Chapter

Natural Polymers in Micro- and Nanoencapsulation for Therapeutic and Diagnostic Applications: Part I: Lipids and Fabrication Techniques

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Abstract

Encapsulation, specifically microencapsulation is an old technology with increasing applications in pharmaceutical, agrochemical, environmental, food, and cosmetic spaces. In the past two decades, the advancements in the field of nanotechnology opened the door for applying the encapsulation technology at the nanoscale level. Nanoencapsulation is highly utilized in designing effective drug delivery systems (DDSs) due to the fact that delivery of the encapsulated therapeutic/diagnostic agents to various sites in the human body depends on the size of the nanoparticles. Compared to microencapsulation, nanoencapsulation has superior performance which can improve bioavailability, increase drug solubility, delay or control drug release and enhance active/passive targeting of bioactive agents to the sites of action. Encapsulation, either micro- or nanoencapsulation is employed for the conventional pharmaceuticals, biopharmaceuticals, biologics, or bioactive drugs from natural sources as well as for diagnostics such as biomarkers. The outcome of any encapsulation process depends on the technique employed and the encapsulating material. This chapter discusses in details (1) various physical, mechanical, thermal, chemical, and physicochemical encapsulation techniques, (2) types and classifications of natural polymers (polysaccharides, proteins, and lipids) as safer, biocompatible and biodegradable encapsulating materials, and (3) the recent advances in using lipids for therapeutic and diagnostic applications. Polysaccharides and proteins are covered in the second part of this chapter.

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

1. Introduction

Encapsulation, a process involving entrapment of an active ingredient or diagnostic tool within a carrier (capsule, shell, coating material or a matrix) is an old technology that has gained traction over the years with advances in polymer science and encapsulation technologies. The applications of encapsulation span pharmaceuticals, biotechnology, agrochemical, environmental, food and cosmetic spaces with immense benefits found in the pharmaceutical and biotechnology spaces. Encapsulation is used for immobilization of volatile compounds, enzymes, and microorganisms; protection and stabilization from environmental factors; safe handling of hazardous but useful materials; controlled release; taste, odor and color masking; site-specific delivery and solidifying of liquid droplets. Encapsulation improves on the challenges of conventional dosage forms in enhancing stability, taste, bioavailability and biodistribution. Some drugs such as insulin are not given orally because of degradation in the GIT before absorption. Encapsulation may be an approach to change route of administration from intravenous to oral.

The parameters for encapsulation of active ingredients depend on the physicochemical properties of the active ingredient such as solubility, thermal and redox stability; however, the release of the active is then modulated by mechanical process, pH variations, enzymatic actions or other external stimuli [1]. The encapsulation methods classified as chemical, physicochemical and physicomechanical methods are used to encapsulate an active ingredient with the specific method chosen based on application and desired outcomes. The choice of materials to be used for encapsulation to accommodate the physicochemical behavior of the active in order to produce the desire encapsulation efficiency, shell or capsule size, surface morphology and functionalities of the capsule and the behavior of encapsulated active ingredient are fundamental preformulation studies before a new encapsulated product is developed. The bioavailability of existing poorly soluble drugs and those in the development pipeline can be significantly enhanced by encapsulation with the right encapsulating material(s).

Natural polymers are choice materials for encapsulation. Natural polymers are macromolecules of large molecular weights obtained from nature and are preferred due to their flexibility to modification, biocompatibility, biodegradability, renewability, and low toxicity [2]. Being of natural origins such as plants, animals and microorganisms, they are able to interact with tissues and cells displaying some properties the body identifies with and as a result do not treat them as foreign bodies [3]. Natural polymers such as proteins, polysaccharides and lipids have been employed as encapsulation materials for encapsulating hydrophilic or hydrophobic active ingredients which may be in liquid, solid and gaseous states for transport and delivery to the sites they are needed. The chapter reviews fabrication techniques, lipids, and their applications in micro- and nanoencapsulation elucidating their functionalities which enable them to be utilized for encapsulation of therapeutics and diagnostics producing delivery systems with the desired outcomes. Polysaccharides and proteins are covered in part (II) of this chapter.

2. Encapsulation

Encapsulation is the process of enclosing, entrapping, coating, or surrounding a liquid, solid or gas active compound within a material to achieve a more controlled/sustained release, protect the active compound/active pharmaceutical ingredient from degradation before reaching the site of absorption or before reaching the site of action as well as reducing the associated adverse effects that go along with some non-encapsulated compounds like NSAIDS [4, 5]. Research on encapsulation utilizing natural polymers and their derivatives (semi-synthetic polymers) has evolved

over time with the particle size as the main difference. Encapsulation on the micro scale is referred to as microencapsulation while when encapsulation is done on the nanoscale it is referred to as nanoencapsulation [6].

2.1 Microencapsulation

Microencapsulation refers to the formulation process of encapsulating a bioactive compound in a particle size that is 1–1000 μm in diameter for the purpose of controlled and sustained delivery as well as protection of the encapsulated bioactive compound from the surrounding environment [7, 8]. Microencapsulation technology came into existence with the focus of achieving controlled and extended release profiles. Due to the size of micro carriers, encapsulation of macromolecules with large molecular weight such as proteins for controlled release can be encapsulated. Bochenek *et al.,* [9] utilized chemically modified alginate microsphere formulations to encapsulate allogeneic pancreatic islet cells for transient islet-graft function that have reached clinical trial stage for management insulin deficiency in diabetic populations. This was due to the fact that the immune-modulating alginate copolymer employed had controlled release profile that caused encapsulated islet cells to remain viable after transplantation into the general intraperitoneal (IP) space of human subjects, while also exhibiting lowered foreign-body reaction (FBR) compared to previous formulations. Chitosan-alginate microcapsules encapsulating biologically active compounds from aqueous extracts of *Garcinia kola* (GK) and *Hunteria umbellata* (HU) seeds, have also been shown to have selective release patterns depending on the pH of the medium [10]. Slower release of the GK and HU microcapsules of the active compounds was observed at pH 1.2, but increased controlled extended release profiles were observed to occur at pH 6.8 unlike conventional tablets that did not show controlled extended release profiles [10].

2.2 Nanoencapsulation

Nanoencapsulation can be defined as the entrapment, enclosure, or coating of a bioactive compound within a carrier that is on the nanoscale dimension [5]. Nanoscale dimension is seen as particle sizes 1–100 nm [6]. However more recent definitions have given room for 1–300 nm and others 1–1000 nm [11]. The advancement of encapsulation from the micro scale to the nano scale was driven by the need for more site selective targeting purposes such as the use of chemotherapeutics in cancer. The main draw back in cancer chemotherapeutics has been that severe adverse effects occur due to toxicity caused by the non-selective action on both cancer cells and healthy cells at therapeutic doses. Hence, the inception of nanomedicines that could achieve active targeting was born. Nanoencapsulation in drug delivery has the merit of having a higher encapsulation efficiency, due to enhanced drug solubility of bioactive molecules in the core [12].

Silk fibroin nanoparticles encapsulating curcumin were found to demonstrate selective cytotoxicity for cancer therapy in neuroblastoma cells and hepatocarcinoma cells while not adversely affecting the healthy cells [13]. Diagnostics are also gaining from the merits of encapsulation. This was demonstrated in the simultaneous co-encapsulation of MRI contrast agent Gd-DTPA and fluorescent label ATTO488 in multimodal PEG – crosslinked hyaluronic acid nanoparticles (PEG-cHANPs) to formulate a probe for diagnostic purposes [14]. The PEG-cHANPs were observed to improve MR signals while concurrently magnifying the relaxation time, T_1 5 times due to the presence of the ATTO 488 in the human glioma U87 MG cell line. Tammaro *et al.,* [14] implied that this could lead to the decrease in the administered dose of the probe, thereby resulting in a better resolution and higher quality images.

3. Merits and demerits of encapsulation

The merits and demerits of encapsulation are viewed from the expected outcomes of encapsulation and are based on physicochemical properties of core materials (small molecules, biologics, or diagnostics), encapsulating techniques and materials. Encapsulating materials such as natural and semi-synthetic polymers have many advantages because they are obtained from natural sources. Immunogenicity issues when natural polymers are used as the encapsulating materials are greatly reduced compared to synthetic polymers. Encapsulation using natural polymers can be done without high temperatures thus preventing degradation due to high temperatures as seen in alginate–chitosan micro/nanoparticles which were successfully fabricated at room temperature excluding the utilization of organic solvents [4].

Encapsulation of an active compound using pegylated phospholipids also known as lipopolymers such as 2000 Da PEG-DSPE have demonstrated the merit of prolonging circulation time of active compounds when administered as nanoliposomes. This led to a reduction in the dosage frequency and reduction in uptake by the RES, thus leading to an increase in patient compliance as was observed in the first FDA approved nanomedicine; liposomal doxorubicin Doxil® in 1995 [15].

Efficacious poorly water-soluble drug candidates for therapeutics before now posed a challenge in formulation due to their low solubility profile and pharmacokinetic characteristics [16]. Micro- and nanoencapsulation technologies such as supramolecular hydrogels formulated with natural cyclic oligosaccharides also called cyclodextrins have successfully been able to encapsulate and deliver such drug candidates. This was demonstrated when lipophilic non-hydroxylated coumarins were encapsulated in the core of β-cyclodextrin hydrogels for trypanocidal activity via mitochondrial membrane potential studies for Chagas disease caused by the protozoan parasite, *Trypanosoma cruzi*. Trypanocidal activity was increased by 10% with the Supramolecular hydrogels of β-cyclodextrin linked to calcium homopoly-L-guluronate as compared to the free corresponding amidocoumarins [17, 18].

Demerits of natural and semi-synthetic polymers in micro- and nanoencapsulation mainly depends on the individual materials. However, a major drawback observed with natural polymers and their derivatives is batch to batch variation depending on regions/environments that these polymers were sourced from. Plant and animals of the same species have been found to have some slight differences in their composition based on factors such as type of soil or geographical regions [19, 20]. Low mechanical strength leading to weak wall formation, susceptibility to change in pH causing a reduction in stability, highly hygroscopic leading to denaturation are challenges that are observed with such polymers as alginate, gelatin and sodium hyaluronate [8].

To overcome the demerits of these polymers, physical and chemical modifications are undertaken. Extra care should be taken during storage of natural polymers to reduce degradation and denaturation that occur during storage. Despite any demerits that natural polymers and their derivatives may have, the application of natural polymers and their derivatives for micro- and nanoencapsulation will continue to increase because of their immense merits in therapeutics and diagnostics.

4. Encapsulation techniques

The process of enclosing vesicles in a thin continuous film of a natural or semi synthetic polymer has been accomplished using a variety of both physical and chemical methods or a combination of both depending on the size of the targeted

capsules either in the nanometer, micrometer or millimeter range. Over the years and as a result of continuous innovation, these techniques have evolved from the earliest relatively simple coacervation phase separation used for making only microcapsules to current comparatively complex techniques capable of making both microcapsules and nanocapsules with a careful tuning of process parameters. The choice of a technique not only determines the size but also the morphology and probably the stability expected of the targeted capsules [21]. The choice of technique stems from other parameters such as the physicochemical fingerprint of both shell and core material, the objective of the encapsulation process, the expected release profile/mechanism, the intended application of the final capsules, need for scale up, and of course, processing cost for large scale manufacture. An ideal technique aims at achieving monodispersed capsules with great stability to aggregation, adherence and other destabilizing factors, and high loading and encapsulation efficiency for the cargo. The changing landscape of expectations for drug delivery and other applications is driving the application of more than one encapsulation technique towards achieving goals such as in theragnostic applications. Bazylińska and colleagues [22] combined two encapsulation techniques (**Figure 1**), emulsion solvent evaporation and Layer by layer assembly (LbL) to engineer nanocapsules for dual fluorescence bioimaging and drug delivery.

Encapsulation processes have been classified broadly into physical and chemical techniques. There is no consensus over the third class, physicochemical techniques, and may be because the techniques only take the mechanism of capsule formation into consideration. However, each encapsulation technique involves processing that may involve mechanism not inherent in the name. For instance, emulsion solvent evaporation or in situ polymerization techniques are core chemical techniques but may involve physical processes such as mechanical stirring, homogenization, and sonication in achieving solvent evaporation. **Table 1** outlines the different classification of encapsulation techniques.

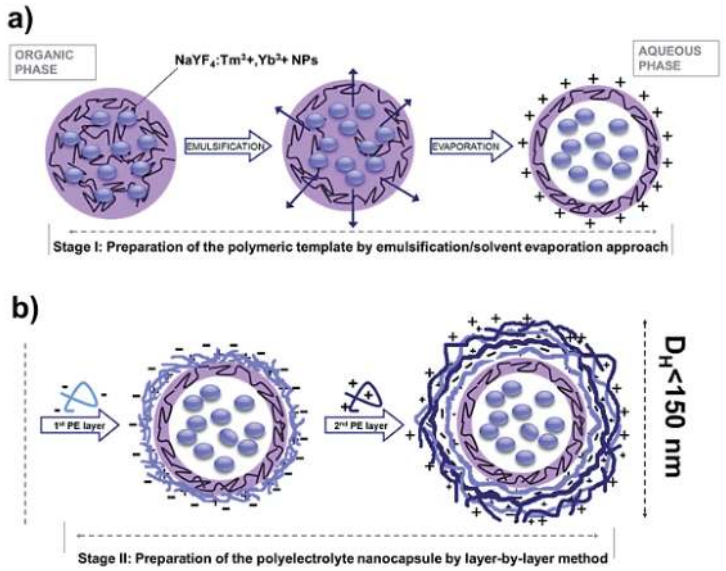

Figure 1.
Combination of two encapsulation techniques for microcapsule formation [22].

