Journal of Innovations in Applied Pharmaceutical Science [JIAPS]

Content available at: www.saap.org.in ISSN: 2455-5177

COMPREHENSIVE CHARACTERIZATION TECHNIQUES FOR NANOEMULSION-BASED SYSTEMS

Shanmugarathinam Alagarsamy[1]*, RAJEEVKUMAR PAZHANIMUTHU[2], DHANISHKA BALRAJS[1], SANTHOSH KUMAR GNANASEKAR[1]

[1]Department of Pharmaceutical Technology, University College of Engineering, Bharathidasan Institute of Technology Campus, Anna University, Tiruchirappalli – 620024, Tamil Nadu, India.

[2]Kanachur College of Pharmacy, University Road, Natekal, Mangalore, Karnataka-575018.

DOI: https://doi.org/10.37022/jiaps.v11i2.922

Article History

Received: 14-12-2025
Revised: 06-01-2026
Accepted: 15-02-2026

Keywords: Nanoemulsions, Drug Delivery Systems, Characterization Techniques, Droplet Size, Analysis, Zeta Potential, Thermodynamic Stability.

***Corresponding Author**
Dr. Shanmugarathinam Alagarsamy

Abstract

Nanoemulsions have gained significant attention in pharmaceutical research due to their ability to enhance the solubility, stability, and bioavailability of poorly water-soluble drugs. Proper characterization of nanoemulsion systems is essential to ensure their quality, stability, and effectiveness as drug delivery carriers. This article provides a comprehensive overview of the major characterization techniques used to evaluate nanoemulsion formulations. Key parameters such as droplet size, polydispersity index (PDI), zeta potential, morphology, viscosity, pH, and refractive index are discussed in detail. These parameters help in understanding the physical properties, uniformity, stability, and performance of nanoemulsions. Advanced analytical techniques, including Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Brookfield viscometry, play an important role in determining structural and physicochemical characteristics. In addition, thermodynamic stability studies are essential to identify potential instability mechanisms such as coalescence, flocculation, and Ostwald ripening. A systematic evaluation of these parameters ensures the development of stable, uniform, and efficient nanoemulsion systems. Overall, comprehensive characterization supports formulation optimization and improves the reliability of nanoemulsions as advanced drug delivery systems.

INTRODUCTION

Characterization of nanoemulsion systems is a very important step in formulation development because it helps us understand whether the prepared system is suitable for drug delivery. In simple terms, characterization means carefully studying and measuring different physical and chemical properties of the nanoemulsion. Important parameters such as droplet size, polydispersity index (PDI), zeta potential, morphology, viscosity, pH, refractive index, and thermodynamic stability are evaluated. Droplet size and PDI help in understanding how small and uniform the droplets are. Zeta potential gives information about the surface charge and stability of the droplets. Morphology confirms the shape and structure of the droplets. Viscosity tells us about the flow behavior of the formulation, while pH ensures compatibility with the body and drug stability. Refractive index helps confirm the transparency and uniform nature of the system. Together, these tests give a complete idea about the overall quality of the nanoemulsion [1]. These characterization studies are also essential to ensure that the nanoemulsion remains stable during storage and use. Without proper evaluation, the formulation may undergo instability problems such as coalescence (fusion of droplets), flocculation (clustering of droplets without merging), Ostwald ripening (growth of larger droplets at the expense of smaller ones), creaming, or sedimentation. By performing detailed testing, these instability mechanisms can be identified at an early stage and corrected through optimization. Proper characterization, therefore, confirms that nanoemulsion is stable, uniform, safe, and effective for delivering the drug. In short, a detailed evaluation improves formulation quality, ensures long-term stability, and supports reliable therapeutic performance [2].

DROPLET SIZE ANALYSIS

Importance of Droplet Size in Nanoemulsion Systems

Droplet size is considered the most critical parameter in nanoemulsion characterization because it directly influences the formulation's stability, appearance, drug release behavior, and bioavailability. In nanoemulsions, the droplet size typically ranges between 20 and 200 nm, placing them in the colloidal dimension. At this nanoscale level, the surface area of the dispersed phase increases dramatically, which enhances drug dissolution and absorption. Smaller droplets also reduce gravitational separation phenomena such as creaming or sedimentation, thereby improving physical stability. Moreover, droplet size determines whether the formulation appears

transparent, translucent, or milky, as nanosized droplets scatter light minimally compared to conventional emulsions [3].

PRINCIPLE OF DROPLET SIZE MEASUREMENT

Droplet size in nanoemulsions is commonly determined using Dynamic Light Scattering (DLS), also known as photon correlation spectroscopy. The principle of DLS is based on the Brownian motion of particles suspended in a liquid medium. When a laser beam passes through the nanoemulsion sample, the droplets scatter light, and the fluctuations in scattered light intensity are measured over time. These fluctuations are related to the diffusion rate of the droplets, which is then converted into particle size using the Stokes–Einstein equation. This technique provides the average droplet diameter (Z-average) along with size distribution data. DLS is widely preferred because it is rapid, non-destructive, and highly sensitive to nanoscale particles [4].

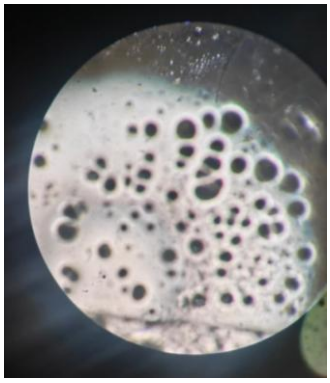

Figure 01: Globule Size

FACTORS AFFECTING DROPLET SIZE

Several formulation and processing parameters influence droplet size in nanoemulsions. The type and concentration of surfactant and co-surfactant play a crucial role in reducing interfacial tension between oil and water phases, thereby enabling the formation of smaller droplets. Higher surfactant concentration generally leads to smaller droplet sizes due to improved stabilization. The oil phase composition also affects droplet size, as oils with different viscosities and solubility characteristics behave differently during emulsification. Additionally, preparation methods such as high-pressure homogenization, ultrasonication, or spontaneous emulsification significantly impact droplet size. Increased homogenization time and energy input typically reduce droplet size by breaking larger droplets into nanoscale dimensions [5].

