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Development and Characterization of Mucoadhesive Nanoparticles of Topiramate for Nasal Administration

Shwetal Nilkanth Chopade*, Shivakant Shukla, Akhilesh Kumar Singhai

Lakshmi Narain College of Pharmacy- [LNCP], Bhopal (M.P.)

ABSTRACT

Nasal drug delivery has been recognized as a promising route for delivery of therapeutic compounds including biopharmaceuticals. It has been demonstrated that absorption of drugs can enhance by using absorption enhancers or increasing the drug residence time in the nasal cavity. Mucoadhesive polymers can serve both functions. The residence time in the nasal cavity is considerably increased for nanoparticles compared to solutions. Topiramate (TPM) is used alone or with other medications to prevent and control seizures (epilepsy). This medication is also used to prevent migraine headaches and decrease how often you get them. TPM will not treat a migraine headache once it occurs. The purpose of the present study was to prepare and evaluate mucoadhesive chitosan nanoparticles of TPM for the nasal administration by ionic gelation method. Nanoparticles were subjected to various characterization techniques such as FTIR, particle size, scanning electron microscopy (SEM), drug entrapment efficiency and zeta potential are also determined for the developed formulations. The nanoparticles exhibited mucoadhesive properties, which diminished with increasing drug content. The nanoparticles with a particle size range between 45and 62nm exhibited excellent mucoadhesive properties. Stability study at various storage temperature was also done in which the prepared formulation showed an improved stability. The zeta potential of the best chitosan preparation (F4) was found to be -35.5mV, which confirms the stability of prepared nanosuspension. Mucoadhesive chitosan nanoparticles can be a promising carrier for nasal delivery of TPM found to have high encapsulation efficiency and predetermined *in vitro* release profile.

Keywords: Nasal drug delivery, Topiramate, Nanoparticles, ionic gelation method, Migraine headache

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***Address for Correspondence:**

Shwetal Nilkanth Chopade, Lakshmi Narain College of Pharmacy-[LNCP], Bhopal (M.P.)

INTRODUCTION

Nasal administration can be used to deliver drugs for either local or systemic effect. The nasal route circumvents hepatic first pass elimination associated with oral delivery. Rapid mucociliary clearance of t drug formulation is responsible for the low bioavailability of drugs administered by nasal route. To circumvent these problems, mucoadhesive drug delivery systems are being developed to provide a long term therapeutic concentration of the drug[1]. The use of mucoadhesive polymers for the development of delivery system maximizes the residence time of the drug formulation in the nasal cavity and hence prolonging the period of contact with the nasal mucosa thereby improving drug absorption[2]. Multiparticulate systems like nanoparticles, microparticles provide controlled release of the drugs. Nanoparticles are colloidal sized particles, possessing diameters ranging between 10 and 1000 nm, and drugs may be encapsulated, adsorbed or dispersed in them. Properties of the nanoparticles are largely dependent on the polymers used to prepare it. Chitosan has been shown to have mucoadhesive properties because of its viscosity and interaction of the positively charged amino group with the negatively charged sites on the mucosa surface. Chitosan (CS) is a deacetylation derivative of chitin, and is biocompatible, biodegradable and non toxic in nature. Investigations have suggested that there are two effects of chitosan delivery systems on nasal mucosa. The mucoadhesive properties of the polymer can reduce the clearance rate of drugs from nasal cavity, thereby prolonging the contact time of chitosan delivery system with nasal epithelium. In addition, it has been shown that the interaction of the positively charged amino group of chitosan with the negatively charged sialic acid residues in mucus causes the transient opening of the tight junctions and allows large hydrophilic compounds to be transported across the epithelium[3]. Topiramate (TPM) is a novel antiepileptic drug derived from the naturally occurring monosaccharide D fructose. It is not structurally related to other antiepileptic drugs and was originally synthesized as

the part of a search for fructose related compounds with hypoglycemic activity[4]. It has multiple mechanisms of action such as sodium and calcium channel blockade; potassium channel activation; glutamate receptor antagonism; gamma-aminobutyric acid potentiation and carbonic anhydrase inhibition[5]. In the present research work, an attempt has been made to prepare mucoadhesive nanoparticulate system of TPM for nasal delivery by ionic gelation method.

