Recent Developments in
APPLIED MICROBIOLOGY AND BIOCHEMISTRY

VOLUME 2

Edited by Buddolla Viswanath

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Section A

Medical microbiology and biochemistry

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Recent Developments in Applied Microbiology and Biochemistry

Volume 2

Edited by

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Contents

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Section B
Microbial biotechnology

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Chapter 14

Recent developments in nanoencapsulation of bioactive compounds of microbial sources and their biomedical applications

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1 Introduction

There are several hydrophobic or poorly soluble nutrients and bioactive food compounds important to human health. The use of these ingredients in food is limited by a variety of factors including low stability due to susceptibility to oxygen, light and temperature, poor solubility, and low bioavailability (Rezaei et al., 2019). An alternative for increasing their solubility and bioavailability is nanoencapsulation with hydrophilic carriers (Suganya and Anuradha, 2017). Nanoencapsulation can also protect unstable compounds from unfavorable conditions during processing, storage, and transportation to boost their consistency and stability and increase their binding affinities, along with controlled release of bioactive compounds (Garavand et al., 2019). Nanoencapsulation can therefore be described as a technique for enclosing a bioactive compound in an inert material, typically with molecules bound together in long repeating chains (Leena et al., 2020). There are two types of encapsulation currently producing the best results, namely micro and nanoencapsulation (Shishir et al., 2018). Nanoencapsulation involves coating at the nanoscale to form particles of <100 nm and microencapsulation forms particles larger than 100 nm.

Most natural molecules have low solubility due to their nonpolar nature and degrade upon entry into the body that restricts their successful usage (Yang and Hinner, 2015). Some studies have attempted to improve drug bioavailability through fixing compound physical properties with limited success through victimization, adjuvants, and other alternative approaches (Andrysek, 2003). In order to tackle these problems, nanotechnology is gaining attention in the food and health-care sectors due to improved solubility, bioavailability, and reduced dose of bioactive molecules, especially for toxic and precious compounds, and targeted delivery of active molecules (Patra et al., 2018a,b). Nanotechnology enables the application of biodegradable, nontoxic nanoparticles to bind or encapsulate insoluble natural or synthetic hydrophobic compounds utilizing various techniques (McNeil, 2005). Formulations of nanomaterials can greatly improve the therapeutic index and pharmacokinetic parameters of drugs, especially those of plant origin (Khan and Gurav, 2018; Patra et al., 2018a, b). Other techniques such as nano-micellular delivery of natural compounds to the target site are highly promising, because they can potentially minimize toxicity to other tissues or body parts (Kumari et al., 2012). Nanoencapsulation can supply specific nutrients in small quantities for food, and allow slow and sustained delivery of flavor and spice ingredients.

The current system for targeted drug delivery includes liposomes, dendrimers, nanotubes, polymeric micelles, diblock and triblock copolymers, polymer drug conjugates, magnetic nanoparticles, and drugs encapsulated in biodegradable polymers. Some encapsulating polymers are easily degraded in the body by hydrolytic enzymes, thereby reducing adverse effects and related toxicity. In the above methods, nanoparticles are encapsulated or conjugated in nano-size casings, thus reducing the need for high concentrations of the drug. One present problem is whether these nanoencapsule systems are capable of therapeutic effects at low concentrations because most novel anticancer drugs require high concentrations to show an appreciable effect. Hence, major research efforts are focused on improving

Recent Developments in Applied Microbiology and Biochemistry. https://doi.org/10.1016/B978-0-12-821406-0.00014-X
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the pharmacokinetic parameters, solubility, stability, bioavailability, bio-efficacy, drug-loading capacity, therapeutic index, and antimicrobial resistance using nanotechnology (Kumari et al., 2012). Based on the partition coefficients of the drug, a suitable formulation will help enhance solubilization and absorption of drugs such as curcumin. For example, encapsulation of curcumin in a lipid-based nanoemulsion significantly increased the bioavailability (Yu and Huang, 2012). Based on these urgent needs and promising results, the exploration of nanoparticles as drug delivery methods has emerged as an important concern for research.

2 Nanoencapsulation and its importance in enhancing properties of bioactive compounds

As previously mentioned, nanoencapsulation is the containment of a molecule or substance within a cavity made of nanoscale material that provides the agent with overall protection from harsh environments, enhances stabilization, operation, targeted delivery, and controlled release (Patra et al., 2018a,b). These properties offer especially huge advantages in the area of pharmaceutical applications, where traits like drug delivery efficiency, targeted delivery, sustained activity, extended release, and helps in immobilizing poorly soluble drugs (Florence and Siepmann, 2009). Furthermore, encapsulation allows coating or modifications of the outer surfaces improving surface properties including charge, solubility, etc. In pharmaceutical applications, drug delivery is the most crucial step to determine whether a drug is effective or ineffective (Florence and Siepmann, 2009).

2.1 Improved release and delivery by encapsulation

Drug release cannot be controlled without coating or encapsulation, as an orally administered drug will be dissolved when it interacts with the digestive tract enzymes. Thus, drug encapsulation provides a layer of protection for gradual release of its drug content when it is naturally dissolved, solubilized, diffused, or degraded (Singh and Lillard, 2009; O'Sullivan et al., 2016). A more stable, large-sized encapsulation has a longer release rate of up to weeks and months. Sustained release in case vaccines provides a prolonged immune system stimulation (Kreuter, 2007). Besides vaccines, sustained release was also applied to other drugs such as bioactive compound (Zeisser-Labouèbe et al., 2006), protein (Jung et al., 2000), DNA (Borodina et al., 2007), stem cell (Kauer et al., 2011), and so on.

