Chapter

Natural Polymers in Micro- and Nanoencapsulation for Therapeutic and Diagnostic Applications: Part II - Polysaccharides and Proteins

Ndidi C. Ngwuluka, Nedal Y. Abu-Thabit, Onyinye J. Uwaezuoke, Joan O. Erebor, Margaret O. Ilomuanya, Riham R. Mohamed, Soliman M.A. Soliman, Mahmoud H. Abu Elella and Noura A.A. Ebrahim

Abstract

Encapsulation remains a fundamental and consistent approach of fabrication of drug and diagnostic delivery systems in the health space and natural polymers such as polysaccharides and proteins continue to play significant roles. Micro- or nanoencapsulation is employed for the conventional pharmaceuticals, biopharmaceuticals, or biologics, bioactives from natural sources and diagnostics such as biomarkers. The outcome of any encapsulation depends on the technique employed and the encapsulating material. The encapsulating materials employed influence the physical and chemical attributes of the fabricated micro- and nanocapsules. The encapsulating materials could be natural or synthetic, however, natural polymers are preferred because they are human and environmentally friendly. Polysaccharides and proteins are abundant in nature, biogenic, biocompatible, biodegradable and possess biological functions making them materials of choice for encapsulation of drugs and diagnostics. This chapter reviews the recent and advanced applications of polysaccharides and proteins as nanocarrier materials for micro- and nanoencapsulation of therapeutics and diagnostics.

Keywords: natural polymers, biopolymers, drug delivery, nanoencapsulation, microencapsulation, polysaccharides, proteins, therapeutic, diagnostic

1. Introduction

Encapsulation remains a fundamental and consistent approach of fabrication of drug and diagnostic delivery systems in the health space and natural polymers such as polysaccharides and proteins continue to play significant roles. Natural polymers

(polysaccharides, proteins, and lipids), the macromolecules found in nature are integral in the existence of living organisms especially polysaccharides and proteins. Natural polymers constitute a huge portion of the earth's organic matter because they are synthesized by living organisms such as plants, animals, bacteria, and fungi during their entire life cycle. Polysaccharides also referred to as glycans are the most abundant organic compounds on earth. Polysaccharides are involved in many vital functions in nature such as provision of support and stability for cells and tissues, facilitation of cell communications, storage of energy, protection, lubrication, and cell recognition. Proteins are regarded as the 'workhorses' of cells being involved in many processes necessary for life including being the expressions of genetic information.

Polysaccharides, proteins, and lipids interact in nature to enhance day to day functions in living organisms. Polysaccharides bind with protein and lipids to form glycoproteins and glycolipids respectively which can be used for cell communications. Other processes modulated by glycoconjugates (glycoproteins and glycolipids) include molecular targeting, cell migration, cell–cell interactions, immune responses, and blood clotting. Polysaccharides influence how proteins function and how cells respond to stimuli. The behavior of a protein is affected by which glycan is attached to it. Glycoproteins are abundant in the cells where they can serve as regulatory switches.

Since natural polymers are biogenic, when used for therapeutic applications, the body would usually identify with them and not treat as foreign bodies thereby bypassing the body's defense mechanisms leading to long circulation of the delivery system and possible targeting to the site of action. The biological properties of polysaccharides and proteins such as cell recognition and interactions, enzymatic degradability, semblance to extracellular matrix and their chemical flexibility [1] make them materials of choice for encapsulation of drugs and diagnostics. In addition, they are preferred to synthetic polymers because they are less toxic, eco-friendly, biodegradable, biocompatible and renewable. Polysaccharides and proteins are used for micro- and nanoencapsulation because they exhibit good process efficiency, are modifiable and can be tailored to target the desired site of action, have good rheological and emulsification/emulsion stabilizing properties, gelling and film forming [2, 3]. In addition, use of polysaccharides and proteins align with the interest and advocacy for 'green' production of drugs and diagnostics. This chapter reviews the applications of polysaccharides and proteins as preferred encapsulating materials in micro- and nanoencapsulation of therapeutics and diagnostics.

2. Polysaccharide-based encapsulation

Carbohydrate monosaccharide molecules that are cohesively bound together by glycosidic chains are termed polysaccharides [4]. The nature, sequence, and glycosidic monosaccharide chains inherent in polysaccharides influence the molecular and structural properties of polysaccharides. Water retention ability, digestibility, gelation, and solubility properties of polysaccharides are dependent on the composition of their glycosidic monosaccharide chains [5–7]. Polysaccharides are usually obtained through low cost production techniques with raw materials obtained from natural sources. These polymers possess essential properties critical for drug delivery systems [8–11]. Polysaccharides are macromolecules structured in a linear or branched pattern extensively used in both conventional and advanced drug delivery systems as carriers, building blocks, bioactive materials and excipients. Due to their flexibility, they can be derivatized and tailored to achieve certain functionalities

that can enhance targeting and delivery properties. Some of the derivatives of polysaccharides called semi-synthetic polysaccharides include carboxymethylcellulose, starch acetate, methylcellulose, ethylpullulan, and chitosan sulfate. Polysaccharides and their derivatives enhance delivery and diagnostic properties such as mechanical strength, stability, protection, solubility, targeting, stimuli responsiveness, controlled release, self-regulation, adhesion, bioimaging, labeling, site-specificity and multifuntionality which equip them to respond to stimuli, diagnose, image, target and treat as single devices [12]. These potentials of polysaccharides make them convenient materials for encapsulation conferring on the encapsulated product the various benefits of encapsulation.

2.1 Polysaccharides in micro- and nanoencapsulation

Drug delivery systems make use of a wide range of polysaccharide-based delivery systems from plant (khaya gum, starch and cellulose) [13, 14], animal (chitosan) [15], algae (alginate and carrageenan) and microbial (dextran and xanthan gum) sources [16, 17] and cyclodextrins [18]. These polysaccharides delivery systems can interact with bioactive compounds ensuring that they act as innate drug carriers which bind and encapsulate hydrophilic and hydrophobic functional compounds [19]. The size, shape, and internal structure of these polysaccharide delivery systems differ depending on several factors including the method of formulation and the polysaccharide used in formulation [9]. Size is an integral component of drug delivery systems since it affects their physicochemical stability, encapsulation and release characteristics, and biological activity [9]. Encapsulation of bioactive drug compounds can be achieved via a single or a combination of polysaccharides.

Polysaccharides used for micro- or nanoencapsulation include varied types of polysaccharides and utilization of specific polysaccharides for encapsulation is dependent on its chemical non reactivity with active pharmaceutical ingredient, chemical compatibility as well as stability target-selected delivery [20]. Micro and nanoencapsulation are achieved majorly via chemical (emulsification, polymerization, and liposomes) or physical (freeze-drying/lyophilization, spray drying, co-crystallization, fluidized-bed coating encapsulation processes). Drugs with low solubility and high permeability i.e. BCS class 2 drugs are usually formulated via encapsulation to optimize bioavailability, stability, and controlled release of drugs [20].

2.2 Merits and demerits in therapeutic delivery

Despite the many advances of encapsulation process, relatively few of these products have been made commercially available due to many reasons which include degradation of highly temperature sensitive compounds, difficulty in controlling the particle size especially as the size of the yield is usually small [21]. Long processing times, expensive costs of production and storage of these dosage forms are also of concern. **Table 1** shows varying polysaccharides utilized on the encapsulation process stating the merits and demerits of each application.

2.3 Case studies/applications

2.3.1 Encapsulation of small molecules

Mankala *et al.,* [19] incorporated aceclofenac, a nonsteroidal anti-inflammatory drug (NSAID) with biological half-life of 4.3 h and a BCS class 2 drug into

	Polysaccharide	Encapsulation type	API	Significance of the study	Demerits of the encapsulation process/study set back	Reference
1.	Chitosan	Microencapsulation	Isoniazid and rifabutin	Spray-dried chitosan microparticles inhibited bacterial growth by 96%, demonstrating that microencapsulation preserved drug antibacterial activity *in vitro*. Overall, the obtained data suggest the potential of chitosan microparticles for inhalable lung tuberculosis therapy.	Microparticles were sensitive to high temperatures and the yield was extremely small. Control of the particle size during the production process was difficult.	[22]
		Nanoencapsulation	Tamoxifen	Compared to tamoxifen citrate suspension, the amount of the drug permeated using the nano formulation was increased from 1.5 to 90 times, in absence or in presence of pancreatin or lipase. The encapsulation of tamoxifen in lecithin/chitosan nanoparticles improved the non-metabolized drug passing through the rat intestinal tissue via paracellular transport.	The effect of enzymes on intestinal permeation of tamoxifen was shown only when tamoxifen-loaded nanoparticles were in intimate contact with the mucosal surface. The nanoparticle yield was extremely small.	[23]
		Nanoencapsulation	Gallic acid	Gallic acid (GA) loaded chitosan nanoparticles (GANP) treated mice, reversed the scopolamine induced amnesia in mice which may be attributed to its antioxidant properties and improved cholinergic functions. These effects were significantly increased by the administration of GANP compared with pure GA administration, but no significant change was observed for GANP.	The nanoparticles required coating with Tween 80 to enhance the stability of the product. The yield of the nanoparticles was extremely small.	[24, 25]
2.	Alginate	Microencapsulation	Gallic acid	Electro-spraying microencapsulation of Gallic acid calcium alginate for use in management of dementia provided alginate beads with a size of 200 μm - 1.3 mm. Loading capacity acid varied from 7 to 12 g/100 g. There was a faster release profile in simulated intestinal fluid than in gastric fluid.	Difficulty in controlling particle size, moderate yields for small batches was experienced. The need for special handling and storage conditions was required.	[26, 27]
		Nanoencapsulation	IgY	Controlled delivery of therapeutic agents by alginate nanoparticles became an attractive issue in the gastric organ. Some therapeutic agents such as proteins could not tolerate severe conditions in the gastrointestinal tract. Concentrations of a specific IgY as a prophylactic	The yield was small, and the formulation was not stable at high temperatures. Degradation of highly temperature- sensitive compounds was also experienced because of the nanoencapsulation process.	[28]

	Polysaccharide	Encapsulation type	API	Significance of the study	Demerits of the encapsulation process/study set back	Reference
				agent against *E. coli* O157: H7 was entrapped in 0.2% $^{w}/_{v}$ sodium alginate nanoparticles by ionic gelation method. Alg NPs prepared from 0.2%$^{w}/_{v}$ stock solution could be appropriate candidates for efficient and safe delivery of IgY through the gastrointestinal tract.		
		Microencapsulation	Paclitaxel	Alginate microparticles were fabricated by an emulsification technique and characterized. The *in vitro* cytotoxicity activity of paclitaxel loaded microparticles was assessed using human non-small cell lung cancer cell lines (A549 and Calu-6). Results showed that exposure of cells to pure paclitaxel and paclitaxel loaded microparticles effectively inhibited the growth of A549 and Calu-6 cells similarly in a concentration- and time-dependent manner thus its use in management of primary or metastatic lung cancer.	The yield was small, and the formulation was not stable at high temperatures. Clearance of the microparticles by macrophages is expected to be high. This will alter its overall bioavailability.	[29]
3.	Hyaluronate	Nanoencapsulation	Recombinant neuroglobin	The formulation of sodium hyaluronate nanoparticles loaded with neuroglobin, showed that the formulation is highly compatible for pharmaceutical use and may act as a delivery system to transport neuroglobin within the blood. After a stroke, the sodium hyaluronate nanoparticles loaded with neuroglobin injected intravenously at the onset of the reperfusion period, can cross the BBB. They quickly reached the damaged nerve cells, being detected inside cytoplasm. This delivery system successfully carried the neuroprotective oxygen-sensing protein NGB to the damaged ischemic brain after 2 hr. and was retained after 24 hr. of reperfusion.	The nano particles must be preserved at low temperatures.	[30]
		Microencapsulation	—	Sodium hyaluronate was evaluated as a material for inhalation due to its therapeutic potential, utility as a formulation excipient or drug carrier, and ability to target lung inflammation and cancer.	The microparticles was fabricated over long processing time and incurred expensive process costs.	[31]