Physical/mechanical/thermal techniques				Physicochemical techniques	Chemical techniques
Coating	**Atomization**	**Extrusion**	**Thermal**	**Coacervation**	**Polymerization**
• Pan Coating • Fluid bed coating	• Spinning disc	• Stationary nozzle • Centrifugal extrusion • Vibrating nozzle/annular jet (Coextrusion) • Electrohydrodynamics • Microextrusion • Single or twin screwed extrusion	• Spray drying • Spray chilling • Spray congealing • Phase inversion	• Layer by Layer deposition • Solvent evaporation • Solvent extraction • Molecular inclusion • Sol–gel	• Suspension • Emulsion • Interfacial

Table 1.
Classification of encapsulation techniques.

4.1 Physical encapsulation techniques

Physical/mechanical encapsulation techniques involve formation of micro or nanocapsules by a transformation in the physical attributes of a droplet such as its size or a change from a liquid droplet to a solid droplet. Some steps as discussed below are common to most physical encapsulation techniques.

Atomization: This a process whereby tiny droplets are created from a liquid by dispersion in a gas phase. A range of atomization methods are available and can be adapted to various techniques. These are pressure nozzles, vibrating nozzles, and spinning disc atomizers. There are a variety of archetypes for each.

4.1.1 Spray drying

Spray drying is a widely used encapsulation method that dates to the early fifties. It also serves as a means of microcapsule recovery for many other encapsulation methods. The process involves dispersion of the core material in a solution of the shell material (most commonly water or cosolvents) to form a dispersion, emulsion or suspension [23]. The resulting liquid is fed into the drying chamber at the same time atomized with hot air (nitrogen in rare gases) coming from sonic energy, pressure nozzle, two-fluid nozzle or veined wheel. The solvent is flash evaporated in the hot air stream leaving a free flowing solid of core encapsulated in the shell. It is a simple, flexible encapsulation method that yields consistently distributed particles size between 10 and 40 μm range and is amenable to automation [24]. The first step in the process is to dissolve the shell material in a solvent, most commonly water, and homogenize with the core active ingredient, most commonly hydrophobic. The film forming materials predominantly used in spray drying are hydrophilic natural polymers such as modified starch, gelatin, gum Arabic and maltodextrin. It is not uncommon to use blends of these polymers. The second step is to feed this dispersion into the drying chamber using a sprayer that atomizes it to droplets. Hot air fed into the chamber quickly evaporates the solvent leaving a deposit of the shell forming material around the core droplet. The encapsulated material is then collected through a separator that separates the product from the exhaust air. Even though spray coating is one of the most common and industrialized encapsulation methods particularly for lipids, flavors, aromas and pigment, the process is beset with low thermal efficiency, nozzle clogging, high maintenance cost and product loss [21]. Optimization of process parameters such as inlet temperature, nozzle diameter, liquid feed viscosity and flow rate, gas flow rate, atomization pressure, temperature distribution efficiency and drying rate can minimize negative outcomes in terms of morphology, size and size distribution of the product [25]. Zang and coworkers [26] explored the influence of process parameters on the physical characteristics of tea tree oil microcapsules. They found that there is a need to strike a balance in inlet temperature as an extremely high temperature cracked the microcapsules while an extremely low temperature led to the formation of droplets instead of microcapsules. More recently, Wei and colleagues [27] studied the influence of inlet temperature and precursor concentration among other parameters on the physicochemical properties of theophylline loaded chitosan-triphosphate particles prepared by spray drying. They showed that particle size increased with precursor concentration. In their study, the optimum temperature for making the targeted size of microcapsules was 130° C. Zhang and coworkers [26] found the optimum temperature for their targeted application in the range of 210°C - 215° C buttressing the need for personalizing each process.

4.1.2 Prilling (spray congealing/spray chilling/spray cooling)

This is an encapsulation technique in which a homogenized dispersion of core material in a molten shell material (spray congealing, Prilling) or thermally gelling matrix (spray chilling) is atomized by suspension in a gas phase at ambient or low temperature (usually air or nitrogen (gas or liquid) that causes rapid solidification of the shell material around the core material [28, 29]. When the melting point of the lipophilic matrix is above 45° C, solidification is brought about at ambient temperature in a spray cooling process but for matrixes with lower melting point, frozen gases are used, and the process known as two major steps are involved firstly of which is creating free falling drops from molten solid, strong solutions or slurries using spinning discs and baskets. The second is solidifying the drops individually in a countercurrent of cold air. The size of each droplet determines the final size of each sphere. The resulting encapsulates of this high productivity, grossly monodispersity technique are microspheres with sizes in the range of 60 to 2000 μm [28]. Prilling is a high throughput, relatively inexpensive, easy-to-operate technique that has found extensive use in the fertilizer industry. When used for food encapsulation, the process is limited by the possibility of granule agglomeration due to high temperatures. Most used shell materials are lipids, waxes, fats and gelling hydrocolloids. Russo and colleagues [30] recently explored the possibility of combining Zn and Ca cations as an ionic gelation agents for prednisolone encapsulated alginate beads developed with the Prilling technique. The calcium carbonate decomposed internally in the acidic environment releasing a gas that increased porosity of the microcapsules ultimately translating to buoyancy in the gastrointestinal fluid and extended hours of anti-inflammatory effect.

4.1.3 Coating

Coating as an encapsulation technique involves the deposition of a thin film of membrane around a solid particle or a liquid adsorbed onto a solid. Two approaches have been used. The traditional older pan coating and the air suspension coating or fluid bed coating.

Pan coating: Pan coating is old coating technique that dates to the 18th century and traditionally used for applying sugar and film coats to tablets and pellets measuring several millimeters. In encapsulation, it is generally used for core material measuring above 600 μm [31]. The process involves the application of a coating solution through a spray unto the granule bed in a rotating coating pan inclined at an angle and fitted on the inside with anti-slip bars or angled blade that enable circulation of the core material. Warm or room temperature air is continually introduced and removed through exhaust pipes to facilitate the drying of the coating solution. Coating pans can be conventional or vented with perforations that allow for the escape of the drying air through the powder bed [32].

Air suspension technique: This technique also known as fluid bed coating is the gold standard in coating. The core material is suspended in a stream of hot or ambient air (depending on the coating solution) in relation to a coating spray that can be applied in the same direction as the fluidized air, tangentially or in opposite direction. **Figure 2** shows the application from the bottom of the chamber also known as the Wurster set up. The air suspension technique has also been used for both drying and granulation. For encapsulation purposes, a powder bed is initially fluidized by a jet of hot or ambient air. Subsequently, a coating solution of the shell material is sprayed through an atomizing nozzle onto the fluidized particles depositing a coat, consequent to the evaporation of the solvent, on individual particles

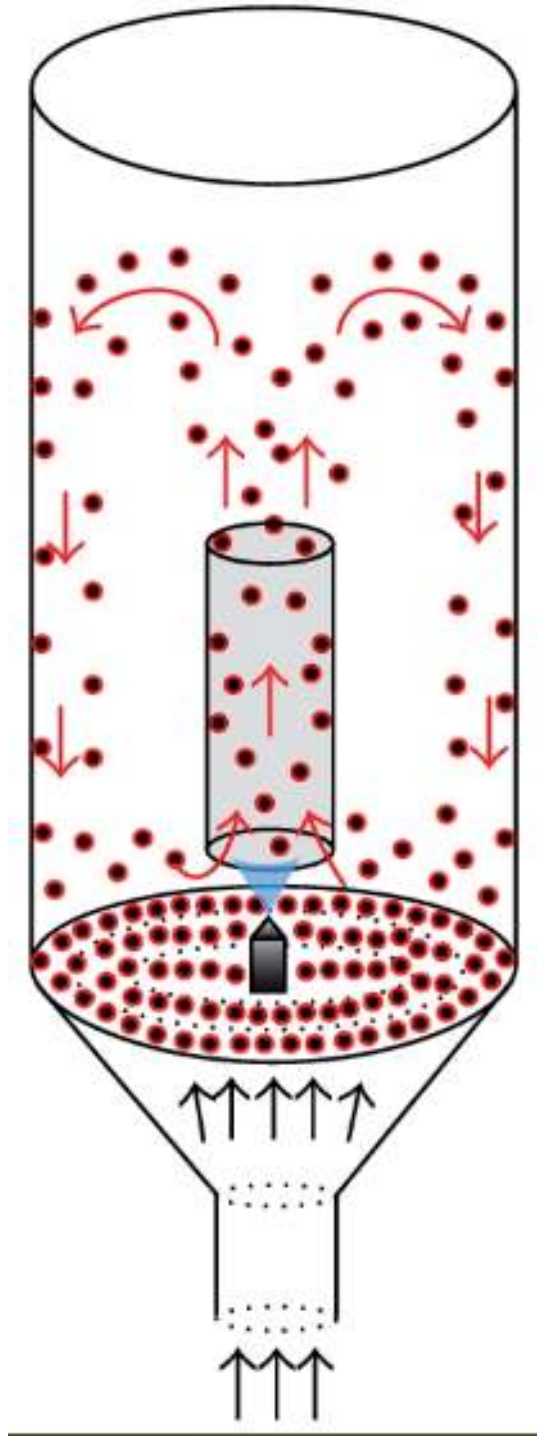

Figure 2.
Schematic representation of the Wurster set up [34].

as they get to the top of the chamber. The exhaust air passes through a filter to the outside while the particles recycle to the base of the chamber and the coating cycle continues till adequately coated. Almost any type of wall material can be applied in the Wurster process [33]. The particle size ranges from less than 100 μm to 150 μm [24]. Uniformity of the coat and the size of the capsules depend on the size and type of spraying nozzle. The viscosity of the coating liquid, air inlet temperature and flow rate must be optimized for each application.

4.1.4 Extrusion

Generally, extrusion is a process in which a material is subjected to some form of compression that bring about a change in its physical properties as it is pushed through the orifice or die of an extruder, that is made up of one or two screws, under controlled conditions [35]. The core material is blended with the polymeric shell material in a molecular mixing to form a solid dispersion or solution. The solid dispersion is then passed through extruders to produce submicron capsules. A variety of extruders and nozzles configuration exists for different applications.

Extrusion-spheronisation: In this technique, the core material is intimately combined with the shell material and extruded into cylindrical mass that is subsequently broken up and rounded into spheres [36]. Muley and coworkers [36] described a variety of extruders to include sieve, basket, ram, screw and roll extruders.

Hot melt extrusion (HME): This continuous process technique originated for the food, plastic and rubber industries in the early nineteenth century but was applied much later in the pharmaceutical industry for product development and manufacturing of poorly soluble drugs. It involves pumping polymeric material that serves as the shell and the API through screw extruders at temperatures above their glass transition or gelling (and sometimes, melting) temperature to achieve molecular mixing of the component as shown in **Figure 3** [37].

The rotating screw pushes the feed towards the orifice whilst generating frictional heat that increases the viscosity of the feed as it melts. The extrudate is shaped by passing through a flake forming calendar roll or a pellet forming rotary knives, traveling shears or saws as it leaves the orifice. Materials capable of HME processing must be capable of deformation inside the extruder and individually capable of physically and chemically withstanding high temperatures. Waxes find extensive use as inert carrier materials for HME process. Starches, sugars, and sugar alcohols have also been used. Plasticizers such as acetic acid, stearic acid, citric acid, salicylic acid and triethyl citrate are used to alleviate the temperature effects in the HME process.