By encapsulating a drug in a biopolymer, drug release can be controlled by fine-tuning its release system. For example, multivesicular liposome (MVL) is a unique lipid encapsulation biomaterial for the purpose of controlling the drug release rate and dose effectively. These two properties are due to the packed internal aqueous chamber of the substance, which produces a higher mechanical strength, and the presence of triglyceride at the junction stabilizer as a hydrophobic space filler, which is used to change its drug release rate (Zhong et al., 2005). Encapsulation may also create a mechanism for the release of drugs through either an external or internal trigger switch which can be used to regulate the release at a specified time (after drug administration). A simple trigger is the self-degrading microcapsules which degrade by releasing the encapsulated agent via proteolysis. Certain methods for switch regulated self-degradation systems include external physical triggers such as ultrasound, magnetic field, and infrared laser, while internal triggers such as hydrolytic cleavable polyelectrolytes and chitosanase are reported (Borodina et al., 2007).

2.2 Improved targeting and permeability

Common drug delivery routes are dependent on required therapy, feasibility, cost and include parenteral, transdermal, inhalation, etc. (Hassan, 2012). Oral route is the most common, preferred, and widely used route for the delivery of drugs, but it faces barriers to environment degradation, elimination, inactivation, loss, unspecific, and weak adsorption. In the case of oral drug administration, encapsulation would protect the product as it provides isolation and removes interaction between the sensitive and active product agents along the oral delivery path, especially in the areas of mouth, stomach, duodenum, jejunum, and proximal ileum, exposure of antioxidants to free radicals, enzymatic degradation, and acidic environment. Drugs such as epigallocatechin 3-gallate, epicatechin 3-gallate, epigallocatechin, and epicatechin were successfully encapsulated in biopolymer and have been reported to be effective against free radical and enzymatic degradation (Tang et al., 2013). Drug delivery in organs like the brain is restricted by the blood-brain barrier (BBB) (Brasnjevic et al., 2009). However, the brain itself originates from many neurological disorders such as brain cancer, neurovascular, neurodegeneration, and so on. Due to its failure to crack the BBB and hit the target cell, neurological drugs administered orally are less effective. But encapsulation only protects the medication; its permeability is improved with surface modifications to hit through BBB (Faraji and Wipf, 2009). The first surface-coated encapsulated nanoparticle reported to demonstrate efficient trans BBB transport was polysorbate 80-coated poly(butyl cyanoacrylate) (Kreuter, 2007). Later, various other encapsulation nanoparticles were prepared with a surface coating of polysorbate 80 (Tween 80), especially for nootropic delivery into the brain (Jose et al., 2014). In addition to the surface coating process, the selection of an effective encapsulation biopolymer often allows for improved permeability of the BBB. For example, liposomes for neurological drugs such as calpain inhibitor,

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GABA, and phenytoin have been documented as encapsulation biopolymers. However, liposomes are biocompatible and biodegradable but suffer from low stability and large volumes (Brasnjevic et al., 2009). The oral delivery of heparin, calcitonin, and insulin has been improved by the viapara-cellular transport achieved by thiolated polycarbophil encapsulation. Besides, it also provides sustained release, enzymatic inhibition, and zero side effects (Bernkop-Schnürch, 2005). Enhanced paracellular transport effectiveness has been due to encapsulation with mucoadhesive biopolymer, which mimics naturally secreted mucus glycoprotein, providing stability and greater adhesive power. Improving cell permeability by encapsulation approach is more beneficial than a more condensed and safe structural alteration of drugs (Salamat-Miller and Johnston, 2005).

Drug targeting is enhanced for delivery by encapsulation into particles. The encapsulation of liposomes and nanoparticles has been shown to be a good biomaterial for controlled drug delivery by either active or passive targeting (Singh and Lillard, 2009; Patra et al., 2018a,b). Drugs like Insulin cannot be taken orally and needs to be specifically delivered to inhibit hepatic glucose production (Agarwal and Khan, 2001). Therapeutic stem cells need to be delivered to the glial tissue of the nervous system (Kauer et al., 2011). Metformin hydrochloride, an oral antidiabetic drug, was aimed at a targeted and constant release into the upper part of the gastrointestinal tract (Pandit et al., 2013). Insulin encapsulation strategies enable oral drug delivery to overcome enzymatic degradation, intestinal transport, stability issues, and the ability to target hepatic cells. Besides, encapsulation also provides an alternative administration route to the current subcutaneous route (Agarwal and Khan, 2001). Encapsulation of the therapeutic stem cell in the synthetic extracellular matrix (sECM) is a recent noninvasive alternate therapeutic treatment for cancer treatment (Kauer et al., 2011). Encapsulation of metformin hydrochloride in the ethyl cellulose microsphere increased residence time while maintaining bioavailability up to 24 h after administration (Pandit et al., 2013).

3 Nanoencapsulation techniques for bioactive compounds

Methods of encapsulation can be used to preserve and monitor the release of bioactive compounds. Nanoencapsulation is one of the most effective strategies for bioactivity protection (Bayraktar et al., 2017). The extensive advantages of nanoencapsulation are guided movement of chemicals and efficient cell absorption. Nanoencapsulation can be divided into two main weather-based categories. The formulation includes polymerization reaction or is obtained directly from a macromolecule or preformed polymer (Couvreur et al., 1995). The techniques of polymerization can also be categorized as emulsion and interfacial polymerization, and there are two forms of emulsion polymerization: organic and aqueous, depending on the continuous process. Nanoencapsulations are often made directly from synthetic or natural polymers, natural macromolecules, and recently nebulization process is in use for nanoencapsulation. Fig. 1 is an overview of nanoencapsulation strategies and applications.

