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PREPARATION AND EVALUATION OF NANOPARTICLES TOPIRAMATE BY ION GELATION METHOD

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Abstract: The present work was designed to formulate mucoadhesive nanoparticles of anti-epileptic agent (topiramate) and chitosan using byionotropic gelation method and its pharmaceutical evaluation chitosan was evaluated for percentage yield, melting point, zeta potential, aqueous solubility and rheological investigation. The formation of chitosan was characterized by FTIR, DSC and XRD analysis. The chitosan obtained by reductive methylation of chitosan was white to off-white odorless powder; having increased saturation aqueous solubility than chitosan. The melting point of chitosan was found in the range of 183-185˚C. Results of rheological investigation revealed that the viscosity of chitosan solution was increased as compared to chitosan and this might be attributed to the incorporation of methyl groups to the amino group of chitosan. , FTIR spectra of chitosan, topiramate and physical mixture of topiramate and chitosan in depected in 7.3, 7.4, 7.5, 7.6. In the current study indicate Nanoparticles prepared using chitosan as mucoadhesive polymer showed increase in drug release as compared to nanoparticle prepared by using chitosan. The permeation (drug release) of topiramate from nanoparticles of batch F5 was found to be 92.19 % respectively after 180 min.

Keywords: Chitosan, Nanoparticles, Topiramate, Calibration Curve, Ion Gelation.

INTRODUCTION

Nanoparticles are solid colloidal particles ranging from 1nm to1000nm in size ,they consist of macromolecular material in whichthe active ingredients (drug or biologically active material)is dissolved,entrapped,or encapsulated,or absorbed. Nano derives from the Greek word “nanos”,which means dwarf or extremely small. [1] It can be use as prefix for any unit to mean abillionth of that unit. There are two types of microspheres i)Nanospheres :matrix type structure in which a drug is dispersed ii)Nanocapsules: membrane wall structure with an oil core containing drug.[2]

Ideal characteristics of Nanoparticles:

- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and dispersibility in aqueous vehicles for injection.
- Particle size reduction for enhancing solubility of the poorly soluble drug.
- Provide constant and prolonged therapeutic effect.
- provide constant drug concentration in blood there by increasing patent compliance,

Material and Method

Topiramate is a gift sample obtain from global calcium pharmaceutical and other reagent from the SPU central chemical store.

Calibration curve

Calibration curve was prepared by plotting concentration on X-axis and the respective absorbance on Y-axis.

Measurment of pH

pH of 1% w/v aqueous solution of polymer was measured by pH meter.

Viscosity

Viscosity of chitosan was determined using Brookfield's Viscometer For the measurment varying the concentration (0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5% w/v) of chitosan were prepared in bidistilled water, following condition were set during measurment equilibration time: 5 min., spindle: 63, shear rate: 100 rpm and viscosity was determined.[3]

Solubility

Accurately weighed 100 mg of polymer was suspended in 10 ml of distilled water and stirred at room temperature for 6 h to obtain a saturated solution.[4] The insoluble chitosan was collected by filtration, washed with acetone and dried in oven at 40°C. The percent saturation solubility was determined using following formula.

Saturation solubility (%) $= [\frac{100-W1}{100}] \times 100$

Where W1 is the weight of undissolved polymer (mg)

FTIR Drug polymer compatibility study

The compatibility studies between Topiramate and chitosan were conducted by FTIR (Thermo Nicolet, Avatar 370) to detect drug-excipient interactions, if any. Approximately 2 mg sample was powdered uniformly with 200 mg of KBr for the production of KBr compacts. The samples were previously triturated and mixed thoroughly with KBr in 1:1 (sample: KBr) ratio, KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. Scans were obtained at a resolution of 4 cm^{-1} from 4000 to 400cm^{-1}.The IR spectra of the physical mixture of the drug and polymer were compared with the spectrum of drug and polymer to determine the compatibility of drug and excipient.

Preparation of BioadhesiveNanoparticles by Ion gelation method

TopiramateNanoparticles were prepared by Ion gelation gelation method using chitosan and sodium tri-poly phosphate as crosslinking agent. 500 mg of chitosan and was dissolved in 50 ml of 2% aqueous acetic acid and distilled water respectively. This solution was then added dropwise to solution of crosslinking agent sodium tri-poly phosphate 2% w/v under magnetic stirring at room temperature. The nanoparticles formed were separated after 30 min, washed with deionized water and then subsequently dried at 60° C for 3 h.

