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Formulation and Evaluation of Brivaracetam Loaded Nanospheres

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A B S T R A C T

The present study focused on the development and evaluation of Brivaracetam-loaded nanospheres using poly (lactic-co-glycolic acid) (PLGA) as the polymer via the solvent evaporation method, aiming to enhance drug solubility, control release, and improve therapeutic efficacy. Preformulation studies confirmed that Brivaracetam is a white to off-white, bitter-tasting, odourless drug with high solubility in water and various organic solvents. The melting point (131.97°C) and FTIR, along with DSC analyses, confirmed the drug's purity and compatibility with PLGA, showing no significant interactions between drug and polymer.

Nine nanosphere formulations (F1–F9) were developed and evaluated. Particle size analysis revealed that formulation F5 had the smallest particle size (149.2 nm), contributing to its improved surface area and stability. Zeta potential analysis showed F5 possessed the most negative value (–27.8 mV), indicating excellent colloidal stability. SEM imaging of F5 confirmed uniform spherical morphology with minimal aggregation. Drug entrapment efficiency and drug loading were highest in F5, at 79.4% and 12.3%, respectively, signifying effective drug encapsulation and optimal polymer interaction.

In vitro dissolution studies demonstrated sustained drug release across all formulations over 12 hours, with F5 showing the highest cumulative release of 85.3% for nanospheres and 95.6% for capsule formulations, confirming its superior release profile. Capsule evaluation parameters including weight variation, disintegration time, and drug content uniformity remained within pharmacopeial limits, with F5 again displaying the most favourable results (disintegration time: 18 min; drug content: 98.7%).

Overall, the data validate the success of the solvent evaporation method for formulating Brivaracetam nanospheres using PLGA, and among all the developed formulations, F5 emerged as the most optimized formulation.

Keywords: Brivaracetam, PLGA, Nanospheres, Encapsulation

1. INTRODUCTION

The Nano Drug Delivery System (NDDS) is a dynamic area of scientific research, driven by the rapid advancements in nanotechnology. This field focuses on the innovative development of drug delivery methods that leverage nanoscale materials to enhance therapeutic efficacy and precision.[1] It involves the investigation of individual molecules, atoms, or compounds to create structures that exhibit unique properties.[2] Nanotechnology with a wide range of nanocarriers such as liposomes and nanoparticles focused on targeted drug delivery has been expanding quickly. [3] Nanoparticles (NPs) are one of the nano system delivery methods which have been developed to accomplish extended or organized drug delivery, to increase the bioavailability, drug stability, and drug targeting to the site of action. Nanospheres are small particles with a size range of 10 to 200 nm.[4]

Epilepsy is considered one of the most prevalent neurological illnesses, affects more than 50 million people worldwide, and each year approximately 5 million new cases are diagnosed. Epilepsy is characterized by aberrant brain activity resulting in convulsions. Depending on basic brain dysfunctions, this neurological disorder comprises numerous etiologies including abrupt and excessive neuronal discharges that result in epileptogenesis. Although it affects people of all ages, the rate of disease is high in children and elderly persons.[5]

Nano Drug Delivery Systems (NDDS) can prolong the presence of drugs in the bloodstream, resulting in reduced fluctuations in plasma levels and consequently minimizing side effects. These nanospheres enable targeted drug delivery, which is crucial for effectively managing epilepsy.[6][7]

1.1 Advantages of Nanospheres

- Efficient Penetration: Nanospheres can readily traverse even the smallest capillary vessels, ensuring effective distribution throughout the body.
- Targeted Organ Delivery: They can be utilized to specifically target organs such as the liver, spleen, lungs, and spinal cord, enhancing therapeutic precision.[8]

- Decreased Toxicity: Nanospheres help lower toxicity levels and reduce the frequency of required dosages.
- Versatile Administration Methods: They can be administered through multiple routes, including oral, nasal, and parenteral options.
- Quick Clearance and Targeted Delivery: Nanospheres enable rapid clearance from the bloodstream while allowing for precise targeting at specific sites within the body.[9]

1.2 Disadvantages of Nanospheres

- Handling Challenges: Nanospheres can be difficult to manage in both liquid and dry forms.
- Manufacturing Expertise Required: The production of nanospheres demands specialized skills and techniques.
- Susceptibility to Aggregation: Due to their small size and larger surface area, nanospheres are prone to particle aggregation.[10]

1.3 EPILEPSY

Epilepsy is a neurological disorder which is characterized by repeated seizures and known to affects people of all age group. Seizures are electrical impulses discharged by nerve cells present in the brain. Possible reasons of seizures are head injuries, abnormal brain development, genetic and infectious illness (meningitis). The drugs used for the treatment of epileptic seizures are known as antiepileptic drugs which are known to decrease the frequency or severity of seizures.

Most of the antiepileptic drug's act by any of these three mechanisms;

- ✓ Modulation of voltage gated ion channels,
- ✓ Enhancement of gamma amino butyric acid (GABA) mediated inhibitory neurotransmission
- ✓ By glutamate mediated excitatory neurotransmission.

1.4 Capsules

Capsules are unit solid dosage forms that serve as small containers (shells) made primarily of gelatine, enclosing accurately measured amounts of drug substances. The term "capsule" comes from the Latin word *capsula*, meaning "small container." Capsules hold a significant role in drug development and are often considered a primary oral dosage form due to the simplicity of their manufacturing process compared to other dosage forms. Besides gelatine, materials such as denatured gelatine, methylcellulose, and polyvinyl alcohol can be used for capsule shells.

Capsules are mainly of two types: hard-shelled capsules, containing dry powders or pellets produced through processes like extrusion or spheronization, and soft-shelled capsules, containing liquids or semi-solids. Hard capsules consist of two parts a smaller "body" and a larger "cap" which fit together. Both types are made from aqueous solutions of gelling agents (animal proteins like gelatine or plant polysaccharides), with added plasticizers (e.g., glycerine, sorbitol) to adjust shell hardness**.** [11,12]

Advantages of Capsules

- Fewer developmental issues, enabling quicker submission of new drugs for clinical trials.
- Easier to vary the dose compared to other dosage forms.\
- Require fewer excipients than tablets.
- Manufacturing involves fewer steps than tablet production.
- Easy to swallow, enhancing patient compliance.
- Allow simple separation of two incompatible drugs within the same dosage unit.
- Offer greater possibilities for product identification through printing.
- Suitable for incorporating drugs with high doses and low compressibility. [13]

Disadvantages of Capsules

- Capsules are unsuitable for liquids that can dissolve gelatin, such as aqueous or hydroalcoholic solutions.
- Concentrated solutions requiring prior dilution are not suitable for encapsulation, as direct administration may cause gastric irritation.

Types of capsules

I. Hard gelatine capsule.

II. Soft gelatine capsule.

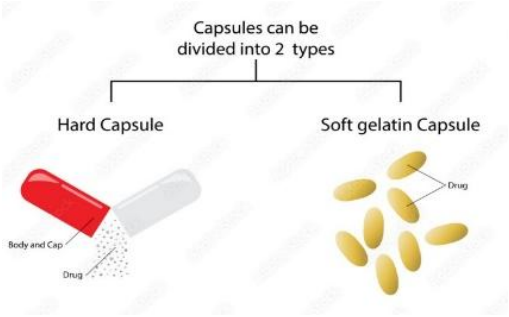


Fig 4: types of capsules

1.5 Filling of hard gelatine capsules

The process of filling hard gelatine capsules is a well-established technology, with equipment options ranging from small-scale manual devices (e.g., Feton capsule filling machine) to medium-scale semi-automatic machines and large-scale fully automatic systems. In compounding pharmacies, capsules may also be filled manually, one at a time.