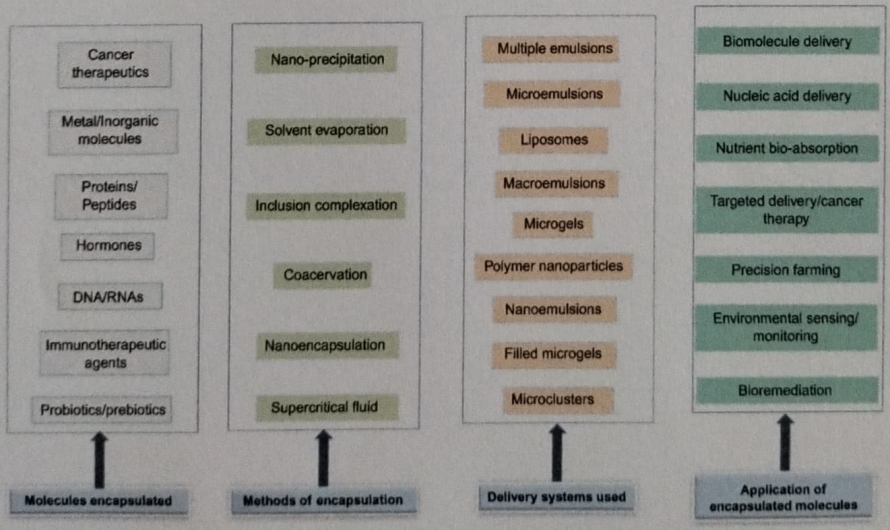

FIG. 1 An overview of nanoencapsulation-molecules encapsulated, methods used, delivery systems, and applications.

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3.1 Nanoencapsulation by emulsion polymerization

Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable where the monomer is continuously spread into an inverse microemulsion or a nonsolvent and surfactants or soluble polymers are used to prevent aggregation (Kreuter, 1991). Due to the nonbiodegradable nature of the polymer as well as the difficult procedure, alternate materials like poly(ethylcyanoacrylate) (PECA), poly(methylmethacrylate) (PMMA), poly(butylcyanoacrylate), etc. were used for nanoencapsulation synthesis by dispersion via a surfactant into solvents such as cyclohexane (ICH, class 2), *n*-pentane (ICH, class 3), and toluene (ICH, class 2) as the organic phase (El-Samaligy et al., 1986) for encapsulating triamcinolone (Krause et al., 1986), fluorescein (El-Samaligy et al., 1986), pilocarpine (Harmia-Pulkkinen et al., 1989), and timolol (Harmia-Pulkkinen et al., 1989).

PMMA nanoparticles are suitable adjuvants for vaccines and are produced by the radical emulsion polymerization mechanism without emulsifiers (Kreuter, 1991). The PMMA nanospheres were produced with a single monomer methylmethacrylate. Then copolymerization of MMA, hydroxypropylmethacrylate, methacrylic acid, and ethylene glycol dimethacrylate was reported, yet it possesses drawbacks like chemical or physical initiation and the PMMA nanospheres are nonbiodegradable. Poly(alkylcyanoacrylate) (PACA) is biodegradable and polymerization is via the anionic process occurring at room temperature and effective in encapsulating hydrophilic drugs like ampicillin and doxorubicin and sparingly soluble compounds (Seijo et al., 1990).

3.2 Encapsulation by interfacial polymerization

Nanoencapsules are created by spontaneous polymerizing when cyanoacrylate after contacts with initiating ions in water result in colloidal suspensions harvested by evaporation under vacuum. The advantages of interfacial polymerization include high-efficiency drug encapsulation; the derived nanoencapsules can be controlled to fit the contour of the inner phase and the process is in situ (Couvreur et al., 1995). PECA (Watnasirichaikul et al., 2000), poly(isobutylcyanoacrylate) (Lambert et al., 2000), and poly(isohexylcyanoacrylate) (Lenaerts et al., 1995) were used in production of nanocapsules containing insulin (Watnasirichaikul et al., 2000), calcitonin (Lowe and Temple, 1994), octreotide (Damgé et al., 1997), darodipine (Hubert et al., 1991), indomethacin, and phthalocyanines in an injectable vehicle by this process. However, an organic solvent is required as the external phase (Lenaerts et al., 1995). Similarly, interfacial polycondensation nanoparticles can be produced with the help of a lipophilic monomer: phtaloyldichloride and hydrophilic monomer: diethylenetriaminel the encapsulation can be produced with or without a surfactant (Montasser et al., 2002).

3.3 Production of nanoparticles using natural macromolecules

3.3.1 *Albumin*

Albumin microspheres are prepared by either stabilization in thermal treatment at elevated temperatures (95–170°C) or chemical treatment in vegetable oil, isooctane emulsions, or aqueous medium with slight modifications. Albumin nanospheres are shaped by homogenizing the oil-containing albumin droplets and thermally stabilized by heating at 175–180°C for 10 min, then diluted with diethyl ether to allow separation (Patil, 2003). The particles were agitated, extracted through centrifugation, and lyophilized, but Heat-labile molecules are not suitable for this process. As a modification of this method (Longo et al., 1982), an aqueous solution of albumin was emulsified in chloroform containing hydroxypropyl cellulose and ethyl cellulose (EC) as stabilizers. The blended macromolecule is later cross-linked with glutaraldehyde and washed.

3.3.2 *Gelatin*

Emulsified gelatin mixture droplets were hardened by cooling the emulsion extremely in an ice bath, resulting in gelation of the gelatin droplets. Gelatin nanodroplets were filtered, washed, and cross-linked with formaldehyde. This technique is applicable to heat-sensitive drugs; however, drugs can be covalently bound to the gelatin by formaldehyde treatment (Kreuter, 1992). Another similar system for drug delivery is vegetable macromolecule fractions, gliadins from wheat gluten, efficient in encapsulating lipophilic substances providing controlled release of the drug (Duclairoir et al., 2002).

3.3.3 *Alginate nanoparticles*

Sodium alginate is a water-soluble polymer that gels in the presence of cations such as calcium (Aslani and Kennedy, 1996). Alginate particles are typically made by dropwise extrusion of sodium alginate solution into calcium chloride solution. An alginate particle scale depends on the size of the initial extruded droplet. The smallest particles produced had a size of 1–5 μm, obtained by air atomization (Kwok et al., 1991). The preparation of alginate nanoparticles with an exceedingly diluted sodium alginate solution added to the low concentration calcium formed invisible clusters of calcium alginate gels. Later, alginate particles have been produced by using a modified emulsification/internal

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gelation method. The preparation of alginate nanoparticles via this method requires the elimination of residual oil droplets (Reis et al., 2005).

