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RESEARCH ARTICLE

PREPARATION OF CARBAMAZEPINE CHITOSAN NANOPARTICLES FOR IMPROVING NASAL ABSORPTION

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ABSTRACT

In this study the nasal administration of carbamazepine has been studied using chitosan nanopartcles. The chitosan nanoparticles were prepared by ionic gelation of chitosan (100-300mg) with Tripolyphosphate sod (100-300mg in 100ml. Nine formulations were prepared, characterized and compared in terms of morphology (Transmission electron microscopy), drug content, particle size (zetasizer) and *In-vitro* drug release. *In-vitro* drug release studies were performed in Franz diffusion cell using phosphate buffer (buffer pH 5.5) as dissolution medium. The Chitosan nanoparticles had a mean size of 124.2±05 to 580±13nm, zeta potential were found to be +21 to 26.6 mV and the entrapment efficiency were found to be 65 to 72.7%. The *in-vivo* study was performed on Wistar rat, nanoparticles were administered through nasal route and compared with carbamazepine given by i.v. route, the results indicate that carbamazepine loaded chitosan nanoparticles enhances the drug absorption through nose. The results showed that the carbamazepine could be directly transported into the rat brain through nose and the possible side effects could be minimized.

Key Words: Carbamazepine, Chitosan Nanopartcles, Ionic Gelation, Epilepsy

1. INTRODUCTION

Epilepsy is a common neurological disorder affecting approx. 0.5-1% population in all over the world. There are several formulations in the market those are employed for the treatment of epilepsy. Antiepileptic drug after oral or intravenous administration exhibits high distribution of drug into brain and other non-targeted organs which can precipitate the undesirable side effects e.g. somnolence, dizziness and ataxia to severe peripheral pathologic conditions like dermatologic and hematologic, renal and hepatic dysfunctions.[1,2,3]The prevention of such kind of condition is very difficult but a much needed work, for this the drug should be targeted directly into the brain. But to deliver the drugs directly into the brain is a very difficult task because the penetration of drugs into brain is a great challenge, due to the functionality and structure of the BBB. The blood brain barrier is a unique system of capillary endothelial cell which prevent foreign material to permeate into the brain. BBB limits the transport of drugs by tight junction or physical barrier and the transendothelial electron resistance (TEER), enzymes or metabolic barrier also limits the penetration into brain.[4] In last 10 years, different techniques have been attempted to target the brain to deliver drugs directly into the brain. Nowadays alternative route of drug delivery is in practice, nasal route has attracted scientist's attention to develop new drug delivery system.[5] Nasal route appears to be an ideal alternative to parenterals for systemic drug delivery as well as for brain tageting.[6] Nasal drug delivery is a non-invasive method and it also avoids gastrointestinal and hepatic first-pass metabolism. It exhibit rapid-onset of action, patient compliance, self administrable, which make it a route of choice for the management of emergency situations.[7] Despite these advantage nasal drugs delivery has some limitations for drug penetration like size, molecular weight and low residence time of drugs in the nasal cavity.[4,7] To overcome these problems particulate carrier like nanoparticles made up of mucoadhesive biomaterial can be used to target brain via nasal route due to their small size and mucoadhesive properties.[8] Polymeric nanoparticles are solid colloids having a size of 10-1000nm. Chitosan is widely used polymer for preparation of mucoadhesive nanoparticles. Chitosan shows very good entrapment of drugs. Various studies have shown the successful use of chitosan nanoparticles for better drug targeting in brain as well as systemic circulation.[8]

Carbamazepine is first-line antiepileptic drugs with narrow therapeutic window, complex pharmacokinetics, it has chances of interacting with other drugs and causing side effects, it is absorbed slowly from the oral route, its oral bioavailability is 75% and the peak plasma concentration is achieved in about 4-8h after dosing, it may be vary to 24h at high doses.[2] Carbamazepine metabolized by liver and the enzymatic induction can

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cause the unpredictable fluctuation in plasma and an increase in unexpected clearance. For this the dose adjustment is required. All these pharmacokinetics limitation of orally administered carbamazepine make it, a suitable candidate for making nanoparticles using chitosan polymer for nasal administration.