2. MATERIALS AND METHODS

SL.NO	Ingredients	Manufacturer
1	Brivaracetam	Micro labs, Bangalore.
2	PLGA (poly lactic co glycolic acid)	Venus Lab Products, Bangalore**.**
3	PVA (Poly Vinyl Alcohol)	Karnataka Fine Chem, Bangalore.
4	(DCM)Dichloromethane	Sisco Research Laboratories Pvt. Ltd, New Mumbai**.**

2.1 PRE-FORMULATION STUDIES:

Pre-formulation may be described as the stage of development during which the physicochemical and biopharmaceutical properties of a drug substance are characterized. It is an important part of the drug development process. The information relating to drug development acquired during this phase is used for making critical decisions in subsequent stages of development. A wide variety of information must be generated to develop formulations rationally. Characterization of the drug is a very important step at the pre-formulation phase of product development followed by studying the properties of the excipients on their compatibility.

2.1.1 Organoleptic properties of Brivaracetam:

Small sample of Brivaracetam was taken in Petri dish, spread carefully and determine the colour, odour and texture. and the observations are shown in table 4.

2.1.2 Solubility:

Solubility is expressed in terms of parts per million of solvent in which 1g of solid is soluble. Solubility of the powder in various solvents at 20°C. [14]

2.1.3 Melting point:

The melting point was carried out by using capillary tube method. A small sample of Brivaracetam was placed in a glass capillary tube that had been previously sealed on one end. The drug-filled capillary tube was then placed inside the melting point apparatus, and the temperature at which the drug began to melt was monitored using a thermometer.[15]

2.2 Compatibility Studies

2.2.1 FTIR Spectroscopy

FTIR study was carried out to check the compatibility of drug with polymers. Infrared spectrum of Brivaracetam was determined on Fourier transform Infrared spectrophotometer using KBr dispersion method. The baseline correlation was done using dried potassium bromide. Then the spectrum of dried mixture of drug and Potassium bromide was run followed by drug with various polymers by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum. [16] and the observations are shown in Figure11 and 12.

2.2.2 Differential Scanning Calorimetry studies of Brivaracetam

To investigate the physical and chemical interactions between the medicine and the applied excipients, Differential Scanning Calorimetry (DSC) was used. On the DSC-60 equipment, DSC spectra of pure drugs and drug composite mixtures were captured. The drug-excipient mixture was scanned in a nitrogen-filled environment between 50 and 400°C. All samples were prepared in aluminium pans with aluminium covers. The thermograms were observed for any kind of interaction at a heating rate of 20 °C/min. [17] and the observations are shown in Figure 13 and 14.

2.3 Analytical method used in determination of Brivaracetam

Standard Curve of Brivaracetam

Stock solution was prepared by using 50 mg of Brivaracetam in 100 ml of phosphate buffer (pH 6.8). From this stock solution 10 ml was withdrawn and diluted up to 100 ml using water. Calibration curve was prepared by using different concentration (5µg/ml-30 µg/ml) by appropriate dilution of stock solution. The absorbance was measured at 272 nm. [18] and the observations are shown in Figure 15.

2.4 FORMULATION DEVELOPMENT OF BRIVARACETAM BY SOLVENT EVAPORATION METHOD

Table-3: Formulation trials of nanosphere with Brivaracetam

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Brivaracetam in (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Poly lactic co glycolic acid (PLGA) (g)	5.0	6.0	7.0	5.0	6.0	7.0	5.0	6.0	7.0
Poly Vinyl Alcohol (PVA) (g)	0.1	0.1	0.1	0.2	0.2	0.2	0.25	0.25	0.25
Dichloromethane (DCM) (ml)	5	5	5	5	5	5	5	5	5
Distilled water (ml)	25	25	25	25	25	25	25	25	25
Total (g)	35.2	36.2	37.2	35.3	36.3	37.3	35.35	36.35	37.35

Procedure:

2.4.1 Preparation of Organic Phase:

- Dissolve Brivaracetam (100 mg) and PLGA (600 mg) in 5mL of DCM.
- Mix thoroughly until a clear organic solution is formed.

2.4.2Preparation of Aqueous Phase:

- Prepare 1% PVA solution by dissolving 1 g of PVA in 100 mL distilled water while continuously heating and stirring.

2.4.3 Emulsification:

- Slowly add the organic phase dropwise into the aqueous phase (under magnetic stirring at 1000–1500 rpm).
- Continue stirring for 2–3 hours at room temperature to allow DCM to evaporate.

2.4.4 Collection of Nanospheres:

- Centrifuge the resulting emulsion at 15,000 rpm for 30 minutes.

Discard the supernatant and wash the nanospheres with distilled water 2–3 times.

2.5 Capsule Filling Procedure

The optimized nano spheric formulation was dried completely and weighed accurately. The amount equivalent to the required dose of Brivaracetam was filled into size '0' hard gelatine capsules manually using a capsule filling machine. Care was taken to ensure uniformity in capsule weight and content. Each capsule was stored in a desiccator until evaluation.[19]