3.3.4 Chitosan nanoparticles

Chitosan nanoparticles have been developed to encapsulate proteins such as bovine serum albumin, tetanus, and diphtheria toxoid (Soppimath et al., 2001; Akbari-Alavijeh et al., 2020), vaccines (Vila et al., 2004), anticancer agents (Janes et al., 2001), insulin (Ma et al., 2005), and nucleic acids (Mansouri et al., 2004). Chitosan considerably enhanced the absorption of peptides such as insulin and calcitonin across the nasal epithelium. Chitosan nanoparticles obtained by formation of a spontaneous complex between chitosan and polyanions such as tripolyphosphate (Calvo et al., 1997) have small diameters (200–500 nm) and show a quasi-spherical shape. Chitosan nanoparticles can also be created by gelation in an emulsification-based technique.

3.3.5 Agarose nanoparticles

Agarose nanoparticles were developed for the administration of therapeutic proteins and peptides. Agarose solution forms thermally reversible hydrogels when cooled below 31–36°C. Thermal gelation results in the formation of three-dimensional helical structure. The polymeric product of hydrogel, being hydrophilic, inert, and biocompatible, forms a suitable matrix for proteins and peptides which will be entrapped within the gel throughout formation (Vauthier and Couvreur, 2000).

3.4 Nanoparticles produced by desolvation of macromolecules

One more technology applicable to a large variety of polymers is predicated on desolvation by charge and pH changes, or by addition of a desolvating agent (ethanol or concentrated inorganic salt solutions). The advantage is, this process does not require an increase in temperature, and therefore may be useful when heat-sensitive drugs are used. Nanoparticles were prepared by reversible swelling of macromolecules using gelatin, human serum albumin, bovine serum albumin, and casein as the macromolecular materials (Weber et al., 2000). This offers the advantage of manufacturing nanoparticles in liquid suspension; however, the employment of glutaraldehyde and desolvating agents requires subsequent purification (Couvreur et al., 1995). In the case of gelatin, different methods such as the two-step desolvation method (Zillies and Coester, 2004) have been applied to produce nanoparticles.

4 Nanoencapsulation of bioactive compounds of microbial origin

4.1 Organic-organic nanoparticle conjugates

Biological nanoparticles are a part of a number of industrial products, particularly in the pharmaceutical, food, and cosmetic industry. Biological nanoparticles derived from a single biomolecule element have been commonly used as both anticancer and therapeutic agents (Patra et al., 2018a,b). For example, the encapsulation of β-carotene into whey protein concentrate (WPC) by electro-spraying resulted in a nanocapsule with high encapsulation efficiency and photooxidation stability (López-Rubio and Lagaron, 2012). Liao et al. (2016) suggested that selenium nanoparticles with a dictyophoraindusiate polysaccharide formed monodispersed nanoparticles with high stability due to hydrophilic repulsion on the polysaccharide surface as opposed to naked selenium. Moreover, the nanoparticle conjugate exhibited enhanced selectivity and antiproliferative activity through inducing apoptosis. Use of two or more organic hybrid nanoparticles to encapsulate biomolecules and bioactives such as curcumin (Huang and Kuo, 2014), quercetin (Ha et al., 2013), and epigallocatechin-3-gallate (Hu et al., 2012) into chitosan-based nanoparticles has been reported as naked chitosan is unstable and can be easily dissociated at low pH. Coating the protein nanoparticles with polysaccharide molecules enables control of an electrostatic interplay and steric repulsion between the particles, thereby increasing the stability of the nanoparticles, resulting in greater water dispersibility and antioxidant activity (Huang et al., 2016).

4.2 Applications of biomolecule-nanoparticle conjugates

4.2.1 Medical and pharmaceutical

Nanoparticular-based treatments are widely used to diagnose and treat diseases such as cancer, diabetes, and allergies. In addition, the nanoparticles-based drug delivery system allows better control of drug release to the target area by reducing the administration frequency and minimizing the risk of systemic side effects (Mahapatro and Singh, 2011; Patra et al., 2018a,b). Attempts to boost biomolecule-nanoparticle conjugates include mimicking the ability of *Salmonella enterica* serotype Typhimurium pathogen to reverse multidrug resistance. Mercado-Lubo et al. (2016) has constructed a semisynthetic 'Salmonella nanoparticle mimic' based primarily on gold nanoparticles packed with effector protein (SipA). In another study, conjugation of prostate-specific antigen (PSA) to gold nanoparticles has enhanced the efficacy and sensitivity of PSA for diagnosis of prostate

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cancer based on localized surface plasmon resonance (Jazayeri et al., 2016). Enzyme prodrug remedy based on horseradish peroxidase (HRP) immobilized onto mesoporous silica nanoparticles converts a prodrug [indole-3-acetic acid (IAA)] into cytotoxic radicals, which prompts apoptotic tumor cell death in human colon carcinoma cells (Hung et al., 2015). In addition, the incorporation of biomolecule nanoparticles enables the creation of three-dimensional scaffolds based on gelatin-hydroxyapatite hybrid nanoparticles with uniformly disbursed nano-topologies for use in osteogenesis (Yang et al., 2017). Nanoparticles derived from proteins and polysaccharides are increasingly favored for reducing carrier-induced cytotoxicity (Nitta and Numata, 2013). The protein nanoparticle conjugate serves in breaching the BBB (Sundar et al., 2010).

