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A technical review on characterization methods for structures and properties of emulsion

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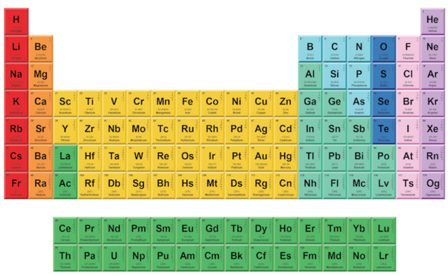

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ABSTRACT

Emulsions, particularly in the pharmaceutical, cosmetic, and food industries, are essential for the delivery and stabilization of active ingredients. Due to their structural complexity—including variations in droplet size, phase distribution, and interfacial properties—characterizing emulsions is essential for optimizing their performance and stability. Existing reviews tend to focus on specific emulsion types, properties, or individual characterization techniques, often failing to provide a holistic assessment. Consequently, there is a critical need for a comprehensive review that integrates various characterization methods. This review addresses this gap by systematically evaluating key techniques, including scattering methods (dynamic light scattering, small-angle x-ray scattering), spectroscopic techniques (Fourier transform infrared and nuclear magnetic resonance spectroscopy), microscopy methods (scanning electron microscopy, confocal laser scanning microscopy), and rheometry. By consolidating the strengths and limitations of each method, this review offers a unified framework to guide researchers in selecting appropriate techniques for characterizing emulsions, ultimately contributing to the optimization of their structure, properties, and performance across diverse applications.

I. INTRODUCTION

Emulsions, comprising two immiscible liquids such as oil and water, are a cornerstone of formulations across various industries, including pharmaceuticals, cosmetics, and food science. These systems are typically stabilized by surfactants or particles, which prevent the dispersed phase from coalescing, thereby maintaining the emulsion's structure and stability.[1,2] The diverse applications of emulsions—from drug delivery vehicles to food emulsifiers—stem from their ability to encapsulate, protect, and deliver active ingredients in a controlled manner.[3]

However, the structural complexity of emulsions, including variations in droplet size, phase distribution, and interfacial properties, presents significant challenges in their characterization.[3] Precise and comprehensive characterization is crucial not only for understanding the fundamental properties of emulsions but also for optimizing their performance and stability in practical applications.[1]

In recent years, a variety of experimental techniques have been developed and refined to probe the intricate details of emulsion systems. These techniques include scattering methods, such as Dynamic Light Scattering (DLS) and Small-Angle X-ray Scattering (SAXS), which provide insights into droplet size distribution and internal structure.[4,5] Microscopy techniques, including Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM), offer direct visualization of emulsion morphology and interfacial characteristics.[6,7] Spectroscopic methods, like Fourier transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy, reveal the molecular interactions and chemical environment within emulsions.[8,9] Additionally, bulk rheometry and interfacial rheometry are critical for understanding the mechanical properties and stability of emulsions under various conditions.[10,11]

Existing reviews in the field of emulsions tend to focus on specific emulsion types, certain properties, or individual techniques, with limited coverage on a holistic assessment of characterization methods. Most studies emphasize either a single performance parameter or specific emulsion types (such as Pickering or multiple emulsions). However, the complexity of emulsion systems makes it difficult to fully understand their behavior using a single method. Factors such as droplet size, phase distribution, and interfacial properties are often interrelated, and a combination of various characterization techniques is crucial for a more comprehensive understanding.[12]

Therefore, there is a critical need for a comprehensive review centered on the characterization methods themselves. Understanding these methods is crucial for accurately assessing the structure, properties, and performance of emulsions. This review addresses this gap by adopting a novel approach: it systematically integrates the strengths of various characterization techniques to provide a unified framework. By summarizing the applications and limitations of these methods in emulsion research, the review aims to assist researchers and formulators in making informed decisions when selecting appropriate techniques for their specific objectives. Characterization techniques play a pivotal role in revealing the microstructural and interfacial properties of emulsions, which directly impact their stability, functional performance, and applications across various industries.

II. OVERVIEW OF EMULSION TYPES

Emulsions can be categorized based on their composition, the size of the dispersed droplets, and the nature of their stabilization. Each type of emulsion presents unique characteristics and challenges that influence the choice of characterization techniques.

