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Research Article

Formulation and Characterization of Edaravone-loaded Chitosan Nanoparticles for Treatment of Amyotrophic Lateral Sclerosis

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ABSTRACT

Owing to extensive hepatic first pass metabolism, Edaravone (EDV) shows low bioavailability (60 %) when administered orally, this leads to frequent administration of drug which may show toxicity. To overcome the above demerits of oral drug delivery of EDV, the present study is intended to prepare and evaluate EDV-loaded chitosan (CS) nanoparticles (EDV@CS-NPs) for the nose to brain delivery of EDV by ionic gelation technique. Chitosan, a biodegradable and mucoadhesive polymer, was selected for entrapment of EDV. The formulated, EDV@CS-NPs were characterized by UV-Visible spectroscopy, FT-IR spectroscopy, particle size analyser, zeta sizer, DSC, XRD, SEM, EDX, *In vitro-Ex-vivo* drug diffusion across goat nasal mucosa and *Ex-vivo* mucoadhesive study on goat nasal mucosa. The developed EDV@CS-NPs showed particle size of 200 ± 0.273 nm, polydispersity index (PDI) of 0.386, zeta potential -29 mV, an entrapment efficiency (EF) 97.52 ± 0.742%, and drug loading (DL) 22.72 ± 0.754%. The *in vitro* drug diffusion study displayed 91.80% of the drug release within 3 hours. The optimized batch (F2) showed the highest *Ex-vivo* drug diffusion up to 37.09 ± 0.24 % at the end of 3 hours.

Further, DSC studies reveal absence of any incompatible interaction between EDV, and CS. SEM demonstrated a smooth morphology with an uneven shape. Elemental analysis reveals successful formulation of EDV@CS-NPs. Most importantly, better mucoadhesive strength of formulated nanoparticles was observed in *Ex-vivo* mucoadhesive study on goat nasal mucosa. Formulated EDV@CS-NPs may present a promising drug delivery system to treat many neurodegenerative illnesses like amyotrophic lateral sclerosis (ALS).

Introduction

Amyotrophic lateral sclerosis (ALS), also recognized as motor neuron disease, is a rapidly escalating neurodegenerative disease that causes dysfunction of the nerves that regulate muscle variation.[1,2] ALS principally affects motor neurons in the brain, spinal cord, and brain stem.[3] It may lead to progressive motor neuron deprivation and muscle atrophy, which finally results in paralysis and ultimately, loss of life owing to respiratory failure.[4] There are no operative actions for ALS, attributable to deprived understanding of the mechanisms, incorrect animal models, defective clinical trial designs, absence of active and effective biomarkers, late analysis and diagnosis, inadequate bioavailability of drugs, and short efficiency of delivering ALS drugs to the central nervous system (CNS). There are certain potential reasons hampering the substantial translational growth in ALS clinical trials.[5,6] Only two medications, Rituzole and Edaravone (EDV), have been approved by the US Food and Drug Administration (USFDA) to reduce ALS progression modestly.[4] In order to address the foregoing limitation in ALS management, current approaches are critical. In ALS clinical trials, the successes of nanotechnology-based technologies in treating neurodegenerative disorders may be advantageous to inflate therapeutic competence of medications.

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Drug management for various neurodegenerative illnesses, whether chronic or acute, is difficult for a variety of reasons. Low bioavailability and insufficient brain familiarity of oral medications, rapid metabolism, eradication, undesired side effects, and a high dose to be added all mean hassles for patients and large costs for patients, their families, and society.[7] Drug administration through the nasal cavity has been used steadily as a route for local and systemic delivery of numerous therapeutic compounds.[8] Intranasal drug management is unique due to the auspicious choices to avoid the blood brain barrier (BBB), decrease the systemic antagonistic effects of drugs, and lower the doses to be supervised. The intranasal administration is painless, non-invasive, does not require a sterile preparation, and drug could be readily and easily administered.[9] Furthermore, medications administered through the nasal cavity have higher absorption, fewer adverse effects, and some consequences in terms of advanced brain coverage than oral treatments. The presence of numerous microvilli, increased vascularization of the subepithelial layer, escape of hepatic first-pass metabolism of drugs, and the possibility of direct drug transference to the brain are all factors that contribute to the large exterior zone available for drug absorption during nasal administration.[10] As a result, a new technique is required to address these flaws. A lot of pharmaceutical research thoughts have been intrigued by drug distribution coordination due to adaptability in the directing tissues, recovering profound molecular targets, and supervisory control of drug release. Nanoparticles are solid colloidal drug encapsulates with diameters ranging from 10 to 1000 nm, made up of synthetic, semi-synthetic, or natural polymers that encase the drug molecule. Because of its biocompatibility, biodegradability, gentler preparation methods, and adaptability in application have low toxicity.[11] Chitosan (CS) has proven to be a good material for making micro and nanoparticles with controlled medication release. Chitosan nanoparticles have attracted increased attention as a drug delivery vehicle due to their superior consistency, low toxicity, simple and easy manufacturing procedures, and customizable administration routes.[12] They can regulate the release of active substances. Although they are soluble in aqueous acidic solutions, they avoid using harmful organic solvents by building particles. Furthermore, chitosan is a lined polyamine with a variety of amine free groups that are readily available for crosslinking, and its cationic character allows for ionic crosslinking with multivalent anions.[13] While nanoparticles may provide an increase in nose-to-brain medication delivery, protecting the encapsulated drug from chemical and biological degradation, extracellular transport of the drug via P-glycoprotein efflux proteins may result in effective brain targeting.[14]

