# Chitosan-Based Polymeric Nanoparticles of Lamotrigine for Solubility Enhancement

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**ABSTRACT**

A naturally occurring polymer, chitosan is mostly extracted from the shell of marine organisms. Due to its unique properties of low toxicity, biocompatibility, biodegradation, and low immunogenicity, chitosan is being employed extensively in the field of biomedicine. Chitosan nanoparticles can be easily formulated. In this work Development of nanoparticles and scaling up the formulation with its size atoms and molecules has been focused on. The achievement in therapeutic efficacy and fewer side effect has been achieved. In this current work, the targeted drug delivery for various diseases has been achieved by preparing the nanoparticles. The drug lamotrigine is used in increasing the efficacy of exerting cellular activities, to increase the adsorption of lamotrigine we have developed lamotrigine nanoparticles and we have confirmed that Nano formulation with analyzing FTIR, DSC, and solubility. Also, we have studied the same and particle size analysis with entrapment efficacy. The F2 formulation had shown the highest percent drug content with the highest entrapment efficacy. Lamotrigine showed 43.59% and formulation F2 showed 83.12% drug release at 90 min., which was linearly increasing concerning time. The release rate was increased almost by 44% significantly higher than that of pure Lamotrigine.

## INTRODUCTION

Nanotechnology is getting developed at various levels like materials, systems, and devices. At present in commercial applications as well as scientific information the most innovative level is nanomaterials. Nanotechnology is a minor object that acts as a complete unit in terms of its properties or transport and is called a particle. They can be classified according to their sizes as fine particles and ultrafine particles [1]. Nanotechnology is the science of the small; the very small. It is the use and manipulation of matter on a tiny scale. At this size, atoms and molecules work differently and provide a variety of surprising and interesting uses. Nanotechnology as well as nanoscience studies have emerged rapidly during the past years in a broad range of product domains. It provides opportunities for the development of materials including those

for medical applications, where conventional techniques may reach their limits. Nanotechnology should not be viewed as a single technique that only affects a specific area. Nanotechnology shows the design, production, and application of materials at atomic, molecular, and macromolecular scales, to produce new Nano-sized materials. Nanoparticles are defined as it is solid, submicron-sized (less than 100 nm in diameter) drug carriers that may or may not be biodegradable. It is a combined name for both nanospheres as well as Nanocapsules. Nanospheres are matrix systems in which the drug is uniformly dispersed while Nanocapsules the system in which the drug is surrounded by a unique polymeric membrane called Nanocapsules [2, 3]. Nanoparticles are not simple molecules themselves and therefore composed of three layers (A) The surface layer which may be functionalized with a variety of small molecules, metal ions, surfactants, and polymers. (B) The shell layer is a chemically different material from the core in all aspects, and (C) The core is essentially the central portion of the nanoparticles and usually refers to the nanoparticles themselves. Nanoparticles possess unique physical and chemical properties due to their high surface area and nanoscale size [4, 5].

Polymeric nanoparticulate systems from biodegradable and biocompatible polymers are interesting options for controlled drug delivery as well as drug targeting. "The polymeric nanoparticles are solid colloidal particles with a diameter ranging from 1 to 1000 nm in size and composed by natural and synthetic polymer called as polymeric nanoparticle" [6]. The utility of the nanoparticles delivery system is dependent upon the bio acceptability of the carrier polymer, which in turn is affected by the particle size and physicochemical properties of the polymer. The additional advantages of nanoparticles when compared with the other colloidal carriers, such as higher stability when in contact with the biological fluids, high drug-loading capacities, and protection by the solid matrix of the incorporated drug against degradation, thus leading to the increased intracellular concentration of the drug. The surface of the polymeric Nanoparticles can be covalently conjugated to folic acid, and monoclonal antibodies, to achieve targeted delivery and cell-specific uptake [7]. Polymers are the most common materials for constructing nanoparticle-based drug carriers. Polymers used to form nanoparticles can be both synthetic and natural polymers [8]

Chitosan is a molecule with a carbohydrate backbone structure and is similar to cellulose chitosan consists of two types of repeating units, N-acetyl-d-glucosamine and d-glucosamine they are linked with (1-4)-β-glycosidic linkage [9]. Chitosan is a biopolyamino saccharide cationic polymer that is obtained from chitin by alkaline deacetylation and characterized by the presence of a large number of amino groups on its chain. Although chitosan is obtained from chitin, the applications of the latter compared to chitosan are limited because it is chemically inert. A common method for chitosan synthesis is the deacetylation of chitin, usually derived from the shells of shrimp as well as other sea crustaceans, using excess aqueous sodium hydroxide solution as a reagent [10].

