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Formulation of Zonisamide Nanoemulsion for Brain Targeted Drug Delivery Via Intranasal Route

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ABSTRACT: The development of a Zonisamide nanoemulsion designed for brain-targeted drug delivery through the intranasal route represents a significant advancement in overcoming the challenges of the blood-brain barrier (BBB). This technique utilizes the direct nose-to-brain pathway, effectively avoiding systemic circulation and first-pass hepatic metabolism. Nanoemulsions, characterized by their minute droplet size and extensive surface area, improve drug solubility, stability, and bioavailability. They also facilitate controlled and site -specific drug release, thereby minimizing systemic side effects.
As an antiepileptic medication, Zonisamide is well-suited for this delivery system, which ensures efficient transport to the central nervous system (CNS) and may enhance treatment outcomes for neurological conditions. The formulation process focuses on optimizing the ratios of oil, surfactant, and co-surfactant to produce a stable, functional nanoemulsion. Crucial characterization methods, such as particle size measurement, zeta potential analysis, and in vitro release studies, are employed to confirm the formulation's effectiveness.

Keywords: Nanoemulsion, blood-brain barrier, central nervous system, HPLC.

INTRODUCTION: Brain diseases such as dementia, epilepsy, migraines, autoimmune disorders (including Parkinson's, Alzheimer's, and prion diseases), brain tumors, and acute ischemic brain hemorrhages demand significant clinical attention due to their high rates of morbidity and mortality globally. Many existing brain-targeting medications primarily address symptomatic brain dysfunctions but often fail to deliver satisfactory therapeutic outcomes. Key challenges include (i) the lipophilic nature of the blood-brain barrier (BBB), (ii) the complexity of the brain's microenvironment, and (iii) abnormal protein dynamics. The central nervous system (CNS) vessels, comprising arterioles and venules, are continuous and non-aperture, facilitating the regulation and exchange of ions and molecules within brain cells. The unique structure of CNS vessels and the protective role of the BBB prevent the entry of antigens, toxins, and pathogens. The BBB acts as a barrier, diverting blood from the interstitial fluid and restricting the diffusion of most active substances to the brain's receptors. It functions as a dynamic regulator, transporting nutrients while blocking heavy and undesirable lipophilic molecules from entering the brain's extracellular fluid. Lipophilic molecules with an optimal Log P (approximately

1.5–2.7) and a molecular weight of up to 600 Daltons can permeate the BBB. Brain endothelial cells (BECs), which form the walls of brain blood vessels, exhibit high polarization compared to endothelial cells in other tissues.The nasal route, often referred to as "the door to the brain" by Ayurveda practitioners, has been utilized for administering nasal drops (Nasya) to enhance voice, vision, and mental clarity. Natural substances such as calamus oil, coconut oil, ghee (clarified butter), sunflower oil, and sesame oil have demonstrated therapeutic benefits when administered intranasally. Unlike invasive techniques, this route does not require coupling therapeutic agents with carriers or modifying the drug's structure. A wide range of therapeutic agents has been successfully delivered to the CNS via the nasal route. This pathway bypasses the BBB due to the unique connection between the nose and the brain. Drug administration through the nasal route offers a distinct advantage and an effective option for targeting medications to the brain.

Nanoparticles for Drug Delivery to the Brain

The delivery of therapeutic agents to the brain remains a major challenge due to the presence of the blood-brain barrier (BBB), which tightly regulates the movement of substances between the bloodstream and the central nervous system (CNS). To overcome this limitation, nanotechnology has emerged as a powerful tool in enhancing drug delivery to the brain. Among various approaches, nanoparticles have shown great promise in improving drug transport, targeting, and therapeutic efficacy for a wide range of neurological disorders.

1. Characteristics of Nanoparticles

Nanoparticles are colloidal carriers with sizes typically ranging from 1 to 1000 nanometers. Their small size, large surface area, and modifiable surface properties allow them to penetrate biological barriers and interact with cells at the molecular level. Nanoparticles can be engineered to improve solubility, protect drugs from enzymatic degradation, and enable controlled or sustained release, making them highly suitable for CNS drug delivery.