Edaravone has been chosen as a potential treatment for amyotrophic lateral sclerosis. EDV is (3-methyl-1-phenyl-2-pyrazolin-5-one, Radicut, MCI-186) low molecular weight free radical scavenger having BCS class IV category.[15] Although the effective neuroprotection by EDV has been proved, several critical problems remain, such as the ineffectiveness in crossing the blood-brain barrier (BBB), repetitive and over-high dosage, and severe side effects. Given above major hurdle in the delivery of EDV, an alternate drug delivery system is a need of the hour to treat neuro-degenerative disorders like ALS. Research groups like *Jianlin Shi et al.*, 2018 formulated Edaravone-Loaded Ceria Nanoparticles for Simultaneous Blood-Brain Barrier Crossing and Protection for Stroke Treatment.[16]

EDV@CS-NPs, which are innovative nanoparticulate systems, can be used for intranasal administration. The CS-NPs are gaining popularity due to numerous characteristics such as biodegradability and the ability to fool a variety of physically active molecules as well as the possibility of measured release, drug targeting, increased drug stability, and high drug payload.[17]

Delivering drugs having first pass metabolism through the nose to brain pathway always proven beneficial as there is a direct transport of drugs from olfactory nerve connected to nose and brain. Many drugs that are either metabolized by hepatic pathway or showing toxicity due to high dose can be delivered by nose to brain pathway with high bioavailability and less tissue toxicity. Hence, the current research intended to prepare EDV@CS-NPs using the ionotropic gelation technique. To explore the possibility of brain targeting through the nasal route to evade the first-pass metabolism and evade the delivery and distribution to non-targeted sites, thus reducing peripheral side effects. The mucoadhesive EDV@CS-NPs are believed to provide a number of advantages over conventional nasal dose forms, including increased nasal residence and the ability to release drugs at a slow and consistent rate.

Materials and Methods

Materials and Reagents

A gift sample of Edaravone was obtained as a gift from BDR Pharmaceuticals International Pvt. Ltd., Ankleshwar. Chitosan was purchased from Sigma Aldrich, Mumbai, India. Acetic acid, sodium hydroxide, potassium dihydrogen orthophosphate, pentasodium tripolyphosphate, and glycerol were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals and solvents were of analytical grade and used without purification.

Methods

To calculate the lambda max, the UV spectrum of EDV in water was scanned at 800 to 200 nm using a UV-visible spectrophotometer (Shimadzu-1800, Japan). 10 mg of EDV was accurately weighed and diluted in 100 mL water to make a stock solution (100 µg/mL), from which various

dilutions were created. A UV-spectrophotometer was used to measure the absorption.

Preparation of CS-NPs By Ionotropic Gelation Technique

Chitosan nanoparticles were prepared as per the ionic gelation method. Chitosan (0.20% w/v) dissolved 1% (v/v) acetic acid by using a magnetic stirrer to produce a clear chitosan solution. Then the pH was raised to 4.7 using 1 N NaOH solution, and the pH was checked by a digital pH meter. On the other hand, the sodium tripolyphosphate (STTP) solution was prepared (0.5 mL, 0.2% w/v in 0.1 N NaOH). The nanoparticles were obtained upon adding TTP aqueous solution to the chitosan solution (2 mL, 0.2% w/v) under a magnetic stirrer at room temperature for 1-hour.[18,19]

Preparation of EDV@CS-NPs

Using a magnetic stirrer, chitosan (0.2% w/v) was dissolved in 1% (v/v) acetic acid to generate a transparent chitosan solution. Dissolve 10 milligrams of edaravone in 10 ml of warm distilled water to obtain a clear, aqueous edaravone solution. Then, the produced edaravone solution is introduced to the chitosan solution (drug loading). The pH was then increased to 4.7 using a 1 N NaOH solution and measured using a digital pH meter. The nanoparticles were made by mixing aqueous STTP (0.5 mL, 0.2% w/v in 0.1 N NaOH) solution with chitosan solution (2 mL, 0.2% w/v) for 1 hour at room temperature using a magnetic stirrer. The aforementioned solution was concentrated by spinning at 8,000/gm for 30 minutes in centrifuge tubes with a 10 µL glycerol bed at the bottom to prevent nanoparticle aggregation. The supernatant was discarded after that, and the nanoparticles were re-suspended in phosphate buffer (pH 6.4). This was utilized to control the amount of drug-loaded and the effectiveness of the entrapment. The undiluted nanoparticles were lyophilized using Lyophilizer (Bioasset, Virar, India) after adding D-mannitol (5%) as a cryoprotectant. The cryoprotectant was required to avoid particle aggregation during lyophilization, resulting in better re-dispersibility, injectability, and syringe-ability following reconstitution.