A. Conventional emulsions (O/W, W/O)

Conventional emulsions consist of oil droplets dispersed in water (O/W) or water droplets dispersed in oil (W/O), stabilized by surfactants that reduce the interfacial tension between the immiscible phases, preventing droplet coalescence.[1] The droplet size in conventional emulsions typically ranges from 0.1 to 100 μm, and their stability is heavily influenced by factors such as surfactant concentration, droplet size distribution, and the physicochemical properties of the dispersed and continuous phases.[3] These emulsions are widely used in food products, cosmetics, and pharmaceuticals due to their ability to encapsulate and deliver both hydrophobic and hydrophilic compounds.[1]

B. Pickering emulsions

Pickering emulsions are unique in that they are stabilized by solid particles rather than traditional surfactants.[13] These particles adsorb at the oil–water interface, creating a mechanical barrier that prevents droplet coalescence, leading to highly stable emulsions.[14] The stability and behavior of Pickering emulsions are significantly influenced by the size, shape, and surface properties of the stabilizing particles, which can be inorganic materials like silica or organic materials like cellulose.[13] Pickering emulsions are increasingly studied for applications in drug delivery, food science, and material synthesis due to their robust stability and potential for reducing surfactant use.[14]

C. Multiple emulsions (W/O/W, O/W/O)

Multiple emulsions are complex systems where emulsions are nested within other emulsions, such as water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O). These systems allow for the encapsulation and controlled release of substances, making them valuable in drug delivery, food science, and cosmetics.[15] However, their complexity also presents significant challenges for stability and characterization, as these emulsions are sensitive to environmental conditions, and maintaining the stability of both the internal and external interfaces requires careful selection of surfactants and stabilizers.[16]

III. CHARACTERIZATION TECHNIQUES

A. Scattering techniques

1. Light scattering

Dynamic Light Scattering (DLS) is based on the analysis of time-dependent fluctuations in the intensity of scattered light, which result from the Brownian motion of particles. When a laser beam passes through a sample, the particles scatter light in all directions. The intensity of this scattered light fluctuates over time, and these fluctuations are correlated with the diffusion coefficient of the particles. The diffusion coefficient is inversely related to the particle size, as described by the Stokes–Einstein equation[17,18]

$$D = \frac{k_B T}{6\pi\eta R_h},$$

where

- D is the diffusion coefficient,
- k_B is the Boltzmann constant,
- T is the absolute temperature,
- η is the viscosity of the solvent and,
- R_h is the hydrodynamic radius of the particles.

The core of the DLS measurement involves calculating the autocorrelation function of the scattered light intensity, which gives information about the time scale over which these fluctuations occur. From this, the diffusion coefficient can be determined and, consequently, the hydrodynamic radius of the particles can be inferred.[18]

DLS is particularly useful to characterize the size distribution of the emulsion droplets.[19–22] By monitoring changes in size over time, an insight into the stability of the emulsions can be provided.[19] Zeta potential correlation[21,23] and molecular weight estimation. While DLS is a powerful tool, it has certain limitations. When dealing with highly polydisperse samples as emulsion, especially for Pickering emulsion, DLS can encounter significant challenges. This is primarily because DLS measures the intensity of scattered light. The intensity of light scattered by a particle is proportional to the sixth power of its radius, as described by Rayleigh scattering. This means that larger particles in a sample will scatter light much more intensely than smaller particles. In a polydisperse sample, even a small fraction of large particles can dominate the scattering signal, overshadowing the contribution from smaller particles. As a result, the DLS measurement may disproportionately reflect the polydispersity of emulsion, leading to a misrepresentation of the true size distribution.[4,24,25]

Static Light Scattering (SLS) complements Dynamic Light Scattering (DLS) by providing static measurements that are particularly valuable for characterizing larger droplets in emulsions. While DLS analyzes fluctuations in scattered light intensity over time to determine particle size distribution, SLS examines the time-averaged intensity of scattered light to obtain information about particle size, molecular weight, and interparticle interactions. In SLS, the intensity data are interpreted using theoretical models such as the Rayleigh–Gans–Debye approximation or Lorenz–Mie theory, depending on the expected droplet size. The Rayleigh–Gans–Debye theory is appropriate for describing the scattering of droplets that are small relative to the wavelength of light, typically nanometer-sized particles. Conversely, the Lorenz–Mie theory is applied to droplets of micrometer size.[26–28] Both models assume that the particles are spherical and uniform. Any deviation from these assumptions, such as non-spherical shapes or highly polydisperse emulsion droplets, can generate complex scattering patterns, introducing errors in size and stability measurements.[29,30] For polydisperse emulsions, a recent study shows that more complex models like the T-matrix method[31,32] or discrete dipole approximation[33] may be necessary for more accurate interpretation. Furthermore, multiple scattering effects become significant at higher concentrations, where scattered light from one particle is further scattered by others, distorting the intensity data.[26]

Static Multiple Light Scattering (SMLS) goes a step further by addressing some of the limitations encountered in traditional light scattering techniques. In SMLS, the sample is illuminated by a laser or light source, and multiple scattering events are analyzed to obtain information about both droplet size and their distribution in the emulsion. Unlike SLS and DLS, which can be affected by high particle concentration leading to secondary scattering effects, SMLS utilizes backscattering and transmission data to mitigate these effects. The principle behind SMLS is closely related to the phenomena of coherent backscattering.[34] The interference between multiple scattering paths, especially time-reversed paths, results in enhanced backscattering intensity. This is particularly valuable for investigating systems where scattering events are numerous, as in dense colloidal suspensions or emulsions. By studying the angular shape of backscattering, SMLS provides insights into particle size, distribution, and dynamics, even under conditions of strong multiple scattering, where traditional single-scattering methods fall short.