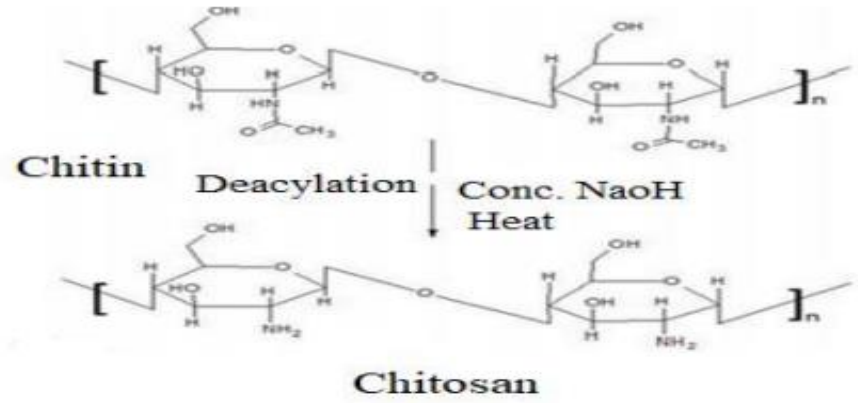


**Figure 1. Deacylation of chitin to chitosan**

The presence of this much high amines provides important functional properties to chitosan. The degree of deacetylation as well as the molecular weight of chitosan affect its physicomechanical properties. The degree of deacetylation of chitosan affects its hydrophobicity, solubility, and toxicity. The chitosan having a higher degree of deacetylation shows toxicity according to their molecular weights which are less toxic for low molecular weight and highly toxic for high molecular weights. Chitosan having a lower degree of deacetylation acts as an absorption enhancer at both high and low molecular weights [11]. Chitosan nanoparticles have the characteristics of chitosan and the properties of nanoparticles such as surface and interface effect, small size, and quantum size effects [12]. Chitosan nanoparticles have the characteristics of chitosan and the properties of nanoparticles such as surface and interface effect, small size as well as quantum size effects [13]. Chitosan acts as a penetration enhancer by opening the tight junctions of the epithelium. Chitosan facilitates both paracellular and transcellular transport of drugs they are shown diagrammatically follows by

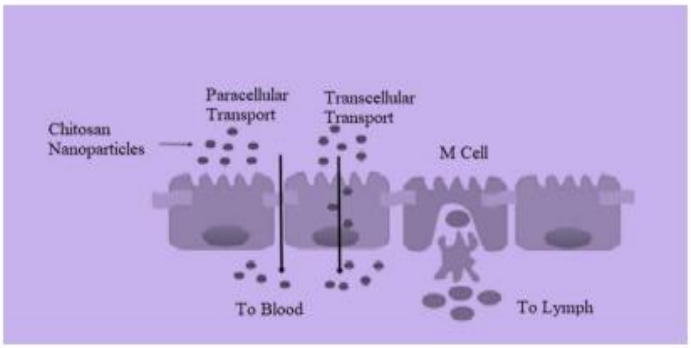


**Figure 2. Diagrammatic representation of the mechanism of transcellular and paracellular transport of Chitosan Nanoparticles across the epithelium**

## MATERIALS AND METHODS

### Materials

Lamotrigine is taken as API, Chitosan, Acetic Acid, Tripolyphosphate (TPP), Mannitol are the excipients. All ingredients are purchased from authorized vendors.

### Methods

#### a. Preformulation study

The formulation studies like qualitative analysis of the drug which includes physical characterization, Drug-Excipient compatibility studies, and solubility study of the drug.

**b. API Characterization**

The API is characterized by using methods and procedures given as per USP and certificate of analysis (COA) provided by the manufacturer of the API. The following tests were performed- Description- Colour, Nature, Odour, and Taste.

**c. Identification of pure drug**

Identification of Lamotrigine was carried out by melting point determination, UV spectroscopy, FTIR, and DSC.

**d. Melting point determination**

The melting point of the drug is determined by taking a small amount of the drug in a capillary tube closed at one end. The capillary tube was placed in the melting point apparatus and the temperature at which the drug melted has recorded this procedure was performed thrice and the average value was noted.

**UV Spectroscopy**

**a. Determination of λ max and a calibration curve of Model drug**

Accurately weighed 1mg of drug was transferred to 100 ml of volumetric flask. Add Methanol and the volume was made up to 100ml and the solution was scanned on a UV spectrometer in the range 200-400nm [14]

**b. Calibration curve of Lamotrigine**

The stock solution for the standard drug of 1 mg was prepared using 100 ml of methanol. The maximum absorbance for the drug solution of 10 mcg/ml was found to be 224 nm. The linearity was found between the concentration ranges of 2-10 mcg/ml for UV Spectroscopy. Methanol was used as the diluent solvent for the dilutions [14]

**Solubility study**

The aqueous solubilities of Lamotrigine in the different solvents were determined by the Higuchi and Connors method [15]. Briefly, an excess amount of Lamotrigine was added in various solvents, in the volumetric flask at room temperature. The solutions were agitated using the mechanical shaker for 48 h, at 200 rpm. The supernatant was filtered through a membrane filter (0.45 μ). 1 mL of this filtrate, after appropriate dilutions, was assayed using a UV–visible spectrophotometer at the respective wavelength.

**Drug and excipient compatibility study**

**a. Fourier Transformation Infrared Spectroscopy (FTIR)**

For determination of the presence of structure claiming functional groups of Lamotrigine, its analysis was done using an FTIR spectrophotometer (Model: IR Affinity, Shimadzu Corporation, Kyoto, Japan). Briefly, Lamotrigine (2 mg) was uniformly mixed with potassium bromide (KBR, 200mg). Each analysis included 45 scans, at a resolution of 4($cm^{-1}$) in the wavelength range of 4000 to 400 ($cm^{-1}$) [16].

**b. Differential scanning calorimetry (DSC)**

Differential scanning calorimetry is a well-established technique for the analysis of the thermal behavior of a wide variety of materials. Measuring the changes in material properties as a function of controlled changes in temperature can provide useful information regarding the melting, degradation, compatibility, stability, and other related properties of test materials. In DSC thermograms these changes exhibit themselves as enthalpy changes, appearance/disappearance of peaks, and changes to a peak's onset time, shape, and relative area. It also provides information on drug-excipient interactions and the formation of new entities. The pure sample of Lamotrigine was taken in a standard aluminum pan and heated from 20

°C to 140 °C at a constant rate of 10 °C per minute under a nitrogen atmosphere by using Differential scanning calorimetry (Model: DSC-1 821e, Mettler-Toledo AG, Analytical, Schwerzenbach, Switzerland) Dried nitrogen was used as a purge gas [17].