Physiochemical Characterization of CS-NPs, EDV@ CS-NPs

Fourier Transform Infra-Red (FT-IR) Spectroscopy

The FTIR spectra of edaravone and EDV@CS-NPs were scanned over a wave range of 4000-400 cm^{-1} and spectra were obtained by FTIR spectrometer-430 (FTIR-8400S, Shimadzu, Japan). Edaravone was mixed thoroughly with KBr individually in a 1:100 (Edaravone: KBr) ratio.

Differential Scanning Colorimeter (DSC)

DSC analysis of EDV, CS-NPs, physical mixture, and EDV@ CS-NPs were carried out using DSC 822c, Mettler Toledo, Switzerland. Each sample was placed in an aluminium pan and then crimped with an aluminium cover. The heating and cooling rates were 20 °C/min, and all measurements were performed over the temperature range of 40-300°C.

Particle Size and Polydispersity Index (PDI) Analysis

The polydispersity index and analysis of nanoparticle size were resolved by using a Zeta sizer (Nanoplus 3 Particulate System Micromeretics, USA). Every sample was appropriately diluted with sieved purified distilled water up to 2 mL to escape multi-scattering marvels and was placed in a disposable sizing cuvette. The PDI was done to study the distribution of particle size. The size examination of a sample has 3 dimensions, and the results are the average particle size (n = 100) of the nanoparticles, and the standard deviation (σ) calculated for the optimized batch.

Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray Spectroscopy (EDAX)

The surface morphology and elemental analysis of the optimized formulation were examined using scanning electron microscopy (JSM-7900F, Japan). Samples were scaled on the aluminum stub and covered with a tiny gold palladium coat layer by an auto-fine coater to a thickness of 400 Å. Images were taken at different random locations and at four magnifications (×100, ×500, ×1000, and ×3000) and examined with a scanning electron microscope operated at a 15 kV acceleration voltage.

X-Ray Diffraction Study (XRD)

The XRD pattern was useful for analyzing the size of crystals of plane drugs and freeze-dried nanoparticles were noted on an X-ray diffractometer (D8 advanced Bruker, Germany). The samples were illumined with monochromatic Cu Kα radiations and analyzed between the 3 and 40° (2θ). The current and voltage used were 40 kV and 40 mA, correspondingly.

Entrapment Efficiency (EE) and Drug Loading (DL)

The edaravone entrapped in CS-NPs formulations was determined indirectly by the ultracentrifugation method using an ultracentrifuge (Eltek Rc4100 Electrocraft, India) centrifugation at 4°C at 25,000 rpm for 30 min. The gained supernatant was measured by a UV-spectrophotometer at 242 nm for free drug content. Loading efficiency (LE) of the drug-loaded system was also calculated concerning the yield of the nanoparticles obtained after centrifugation. The %EE and %DL were calculated as per the equation given below, with all the measurements being performed in triplicate.

$$\%\ EE = \frac{Total\ amount\ of\ drug - Free\ drug\ in\ supernant}{Total\ amount\ of\ drug} x\ 100$$

...equation 1

The % DL was calculated using,

$$\%\ DL = \frac{Total\ amount\ of\ drug - Free\ drug\ in\ supernant}{Total\ amount\ of\ polymer} x\ 100$$

...equation 2

Product Yield

The product yield of nanoparticles of various batches was calculated using the weight of the last product after dehydrating, concerning the initial total weight of the drug and polymer used for the composition of nanoparticles, and percent production yields were calculated as per the formula mentioned below.

In-vitro Drug Release Study

In vitro release of EDV from EDV@CS-NPs was assessed by the dialysis bag diffusion method.[20] The release study of EDV from nanoparticles were done in PBS (phosphate-buffered saline) at pH 6.4 to make an ideal sink condition since EDV has inadequate solubility in the buffer. The dispersion equivalent of aqueous nanoparticulate to the 10 mg of EDV was employed in the dialysis bag (cut-off 12,000 Da; Himedia), which was previously drenched overnight in water, cleaned the next morning, and airtight at both ends. The dialysis bags are immersed in the receptor compartment containing 100 mL of PBS (pH 6.4), stirred at 100 rpm, and maintained at 37 ± 2°C. The receptor compartments are covered to avoid desertion and evaporation of the release medium. Samples were withdrawn at regular time intervals, and a fresh release medium replaced the same volume, and then samples were analyzed with a UV-spectrophotometer at 242 nm wavelength.

In-vitro Diffusion Studies

In-vitro drug release from EDV@CS-NPs was performed in phosphate buffer (pH 6.4) by the goat mucosal membrane at 37 ± 0.5 °C. The goat mucosal membrane was purchased from the slaughterhouse. Adding 10 mg of EDV@CS-NPs to the mucosal membrane in the donor compartment and withdrawing solution at different time intervals was undertaken. Each sample's UV spectrum was taken for drug content at 242 nm.