Intracellular delivery of anticancer drugs to cancer cells is a major challenge due to rapid modifications in pH occurring endosomal compartments. Kim et al. (2015) proposed the synthesis of pH-responsive drug transport system using mussel adhesive proteins (MAPs)-based iron (III)—3,4-dihydroxyphenylalanine (DOPA) nanoparticles. This newly developed system has effective cytotoxicity toward cancer cells. With the enormous benefits of the nanoscale materials and the development made in the drug delivery, many drug-bound organic nanoparticles are presently under clinical trial and a small number is already commercialized like albumin-bound paclitaxel, which is marketed as Abraxane, for use in metatstatic breast cancer treatment. Most recently, Bhattacharyya et al. (2015) has designed a new drug transport system for paclitaxel that outperforms Abraxane. The system was prepared with a chimeric polypeptide that spontaneously self-assembled, producing 60 nm of monodispersed nanoparticles. The chimeric polypeptide-paclitaxel conjugate has demonstrated whole tumor regression in single-dose breast and prostate cancers. Fig. 2 summarizes the advantages of nanoencapsulation of microbial bioactive compounds.

5 Recent advances of nanoencapsulated bioactive compounds in biomedical research

5.1 Virus encapsulation by polymethylmethacrylate and polyacrylamide

While viruses are usually considered pathogenic biological entities that cause a multitude of diseases, they may also be used in certain applications with potentially beneficial effects including encapsulation. For instance, influenza vaccine using the inactivated influenza virus was the first encapsulation prepared by polymethylmethacrylate and polyacrylamide copolymerization. After co-polymerization, the virus was encapsulated by adsorption or mixed with the monomers and co-polymerized with the biomaterial for encapsulation. Immunization of the influenza after encapsulation demonstrated greater immunity than free vaccination (Pandit et al., 2013).

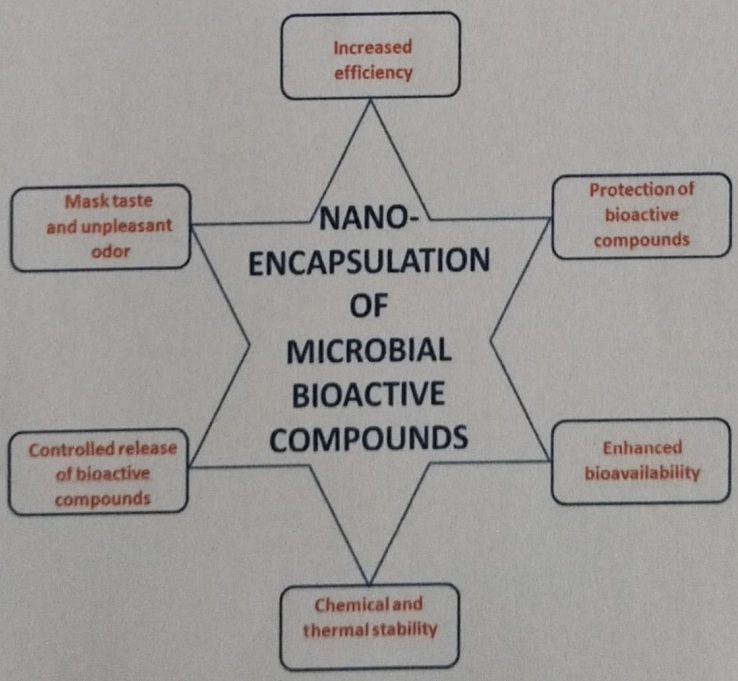

FIG. 2 Advantages of nanoencapsulating bioactive compounds.

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5.2 Active agent encapsulation with PLA and PLGA

Active agents like hypericin encapsulation were prepared in polylactic acid (PLA) polymer such as (PLA) and poly(lactic-*co*-glycolic) acid (PLGA) due to their biodegradability and biocompatibility by nano-precipitation, also known as solvent displacement/diffusion/shifting, a fast, easy, and simple method (Zeisser-Labouèbe et al., 2006). Polymers and hypericin were dissolved together in acetone to obtain the organic phase and the addition of poly(vinyl alcohol) (PVAL) surfactant forces the polymerization and sphere particulate formation of PLA. Afterward, the solvent was removed with evaporation and unpolymerized polymer and free hypericin were removed by centrifugation. Hypericin encapsulated PLA or PLGA was used to deliver the agent to ovarian cancer for PD and PDT treatment (Schubert et al., 2011).

5.3 Tetanus toxoid encapsulation with synthetic biomaterial

The new synthetic biomaterial, known as poly(2-sulfobutyl-vinyl alcohol)-*graft*-poly(D,L-lactic-*co*-glycolic acid) (SB-PVAL-γ-PLGA), was used to overcome certain shortcomings of conventional biomaterials like charge modification. The biomaterial was prepared into a nanoparticle by the solvent displacement method. Then, the protein drug for the vaccination: tetanus toxoid was encapsulated by adsorption (Jung et al., 2000).

5.4 DNA encapsulation in $CaCO_3$

DNA and Pronase were encapsulated inside a $CaCO_3$ spherical microparticle template via the coprecipitation method. Nano-sized calcium carbonate shows special properties such as a large surface area, strength, thermal stability, and suitable immobilization. The preparation of DNA encapsulation in $CaCO_3$ involves the rapid mixing of H_2O, $CaCl_2$, Na_2CO_3, Pronase, and dsDNA, under stirring. The novelty of this encapsulation preparation is the inclusion of Pronase in the encapsulation of the nanoparticle. The Pronase acts as a DNA remote release control switch with an internal trigger, lysing the encapsulation and releasing DNA. The lysis is complete and ensures complete release of DNA content from the encapsulation.

5.5 Breviscapine encapsulation in multivesicular liposome

A well-known, bioactive drug used to treat ischemic, cerebrovascular, and cardiovascular disease breviscapine is encapsulated to extend its circulation time in blood, reduce administration frequency, and improve patient compliance to the drug. Encapsulation of breviscapine inside MVL was performed via the double emulsification process, a convenient and widely used method to produce nanoparticles in the microparticle (NIM) encapsulation system (Borodina et al., 2007). The emulsification of water-in-oil (W/O) emulsion was performed by centrifugation at high speed and subsequent emulsification was performed with glucose and L-Lysine solution, yielding water-in-oil-in water (W/O/W) double emulsion (second emulsion). Chloroform and diethyl ether were removed from the second emulsion by flushing nitrogen over the surface of the mixture and an encapsulated drug is obtained after centrifugation.