	Polysaccharide	Encapsulation type	API	Significance of the study	Demerits of the encapsulation process/study set back	Reference
4.	Cellulose derivatives	Nanoencapsulation	—	Tempo-oxidized bacterial cellulose (TOBC) and sodium alginate (SA) composites were prepared to improve the properties of hydrogel for cell encapsulation. The compression strength and chemical stability of the TOBC/SA composites were increased compared with the SA hydrogel, which indicated that TOBC performed an important function in enhancing the structural, mechanical and chemical stability of the composites.	Nanocellulose-alginate hydrogel for cell encapsulation are not easily adapted for encapsulation of biologics. The encapsulation process is time consuming.	[32]
		Nanoencapsulation	Celecoxib	Amorphous drug/polymer nanoparticles containing celecoxib were prepared using ethyl cellulose. Nanoparticles released celecoxib rapidly and provided higher dissolved-drug concentrations than micronized crystalline drug. Nanoparticle suspensions were stable for several days and can be spray-dried to form dry powders resuspendable in water. Drug/polymer nanoparticles are well suited for providing rapid oral absorption and increased bioavailability of BCS Class II drugs.	Absorption profile must be monitored as the formulation showed enhanced bioavailability of the API to avoid dose dumping.	[33]
		Microencapsulation	Probiotic bacteria	Novel carboxymethyl cellulose-chitosan hybrid microparticles were prepared in aqueous media for delivery of probiotic bacteria. The model probiotic bacteria (*Lactobacillus rhamnosus*) was successfully encapsulated in carboxymethyl cellulose based particles with acceptable viability count for its potential delivery in the intestinal tract with the purpose of modulating gut microbiota and improving human health.	The microparticles produced must be protected from environmental stresses i.e. heat	[34]

Table 1.
Polysaccharides used in encapsulation of various active pharmaceutical ingredients showing the merits and demerits in therapeutic delivery.

polymeric microcapsules. Aceclofenac-loaded microcapsules was formulated using ionic gelation technique employing sodium alginate as the coat material in combination with some mucoadhesive polysaccharide derivatives such as hydroxypropyl methyl cellulose (HPMC), sodium carboxymethyl cellulose (SCMC) and methylcellulose. The microcapsules were spherical (**Figure 1**) with microencapsulation efficiency of 83.25–99.94%, good mucoadhesive property to enhance bioavailability and ensured over 15 hr. sustained release of aceclofenac via zero order kinetic super case 2 transport [19]. The formulation composition of drug:sodium alginate:HPMC in the ratio of 2:4:1 displayed a sustained release of up to 24 hr. In another study using aceclofenac, Dharmendra *et al.,* [20] developed a LbL self-assembly which was utilized to make aceclofenac single bilayer microcapsules produced by sequential adsorption of positively charged chitosan and negatively charged pectin, a polysaccharide on the external surface of negatively charged aceclofenac microcrystals. This enabled targeted release of aceclofenac in the colon.

Glipizide an antidiabetic agent with short biological half-life was microencapsulated using polysaccharide coat comprising alginate alone or in combination with chitosan via ionotropic gelation process [35]. Abdelbary *et al.,* [35] showed that the microencapsulated glipizide enhanced drug bioavailability causing significant hypoglycaemic activity compared to innovator product. Microencapsulation provides a physical barrier against digestive enzymes, whilst offering protection against the acidic gastric environment. Cholesterol-lowering efficacy of yoghurt formulation containing microencapsulated bile salt hydrolase (BSH)-active *Lactobacillus reuteri* for management of hypercholesterolaemia adults was evaluated by Martoni *et al.* [36]. Microencapsulation of bile salt hydrolase-active *Lactobacillus reuteri* using sodium alginate showed superiority over traditional probiotic therapy and may be an exceptional choice as a cholesterol-lowering agent to be administered alone or in combination with other cholesterol-lowering agents [36].

Karan *et al.,* [37] developed novel polymeric microspheres of 5-fluorouracil (5-FU) using natural polysaccharide gum katira via microencapsulation to obtain an optimal therapeutic response at the colon. This controlled release delivery system of 5FU released the chemotherapeutic agent at a controlled rate whilst retarding gastric degradation of the drug. Utilization of natural polysaccharides in microencapsulation of 5FU via optimized katira gum microsphere ensured that a micro-carrier for efficient colon drug targeting was developed.

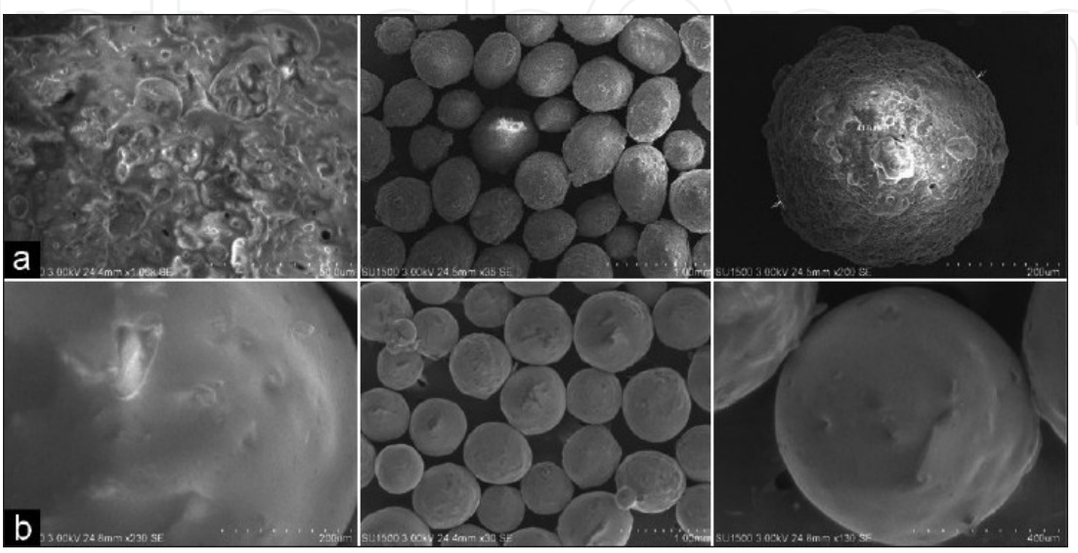

Figure 1.
(a) SEM images of aceclofenac mucoadhesive microcapsules formulated with HPMC; (b) SEM images of aceclofenac mucoadhesive microcapsules formulated with SCMC [19].

The use of polysaccharides for microencapsulation of bicalutamide was utilized by Tekade *et al.,* [38] to develop bicalutamide microspheres using guar gum as a polymer via oil-in-water emulsion solvent diffusion method. The microencapsulated dosage forms containing 2.5% of guar gum, 0.25% span 80 as dispersing agent showed the optimum drug release of 94.22% within 24 hrs, devoid of drug excipient interactions. The potential to enhance bicalutamide bioavailability and sustained release especially due to the polymorphic nature of the drug could be further optimized using nanoencapsulation. This will create a channel for precision targeting of the non-steroidal antiandrogen to a greater extent than microencapsulation [39] especially where these microencapsulated solid dosage forms have not met the pharmacological need of the patient.

Molecular Envelope Technology (MET) nanoparticles fabricated from complex polysaccharide chains of N-palmitoyl-N monomethyl- N, N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan, a self-assembling polymer amphiphile has been utilized in delivery of formulations to target sites of action across the blood brain barrier [40]. Fisusi *et al.,* [40] developed drug loaded MET formulations containing Lomustine for management of Glioblastoma multiforme. The MET envelope utilizing the complex polysaccharide for nanoencapsulation optimized biodistribution and pharmacodynamics whilst reducing the toxic effects of the active drug, lomustine thereby providing better outcomes for patients managed for brain cancer. As the active pharmaceutical ingredient is protected from degradation, the MET envelope ensures targeted drug delivery due to PEGylation of the polysaccharide to facilitate extended circulation time within the body [40, 41]. Lekshmi *et al.,* [42] prepared and characterized repaglinide loaded ethylcellulose nanoparticles by the solvent evaporation method for the management of type 2 diabetes. The polysaccharide encapsulated nanoparticles showed high encapsulation efficiency suggesting that nanoencapsulation of repaglinide in biodegradable, biocompatible polymer was able to improve its pharmacological activity via modification of surface function and charge to promote cell entry.

Di Martino and co-workers fabricated polysaccharide-based polyelectrolyte nanocomplexes which exhibited the several benefits of encapsulation [43]. Polyelectrolyte nanocomplexes were formed between chitosan (CS) and alginic acid (ALG) and then chitosan and polygalacturonic acid (PGA). Solutions of the polycation (CS in acidic medium) and the polyanions (ALG and PGA in alkaline medium separately) were prepared. The drugs, temozolomide (TMZ) and fluorouracil (5-FU) were dissolved in aqueous solution and added to the separate solutions of the polyanions. The drug(s)-polyanions solutions were added dropwise into increasing concentrations of CS. Characterization of the encapsulated product revealed spherical nanoparticles with diameters within 100–200 nm, increased encapsulation efficiency with increasing concentration of CS, controlled release of drugs, pH sensitivity making it a possible system for colon delivery, stability of drugs especially TMZ. A setback is the burst release which may be due to several factors such as drying method (freeze drying), and degree of complexation. This setback can be modulated by adjusting the ratio of CS:ALG or CS:PGA, harvesting of the nanoparticles early from the fabrication medium. Spray drying may be an alternative to freeze drying to reduce migration of the drugs to the surface during drying. In addition, derivatization of the polysaccharides without loss of their polyelectrolytic nature may increase their mechanical strength, reduce pores within and enhance entrapment.

Gastrointestinal intolerance of metformin HCl may be reduced by encapsulation which provides controlled release of the drug ensuring therapeutic efficacy and minimizing adverse effects. Extended release formulation became necessary to improve patient adherence and reduce gastrointestinal intolerance (GI)

experienced by the patients when on immediate release formulation [44, 45]. It is envisaged the metformin HCl-loaded tamarind seed polysaccharide-alginate encapsulated beads fabricated by Nayak and co-workers [46] will control the release of metformin HCl, improve GI tolerability and more. The drug release was pH sensitive as less than 20% of metformin released in two hours while in acidic medium. Most of the drug was released in pH 7.4, suggesting the maximal absorption may occur in the duodenum and jejunum and possibly increasing the bioavailability of metformin. The release of metformin followed a zero-order pattern which suggests that metformin will be released at a constant rate thereby maximizing its therapeutic efficacy and minimizing adverse effects.

2.3.2 Encapsulation of biologics

In a study undertaken by Sari and colleagues [47], chitosan was used as adjuvant and encapsulating material for the formulation of an anti-botulism single shot vaccine. The toxoids type C and D were encapsulated in chitosan by coacervation method using sodium sulfate as the precipitating agent. The toxoids-loaded chitosan microspheres were compared with the conventional method of mixing the toxoids with aluminum hydroxide which served as an adjuvant. The protein encapsulation efficiency obtained was 41.03% for toxoid C and 32.3% for toxoid D. It is envisaged that modulation of parameters such as protein and chitosan concentration may enhance the encapsulation efficiency. The comparative vaccination in guinea pigs and the neutralization bioassay indicated that the animals were able to develop titers of 10 and 2 IU/mL against *C. botulinum* type C and D respectively for both toxoid-loaded chitosan microspheres and the conventional method of delivery. However, aluminum hydroxide is fraught with adverse reactions such as local pain, swelling, irritation at the injection site, erythema, subcutaneous nodules, contact hypersensitivity and granuloma and allergic reactions [48, 49] making chitosan as an adjuvant a better alternative. Chitosan enables humoral and cellular immune responses and so it is efficient and safe compared to aluminum hydroxide [47].

