FORMULATION DEVELOPMENT AND EVALUATION OF BRAIN TARGETED ESLICARBAMAZEPINE ACETATE NANOSUSPENSION FOR EPILEPSY

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ABSTRACT

Epilepsy is a major neurological disorder affecting people of all age groups; around 50 million people worldwide are suffering from it. A combination of nanosuspension along with a nose-to-brain approach can help in promoting the treatment efficacy by site specific delivery. Eslicarbamazepine acetate (ESL) is a novel drug with dual mechanism of action i.e voltage-gated sodium channel and t-type calcium channel blocker with anticonvulsant activity. It is a prodrug, which is the drug entity responsible for the pharmacological effect with high potency and efficient safety as well as efficacy. Nanosuspensions have emerged as an attractive and promising approach to improve stability and bioavailability of poorly soluble drugs of size below 1μm, which is prepared by media milling method. The current research efforts are being directed for extending their applications in site-specific (Nasal) drug delivery system. Nanosuspensions generally have the ability to deliver the drug to the brain through olfactory and trigeminal nerves pathways circumventing the blood brain barrier. Nasal drug delivery has received a great deal of attention as a convenient, reliable and promising method for the systemic administration of drugs. The prepared formulations were then evaluated for particle size distribution (PDI), Zeta Potential, drug content, % Entrapment Efficiency and Invitro drug release.

Keyword:- *Eslicarbamazepine acetate, nose-to-brain, Nanosuspensions Zeta Potential, drug content, % Entrapment Efficiency and Invitro drug release*

1. INTRODUCTION

1.1 Introduction To Nasal to Brain Drug Delivery System

Nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption because it is permeable to more com-pounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus and less dilution by gastrointestinal contents. In recent years many drugs have been shown to achieve better systemic bioavailability through nasal route than by oral administration. In general, the nasal cavity is highly suitable for drug delivery as the nasal mucosa presents an efficient absorption and a very good permeability of both: smallmolecule drugs and biopharmaceuticals. This strategy is useful to treat variety of CNS disorders including, Epilepsy, Brain tumors, Parkinson disorder, Multiple

Sclerosis, Alzheimer disorder and Psychiatric disorders Furthermore, the roof of the nasal cavity is located in very close vicinity to the brain (skull base) and harbours nerves that project to the brain. Therefore, a promising strategy to bypass the blood-brain barrier and blood cerebrospinal-fluid barrier is the delivery of drugs from the nose to the brain (Fig 1.1). Nasal to Brain delivery is minimally invasive with a decent patient compliance and the potential for self-medication. The Nasal to Brain drug route is discussed to deliver substances to the brain via the olfactory and trigeminal nerve. Nasal to Brain is not limited to small molecule drugs, as peptides or proteins, even stem cells, viruses and nucleotides have already been proven to pass from the nose to the brain. [1]