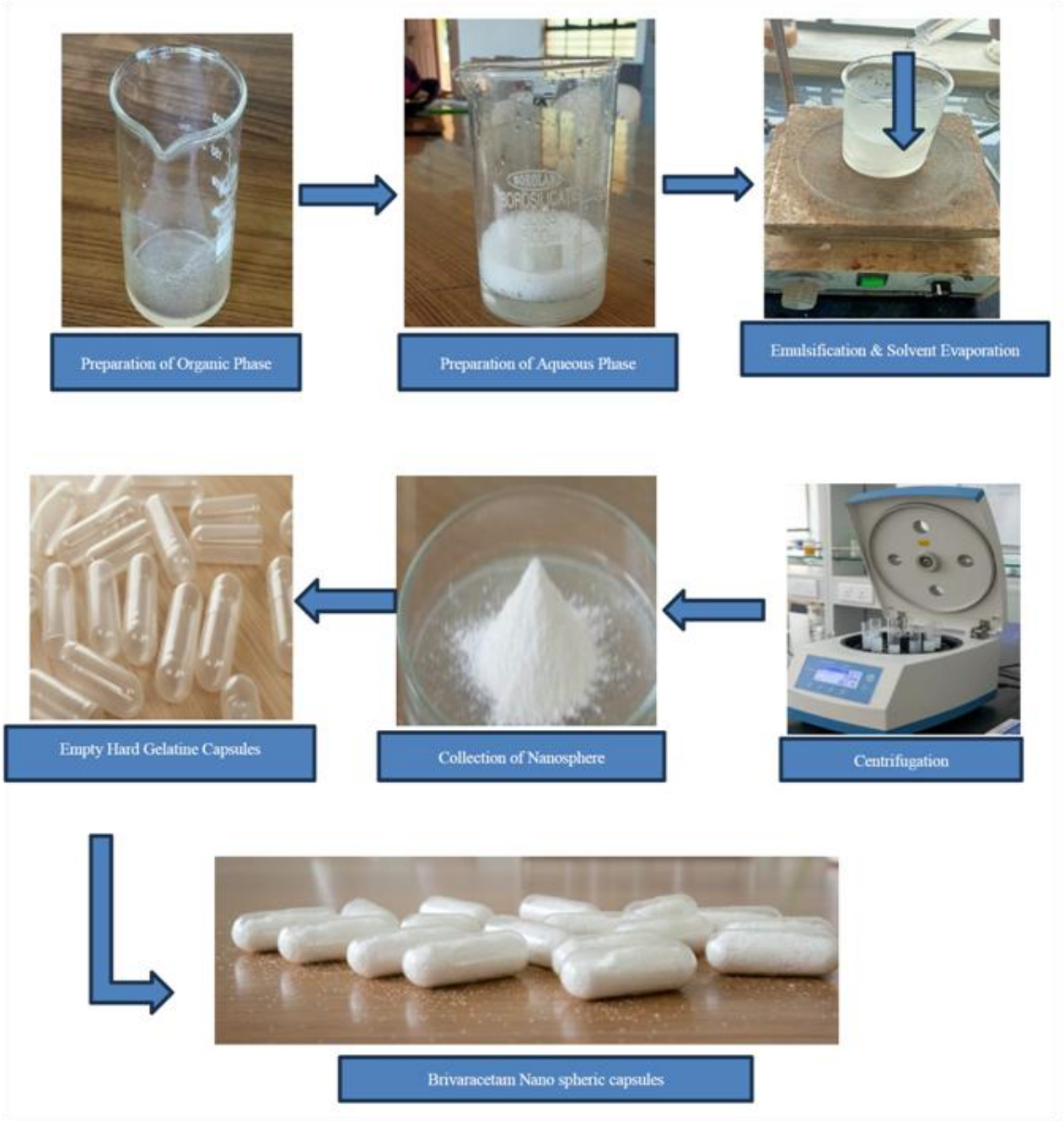


Figure 10: Formulation of Brivaracetam loaded nanosphere.

2.6 EVALUATION OF NANOSPHERE

2.6.1 Particle Size Distribution

The particle size and distribution are one of the most important characteristics of nanoparticle systems. Immediately after precipitation, the size of the drug nanospheres was measured using dynamic laser light scattering with a nanoparticle size analyzer (Malvern). The drug solution was diluted to a

concentration of 0.2 mg/mL with purified water prior to analysis. The results from the particle size study were interpreted using the graphic mean size (Mz) and calculated surface area. [20][21] and the observations are shown in Table 08 and Figure 16.

2.6.2 Zeta Potential

The size, size distribution, and zeta potential of the nanospheres were assessed using a zeta sizer (ZS 90 Malvern). Prior to testing, the lyophilized samples were diluted with PBS to achieve a concentration of 1 mg/mL and a pH of 6.0. These samples were placed in a clean cuvette during the size analysis to obtain multiple peaks, which were then used to calculate the average zeta size. For the zeta potential measurements, the samples were kept in the analysis chamber of the zeta sizer while it was operational to collect accurate data. Typically, the focus is on the monodisperse characteristics of this data rather than its polydisperse aspects.[22][23] and the observations are shown in Table 09 and Figure 17,18 &19.

2.6.3 Drug Entrapment Efficiency (EE%) and Drug Loading (DL%)

To determine the drug entrapment efficiency (EE%) and drug loading (DL%), the nanospheres undergo a process of centrifugation, washing, re-centrifugation, and subsequent filtration. An aliquot of the supernatant is taken and diluted for analysis. The concentration of the free drug is measured using a UV-Visible spectrophotometer. The amount of entrapped drug is calculated by subtracting the quantity of free drug from the total amount of drug initially added to the formulation. [24] and the observations are shown in Table 10 & 11 and Figure 22 & 23.

Calculating the formula:

$$\textbf{EE (W/W) \%} = \frac{\textbf{Amount of entrapped drug}}{\textbf{Total amount of the drug added}} \times 100$$

$$\textbf{DL (W/W) \%} = \frac{\textbf{Amount of entrapped drug}}{\textbf{(amount of polymer + entrapped drug)}} \times 100$$

2.6.4 Scanning Electron Microscopy (SEM)

The particle morphology of both untreated and treated drug nanospheres was analysed using scanning electron microscopy. Each drug powder sample was divided into small pieces and affixed to double-sided carbon conductive tape. A Pt-Pd alloy coating, approximately 5 nm thick, was then applied to cover the entire surface of the tape. Micrographs were captured using a Zeiss DSM 982 Field Emission Gun Scanning Electron Microscope (Carl Zeiss AG, Germany). [25] and the observations are shown in Figure 20 & 21.

2.6.5 Modified Dissolution Test

The in vitro drug release study by the membrane diffusion method involves soaking a dialysis membrane in distilled water overnight, then rinsing with phosphate buffer. A fixed volume of the nanosphere formulation is placed inside the membrane, which is sealed and immersed in 100 mL of phosphate buffer (pH 6.8 or 7.4) at 37 ± 0.5 °C with continuous stirring. At specific time intervals, 1 mL of the sample is withdrawn and replaced with fresh buffer. Drug release is analyzed using a UV spectrophotometer, and cumulative percentage release is calculated and plotted against time to study the release kinetics and profile. [26] and the observations are shown in Table 12 and Figure 24,25 & 26.

2.7 EVALUATION OF BRIVARACETAM LOADED NANOSPHERE CAPSULES

Weight Variation

Disintegration Time

Drug Content Uniformity

Invitro Drug Release

2.7.1 Weight Variation:

20 capsules are selected or taken at randomly and weighed individually, take average and compare each capsule weight with average. Then test passes if none of the individual weights are less than 90% and more than 110% of average. If test requirements are not met, we have to remove the powder, net content of powder can be weighed individually. They have to be averaged. Test requirements are met if not more than 2 of the individual's difference is not greater than10 of average. In any case difference should not be more than or equal to 25%. If more than 2 and less than 6 net weights determined, they deviate 10% Then we go for additional 40 capsules. The average of 60 capsules is determined by weighing capsules individually and compared with average.[27] and the observations are shown in Table 13.

$$\textbf{Percentage Deviation (\%)} = \frac{\textbf{Individual Capsule Weight} - \textbf{Average Weight}}{\textbf{Average Weigh}} \times 100$$

2.7.2 Disintegration Time:

The capsules are placed in the basket-rack assembly, which is repeatedly lowered 30 times per minute into a thermostatically controlled bath of fluid at 37 +2°C and observed over the time described in the individual monograph. [27] and the observations are shown in Table 14 and Figure 27.

2.7.3 Drug Content Uniformity:

This test is required only when specified in individual monographs or when capsules fail the weight variation test. If the capsules are completely filled, the test is not necessary. Unless otherwise stated in the monograph for a specific capsule, the drug content determined by assay should fall within 85.0% to 115.0% of the labelled claim in at least nine out of ten dosage units, with no single unit outside 75.0% to 125% of the labelled content. Additional testing is required if two or three dosage units fall outside the acceptable range but remain within the stated limits.[27] and the observations are shown in Table 15.

2.7. 4 Invitro Drug Release:

The release profile was studied using the IP Dissolution Test Apparatus Type II (basket type). The basket was immersed in 900 ml of phosphate buffer (pH 6.8) maintained at 37 ± 0.5°C, with the apparatus operated at 50 rpm. At five-minute intervals for up to 60 minutes, 5 ml of the medium was withdrawn, filtered through Whatman No. 41 filter paper, and replaced with fresh buffer. Absorbance was recorded at 272 nm, and cumulative drug release was calculated from the calibration curve.[27] and the observations are shown in Table 16 Figure 28,29& 30.

3. RESULTS AND DISCUSSION

3.1 PREFORMULATION STUDIES

3.1.1 Organoleptic property of Brivaracetam

The organoleptic properties like colour, odour, and taste of the excipients were evaluated and the observation shown in table 4.