Sodium alginate was used as an encapsulating material for the encapsulation of mesenchymal stem cells (MSCs), a promising cell-based therapeutic agent for the treatment of cancers, tissue injury, immune disorders, cardiovascular and neurological diseases [50]. MSCs were mixed with alginate solution and cell-encapsulated alginate beads were fabricated by ionotropic gelation with calcium chloride as the crosslinking agent. The cell-encapsulated beads were characterized to assess the ability of the cells to execute their functions despite encapsulation. MSCs were able to proliferate within the alginate beads at different times. Expression of genes proceeded unhindered. In comparison to 2D cultured cells, the 3D (three-dimensional microenvironment provided by the alginate microbeads) cells showed a significant increase in expression of pro-angiogenic genes hypoxia-inducible factor-1 (HIF-1 – 80.4%) and VEGF (74%). Seven paracrine signaling factors such as VEGF, TGF-β, TNF-α, IFN-γ, IL-10, IL-6, and IL-1β were secreted. There was an indication that the 3D microenvironment could enhance pluripotency of MSCs. The alginate beads facilitated proper growth and viability of MSCs contributing to the higher therapeutic efficiency of MSCs in vivo. Encapsulated MSCs exhibited anticancer activity against breast cancer stem cells, suppressed cancer-associated genes, inhibited migration and angiogenesis of breast CSCs among other activities making encapsulated MSCs a promising cell-based therapy for targeting cancer cells and reducing the burden of cancer.

Insulin-loaded arabinoxylan microspheres were fabricated by crosslinking of arabinoxylan employing enzymatic reaction and characterized [51]. Insulin solution was prepared in 0.25 mM HCl and thereafter, glutamic acid was added, and pH

adjusted to 4. The insulin solution was added to a solution of arabinoxylan in 0.1 M acetate buffer and then agitated. The enzyme, laccase was added as the crosslinking agent and dropwise extrusion into a hydrophobic liquid, and the microspheres were formed and harvested after 6 hr. The insulin-loaded arabinoxylan microspheres were characterized extensively in vitro and in vivo in diabetic induced Wistar rats. Average size of the spherical shaped and smooth surfaced microspheres was 322 μm having irregular pore sizes and geometries. Insulin aggregates in the microspheres were stabilized by presence of glutamic acid yielding a homogenously distribution of insulin. However, at higher insulin/arabinoxylan mass ratio, micro-phase separation occurred. Arabinoxylan microspheres minimized release of insulin in the gastric and small intestine facilitating delivery of insulin to the colon and limiting degradation by the digestive enzymes. Controlled release of insulin over 10 hr. was observed in vitro. For in vivo studies, insulin was first labeled with RITC before encapsulation. It was observed that the insulin-RITC-loaded arabinoxylan microspheres were relatively intact in the upper GIT releasing about 13–21% of the total RITC load. Maximum amount of RITC was found in the colon, about 78.8% after 8 hr. possibly due to the degradation of arabinoxylan microspheres by the colonic microflora. The blood glucose in the diabetic induced rats decreased by 70% between 9 and 12 hr. after three treatments orally while hyperglycemia was sustained in the control groups. Arabinoxylan microspheres protected insulin from enzymatic degradation, retained a high percentage of insulin for delivery and release in the colon exerting significant hypoglycemic effect.

Microneedle technology, an encapsulation technology used for biologics and small molecules as an alternative to hypodermic injection and implantation was used to encapsulate etanercept, for transdermal delivery for rheumatoid arthritis [52]. Microneedles fabricated are microscopic needles of lengths 50–900 μm (**Figure 2**) which pierces the stratum corneum barrier generating transient microchannels for delivery of encapsulated biologic or small molecule without triggering the nerves and injuring the blood vessels. Etanercept is a human dimeric fusion protein which is fully soluble and is a tumor necrosis factor (TNF) inhibitor as it binds to TNF preventing the activation of the inflammatory cascade. It is a fusion protein of recombinant human TNF-receptor p75 fused with the Fc domain of human Immunoglobulin G1 (IgG1).

Acrylate modified-hyaluronic acid was used to fabricate the microneedles and on application to the skin released etanercept which was absorbed by the blood capillary and etanercept was transported to the arthritic tissue where it exerted therapeutic effect by binding to TNF (**Figure 3**). The etanercept-loaded microneedles were fabricated by micromoulding method. Thereafter, the microneedles were detached from the mold and crosslinked by exposing to UV light to enhance mechanical strength. Drug loading and in vitro bioactivity were evaluated. Skin penetration, microneedle dissolution and skin recovery, therapeutic

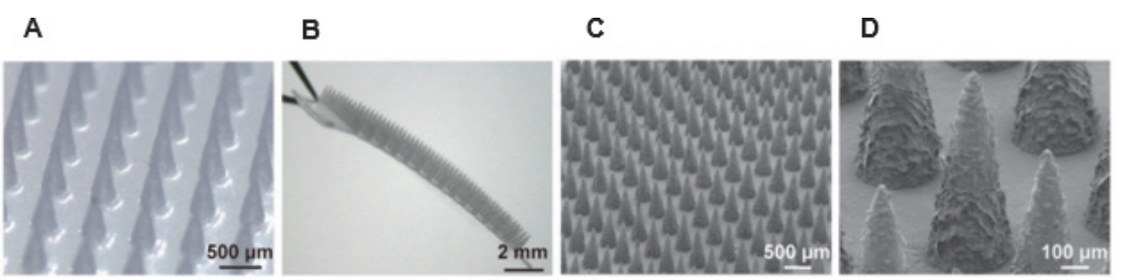

Figure 2.
Images of microneedles: (A) microscopic image – 500 μm, (B) microscopic image – 2 mm, (C) scanning electron microscopic (SEM) image – 500 μm, and (D) SEM image – 100 μm [52].

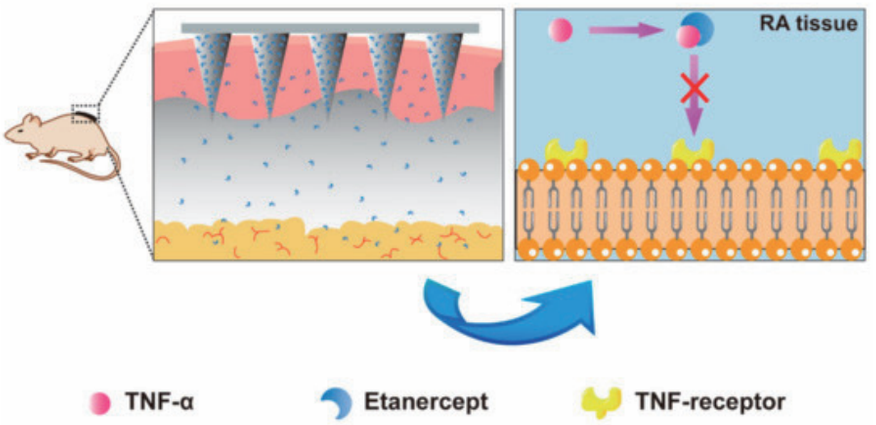

Figure 3.
Schematic illustration of microneedle-assisted transdermal delivery of etanercept from application on the dorsal skin of the mouse to binding of etanercept to TNF [52].

effect were determined with mice. Average etanercept per microneedle was 42.72 ± 5.81 μg which was sufficient for in vivo evaluation. The microneedles exhibited sufficient mechanical strength, complete dissolution of microneedles in the skin after 90 min, quick recovery of skin after 120 min, good biocompatibility, little interference with bioactivity of etanercept and high anti-inflammatory efficacy. There was evidence of reduction of TNF-α and IL-6 in serum, protection of the joint from erosion and microneedle system showed good bioequivalence to the classical subcutaneous route.

Hydrogel encapsulation systems compare better than use of autologous chondrocytes and the marrow stimulating technique for cartilage repair because hydrogel encapsulation systems do not just encapsulate chondrocytes but also maintain both cell viability and phenotype and support neocartiliage formation [53]. Fenbo and co-workers [54] fabricated chondrocytes-loaded alginate-chondroitin sulfate hydrogel beads by mixing solutions of sodium alginate and chondroitin sulfate and chondrocytes was added to the mixture which was transferred dropwise into a solution of strontium chloride with a syringe. The beads were harvested, rinsed to remove excess strontium chloride, and then cultured. Characterization of the chondrocytes-loaded hydrogel beads showed that low molecular weight alginate-chondroitin sulfate hydrogel promoted high cell viability and up-regulated the expression of collagen II and B cell leukemia 2 (Bcl-2). The study suggests that low molecular weight alginate-chondroitin sulfate hydrogel beads promotes cartilage formation and decreases inflammation and may be a promising system for cartilage tissue repair. The study observed that molecular weight of encapsulating materials is an important parameter in tissue engineering.

2.3.3 Encapsulation of diagnostics

Encapsulation of biomarkers such as microRNAs (miRNAs or miRs) confer stability on them. MiRNAs are a class of small endogenous non-coding RNAs comprising 18–22 nucleotides regulating various biological processes by preventing expression of target genes [55, 56]. MiRNAs have been suggested and explored as therapeutics and biomarkers for various diseases such as cancer, diabetes, cardiovascular diseases, and other diseases whose etiology is related to atypical gene expression [57]. MiRNAs can be employed in disease environment for diagnosis, treatment, and reoccurrence prediction. Moraes and colleagues [57] modified pullulan by linking quaternized ammonium groups to its backbone. The pullulan derivative interacted with miRNA to form stable polyplexes which were

characterized for physicochemical properties and cellular uptake. Elemental analysis, SEC-MALLS analysis, and IR and NMR spectra confirmed the modification of pullulan. Average size of polyplexes was 130 ± 30 nm, zeta potential was −12 ± 5 mV and morphology study showed homogenous spherical particles. Agarose gel electrophoresis confirmed the presence of miRNA within the polyplexes and loading efficiency was 80%. There was no indication of degradation or fragmentation of miRNA suggesting that cationic quarternized pullulan could protect miRNA. The polyplexes were found to be stable, cytocompatible, and the complexation of miRNA with quartenized pullalan facilitated the uptake of miRNA into the cells.

Cellulose nanofibers are appealing cargo carriers due the unique barrier, chemical, interfacial, mechanical, and optical properties of nanocellulose [58]. Cellulose nanofibers-based microcapsules were fabricated as a diagnostic device with glucose oxidase encapsulated within for glucose monitoring [58]. Cellulose nanofibers (CNF), apple pectin (AP) and xyloglucan-amyloid (XyG) were used to fabricate the microcapsules using layer by layer ((LbL - CNF/XyG/CNF/AP)2CNF) technique on top of fluorescein isothiocyanate (FITC) – labeled glucose oxidase-loaded calcium carbonate particles to build the capsule wall. The FITC-glucose oxidase-$CaCO_3$ particles were crosslinked with glutaraldehyde forming the templates on which LbL microcapsules were fabricated. After LbL fabrication, calcium carbonate was removed with 100 mM EDTA in water. The microcapsules collapsed on drying after removal of the $CaCO_3$ core. The glucose oxidase-loaded microcapsules fabricated were porous, spherical, uniform and structurally stable, and the encapsulation efficiency of glucose oxidase was 68 ± 2%. The microcapsules were used to monitor/measure glucose. An interaction of glucose oxidase and glucose produced hydrogen peroxide which was transported through channels to an external flow-cell where hydrogen peroxide was oxidized electrically producing current that was recorded and used to determine the concentration of glucose. The microcapsules immobilized the enzymes as well as provided a favorable microenvironment for the sustained biocatalytic activity of glucose oxidase. The nanocellulose microcapsules show promise as a device for in vivo monitoring of analytes.

