

## BIOSURFACTANTS AS SMART BIOCOMPATIBLE AGENTS IN PHARMACEUTICAL DRUG DELIVERY: ADVANCES IN FORMULATION, CHARACTERIZATION, AND THERAPEUTIC APPLICATIONS

KASIMANI R<sup>1</sup>, SHARMILA V<sup>2\*</sup>, ESATH NATHEER S<sup>2</sup>, KARUPPASAMY CHELLAPANDI<sup>3</sup>

<sup>1</sup>Department of Biotechnology, Nehru Arts and Science College (Autonomous) Thirumalayampalayam, Coimbatore, Tamil Nadu, India.

<sup>2</sup>Department of Microbiology, Nehru Arts and Science College (Autonomous) Thirumalayampalayam, Coimbatore, Tamil Nadu, India.

<sup>3</sup>Department of Medical Laboratory Technology, Regional Institute of Paramedical and Nursing Sciences, (Under Ministry of H&FW, Government of India), Aizawl, Mizoram, India.

\*Corresponding author: Sharmila V; Email: bioinfosharmi88@gmail.com

Received: 24 July 2025, Revised and Accepted: 06 December 2025

### ABSTRACT

Biosurfactants are a diverse group of amphiphilic biomolecules derived from microbial sources, offering a promising alternative to synthetic surfactants for pharmaceutical applications. Their exceptional biocompatibility, biodegradability, and low toxicity make them attractive candidates for drug delivery systems. This review comprehensively explores the principles, types, and production of biosurfactants, with a specific focus on their integration into advanced pharmaceutical formulations. Various biosurfactant classes – including glycolipids, lipopeptides, and polymeric and particulate biosurfactants – are discussed in detail, highlighting their structural features and functional relevance. The production process, encompassing microbial fermentation, isolation, purification, and formulation strategies, such as micelles, nanoemulsions, liposomes, solid lipid nanoparticles, and hydrogels, is critically analyzed. Characterization techniques, including tensiometry, spectroscopy, electron microscopy, and biocompatibility assays, are also reviewed. Key pharmaceutical applications, such as solubility enhancement, controlled and targeted delivery, gene and protein transport, wound healing, cancer therapy, and vaccine delivery are addressed with current strategic advancements. The review concludes by outlining future perspectives, emphasizing synthetic biology, personalized nanomedicine, and artificial intelligence as tools to enhance biosurfactant utilization in drug delivery. Overall, biosurfactants represent a sustainable, multifunctional platform for next-generation therapeutic systems.

**Keywords:** Biosurfactants, Drug delivery, Glycolipids, Lipopeptides, Micelles, Nanoemulsions, Solid lipid nanoparticles, Hydrogels, Gene therapy, Vaccine delivery, Biocompatibility, Nanomedicine, Sustainable pharmaceuticals.

© 2026 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2026v19i2.56233>. Journal homepage: <https://innovareacademics.in/journals/index.php/ajpcr>

### INTRODUCTION

Biosurfactants, a diverse group of amphiphilic biomolecules produced by microorganisms, have gained tremendous attention in the past decade due to their multifaceted roles across various scientific disciplines [1]. These surface-active compounds composed primarily of peptides, lipids, or polysaccharides, possess the ability to reduce surface and interfacial tension between individual molecules at the surface and interface, respectively [2]. The relevance of biosurfactants has been magnified by the present global shift toward sustainable, biodegradable, and biocompatible technologies, especially within the pharmaceutical and biomedical sectors [3].

Their environmental compatibility, lower toxicity, and functional versatility make biosurfactants ideal alternatives to synthetic surfactants, which are often associated with environmental hazards and cytotoxic effects [4]. Moreover, biosurfactants have demonstrated robust antimicrobial, antiadhesive, and antitumor properties, further solidifying their value in therapeutic formulations [5]. Of particular significance is their role in enhancing drug solubility, bioavailability, and stability, thereby facilitating targeted and controlled drug delivery [6].

In drug delivery systems, biosurfactants have emerged as promising candidates for the formulation of microemulsions, niosomes, solid lipid nanoparticles (SLNs), liposomes, and hydrogels [7]. Their ability to self-assemble into micelles and vesicles under physiological conditions enables the encapsulation and release of poorly soluble drugs, proteins, and genes, ensuring efficient delivery to targeted tissues [8].

Despite their immense potential, the commercialization of biosurfactant-based drug delivery platforms remains limited [9]. Challenges related to cost-effective large-scale production, purification, standardization, and regulatory approval need to be addressed through interdisciplinary research [10]. The integration of synthetic biology, nanotechnology, and process engineering holds the key to overcoming these limitations.

This review provides a comprehensive exploration of biosurfactants with emphasis on their classification, production, formulation strategies, characterization techniques, pharmaceutical applications, and emerging strategies for optimized drug delivery systems [11]. It also outlines future directions for biosurfactant research in the context of personalized and precision medicine.

Although biosurfactants demonstrate versatile pharmaceutical potential, their clinical translation remains hindered by application-specific challenges. In cancer therapy, tumor heterogeneity and biosurfactant-related hemolysis are major barriers. For gene delivery, maintaining stability in blood serum and achieving efficient endosomal escape remain unresolved. Vaccine applications demand careful optimization to avoid excessive immune activation while maintaining safety. Topical wound healing requires balancing sustained release with antimicrobial efficacy. Furthermore, large-scale production, regulatory approval, and reproducibility of biosurfactant-based formulations are ongoing hurdles. Comparative analysis suggests that no single biosurfactant class is universally superior; rather, each application requires tailored design and hybrid strategies to overcome these limitations.

## PRINCIPLE OF BIOSURFACTANTS AND THEIR TYPES

### Principle of biosurfactants

Biosurfactants operate on the fundamental principle of amphiphilicity. The dual affinity of biosurfactant molecules – comprising hydrophilic (water-attracting) and hydrophobic (water-repelling) domains – facilitates their alignment at interfaces, such as oil-water or air-water boundaries [12,13]. This alignment disrupts the cohesive forces between molecules at the surface, effectively lowering the surface and interfacial tension [14].

The amphiphilic structure enables biosurfactants to form a variety of supramolecular assemblies, including micelles, bilayers, and vesicles, which are crucial for encapsulating and transporting therapeutic agents [15,16]. These self-assembled structures play a vital role in solubilizing hydrophobic drugs, protecting labile molecules from degradation, and enhancing cellular uptake [17].

In addition, biosurfactants exhibit a critical micelle concentration (CMC), above which micelles spontaneously form [18]. This property is exploited in drug delivery systems to modulate the release

rate and enhance solubilization [19]. Their interaction with cell membranes, ability to modulate permeability, and potential to trigger immunological responses form the mechanistic basis of their action in drug delivery [20].

Fig. 1 shows that Representative chemical structures of biosurfactants and phospholipids. Rhamnolipid and sophorolipid are glycolipid biosurfactants; surfactin and mycosubtilin are lipopeptide biosurfactants; sphingomyelin and lecithin are phospholipids commonly found in biological membranes. These amphiphilic molecules exhibit surface-active properties that are exploited in drug delivery and therapeutic applications. CID for PubChem: 5458394.

Fig. 2 represents the biosurfactants, which are amphiphilic compounds containing hydrophilic sugar moieties and hydrophobic fatty acid chains, enabling surface tension reduction and emulsification. Rhamnolipids (mono- and di-) have one or two rhamnose units linked to  $\beta$ -hydroxy fatty acids. Sophorolipids exist in lactonic (closed-ring) and acidic (open-chain) forms. Trehalolipids consist of a trehalose disaccharide esterified with long-chain fatty acids. These structural

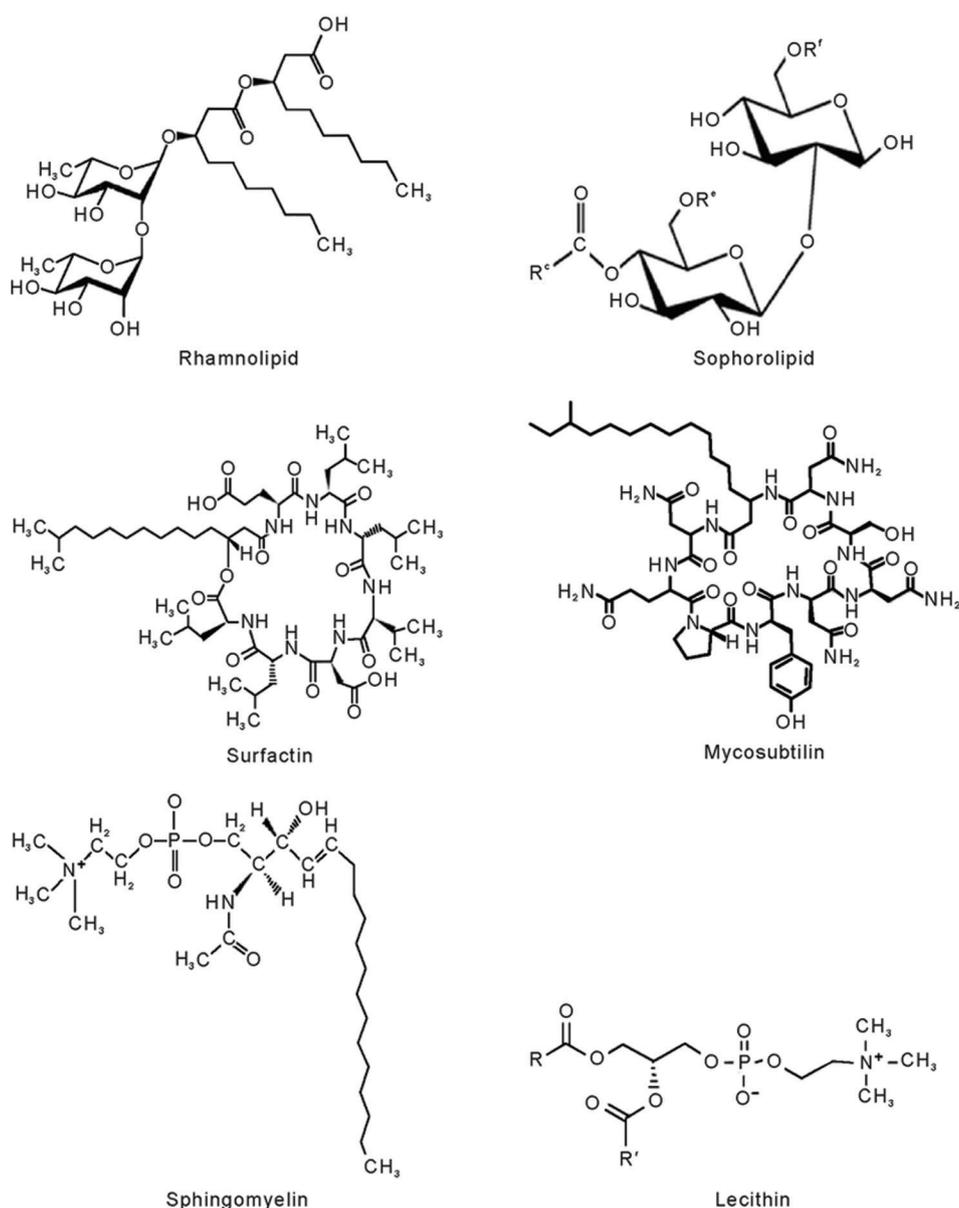
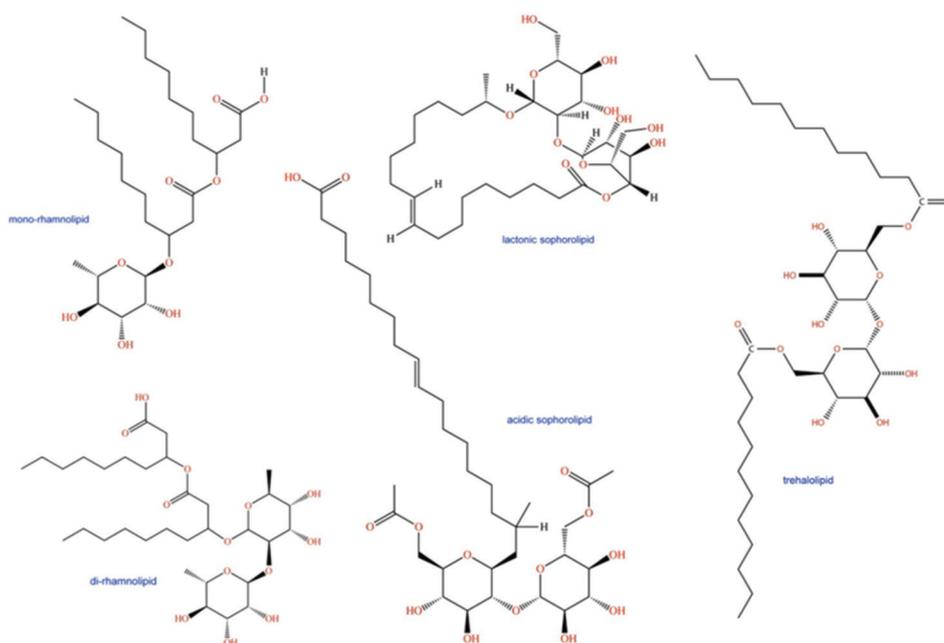


Fig. 1: Chemical structure of various biosurfactant



**Fig. 2: Representative chemical structures of major biosurfactants includes mono-rhamnolipid and di-rhamnolipid (glycolipids produced by *Pseudomonas* spp.), lactonic and acidic sophorolipids (produced by *Starmerella bombicola*), and trehalolipids (produced by *Rhodococcus* spp.)**

differences influence their physicochemical properties and potential applications in pharmaceuticals, cosmetics, and bioremediation.

