotrigine, Oxcarbazepine, Valproate, Lacosamide), Drugs that affect Glutamate receptors (Perampanel). Antiepileptic drugs can act by directly affecting specific ion channels or neurotransmitters or receptors. GABA and glutamate are the most important inhibitory neurotransmitters and excitatory neurotransmitters respectively. Tiagabine is used for controlling seizures in certain types of epilepsy. However, Tiagabine sometimes produces uncomfortable side-effects, which if severe, may lead to discontinuation of anti-epileptic therapy with the compound. Certain of the side-effects are related to the

central nervous system that is associated with reduced tolerance for the drug. PLGA (Poly Lactic and Glycolic Acid) has been widely explored for preparation of polymeric nanoparticles and is well reported for mucoadhesive properties, improved drug stability, and enhanced entrapment efficiencies.

In this present study PLGA was used for the preparation of Nanoparticles of Tiagabine Hydrochloride, because of its biodegradability and biocompatibility. It degraded by hydrolysis of ester linkages in the presence of water in to two monomers lactic acid and glycolic acid, which under normal physiological condition, are by – products of various metabolic pathways in the body. PLGA with 50: 50 monomers and molecular weight 17000 was used in this work. It has very good mechanical properties and long shelf-life.

MATERIALS AND METHODS

The drug Tiagabine Hydrochloride [4,4-Bis(3-methyl-2-thienyl)-3-butenyljnipecotic acid hydrochloride) was received as a gift sample from Sun Pharma Limited, Delhi, India. PLGA, PLA and Pluronic F68 were purchased from Sigma-Aldrich Pvt Ltd. All the other materials like Dichloro methane, Sodium chloride, Potassium hydroxide, Potassium dihydrogen ortho phosphate were purchased from Sisco Research Laboratories.

Preparation

Nanoparticles were prepared by Emulsification Solvent Evaporation method. Tiagabine Hydrochloride and PLGA were dissolved in a mixture of 20 ml of Acetone and Dichloromethane. This organic phase was injected at the rate of 10 ml/min in 20 ml of water containing Pluronic F-68 under stirring at room temperature. Organic phase was evaporated under reduced pressure. After that the aqueous colloidal mixture was centrifuged and lyophilized to obtain the dry powder nanoparticles [3].

Taking into account various formulation variables like amount of drug, polymer concentration and stabilizer concentration and process variables include stirring speed, stirring time and sonication time six optimized formulations were prepared, named as F1, F2, F3, F4, F5 and F6. All the formulations were characterized for their physiochemical parameters and in-vitro release studies.

Physiochemical Characterization of Nanoparticles:

Differential Scanning Calorimetry

The Differential Scanning Calorimetry analysis was performed for the compatibility studies between the drug and the polymer. Each sample was sealed in Aluminium disc and purged with air at a flow rate of 40ml/min and maintain the temperature at25^0C-200^0C [4]. The DSC spectrum of the pure Tiagabine was compared with mixture of the Tiagabine and PLGA.

Particle size

Nanoparticle size was determined using Photon Correlation Spectroscopy (PCS). All samples were diluted with ultra-purified water. The analysis was performed at a scattering angle of 25° to 90°scaterring angle and it was recorded for 180 s for each measurement. The mean diameter for each sample and mean hydro--dynamic diameter was generated by cumulative analysis in triplicate [5].

Polydispersity studies:

Polydispersity was determined according to the equation,

$$\text{Polydispersity} = \frac{D(0.9) - D(0.1)}{D(0.9)}$$

Where, D(0.9) corresponds to particle size immediately above 90% of the sample, D(0.5) corresponds to particle size immediately above 50% of the sample. D(0.1) corresponds to particle size immediately above 10% of the sample [5].

Surface Charge determination

Nanoparticles were characterized with Zeta potential (ζ) using a Zeta Sizer 4 (Malvern Instruments ltd., Malvern UK). The zeta potential measurements were performed by using an aquous dip cell in an automatic mode. The prepared nanopriculate samples were diluted with ultrapurified water and keep in the measurement cell for potential energy analysis, time after the position adjusted and fix suitable position [6].