Table 4: Organoleptic property of Brivaracetam

Test	Observation
Colour	White to off white
Odour	Odourless
Taste	Bitter

3.1.2 Solubility

Solubility is expressed in terms of parts per million of solvent in which 1g of solid is soluble. Solubility of the powder in different solvents like ethanol was determined at 20°C. The solubility studies of drug revealed that, Brivaracetam is highly soluble in various solvents. It is freely soluble in water, buffer (pH 1.2, 4.5, 7.4), Ethanol, Dichloromethane, Methanol and Glacial acetic acid, Soluble in Acetone, Toluene and slightly soluble in n- hexane. Brivaracetam has a solubility of 21.23 mg/ml in both DMSO and water. Hence Brivaracetam exhibits excellent solubility in water and common organic solvents, which facilitates its formulation and administration.

3.1.3 Melting point determination

The melting point was carried out by using capillary tube method. The result found to be 131.97°C. Hence complies with USP standard, thus indicates the purity of the drug sample.

3.1.4 FTIR studies of Brivaracetam

The active component Brivaracetam and physical mixture with different polymers were taken for FTIR. The FTIR spectrum of Brivaracetam and mixture of Brivaracetam with polymer was given in the fig 11 and 12

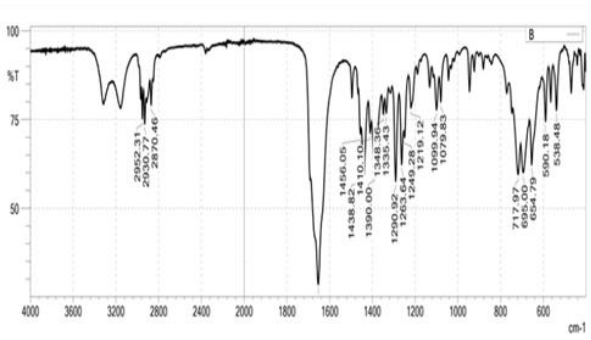


Figure 11: FTIR Spectra of Brivaracetam

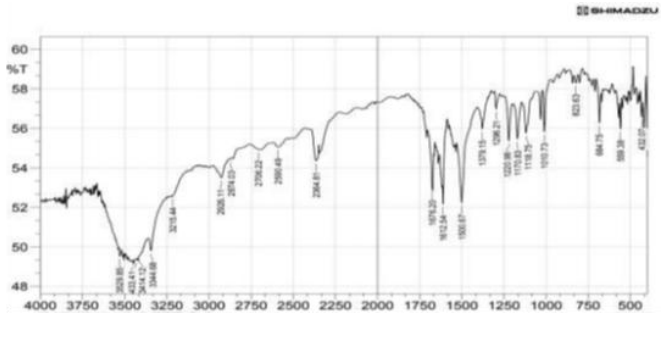


Figure 12: FTIR Spectra of Brivaracetam and PLGA

Table 5: Comparison peak of functional groups of Brivaracetam observed in FTIR spectra of compatibility studies

Sl. No	Types of Vibration	Brivaracetam (cm^{-1})	Physical Mixture (Drug+ PLGA) (cm^{-1})
1	O-H Stretching	3292.60	3315.74
2	N-H Stretching	2914.54	2831.76
3	C-H Stretching	2848.96	2819.68
4	C-N Stretching	2237.50	2249.61
5	C=O stretching	1656.91	1659.79
6	C-C stretching	1406.15	1401.25

The FTIR compatibility study between Brivaracetam and PLGA was evaluated by comparing the characteristic peaks of the drug, physical mixture, and formulation. The O–H stretching vibration appeared at 3292.60 cm^{-1} for Brivaracetam, 3315.74 cm^{-1} in the physical mixture, and 3310.10 cm^{-1} in the formulation, indicating slight shifts. N–H stretching was observed at 2914.54 cm^{-1} in the drug, 2831.76 cm^{-1} in the mixture, and 2842.45 cm^{-1} in the formulation. C–H stretching showed peaks at 2848.96 cm^{-1} for Brivaracetam, 2819.68 cm^{-1} in the mixture, and 2814.76 cm^{-1} in the formulation. The C–N stretching vibration was noted at 2237.50 cm^{-1} for the drug, 2249.61 cm^{-1} in the mixture, and 2247.81 cm^{-1} in the formulation. The C=O stretching vibration, a key functional group in amide bonds, appeared at 1656.91 cm^{-1} in the drug, 1659.79 cm^{-1} in the mixture, and 1654.31 cm^{-1} in the formulation. Lastly, C–C stretching appeared around 1406–1401 cm^{-1} across all samples. The retention of all major peaks with minor shifts indicates no significant chemical interaction, confirming good compatibility between Brivaracetam and PLGA.

3.1.5 Differential Scanning Calorimetry studies of Brivaracetam

Physical incompatibility study was carried out by DSC. Due to its value in studying solid-state interactions, thermograms were produced for mixtures containing both pure drugs and drug excipients.

There was no interaction between the medication and the polymers, according to the DSC study. There was no discernible variation in the melting endotherms of the drugs physical combination containing all polymers, and the thermograms of DSC revealed the endothermic peak of the pure drug at 102°C.

The results showed that the selected drug and the polymers were physically compatible. The DSC results were represented in Table 6 and DSC thermograms are shown in fig.13 And 14.

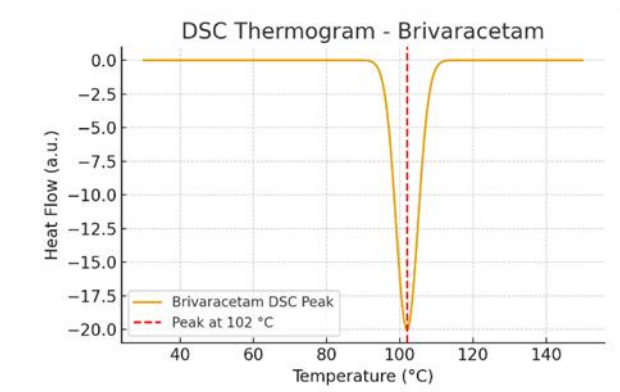


Figure 13: DSC of Brivaracetam

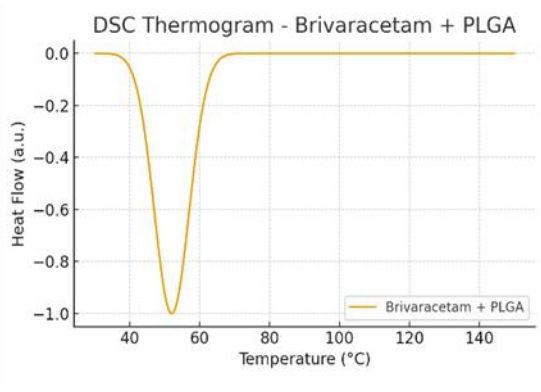


Figure 14: DSC of Brivaracetam and PLGA

The DSC thermogram of pure Brivaracetam shows a sharp endothermic peak at approximately 102°C, corresponding to its melting point and confirming its crystalline nature and purity. PLGA exhibits a glass transition temperature (Tg) around 47.4°C, with a minor transition near 52.0°C, indicating its amorphous character and polymer mobility. In the physical mixture of Brivaracetam and PLGA, the melting peak of the drug is significantly reduced or absent, this shift indicates successful molecular dispersion of Brivaracetam within the PLGA matrix, supporting the formation of a solid dispersion or encapsulated system.