Table. No: 1 Composition of Topiramate chitosan nanoparticles

Sr. No.	Formulation code	Drug (mg)	Chitosan (mg)	Concentration of crosslinking agent (%)
1	F1	100	300	2
2	F2	100	350	2
3	F3	100	400	3
4	F4	100	450	3
5	F5	100	500	3
6	F6	100	550	3
7	F7	100	550	4

Characterization of Nanoparticle

Determination of percentage yield

Percentage yield of nanoparticles was calculated as a percentage weight of the final product after drying with respect to initial weight of polymer and drug taken for the preparation of nanoparticles. Percentage yield was calculated by using the following formula,

$$\text{Percentage yield} = \frac{\text{weightofmicrospheres}}{\text{weightofdrug+weightofpolymer}} \times 100$$

Particle size determination

The particle size of the nanoparticle was measured usinga stage micrometer scale. For optical microscopy the nanoparticleswere directly observed under magnification. Instrument was calibratedand found that 1 unit of eyepiece micrometer was equal to 11.76µm.Dry nanoparticles (3 mg) were suspended in distilled water andultrasonicated for 10 minutes. A drop of suspension was placed on aclean glass slide, and the nanoparticles were counted under opticalmicroscopy. A minimum of 100 nanoparticles was counted per batcwith a magnification of 45X. The average size of 100 particles was determined by the given equation(s): Size of individual particle (µm) = Number of division on eye piece ×

Swelling Index

This technique is used for characterization of nanoparticles. Different solution (100mL) are taken such as [distilled water, buffer solution of Ph (1.2, 4.5, 7.4)] and nanoparticles (100mg) are placed in a wire basket and kept on the above solution and swelling is allowed at 37ºC. Thus, changes in weight variation between initial weight of nanoparticles and weight due to swelling is measured by taking weight periodically and soaking with filter paper.

Zeta potential and zeta sizer

Zeta potential (surface electric charge) was determined by photon correlation spectroscopy (PCS) using Zetasizer (Malvern Instruments Ltd, Ver. 6.2) at 25°C and at a scattering angle of 90°, maintaining electric field strength of 25 V m^{-1}. Briefly, weighed amount of sample (5 mg) was dispersed in 10 ml of bidistilled water to get optimum 100–200 kilo-counts s^{-1} (kcps) for measurements and zeta potential in the range of +200 mv to −200 mv.

Surface morphology

Scanning electron microscopy (SEM) was used to study the surface topology of the nanoparticles. SEM imaging of the prepared nanoparticles was performed by scanning electron microscope- energy dispersive spectrometer (JEOL Model JSM - 6390LV).The samples were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminumstub. The stubs were then coated with gold to a thickness ~300 Aº under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with SEM.

Drug content and entrapment efficiency

Drug content and entrapment efficiency of the nanoparticles was analyzed to determine the amount of drug incorporation into the nanoparticles. Nanoparticle equivalent to 10 mg of drug were dissolved in 20 ml solvent (phosphate buffer solution 6.8 for topiramate nanoparticles) and kept overnight to extract the drug. The samples were centrifuge at 560 rpm for 10 min to eliminate the non soluble residue. The resultant solution was filtered and 1 ml of filtrate was analyzed for the drug content by UV- Visible spectrophotometer (Analytical Technologies Ltd., Gujarat, India) at respective wavelength (260 nm for topiramate loaded nanoparticles). Phosphate buffer solution was used as a blank for topiramate determination. The data was collected by repeating the procedure in triplicate. Drug content was determined by the following formula

$$Drugcontent\ \% = \frac{Qp}{Q} \times 100$$

Where, Qp = quantity of drug encapsulated in nanoparticles

Q = weighed quantity of powder of nanoparticles

The entrapment efficiency (%) of drug was calculated using the formula:

$$PercentEncapsulation(E) = \frac{Qp}{Qt} \times 100$$

Where, E = percentage of encapsulation of nanoparticles
Qp = quantity of drug encapsulated in nanoparticles
Qt = quantity of the drug added for encapsulation

RESULTS

Drug characterization

Melting Point

The melting point of the topiramate was found to be 122°C - 123°C.

Solubility

From the solubility study (Table No. 2) it was conformed that the drug was freely soluble in 95% ethanol, water and pH 6.8 phosphate buffer.

Table No.2: Solubility of Topiramate in different solvent

Sr. No.	Solvent	Solubility (mg/ml)
1	pH 6.6 phosphate buffer	1.28
2	Water	9.8
3	Ethanol	2.05