Recent discoveries show that SMLS is particularly valuable for stability monitoring of emulsions. By measuring scattering intensity over time, it is possible to detect processes like coalescence or Ostwald ripening, providing insights into the emulsion's stability and shelf life.[35,36] The development of a predictive algorithm for static multiple light scattering allows for the prediction of stability over time based on scattering profiles.[37] The algorithms correlate scattering data with empirical observations, simulating the time at which destabilization events such as sedimentation or phase separation are likely to occur. The models incorporate various physicochemical parameters like particle size, refractive index, and density, enabling more accurate predictions of long-term stability.

2. Small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS)

Small-Angle X-ray Scattering (SAXS) and Small-Angle Neutron Scattering (SANS) are powerful techniques that provide insight into the structure and dynamics of materials on length scales ranging from 1 to 100 nm. SAXS and SANS are based on the elastic scattering of x-rays and neutrons, respectively, and are particularly valuable for studying the size distribution of particles or droplets in emulsions.

Both SAXS and SANS rely on the measurement of scattering intensity $I(q)$ as a function of the scattering vector q, where

$$q = \frac{4\pi \sin \theta}{\lambda}.$$

Here, θ is the scattering angle, and λ is the wavelength of the incident beam (x-rays in SAXS, neutrons in SANS). The scattering vector q is inversely proportional to the characteristic length scales in the sample, making these techniques particularly suitable for studying features ranging from a few nanometers to several hundred nanometers.[38,39]

In SAXS, x-rays interact with the electron clouds of atoms, and the scattering intensity is proportional to the electron density variations within the sample. Therefore, SAXS experiments will only be useful when there is a large difference in electron density between the particles and the fluid phases. On the contrary, in SANS, neutrons interact with atomic nuclei, and the scattering intensity depends on the differences in scattering length density (SLD), making SANS particularly useful for studying materials with light elements or for exploiting contrast variation by isotopic substitution (e.g., deuterium for hydrogen).[39]

The scattering intensity $I(q)$ provides information about the size, shape, and distribution of scattering entities (such as particles or droplets) within the sample. For monodisperse systems (where all entities are of the same size and shape), the scattering intensity can be described using a form factor $P(q)$ for individual particles,

$$I(q) \approx P(q)S(q),$$

where

- $P(q)$ is the form factor that describes the scattering from an individual particle and,
- $S(q)$ is the structure factor that accounts for interactions between particles.

To study complex emulsions, researchers have developed advanced models to interpret scattering data over a wide q range. For instance, Jestin *et al.*[40] utilized SANS to investigate water-in-oil emulsions stabilized by asphaltene particles. The study exploited the unique capabilities of SANS, particularly contrast variation, to isolate scattering contributions from specific components. By adjusting the ratio of hydrogenated to deuterated solvents, the researchers minimized scattering from the bulk oil and water phases, allowing a focus on the interfacial layer where the asphaltene aggregates are located.

The SANS data were analyzed using a flat disk form factor model, which facilitated the determination of the interfacial film's thickness and the organization of the aggregates. Additionally, Porod's approximation was applied in the intermediate q range to derive the surface-to-volume ratio of the emulsions, while the radius of gyration of the aggregates was calculated in the Guinier region using Zimm's method. However, this analysis did not cover the full q range. For emulsion systems that span a wide range of relevant length scales, a comprehensive model is necessary to fully utilize the entire range of scattering data.

Verruto and Kilpatrick[41] developed a more detailed model capable of fitting over an extended q range. They introduced a polydisperse core/shell form factor model to interpret the SANS data and extract detailed information about the thickness and composition of the interfacial films in water-in-oil emulsions stabilized by asphaltenes. This model enables the deduction of film thickness and the asphaltene volume fraction within the films, offering a more comprehensive understanding of the emulsion structure.

Larson-Smith *et al.*[5] further advanced this work by developing a comprehensive structure factor model based on the classic Debye equation, adapted to account for the spherical nature of both the core and the adsorbed particles. This model introduces the concept of an effective radius, which includes the penetration depth of particles into the oil phase. Recognizing the variability in core sizes in real-world systems, the model also accounts for polydispersity, enhancing its applicability to practical scenarios. The model was validated using data from SANS and USANS experiments on a 1 wt. % hexadecane-in-water emulsion stabilized by 0.25 wt. % hydrophobically functionalized silica nanoparticles. The scattering data, covering a wide range of q-values (0.000 04–0.4 $Å^{-1}$), demonstrated that the model accurately captured the experimental scattering profile, confirming its validity across the full q range.