Table 6: DSC results from the thermograms of both drug and physical mixture of drug- excipients

Sl.no	Sample	Endothermic peak
1	Brivaracetam	102 °C
2	Brivaracetam + PLGA	52 °C

DSC analysis showed that Brivaracetam has a sharp melting peak at ~101°C, confirming its crystalline nature, while PLGA displayed a glass transition around 52°C. In the physical mixture, the drug's peak was slightly shifted and reduced, indicating no major interaction but good compatibility. These results confirm the thermal stability of Brivaracetam with PLGA.

3.1.6 Standard Graph of Brivaracetam

Stock solution was prepared by using 50 mg of Brivaracetam in 100 ml of phosphate buffer (pH 6.8). From this stock solution 10 ml was withdrawn and diluted up to 100 ml using water. Calibration curve was prepared by using different concentration (5µg/ml-30 µg/ml) by appropriate dilution of stock solution. The absorbance was measured at 272 nm.

Table 7: Standard Graph of Brivaracetam

Sl no	Concentration µg/ml	Absorbance
1	0	0
2	5	0.159 ± 0.03
3	10	0.326 ± 0.01
4	15	0.525 ± 0.05
5	20	0.661 ± 0.02
6	25	0.821 ± 0.01
7	30	0.941 ± 0.04

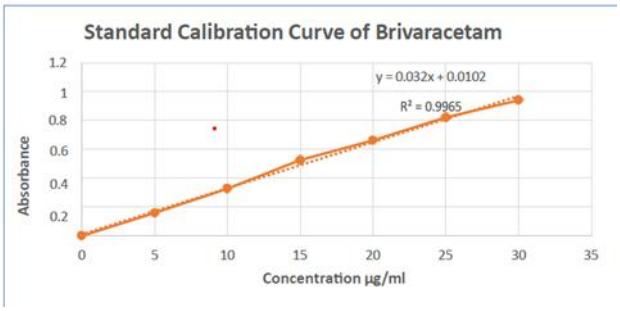


Figure 15: Standard graph of Brivaracetam

It was found that the solution of Brivaracetam in phosphate buffer show linearity (R^2 =0.9965) in absorbance at concentration of 5-30(µg/ml) and obeys Beer Lamberts Law.

3.2 EVALUATION PARAMETERS:

3.2.1 PARTICLE SIZE:

The particle size analysis for Brivaracetam-loaded nanospheres formulated using the solvent evaporation method with PLGA as the polymer was carried out using Malvern Zetasizer revealed significant variation across formulations F1 to F9. The particle sizes ranged from 149.2 nm to 213.5 nm.

Among all formulations, **F5** exhibited the smallest particle size at 149.2 nm, indicating a potentially more stable and efficient nanosphere system due to its reduced size and higher surface area. In contrast, F3 recorded the largest particle size at 213.5 nm, which may influence drug release and stability. The data suggest that formulation parameters significantly influenced particle size, with F5 showing optimal characteristics for enhanced drug delivery. This evaluation supports the effectiveness of PLGA-based nanospheres in controlling particle size for improved therapeutic outcomes.

Table 8: Particle size (nm)

FORMULATIONS	PARTICLE SIZE (nm)
F1	205.3
F2	187.6
F3	213.5
F4	175.9
F5	149.2
F6	162.3
F7	195.7
F8	181.6
F9	207.4

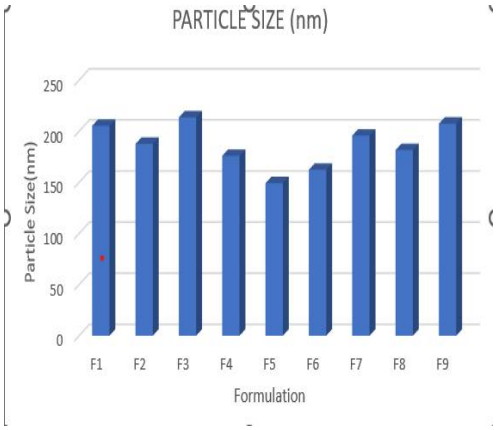


Figure:16: Particle size (nm) of the Formulations F1-F9

3.2.2 ZETA POTENTIAL

The zeta potential evaluation of Brivaracetam-loaded nanospheres prepared by the solvent evaporation method using PLGA as the polymer was carried out using Malvern Zetasizer showed negative surface charges across all formulations, indicating good colloidal stability. The zeta potential values ranged from –19.5 mV to –27.8 mV. Among the nine formulations (F1–F9), formulation F5 exhibited the highest negative zeta potential of –27.8 mV, suggesting superior stability due to stronger repulsive forces between particles.

Table 9: Zeta potential (Mv)

FORMULATIONS	Zeta Potential (Mv)
F1	**-21.4**
F2	**-23.1**
F3	**-19.5**
F4	**-24.2**
F5	**-27.8**
F6	**-25.6**
F7	**-22.3**
F8	**-23.7**
F9	**-20.1**

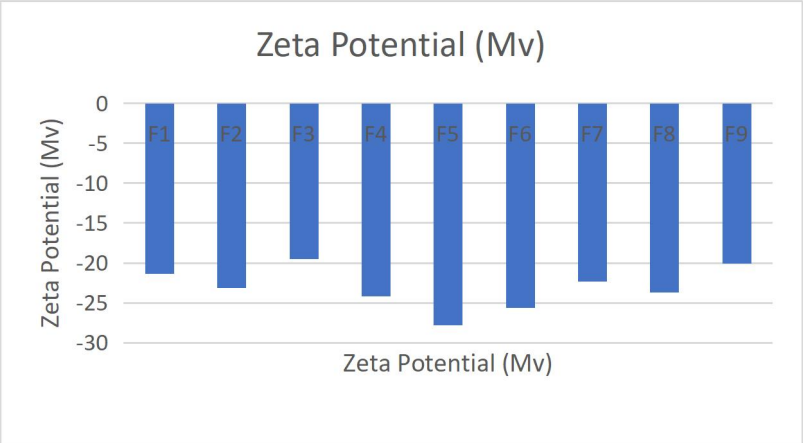
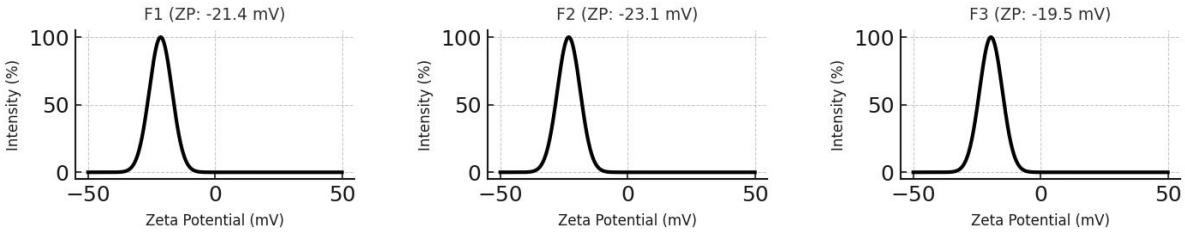
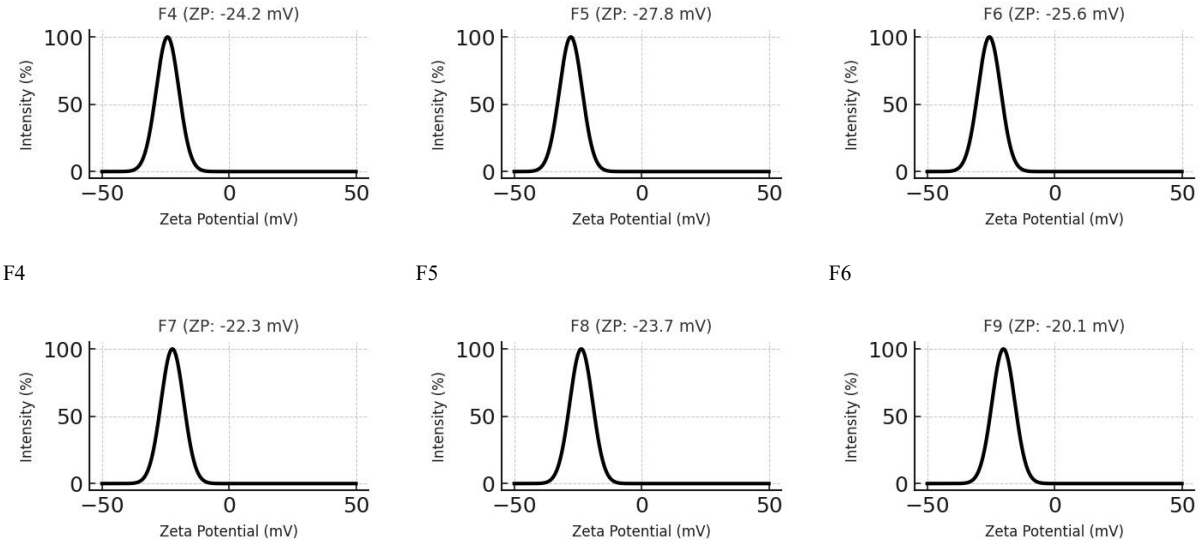