2. Mechanisms of Brain Delivery

Nanoparticles can reach the brain via two primary mechanisms:

- **Transcytosis across the BBB**: Certain surface-modified nanoparticles can interact with receptors or transporters on the endothelial cells of the BBB, facilitating transport through endocytosis.
- **Nose-to-brain route**: When administered intranasally, nanoparticles can bypass the BBB entirely by utilizing the olfactory and trigeminal nerve pathways, delivering the drug directly to the brain.

3. Types of Nanoparticles Used

Several types of nanoparticles are explored for brain-targeted delivery:

- **Polymeric nanoparticles**: Biodegradable polymers like PLGA (poly(lactic-co- glycolic acid)) offer controlled release and high drug loading.
- **Lipid-based nanoparticles**: Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) enhance lipophilic drug delivery and biocompatibility.
- **Nanoemulsions**: These are oil-in-water systems that improve the solubility and nasal absorption of hydrophobic drugs.
- **Metallic nanoparticles**: Gold and silver nanoparticles are being investigated for theranostic (therapeutic + diagnostic) applications in brain diseases.
- **Dendrimers**: Branched polymers with high surface functionality that can carry drugs and targeting ligands.

4. Advantages of Nanoparticle-Based Delivery

- **Enhanced brain uptake** due to improved penetration and retention.
- **Controlled and sustained release**, reducing dosing frequency.
- **Targeted delivery**, minimizing off-target effects and systemic toxicity.
- **Protection of the drug** from premature degradation.

5. Applications in Neurological Disorders

Nanoparticles have been used to deliver drugs for the treatment of various CNS conditions, including:

- **Epilepsy** (e.g., Zonisamide-loaded nanoparticles)
- **Alzheimer's disease**
- **Parkinson's disease**
- **Brain tumors**
- **Stroke and traumatic brain injury**

6. Challenges and Future Perspectives

Despite their potential, nanoparticle-based delivery systems face challenges such as:

- Ensuring safety and avoiding long-term toxicity.
- Scaling up production with reproducibility.
- Navigating regulatory approval for clinical use.

MATERIALS AND METHODS:

Physical Characterization and Identification of Zonisamide

The Zonisamide sample was analyzed based on its physicochemical properties, including color, odor, and solubility in water and other solvents. Techniques such as melting point determination, differential scanning calorimetry (DSC), UV spectroscopy, and Fourier

Transform Infrared (FTIR) spectroscopy were employed. The results were compared with previously reported data in the literature.

Organoleptic Properties

The organoleptic characteristics of Zonisamide, including its nature, color, and odor, were evaluated.

Melting Point Determination

The melting point of Zonisamide was measured using a melting point determination apparatus. The drug sample was packed into a capillary glass tube, forming a compact column of 4–6 mm in height. This tube was placed in a HICON melting point apparatus (Ningbo Hicon Industry Co. Ltd, Zhouxiang, China) alongside a calibrated thermometer, and the melting temperature was recorded.

Differential Scanning Calorimetry (DSC)

Approximately 5 mg of Zonisamide was sealed in a DSC pan using a loading puncher. The sample was scanned from 30°C to 350°C at a heating rate of 10°C per minute under a nitrogen atmosphere (flow rate: 60 ml/min) using a Perkin Elmer Pyris 6 DSC (Massachusetts, USA). An empty pan served as the reference.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis of Zonisamide was conducted using the Potassium Bromide (KBr) pellet technique. A precisely weighed amount of Zonisamide (5 mg) was mixed with KBr in a 1:1 ratio and compressed into a pellet using a hydraulic press.

UV Spectroscopy

The UV spectrum of Zonisamide was recorded in methanol, phosphate buffer at pH 6.4, and pH 7.4 using a Shimadzu UV spectrophotometer (Kyoto, Japan).