5.6 Catechins encapsulation in γ-PGA

Catechin is a polyphenolic compound with antioxidant, metal chelation, free radical scavenging, neuroprotection, and cholesterol metabolism modulation properties, but is sensitive to lysis and has low bioavailability. The encapsulation was carried out in chitosan γ-PGA as it is an anionic, nontoxic, and edible polypeptide found in natto. The preparation involves a simple polyelectrolyte self-assembly method involving an electrostatic interaction of aqueous γ-PGA added into aqueous chitosan to build up a multilayered particle with unique properties. Catechin encapsulation was prepared by dissolving the agent with γ-PGA prior to polyelectrolyte self-assembly. The encapsulated agent was collected in a pallet after centrifugation (Tang et al., 2013).

5.7 Bacoside encapsulation in PLGA

Bacoside is a potential Alzheimer's disease cure, memory and cognition enhancing agent besides being an analgesic, hepatic protectant and anti-inflammation agent. Conventional administration of the supplement is via oral delivery of the free unencapsulated agent in tablet form. The agent delivery was suggested for improvement via encapsulation, especially in oral delivery where it is known to involve many challenges and exposure to the harsh environment. Additionally, drug delivery to nerve cell requires trans BBB crossing of the agent, which is another major hurdle (Dong, 2018). Bacoside was encapsulated in a PLGA nanoparticle via the o/w emulsion solvent evaporation process resulting in hardened microsphere, which contains the drug inside the structure. Furthermore, the encapsulated nanoparticle was surface modified by coating with polysorbate 80 (Jose et al., 2014).

5.8 Loperamide encapsulation and coating with alipoprotein E

To improve delivery across BBB, the drug was encapsulated in the serum albumin nanoparticle (HSA-NP) and the surface was modified with apolipoprotein E coating

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by covalent bonding. HSA-NP weas prepared by desolvation (Jose et al., 2014). HSA was initially dissolved in purified water or salt (NaCl) solution with pH 7–10. Then, the solution was transformed into nanoparticles when the desolvating agent, ethanol, was continuously added and stirred at room temperature. Nanoparticles were formed and cross-linked by the addition of glutahydrate in water and continually stirred overnight and nanoencapsulated particles were obtained by centrifugation (Michaelis et al., 2006).

5.9 Heparin encapsulation

Oral delivery of heparin is a most affordable and convenient administration method. Thus, encapsulation was aimed at improving its efficiency and protection along the oral drug delivery pathway. Encapsulation can improve the bioavailability of heparin in the patient's bloodstream, reduce frequent hospital visits, and provide compliance and convenience to the patient. Mucoadhesive polymers like poly(acrylic acid)-cysteine as an encapsulation carrier for heparin resulted in an efficient drug delivery system with muco-adhesive surfaces and sustained drug release, significantly increasing absorbed heparin. In the polymercysteine preparation, initially polycarbophil was neutralized with NaOH and then hydrated with demineralized water. The polymer's carboxylic acid moieties were activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Then, at pH 4–5, L-cysteine hydrochloride was added, incubated with stirring for conjugation with the polymer. Conjugated polymer was isolated by dialysis and lyophilized by drying frozen aqueous polymer solution (Michaelis et al., 2006).

5.10 Insulin encapsulation

Various insulin encapsulation systems have been prepared to enhance insulin oral delivery. The encapsulation preparation methods must consider the effect of the organic solvent used in the preparation on insulin activity. Polymers such as PLA, PLGA, lipid, Eudragit, poly(methacrylic acid), poly(ethylene glycol), hydrogel, chitosan, and alginate are reported (Gholamipour-Shirazi, 2008). To encapsulate insulin, emulsification, internal polymerization, polymer complexation, phase inversion, reverse micellar, sol-gel, layer-by-layer assembly, membrane emulsification, coacervation, and spraying methods were applied. PLGA and multiple emulsion nanoparticle preparation method is reported as the most promising polymer for insulin encapsulation for oral delivery (Gholamipour-Shirazi, 2008).

5.11 Metformin hydrochloride encapsulation in ethyl cellulose

Metformin hydrochloride is a biguanide, an oral antidiabetic drug for type-1 diabetes, and has poor bioavailability. Metformin encapsulation was prepared by the emulsion solvent evaporation method. Biopolymer, ethyl cellulose solution was dissolved in acetone and metformin drug was dispersed in the polymer solution with continuous stirring. Then, the dispersion was added into liquid paraffin and Span 60 (continuous phase) while it was continuously stirred. Continue stirring until all the solvent is removed, to obtain a spherical microsphere. The microsphere was washed with petroleum ether and air-dried to improve its rigidity (Pandit et al., 2013).

6 Limitation and future prospective

As per the World Health Organization (WHO) report, in developing countries, the basic health needs of approximately 80% of the population are met and/or complemented by traditional medicine (Robinson and Zhang, 2011). Currently, the scientific community is focusing on the studies related to bioactive compounds to produce innovative active ingredients that present relatively minor side effects (Atanasov et al., 2015). Most of the natural compounds of financial significance with medicinal doables that are already being marketed have been observed in greater flowers (Atanasov et al., 2015; Kinghorn et al., 2011). Several drugs that possess natural therapeutics in their composition are already commercialized; some are as follows: malaria therapy (Artemotil derived from *Artemisia annua* L.,), Alzheimer's sickness treatment (Reminyl, an acetylcholinesterase inhibitor from Galanthus woronowiiLosinsk), cancer therapy (Paclitaxel and its analogues derived from the *Taxus brevifolia* plant; vinblastine and vincristine extracted from *Catharanthus roseus*; camptothecin and its analogs derived from Camptotheca acuminate Decne), liver disorder treatment (silymarin from Silybummarianum) (Atanasov et al., 2015).

