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BIOPOLYMER FORM CESTRUM NOCTURNUM FLOWER: ISOLATION, CHARACTERIZATION AND ITS UTILIZATION IN PREPARATION OF PHENYTOIN LOADED BIO-NANOSUSPENSION

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ABSTRACT

The aim of this research was to formulate phenytoin loaded bio-nanosuspension for targeting to brain via ear using novel isolated biopolymer form *Cestrum nocturnum* flower. The significance of this research was to isolate the bio-polymer by using an economical and simple procedure. Bio-polymer was subjected for various physicochemical characterizations with spectral analysis like UV, FT-IR, mass and H-NMR, DSC was done. The phenytoin loaded bio-nanosuspension (PCN1-PCN8) were prepared by bath sonication method using isolated stabilizer cum retardant novel bio-polymer in different ratio. Various formulations were evaluated for different parameters like pH study, dispersibility study, entrapment efficacy, zeta particle size, zeta potential, *In-vitro* release study, stability studies and *In-Vivo* study. The formulation PCN8 was found to be the best formulation having t50% of 17.06 hours and t80% of 29.06 hours with r2 value of 0.9899. The best formulation PCN8 showed up to 90.87% drug release over 36 hours. According to the release kinetic study the best fit model was found to be Korsmeyer Peppas and the mechanism of drug release was found to be Anomalous transport. The isolated biopolymer was found to have a promising, novel inbuilt stabilizer cum retardant polymeric properties for developing stable phenytoin loaded bionanosuspension. The formulated bionanosuspension was found to be safe and compatible for targeting to brain via ear.

KEYWORDS: Biopolymer, Bio-nanoparticles, bionanosuspension, Phenytoin, Nanosizing, drug targeting.

INTRODUCTION

A number of researches have been done and proved the brain drug targeting via various routes. The diseases related with C.N.S. are challenging to treat effectively. The targeting of drug to brain by using the various carriers systems is now very interesting field of drug delivery.

Now days various rotes are there for drug targeting to brain, one of them the most challenging and interesting route is ear.

Ear may be an alternative way for brain drug targeting. A number of methods are there by which we can delivery,[11,12] the brain-targeted drugs into cerebrospinal fluid via ear.

There is a cochlear aqueduct which pass from the inner ear to the brain subarachnoid space which may be used as a novel drug delivery route. Many animal studies has proven that ear may be used as a novel platform for brain drug targeting. Thus physiological flow of fluid from inner ear compartment to CSF in subarachnoid space of brain may be used as an excellent way for targeting of drug to brain[13] via ear.

The neural connection between ear and brain may also impart a way for brain drug targeting. The surface of auricle are supplied with the great auricular nerve and occipital nerves from the cervical plexus and auriculotemporal branch of mandibular nerve (V3)and deeper parts of auricle are supplied with vagus nerve(X) and also facial nerve (VII). Vestibulocochlear nerve (VIII-cranial nerve) a greater nerve which connects ear with brain. Such unique features of ear may be used as novel platform for targeting of drug to brain.[13]

Biopolymers isolated[1] from natural sources may be used as novel excipients[3] having the polymeric nature. These isolated biopolymers[4] have excellent bioretardant, biostabilizer and mucoadhesive property. It has excellent film forming ability, and bio-stability properties. The

isolated bio-polymers have excellent drug release rate controlling abilities. Since these are natural and edible in nature and these are easily biodegradable[6] and may be used as an alternative to conventional synthetic and semi synthetic polymers.

The isolated biopolymer shows the significant biodegradable, mucoadhesive, filmability and retardibility properties which are similar to properties of synthetic standard polymers. They have most of the novel properties which can be safely used for drug delivery. The biopolymers are isolated from the natural sources which are economical. The synthetic polymers are prepared by using the different chemical treatment which have many harmful effects. The biopolymers have unique novel properties. The biopolymers may be used as for controlling the dug release in sustained way, controlled way, extended way, prolonged way and thus are used as drug carrier bioexcipients. Since they are having natural origin and biodegradable in nature can be sued for minimizing the unwanted effects with synthetic polymers. In this research the biopolymer was isolated from Cestrum nocturnum flower petals by simple economical method having various novel properties like natural origin and biodegradable properties.

Since these unique features of isolated biopolymers are prime characteristics for design of novel drug delivery system.

Bionanoparticles[2] are the nanoparticles which are prepared by using the novel biocompatible and biodegradable biopolymers. We can use the novel polymeric properties in developing the bio-nanoparticles for targeting the drug to brain via blood brain barrier. The bionanoparticles may release the drug to the target in significant amount. The bionanoparticles are stable and its excellent release rate controlling properties makes it novel.

The central nervous system is most critical part of the body which is surrounded by blood brain barrier and protected. The blood brain barrier protects the brain from movement of ions of limited number of small molecule and macromolecules from blood into the brain. The blood brain barrier also protects the brain from any shocks and diseases. Neurological disorders are the major cause of mortality globally. Now days there are very less therapies for treatment of neurological disorders associated with brain.

Epilepsy[14] is a neurological disorder of brain. In epilepsy brain does not act normally which causes seizures or episode of abnormal behavior, sensations and may loss awareness sometimes. Epilepsy may occur due to genetic disorder or may be the result of acquired brain injury like trauma or stroke. The epileptic patient act abnormal and experiences abnormal behavior and symptoms. Sometime the patient may also lose consciousness.

