Journal of Molecular and Pharmaceutical Sciences

Review

An overview of bioactive biomolecule encapsulation: techniques, material characterization, and activity optimization

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Abstract

The encapsulation of bioactive biomolecules is an important strategy designed to enhance the protection, stability, and functional performance of sensitive compounds while maintaining their biological activity. This review discusses a range of encapsulation techniques such as coacervation, solvent-based methods, gelation, polymerization processes, spray drying, fluidized bed coating, and extrusion, which are widely used to produce micro- and nanoscale delivery systems. These technologies enable multiple functions, including safeguarding active compounds from environmental degradation, improving their handling properties, and allowing controlled or targeted release. However, their large-scale application is still constrained by economic factors, limited availability of standardized materials, and technological challenges in industrial processing. Recent developments in advanced materials such as nanostructured carriers, modified natural polymers, hybrid matrices, and stimuli-responsive systems have shown improved efficiency in encapsulation and release control. Ongoing research is increasingly focused on improving process efficiency, ensuring safety, and developing scalable systems, including multi-component encapsulation strategies. These advances are expected to expand the potential use of encapsulated biomolecules in food systems, biomedical applications, and pharmaceutical formulations.

Keywords:

Encapsulation; bioactive biomolecules; drug delivery systems; enhanced efficacy

★ Received May 06, 2026; accepted June 06, 2026

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Cited as: Srief M. An overview of bioactive biomolecule encapsulation: techniques, material characterization, and activity optimization. J. Mol. Pharm. Sci. 05 (01), 2026, 29-38.

1. Introduction

Biomolecules such as proteins, enzymes, nucleic acids, and naturally derived bioactive compounds play a central role in many fields. Their importance lies in the diversity of their biological properties, including therapeutic, antioxidant, antimicrobial, and functional activities. As such, they are attracting increasing interest, particularly in the development of new strategies for disease prevention and treatment. Moreover, the growing demand for natural products and biotherapies has reinforced the need to optimize the exploitation of these high-potential molecules.

However, the direct use of these molecules remains limited due to their instability and sensitivity to environmental conditions, particularly variations in temperature, pH, exposure to light, oxygen, and enzymatic activity. These factors can lead to their degradation, structural alteration, and consequently a significant reduction in their biological activity. These limitations have driven the development of advanced delivery systems aimed at improving their stability, transport, and biological effectiveness [1].

Today, encapsulation represents a technology widely used across multiple industries involved in the production of formulated products, including the chemical, food, pharmaceutical, cosmetic, nutraceutical, agricultural, textile, and coating sectors. This technique consists of entrapping biomolecules within microstructures made of suitable materials, forming a protective barrier that mitigates the impact of external environmental factors. It is a method used to produce high value-added products, regardless of whether the core material is solid, liquid, or gaseous within a carrier matrix [2]. Encapsulation thereby protects biomolecules against degradation factors such as light, oxidation, temperature, or pH variations. Furthermore, it enables controlled release of the encapsulated molecules and promotes their targeting toward specific sites, fitting within the framework of advanced drug delivery systems.

In recent years, significant advances in encapsulation materials and techniques, particularly through nanotechnologies and stimuli-responsive systems (pH, temperature, enzymes), have expanded its applications from targeted drug delivery to the protection of sensitive compounds in food and cosmetic products [3].

In this context, this review aims to provide a comprehensive overview of current knowledge related to biomolecule encapsulation. The main materials and encapsulation techniques will be described, along with their applications across various fields. A critical analysis of the advantages, limitations, and future perspectives will also be presented in order to better understand the potential of this technology in current research and its future development.

2. Principles of Biomolecule Encapsulation

Encapsulation is defined as a process consisting of trapping or coating solid, liquid, or gaseous materials within individualized miniature membranes or matrices. In other words, it involves incorporating one or more molecules into small hollow or porous spheres of nano-, micro-, or millimeter size, thereby protecting them with a barrier that inhibits interactions with the surrounding environment. These encapsulated molecules are subsequently released in a controlled manner, at variable rates over defined periods of time, under specific conditions such as pH, temperature, enzymatic activity, oxygen, or other particular stimuli. Depending on the size of these particles, the outcome of encapsulation may be at the micrometer scale (microparticles), nanometric scale (nanoparticles, mainly liposomes), or even millimetric scale, especially when presented as aggregates [1,2].