**Preparation of chitosan nanoparticles by using the Ionic gelation Method**

Chitosan nanoparticles were prepared using ionic gelation techniques. Briefly, chitosan (3–6 mg/ml) was incorporated in a 2 % acetic acid aqueous solution maintaining 100 rpm magnetic stirring for 5 h at 25 °C. After 5 h of constant stirring, the desired quantity of Lamotrigine (drug) was incorporated slowly in the chitosan (polymer) solution containing the desired percentage of TPP (anionic surfactant) and stirred for 25 min. The resultant solution was further homogenized at a specific rpm using a high share homogenizer for 15 min. Further, the nanosuspension was centrifuged at a specified rpm. The suspension was further mixed with cryoprotectant using a magnetic stirrer at 500 rpm for 15 min and stored at −80 °C for 48 h. After 48 h, the frizzed suspension was lyophilized using Lyophilizer at b100mTorr vacuum pressure and maintained condenser temperature at −78 °C for 8 h. The dried powder was used for further characterization [18,19]

**Optimization of chitosan nanoparticles of Lamotrigine by Ionic gelation method**

Using the different concentrations of chitosan various formulations were taken placed as shown in the following table and subjected to characterization to get the optimized one

**Table 1. Formulation of chitosan nanoparticles**

| **Sr.** | **Ingredients** | **F1** | **F2** | **F3** | **F4** |
|---|---|---|---|---|---|
| 1. | Lamotrigine | 10 mg | 10 mg | 10 mg | - |
| 2. | Chitosan | 120 mg | 180 mg | 240 mg | 240 mg |
| 3. | 2% Acetic acid | 40 ml | 40 ml | 40 ml | 40 ml |
| 4. | Tripolyphosphate | 05 mg | 05 mg | 05 mg | 05 mg |
| 5. | Mannitol | 1.25mg | 1.25mg | 1.25mg | 1.25mg |

**Solid state characterization of nanoparticles**

**a. X-ray diffraction study (XRD)**

To understand the crystallinity and the dimension of the polymeric nanoparticles, an X-ray diffraction study was performed by (Model: D8 ADVANCE, Bruker AXS, Inc., Madison, WI, USA) X-ray diffractometer where pure drug (Lamotrigine), physical mixture & lyophilized chitosan conjugated Lamotrigine Polymeric nanoparticles was analyzed. During the operation, the overall voltage was maintained at 30 Mv and a 10mA monochromator slit was used to take the pattern of samples. Using silicon as a standard, Lamotrigine & lyophilized chitosan conjugated Lamotrigine polymeric nanoparticles were scanned at 2θ extending from 10 to 80° utilizing an Xbeam diffractometer with a step size of 0.02°and count time of 2 s per each step [20]

**b. Scanning Electron Microscopy (SEM)**

For studying the surface morphology of Lamotrigine-loaded chitosan nanoparticles, Scanning Electron Microscopy (Jeol JFC1600, Japan) was used. After lyophilization of the sample, it was gold coated using a sputter coater for 4 min at 10 mA current. After the gold coating, samples were attached to the aluminum stubs and then viewed using an accelerating voltage of 15.00 kV at the different magnifications [20]

**c. Transmission Electron Microscopy (TEM)**

The Transmission electron microscopy analysis was done to find out the morphology of the chitosan nanoparticles it confirms the size range of the drug-loaded nanoparticles. The optimized Lamotrigine-loaded nanoparticle was further diluted (1: 50) with distilled water and ultrasonicated for 15 min. It was then stained with 2% Phosphotungstic Acid and a drop of the sample was then fixed on a 300 mesh carbon-coated copper grid. The images of representative areas were taken at suitable magnifications (200nm) [20]

**Physicochemical characterization of nanoparticles**

**a. Determination of drug content**

10mg of Lamotrigine was dissolved in 10mL of methanol, and the solution was considered standard. Similarly, the 10mg formulation was dissolved in 10mL of methanol and considered as a test. The absorbance of both the standard and test was measured out, by using the UV–visible spectrophotometer (UV-1800, Shimadzu, Japan) at a particular wavelength [21]. By using the following formula and comparing the test with the standard, the % drug content was calculated.

$$\textit{\% drug content} = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times 100$$

**b. Particle size, Zeta potential, and Polydispersity index**

Photon Cross-Correlation Spectroscopy (PCCS) with dynamic light scattering was used to analyze the particle size distribution of the prepared Chitosan nanoparticles. Which is based on the Brownian motion of molecules, dispersed in a liquid and relates this to the size of the particles by illuminating the particles with laser light and analyzing the intensity fluctuations in the scattered by using (Model: Malvern Instruments Ltd) with sensitivity range is 1nm to 10 μm. Zeta potential (ZP) shows the electrophoretic particle velocity in an electrical field where the particle obtains a charge due to the dissipation of the counterions on the surface of the molecule and zeta potential of the chitosan nanoparticles was measured by using a Dynamic Light Scattering (DLS) zeta potential as well as nanoparticle analyzer (Model: Malvern Instruments Ltd) with a zeta potential range of −200 to +200 mV and polydispersity index can be measured by using the mean diameter [21].