INFLUENCE OF DROPLET SIZE ON STABILITY

Droplet size has a strong correlation with the thermodynamic and kinetic stability of nanoemulsions. Smaller droplets exhibit reduced tendency toward coalescence because the surfactant layer surrounding them provides effective steric or electrostatic stabilization. Larger droplets, on the other hand, are more prone to aggregation, flocculation, and eventual separation. Uniform and small droplet size distribution ensures minimal Ostwald ripening, a phenomenon where smaller droplets dissolve and redeposit onto larger ones, leading to instability. Therefore, maintaining a narrow and small droplet size range is essential for long-term storage stability.

EFFECT OF DROPLET SIZE ON DRUG RELEASE AND BIOAVAILABILITY

The droplet size of nanoemulsion significantly affects drug release kinetics and absorption. Smaller droplets offer a larger interfacial surface area for drug diffusion into the surrounding medium, which enhances dissolution rate. This is particularly beneficial for poorly water-soluble drugs, as nanoemulsions improve their apparent solubility and gastrointestinal absorption. In oral delivery systems, smaller droplets facilitate better lymphatic uptake and reduce first-pass metabolism. For topical formulations, reduced droplet size enhances skin penetration by increasing contact surface area. Thus, droplet size optimization is directly linked to therapeutic effectiveness.
Optical Properties and Transparency

One of the distinguishing features of nanoemulsions is their optical clarity. Droplet size below the wavelength of visible light (approximately 400–700 nm) results in minimal light scattering, giving the system a transparent or translucent appearance. As droplet size increases beyond the nanoscale, light scattering intensifies, and the formulation becomes turbid or milky. Therefore, visual appearance indirectly indicates droplet size range and homogeneity.Fig.2 Transparent nanoemulsions are often preferred in pharmaceutical and cosmetic applications due to their aesthetic appeal and consumer acceptability [6].

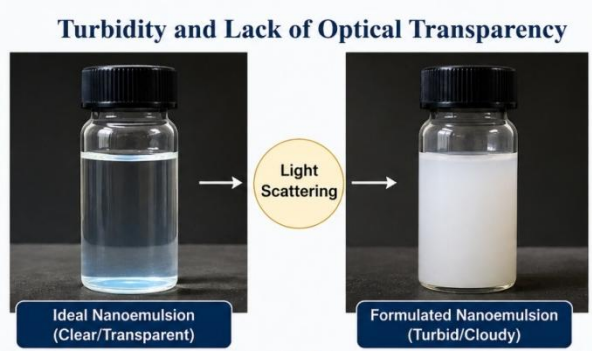

Figure 02: Optical properties and Transparenc

REPORTING AND INTERPRETATION OF RESULTS

Droplet size results are generally reported as mean droplet diameter along with standard deviation. The Z-average value obtained from DLS provides an intensity-weighted mean size. For pharmaceutical nanoemulsions, droplet sizes below 200 nm are typically considered ideal. Consistency in repeated measurements confirms formulation reproducibility. It is also important to measure droplet size under controlled temperature conditions, as viscosity and diffusion properties of the medium can influence results.

Table 01: Instability Mechanisms in Nanoemulsion Systems [7]

Instability Mechanism	Description	Scientific Mechanism	Contributing Factors	Visual Observation	Prevention Strategy
Coalescence	Fusion of droplets into larger droplets	Breakdown of interfacial film	Insufficient surfactant, mechanical stress	Oil layer formation	Increase surfactant concentration and interfacial strength
Flocculation	Droplets aggregate without merging	Weak repulsive forces	Low zeta potential, high ionic strength	Cluster formation	Improve electrostatic or steric stabilization
Ostwald Ripening	Growth of large droplets at expense of small ones	Molecular diffusion driven by solubility differences	High oil solubility in water	Gradual increase in droplet size	Use ripening inhibitors or mixed oils
Creaming	Upward movement of droplets	Density difference between phases	Large droplet size, low viscosity	Cream layer at top	Reduce droplet size, increase viscosity
Sedimentation	Downward settling of droplets	Gravitational separation	High-density oil phase	Layer formation at bottom	Increase viscosity and reduce droplet size
Phase Separation	Complete separation of phases.Fig.3	Loss of emulsion stability	Poor formulation optimization	Clear phase boundary	Optimize Smix ratio and processing energy
Aggregation	Droplet clustering over time	Attractive van der Waals forces	Low surface charge	Turbidity increase	Maintain adequate zeta potential