Ex-vivo Mucoadhesive Test by Texture Analyzer

The tensile strength measurements were conducted with the CT3 Texture Analyzer (Brookfield, USA). The mucosa model was secured to the upper movable probe as described above, and the formulation sample was placed on the lower platform with a specific assembly, and the CS-NP_S was pressed to the mucosal membrane to test the mucoadhesive property of CS-NPs.[21] The measurement was activated to begin as the upper probe encountered a force of 2 mN upon contact with the sample. The probe was reserved in connection with no force pragmatic for 60 s, then it was elevated at the speed of 0.5 mm/s and the force required for objectivity was recorded. The tensile work, proportional to the area under the force-time curve, was used to express the mucoadhesive characteristics.

Results and Discussion

FTIR Spectra of Chitosan Nanoparticles

The FT-IR spectra of the blank CS-NPs formulation are shown in Fig. 1, It shows characteristic band at 3419.90 cm^{-1} with a shift of 2922.25 cm^{-1} attributed to $-NH_2$ and –OH groups stretching vibration, related to extra-molecular hydrogen bonding of the molecule and the IR spectra of chitosan at 2852.81 cm^{-1} with a shift of 2378.31 cm^{-1} showing the O=C=O stretching vibration. The characteristic absorption bands appeared at 1710.92 cm^{-1} (Amide-I), 1656.91 cm^{-1} C=C stretching vibrations, and 1408.08 cm^{-1} O-H bending vibration. According to other investigations, the only variation was the peak of P=O at 1060 cm^{-1}. The association between the phosphoric and ammonium ions could explain this band. The amine group's vibration range is 923.93 cm^{-1}, and the C-O asymmetric vibration range is 750.33 cm^{-1}. The peak at 651.96 cm^{-1} relates to P=O group stretching vibration after the reaction between chitosan and sodium tripolyphosphate (STPP).

The FTIR spectrum of EDV@CS-NPs is depicted in Fig. 2. This illustrates the distinctive absorption band of a primary amine, which is 3468.13 cm^{-1}. The peak at 3286.81 cm^{-1} relates to the aliphatic primary amine which confirms the loading of drugs in the nanoparticles. The broad peak at 1602 cm^{-1} corresponds to the conjugated alkenes. The amine and sulfate absorption bands in chitosan nanoparticles show slight variations at the 1624.12 and 1026.52 cm^{-1}, indicating that the presence of both polyelectrolytes in the final nanoparticle composition results in electrostatic interaction. According to the results, there was no interaction between the drug and the excipients found.

Differential Scanning Calorimetry (DSC)

The melting point and crystalline nature of edaravone were determined using differential scanning calorimetry.

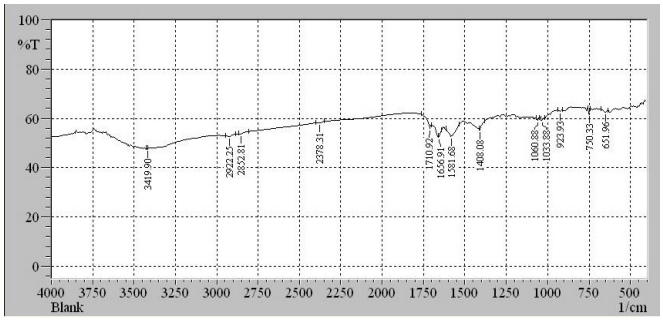

Fig. 1: FT-IR spectra of blank CS-NPs

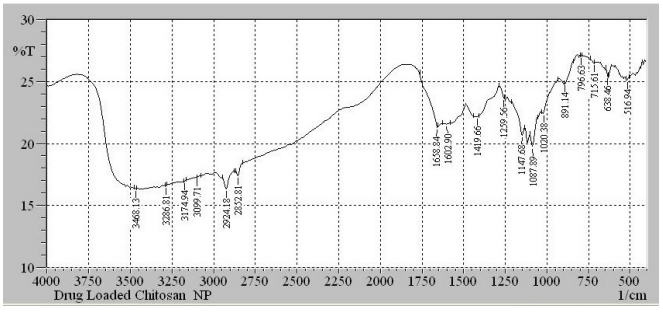

Fig. 2: FT-IR spectra of EDV@CS-NPs

Fig. 3 shows the DSC thermogram of pure edaravone in its purest form. It has a pronounced endothermic peak at 129.09°C, which was corroborated by the melting point of edaravone, which is between 127.70 and 129.55°C.[22] To further understand the behavior of crosslinked chitosan when heat energy is applied, DSC tests on blank CS-NPs were conducted. Polysaccharides have a great attraction for water molecules in general, and in their solid state, these macromolecules exhibit disordered configurations that may be easily hydrated. The endotherm associated with water evaporation is predictable, revealing the chemical changes that occur after cross-linking. At different pH levels, the water-holding capacity of chitosan and cross-linked chitosan differs. Fig. 4 illustrates the DSC thermogram of blank CS-NPs. In DSC of chitosan nanoparticles, an endothermic peak at 57.74°C was observed with an onset point of 53.89°C and an end setpoint of 60.04°C. Fig. 5 shows the DSC thermogram of EDV@CS-NPs. At 208.63°C, there is an endothermic peak. Because of the *in-situ* drug loading of drug in preparation NPs, the EDV@CS-NPs do not exhibit a drug peak.