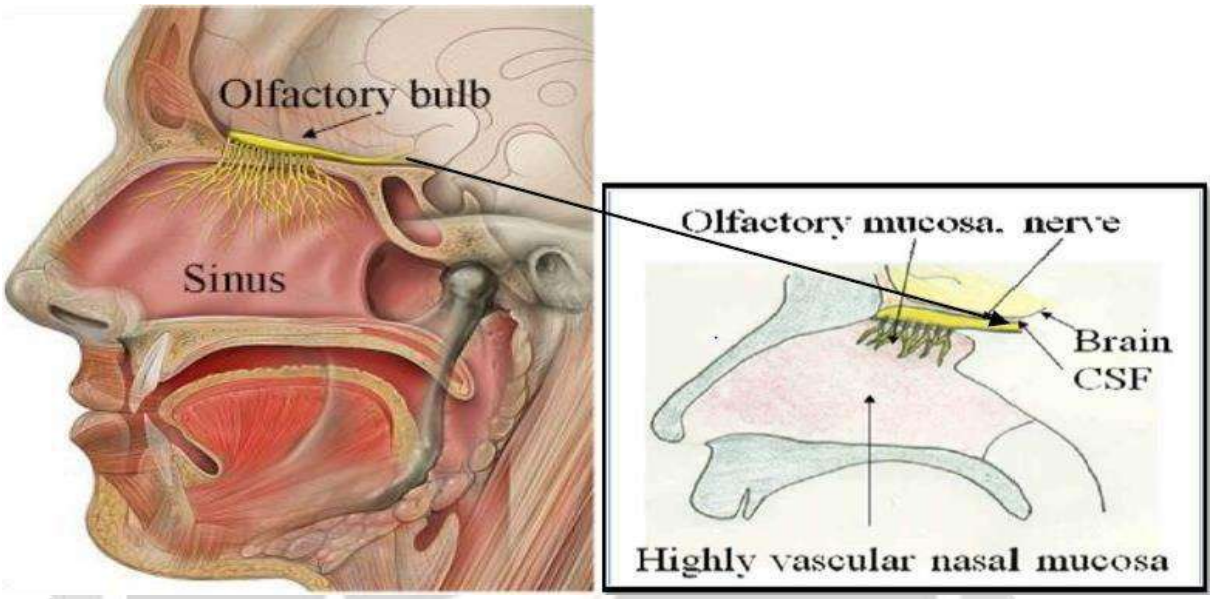

Fig-1: Anatomy and Physiology of Nasal Cavity

1.2 Advantages of intranasal drug delivery

- Rapid drug absorption via highly vascularized mucosa
- Ease of administration, non-invasive
- Improved bioavailability
- Improved convenience and compliance
- Self-administration
- Large nasal mucosal surface area for dose absorption
- Avoidance of the gastrointestinal tract and first-pass metabolism
- Rapid onset of action[2]
- Lower side effects
- Drugs which cannot be absorbed orally may be delivered to the Systemic circulation through nasal drug delivery system.
- Convenient route when compared with parenteral route for long term therapy.
- Bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach.

1.3 Disadvantages of intranasal drug delivery

- Some drugs may cause irritation to the nasal mucosa
- Nasal congestion due to cold or allergies may interfere with absorption of drug.
- Drug delivery is expected to decrease with increasing molecular weight.
- Frequent use of this route leads to mucosal damage.[3]
- The amount of drug reaches to different regions of the brain and spinal cord, varies with each agent.

1.4 Factors Influencing Nasal Drug Absorption

Several factors affect the systemic bioavailability of drugs which are administered through the nasal route.[4] The factors influencing nasal drug absorption are described as follows.

1) Physiochemical properties of drug

- Molecular size
- Lipophilic-hydrophilic balance
- Enzymatic degradation in nasal cavity

2) Nasal Effect

- Membrane permeability
- Environmental pH
- Mucociliary clearance
- Cold, rhinitis

3) Delivery Effect

- Formulation (Concentration, pH, osmolarity)
- Delivery effects
- Drugs distribution and deposition
- Viscosity

Table-1: Nasal DDS Comparison between Oral, Parenteral and Transdermal DDS

Parameters	Nasal	Oral	Parenteral	Transdermal
Higher plasma drug levels	Yes	No	Yes	Yes
BBB and CSF bypass	Yes	No	No	No
Rapid onset	Yes	No	Yes	Yes
Pain at the site of administration	No	No	Yes	No
Mucosal irritation	No	Yes	No	Yes
Systematic activity	Yes	No	Yes	Yes
Self-administration	Yes	Yes	No	Yes
Patient compliance	High	High	Low	Low
Drug degradation	No	High	No	Low
Hepatic first pass metabolism	No	Yes	No	No
Targeted delivery	Yes	No	Yes	Yes

1.6 Nanosuspensions

- Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1 μm in size.[5]
- Nanosuspensions can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result, the rate of flooding of the active compound increases and the maximum plasma level is reached faster. This is one of the unique advantages that it has over other approaches for enhancing solubility.
- The particle size distribution of the solid particles in nanosuspensions are usually less than one micron with an average particle size ranging between 200 and 600 nm.
- Nano size particles can increase dissolution velocity and saturation solubility because of the vapour pressure effect.

1.7 Advantages of nanosuspensions

The major advantages of nanosuspension technology are:

- Its general applicability to most drugs and its simplicity.
- Can be applied for the poorly water soluble drugs.
- Can be given by any route.
- Reduced tissue irritation in case of subcutaneous/intramuscular administration.

- Rapid dissolution and tissue targeting can be achieved by IV route of administration.
- Oral administration of Nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability.
- The absorption from absorption window of the drugs can be increased, due to reduction in the particle size.
- Higher bioavailability and more consistent dosing in case of ocular administration and inhalation delivery.
- Drugs with high log P value can be formulated as Nanosuspensions to increase the bioavailability of such drugs.
- Improvement in biological performance due to high dissolution rate and saturation solubility of the drug.
- Ease of manufacture and little batch-to-batch variation.
- Long term physical stability (Due to absence of Ostwald ripening).
- Nanosuspensions can be incorporated in tablets, pellets, hydrogel and suppositories are suitable for various routes of administration.[6]

1.8 Properties of Nanosuspension

1. Physical long-term stability
2. Internal structure of Nanosuspensions
3. Adhesiveness
4. Crystalline state and morphology
5. Increase in Saturation Solubility and Dissolution Velocity of drug.

2.0 METHODOLOGY

Materials: Eslicarbamazepine acetate was gifted from CTX Life sciences Pvt. Ltd, Udhana, Surat. Poloxamer 407 was obtained from Zeel Pharmaceuticals Ltd Mumbai, Poloxamer 188 was obtained from Balaji Drugs Surat, Glycerol was obtained from Vishal Chemicals Mumbai, Zirconium dioxide beads was obtained from Jyoti Ceramics Nasik.All the chemicals and excipients used were of analytical grade.