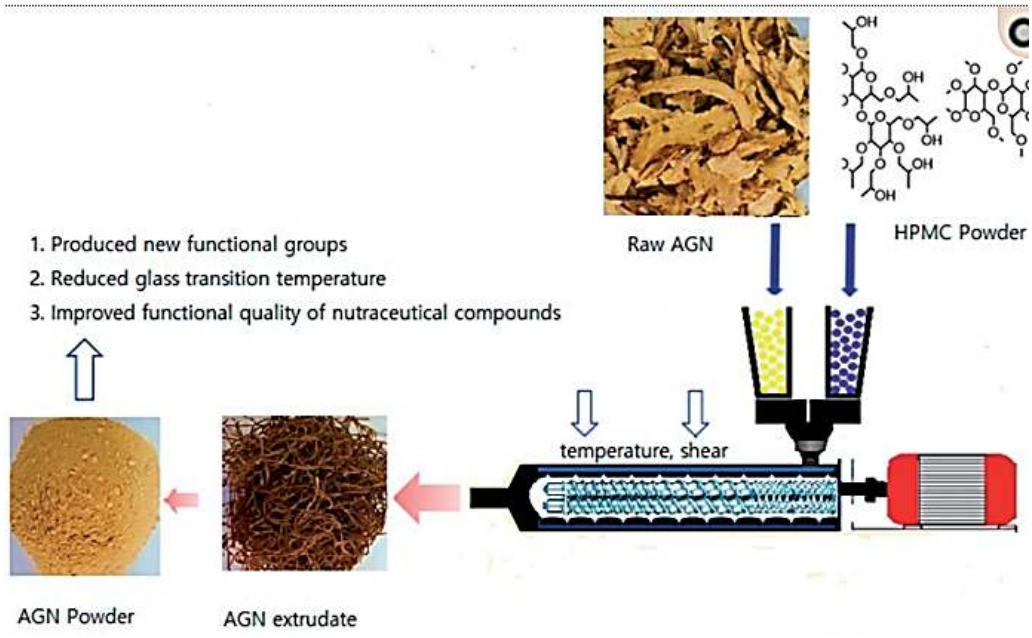

Figure 3.
A schematic diagram of the hot melt extrusion process used in the encapsulation of Angelica gigas Nakai (AGN) [38].

Gately and colleague [39] explored the possibility of using a natural polymer, shellac as a low temperature extrudable polymer in the encapsulation of a probiotic powder. They found that not only was it possible, but the probiotic powder had an additional plasticizing effect on the extrudates. Melt extrusion and its earlier variant, melt injection has found extensive use as an encapsulation technique especially for fragrances and flavors due to the lower energy requirement, minimal emission of odor fouled exhaust, no requirement for solvent, and possibility for large volume encapsulation [40]. In addition, extrusion encapsulations impart longer stability on flavors and lower degradation for enzymes, when compared to other encapsulation methods like spray drying [39]. Glassy carbohydrates, polysaccharides, proteins and their blends have all been used as carrier polymers for melt extrusion encapsulated flavors [41]. Carnauba wax was also recently used for melt encapsulation of Quercetin [42].

4.1.5 Coextrusion

Coextrusion is a variation of the extrusion technique that involves two concentric nozzles through which the core and shell material are extruded individually and exiting the nozzle as a single drop of core material encapsulated in the shell. It is designed primarily for liquid materials and the process schematically represented in **Figure 4**. The core material and the shell material do not mix unlike in the extrusion technique. The liquid shell material is pumped through the outer nozzle while the core material is extruded through the inner nozzle. The stream of liquid forms a laminar that is broken into discrete drops of the core enveloped by the shell. The drops are received in a curing liquid that hardens the encapsulated product [43]. It has been shown that coextrusion encapsulation technique offers better protection against instability in encapsulated aroma oils than extrusion technique [44].

Additionally, extrusion yields matrix spheres in which there is an intimate mixture between the shell and the core. Whereas in coextrusion, the core is separated from and covered by the shell. Sodium alginate has extensively been used as a shell

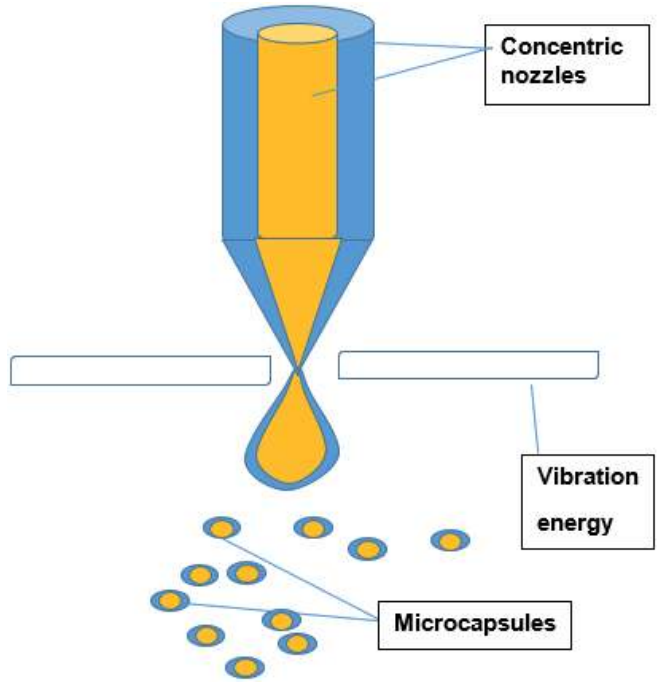

Figure 4.
Schematic representation of the co-extrusion technique.

forming polymer which is usually cured by ionic interactions with divalent cations. Silva and colleagues [44] compared the extrusion technique with co-extrusion for the encapsulation of probiotic, *Lactobacillus acidophilus* LA3 using a blend of alginate and shellac. They found that co-extrusion using sunflower oil as a carrier for the probiotic provided additional stability.

Centrifugal extrusion: This variation of co-extrusion is a liquid extrusion technique that makes use of a spinning extrusion head that carries the concentric nozzles. The concentric feeding tube serves as a tributary to the many concentric nozzles located at the surface of the device. As the spinning head rotates, the inner core and the outer shell material are extruded in flow that break into droplets as it makes its way from the nozzles (**Figure 5**). The particle size of extrudates can be as small as 150 μm. The particles harden by solvent evaporation as they take flight from the device.

4.1.6 Techniques based on drop generation method

Mechanical means are used for droplets generation during co-extrusion and has given rise to many modern encapsulation techniques. These all depend on the dripping and jet break up principle for droplet generation at an orifice or from a laminar jet. A droplet that forms at an orifice and is discharged is a result of a formation process that depends on the interplay of surface tension of the extruded liquid, velocity of extrusion, gravitational force, impulse and frictional forces. Configurations for droplet generation are based on five mechanisms.

A. At extremely low velocity, single droplet form at the orifice. The drop detaches under gravity as gravity overcomes surface tension and frictional forces.

B. As the velocity increases, the number of drops ejected from the orifice increases marginally, leading to increased coalescence and polydispersity of the drops.

C. Co-axial flow: A higher increase in velocity results in the formation of a continuous liquid laminar jet that breaks by surface tension and axial symmetrical vibration.

D. Further increase in velocity causes normal distribution of droplets because of spiral symmetrical vibrations.

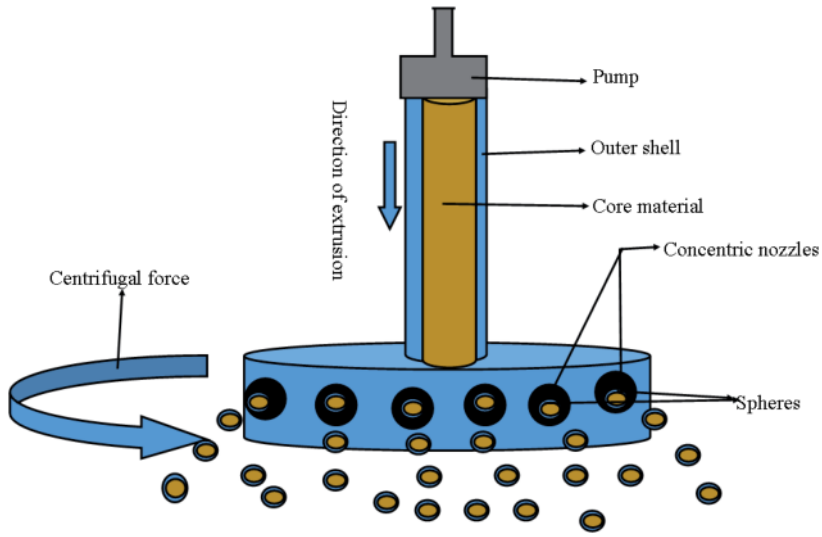

Figure 5.
A schematic representation of the centrifugal extrusion device.

E. C could also result in droplet formation because of high frictional forces when a jet is sprayed.

Encapsulation methods based on these mechanisms and on the droplet generation method are elucidated below.

Vibrating nozzle/jet: This encapsulation technique, commercialized by Inotech Biotechnoly Ltd. and Nisco Ltd., makes use of permanent vibrational or sinusoidal frequencies of definite amplitude to break up a laminar jet into equally sized droplets stabilized by electrostatic repulsion and achieved by application of an electric field [45]. Two variations are the vibrating nozzle and the vibrating chamber techniques. The size distribution of the droplets is narrow and the size generated with a given amplitude is in the range of 0.10–1.50 mm and depends on the nozzle diameter, jet velocity, rheology and surface tension of the liquid [46]. It is predominantly used in cell immobilization with Newtonian systems. Dorati and coworkers undertook an assessment of the vibrating nozzle technique combined with freeze drying technique in the encapsulation of a model hydrophilic molecules in a hydrophilic polymer, alginate. They concluded that vibrating nozzle technique is an easy and scalable process for microencapsulation of hydrophilic drugs [47].

Simple dripping: This simple method involves the free formation of a droplet at the orifice. The drop continues to increase in volume until the weight of the liquid just exceed the capillary force. The drop detaches and forms a sphere due to surface tension. This method of low droplet production rate is mostly applicable to laboratory encapsulation with droplets sizes approximately 1000 μm. It has found use in microfluidic devices and in porous membranes such as Shirasu porous glass in which high pressure is applied to cause the droplet generation of a disperse phase directly into the continuous phase held in the membrane [48]. These membranes are used for generation of emulsions and mini emulsions on a lab scale. The membrane pore size directly controls the droplet size. The membranes are hydrophilic and therefore, favors oil-in- water emulsions formation. For an oil continuous phase, there may be a need to coat the membrane using silicone resins [46].

Generally, in microfluidic devices, several configurations are obtainable for droplet generation and include co-flowing, T-junction and fluid focusing [49]. The fluid focusing configuration is particularly advantageous since it is passive and droplet generation and cell encapsulation depends on hydrodynamically pumping fluid adjacent to an outflowing cell and can therefore prove useful for sensitive materials such as living cells for probiotics [46, 50]. In fluid focusing, the focused fluid (disperse phase) is introduced into a capillary tube enclosed in a chamber containing the focusing fluid (carrier/continuous phase) which exerts pressure on the focused fluid as it exits the orifice facing the feeding tube. The pressure exerted on the disperse phase, compounded by fluid instabilities, is sufficient to cause it to break into droplets as it squeezes pass the orifice [51]. The droplet size of the internal phase does not depend on the orifice diameter. Device geometry, fluid properties and the process parameters such as flow rate and pressure drop determine what happens as the internal phase emerges.

For microencapsulation applications requiring the generation of microsized droplets of narrow size distribution, it is important that these parameters be tuned such that the carrier phase acts as micro tweezers that pressure the tip of the disperse phase meniscus at the orifice causing it to break into a microjet that eventually breaks into homogenous small droplets [52]. Microfluidic devices have found application in the facile preparation of double emulsions.

Spinning disc: In this technique, also known as centrifugal suspension separation, drops are generated when coated particles are flung off a rotating disc by the generated centrifugal force. The core material usually in solid form is suspended in a viscous coating liquid and poured on top of the rotating disc. The suspension

spreads out to form a thin film on the disc and subsequently gathers momentum as it moves towards the edge of the disc. At the edge, the droplets hold unto the rim due to interfacial tension and viscosity. It is detached when the centrifugal force overcomes the interfacial tension. The drop is detached an angle to the disc and to a distance from the disc depending on their size. This separation by distance traveled as per size is used to sort different size ranges enabling the collection of monodispersed capsules in the solidifying chamber. The rotary speed and geometry of the disc, alongside the viscosity of the suspension and the feed flow rate determine the size of the droplet which usually ranges from 1 to 200 μm [45].

Spinning disc method represented in **Figure 6**, is an easily scalable method for producing large quantities of spherical beads with a narrow size distribution, using liquids of varying viscosities, in minutes. However, product recovery usually requires large space for the gelling bath which makes sterilization difficult. Even though it is amenable to continuous manufacturing, it is expensive comparatively. The coating materials are usually meltable waxes, diglycerides that solidify on cooling.