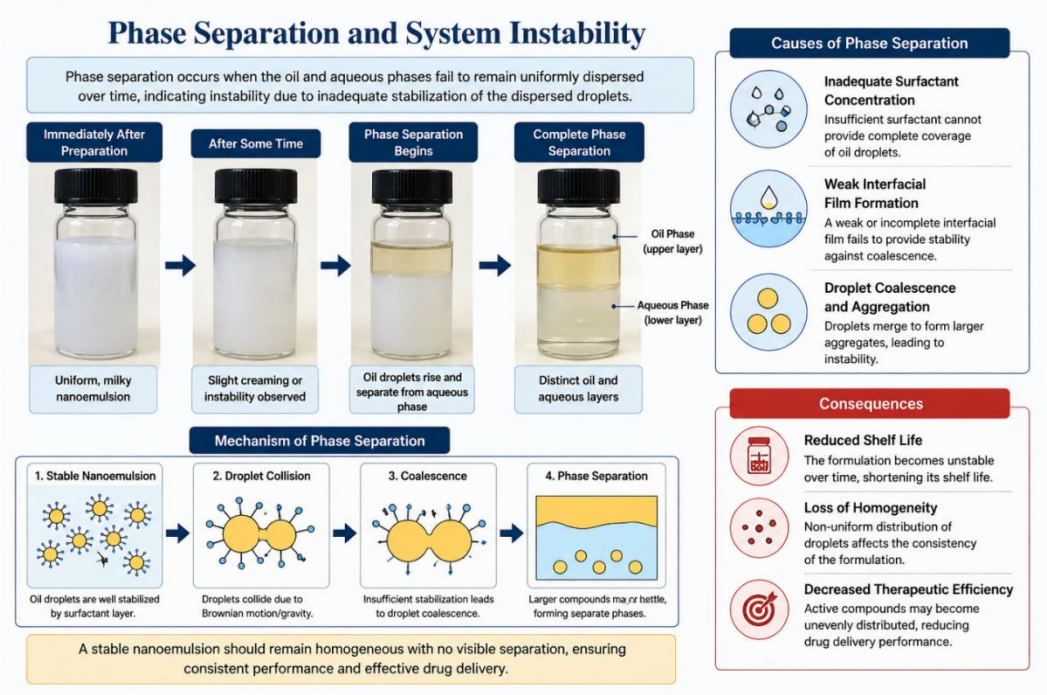

Figure 03: Phase Seperation

POLYDISPERSITY INDEX (PDI)

Concept and Significance of Polydispersity Index

Polydispersity Index (PDI) is a critical parameter used to evaluate the uniformity of droplet size distribution in nanoemulsion systems. While average droplet size provides information about the mean particle diameter, it does not indicate whether all droplets are of similar size. PDI addresses this limitation by describing the degree of size variation within the formulation. In simple terms, PDI reflects how homogeneous or heterogeneous the nanoemulsion system is. A lower PDI value indicates narrow size distribution and better formulation consistency, whereas a higher PDI suggests broad size variation, which may compromise stability and performance. Therefore, PDI serves as an essential indicator of formulation quality and optimization efficiency.

PRINCIPLE OF PDI MEASUREMENT

Polydispersity Index is commonly determined using Dynamic Light Scattering (DLS), the same technique employed for droplet size analysis. During DLS measurement, fluctuations in scattered light intensity caused by Brownian motion of droplets are analyzed. The instrument calculates not only the average droplet size but also the distribution pattern of particle sizes in the sample. PDI is derived from the cumulant analysis of the correlation function obtained during DLS measurement. It is a dimensionless value that ranges from 0 to 1. A value closer to zero indicates a monodisperse system, while a value approaching one suggests a highly polydisperse system with significant size variation.

INTERPRETATION OF PDI VALUES

Understanding PDI values is essential for evaluating nanoemulsion stability and uniformity. Generally, a PDI value below 0.3 indicates a uniform and stable nanoemulsion with narrow size distribution, which is ideal for pharmaceutical applications. Values between 0.3 and 0.5 suggest moderate uniformity and may still be acceptable depending on the formulation purpose. Figure 04 However, a PDI value above 0.5 indicates high heterogeneity and poor size distribution, which may lead to instability issues such as aggregation, coalescence, or phase separation. Therefore, maintaining a low PDI is crucial to ensure reproducibility and long-term stability of nanoemulsion formulations [8].

FACTORS INFLUENCING POLYDISPERSITY INDEX

Several formulation and processing variables influence the PDI of nanoemulsions. The concentration and type of surfactant and co-surfactant significantly affect droplet stabilization and uniformity. Adequate surfactant concentration helps in forming evenly sized droplets and prevents coalescence, thereby reducing PDI. The method of preparation also plays an important role; high-energy techniques such as ultrasonication and high-pressure homogenization generally produce narrower size distributions compared to low-energy methods. Additionally, mixing speed, temperature, oil phase composition, and homogenization time can impact droplet breakup efficiency and distribution uniformity. Improper optimization of these parameters may result in broad size distribution and increased PDI.

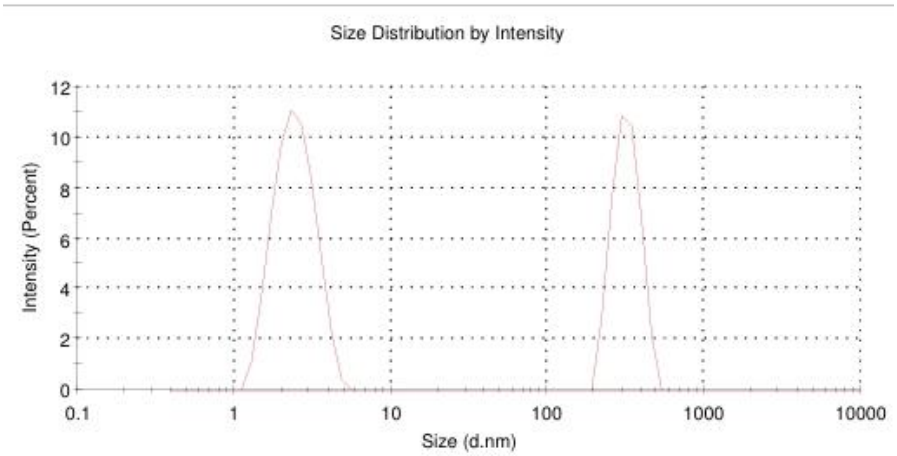

Figure 04: PDI (Polydispersity Index)

RELATIONSHIP BETWEEN PDI AND STABILITY

Polydispersity Index is closely associated with the physical stability of nanoemulsion systems. A narrow size distribution minimizes the chances of instability phenomena such as Ostwald ripening, where smaller droplets dissolve and redeposit onto larger ones. When droplet sizes vary widely, the system becomes thermodynamically unstable, accelerating growth of larger droplets and leading to eventual phase separation. A low PDI indicates uniform droplet population, which enhances steric and electrostatic stabilization provided by surfactants. Hence, nanoemulsions with low PDI values are generally more resistant to aggregation and coalescence during storage.