2 MATERIALS AND METHOD

2.1 Material

Chitosan was obtained as a gift sample from CIFT, Kocchi, and Carbamazepine was purchased from Sigma Alderich Mumbai, Tween80 was purchased from Central Drug House, Mumbai, Dialysis membrane-70 was purchased from Hi Media, Mumbai. All other chemicals and solvents used in the study were of analytical or HPLC grade.

2.2 Method

Nanoparticles were prepared by using ionic gelatin technique, which was described by Calvo,[11] in which 100-300mg chitosan was dissolved in 100ml aq. solution of 0.5% acetic acid. The aq. solution of 100-300mg TPP sod was prepared separately. Then the required amount of drug was dissolved in 10ml methanol and mixed into chitosan solution. Then 25ml of the aq. solution of TPP sod was added to 100ml of chitosan solution and stirred for 2hr at 4000rpm using Remi mechanical stirrer. The formed nanoparticles were collected by centrifuging the system for 10min at 5000rpm and then air dried. Nine formulations were prepared by varying chitosan and TPP sod conc.

2.2.1 Particle size:

The average diameter and Polydispersity Index (PDI) of prepared batches of carbamazepine loaded chitosan nanoparticles were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer (Malvern, Ver. 6.01) at a fixed angle at 25°C. Sample was diluted 10 times with distilled water and then it was analyzed for particle size. The readings were recorded in triplicate.

2. 2.2 Zeta potential:

The zeta potential can be measured by determination of the movement velocity of the particles in an electric field and the particle charge, the chitosan nanoparticles dispersion was diluted 10 times with distilled water and analyzed by Zetasizer (Malvern, Ver. 6.01).

2.2.3 Drug Entrapment Efficiency

The entrapment efficiency (EE), which corresponds to the percentage of carbamazepine encapsulated within and adsorbed on to the nanoparticles, was determined by measuring the concentration of free carbamazepine in the dispersion medium. 10 mg of carbamazepine loaded nanoparticles was dissolved into 10ml methanol and kept for overnight then centrifuged at 5000rpm for 10min. Supernatant was than filtered by 0.2µ membrane filter and analyzed by UV-VIS spectroscopy at 280.4 nm.

$$\% EE = \frac{[\text{Initial drug} - \text{Free drug}]}{\text{Initial drug}} \times 100 \qquad \text{eq.1}$$

Where, Initial drug is the mass of initial drug used for the assay.

Free drug is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

2.2.4 Drug Content

10mg of carbamazepine nanoparticles was dissolved into 10ml methanol and kept for overnight. The soaked solution was centrifuged at 5000 rpm for 10min to separate the polymer. Supernatant was than filtered by 0.2µ membrane filter and analyzed by UV-VIS spectroscopy at 280.4 nm.

2.2.5 *In-Vitro* Drug Release Study nanoparticles[12]

The *in-vitro* release study was performed using previously overnight soaked dialysis membrane-70 mounted over the receptor compartment of Franz diffusion cell. The donor compartment was fixed and 37°C±0.5°C and stirred at 100rpm with Teflon-coated magnetic stirring bars. The formulations were placed in donor compartment and dialysis medium (buffer pH 5.5) was filled in receptor compartment. At predetermined time intervals (0.5, 1, 2, 3, for 9 hrs.), 5 ml aliquots was withdrawn and replaced with the same amount of fresh medium. Sink condition was maintained throughout the experiment. The amount of carbamazepine released from the nanoparticles was measured by UV spectrophotometer at 280.4 nm.

2.2.6 *In-Vitro* Drug Release Kinetics[13, 14]

In order to investigate the mechanism of release, the release data were analyzed with the following mathematical models: zero-order kinetic, first-order kinetic, Korsmeyer-Peppas kinetic model and Higuchi kinetic model.