**c. Entrapment efficiency**

The entrapment efficiency (EE %) of Lamotrigine was determined by an indirect method. The centrifugation of Chitosan nanoparticles at 15,000 rpm for 40 min at room temperature after centrifugation the supernatant solution was collected and filtered through a 0.22m membrane filter and the amount of drug present was measured at a particular wavelength by UV–Visible spectrophotometer (UV-1800, Shimadzu, Japan). The amount of drug in the supernatant was calculated by using the equation y= mx+c, where y shows the

absorbance and x shows concentration (mg/ml). The amount of drug present in the supernatant was subtracted from the total amount of drug added and accordingly, Drug Entrapment Efficiency was calculated by following the equation [22].

$$\% \ Entrapment \ efficiency = \frac{Total\ added\ drug - free\ drug}{Total\ added\ drug} \times 100$$

**d. Drug loading**

The proportion of encapsulated Lamotrigine was determined by centrifuging 10 mL of chitosan nanoparticles formulation at 15,000 rpm for 60 minutes at room temperature. The supernatant was taken carefully using a micropipette. Pure supernatant was then dissolved in methanol to disrupt the vesicles and appropriate dilution was made to measure the Lamotrigine content using UV spectrophotometry (UV-1800, Shimadzu, Japan) at a particular wavelength [23]. % Drug loading capacity was calculated by the equation below

$$\% \ Drug \ loading = \frac{Amount\ of\ encapsulated\ drug}{Amount\ of\ total\ drug} \times 100$$

**e. Dissolution study**

The in vitro dissolution study of Lamotrigine and Chitosan nanoparticles formulation was performed in dissolution test apparatus, USP standard type II. The study was carried out in 0.1N Hcl, Acetate buffer pH4, Phosphate buffer pH 6.8, and Phosphate buffer 7.4 by taking quantity to dose equivalent to a single dose of Lamotrigine in a muslin cloth and placed in 900 ml dissolution media rotated at 50 rpm and maintained at 37±0.5˚C. Aliquots were withdrawn at intervals of 15 mins, for 1hrs, and analyzed by UV spectroscopy at respective wavelengths [24, 25].

## RESULTS AND DISCUSSION

**Preformulation study**:

**a. API characterization**

**Table 2. Organoleptic properties of Lamotrigine**

| Sr. No. | Name of property | Specification |
|---|---|---|
| 1. | Color | White |
| 2. | Odour | Unpleasant |
| 3. | Nature | Amorphous |

**Identification of pure drug**

**a. Melting Point**

The melting point of Lamotrigine was found to be 217 ˚C, which is in the range given in the literature (216-218˚C). Hence the drug can be stated as pure

**b. UV Spectroscopy**

**Determination of λ max**

Accurately weighed 1 mg of the drug was transferred to 100 ml of a volumetric flask add dissolved in methanol volume was made up to 100 ml and the solution was scanned on a UV spectrometer in the range 200-400nm.

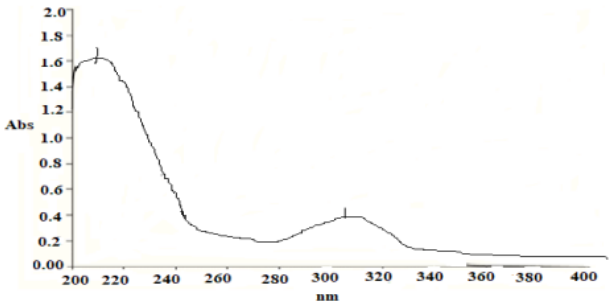


**Figure 3. UV Spectrum of Lamotrigine**.

An absorption maximum was found to be at 307nm. Hence 307nm was selected as λ max for further studies

**Calibration curve of Lamotrigine in methanol**

The stock solution for the standard drug of 1 mg was prepared using 100 ml of methanol. The maximum absorbance for

the drug solution of 10 mcg/ml was found to be 307 nm. The linearity was found between the concentration range of 10-35 mcg/ml for UV spectroscopy.

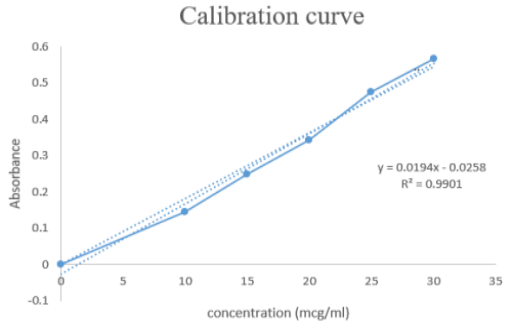


**Figure 4. Calibration curve of Lamotrigine in Methanol**

**Table 3. Parameters found in the calibration curve**

| Sr.No. | Parameter | Finding |
|---|---|---|
| 1. | Wavelength detection | 307 nm |
| 2. | Regression equation | y = 0.0194x – 0.0258 |
| 3. | Correlation coefficient | $R^2$ = 0.9901 |

**Solubility study:**

**Table 4. Solubility study of Lamotrigine**

| Sr.no. | Different buffers | % Solubility |
|---|---|---|
| **1.** | Water | 11.45% |
| **2.** | 0.1 N HCl (pH 1.2) | 29.00% |
| **3.** | Acetate buffer (pH 4) | 19.99% |
| **4.** | Phosphate buffer solution (pH 6.8) | 24.95% |
| **5.** | Phosphate buffer solution (pH 7.4) | 15.78% |

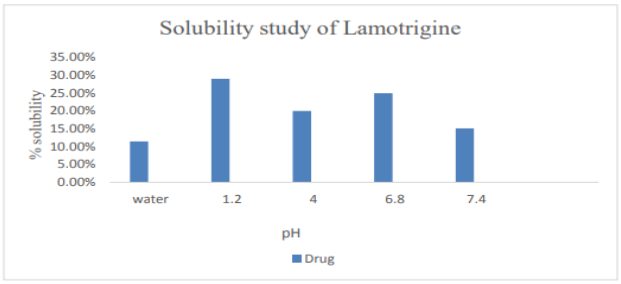


**Figure 5. Solubility study of Lamotrigine in water and different buffer**

**Drug and excipient compatibility study**

**a) Fourier Transformation Infrared Spectroscopy (FTIR)**

FTIR spectrum of Lamotrigine was shown in following Fig. 7.4 revealed that the characteristic peaks representing the presence of functional groups claim by its chemical structure. From this, we can consider that the Lamotrigine was of pure quality.