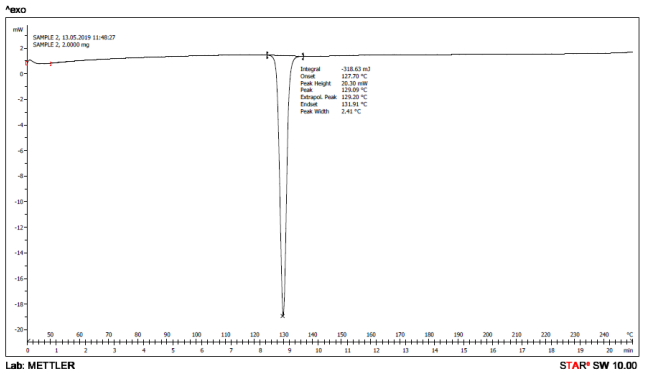

Fig. 3: DSC spectra of pure drug (EDV)

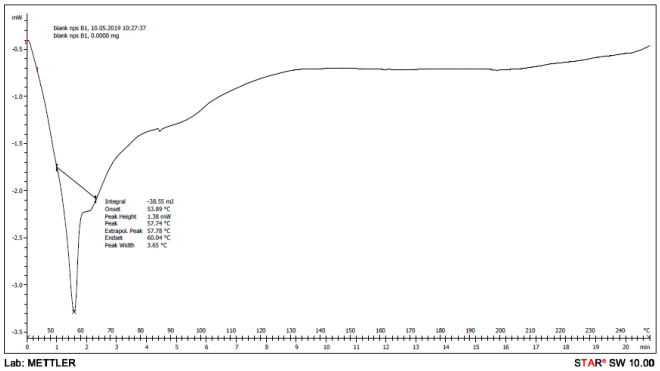

Fig. 4: DSC of blank CS-NPs.

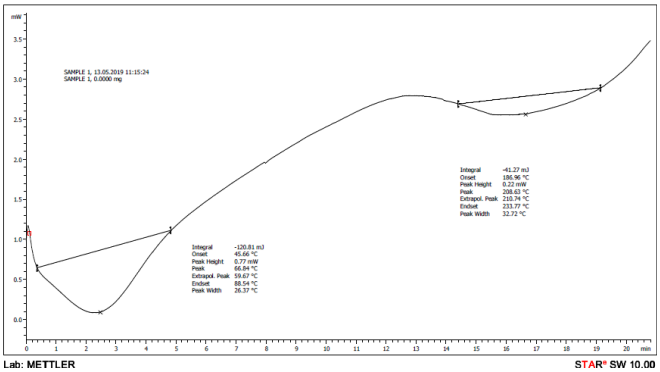

Fig. 5: DSC of EDV@CS-NPs.

X-ray Diffraction Study (XRD)

The X-ray powder diffraction patterns of blank CS-NPs and the EDV-loaded CS-NPs are shown in Figs. 6 and 7, respectively. The diffractogram of chitosan has distinguishing dissimilar peaks appearing at angles of 2θ = 10.72 Å, and 12.84 Å, indicating the semi-crystalline feature of chitosan.[23] The EDV@CS-NPs, shown in Fig. 7, have less intense peaks at 11.44 Å and 18.28 Å and reveal that some of the crystalline peaks of the drug were still detectable but with reduced intensity and fewer numbers in the diffractogram.

Scanning Electron Microscopy (SEM)

SEM was used to examine the surface morphology of blank and formulated EDV@CS-NPs. Figs. 8 and 9 show SEM images of blank CS-NPs and EDV@CS-NPs (a and b, respectively). SEM images