2.2.7 Pharmacokinetic studies[1]

The rats were divided into two groups (A and B), Group A containing 18 animals which received the drug by i.v. administration and group B containing 18 animals received nanoparticles by nasal route. At a set time interval (5, 15,30,45,60,120 min) after dosing 3 animal per time point were sacrificed by cervical dislocation then decapitated. The blood was then immediately collected in tube containing heparin. The brain was removed and weighed. The blood sample were centrifuged at 4^0C at 4000rpm for 10min. the plasma were stored at -30 0 C for further analysis. The brain was homogenized with 0.1M sod phosphate buffer pH5.0 (4ml per gram) of tissue. The Teflon pestle tissue homogenizer was used. Tissue homogenate was then centrifuged at 4000rpm for 15min at 4^0C and the supernatant was then kept at -30 0 C for further analysis.

2.2.8 In-vivo experiments in rat

The *in-vivo* experiments were performed on Wistar rat at Deshpande Lab Bhopal, India. All animal studies were performed according to CPCSEA. The CPCSEA/IAEC approval No. **IAEC /DL/ 2015/RK/012**.

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2.2.9 Preparation of IV Drug solution for i.v. Administration[1]

For i.v. administration, the drug was dissolved in a mixture of propylene glycol-physiologic saline (0.9%NaCl)-ethanol in a ratio of 5:3:2 to make a final conc. of 1mg per ml.

2.2.10 Intranasal and i.v. administration[1]

The rats were anaesthetized with an intraperitoneal injection of a mixture of ketamine (100mg/ kg) and xylazine (10mg/kg) and the temperature of room was maintained warm. The prepared i.v. solution at dose of 0.1mg/kg was administered by injection on lateral tail vein. The prepared nanoparticles were administered at a dose equivalent to 1mg/kg to rat. The rat was placed on one side and the formulation was instilled using a polyurethane tube attached to a syringe. The tube inserted to 10mm deep into one the nares, to deliver the formulation to roof of the nasal cavity.

2.2.11 Drug Analysis:

Determination of drug was performed by a liquid chromatography system equipped with DAD detector 1100 at 285 nm (Agilent Technologies, Milano, Italy). A Luna® phenyl hexyl 5-m (Phenomenex, Torrance, USA) 250mm×4.6mm column with cartridge precolumn was used for separation at a flow rate of 0.60 ml/min. The mobile phase consisted of acetonitrile, methanol and water (0.1%) 30:60:10 (v/v) and the injection volume was 100µl. The amount of carbamazepine in serum and brain was expressed as ng/ml serum.

2.2.12 Pharmacokinetic analysis[13]

The peak plasma conc. C_{max} of drug was directly observed from plasma or brain and the time (T_{max}) to reach C_{max} was directly estimated from the data received by experiments, other Pharmacokinetic parameter were calculated based on the SEM (n=3) at each time point by a non compartment pharmacokinetics analysis. The pharmacokinetics parameters like AUC were evaluated, from t_0 to the last quantifiable conc. t_{last} by linear trapezoidal rule.

To assess the brain targeting efficiency of nasal formulations the drug targeting efficiency (DTE) index was calculated. It is a ratio of nasal and i.v.

$$DTE = \frac{(AUC\ brain/AUCplasma)\ nasal}{(AUC\ brain/AUCplasma)iv} \quad \text{(eq. 2)}$$

Where AUCbrain and AUCplasma are the area under the drug conc. time curve for brain and plasma after nasal and i.v. administration. For an effective drug targeting the ratio should be more than one.

2.2.13 Statistical analysis

The data were expressed as SEM, the comparative studies done between i.v. and nasally delivered formulation using single unpaired one tail ANOVA, difference was considered significant for a p- value $p<0.05$.

3. RESULT AND DISCUSSION

3.1 Preparation of Chitosan Nanoparticles

In present study, ionic gelation method was used to prepare nanoparticles, a simple and reproducible method for preparation of polymeric nanoparticles

3.2. Characterization of Chitosan Nanoparticles:

3.2.1 Particle Size:

In all the formulations, the particles size ranged from 124.2±05 to 580±13nm, and polydispersibility index were found to be 0.261±0.15 to 0.656±0.073. The results are given in table no.1. The study reveals that, polymer conc. have a significant effect on size, at the lower conc. of polymer, small size nanoparticles formed, but on increasing the conc. of polymer the size of nanoparticles increased, at higher conc. the chitosan forms a viscous gel which would not break down into small particles at given stirring speed, therefore a high particle size was observed. The conc. of sod TPP also plays effective role in size controlling, as the conc. of TPP sod was increased the particles size was decreased.

