2026 Volume: 02 Issue: 05 www.ijrpa.com ISSN 2456-9995 Research Article

International Journal Research Publication Analysis

Page: 01-33

FORMULATION AND CHARACTERIZATION OF ESKETAMINE LOADED NANOGLOBULES FOR IMPROVED NANOTHERAPEUTIC DELIVERY IN DEPRESSION

***Pooja Prashant Kharosekar, Dr. Sachin R. Hangargekar**

M. Pharmacy Pharmaceutics, Name of organization: Shivlingeshwar College of pharmacy Almala.

Article Received: 28 March 2026
Article Revised: 18 April 2026
Published on: 08 May 2026

***Corresponding Author: Pooja Prashant Kharosekar**
M. Pharmacy Pharmaceutics, Name of organization: Shivlingeshwar College of pharmacy Almala.

DOI: https://doi-doi.org/101555/ijrpa.1858

ABSTRACT:

Depression is a severe and debilitating mental disorder affecting millions worldwide, with limitations in conventional pharmacotherapy such as delayed onset of action and poor brain targeting. Esketamine, the S-enantiomer of ketamine, has emerged as a rapid-acting antidepressant; however, its therapeutic efficacy is constrained by poor bioavailability and systemic side effects. The present study aims to formulate and characterize esketamine-loaded Nanoglobules to enhance brain delivery and improve therapeutic outcomes.Nanoglobules were prepared using high-speed homogenization followed by ultrasonication technique. Various formulations (NG1–NG4) were developed using different concentrations of lipids and surfactants. The prepared Nanoglobules were evaluated for particle size, polydispersity index (PDI), zeta potential, drug entrapment efficiency, drug content, and in vitro drug release. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) were employed for compatibility and morphological analysis.The optimized formulation exhibited a particle size of approximately 160 nm with a low PDI, indicating uniform distribution. Zeta potential values confirmed good stability of the formulation. High entrapment efficiency and controlled drug release profile were observed over 12 hours, demonstrating sustained drug delivery. FTIR studies confirmed the absence of drug-excipient interactions, while SEM analysis revealed spherical Nanoglobules with smooth surfaces.The developed esketamine-loaded Nanoglobules significantly enhance drug delivery efficiency, offering sustained release and improved targeting potential for depression treatment.

.

KEYWORDS: Nanoglobules; Depression;Nanotherapeutics;Drug Delivery; Controlled Release.

INTRODUCTION

Depression is a chronic and debilitating psychiatric disorder characterized by persistent low mood, loss of interest, cognitive impairment, and reduced quality of life. It affects millions of individuals worldwide and represents a significant public health burden due to its high prevalence and association with suicide and disability. Conventional antidepressant therapies, such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants, often require several weeks to exhibit therapeutic effects and are ineffective in a substantial proportion of patients, leading to treatment-resistant depression (TRD). In recent years, esketamine, the S-enantiomer of ketamine, has emerged as a breakthrough therapy due to its rapid onset of antidepressant action mediated through N-methyl-D-aspartate (NMDA) receptor antagonism and modulation of glutamatergic neurotransmission. Despite its clinical efficacy, the conventional routes of esketamine administration, including intravenous and intranasal delivery, present limitations such as invasiveness, variable bioavailability, and potential systemic side effects.

To overcome these challenges, nanotechnology-based drug delivery systems have gained considerable attention for enhancing the therapeutic performance of central nervous system (CNS) drugs. Among these, nanoglobules, which are submicron-sized lipid-based carriers, offer several advantages including improved drug solubility, enhanced permeability across the blood-brain barrier, controlled drug release, and increased stability. The small particle size and large surface area of nanoglobules facilitate efficient drug transport and targeted delivery to the brain, thereby potentially improving therapeutic outcomes while minimizing adverse effects. Therefore, the present study focuses on the formulation and characterization of esketamine-loaded nanoglobules as a novel nanotherapeutic approach to enhance drug delivery and efficacy in the treatment of depression.

.

MATERIALS AND METHODS

Materials

Table.No.1. Material Used in Formulation.

Sr.No	Materials	Supplier of Materials
1	Esketamine	Dhamtec pharma and consultants, Navi Mumbai
2	Oleic Acid	Research lab fine chem industries Mumbai
3	Tween 80	Research lab fine chem industries Mumbai
4	Propylene Glycol	Research lab fine chem industries Mumbai
5	Distilled Water	Wkm 4L water distillation unit

Table No.2List of Instruments

Sr.No	Instruments	Model &Make
1	Digital Balance	Electro lab
2	pH Meter	Lab India
3	Homogenizer	Remi
4	Ultra Sonicator	Electro lab
5	Ultraviolet spectrophotometer	Shimadzu 1800 UV single bean spectrophotometer
6	Electronic Microscope	Micro lab
7	FTIR instrument	Icon lab
8	Malvern instruments	Icon Lab
9	Scanning Electron Microscopy	Icon lab

METHODS

Preformulation Studies

Organoleptic Evaluation

Organoleptic evaluation is an essential part of preformulation studies in which the drug substance is examined using sensory organs to determine its physical characteristics. In this method, a small quantity of the sample Esketamine is placed on a clean, dry surface like a watch glass and observed under natural or adequate light for its color and appearance, including whether it is crystalline or amorphous. The odor is assessed by gently wafting the vapors toward the nose to identify if it is odorless or possesses any characteristic smell. [68]

Taste evaluation is performed only if the drug is safe and non-toxic, by placing a minute quantity on the tongue to determine whether it is bitter, sweet, or tasteless; however, this step is generally avoided for potent drugs. Additionally, the texture of the sample is evaluated by rubbing it between fingers to determine whether it is smooth, coarse, or gritty. All observations are carefully recorded. This study provides preliminary information about the identity, purity, and quality of the drug substance and helps in the selection of suitable excipients during formulation development.[69]

.

Solubility Studies

The solubility of Esketamine was determined using the shake flask method in various solvents, including distilled water, methanol, ethanol, different lipids, and surfactants. An excess amount of the drug was added to each solvent and shaken continuously until equilibrium was achieved. The samples were then filtered, and the concentration of dissolved drug was analyzed using UV–Visible spectrophotometry to obtain accurate solubility data in each medium. The purpose of this study was to select a suitable lipid and surfactant system for the formulation of Nanoglobules and to ensure maximum drug solubilization. Determining the solubility also helps in evaluating the drug loading capacity, which is essential for achieving efficient encapsulation, stability, and enhanced therapeutic performance of the final formulation.[70]

Melting Point

Melting point determination is an important preformulation study used to assess the purity and identity of a drug substance. In this method, a small quantity of the Esketamine is finely powdered and filled into a capillary tube sealed at one end, ensuring the sample height is about 2–3 mm. The capillary tube is then attached to a thermometer and placed in a melting point apparatus or Thiele tube containing a suitable heating medium such as liquid paraffin. The temperature is gradually increased at a controlled rate, usually 1–2°C per minute, and the sample is carefully observed. The temperature at which the drug begins to melt is noted as the initial melting point, and the temperature at which it completely turns into a clear liquid is recorded as the final melting point. The melting point range is then determined. A sharp and narrow melting point range indicates purity of the drug, whereas a broad range suggests the presence of impurities. This method is simple, accurate, and widely used in pharmaceutical analysis for characterization of drug substances.[71]

Determination of λmax

Determination of λmax (maximum absorption wavelength) is an important step in preformulation studies used for the quantitative analysis of a drug using UV-Visible spectrophotometry. In this method, an accurately weighed quantity of the drug (such as Esketamine) is dissolved in a suitable solvent like methanol to prepare a stock solution. This stock solution is then diluted appropriately to obtain a working solution of suitable concentration. The prepared solution is filled into a quartz cuvette, while the solvent is used as a blank. The sample is scanned over a wavelength range, typically between 200–400 nm,

using a UV-Visible spectrophotometer. During scanning, the instrument records the absorbance at each wavelength and generates an absorption spectrum. The wavelength at which the drug shows maximum absorbance is identified as λmax. This value is crucial for further analytical studies such as calibration curve preparation and drug content estimation, as it provides maximum sensitivity and accuracy.[72]

Calibration Carve of Esketamine

A calibration curve of Esketamine was prepared using UV–Visible spectrophotometry to determine drug concentration.A standard stock solution of Esketamine was prepared by dissolving an accurately weighed quantity of drug (e.g., 10 mg) in a suitable solvent such as methanol and making up the volume to 100 mL to obtain a concentration of 100 µg/mL. From this stock solution, a series of dilutions were prepared (e.g., 2, 4, 6, 8, 10 µg/mL) using phosphate buffer (pH 7.4) or distilled water.The absorbance of each solution was measured at the λmax of Esketamine (approximately 270 nm) using a UV–Visible spectrophotometer, with the respective solvent as blank. A graph was plotted between concentration (µg/mL) on the X-axis and absorbance on the Y-axis**.**The calibration curve showed a linear relationship obeying Beer-Lambert's law within the selected concentration range, and the regression equation (y = mx + c) was used for further drug content and release[73]

Method of preparation of Nanoglobules

High Speed Homogenization

High-speed homogenization is a widely used technique for the preparation of Nanoemulsions, Nanoglobules, and other nanoparticulate drug delivery systems. In this method, the drug Esketamineis first dissolved in the oil phase along with suitable lipids, while the aqueous phase is prepared separately by dissolving surfactants and co-surfactants in distilled water. Both phases are heated to the same temperature (typically 40–60°C) to ensure uniform mixing. The oil phase is then slowly added to the aqueous phase under continuous stirring to form a coarse emulsion.