Phenytoin is an antiepileptic drug which are available inform of tablets, capsules and in oral suspension. Epilepsy is a neurological disorder of brain. In epilepsy brain does not act normally which causes seizures or episode of abnormal behavior, sensations and may loss awareness sometimes. Epilepsy[24] may occur due to genetic disorder or may be the result of acquired brain injury like trauma or stroke. The epileptic patient act abnormal and experiences abnormal behavior and symptoms. Sometime the patient may also lose consciousness.

In this research the bionanosuspension[2] was prepared. A number of researches have proven the formulation of phenytoin loaded nanoparticles by using the synthetic polymers. Here the nanoparticles were prepared by using the biodegradable biomaterial isolated from Cestrum nocturnum. The prepared bionanoparticles showed a number of advantages over other nanoparticles and other marketed formulation also. Its preparations by using the natural, biodegradable biopolymer minimizes the problems associated with other formulated nanoparticles using synthetic polymers and other conventional phenytoin formulations.

MATERIAL AND METHODS

Phenytoin was obtained as a gift sample from Affy pharma private limited, Baddi. The *Cestrum nocturnum* flower was purchased from the local market of Lucknow. All other chemicals used were of analytical grade.

Isolation of biopolymer

100 gm of *Cestrum nocturnum* flower was taken and petals were washed with purified water. About 100ml of water was added in this and this was mixed in mixer. This mixture was filtered with the help of muslin cloth. The resultant was filtered and juice of *Cestrum nocturnum* was obtained as filtrate. After that the mixture was subjected to centrifugation at 4000rpm for 15 minutes and the resultant supernatant layer was separated. Methanol was added in the ratio of 1:2 and mixed properly. This mixture was kept in refrigerator overnight and the product obtained was centrifuged at 4000rpm for 30minutes. Residue was collected having biopolymer and dried in desiccators. This residue of biopolymer was washed with chloroform and acetone and dried properly for getting free flowing powder. The collected biomaterial was passed through sieve no 120 and stored in airtight containers for further use. This procedure was optimized by repeating it for six times and then percentage yield was calculated.[16]

Characterization of isolated biopolymer

The physico-chemical properties of isolated biopolymer were characterized for color, odor, taste and solubility[17]. The chemical tests for presence of carbohydrate, starch and proteins were also performed. The isolated biopolymer was also characterized for SEM analysis, DSC testing, IR spectroscopy, mass spectroscopy and NMR spectroscopy.[18]

Physical characterization

The color, odor, texture of isolated biopolymer were physically evaluated. The color changing point was determined by using the melting point test apparatus. The isolated biopolymer was filled in the capillary tube and it was kept in melting point apparatus. The apparatus was switch on and observed for the temperature at which there was change in the color and melting of biopolymer starts. The temperature was observed with the help of thermometer.

CHEMICAL TESTS FOR CHEMICAL CONSTITUENTS

Chemical test for carbohydrate

1ml of biopolymer solution (5% biopolymer solution in distilled water) was taken in test tube. Add two drops of Molisch reagent .Add 1-2 ml of conc. Sulfuric acid in the test tube and observe for the formation of purple color at the at the interface of two layers formed.[16]

Chemical test for proteins

Biuret test was performed for the confirmation of proteins. 2 ml of *Cestrum nocturnum* was taken in test tube (5% biopolymer solution in distilled water), add 1 ml of sodium hydroxide solution with addition of copper sulphate solution drops. The mixture was kept aside for five minutes and observe any color changes. The appearance of violet color confirms the presence of proteins.

SEM(Scanning electron microscopy) analysis

The isolated biopolymer was analyzed by scanning electron microscope. In SEM analysis the external surface and internal structure was characterized. The small quantity of biopolymer was taken and fixed on aluminum studs and the coated with gold with the help of coater sputter under vacuum. Then the scanning electron micrograph as taken for the biopolymer under observation.

SPECTRAL ANALYSIS

FTIR spectroscopy of isolated biopolymer

The FTIR spectroscopy[13] was done by preparing the KBr discs. 1mg of isolated biopolymer was taken and mixed with 100mg of dried and desiccated solid KBr (Potassium bromide). The mixture was mixed in mortar and pestle and placed in IR lamp to remove any moisture. The mixture was converted into disc by using the hydraulic pump under the pressure of 10 tons. The prepared KBr disc was placed in disc holder in the path of IR radiation.[18] The spectrum was recorded into the range of 4000-200cm^{-1}.

DSC testing (Differential scanning calorimetry)

In DSC testing is the thermal analysis technique in which the heat flow into or out of the sample is determined as the function of temperature. Here the sample was taken and exposed to controlled temperature program. The glass transition temperature was determined. The heat flow range was 50-300℃. The DSC thermogram was recorded.

Mass spectroscopy

This is the laboratory technique in which the sample of biopolymers was introduced in the through the inlet system. The gas phase ions of the compound were produced. Then molecular ion fragmentation, the ions separated in mass spectrometer according to their mass to charge ratio. A mass spectrum of ion abundance versus mass to charge ratio was obtained.

NMR spectral analysis

The NMR spectroscopy was done for spectral analysis of isolated biopolymer. The sample was dissolved in specific solvent like CDCl3. The mixture was pumped in the instrument at high rate flow. The valve switch was used to stop the flow. The measurement was performed. After the finishing of measurement the spectrum was processed and analyzed in automation computer.