B. Spectroscopic techniques

1. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy is a widely recognized technique for identifying and analyzing the structure of chemical compounds.[42,43] The technique relies on the excitation of vibrational modes of molecules, which are unique to different chemical bonds and functional groups, making the IR spectrum a "fingerprint" for compounds.[44,45] Fourier transform infrared (FTIR) spectroscopy is well known as a fast, nondestructive, and simple analysis. FTIR can provide valuable insights into molecular interactions, such as hydrogen bonding, van der Waals forces, and dipole–dipole interactions. These interactions affect the environment surrounding specific functional groups within a molecule, altering the electron distribution around a bond and, therefore, changing its vibrational frequency. This makes FTIR a useful technique for studying the interfacial structure in emulsions since the droplets are stabilized by surfactants or solid particles adsorbed and aligned at the interface. Certain phenomena can also provide critical insights into the differences between interfacial water (located near the interface) and bulk-like water[8,46,47] in emulsion. Furthermore, the intensity of a particular vibrational mode can also change when molecules interact. This is because the interaction can either enhance or weaken the bond strength, affecting the dipole moment change during vibration.[48–50]

However, analyzing emulsions with FTIR presents certain challenges, such as multiple vibrational transitions, peak overlap, and peak lengthening. Recent research shows that advanced data analysis techniques, like chemometric methods,[51] Principal Component Analysis (PCA)[51] or Partial Least Squares (PLS)[43,52] regression, can be used to tackle the challenge and enhance the interpretation of complex spectra obtained from emulsions, enabling assessment of emulsion stability and monitoring of compositional changes over time.

2. Raman spectroscopy

Raman spectroscopy can be a good complement technique for FTIR; as FTIR is sensitive to dipole changes in molecules, Raman spectroscopy is sensitive to changes in polarizability.[53] Raman spectroscopy is based on the inelastic scattering of monochromatic light, usually from a laser source. When light interacts with a molecule, most photons are elastically scattered (Rayleigh scattering), meaning they scatter with the same energy (or wavelength) as the incident light. However, a small fraction of light (about 1 in 10×10^6 photons) is inelastically scattered, where the scattered photons have different energies than the incident photons. This shift in energy is due to the interaction with molecular vibrations within the sample, leading to either an increase or decrease in energy. The difference in energy between the incident photons and the inelastically scattered photons is called the Raman shift. This shift is typically expressed in wavenumbers (cm^{-1}) and corresponds to the vibrational modes of the molecules in the sample. The Raman spectrum is a plot of intensity vs Raman shift. Raman spectroscopy allows us to identify and characterize the chemical structure of emulsifiers and stabilizers, which are crucial in determining the stability and properties of emulsions.[54,55] Because Raman measurement is quick and nondestructive, it can also be used to monitor chemical and structural changes in real-time during the emulsification process.[56,57]

Raman microscopy is a powerful technique that combines Raman spectroscopy with optical microscopy to provide detailed chemical and structural information about the sample. In the context of emulsion, Raman microscopy is used to identify the chemical composition and spatial distribution of particles at the emulsion interface without the need for additional staining or labeling. Raman microscopy not only offers high-resolution 2D images but can also be extended to 3D imaging.[58] This enables a more comprehensive analysis of the emulsion structure, including the distribution and orientation of particles throughout the entire droplet. One of the challenges mentioned is that Raman microscopy requires the emulsion sample to be stationary during the measurement process. Since the emulsions are inherently dynamic, this necessitates special sample handling techniques, such as using a special sample holder to immobilize the emulsion droplets during analysis.[59]

3. Nuclear magnetic resonance (NMR) spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for the characterization of emulsions, providing detailed information about the molecular environment, structure, and dynamics of the components within the emulsion. When a sample is placed in a magnetic field, these nuclei align and can be excited by radiofrequency pulses. As they return to their original state, they emit signals that depend on their chemical environment. Basic 1D NMR, e.g., ^{1}H-NMR, ^{13}C-NMR, can be used to identify and quantify the different components (e.g., water, oil, surfactants) within the emulsion. Components can be identified by their distinct chemical shifts, coupling, and integration.[60] Relaxation times (T1 and T2) describe how nuclear spins return to equilibrium after being disturbed by a radiofrequency pulse. T1 (spin–lattice relaxation) provides information about how energy is exchanged between spins and their surroundings, while T2 (spin–spin relaxation) reveals how interactions between spins cause dephasing. The relaxation times provide information about the mobility of molecules in different phases (e.g., dispersed phase, continuous phase). Water in a denser molecular environment can have a shorter T2 relaxation time compared to water in the continuous phase. These relaxation properties are crucial to gain deeper insights into the mobility and distribution of these components within different phases of the emulsion.[9]