#### Biosurfactant types

Biosurfactants can be broadly categorized according to their microbial source, structure, and chemical makeup [21]. Fatty acids and phospholipids, polymeric biosurfactants, particulate biosurfactants, glycolipids, and lipopeptides/lipoproteins are the main categories [22]. The distinct physicochemical and biological characteristics of each category dictate its appropriateness for particular industrial and pharmaceutical uses [23].

#### Glycolipids

One of the most researched classes of biosurfactants is glycolipids. These are molecules based on carbohydrates that have long-chain hydroxy fatty acids joined to monosaccharide or disaccharide moieties [24]. Glycolipids are prized for their superior emulsifying, surface-active, and antimicrobial qualities and are primarily produced by bacterial and yeast strains [25].

*Pseudomonas aeruginosa* is the primary producer of rhamnolipids, which are among the best-characterized biosurfactants [26]. They are made up of one rhamnose sugar molecule (mono-rhamnolipid) or two (di-rhamnolipid) linked to one or two chains of β-hydroxy fatty acids [27]. Excellent emulsification ability and bioactivity, including antimicrobial and anti-adhesive properties, have been demonstrated by rhamnolipids [28]. They are thought to be very appropriate for drug delivery because of their low toxicity and biodegradability, especially in topical and pulmonary formulations [29].

Yeast species, such as *Candida bombicola* secrete sophorolipids, which are made up of a long-chain hydroxylated fatty acid joined to a sophorose sugar, a glucose disaccharide [30]. Depending on how the carboxylic group is esterified, these biosurfactants can be either lactonic or acidic [31,32]. It has been investigated how they interact with immune cells, especially macrophages, to improve antigen presentation in the treatment of infectious diseases [33].

#### Lipoproteins and lipopeptides

Biosurfactants made of peptides or proteins coupled with lipid moieties are known as lipopeptides and lipoproteins. These biosurfactants,

which are mostly produced by *Streptomyces* and *Bacillus* species, are well-known for their diverse structural makeup and strong biological activity [34].

*Bacillus subtilis* produces surfactin, one of the most potent biosurfactants on the market. It is made up of a chain of β-hydroxy fatty acids connected to a cyclic heptapeptide. At very low concentrations, surfactin can lower the surface tension of water from 72 to 27 mN/m, demonstrating its exceptional surface activity. Surfactin is a promising agent for pharmaceutical applications, especially in drug solubilization and gene delivery, because it has been demonstrated to have antibacterial, antifungal, antiviral, and antitumor activities in addition to its emulsifying qualities [35].

Iturins and Fengycins are cyclic lipopeptides with potent antifungal effects that are also produced by *Bacillus* species [36]. Fengycins have a decapeptide with a fatty acid tail, while iturins are made up of a heptapeptide ring connected to a β-amino fatty acid chain [37]. These substances are useful for antifungal medication formulations because they compromise the integrity of fungal membranes. In addition, their cytolytic and hemolytic properties are being used to create biosurfactant-based anticancer treatments [38].

#### Neutral lipids, phospholipids, and fatty acids

Microorganisms, such as *Corynebacterium lepus* frequently produce these simpler biosurfactants as secondary metabolites. They have a moderate level of surface activity and are usually made up of phospholipid molecules or long-chain fatty acids [39].

Under specific stress conditions, these molecules, which are essential parts of microbial membranes, can be released into the surrounding medium. Their capacity to alter lipid bilayer structures and take part in emulsification is their main advantage. They are especially helpful as co-surfactants in emulsions and liposomal drug delivery systems in pharmaceutical formulations because they increase the stability of lipid-based carriers and improve membrane permeability [40].

#### Biosurfactants made of polymers

High molecular weight amphiphilic macromolecules made of repeating monomeric units of lipids, proteins, or carbohydrates are known as

polymeric biosurfactants [41]. Their superior emulsifying ability and thermal stability are frequently attributed to their complex structure [42].

The most well-known type of polymeric biosurfactant is emulsan, which is made by the bacteria *Acinetobacter calcoaceticus*. It consists of a heteropolysaccharide backbone that is covalently bonded to proteins and fatty acids [43]. Even at high temperatures and low concentrations, emulsan is especially effective at stabilizing oil-in-water emulsions [44]. Because of its strong emulsifying ability, it can be used to create sustained-release formulations and stabilize hydrophobic drug suspensions. Furthermore, polymeric biosurfactants' low toxicity and biodegradability increase their attractiveness for use in cosmetic and pharmaceutical applications [45].

### Biosurfactants in particulate form

Bioemulsifiers and particles coated with biosurfactants are examples of the special class of surface-active agents known as particulate biosurfactants. These are typically made up of polysaccharides or lipoprotein complexes with particulate properties that stabilize rather than significantly reduce surface tension [46].

The protein-polysaccharide complex that makes up Liposan, a well-researched particulate biosurfactant made by *Candida lipolytica*, is excellent at stabilizing oil-in-water emulsions. Because of their emulsifying power and biocompatibility, liposan and other bioemulsifiers are especially helpful in the food, pharmaceutical, and cosmetic industries [47]. Particulate biosurfactants, in contrast to low-molecular-weight surfactants, offer steric stabilization, which is essential for avoiding droplet coalescence in emulsions and suspensions, but they do not considerably reduce surface tension [48].

### FORMATION AND PRODUCTION OF BIOSURFACTANTS

For biosurfactants to be used in pharmaceutical drug delivery, their formulation and efficient production are essential [49]. Under ideal fermentation conditions, biosurfactants can be made from a range of microbial sources. Following production, these compounds go through a number of downstream procedures, including formulation into various delivery systems, isolation, and purification. To preserve the biosurfactants' structural integrity and functionality throughout storage and use, stabilization techniques are also essential [50-52].

### Microbial origins and culture

Numerous microorganisms, such as bacteria, yeasts, and fungi, naturally produce biosurfactants [53-56]. The most common producers among bacterial species are *Rhodococcus*, *Bacillus*, and *Pseudomonas* [57-60]. For example, *B. subtilis* produces strong lipopeptides, such as iturin and surfactin, while *P. aeruginosa* is known to produce rhamnolipids [61-65]. However, yeasts and fungi, such as *Aspergillus niger*, *Torulopsis bombicola*, and *C. bombicola* are efficient in producing mannosylerythritol lipids and sophorolipids [66].

Usually, solid-state fermentation (SSF) or submerged fermentation (SmF) is used to cultivate these microorganisms [67]. Microorganisms are grown in a liquid nutrient medium during SmF, which allows for exact control over environmental factors through regulated aeration and agitation. SSF is more cost-effective and ecologically friendly,

particularly for large-scale production, since it uses solid materials, such as bran or agro-waste instead of free-flowing water [68]. Researchers frequently use low-cost, renewable feedstocks, such as sugarcane bagasse, whey, molasses, and other agro-industrial wastes to lower production costs. These feedstocks also support sustainable manufacturing practices [69-72].

### Conditions of fermentation

To increase the yield and activity of biosurfactants, fermentation conditions must be optimized. A number of factors, such as pH, temperature, the carbon-to-nitrogen (C/N) ratio, dissolved oxygen levels, and agitation speed, have a major impact on microbial growth and biosurfactant synthesis [73]. Because it directly affects the kind and amount of biosurfactant produced, choosing the right carbon source is especially important. Many species tend to produce more biosurfactants when exposed to hydrophobic carbon sources, such as vegetable oils and alkanes.

Bioreactor systems, such as airlift fermenters or stirred tank reactors are usually needed for industrial-scale production. These systems make it possible to effectively regulate temperature, mixing, oxygen transfer rates, and foam management. Replicating lab-scale fermentation conditions while increasing volumetric productivity and decreasing batch-to-batch variability is a common goal of scale-up studies. Substrate feeding techniques are optimized in fed-batch or continuous bioreactor systems to extend the production phase and prevent substrate inhibition [74].

Although many studies describe biosurfactant-producing microbes, few provide reproducible quantitative yields under defined conditions. Table 2 summarizes representative examples from the literature, highlighting microbial species, substrates, and typical yields. For instance, *P. aeruginosa* commonly produces 2–10 g/L rhamnolipids in shake flasks using glucose or vegetable oils, while optimized fed-batch fermentations can exceed 40–50 g/L. Similarly, *C. bombicola* has been reported to produce sophorolipids up to 100–300 g/L using renewable substrates, such as waste oils.

Table 2 shows the typical fermentation parameters also influence yield: For *P. aeruginosa*, a C/N ratio of ~20:1, pH 6.5–7.0, and 30°C are frequently optimal, while aeration rates of 0.5–1.0 vvm and agitation at 200–300 rpm are commonly used in bioreactors. *B. subtilis* lipopeptides generally reach 2–5 g/L in submerged culture, with productivity strongly dependent on oxygen transfer and trace mineral availability.

### Purification and isolation

Following fermentation, a number of separation and purification procedures are needed to recover biosurfactants and produce high-purity, biologically active products [80]. To get rid of the microbial cells, the culture broth is first centrifuged. The biosurfactant is then precipitated by acidifying the supernatant to a pH of 2–4. It is then extracted using organic solvents, such as ethyl acetate, methanol, or chloroform [81,82].

Advanced purification techniques include chromatographic methods, such as high-performance liquid chromatography and ion-exchange

**Table 1: Summary of types and classes of biosurfactants**

Category	Compound	PubChem CID(s)	Structural Info
Glycolipid	Rhamnolipid	425975, 5458394	Sugars+fatty acids
	Sophorolipid	11856871	Sophorose+FA [49]
	Trehalolipid	-	Trehalose+FA
Lipopeptide	Surfactin	73170 (example isoform)	Cyclic peptide+FA [50]
	Iturin	various CIDs	Cyclic lipopeptide
	Fengycin	various CIDs	Cyclic lipopeptide
Fatty acids/phospholipids	-	-	Modular lipid structures
Polymeric biosurfactant	Emulsan	-	Polysaccharide-lipid complex [51,52]
Particulate	Liposan	-	Protein-polysaccharide mix [53]

chromatography for finer purification, as well as ultrafiltration, which enables the separation of biosurfactants according to molecular weight [83]. Because downstream processing is expensive and yields low, isolation and purification continue to be bottlenecks in the commercialization of biosurfactants, despite the availability of these techniques. Innovations in membrane technology and solvent-free extraction methods are currently being explored to enhance economic viability [84].

#### Methods of formulation

Biosurfactants are added to a variety of drug delivery systems that enhance drug solubility, stability, and bioavailability to make their use in pharmaceutical applications easier [85]. Various formulation techniques have been used, depending on the therapeutic agent's and the biosurfactant's physicochemical characteristics [86,87].

Drugs that are poorly soluble in water can be effectively dissolved by micelles and nanoemulsions [88]. To improve drug dispersion and absorption, biosurfactants in these systems self-assemble into micellar structures that contain hydrophobic drugs at their core [89]. Additional benefits of nanoemulsions include enhanced skin or mucosal penetration and quick drug release, with droplet sizes ranging from 20 to 200 nm [90,91].

Both hydrophilic and lipophilic medications are delivered by liposomes and niosomes, which are vesicular systems made of phospholipids and non-ionic surfactants, respectively [92,93]. By adding biosurfactants to these vesicles, the fluidity and stability of the membrane can be increased, allowing for targeted and regulated drug release [94]. These vesicles are especially helpful for pulmonary and transdermal delivery systems [95].

Biosurfactants can be added to hydrogels and films, which are networks of polymers, to deliver drugs topically. These formulations support tissue regeneration and wound healing by delivering sustained drug release and preserving a moist wound environment. These systems' antimicrobial and anti-inflammatory qualities are improved by the addition of biosurfactants [96].

Submicron colloidal carriers made of solid lipids are known as SLNs [97]. The utilization of biosurfactants as emulsifying agents in SLNs enhances sustained release, drug loading effectiveness, and particle stability. Because of their controlled drug release profile and safety, these systems are becoming more and more popular for parenteral and oral drug delivery [98].

Table 3 shows practice, biosurfactant-stabilized formulations exhibit defined physicochemical ranges critical for reproducibility and scale-up. For example, SLNs prepared with rhamnolipids typically yield particle sizes of 120–200 nm with polydispersity index (PDI) between 0.2 and

0.3 and zeta potentials of –25––40 mV, ensuring colloidal stability. Nanoemulsions stabilized with sophorolipids or surfactin often have droplet sizes of 50–150 nm, with PDIs <0.25. Liposomes incorporating glycolipids frequently display mean diameters of 100–200 nm and zeta potentials near neutrality or slightly negative, depending on the formulation.

#### Strategies for stabilization

The shelf-life and functional effectiveness of formulations based on biosurfactants are significantly impacted by stability. To prevent biosurfactants from deteriorating in the environment while being stored and used, a variety of stabilization techniques are used [103].