Nanosizing of Phenytoin by modified solvent evaporation method

500 mg of **Phenytoin** was taken and mixed with dextrose 50mg in mortar in mortar and pestle and the 25ml of methanol was added to mixture. The clear solution mixture transferred in 50 ml beaker and sonicated for 5cycles (One cycle was for three minutes). This mixture was bath sonicated for optimized sonication cycle for 15 cycles (One cycle was for three minutes) continuously. The bath sonication was done at 250 hertz frequency at room temperature. During sonication 25ml of purified water as added slowly drop by drop till turbidity was observed. The obtained residue was subjected for centrifugation for 30 minutes at 4000 rpm for complete recovery of nanosized drug.[17] [20] During the optimization process at each cycle % transmittance was noted in order to know the minimum cycles required for nanosizing of the drug. After the completion of 15 cycles sonication and centrifugation the nanoparticles were collected and supernatant was discarded. The collected nanoparticles were washed with water, dried and packed in air tight container.[8]

The recovered nanoparticles were observed for physical appearance, permeability through egg membrane. Further the nanoparticles were stored in air tight container for further use. Its particle size was confirmed by SEM testing image for their in nano range particle size. Then its permeability study was conducted for confirmation of its suitability for passage through the biomembrane in nano range.

Permeability study through the egg membrane

The 5mg/ml pure phenytoin solution was prepared in phosphate buffer solution pH 7.4 and taken (4ml) in donor compartment and tied the donor compartment with the egg membrane. The pH7.4 solution was prepared and taken in receptor compartment. The donor with drug solution was fitted in receptor compartment for

permeation of drug. At specific time interval the receptor compartment sample was completely replaced with the fresh 100 ml of the buffer solution. The sampling was done at 1,2,4,6,8,10 24, 36 and 48 hours. A graph was plotted between the time and concentration of drug permeated at specific time interval.

Drug excipient interaction study

The drug-biopolymer interaction study was performed by the U.V. spectroscopy method. The drug-biopolymer mixture was prepared in ratio of 1:1, 1:3 and 3:1 by wet and dry mixing. After mixing the drug and polymer mixtures were stored at 50℃ for three days in wet method and then the mixture was extracted with solvent and scanned for the absorption maxima (λmax). In dry method the three different ration of drug-biopolymer was prepared in their physical form and then after storage at room temperature this was diluted with 2ml of methanol and then scanned by UV spectrophotometer for any change in λmax.

Formulation of phenytoin loaded Bio-nanosuspension

The bio-nanosuspension was prepared by using different drug –biopolymer ratio as given in the Table 1. The bionanosuspension was prepared by sonication of the mixture of drug and biopolymer along with other excipients like polyvinyl alcohol as suspending agent, sodium benzoate as the preservative, purified water and dextrose as nanosizent. The drug, *Cestrum nocturnum* biopolymer and other excipients were accurately weighed and triturated with addition of the double distilled water. This mixture was sonicated for 3 cycles. Then 0.5ml of 0.5 % polyvinyl alcohol was added during sonication. The volume was made up to 10 ml with double distilled water having sodium benzoate (0.1-0.5%). Add dextrose if necessary as nanosizing agent and allowed for sonication for 15 cycles at 4000rpm. After sonication the bionanosuspension,[14] was refrigerated for two days. If no settlement is there then it means the formulation is optimized. If settlement is there the 0.5 ml of 0.5 % polyvinyl alcohol was again added and allowed for sonication for 10 cycles and refrigerated for 48 hours. The different formulations PCN1- PCN8 were prepared and then formulations were optimized according to different stability parameters and selected for further evaluation.[1]

Table 1: Phenytoin loaded bio-nanosuspension using *Cestrum Nocturnum* biopolymer.

S.NO.	Formulations Codes	PCN1	PCN2	PCN3	PCN4	PCN5	PCN6	PCN7	PCN8
	Drug : Biopolymer ratio	**01:00.3**	**01:00.5**	**01:00.8**	**01:1**	**01:02**	**01:03**	**01:04**	**01:05**
1	Phenytoin (mg)	10	10	10	10	10	10	10	10
2	***Cestrum Nocturnum*** (Biopolymer) (mg)	3	5	8	10	20	30	40	50
3	Polyvinyl alcohol(ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
4	Sodium benzoate (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
5	Double distilled Water (ml)	10	10	10	10	10	10	10	10

Characterization of Phenytoin loaded bio-nanosuspension

Dispersiblity study of bio-nanosuspension

5ml of the formulated bionanosuspension was taken and dispersed in 20ml of the distilled water in a test tube. The time for settling of the dispersed nanoparticles in the bottom was noted and then again the nanoparticles was redispersed and noticed for the redispersion. After shaking any lump or aggregates or any precipitation formation was observed.

pH study of bio-nanosuspension

The ph of formulated bionanosuspension was evaluated with digital pH meter. The study was done in triplicate and the mean was taken and checked that the pH of the bionanosuspension is in required range or not.