Diffusion-Ordered Spectroscopy (DOSY-NMR) is an invaluable technique to evaluate the size and stability of the emulsion. DOSY-NMR measures the diffusion coefficients of molecules within the emulsion, which are directly related to the size of the dispersed droplets.[9,61,62] By observing how these coefficients change over time or under different conditions, it is possible to assess the stability of the emulsion.[63,64]

C. Microscopy techniques

Microscopy techniques allow for the direct visualization of emulsion structures, offering insights into droplet morphology, interfacial characteristics, and internal organization. Optical microscopy is the most commonly used approach for emulsion viewing for its simplicity and ease of use, allowing for the quick visualization of emulsion morphology. It is particularly useful for observing the overall shape and size of droplets. However, optical microscopy has limitations in terms of resolution (down to ~1 μm) and contrast, making it difficult to distinguish between different phases of the emulsion or to observe nano-sized structures. Therefore, advanced microscopy is used for further characterization.

1. Confocal laser scanning microscopy (CLSM)

Confocal Laser Scanning Microscopy (CLSM) represents a significant advancement over traditional optical microscopy, particularly in its ability to achieve high-resolution imaging with enhanced contrast. This technique is particularly well-suited for studying the three-dimensional architecture of emulsions, providing a window into their internal structures that was previously difficult to access.

The principle behind CLSM is its use of point illumination and a spatial pinhole, which work together to eliminate out-of-focus light from thicker specimens.[65] This allows researchers to obtain sharp, high-contrast images of emulsions at different depths, creating a three-dimensional reconstruction of the sample. This capability is particularly important in emulsion studies, where understanding the spatial distribution of different phases—such as oil, water, and emulsifiers—is critical.

One of the key strengths of CLSM lies in its ability to use fluorescent dyes to selectively label different components within an emulsion. For instance, lipophilic dyes like Nile Red can stain the oil phase, while other dyes can be used to highlight the water phase. This selective staining enables researchers to observe how different components are distributed within the emulsion.[66] For Pickering emulsion, it enables identifying the location and morphology of solid particles located at the interface.[67–71] The three-dimensional imaging capability of CLSM allows for the detailed study of droplet interfaces, interfacial thickness, and even the encapsulation of active ingredients within the emulsion.

While CLSM offers significant advantages, it also has certain limitations. The technique necessitates fluorescent labeling, which might not be appropriate for all types of samples. Furthermore, confocal microscopy may not be ideal for samples that are light-sensitive or prone to continuous changes, as it requires prolonged exposure to achieve sufficient signal intensity.

2. Electron microscopy

For those seeking even higher resolution and more detailed structural information, electron microscopy provides an unparalleled view into the nanoscale features of emulsions. Electron microscopy encompasses both Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), each offering distinct advantages for studying emulsions.

SEM is primarily used to explore the surface morphology of emulsion droplets. By scanning the surface with a focused electron beam, SEM produces detailed topographical images that reveal the presence of stabilizing particles and other features that are critical to understanding the physical properties of the emulsion.[72,73] SEM's ability to combine imaging with techniques like energy-dispersive x-ray spectroscopy (EDS) also allows for the analysis of elemental composition, adding another layer of information about the materials involved in the emulsion.

TEM, on the other hand, provides insights into the internal structure of emulsions at an even finer scale. By transmitting a beam of electrons through an ultrathin sample, TEM generates images that reveal the internal morphology of droplets, down to the nanometer level.[74] This makes TEM particularly useful for analyzing complex emulsion systems, such as Pickering emulsion or multiple emulsion. Its ability to resolve fine details at the nanoscale makes it an essential tool for examining the arrangement of surfactants, the presence of nanoparticles, and the overall internal structure of the emulsion.[20]

However, both SEM and TEM require extensive sample preparation. Both SEM and TEM operate under high vacuum. Therefore, the samples often need to be dehydrated. For SEM, it is also needed to be coated with a conductive material, such as gold or carbon, to prevent charging under the electron beam. For TEM, samples must be prepared as extremely thin sections—typically less than 200 nm thick—and often require staining to enhance contrast.[74] All those preparation processes can potentially obscure or distort the emulsion's structure, making it less representative of the native state.

Earlier methods for visualizing emulsion droplets relied on fixation techniques,[75,76] which can introduce artifacts and potentially distort the observed structures.[77] In contrast, modern approaches employ cryo-techniques for sample preparation in both TEM and SEM. These involve rapid freezing of the emulsion droplets to preserve their native hydrated state. However, one of the biggest challenges with cryo-techniques is the potential for water crystallization, which can disrupt the emulsion's native structure. To address this, vitrification is used during sample preparation, where the emulsion is cooled so rapidly that water transitions directly from a liquid to a glass-like amorphous solid state without crystallizing.[78] Following vitrification, the sample may undergo freeze-fracturing to expose the internal microstructure. This allows for high-resolution visualization of the emulsion's fine details, including morphology,[66,74] interfacial structures,[66,79] and, in the case of Pickering emulsions, the distribution of solid particles.[20,45]