To maintain the structural integrity of biosurfactants, cryoprotectants, such as mannitol, sucrose, and trehalose are frequently added before lyophilization, also known as freeze-drying [104]. When it comes to transforming biosurfactants into a stable, dry form that can be reconstituted before use, lyophilization is especially efficient [105]. Another successful tactic is polymer-based encapsulation, in which biosurfactants are trapped in biodegradable polymer matrices, such as poly (lactic-co-glycolic acid), chitosan, or alginate [106]. This encapsulation prevents the biosurfactant from being broken down by enzymes, enhances mechanical stability, and regulates drug release [107]. To create stable biosurfactant-loaded particles appropriate for a range of drug delivery methods, spray-drying and microencapsulation techniques are also employed [108,109].

Biosurfactants can be efficiently used for cutting-edge pharmaceutical applications with the help of these production and formulation techniques [110]. To overcome present obstacles and increase the clinical utility of these multifunctional biomolecules, ongoing innovation in formulation science, downstream processing, and microbial engineering is crucial [111,112].

The flowchart that depicts the multistep process for producing biosurfactants is displayed in Fig. 3 and Table 4. First, microbial producers, such as *Pseudomonas*, *Bacillus*, and *Candida* species are chosen, and then they are cultivated in ideal fermentation conditions (such as pH, temperature, and nutrient ratios). Purification procedures, such as solvent extraction and acid precipitation are part of the downstream processing. SLNs, hydrogels, liposomes, micelles, and nanoemulsions are among the pharmaceutical formulations that incorporate the purified biosurfactants. These formulations are designed to improve drug solubility, stability, and controlled release [114].

#### The properties of biosurfactants

To assess biosurfactants' effectiveness, stability, safety, and suitability – especially in pharmaceutical drug delivery – exact characterization is crucial. Using a variety of analytical and instrumental techniques, the

**Table 2: Representative biosurfactant production yields**

Microorganism	Biosurfactant Type	Substrate(s)	Yield (g/L)
<i>Pseudomonas aeruginosa</i> [75]	Rhamnolipid	Glucose, vegetable oil	2–10 (shake flask); up to 50 (fed-batch)
<i>Candida bombicola</i> [76]	Sophorolipid	Waste frying oil, glucose	100–300
<i>Bacillus subtilis</i> [77]	Surfactin	Glucose, peptone	2–5
<i>Rhodococcus erythropolis</i> [78]	Trehalolipid	n-alkanes	1–3
<i>Acinetobacter calcoaceticus</i> [79]	Emulsan	Ethanol, hydrocarbons	2–10

**Table 3: Representative physicochemical properties of biosurfactant-based formulations**

Formulation type	Biosurfactant used	Particle size (nm)	PDI	Zeta potential (mV)
Solid Lipid Nanoparticles [99]	Rhamnolipid	120–200	0.2–0.3	–25––40
Nanoemulsion [100]	Sophorolipid	50–150	<0.25	–20––35
Liposome [97]	Glycolipid (rhamnolipid)	100–200	0.2–0.3	–5––15
Hydrogel composite [101]	Surfactin-loaded	200–400 (swelling dependent)	–	Neutral
Niosome [102]	Sophorolipid+non-ionic surfactant	100–180	<0.3	–10––20

Table 4: Summary of biosurfactant production and formulation techniques

Stage	Description	Techniques/Examples
Microbial sources	Biosurfactants produced by bacteria, fungi, and yeasts.	<i>Pseudomonas, Bacillus, Candida, Aspergillus</i> [113]
Cultivation methods	Microbial growth on suitable substrates.	Submerged fermentation, Solid-state fermentation
Fermentation parameters	Optimized to enhance yield and activity.	pH, temperature, C/N ratio, aeration, and agitation
Bioreactor systems	Used for controlled and scalable biosurfactant production.	Stirred-tank, airlift, bubble column reactors
Isolation techniques	Recovery of crude biosurfactants post-fermentation.	Centrifugation, acid precipitation, solvent extraction
Purification methods	Enhancement of biosurfactant purity and quality.	Ultrafiltration, high-performance liquid chromatography, chromatography
Formulation strategies	Incorporation into drug delivery systems.	Micelles, liposomes, solid lipid nanoparticles, hydrogels, nanoemulsions
Stabilization techniques	Preserve structural integrity and bioactivity.	Lyophilization, cryoprotectants, polymer encapsulation

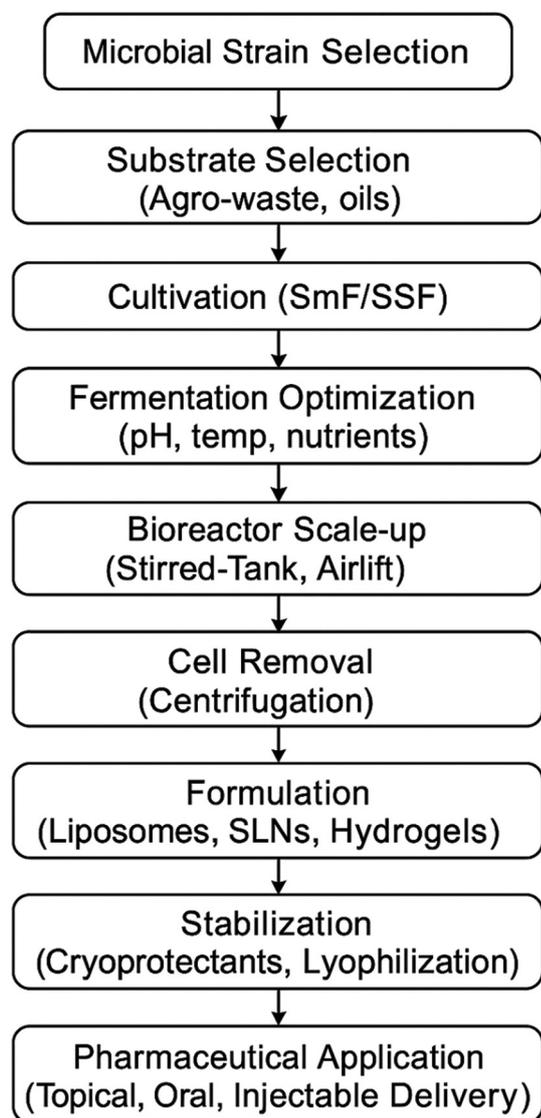


Fig. 3: Schematic representation of the biosurfactant production process involving microbial source selection, fermentation optimization, isolation and purification methods, followed by advanced formulation strategies, including nanoemulsions, liposomes, hydrogels, and solid lipid nanoparticles

process includes physicochemical, structural, morphological, thermal, and biocompatibility analyses [115-117].

#### Properties of physicochemistry

The capacity of biosurfactants to lower surface and interfacial tension is one of the main markers of their activity [118].

Tensiometric techniques, such as the Wilhelmy plate method and the Du Noüy ring, are frequently used to measure this. Water's surface tension can usually be lowered by effective biosurfactants from 72 mN/m to levels below 30 mN/m [119]. To assess emulsification capabilities, the interfacial tension between two immiscible liquids – such as water and oil – is also measured. Another important physicochemical parameter is the CMC, which is the concentration at which biosurfactant molecules self-assemble into micelles [120]. When creating dosage forms, such as micelles, liposomes, and nanoemulsions, figuring out CMC is essential. In pharmaceutical formulations, a lower CMC typically denotes higher surface activity and greater efficiency at lower concentrations [121-123].

In addition, the colloidal stability and homogeneity of biosurfactant dispersions are evaluated by measuring the zeta potential and particle size using Dynamic Light Scattering [124]. Strong electrostatic repulsion between particles is implied by a high absolute value of zeta potential, either positive or negative, which reduces aggregation and thus indicates formulation stability [125].

#### Clarification of structure

Understanding the functional groups of biosurfactants and how they interact with biological targets requires an understanding of their chemical structure [126]. Functional groups, such as hydroxyl, carboxyl, ester, and amide are frequently identified using Fourier Transform Infrared (FTIR) Spectroscopy. These groups are important for surface activity and biological compatibility [127-129].

Both <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy offer comprehensive details about the molecular structure of the biosurfactant [130]. It makes it possible to identify the hydrophilic (like sugar or amino acids) and hydrophobic (like fatty acids) components, as well as their connections. These data are useful for customizing particular formulations to improve their interaction with target tissues or pharmaceutical carriers [131-133].

The molecular weight and fragmentation pattern of the biosurfactant are verified by mass spectrometry (MS), such as matrix-assisted laser desorption/ionization-time of flight or electrospray ionization-MS [134]. It is particularly helpful for verifying the identity of complex polymeric biosurfactants, lipopeptides, or glycolipids [135]. Understanding the mechanism of action in drug delivery systems and maintaining quality control depend on these structural details [136-138].

#### Analysis of morphology

The way biosurfactants and their prepared nanoparticles or emulsions behave in drug delivery applications are greatly influenced by their morphological properties [139]. The internal structure and surface topography of biosurfactant assemblies are visualized using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) [140]. While TEM gives details on internal organization and vesicular structures at the nanoscale, SEM provides detailed surface images that show the texture and distribution of materials coated with biosurfactants.

Furthermore, 3D nanoscale topography and surface roughness analysis are provided by Atomic Force Microscopy (AFM). Because AFM doesn't require a vacuum environment, such as SEM or TEM does, samples can be observed in their naturally hydrated state. Aggregation tendencies, film homogeneity, and surface adhesion can all be assessed by correlating AFM data with physicochemical measurements [141-143].

#### Crystalline and thermal characteristics

For biosurfactants to remain stable during processing and storage, it is essential to comprehend their thermal behavior. The heat flow connected to thermal transitions, such as melting, crystallization, or glass transition, is examined using Differential Scanning Calorimetry (DSC). For drug delivery systems based on biosurfactants, this information aids in the selection of appropriate excipients and storage conditions [144].

By tracking weight loss as a function of temperature, thermogravimetric analysis, or TGA, is used to assess the degradation profile and thermal stability. This information is particularly crucial for guaranteeing the stability of biosurfactants during lyophilization and formulation [145].

The crystalline or amorphous nature of biosurfactants and their formulations is evaluated using X-ray diffraction (XRD). Although amorphous structures can be less stable than crystalline forms, they are usually more soluble and bioavailable. Therefore, maintaining crystallinity balance is essential for creating stable and effective pharmaceutical systems [146-148].

#### Testing for biocompatibility

Biosurfactants need to be assessed for safety and biocompatibility before being used in pharmaceutical applications. Mammalian cell line viability in the presence of biosurfactants is frequently evaluated using cytotoxicity tests, such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase release assays. For formulations based on biosurfactants, these tests aid in determining the right dosage and therapeutic window [149-152].

An important safety concern for injectable formulations is the ability of biosurfactants to lyse red blood cells, which is measured by the

hemolysis assay. For pharmaceutical applications, a hemolysis rate of <5% is usually regarded as acceptable [153].

Additional functional tests that shed light on the therapeutic potential of biosurfactants include antimicrobial and antioxidant tests [154]. When biosurfactants are utilized for oral care, wound healing, or dermatological formulations, these activities can work in concert to provide both delivery and therapeutic benefits [155,156].

The main analytical methods for biosurfactant characterization are categorized in Table 5 and Fig. 4. Tensiometry is used to assess surface characteristics, such as surface tension and CMC. FTIR, NMR, and MS are used to clarify structural features, allowing for a comprehensive molecular profile. Imaging methods, such as SEM, TEM, and AFM are used to record morphological features. To ascertain material stability, DSC, TGA, and XRD are used to evaluate thermal and crystalline behaviors. Finally, cytotoxicity and hemolysis assays are used to test biocompatibility, guaranteeing safety for pharmaceutical applications [157].

#### USE OF BIOSURFACTANTS IN THE DELIVERY OF PHARMACY

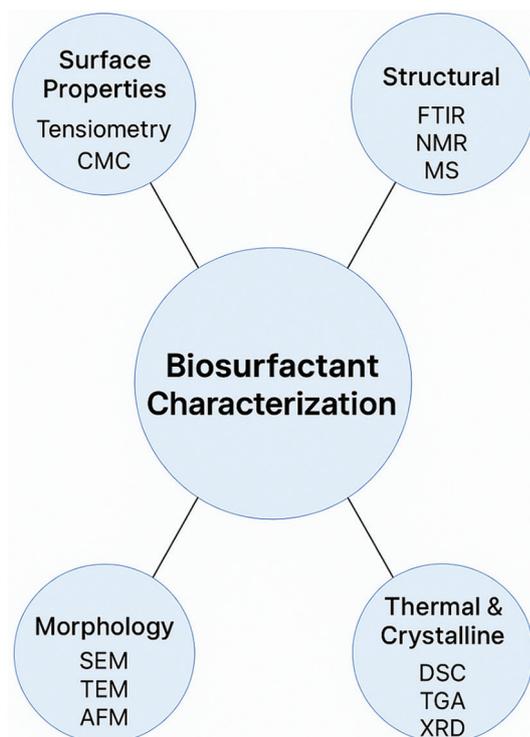
Because of their surface activity, amphiphilia, and biocompatibility, biosurfactants have drawn a lot of attention in pharmaceutical drug delivery systems. The solubility, bioavailability, and controlled, targeted delivery of therapeutic agents can all be improved by these biomolecules [158]. They are extremely valuable in a variety of delivery platforms, such as hydrogels, liposomes, nanoparticles, and micelles, due to their distinct functional properties [4]. Biosurfactants are being incorporated more and more into novel drug delivery systems in the current research environment in an effort to get around traditional pharmacokinetic and pharmacodynamic constraints [159,160].