A glucose biosensor fabricated based on gum tragacanth was tested on actual blood samples. Cadmium Telluride Quantum Dots (CdTe QDs) and glucose oxidase were encapsulated in tragacanth gum for glucose detection [59]. Tragacanth gum nanohydrogels were prepared by adjustment of pH, sonication followed by precipitation. Modified tragacanth gum was prepared by radial graft copolymerization of acrylic acid (AA: 0–5 mL), using N,N′-methylenebisacrylamide (MBA: 0.1–0.3 g) as a crosslinker in potassium persulfate solution (initiator) followed by precipitation after agitation for 4 hr. at 70°C. The nanohydrogels were characterized and the composition with the desired mechanical strength and highest swelling ratio was used in fabrication of superabsorbent biosensor nanohydrogels. The biosensor nanohydrogels were tested for leakage of CdTe QDs and glucose oxidase, encapsulation efficiency and glucose detection. The QDs leakage was about 1.48% for 2 hr. There was insignificant change in fluorescence intensity of QDs after 45 days at 4°C and 17% decrease in fluorescence intensity at ambient temperature after 45 days. Enzyme was stable at 4°C and unstable with time at ambient temperature. Fluorescence intensity decreased significantly with increase in hydrogen peroxide concentration indicating the encapsulated glucose oxidase was able to catalyze the oxidation of glucose to hydrogen peroxide and gluconic acid.

Enzymatic reaction, base stacking (aptamers) and antigen–antibody linkers are possible approaches to cholesterol detection; however, they are fraught with some limitations and foreign interference [60]. Chebi and co-workers [60] fabricated a nano-sensor for cholesterol sensing without enzymatic reaction using curcumin

(CUR) as a fluorescence probe, polyelectrolyte, chitosan oligosaccharide lactate (COL) as the encapsulating material and silica nanoparticles as the core (**Figure 4**). The nanohybrid particles were fabricated by precipitation technique. Solutions of COL and curcumin were mixed under agitation and a dispersion of silica nanoparticles was added dropwise and agitated overnight. The nanocapsules formed were harvested and characterized. When not aggregated, the sizes of the spherical particles were 25–35 nm. In the presence of cholesterol, a large blue shift > 100 nm was observed in the fluorescence intensity of nanohybrid particles. The fluorescence intensity of the nanohybrid particles were not affected by interfering substances such as ascorbic acid, uric acid and glucose indicating specificity, selectivity and sensitivity of cholesterol determination using the fabricated nano-sensor.

Urea is a waste product of metabolism and is eliminated from the body through the kidney. Evaluation of urea content is used to assess kidney function and other possible implications. Increased level of urea in the urine and blood indicates the presence of some acute and chronic diseases such as kidney failure, and myocardial infarction, or dehydration, gastrointestinal hemorrhage, high protein diet, aging and catabolic states such as trauma, severe infection, starvation and drugs. Decreased urea content is indicative of pregnancy, low protein diet, overhydration, advanced liver disease and reduced urea synthesis. While there are several techniques for determining urea content, there is a growing need for easy to fabricate, easy to use and cheap diagnostic tools. Khattab and co-workers [61] fabricated crosslinked calcium alginate microcapsules containing urease and tricyanofuran hydrazone fixed on cotton fibers to create a colorimetric cotton strip as a sensor for determining urea content. Solutions of sodium alginate, urease and tricyanofuran hydrazone was mixed and overlaid on cotton fiber strips and dried. Thereafter the dried cotton fiber strips were immersed in a solution of calcium choride for the crosslinking process. The microcapsules were characterized, and the sensor was used to determine urea content. The urea content assay using the sensor fabricated, displayed a visual color change from light yellow to purple indicating the presence

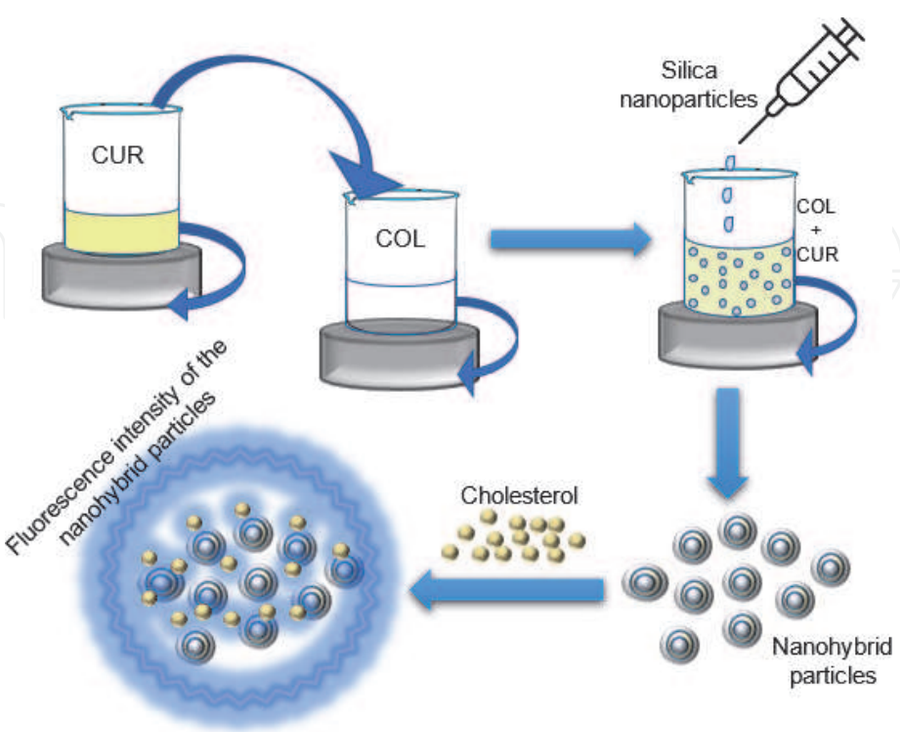

Figure 4.
Schematic illustration of the fabrication of nanohybrid particles and the fluorescence intensity in the presence of cholesterol.

Micro-encapsulating materials		Active compounds	Micro-encapsulation technique	Application	EE %	Ref.
Polysaccharide	**Other materials**					
Gum katira		5-fluorouracil	Emulsion solvent evaporation	Treatment of colon cancer	59.45 ± 3.18–79.25 ± 4.25	[37]
Guar gum		Bicalutamide	Emulsion solvent diffusion	Treatment of prostate cancer	69.43 ± 1.06–74 ± 1.07	[38]
Tamarind seed polysaccharide/alginate		Metformin HCl	Ionotropic gelation	Diabetes	94.86 ± 3.92	[46]
Arabinoxylan		Insulin	Enzymatic crosslinking	Diabetes	—	[51]
Hyaluronic acid	Methacrylic anhydride	Etanercept	Micromoulding	Rheumatoid arthritis	—	[52]
Alginate	Eudragit E100 Eudragit L30D-55	Indomethacin	Ultrasonic atomization/ polyelectrolyte complexation	Inflammation, pain	74 ± 1	[62]
Gellan gum		Methotrexate	Emulsion solvent diffusion method	Cancers and auto-immune diseases	50.78–84.8	[63]
Esterified Agave Fructans		Ibuprofen	Coacervation	Inflammation, pain	0.8–21.5	[64]
Chitosan		Astragalus Polysaccharide	Spray-drying	Allergic rhinitis		[65]
Galactomannan ***(Delonix regia)***	pluronic® F127	Riboflavin	Spray-drying	Energy conversion, growth of cells and health of eyes and skin	87.14–88.53	[66]
β-glucan		Anthocyanins	Spray-drying	Antioxidant/therapeutic	45	[67]
psyllium husk mucilage		Curcumin	Precipitation	Antioxidant, antifungal and antibacterial	56	[68]
Cyanobacterial polysaccharide		Vitamin B12	Spray drying	Cell metabolism, blood cell production		[69]
Pectin-Alginate		Vitamin E	Emulsification-ionic gelation	Antioxidant	52.91	[70]
β-cyclodextrin		Caffeine	Freeze drying	Psychostimulant	74	[71]
Sodium alginate		Urease/tricyanofuran hydrazone	Ionotropic gelation	Urea detection	38.5–55.5	[61]

Table 2.
Polysaccharide-based micro-encapsulating carriers for delivery of bioactive compounds.

Nano-encapsulating materials		Active compounds	Micro-encapsulation techniques	Applications	EE %	Ref.
Polysaccharide	**Other materials**					
Chitosan derivative		Lomustine	Molecular Envelope Technology/ Probe sonication	Treatment of Glioblastoma multiforme		[40]
Ethyl cellulose		Repaglinde	Solvent evaporation	Diabetes Mellitus	58.8 ± 0.724–86.4 ± 0.31	[42]
Chitosan/alginic acid		Temozolomide/fluorouracil	Polyelectrolyte complexation	Cancer	43 ± 3–76 ± 8	[43]
Pullulan derivative		miRNA	Polyelectrolyte complexation	Gene delivery/biomarker	80	[57]
Cellulose/pectin/xyloglucan		Glucose oxidase	Layer by layer	Glucose detection	68 ± 2	[58]
Tragacanth gum	Acrylic acid	Glucose oxidase/cadmium telluride quantum dots	Copolymerization-precipitation	Glucose detection		[59]
Chitosan oligosaccharide lactate	Silica	Curcumin	Precipitation	Cholesterol detection		[60]
Auricularia auricular polysaccharide/chitosan		Doxorubicin HCl	Polyelectrolyte complexation	CANCER	74.1 ± 2.2	[72]
Pectin-chitosan		Nisin	Polyelectrolyte complexation	Antimicrobial	42.3 ± 3.7–65.9 ± 6.1	[73]
Depolymerized holothurian glycosaminoglycan		Doxorubicin	Polyelectrolyte complexation	Cancers	55–64.2	[74]
Mango gum		Donezepil	Emulsion crosslinking	CNS - dementia	85 ± 2.14	[75]
Azivash gum	Polyvinyl alcohol	Catechin	Electrospinning	Antioxidant	87.4–99	[76]
Cashew gum	N-isopropylacrylamide	Epirubicin	Self-assembly	Cancer	63.67 ± 1.4%	[77]
Chitosan-fucoidan		Red ginseng extract	Nanoprecipitation/ionic gelation	Thrombosis	22.63 40.13	[78]
Cactus mucilage (*Opuntia monacantha*)		Zeaxanthin	Nano emulsification	Antioxidant	96.57	[79]

Table 3.
Polysaccharide-based nano-encapsulating carriers for delivery of bioactive compounds.

of urea. The detection ability of the sensor was determined by the color strength and the International Commission on Illumination – CIE L*, a*, and b* color coordinates. The dye and enzyme-loaded crosslinked alginate microparticles coated cotton sensor strips were effectively employed to determine unknown concentrations of urea. The spectroscopic parameters indicated the microencapsulated sensor displayed a detection range of 0.1 ppm to 250 ppm. **Tables 2** and **3** indicate applications based on micro- and nano-encapsulation utilizing natural polysaccharides as encapsulating materials.