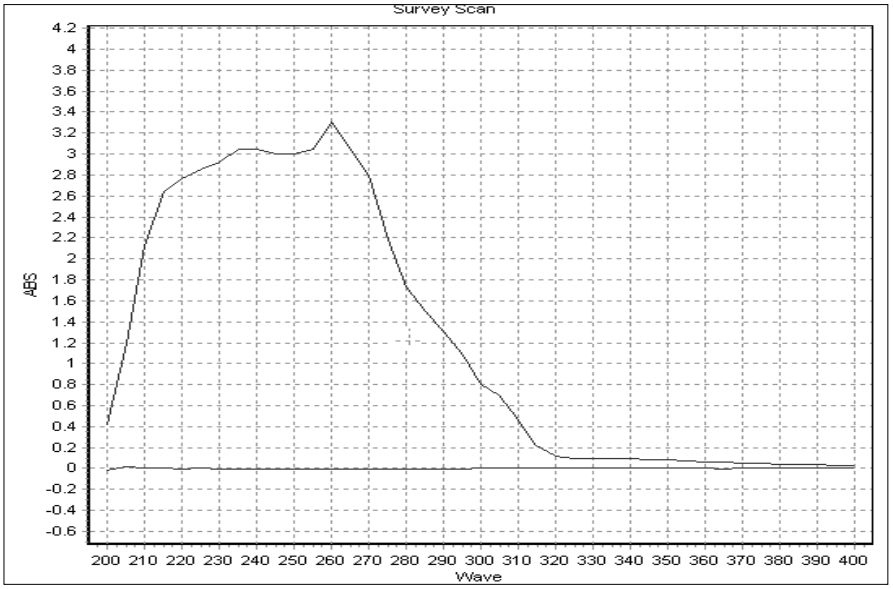

Fig.1 UV Spectrum of Topiramate in pH 6.8 Phosphate Buffer

UV Scanning

The UV scanning of topiramate showed maximum absorbance at 260 nm (λ_{max}) as shown in Fig 1. so all the spectrophotometric determination were carried out at this obtained maximum wavelength.

Standard calibration curve

Absorbance for topiramate in pH 6.8 phosphate buffer. The absorbance was measured at λmax 260 nm in the concentration range 2-12 µg/ml. The calculation of in-vitro drug diffusion, drug content and drug permeation study were based on the calibration curve. The curve obeys Beer-Lambert's law within concentration range of 2-12 µg/ml of topiramate in pH 6.8 phosphate buffer. The correlation coefficient (R^2) value for topiramate calibration curve was 0.998 in pH 6.8 phosphate buffer.

Table. No.3: Absorbance for calibration curve for Topiramate in pH 6.8 phosphate buffer

Sr. No	Conc. µg/ml	Absorbance
1	2	0.022
2	4	0.038
3	6	0.052
4	8	0.068
5	10	0.088
6	12	0.102

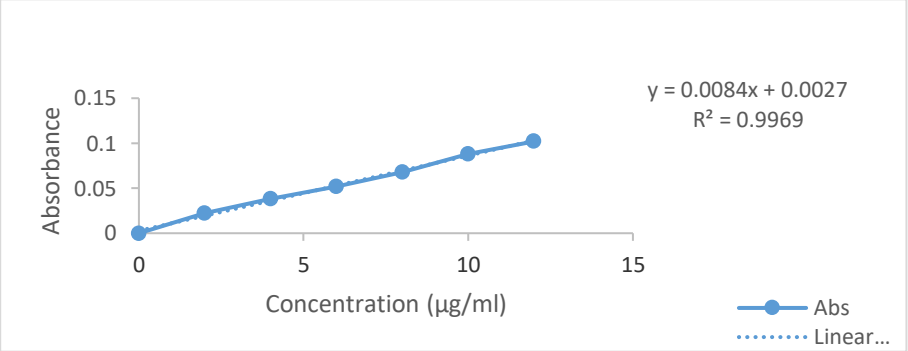

Fig 2: Calibration curve of topiramate in pH 6.8 Phosphate buffer

FTIR Study

For the determination af any kind of interaction between drug and excipient FTIR spectra of physical mixture of topiramate and chitosan was compaired with the spectra of physical mixture of both. The spectrum of chitosan was characterized by the presence of the band at 2890 cm^{-1} of C-H bonds. The band assigned to O-H (3000–3700 cm^{-1}) on the chitosan spectrum. The vibrational peak at 3443 cm^{-1} was indicative of the O-H group. The characteristic bands at 1307 cm^{-1} and 1091 cm^{-1} on chitosan spectrum indicated primary and secondary alcohols (Table 7.5). The intense band centered at 1664 cm^{-1} on chitosan spectrum is assigned to the C- O bonds of the acetamide groups, referred to as amide band. The obtained FTIR spectra also showed the same absorption peaks as mentioned above confirms the polymer was chitosan. The FTIR spectrum of topiramate (Fig 4) showed the characteristics peaks of absorption at 1072.47 cm^{-1} (S=0 Stretching), 1552.94 cm^{-1} (N-H-stretching) and 1648.25 cm^{-1} (C O stretching) illustrated in table 6.6. All the absorption peaks are similar as observed in the reference spectra for the drug topiramate(I.P. 2014). The spectrum of

topiramate and chitosan physical mixture was characterized by the presence of strong absorption band absorption at 2956.48 cm^{-1} (C- H Stretching), 1552.94 cm^{-1} (N-H-stretching) and 1648.25 cm^{-1} (C O stretching)