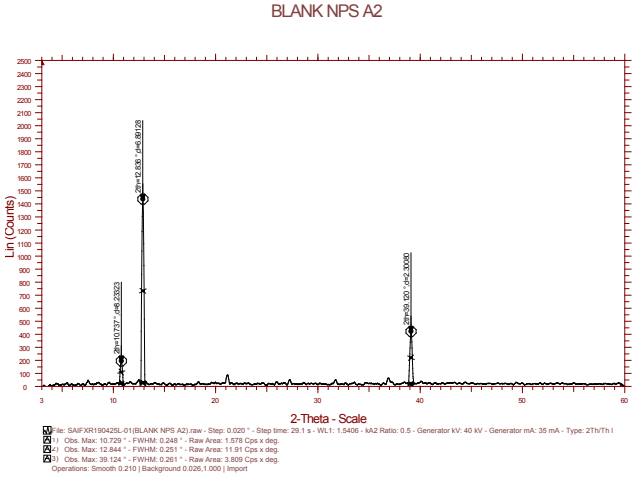

Fig. 6: X-ray Blank CS-NP$_S$

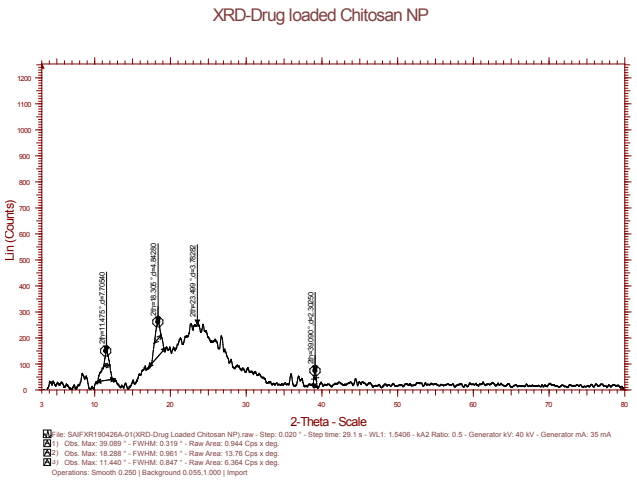

Fig. 7: X-ray EDV@CS-NPs

of blank CS-NPs demonstrated a smooth morphology with an uneven shape, whereas SEM images of EDV@CS-NPs exhibited consistent drug distribution within the CS-NP. Studies also verified that the crystalline edaravone was encapsulated by the polymer with a rough surface morphology.

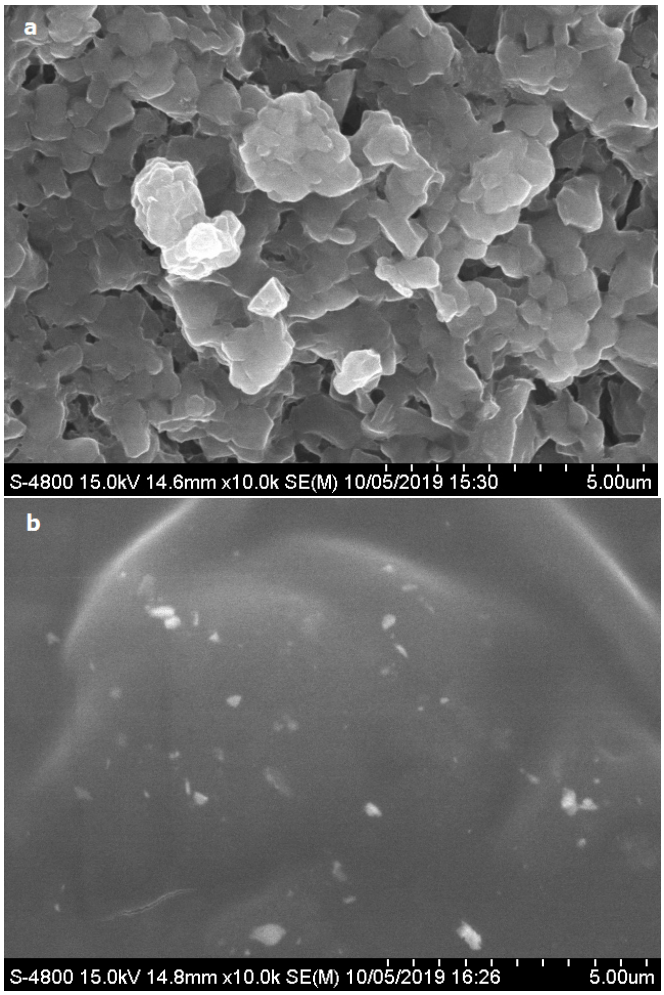

Fig. 8: Photomicrographic images of blank CS-NPs and optimized EDV@CS-NPs

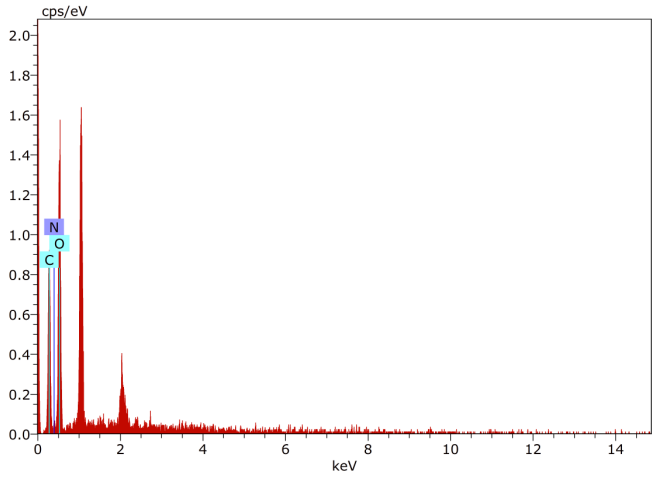

Fig. 9: EDX of blank CS-NPs.

Elemental Analysis by using Energy Dispersive X-ray (EDX)

The elemental analysis of blank chitosan nanoparticles (Fig. 9) shows the elemental concentration of C (35.09), O (64.14) and N (0.77). Due to the nature of chitosan, it has a high concentration of C and O. The EDX spectrum of EDV@CS-NPs (Fig. 10) shows the elemental concentration of C (10.9%) and O (13.06%). Other peaks in nanoparticles, on the other hand, reveal impurities such as Na (1.03 percent), Ca (0.08 percent), Si (0.1 percent), and P (1.74 percent), indicating that EDV@CS-NPs were successfully formulated.