Environmental Scanning Electron Microscopy (ESEM), derived from conventional scanning electron microscopy, represents a significant advancement in electron microscopy, particularly for *in situ* imaging of non-conductive and hydrated samples without prior preparation. In ESEM, a differential pumping system is introduced to maintain a high vacuum in the electron gun while allowing for a higher pressure of up to 20 Torr in the specimen chamber. This maintains a controlled environment around the sample, which can include variable pressure and the presence of water vapor. Imaging in ESEM uses a gaseous secondary electron detector (GSED), which relies on the interaction between secondary electrons and the imaging gas. As secondary electrons are emitted from the sample, they ionize the gas molecules, leading to a cascade of further electron emissions that amplify the signal.[80] This process is essential for obtaining images with sufficient contrast and resolution in the presence of gas. ESEM can not only be used on the emulsion to examine the true morphology, size distribution, and stability of the droplets like conventional SEM;[81] it is also useful to differentiate the water and unsaturated oil phases in the emulsion. A reduction of secondary electron emission from unsaturated oil phase can be observed owing to additional energy-absorbing mechanisms that are caused by the presence of π-bonds (double bonds between carbon atoms), reducing the number of electrons that escape and are detected, thus resulting in lower intensity in the images.[82]

Recently, liquid phase transmission electron microscopy (LPTEM) has emerged as a powerful tool for the *in situ* observation of dynamic processes in liquid environments, making it invaluable for the characterization of emulsions.[83–85] Unlike traditional TEM, which requires samples to be dehydrated and placed in a high vacuum, LPTEM uses specialized liquid cells to encapsulate the emulsion, maintaining its natural hydrated state during imaging. Recent research explores the use of LPTEM to directly observe and quantify the demulsification of water-in-oil emulsions.[86] Traditional techniques like light scattering and rheometry, while useful for ensemble measurements, do not capture the detailed behavior of individual droplets during phase separation. LPTEM overcomes this limitation by allowing real-time observation of processes such as droplet coalescence, Ostwald ripening, and flocculation with high temporal and spatial resolution. While LPTEM excels in capturing dynamic processes, it is limited by the need for continuous electron beam exposure, which can alter sample behavior through radiolysis. Cryo-TEM, on the other hand, offers static high-resolution snapshots of emulsions with a lower risk of beam-induced changes, preserving their structure through rapid vitrification. However, cryo-TEM lacks the ability to capture real-time dynamics, which limits its use for studying processes like coalescence and Ostwald ripening in emulsions.[85]

3. Atomic force microscopy (AFM)

The Atomic Force Microscope (AFM) is a high-resolution scanning probe microscope extensively used to analyze surfaces at the atomic or molecular level.[87] AFM imaging employs a cantilever with an extremely sharp tip, typically made from silicon or silicon nitride, which scans and interacts with the sample surface. This cantilever, usually a few micrometers long, has a tip with a nanometer-scale radius of curvature. A detector captures the cantilever's movements, providing detailed information about the surface topography.

AFM operates in various modes, including contact mode, tapping mode, and non-contact mode. In both contact and tapping modes, the cantilever maintains direct interaction with the emulsion droplets.[88–92] In tapping mode, however, the cantilever oscillates near its resonance frequency, intermittently contacting the sample surface. This intermittent contact minimizes lateral forces on the sample, reducing the risk of deforming or damaging the delicate droplet surfaces and preserving the sharpness of the AFM tip.[93,94] Recent studies have successfully employed tapping mode AFM to image nanoemulsion droplets, determining their size, shape, and distribution.[95] In contrast, the non-contact mode often yields less reliable and less detailed images due to the continuous phase surrounding the emulsion droplets. The difficulty in maintaining the correct distance between the tip and sample in non-contact mode often leads to reduced resolution and less accurate data, limiting its use for soft materials like emulsions.

Recent developments in AFM have expanded its utility beyond surface topography imaging. AFM is now being used to investigate interfacial mechanical properties[59,88,90,93,96] such as elasticity, hardness, and adhesion, which are critical for understanding the stability of emulsion. For instance, force-distance measurements can provide insight into the strength and behavior of the interfacial layers surrounding nanoemulsion droplets.[97] However, complex sample preparation can make it challenging to avoid distortion of droplets during the process. By pre-attached the droplets to cantilever tips, it is possible to directly measure the surface forces between a pair of droplets under aqueous conditions.[96,98] The deformability of oil droplets is linked to the structure and composition of their interfacial layers. By analyzing alterations in the force-distance curve slopes, scientists can quantify droplet deformability and monitor changes in interfacial structures.[98] Nevertheless, significant challenges persist in using AFM due to the difficulties associated with pre-attaching single droplets to the cantilever tips.