Analytical Methodology

To quantify Zonisamide at various stages and in different samples, analytical methods were developed and validated using UV spectrophotometry and high-pressure liquid chromatography (HPLC).

Method Validation of UV Spectrophotometer in Methanol

The validation of the method was conducted in accordance with ICH guidelines, Q2 (R1), focusing on parameters such as linearity and range, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

(i) Linearity and Range

A calibration curve was created by transferring accurately measured volumes of Zonisamide stock solution into a series of 10 ml volumetric flasks, followed by dilution with the specified solvent. The absorbance of these solutions was measured at 224 nm (λmax for methanol).

(ii) Precision

The precision of the method was evaluated using three different concentrations of Zonisamide: 2, 4, and 10 μg/ml.

- **(a) Repeatability:** Intra-day precision was assessed by analyzing the three concentrations (2, 4, and 10 μg/ml) three times within a single day.
- **(b) Intermediate Precision:** Inter-day precision was determined by analyzing the same three concentrations (2, 4, and 10 μg/ml) three times a day over three consecutive days.

(iii) Accuracy (Recovery Studies)

Accuracy was assessed through recovery studies using the standard addition method. Pre- analyzed samples were spiked with 50%, 100%, and 150% of the standard Zonisamide, and the mixtures were analyzed using the proposed method.

(iv) Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the following formulas as per ICH guidelines:

- **LOD = 3.3 × σ/S**
- **LOQ = 10 × σ/S** Where:
- **σ** is the standard deviation of the intercept of the calibration curve.
- **S** is the slope of the regression line.

RESULTS AND DISCUSSION

Physical characterization and identification of Zonisamide
Organoleptic properties

Table 1: Zonisamide was observed for its nature, colour, odour and taste.

S.No.	Parameters	Inference
1	Nature	Fine crystalline powder
2	Color	White
3	Odor	Odorless
4	Taste	Tasteless

Melting point determination

The melting point was determined by capillary method and was found to be 124ºC that was close to reported value 123-125ºC.

Differential Scanning Calorimeter (DSC)

The DSC thermogram of pure Zonisamide is depicted. It exhibited a distinct endothermic peak at 125.08°C, which aligns with the reported range of 123–125°C. This confirms that the Zonisamide sample was both authentic and of high purity.

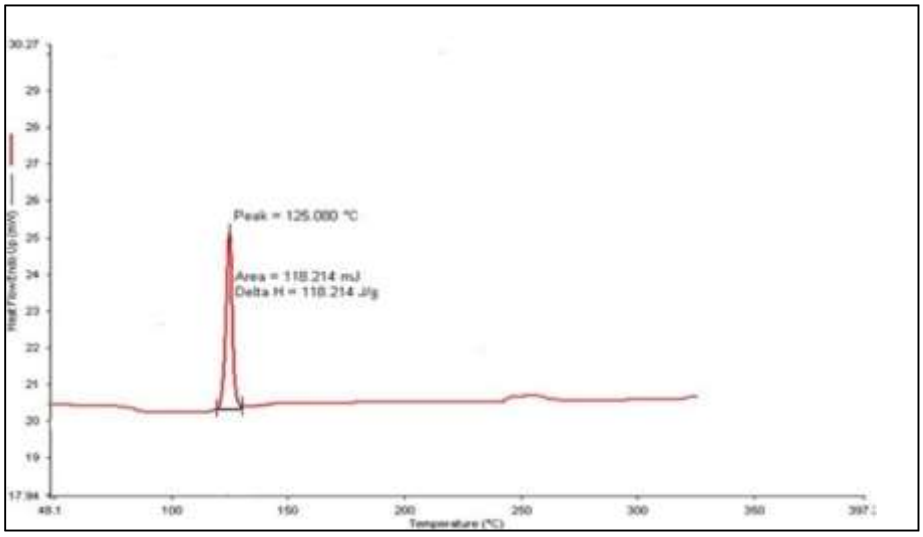

Figure 1: DSC Thermogram of Zonisamide

Fourier Transform Infrared (FTIR) spectroscopy

The FTIR spectra of Zonisamide were acquired using the KBr pellet technique. The spectra displayed characteristic peaks at 3526, 2926, 2858, 2090, 1736, 1638, 1461, 1299, 948, and 588 cm^{-1}, which corresponded well with the reported spectra for Zonisamide.