Currently, many nanomedications are modified release systems for active ingredients (AI) that are already approved (Ventola, 2017). It can be ascertained that the nanoformulations present benefits over the existing formulation if the AI (berberine, curcumin, ellagic acid, resveratrol, curcumin, and quercetin) is directed toward the targeted tissue, with accelerated uptake/absorption by the cells and lower toxicity (Havel et al., 2016; Kumar et al., 2013). Presently, natural product-based materials are considered as the key ingredients in the preparation and processing of new nano-formulations as they are biodegradable, biocompatible, renewable, and present low toxicity (Khan et al., 2018). In addition, biomaterials can undergo chemical modifications to be used in the field of nanomedicine

Principal
D.K.Govt. College for Women (A)
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(Aravamudhan et al., 2014). Gold, silver, cadmium sulfide, and titanium dioxide of different morphological characteristics have been synthesized using several bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and Klebsiella pneumoniae (Iravani, 2014). These nanoparticles, especially the silver nanoparticles, have been abundantly studied in vitro (Pajardi et al., 2016; Patra et al., 2016; Patra and Baek, 2017). The sustained release systems of naturally occurring AIs are key tools for improving the biological activity as well as minimizing their limitations (Bilia et al., 2014).

In the manufacturing, refining, and active packaging and use of nanoprocessed products, safety and health issues as well as regulatory policies should be addressed. No specific legislation is implemented globally concerning the regulatory dimensions of nanostructures in the food and medical sectors. Most countries also lack clear risk assessment legislation for encapsulated nano-products. The lack of continuity in knowledge sharing between countries constitutes a documented risk to human health and the environment and can restrict the promotion of innovative and beneficial products around the world.

6.1 Regulations and reality

Currently, in the nanotechnology scenario market, there are 50 plus products (Hassan et al., 2017; Ventola, 2017; Agrahari and Agrahari, 2018) utilized in clinical practice, primarily developed for drugs, which have low aqueous solubility and high toxicity. A recent review by Caster et al. (2016) reports FDA regulations and clinical trial initiatives suggesting many nanotechnology-based new drugs will soon be available. Among these nanomaterials that are under study, 18 are directed chemotherapeutics; 15 are antimicrobial agents; 28 are for different psychological diseases, autoimmune conditions; 30 are aimed at nucleic acid-based therapies. However, there are high levels of uncertainties regarding definition, characterization, safety, toxicity, and effective regulation. It is important to evaluate structure/function relationships of nanomaterials, as well as their characteristics, composition, and surface coat interactions with biological systems as the aggregate and agglomerate formation in the course do not reflect the properties of the individual particle (Wacker et al., 2016). There is also a lack of standard protocols for nanomedicine characterization at physicochemical and physiological/biological levels limiting the efforts to determine the toxic potential of nanodrugs (Ventola, 2017). Due to the rapid development of nanotechnology as well as its potential use in nanomedicine, a reformed and more integrated regulatory approach is urgently required to develop new protocols that must be specific and sufficiently rigorous to address any safety concerns, thus ensuring the release of safe and beneficial nanomedicine (Grossman et al., 2017; Ventola, 2017).

Further studies are required to overcome the limitations of nanoencapsulation processes and develop existing methods, formulations, and encapsulation systems, meet commercial demands, and explore the application and industrial production of nano-products (Bazana et al., 2019a, b). Additionally, studies will emphasize the application of bioactive compound nanocarriers in food and biological systems, investigating their effects on cell viability and their profiles of adsorption, delivery, metabolism, and excretion in humans and other living systems. The prospects for bioactive compounds nanoencapsulation highlight the need for their worldwide control to promote their safe use and marketing. This phenomenon is backed by numerous studies documenting the beneficial effects of bioactive nanostructured compounds and providing a promising future path for science (Bazana et al., 2019a, b).

6.2 Future of nanomedicine and drug delivery systems

In the last two decades, a lot of work in this area has already led to 1500 patents being filled out and several dozen clinical trials completed (Pandit and Zeugolis, 2016). Cancer is the best case of a disease where nonmedicine has benefited from treatment and therapy. The application of nanomedicine and a nano-drug delivery method is definitely the phenomenon that will stay in the future arena by using different forms of nanoparticles for the precise delivery of medication to the affected cells, without harming normal cells. The future area of research will be more research materials with clear uniformity and drug loading and release efficiency. The use of metals in treatment and therapy, like gold and silver, is a research field that may potentially contribute to broader use of nanomedicines in the future. Despite the overwhelming awareness of nanomedicine and nano-drug delivery, its real effect on the healthcare system, including cancer therapy/diagnosis, remains limited (Bazana et al., 2019a, b). In the end, nanomedicine technology will advance with the implementation of marker recognition capability. The principle of controlled release of different drugs at the beleaguered sites, technology for evaluating such events, the impact of drugs on a tissue/cellular level, as well as theoretical mathematical models of prediction, have not been mastered yet.

7 Conclusion

Nanoencapsulation of microbial bioactive compounds with different carriers is a promising approach to boost their bioavailability and stability and also to increase their use in the pharmaceutical industry. The nanotechnology applied to the encapsulation of microbial bioactive compounds has many apparent advantages from food processing to packaging, such as stability improvements and bioavailability

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improvements. This also offers benefits for safety through the defense and controlled release of bioactive compounds. Nevertheless, recognizing the safety and toxicity of these nanomaterials needs further work, and global legislation needs to be implemented to facilitate the safe marketing of new health-beneficial nanotechnology products. In other words, novel extraction and encapsulation techniques for the development of highly efficient microbial bioactives applicable in various industries such as food, pharmaceutical, and cosmetic industries need to be developed and implemented. Furthermore, understanding the safety and toxicity of these nanoencapsulations needs further work, and global legislation needs to be implemented to facilitate the safe marketing and utilization.