This coarse emulsion is subjected to high-speed homogenization using a rotor-stator homogenizer at speeds ranging from 1,000 to 2,000 rpm for a specific time (usually 10–30 minutes). The intense mechanical shear forces generated during homogenization reduce the droplet size and produce a fine, stable Nanoemulsion or Nanoglobules system. After homogenization, the formulation is allowed to cool to room temperature, resulting in the formation of stable nanoparticles. This method is simple, efficient, and suitable for large-

scale production, and it plays a crucial role in improving drug solubility, bioavailability, and study[74,75]

Ultrasonication (size reduction step)

Ultrasonication is a widely used technique for the preparation of Nanoemulsions and Nanoglobules, based on the principle of acoustic cavitation. In this method, the drug Esketamine is first dissolved in the oil phase, while the aqueous phase containing surfactants is prepared separately. The oil phase is then added to the aqueous phase under stirring to form a coarse emulsion. This emulsion is subjected to ultrasonication using a probe sonicator or bath sonicator.During ultrasonication, high-frequency sound waves (typically 20–25 kHz) pass through the liquid medium, creating microscopic bubbles that grow and collapse violently. This phenomenon, known as cavitation, generates intense shear forces that break down larger droplets into nanosized particles,resulting in a fine and stable Nanoemulsion.

The process is usually carried out for a specific time (e.g., 5–15 minutes) with controlled amplitude and pulse cycles to avoid overheating. In the case of probe sonication, the probe is directly immersed in the sample, while in bath sonication, the sample container is placed in a water bath. After sonication, the formulation is allowed to cool to room temperature to stabilize the system. Ultrasonication is a simple, efficient, and rapid method that produces uniform particle size distribution and enhances drug solubility and bioavailability, making it highly suitable for nanoparticle formulation development [76]

Solidification

The solidification method is an important technique used in the preparation of lipid-based nanoparticles, where a liquid emulsion is converted into solid particles by controlled cooling. In this method, the drug Esketamine is first dissolved or dispersed in a melted lipid phase at a temperature above its melting point. Separately, an aqueous phase containing suitable surfactants is prepared and heated to the same temperature. The molten lipid phase is then added to the aqueous phase under continuous stirring or homogenization to form a hot oil-in-water emulsion.

This hot emulsion is subsequently subjected to high-speed homogenization or ultrasonication to reduce particle size and obtain a fine dispersion. After achieving the desired droplet size, the emulsion is rapidly cooled to room temperature or below, often using an ice bath. During this cooling process, the lipid phase solidifies, leading to the formation of solid lipid nanoparticles or Nanoglobules. The drug gets entrapped within the solidified lipid matrix, enhancing its stability and controlled release properties. The solidification method is simple,

.

efficient, and widely used in pharmaceutical formulation for improving drug stability, bioavailability, and sustained release behavior.[77]

Filtration

Filtration is a simple and essential method used in pharmaceutical formulation to remove undissolved particles, aggregates, and impurities from a solution or dispersion. In this method, the prepared formulation Esketamine Nanoglobules is passed through a suitable filter medium like Whatman filter paper or a membrane filter (commonly 0.45 μm or 0.22 μm).

The filtration can be carried out using a simple gravity filtration setup with a funnel or by using vacuum filtration for faster and more efficient separation.

The formulation is carefully poured into the filtration apparatus, and the liquid passes through the filter while solid particles and aggregates are retained on the filter surface. For nanoparticle formulations, membrane filtration is often preferred to ensure removal of larger particles and to obtain a uniform dispersion. The filtrate collected is clear and free from particulate matter, which improves the quality, stability, and performance of the formulation.

Filtration is a crucial step in formulation development as it ensures clarity, sterility (in case of fine membrane filters), and uniformity of the final product. It is widely used before analytical studies, filling, or further processing of pharmaceutical formulation [77,78]

(Fourier Transform Infrared spectroscopy) FTIR

Fourier Transform Infrared (FTIR) spectroscopy is a widely used analytical technique in preformulation studies to identify functional groups and to evaluate drug–excipient compatibility. In this method, the sample Esketamine or its formulation is prepared either by the KBr pellet method or by using the ATR (Attenuated Total Reflectance) technique. In the KBr pellet method, a small quantity of the finely powdered drug is mixed with dry potassium bromide and compressed into a transparent pellet using a hydraulic press. In the ATR method, a small amount of sample is directly placed on the crystal surface without complex preparation.

The prepared sample is then placed in the FTIR spectrophotometer, and the spectrum is recorded over a range of 4000–400 cm^{-1}**.** The instrument measures the absorption of infrared radiation by the sample at different wavelengths, producing a spectrum with characteristic peaks corresponding to specific functional groups present in the molecule. The obtained spectrum is then compared with standard reference spectra to confirm the identity of the drug

and to detect any possible interactions between drug and excipients. The presence or absence of significant shifts, disappearance, or appearance of new peaks indicates compatibility or incompatibility. FTIR is a rapid, reliable, and non-destructive technique widely used for qualitative analysis in pharmaceutical research.

the sample, while in bath sonication, the sample container is placed in a water bath. After sonication, the formulation is allowed to cool to room temperature to stabilize the system. Ultrasonication is a simple, efficient, and rapid method that produces uniform particle size distribution and enhances drug solubility and bioavailability, making it highly suitable for nanoparticle formulation development [79,80]

Characterization and Evaluation of Nanoglobules

SEM (Scanning Electron Microscopy)

Scanning Electron Microscopy (SEM) is an advanced analytical technique used to study the surface morphology, (shape), and (size distribution) of drug particles and nanoparticle formulations. In this method, a small amount of the dried sample Esketamine Nanoglobules is mounted on an aluminum stub using double-sided adhesive tape. The sample is then coated with a thin layer of a conductive material, usually gold or platinum, using a sputter coater to prevent charging under the electron beam.

The prepared sample is placed inside the SEM instrument, where it is scanned with a focused beam of high-energy electrons under vacuum conditions. When the electron beam interacts with the surface of the sample, it produces secondary electrons that are detected to form high-resolution images. These images provide detailed information about the surface characteristics, including particle , shape (spherical, irregular), and surface texture (smooth or rough).SEM analysis is highly useful in pharmaceutical research for confirming the formation of nanoparticles, evaluating surface morphology, and ensuring uniformity of the formulation. It is a powerful, precise, and widely used technique for the characterization of nanocarrier systems.[81]

In Vitro Drug Release Study (Dialysis Bag Method)

The dialysis bag method is a widely used technique for evaluating the in vitro drug release profile of nanoparticle and Nanoglobules formulations. In this method, a pre-treated dialysis membrane (semi-permeable membrane) is soaked in distilled water or buffer solution to remove preservatives and to hydrate it properly. A known quantity of the formulation Esketamine Nanoglobules is then placed inside the dialysis bag, and both ends of the bag are

.

securely tied to prevent leakage.The filled dialysis bag is immersed in a beaker containing a suitable dissolution medium, commonly phosphate buffer saline (PBS, pH 6.8 or 7.4), which simulates physiological conditions. The entire setup is maintained at 37 ± 0.5°C and stirred continuously using a magnetic stirrer at a constant speed (e.g., 50–100 rpm) to ensure uniform distribution. At predetermined time intervals, a specific volume of the external medium (e.g., 2 mL) is withdrawn and replaced with fresh buffer to maintain sink conditions. The collected samples are analyzed using a UV-Visible spectrophotometer at the determined λmax to measure the amount of drug released. The cumulative drug release is then calculated and plotted against time to obtain the release profile. This method is simple, reliable, and effective for studying controlled and sustained drug release behavior of pharmaceutical formulations.[82]

Drug Release Calculation

$$\text{Amount of Drug Released} = \frac{\text{Absorbance} \times V_t \times D_f}{\text{Slope} \times 1000}$$

% Drug Release

$$\%\textbf{Drug Release} = \frac{\textbf{Amount of Drug Released}}{\textbf{Total Drug}} \times \mathbf{10}$$

Entrapment Efficiency (EE%)

Entrapment Efficiency (EE%) is an important parameter used to determine the amount of drug encapsulated within a nanoparticle system compared to the total drug used during formulation. In this method, a known quantity of the nanoparticle formulation Esketamine nanoglobules is subjected to centrifugation at high speed (e.g., 1,000–2,000 rpm) for a specific time to separate the entrapped drug (pellet) from the unentrapped/free drug (supernatant).