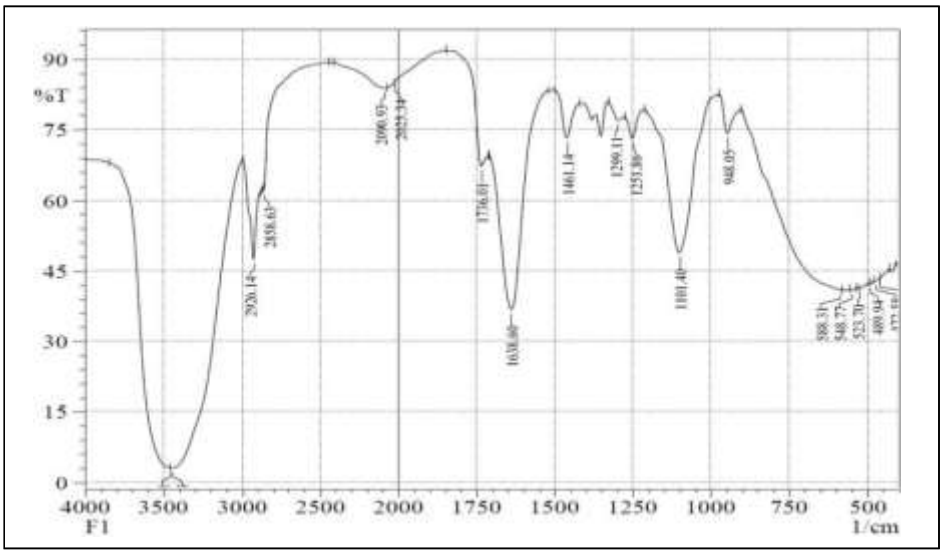

Figure 2: FTIR graph of Zonisamide

UV Spectroscopy

The UV spectra of Zonisamide were recorded at a concentration of 10 µg/ml in methanol, as well as in phosphate buffers with pH values of 6.4 and 7.4. The λmax values observed for Zonisamide in methanol, phosphate buffer pH 6.4, and phosphate buffer pH 7.4 were
224.5 nm, 261 nm, and 264 nm, respectively. These values were consistent with the previously reported values of 224 nm, 261 nm, and 264 nm.

Preparation of calibration curves in methanol

Table 2: Calibration curve data for Zonisamide in methanol (n=3)

Sr. No.	Concentration (µg/ml)	Absorbance at λ_{max} 224 nm Mean ± SD (n = 3)	Regressed Absorbance
1	2	0.176 ± 0.0027	0.175
2	4	0.321 ± 0.0024	0.386
3	6	0.571 ± 0.0052	0.563
4	8	0.785 ± 0.0059	0.757
5	10	0.947 ± 0.0086	0.951

Method Validation of UV Spectrophotometer in Methanol

(i) Linearity and Range The absorbance of the prepared standard solution was measured using a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The sample was scanned across the wavelength range of 200–400 nm, with absorption maxima observed at 224 nm.

(ii) Precision The precision of the method was evaluated by analyzing Zonisamide at three different concentrations: 2, 4, and 10 µg/ml**.**

(a) Repeatability Repeatability (intra-day precision) was assessed by analyzing Zonisamide at the specified concentrations (2, 4, and 10 µg/ml) three times within a single day

(b) Intermediate Precision Intermediate precision (inter-day precision) was evaluated by analyzing Zonisamide at the same concentrations (2, 4, and 10 µg/ml) three times daily over three consecutive days.