Figure 17: Zeta potential (Mv) of the Formulations F1-F9



F1 F2 F3



F4 F5 F6

F7 F8 F9

Figure 17: Zeta potential (Mv) of the Formulations F1-F9

3.2.3 Scanning Electron Microscopy (SEM):

The below SEM image represents Formulation F5 of Brivaracetam-loaded nanospheres prepared using PLGA as a polymer. The nanospheres exhibit a nearly spherical morphology with smooth surfaces, indicating successful encapsulation. The particles appear to be uniformly distributed with minimal aggregation, confirming good stability and optimized formulation characteristics. This morphology supports effective drug entrapment and sustained release potential**.**

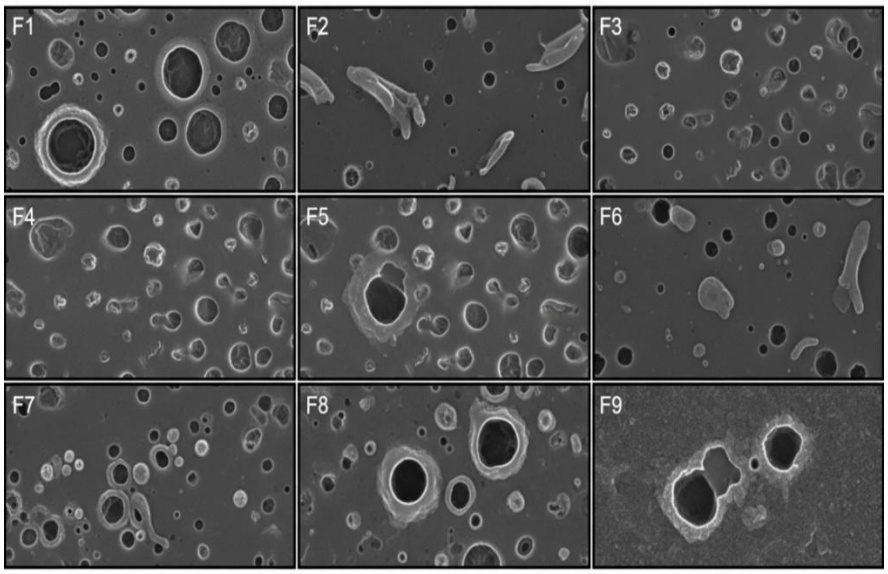


Figure 20: SEM image of of the Formulations F1-F9

3.2.4 Drug Entrapment Efficiency

The entrapment efficiency of Brivaracetam-loaded nanospheres formulated using the solvent evaporation method with PLGA varied between 56.4% and 79.4% across formulations F1 to F9. Formulation F1 showed an efficiency of 58.2%, while F2 and F3 exhibited 61.7% and 56.4%, respectively. A significant improvement was observed in F4 and F5, with values of 68.1% and 79.4%, respectively, indicating enhanced drug encapsulation due to better polymer–drug interaction. F6 also demonstrated a high entrapment efficiency of 73.2%. Formulations F7 and F8 recorded 65.5% and 69.8%, respectively. F9 showed 60.3%. Among all, F5 was found to be the most optimized formulation.

Figure 22: Entrapment Efficiency (%) of the Formulations F1-F9

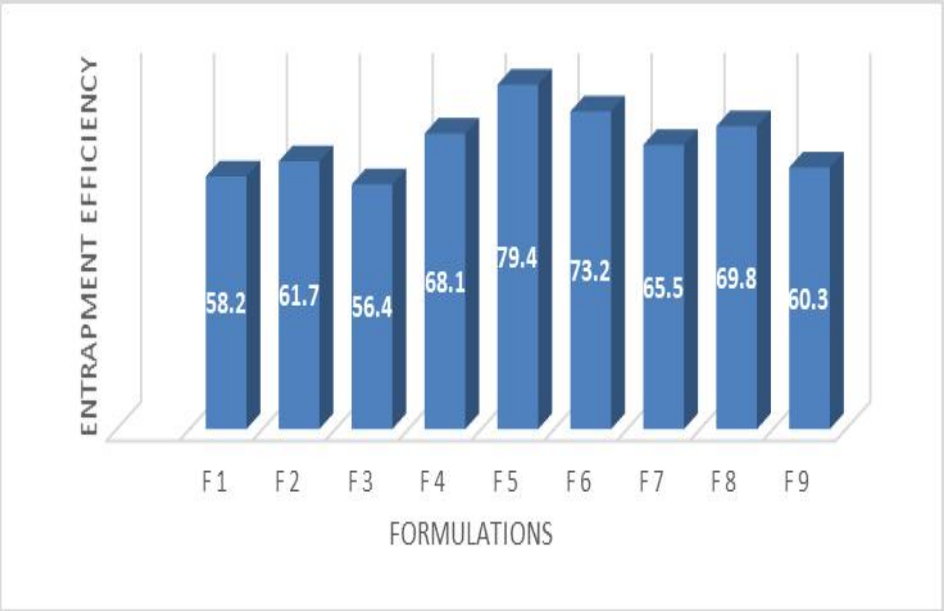


Table 10: Entrapment Efficiency (%)

FORMULATIONS	Entrapment Efficiency (%)
F1	58.2
F2	61.7
F3	56.4
F4	68.1
F5	**79.4**
F6	73.2
F7	65.5
F8	69.8
F9	60.3

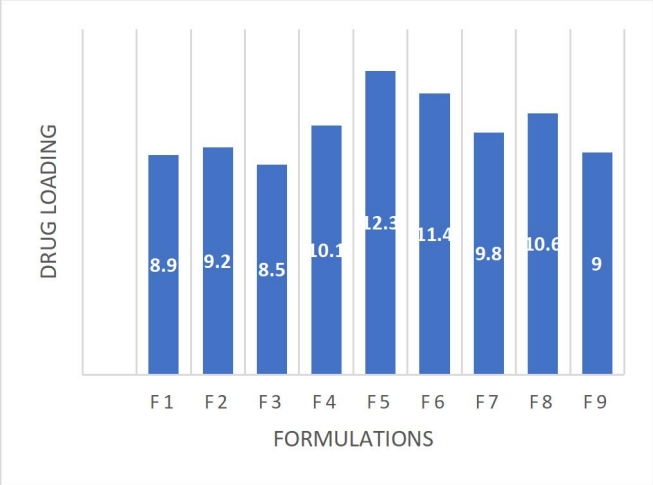
3.2.5 Drug Loading

The drug loading of Brivaracetam-loaded nanospheres prepared by the solvent evaporation method using PLGA varied across formulations F1 to F9, ranging from 8.5% to 12.3%. Formulation F1 showed a drug loading of 8.9%, while F2 and F3 exhibited 9.2% and 8.5%, respectively. An increase was observed in F4 and F5, with values of 10.1% and 12.3%, respectively, indicating enhanced drug incorporation. F6 also demonstrated a high drug loading of 11.4%. F7 and F8 recorded 9.8% and 10.6%, respectively, while F9 had a loading of 9.0%. Among all, F5 exhibited the highest drug loading, suggesting optimal formulation efficiency.