After centrifugation, the clear supernatant is carefully collected and analyzed using a UV-Visible spectrophotometer at the predetermined λmax to determine the concentration of free (unentrapped) drug. The amount of drug entrapped in the nanoparticles is calculated by subtracting the amount of free drug from the total drug initially added. The entrapment efficiency is then calculated using the formula:

$$\text{EE\%} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

.

A higher EE% indicates better drug incorporation within the nanoparticles, which is desirable for controlled drug delivery systems. This method is simple, reliable, and widely used to evaluate the efficiency of nanoparticle formulations in encapsulating drugs[83]

Drug Content

Drug content determination is an important analytical method used to quantify the actual amount of drug present in a formulation**.** In this method, an accurately weighed quantity of the formulation Esketamine Nanoglobules is taken and dissolved in a suitable solvent like methanol or another appropriate medium to extract the drug completely. The solution is then subjected to sonication or vigorous shaking to ensure complete drug release from the formulation matrix.

The resulting solution is filtered or centrifuged to remove any undissolved particles, and the clear supernatant is collected. This solution is further diluted appropriately to bring the concentration within the measurable range. The absorbance of the sample is then measured using a UV-Visible spectrophotometer at the predetermined λmax of the drug.

The drug content is calculated using a previously prepared calibration curve. The amount of drug present in the formulation is expressed as a percentage of the theoretical drug content. This method is simple, accurate, and essential for ensuring uniformity, quality, and consistency of pharmaceutical formulations.[84]

Formula

$$\text{Drug Content (\%)} = \frac{\text{Actual Drug Present}}{\text{Theoretical Drug Amount}} \times 100$$

pH Determination

pH determination is an essential evaluation parameter in pharmaceutical formulations to ensure stability, compatibility, and safety of the product. In this method, the pH of the formulation (such as Esketamine Nanoglobules) is measured using a digital pH meter**.** Before measurement, the pH meter is calibrated using standard buffer solutions of known pH values, typically pH 4.0, 7.0, and 9.2, to ensure accuracy.

A small quantity of the formulation is taken in a clean beaker, and if required, it is diluted with distilled water to obtain a uniform solution. The electrode of the pH meter is then immersed in the sample solution, ensuring proper contact without touching the walls or bottom of the container. The reading is allowed to stabilize, and the pH value is recorded. After measurement, the electrode is rinsed with distilled water to avoid contamination. This

method is simple, rapid, and reliable. Maintaining an appropriate pH is crucial as it affects drug stability, solubility, and patient acceptability, especially in formulations intended for oral, topical, or parenteral administration.[85]

Viscosity

Viscosity determination is an important parameter used to evaluate the flow behavior and consistency of pharmaceutical formulations. In this method, the viscosity of the formulation Esketamine Nanoglobules is measured using a Brookfield viscometer or similar rotational viscometer. Before measurement, the instrument is properly calibrated and a suitable spindle is selected based on the viscosity range of the sample.A specific quantity of the formulation is taken in a clean, dry beaker, and the spindle is immersed into the sample without touching the bottom or sides. The viscometer is then operated at a predetermined speed (rpm), and the resistance offered by the fluid to the rotating spindle is measured. The reading is displayed directly in units such as centipoise (cP). The measurement is usually performed at controlled temperature (e.g., 25°C) to ensure consistency.Viscosity plays a crucial role in determining the stability, spreadability, and drug release behavior of the formulation. This method is simple, accurate, and widely used in pharmaceutical research to ensure uniformity and quality of liquid and semi-solid dosage forms.[86]

Zeta Potential

The zeta potential of Esketamine-loaded Nanoglobules was measured using a zeta potential analyzer based on electrophoretic light scattering**.** The formulation was diluted appropriately with distilled water to obtain a clear and stable dispersion.

The diluted sample was transferred into a clean zeta cell, ensuring the absence of air bubbles. Measurements were carried out at 25°C by applying an electric field, which caused the charged particles to migrate. The electrophoretic mobility of particles was recorded and converted into zeta potential values using the Smoluchowski equation. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation**.**[87]

Particle Size and Polydispersity Index (PDI)

Particle size and polydispersity index (PDI) are critical parameters in the characterization of nanoparticulate drug delivery systems. These are commonly determined using Dynamic Light Scattering (DLS) technique with instruments such as a zeta sizer. In this method, a small amount of the nanoparticle formulation (e.g., Esketamine Nanoglobules) is diluted with distilled water or an appropriate medium to avoid multiple scattering effects. The diluted

sample is then transferred into a clean, dust-free cuvette and placed in the instrument. The system measures fluctuations in light scattering caused by the Brownian motion of particles and calculates the average particle size and size distribution.The particle size is usually expressed in nanometers (nm), representing the mean diameter of particles, while the PDI indicates the uniformity of particle size distribution. A PDI value below 0.3 suggests a narrow and uniform distribution, whereas values above 0.5 indicate a broad and heterogeneous system. The analysis is performed at a controlled temperature, typically around 25°C, and results are obtained in the form of size distribution graphs and numerical values. This method is rapid, non-invasive, and essential for ensuring stability, reproducibility, and performance of nanoparticle formulations.

Stability studies are conducted to evaluate the physical, chemical, and microbiological stability of a pharmaceutical formulation over time under different environmental conditions. In this method, the prepared formulation Esketamine Nanoglobules is packed in suitable containers and stored under specified conditions according to guidelines provided by the International Council for Harmonisation (ICH). The samples are typically stored at different conditions such as 25 ± 2°C / 60 ± 5% RH (room temperature) and 40 ± 2°C / 75 ± 5% RH (accelerated conditions) for a defined period (e.g., 1, 2, and 3 months or longer). At predetermined time intervals, samples are withdrawn and evaluated for various parameters such as appearance, pH, drug content, particle size, and in vitro drug release.The obtained data are compared with initial values to detect any significant changes in the formulation. Stability indicating assays are performed, often using UV-Visible spectrophotometry. The results help in determining the shelf life, storage conditions, and overall stability profile of the formulation. Stability studies are essential to ensure the safety, efficacy, and quality of pharmaceutical products throughout their shelf life.[89,90]

The samples are typically stored at different conditions such as 25 ± 2°C / 60 ± 5% RH (room temperature) and 40 ± 2°C / 75 ± 5% RH (accelerated conditions) for a defined period (e.g., 1, 2, and 3 months or longer). At predetermined time intervals, samples are withdrawn and evaluated for various parameters such as appearance, pH, drug content, particle size, and in vitro drug release.The obtained data are compared with initial values to detect any significant changes in the formulation. Stability indicating assays are performed, often using UV-Visible spectrophotometry. The results help in determining the shelf life, storage conditions, and overall stability profile of the formulation. Stability studies are essential to ensure the safety, efficacy, and quality of pharmaceutical products throughout their shelf life.[89,90]

.

Stability Studies

Stability studies are conducted to evaluate the physical, chemical, and microbiological stability of a pharmaceutical formulation over time under different environmental conditions.

In this method, the prepared formulation Esketamine Nanoglobules is packed in suitable containers and stored under specified conditions according to guidelines provided by the International Council for Harmonisation (ICH).