Particle Size and Particle Size Distribution Analysis

Particle size is a crucial factor because it determines the rate and extent of drug release and drug absorption. The particle size of different batches was found to be in the range 200 ± 0.273 to 480 ± 0.281 nm. It is evident from the results of particle size, the concentration of chitosan plays an important role, as the chitosan concentration is increased, the particle size also increases, and the reason behind this is attributed to bulkiness offered by increasing concentration of chitosan on size of nanoparticles. The PDI of all the formulations was found in the range of 0.325 ± 0.053 to 0.643 ± 0.035. The particle size of the optimized batch (F2) was 200 ± 0.273 nm. From the above results, EDV@CS-NPs give better particle size and particle size distribution during analysis.

Zeta Potential Measurement

The zeta potential was determined by the zeta-size analyser. It designates the stability of the prepared formulation. A high zeta potential indicates extremely charged particles. Generally, high zeta potential (positive or negative) stops the accumulation of the particles due to electrical repulsion and electrically stabilizes the nanoparticular dispersion. Negative zeta values are observed in the formulation due to negative charges on the chitosan. The zeta potential value of optimized batch F2 was determined to be -29.33 mV in this investigation, indicating that the nanoparticles are uniformly distributed. Polymers having a high charge density have been demonstrated in studies to be effective

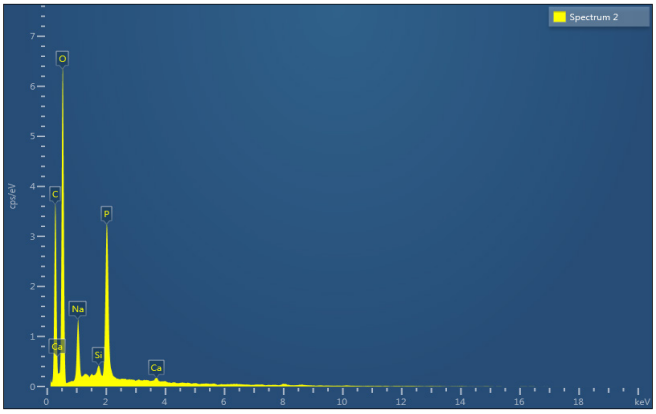

Fig. 10: EDX of EDV@CS-NPs

Table 1: Drug loading and entrapment efficiency

Batch code	*Conc. of chitosan (mg)*	*Conc. of TPP (%)*	*Conc. of Drug (mg)*	*% Entrapment efficiency (EF)*	*% Drug loading (DL)*
F1	150	0.25	10	96.47 ± 1.341	17.16 ± 0.193
F2	200	0.50	10	97.52 ± 0.742	22.72 ± 0.754
F3	250	1.00	10	97.45 ± 0.559	19.71 ± 0.556

Table 2: Product yield of the different batches

Batch Code	*Conc. of chitosan (mg)*	*Conc. of TPP (%)*	*Conc. of Drug (mg)*	*Production Yield (%)*
F1	150	0.25	10	76.38 ± 1.174
F2	200	0.50	10	82.13 ± 2.577
F3	250	1.00	10	79.62 ± 2.769

Table 3: *In-vitro* release of EDV from CS-NPS of different batches

S. N.	*Formulation*	*In vitro release*
1	F1	85.48%
2	F2	91.80%
3	F3	71.94%

mucoadhesive agents. The zeta potential of all batches is between -20 and -30 mV.

Drug Loading and Entrapment Efficiency

The entrapment efficiency of EDV@CS-NPs was investigated by varying the chitosan polymer (CS) concentration while maintaining a consistent drug concentration. Centrifugation of colloidal samples at 6000 rpm at 20°C for 10 minutes was used to measure drug loading and entrapment efficiency of EDV@CS-NPs. As due to the physical entrapment of EDV in core of chitosan, the maximum percent entrapment efficiency of 97.52 ± 0.742 was achieved at 200 mg/mL of CS concentration and 10 mg/ml of EDV for batch F2. Whereas percent drug loading was found to be 22.72 ± 0.754 for the same batch as shown in Table 1. Several batches were created by varying the concentration of chitosan polymer while keeping the medication concentration constant. The F2 batch performed well in entrapment efficiency and drug loading experiments.

Product Yield

All the formulated batches shown good product yield in between 76.38 - 82.13 % as shown in Table 2. Because of optimization in the concentration of crosslinker, product yield varies with the quantity of crosslinker (triphosphate) utilized, the F2 batch has exhibited the largest extent of product yield out of all batches. The required production yield implies that the approach may be used for large-scale technology transfer.

In-vitro Drug Release of EDV@CS-NPs

Using the dialysis bag technique, the in-vitro release of Edaravone from CS-NPs formulations was investigated.