% Entrapment efficacy of loaded bionanosuspension

The freshly formulated bionanosuspension was taken and centrifuged at 2000rpm in ultracentrifuge .After centrifugation the supernatant was taken and diluted up to 10µg/ml and the amount of drug unincorporated was measured by determining the absorbance under UV spectroscopy at 216nm.The amount of the drug loaded in nanoparticles was calculated by subtracting the amount of free drug in supernatant from the initial amount of drug taken in formulation. This determination was done in triplicate and average was calculated by using the following formula:

$$\% \text{ Entrapment efficacy} = \frac{\text{Amount of the drug loaded in nanoparticles}}{\text{Initial amount of the drug taken in formulation}} \text{X } 100$$

Particle size screening of the nanoparticles in bionanosuspension by UV method

The bionanosuspension was evaluated by measuring the %transmittance of the bionanosuspension. The %transmittance was measured as a function of the particle size in nano range which was achieved by sonication method. The % transmittance depend on the particle size range at the particular wavelength that defines that the size of the particles are below the range and size of the particles are beyond the range required. The % transmittance at different wavelength revelas that when the light is passed through the particles means the particle size is below that wavelength which indicates that % of the particles is below 400nm in the mixture and the % blockage shows that the % of particles is above 400nm. The % transmittance was measured by using the UV spectrophotometer. The effect of sonication on %

transmittance was observed after measuring the % transmittance after each sonication cycle.

Particle size analysis

The particle size of the bionanosuspension was studied by characterizing with the Malvern zetasizer. The particle size distribution by intensity was confirmed by using the zetasizer. Zeta potential was also measured for the prepared bionanosuspension.

***In-vitro* release study of bio-nanosuspension**

The In-vitro release study was performed for the all formulation bionanosuspension (PCN1-PCN8). In-Vitro release study was performed by novel static method by using modified M.S. Diffusion apparatus. It consists of two compartment one donor and one receiver compartment. The formulation for release study was taken in donor compartment (1ml) and the end of the donor is tied with the egg biomembrane. This donor compartment was immersed in the receiver compartment having 13 ml of pH 7.4 phosphate buffer solution. Sampling was done at different regular time interval for 36 hours. The samples were withdrawn completely and replaced with the fresh phosphate buffer solutions after every sampling. The samples were analyzed by UV Spectrophotometer for determining the released amount of the drug. The graph was plotted between the %CDR and time. The other parameters like r^2, t50 and t80% were calculated. On the basis of obtained results of *In-vitro* release study of different formulated bionanosuspension (PCN1-PCN8).

Stability study

The stability study was performed as per ICH guidelines. The formulations were stored at different temperatures like at freeze temperature, room temperature, 25℃±2 ℃, 60%±5% RH and 40℃±2 ℃, 75%±5%RH for six month in humidity chamber. The samples under evaluation were observed for the drug content, pH changes and also any changes in color, odor and taste, its entrapment efficacy and in-vitro release study.

RESULTS AND DISCUSSION

Isolation of bio-polymer

The *Cestrum nocturnum* bio-polymer Brwnish-green in color with %yield of 4±1.2% .The color changing point was found to be 205°C±5℃.

Characterization of isolated biopolymer of *Cestrum nocturnum*

The isolated biopolymer was Brwnish-green color in appearance. The biopolymer was odorless with characteristic taste. It was found to be sparingly soluble in water. It showed the positive test for carbohydrate and protein. The findings of Physico-chemical Characterization of isolated biopolymer of *Cestrum nocturnum* has been shown in Table 2.

Table 2: Physico-chemical Characteristics of isolated biopolymer of *Cestrum nocturnum*.

Parameters evaluated	Observation
Color	Brwnish-green
Odor	Characteristic
Taste	Characteristic
Melting Point	205°C±5℃
Solubility Carbohydrate Protein	soluble in water Present Present

SEM (Scanning electron microscopy) analysis of biopolymer

The isolated biopolymer was analyzed by scanning electron microscopy for surface characterization. The SEM analysis of the isolated biopolymer shows the rough, granular and flaky surface. This flaky appearance reveals its polymeric nature. The SEM image at has been shown in Figure 1.

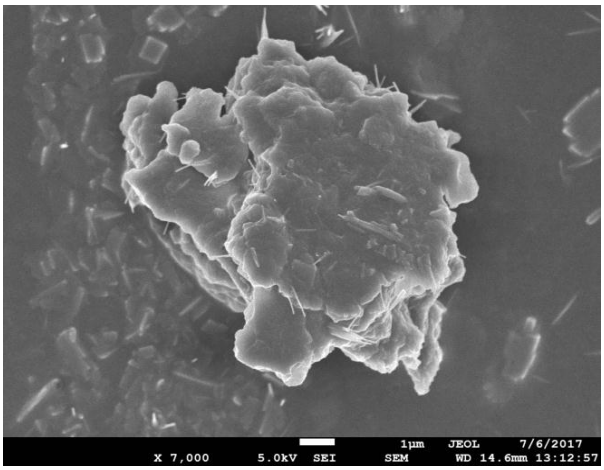

Figure 1: Surface morphology study of *Cestrum Nocturnum* Biopolymer

DIFFERENT SPECTRAL ANALYSIS AND THEIR FINDINGS

FTIR spectroscopy of isolated biopolymer

FTIR spectroscopy of ***Cestrum nocturnum*** isolated biopolymer shows the presence of different functional groups like alcoholic O-H stretching at 3400.22 cm^{-1}, Carboxylic O-H stretching at 3019.48 cm^{-1}, C-H bending at 758.04cm^{-1},C-O stretching at 1215.51 cm^{-1}, C=C stretching at 1621.37cm^{-1}, C-N stretching at 1406.72 $cm^{-1}$and R-NH_2 at 1068.67 cm^{-1}. The presence of the hydroxyl and carboxylic group defines its polymeric nature. The presence of amine and other above groups also reveals its polymeric nature. The FTIR spectra has been shown in Figure 2.