The samples are typically stored at different conditions such as 25 ± 2°C / 60 ± 5% RH (room temperature) and 40 ± 2°C / 75 ± 5% RH (accelerated conditions) for a defined period (e.g., 1, 2, and 3 months or longer). At predetermined time intervals, samples are withdrawn and evaluated for various parameters such as appearance, pH, drug content, particle size, and in vitro drug release.The obtained data are compared with initial values to detect any significant changes in the formulation. Stability indicating assays are performed, often using UV-Visible spectrophotometry. The results help in determining the shelf life, storage conditions, and overall stability profile of the formulation. Stability studies are essential to ensure the safety, efficacy, and quality of pharmaceutical products throughout their shelf life.[89,90]

FORMULATION

Batch	NG1	NG2	NG3	NG4	Total wt
Esketamine	10mg(0.01g)	10mg(0.01g)	10mg(0.01g)	10mg(0.01g)	0.04g
Oleic acid	1ml	1ml	1ml	1ml	4ml
Tween80	3ml	3ml	3ml	3ml	12ml
Propylene glycol	1ml	1ml	1ml	1ml	4ml
Distilled water	5ml	5ml	5ml	5ml	20ml
Total	10.01	10.01	10.01	10.01	40.04

RESULTS AND DISCUSSION

Preformulation Studies

Organoleptic Characteristics

Table No.8. The physical characterization (color ,odor and appearance) of esketamine was done.

Sr.No	Organoleptic properties	Esketamine
1	color	White to off -white crystalline powder
2	odor	odorless
3	Appearance	Crystalline powder

.

Determination of melting point

Table No.9 Melting Point

Sr.No.	Melting point	Average Melting Point (°C)
1	93	
2	94	94 ± 1°C
3	95	

The melting point of esketamine was found to be 94 ± 1°C

Solubility

Table No. 10. solubility

The solubility study of esketamine was determined using capillary tube method

Sr.No	Name of solvent	Solubility
1	Water	Sparingly solubles
2	Phosphate Buffer pH 7.4	Slightly soluble
3	Ethanol	Freely soluble
4	Methanol	Soluble
5	Chloroform	Soluble

U.V Spectroscopy

Calibration curve of esketamine

Table No.11. Calibration curve of esketamine

Sr.No	Concentration	Absorbance
1	**0**	**0.000**
2	**2**	**0.285**
3	**4**	**0.567**
4	**6**	**0.842**
5	**8**	**1.126**
6	**10**	**1.405**

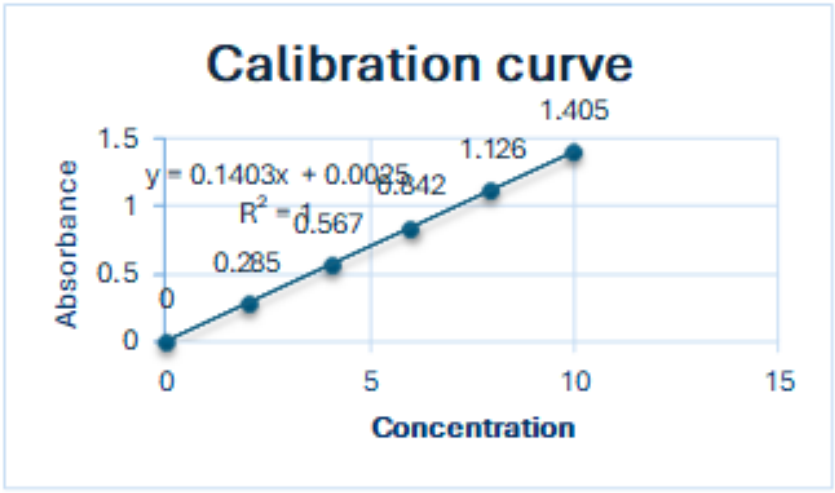


Fig.No.6. Callibration curve of Esketamine

.

Table No. 12. Results of Regression Analysis of UV Method of Esketamine

Statistical Parameters	Results
λmax	270nm
Regression Equation	Y = 0.1403x + 0.001
Slope (m)	0.140
Intercept (C)	0.001
Correlation Coefficient (R^2)	0.999

The provided statistical parameters for the analytical method indicate a high degree of precision and linearity. The maximum absorption wavelength was determined to be 270 nm. The regression analysis yielded the equation Y = 0.1403x + 0.001, featuring a slope (m)of 0.140 and an intercept (C) of 0.001. Notably, the correlation coefficient (R^2) of 0.999 demonstrates a near-perfect linear relationship, which is essential for accurate data visualization and pharmacological studies.

FTIR (Fourier Transform Infrared spectroscopy)

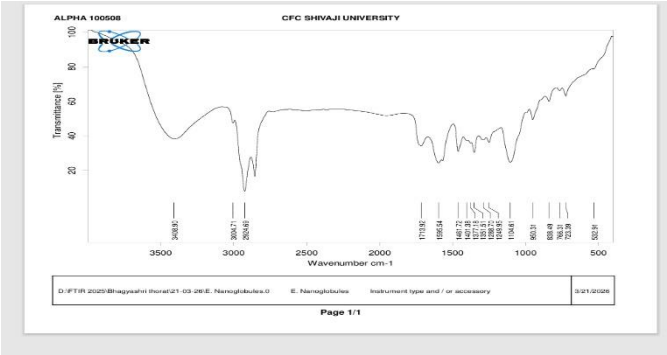
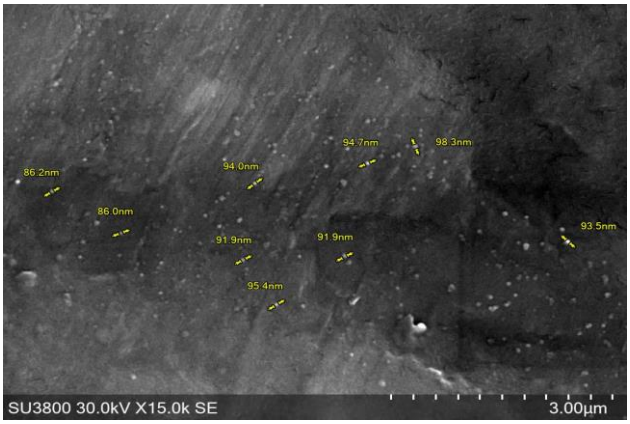
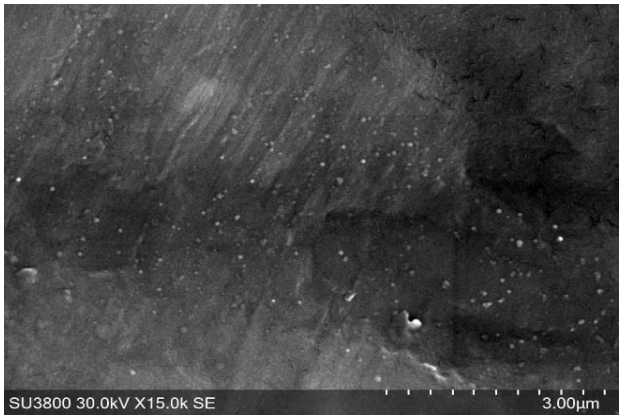


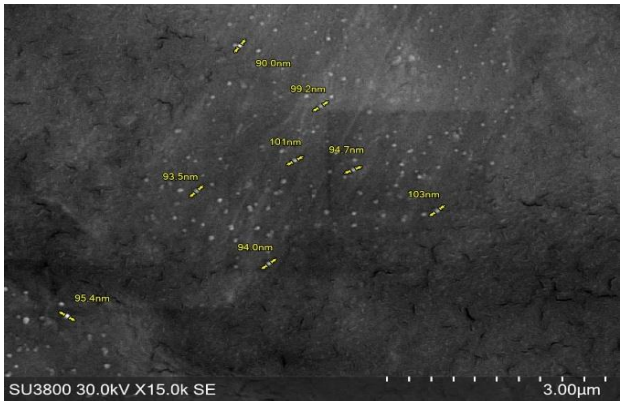
Fig.No.7 FTIR.

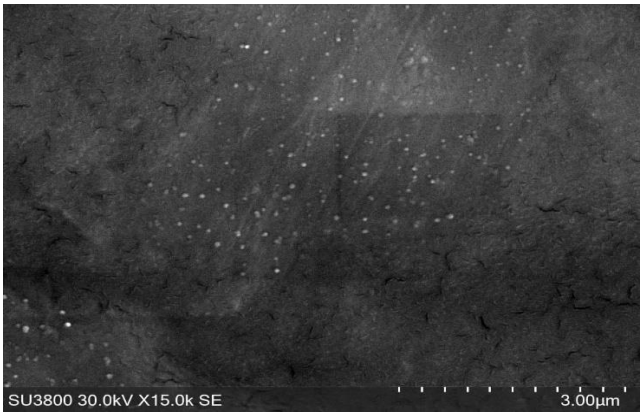
SEM (Scanning Electron Microscopy)



.