As demonstrated in Fig. 11, diffusion of pure drug was found to be 21.32% at the end of 3 hours, while the

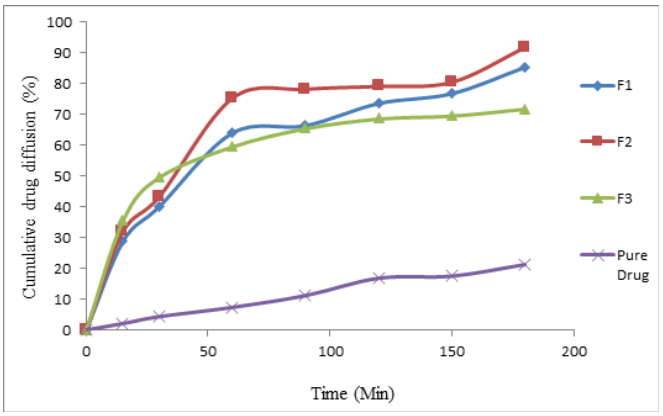

Fig. 11: *In-vitro* study of EDV@CS-NPs

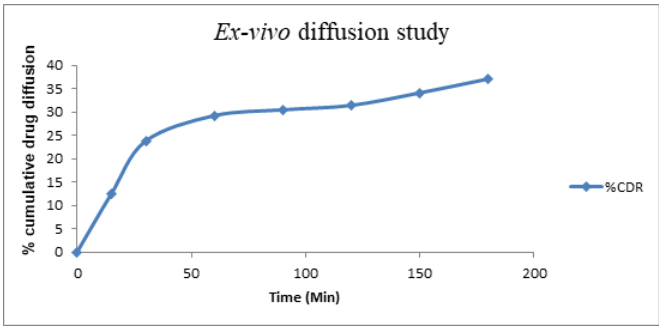

Fig. 12: Drug diffusion profile of EDV@CS-NPs across goat nasal mucosa

established formulation released 91.80% of the drug in 3 hours (F2 optimized batch). The release pattern indicates that the medication will be released in the brain for a long time after passing through the barrier and accumulating in the brain. As a result, EDV from CS-NPs continues to release for another 3 hours, indicating sustained release. The F2 batch had the maximum medication release due to the highest polymer concentration applied. The EDV release is delayed in the F2 batch due to the increased concentration of crosslinker utilized in the formulation. The release rate of EDV from various prepared batches is shown in Table 3.

Ex-Vivo diffusion Studies

Ex-vivo drug diffusion experiments were run, as shown in Fig. 12, to assess the rate of drug diffusion from the

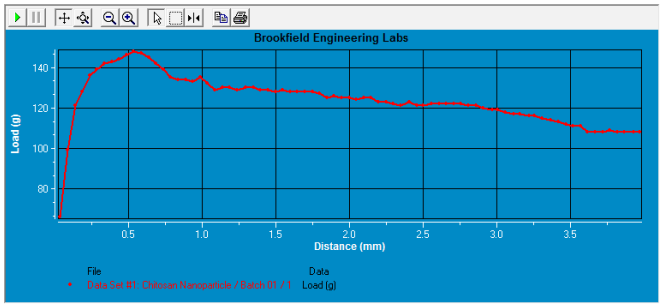

Fig. 13: Graph of peak deformation of EDV@CS-NPs by texture analyzer

preparation over the goat nasal mucosa. The *ex-vivo* diffusion study, of EDV@CS-NPs was performed in phosphate buffer (pH 6.4) using goat nasal mucosal membrane at 37 ± 0.5°C. The optimized batch (F2) showed the highest drug diffusion rate, up to 37.09 ± 0.24% at the end of 3 hours. According to the results, the reduction in the particle size of EDV@CS-NPs increases the diffusion of EDV across goat nasal mucosa.

Ex-vivo Mucoadhesive test of CS-NPs on Goat Mucosa using Texture Analyzer

Using a texture analyzer, the mucoadhesive strength of the formulations was determined by calculating the force required to separate the formulation from the mucosal model. The mucoadhesive strength of improved EDV@CS-NPs is estimated in Fig. 13 by considering the mucin binding efficiency to the optimized formulation. The adhering particles to tissue were analyzed visually for up to 10 min, where EDV@CS-NPs were found to adhere to the mucosal membrane even after 3 hours, indicating the better mucoadhevsive strength of formulated nanoparticles. The study showed the following outputs: Peak Load: 148 g, deformation at Peak Load: 0.54 mm Work: 4.8 Mj, Final Load: 108 g.

Conclusions

Edaravone is used to treat amyotrophic lateral sclerosis because it has a dynamic free radical scavenging capacity (ALS). EDV@CS-NPs were developed in this study to employ them to treat ALS, overcoming the limitations of commercially available edaravone intravenous formulations. An ionotropic gelation method has been adopted to successfully formulate EDV@CS-NPs, which were characterized by various sophisticated analytical instruments and the drug release profile was also studied. The particle size of formulated nanoparticles was 200 ± 0.273 nm, which is an acceptable limit for the nasal route of drug delivery. Almost 91.80% of drug release was observed in the optimized formulation within 3 hrs, which proves that the nanoparticles had a good release profile. All the results confirmed that the formulated nanoparticles can be used for the effective treatment of ALS using the nasal delivery route.

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