D. Rheometry

Rheology is the study of the flow and deformation of matter, primarily focusing on the behavior of complex fluids and soft solids.[99,100] It provides insights into how materials respond to applied forces, helping to understand the mechanical behavior, stability, and microstructure of emulsions. A solid understanding of emulsion rheology is also essential for understanding and predicting their performance in various applications.

Shear modulus is a measure of the emulsion's ability to resist deformation under shear stress, reflecting its viscoelastic properties. For emulsions, the shear modulus reflects the interfacial properties of the droplets and the strength of the interactions between the phases.[101] The shear-rheological properties were measured by applying oscillatory frequency-sweep or strain-sweep and measuring the stress. Linear Viscoelastic Region (LVR) is a crucial aspect in rheology and dynamic mechanical analysis (DMA). The LVR is the range in which the viscoelastic properties of a material, such as modulus and viscosity, remain independent of the applied strain. Maintaining measurements within the LVR is essential for obtaining accurate and representative data, as experiments outside this region can alter the

TABLE I. Advantages, disadvantages, and application of various techniques for characterizing emulsion.

Characterization technique	Application	Advantages	Disadvantages	Comments
Dynamic light scattering (DLS)	Analyzing particle/droplet size distribution, monitoring emulsion stability	Quick, suitable for small particle size distribution	Not ideal for high polydisperse sample, large particles/droplets can dominate the signal	Challenging for most emulsions due to polydispersity
Static light scattering (SLS)	Size distribution of large particles/droplets, molecular weight analysis, and interparticle interactions	Suitable for larger particle sizes, better for emulsion with polydisperse	Relies on theoretical models, multiple scattering effects at high concentrations	Limited use in Pickering emulsions due to high particle concentration
Small-angle x-ray scattering (SAXS)	Analyzing the internal structure of particles/droplets, suitable for structures within the 1–100 nm range	High-resolution structural information at the nanoscale	Requires high density contrast and complex data modeling for complex samples	Highly suitable for Pickering and multiple emulsions
Small-angle neutron scattering (SANS)	Similar to SAXS but better for light-element materials and contrast enhancement through isotopic substitution	Allows component contrast through isotope substitution, suitable for complex systems	Expensive equipment, requires radiation protection	Ideal for Pickering and multiple emulsions due to structural complexity
Fourier transform infrared (FTIR) spectroscopy	Analyzing molecular interactions and interfacial structures in emulsions	Fast, non-destructive, provides detailed information on molecular interactions	Overlapping peaks are common, requires chemometric methods for complex spectra	Beneficial for Pickering emulsions to understand interfacial interactions
Raman spectroscopy	Surface and structural analysis of emulsions, real-time monitoring of emulsion formation	Component distribution analysis without staining, suitable for real-time monitoring	Sensitive to sample movement, signal can be affected by background light	Useful for all type of emulsions to analyze interfacial layers
Nuclear magnetic resonance (NMR)	Analyzing component distribution, particle diffusion, and emulsion stability	Provides molecular-level dynamic information suitable for stability monitoring	Expensive equipment, complex data interpretation	DOSY-NMR especially beneficial for size and stability analysis in multiple and Pickering emulsions
Confocal laser scanning microscopy (CLSM)	Observing internal 3D structure of emulsions	High-resolution 3D imaging, can distinguish different components	Requires fluorescent dyes; samples may be damaged by prolonged laser exposure	Highly effective for Pickering and multiple emulsions to examine particle and droplet distribution
Scanning electron microscopy (SEM)	Observing emulsion surface morphology	High-resolution surface morphology images	Requires vacuum conditions, may require coating	Environmental SEM (ESEM) is particularly useful for Pickering emulsions

TABLE I. (*Continued*.)

Characterization technique	Application	Advantages	Disadvantages	Comments
Transmission electron microscopy (TEM)	Observing internal structure of emulsions, suitable for Pickering emulsion or multiple emulsion	Provides nanoscale internal structural information	Requires ultra-thin slicing or cryo-preparation, complex sample preparation	Cryo-TEM is especially beneficial for Pickering emulsions to avoid artifact formation
Rheometry	Analyzing mechanical properties, stability, and flow behavior of emulsions	Suitable for analyzing mechanical properties and understanding structure-stability relationships	Results highly dependent on measurement conditions, requires integration with other techniques	Essential for all emulsion types; particularly relevant for Pickering and multiple emulsions due to complex interfacial structures

material's microstructure, leading to misleading results. The LVR can be determined in the strain-sweep measurement.

After a deformation is applied to an emulsion, its ability to return to its original state over time is known as relaxation behavior. In stress relaxation tests, the material's stress is observed as it relaxes under a constant strain.[102] In creep recovery tests, the material's ability to recover after a constant strain is removed is measured.[101,103] Relaxation behavior gives insights into the time-dependent stability of an emulsion. Slow relaxation indicates a stable internal structure, where the droplets or the network within the continuous phase are less likely to change over time. Fast relaxation, on the other hand, might suggest that the emulsion will lose its structure or separate more quickly. Relaxation behavior gives a macroscopic reflection of material's internal structure and how it rearranges or relaxes after being disturbed.