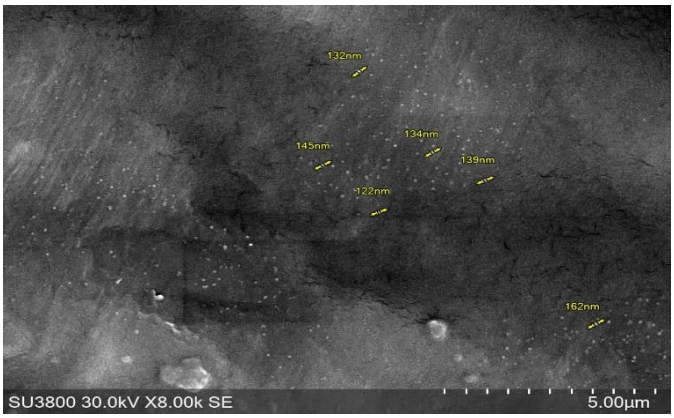


Fig.No. 8. SEM Esketamine Nanoglobules.

.

The scanning electron microscopy (SEM) image illustrates the surface morphology and particle size distribution of the formulated nanoglobules. The micrograph reveals spherical particles dispersed throughout the matrix, with specific measurements highlighting sizes such as 122 nm, 132 nm, 134 nm, 139 nm, 145 nm, and 162 nm. These findings are consistent with the previously recorded mean particle sizes for batches NG1 through NG4, which ranged approximately between 155 nm and 178 nm. The uniformity in shape and the nanoscale dimensions confirmed by the 5.00 µm scale bar suggest a successful formulation suitable for targeted drug delivery applications.

In Vitro Drug Release Studies

Table No.14 . % Drug Release of NG1

Time (hr)	%Drug Release
0	0 %
1	32 %
2	40%
4	52%
6	60 %
8	66%
12	72%

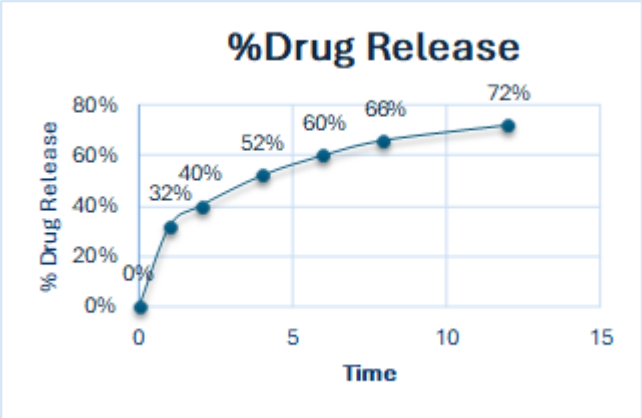


Fig.No.9 NG 1 graph.

.

Fig.No.10 NG 1.

Table No. 15 .%Drug Release NG 2

Time(hr)	%Drug Release
0	0%
1	38%
2	46%
4	60%
6	68%
8	75%
12	82%

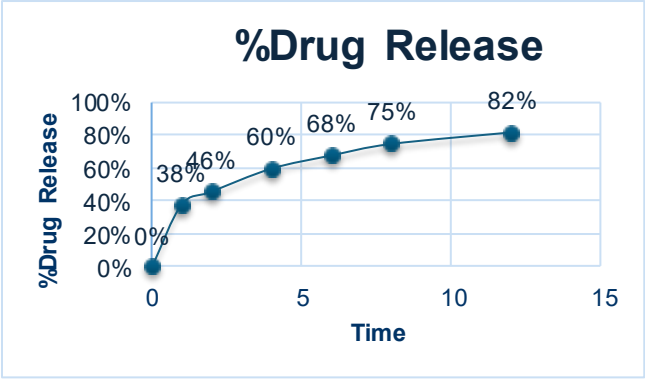


Fig.No.12 NG 2 Graph.

.

Fig.No.13 NG 2.

Table No.16. % Drug Release NG 3.

Time(hr)	%Drug Release
0	0%
1	42%
2	52%
4	68%
6	75%
8	82%
12	90%

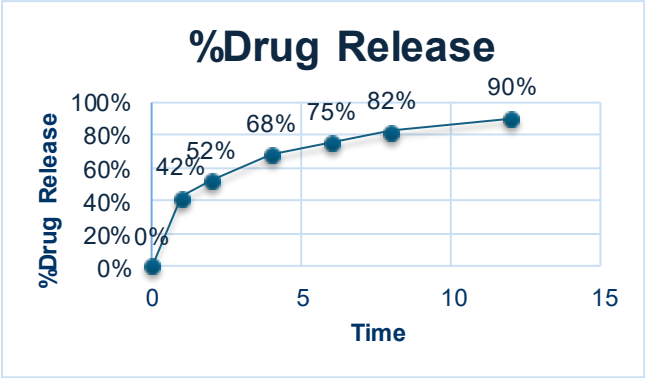


Fig.No.14 NG 3 Graph.

.

Fig.No.16. NG 3.

Table No .17 .% Drug Release NG 4.

Time	%Drug Release
0	0 %
1	48%
2	60%
4	75%
6	82%
8	90%
12	96%

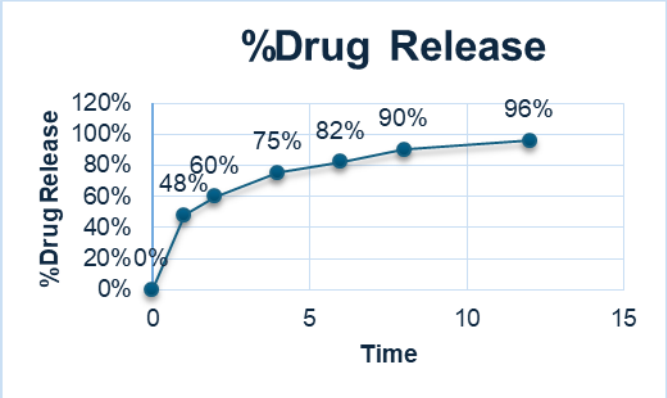


Fig.No.18 .NG 4 Graph.

Table No. 18 . Comparative %Drug Release NG 1 To NG 4.

Time	NG1	NG2	NG3	NG4
0	0%	0%	0%	0%
1	32 %	38%	42%	48%
2	40%	46%	52%	60%
4	52%	60%	68%	75%
6	60 %	68%	75%	82%
8	66%	75%	82%	90%
12	72%	82%	90%	96%

.

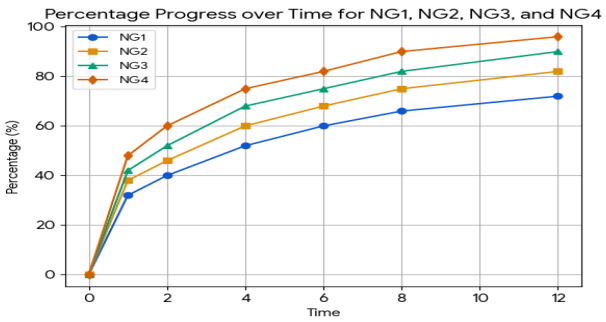


Fig.No.21.% Drug Release NG1 to NG 4.

The in vitro drug release study conducted over 12 hours reveals a distinct biphasic release pattern across all nanoglobule batches. Initially, all formulations exhibited a relatively rapid release within the first 2 hours, with batch NG4 showing the highest initial burst at 60%, while NG1 showed the lowest at 40%. As the study progressed to the 12-hour mark, a more sustained release profile was observed. NG4 achieved the most extensive cumulative drug release at 96%, followed by NG3 (90%) and NG2 (82%), with NG1 reaching 72%. This variation across batches suggests that the formulation parameters significantly influence the release kinetics, providing a range of options for tailoring the delivery speed of the therapeutic agent.