IMPACT OF PDI ON DRUG DELIVERY PERFORMANCE

PDI not only affects stability but also influences drug release and therapeutic efficiency. Uniform droplet size ensures consistent drug distribution within the formulation, leading to predictable and reproducible drug release profiles. In contrast, highly polydisperse systems may exhibit uneven drug loading and inconsistent release behavior. For oral and topical drug delivery systems, homogeneous droplet size distribution enhances absorption and bioavailability. Therefore, achieving an optimal PDI is essential to ensure both formulation stability and effective drug delivery [9].

REPORTING AND QUALITY CONSIDERATIONS

In research articles and thesis work, PDI values are typically reported along with average droplet size and zeta potential to provide a comprehensive understanding of nanoemulsion characteristics. Measurements should be performed under controlled temperature

conditions and repeated to confirm reproducibility. A consistently low PDI across multiple batches indicates successful formulation, optimization and process reliability. Thus, PDI serves as a fundamental quality control parameter in nanoemulsion development.

ZETA POTENTIAL MEASUREMENT

Zeta potential is a fundamental parameter used to determine the surface charge of droplets in a nanoemulsion system. It represents the electrical potential at the slipping plane surrounding dispersed droplets in a colloidal system. In simple terms, zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in dispersion. Since nanoemulsions consist of nanosized oil droplets dispersed in a continuous phase, surface charge plays a crucial role in maintaining physical stability. A sufficiently high zeta potential value prevents droplet aggregation by generating repulsive forces, thereby reducing the chances of coalescence and phase separation. Thus, zeta potential measurement is essential to predict the long-term stability of nanoemulsion formulations.

PRINCIPLE OF ZETA POTENTIAL MEASUREMENT

Zeta potential is commonly measured using electrophoretic light scattering (ELS). The principle is based on the movement of charged droplets under the influence of an applied electric field. When an electric field is applied to the nanoemulsion sample, positively or negatively charged droplets migrate toward the oppositely charged electrode. The velocity of this movement, known as electrophoretic mobility, is measured by analyzing the Doppler shift in scattered light. This mobility is then converted into zeta potential values using the Henry equation. The measurement is usually performed using specialized instruments such as a zeta sizer, which provides accurate and reproducible results [10].

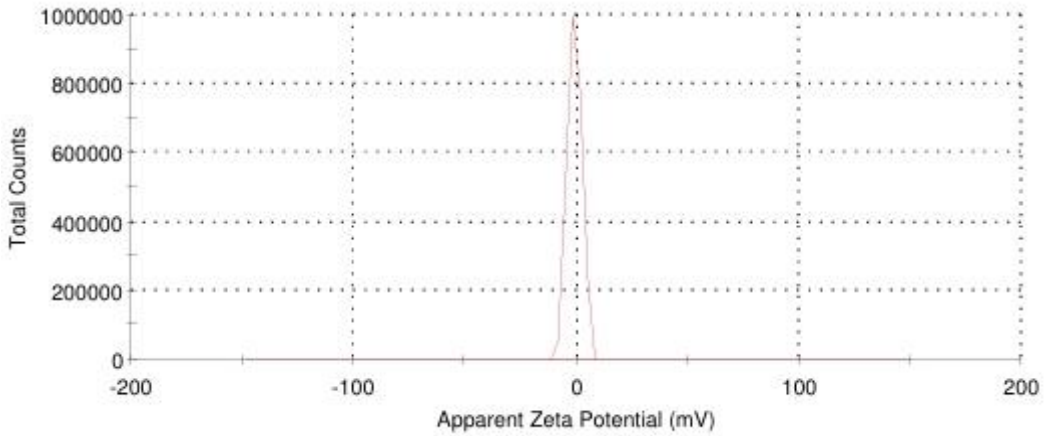

Figure 05: Zeta Potential

INTERPRETATION OF ZETA POTENTIAL VALUES

The magnitude of zeta potential provides insight into the stability of nanoemulsion systems. Generally, zeta potential values greater than +30 mV or less than −30 mV indicate good electrostatic stability due to strong repulsive forces between droplets. Values between +20 mV and −20 mV suggest moderate stability, whereas values close to zero indicate weak repulsion and a higher risk of aggregation. However, in some nanoemulsions stabilized by non-ionic surfactants, stability may still be achieved through steric hindrance even if zeta potential values are relatively low. Therefore, interpretation should consider both electrostatic and steric stabilization mechanisms.

FACTORS AFFECTING ZETA POTENTIAL

Several formulation variables influence the zeta potential of nanoemulsions. The type of surfactant used plays a major role; ionic surfactants impart significant surface charge, whereas non-ionic surfactants mainly provide steric stabilization. The pH of the formulation can alter the ionization state of components, thereby affecting surface charge and zeta potential values. Electrolyte concentration in the medium also impacts zeta potential, as increased ionic strength may compress the electrical double layer and reduce repulsive forces. Additionally, oil phase composition and drug characteristics can contribute to surface charge modification. Careful optimization of these factors is necessary to achieve desirable zeta potential values.