#### Improving hydrophobic drug solubility

The improvement of drug solubility and bioavailability is one of the most well-known uses of biosurfactants in drug delivery. Many recently developed medications, such as ibuprofen, paclitaxel, and curcumin, have poor aqueous solubility, which results in poor absorption and uneven therapeutic effects. These hydrophobic substances can be

Table 5: Summary of biosurfactant characterization techniques

Characterization parameter	Purpose	Technique(s)	Outcome
Surface and interfacial tension	Measure surfactant efficiency	Tensiometry (Du Noüy, Wilhelmy plate)	Reduced surface tension, enhanced solubilization
CMC determination	Determine optimal concentration for micelle formation	Surface tension versus concentration plot	Ideal formulation dosage
Zeta potential and particle size	Assess stability and dispersion	Dynamic light scattering	Colloidal stability, uniformity
Functional group identification	Understand chemical composition	Fourier transform infrared Spectroscopy	Identification of carboxyl, hydroxyl, and ester groups
Molecular structure	Confirm biosurfactant framework	nuclear magnetic resonance ( <sup>1</sup> H and <sup>13</sup> C) Spectroscopy	Backbone structure confirmation
Molecular weight	Analyze purity and structural identity	Mass spectrometry (electrospray ionization-mass spectrometry, matrix-assisted laser desorption/ionization-time of flight)	Molecular identity validation
Surface morphology	Visualize surface and particle features	Scanning electron microscopy, transmission electron microscopy	Nanostructure analysis
Topography at nanoscale	Assess height, roughness, and interactions	Atomic Force Microscopy	Nanoscale structure imaging
Thermal stability	Detect melting, degradation, and crystallization points	differential scanning calorimetry, thermogravimetric analysis	Storage and processing temperature limits
Crystalline nature	Examine solid-state characteristics	X-Ray Diffraction	Crystalline versus amorphous state
Cytotoxicity and biocompatibility	Assess safety on cell lines and tissues	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, lactate dehydrogenase assays	Suitable dose range and safety profile
Hemolysis potential	Evaluate RBC lysis risk	Hemolysis Assay	Safety for injectable use
Additional bioactivity	Assess antioxidant or antimicrobial action	DPPH, ABTS, MIC assays	Functional benefits in pharma applications



**Fig. 4: Diagrammatic overview of key techniques used for biosurfactant characterization, grouped under surface properties, structural elucidation, morphological analysis, thermal/crystalline evaluation, and biocompatibility assessment**

encapsulated in micelles and nanoemulsions by biosurfactants, such as rhamnolipids and sophorolipids, which increases the solubility of these substances in biological fluids [161].

The process depends on biosurfactant molecules self-assembling into micellar structures at concentrations higher than their CMC. By retaining an aqueous-compatible exterior and efficiently solubilizing lipophilic drug molecules in their hydrophobic core, these micelles can improve systemic delivery [162]. Biosurfactant-based nanoemulsions co-loaded with hydrophilic and hydrophobic medications are frequently used in current formulation strategies because they provide dual solubility and enhanced permeability across epithelial barriers [163].

#### Targeted and managed drug administration

Using biosurfactants as carriers for targeted and regulated drug delivery is another essential use for them. Biosurfactants can be designed into intelligent delivery systems that release medications selectively at specific locations because of their capacity to react to environmental cues, such as pH or temperature. For example, by functionalizing liposomes with ligands that recognize hepatocyte-specific receptors, rhamnolipids have been integrated into liposomes for liver-targeted drug delivery [164].

For illnesses, such as cancer and inflammation that are marked by localized pH changes, the use of biosurfactants in the creation of pH-responsive or enzyme-triggered systems is also being investigated. The formation of vesicles or micelles that are stable at physiological pH but break down in the acidic tumor microenvironment to release their cargo is made possible in these systems by biosurfactants. This guarantees a better therapeutic index and less systemic toxicity [165]. Furthermore, the enhanced retention of surfactin-loaded nanocarriers in tumor tissues through the Enhanced Permeability and Retention (EPR) effect is being studied [166].

#### Delivery of genes and proteins

Biosurfactants are appropriate for the delivery of proteins and genetic materials due to their membrane-interactive characteristics and biocompatibility. It has been demonstrated that lipopeptide-based biosurfactants can combine with therapeutic proteins, siRNA, and plasmid DNA to form stable complexes that protect against enzymatic degradation and make transfection across cell membranes easier. After internalization, these carriers have the ability to exit endosomal compartments and release their genetic cargo into the nucleus or cytoplasm, which is essential for efficient gene expression [167].

The development of biosurfactant-based cationic liposomes and nanocarriers for CRISPR-Cas9 delivery and gene silencing in viral infections and cancer is an example of a recent tactic [168]. These materials' inherent biodegradability also allays worries about long-term cytotoxicity or tissue accumulation, which is frequently a drawback of synthetic gene carriers [169].

Despite encouraging *in vitro* results, serum instability, enzymatic degradation, and immune activation remain key hurdles for biosurfactant-mediated gene/protein delivery. Glycolipid-based carriers offer stable micellar complexes but suffer from poor endosomal escape, limiting transfection efficiency. Lipopeptides facilitate membrane fusion and endosomal escape, improving transfection rates, but cytotoxicity and batch variability require attention. Polymeric biosurfactants enhance stability in circulation and enable sustained release but often display reduced cellular uptake compared to lipopeptides. Overcoming these limitations requires hybrid strategies (e.g., glycolipid-lipopeptide conjugates) to integrate stability with efficient intracellular delivery.

#### Topical medication delivery and wound healing

Biosurfactants also show great promise in topical application, especially in dermal therapy and wound healing. Hydrogels, films, and ointments loaded with biosurfactants have been created to take advantage of their natural antimicrobial, anti-inflammatory, and wound-healing qualities [170]. For instance, it has been observed that sophorolipid and surfactin formulations prevent the formation of biofilms by pathogens, such as *P. aeruginosa* and *Staphylococcus aureus*, which are major contributors to chronic wound infections [171].

In addition, biosurfactants enhance transdermal absorption by interacting with the lipids in the stratum corneum to improve the penetration of therapeutic agents through the skin [172]. The goal of present research is to develop multifunctional wound dressings that can deliver growth factors, antibiotics, or even stem cell-derived vesicles to speed up tissue regeneration by combining biosurfactants with polymeric matrices, such as chitosan and alginate [173].

Topical delivery systems benefit from the antimicrobial and biofilm-disrupting properties of biosurfactants, yet several formulation challenges persist. Maintaining sustained release while preventing microbial colonization is particularly difficult. Glycolipids are highly skin-compatible and display antimicrobial activity, but their ability to deliver growth factors remains limited. Lipopeptides effectively disrupt biofilms and accelerate wound healing, though irritation at higher doses has been reported. Polymeric biosurfactants integrate well into hydrogels, enabling stable, long-term release, but they provide weaker antimicrobial activity compared to glycolipids or lipopeptides.

#### Treatment for cancer

Biosurfactant-stabilized nanoparticles have been investigated in oncology as chemotherapeutic agent carriers to increase cellular uptake, lower systemic toxicity, and boost therapeutic efficacy [174]. Hydrophobic anticancer medications, such as doxorubicin and paclitaxel can be encapsulated thanks to their capacity to form nanostructures, such as liposomes or micelles. These biosurfactant-based carriers allow endosomal escape, which is essential for intracellular drug release, and facilitate effective tumor penetration, which is frequently facilitated by the EPR effect [175].

**Table 6: Advantages and disadvantages of biosurfactant classes in major pharmaceutical applications**

Application	Glycolipids (e.g., Rhamnolipids, Sphorolipids)	Lipopeptides (e.g., Surfactin, Iturin)	Polymeric biosurfactants (e.g., Emulsan)
Cancer therapy	Stable micelles/liposomes; low toxicity; weak intrinsic anticancer effect	Strong anticancer synergy; hemolytic and immunogenic risk	Good sustained release; scale-up difficult
Gene/protein delivery	Serum-stable micelles; limited endosomal escape	Efficient membrane fusion and transfection; cytotoxicity risk	Excellent stability; lower cellular uptake
Vaccine/ADJUVANT	Safe; moderate immune stimulation	Strong immune boost; risk of inflammation	Long release and safe; weaker immunogenicity
Wound healing/topical	Antimicrobial; skin-compatible	Potent biofilm disruption; irritation risk	Excellent hydrogel integration; weaker antimicrobial effect

In addition, certain biosurfactants, such as surfactin, have inherent anticancer qualities that work in concert to increase the cytotoxic effect of loaded medications. Folate, transferrin, or antibodies have been added to biosurfactant-based nanoparticles to enable receptor-mediated targeting of cancer cells, providing site-specificity and reducing off-target effects [176].

Biosurfactant-stabilized nanocarriers hold promise in oncology, but their translation faces critical barriers. The EPR effect varies across tumor types, limiting universal applicability. Moreover, rapid clearance and potential hemolysis at higher biosurfactant concentrations restrict clinical use. Glycolipids, such as rhamnolipids form stable micelles and liposomes, improving hydrophobic drug solubility, but their intrinsic anticancer effect is relatively weak. Lipopeptides, particularly surfactin, display inherent anticancer activity and synergize with chemotherapeutics; however, their hemolytic potential and immunogenicity during repeated dosing pose safety challenges. Polymeric biosurfactants (e.g., emulsan) provide superior stability and controlled release but remain difficult to scale up economically. Thus, application-specific tailoring is essential to balance efficacy, safety, and manufacturability.

#### Adjuvant activity and vaccine delivery

The potential of biosurfactants as immunomodulatory adjuvants and vaccine delivery vehicles is becoming more widely acknowledged [177]. Surfactin and other lipopeptides can increase humoral and cellular immune responses, stimulate antigen-presenting cells, and improve mucosal absorption of antigens [178]. Biosurfactants enhance mucosal penetration and antigen stability when added to oral or nasal vaccine formulations [179].

The creation of biosurfactant-containing nanocarriers that encapsulate antigens for subunit vaccines – specifically for pathogens, such as influenza and HPV – is one recent tactic. By providing the benefits of prolonged antigen release, adjuvant qualities, and non-invasive administration, these systems lessen the need for potentially harmful chemical adjuvants [180].

In vaccine formulations, biosurfactants must balance safety with immune activation. Glycolipids, such as sphorolipids provide moderate adjuvanticity with minimal toxicity, making them suitable for mucosal vaccines. Lipopeptides strongly activate antigen-presenting cells and enhance both humoral and cellular responses, but their potency raises concerns of local inflammation and requires precise dose optimization. Polymeric biosurfactants contribute to prolonged antigen release and mucosal adhesion but may lack sufficient intrinsic immunogenicity. Comparative studies suggest that combining biosurfactants with conventional adjuvants could help achieve a safer and more balanced immune response [181,182]. The overall advantages and disadvantages of biosurfactant in major pharmaceutical applications shown in Table 6.

#### Present pharmaceutical application strategies

Green synthesis, customized delivery methods, and the integration of nanotechnology are the main areas of recent developments in the pharmaceutical use of biosurfactants. To create hybrid nanoparticles with improved stability and multifunctionality, researchers are now mixing biosurfactants with other natural or synthetic polymers. For

example, nanostructured lipid carriers and biosurfactant-stabilized SLNs are being developed for both imaging and drug delivery [183].

Furthermore, biosurfactant-coated vectors are being used in the development of CRISPR-based gene editing tools to improve genome editing accuracy and intracellular delivery. To precisely control the timing and location of drug release, there is also increasing interest in creating biosurfactant-based smart drug delivery systems that use stimuli-responsive materials (such as thermo-, pH-, or enzyme-sensitive) [1].

From the perspective of formulation, present trends highlight scalable and environmentally friendly production methods that use agro-industrial waste as substrates, which is consistent with the ideas of green chemistry and sustainable pharmacy. All things considered, the versatility and functionality of biosurfactants are pushing the limits of pharmaceutical formulation science [184].

#### FUTURE THOUGHTS

Future research should shift from generalized optimism toward targeted problem-solving. Synthetic biology and metabolic engineering can help reduce production costs and tailor biosurfactant structures for specific applications. Hybrid systems combining glycolipids, lipopeptides, and polymers may overcome limitations of individual classes, e.g., achieving both stability (polymeric) and efficient transfection (lipopeptides) in gene delivery. Advanced delivery platforms – such as exosomes, dendrimers, and 3D-printed scaffolds – offer opportunities to integrate biosurfactants into multifunctional carriers. Clinical translation requires robust toxicological studies, especially regarding immunogenicity and hemolysis in chronic or repeated dosing. Artificial intelligence and machine learning should be leveraged to predict biosurfactant–drug interactions and optimize formulation parameters. Addressing these hurdles with interdisciplinary strategies will accelerate the transition of biosurfactants from experimental platforms to clinically approved pharmaceutical agents [185].

#### CONCLUSION

Biosurfactants represent a powerful and versatile class of biomolecules with far-reaching implications in pharmaceutical drug delivery. Their unique physicochemical and biological properties – such as amphiphilicity, surface activity, biodegradability, and biocompatibility – make them highly suitable for addressing many of the limitations associated with conventional surfactants and drug carriers.

Across various mechanisms, including solubility enhancement, controlled release, targeted delivery, and biofilm disruption, biosurfactants have demonstrated superior performance in diverse pharmaceutical applications. Their roles in cancer therapy, gene and protein delivery, wound healing, and vaccine adjuvant systems are expanding rapidly, supported by novel formulation strategies, such as micelles, liposomes, nanoemulsions, and SLNs.