FORMULATIONS	Drug Loading (%)
F1	8.9
F2	9.2
F3	8.5
F4	10.1
F5	12.3
F6	11.4
F7	9.8
F8	10.6
F9	9.0

Table 11: Drug Loading (%)

Figure 23: Drug Loading (%) of the Formulations F1-F9



3.2.6 In Vitro Dissolution Study

An *in vitro* dissolution study was conducted for Brivaracetam-loaded PLGA nanospheres (F1–F9) using the dialysis bag diffusion technique in phosphate buffer saline (PBS, pH 7.4). Nanosphere formulations equivalent to 10 mg of Brivaracetam were enclosed in dialysis bags and immersed in 100 mL PBS at 37 ± 0.5°C with constant stirring at 100 rpm. At specific time intervals (0.5, 1, 2,3,4, 6, 8,10 and 12 hours), 5 mL samples were withdrawn and replaced with fresh medium. Drug release was quantified using a UV-Visible spectrophotometer at 272 nm

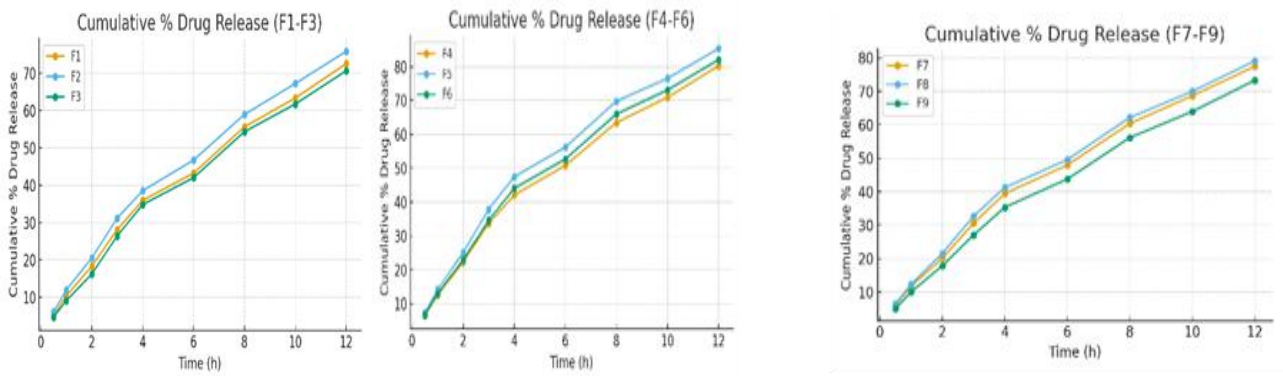


Figure 24,25,26 : *in-vitro* dissolution profile of Brivaracetam-loaded nanospheres for formulations F1–F3, F4-F6, F7-F9

Table12: *in vitro* dissolution test of brivaracetam loaded nanospheres

Time(h)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	5.2	6.1	4.7	6.5	7.3	6.8	5.9	6.4	5.0
1.0	10.4	12.0	9.1	12.6	14.2	13.0	11.7	12.3	10.0
2.0	18.3	20.5	16.2	22.3	25.1	23.0	20.1	21.5	17.8
3.0	28.1	31.2	26.4	33.7	37.9	34.6	30.5	32.6	27.0
4.0	35.9	38.5	34.8	42.1	47.5	44.1	39.4	41.2	35.3
6.0	43.2	46.7	42.0	50.8	56.2	52.7	47.9	49.5	43.7
8.0	55.6	58.9	54.3	63.5	69.8	66.0	60.3	62.1	56.1
10.0	63.3	67.2	61.7	70.9	76.5	73.1	68.6	69.9	63.9
12.0	72.5	75.8	70.6	80.2	85.3	82.0	77.4	79.0	73.2

The in-vitro drug release of Brivaracetam-loaded nanospheres was evaluated using the membrane diffusion technique. Dialysis membranes (previously soaked in distilled water overnight and rinsed with phosphate buffer pH 6.8) were used as diffusion barriers. A fixed volume of each nanosphere formulation (F1–F9) was placed inside the membrane, which was tightly sealed and immersed in 100 mL of phosphate buffer maintained at 37 ± 0.5 °C with constant stirring at 50 rpm. At predetermined intervals (0.5, 1, 2,3, 4, 6, 8, 10, and 12 hours), 1 mL aliquots were withdrawn and replaced with fresh buffer to maintain sink conditions. The samples were analysed at λmax of Brivaracetam using a UV spectrophotometer, and cumulative percentage release was calculated.

The results confirmed that all formulations followed a sustained release pattern, with F5 (85.3% at 12 h) showing the highest release, while F3 and F9 exhibited comparatively slower drug diffusion. This validates the efficiency of the membrane diffusion method in assessing controlled drug release from nanospheres.

3.3 The filled capsules were evaluated for the following parameters

3.3.1 Weight Variation:

The weight variation analysis of Brivaracetam nanosphere-loaded capsules indicated that all formulations were within the acceptable pharmacopeial limits, thereby confirming consistency in dosage unit uniformity. Formulation F1 exhibited a mean weight of 502.4 ± 2.6 mg, whereas F2 and F3 showed 498.7 ± 2.3 mg and 505.9 ± 2.4 mg, respectively. Similarly, F4 demonstrated a mean capsule weight of 500.5 ± 2.8 mg, F5 was 507.3 ± 2.7 mg, and F6 recorded 495.1 ± 2.5 mg. Among the later batches, F7 showed 503.2 ± 2.2 mg, F8 exhibited 499.7 ± 2.9 mg, and F9 was found to be 504.3 ± 2.6 mg. The minimal deviation observed among all formulations confirms the precision of the encapsulation process and indicates excellent reproducibility and uniformity in capsule filling, which are critical parameters for ensuring dose accuracy and product quality.