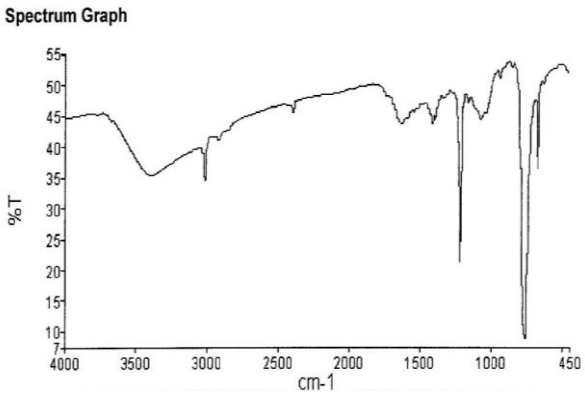

Figure 2: FT IR Spectra of biopolymer from *Cestrum nocturnum*.

DSC testing (Differential scanning calorimetry)

DSC thermogram of biopolymer isolated from *Cestrum nocturnum* showed the endothermic peak at 63.83°C with 98.71mJ/mg. The DSC spectra with broad endothermic peak reveals about the amorphous nature of biopolymer. The DSC spectra of the isolated biopolymer is shown in Figure 3.

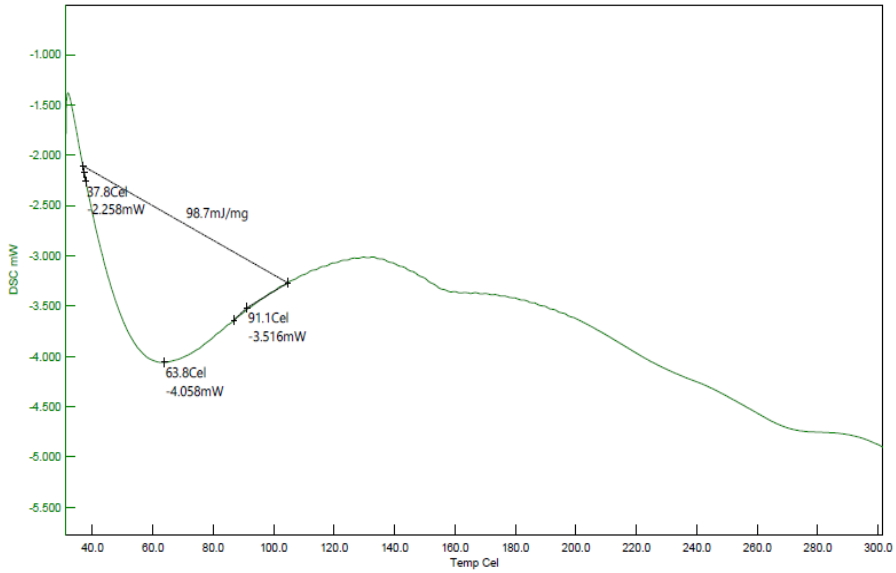

Figure 3: DSC of biopolymer from Cestrum nocturnum.

Mass spectroscopy

The mass spectra of biopolymer from *Cestrum nocturum* showed parent peak at m/z ratio 171.19 m/z ratio. This confirms its polymeric nature. The mass spectra is shown in Figure 4.

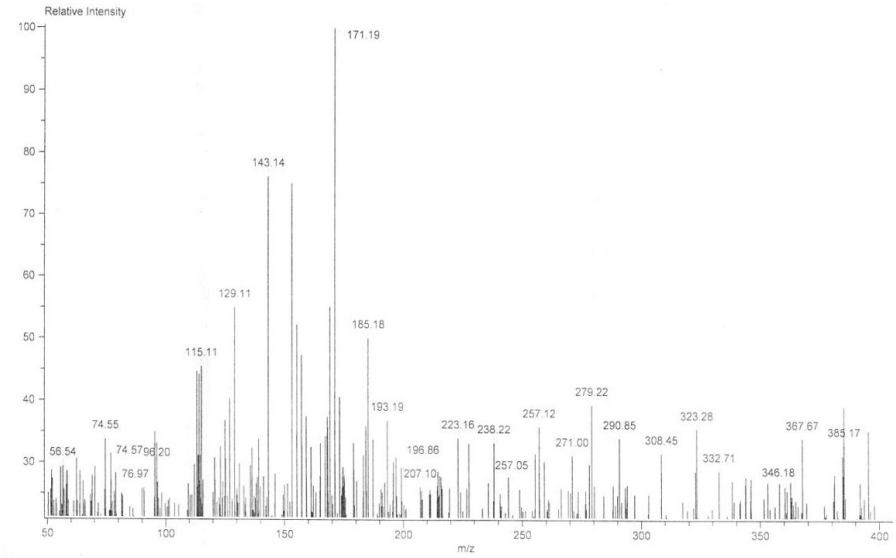

Figure 4: High resolution Mass Spectrum of isolated biopolymer from Cestrum nocturnum.

NMR spectral analysis

The NMR spectra of ***Cestrum nocturnum*** biopolymer show the presence of different peaks like hydroxyl at 1.255ppm, aromatic at 7.26, methyl -CH_3 at 0.901ppm, -CH2 at 1.333ppm as methylene, R-NH_2 at 1.557ppm. The presence of these groups like methyl, methylene, hydroxyl aromatic group reveals its polymeric nature. The NMR spectra is shown in Figure 5.