2.1 Method of Preparation

Formulation of *Nanosuspension*: Accurately weighed amount of Eslicarbamazepine Acetate API was dispersed in an aqueous solution (Distilled water 10ml) containing Glycerol and different ratio of Poloxamer in 30ml vial. The resulting coarse pre-dispersion was continued using Zirconium oxide beads i.e milling media on a magnetic stirrer at about 1000rpm for 24hrs. The prepared Nanosuspension was separated from Zirconium oxide beads by decanting the suspension followed by washing the beads with Distilled water. Various formulations were prepared by varying the sizes of Zirconium oxide beads, concentrations of Poloxamers, types of Poloxamers (407 and 188), volumes of Zirconium oxide beads.[7]

Table-2: Formulation of Brain Targeted Eslicarbamazepine Acetate Nanosuspension

Batches	Drug (%w/v)	Poloxamer 407 (%w/v)	Poloxamer 188 (%w/v)	Glycerol (%w/v)	Milling media (%v/v)	Water (% q.s)	Ratio of beads of different sizes	
							0.4-0.7	1.2 – 1.7
F_1	0.07	0.1	--	2	25	11.25	25	75
F_2	0.07	0.1	0.2	2	50	11.25	50	50
F_3	0.07	0.1	--	2	25	11.25	75	25
F_4	0.07	0.2	--	2	25	11.25	25	75
F_5	0.07	0.2	0.1	2	50	11.25	50	50
F_6	0.07	0.2	--	2	25	11.25	75	25
F_7	0.07	0.1	--	2	50	11.25	25	75
F_8	0.07	0.1	0.2	2	25	11.25	50	50
F_9	0.07	0.1	--	2	50	11.25	75	25

2.2 Determination of λmax

2.2.1 Preparation of stock solution

In order to confirm λmax of 100 mg Eslicarbamazepine Acetate of was dissolved in about 100 ml phosphate buffer pH-6.4 (1000 µg/ml). The solution was sonicated for 1-2 hours and then final volume upto 100ml was adjusted with phosphate buffer 6.4.[8] 10 ml of this solution was further diluted with phosphate buffer pH-6.4 upto 100 ml to obtain (100 µg/ml). A standard stock solution having concentration of 10 µg/ml of Eslicarbamazepine Acetate was further prepared by withdrawing 1ml from 100 µg/ml.

2.2.2 Preparation of working sample solutions

From stock solution (10 µg/ml), accurately measured standard working sample solutions of Eslicarbamazepine Acetate (1, 2, 3, 4 and 5 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with phosphate buffer pH-6.4 to obtain the concentration of 5,10,15, 20 and 25µg/ml. The absorbance of prepared solutions of Eslicarbamazepine Acetate in phosphate buffer pH-6.4 was measured at 225 nm using UV-visible spectrophotometer against phosphate buffer 6.4 as blank. The experiment was performed in triplicate and based on average absorbance, the equation for best line was generated.

2.3 Evaluation of Nanosuspension

2.3.1 Particle Size & Size Distribution (PDI)

The mean particle diameter and size distribution of the prepared nanosuspension was measured using Malvern particle size analyzer.

2.3.1.1 Polydispersity Index

Polydispersity Index is an index of width or spread or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas higher value of PDI indicates a wider particle size distribution and the

polydisperse nature of the sample. PDI is calculated by the using following formula:

PDI = Δd/davg

where, Δd is the width of distribution and d avg is the average particle size. The usual range of PDI values are as follows,

Table-3: Standard PDI Values for Nanosusspension

Polydispersity Index	Type of dispersion
0 – 0.05	Monodisperse standard
0.05 – 0.08	Nearly monodisperse
0.08 – 0.7	Midrange polydispersity
> 0.7	Very polydisperse

2.3.2 Zeta potential determination :-

Zeta potential of the suspension is measured by Malvern Zetasizer using the Helmholtz-Smoluchowski equation. The Zetasizer mainly consists of laser which is used to provide a light source to illuminate the particles within the sample. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the centre of the sample cell and the scattered light at an angle of about 130 is detected. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility occurs. Hence the zeta potential is calculated and stability is checked by comparing with the Table 4.

Table-4: Zeta potential for Nanosuspensions with their stability

Zeta potential (mv)	Stability behaviour
From 0 to ± 5	Rapid coagulation or flocculation
From ± 10 to ± 30	Incipient stability
From ± 30 to ± 40	Moderate stability
From ± 40 to ± 50	Good stability
More than ± 61	Excellent stability

Zeta potential is an important parameter in the assessment of the physical stability of colloidal dispersions. The zeta potential only exists when the surface of a particle is in contact with a liquid. A good physical long-term stability of the dispersion is normally predicted by a zeta potential higher than +30 mV or lower than -30 mV. Zeta potential was measured by Zeta sizer.