Despite the present challenges in large-scale production, regulatory acceptance, and clinical validation, the future of biosurfactants in drug delivery is bright. Ongoing advancements in microbial biotechnology, nanoscience, and pharmaceutical formulation, coupled with a strong push toward green and sustainable technologies, are expected to propel

biosurfactants into the mainstream of next-generation drug delivery systems. As our understanding deepens, biosurfactants are poised to transition from niche biomaterials to essential components of modern pharmaceuticals.

#### AUTHOR'S CONTRIBUTION

Conceptualization: Sharmila. V and Kasimani. R; Methodology and data collection: Sharmila. V and EsathNatheer. S; Literature review and analysis: Karuppasamy Chellapandi; Writing - original draft/preparation: Sharmila. V and Kasimani. R; Writing review and editing: Sharmila. V, Kasimani. R, EsathNatheer. S, and Karuppasamy Chellapandi; Supervision and project administration: Sharmila. V.

#### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

#### FUNDING

The authors declare that no specific grant from funding agencies in the public, commercial, or not-for-profit sectors was received for this research

#### REFERENCES

- Barbosa FG, Ribeaux DR, Rocha T, Costa RA, Guzmán RR, Marcelino P, et al. Biosurfactants: Sustainable and versatile molecules. *J Braz Chem Soc.* 2022;33(8):870-93. doi: 10.21577/0103-5053.20220074
- Ceresa C, Fracchia L, Sansotera AC, De Rienzo MA, Banat IM. Harnessing the potential of biosurfactants for biomedical and pharmaceutical applications. *Pharmaceutics.* 2023;15(8):2156. doi: 10.3390/pharmaceutics15082156, PMID 37631370
- Ceresa C, Fracchia L, Fedeli E, Porta C, Banat IM. Recent advances in biomedical, therapeutic and pharmaceutical applications of microbial surfactants. *Pharmaceutics.* 2021;13(4):466. doi: 10.3390/pharmaceutics13040466, PMID 33808361
- Banat IM, Carboué Q, Saucedo-Castañeda G, De Jesús Cázares-Marinero J. Biosurfactants: The green generation of speciality chemicals and potential production using solid-state fermentation (SSF) technology. *Bioresour Technol.* 2021;320(Pt A):124222. doi: 10.1016/j.biortech.2020.124222, PMID 33171346
- Ohadi M, Shahravan A, Dehghannoudeh N, Eslaminejad T, Banat IM, Dehghannoudeh G. Potential use of microbial surfactant in microemulsion drug delivery system: A systematic review. *Drug Des Devel Ther.* 2020;14:541-50. doi: 10.2147/DDDT.S232325, PMID 32103896
- Bjerk TR, Severino P, Jain S, Marques C, Silva AM, Pashirova T, et al. Biosurfactants: Properties and applications in drug delivery, biotechnology and ecotoxicology. *Bioengineering (Basel).* 2021;8(8):115. doi: 10.3390/bioengineering8080115, PMID 34436118
- Hellweg T, Oberdisse J, Sottmann T. Recent advances in biosurfactant-based association colloids - formation of microemulsions. *Front Soft Matter.* 2023;3:1260211. doi: 10.3389/frsfm.2023.1260211
- Wang X, An J, Cao T, Guo M, Han F. Application of biosurfactants in medical sciences. *Molecules.* 2024;29(11):2606. doi: 10.3390/molecules29112606, PMID 38893481
- Naughton PJ, Marchant R, Naughton V, Banat IM. Microbial biosurfactants: Current trends and applications in agricultural and biomedical industries. *J Appl Microbiol.* 2019;127(1):12-28. doi: 10.1111/jam.14243, PMID 30828919
- Thakur V, Baghmare P, Verma A, Verma JS, Geed SR. Recent progress in microbial biosurfactants production strategies: Applications, technological bottlenecks, and future outlook. *Bioresour Technol.* 2024;408:131211. doi: 10.1016/j.biortech.2024.131211, PMID 39102966
- Sarubbo LA, Silva MG, Durval IJ, Bezerra KG, Ribeiro BG, Silva IA, et al. Biosurfactants: Production, properties, applications, trends, and general perspectives. *Biochem Eng J.* 2022;181:108377. doi: 10.1016/j.bej.2022.108377
- Maibaum L, Dinner AR, Chandler D. Micelle formation and the hydrophobic effect. *J Phys Chem B.* 2004;108(21):6778-81. doi: 10.1021/jp037487t
- Shaimerdenova U, Kaiymanova G, Lewandowska W, Bartoszewicz M, Swiecicka I, Yernazarova A. Biosurfactant and biopolymer producing microorganisms from West Kazakhstan oilfield. *Sci Rep.* 2024;14(1):2294. doi: 10.1038/s41598-024-52906-7, PMID 38280982
- Nayariseri A, Singh P, Singh SK. Screening, isolation and characterization of biosurfactant producing *Bacillus subtilis* strain ANSKLAB03. *Bioinformation.* 2018;14(6):304-14. doi: 10.6026/97320630014304, PMID 30237676
- El-Moslemany RM, El-Kamel A, Allam EA, Khalifa HM, Hussein A, Ashour AA. Tanshinone IIA loaded bioactive nanoemulsion for alleviation of lipopolysaccharide induced acute lung injury via inhibition of endothelial glycocalyx shedding. *Biomed Pharmacotherapy.* 2022;155:113666. doi: 10.1016/j.biopha.2022.113666
- Hellweg T, Sottmann T, Oberdisse J. Recent advances in biosurfactant-based association colloids - self-assembly in water. *Front Soft Matter.* 2023;2:1081877. doi: 10.3389/frsfm.2022.1081877
- Penfold J, Thompson L, Tucker I, Thomas RK, Li P, Dong Z, et al. Self-assembly and interfacial adsorption of biosurfactants: Recent insights from scattering techniques. *Colloids Surf B Biointerfaces.* 2023;222:113091. doi: 10.1016/j.colsurfb.2023.113091
- Sharma J, Durai S, Srivastava P. Biosurfactants: Potential agents for controlling cellular communication, motility, and antagonism. *Front Mol Biosci.* 2021;8:727070. doi: 10.3389/fmolb.2021.727070, PMID 34708073
- Akbari S, Rahimnejad M, Mokhtari-Hosseini ZB. Biotechnological production of biosurfactants for medical and pharmaceutical applications. *Biotechnol Adv.* ???;42:107579. doi: 10.1016/j.biotechadv.2020.107579
- Datta M, Chattopadhyay I. Applications of microbial biosurfactants in human health and environmental sustainability: A narrative review. *Discov Med.* 2024;1(1):160. doi: 10.1007/s44337-024-00181-w
- Plaža GA, Chojniak J, Banat IM. Biosurfactant mediated biosynthesis of selected metallic nanoparticles. *Int J Mol Sci.* 2014;15(4):13720-37. doi: 10.3390/ijms150813720, PMID 25110864
- Romero Vega G, Gallo Stampino P. Bio-based surfactants and biosurfactants: An overview and main characteristics. *Molecules.* 2025;30(4):863. doi: 10.3390/molecules30040863, PMID 40005173
- Mulligan CN. Recent advances in the environmental applications of biosurfactants. *Curr Opin Colloid Interface Sci.* 2009;14(5):372-8. doi: 10.1016/j.cocis.2009.06.005
- Thakur P, Saini NK, Thakur VK, Gupta VK, Saini RV, Saini AK. Rhamnolipid the glycolipid biosurfactant: Emerging trends and promising strategies in the field of biotechnology and biomedicine. *Microb Cell Fact.* 2021;20(1):1. doi: 10.1186/s12934-020-01497-9, PMID 33397389
- Lourenço M, Duarte N, Ribeiro IA. Exploring biosurfactants as antimicrobial approaches. *Pharmaceutics (Basel).* 2024;17(9):1239. doi: 10.3390/ph17091239, PMID 39338401
- Moussa TA, Mohamed MS, Samak N. Production and characterization of di-rhamnolipid produced by *Pseudomonas aeruginosa* TMN. *Braz J Chem Eng.* 2014;31(4):867-80. doi: 10.1590/0104-6632.20140314s00002473
- Carolin FC, Kamalesh T. Advances in stabilization of metallic nanoparticle with biosurfactants- a review on current trends. *Heliyon.* 2024;10:e29773. https://doi.org/10.1016/j.heliyon.2024.e29773
- El-Housseiny GS, Aboshanab KM, Aboulwafa MM, Hassouna NA. Structural and physicochemical characterization of rhamnolipids produced by *Pseudomonas aeruginosa* P6. *AMB Express.* 2020;10(1):201. doi: 10.1186/s13568-020-01141-0, PMID 33146788
- Eslami P, Hajfarajollah H, Bazsefidpar S. Recent advancements in the production of rhamnolipid biosurfactants by *Pseudomonas aeruginosa*. *RSC Adv.* 2020;10(56):34014-32. doi: 10.1039/D0RA04953K, PMID 35519061
- Cho WY, Ng JF, Yap WH, Goh BH. Sophorolipids - bio-based antimicrobial formulating agents for applications in food and health. *Molecules.* 2022;27(17):5556. doi: 10.3390/molecules27175556, PMID 36080322
- Elshafie AE, Joshi SJ, Al-Wahaibi YM, Al-Bemani AS, Al-Bahry SN, Al-Maqbali D, et al. Sophorolipids production by *Candida bombicola* ATCC 22214 and its potential application in microbial enhanced oil recovery. *Front Microbiol.* 2015;6:1324. doi: 10.3389/fmicb.2015.01324, PMID 26635782
- Rodrigues LR, Banat IM. Biosurfactants: Promising molecules for therapeutic and pharmaceutical applications. *Trends Biotechnol.* 2020;38(10):1129-41. doi: 10.1016/j.tibtech.2020.02.015
- Gupta R, Prabhune AA. Structural determination and chemical esterification of the sophorolipids produced by *Candida bombicola* grown on glucose and  $\alpha$ -linolenic acid. *Biotechnol Lett.* 2012;34(4):701-7. doi: 10.1007/s10529-011-0818-y, PMID 22167634
- Hu F, Cai W, Lin J, Wang W, Li S. Genetic engineering of the precursor supply pathway for the overproduction of the nC14-surfactin isoform with promising MEOR applications. *Microb Cell Fact.* 2021;20(1):96. doi: 10.1186/s12934-021-01585-4, PMID 33964901
- Das B, Kumar B, Begum W, Bhattarai A, Mondal MH, Saha B. Comprehensive review on applications of surfactants in vaccine formulation, therapeutic and cosmetic pharmacy and prevention of