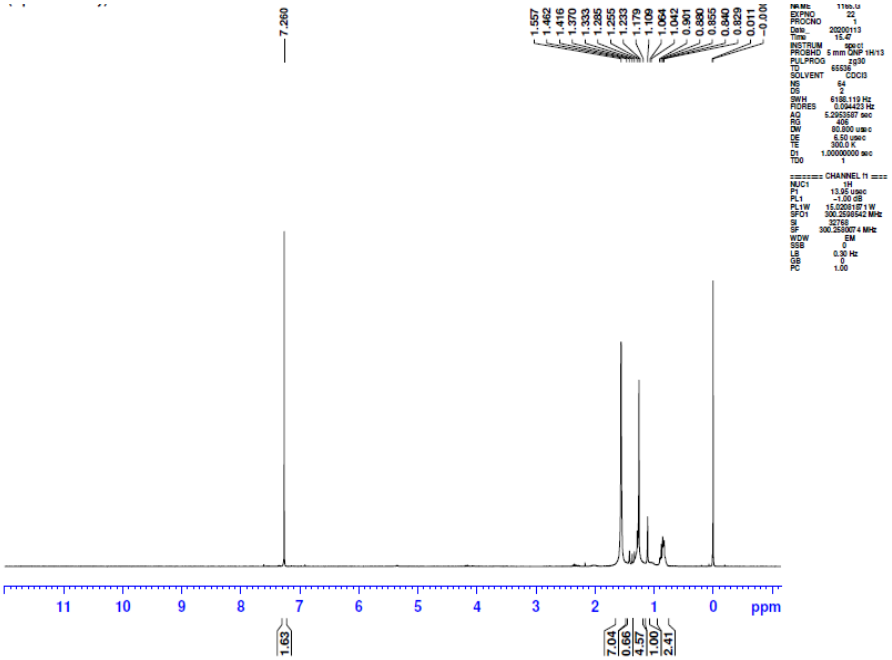

Figure 5: NMR Spectra of biopolymer from *Cestrum nocturnum*.

Nanosizing of Phenytoin

During the nanosizing of phenytoin after each sonication cycle the sample was observed for %transmittance which confirms that as the number of sonication cycle increases the % transmittance was found to be increased. This was due to decrease in particle size and particles are now are in nanorange. Thus % transmittance shows the % of particles below 400nm in bionanosuspension and % blockade give an idea about the % of particles which are above 400nm. Thus the UV method has given an idea about particles in nanorange. Thus UV method can be used for screening of the nano particles size. Figure 6.

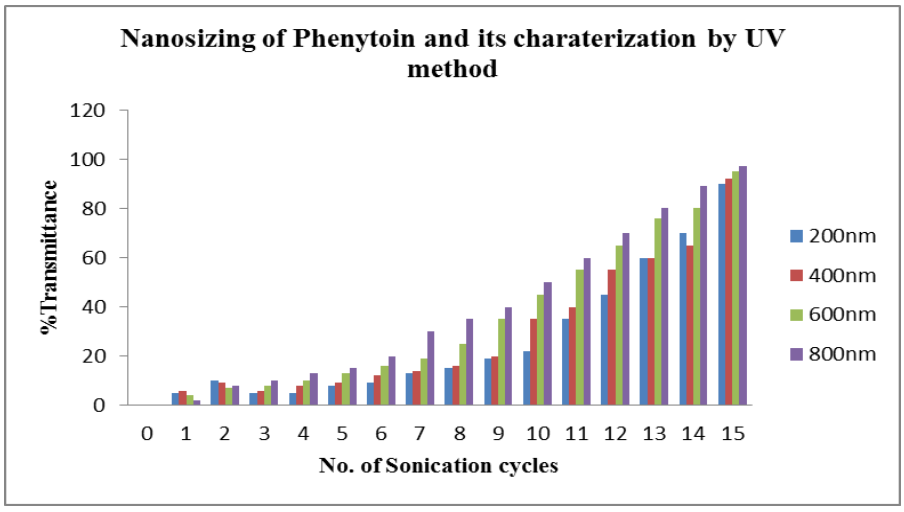

Figure 6: Nanosizing of Phenytoin and its charaterization by UV method.

Permeability study through the egg membrane

After analysis by UV spectrophotometer, a graph was plotted between concentration and time which shows the amount of the drug permeated at different time interval through the egg shell membrane. The egg membrane was used as the permeation membrane which mimics the physiology of ear biomembrane. So the permeation of phenytoin reveals that egg membrane mimics the ear physiological membrane and can be used for the drug release study. The graph of drug permeation versus time is shown in Figure 7.