2.3.3 Drug content:-

An aliquot of 3 ml of nanosuspension was transferred to a china dish. The suspension was subjected to drying. The remaining solid was taken. It was dissolved in 10 ml of methanol to dissolve drug. It was stirred on magnetic stirrer for 1 hr. The solution was than filtered and absorbance was measured by UV Spectrophotometer. Percentage drug content was calculated using following formula:-

% DC = Amount of drug obtained x 100 /(Initial amount of drug)

2.3.4 Entrapment Efficiency (%):-

An aliquot of 1ml of nanosuspension was transferred to 10ml volumetric flask.Methanol was used to make up the volume and then the flask was subjected to stirring at 800rpm for 24hrs. Then 1ml aliquot was taken from this flask and then made upto 10ml with methanol. Absorbance at 256nm was taken on UV Spectrophotmeter of the dilution. % Entrapment [8]

Efficiency was then calculated using following formula:-

% EE = (Total amount of Drug– Free dissolved drug) / (Total amount of Drug) x 100

2.3.5 In-Vitro Drug Release Study of Nanosuspension:

In vitro release of Eslicarbamazepine acetate Nanosuspensions was assess by the dialysis bag diffusion technique.

The drug release studies of Nanosuspension were performed in PBS (pH6.4).[11]The Nanosuspension was introduced in a cellulose dialysis bag and then tied at both ends. The dialysis bag was immersed in the receptor compartment containing 50 ml of in PBS (pH 6.4) and this dialysis bag immersed in to the dissolution apparatus USP-II Type which was stirred at 100 rpm and maintained at 37 ± 0.5°C. The receptor chamber was covered for the prevention the evaporation of dissolution medium. Samples were withdrawn (5ml) at regular time intervals (2hours),and the same volume was replaced by freshly prepared dissolution medium. The samples were analyzed using a UV-visible spectrophotometer set at 210nm and 240 nm respectively.

3. RESULTS:

3.1 Calibration curve of Eslicarbamazepine Acetate:

Eslicarbamazepine Acetate shows maximum absorbance at 225 nm in phosphate buffer pH-6.4 respectively and shows linearity in range of 5-25 µg/ml for phosphate buffer pH-6.4.