Drug Content and Drug entrapment efficiency:

A known amount of freeze-dried nanoparticles was taken in triplicate, and dissolved in a known volume of acetone. The amount of Tiagabine was quantified by Spectrophotometer by measuring the absorbance at 257 nm.

Entrapment efficiency was calculated as follows [7].

$$\text{DEE\%} = \frac{\text{Amount of Drug actually present x 100}}{\text{Theoretical Drug load expected}}$$

External Morphological Studies (TEM):

External morphological of nanoparticles was determined using Transmission Electron Microscopy (TEM) with Philips EM-CM 12, 120 kr. Sample were prepared by placing one drop on a copper grid, dried under vacuum pressure before being examined using a TEM without being stained [8].

In – Vitro Drug Release Studies:

The dialysis bag diffusion technique was used to study the *in-vitro* drug release of Tiagabine nanoparticles. The prepared nanoparticles were placed in the dialysis bag and immersed in to 50ml of Phosphate Buffer (7.4). The entire system was kept at 37±0.5ºC. With the continuous magnetic stirring at 200 rpm/min. Samples were withdrawn from the receptor compartment at predetermined intervals and replaced by fresh medium. The amount of drug dissolved was determined with UV-Spectrophotometry at 257 nm [9].

Table 1: Formulation of PLGA Nanoparticles

Formulation Code	Amount of Drug (mg)	Amount of PLGA (mg)	Amount of Pluronic F-68 (mg)	Amount of Acetone/ Dichloromethane (ml)	Amount of Water (ml)
F1	10	100	100	20	50
F2	10	125	100	20	50
F3	10	150	100	20	50
F4	10	100	150	20	50
F5	10	125	150	20	50
F6	10	150	150	20	50

Table 2: Evaluation of PLGA Nanoparticles

Formulation Code	Drug Content (mg)	Entrapment Efficiency (%)	Polydispersity	Particle Size (nm)	Zeta Potential
F1	6.22	62.2	0.098	0.132	-16
F2	6.82	68.2	0.132	0.148	-17
F3	7.21	72.1	0.113	0.164	-15
F4	6.68	66.8	0.157	0.171	-16
F5	7.11	71.1	0.131	0.189	-18
F6	7.43	74.3	0.128	0.204	-17

Table 3: In-Vitro Release studies of PLGA Nanoparticles

Time (In hrs)	1	2	4	6	8	10	12	24
F1	13.13±1.23	23.31±1.43	32.25±1.34	41.33±1.41	47.29±1.37	53.35±1.48	59.21±1.36	63.13±1.17
F2	11.26±1.45	21.18±1.31	29.21±1.22	35.31±1.21	40.26±1.28	45.28±1.27	50.18±1.39	59.21±1.23
F3	9.35±1.54	18.32±1.61	25.56±1.19	32.58±1.68	37.51±1.21	41.33±1.52	46.38±1.51	55.35±1.61
F4	14.51±1.29	25.32±1.21	35.63±1.21	45.23±1.57	51.29±1.43	59.31±1.32	65.34±1.34	72.63±1.37
F5	12.24±1.31	22.21±1.54	32.18±1.32	42.28±1.39	49.21±1.17	54.41±1.54	60.93±1.12	65.24±1.19
F6	10.44±1.24	20.38±1.21	30.45±1.12	36.35±1.32	42.23±1.28	46.51±1.32	55.34±1.25	62.45±1.32