- pulmonary failure due to COVID-19. Chem Afr. 2022;5(3):459-80. doi: 10.1007/s42250-022-00345-0
36. Das S, Rao KV. A comprehensive review of biosurfactant production and its uses in the pharmaceutical industry. Arch Microbiol. 2024;206(2):60. doi: 10.1007/s00203-023-03786-4, PMID 38197951
  37. Ali N, Pang Z, Wang F, Xu B, El-Seedi HR. Lipopeptide biosurfactants from *Bacillus* spp.: Types, production, biological activities, and applications in food. J Food Qual. 2022;2022:1-19. doi: 10.1155/2022/3930112
  38. Dunlap CA, Bowman MJ, Rooney AP. Iturinic lipopeptide diversity in the *Bacillus subtilis* species group - important antifungals for plant disease biocontrol applications. Front Microbiol. 2019;10:1794. doi: 10.3389/fmicb.2019.01794, PMID 31440222
  39. Cooper DG, Zajic JE, Gerson DF. Production of surface-active lipids by *Corynebacterium lepus*. Appl Environ Microbiol. 1979;37(1):4-10. doi: 10.1128/AEM.37.1.4-10.1979, PMID 760639
  40. Ahmadi-Ashtiani HR, Baldissarotto A, Cesa E, Manfredini S, Sedghi Zadeh H, Ghafori Gorab M, et al. Microbial biosurfactants as key multifunctional ingredients for sustainable cosmetics. Cosmetics. 2020;7(2):46. doi: 10.3390/cosmetics7020046
  41. Sanjai C, Gaonkar S, Hakkimane S. Harnessing nature's toolbox: Naturally derived bioactive compounds in nanotechnology enhanced formulations. ACS Omega. 2024;9:43302-43318. doi: 10.1021/acsomega.4c07756
  42. Afolabi F, Mahmood SM, Yekeen N, Akbari S, Sharifigaliuk H. Polymeric surfactants for enhanced oil recovery: A review of recent progress. J Petrol Sci Eng. 2022;208:109358. doi: 10.1016/j.petrol.2021.109358
  43. Alizadeh-Sani M, Hamishehkar H, Khezerlou A, Azizi-Lalabadi M, Azadi Y, Nattagh-Eshtivani E, et al. Bioemulsifiers derived from microorganisms: Applications in the drug and food industry. Adv Pharm Bull. 2018;8(2):191-9. doi: 10.15171/apb.2018.023, PMID 30023320
  44. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev. 1997;61(1):47-64. doi: 10.1128/mmbr.61.1.47-64.1997, PMID 9106364
  45. Yi G, Son J, Yoo J, Park C, Koo H. Emulsan-based nanoparticles for *in vivo* drug delivery to tumors. Biochem Biophys Res Commun. 2019;508(1):326-31. doi: 10.1016/j.bbrc.2018.11.106, PMID 30502086
  46. Antonioli Júnior R, Poloni JF, Pinto ES, Dorn M. Interdisciplinary overview of lipopeptide and protein-containing biosurfactants. Genes (Basel). 2022;14(1):76. doi: 10.3390/genes14010076, PMID 36672817
  47. Cirigliano MC, Caman GM. Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*. Appl Environ Microbiol. 1985;50(4):846-50. doi: 10.1128/AEM.50.4.846-850.1985, PMID 16346917
  48. Santos DK, Rufino RD, Luna JM, Santos VA, Sarubbo LA. Biosurfactants: Multifunctional biomolecules of the 21<sup>st</sup> century. Int J Mol Sci. 2016;17(3):401. doi: 10.3390/ijms17030401, PMID 26999123
  49. Thacharodi A, Lamont IL. Gene-gene interactions reduce aminoglycoside susceptibility of *Pseudomonas aeruginosa* through efflux pump-dependent and -independent mechanisms. Antibiotics (Basel). 2023;12(1):152. doi: 10.3390/antibiotics12010152, PMID 36671353
  50. Ongena M, Jacques P. Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. Trends Microbiol. 2008;16(3):115-25. doi: 10.1016/j.tim.2007.12.009, PMID 18289856
  51. Bhattacharya A, Roy P. Recent trends in biosurfactant research: Applications in drug delivery and nanomedicine. Colloids Surf A Physicochem Eng Aspects. 2021;630:127561.
  52. Goldman S, Shabtai Y, Rubinovitz C, Rosenberg E, Gutnick DL. Emulsan in *Acinetobacter calcoaceticus* RAG-1: Distribution of cell-free and cell-associated cross-reacting material. Appl Environ Microbiol. 1982;44(1):165-70. doi: 10.1128/AEM.44.1.165-170.1982, PMID 16346052
  53. Bhatnagar A, Kaur H. Rhamnolipids in drug delivery: Emerging opportunities and design strategies. Pharm Nanotechnol. 2021;9(2):147-60.
  54. Kathole A, Hatwar P. Nanotechnology-based drug delivery systems and herbal medicine. J Drug Deliv Ther. 2025;15:133-41. doi: 10.22270/jddt.v15i3.7017
  55. Chong H, Li Q. Microbial production of rhamnolipids: Opportunities, challenges and strategies. Microb Cell Fact. 2017;16(1):137. doi: 10.1186/s12934-017-0753-2, PMID 28779757
  56. Zhou J, Xue R, Liu S, Xu N, Xin F, Zhang W, et al. High di-rhamnolipid production using *Pseudomonas aeruginosa* KT1115, separation of mono/di-rhamnolipids, and evaluation of their properties. Front Bioeng Biotechnol. 2019;7:245. doi: 10.3389/fbioe.2019.00245, PMID 31696112
  57. Abdel-Mawgoud AM, Lépine F, Déziel E. Rhamnolipids: Diversity of structures, microbial origins and roles. Appl Microbiol Biotechnol. 2010;86(5):1323-36. doi: 10.1007/s00253-010-2498-2, PMID 20336292
  58. Belisle JT, et al. Structure and function of mycobacterial lipids: Pathogenicity and immune response. Nat Rev Microbiol. 2009;7(7):543-55. doi: 10.1038/nrmicro2168
  59. Sachin K, Karn SK. Microbial fabricated nanosystems: Applications in drug delivery and targeting. Front Chem. 2021;9:617353. doi: 10.3389/fchem.2021.617353
  60. Thakur S, Singh A, Sharma R, Aurora R, Jain SK. Biosurfactants as a novel additive in pharmaceutical formulations: Current trends and future implications. Curr Drug Metab. 2020;21(11):885-901. doi: 10.2174/138920021666201008143238, PMID 33032505
  61. Satpute SK, Banpurkar AG, Dhakephalkar PK, Banat IM. Eco-friendly biosurfactants: New insights into their biomedical and biotechnological applications. Front Microbiol. 2020;11:437. doi: 10.3389/fmicb.2020.00437
  62. Sultan F, Chattopadhyay I, Maji D, Phatake RS, Kumar K. Pharmaceutical applications of microbial biosurfactants: A comprehensive review. Int J Pharm. 2025;681:125887.
  63. Madankar CS, Meshram A. Review on classification, physicochemical properties and applications of microbial surfactants. Tenside Surfactants Deterg. 2022;59(1):1-16. doi: 10.1515/tsd-2021-2353
  64. Adetunji AI, Olaniran AO. Production and potential biotechnological applications of microbial surfactants: An overview. Saudi J Biol Sci. 2021;28(1):669-79. doi: 10.1016/j.sjbs.2020.10.058, PMID 33424354
  65. Pardhi DS, Panchal RR, Raval VH, Joshi RG, Poccai P, Almalki WH, et al. Microbial surfactants: A journey from fundamentals to recent advances. Front Microbiol. 2022;13:982603. doi: 10.3389/fmicb.2022.982603, PMID 35992692
  66. Gayathiri E, Prakash P, Karmegam N, Varjani S, Awasthi M, Ravindran B. Biosurfactants: Potential and eco-friendly material for sustainable agriculture and environmental safety-a review. Agronomy. 2022;12(3):662. doi: 10.3390/agronomy12030662
  67. Eras-Muñoz E, Farré A, Sánchez A, Font X, Gea T. Microbial biosurfactants: A review of recent environmental applications. Bioengineered. 2022;13(5):12365-91. doi: 10.1080/21655979.2022.2074621, PMID 35674010
  68. Abu Yazid N, Barrena R, Komilis D, Sánchez A. Solid-state fermentation as a novel paradigm for organic waste valorization: A review. Sustainability. 2017;9(2):224. doi: 10.3390/su9020224
  69. Sundaram T, Govindarajan RK, Vinayagam S, Krishnan V, Nagarajan S, Gnanasekaran GR, et al. Advancements in biosurfactant production using agro-industrial waste for industrial and environmental applications. Front Microbiol. 2024;15:1357302. doi: 10.3389/fmicb.2024.1357302, PMID 38374917
  70. Miao Y, To MH, Siddiqui MA, Wang H, Lodens S, Chopra SS, et al. Sustainable biosurfactant production from secondary feedstock - recent advances, process optimization and perspectives. Front Chem. 2024;12:1327113. doi: 10.3389/fchem.2024.1327113, PMID 38312346
  71. Rane AN, Baikar VV, Ravi Kumar V, Deopurkar RL. Agro-industrial wastes for production of biosurfactant by *Bacillus subtilis* ANR 88 and its application in synthesis of silver and gold nanoparticles. Front Microbiol. 2017;8:492. doi: 10.3389/fmicb.2017.00492, PMID 28392783
  72. Mohanty SS, Koul Y, Varjani S, Pandey A, Ngo HH, Chang JS, et al. A critical review on various feedstocks as sustainable substrates for biosurfactants production: A way towards cleaner production. Microb Cell Fact. 2021;20(1):120. doi: 10.1186/s12934-021-01613-3, PMID 34174898
  73. Gurkok S, Ozdal M. Microbial biosurfactants: Properties, types, and production. J Pharm Biol Sci. 2021;2:2-7.
  74. Albasri HM, Almohammadi AA, Alhazmi A, Bukhari DA, Waznah MS, Mawad AM. Production and characterization of rhamnolipid biosurfactant from thermophilic *Geobacillus stearothermophilus* bacterium isolated from Uhud mountain. Front Microbiol. 2024;15:1358175. doi: 10.3389/fmicb.2024.1358175, PMID 38873141
  75. Kumar AM, Singh S, Kant C, Verma H, Kesawat MS, Bhatia S, et al. Microbial biosurfactant: A new frontier for sustainable agriculture and pharmaceutical industries. Antioxidants 2021;10(9):1472. doi: 10.3390/antiox10091472
  76. Qu D, Show PL, Miao X. Transcription factor ChbZIP1 from alkaliphilic microalgae *Chlorella* sp. BLD enhancing alkaline tolerance in transgenic *Arabidopsis thaliana*. Int J Mol Sci. 2016;22(5):2387. doi: 10.3390/ijms22052387
  77. Pacwa-Płociniczak M, Plaza GA, Piotrowska-Seget Z, Cameotra SS. Environmental applications of biosurfactants: Recent advances. Int J Mol Sci. 2021;22(19):10444. doi: 10.3390/ijms221910444
  78. Chen Y, Tang Y, Li Y, Rui Y, Zhang P. Enhancing the efficacy of active pharmaceutical ingredients in medicinal plants through nanoformulations: A promising field. Nanomaterials (Basel). 2024;14(19):1598. doi: 10.3390/nano14191598
  79. Patel P, Garala K, Singh S, Prajapati B, Chittasupho C. Lipid-based nanoparticles in delivering bioactive compounds for improving therapeutic efficacy. Pharmaceuticals. 2024;17(3):329. doi: 10.3390/ph17030329