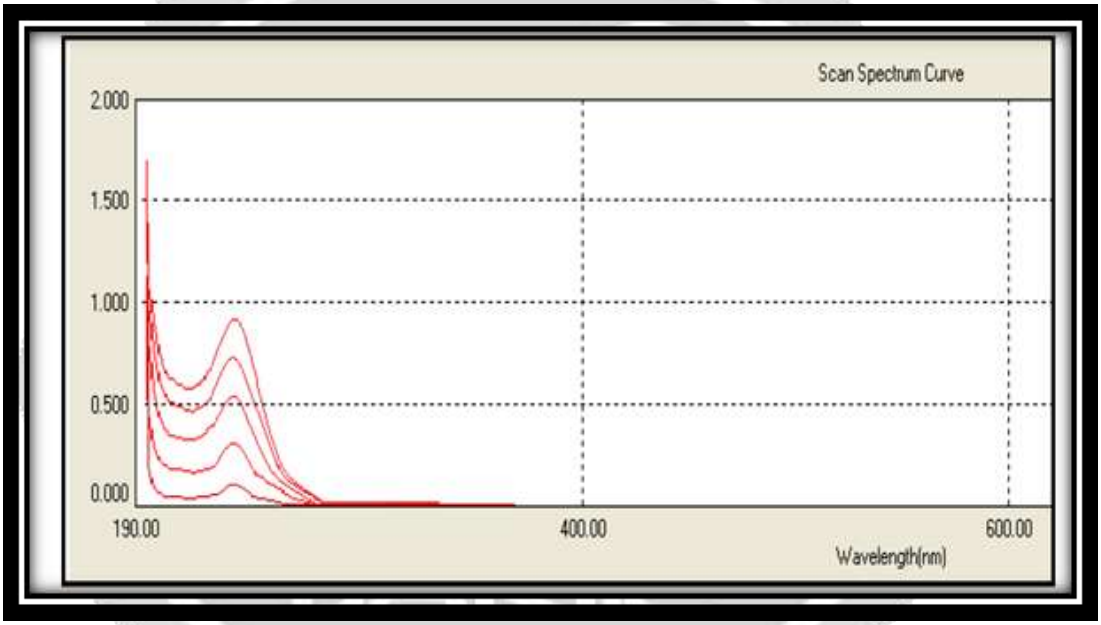

Fig-2: UV Visible spectra of Eslicarbamazepine Acetate at 225 nm

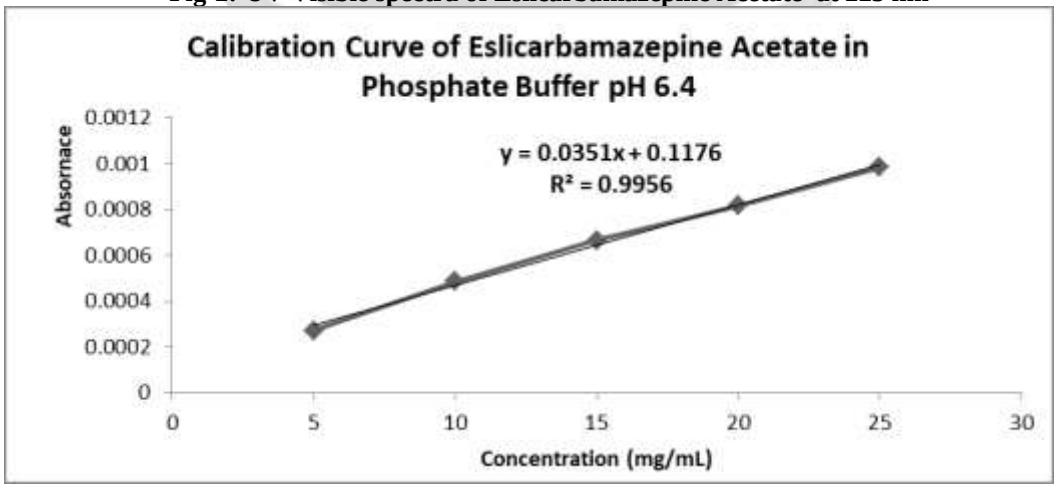

Fig-3: Calibration Curve of Eslicarbamazepine Acetate in Phosphate buffer

pH 6.4 at 225 nm

Table-5: Calibration Curve of Eslicarbamazepine Acetate in Phosphate buffer (pH 6.4)

Sr. No.	Concentration (mg/ml)	Absorbance (cm^{-1})
1	5	0.271
2	10	0.486
3	15	0.665
4	20	0.816
5	25	0.984

3.2 FT-IR studies:

IR spectra of drug were shown as the peaks obtained in the spectra of drug correlates with functional groups of Eslicarbamazepine Acetate which confirms purity of drug.[12] All the characteristic peaks respective to their functional groups of drug are shown and comparison of graph done which reveal no interaction with polymer and drug mixture. Figure 4, 5, 6,7 and 8 are shown below.