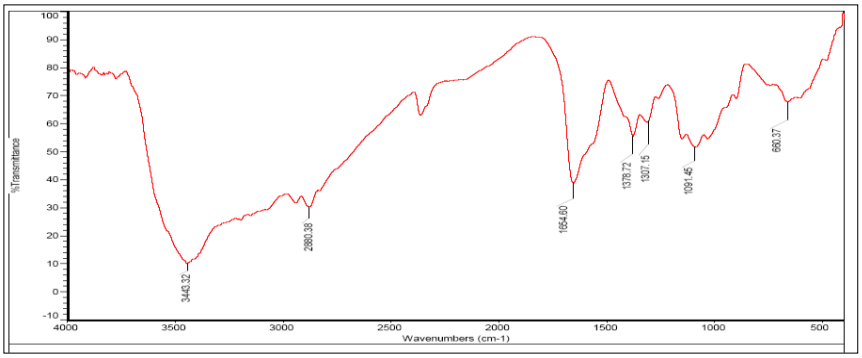

Fig 7.3 : FTIR spectrum of chitosan

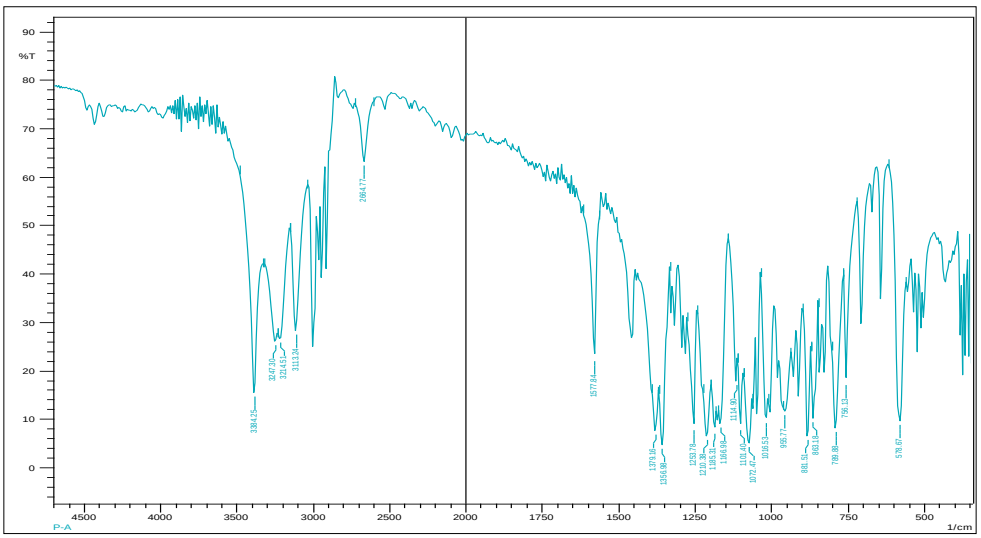

Fig. 4. FTIR spectrum of topiramate

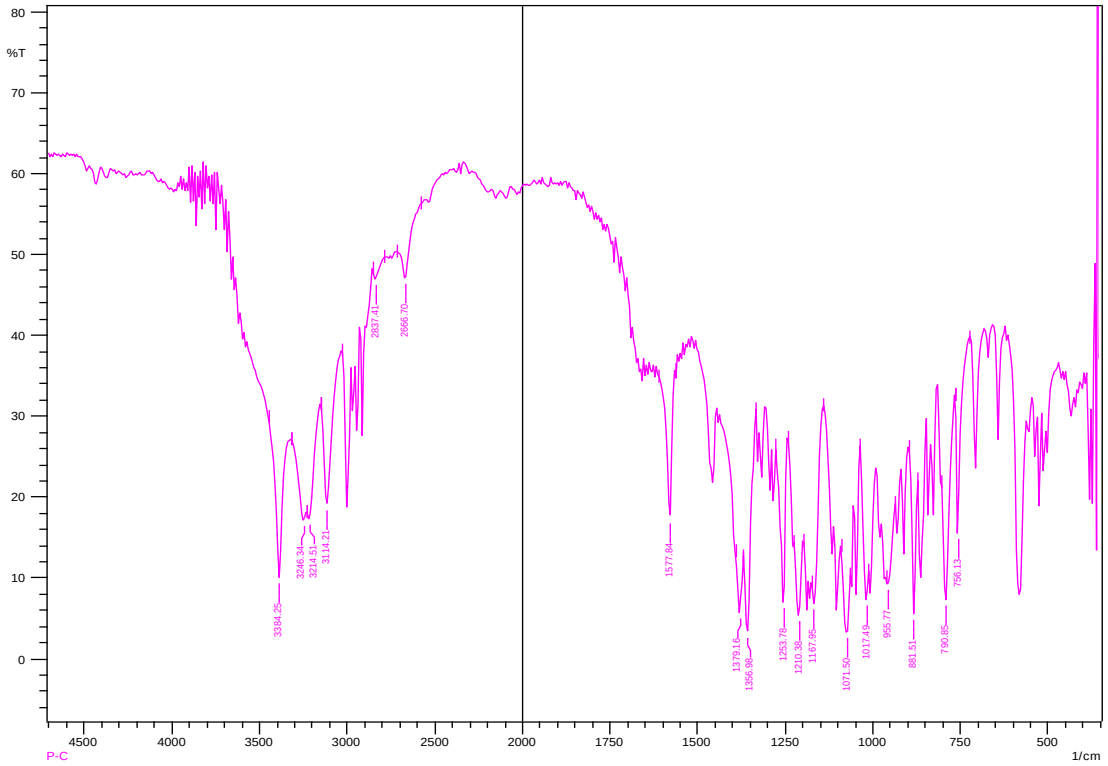

Fig. 5 : FTIR of Formulation

Evaluation of topiramate Nanoparticles

Percentage yield

The percentage yield was calculated to determine the yield of Nanoparticles prepared by the ionotropic gelation method. All the batches of topiramate loaded chitosan Nanoparticlesshowed percentage yield from the result it was observed that at the concentration of chitosan was increased, increase in percentage yield was observed.

Table.No.4 : Percentage of Topiramate loaded nanoparticle

Batch	% yield
F1	73.66
F2	75.48
F3	82.85
F4	72.02
F5	69.85
F6	83.73
F7	93.25

Particle size

The particle size of different Nanoparticles is shown in Table 7.10. The particle size of Nanoparticles varies with the concentration of chitosan used. At 0.55% w/v chitosan concentration, the particles of Nanoparticles were irregular with mean size 246.96nm, which increase for Nanoparticles prepared with chitosan respectively.

Table.No.5. Result of partical size

Batch	Partical size
F5	223.44
F6	246.96

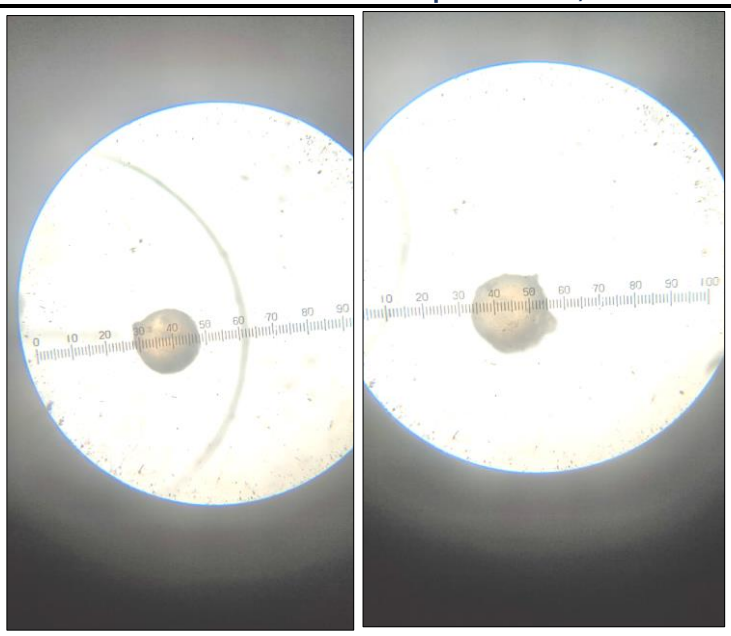

Fig.no 6. Partical size by stage micrometer

Swelling index of Topiramate loaded nanoparticle