Entrapment Efficiency (EE%)

Table No.19. (NG 1–NG 4)

Batch	Entrapment Efficiency (EE%)
NG1	72.4 ± 1.5
NG2	78.6 ± 1.2
NG3	83.2 ± 1.1
NG4	87.8 ± 1.0

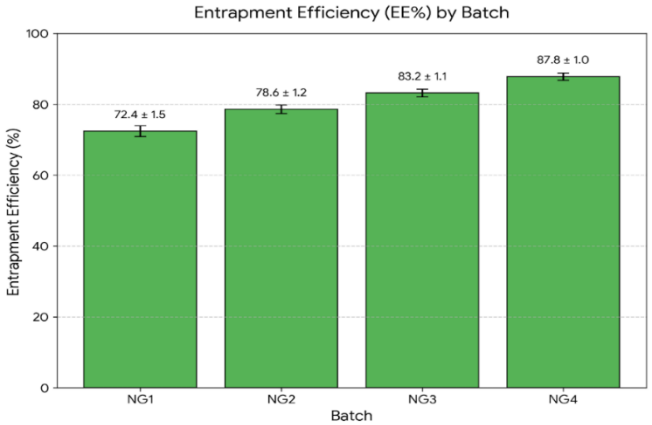


Fig.No.22. Entrapment Efficiency(EE%)

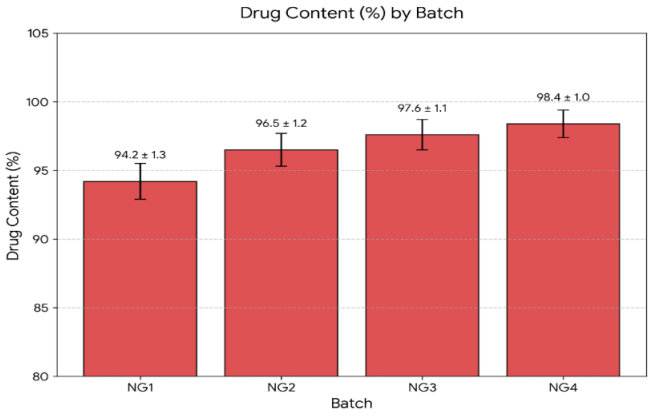
.

The assessment of Entrapment Efficiency (EE%) across the four Nanoglobules batches reveals a progressive increase in the formulation's ability to encapsulate the drug. Batch NG1 exhibited the lowest entrapment at 72.4 ± 1.5 while subsequent modifications in the formulation led to improved results in NG2 78.6 ± 1.2 and NG3 83.2 ± 1.1 The highest efficiency was achieved by batch NG4, which successfully entrapped 87.8 ± 1.0 of the drug. The consistently low standard deviation values across all batches indicate a high degree of reproducibility in the preparation process, suggesting that the optimized conditions for NG4 are particularly effective for maximizing drug loading within the Nanoglobules structure

Drug Content

Table No.20. (NG 1–NG 4)

Batch	Drug Content (%)
NG1	94.2 ± 1.3
NG2	96.5 ± 1.2
NG3	97.6 ± 1.1
NG4	98.4 ± 1.0



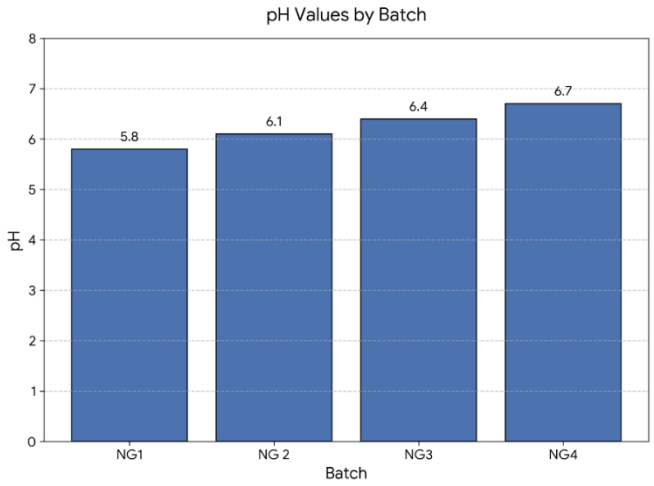
The analysis of Drug Content (%) across the nanoglobule formulations indicates a high level of uniformity and successful drug incorporation within all tested batches. Batch NG1 showed a drug content of 94.2 ± 1.3 while successive batches demonstrated incremental improvements, with NG2 at 96.5 ± 1.2 and NG3 at 97.6 ± 1.1 The highest drug content was observed in batch NG4, reaching 98.4 ± 1.0 suggesting that this specific formulation optimization provides the most efficient drug loading. These high percentage values, accompanied by low standard deviations, confirm the reliability of the preparation method and ensure that nearly the entire intended dose is present within the nanoglobule carrier system.

.

pH Determination

Table No.21. pH Determination

Batch	pH
NG1	5.8
NG2	6.1
NG3	6.4
NG4	6.7



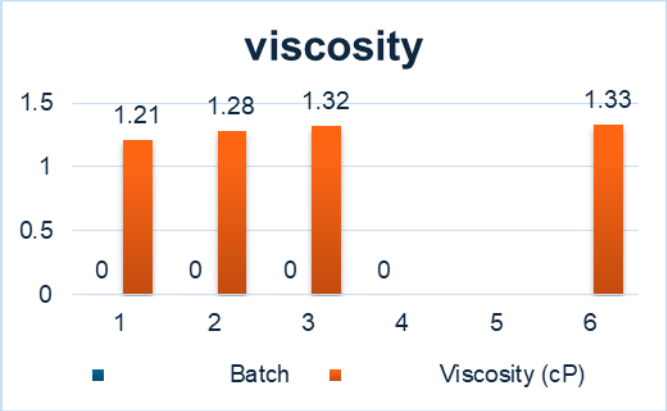
The pH evaluation of the nanoglobule batches reveals a gradual shift toward more neutral values across the formulation series. Batch NG1 recorded the lowest pH at 5.8, followed by incremental increases in NG2 at 6.1 and NG3 at 6.4. The final batch, NG4, exhibited the highest pH value of 6.7. These results are particularly significant for intranasal delivery, as the measured pH range of 5.8 to 6.7 is generally compatible with the physiological environment of the nasal mucosa, which typically maintains a pH near 5.5 to 6.5. This ensures that the formulations are likely to be non-irritating and suitable for nose-to-brain drug transport.

Viscosity

Table No.22.Viscosity

Batch	Viscosity (cP)
NG1	1.21
NG2	1.28
NG	1.32
NG4	1.33

.



The viscosity measurements for the nanoglobule batches indicate a slight, consistent increase in resistance to flow as the formulations were optimized. Batch NG1 exhibited the lowest viscosity at 1.21 cP, while NG2 and NG3 (identified as NG) showed moderate increases to 1.28 cP and 1.32 cP, respectively. The final formulation, NG4, reached a peak viscosity of 1.33 cP. Maintaining a low viscosity profile, particularly within the range of 1.21 to 1.33 cP, is critical for intranasal drug delivery. These values ensure that the nanoglobules remain easily sprayable and capable of efficient distribution across the nasal mucosa, facilitating effective nose-to-brain drug transport without being hindered by excessive thickness.

Zeta Potential

Table No.23.Sample Details

Sample Name	**E. Nanoglobules 2**
SOP Name	mansettings. Nano
File Name	Zeta Potential. Dts
Record Number	5
Dispersant Name	Water
Dispersant RI	1.330
Viscosity (cP)	0.8872
Dispersant Dielectric Constant	78.5

The Zeta Potential Distribution graph for the nanoglobule formulation (Record 5: E. Nanoglobules 2) displays a sharp, well-defined peak centered at approximately -50 mV. This significant negative surface charge indicates strong electrostatic repulsion between the particles, which is a key factor in preventing aggregation and ensuring the long-term physical stability of the nanoglobules in suspension. The high intensity of the peak, reaching nearly 150,000 counts, further suggests a high concentration of particles with a uniform surface charge distribution. Such a robust zeta potential value is essential for maintaining the

integrity of the delivery system, particularly for pharmacological applications requiring stable drug entrapment and consistent release.

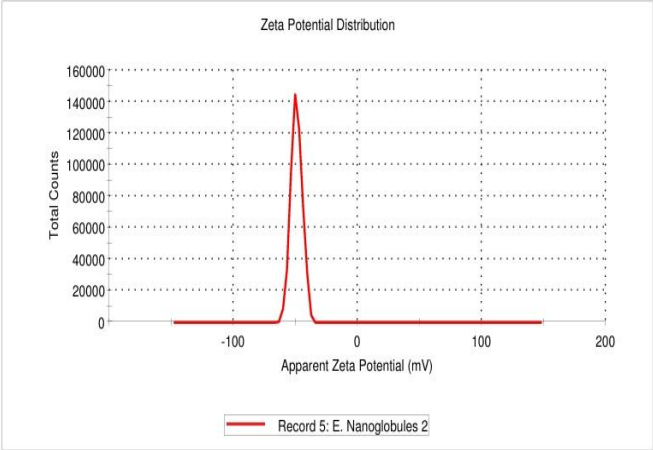


Table No. 25. Results.