3.2.2 Zeta Potential

In all the formulations, the zeta potential ranged from +32.1 to +15.1. The chitosan is positively charged polymer therefore as the conc. of chitosan increased the zeta potential increases, and because the TPP sod is negatively charged therefore on increasing the conc. of TPP sod the zeta potential decreased. The results are given in table no.1

The higher zeta potential shows that all formulation are stable because the high zeta potential would not allow the particles to get aggregate or to form a big colloid due to electrical repulsion between particles. The Tween-80 would also provide stabilization to the formulations. The final results are shown in table no.1

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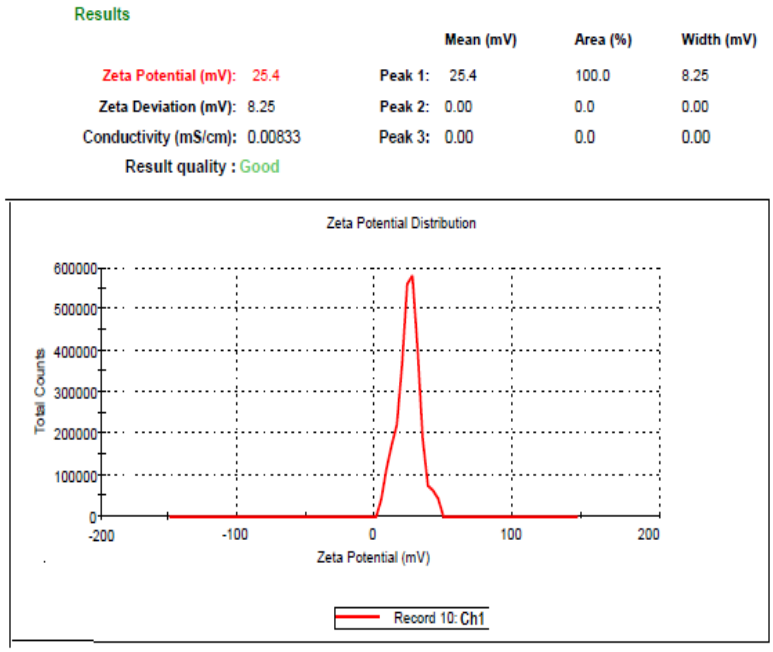

Figure 1: showing particle size of formulation Ch1

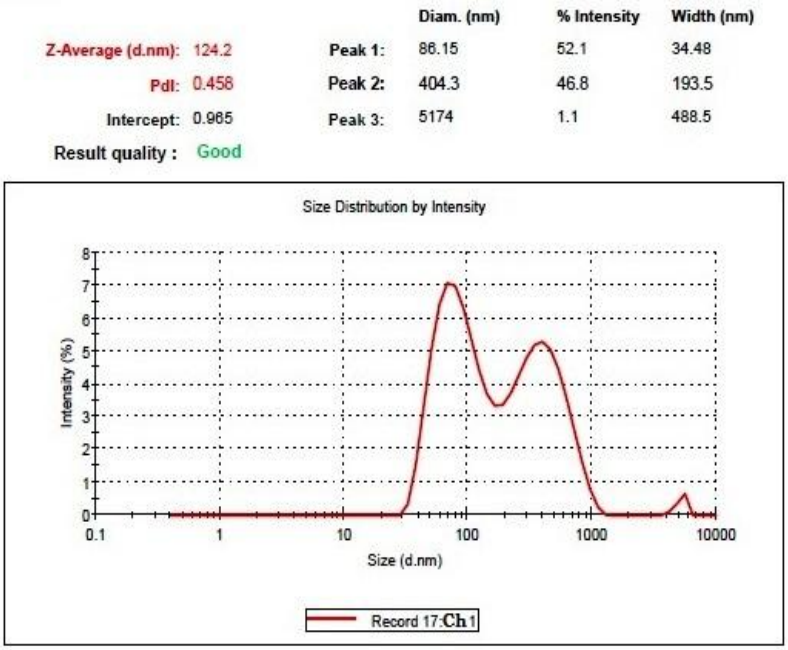

Figure 2: showing zeta potential of formulation Ch1

3.2.3 Drug Entrapment Efficiency

Percentage entrapment efficiency of all the formulations was found to be 40±1.25 to 69±0.85. The results are given in table no.1