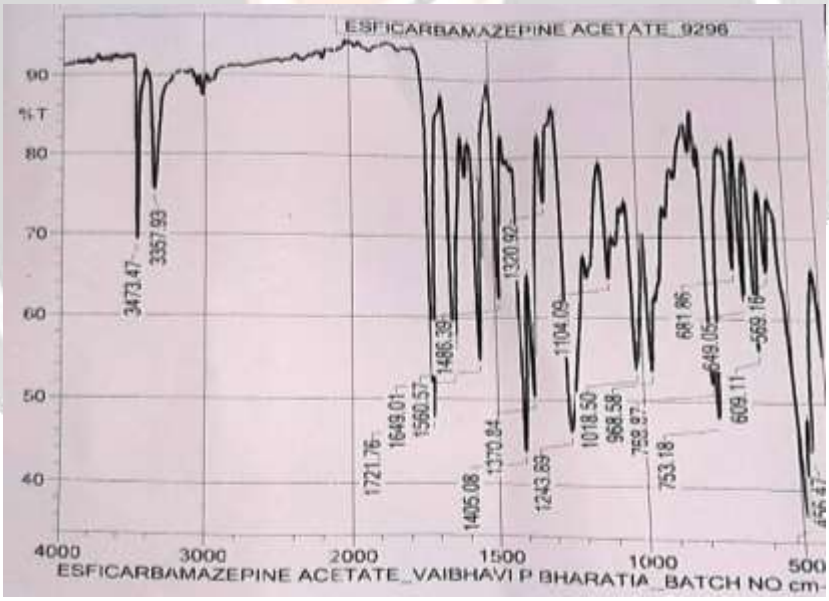

Fig-4: FTIR of Eslicarbamazepine acetate pure drug

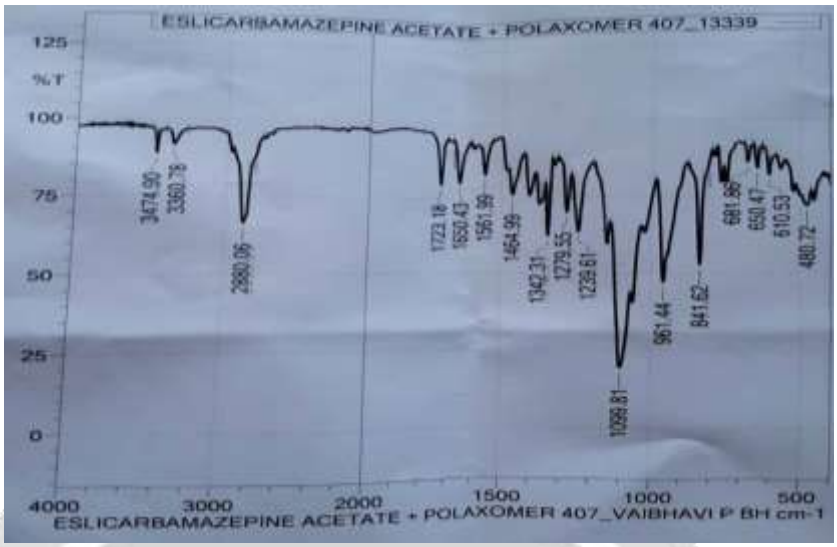

Fig-5: FT-IR spectrum of Eslicarbamazepine Acetate +Poloxamer 407

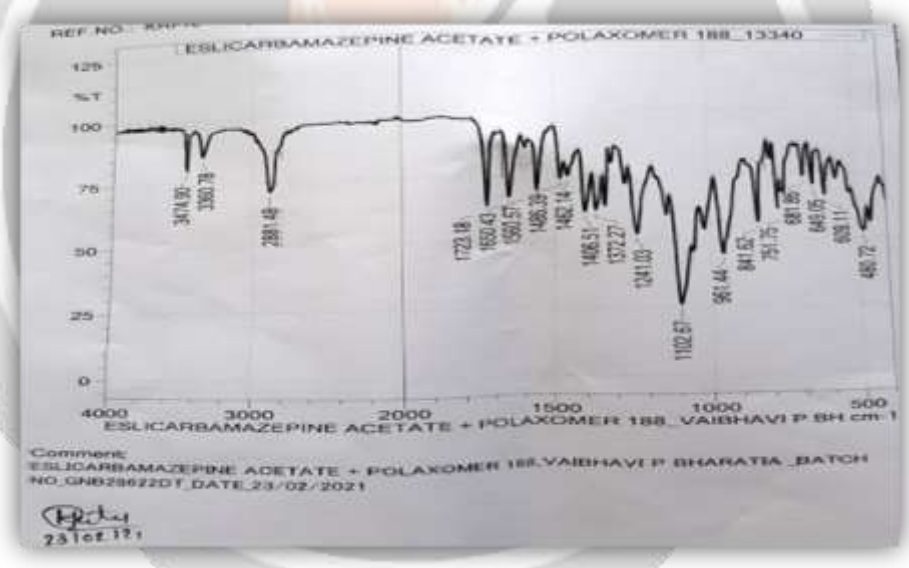

Fig-6: FT-IR Spectrum of Eslicarbamazepine Acetate +Poloxamer 188

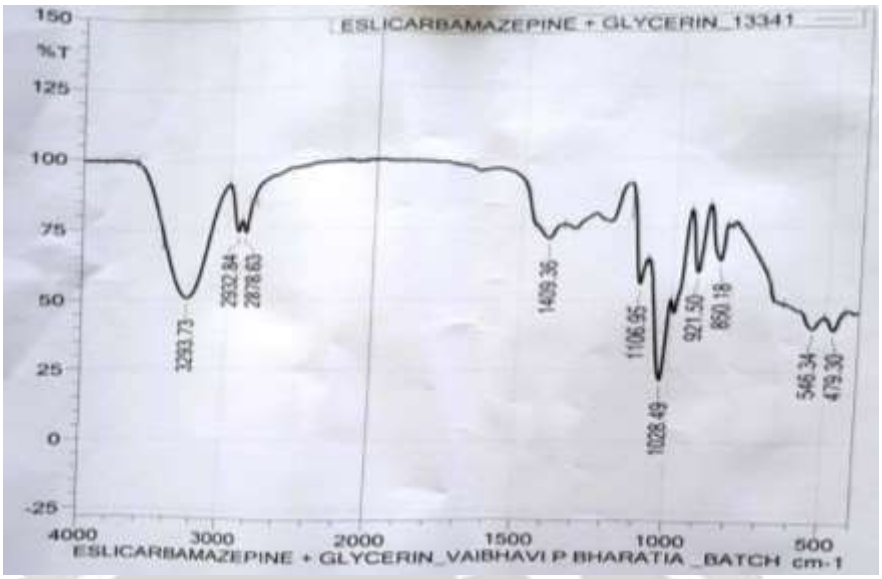

Fig-7: FT-IR spectrum of Eslicarbamazepine acetate

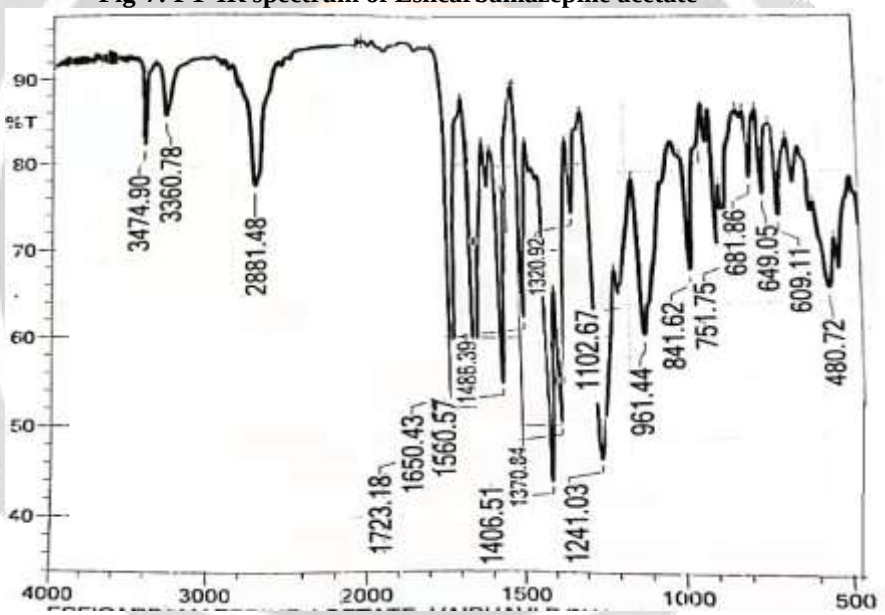

Fig-8: FT-IR Spectra of Eslicarbamazepine Acetate with all excipients

3.3 Evaluation of Nanosuspension

3.3.1 Particle size and Size distribution:-

Particle size analysis was performed for nanosuspension formulationsF1-F9 as shown in Table 6.48. From the above graph it can be concluded that result of F8 Batch Particle Size is optimum i.e 437.1nm.