Microparticles can be classified into two categories according to their microstructure (Figure 1):

- ✓ **Microcapsule:** a particle or reservoir-type system, which may have a non-spherical shape, consisting of a core containing an active substance in liquid or solid form, surrounded by a thin solid wall made of a coating material. The active substance may be located inside the microcapsule or adsorbed onto its surface.
- ✓ **Microsphere:** a structure composed of a continuous polymer network forming a matrix in which a bioactive substance is dispersed in the form of molecules, fine solid particles, or solution droplets. This system is referred to as a matrix system, as the bioactive substance is embedded within the polymer matrix [2].

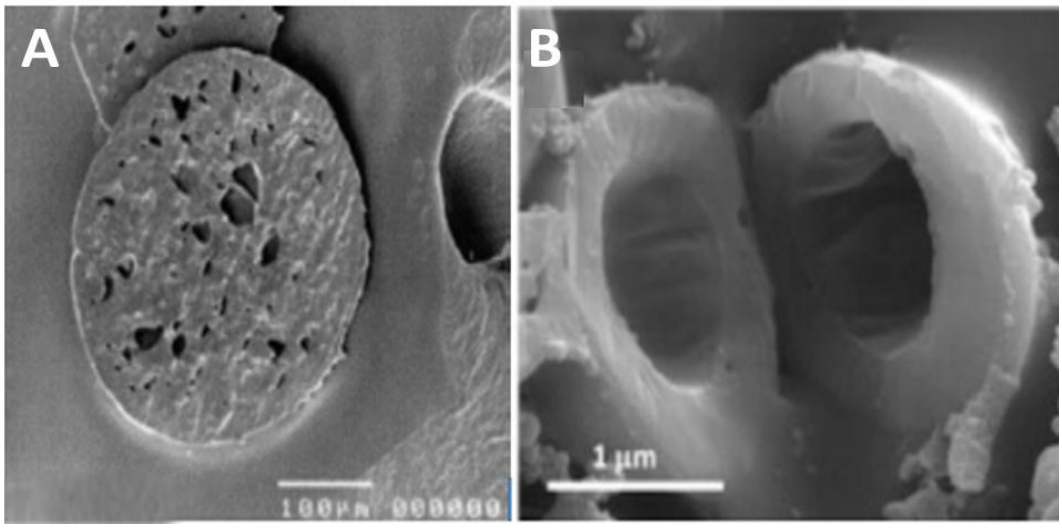

Figure 1: Comparison of images of microspheres (a) and microcapsules (b) containing a dispersed active compound, observed by scanning electron microscopy [4].

3. Encapsulation Materials

The coating materials used in the encapsulation process can originate from different sources, namely natural, synthetic, or semi-synthetic. They must be biocompatible, biodegradable, non-toxic, and capable of forming a protective barrier between the internal phase and its environment. The selection of coating materials is generally based on criteria such as [1]:

Physicochemical properties (such as oxidation, stability, moisture, pH), as well as thermal and mechanical properties of the coating materials

- ✓ The type of active compound and its characteristics
- ✓ The intended application of the encapsulated products
- ✓ Cost constraints, which are considered a key factor in selecting the most appropriate materials

The most commonly used base materials include [2,5,6]:

3.1. Natural Polymers

Natural polymers, such as polysaccharides and polypeptides, are promising candidates as carriers for biomedical compounds. They are widely used in encapsulation due to their biocompatibility, biodegradability, and low toxicity. Derived from biological sources (plants, animals, or microorganisms), they are particularly suitable for pharmaceutical, food, and biomedical applications. Natural polymer-based carriers, often exhibiting a core-shell structure, offer numerous possibilities for introducing multifunctional features and enable the simultaneous encapsulation of active materials with different physicochemical properties for targeted delivery and prolonged and/or stimuli-responsive release [7,8].

Polysaccharides are natural polymers composed of monosaccharide units linked by glycosidic bonds [8,9]. They are attractive encapsulation materials due to their good solubility, low viscosity at high concentrations, and low cost. They include starch and its derivatives, plant-derived gums and pectins, marine polysaccharides such as alginate and carrageenans, as well as microbial and animal polysaccharides such as dextran, xanthan, and chitosan.