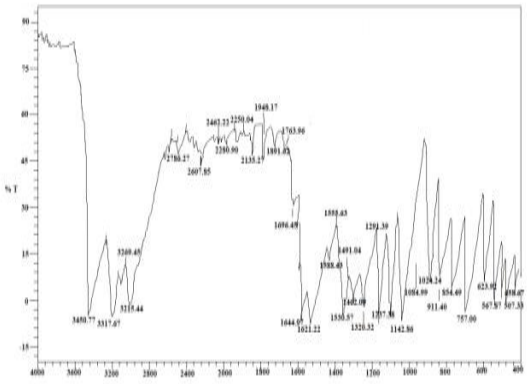

**Figure 6. FTIR spectra of Lamotrigine**

**Table 5: Interpretation data of Lamotrigine**

| Material | Functional group | Standard IR Ranges ($cm^{-1}$) | Observed IR Ranges ($cm^{-1}$) |
|---|---|---|---|
| Lamotrigine | C=C Stretching | 1680-1640 | 1644.97 |

| | | | |
|---|---|---|---|
| | C-N Stretching C-C Stretching C-Cl Stretching | 1200-1350 2100-2200 850-550 | 1320.52,1237.88 2135.27 757.00,623.92, 567.5 |

After interpretation of the FT-IR Spectrum of Lamotrigine, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of Lamotrigine were found within the reference range and confirming its identity.

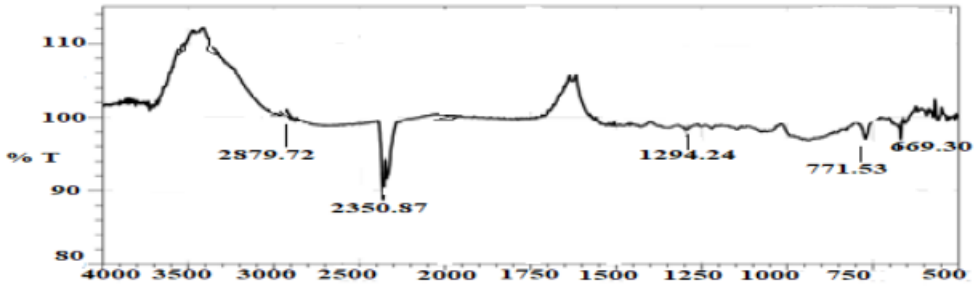


**Figure 7. FTIR spectra of Chitosan**

**Table 6. Interpretation data of Chitosan**

| **Material** | **Functional group** | **Standard IR Ranges ($cm^{-1}$)** | **Observed IR Ranges ($cm^{-1}$)** |
|---|---|---|---|
| Chitosan | O-H Stretching | 3300-2500 | 2879.72 |
| | | 1382-1036 | 1294.24 |
| | C-O Stretching | | |

After the interpretation of the FT-IR Spectrum of Chitosan, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of Chitosan were found within the reference range, confirming its identity.

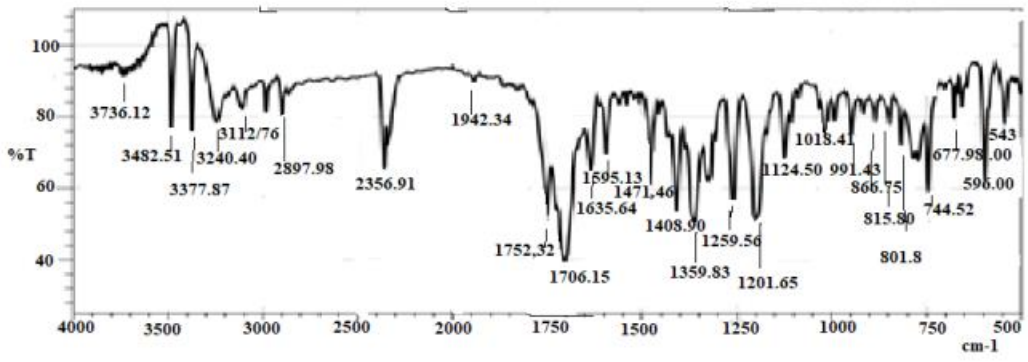


**Figure 8 . FTIR spectra of physical mixture**

After interpretation of the FT-IR Spectrum of Chitosan and its physical mixture with the drug Lamotrigine, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of Lamotrigine were

not found intact within the reference range, confirming its reactivity with chitosan. This interaction further supports the selection of polymer.