Ability of nanoparticles to swell in presence of suitable medium is also a matter of prime importance to determine its capacity to liberate entrapped drug into release medium. Swelling behavior of nanoparticles predict drug release profile facilitating the requirement of optimum drug action. Data revealed that variation chitosan ratio affected a lot the degree of swelling of each batch of nanoparticle where it was found to increase the value with change in polymer proportion in formula and 0.55 %w/w of chitosan was not so sharp as shown in batch F6. However a satisfactory value was found for the batch F5 that was considered optimum because efficient control of electrostatic charges on the surface achieved by using optimum proportion of polymers for controlled interaction at predetermined pH.

Table .6: Swelling index

Time (Min)	F5 % Swell	F6 % Swell
00	00	00
10	2.1	1.9
20	7.1	5.1
30	11.3	9.8
40	15.9	14.1
50	18.1	17.2
60	20.1	19
70	21.2	21.01
80	22.1	21.93
90	24.9	23.1

Zeta Potential and Zeta Sizer

The zeta potential of topiramate and chitosan loaded Nanoparticles was found to be -5.21 mv (fig 7,8).it showed negative charge.the formulation displayed negative zeta potential due to the introduction of reduction of droplets size on particals,which increses the anionic property of formulation this permenent negative charge carried by the protonated amino group of formulation is driving force to the high solubility.