Proteins, composed of amino acids, are also widely used as encapsulation materials. Their amphiphilic nature allows them to encapsulate both hydrophilic and hydrophobic compounds while providing emulsifying, gelling, and film-forming properties. These include plant proteins (soy, wheat) and animal proteins (casein, egg proteins, gelatin) [9,10].

3.2. Synthetic Polymers

Synthetic polymers are macromolecular materials produced by chemical synthesis in the laboratory and do not occur naturally. They are obtained through the polymerization of specific monomers, allowing precise control over their physicochemical properties. Among them, acrylic polymers, PLGA, and PEG are widely used in biomedical and pharmaceutical applications.

Due to their high design flexibility, they can be tailored in terms of structure, stability, hydrophilicity, and biodegradability. In encapsulation, they are particularly valued for their ability to form stable matrices and protect biomolecules from degradation.

However, certain limitations related to the biocompatibility and biodegradability of some polymers still require improvement for optimal biological applications [10,11].

3.3. Lipids

The use of lipids as coating materials for microencapsulation is relatively recent. The main advantage of lipid-based systems is their strong affinity with cell membranes, which enhances the bioavailability of the encapsulated active substance. Lipids are often used in solid form (fatty acids, fatty alcohols, glycerides, phospholipids) or as waxes (beeswax, carnauba wax, candelilla wax) [2,6,7,12].

3.4. Emerging Materials

Emerging materials represent an innovative class of encapsulation supports developed to overcome the limitations of conventional systems and improve biomolecule delivery. They offer advanced properties such as enhanced stability, high biocompatibility, and responsiveness to various stimuli (pH, temperature, enzymes) [11,12,13].

Among these, nanomaterials occupy a prominent place. They include polymeric nanoparticles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and nanogels. These systems enable efficient encapsulation and controlled release due to their nanoscale size and large specific surface area [14].

Modified or recombinant biopolymers also represent an emerging category. These include modified polysaccharides and recombinant proteins, offering tunable properties depending on biomedical and pharmaceutical applications [14,15].

In parallel, hybrid materials combining natural and synthetic polymers or lipid matrices improve the stability and functionality of encapsulation systems [16].

Smart or stimuli-responsive materials represent a major advancement in encapsulation science. These systems respond to specific internal or external triggers such as pH, temperature, redox potential, light, or enzymatic activity to release their payload in a spatially and temporally controlled manner. pH-responsive systems are particularly valuable for oral drug delivery, where the acidic gastric environment (pH ~1.2-2) can be exploited to protect cargo in the stomach while releasing it in the intestine (pH ~6.8-7.4). Temperature-sensitive hydrogels based on poly(N-isopropylacrylamide) (PNIPAM) undergo reversible sol-gel transitions near physiological temperature, making them attractive for localized drug depots. Redox-responsive carriers exploit the elevated glutathione concentration in tumor cells (~10 mM intracellular vs. ~2 μM extracellular) to trigger disulfide bond cleavage and selective cargo release at the tumor site [16,17].

Among the most clinically significant recent advances are lipid nanoparticles (LNPs), which have emerged as the leading delivery platform for nucleic acid therapeutics. The successful deployment of mRNA-LNP vaccines against COVID-19 (BNT162b2 and mRNA-1273) demonstrated the scalability and clinical viability of this technology, achieving encapsulation efficiencies exceeding 90% and enabling systemic mRNA delivery at unprecedented scale [16,17]. Beyond vaccines, LNPs are now being investigated for the delivery of siRNA, CRISPR-Cas9 components, and cancer immunotherapy payloads. Similarly, polymeric nanoparticles such as PLGA-based carriers have been approved or are in advanced clinical trials for the delivery of proteins, peptides, and small-molecule drugs, demonstrating encapsulation efficiencies of 60-85% with tunable release kinetics from days to months.

Overall, these emerging materials open significant prospects for the development of advanced encapsulation systems suited to biomedical, pharmaceutical, and food applications. However, their translation from bench to clinic remains challenged by manufacturing complexity, regulatory requirements for novel excipients, and

the need for robust long-term stability data. Critical evaluation of these systems must therefore consider not only their in vitro performance but also their scalability, safety profile, and cost-effectiveness in real-world production contexts.