80. Fernandes NA, Simões LA, Dias DR. Biosurfactants produced by yeasts: Fermentation, screening, recovery, purification, characterization, and applications. *Fermentation*. 2023;9(3):207. doi: 10.3390/fermentation9030207
81. Marchut-Mikolajczyk O, Drożdżyński P, Polewczyk A, Smulek W, Antczak T. Biosurfactant from endophytic *Bacillus pumilus* 2A: Physicochemical characterization, production and optimization and potential for plant growth promotion. *Microb Cell Fact*. 2021;20(1):40. doi: 10.1186/s12934-021-01533-2, PMID 33557838
82. Zeb A, Rana I, Choi HI, Lee CH, Baek SW, Lim CW, et al. Potential and applications of nanocarriers for efficient delivery of biopharmaceuticals. *Pharmaceutics*. 2020;12(12):1184. doi: 10.3390/pharmaceutics12121184
83. Almeida C, Pedro AQ, Tavares AP, Neves MC, Freire MG. Ionic-liquid-based approaches to improve biopharmaceuticals downstream processing and formulation. *Front Bioeng Biotechnol*. 2023;11:1037436. doi: 10.3389/fbioe.2023.1037436, PMID 36824351
84. Nascimento MF, Keković P, Ribeiro IA, Faria NT, Ferreira FC. Novel organic solvent nanofiltration approaches for microbial biosurfactants downstream processing. *Membranes (Basel)*. 2023;13(1):81. doi: 10.3390/membranes13010081, PMID 36676888
85. Talla S, Wadher K, Umekar M, Lohiya R. Recent and relevant methodology in the advancement of solid dispersion. *J Drug Deliv Ther*. 2021;11(4):1-7.
86. Mahanty R, et al. Biosurfactant-based metallic nanoparticles in antimicrobial therapy: Recent advances and applications. *Colloids Surf B Biointerfaces*. 2024;235:112561. doi: 10.1016/j.colsurfb.2024.112561
87. Alharbi WS, Almughem FA, Almeahady AM, Jarallah SJ, Alsharif WK, Alzahrani N, et al. Phytosomes as an emerging nanotechnology platform for the topical delivery of bioactive phytochemicals. *Pharmaceutics*. 2021;13(9):1475. doi: 10.3390/pharmaceutics13091475
88. Hwang D, Ramsey JD, Kabanov AV. Polymeric micelles for the delivery of poorly soluble drugs: From nanoformulation to clinical approval. *Adv Drug Deliv Rev*. 2020;156:80-118. doi: 10.1016/j.addr.2020.09.009, PMID 32980449
89. Jacob S, Kather FS, Boddu SH, Shah J, Nair AB. Innovations in nanoemulsion technology: Enhancing drug delivery for oral, parenteral, and ophthalmic applications. *Pharmaceutics*. 2024;16(10):1333. doi: 10.3390/pharmaceutics16101333, PMID 39458662
90. Preeti S, Sambhakar S, Malik R, Bhatia S, Al Harrasi A, Rani C, et al. Nanoemulsion: An emerging novel technology for improving the bioavailability of drugs. *Scientifica (Cairo)*. 2023;2023:6640103. doi: 10.1155/2023/6640103, PMID 37928749
91. Salim N, Ahmad N, Musa SH, Hashim R, Tadros TF, Basri M. Nanoemulsion as a topical delivery system of antipsoriatic drugs. *RSC Adv*. 2016;6(8):6234-50. doi: 10.1039/c5ra14946k
92. Aimonen K, Suhonen S, Hartikainen M, Lopes VR, Norppa H, Ferraz N, et al. Role of surface chemistry in the *in vitro* lung response to nanofibrillated cellulose. *Nanomaterials (Basel)*. 2021;11(2):389. doi: 10.3390/nano11020389, PMID 33546402
93. Priya S, Desai VM, Singhvi G. Surface modification of lipid-based nanocarriers: A potential approach to enhance targeted drug delivery. *ACS Omega*. 2023;8(1):74-86. doi: 10.1021/acsomega.2c05976, PMID 36643539
94. Zhao J, Su J, Qin L, Zhang X, Mao Y, et al. Exploring the influence of inhaled liposome membrane fluidity on its interaction with pulmonary physiological barriers. *Biomater Sci*. 2020;8(23):6592-605. doi: 10.1039/D0BM01128C
95. Sharma VK, Sarwa KK, Mazumder B. Fluidity enhancement: A critical factor for performance of liposomal transdermal drug delivery system. *J Liposome Res*. 2014;24(2):83-9. doi: 10.3109/08982104.2013.847956, PMID 24160895
96. Elhissi A. Liposomes for pulmonary drug delivery: The role of formulation and inhalation device design. *Curr Pharm Des*. 2017;23(3):362-72. doi: 10.2174/1381612823666161116114732, PMID 27848886
97. Adu SA, Twigg MS, Naughton PJ, Marchant R, Banat IM. Characterisation of cytotoxicity and immunomodulatory effects of glycolipid biosurfactants on human keratinocytes. *Appl Microbiol Biotechnol*. 2023;107(1):137-52. doi: 10.1007/s00253-022-12302-5, PMID 36441210
98. Zouari R, Moalla-Rekik D, Sahnoun Z, Rebai T, Ellouze-Chaabouni S, Ghribi-Aydi D. Evaluation of dermal wound healing and *in vitro* antioxidant efficiency of *Bacillus subtilis* SPB1 biosurfactant. *Biomed Pharmacother*. 2016;84:878-91. doi: 10.1016/j.biopha.2016.09.084, PMID 27750203
99. Haddaji N, Bahloul B, Bahia W, Bechambi O, Mahdhi A. Development of nanotechnology-based drug delivery systems for controlling clinical multidrug-resistant *Staphylococcus aureus* and *Escherichia coli* associated with aerobic vaginitis. *Pharmaceutics*. 2023;15(8):2133. doi: 10.3390/pharmaceutics15082133
100. Adejumo S, Oli A, Okoye E, Nwakile C, Ojiako CM, Okezie U, et al. Biosurfactant production using mutant strains of *Pseudomonas aeruginosa* and *Bacillus subtilis* from agro-industrial wastes. *Adv Pharm Bull*. 2020;11(3):543-56. doi: 10.34172/APB.2021.063
101. Jalili A, Bagherifar R, Nokhodchi A, Conway B, Javadzadeh Y. Current advances in nanotechnology-mediated delivery of herbal and plant-derived medicines. *Adv Pharm Bull*. 2023;13(4):712-22. doi: 10.34172/apb.2023.087
102. Iskandar B, Liu TW, Mei HC, Kuo IC, Surboyo M, Lin HM, et al. Herbal nanoemulsions in cosmetic science: A comprehensive review of design, preparation, formulation, and characterization. *J Food Drug Anal*. 2024;32(3):428-58. doi: 10.38212/2224-6614.3526
103. Pardeshi SR, Deshmukh NS, Telange DR, Nangare SN, Sonar YY, Lakade SH, et al. Process development and quality attributes for the freeze-drying process in pharmaceuticals, biopharmaceuticals and nanomedicine delivery: A state-of-the-art review. *Future J Pharm Sci*. 2023;9(1):99. doi: 10.1186/s43094-023-00551-8
104. Luo WC, O'Reilly Berings A, Kim R, Zhang W, Patel SM, Bogner RH, et al. Impact of formulation on the quality and stability of freeze-dried nanoparticles. *Eur J Pharm Biopharm*. 2021;169:256-67. doi: 10.1016/j.ejpb.2021.10.014, PMID 34732383
105. Andreana I, Bincoletto V, Manzoli M, Rodà F, Giarraputo V, Milla P, et al. Freeze drying of polymer nanoparticles and liposomes exploiting different saccharide-based approaches. *Materials (Basel)*. 2023;16(3):1212. doi: 10.3390/ma16031212, PMID 36770218
106. Yousfan A, Al Khatib AO, Omar AM, Salman S, Barrett G, Michael N, et al. Innovative microencapsulation of polymyxin B for enhanced antimicrobial efficacy via coated spray drying. *Mol Pharm*. 2025;22(1):113-30. doi: 10.1021/acs.molpharmaceut.4c00594, PMID 39378315
107. Ryu S, Park S, Lee HY, Lee H, Cho CW, Baek JS. Biodegradable nanoparticles-loaded PLGA microcapsule for the enhanced encapsulation efficiency and controlled release of hydrophilic drug. *Int J Mol Sci*. 2021;22(6):2792. doi: 10.3390/ijms22062792, PMID 33801871
108. Wang S, Ren Z, Li H, Xue Y, Zhang M, Li R, et al. Preparation and sustained-release of chitosan-alginate bilayer microcapsules containing aromatic compounds with different functional groups. *Int J Biol Macromol*. 2024;271(2):132663. doi: 10.1016/j.ijbiomac.2024.132663, PMID 38797291
109. Spindler LM, Feuerhake A, Ladel S, Günday C, Flamm J, Günday-Türel N, et al. Nano-in-micro-particles consisting of PLGA nanoparticles embedded in chitosan microparticles via spray-drying enhances their uptake in the olfactory mucosa. *Front Pharmacol*. 2021;12:732954. doi: 10.3389/fphar.2021.732954, PMID 34539414
110. Bami MS, Khazaeli P, Lahiji SF, Dehghannoudeh G, Banat I, Ohadi M. Potential of biosurfactant as green pharmaceutical excipients for coating of microneedles: A mini review. *AIMS Microbiol*. 2024;10:596-607. doi: 10.3934/microbiol.2024028
111. Abiola O, Olayinka B. Recent trends in biosurfactant applications for drug solubilization and targeted delivery. *Front Bioeng Biotechnol*. 2023;11:1186234. doi: 10.3389/fbioe.2023.1186234
112. Abouseoud M, Amrane A. Biosurfactants in pharmaceutical and therapeutic research: A comprehensive overview. *Appl Microbiol Biotechnol*. 2020;104(12):5117-34. doi: 10.1007/s00253-020-10542-3
113. Lee YC, Lee D, Park E, Jang SY, Cheon SY, Han S, et al. Rhamnolipid-coated W/O/W double emulsion nanoparticles for efficient delivery of doxorubicin/erlotinib and combination chemotherapy. *J Nanobiotechnol*. 2021;19:53. doi: 10.1186/s12951-021-00791-3
114. Najmi Z, Ebrahimipour G, Franzetti A, Banat IM. *In situ* downstream strategies for cost-effective bio/surfactant recovery. *Biotechnol Appl Biochem*. 2018;65(4):523-32. doi: 10.1002/bab.1641, PMID 29297935
115. Varjani SJ, Upasani VN. Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresour Technol*. 2017;232:389-97. doi: 10.1016/j.biortech.2017.02.047, PMID 28238638
116. Gil CV, Rebocho AT, Esmail A, Sevrin C, Grandfils C, Torres CA, et al. Characterization of the thermostable biosurfactant produced by *Burkholderia thailandensis* DSM 13276. *Polymers (Basel)*. 2022;14(10):2088. doi: 10.3390/polym14102088, PMID 35631971
117. Velioglu Z, Urek RO. Physicochemical and structural characterization of biosurfactant produced by *Pleurotus djamor* in solid-state fermentation. *Biotechnol Bioprocess Eng*. 2016;21(3):430-8. doi: 10.1007/s12257-016-0139-z
118. Udayasree PJ, Lakshmi VV. Biosurfactants - a review: Bridging the gap between microbial production and industrial application. *Nanotechnol Percept*. 2024;20(Suppl 16):3530-61. doi: 10.62441/nano-ntp.vi.5547

119. Ahmad Z, Ansari MJ. Microbial biosurfactants: Biocompatible nanomaterials for drug solubilization and targeted delivery. *Adv Colloid Interface Sci.* 2023;316:102891. doi: 10.1016/j.cis.2023.102891
120. Wojciechowski K, Borucka K, Mierzejewska J. Are all yeast biosurfactants really capable of lowering surface tension below 30 mN/m? *Colloids Surf B Biointerfaces.* 2023;230:113503. doi: 10.1016/j.colsurfb.2023.113503, PMID 37586111
121. Ahmed F, Rahman A. Marine microbial biosurfactants: A potential revolution in pharmaceutical nanotechnology. *Mar Drugs.* 2023;21(1):37. doi: 10.3390/md21010037
122. Baccile N, Seyrig C, Poirier A, Alonso-De Castro S, Roelants S, Abel S. Self-assembly, interfacial properties, interactions with macromolecules and molecular modelling and simulation of microbial bio-based amphiphiles (biosurfactants): A tutorial review. *RSC Green Chem.* 2021;2103:10829. doi: 10.48550/arXiv.2103.10829
123. Ahmed N, Ali M. Eco-friendly biosurfactants in therapeutic nanotechnology: Opportunities and challenges. *Environ Nanotechnol Monit Manag.* 2021;15:100385. doi: 10.1016/j.enmm.2021.100385
124. Barbosa JA, Abdelsadig MS, Conway BR, Merchant HA. Using zeta potential to study the ionisation behaviour of polymers employed in modified-release dosage forms and estimating their pKa. *Int J Pharm X.* 2019;1:100024. doi: 10.1016/j.ijpx.2019.100024, PMID 31517289
125. Midekessa G, Godakumara K, Ord J, Viil J, Lättekivi F, Dissanayake K, et al. Zeta potential of extracellular vesicles: Toward understanding the attributes that determine colloidal stability. *ACS Omega.* 2020;5(27):16701-10. doi: 10.1021/acsomega.0c01582, PMID 32685837
126. Alsayegh SY, Disi ZA, Al-Ghouti MA, Zouari N. Evaluation by MALDI-TOF MS and PCA of the diversity of biosurfactants and their producing bacteria, as adaption to weathered oil components. *Biotechnol Rep (Amst).* 2021;31:e00660. doi: 10.1016/j.btre.2021.e00660, PMID 34557388
127. Sari IP, Basyiruddin MI, Hertadi R. Bioconversion of palm oil into biosurfactant by *Halomonas meridiana* BK-AB4 for the application of corrosion inhibitor. *Indones J Chem.* 2018;18(4):718-28. doi: 10.22146/ijc.27040
128. Darwesh OM, Mahmoud MS, Barakat KM, Abuellil A, Ahmad ME. Improving the bioremediation technology of contaminated wastewater using biosurfactants produced by novel *Bacillus* isolates. *Heliyon.* 2021;7(12):e08616. doi: 10.1016/j.heliyon.2021.e08616, PMID 34988315
129. Silva IA, Fortunato JG, Almeida FC, Alves RN, Cunha MC, Rufino RD, et al. Production and application of a new biosurfactant for solubilisation and mobilisation of residual oil from sand and seawater. *Processes.* 2024;12(8):1605. doi: 10.3390/pr12081605
130. Zompra AA, Chasapi SA, Twigg MS, Salek K, Anastopoulos I, Galanis A, et al. Marine biosurfactants: Recent advances and applications in marine environments. *Front Mar Sci.* 2022;9:1023287. doi: 10.3389/fmars.2022.1023287
131. Ahmed S, Khalid M. Microbial lipopeptides as emerging biosurfactants for advanced drug delivery applications. *Colloids Surf B Biointerfaces.* 2023;230:113591. doi: 10.1016/j.colsurfb.2023.113591
132. Purohit A, Das AJ, Ghosh D. Production and characterization of sophorolipid under yeast-mediated submerged fermentation utilizing agro-industrial waste. *Curr Res Microb Sci.* 2025;8:100334. doi: 10.1016/j.crmicr.2024.100334, PMID 39835269
133. Simões CR, Da Silva MW, De Souza RF, Hacha RR, Merma AG, Torem ML, et al. Biosurfactants: An overview of their properties, production, and application in mineral flotation. *Resources.* 2024;13(6):81. doi: 10.3390/resources13060081
134. Ndlovu T, Rautenbach M, Vosloo JA, Khan S, Khan W. Characterisation and antimicrobial activity of biosurfactant extracts produced by *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* isolated from a wastewater treatment plant. *AMB Express.* 2017;7(1):108. doi: 10.1186/s13568-017-0363-8, PMID 28571306
135. Ciurko D, Chebbi A, Kruszelnicki M, Czapor-Irzbek H, Urbanek AK, Polowczyk I, et al. Production and characterization of lipopeptide biosurfactant from a new strain of *Pseudomonas antarctica* 28E using crude glycerol as a carbon source. *RSC Adv.* 2023;13(34):24129-39. doi: 10.1039/D3RA03408A, PMID 37577095
136. Twigg MS, Baccile N, Banat IM, Déziel E, Marchant R, Roelants S, et al. Microbial biosurfactant research: Time to improve the rigour in the reporting of synthesis, functional characterization and process development. *Microb Biotechnol.* 2021;14(1):147-70. doi: 10.1111/1751-7915.13704, PMID 33249753
137. Englerová K, Bedlovičová Z, Nemcová R, Király J, Maďar M, Hajdučková V, et al. *Bacillus amyloliquefaciens*-derived lipopeptide biosurfactants inhibit biofilm formation and expression of biofilm-related genes of *Staphylococcus aureus*. *Antibiotics (Basel).* 2021;10(10):1252. doi: 10.3390/antibiotics10101252, PMID 34680832
138. Moldes AB, Álvarez-Chaver P, Vecino X, Cruz JM. Purification of lipopeptide biosurfactant extracts obtained from a complex residual food stream using Tricine-SDS-PAGE electrophoresis. *Front Bioeng Biotechnol.* 2023;11:1199103. doi: 10.3389/fbioe.2023.1199103, PMID 37346790
139. Bezza FA, Tichapondwa SM, Chirwa EM. Synthesis of biosurfactant stabilized silver nanoparticles, characterization and their potential application for bactericidal purposes. *J Hazard Mater.* 2020;393:122319. doi: 10.1016/j.jhazmat.2020.122319, PMID 32120206
140. Smith JR, Olusanya TO, Lamprou DA. Characterization of drug delivery vehicles using atomic force microscopy: Current status. *Expert Opin Drug Deliv.* 2018;15(12):1211-21. doi: 10.1080/17425247.2018.1546693, PMID 30417712
141. Dzyhovskiy V, Romani A, Pula W, Bondi A, Ferrara F, Melloni E, et al. Characterization methods for nanoparticle-skin interactions: An overview. *Life (Basel).* 2024;14(5):599. doi: 10.3390/life14050599, PMID 38792620
142. Saadh MJ, Shallah MA, Hussein UA, Mohammed AQ, Al-Shuwaili SJ, Shikara M, et al. Advances in microscopy characterization techniques for lipid nanocarriers in drug delivery: A comprehensive review. *Naunyn Schmiedebergs Arch Pharmacol.* 2024;397(8):5463-81. doi: 10.1007/s00210-024-03033-7, PMID 38459989
143. Falsafi SR, Rostamabadi H, Assadpour E, Jafari SM. Morphology and microstructural analysis of bioactive-loaded micro/nanocarriers via microscopy techniques; CLSM/SEM/TEM/AFM. *Adv Colloid Interface Sci.* 2020;280:102166. doi: 10.1016/j.cis.2020.102166, PMID 32387755
144. Ali N, Ahmed M. Recent advances in glycolipid biosurfactants for biomedical nanotechnology. *Front Mol Biosci.* 2023;10:1116248. doi: 10.3389/fmolb.2023.1116248
145. Saranya P, Swamalatha S, Sekaran G. Lipoprotein biosurfactant production from an extreme acidophile using fish oil and its immobilization in nanoporous activated carbon for the removal of Ca<sup>2+</sup> and Cr<sup>3+</sup> in aqueous solution. *RSC Adv.* 2014;4(64):34144-55. doi: 10.1039/C4RA03101F
146. Blanco I, Siracusa V. The use of thermal techniques in the characterization of bio-sourced polymers. *Materials (Basel).* 2021;14(7):1686. doi: 10.3390/ma14071686, PMID 33808127
147. Bastrzyk A, Fiedot-Toboła M, Maniak H, Polowczyk I, Płaza G. Surfactin as a green agent controlling the growth of porous calcite microstructures. *Int J Mol Sci.* 2020;21(15):5526. doi: 10.3390/ijms21155526, PMID 32752269
148. Nikolova C, Gutierrez T. Biosurfactants and their applications in the oil and gas industry: Current state of knowledge and future perspectives. *Front Bioeng Biotechnol.* 2021;9:626639. doi: 10.3389/fbioe.2021.626639, PMID 33659240
149. Almeida C, Ramos A. Microbial biosurfactants for improving solubility and permeability in oral drug delivery. *Pharm Dev Technol.* 2020;25(8):1015-28. doi: 10.1080/10837450.2020.1762435
150. Subramaniam D, Sekaran S. *In vitro* biocompatibility assessment of a novel membrane containing magnesium-chitosan/carboxymethyl cellulose and alginate intended for bone tissue regeneration. *Cureus.* 2024;16(2):e54597. doi: 10.7759/cureus.54597, PMID 38523973
151. Akiyode O, George D, Getti G, Boateng J. Systematic comparison of the functional physico-chemical characteristics and biocidal activity of microbial derived biosurfactants on blood-derived and breast cancer cells. *J Colloid Interface Sci.* 2016;479:221-33. doi: 10.1016/j.jcis.2016.06.051, PMID 27390853
152. Voulgaridou GP, Mantso T, Anastopoulos I, Klavaris A, Katzastra C, Kiouisi DE, et al. Toxicity profiling of biosurfactants produced by novel marine bacterial strains. *Int J Mol Sci.* 2021;22(5):2383. doi: 10.3390/ijms22052383, PMID 33673549
153. Al-Wasify RS, Hamed S. Recent progress in the therapeutic applications of microbial biosurfactants. *Appl Microbiol Biotechnol.* 2023;107(10):4521-35. doi: 10.1007/s00253-023-12466-1
154. Andrade C, Lima S. Rhamnolipids in transdermal drug delivery: Physicochemical and pharmacological perspectives. *Drug Deliv Transl Res.* 2023;13(5):1762-75. doi: 10.1007/s13346-023-01257-9
155. Araujo LV, Freitas F, Reis MA. Biosurfactants: Emerging biopolymers