Electrospraying (Electrohydrodynamic atomization)/Electrospinning: This technique of droplet generation depends on electrohydrodynamics which deals with interaction of fluid and electric field. Electrospraying depends on the principle of charged droplet which states that when an electric field is applied to a drop of liquid, it acquires an electrostatic force which competes with the cohesive force due to surface tension. If this coulomb force is large enough to overcome the surface tension force, the drop detaches and breaks up into submicron droplets which quickly solidify into self-dispersing nano and micro capsules with limited agglomeration and

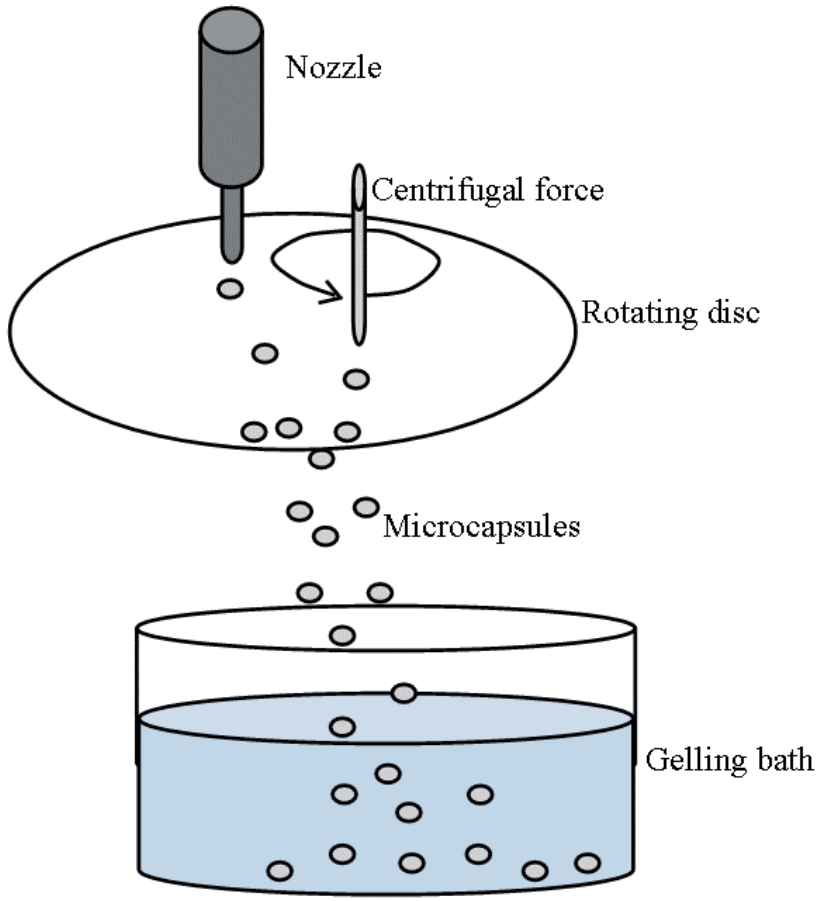

Figure 6.
Schematic representation of the spinning disc assembly.

coagulation. The technique just like its better applied counterpart, electrospinning, is a facile relatively inexpensive, flexible, easy-to set-up and versatile (in terms of processable materials, set up configuration) process that is amenable to continuous manufacture of tunable compositions, and customized properties of size, and morphology [53]. Droplet generation starts with pushing the liquid in the syringe to flow through the nozzle to the metallic capillary which is connected to the collector through a voltage generating unit. As the liquid passes the electric field, electric charges are inducted leading to the formation of a conically shaped lower meniscus (also known as the Taylor's cone) at which tip the acquired charges are concentrated as a result of equilibrium between capillary forces and electrodynamics [54]. The liquid then accelerates away from the nozzle in a tiny thread tip leading to formation of a jet with high charge density. What happens next determines whether electrospinning or electrospraying will occur. The former occurs if the jet experiences sufficiently high axial tension such that the jet undergoes a whipping instability and elongates to reach the collector instead of breaking up. This high axial tension usually results from a high concentration of high molecular weight polymers. Alternatively, the liquid jet breaks up into primary droplets that could experience the so-called Coulombs fission on their way to the collector. This occurs due to solvent evaporation, droplet shrinkage and subsequent break up again into submicron encapsulation due to charge density. Subsequent break ups that could lead to polydispersity could be prevented by a secondary voltage set up known as corona neutralizer.

Other electrospraying modes, other than the Taylor's cone, is possible as the applied voltage gradually increases. The dripping mode gives way to micro dripping, then spindle, Taylor cone jet, and multiplet mode. Obtaining a continuous jet is important for determining the droplet size and morphology which depends on interplay of factors related to the polymer liquid such as density, concentration, surface tension, conductivity [55], molecular weight, viscosity and solvent; and process parameters such as gravity, applied voltage, flow rate, capillary diameter, and distance of the collector from the capillary tip [56]. Among natural polymers that have been applied in electrospraying are chitosan, cellulose and alginate [57].

Different configurations have been used in electrospraying due to the wide range of factors that needs optimization for droplet size and morphology. One of these is the coaxial assembly which uses two concentric capillaries, the inner and the outer for pushing two different liquid compositions. A typical coaxial set up is shown in **Figure 7**. Shams and colleagues [58] developed pH responsive prednisolone loaded Eudragit L100–55 microparticles for colon specific delivery using single and coaxial electrospraying. In vitro assessment of the five developed formulations showed that careful selection of polymeric system alongside process parameters in electrospraying technique can yield site specific delivery.

Yuan and colleagues [59] also used the coaxial electrospray assembly in the fabrication of curcumin-loaded microcapsules aimed at improving the release profile of curcumin. The improved coaxial electrospray was able to generate stable Taylor's cone mode under a variety of operating condition that yielded an obvious core-shell structure of targeted size and morphology [59]. Likewise, Gómez-Mascaraque and colleagues [60], for the first time, encapsulated probiotic, *Lactobacillus plantarium* with a whey protein inner core and a gelatin outer shell using acetic acid as an external gelling agent. They found that the application of high voltage alongside the presence of acetic acid negatively impacted the viability of the probiotic [60].

Another configuration that has been explored to overcome the low throughput of the stable Taylor's cone mode in electrospray is the multiple capillary assembly. Though not without challenges, Parhizkar and colleagues [55] designed and tested two multiple needle electrospraying geometries with each consisting of four needles. The challenge was to operate all four needles at stable cone mode. Higher particles

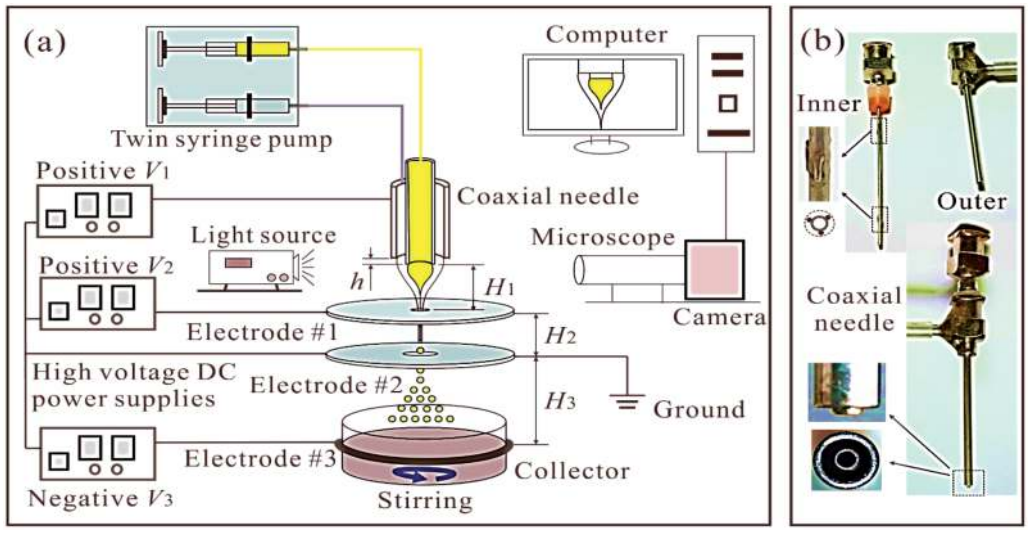

Figure 7.
(a) Coaxial assembly for electrospraying. (b) Inner and outer coaxial needles [59].

recovery rate was recorded for the assemble comparatively for the same collection time with no significant changes in size and morphology [55]. Also, Lee and coworkers [61] designed functionable poly-styrene-random-glycidyl methacrylate that was used to fabricate microparticles via electrospraying. They further studied the influence of both polymer factors and process parameters on the size and morphology of the fabricated microparticles. Their results showed that polymer structure and properties can be used to tune the structural parameters of the capsules [61].

Jet cutting method for droplet generation: This technique commercialized by geniaLab is a rarely used but cost effective technique that depends on a set of cutting wires that serve as a cutting tool for a jet of liquid as it rotates about its axis to generate uniformly sized droplets that is shaped as a result of surface tension. It is suited for cutting high viscosity liquids that harden on cooling or by ionotropic gelation. The drops generated are generally in the size range of 120 μm to 3 mm. Paulo and colleagues [62] recently x-rayed the process parameters requisite for the generation of optimally suited calcium alginate beads using the jet cutter. A maximum flow rate of 49 mL/minute yielded beads of about 2 mm size. Increasing the rotational speed of the cutter decreased the bead size by 50% though increased the tangential velocity of the droplets leading to a larger space requirement for product collection [62]. Other parameters such as gravitational force, surface tension, viscosity and flow rate were also noted. A major limitation is the cutting loss occurring with each cut of the liquid jet.

4.1.7 Phase inversion

Phase inversion and separation occurs in a system due to mass transfer. Usually, for phase inversion to be induced, a polymer solution is exposed to a miscible non-solvent. When a polymer solution is exposed to its non-solvent, the solvent molecules would move out of the polymer while the non-solvent will move in. The first step in the process is to dissolve a polymer in its solvent. The second step is to cast the polymer solution. The third step is to initiate phase separation by immersion of the cast polymer in a coagulation bath containing the non-solvent. Other methods that have been used to induce phase separation is non-solvent vapor [63]. Ammendola and colleagues [63] used the phase inversion technique to prepare fragrance loaded cellulose acetate microcapsules. They then compared the vapor induced phase separation with immersion induced phase separation. Their study showed that the

relatively uncommon vapor induced phase separation yielded microcapsules with more controllable characteristics in terms of structure.

4.2 Encapsulation techniques based on chemical mechanisms

Chemical methods of encapsulation generally depend on chemical interactions for encapsulation to occur. These involve predominantly polymerization reactions involving monomer dispersions. The major chemical methods are interfacial polymerization, interfacial polycondensation polymerization, emulsion polymerization and in-situ polymerization.

4.2.1 Interfacial polymerization

In this technique, the wall material is made to form at the oil-in-water interface of dispersed oil drops. Monomers of the wall forming polymer (usually multifunctional) is first dissolved in the core material and then emulsified in the aqueous continuous phase containing other polymerization reactant. Polymerization ensues right after on both sides of the interface of the dispersed oil drops with water leading to the formation of rigid capsule walls [64]. Particle sizes as low as 3 μm can be achieved though most commercialized capsules from this technique are in the range of 20–60 μm. This technique can also be employed for reverse emulsions. The polymerization occurs across the interface of the droplets. Four major groups of polymers have been employed and include polyamides, polyurea, polyurethane and polyesters in applications that spans the fields of agriculture, pharmaceutics, cosmetics, and energy storage materials. Interfacial polymerization is a well-controlled technique capable of delivering targeted sizes and morphology. An interfacial polymerization approach has been developed that makes use of safer polymers for cosmetic and internal use is the transacylation interfacial polymerization. In this approach, biodegradable oligosaccharides, polysaccharides such as acacia; and polyethylene glycol, and alginate are used in the internal and external phases respectively or vice versa. On mixing the two phases, acacia reacts with the carboxylic acid group of the propylene glycol leading to the overall attachment of alginate and release of polyethylene glycol. The operational shell material is made up of acacia-alginate polymer that does not require further crosslinking [65].

4.2.2 In-situ polymerization

This technique is very much like interfacial polymerization. The difference is that the polymerization occurs entirely in the one phase. This term includes suspension polymerization, emulsion polymerization, and dispersion polymerization. In a typical process, the wall forming monomer or pre-polymer is dissolved in the continuous phase and used to emulsify the external phase under high pressure homogenization. Thereafter, an initiator for polycondensation soluble in the continuous phase is added to initiate polycondensation. Acids are normally added to reduce pH and trigger polycondensation which leads to crosslinking and the deposition of crosslinked wall material round the oil drops [66]. Material used, stirring speed, pH, and curing temperature are some of the factors for optimization. Urea-formaldehyde and melamine—formaldehyde are well known examples developed with this method. Ishizuka and colleagues [67] recently prepared microcapsules by this technique with an amphiphilic macro RAFT wall material they synthesized. Their procedure eliminated the use of toxic solvents. The wall monomer was introduced into the rice bran oil continuous phase which was then emulsified with the aqueous phase containing sodium chloride in a shirasu porous glass membrane.

The crosslinkers, ethyleneglycoldimethacrylate, was added to the formed emulsion to bring about polymerization [67].

Emulsion polymerization: In this procedure, the core material is dissolved in a surfactant. The monomer solution is then added to it dropwise.

Dispersion polycondensation: In the category, all the components including the monomer, the dispersant and the initiator are present in a solvent in which the polymer to be formed is insoluble. Here, swelling of the polymer occur leading to growth of microcapsules which is sustained by continued addition of monomer and oligomer [68]. Jiang and colleagues [69] used this method to prepare a core shell for site specific delivery of a small molecule, doxorubicin and a protein drug, TRAIL, for cancer therapy.

Suspension polymerization: In this approach, the monomers used are insoluble in the continuous phase hence, they are dispersed as liquid droplets, in the aqueous phase, in the presence of a stabilizer using high pressure homogenization. The polymer is obtained as dispersed solid in the continuous phase. Racoti and coworkers [70] recently used suspension polymerization for the microencapsulation of ginger oil in polymethyl methacrylate shell using triethyleneglycol dimethachrylate as a monomer and Azobisisobutyronitrile (AIBN) as initiator. Their study showed that particle size increased with initiator concentration while encapsulation efficiency decreased with increasing oil concentration.