4. Encapsulation Processes

As previously mentioned, encapsulation refers to all methods used to entrap an active compound within a membrane or a solid or liquid matrix. These processes enable the production of particles of various sizes at the millimeter, micrometer, or nanometer scale. These particles can release their contents in a controlled manner depending on various factors such as the nature of the wall materials, pH, enzyme activity, and chelating agents, at predetermined rates.

Encapsulation efficiency and yield can be influenced by several parameters governing the interaction between the active compound and the coating materials. These include the chemical nature of the core, such as its molecular weight, degree of hydrophilicity/hydrophobicity, functional groups, polarity, and volatility. The properties of the base material used for encapsulation, as well as the chosen encapsulation technique, are also important factors to consider [6,7].

To meet the requirements of various active substances and coating materials, a wide range of encapsulation methods has been developed. Industrial encapsulation processes can be classified according to different criteria, such as:

- ✓ The use or absence of organic solvents
- ✓ The state of the dispersing medium (liquid, gaseous, or supercritical state)
- ✓ The use of preformed polymers, lipids, or monomers
- ✓ The process used to achieve microencapsulation

They can be broadly classified into three groups (Table 1) according to the technological processes used: physicochemical, mechanical, and chemical [17].

Table 1: Different encapsulation processes [17].

Microencapsulation Processes	Principle	Techniques
Physicochemical	Based on changes in the physical state of polymers, such as melting and solidification, as well as on parameters like solubility and precipitation, which can vary depending on the addition of a non-solvent, pH modifications, or temperature changes.	Coacervation (simple or complex) Solvent evaporation/extraction Thermal gelation
Chemical	Involve the in situ formation of the coating material through polycondensation or polymerization of monomer units. Unlike physicochemical and mechanical methods, which use pre-existing coating materials such as polymers or lipids.	Interfacial polymerization In situ polymerization (radical or anionic) Polycondensation
Mechanical	Based on spraying techniques, droplet or bead formation, as well as extrusion processes.	Supercritical fluid technology Spray drying Droplet gelation or freezing Fluidized bed coating Extrusion

A critical comparison of encapsulation techniques reveals significant differences in terms of efficiency, scalability, cost, and suitability for specific biomolecules (Table 3). Physicochemical methods such as coacervation offer high encapsulation efficiency (60-95%) and are well suited for sensitive proteins and

peptides; however, they require careful pH and temperature control and may involve complex, multi-step processes that limit industrial throughput. Spray drying is the most widely industrialized technique due to its continuous operation mode, low cost per unit, and compatibility with heat-stable compounds, but it suffers from reduced efficiency (40-80%) for heat-sensitive biomolecules and can generate particles with broad size distributions. Chemical methods such as interfacial polymerization yield particles with excellent membrane integrity and sustained-release profiles, yet the use of organic solvents and monomers raises safety and regulatory concerns that complicate scale-up. Emerging nanoencapsulation approaches (LNPs, polymeric nanoparticles) achieve superior control over particle size (50-500 nm) and release kinetics, and are increasingly used in clinical applications; nevertheless, their production requires sophisticated equipment, stringent quality control, and represents a substantially higher manufacturing cost compared to conventional microencapsulation.

Table 3: Quantitative comparison of major encapsulation methods — efficiency, particle size, release behavior, advantages, and limitations.

Method	**EE (%) / Size**	**Release Profile**	**Scalability / Cost**	**Key Advantages**	**Key Limitations**
Coacervation	60-95% / 1-1000 µm	Sustained; pH-triggered	Moderate / Medium	High EE; mild conditions; suitable for proteins and peptides	Multi-step process; sensitive to ionic strength and pH; limited industrial throughput
Spray Drying	40-80% / 10-400 µm	Immediate to sustained	High / Low	Continuous process; low cost; easily scalable; produces free-flowing powder	Thermal degradation of heat-sensitive compounds; broad particle size distribution
Interfacial Polymerization	50-90% / 0.5-500 µm	Sustained; membrane-controlled	Low-Moderate / High	Excellent membrane integrity; tunable wall thickness; suitable for reactive core materials	Use of organic solvents and monomers; residual monomer toxicity; complex regulatory approval
Lipid Nanoparticles (LNPs)	>90% / 50-300 nm	Rapid endosomal release; on-demand	Moderate-High / High	Clinically validated (mRNA vaccines); high EE for nucleic acids; biocompatible lipid components	Cold chain storage required; specialized microfluidic manufacturing; high cost per dose
PLGA Nanoparticles	60-85% / 100-500 nm	Sustained; days to months	Moderate / High	FDA-approved excipient; biodegradable; tunable degradation kinetics; clinical track record	Acidic microenvironment upon degradation (can denature proteins); burst release; organic solvent use
Fluidized Bed Coating	70-95% / 100 µm-3 mm	Sustained; moisture-triggered	High / Low-Medium	Scalable; continuous operation; uniform coating; food and pharma approved	Limited to solid or granulated cores; particle attrition; high energy consumption