3. Protein-based encapsulation

3.1 Proteins in micro- and nanoencapsulation

Protein-based delivery systems can be synthesized via different kinds of animal and plant proteins using a range of different production methods. A fabricator should pick the most fitting protein for a certain application, after making sure of all safety requirements. The main elements influencing the selection of the protein and encapsulation methods are:

a. Nanocarrier chemical or physical compatibility with food components.

b. Nanocarrier stability under processing, storage, or during its application.

c. Possible release mechanism(s) and conditions affecting the rate of release.

d. Biodegradability of the protein-based nanocarrier in the body.

e. Cost-effectiveness of nanocarriers when synthesized on a large scale for real applications.

Figure 5 is a schematic illustration of polymeric encapsulation of bioactive agents.

3.1.1 Applications and clinical usage

Protein-based nanoparticles (PBNs), recently reported, are of great interest due to their various advantages. They confirmed their high activity in both clinical and medicinal fields. Several formulations have been developed and suggested as potential future therapeutic products [80]. Besides, some PBNs have been officially accepted by US food and drug administration [81]. In addition, protein-based nanoparticles functional groups (e.g. carboxylic and amino groups) facilitate the particles surface modification, which makes them suitable for tumor targeting strategies. On the other hand, protein-based nanoparticles (PBNs) surface can be modified by attachment of targeting ligands such as peptides, antibodies, vitamins, hormones, and enzymes. These surface modifications allow specific targeting and accumulation of the particles at the desired site such as a tumor. Each protein tends to encapsulate either hydrophobic or hydrophilic molecules. Gelatin, silk, gliadin, and legumin have higher encapsulation efficiency for hydrophilic drugs. While collagen, casein, and zein proteins have higher encapsulation efficiency for hydrophobic drugs. Albumin, however, can bioconjugate with hydrophilic drugs and interact with highly hydrophobic drugs. Besides, each protein has some characteristics that enhance its selectivity to be a better carrier for a certain drug. Albumin,

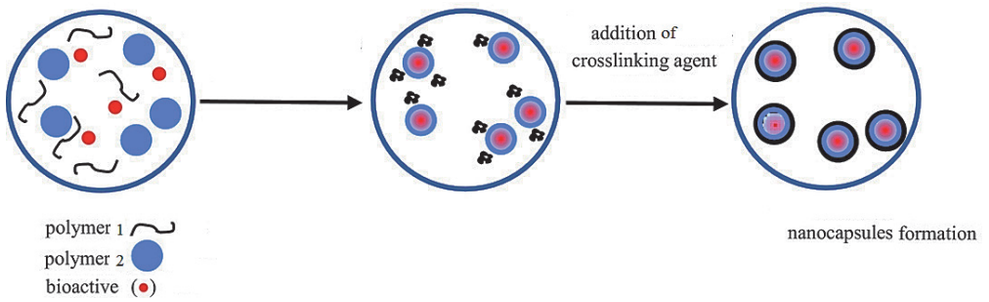

Figure 5.
Schematic illustration of encapsulation of bioactive agents.

for example, is the most abundant plasma protein, which makes it non-toxic, biodegradable, and non-immunogenic. It has also good connectivity to many drugs and it is extremely robust to various conditions. Gelatin and collagen possess many carboxyl groups with possible crosslinking functions. These parameters are important for selecting the nanoparticles synthesis method. The protein selection depends on drug properties, and on the target of the nanoparticles to be prepared. The selected protein properties such as functionality, molecular weight, and hydrophobicity can affect particle size, drug loading and loading efficiency, and dissolution or release profile of the drug to the surrounding environment of the nanoparticles. The proteins have the chance to target a specific place in vivo and secure the encapsulated active molecules from biodegradation and undesirable metabolism - **Figure 6**. Protein nanoparticles, however, have unique properties when compared to other nanoparticles since they are extracted from natural origins that exist in nature, easy to handle, and most importantly they are non-toxic as they do not leave undesirable biodegradation products.

3.1.2 Physicochemical properties of proteins

The physicochemical properties of proteins such as isoelectric point (pI), chemical compositions, denaturation thermal temperature (Tm), and solubility are necessary for the fabrication of the protein-based micro- and nanoencapsulation delivery systems (**Table 4**) [82–90]. Some micro- and nanoencapsulation processes use protein as a wall material to act as a barrier which is used to protect bioactive agents against the surrounded environmental conditions including pH, temperature, moisture, and oxygen and form stable capsules (they are in a range size between few micrometers and millimeters in microencapsulation methods and from 10 nanometers to one micrometer in nanoencapsulation methods) with high encapsulation efficiency (EE) due to their excellent gel, film and emulsifying formation properties, and promising improvements such as water solubility, stability, and bioavailability [91, 92]. Besides,

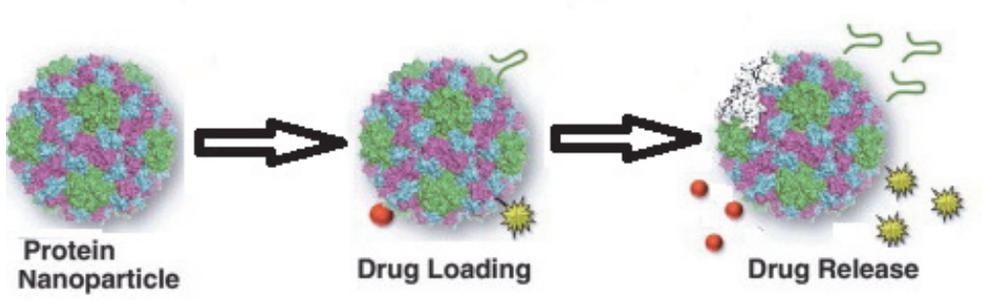

Figure 6.
Drug loading and release from protein-based nanoparticles.

their functional groups possess the ability to protect, reverse binding, and interact with numerous bioactive compounds. Nowadays, two sources of proteins; plant proteins such as soy, pulses, and cereals proteins and animal proteins such as bovine serum albumin, casein and whey proteins are widely used as encapsulating agents in micro- and nanoencapsulation techniques. Proteins are employed to encapsulate solids and liquids including oils. Their ability to encapsulate oils depends on their capacity to adsorb at the interface and form stable emulsions. The following factors affect their emulsifying properties [93]:

- The molecular size of proteins
- Hydrophobicity of the protein surface
- The flexibility of protein compounds
- Protein solubility

Protein	Source	Main composition	Properties	pI	Tm (°C)
1.1. Soy protein	Soybean	Albumins and globulins	Globular and water soluble	4.5	67–87
1.2. Cereal proteins 1.2.1. Zein protein	Maize (corn)	Glutamic acid, leucine, alanine, and proline	Soluble in aqueous alcohol	6–7	75–95
1.2.2. Wheat protein	Wheat	Gliadin and glutenin	Soluble in aqueous alcohol	5–8	90
1.2.3. Barley protein	Barley	Hordein and glutelin	Soluble in alkali	5	115
1.3. Pulse proteins					
1.3.1. Pea protein	Pea	Albumins,glutelins and globulins	Water soluble	4.3–4.5	85–90
1.3.2. Chickpea	Chickpea	Glutein, albumin, prolamine, and globulin	Water soluble	4.5	90
1.3.3. Lentil	Lentil	Globulins, albumin, glutelins, and prolamins	Water soluble	4.5	90
2.1. Whey protein	Cheese or casein	β-lactoglubulin, α-lactalbumin, and serum albumin	Globular and water soluble	4.4–5.5	60–90
2.2. Casein protein	Milk	94% protein and 6% low Mwt colloidal calcium phosphate	Rheomorphic, poorly water soluble, and highly stable	4.6–4.8	125–140
2.3. Bovine serum albumin	Bovine serum or milk	583 amino acids and contains three domains: I, II, and III	Globular, water soluble, and antioxidant	4.7	70–90
2.4. Gelatin	Collagen	Acid (Type-A) gelatin and basic (Type-B) gelatin	Linear, soluble in hot water, and highly stable	4.8	40

pI: Isoelectric point and Tm: denaturation thermal temperature.

Table 4.
Physicochemical properties of plant and animal proteins used for the delivery of bioactive ingredients.

- Preparation methods of protein compounds
- Environmental conditions include pH of solvent and ionic strength

Protein ingredients have been vastly used as good encapsulating agents alone or in combination with either another protein or polysaccharides which occurs through different possible interactions (such as covalent, electrostatic, H-bonding, hydrophobic, van der Waals, and disulfide interactions) for delivery of various bioactive compounds (**Figure 7**) [94]. Different polysaccharides can be used to fabricate bilayer on the oil droplets surface with protein compounds to increase the physical stability of both emulsion and the interfacial modifying properties [95].

3.1.3 Plant proteins

Plant proteins have different types such as soy proteins, cereals proteins (e.g., zein, wheat, and barley proteins), and pulses proteins (e.g., pea, chickpea, and lentil proteins).

3.1.3.1 Soy proteins

Soy proteins are globular proteins and are considered as one of the most important food proteins that are applied significantly in human diets because they have good nutritional values, health-benefiting effects, and high functionalities. They are mainly composed of albumins and globulins which represent around 50–90% of total seed proteins. They are divided according to their sedimentation coefficients into four fractions: 2S, 7S, 11S, and 15S fractions. The 2S fraction corresponds to albumins, while the globulins are present in 7S, 11S, and 15S fractions. Also, glycinin (named as 11S or SG, and Mwt 350 kDa) and β-conglycinin (named as 7S or SC, and Mwt of about 70 kDa are two globulin forms). Soy protein isolate (SPI) is considered as an essential product of soy proteins and contains protein range from 85 to 90% as a dry basis [96, 97]. Due to the good properties of soy proteins such as water solubility, fat and water absorption, gel, film, and foaming-formation and emulsion stabilization properties, they have been used as wall materials for micro- and nanoencapsulation of different bioactive ingredients alone or in combination with either proteins or polysaccharides [98].

3.1.3.2 Cereal proteins

Cereal grains are grown worldwide and they comprise three main biopolymers which are roughly classified into three groups - protein, starch, and non-starch polysaccharides [99]. Cereal micro- and nanotechnology for biomedical, food, and pharmaceutical applications focus on fabricating highly functional micro- and nanostructures from cereal biopolymers. Cereal proteins (e.g., zein, barley, and wheat proteins) are considered a vital protein source in the diet. Cereal proteins have excellent properties such as low cost, widely available, and versatile molecules which are valuable compounds for nano- and microtechnology applications. They are classified into four classes according to the solubility of fractionations: (a) albumins; proteins soluble in water, (b) globulins; proteins soluble in the diluted salt solution, (c) prolamins; proteins soluble in the aqueous alcohol, and (d) glutelins; proteins soluble in the diluted acid or alkali [100, 101].