4.3 Physicochemical techniques

The physicochemical techniques discussed here are classified as chemical methods by some authors. However, they are classified as physicochemical techniques here because each technique involves one or two physical steps. Such techniques are solvent evaporation, coacervation, layer by layer deposition and liposomes.

4.3.1 Solvent evaporation

The first step is the dispersion of the core material in the coating solution to form an oil-in-water emulsion. The mixture is then homogenized in the presence of stabilizers such as polyvinyl alcohol (PVA), tween 80 and span 80 to obtain appropriately sized microcapsules. The last step is to evaporate the solvent off either at ambient or elevated temperatures depending on the solvent. For double emulsion solvent evaporation, the formed oil-in-water emulsion is emulsified again, homogenized before solvent evaporation [71]. The type of emulsion chosen will be dependent on the lipophilicity or hydrophilicity of the core material. Double emulsions of the w/o/w type are usually used for highly hydrophilic materials in order to improve their encapsulation efficiency and limit their diffusion out of the capsule into the continuous phase of oil-in-water emulsions [71]. Another approach that has been used for hydrophilic payloads is the suspension in organic phase template [72]. Solvent evaporation is the common method for preparing nanoparticles. Hoa and coworkers [73] prepared PVA stabilized ketoprofen loaded Eudragit E100-Eudragit RS nanoparticles using the solvent evaporation method. They studied effect of process and formulation parameters on the properties of the nanoparticles. They confirmed that the size and morphology of the particles depended on polymer and surfactant concentration, power and duration of applied energy, and volume ratio of water to oil phases. More recently, Jiang and colleagues [74] developed nanoparticles of Ginkoglide using solvent evaporation method. Likewise, Urbaniak and Musial [72], using solvent evaporation technique, prepared submicron sized capsules from lamivudine conjugated poly-ε-caprolactone polymer and studied the influencing parameters such as concentration and type, homogenization time and

rate on the particle size. Surfactant concentration and homogenization rate were identified as the most important factors affecting particle size. Solvent evaporation method is advantageous in that it limits the use of toxic solvents, proceeds rapidly yielding particles in the size range of 10–100 nm.

4.3.2 Nanoprecipitation

This technique also known as solvent displacement technique was patented by Fessi in 1989 [75] for making nanospheres and nanocapsules. It has close resemblance to solvent evaporation technique. Here, the solvent phase containing the film forming polymer, and the drug to be encapsulated is a water miscible solvent such as acetone or methanol, and the non-solvent phase which is a water immiscible solvent such as chloroform or dichloromethane, also called the oil phase, are mixed under stirring. Thereafter, the solvent is removed to yield nanoparticles suspension or nanocapsules if a mineral oil was added. Centrifuging and freeze drying will yield the powder. Chitosan, starch, and gelatin are among the commonly used natural polymer film formers. Many studies have tried to analyze the difference in nanoparticles generated by solvent evaporation and solvent displacement. Hernández-Giottonini and colleagues [76] evaluated the effect of process parameters and formulation parameters on polylactic-co-glycolic acid (PLGA) nanoparticles prepared by both techniques. While particle size was dominantly affected by PLGA and PVA concentrations for the nanoprecipitation method, solvent fraction had the most effect of the particle size for the solvent evaporation technique. However, the influence of agitation speed in both techniques was the same- a decrease in average particle diameter [76].

4.3.3 Coacervation

This technique involves the phase separation of one or more hydrocolloids from its initial solution brought about by changes such as pH, ionic strength, temperature, solvent type or polarity and the subsequent deposition of the separated coacervate on the core droplets in the solution [77]. The lower particle diameter obtainable from simple coacervation is 20 μm while that for complex coacervation is 1 μm; and 500 μm capsules are also possible from both [33]. Generally, the first step in any coacervation process is the dispersion of the oil phase in the solution of the hydrocolloid (formation of oil-in-water emulsion). The next step involves the precipitation of the hydrocolloid by temperature, polarity, pH, or ionic strength change (polyelectrolyte complex formation). This is usually achieved by addition of a salt such as sodium sulphate, or desolvation with water miscible non-solvent, in simple coacervation [78]. Induction of polymer-polymer gelling by addition of a second oppositely charged hydrocolloid happens only in complex coacervation. The resulting complex is stabilized by cross-linking (usually glutaraldehyde, transglutaminase, calcium ions or tripolyphosphate) and the harvested microcapsules washed and dried. Complex coacervation is advantageous due to the high loading of payload up to 99%. From the relatively simple and early use of pork skin gelatin and gum arabic, many other variations have emerged including patented deviations. Majority of the polymers used are natural polysaccharides such as starches, maltodextrins, and gum arabic; and proteins such as albumin, gelatin, and casein; and lipids such as diglycerides [77].

Brito de Souza and coworkers [79] used complex coacervation as a tool to protect the phenolic compounds and mask the astringent taste of spray dried hydrophilic proanthocyanidins-rich cinnamon using a combination of various polysaccharides and gelatin as the coacervate wall material. They also evaluated the stability of the microcapsules under various storage conditions. Their study showed that

gelatin/k-carrageenan and gelatin/cashew tree gum were exceptional in maintaining the stability of the microcapsules as wall material. Complex coacervation using these combinations enabled the efficient use of proanthocyanidins-rich cinnamon extract in ice cream formulation while keeping the taste masked [79]. Lemos and colleagues [80] evaluated the effect of homogenizing speed and the hydrodynamics int. coacervation medium on the carotenoid rich Buriti oil microcapsules formulated using gelatin-alginate wall material. They found that as the Reynold number increased beyond 70,000, the particle size reduced to 200 μm. With about 80% encapsulation efficiency, the hydrodynamic conditions affected the particle size of the complex coacervates [80].

4.3.4 Layer by layer (LbL) deposition

This encapsulation technique is a straightforward versatile technique that involves the serial alternate deposition of oppositely charged polyelectrolytes films on a colloidal particle used commonly as a sacrificial template that is later eliminated or calcined. The technique permits the assembly of different compounds that interact through primary electrostatic interactions, though other bonds such as dipole–dipole moment, hydrogen bonds, host-guest interaction, acid–base interaction, and Van der Waals forces are possible. In the preparation of LbL capsules, layers have been deposited by dipping, spraying, and spin coating with the polyelectrolyte [81]. It is important to wash with distilled water after each layer deposition to minimize cross contamination by polyelectrolytes. Parameters that require monitoring include number of deposition cycles, ionic strength, pH, polyelectrolyte concentration to tune the thickness, roughness and porosity of the product [82]. Both nanocapsules and microcapsules can be prepared by this technique. The availability of wide permeability coefficient spectrum permits tuning to achieve specific application targets which could be biosensing, drug delivery, bioreactor, or biogenic application. With careful selection of the layering material, and assembly conditions final properties of the capsule can be determined. A major drawback is the lengthy fabrication process though this has drastically been reduced by the spraying approach [81]. Piccinino and colleagues [83] prepared micro- and nanocapsules of mixed polyphenols, tannic acid and sulfonate lignin using Manganese carbonate ($MnCO_3$) and organosolv lignin nanoparticles as a template and polydiallyldimethylammonium chloride and chitosan as supporting layers. The prepared nano and microcapsules displayed good antioxidant activity and photo-shielding and electrochemical responsiveness that was higher than that possible from the individual homopolymers. Rochín-Wong and coworkers [84] recently developed a LbL assembly of two natural polymers, κ-carrageenan and chitosan on diflunisal nanoemulsion droplets with the aim of studying the release properties. They reported the formation of stable 300 nm-sized particles that demonstrated controlled released of diflunisal in proportion to the number of adsorbed layers. Paşcalău and colleagues [85] recently developed Sorafenib nanocapsules using the LbL deposition. They initially co-precipitated bovine serum, BSA, with the sacrificial calcium carbonate ($CaCO_3$) porous templates to form the BSA gel core microtemplate. The microtemplate was then coated Ca^{2+} crosslinked hyaluronic acid hydrogel and subsequently alternated with chitosan in a multilayer assembly. Subsequently, the sacrificial template was removed through a semipermeable membrane and the BSA thermo-gelled. The sorafenib was then loaded into the microcapsule by diffusion to yield a delivery system that was thermo-responsive.

4.3.5 Supercritical fluids (SCF)

This technique involves the use of SCFs which are substances that exist above their critical temperature and pressure (at this point they exist in single phase and exhibit properties of both gases and liquids) and therefore can diffuse through solids

like a gas would and dissolve solids and liquids like a liquid. They exhibit densities close to that of a liquid but with viscosities and diffusion coefficients like that of a gas as shown in **Figure 8**. Supercritical carbon dioxide is the commonly used SCF for encapsulation because it is cheap, easily available, inert, non-toxic, uninflammable, with low critical temperature of 31.06°C. The technique may involve supercritical CO_2 ($SCCO_2$) as a solvent, antisolvent, co-solvent, or nebulizer. The process generally involves dissolution of both the core material and wall material in the SCF. Then, the solution is released through a small nozzle and the rapid reduction in pressure caused desolvation and deposition of polymer material shell on the core.

Approaches using $SCCO_2$ as a solvent involves rapid expansion of a supercritical solution (RESS) as shown in **Figure 9**. In this process, components are dissolved in $SCCO_2$ and the solution is then released into a collector through tiny nozzles at atmospheric pressure. The rapid decrease in pressure brings about the desolvation of $SCCO_2$

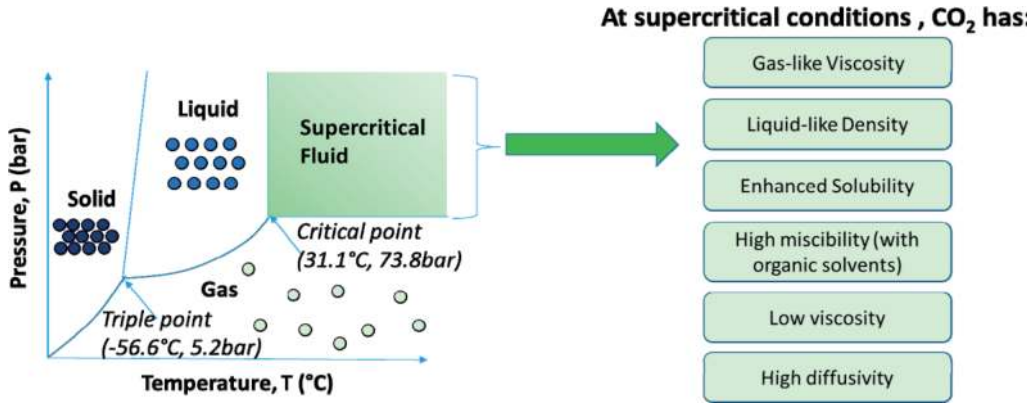

Figure 8.
Phase diagram of $SCCO_2$ (not to scale) [86].

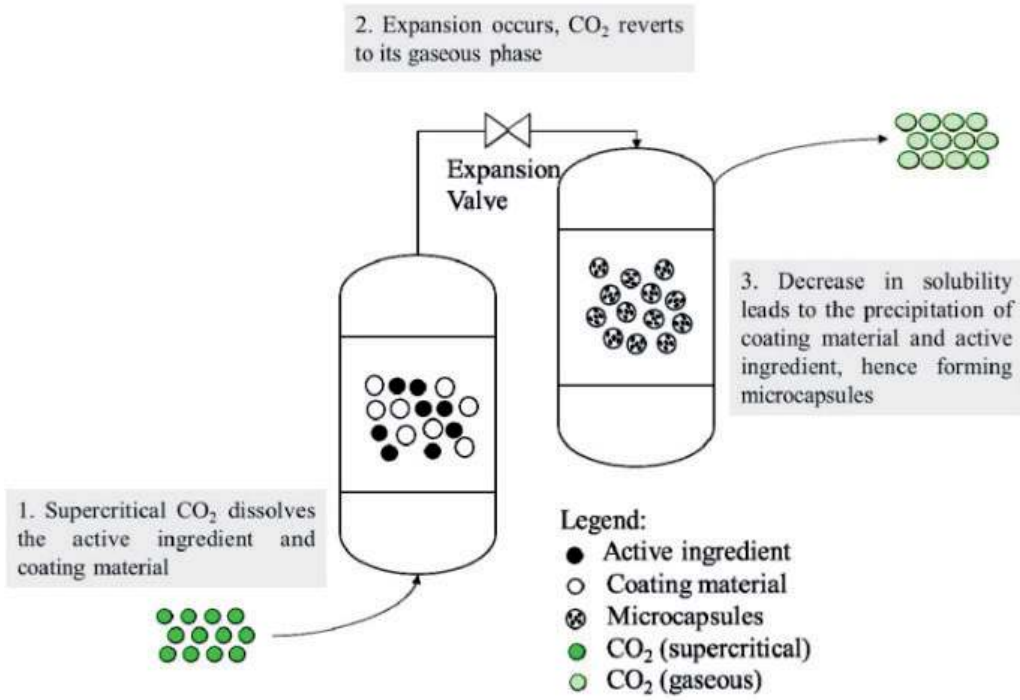

Figure 9.
Schematic representation of the RESS process [86].

and the solution components deposited as submicron particles. The major setback is that many polymer materials, fats, and encapsulants are poorly soluble in $SCCO_2$.