5. Characterization of Encapsulated Systems

The characterization of encapsulated systems is an essential step to evaluate their physicochemical properties, performance, and long-term stability. Several key parameters are generally studied.

Particle size and distribution

Particle size and its distribution directly influence the stability of encapsulated systems. A decrease in particle size increases the surface-to-volume ratio, thereby improving bioavailability and functional properties, while reducing interactions between particles. Conversely, larger particles may promote degradation of the encapsulating material.

5.1. Particle sizes

generally range from 1.02 to 1880 μm for microparticles and from 10 to 811 nm for nanoparticles. The polydispersity index (PDI) is used to assess homogeneity: values below 0.3 indicate a uniform distribution and good stability, whereas higher values indicate heterogeneity.

Particle size and PDI are mainly measured by dynamic light scattering (DLS), complemented by other techniques such as photon correlation spectroscopy and laser diffraction [18].

5.2. Surface charge (zeta potential)

In ionic media, particles are surrounded by oppositely charged ions forming an electrical double layer. The potential measured at the interface of this layer, known as the zeta potential, is a key indicator of colloidal stability.

High zeta potential values (greater than ±30 mV) indicate good stability due to electrostatic repulsion forces that prevent particle aggregation. Conversely, lower values are associated with reduced stability and a higher tendency to agglomerate.

Zeta potential is generally measured using a zeta sizer, as well as techniques such as phase analysis light scattering (PALS) or electrophoretic mobility measurement [18].

5.3. Morphology (SEM, TEM)

Particle morphology corresponds to their external and internal structure, strongly influenced by the nature of encapsulation materials and preparation conditions. Particles are generally spherical but may also present cylindrical, ellipsoidal, or irregular shapes. Particle shape plays an important role as it can affect stability, aggregation tendency, optical properties, and the release profile of encapsulated compounds.

Morphological characterization is performed using different microscopy techniques, including phase contrast microscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). These methods allow observation of particle topography, structure, and shape, providing essential information about their organization and integrity [18].

5.4. Encapsulation efficiency

Encapsulation efficiency (EE%) corresponds to the percentage of active compound effectively entrapped within the system compared to the initial amount used. It is generally determined by analytical methods such as high-performance liquid chromatography (HPLC) after separation of the free active compound. High efficiency is desired to optimize therapeutic loading and minimize losses [19].

5.5. Controlled release

The study of the active compound release profile allows evaluation of the system's ability to provide sustained or targeted delivery. These studies are often performed in vitro in media simulating physiological conditions. Encapsulated systems may exhibit different release profiles (immediate, sustained, or biphasic), depending on the nature of the polymer and matrix-active compound interactions [20].

5.6. Stability

Stability is a critical parameter for the development and storage of encapsulated systems. It includes physical stability (aggregation, particle size changes), chemical stability (active compound degradation), and colloidal stability. Stability studies are conducted under different temperature, pH, and humidity conditions to predict shelf life and optimal storage conditions [19,21].

6. Objectives of Encapsulation

Encapsulation acts as a barrier surrounding active compounds, reducing chemical interactions and providing protection against harmful environmental influences, especially for sensitive, volatile, or unstable compounds, or when a change in physical state is desired (e.g., liquid to solid) [1]. According to Poncelet (2006), the objectives of encapsulation can be summarized into five categories:

- ✓ Immobilization or entrapment: to reduce proximity or contact with specific elements of a system. For example, immobilization of cells or enzymes enables continuous processing while preventing washout.
- ✓ Protection or stabilization: to limit evaporation of volatile active agents and protect fragile ingredients from unfavorable chemical, physicochemical, and mechanical conditions. For example, vitamins or polyunsaturated fatty acids can be degraded by oxygen, while some drugs and probiotics are destroyed during gastric transit.
- ✓ Controlled or delayed release: to ensure prolonged release of substances at a targeted site, reducing immediate release (e.g., aromas, vitamins, therapeutic bioactives).
- ✓ Modification of structural properties: microencapsulation can transform a liquid or viscous solution into a powder, which is easier to handle pharmaceutically. It can also modify organoleptic properties, such as masking undesirable taste and odor.
- ✓ Functionalization: microencapsulation can be used to develop new functions such as regulation of biocatalyst activity and control of membrane permeability.