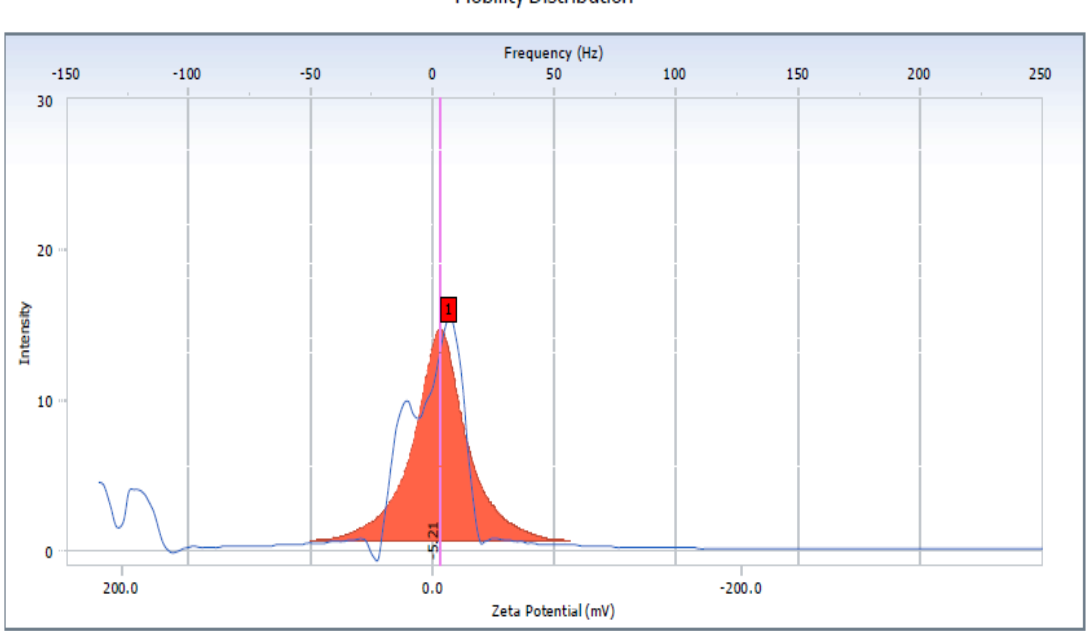

Fig 7. zeta potential of topiramate and chitosan loaded Nanoparticles.

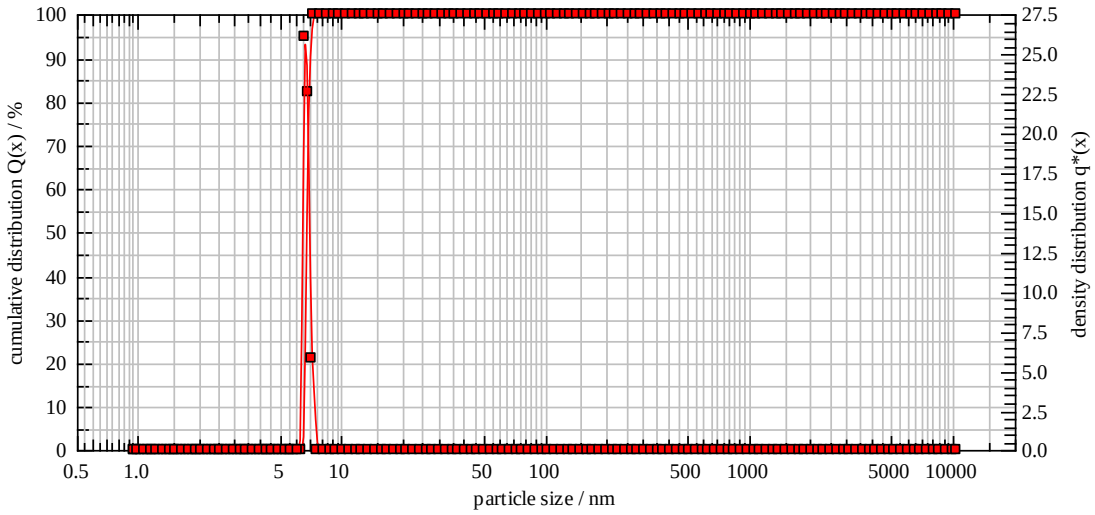

Fig .8. Zeta sizer

Surface morphology of Nanoparticles

Morphology of prepared Nanoparticles was determined by SEM and photomicrographs of topiramate loaded chitosan Nanoparticles are displayed in Fig 9. The drug loaded Nanoparticles prepared by ionotropic gelation method showed regular shape and smooth surface. The resultant Nanoparticles did not show any ruptures on surface.