Another approach is the antisolvent gas, GAS approach (**Figure 10**). In this approach, the components of microcapsules are dissolved in a suitable (primary) organic solvent and then introduced into $SCCO_2$ which reduces the solubility of the components in the organic solvent. $SCCO_2$ does this by rapidly permeating through the solution due to its high diffusion coefficient effecting a mass transfer process that is evidenced by increase in volume, decrease in viscosity and density. Decrease in density significantly reduces the solubility of the components in the solvent, producing a supersaturated solution from which the components precipitate as micro and nano particles. The solutes used in this process must have minimal solubility in $SCCO_2$. The GAS technique is advantageous for the encapsulation of polar compounds and compounds not soluble in $SCCO_2$ using organic solvents may leave worrisome traces in the capsules.

The third approach is particles from gas saturated solution, PGSS, (**Figure 11**) that depends on the high solubility of $SCCO_2$ in materials such as molten fats, lipids and polymers at relatively low pressures, and the cooling effect of depressurization (Joule- Thompson effect). In this procedure, $SCCO_2$ is introduced into a substrate, its suspension or solution in an organic solvent at high pressure. The resulting saturated solution is rapidly expanded through a tiny nozzle using moderate pressure which leads to reduction in temperature and the formation of particles due to the cooling effect. Zhu and colleagues [87] encapsulated menthol in beeswax using the PGSS.

Equipment required for SCF encapsulation include a compressed $SCCO_2$ cylinder, two high pressure liquid pumps for $SCCO_2$ and the other solvents, high pressure chambers, product separation units, liquefying units, recirculating pumps, manometers, in-line filters, thermocouples, and a host of others. Parameters that needs to be optimized for each application include temperature, pressure, and feed emulsion rate. Karim and coworkers [88] used the GAS process to microencapsulate fish oil using a semi synthetic polymer, ethyl cellulose.

4.3.6 Sol: Gel technique

Translated literally means solution gelling and basically refers to an encapsulation method involving solutions (sol) that transform to a gel in response to alternating

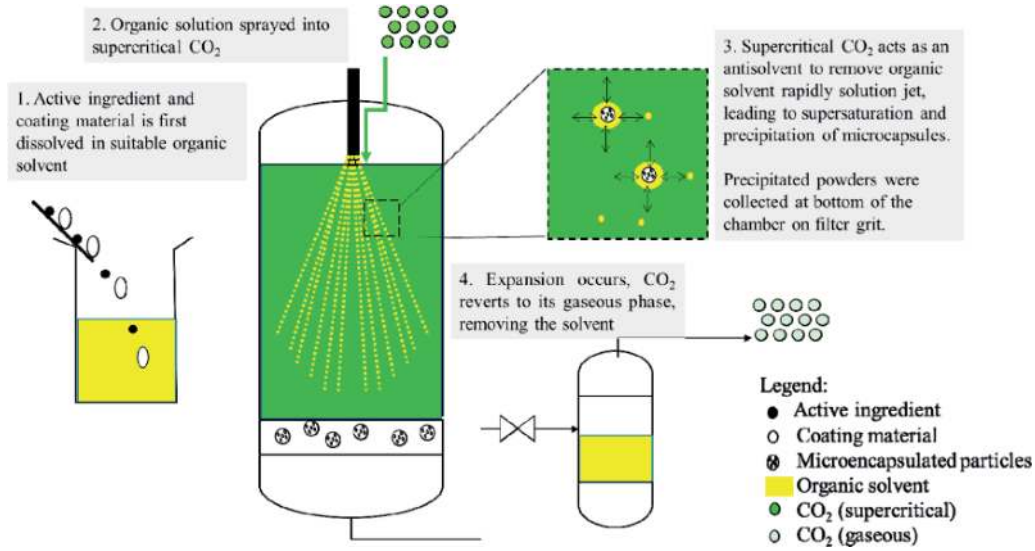

Figure 10.
Schematic representation of the GAS process [86].

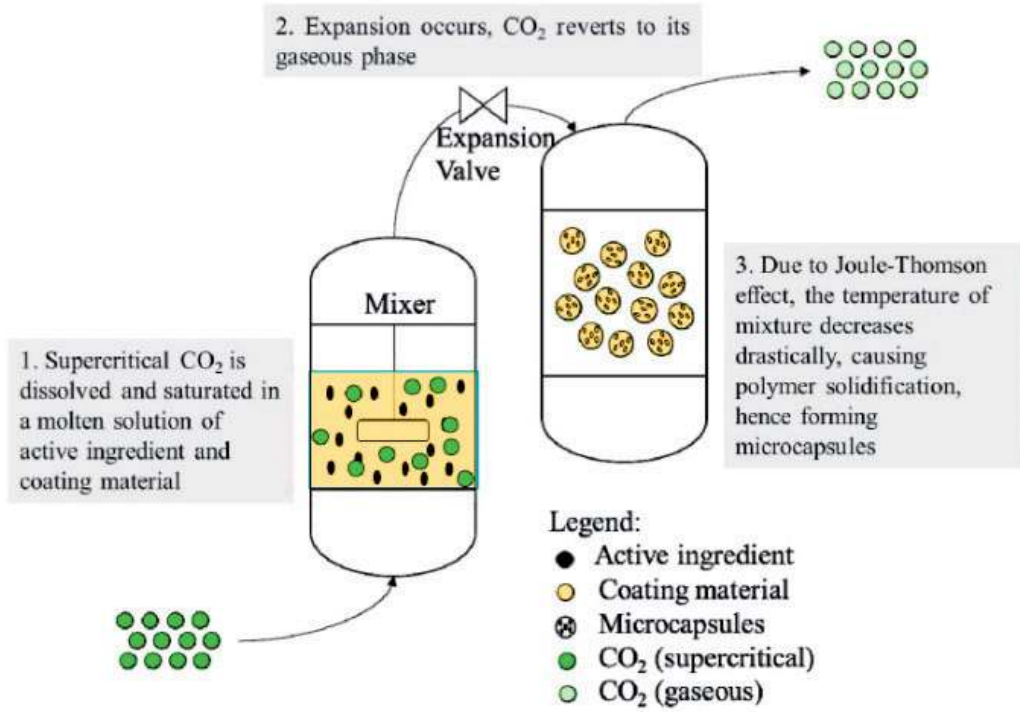

Figure 11.
Schematic representation of the PGSS approach [86].

physicochemical changes. In this process, the sol precursor is added to water which gels or hardens to capsules enclosing any included cargo. Generally, the steps involved are hydrolysis, condensation, gelling, aging, drying and densification. Organosilanes are the most commonly used encapsulation sols in contemporary times [89]. Their properties including surface functionalities, biocompatibility with many drugs and biomolecules, mild processing conditions of temperature and pH. This technique is commonly used for encapsulation of biomolecules, enzymes, and drugs. The alkoxysilanes precursors such as tetraethyl orthosilicate, triethoxysilane, trimethoxysiliane, methyltrimethoxysilane, tetraethylorthosilicate are insoluble in water so their dispersion in water in the presence of a surfactant and possibly a hydrophobic cargo results in the formation of emulsion droplets that serve as templates from which hydrolysis, condensation and polycondensation occurs at room temperature in the presence of water and an acid to form silanol groups which subsequently condense at basic pH to form organosillane matrixes or cages of different porosity and size ranging from 1 to 40 μm [90]. The general equation is given in Eq. (1).

$$\text{Precursor} + \text{Si(OR)}_4 + 2\text{H}_2\text{O} \rightarrow \text{Active} + \text{SiO}_2 + 2\text{ROH} \quad (1)$$

One byproduct of that reaction is ethanol which acts as a preservative for enclosed biomolecules. The matrix can be dried to form xerogels and can serves as a container for the enclosed biomolecule since there is no covalent relationship between them. Although rotation and unfolding movements are restricted for proteins, their inclusions can still be detected in appropriate setting by the target receptors.

4.3.7 Liposomes

Liposomes are lipid bilayer phospholipid vesicles with diameters ranging from 25 nm to 10 μm. They form spontaneously when disrupted in water. They can

encapsulate polar materials in their core while keeping hydrophobic materials in their lipid bilayer. Liposomes are traditionally made by the film hydration method with constituents like lipid, cholesterol, and solvent. Film hydration involves the dissolution of the lipid components in a suitable solvent most commonly ethanol and chloroform. The solvent is removed in a rotary evaporator leaving behind a thin film which is rehydrated to yield large multilamellar vesicles liposomes. The size of the liposomes can be reduced by passing through successively smaller sized polycarbonate filters. Ultrasonication method of preparing liposomes involves an aqueous dispersion of lipids using a strong sonicator probe and usually yields small unilamellar vesicles. Reverse phase evaporation is another method for liposome preparation [91]. In this method, a mixture of lipids and cholesterol dissolved in an appropriate solvent is subjected to the rotary evaporator for solvent removal. The residue is dried with hydrogen and resuspended in an organic solvent usually diethyl ether. An aqueous solution of the drug to be encapsulated is added to the lipid solution and sonicated under nitrogen until a homogenous mixture result. The solvent is then removed to yield large unilamelar vesicles usually used to encapsulate large molecular weight biomolecules. Ether vaporization method involves a mixture of lipids dissolved in an organic solvent such as ether and subsequently injected into a hot aqueous solution resulting in osmotic liposomes [92].

Major instability issues with liposomes is related to hydrolysis, oxidation, aggregation, and fusion. Appropriate buffer inclusion is necessary to limit oxidation of liposome phospholipids. Freeze drying has also been used to overcome the effect of temperature on liposomes. Such proliposomes are then reconstituted in water just before use. Research by Gomez and coworkers [91] showed that the encapsulation efficiency of any liposome preparation depend on the encapsulated molecule.

4.3.8 Molecular inclusion complexes

Inclusion complexes are microcapsules made by including a material to be encapsulated into the cavity of cyclodextrin molecule. Cyclodextrins are a family of cyclic oligosaccharides made up of glucopyranosyl linked by α (1,4) bonds. The most common members of the family are α-, β-, and γ- cyclodextrins consisting of 6, 7, and 8 glucopyranose units respectively. The most frequently used is β-cyclodextrin. The unique nature of a cyclodextrin molecule with a hydrophobic cavity enclosed by a hydrophilic container makes them targets for encapsulation of hydrophobic molecules. They serve as host to a great variety of hydrophobic compounds. Materials are enclosed into their cavity through different means.

Physical mixing through a kneading action of a solution of guest molecule with a slurry of cyclodextrin. The kneaded paste is dried and washed with a solvent. This is usually reserved for very poorly soluble materials and unsuitable for large scale production. In co-precipitation method, the guest molecule is dissolved is a suitable organic solvent such as diethyl ether, chloroform. Then, an aqueous solution of the cyclodextrin is added under agitation. The complex formed is precipitated out of solution using temperature reduction. The crystals are collected, washed with organic solvent and dried at 50°C. This method is usually reserved for payloads not too soluble in water [93].

Heating can also be used for inclusion complex formation. For this procedure, the physical mixture of the guest and the host can adsorb water and thereafter is heated in an enclosed vessel at a temperature of 40–145°C. This process yields crystalline complexes but can only be used for payloads stable at such temperature range [93]. Freeze drying is usually reserved for heat labile water-soluble cargoes. The required quantities of both guest and host materials are dissolved in water with stirring and then freeze dried. The obtained crystals are then washed with an organic solvent and dried in vacuum. This method is scalable and gives good yields [93].

Spray drying has also been used to obtain host-guest complexes. The host and guest molecules are dissolved in deionized water under agitation and subsequently dried in a spray dryer. There is need to optimize the operation conditions and this process may be unsuitable for heat labile materials [93].

5. Natural polymers in encapsulation

In 1953, Hermann Staudinger was awarded the Nobel Prize in chemistry for demonstrating the existence of "*Makromoleküle*" macromolecules which led to the birth of the polymer chemistry field [94]. In the past 50 years, various natural, synthetic and semi-synthetic polymers have been investigated for developing diverse nano-, micro-, and macroscale drug delivery system (DDSs) for various therapeutic and diagnostic applications [94–96]. Natural polymers along with their derivatives (semi-synthetic polymers) are the safest micro- and nanocarriers due to their low toxicity, biocompatibility and intrinsic biodegradability by enzymes [97, 98]. This section highlights the main types of natural polysaccharides, proteins and lipids that have been employed as nanocarriers for therapeutic and theranostic applications.