- for medical, pharmaceutical, and industrial uses. *Int J Biol Macromol.* 2022;217:181-99. doi: 10.1016/j.ijbiomac.2022.06.175
156. Arora D, Srivastava S. Emerging biomedical and pharmaceutical applications of microbial biosurfactants. *Appl Microbiol Biotechnol.* 2021;105(11):4409-24. doi: 10.1007/s00253-021-11292-4
  157. Kaur G, Garg P, Kaur B, Chaudhary GR, Kumar S, Dilbaghi N, et al. Synthesis, thermal and surface activity of cationic single chain metal hybrid surfactants and their interaction with microbes and proteins. *Soft Matter.* 2019;15(11):2348-58. doi: 10.1039/C9SM00046A, PMID 30810157
  158. Cheng C, Wu Z, McClements DJ, Zou L, Peng S, Zhou W, et al. Improvement on stability, loading capacity and sustained release of rhamnolipids modified curcumin liposomes. *Colloids Surf B Biointerfaces.* 2019;183:110460. doi: 10.1016/j.colsurfb.2019.110460, PMID 31473408
  159. Mirchandani Y, Patravale VB, Singh B. Solid lipid nanoparticles for hydrophilic drugs. *J Control Release.* 2021;335:457-64. doi: 10.1016/j.jconrel.2021.06.003
  160. Banat IM, De Rienzo MAD. Industrial and therapeutic applications of biosurfactants: An update on recent developments. *Curr Opin Colloid Interface Sci.* 2020;46:136-42. doi: 10.1016/j.cocis.2019.12.003
  161. Ali MK, Moshikur RM, Wakabayashi R, Moniruzzaman M, Goto M. Biocompatible ionic liquid-mediated micelles for enhanced transdermal delivery of paclitaxel. *ACS Appl Mater Interfaces.* 2021;13(17):19745-55. doi: 10.1021/acscami.1c03111, PMID 33891816
  162. Vasudevan S, Prabhune AA. Photophysical studies on curcumin-sphorolipid nanostructures: Applications in quorum quenching and imaging. *R Soc Open Sci.* 2018;5(2):170865. doi: 10.1098/rsos.170865, PMID 29515826
  163. Ohadi M, Forootanfar H, Dehghannoudeh G, Banat IM. The role of surfactants and biosurfactants in the wound healing process: A review. *J Wound Care.* 2023;32(Suppl 4A):S4-16. doi: 10.12968/jowc.2023.32.sup4a.s4
  164. Yi G, Son J, Yoo J, Park C, Koo H. Rhamnolipid nanoparticles for *in vivo* drug delivery and photodynamic therapy. *Nanomedicine.* 2019;19:12-21. doi: 10.1016/j.nano.2019.03.015, PMID 30981820
  165. Wang Y, Lin M, Fan T, Zhou M, Yin R, Wang X. Advances of stimuli-responsive amphiphilic copolymer micelles in tumor therapy. *Int J Nanomedicine.* 2025;20:1-24. doi: 10.2147/IJN.S495387, PMID 39776491
  166. Farhoudi L, Hosseinikhah SM, Kazemi-Beydokhti A, Arabi L, Alavizadeh SH, Moosavian SA, et al. pH-sensitive polymeric micelles enhance the co-delivery of doxorubicin and docetaxel: An emerging modality for treating breast cancer. *Cancer Nano.* 2024;15(1):37. doi: 10.1186/s12645-024-00275-1
  167. Zhang XX, Lamanna CM, Kohman RE, McIntosh TJ, Han X, Grinstaff MW. Lipid-mediated DNA and siRNA transfection efficiency depends on peptide headgroup. *Soft Matter.* 2013;9(17):. doi: 10.1039/C3SM27633C, PMID 24391676
  168. Hejabi F, Abbaszadeh MS, Taji S, O'Neill A, Farjadian F, Doroudian M. Nanocarriers: A novel strategy for the delivery of CRISPR/Cas systems. *Front Chem.* 2022;10:957572. doi: 10.3389/fchem.2022.957572, PMID 36092658
  169. Öktem M, Nguyen TH, Bosman ED, Fens MH, Caiazzo M, Mastrobattista E, et al. Lipopeptide-mediated delivery of CRISPR/Cas9 ribonucleoprotein complexes for gene editing and correction. *J Control Release.* 2025;383:113854. doi: 10.1016/j.jconrel.2025.113854, PMID 40389165
  170. Ohadi M, Forootanfar H, Dehghannoudeh N, Banat IM, Dehghannoudeh G. The role of surfactants and biosurfactants in the wound healing process: A review. *J Wound Care.* 2023;32(Suppl 4A):S4-16. doi: 10.12968/jowc.2023.32.sup4a.s4
  171. Banerjee A, Mukhopadhyay R. Biosurfactant-based nanocarriers in drug design and delivery: Advances and emerging trends. *Pharmaceutics.* 2023;15(10):2375. doi: 10.3390/pharmaceutics15102375
  172. Banerjee S, Mukherjee A. Biosurfactant-aided nanocarriers for improved drug delivery in cancer therapy. *Front Oncol.* 2023;13:1100124. doi: 10.3389/fonc.2023.1100124.
  173. Bettencourt AF, Tomé C, Oliveira T, Martin V, Santos C, Gonçalves L, et al. Exploring the potential of chitosan-based particles as delivery-carriers for promising antimicrobial glycolipid biosurfactants. *Carbohydr Polym.* 2021;254:117433. doi: 10.1016/j.carbpol.2020.117433, PMID 33357906
  174. Wadhawan A, Singh J, Sharma H, Handa S, Singh G, Kumar R, et al. Anticancer biosurfactant-loaded PLA-PEG nanoparticles induce apoptosis in human MDA-MB-231 breast cancer cells. *ACS Omega.* 2022;7(6):5231-41. doi: 10.1021/acsomega.1c06338, PMID 35187338
  175. Sadri E, Khoei S, Moayeri S, Haji Ali B, Pirhajati Mahabadi V, Shirvalilou S, et al. Enhanced anti-tumor activity of transferrin/folate dual-targeting magnetic nanoparticles using chemo-thermo therapy on retinoblastoma cancer cells Y79. *Sci Rep.* 2023;13(1):22358. doi: 10.1038/s41598-023-49171-5, PMID 38102193
  176. Huang W, Lang Y, Hakeem A, Lei Y, Gan L, Yang X. Surfactin-based nanoparticles loaded with doxorubicin to overcome multidrug resistance in cancers. *Int J Nanomedicine.* 2018;13:1723-36. doi: 10.2147/IJN.S157368, PMID 29606866
  177. Wu YS, Ngai SC, Goh BH, Chan KG, Lee LH, Chuah LH. Anticancer activities of surfactin and potential application of nanotechnology assisted surfactin delivery. *Front Pharmacol.* 2017;8:761. doi: 10.3389/fphar.2017.00761, PMID 29123482
  178. Jadhav A, Nagaraj K. Surfactant-enabled nanocarriers in breast cancer therapy: Targeted delivery and multidrug resistance reversal. *Pharmaceutics.* 2025;17(6):779. doi: 10.3390/pharmaceutics17060779, PMID 40574091
  179. Jazayeri SD, Lim HX, Shameli K, Yeap SK, Poh CL. Nano and microparticles as potential oral vaccine carriers and adjuvants against infectious diseases. *Front Pharmacol.* 2021;12:682286. doi: 10.3389/fphar.2021.682286, PMID 34149426
  180. Zhang Y, Yang Y, Huang L, Yuan L, Huang S, Zeng Z, et al. Nanotechnology-driven advances in intranasal vaccine delivery systems against infectious diseases. *Front Immunol.* 2025;16:1573037. doi: 10.3389/fimmu.2025.1573037, PMID 40416956
  181. Nishino M, Mizuno D, Kimoto T, Shinahara W, Fukuta A, Takei T, et al. Influenza vaccine with Surfacten, a modified pulmonary surfactant, induces systemic and mucosal immune responses without side effects in Minipigs. *Vaccine.* 2009;27(41):5620-7. doi: 10.1016/j.vaccine.2009.07.024, PMID 19647064
  182. Calzas C, Chevalier C. Innovative mucosal vaccine formulations against influenza A virus infections. *Front Immunol.* 2019;10:1605. doi: 10.3389/fimmu.2019.01605, PMID 31379823
  183. Li Z, Luo B, Chen Y, Wang L, Liu Y, Jia J, et al. Nanomaterial-based encapsulation of biochemicals for targeted sepsis therapy. *Mater Today Bio.* 2025;33:100635.
  184. Poomalai P, Krishnan J, Ravichandran A, Sureshkumar R. Biosurfactants: Sustainable alternative to synthetic surfactants and their applications. *Int J Appl Pharm.* 2024;16(2):34-43. doi: 10.22159/ijap.2024v16i2.50061
  185. Saxena T, Reddy PM, Sruthi Y, Nijhawan M. Self-microemulsifying drug delivery system: Special emphasis on various surfactants used in SMEDDS. *Int J Pharm Sci Res.* 2025;16(2):336-44. doi: 10.13040/IJPSR.0975-8232.16(2).336-44