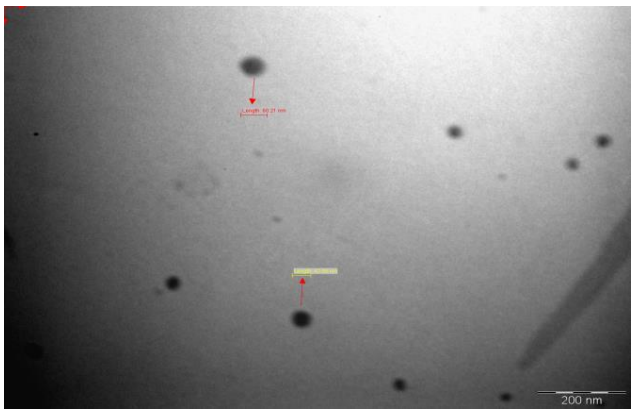

Figure 1: TEM Image of PLGA Nanoparticles

DISCUSSION

Preparation of Tiagabine Loaded PLGA Nanoparticles

Tiagabine loaded PLGA Nanoparticles were prepared by emulsification solvent evaporation method. Poly Vinyl Alcohol was used as a copolymer. Pluronic F-68 was used as a stabilizer. The prepared particles were stored in lyophilized form for their long storage and stability.

Differential Scanning Calorimetry

DSC thermograms of Tiagabine, PLGA, Pluronic F-68 and Physical admixtures of Tiagabine with other excipients were recorded. The pure drug and the physical admixtures of drug showed the same peak at 84.6°C in the thermograms. The peak showed that there were no significant compatibility problems between the drug and the polymer.

Particle Size Distribution:
The mean particle size of Tiagabine loaded nanoparticles is shown in Table 2. The particle size distribution curves for all the samples were unimodel. Average nanoparticles sizes of the formulations were range from 132-204 nm (F1-F6) respectively. The nanoparticle size is dependant on PLGA concentration. The smallest particle size of were found in batch F1 (132 nm) and largest particle of was found in batch F6 (204 nm). The data suggested that in an increase in polymer concentration increase the particle size.

Polydispersity Index
The Polydispersity index of the data revealed that the particle size distributions of all the formulations were unimodel. The results were shown in Table 2.

Surface Charge Determination:
The zeta potential measure the surface charge of the particle. The zeta potential can greatly involved the stability of suspension through the electrostatic repelsion and attraction between the particles. The repulsive interaction will be higher between the particles as the zeta potential increases. It leads to the formation of more stable particles with more uniform size distribution.[10] The Zeta Potential of PLGA nanoparticles of Tiagabine were shown in Table 2.

Drug Content and Drug entrapment efficiency:
The total drug content in nanoparticle were varied from 62.2-7.43 mg for F1 to F6 and shown in Table 2. The percentage entrapment efficiency varied from 62.2-74.3%. Entrapment efficiency of the formulation increased due to the high concentration of drug. [11]

External Morphological Studies:
The External Morphological Studies revealed that maximum nanoparticles were nearly spherical or crystal.(Figure 1). The nanoparticle size observed by TEM correlated well with the particle size distribution measured by Master sizer (Malvern Instrument).

***In-Vitro* Release Studies**
In-Vitro release studies were carried out by dialysis bag method. Figure 1 showed that the release profile of Tiagabine from PLGA nanoparticles. The release of drug from the polymer varied from 63% to 72% for 24 hours (Table-3). In the *in-vitro* release profile of the drug loaded nanoparticles showed 50 – 60% of drug release in a sustained manner within a period of 12 hours remaining quantity slowly elute from the polymer. [12] The cumulative percentage of drug release from the nanoparticle formulation F3 was 72.63±1.37 for 24 hours.

CONCLUSION

This study confirms that the Emulsification- Solvent Evaporation technique is suitable for the preparation of Tiagabine nanoparticles with high encapsulation efficiency. This formulation approach can be used to improve the therapeutic efficacy of poorly soluble drugs. The changes in nanoparticle size and release kinetics were affected by changes in polymer and stabilizer concentration. The sustained release of drug from the Tiagabine nanoparticles suggested that the frequency of administration, dose and adverse effects of this molecule could be reduced. We can conclude that there is large scope for improving the use of Tiagabine in hypertensive treatments through nanoparticle as a drug delivery system.

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