RELATIONSHIP BETWEEN ZETA POTENTIAL AND STABILITY

Zeta potential is directly related to the physical stability of nanoemulsion systems. High surface charge creates strong repulsive forces that prevent droplets from approaching each other closely, thus minimizing flocculation and coalescence. When zeta potential is low, attractive forces may dominate, leading to droplet aggregation and eventual phase separation. Monitoring zeta potential during stability studies helps assess whether the formulation maintains its integrity over time. A

stable nanoemulsion should retain consistent zeta potential values throughout storage under different environmental conditions.

INFLUENCE ON DRUG DELIVERY PERFORMANCE

Beyond stability, zeta potential can influence biological interactions and drug delivery efficiency. Surface charge affects the interaction of nanoemulsion droplets with biological membranes, mucosal surfaces, and skin layers. For instance, positively charged droplets may exhibit enhanced interaction with negatively charged biological membranes, potentially improving drug absorption. However, excessively high surface charge may also increase the risk of irritation or toxicity in certain routes of administration. Therefore, optimizing zeta potential is crucial not only for physical stability but also for therapeutic safety and effectiveness.

MORPHOLOGICAL CHARACTERIZATION

Morphological characterization plays a vital role in confirming the structural integrity and nanoscale architecture of a nanoemulsion system. While droplet size analysis provides numerical data, morphological evaluation offers visual confirmation of droplet shape, surface characteristics, and distribution pattern. It helps in understanding whether the prepared nanoemulsion truly possesses nanosized, uniformly dispersed droplets without aggregation or structural deformities. This analysis strengthens the reliability of the formulation and supports other characterization findings.[11]

IMPORTANCE OF MORPHOLOGY IN NANOEMULSION STABILITY

The morphology of droplets directly influences the physical stability and drug delivery performance of nanoemulsions. Ideally, nanoemulsion droplets should exhibit a spherical shape with smooth surfaces. Spherical droplets minimize surface free energy, thereby enhancing thermodynamic stability. Any irregularity in shape, droplet fusion, or clustering may indicate instability, poor surfactant efficiency, or improper formulation technique. Therefore, morphological studies help predict long-term stability and ensure consistency in pharmaceutical performance.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is one of the most widely used techniques for evaluating nanoemulsion morphology. TEM provides high-resolution images that allow visualization of individual droplets at the nanometer scale.

During analysis

A diluted sample of nanoemulsion is placed on a copper grid. It is stained (commonly with phosphotungstic acid) to enhance contrast. The sample is observed under high vacuum conditions.TEM images typically reveal spherical droplets with uniform distribution in a well-formulated nanoemulsion. The observed droplet size in TEM may appear slightly smaller compared to Dynamic Light Scattering results because TEM measures the actual core size without the hydration layer.

Table 02: Comparison of Nanoemulsion Characterization Techniques [12]

Technique	Working Principle	Type of Information	Advantages	Limitations	Typical Application
Dynamic Light Scattering (DLS)	Measures fluctuation of scattered laser light due to Brownian motion	Average droplet size and PDI	Rapid, non-destructive, highly sensitive	Sensitive to dust and dilution errors	Routine size characterization
Transmission Electron Microscopy (TEM)	Electron beam passes through thin sample	High-resolution internal structure	Direct nanoscale visualization	Complex sample preparation	Morphological confirmation
Scanning Electron Microscopy (SEM)	Electron beam scans sample surface	Surface morphology and texture	Produces 3D-like images	Requires drying and vacuum	Solidified nanoemulsions
Cryo-TEM	Rapid freezing preserves hydrated state	Native droplet structure	Prevents drying artifacts	Expensive instrumentation	Advanced structural studies
Atomic Force Microscopy (AFM)	Probe scans surface mechanically	Surface roughness & topology	Minimal sample preparation	Small scan area	Droplet interaction analysis

Electrophoretic Light Scattering	Movement of charged droplets under electric field	Zeta potential	Accurate stability prediction	Influenced by ionic strength	Stability evaluation
Brookfield Viscometry	Measures resistance to spindle rotation	Rheological behavior	Simple and reproducible	Temperature sensitive	Flow property analysis
Abbe Refractometry	Light refraction measurement	Optical homogeneity	Quick assessment	Limited structural info	Transparency confirmation

SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) is another imaging technique used to study surface morphology. SEM provides three-dimensional surface images and helps assess surface smoothness and aggregation tendencies.

Although SEM is less commonly used than TEM for liquid nanoemulsions, it can be applied after converting the nanoemulsion into a dried form (such as freeze-dried samples). SEM analysis helps confirm: Surfacetexture, Absence of cracks, Structuraluniformity. This technique is particularly useful when nanoemulsions are transformed into nanoemulgels or solidified delivery systems.

CRYO-TRANSMISSION ELECTRON MICROSCOPY (CRYO-TEM)

Cryo-TEM is an advanced technique that allows visualization of nanoemulsion droplets in their natural hydrated state. Unlike conventional TEM, Cryo-TEM preserves the native structure by rapidly freezing the sample. This methodPrevents structural deformation, avoids drying artifacts, Provides more accurate morphological representation. Cryo-TEM is especially useful in research-level studies where precise structural validation is required.

ATOMIC FORCE MICROSCOPY (AFM)

Atomic Force Microscopy (AFM) provides three-dimensional topographical information about nanoemulsion droplets. It operates by scanning a sharp probe over the sample surface and measuring surface interactions.

AFM helps inDetermining surface roughness, Evaluating droplet height and width, studying particle interaction behavior Although less frequently used than TEM, AFM offers additional structural insight without requiring extensive sample preparation, Interpretation of Morphological Results

A well-prepared nanoemulsion typically exhibitsSpherical droplets, Uniform size distribution, Noaggregation, Clear separation between droplets. Any presence of flocculation, coalescence, or irregular structures suggests instability or improper formulation parameters. Morphological data should always correlate with droplet size and PDI results to confirm overall formulation quality.