Table 3: Intra-day data for validating repeatability in methanol (n=3)

Sr. No	Concentration (µg/ml)	Absorbance at λ_{max} 224 nm Mean ± SD (n = 3)	% RSD
1	2	0.179 ± 0.0028	1.56
2	4	0.318 ± 0.0024	0.75
3	10	0.953 ± 0.0089	0.93

Table 4: Inter-day data for validating intermediate precision in methanol (n=3)

Concentration (µg/ml)	Day	Absorbance at λ_{max} 224 nm Mean ± SD (n = 3)	% RSD
2	1	0.182 ± 0.0028	1.53
2	2	0.179 ± 0.0027	1.50
2	3	0.184 ± 0.0024	1.30
4	1	0.316 ± 0.0024	0.75
4	2	0.319 ± 0.0021	0.65
4	3	0.321 ± 0.0025	0.77
10	1	0.948 ± 0.0089	0.93
10	2	0.951 ± 0.0083	0.87
10	3	0.953 ± 0.0086	0.90

(iii) Accuracy Through Recovery Studies The accuracy of the method was evaluated by analyzing three concentrations of the standard drug solution using the standard addition technique.

HPLC method validation for determination of Zonisamide in rat plasma and brain homogenate

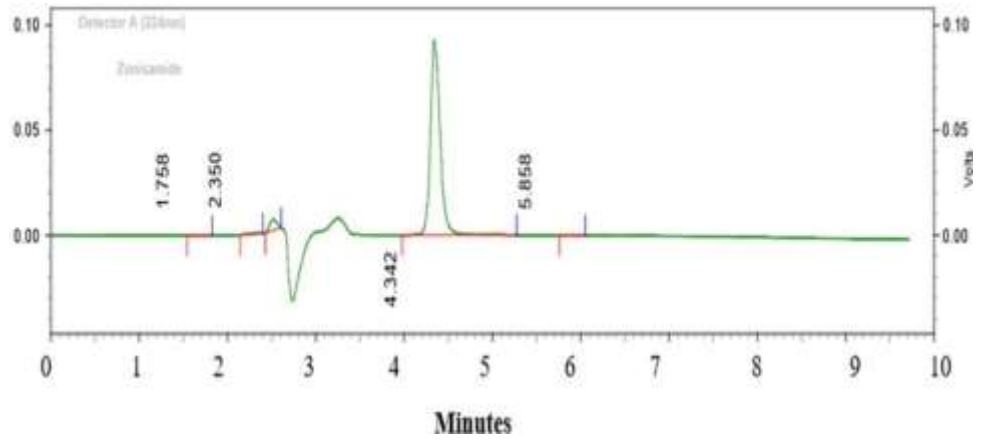

Figure 3: HPLC chromatogram of Zonisamide

CONCLUSION:

Aim of the Study The primary objective of this study was to formulate a nanoemulsion of Zonisamide for brain-targeted delivery via the nasal route. This approach aimed to bypass first-pass metabolism and minimize drug distribution to non-targeted sites.

- **Drug Identification and Purity:** Physical property analysis and identification tests confirmed that the Zonisamide sample was authentic, pure, and met established standards.
- **UV Spectrophotometric Method Validation:** A UV method for estimating Zonisamide in methanol was validated. The low % RSD values for repeatability and intermediate precision demonstrated the method's excellent precision. The limit of detection (LOD) and limit of quantification (LOQ) were determined as per ICH guidelines, yielding values of 0.47 µg/ml and 1.44 µg/ml, respectively.
- **HPLC Method Development and Validation:** A stability-indicating HPLC method for Zonisamide estimation in rat plasma and brain homogenate was developed and validated. Chromatographic separation was achieved using a C18 reverse-phase column with a mobile phase of water: acetonitrile (70:30 v/v) containing 0.1% v/v trifluoroacetic acid. The retention time for Zonisamide was
  4.342 minutes. Recovery values were close to 100%, and low % RSD values indicated high accuracy and precision. The LOD and LOQ for Zonisamide in plasma were determined as per ICH guidelines, with values of 10.51 ng/ml and
  31.87 ng/ml, respectively.

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