7. Applications of Encapsulation

Encapsulation has numerous applications across various fields, as shown in Table 2. This table also provides examples of compounds that can be encapsulated [22].

Table 2: Fields of application of encapsulation [22].

Field	Examples of encapsulated compounds
Medical and pharmaceutical	Antibiotics, enzymes, vaccines
Food	Essential oils, fats, spices, flavors, colorants, yeasts, microorganisms
Cosmetic and parapharmaceutical	Perfumes, detangling balms, essential oils, shaving foams
Biotechnology	Immobilized enzymes, tissue cultures, microorganisms, living cells
Chemistry	Catalysts, plastic additives, lubricants, colorants and pigments
Agriculture	Crop protection (herbicides, insecticides), fertilization (fertilizers), pest control (repellents), plant growth regulation (plant hormones)
Detergents	Bleaching and foaming agents, silicones, waxes, stain removers, disinfectants, fabric softeners and conditioning agents
Textile	Finishing agents (stain resistance, water repellency, wrinkle resistance), UV protection agents, antimicrobial agents, controlled release systems (cosmetic, medical, repellents), flame retardants
Graphics and printing	Colorants, protective agents (scratch resistance, UV protection, environmental protection), developers, toners
Waste treatment	Encapsulation of hazardous waste, radioactive waste, organic waste
Electronics	Encapsulation of flexible electronic devices and integrated circuits for environmental isolation and protection against physical shocks

8.Conclusions and Future Perspectives

The encapsulation of bioactive biomolecules represents an essential approach to improving their stability, bioavailability, and effectiveness while preserving their biological activities. It is based on various techniques such as coacervation, solvent extraction, gelation, polymerization, spray drying, fluidized bed coating, and extrusion, enabling the production of micro- and nanoparticles suitable for numerous applications. These systems perform several key functions, including the protection and stabilization of sensitive compounds, their immobilization, the modification of their physical and organoleptic properties, and their functionalization to enhance or control their activity.

A critical point that merits greater emphasis is the direct relationship between encapsulation strategy and the optimization of biological activity. Encapsulation does not merely protect biomolecules passively; it actively modulates their bioavailability, pharmacokinetics, and therapeutic efficacy. For example, encapsulation of insulin in chitosan nanoparticles has been shown to increase oral bioavailability from less than 1% to 4-10% by protecting the protein from gastric proteolysis and enabling paracellular transport across intestinal epithelium. Similarly, the encapsulation of curcumin a hydrophobic polyphenol with near-zero oral bioavailability—in PLGA or lipid nanoparticles has improved its plasma AUC by up to 10-fold in preclinical models. These examples illustrate that the choice of encapsulation system must be driven not only by protection criteria but by a rigorous understanding of the target site, desired release kinetics, and the biological barriers the carrier must overcome.

Despite the progress made, significant industrial and translational challenges remain. Scale-up from laboratory to industrial production is a critical bottleneck: processes that yield high encapsulation efficiency at the milligram scale often suffer from reduced performance, batch-to-batch variability, and increased costs at pilot or commercial scale. Continuous manufacturing approaches, including microfluidics-assisted production of LNPs and continuous spray-drying lines, are emerging as promising solutions but require substantial capital investment and process validation. From a regulatory standpoint, novel excipients and nanomaterials face stringent requirements from agencies such as the FDA and EMA, including comprehensive toxicological characterization, long-term stability data, and demonstration of manufacturing reproducibility according to Good Manufacturing Practice (GMP) guidelines. The regulatory pathway for nanomedicines remains complex and varies between jurisdictions, which slows commercialization. Future perspectives should therefore focus not only on scientific innovation but also on building regulatory science frameworks, harmonizing characterization standards, and developing cost-effective manufacturing processes that can bridge the gap between promising laboratory results and commercially viable, safe encapsulation systems for food, biomedical, and pharmaceutical applications.

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