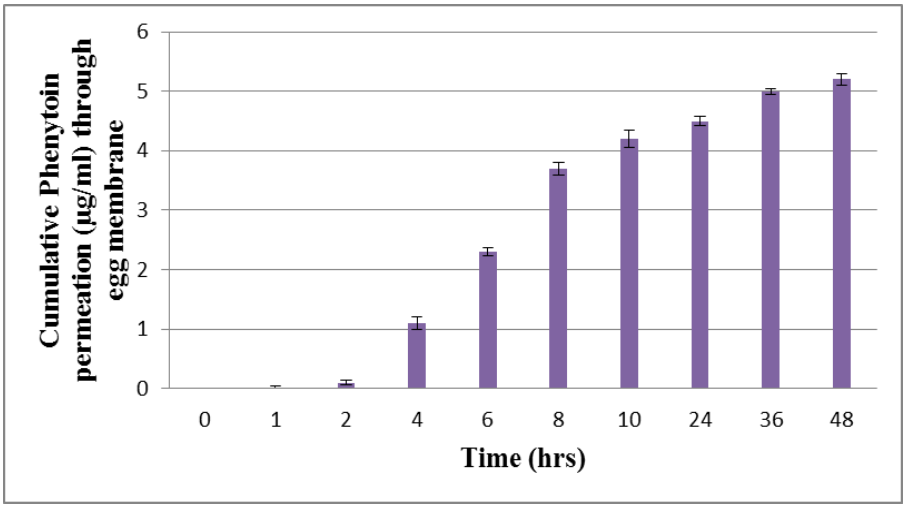

Figure 7: Cumulative Phenytoin permeation (µg/ml) through egg membrane.

Drug-excipient interaction study

There was no any change in λmax before (216nm) and after the test (216nm) in drug excipient study. The λmax was found to be same of the drug-biopolymer mixture as that of pure drug. It means it confirms that there was no any interaction between drug and biopolymer and other excipients also. It was observed and confirmed that excipients is not interacting and not producing any changes in drug properties so the isolated biopolymer can be used for the preparation of bionanosuspension.

Formulation of phenytoin loaded Bio-nanosuspension

The different formulations in different ratios were prepared as given in Table 1. After formulation of bionanosuspension they were evaluated for different parameters and their finding are described below.

Dispersiblity study of bio-nanosuspension

The dispersiblity of the formulated bio-nanoparticles was found to be excellent. The redispersion was also found to be good. The all nanoparticles were in dispersed state during dispersion. No aggregation or lump formation was observed Table 3.

pH study of bio-nanosuspension

The pH of the bionanosuspension was found to be in range of pH 6.4±0.04 to pH 7.4±0.12. This means the formulations were in desired ph range that is suitable for the stability of the bionanosuspension. The ph of different bionanosuspension obtained has been given in Table 3.

% Entrapment efficacy of loaded bionanosuspension

The entrapment efficacy of the formulated bionanosuspension was found to be 67.26±3.1 % to 84.82±4 %. Thus the formulated bionanosuspension showed the maximum entrapment efficacy up to 84.82±4 %. % Entrapment efficacy of the phenytoin loaded bionanosuspension has been given in Table 3.

Table 3: Observed pH, Dispersibility and % Entrapment Efficacy of PCN1-PCN8.

Formulations	Observed pH	Dispersibility	Entrapment Efficacy (%)
PCN1	6.6±0.18	-	67.26±3.1
PCN2	6.7±0.25	+	69.12±3.3
PCN3	7.3±0.09	+	80.45±322
PCN4	6.5±0.4	+	79.89±4.8
PCN5	7.4±0.12	-	83.12±2.7
PCN6	6.9±0.23	+	82.59±2.2
PCN7	6.4±0.04	+	83.61±1
PCN8	7.4±0.05	+	84.82±4

Particle size screening of the nanoparticles in bionanosuspension by UV method

Here UV method has been used for screening the nanoparticles in bionanosuspension. As the sonication cycle was increased the % transmittance was found to be increased because the particle size after sonication has come in nanorange. The% transmittance indicated about the % of particles below 400nm and the % blockade showed the % of particles above the 400nm when screened by UV spectrophotometry method. Thus UV method can be used as a screening method for the evaluation of nanoparticles in bionanosuspension.

Particle size analysis

The nanoparticles size in bionanosuspension was found to be 1339nm after evaluation with Malvern Zetasizer. Thus the obtained size with the zeta potential of -7.09mV which confirms that the nanoparticles are in nanorange

which is responsible for the stability of nanosuspension. It also confirms that the stable bionanosuspension loaded with phenytoin prepared by using *Cestrum nocturnum* biopolymers can be safely used for targeting to brain. The particle size distribution and Zeta potential distribution of bio-nanosuspension has been shown in Figure 8,9.

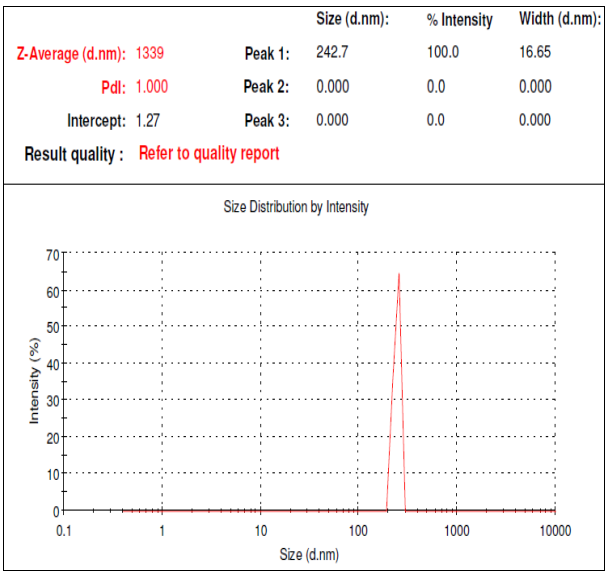

Figure 8: Zeta particle size of PCN8 Bionanosuspension.