SIGNIFICANCE IN DRUG DELIVERY APPLICATIONS

Morphological characterization is essential because droplet shape and uniformity directly affect drug release kinetics, bioavailability, and absorption. Uniform spherical droplets provide consistent drug diffusion pathways and predictable release profiles. Furthermore, stable morphology enhances shelf life and reduces the risk of phase separation during storage.

Thus, morphological analysis not only confirms nanoscale structure but also validates the suitability of nanoemulsion as an effective drug delivery system.

VISCOSITY DETERMINATION

Viscosity is an essential physicochemical parameter in the characterization of nanoemulsions. It refers to the internal resistance of a liquid to flow and significantly influences stability, drug release behavior, and patient acceptability of the formulation. In nanoemulsion systems, viscosity determines how easily the formulation spreads, pours, or flows, depending on its intended route of administration. Therefore, measuring viscosity provides valuable insight into formulation consistency and performance.

IMPORTANCE OF VISCOSITY IN DRUG DELIVERY

The viscosity of nanoemulsion directly impacts its therapeutic efficiency and usability. In topical formulations, appropriate viscosity ensures good spread ability, prolonged residence time on the skin, and better drug absorption. For oral nanoemulsions, lower viscosity facilitates easy swallowing and uniform dosing.

Additionally, viscosity influencesDroplet movement within the system, Creaming or sedimentation rate, Stability against phase separation, Drug diffusion rate, Higher viscosity can reduce droplet mobility and improve physical stability, while very low viscosity may increase the risk of instability under storage conditions [13].

MEASUREMENT TECHNIQUES

Viscosity is commonly measured using a Brookfield viscometer, which operates by rotating a spindle in the nanoemulsion sample and measuring the resistance to rotation. The measurement is usually performed at controlled temperature conditions (typically 25°C) because viscosity is temperature dependent.

During analysis the sample is placed in a suitable container specific spindle is selected based on viscosity range then the rotational speed is adjusted to obtain accurate readings. Viscosity is expressed in centipoise (cP). Repeated measurements are often taken to ensure reproducibility and reliability of the data.

INFLUENCE OF COMPOSITION ON VISCOSITY

The viscosity of nanoemulsion depends largely on its composition. Factors affecting viscosity include:

Oil phase concentration

Surfactant and co-surfactant ratio (Smix ratio)
Droplet size distribution
Presence of thickening agents

An increase in surfactant concentration may either increase or decrease viscosity depending on the interaction between components. Similarly, higher oil content generally leads to increased viscosity due to enhanced internal phase volume.

The formulation design must balance viscosity to maintain both stability and ease of application.

Rheological Behavior:

Nanoemulsions may exhibit different rheological behaviors, such asNewtonian flow, non-Newtonianflow. Most oil-in-water nanoemulsions show Newtonian behavior due to their low internal phase volume. However, when converted into nanoemulgels, the system may display pseudoplastic behavior, which is desirable for topical applications as it spreads easily under stress but retains viscosity at rest. Rheological analysis helps predict how nanoemulsion behaves during manufacturing, storage, and administration [14].

EFFECT OF TEMPERATURE ON VISCOSITY

Temperature significantly affects viscosity. As temperature increases, intermolecular forces decrease, leading to lower viscosity. Therefore, viscosity measurements should always be performed under controlled temperature conditions to maintain consistency.

Studying viscosity at different temperatures also helps evaluate formulation stability under varying storage conditions.

pH Measurement

pH is a fundamental physicochemical parameter that plays a crucial role in determining the stability, safety, and compatibility of nanoemulsion formulations. Since nanoemulsions are commonly developed for oral, topical, ocular, or parenteral drug delivery, maintaining an appropriate pH range is essential to prevent irritation, degradation, or reduced therapeutic efficacy. The pH of a nanoemulsion reflects the interaction between its oil phase, aqueous phase, surfactants, and incorporated drug. Therefore, systematic pH evaluation ensures that the formulation is both pharmaceutically stable and physiologically acceptable.

Importance of pH in Drug Stability

The stability of many drugs is highly dependent on pH conditions. Changes in pH can accelerate hydrolysis, oxidation, or other degradation reactions, leading to reduced potency. For example, certain drugs remain stable only within a narrow pH range. If the nanoemulsion environment becomes too acidic or alkaline, drug degradation may occur over time.

Thus, monitoring pH helps inEnsuring chemical stability of the drug, Preventing precipitation or phase separation, maintaining consistent therapeutic performance, an optimized pH value enhances both shelf life and formulation reliability [15].

MEASUREMENT METHOD

The pH of nanoemulsion is commonly measured using a calibrated digital pH meter. Before measurement, the instrument is standardized using buffer solutions of known pH (usually pH 4.0, 7.0, and 9.2).

During analysis:

A small quantity of nanoemulsion is taken in a beaker.
The electrode is immersed completely in the sample.
The reading is recorded once stabilized.

Measurements are generally performed at room temperature (25°C) to ensure consistency. In some cases, dilution with distilled water may be done if the formulation is highly viscous.

Ideal pH Range Based on Route of Administration

The acceptable pH range of nanoemulsion depends on its intended route of delivery:

Topical formulations: pH 5.0–7.0 (compatible with skin pH
Oral formulations: near neutral pH to avoid gastric irritation
Ocular formulations: pH 6.5–7.5 to match tear fluid
Parenteral formulations: approximately 7.4 to match blood pH

Maintaining pH within these physiological limits minimizes irritation, enhances patient compliance, and ensures safety.