The results shows that the polymer conc. play an important role in drug entrapment, on increasing the conc. of chitosan initially favors increase in the entrapment efficiency up to a certain level and then a decrease in the entrapment efficiency was observed may be due to the formation of viscous gel at higher polymer conc. i.e. 0.3%, the gel might hinder the permeation of drug into chitosan polymer matrix during formation of nanoparticles.

The conc. of TPP sod also affects the entrapment efficiency, at higher conc. of TPP sod the entrapment efficiency also decreased due to formation of a cross linked network which would not allow the drug to get diffuse into the polymer-TPP network.

3.2.4 Drug Content

Percentage drug content of all the formulation was found to be 84.69% to 98.85%. The final results are shown in table no.1

Table 1:

S.NO.	Batch code	Zeta size (nm)	PDI	Zeta potential (mV)	% EE	%Drug content
1	Ch1	124.2±05	0.368±0.12	+25.4	65±0.12	84.69%
2	Ch2	370±07	0.258±0.154	+28.9	69±1.02	98.85%.
3	Ch3	580±13	0.431±0.09	+32.1	56±0.98	88.34%
4	Ch4	108±10	0.368±0.65	+17.2	58±0.56	88.25%
5	Ch5	310±05	0.261±0.15	+19.2	61±0.80	86.05%
6	Ch6	410±09	0.461±0.106	+20.1	51±1.07	85.85%
7	Ch7	89±03	0.638±0.170	+15.1	48±1.10	90.85%
8	Ch8	250±10	0.656±0.073	+17.5	53±0.85	92.85%
9	Ch9	325±12	0.361±0.08	+19.8	40±1.25	91.85%

3.2.5. *In-vitro* drug release

The *in-vitro* cumulative drug release was found to be 69.8% to 82.8%. The formulation Ch2 shows the highest drug release in 9hr i.e. 86.3% where as Ch9 shows the lowest drug release i.e. 69.8 % in 9hr. it was observed in the study that the conc. of chitosan and the conc. of TPP sod plays important role in drug release from the formulations. Initially in the first 120min the drug released highest from the Ch3 having the highest conc. of chitosan but after 120 min the release is retarded because its forms gel, which retarded the drug release. On the other hand the increase in TPP sod conc. also retarded drug release because higher conc. of TPP sod makes a close network within the chitosan polymeric network.

3.2.5.1. Effect of chitosan conc. on drug release

Statistical analysis was used to analyze and check, whether there was any significant difference present in drug release pattern of different formulations or not. The single factor ANOVA was done with the help of MS Excel 2007 Software for a time period of 9hr applied for drug release, with increase in conc. of chitosan, a significant decrease $p<0.05$ (0.009335) was observed in *in-vitro* release of carbamazepine from nanoparticles, as the conc. of chitosan increased from 0.1% to 0.2% the drug release increased from 82.8 % to 86.3 but on increasing the conc. of chitosan to 0.3% from 0.2% the drug release was decreased.