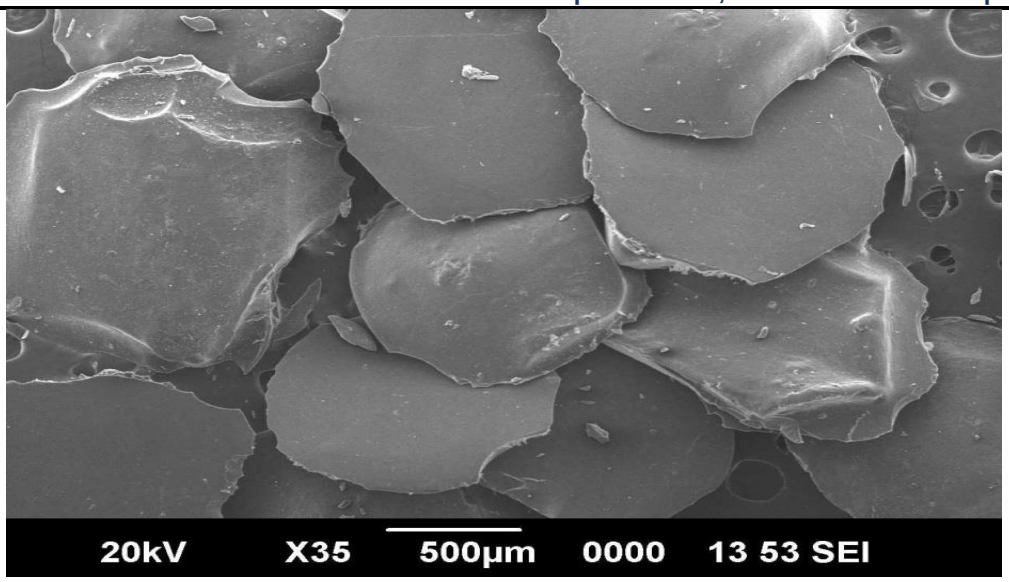

Fig. 9. SEM images of topiramate loaded chitosan Nanoparticles

The resultant topiramate loaded chitosan nanoparticles showed drug content in the range from 94.19 % and high drug entrapment efficiency from 96.46%. As shown in Table 7, the drug entrapment in the chitosan nanoparticles containing topiramate was found to be increased with increased proportion of drug polymer ratio i.e. as the concentration of chitosan increased the entrapment efficiency was found to decrease.

Table no 7.Drug content and Entrapment efficiency of chitosan

Formulation code	Drug content (%)	Entrapment Efficiency (%)
F1	68.35±1.5	76.12±1.2
F2	62.32 ±2.5	85.64±1.5
F3	61.02 ±1.0	82.68±1.0
F4	70.69 ±0.9	90.42±2.8
F5	62.31 ±0.8	89.39±1.4
F6	94.19 ±0.1	96.46±0.5
F7	92.19±2.8	80.46±1.5

In-vitro drug diffusion studies

The release profile of batch F5 to F6 was shown in table results indicated that the rate and extent of drug release from nanoparticles were significantly retarded with an increase in chitosan concentration. Maximum drug release was obtained for the F5 batch i.e. 94.19 % respectively after 180 min. Release of topiramate from the nanoparticles also changes with the crosslinking of polymer. Increase in the amount of crosslinking agent reduces the drug release. So rate of drug release from the nanoparticles prepared by ionotropic gelation method can be modified by altering the concentration of chitosan and crosslinking agent sodium tri poly phosphate. the effect of chitosan concentration on the drug release through diffusion membrane of prepared formulations.. The release of F5 formulations was relatively high compared to other batches

Table no. 8. Drug diffusion of topiramate chitosan Nanoparticles

Sr. no.	Time (Min)	Percentage release of drug (%)	
		F5	F6
1.	15	21.8±1.4	16.99±1.7
2.	30	25.2±1.7	22.26±1.5
3.	45	30.7±2.5	28.17±3.2
4.	60	35.7±3.1	32.80±2.1
5.	75	45.1±2.5	40.98±2.0
6.	90	55.5±3.4	45.28±1.4
7.	105	60.4±2.5	62.52±3.2
8.	120	72.2±3.4	65.52±1.2
9.	135	85.1±2.4	69.55±3.1
10.	150	89.8±1.8	72.33±3.1
11.	165	90.8±2.5	75.24±2.8
12.	180	94.19±3.1	75.99±3.2

Table no 9. Cumulative drug release of Topiramate& chitosan

Sr. No	Time	Cumulative drug release (%) of F5
1	15	10.56
2	30	12.57
3	45	20.30
4	60	26.75
5	75	33.45
6	90	37.75
7	105	42.68
8	120	55.23
9	135	60.50
10	150	65.07
11	165	78.09
12	180	92.19