5.1 Polysaccharides

Polysaccharides are the most abundant natural biopolymers derived from diverse bioresources, **Figure 12**. Polysaccharides are different from proteins, nucleic acids,

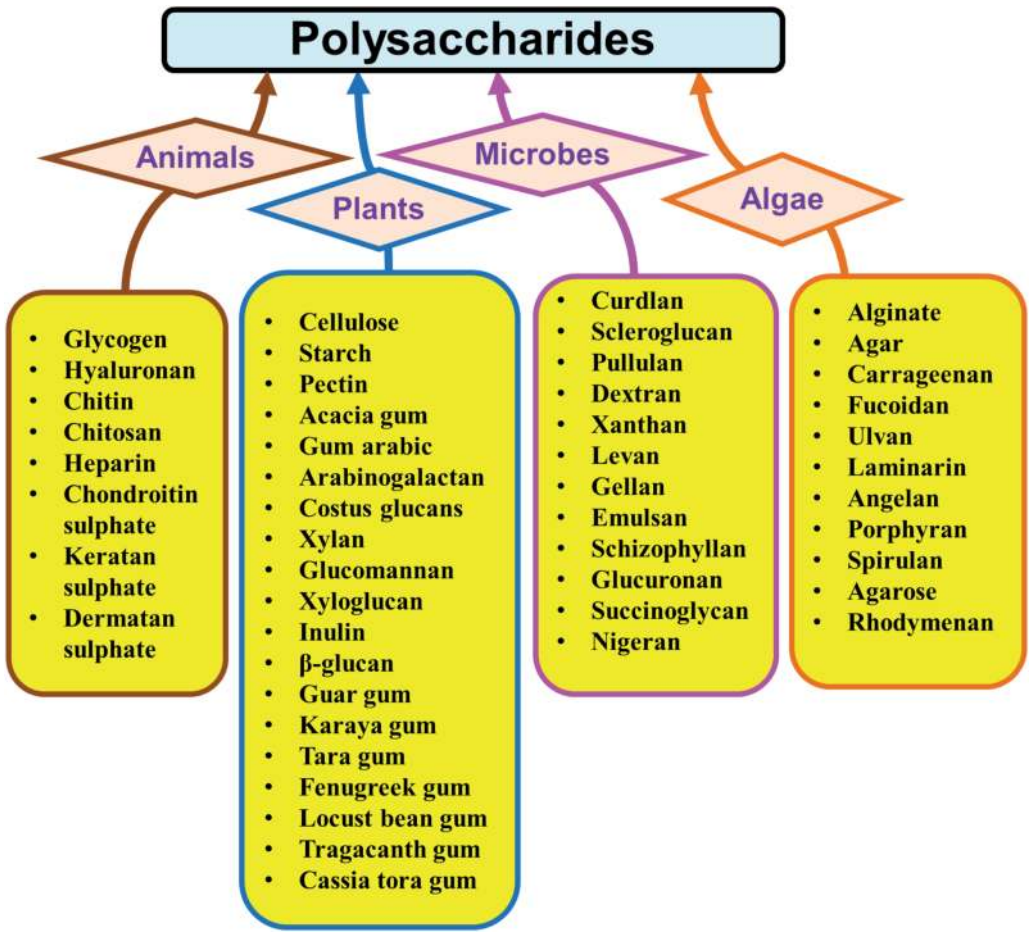

Figure 12.
Classification of polysaccharides based on their origin [100].

glycoproteins and glycolipids, in that they contain repetitive structural features [99]. Polysaccharides have been employed as responsive nanocarriers for targeted and controlled gene delivery and drug delivery of small molecules, proteins, peptides, nucleic acids, and antibiotics [100]. Among various polysaccharides, cellulose is the most abundant renewable natural polymer on earth, which is unbranched, linear homopolysaccharide, composed of repeating β-(1 → 4) linked D-glucose units [101]. However, since cellulose is water-insoluble, various water-soluble and hydrophilic cellulose-based derivatives have been used for creating macroscale DDSs and devices for oral drug delivery to the gastrointestinal (GI) tract due to their good compression characteristics and adequate water-swelling property which allows for controlled release drugs through rapid formation of an external gel layer [102]. Examples of commercialized macroscale DDSs based on cellulose acetate and hydroxypropyl methylcellulose (HPMC) are shown in **Figure 13** (I, II). The details about the former DDSs/devices and the mechanisms of drug release are described by Abu-Thabit and Makhlouf [94].

Another important polysaccharide is starch. Starches are made from 300 to 1000 glucose monomeric units. The main components of starch are amylose (~20%) and amylopectin (~80%) macromolecules. Amylose is unbranched, linear homopolysaccharide, composed of repeating α-(1 → 4) linked D-glucose units. Amylose adapts helical structure due to the formation of hydrogen bonding among D-glucose monomeric units. The helical conformation of amylose provides room to accommodate the iodine molecules in its core, and results in the formation of iodine-amylose complex with the characteristic blue-violet color as a strong indication for the presence of tiny amounts of starch. Amylopectin is a branched polysaccharide that is composed of repeating

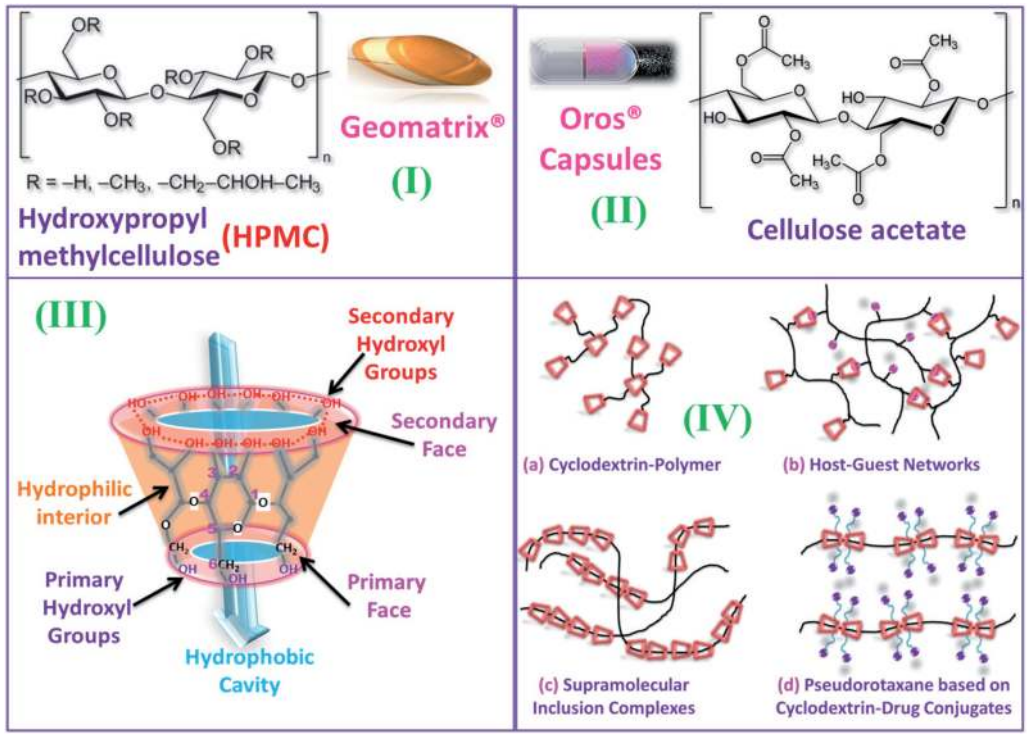

Figure 13.
(I) And (II) represent the chemical structures and examples of macroscale-based DDSs using HPMC, and cellulose acetate; reproduced with permission from ref. [94]. (III) schematic illustration of (a) structure of cyclodextrin polymer; diversity of using cyclodextrins for drug delivery systems via (b) host-guest interactions; (c) formation of supramolecular inclusion complexes (e.g. with PEG); and (d) cyclodextrin-drug conjugates; (IV) conventional representation for native cyclodextrins (CDs) as a truncated cone with "hydrophobic" cavity (blue color) that can accommodate hydrophobic drugs; reproduced with permissions from ref. [103].

α-(1 → 4) linked D-glucose units with occasional α-1,6-glycosidic bonds, which are responsible for the branching. The helical structure of amylopectin is disrupted by the available branched side chains which yield less intense reddish-brown color for the formed amylopectin-iodine complex instead of intense blue-violet color. Another class of polysaccharides nanocarriers is cyclodextrins (CDs) which are crystalline cyclic oligosaccharides consisting of α-1,4-glycosidic bonded D-glycopyranose units with glucose units arranged in a donut shape ring [103]. CDs are produced by enzymatic degradation of starch. CDs are classified as cage molecules with hydrophilic exterior and hydrophobic inner cavity which enables the formation of inclusion complexes with a variety of hydrophobic drug molecules [103], **Figure 13** (III). CDs are categorized based on the number of glucose residues in their structure, for example, CD with the glucose hexamer is named as α-CD, the heptamer as β-CD and the octomer as γ-CD [104]. The designs of supramolecular systems with CD are very diverse; since CDs can be used alone, grafted to other molecules or linked to each other [105], **Figure 13** (IV).

Chitin is the second most abundant natural polysaccharide which can be described as cellulose with one hydroxyl group on each monomer replaced by acetyl amine group. Chitin is abundant in invertebrates, mollusks, the cell walls of fungi, and the exoskeletons of arthropods. Like cellulose, chitin is a hydrophobic and water-insoluble biopolymer with limited application for fabricating DDSs. Chitosan, which is prepared by alkaline or enzymatic hydrolysis of chitin, is considered as the most important derivative of chitin due to its biocompatibility, biodegradability and non-toxic nature [106]. Unlike most anionic polysaccharides, chitosan is classified as a cationic polymer due to the presence of amine group which can be protonated upon dissolving chitosan in dilute acidic solutions such as acetic acid or hydrochloric acid. This unique character allowed chitosan to be used for fabricating various DDSs, such as micro/nanoparticles and hydrogels, *via* formation of polyelectrolyte complexes with various anionic polysaccharides [107, 108]. Another naturally occurring linear polysaccharide is hyaluronic acid (HA) (also called hyaluronan) which is composed of D-glucuronic acid and N-acetyl-D-glucosamine disaccharide. HA acts as an anionic polyelectrolyte at neutral pH, as the pKa of the carboxylic acid groups is ≈ 3–4, which makes HA highly hydrophilic and superabsorbent for water, with ability to expand up to 1000 times its solid volume, leading to loose, hydrated network [97]. Dextran is a water-soluble branched polysaccharide with varied chain lengths and molecular weight in the range of 30,000 – 2,000,000 g/mole [106]. In 1861, Louis Pasteur isolated dextran from wine as a microbial product [109]. Dextran has been applied in nanomedicine, a novel discipline that applies submicron particles for therapeutic and diagnostic purposes. Dextran has been applied in nanomedicine as encapsulating matrix for therapeutic and diagnostic purposes [94], and as an alternative to PEGylation to avoid nanoparticles (NPs) [95] and opsonin interactions [110]. Dextran has been employed for preparing pH-sensitive NPs by polyelectrolyte complexation between dextran sulfates and chitosan [108, 111].

5.2 Proteins

The term protein *was coined in 1838 by the* Swedish chemist Jöns Jacob Berzelius, which was derived from the Greek *prōteios*, meaning "holding first place" [112]. Proteins are versatile biomacromolecules with large and diverse functions in living organisms such as transcription, translation, transport and metabolism [113]. Proteins are key class of biopolymers that have been extensively used as nanocarriers for therapeutic and diagnostic drug delivery applications [114]. Proteins can be classified based on their origin as plant-based proteins and animal-based proteins, **Figure 14**. Detailed description and characteristic for each protein type is provided in the next chapter on proteins. This section provides brief idea about animal-based proteins including gelatin, casein, and albumin.

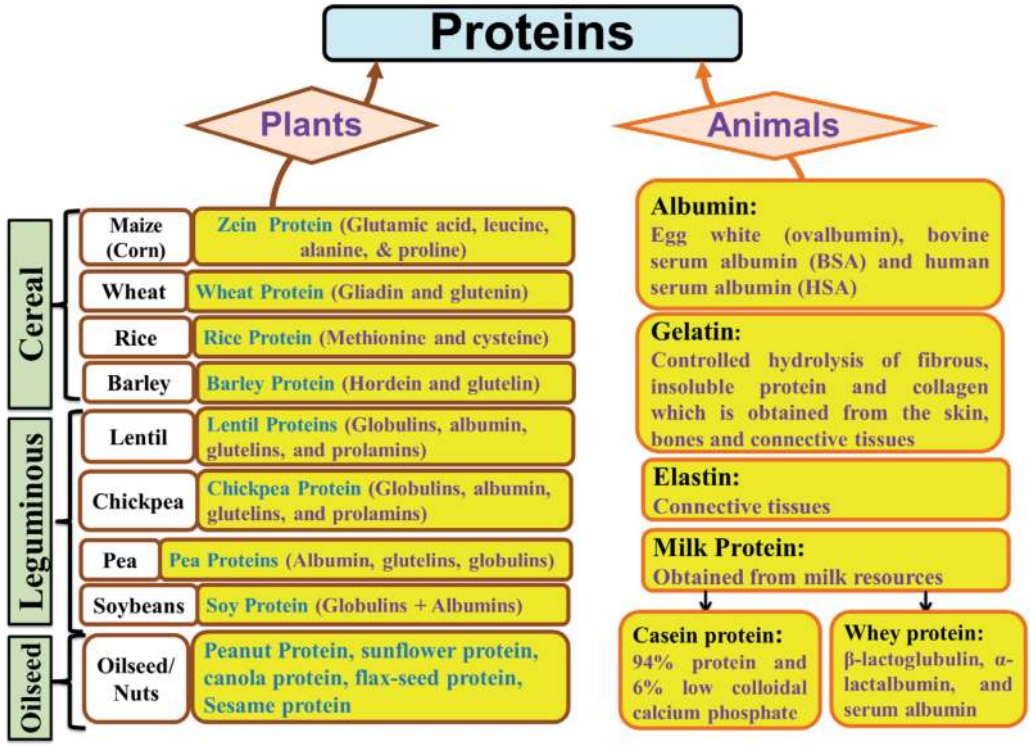

Figure 14.
Classification of proteins based on their origin with the list of the main components.