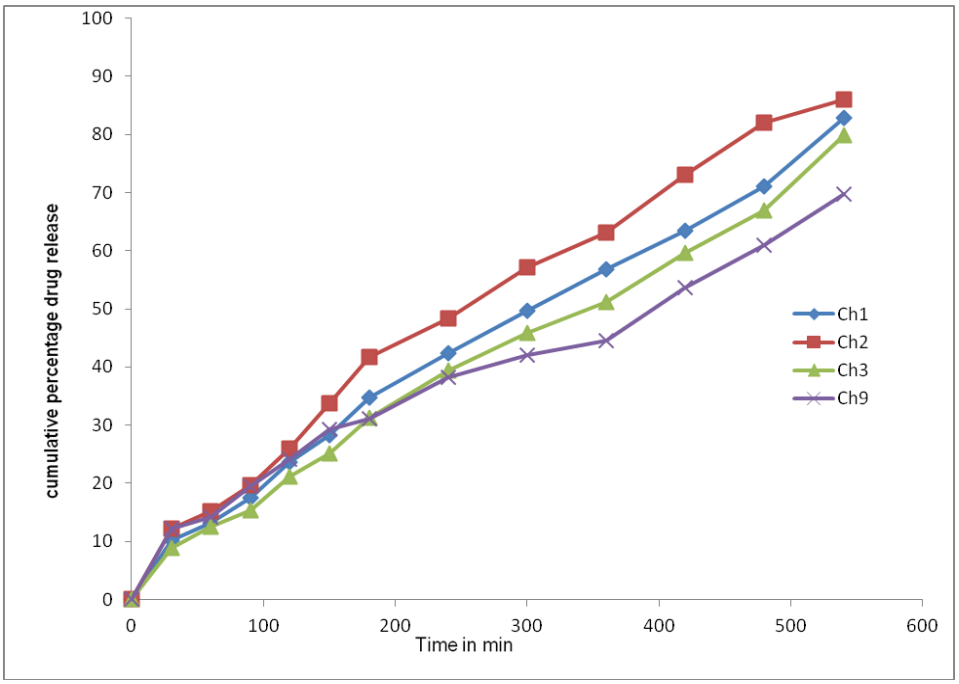

Figure 3: showing *In-vitro* drug release of formulation Ch1, Ch2, Ch3 and Ch9

In first 2hr, formulation Ch3 has higher chitosan conc. shows faster release about 31.3% than Ch1 and Ch2 but the drug release then decreased in comparison to Ch1 and Ch2. This is may be due to the formation of gel at higher chitosan conc.

3.2.5.2. Effect of cross linking agent

To understand the effect of cross linking agent on the drug release, formulation Ch1,Ch2 and Ch3 were analyzed with increasing conc. of TPP sod a significant

p<0.05 (0.006163) decrease in release rate was observed which revealed that the higher conc. of cross linking agent retards the drug release. The 80.7%, 84.23% and 86.51% carbamazepine was released from Ch1, Ch2 and Ch3 respectively.

3.2.6. Kinetics modeling of all formulation

The drug release kinetics of all formulations was evaluated to understand the drug release behavior from the chitosan nanoparticles. The drug release data were evaluated for a best fit equation of Zero order, First order, Koresymer Papas and Higuchi model. From the above study, the drug release data of most of the formulations fits into Korsmeyer Papps model where r^2 value were found near to 0.999, for the drug release of formulations Ch1, Ch2, Ch3, Ch4, Ch6 and Ch9. The formulations Ch5, Ch7, and Ch8 have shown a zero order drug release. But the drug release data of formulation Ch1 has shown a perfect Korsmeyer Papps model where the r^2 value was found to be 0.9904 and the K value was found to be 0.6059, which shows that the drug release follows Korsmeyer Papps model with non-Fickian diffusion super class II (0.45 < n< 0 8.9). The drug release from the chitosan nanoparticles may be due to the diffusion of water into the nanoparticles followed by swelling and finally formation of gel. The non-fickian case-II transport mechanism associated with formation of water soluble glassy polymer. The first 60% drug release data fits into Korsmeyer Papps from the above studies formulation Ch1 is selected for further study because it has narrow size range 124.2nm with good entrapment efficiency 65% and a good release profile. In this formulation the drug release initially showed a burst release and then a constant release for a period of 9hr.

3.2.7. Transmission Electron Microscopy:

The TEM study of the optimized formulation Ch1 was done, which also confirms the size of the prepared nanoparticles.