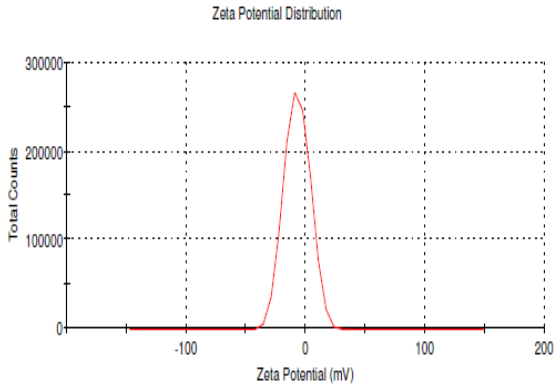

Figure 9: Zeta potential of PCN8 Bionanosuspension.

***In-Vitro* release study of bio-nanosuspension**

The In-vitro release study was done by using M.S. diffusion apparatus. The release kinetic study was done by using the BIT- 1.12 software and t50% and t80%, r2 were calculated. All the formulation showed more than 90.87% drug release (Figure 10). The In-vitro release study of different formulations showed the % drug release from 90.87% to 99.126%. The different formulations were evaluated for the *In-vitro* release study and release kinetic was studied. The formulation PCN8 was found to be the best formulation having t50% of 17.06 hours and t80% of 29.06 hours with r2 value of 0.9899. The best formulation PCN8 showed up to 90.87% drug release over 36 hours. According to the release kinetic study the best fit model was found to be Korsmeyer Peppas and the mechanism of drug release was found to be anomaolus transport. The result obtained from In-vitro release study and analysis of the release kinetic of the all formulations indicates the sustained release of the phenytoin from the bionanosuspension.[14]

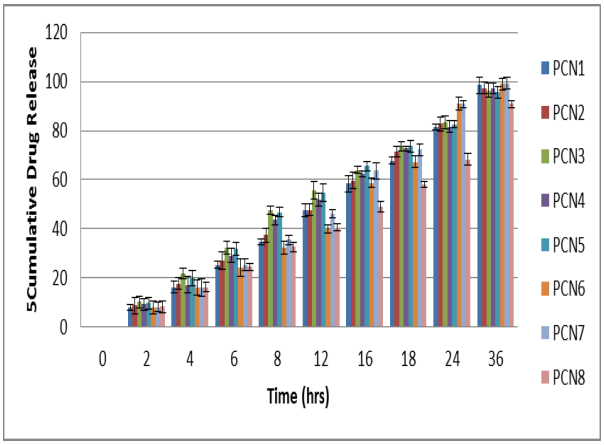

Figure 10: *In-vitro* release of PCN1-PCN8 using % Cumulative Drug Release.

According to the result of In-vitro release and release kinetic study it has been confirmed that the isolated *Cestrum nocturnum* biopolymer consist of the desired bio-retardant and bio-stabilizer novel properties. So it can be safely used for the formulation of stable bionanosuspension. The isolated *Cestrum nocturnum* biopolymer was found to be novel, non- toxic, non-reactive, biocompatible, inert and biodegradable. So the biopolymer can be used as the novel bio-excipient for novel drug delivery formulations.

Stability study

The optimized formulations showed no any change in λmax, entrapment efficacy and in drug release. So there was no drug loss during the study period. The other evaluation parameters also showed the satisfactory result. The best optimized formulation was found to be stable during study period. There was no change in color, odor, pH and physical appearance. During the stability study period all the results obtained from different parameters were satisfactory and the formulation PCN8 was found to be the best optimized stable formulation. During the study obtained results confirmed that the formulation was physically and chemically stable.

Here this research concludes about the isolation of novel bio-polymer, preparation of bio-nanosuspension, their evaluation for suitability to treat the CNS disease[7] by overcoming and minimizing all above mentioned problems. The CNS disease like epilepsy can be treated by drug targeting[5,9,10] to brain by using the novel intelligent biopolymer isolated from the natural source like flower petals of Cestrum nocturnum. The isolated bio-polymer from Cestrum nocturnum confirms its novel characteristics like sustainability, retardibility, stability and intelligent polymeric characteristics in release of phenytoin. The phenytoin was nano-sized to bring in

nano range. The formulated bio-nanosuspension using the naonosized phenytoin can be administered for the treatment of epilepsy. Thus the isolated biopolymer form *Cestrum nocturnum* was found to have promising inbuilt bioretardant cum biostabilizer properties which was found to be able to deliver the nanosized phenytoin in sustained manner over 36 hours.

CONCLUSION

The research reveals the suitability of isolated biopolymer from *Cestrum nocturnum* for the preparation of stable bionanosuspension loaded with nanosized phenytoin for treatment of epilepsy without any sides effects in very low dose. LCN8 was found to be best stable formulation among all the formulations of phenytoin loaded bionanoparticles. This showed a significant release in In-vitro release study. It also showed the significant satisfactory result in pharmacodynamic and pharmacokinetic studies. So these developed and evaluated bionanoparticles by using biopolymer[15,17] from *Cestrum nocturnum* can be safely and economically used for the treatment of epilepsy in very low dose as the nanosized phenytoin was used. So in very economical way the isolated biopolymer form natural source may be used in development of phenytoin loaded bionanosuspension for targeting to brain via ear. Thus in future ear may be one of the wonderful novel platforms for drug targeting to brain.

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