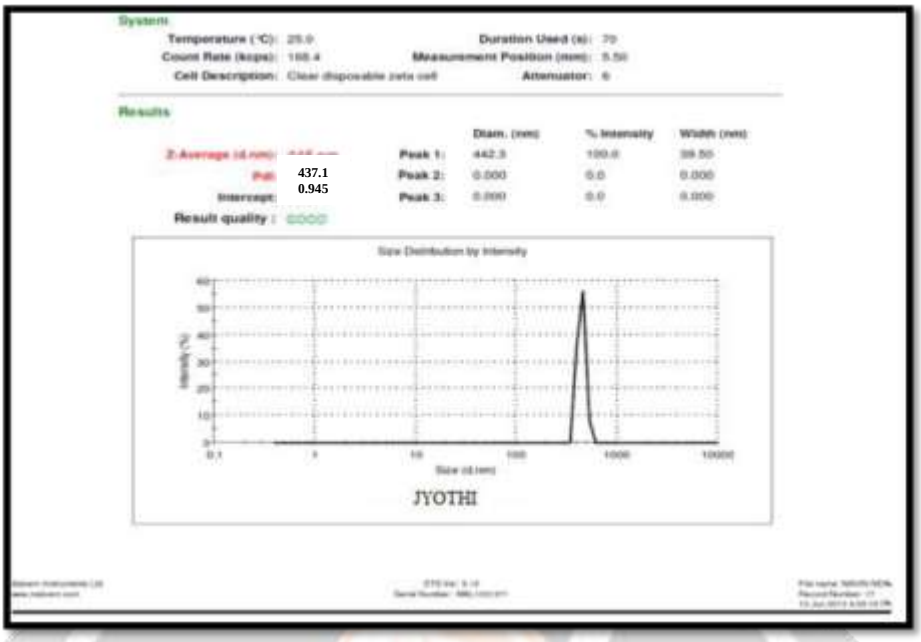

Fig -9 :-Particle size distribution curve F8

437.1
0.945

437.1
0.945

3.3.1.1 PDI (Polydispersity Index)

Polydispersity index (PDI) is a measure of particle size homogeneity and it varies from 0.0 to 1.0. Polydispersity is the ratio of standard deviation to mean particle size; hence, it indicates the uniformity of particle size within the formulation. The higher the polydispersity, the lower the uniformity of the particle size in the formulation. The polydispersity index was found to be 0.954 for formulations F8.[13]

3.3.2 Zeta potential

Physical instability leads to a size increase either by crystal growth due to Ostwald ripening or alternatively due to aggregation caused by insufficient stabilization. The nanosuspensions were stabilized by different, non-ionic surfactants. The Zeta potential was found to be -14.8 for formulations F8.