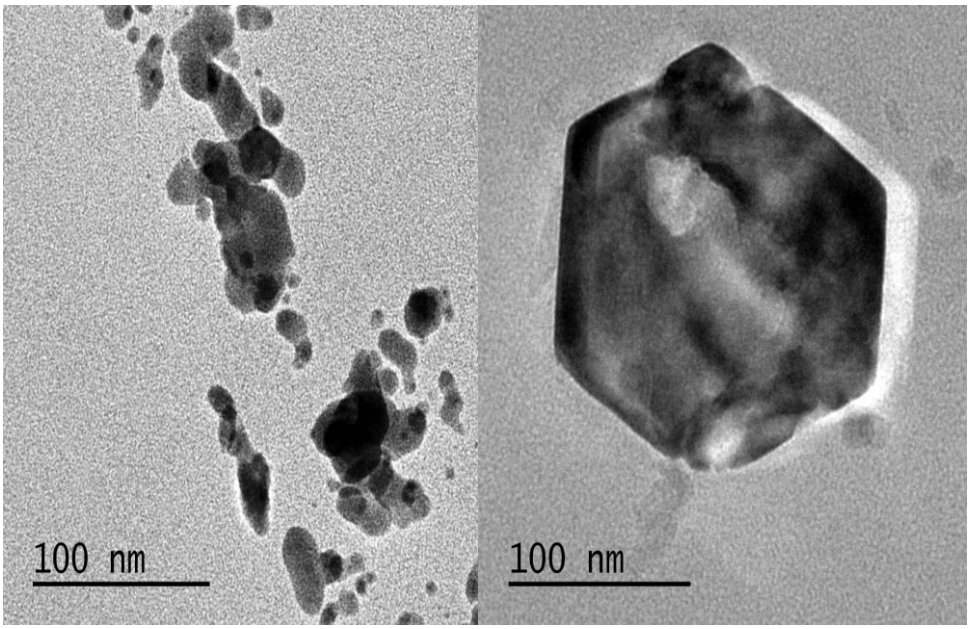

Figure 4: Showing TEM photograph (A) of Formulation Ch1 and B showing single particle of Formulation Ch1

3.2.8. Pharmacokinetics study of drug from i.v. and nasally applied chitosan nanoparticles Ch1

The pharmacokinetic data from i.v. administration, the C_{max} and T_{max} in plasma and brain was directly determined, in first 5min the C_{max} was achieved in plasma but in brain it has taken 30min to achieve C_{max}. The AUC in plasma and brain was calculated by trapezoidal method, the i.v. $AUC(Plasma)_{0\text{-}120min}$ was found to be 110.51 μgml^{-1} min^{-1} and $AUC(Brain)_{0\text{-}120min}$ was found to be 78.95 μgml^{-1} min^{-1}. The C_{max} in plasma 1199ng and brain was found to be 1357ng. The ratio of AUC(Brain) and AUC(Plasma) was found as 0.7144, which shows, the distribution of drug into brain (targets) and plasma (non target) slightly equal. The study reveals that the conc. in plasma is initially higher, this higher conc. can precipitate the side effects, after some time the conc. was found higher in brain than plasma.

Then the formulation Ch1 was given nasally to Wister rat after a single dose of drug 1mg/kg, the corresponding pharmacokinetic parameter estimated by non-compartmental analysis and AUC was calculated by trapezoidal method, initial conc. was extrapolated to the origin. In first 5min the C_{max} was achieved in brain but in plasma it has taken 45min to achieve C_{max}. The AUC $(Plasma)_{0\text{-}120min}$ was found to be 104.43 μgml^{-1} min^{-1} and AUC $(Brain)_{0\text{-}120min}$ was found to be 129.53 μgml^{-1} min^{-1}. The C_{max} in plasma 1430ng and brain was found to be 2920ng. The ratio of AUC (Brain) and AUC(Plasma) was found as 1.240, which shows, the high distribution into brain than plasma can be achieved with chitosan nanoparticles.

The drug transport efficiency was found to be 1.735, which shows that a chitosan nanoparticle has good brain targeting efficiency.