The relaxation behavior is always measured within the LVR, where the material's response to stress or strain is linear. Non-linear rheology beyond the LVR, where the stress–strain relationship is no longer proportional, captures the material's response under larger strains or stresses where non-linear effects dominate.[104] Large amplitude oscillatory shear (LAOS) is often used to explore non-linear rheology, where a sinusoidal shear strain or shear stress is applied to the material.[105] The amplitude of this oscillatory input is large enough to push the material into a nonlinear regime. Emulsions exhibit complex non-linear behaviors such as shear thinning and strain hardening or softening depending on the concentration and composition of the dispersed phase. These behaviors are influenced by the droplet size distribution and the rheology of the interface, with more elastic interfaces leading to more pronounced non-linear responses.[16,103]

Viscosity is a key factor in a fluid's resistance to flow. In an emulsion, both the continuous phase (the phase in which droplets are dispersed) and the dispersed phase (the droplets) contribute to the overall viscosity. A higher viscosity of the continuous phase can slow down the movement of droplets, reducing the likelihood of creaming, sedimentation, and coalescence, which is a major cause of emulsion breakdown. Shear viscosity gives a macroscopic reflection of the motion resistance caused by the shearing force or friction between dispersed droplets and the continuous-phase boundary, providing better insights into the internal structure, stability, and flow behavior of the emulsion.[106,107] Emulsion exhibits Newtonian and shearing-thin non-Newtonian behaviors, and a few exhibit thixotropic and rheopexy behavior depending on the composition of the emulsion.[10,11,16,107–110] In a rheometer, shear viscosity is measured by applying a controlled range of shear rate and measuring the corresponding shear stress. The relationship between shear stress (τ) and shear rate ($\dot{\gamma}$) is used to calculate viscosity ($\eta = \frac{\tau}{\dot{\gamma}}$). Geometries need to be carefully selected to match the potential viscosity range.

The properties of the interface between the dispersed droplets and the continuous phase significantly influence the rheology of emulsions. Measurement of the elastic and viscous moduli of the interface and the response of the interface to expansion and compression is crucial for understanding the stability of the emulsion. A more elastic interfacial layer prevents droplet coalescence, thereby enhancing emulsion stability.[11,16,103]

IV. CONCLUSION

In conclusion, the characterization of emulsions is a multi-faceted process requiring a careful selection of techniques tailored to the specific properties, applications, and stability challenges of the emulsion system in question. Advanced scattering techniques like DLS and SAXS offer invaluable insights into droplet size distribution and internal structure. Spectroscopic methods such as Raman spectroscopy and NMR provide detailed information about molecular interactions and the chemical environment within emulsions, which are crucial for understanding interfacial behavior and emulsion stability. Microscopy techniques, ranging from CLSM to SEM or TEM, have proven essential for visualizing emulsion morphology and interfaces, especially when studying complex systems like Pickering emulsions and multiple emulsions. Rheometry further complements these techniques by providing a macroscopic understanding of emulsion stability and mechanical properties under various conditions Table I.

When selecting characterization techniques, it is essential to consider the specific type of emulsion (e.g., conventional, Pickering, or multiple emulsions) and the challenges it presents, such as droplet size distribution, interfacial properties, or phase transitions. Every technique has limitations in providing a complete picture of an emulsion system. Therefore, a combination of methods is often necessary to fully understand its behavior under different conditions. For example, integrating rheometry with scattering analysis techniques such as small-angle neutron scattering (SANS) or small-angle x-ray scattering (SAXS) enables us to link the microstructural characteristics of emulsions with their mechanical behavior under various conditions. Coupling Fourier-transform infrared (FTIR) spectroscopy with rheometry allows for real-time tracking of chemical changes and bond formation within emulsions under shear. The rheo-NMR technique combines rheology with nuclear magnetic resonance, allowing insights into how molecular structures and interactions change during stress.

By providing a detailed overview of each technique's strengths and limitations, this review aims to assist researchers in making informed decisions when selecting the appropriate methods for emulsion characterization. The potential to combine these methods in a complementary manner offers a comprehensive pathway to understanding emulsions at both the molecular and macroscopic levels, guiding innovation in pharmaceuticals, food science, cosmetics, and beyond.

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Xuncheng Shi: Conceptualization (lead); Writing – original draft (lead). **Dawei Qi**: Conceptualization (supporting); Writing – review & editing (supporting). **Caihong Lin**: Writing – review & editing (supporting). **Jianwei Li**: Funding acquisition (lead); Supervision (lead); Writing – review & editing (lead).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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