MATERIALS AND METHODS

Materials

TPM was procured from MSN Organics Pvt. Ltd., Hyderabad as a gift sample. Chitosan was obtained from HiMedia Laboratories Ltd, Mumbai, India. STPP, acetic acid, ethanol, Span 80, acetone, dichloromethane and light liquid paraffin were purchased from Central Drug House Pvt Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade.

Determination of λ_{max} of TPM

Accurately weighed 10 mg of TPM was dissolved in 10 ml of phosphate buffer pH 6.6 in a 10 ml volumetric flask. The resulted solution (1000µg/ml) was used to prepare the concentration 10µg/ml. The spectrum of this solution was recorded in 200-400 nm range using UV spectrophotometer (Labindia-3000+). After the complete scan λ_{max} of TPM was found 260 nm.

Preparation of calibration curve

From stock solutions of TPM 1 ml was taken and diluted up to 10 ml. From this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10ml volumetric flasks and maked up the volume to 10 ml with phosphate buffer pH 6.6, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Preparation of chitosan nanoparticles of TPM

Chitosan nanoparticles were prepared by ionotropic gelation method[6].

Preparation I: Chitosan solution (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 % w/v) was prepared by dissolving chitosan in acetic acid (0.1% v/v) at room temperature.

Preparation II: The drug was dissolved in 5ml of Chitosan solution.

Preparation III: 1% Sodium tripolyphosphate solution was prepared.

Preparation II was added in 50ml of preparation III through a disposable syringe needle by gently agitating. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Nanoparticles were obtained which was air dried for twenty four hours followed by oven drying for six hours at 40˚C Table 1.

Table 1 Composition of mucoadhesive nanoparticles

Components	Formulation code					
	F1	F2	F3	F4	F5	F6
Topiramate (mg)	10	10	10	10	10	10
Chitosan (%)	0.1	0.2	0.3	0.4	0.5	0.6
Acetic acid (%)	0.1	0.1	0.1	0.1	0.1	0.1
Sodium tripolyphosphate (%)	1.0	1.0	1.0	1.0	1.0	1.0
Stirring speed (rpm)	600	600	600	600	600	600
Stirring time (hrs)	2	2	2	2	2	2

Evaluation of nanoparticles

Measurement of mean particle size

The mean particle size of the nanoparticles was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer **(**Malvern particle size analyser**)** at a scattering angle of 90°. A sample (0.5mg) of the nanoparticles suspended in 5 ml of distilled water was used for the measurement.

Determination of zeta potential

The zeta potential of the drug-loaded nanoparticles was measured on a zeta sizer (Malvern particle size analyser) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.

Drug entrapment efficiency

The entrapment efficiency of the drug is defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Chitosan nanoparticles entrapped TPM were separated from the free drug by dialysis method. The above said formulations were filled into dialysis bags and the free TPM dialyzed for 24 hours into 50 ml of phosphate buffer pH 6.6. The absorbance of the dialysate was measured at 260.0 nm against phosphate buffer pH 6.6 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free TPM could be obtained from the absorbance difference based on calibration curve. Calibration curve was made by measuring the absorbance at 260 nm for known concentrations of TPM solution.

Drug content

From the prepared Chitosan nano formulation 1ml of suspension is dissolved in the 10 ml of methanol. The amount of Topiramate was determined using UV spectrophotometer at 260nm.The placebo formulation prepared similarly to drug loaded nanoparticles was used as blank. The total drug content was calculated.

In-vitro wash-off test for chitosan nanoparticles

The mucoadhesive properties of the nanoparticles were evaluated using an *in-vitro* wash-off test. A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch-by-1 inch), using thread. Nanoparticles were spread (approximately 50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegration test apparatus, with continuous oxygen supply. The disintegration test apparatus was operated, giving the tissue specimen was given regular up and down movements within the beaker of the disintegration apparatus, which contained the simulated nasal fluid (pH 6.6). At the end of 30 min, 1 h and at hourly intervals up to

12 h, the number of nanoparticles still adhering onto the tissue was counted.

Shape and Surface Characterization of nanoparticles by Scanning Electron Microscopy (SEM)

From the formulated batches of chitosan nanoparticles optimized formulations which showed an appropriate balance between the percentage releases were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 10 KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.