Table 13: Weight Variation(mg)

Formulation	Weight (mg) ± S.D.	Pharmacopeial Limit (±5%)	Result
F1	502.4 ± 2.6	475 – 525 mg	Complies
F2	498.7 ± 2.3	475 – 525 mg	Complies
F3	505.9 ± 2.4	475 – 525 mg	Complies
F4	500.5 ± 2.8	475 – 525 mg	Complies
F5	507.3 ± 2.7	475 – 525 mg	Complies
F6	495.1 ± 2.5	475 – 525 mg	Complies
F7	503.2 ± 2.2	475 – 525 mg	Complies
F8	499.7 ± 2.9	475 – 525 mg	Complies
F9	504.3 ± 2.6	475 – 525 mg	Complies

3.3.2 Disintegration Time:

The disintegration time study of Brivaracetam-loaded nanospheres encapsulated in hard gelatine capsules was performed in accordance with pharmacopeial guidelines. The results demonstrated that all nine formulations (F1–F9) disintegrated within the acceptable limit of not more than 30 minutes. Among them, formulation F5 showed the shortest disintegration time of 18 minutes, indicating rapid capsule breakdown and efficient release of nanospheres. Formulation F4 also exhibited a favourable profile with 19 minutes, while F2 and F6 disintegrated at 20 and 21 minutes, respectively. Moderate disintegration times were observed for F1 (22 min), F8 (23 min), and F3 (24 min). The slightly longer, yet still acceptable, times were recorded for F9 (25 min) and F7 (26 min). Overall, all formulations complied with pharmacopeial requirements, ensuring consistency and reliability of drug release. Based on these results, F5 can be considered the most optimized formulation in terms of disintegration performance.

Table 14: Disintegration Time (Min)

Formulations	Disintegration Time (Min)	Pharmacopeial Limit (<30 Min)	Result
F1	22	<30 Min	Complies
F2	20	<30 Min	Complies
F3	24	<30 Min	Complies

Formulations	Disintegration Time (Min)	Pharmacopeial Limit (<30 Min)	Result
F4	19	<30 Min	Complies
F5	18	<30 Min	Complies
F6	21	<30 Min	Complies
F7	26	<30 Min	Complies
F8	23	<30 Min	Complies
F9	25	<30 Min	Complies

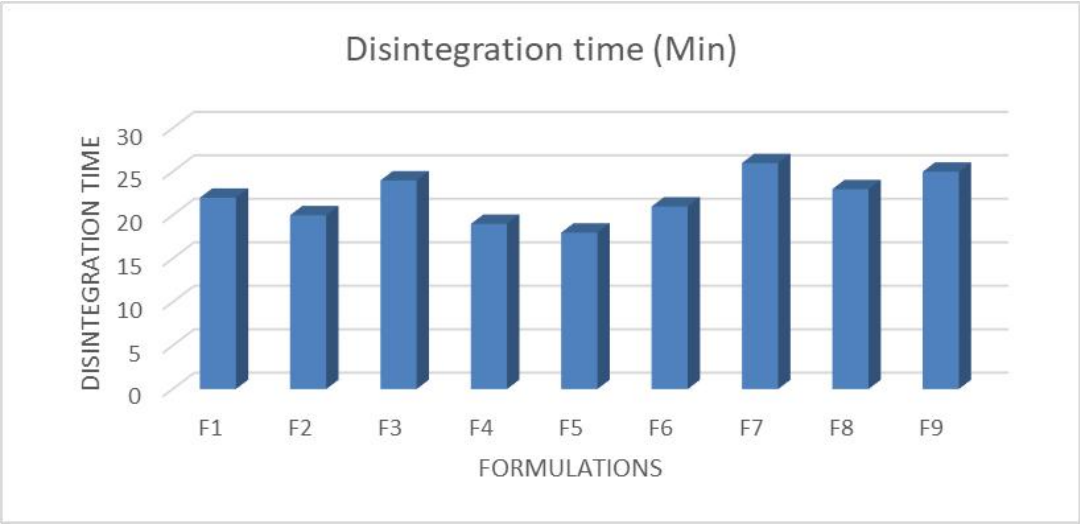


Figure 28: Disintegration Time (Min) of the Formulations F1-F9

3.3.3 Drug Content Uniformity (%):

The drug content uniformity of Brivaracetam nanosphere-loaded capsules was found to be consistent across all formulations, with values ranging from 94.2% to 98.7%. Formulation F1 exhibited a uniformity of 96.2 ± 1.3%, while F2 showed a slightly higher value of 97.1 ± 1.1%. F3 recorded 95.3 ± 1.4%, whereas F4 and F5 demonstrated comparatively higher uniformity at 98.0 ± 1.2% and 98.7 ± 1.0%, respectively. F6 also maintained a satisfactory level with 97.5 ± 1.3%. On the other hand, formulations F7, F8, and F9 showed relatively lower values of 94.8 ± 1.5%, 95.7 ± 1.3%, and 94.2 ± 1.6%, respectively. Overall, all formulations were within the acceptable pharmacopeial limits, ensuring good content uniformity

.**Table 15: Drug Content Uniformity**

Formulations	Drug Content Uniformity (%)	Pharmacopeial Limit (85–115%)	Result
F1	96.2 ± 1.3	85–115%	Complies
F2	97.1 ± 1.1	85–115%	Complies
F3	95.3 ± 1.4	85–115%	Complies
F4	98.0 ± 1.2	85–115%	Complies
F5	98.7 ± 1.0	85–115%	Complies
F6	97.5 ± 1.3	85–115%	Complies
F7	94.8 ± 1.5	85–115%	Complies
F8	95.7 ± 1.3	85–115%	Complies
F9	94.2 ± 1.6	85–115%	Complies

3.3.4 In-vitro Dissolution Study of Brivaracetam Nanosphere Capsules

The *in-vitro* drug release study of Brivaracetam-loaded nanosphere capsules was performed using the USP Type II (paddle) apparatus in 900 mL of phosphate buffer (pH 6.8) at 50 rpm and 37 ± 0.5 °C. Samples were withdrawn at predetermined intervals (0.5–12 h), filtered, and analyzed spectrophotometrically. The results are shown below.

Table 16: *In-vitro* Dissolution Study of Brivaracetam Nanosphere Capsules

Time(h)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	18.5	20.4	17.8	21.1	22.6	20.8	15.6	16.2	14.9
1.0	28.7	30.2	27.5	31.6	33.4	30.8	25.2	26.0	23.8
2.0	41.3	44.6	39.8	46.2	48.7	45.1	36.6	37.8	34.4
3.0	52.5	55.9	50.8	58.4	61.1	57.2	46.2	47.5	43.9
4.0	63.1	66.5	61.7	69.3	72.4	68.2	55.6	56.8	52.7
6.0	74.6	77.8	72.2	80.1	83.6	79.2	65.8	67.4	63.9
8.0	81.2	84.7	79.6	86.4	89.2	85.5	76.8	78.4	74.9
10.0	84.8	87.9	82.6	89.3	92.1	88.6	79.9	81.3	77.8
12.0	88.6	91.3	86.5	92.7	95.6	92.2	83.5	85.1	81.2

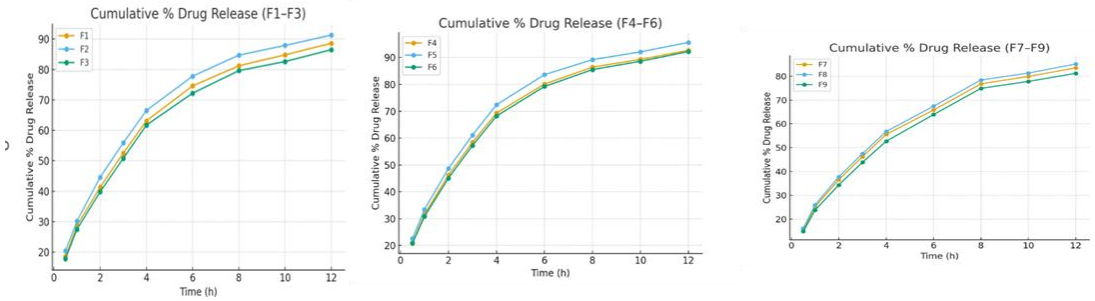


Figure 29: Cumulative % Drug Release (F1–F3), (F4-F6), (F7-F9) showing the comparative drug release profiles.

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