Acknowledgments

The authors are indebted to all the researchers whom we cited in this review for their significant and valuable research. No funding was received to perform this review.

Conflict of interest

The authors declare no relevant competing financial interests.

References

Agarwal, V., Khan, M.A., 2001. Current status of the oral delivery of insulin. Pharm. Technol.

Agrahari, V., Agrahari, V., 2018. Facilitating the translation of nanomedicines to a clinical product: challenges and opportunities. Drug Discov. Today 23 (5), 974–991.

Akbari-Alavijeh, S., Shaddel, R., Jafari, S.M., 2020. Encapsulation of food bioactives and nutraceuticals by various chitosan-based nanocarriers. Food Hydrocoll. 13, 105774.

Andrysek, T., 2003. Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine. Mol. Immunol. 39 (17–18), 1061–1065.

Aravamudhan, A., Ramos, D.M., Nada, A.A., Kumbar, S.G., 2014. Natural polymers: polysaccharides and their derivatives for biomedical applications. In: Natural and Synthetic Biomedical Polymers. Elsevier, New York, pp. 67–89.

Aslani, P., Kennedy, R.A., 1996. Studies on diffusion in alginate gels. I. Effect of cross-linking with calcium or zinc ions on diffusion of acetaminophen. J. Control. Release 42, 75–82.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.-M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol. Adv. 33, 1582–1614.

Bayraktar, O., Erdoğan, İ., Köse, M.D., Kalmaz, G., 2017. Nanocarriers for plant-derived natural compounds. In: Nanostructures for Antimicrobial Therapy. Elsevier, pp. 395–412.

Bazana, M.T., Codevilla, C.F., de Menezes, C.R., 2019a. Nanoencapsulation of bioactive compounds: challenges and perspectives. Curr. Opin. Food Sci. 26, 47–56.

Bazana, M.T., Codevilla, C.F., de Menezes, C.R., 2019b. Nanoencapsulation of bioactive compounds: challenges and perspectives. Curr. Opin. Food Sci. 26, 47–56.

Bernkop-Schnürch, A., 2005. Thiomers: a new generation of mucoadhesive polymers. Adv. Drug Deliv. Rev. 57 (11), 1569–1582.

Bhattacharyya, J., Bellucci, J.J., Weitzhandler, I., McDaniel, J.R., Spasojevic, I., Li, X., Lin, C.-C., Chi, J.-T.A., Chilkoti, A., 2015. A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models. Nat. Commun. 6, 7939 12 pp.

Bilia, A.R., Guccione, C., Isacchi, B., Righeschi, C., Firenzuoli, F., Bergonzi, M.C., 2014. Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evid. Based Complement. Alternat. Med. 2014, 651593.

Borodina, T., Markvicheva, E., Kunizhev, S., Möhwald, H., Sukhorukov, G.B., Kreft, O., 2007. Controlled release of DNA from self-degrading microcapsules. Macromol. Rapid Commun. 28 (18–19), 1894–1899.

Brasnjevic, I., Steinbusch, H.W.M., Schmitz, C., Martinez-Martinez, P., European NanoBioPharmaceutics Research Initiative, 2009. Delivery of peptide and protein drugs over the blood-brain barrier. Prog. Neurobiol. 87 (4), 212–251.

Calvo, P., Remuñan-Lopez, C., Vila-Jato, J.L., Alonso, M.J., 1997. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm. Res. 14, 1431–1436.

Caster, J.M., Patel, A.N., Zhang, T., Wang, A., 2016. Investigational nanomedicines in 2016: a review of nanotherapeutics currently undergoing clinical trials. Wiley Interdiscip. Rev. 2017, 9.

Couvreur, P., Dubernet, C., Puisieux, F., 1995. Controlled drug delivery with nanoparticles: current possibilities and future trends. Eur. J. Pharm. Biopharm. 41, 2–13.

Damgé, C., Vonderscher, J., Marbach, P., Pinget, M., 1997. Poly(alkylcyanoacrylate) nanocapsules as a delivery system in the rat for octreotide, a long-acting somatostatin analogue. J. Pharm. Pharmacol. 49, 949–954.

Dong, X., 2018. Current strategies for brain drug delivery. Theranostics 8 (6), 1481.

Duclairoir, C., Orecchioni, A.M., Depraetere, P., Nakache, E., 2002. a-Tocopherol encapsulation and in vitro release from wheat gliadin nanoparticles. J. Microencapsul. 19, 53–60.

El-Samaligy, M.S., Rohdewald, P., Mahmoud, H.A., 1986. Polyalkylcyanoacrylatenanocapsules. J. Pharm. Pharmacol. 38, 216–218.

Faraji, A.H., Wipf, P., 2009. Nanoparticles in cellular drug delivery. Bioorg. Med. Chem. 17 (8), 2950–2962.

Florence, A.T., Siepmann, J., 2009. Pharmaceutics: New Challenges, New Paradigms. Modern Pharmaceutics.

Garavand, F., Rahaee, S., Vahedikia, N., Jafari, S.M., 2019. Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients. Trends Food Sci. Technol. 89, 26–44.

Gholamipour-Shirazi, A., 2008. Insulin encapsulation. Expert Opin. Drug Deliv. 5 (12), 1335–1355.

Grossman, J.H., Crist, R.M., Clogston, J.D., 2017. Early development challenges for drug products containing nanomaterials. AAPS J. 19, 92–102.

Ha, H.-K., Wook, J., Lee, M.-R., Lee, W.-J., 2013. Formation and characterization of quercetin-loaded chitosan oligosaccharide/β-lactoglobulin nanoparticle. Food Res. Int. 52, 82–90.

Harmia-Pulkkinen, T., Tuomi, A., Kristoffersson, E., 1989. Manufacture of polyalkylcyanoacrylate nanoparticles with pilocarpine and timolol by

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