***In-vitro* diffusion study**

An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. Nanoparticles equivalent to 10 mg of TPM was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 6.6. The diffusion cell was maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer at 260 nm[7-11].

Mathematical treatment of *in-vitro* release data

The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

Zero-order kinetics: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

$$Q_t = Q_o + K_o t$$

where $Q_t$is the amount of drug dissolved in time t, Q_o is the initialamount of drug in the solution (most times, Q_o=0) and $K_o$is the zero order release constant.

First-order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initial amount of drug in the solution and $K_1$is the zero order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

$$Q = K_H . t^{1/2}$$

Where Q is the amount of drug released in time t and K_H is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drugs.

Korsmeyer-Peppas model: Korsmeyer *et al.* used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\, t^n$$

Where $M_t/M_\infty$is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log M_t/M_∞ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of *n* =0.5 for fickian diffusion and higher values of *n*, between 0.5 and 1.0, or *n* =1.0, for mass transfer following a non-fickian model. In case of a cylinder *n* =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent *n* the portion of the release curve where M_t/M_∞< 0.6should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (*l*) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and bvalue would be zero and only $at^n$is used. This mathematical model, also known as *Power Law*, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms[12-14].

Stability studies for optimized formulation

Stability of a formulation on storage is of great concern as it is the major restraint in their development as marketed preparation. Optimized nanoparticle formulation (F4) were stored in amber colored bottles and subjected to exhaustive stability testing at 4±1°C and room temperature for 3 month period. Samples were withdrawn periodically and formulation was observed on the basis of % EE, average particle size and physical appearance.

RESULTS AND DISCUSSIONS

The λ max of TPM was found to be 260 nm by using U.V. spectrophotometer (Labindia-3000+) in linearity range 5-25µg/ml Figure1, 2.

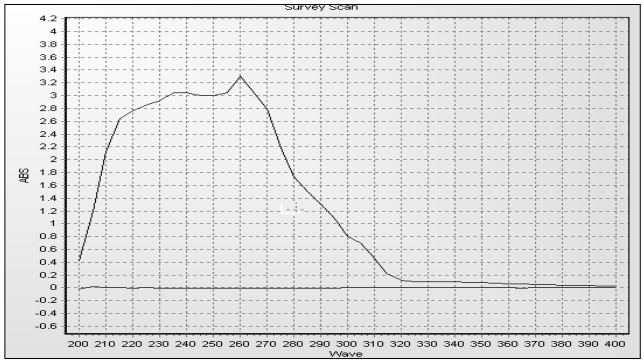

Figure 1 U.V. Spectra of Pure TPM in phosphate buffer pH 6.6

The particle size is an important parameter as it has a direct effect on the stability, cellular uptake, drug release and mucodistribution. The mean particle sizes of the prepared nanoparticles measured by the Horiba Zetasizer were in size range of 45.5±0.9to 62.23±0.7 nm and the distribution of particle sizes are found to be monodispersed as the polydispersity index lies between 0 to 1 (0.642 to 0.965) in all the formulations. There was no noticeable difference between the sizes of nanoparticles obtained with different drug polymer ratio. The particle morphology can be modulated by selecting the agitation speed as well as drug polymer ratio. The drug content and % EE of all formulation was found to be in range of 63.12±0.5 to78.8±0.2 and 49.3±0.25 to 63.1±0.56. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of TPM nanoparticles. The maximum drug content and entrapment efficiency was found formulation F4 Table 2 & Figure 3.