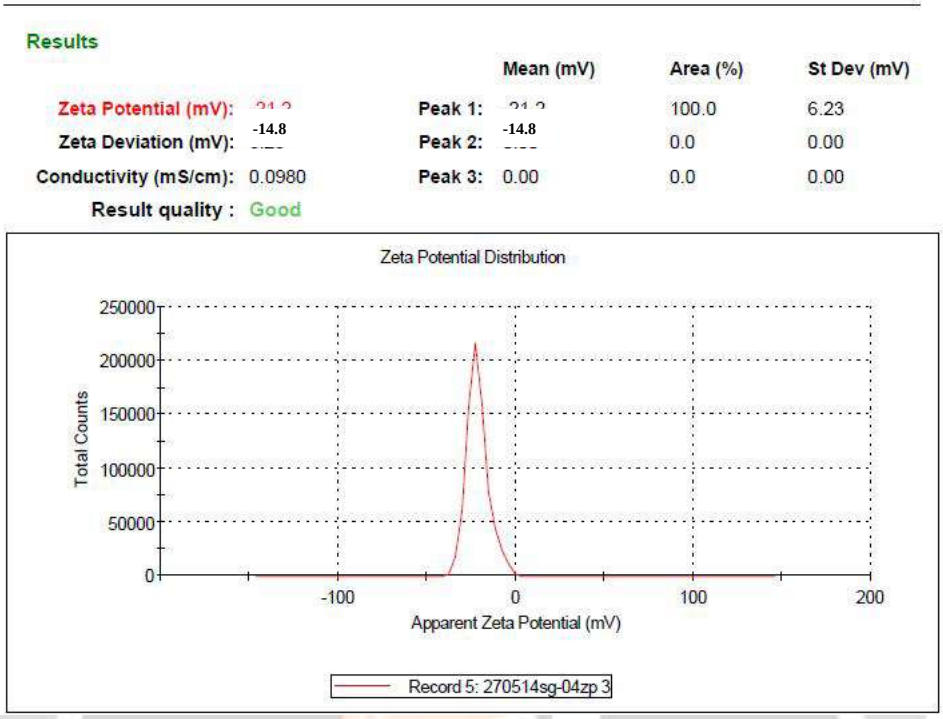

Fig -10:-Zeta potential of F8 Formulation

3.3.3 Drug content

Drug content of nanosuspension was performed by taking dose equivalent nanosuspension and taking absorbance on UV-Vis Spectrometer.[14] The dilution factor was noted and amount was calculated. The drug content of nanosuspension was foumd to be 98.86%.

3.3.4 % Entrapment Efficiency

The entrapment efficiency was calculated by following formula:-

$$\% \text{ EE} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} X\ 100$$

The % EE of nanosuspension was found to be 98.45%.The entrapment efficiency of drug with poloxamer was found in range of 84.66-98.45 %.The high value indicates a high drug poloxamer affinity. Thus it shows that a high drug has been entrapped in poloxamer.[15]

3.3.5 In vitro Drug Release Study of Nanosuspension

Below table shows Invitro drug release of nanosuspension with its graphical representation

Table 5:-Table showing Invitro Drug release of Nanosuspension

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F10	F15
0	1.48± 0.17	0.20± 0.09	0.11± 0.03	0.27± 0.05	0.36± 0.079	2.34± 0.48	0.57± 0.08	**0.15± 1.99**	0.27± 0.04	2.29±0.71
2	51.23± 2.62	28.64 ±1.57	25.59± 4.45	47.44±1.67	52.62± 3.62	48.90± 1.80	28.99± 1.06	**25.18±1.99**	34.4 ±1.92	57.68±1.43
4	78.05± 2.23	54.27 ±1.37	45.55± 2.73	76.34±1.89	82.59± 2.65	79.3± 1.07	43.54± 2.22	**47.81±1.23**	60.65±0.77	79.21±1.34
6	97.23± 0.73	80.02 ±2.14	67.25± 1.89	90.15±0.94	96.55± 0.81	97.5± 1.68	64.82± 1.50	**82.59±2.69**	80.42±1.72	67.25±1.85
8		96.63 ±0.89	88.6± 1.40				89.74± 0.67	**98.72±0.81**	92.33±1.01	88.6±1.4

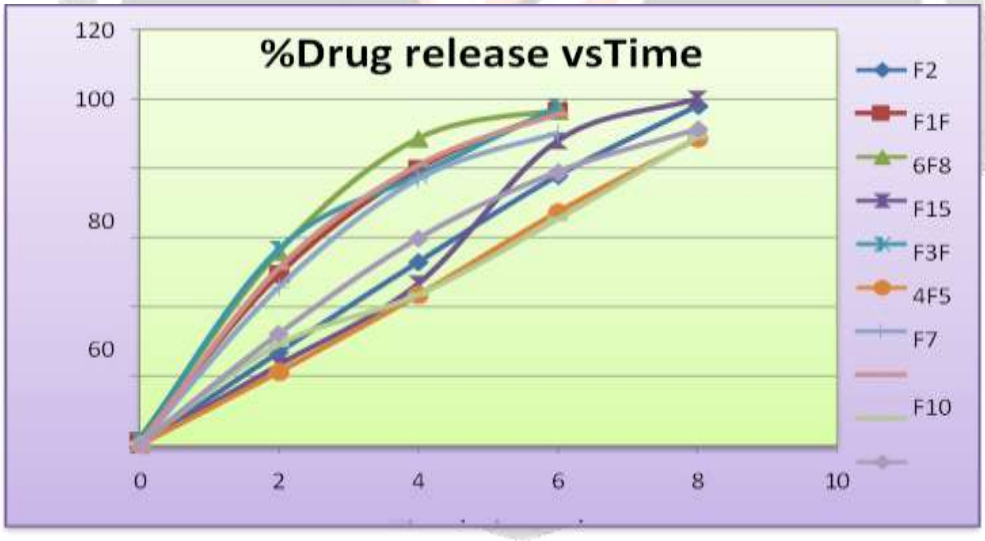

Fig-11:- Graph representing% drug release vs time of nanosuspension

4.0 CONCLUSION

Brain targeted Nanosuspension of Eslicarbamazepine Acetate was successfully formulated by using media milling method and was developed to a satisfactory level, in terms of Particle Size Distribution (PDI),Zeta Potential, Drug Content, %Entrapment Efficiency, Invitro Drug Release. All formulations are translucent in appearance. Formulation F8 with 1:0.2 ratio of Poloxamer 407 and Poloxamer 188 respectively and 25:75 Zirconium dioxide beads shows translucent nanosuspension and retained for extended period. Formulation F8 also shows the highest % drug content of 98.86% along with the drug release of 98.45%.

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