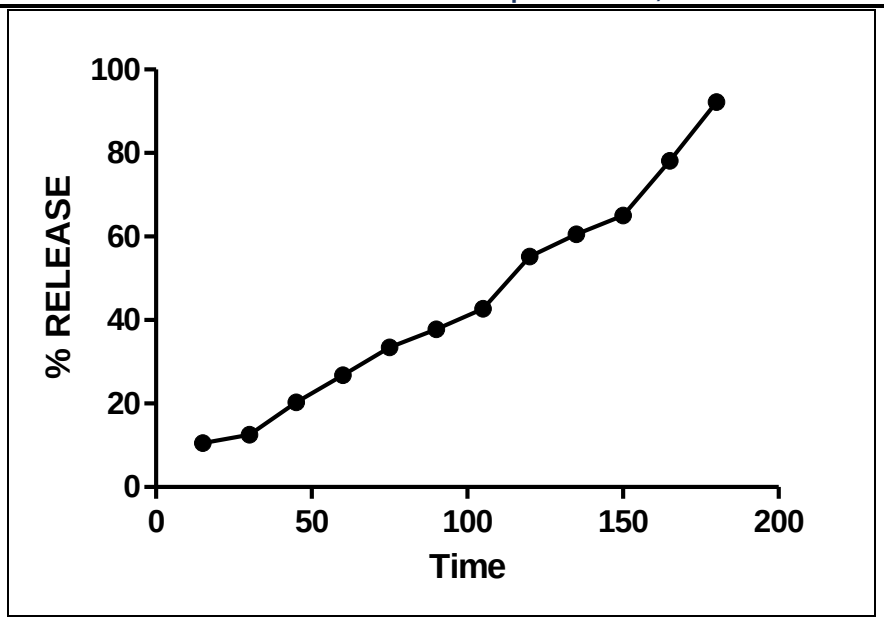

Fig. 10. Cumulative drug release % of F5 batch

DISCUSSION

Results of in vitro drug diffusion studies indicated that the rate and extent of drug release from nanoparticles were significantly retarded with an increase in chitosan concentration. Maximum drug release was obtained for the F5 batch i.e. 94.19 respectively after 180 min. Nanoparticles prepared using chitosan as mucoadhesive polymer showed increase in drug release as compared to nanoparticles prepared by using chitosan. The permeation (drug release) of topiramate from nanoparticles of batch F5 was found to be 92.19 % respectively after 180 min. Topiramate nanoparticles prepared using chitosan showed higher drug permeation. Thus the formulation of topiramate loaded chitosan mucoadhesive nanoparticles could be promising over conventional dosage for the treatment of epilepsy.

CONCLUSION

Topiramate nanoparticles prepared using chitosan showed higher drug permeation. Thus the formulation of topiramate loaded chitosan mucoadhesive nanoparticles could be promising over conventional dosage for the treatment of epilepsy. From the results of the present investigation it may be concluded that drug loaded chitosan nanoparticles can be prepared by a simple technique which avoids the use of complex apparatus and special precautions.

REFERENCES

1. Garg.SA. Visht. P. K. Sharma and N. Kumar "A review formulation charectrization and application on nanoparticals".Der pharmasinica 2011,17-26.

2. Andrew G.P., Laverty T.P., Jones D.S., "Mucoadhesive polymeric for controlled drug delivery. S. J. Pharm."international journal of research in pharmacy and chemistry. 71 (3),(2009) 505-518.

3. BasuS.K,Kavitha K., kumar M R., "Preparation and Evaluation of Trimetazidine Hydrochloride Nanoparticles using Chitosan".International Journal of ChemTech Research. 2 (2),(2010) 1190-1196

4. Cafaggi S., Russo E., Stefani R., Leardi R., Caviglioli G., Parodi B., Bignardi G., Totero D., Aiello C., Viale M.,. "Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin–alginate complex".Journal of Pharmaceutical and Clinical Research. (121). (2007)110 –123.

5. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res. 1997; 14: 1431-1436.;1: 85-90

6. Chen Y, Mohanraj VJ, Parkin JE. "Chitosan-dextran sulfate nanoparticles for delivery of an antiangiogenesis peptide". Letters in Peptide Science 2003; 10: 621-627.

7. Gavini, A.B., Hegge, G. Rassu, V., Sanna, C., "Testa as nasal administration of Carbamazepine using chitosan Microspere" :In-vitro/ In-vivo studies.International Journal of Pharmacy and Biological Sciences. (2011)121-129.

8. Kosta A. K., SolakhiaT. M., Dr. Agrawal S.."Chitosan Nanoparticals- a drug delivery system" .Canadian Journal of Veterinary Research (2012)737-743.

9. KD TRIPATHI, Essentials of Medical Pharmacology Seventh Edition, 411-415 Arshad Ahmed KhanK. *, K. Rajesh Reddy, C. Haranath and C. Suryaprakash Reddy "Preparation and ex vivo evaluation of Nanoparticles for treatment of Urinary tract infections"International Journal of Research and Development in Pharmacy & Life Science; 3164-3172