Influence of Formulation Components on pH

Several formulation variables influence the pH of nanoemulsions, including:

- Nature of surfactant and co-surfactant
- Type of oil phase
- Drug's intrinsic pKa value
- Presence of buffering agents

Surfactants with acidic or basic functional groups can alter the final pH of the system. Similarly, incorporation of ionizable drugs may shift pH depending on their concentration and dissociation characteristics. Therefore, pH adjustment may be performed using suitable buffers to maintain formulation stability.

EFFECT OF PH ON PHYSICAL STABILITY

pH can significantly impact the physical stability of nanoemulsions. Extreme pH conditions may disrupt the interfacial film formed by surfactants, leading to droplet aggregation or coalescence. In addition, changes in pH can alter zeta potential values, affecting electrostatic repulsion between droplets.

A stable nanoemulsion should maintain relatively constant pH over storage time. Significant pH variation during stability studies may indicate chemical degradation or interaction between formulation components.

pH Monitoring During Stability Studies

pH measurement is an important parameter during accelerated and long-term stability testing. Periodic pH evaluation helps detect early signs of instability. A sudden shift in pH may suggest: Drug degradation, Microbial contamination, Chemical interaction among excipients, Consistent pH values throughout storage confirm formulation integrity and compatibility.

REFRACTIVE INDEX DETERMINATION

Refractive index is an important optical parameter used in the characterization of nanoemulsion systems. It indicates how light propagates through a medium and reflects the degree of transparency and isotropy of formulation. Since nanoemulsions are generally transparent or translucent due to their nanosized droplets, measuring the refractive index helps confirm their uniform dispersion and absence of phase separation. This parameter also indirectly supports droplet size analysis and overall formulation consistency.

IMPORTANCE OF REFRACTIVE INDEX IN CHARACTERIZATION

Refractive index measurement provides valuable insight into the homogeneity and clarity of nanoemulsions. A well-formulated nanoemulsion should exhibit a refractive index close to that of the continuous phase, typically water in oil-in-water (O/W) systems.

Significance of refractive index
Confirmation of isotropic nature
Assessment of optical transparency
Detection of phase separation or instability
Evaluation of formulation reproducibility

Any significant deviation in refractive index during storage may indicate instability, such as creaming or coalescence [16].

Principle of Measurement

The refractive index is determined based on the principle of light refraction. When light passes from one medium to another, its speed changes, causing it to bend. The refractive index is defined as the ratio of the speed of light in vacuum to its speed in the sample medium.

In nanoemulsion characterization, this parameter helps assess whether the system behaves as a single, uniform phase or exhibits heterogeneity.

MEASUREMENT METHOD

Refractive index is commonly measured using an Abbe refractometer. The instrument is calibrated using distilled water before analysis.

Procedure includes:

Placing a small quantity of nanoemulsion on the prism surface. Closing the cover plate carefully to avoid air bubbles. Allowing the system to equilibrate at controlled temperature (usually 25°C). Recording the refractive index value once stabilized. Temperature control is essential because refractive index is temperature dependent. Influence of Composition on Refractive Index. Therefractive index of nanoemulsion depends on the proportion and type of formulation components, including:

Oil phase concentration

Surfactant and co-surfactant ratio

Nature of the aqueous phase

DRUG CONCENTRATION

Higher oil content generally increases the refractive index, whereas higher aqueous content lowers it. Therefore, consistent refractive index values confirm uniform mixing and stable composition.

RELATIONSHIP BETWEEN REFRACTIVE INDEX AND STABILITY

Refractive index serves as an indirect stability indicator. A stable nanoemulsion maintains a constant refractive index over time. Changes in value during storage studies may indicate:

Phase separation, Droplet aggregation, Chemical interaction among components. Thus, periodic refractive index monitoring is useful during accelerated and long-term stability testing.

Optical Clarity and Isotropic Nature

Nanoemulsions are thermodynamically stable or kinetically stable isotropic systems. The isotropic nature means that their physical properties remain uniform in all directions. A consistent refractive index confirms this isotropy and transparency.Clear nanoemulsions with nanosized droplets exhibit minimal light scattering, resulting in refractive index values close to that of water (in O/W systems). This optical clarity is often considered a sign of successful formulation.

Thermodynamic Stability Studies

Thermodynamic stability studies are a crucial part of nanoemulsion characterization, as they evaluate the ability of the formulation to remain stable under various stress conditions. Although nanoemulsions are considered kinetically stable systems, they are not always thermodynamically stable like microemulsions. Therefore, stress testing is essential to identify unstable formulations before proceeding to advanced studies. These tests help ensure that the nanoemulsion will maintain its physical integrity, homogeneity, and performance during storage and transportation.

IMPORTANCE OF STABILITY EVALUATION

Stability studies are conducted to predict the long-term behavior of nanoemulsions. A stable nanoemulsion should resist phase separation, creaming, cracking, flocculation, and coalescence. Instability may lead to drug precipitation, reduced bioavailability, and loss of therapeutic efficacy.

Thermodynamic stability testing helps in screening optimized formulations, eliminating metastable systems, confirming robustness of surfactant film,Ensuring shelf-life reliability. Only formulations that pass these tests are considered suitable for further characterization and application.

Heating–Cooling Cycle Test

The heating–cooling cycle test evaluates the formulation's ability to withstand temperature variations.

Procedure

The nanoemulsion is subjected to alternating temperatures, typically between 4°C and 45°C. Each temperature is maintained for at least 48 hours. The cycle is repeated 3–6 times. After completion, the formulation is observed for any signs of instability such as phase separation, turbidity, or precipitation.

Significance

This test simulates environmental temperature fluctuations during storage and transport. A stable nanoemulsion should show no visible changes after repeated cycles.

Centrifugation Test

The centrifugation test accelerates the gravitational separation process to detect physical instability.

Procedure

The nanoemulsion sample is centrifuged at approximately 3000–5000 rpm for 15–30 minutes. The formulation is then examined for phase separation or creaming.