3.1.3.3 Maize (Zein) protein

Zein protein is classified as a storage prolamine protein that makes up 35–60% of total protein in corn (maize). Pure zein protein is one of the important plant

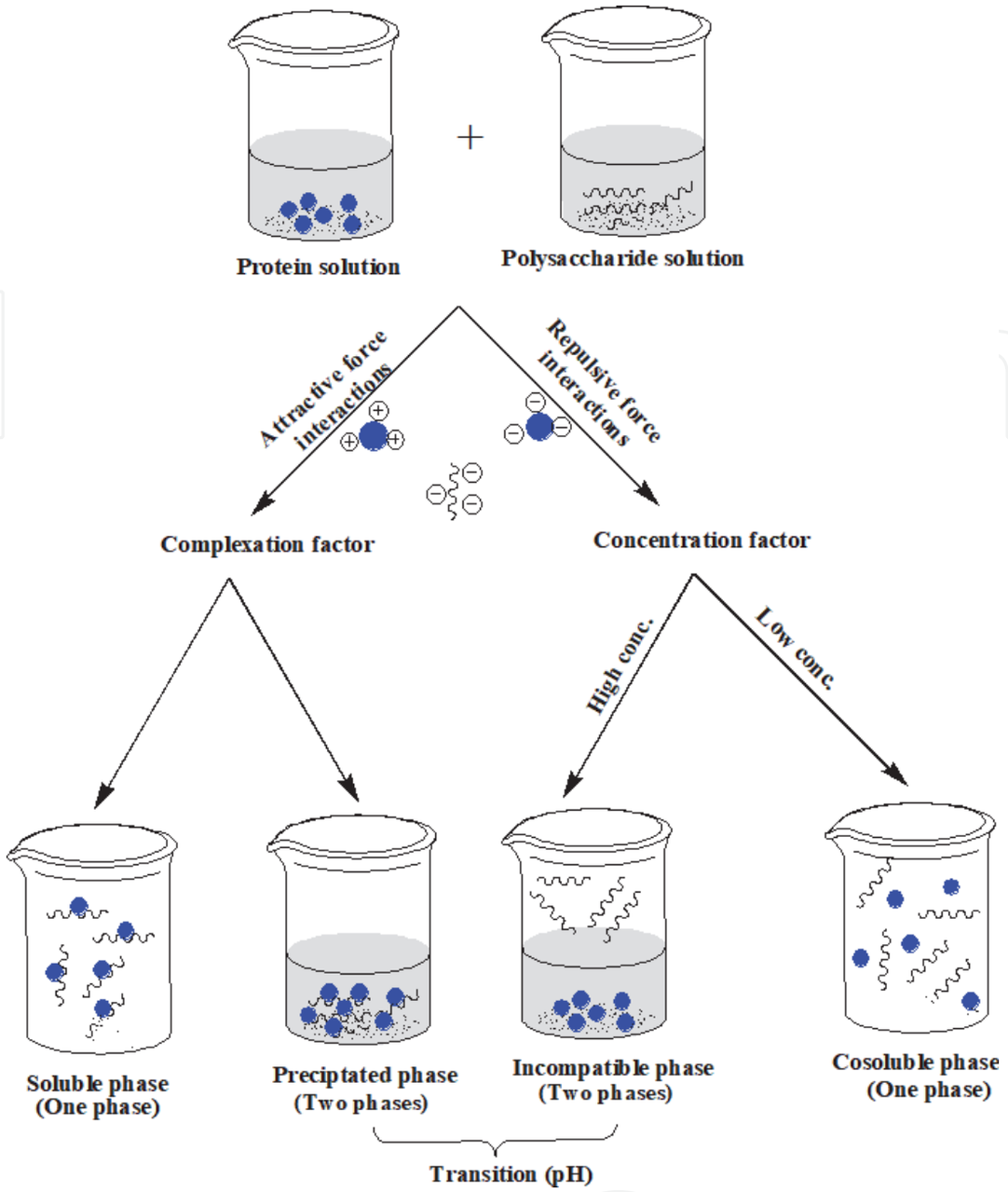

Figure 7.
Suggested interactions in two biopolymers components (e.g., protein and polysaccharide) system.

proteins due to its properties such as clear, tasteless, odorless, and edible properties, making it widely used a protein in different industrial applications [102]. The main composition of zein proteins is amino acids such as glutamic acid, leucine, alanine, and proline that are combined via disulfide bonds. It has four fractions: α, β, and γ as major fractions (accounts for 80% of the total zein protein), while the last one named δ-zein is a minor fraction. α-Zein protein is the main zein type commercially available in the market. It is water-insoluble because of the presence of non-polar amino acids which are previously mentioned but is soluble in the aqueous alcohol (50–95%). Its poor solubility in water due to the absence of essential amino acids including tryptophan and lysine decreased its usage in food products for human consumption, so zein protein nanocarriers have been applied to encapsulate core materials to enhance their distribution in water environment [103, 104]. Prolamine protein as zein protein is a valuable compound used to prepare micro-and nanoscale systems that are stable in water because these systems do not need a post-

production hardening step that stables the integrity of prepared particles, as the constituting protein does not require to re-dissolve in water [105].

3.1.3.4 Wheat proteins

Wheat proteins are obtained during the isolation of starch polysaccharide from wheat flour as a byproduct and account for 80% of total wheat seed proteins; it is also used as an essential food source for both humans and animals [106]. Wheat flour is a complex material which composes of small polysaccharide fraction as starch and protein. The latter composes of two main components as gliadin and glutenin. The constitution of gliadin is single chain polypeptides (average molecular weight, Mwt, ranged from 25 KDa to 100 KDa) which are linked by intramolecular disulfide bonds and soluble in the aqueous alcohol (70% ethanol), while glutenin is a soluble fraction and is similar to gliadin composition but they are linked via intermolecular disulfide bonds with Mwt higher than 105 KDa [106, 107]. Wheat proteins have different interesting physicochemical properties such as gel- and film-forming properties due to its low water solubility and viscoelasticity [108].

3.1.3.5 Barley protein

Barley crop is a very adaptable crop. It is cultivated for both animal feed and brewing industry, in which, the by-product becomes livestock feed. Barley crops and by-products are rich and affordable protein sources which include 8–13% and 20–30%, respectively [109]. Barley proteins composed of two major protein fractions: hordein and glutelin (about 35–55% and 35–40%, respectively). Hordein fraction (alcohol extracted fraction) is divided into five groups - B hordein, C hordein, γ-hordein, D hordein, and A hordein - based on their amino acid compositions and electrophoretic mobility. On the other hand, glutelin is an alkali-soluble protein after hordein extraction, so it is not possible to extract glutelin fraction free from hordein fraction contamination. Barley proteins are highly hydrophobic and they exhibit excellent foaming, emulsifying and film-forming properties indicating that the emulsifying-stabilization process can prepare micro- and nanocapsules from barley protein [110–112].

3.1.3.6 Pulse proteins

Pulse crops are invaluable agricultural commodities that are grown in cool seasons annually. They are grown in many regions around the world such as North America (particularly Canada), Asia, and the Middle East (particularly India and Egypt). They are considered an important source of dietary protein, fiber, essential vitamins, minerals, and carbohydrates. So, they have a health value that relates to decrease of HDL cholesterol, heart disease, and type-2 diabetes. The pulse proteins including lentil, pea, and chickpea proteins which show an attractive alternative to soy proteins because they have a low risk for allergen and non-genetically modified status [113, 114]. Pulse proteins are classified according to their solubility into glutelins (dilute acid and alkaline-soluble and makes up 10–20% of the total pulse protein), globulins (soluble in water-salt solution and accounts for 70% of the total pulse protein), and albumin (water-soluble and represents about 10–20% of the total pulse protein) [113].

3.1.3.7 Pea protein

Pea protein is extracted from pea seeds which represents 18–30% fraction. It is mainly composed of globulins (65–80%) that include three various proteins: legumin, convicilin, and vicilin [115]. Also, it contains albumins and glutelins as two

minority fractions. Pea legumin protein (denoted 11S globulin) has molecular weight ranged 350–400 KDa while convicilin and vicilin (denoted 7S globulin) have a molecular weight of about 150 KDa [98]. Pea proteins have interesting emulsifying and gel-forming properties, so they are used alone or in combination with either proteins or polysaccharides. This interaction creates a stable emulsion that improves the efficiency of a micro/nanoencapsulation technique as it gives good particle size distribution. Also, encapsulation occurs without chemical or enzymatic modification, due to the surfactant, foaming, and solubility properties. Besides, they are cheap and highly nutritious [116, 117].

3.1.3.8 Chickpea protein

Chickpea protein possesses many excellent advantages such as low cost, biodegradable, biocompatible, and non-toxic, hence, its use in the encapsulation field. Moreover, it is generally safe for use in the food industry because of little or no toxicity and side reactions [118–120]. Chickpea protein contains glutein, albumin, prolamine, and globulin with different percentage contents as the main composition: 3.12–6.89%, 8.39–12.31%, 19.38–24.40%, and 53.44–60.29%, respectively, and represents about 28.6% of total chickpea crops [121, 122]. It is a low-cost wall material and possesses high encapsulating ability, emulsifying properties, nutritional value, and beneficial health effects. Additionally, it can form thick viscoelastic films around oil droplets thus enhancing their stability through processing. The chickpea protein is widely used in different culinary applications because it has sustained nutritional benefits, and it is used in stews, soups, and salads [84, 93, 123, 124].

3.1.3.9 Lentil protein

Lentil crops are implanted in over 48 countries around the world and they contain soluble and dietary fiber more than in both pea and chickpea crops, besides, they are rich in protein sources which ranged from 20.6–31.4% of the total lentil plant. Lentil protein comprises globulins (Mwt 320–380 KDa), albumin (Mwt20 KDa), glutelins (Mwt 17–46 KDa), and prolamins (Mwt 16–64 kDa) with different percentages: 70%, 16%, 11%, and 3%, respectively [125–127]. It has good properties such as good solubility, drying, and emulsifying properties that lead to being widely used as a wall material to form stable capsules with high EE % [128].

3.1.4 Animal proteins

Animal proteins are amphiphilic compounds because they compose of block copolymers with both hydrophilic and hydrophobic amino acid residues. There are various types of animal proteins such as milk proteins: whey proteins and casein, gelatin, and bovine serum albumin which can be used in micro- and nanoencapsulation processes as wall materials either alone or in combination with other biopolymers: proteins (as soy protein) and polysaccharides (as chitosan) [91, 129]. Also, they possess many advantages more than plant protein as good wall materials as summarized below:

- More soluble than plant proteins over the wide pH range,
- Lower Mwt than plant proteins (e.g., soy protein has Mwt: 350 kDa, while casein protein has Mwt: 20 kDa)
- More flexible.

3.1.4.1 Milk proteins

Milk proteins can be divided into two groups: casein and whey proteins which can bind their hydrophilic and hydrophobic moieties with different substances with various affinities [130]. They are considered a good choice for micro- and nanoencapsulation of bioactive materials as wall materials due to their physico-chemical properties. They are available commercial products, they are flexible materials to encapsulate hydrophilic, hydrophobic and viable bioactive compounds, and they are rich bioactive peptide sources of various physiological effects. Also, they have a variety of characteristics including pH-responsiveness, self-assembly, and gel swelling behavior that lead to their use as good candidates for bioactive delivery systems [91, 131, 132].

3.1.4.2 Whey proteins

Whey proteins are produced from the manufacture of either cheese or casein as the dairy byproduct. They compose of a mixture of β-lactoglubulin, α-lactalbumin, and serum albumin which are water-soluble, so they have a variety of applications [133]. They are considered complete proteins because they have nine essential amino acids, in addition, low in lactose content. The three forms of whey protein are:

- Whey protein concentrate (WPC) which contains low fat and carbohydrate levels. The protein percentage in WPC is ranged between 30% and 90%.

- Whey protein isolate (WPI) which contains zero fat and lactose contents, and it contains high protein level (≥ 90%)

- Whey protein hydrolysate (WPH) which has been subjected to partial hydrolysis process. So, it is a predigested form of whey protein.

Whey proteins are widely used as good wall coating materials in micro- and nanoencapsulation processes for the controlled release of different bioactive materials such as oils/fats, vitamins, and volatile compounds because of the high encapsulation efficiency and stability during storage [134–137].