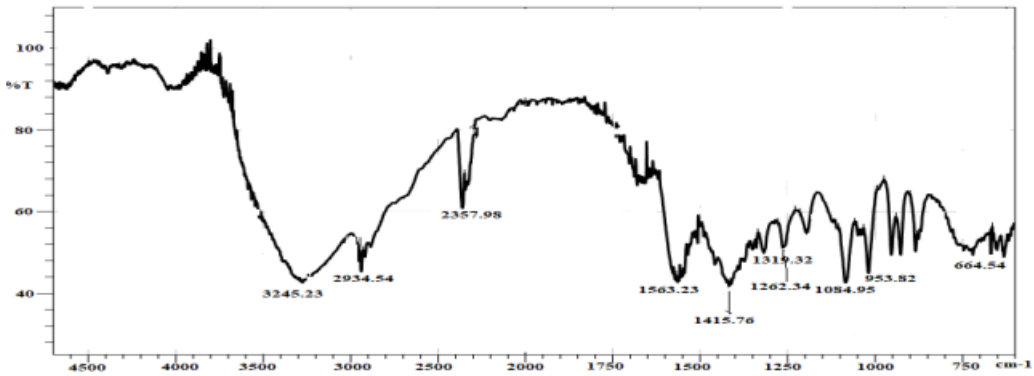


**Figure 9. FTIR Spectra of Formulation (F2)**

**Table 7. Interpretation data of Formulation F2**

| **Material** | **Functional group** | **Standard IR Ranges ($cm^{-1}$)** | **Observed IR Ranges ($cm^{-1}$)** |
|---|---|---|---|
| Formulation F2 | C-N Stretching<br>C-H Stretching<br>O-H Stretching<br>C-O Stretching | 1350-1250<br>3000-2840<br>3300-2500<br>1382-1036 | 1319.32,1262.34<br>2934.54<br>3245.23<br>1084.95 |

There was no considerable change in the positions of characteristic absorption bands and bonds of several functional groups present in the drug. This observation suggests that the Lamotrigine shows no prominent change in its characteristics even in its physical mixture. The results of FTIR spectra indicated the interaction between the drug and polymer. It showed that Lamotrigine was compatible with chitosan.

**b) Differential Scanning Calorimetric analysis (DSC)**

The thermal analysis of Lamotrigine and Chitosan was studied by using DSC as shown in Figures 8 and 9 respectively. The Lamotrigine shows an endothermic peak at approximately 215.34oC and it corresponds to its melting point (fig.7.8). Chitosan shows a sharp endothermic peak at 90.99 C corresponds to its melting point (fig.7.9). And the formulation F2 shows the endothermic peak at 212.98℃ (fig.7.10)

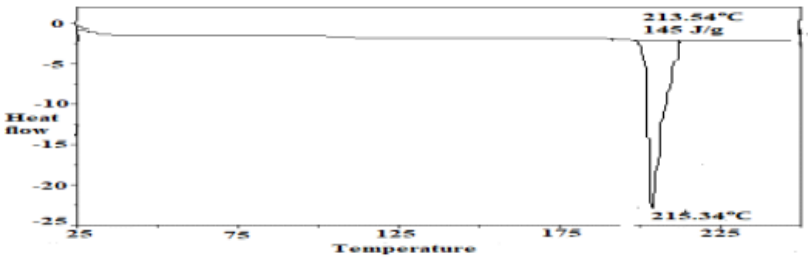

**Figure 10. DSC thermogram of Lamotrigine**

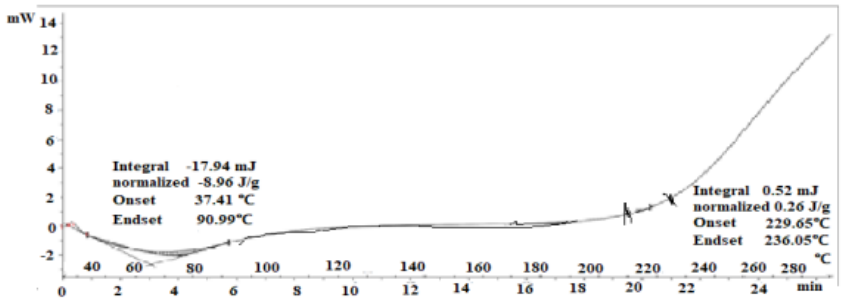


**Figure 11. DSC thermogram of Chitosan**

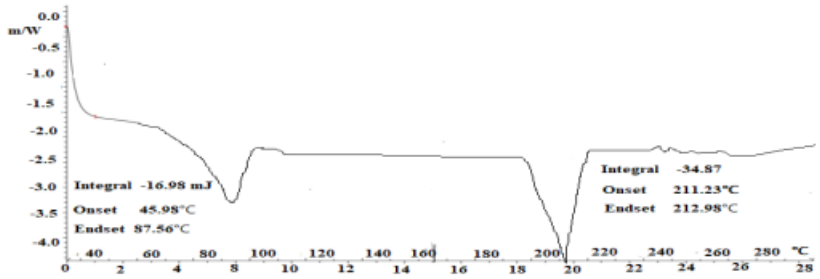


**Figure 12. DSC thermogram of F2 formulation**

**Characterization of nanoparticles**

**a. Determination of drug content**

% Drug content of all chitosan nanoparticles formulations was calculated as shown in the following table 7.10., F2 formulation had shown the highest % drug content (87.45%). Hence based on % drug content, the F2 formulation was selected as an optimized formulation and subjected to further detailed evaluation.