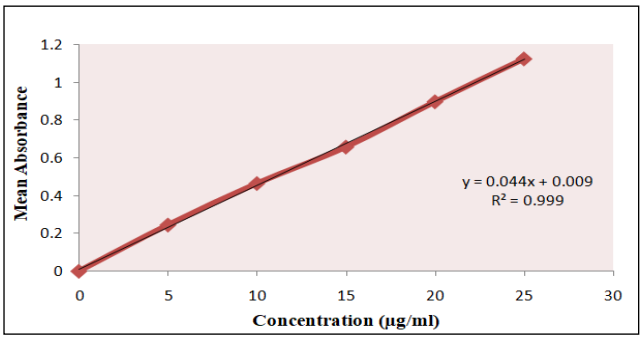

Figure 2 Calibration Curve of TPM in phosphate buffer pH 6.6

Table 2 Evaluation of nanoparticle formulations

Formulation	Particle Size* (nm)	Entrapment efficiency* (%)	Drug content* (%)	Poly-dispersity index*
F1	55.65±0.4	58.2±0.45	69.98±0.5	0.965±0.023
F2	62.23±0.7	55.6±0.32	65.45±0.1	0.912±0.041
F3	52.23±0.5	51.9±0.41	63.12±0.5	0.885±0.036
F4	45.5±0.9	63.1±0.56	78.8±0.2	0.642±0.049
F5	58.89±0.4	49.3±0.25	69.98±0.4	0.756±0.036
F6	62.23±0.3	54.32±0.25	70.12±0.2	0.841±0.041

***Average of three determinations (n=3)**

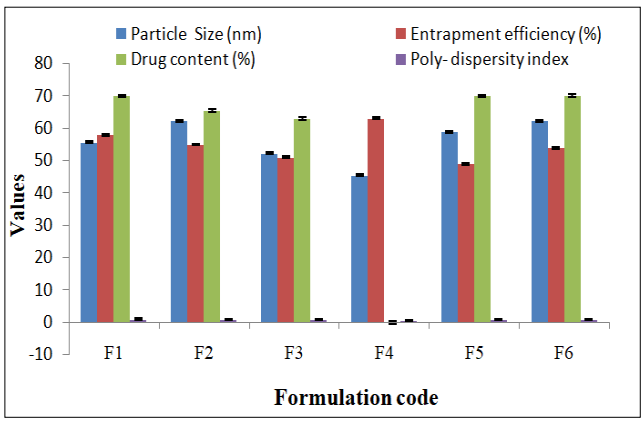

Figure 3 Graph of evaluation of nanoparticle formulations

The results of *in vitro* mucoadhesion carried out showed that all the prepared formulation had good mucoadhesive property. It was found that increase in the concentration of chitosan in the formulation increased the mucoadhesion from 56.65±0.23 to 72.12±0.56% (F1 to F6). Formulation F4 showed maximum mucoadhesion 72.12±0.56 % Table 3 & Figure 4.

The results of measurement of mean particle size of optimized formulation F4 nanoparticles were found 45.5±0.9nm and zeta potential of optimized formulation F4 nanoparticles was found to be -35.5 mV Figure 5.

Table 3 Evaluations of percent mucoadhesion

S. No.	Formulation Code	Percent Mucoadhesion
1	F1	56.65±0.23
2	F2	62.23±0.41
3	F3	63.23±0.32
4	F4	72.12±0.56
5	F5	69.98±0.76
6	F6	59.45±0.34

*Average of three determinations (n=3)

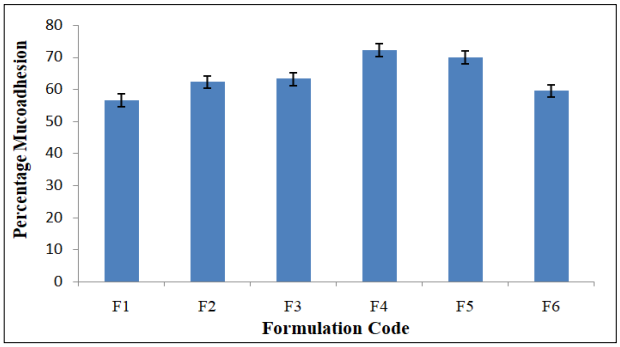

Figure 4 Graph of evaluations of percent mucoadhesion

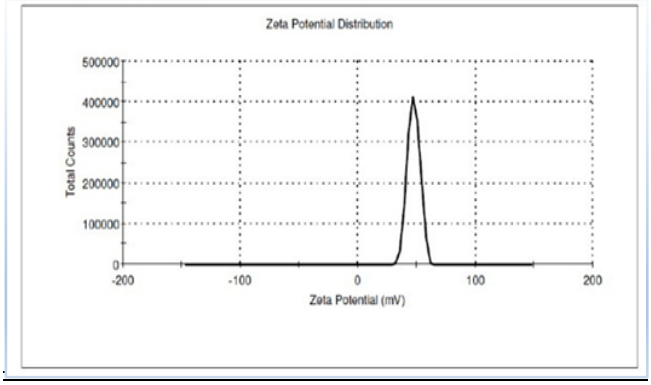

Figure 5 Zeta potential of optimized formulation (F4)