3.1.4.3 Casein proteins

Casein is a major amphiphilic milk protein (it makes up about 80% of total milk protein) which is an essential part of the global daily diet. It has a variety of interesting physicochemical properties such as its availability, low-cost, non-toxicity, high stability, biocompatibility, biodegradability, binding of small and ions molecules, excellent emulsification, and self-assembly that increase its efficacy in both encapsulation and loading efficiency of the loaded bioactive ingredients [130, 138]. Casein protein's composition is 94% protein and 6% low Mwt colloidal calcium phosphate. There are four different casein fractions: αS1-, αS2-, β, and κ-casein which are amphiphilic structures in proportions of 4:1:4:1 by weight, respectively. Mwt is ranged from 19 kDa to 25 kDa [139–141]. Casein micro- and nanoencapsulation carrier systems have attracted attention in recent years for controlled and sustained release delivery of bioactive compounds because of the following advantages: their cheap price, digestibility, good dispersibility in an aqueous system, good amphiphilicity, the capability to encapsulate a variety of drug and nutrients, and form uniform spherical structures [142–144].

3.1.4.4 Bovine serum albumin

Bovine serum albumin (BSA) is a globular natural albumin protein. Its origin is either bovine serum or milk which is transferred between bovine plasma and milk through the lactating cells [131, 145]. Its structure composes a single chain of 583 amino acids with Mwt of 62.2 kDa and contains three domains: I, II, and III which are divided into two helical subdomains (A and B which bond through 17 disulfide bridges) which is specified to bind lipid, nucleotide, and metal ion [130, 146, 147]. Additionally, BSA is negatively charged at physiological pH (pH 7.4). Moreover, BSA is one of the most common protein plasma that is widely used in many applications such as drug and antigen delivery and food industry because it has good features: biocompatibility, biodegradability, non-toxicity, no immunogenicity, good stability, low cost, abundance, ease of purification and unusual ligand-binding. Consequently, its micro- and nano-capsule carriers have gained traction in recent years [145, 148].

3.1.4.5 Gelatin protein

Gelatin protein is not available in nature, but it is extracted from partially hydrolyzed collagen which is the most abundant protein in the skin and bones of the animal bovine or fish. Also, it is a linear denatured protein which is carrying dual charges: positively charged (when it is extracted with acid hydrolysis of collagen, it is known as type –A gelatin and its pI is ranged 7–9.4) and negatively charged (if it is extracted with alkaline hydrolysis, it is known as type –B gelatin and its pI is ranged 4.8–5.5) and its thermal denaturation temperature is about 40°C [149, 150]. In addition, gelatin protein is a good coating material due to its amphoteric nature, and so it is widely used as coating materials in combination with different polysaccharides such as chitosan, pectin, and alginate to form hard and soft capsules (in range of micro- and nanoscale) in food and pharmaceutical applications [151–154].

3.2 Merits and demerits in therapeutic delivery

The link of a drug with a delivery system is named "controlled drug delivery". This link can control drug pharmacokinetics. Various delivery systems were aroused. Among them, we can cite nanoparticles, liposomes, surface-modified nanoparticles, and solid-lipid nanoparticles. Among nanoparticles, protein-based nanoparticles (PBNs) have special merits because they are metabolizable, biodegradable, and can be easily controlled as there are different chances for surface improvement for drug fixation [155].

Many proteins have functional merits making them suitable for encapsulation of bioactive agents, such as pharmaceuticals and nutraceuticals. Natural proteins are biological polymers composed of amino acid chains linked together via peptide bonds, which serve in important biological functions, such as enzyme catalysis, signaling, transport, and structure formation [156]. Some of the proteins' chemical and physical characteristics can be used to construct encapsulation and carrier systems. Proteins used in the nanoparticles field can be classified as animal proteins and plant proteins, and both have advantages and disadvantages.

Low toxicity of the end product using animal proteins gives them an advantage over synthetic polymers. The major drawback of animal proteins is the risk of infection from any pathogenic contamination, although, it is not important as animal proteins can be disinfected. As for plant proteins, their hydrophobic character is the main advantage compared to animal proteins. This could lead to avoiding toxic chemical cross-linkers [157]. Besides, plant proteins are also cheaper than animal proteins.

3.3 Case studies/applications

3.3.1 Encapsulation of small molecules

3.3.1.1 Hydrophobic compounds

It is used with hydrophilic compounds to improve stability and bioavailability of certain compounds such as lipid vitamins (eg: A, D, E, and K). Vitamin A (VA) and VE were also successfully incorporated into biodegradable gelatin nanofibers. Curcumin is a fat-soluble polyphenol that possesses significant antioxidant and anticarcinogenic activities [158]. The release profile showed sustained release behavior of curcumin for over 7 days (around 75%) without significant burst effect when curcumin was encapsulated within amaranth protein isolate (API)/pullulan nanofibers. Dextran and whey protein concentrate (WPC) and chitosan were used as matrix materials to encapsulate lycopene by emulsion electrospinning. WPC afforded the greatest EE (around 75%), and it was also able to protect lycopene against moisture and thermal degradation [158]. Zein nanoparticles were used as delivery nano-system to enhance the oral bioavailability of quercetin (3,3′,4′,5,7-pentahydroxyflavone), which is found in tea, red wine, fruits, and some vegetables. Zein is a protein extracted from corn (with molecular weight usually from 22 to 27 kDa). It has a high content of hydrophobic amino acids, such as proline, glutamine, and asparagine. The encapsulation of quercetin ameliorates its anti-inflammatory effect on endotoxemia was studied in a mouse model [159]. Okagu et al., [160] studied the encapsulation of hydrophobic nutraceuticals (curcumin) by biopolymer nano-complexes based on insect proteins as uncoated or coated with chitosan. The authors explained the interaction between curcumin and insect via hydrophobic forces. They observed under gastrointestinal conditions, over 90% of the nutraceutical was released. Hu et al. [161] formed biopolymer-based nanoparticles through ionic gelation between stearic acid-chitosan conjugate (SA-CS) and sodium caseinate (NaCas) and cross-linked using oxidized dextran (Odex) via Schiff base reaction, as shown in **Figure 8**. The prepared nanoparticles were used to encapsulate Astaxanthin (ASTX) to improve its bioavailability and solubility in an aqueous medium. The authors successfully prepared nanoparticles with a diameter of 120 nm with good dispersity. They estimated the capability of loading ratio of 6% loading ratio and high efficiency of encapsulation.

Xiang et al. [162] formed nanocomplexes composed of ovalbumin (OVA) and methoxy pectin (PEC) to encapsulate Vitamin D3 (VD3). Vitamin D3 is fat-soluble and readily degrades under acidic conditions. The authors observed the efficiency of VD3 encapsulation up to 96.37%. Whey proteins (positive proteins) (4% w/w), and pectin (negatively charged polysaccharides) (1% w/w) were used to form nanocomplexes which were used to encapsulate D-limonene [163]. Resulted nanocomplexes have spherical shaped nanoparticles with an average diameter of 100 nm. The efficiency of D-limonene encapsulation was about 88%.

3.3.1.2 Hydrophilic compounds

Hydrophilic compounds are encapsulated to prevent their interactions with different compounds or to guarantee a certain release pattern. A study was undertaken to assess the release kinetics of prepared nano-encapsulated folic acid using a double W1/O/W2 emulsion [164]. Initially, loaded W1/O nano-emulsions with folic acid were formed and then re-emulsified into an aqueous stage (W2) having a concentrate on a single whey protein (WPC) layer or double-layered complex of WPC-pectin for W1/O/W2 emulsions formation. Single-layer WPC encapsulated

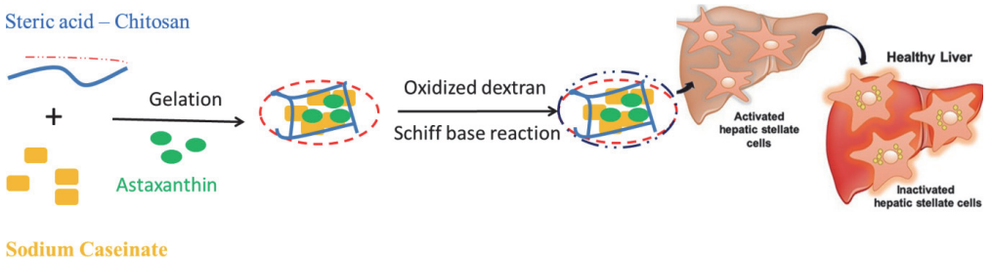

Figure 8.
General mechanism of ASTX loaded SA-CS/NaCas/Odex nanoparticles formulation and its application.

powders was the best model that fits for folic acid release pattern observed with the highest R^2. Enzymes are particularly important in therapeutics because of their catalytic activity and specificity. The challenge in the intracellular delivery of enzymes is that enzymes are unstable and have a huge size. Estrada et al. [165] developed β-Galactosidase delivery nanoparticles based on a protein. β-Galactosidase (β-gal) is an important enzyme, and its deficiency leads to several lysosomal storage disorders. The authors observed that protein-enzyme nanoparticles showed internalization in multiple cell lines in vitro higher than soluble enzyme. Authors based on the result concluded that protein nanoparticles are a biocompatible and display good efficiency for active enzyme therapeutics delivery.

3.3.2 Encapsulation of biologics

Noorani et al. [166] fabricated albumin nanoparticles enhancing anticancer efficiency of albendazole in the xenograft model of ovarian cancer. Nanoparticles based albumin was formulated with the diameter in the range of 7 to 10 nm. Loaded albendazole onto albumin nanoparticles showed the highest killing effect with specificity against ovarian cancer cells studied ex vivo [167].

Trafani de Melo et al. [168] studied the design of whey protein drug delivery system for a photoactive compound, aluminum phthalocyanine chloride for targeting of glioblastoma brain cancer. Nanoparticles were fabricated by spray drying technique with particle size between 100 and 300 nm. Authors based on their results concluded that a combination of hydrophobic drugs and irradiation achieve efficient treatment.

Stein et al. formulated mTHPC-albumin nanoparticles using nab-technology [169]. Nanoparticles showed colloidal stability over a wide range of pH and in physiological NaCl with different concentrations. The authors observed cell culture uptake of mTHPC in a cholangiocarcinoma cell line (TFK-1).

3.3.3 Encapsulation of diagnostics

Nanoscale materials permit nanodevices to enter novel scientific and technological frontiers in different diseases especially cancer diagnosis. Proteinticles are nanoscale protein particles designated by engineering, which are very useful in changing different properties based on surface area and size of various conventional things [170]. Combined detection of two serum biomarkers is also feasible through multiplexed viral detection. The disease detection method is imperative in diseases such as AIDS and hepatitis. Such a protocol is based on lateral flow assay (LFA) for protein nanoparticles. Proteinticles were found to have better biocompatibility and biodegradability with compliance with surface modifications. These nanoparticles are formed by using different proteins like elastin, gliadin, gelatin, zein, legumin,

albumin, soy protein, and milk protein. Various methods used for the formulation include emulsification, desolvation, electrospray, and coacervation. Characterization parameters of these nano-formulations involve morphology of particle, size of a particle, their surface charge, entrapment of drug, loading of a drug, structure of the particle, and in vitro drug release. Different methods for the application of route of administration for protein nanoparticles have been studied by renowned researchers [170]. In nature, particular proteins have a self-assembling property inside cells leading to the formation of nanoscale particles (called "proteinticles") with constant surface topology and structure [171]. Unlike chemically synthesized nano-formulations (e.g., various carbon, metal and polymer nanoparticles), a set of effective proteinticles can be easily produced through genetic modulation of the proteinticles surface, i.e., by inserting or adding specified peptides/proteins to the C- or -N-terminus or the internal region of the modified protein. Proteins/peptides were presented that were proven to recognize specific antibodies in certain diseases that were recognized on the outer surface of human ferritin based proteinticles purposed at accurate 3D diagnosis of human infectious and autoimmune diseases. The surface exhibited the extracellular domain of myelin oligodendrocyte glycoprotein (MOG) with native conformation successfully differentiated between autoantibodies to denatured or native MOG, leading to an accurate diagnosis of multiple sclerosis. Different antigenic peptides from the hepatitis C virus (HCV) were displayed simultaneously on the same proteinticles surface with modification of the composition of each peptide. Proteinticles having heterogeneous peptide surfaces were detected with anti-HCV antibodies in patient serum with 100% accuracy. The desired method of proteinticles engineering can be used in general to the specific and sensitive diagnosis of several human diseases [171].