Significance

Centrifugation increases the rate of droplet movement and sedimentation. If the formulation remains uniform without separation, it indicates good kinetic stability and strong interfacial film formation.

Freeze–Thaw Cycle Test

The freeze–thaw cycle test evaluates the formulation's resistance to extreme temperature stress.

Procedure

The nanoemulsion is stored at freezing temperature (−20°C) for 24 hours. It is then thawed at room temperature or 25°C.The cycle is repeated 3–5 times. After each cycle, the formulation is checked for phase separation, aggregation, or cracking.

Significance

Freezing may cause ice crystal formation in the aqueous phase, which can disturb droplet structure. A stable nanoemulsion should recover its original state after thawing without structural damage.

Observation Parameters

Visual appearance, Phaseseparation, Turbidity, change in droplet size Variation in pH any noticeable change indicates formulation instability and the need for reformulation, Mechanism of Instability.

Nanoemulsion instability may occur due to Coalescence, Flocculation, Ostwald ripening (growth of larger droplets at the expense of smaller ones), Creaming or sedimentation, Thermodynamic stress tests help identify these mechanisms at an early stage.

CONCLUSION

In conclusion, comprehensive characterization of nanoemulsions is essential for ensuring their quality, stability, and effectiveness as drug delivery systems. Parameters such as droplet size, polydispersity index, zeta potential, morphology, viscosity, pH, refractive index, and thermodynamic stability provide critical information regarding formulation performance and reliability. Advanced analytical techniques help confirm nanoscale properties, uniformity, and structural integrity. Stability studies further identify potential instability mechanisms and support formulation optimization. Overall, systematic characterization enables the development of stable, safe, and efficient nanoemulsion systems with enhanced drug delivery, improved bioavailability, and consistent therapeutic outcomes.

ACKNOWLEDGEMENT

Not Declared

FUNDING

Nil

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

INFORM CONSENT AND ETHICAL CNSIDERATIONS

Not applicable

AUTHOR CONTRIBUTIONS

Both are contributed equally.

REFERENCES

1. Rodríguez-Cano A, Cintas P, Fernández-Calderón MC, Pacha-Olivenza MÁ, Crespo L, Saldaña L, et al. Controlled silanization–amination reactions on the Ti6Al4V surface for biomedical applications. *Colloids Surf B Biointerfaces*. 2013;106:248–257. doi:10.1016/j.colsurfb.2013.01.034.
2. Ciron CIE, Gee VL, Kelly AL, Auty MAE. Effect of microfluidization of heat-treated milk on rheology and sensory properties of reduced fat yoghurt. *Food Hydrocoll*. 2011;25(6):1470–1476. doi:10.1016/j.foodhyd.2011.02.012.
3. Qian C, McClements DJ. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocoll*. 2011;25(5):1000–1008. doi:10.1016/j.foodhyd.2010.09.017.
4. Solans C, Solé I. Nano-emulsions: Formation by low-energy methods. *Curr Opin Colloid Interface Sci*. 2012;17(5):246–254. doi:10.1016/j.cocis.2012.07.003.
5. Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. Nanoemulsions: Formation, structure, and physical properties. *J Phys Condens Matter*. 2006;18(41):R635–R666. doi:10.1088/0953-8984/18/41/R01.
6. Anton N, Vandamme TF. The universality of low-energy nano-emulsification. *Int J Pharm*. 2009;377(1–2):142–147. doi:10.1016/j.ijpharm.2009.05.014.
7. L AG, K S, M N. Formulation optimisation and evaluation of a turmeric extract-incorporated emulgel using a simplex lattice approach. *Int J Pharm Drug Anal*. 2026;14(1):1–8. doi:10.47957/ijpda.v14i1.676.
8. Adsorption of gases on heterogeneous surfaces. Amsterdam: Elsevier; 1992. doi:10.1016/C2009-0-21554-6.
9. Shah BR, Li Y, Jin W, An Y, He L, Li Z, et al. Preparation and optimization of Pickering emulsion stabilized by chitosan-tripolyphosphate nanoparticles for curcumin encapsulation. *Food Hydrocoll*. 2016;52:369–377. doi:10.1016/j.foodhyd.2015.07.015.
10. Xiao G, Li X, Kumar A, Cui Z. Transcutaneous DNA immunization following waxing-based hair depilation elicits both humoral and cellular immune responses. *Eur J Pharm Biopharm*. 2012;82(1):212–217. doi:10.1016/j.ejpb.2012.06.012.
11. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev*. 2012;64:175–193. doi:10.1016/j.addr.2012.09.018.
12. Vazir A, Joshi A, Kumar K, Rajput V. Nanoemulgel: For promising topical and systemic delivery. *Int J Pharm Drug Anal*. 2023;11(4):8–13. doi:10.47957/ijpda.v11i4.561.
13. Podkościelny P, Nieszporek K. Adsorption of phenols from aqueous solutions: Equilibria, calorimetry and kinetics of adsorption. *J Colloid Interface Sci*. 2011;354(1):282–291. doi:10.1016/j.jcis.2010.10.034.
14. Shah P, Bhalodia D, Shelat P. Nanoemulsion: A pharmaceutical review. *Syst Rev Pharm*. 2010;1(1):24–32. doi:10.4103/0975-8453.59509.

15. Podkościelny P, Nieszporek K. Adsorption of phenols from aqueous solutions: Equilibria, calorimetry and kinetics of adsorption. *J Colloid Interface Sci*. 2011;354(1):282–291. doi:10.1016/j.jcis.2010.10.034.
16. Israelachvili JN. *Intermolecular and Surface Forces*. 3rd ed. Amsterdam: Elsevier; 2011. doi:10.1016/C2009-0-21560-1.