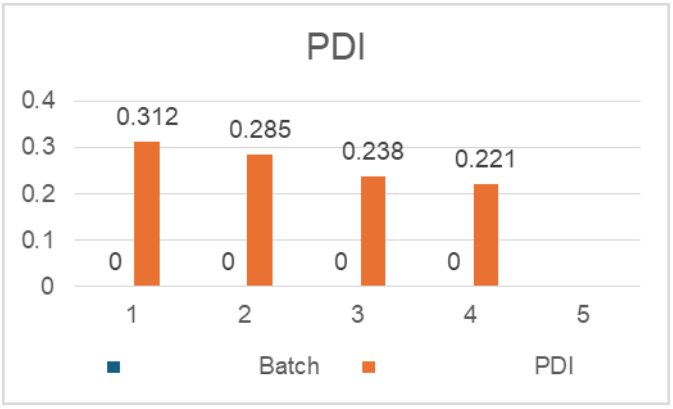
Zeta Potential (mV): -49.0	Mean (mV)	Area (%)	St Dev (mV)
Zeta Deviation (mV): 4.55	Peak 1: -49.0	100.0	4.55
Conductivity (mS/cm): 1.04	Peak 2: 0.00	0.0	0.00
Result quality : Good	Peak 3: 0.00	0.0	0.00

Particle Size and Polydispersity Index (PDI)

Table No. 26.Particle Size Analysis.

Batch	Particle Size(nm)
NG1	178 ± 2.3
NG2	170 ± 2.0
NG3	155 ± 1.8
NG4	160 ± 1.5

Polydispersity Index PDI



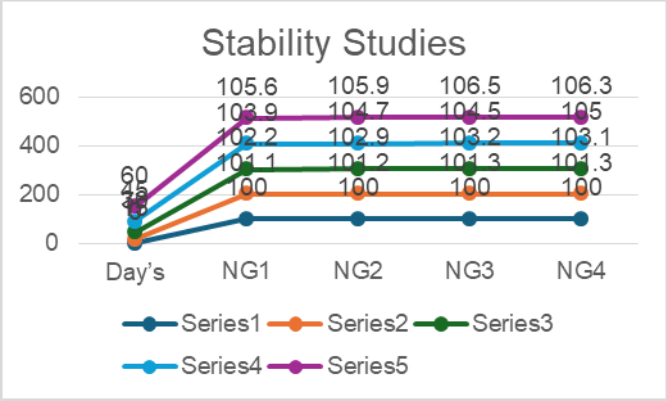
The Polydispersity Index (PDI) of the Nanoglobules batches indicates a high degree of uniformity and monodispersity within the formulations. Batch NG1 exhibited the highest PDI

at 0.312, which gradually decreased through NG2 (0.285) and NG3 (0.238). The lowest PDI was observed in batch NG4 at 0.221. Generally, a PDI value below 0.3 is considered ideal for lipid-based nanocarriers, as it signifies a narrow particle size distribution. These results suggest that the optimization process effectively refined the formulation, leading to a more homogenous system in batch NG4, which is essential for ensuring consistent drug release and stability in nose-to-brain drug transport applications.

Stability studies

Table No. 28. Stability Study Table

Day's	NG1	NG2	NG3	NG4
0	100	100	100	100
15	101.1	101.2	101.3	101.3
30	102.2	102.9	103.2	103.1
45	103.9	104.7	104.5	105.0
60	105.6	105.9	106.5	106.3



Stability study

The stability of Esketamine-loaded Nanoglobules (NG1–NG4) was evaluated over a period of 60 days by monitoring the percentage change in particle size. At day 0, all formulations showed 100%, indicating the initial baseline. A gradual increase in particle size was observed during the study period. By day 15, the values slightly increased to 101.1%, 101.2%, 101.3%, and 101.3% for NG1, NG2, NG3, and NG4 respectively. At day 30, further increases were noted (102.2%, 102.9%, 103.2%, and 103.1%). This trend continued at day 45 with values reaching 103.9%, 104.7%, 104.5%, and 105.0%. At the end of 60 days, the percentage change was found to be 105.6% (NG1), 105.9% (NG2), 106.5% (NG3), and 106.3% (NG4).

.

DISCUSSION

The study demonstrated successful formulation of stable nanoglobules with improved delivery properties. The nanosize enhances permeability, while sustained release improves therapeutic effect and patient compliance.

ACKNOWLEDGMENT

I would like to express my special thanks of gratitude towards **Dr. Sachin R. Hangargekar** for giving opportunity without your support guidance this Research Paper would not have been completed.

REFERENCES

1. World Health Organization. Depression and other common mental disorders: global health estimates. Geneva: WHO; 2017.
2. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 2006;163(11):1905–17.
3. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. Major depressive disorder. Nat Rev Dis Primers. 2016;2:16065
4. Albert PR. Why is depression more prevalent in women? J Psychiatry Neurosci. 2015;40(4):219–21.
5. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 2006;163(11):1905–17.
6. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. Major depressive disorder. Nat Rev Dis Primers. 2016;2:16065
7. Cipriani A, Furukawa TA, Salanti G, Geddes JR, Higgins JP, Churchill R, et al. Comparative efficacy and acceptability of 21 antidepressant drugs for acute treatment of major depressive disorder: a systematic review and network meta-analysis. Lancet. 2018;391(10128):1357–66.
8. Wilkinson ST, Ballard ED, Bloch MH, Mathew SJ, Murrough JW, Feder A, et al. The effect of ketamine on suicidal ideation: a systematic review and meta-analysis. Am J Psychiatry. 2018;175(2):150–58.
9. Hashimoto K. The role of NMDA receptor antagonists in depression: ketamine and beyond. Pharmaceuticals. 2010;3(9):304–20.

.

10. 10.U.S. Food and Drug Administration. FDA approves new nasal spray medication for treatment-resistant depression. Silver Spring (MD): FDA; 2019.
11. Daly EJ, Singh JB, Fedgchin M, Cooper K, Lim P, Shelton RC, et al. Efficacy and safety of intranasal esketamine adjunctive to oral antidepressant therapy in treatment-resistant depression. JAMA Psychiatry. 2018;75(2):139–48.
12. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry. 2000;47(4):351–54.
13. Daly EJ, Singh JB, Fedgchin M, Cooper K, Lim P, Shelton RC, et al. Efficacy and safety of intranasal esketamine adjunctive to oral antidepressant therapy in treatment-resistant depression. JAMA Psychiatry. 2018;75(2):139–48. Wilkinson ST, Ballard ED, Bloch MH, Mathew SJ, Murrough JW, Feder A, et al. The effect of ketamine on suicidal ideation: a systematic review and meta-analysis. Am J Psychiatry. 2018;175(2):150–58.
14. Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, et al. Glutamate and GABA systems as targets for novel antidepressant treatments. Mol Psychiatry. 2002;7(S1):S71–80.
15. Popova V, Daly EJ, Trivedi M, Cooper K, Lane R, Lim P, et al. Efficacy and safety of flexibly dosed intranasal esketamine in treatment-resistant depression. Am J Psychiatry. 2019;176(6):428–38.
16. Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, et al. Glutamate and GABA systems as targets for novel antidepressant treatments. Mol Psychiatry. 2002;7(S1):S71–80.
17. Domino EF. Taming the ketamine tiger. Anesthesiology. 2010;113(3):678–84.
18. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine. J Neurosci. 1997;17(8):2921–27.
19. Maeng S, Zarate CA Jr, Du J, Schloesser RJ, McCammon J, Chen G, et al. Cellular mechanisms underlying the antidepressant effects of ketamine. Biol Psychiatry. 2008;63(4):349–52.
20. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959–64.
21. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68–72.

.

22. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine. J Neurosci. 1997;17(8):2921–27.
23. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci. 2007;27(43):11496–500.
24. Maeng S, Zarate CA Jr, Du J, Schloesser RJ, McCammon J, Chen G, et al. Cellular mechanisms underlying the antidepressant effects of ketamine. Biol Psychiatry. 2008;63(4):349–52.
25. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959–64.
26. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68–72.
27. Maeng S, Zarate CA Jr, Du J, Schloesser RJ, McCammon J, Chen G, et al. Cellular mechanisms underlying the antidepressant effects of ketamine. Biol Psychiatry. 2008;63(4):349–52.
28. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338(6103):68–72.
29. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329(5994):959–64.
30. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci. 2010;33(2):67–75.
31. Abdallah CG, Sanacora G, Duman RS, Krystal JH. Ketamine and rapid-acting antidepressants: a window into a new neurobiology for mood disorder therapeutics. Annu Rev Med. 2015;66:509–23.
32. 32.. Daly EJ, Trivedi MH, Janik A, Li H, Zhang Y, Li X, et al. Efficacy of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. JAMA Psychiatry. 2019;76(9):893–903.
33. Food and Drug Administration. Spravato (esketamine) prescribing information. Silver Spring (MD): FDA; 2019.
34. Domino EF. Taming the ketamine tiger. Anesthesiology. 2010;113(3):678–84.
35. Zanos P, Moaddel R, Morris PJ, Riggs LM, Highland JN, Georgiou P, et al. Ketamine and ketamine metabolite pharmacology. Pharmacol Rev. 2018;70(3):621–60.