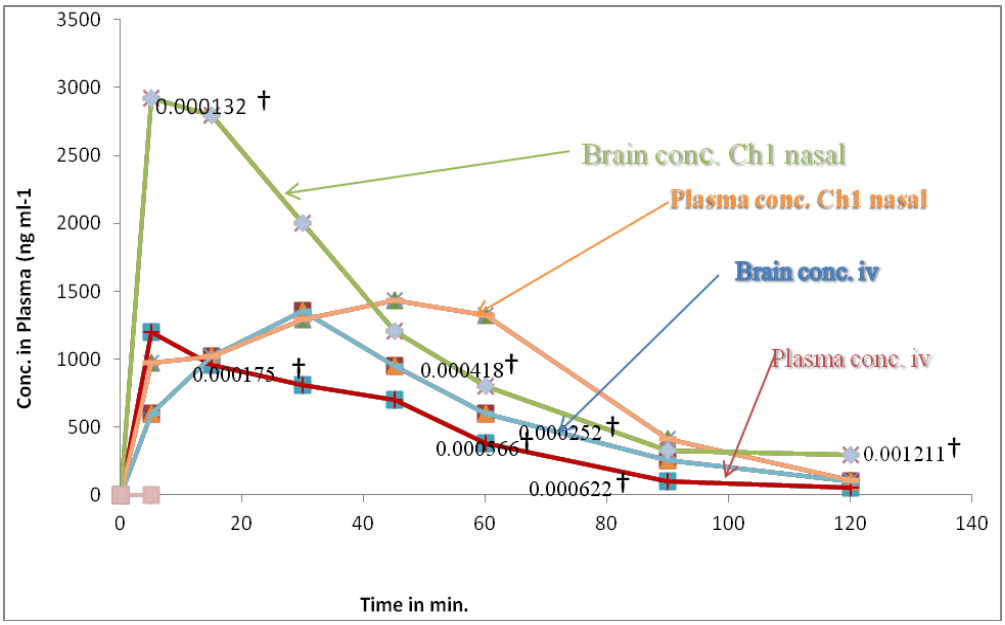

Figure 5: showing plasma and brain concentration- time profile of carbamazepine after i.v and nasally applied nanoparticles, The results are mean values ± SEM, n=3, † represents significant difference at any time point.

Table 2: Showing Pharmacokinetic parameters in brain and plasma after nasal and i.v. administration

Route	Organ/tissue	C_{Max}	T_{Max}	AUC $_{0\text{-}120min}$
i.v.	Plasma	1199ng	5min	110.51 μgml^{-1} min^{-1}
	Brain	1357ng	30min	78.95 μgml^{-1} min^{-1}
Nasal	Plasma	1430ng	45min	104.43 μgml^{-1} min^{-1}
	Brain	2920ng	5min	129.53 μgml^{-1} min^{-1}
AUC (Brain) /AUC(Plasma) nasal	1.240			
AUC (Brain) /AUC (Plasma) i v	0.7144			
DTE	1.735			

SUMMARY AND CONCLUSION

The chitosan nanoparticles of carbamazepine were successfully prepared by ionic gelation method using chitosan as polymer and TPP sod as ionic gelation agent. Nine formulations Ch1, Ch2, Ch3, Ch4, Ch5, Ch6, Ch7, Ch8 and Ch9 were prepared successfully. The conc. of chitosan and TPP sod was varied to understand the behavior of Chitosan and TPP sod on particle size, entrapment efficiency and drug release behavior. Formulation Ch1 has the smallest size and good entrapment efficiency and good *in-vitro* drug release profile therefore Ch1 selected for further *in-vivo* study.

The pharmacokinetic study of Ch1 revealed that drug achieved C_{max} in plasma in 45min and it has taken only 5min to achieve the C_{max} in brain whereas from i.v. route the C_{max} in plasma and brain was achieved in 5min and 30min respectively. The drug targeting efficiency was found to be 1.735, From the above study it was concluded that chitosan nanoparticles are able to transport the drug from nose to brain and can release the drug with enhance residence time and prolong drug release with lowest possible side effects due to lesser amount of drug reaches to the systemic circulation.

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