**Table 8. Drug content**

| Sr. No | Formulation Code | Drug Content |
|---|---|---|
| 1. | F1 | 84.96% |
| 2. | F2 | 87.45% |
| 3. | F3 | 81.56% |

**b. Entrapment efficiency**

% Entrapment efficiency was calculated for the optimized formulation (F2). It was found to be 85.65%. This was the optimum entrapment efficiency, which showed that the amount of chitosan present in the F2 formulation was enough to entrap Lamotrigine present in F2 formulation. As shown in Table 9

**Table 9. Entrapment efficiency**

| Sr. No | Formulation Code | Drug Content |
|---|---|---|
| 1. | F1 | 82.87% |
| 2. | F2 | 85.65% |
| 3. | F3 | 81.56% |

**c. Drug loading**

% Drug loading capacity was calculated for the optimized formulation (F2). It was found to be 81.84%. As shown in Table 7.12

**Table 10. Drug loading**

| Sr. No | Formulation Code | Drug loading |
|---|---|---|
| 1. | F1 | 79.28% |
| 2. | F2 | 81.84% |
| 3. | F3 | 77.19% |

**d. Particle size, Zeta potential, and Polydispersity index**

Particle size analysis provides information on the size distribution of particles. This can be

used to calculate the several properties of a particle and how they will act under certain conditions. The particle size distributions of chitosan nanoparticles were characterized. The particle size of the F2 formulation was found to be 234.6 nm. The polydispersity index (PDI) of the F2 batch was found to be 0.689. PDI is an index width or spread or variation within the particle size distribution. Mono-dispersed samples have a lower PDI value, whereas a higher value of PDI indicates a wider particle size distribution and the poly-dispersed nature of the sample. The zeta potential of the F2 formulation was found to be -24.7 mV. The negative sign indicates the stability of chitosan nanoparticles and the result is shown in the following table 7.14.

**Table 11. Particle size, Zeta potential, Polydispersity index**

| **r. No** | **Formulation Code** | **Particle Size** | **Zeta Potential** | **Polydispersity Index** |
|---|---|---|---|---|
| 1. | F1 | 345.7 | -21.4 | 1.0000 |
| 2. | F2 | 234.6 | -24.7 | 0.689 |
| 3. | F3 | 523.5 | -13 | 0.454 |
| 4. | F4 | 367.9 | -6.49 | 0.512 |

**e. X-ray diffraction study (XRD)**

The X-ray diffraction pattern of pure drug Lamotrigine, chitosan-based Lamotrigine loaded nanoparticles, and polymers i.e., chitosan were recorded on an x-ray diffractometer shown in fig. 7.12,7.13 and 7.14. The distinctive sharp peaks of the drug were observed at diffraction angles, 11.567 o, 31.912 o, on a 2ɵ scale, illustrating the typical crystalline nature of the drug. The nanoparticles showed a broad peak of 20.568 o indicating the amorphous state of the polymer. The absence of crystalline peaks of Lamotrigine in drug-loaded nanoparticles confirmed that the drug was molecularly dispersed in the polymer and conversion of the drug into the amorphous form takes place.

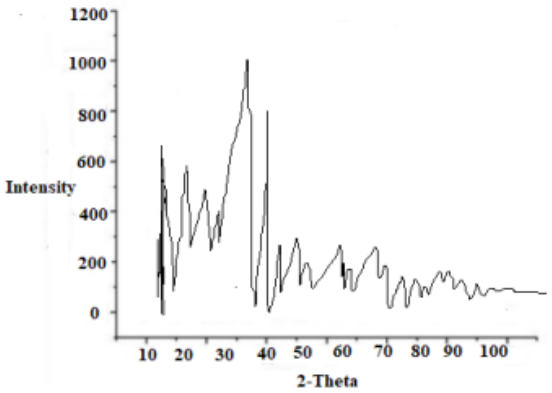


**Figure 13. XRD of pure drug Lamotrigine**

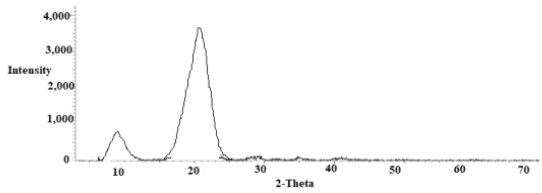


**Figure14. XRD of chitosan**

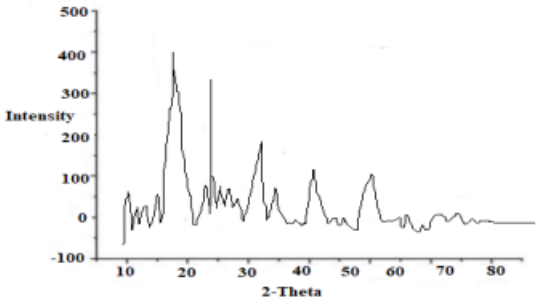


**Figure 15. XRD of F2 formulation**

**f. Scanning Electron Microscopy (SEM)**

Scanning electron microscopy was done for the surface characterization of the F2 formulation. As shown in fig. 7.15 the F2 Formulation was scanned on 5,000x, 15,000x and 3000x

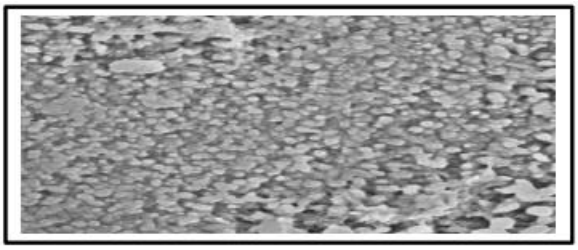

**Figure 16. Scanning electron microscopic images of F2 formulation.**

**g. Transmission Electron Microscopy (TEM)**

Transmission electron microscopy obtained from the imaging showed that the droplet size of the samples was in the nonmetric range as shown in Figure 7.16