The *in vitro* release profiles of the optimized formulation F4 were carried out in pH 6.6 Phosphate buffer for 6 h. Sustained release of the drug from the NPs is important as it would allow for a prolonged residence of the drug at the absorption site, increasing drug bioavailability. The *in vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation, higuchi's and korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values were compared, it was observed that 'r²' values of first order were maximum i.e. 0.946 hence indicating drug releases from formulation follow first order release kinetics. Table 4, 5 & Fig. 7, 8.

Table 4 *In-vitro* drug release data for F4

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	8.23±0.53	0.9154	91.77	1.963
1	1.000	0.000	16.69±0.42	1.2225	83.31	1.921
1.5	1.225	0.176	22.32±0.64	1.3487	77.68	1.890
2	1.414	0.301	44.56±0.13	1.6489	55.44	1.744
2.5	1.581	0.398	56.69±0.27	1.7535	43.31	1.637
3	1.732	0.477	69.98±0.14	1.8450	30.02	1.477
4	2.000	0.602	73.32±0.65	1.8652	26.68	1.426
6	2.449	0.778	83.32±0.89	1.9207	16.68	1.222

*Average of three determinations (n=3)

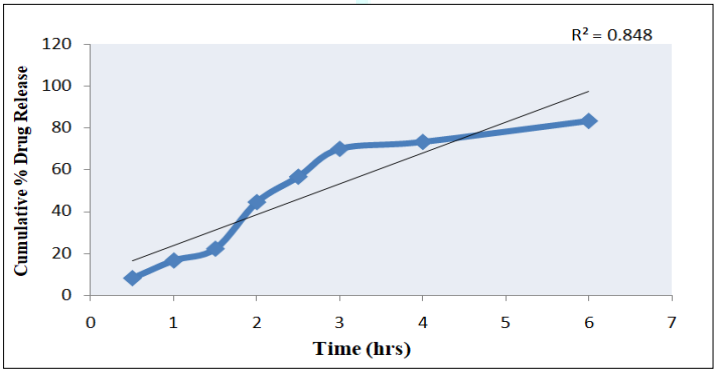

Figure 7 Zero order release kinetics

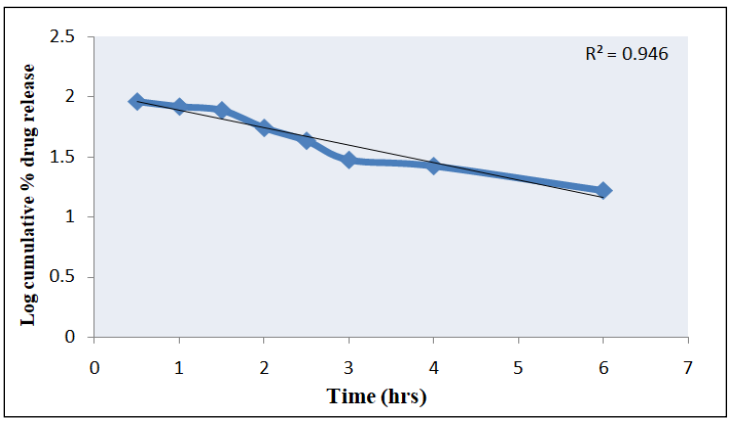

Figure 8 First order release kinetics

Table 5 Regression analysis data of mucoadhesive nanoparticles

Batch	Zero Order	First Order
	r^2	r^2
F4	0.848	0.946

CONCLUSION

Chitosan NPs exhibit significant mucoadhesive properties and could potentially be used for sustained intranasal delivery of antiepileptic drugs. We successfully formulated in the form of a chitosan nanoparticle system and the formulation was optimized by considering the concentration of chitosan, chitosan/TPP ratio and nanoparticle size distribution. In vitro release studies showed that the drug is released from the optimized formulation over a period of 6 hr in a sustained release manner. This produces a formulation which is a viable alternative to conventional methods by virtue of its ability to sustain the drug release and for its ease of administration because of reduced dosing frequency, resulting in better patient compliance.

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