The aptamer is defined as an oligonucleotide-based nano-formulation. Unique characteristics of aptamer exhibit specificity and high-binding affinity with target molecules both intra- and extracellular. It functions as an agonist or antagonist in a biological system [170]. Recently, numerous aptamers were used for the detection of disease, with curative purposes under development for the identification of different molecules of HCC (hepatocellular carcinoma). The aptamer has been proved to improve the effectiveness of conventional chemotherapies and decrease the growth of HCC cells in vitro. Aptamer was proved to elicit antitumor activity and cell death in vivo. The overall data showed that aptamer possessed reduced toxicity levels. Moreover, it may provide a safer base in the field of personalized medicine [170].

Tumor Necrosis Factor-α (TNF-α) by gold protein chip was sensed using a total internal reflection fluorescence microscopy (TIRFM) as a detection method for a nano-based single biomarker for oral cancer diagnosis. Authors observed this method which is an attomolar (aM) concentration level leading to a higher sensitivity of oral cancer detection [172].

Apoferritin Ferritin is a complex of an iron-containing protein having 24 self-assembled polypeptide subunits with external and internal diameters of 12 and 7.6 nm, respectively [173]. These protein-based cage-like networks show three characteristic interfaces, the interior, exterior, and the interface present between the subunits, which exhibit functionalization. When the iron core from the inner cavity is removed, it results in a hollow protein cage-like called the Apoferritin nanocage, which is subjected to assembling and disassembling as a result of the change in the environment surrounding the molecule. Apoferritin nanocage can be utilized to insert inorganic metals inside its cavity purposing at scavenging ROS which are generated during several mechanisms in the cellular environment. This character has been used as a template for the synthesis of an array of nanocomposites for theragnostic applications in cancer treatment. Apoferrtin

Micro-encapsulating materials		Active compounds	Micro-encapsulation techniques	Applications	EE %	Ref.
Protein	**Other materials**					
Barley protein	—	Fish oil	Spray drying	Reducing the inflammation and improving the hypertriglyceridemia	EE: 92.9%	[174]
Chickpea and lentil proteins	Maltodextrin	Flaxseed oil	Spray drying	Reducing the coronary heart risks	EE:88.0% (Lentil) & EE:86.3% (Chickpea)	[175]
WPI protein	—	Docosahexan-oic acid	Spray drying	Improving the hypertriglyceridemia	EE: 93.2%	[176]
Zein and WPI proteins	—	β-carotene	Spray drying	Inhibiting the DNA damage and enhancing the immune system	EE: 74.0%	[177]
Casein protein	Lactose	Oil/milkfat compounds	Spray drying	Reducing the lipid oxidation problem	EE: 95.2%	[178]
Gelatin	—	Ciprofloxacin	Spray drying	Respiratory tract infection treatment	EE: 80.0%	[179]
	Ethyl cellulose	Curcumin	Spray drying	Bladder cancer treatment	—	[180]
	GPTMS	Vancomycin	Emulsification	Skin and joint infections treatment	—	[181]
WPI	—	Riboflavin	Desolvation and Spray drying	Ariboflavinosis treatment	EE: 96.6%	[182]
BSA protein	Ch and HA	Sorafenib	Complexation	Hepatocellular carcinoma treatment	EE: 45.6%	[183]
	—	Hemoglobin	Co-precipitation	Erythrocytes Shortage treatment	EE: 82.0%	[184]
Pea protein	Polyglycerol polyricinoleate	Riboflavin	Emulsification	Ariboflavinosis treatment	EE: 84.0%	[185]

EE: Encapsulation Efficiency; GPTMS: 3-glycidoxypropyltrimethoxysilane; WPI: Whey protein isolate; BSA: Bovine serum albumin; Ch: Chitosan; HA: hyaluronic acid.

Table 5.
Plant and animal proteins-based micro-encapsulated carriers for delivery of bioactive compounds.

nanoparticles enter the targeted tumor cells via clathrin-mediated endocytosis, receptor-mediated endocytosis, and macropinocytosis processes.

A ferritin-based multifunctional nanomaterial was prepared for MR and fluorescence simultaneous imaging of lung cancer cells. Human H-chain ferritin was engineered with green fluorescent protein aiming at stable fluorescence in the cells. Moreover, arginylglycylaspartic acid peptide was fused on the external surface of the ferritin cage for αvβ3 integrin receptors targeting human tumor cells (human glioblastoma U87MG cells and A549 cells) [173]. Multifunctional nanostructures based on ferritin (RGD-GFP-ferritin [RGF]/Fe_3O_4, rHF/Fe_3O_4,

Nano-encapsulating materials		Active compounds	Nano-encapsulation techniques	Applications	Particle size (nm) and EE %	Ref.
Protein	**Other materials**					
Barley protein	—	β-carotene	Emulsification	Inhibiting the DNA damage and enhancing the immune system	90 nm EE: 93.5%	[186]
Zein protein	—	Doxorubicin	Phase separation	Cancer Treatment	247 nm EE: 90.1%	[187]
WPC protein	Maltodextrin	Vitamin B9	Nano-emulsification	Folate deficiency treatment	100 nm EE: 86.6%	[188]
SPI protein	Folic acid	Doxorubicin	Nano-precipitation	Cancer treatment	200 nm EE: 83.8%	[189]
WPI protein	Ch	Trypsin	Nano-precipitation	Satietogenic and inflammation treatment	109 nm EE: 98.5%	[190]
BSA protein	—	Resveratrol	Coacervation	Inflammation and cancer treatment	175 nm EE: 60%	[191]
	Folic acid	Paclitaxel and CDF	Modified desolvation	Ovarian and cervical Cancer treatment	197.8 nm, EE: 78.4% (CDF) & EE:77.4% (Paclitaxel)	[192]
	Starch and chitosan	Curcumin	Coacervation	Cancer treatment	200 nm EE: 92.1%	[193]
Pea Protein	—	Vitamin D	Nano-emulsification	Deficient Vitamin D treatment	233 nm EE: 96.0%	[194]
	CMCFG	Curcumin	Nano-precipitation	Inflammation treatment	100 nm EE: 99.2%	[195]
Casein protein	—	Celecoxib	Coacervation	Inflammation treatment	216.1 nm EE: 90.7%	[196]
	—	Mequindox	Spray drying	Inhibition of pathogenic bacteria	262.5 nm EE: 72.2%	[197]
Gelatin	Folic acid	Irinotecan	Nano-precipitation	Metastatic colorectal cancer	200 nm EE:11.2%	[198]
	—	17β-estradiol (E2)	Modified desolvation	Ischemic stroke treatment	362.3 nm EE: 95.5%	[199]

EE: Encapsulation Efficiency; SPI: Soy protein isolate; WPC: Whey protein concentrate; WPI: Whey protein isolate; Ch:Chitosan; CDF: di-fluorinated curcumin; CMCFG: carboxymethylated corn fiber gum.

Table 6.
Plant and animal proteins-based nano-encapsulated carriers for delivery of bioactive compounds.

and GFP-rFH/Fe_3O_4) were prepared by synthesizing iron oxide (Fe_3O_4) nanoparticles in the previously engineered ferritin cages. Imaging of these cages with fluorescence targeted to αvβ3 integrin-positive A549 and U87MG cells showed higher-intensity fluorescence with RGF, when compared to GFP-rHF control cells. Furthermore, MRI with RGF showed significant enhancement of the signal to facilitate meticulous diagnosis, when compared to GFP-rHF/Fe_3O_4 or without contrast agent. Therefore, efficient targeting and fluorescence imaging of lung cancer cells utilizing engineered nanocages were proved to be a useful vehicle among the different multifunctional, nanostructured, protein-based tools to be used in fluorescent imaging.

The antioxidant enzymes present normally inside the human body, like catalase, superoxide dismutase (SOD), and peroxidase, fail in the protection of the cells under sudden oxidative damage/stress conditions Thus, further studies have developed artificial antioxidants capable of decreasing oxidative stress during lung cancer treatment [173]. Apoferritin-encapsulated protein nanoparticles have been prepared as artificial antioxidants on account of their peroxidase, catalase, and SOD-mimicking activity. Apoferritin-CeO_2 nano-truffle has been used as an artificial redox enzyme owing to its ability to mimic SOD activity. This character can be utilized to combat ROS-mediated lung cancer by scavenging hydrogen superoxide, peroxide, and other small molecules triggered in sudden oxidative damage. Thus, these systems show potential for hopeful application in lung cancer treatment [173]. **Tables 5** and **6** indicate applications based on micro- and nano-encapsulation utilizing animal/plant proteins as encapsulating materials.

4. Conclusions and future trends

This chapter describes in details the applications of polysaccharides, and proteins, as natural nanocarriers for encapsulation and safe delivery of various therapeutic, diagnostic and theragnostic agents. The chapter provides detailed discussion with recent examples and case studies for using polysaccharides and proteins as biocompatible, biodegradable nanocarriers for encapsulation and delivery of small molecules, biologics, and diagnostics.

Encapsulation will remain a valuable process in the design and development of drug delivery systems and fabrication of diagnostic tools. Advances in naturapolyceutics and encapsulation technologies will continue to drive the applicability of natural polymers and encapsulation in drug delivery and diagnostics. More of polymer blending or interactions; increasing combination of the classes of natural polymers will be observed to achieve the evolving need to improve on the delivery of existing drugs and drugs in the development pipeline. The desire to enhance selectivity, specificity and sensitivity of biosensors will continue to drive the innovations and applications of natural polymers in diagnostic space. Filling the gaps in patient related therapies will place encapsulation as the main stay technology in solving delivery related problems and diagnostic challenges. The quest for maximization, cost effectiveness, reducing patient complications, and optimization of systems and devices will lead to increased assembling of multifunctional all-in-one devices. Theragnostics has come to stay and will precipitate combination of natural polymers and encapsulation technologies to achieve the desired theragnostics that will detect biomarkers, bioimage; and target, deliver and monitor drugs at the site of action. As drug delivery and diagnostics advance, natural polymers will remain materials of focus due to their biogenicity, biodegradability, biocompatibility, good interactions with living cells, suitability for long circulation and targeting, and cell recognition.

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Author details

Ndidi C. Ngwuluka[1*], Nedal Y. Abu-Thabit[2*], Onyinye J. Uwaezuoke[3], Joan O. Erebor[4], Margaret O. Ilomuanya[5], Riham R. Mohamed[6], Soliman M.A. Soliman[6], Mahmoud H. Abu Elella[6] and Noura A.A. Ebrahim[7]

1 Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria

2 Department of Chemical and Process Engineering Technology, Jubail Industrial College, Jubail Industrial City 31961, Saudi Arabia

3 Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria

4 Department of Pharmacy Practice, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, Ohio, USA

5 Center for Biomedical Research, Population Council, New York, 10065, USA

6 Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

7 Pathology Department, National Cancer Institute, Cairo University, Giza, Egypt

*Address all correspondence to: ndidi.ngwuluka@biodrudel.com and abuthabit_nidal@yahoo.com

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