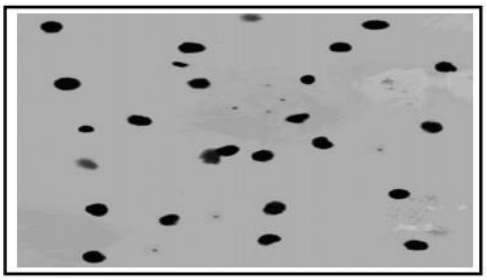

**Figure 17. Transmission electron microscopic images of F2 formulation**

**In-vitro dissolution studies**

**Table 12. In-vitro dissolution studies**

| Sr. No. | Time (min) | % Drug release | |
|---|---|---|---|
| | | Lamotrigine | F2 |
| 1. | 0 | 0 | 0 |
| 2. | 15 | 28.50% | 33.62% |
| 3. | 30 | 31.65% | 44.89% |
| 4. | 45 | 34.89% | 59.61% |
| 5. | 60 | 38.34% | 66.54% |
| 6. | 75 | 40.54% | 71.24% |
| 7. | 90 | 43.59% | 83.12% |

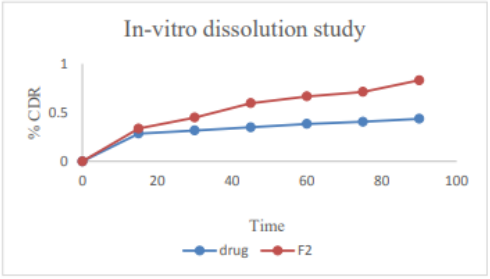


**Figure 18. % Dissolution drug release profile of Lamotrigine and F2**

An In-vitro dissolution study was carried out for Lamotrigine (API) and nanoparticles formulation (F2) in 900 ml of phosphate buffer 0.1N Hcl, using USP paddle type II dissolution apparatus at 37 ± 0.5 °C at 50 rpm for 90 min. The release profile of both was given in table 7.15 and fig 7.17. Lamotrigine showed 43.59% and formulation F2 showed 83.12% drug release at 90 min., which was linearly increasing concerning time. The release rate was increased almost by 44% significantly higher than that of pure Lamotrigine. The release of formulation F2 was attributed to its improved solubility profile. Formulation F2 shows the great result of saturation solubility analysis and % Drug release respectively. Hence F2 formulation was selected for the further evaluations

## CONCLUSION

The study regarding overcoming the resistance issue is a thrust area of research, especially for convulsion disorder i.e. tonic-clonic seizures. Bringing a new drug and its formulation to market to treat the convulsion becomes costly. So overcoming the resistance of already an existing drug in the market is quite time-consuming, cost-effective, and leads to innovation. Hence, an attempt was made to formulate the Anticonvulsant chitosan nanoparticles effectively. The Chitosan Nanoparticle was prepared by ionic gelation Method. Tested for UV Spectrum Analysis the wavelength was found to be 307nm, The FTIR spectra were found to O-H

Stretching observed IR (2879.72cm$^{-1}$) and C-O stretching (1294.24cm$^{-1}$). The Drug percentage yield was found to be F1 84.96%, F2 87.45%, and F3 81.56% the particle size distributions of chitosan nanoparticles were characterized. The particle size of the F2 formulation was found to be 234.6 nm. The polydispersity index (PDI) of the F2 batch was found to be 0.689. PDI is an index width or spread or variation within the particle size distribution. Mono-dispersed samples have a lower PDI value, whereas a higher value of PDI indicates a wider particle size distribution and the poly-dispersed nature of the sample. The zeta potential of the F2 formulation was found to be -24.7 mV. The X-ray diffraction pattern of pure drug Lamotrigine, chitosan-based Lamotrigine loaded nanoparticles, and polymers i.e. the distinctive sharp peaks of the drug were observed at diffraction angles, 11.567º, 31.912º, on 2ɵ scale, illustrating the typical crystalline nature of the drug. The nanoparticles showed a broad peak of 20.568º indicating the amorphous state of the polymer. The absence of crystalline peaks of Lamotrigine in drug-loaded nanoparticles confirmed that the drug was molecularly dispersed in the polymer and conversion of the drug into the amorphous form takes place. Scanning electron microscopy was done for the surface characterization of the F2 formulation. Transmission electron microscopy obtained from the imaging showed that the droplet size of the samples was in the nonmetric range In-vitro dissolution study was carried out for Lamotrigine (API) and nanoparticles formulation (F2) in 900 ml of phosphate buffer 0.1N Hcl, using USP paddle type II dissolution apparatus at 37 ± 0.5 °C at 50 rpm for 90 min. The release profile of both was given in table 7.15 and fig 7.17. Lamotrigine showed 43.59% and formulation F2 showed 83.12% drug release at 90 min., which was linearly increasing concerning time. The release rate was increased almost by 44% significantly higher than that of pure Lamotrigine. The release of formulation F2 was attributed to its improved solubility profile. Formulation F2 shows the great result of saturation solubility analysis and % Drug release respectively. Hence F2 formulation was selected for further evaluation.

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**Abbreviation**

| Sr. No. | Abbreviation | Read as |
|---|---|---|
| 1. | % | Percentage |
| 2. | °C | Degree centigrade |
| 3. | CDR | Cumulative Drug Release |
| 4. | CNPs | Chitosan-based Nanoparticle |
| 5. | FT-IR | Fourier Transform-Infrared Spectroscopy |
| 6. | G | Gram |
| 7. | PBS | Phosphate Buffer Saline |
| 8. | Hr | Hour |
| 9. | Min | Minute |
| 10. | HCl | Hydrochloric Acid |
| 11. | L | Liter |
| 12. | Mg | Milligram |
| 13. | Ml | Milliliter |

| | | |
|---|---|---|
| 14. | $R^2$ | Regression Coefficient Regression Coefficient |
| 15. | RH | Relative Humidity |
| 16. | % EE | Percent Entrapment Efficiency |
| 17. | UV | Ultraviolet |
| 18. | λmax | Maximum Absorbance |
| 19. | µg | Micrograms |

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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