.

36. Popova V, Daly EJ, Trivedi M, Cooper K, Lane R, Lim P, et al. Efficacy and safety of flexibly dosed intranasal esketamine in TRD. Am J Psychiatry. 2019;176(6):428–38.
37. Sweetman SC. Martindale: The Complete Drug Reference. 36th ed. London: Pharmaceutical Press; 2009.
38. 38 Illum L. Nasal drug delivery—possibilities, problems and solutions. J Control Release. 2003;87(1–3):187–98.
39. Daly EJ, Singh JB, Fedgchin M, Cooper K, Lim P, Shelton RC, et al. Efficacy and safety of intranasal esketamine adjunctive to oral antidepressant therapy in treatment-resistant depression. JAMA Psychiatry. 2018;75(2):139–48.
40. Food and Drug Administration. Spravato (esketamine) prescribing information. Silver Spring (MD): FDA; 2019.
41. Domino EF. Taming the ketamine tiger. Anesthesiology. 2010;113(3):678–84.
42. Zanos P, Moaddel R, Morris PJ, Riggs LM, Highland JN, Georgiou P, et al. Ketamine and ketamine metabolite pharmacology. Pharmacol Rev.;70(3):621–201860.
43. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry. 2000;47(4):351–54.
44. Daly EJ, Trivedi MH, Janik A, Li H, Zhang Y, Li X, et al. Efficacy of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. JAMA Psychiatry. 2019;76(9):893–903.
45. Wajs E, Aluisio L, Holder R, Daly EJ, Lane R, Lim P, et al. Esketamine nasal spray plus oral antidepressant in patients with treatment-resistant depression: long-term safety and maintenance. J Clin Psychiatry. 2020;81(3):19m12891.
46. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry. 2000;47(4):351–54.
47. Daly EJ, Trivedi MH, Janik A, Li H, Zhang Y, Li X, et al. Efficacy of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. JAMA Psychiatry. 2019;76(9):893–903.
48. Wajs E, Aluisio L, Holder R, Daly EJ, Lane R, Lim P, et al. Long-term safety of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. J Clin Psychiatry. 2020;81(3):19m12891.

.

49. Daly EJ, Trivedi MH, Janik A, Li H, Zhang Y, Li X, et al. Efficacy of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. JAMA Psychiatry. 2019;76(9):893–903.
50. Food and Drug Administration. Spravato (esketamine) prescribing information. Silver Spring (MD): FDA; 2019.
51. Popova V, Daly EJ, Trivedi M, Cooper K, Lane R, Lim P, et al. Efficacy and safety of flexibly dosed intranasal esketamine in treatment-resistant depression. Am J Psychiatry. 2019;176(6):428–38.
52. Zanos P, Moaddel R, Morris PJ, Riggs LM, Highland JN, Georgiou P, et al. Ketamine and ketamine metabolite pharmacology. Pharmacol Rev. 2018;70(3):621–60.
53. Pardridge WM. The blood–brain barrier: bottleneck in brain drug development. NeuroRx. 2005;2(1):3–14.
54. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurodegenerative diseases. J Control Release. 2016;235:34–47.
55. Illum L. Is nose-to-brain transport of drugs in man a reality? J Pharm Pharmacol. 2004;56(1):3–17.
56. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. Neurobiol Dis. 2010;37(1):13–25.
57. Löscher W, Potschka H. Blood–brain barrier active efflux transporters: ATP-binding cassette gene family. NeuroRx. 2005;2(1):86–98.
58. Kreuter J. Nanoparticles—A historical perspective. Int J Pharm. 2007;331(1):1–10.
59. Kreuter J. Nanoparticles—A historical perspective. Int J Pharm. 2007;331(1):1–10.
60. Illum L. Nasal drug delivery—possibilities, problems and solutions. J Control Release. 2003;87(1–3):187–98.
61. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2012;64:83–101.
62. Patel MM, Patel BM. Crossing the blood–brain barrier: recent advances in drug delivery to the brain. CNS Drugs. 2017;31(2):109–33.
63. Müller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm. 2002;242(1–2):121–28.
64. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurological disorders. J Control Release. 2016;235:34–47.

.

65. Kreuter J. Nanoparticles—A historical perspective. Int J Pharm. 2007;331(1):1–10.
66. Illum L. Nasal drug delivery—possibilities, problems and solutions. J Control Release. 2003;87(1–3):187–98.
67. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2012;64:83–101.
68. Patel MM, Patel BM. Crossing the blood–brain barrier: recent advances in drug delivery to the brain. CNS Drugs. 2017;31(2):109–33.
69. Müller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved drug delivery. Int J Pharm. 2002;242(1–2):121–28.
70. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurological disorders. J Control Release. 2016;235:34–47.
71. Daly EJ, Trivedi MH, Janik A, Li H, Zhang Y, Li X, et al. Efficacy of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. JAMA Psychiatry. 2019;76(9):893–903.
72. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurological disorders. J Control Release. 2016;235:34–47.
73. Kreuter J. Nanoparticles—A historical perspective. Int J Pharm. 2007;331(1):1–10.
74. Wajs E, Aluisio L, Holder R, Daly EJ, Lane R, Lim P, et al. Long-term safety of esketamine nasal spray plus oral antidepressant in treatment-resistant depression. J Clin Psychiatry. 2020;81(3):19m12891.
75. Zanos P, Moaddel R, Morris PJ, Riggs LM, Highland JN, Georgiou P, et al. Ketamine and ketamine metabolite pharmacology. Pharmacol Rev. 2018;70(3):621–60.
76. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2012;64:83–101.
77. Illum L. Nasal drug delivery—possibilities, problems and solutions. J Control Release. 2003;87(1–3):187–98.
78. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2012;64:83–101.
79. Pardridge WM. The blood–brain barrier: bottleneck in brain drug development. NeuroRx. 2005;2(1):3–14.

.

80. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurological disorders. J Control Release. 2016;235:34–47.
81. somani, S.; Robb, G.; Pickard, B.; Dufès, C. Enhanced gene expression in the brain following intravenous administration of lactoferrin-bearing polypropylenimine dendriplex. J. Control. Release 2015,
82. Yin, Y.; Fu, C.; Li, M.; Li, X.; Wang, M.; He, L.; Zhang, L.-M.; Peng, Y. A pH-sensitive hyaluronic acid prodrug modified with lactoferrin for glioma dual-targeted treatment. Mater. Sci. Eng. C 2016,
83. Magro, R.D.; Ornaghi, F.; Cambianica, I.; Beretta, S.; Re, F.; Musicanti, C.; Rigolio, R.; Donzelli, E.; Canta, A.R.; Ballarini, E.; et al. ApoE-modified solid lipid nanoparticles: A feasible strategy to cross the blood-brain barrier. J. Control. Release 2017
84. Neves, A.R.; Queiroz, J.F.; Weksler, B.; Romero, I.; Couraud, P.-O.; Reis, S. Solid lipid nanoparticles as a vehicle for brain-targeted drug delivery: Two new strategies of functionalization with apolipoprotein E. Nanotechnology 2015,
85. Jose, S.; Sowmya, S.; Cinu, T.; Aleykutty, N.; Thomas, S.; Souto, E. Surface modified PLGA nanoparticles for brain targeting of Bacoside-A. Eur. J. Pharm. Sci. 2014,
86. Wang, C.-X.; Huang, L.-S.; Hou, L.-B.; Jiang, L.; Yan, Z.-T.; Wang, Y.-L.; Chen, Z.-L. Antitumor effects of polysorbate-80 coated gemcitabine polybutylcyanoacrylate nanoparticles in vitro and its pharmacodynamics in vivo on C6 glioma cells of a brain tumor model.
87. Wilson, B.; Samanta, M.K.; Santhi, K.; Kumar, K.P.S.; Paramakrishnan, N.; Suresh, B. Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles. Eur. J. Pharm. Biopharm. 2008
88. Ruan, Y.; Yao, L.; Zhang, B.; Zhang, S.; Guo, J. Antinociceptive properties of nasal delivery of Neurotoxin-loaded nanoparticles coated with polysorbate-80. Peptides 2011
89. Shao, K.; Huang, R.; Li, J.; Han, L.; Ye, L.; Lou, J.; Jiang, C. Angiopep-2 modified PE-PEG based polymeric micelles for amphotericin B delivery targeted to the brain. J. Control. Release 2010.