Collagen is the most abundant protein in mammals which forms 30% of all vertebrate body protein with a majority in bone and skin. Gelatin is a denatured collagen which is obtained by either acid hydrolysis (gelatin type A with isoelectric point ≈ 7–9), or alkaline hydrolysis (gelatin type B with isoelectric point ≈ 4.8–5) [115]. Gelatin is biocompatible and biodegradable with high physiological tolerance and low immunogenicity. Gelatin is classified as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA). Therefore, gelatin have been used for vitamin preparation, drug capsules, scaffolding materials to promote cell migration, wound healing, tissue regeneration and as a nanocarrier for drug and gene delivery [106, 115]. Casein and whey proteins are important protein sources for human nutrition. Casein is one of the oldest natural polymers, used for adhesives, dating back to thousands of years [116]. In contrast to whey proteins, caseins are water-insoluble and account for 80% of total bovine milk proteins. Casein protein is found in milk which serves biologically to transfer nutrients from mother to her offspring. Hence, it can be used as a carrier depot for delivery of drugs. Casein has four constituent phosphate-rich sub-units, which are amphiphilic and self-assemble into a micellar structure in the size range 50–300 nm, held together by calcium phosphate nanoclusters acting as bridges connecting these subunits [117]. Although the word "albumin" is usually associated to serum albumin, it is also employed to describe a family of proteins characterized by their solubility in water [114]. Human serum albumin (HSA) and lactalbumin (known as whey protein) are the most popular albumin proteins employed for drug delivery applications [114]. Besides that, albumins can be found in foods, particularly in seeds and nuts. Serum albumin is the major protein constituent in the blood plasma of all vertebrates. The two main exponents are human (HSA) and bovine serum albumin (BSA). Albumin has diverse physiological functions such as maintaining the pH and colloidal osmotic pressure of plasma, its antioxidant effect by trapping free radicals, and its reversible binding ability to variety of important exogenous and endogenous [118, 119]. Albumin has the ability to bind with positively and negatively charged

hydrophobic organic anions such as bilirubin and long-chain fatty acids and divalent cations such as calcium and magnesium [119]. Albumin can bind to different types of compounds including drugs, bile acids, copper, zinc, and even compounds with specific serum binders such as vitamin D and thyroxin [119]. The binding feature of albumin reduces the free concentration of compounds, and hence, limiting their biologic activity, distribution, and rate of clearance [119]. Therefore, HSA is considered an ideal protein for the production of parenteral medications, which has been employed as nanocarrier for drugs, vaccines and genes delivery [120–123].

In 1972, the first protein-based nanoparticle (human serium albumin (HSA) microspheres) was prepared [124]. In January 2005, the first nanotechnology-based drug product, called as Abraxane®, was approved for treatment of metastatic breast cancer [125]. The anticancer and water-insoluble paclitaxel chemotherapeutic agent was easily encapsulated in a shell of protein albumin, where the cancer cells are tricked by the albumin coating into taking the nanospheres embedded with the active cancer-fighting paclitaxel molecules [125]. The encapsulation of paclitaxel drug inside the albumin protein biopolymer provided a harmless way for drug administration as compared to the use of toxic solvents like polyhydroxylated castor oil (Cremophor EL or CrEL), which requires patients to receive premedication for elimination of the allergic reactions and solvent-related hypersensitivity side effects [126]. The FDA approved use of Abraxane® was extended for treatment of non-small-cell lung carcinoma (NSCLC) in 2012, followed by the FDA approval in 2013 for use in treating advanced pancreatic cancer as less toxic alternative to FOLFIRINOX [94]. Gas microbubbles have been encapsulated in the elastic shell of HSA which served as ultrasound contrast agents (e.g. Albunex™ and Optison™ products) for diagnostic applications [127].

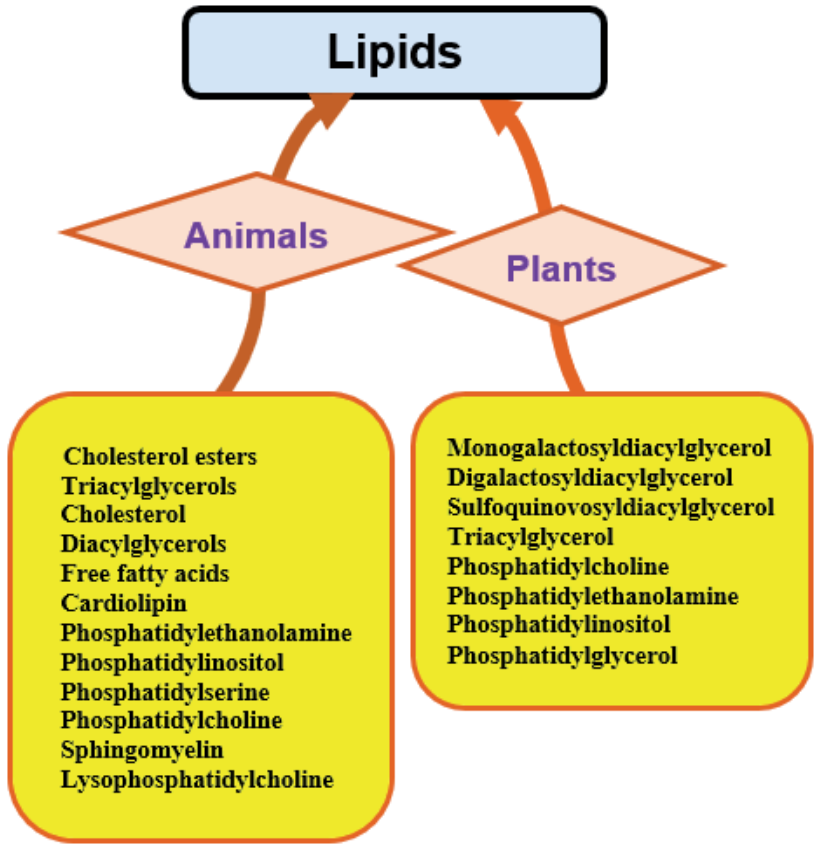

Figure 15.
Classification of lipids based on their origin.

5.3 Lipids

Lipids are heterogenous polymers of fatty acids and in nature occur as fats if solid at ambient temperature, oils if liquids at ambient temperature, fatty acid derivatives, and sterols. A major division among lipids irrespective of their categorization but pertinent for their role in encapsulation and based on polarity divides lipids into polar and non-polar lipids with all types occurring in nature. Polar lipids form aqueous phases with water and occur in nature as constituents of the cell membrane where they form a barrier between the cell and the external water environment. Except for cholesterol, polar lipids have a polar head and a long non-polar tail that aligns itself in a bilayer and include lipids such as glycerophospholipids, sphingolipids and monoglycerides. On the other hand, non-polar lipids such as triglycerides, waxes, are used as energy store and form a solvent for many lyophilic compounds during formulation. Lipids are classified based on origin as shown in **Figure 15**.

6. Lipid-based encapsulation

Lipids are a group of hydrocarbons based organic macromolecules that are rather soluble in non-polar and organic solvents instead of water. Though like carbohydrates in terms of elemental constituents of carbon, hydrogen and oxygen, they differ in containing considerably lower levels of oxygen often attached as part of a single carboxylic acid group at the end of a long hydrocarbon chain. Non-saponifiable lipids such as triglycerides, waxes and phospholipids cannot be hydrolyzed by acid or bases but lipids such as steroids, prostaglandins and terpenes are easily hydrolyzed due to the presence of ester groups. Lipid based encapsulates include liposomes, nanoliposomes, proliposomes, self-assembled micelles, nanostructured lipid carriers, Solid lipid nanoparticles, solid lipid microparticles, liquid lipid nano and micro particles, nanoemulsion, microemulsions, emulsions, nanosuspensions, lipid nanotubes, lipid-polysaccharide complexes and hybrids (**Figure 16**). Some other lipid-based drug delivery systems such as the self-emulsifying microemulsions are not included here because often, they are only encapsulated after ingestion.

A wide range of architectures can be realized depending on the nature and composition of lipids, encapsulation technique, among others. Tuning of process parameters such as pH, temperature, nature and composition of lipid, presence of other constituents such as electrolytes, buffers and sugars will usually determine the size and morphology of the resulting capsules or vesicles. The morphology could be micelles, vesicles, or bilayer sheets. Specific applications will demand specific manipulation of charge, size, pegylation, functionalization, phase transition temperature and drug loading mechanism.

Size: For applications targeting the delivery of macromolecules and tissue penetration, the required size should be below 100 nm but above 5 nm to prevent filtration through the kidney. Multilamellar vesicles are particularly good for depot and sustained release injections while small unilamellar vesicles are good for systemic injections.

Charge: Neutral vesicles usually result in long circulation time and minimal effect of the reticuloenthothelial system. Cationic vesicles however undergo aggregation due to interaction with body protein while anionic vesicles are easily taken up by the liver and spleen.

6.1 Lipids in micro- and nanoencapsulation

Encapsulation processes employed in the use of lipids for formation of micro and nano capsules are similar in principle for both nanocapsules and microcapsules.

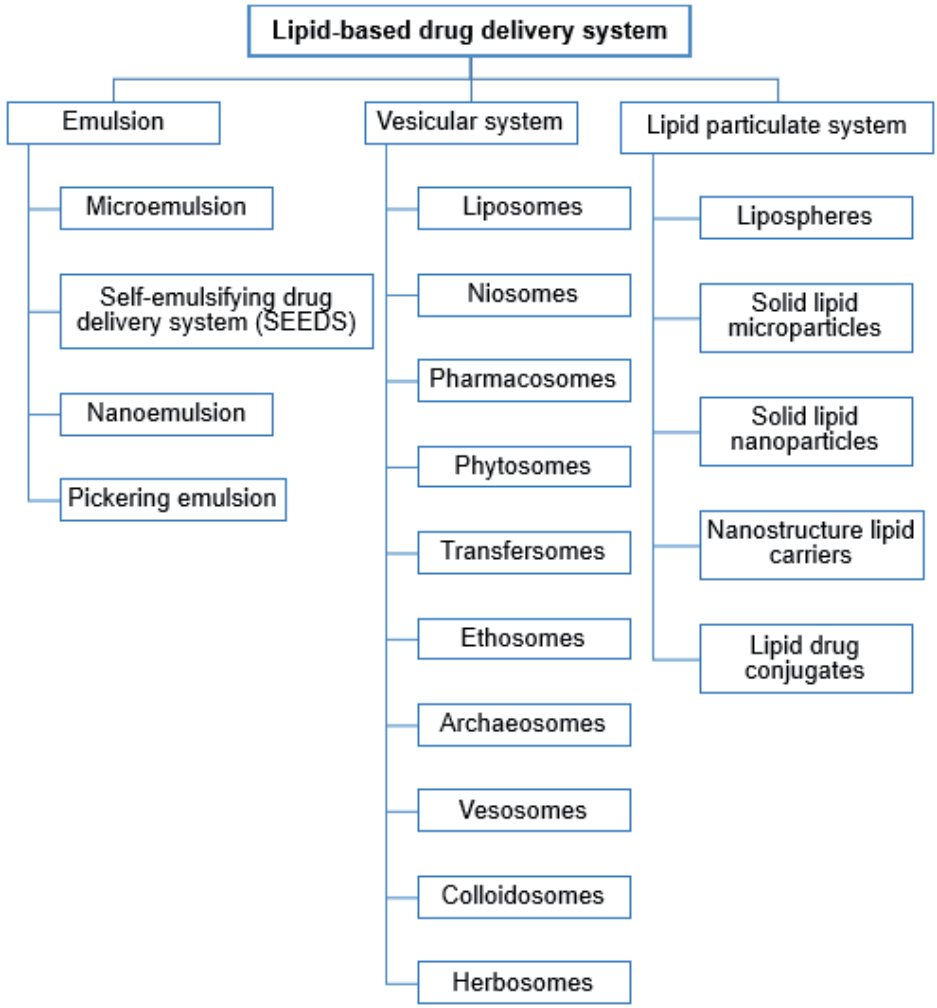

Figure 16.
Classes of lipid-based drug delivery system. Adapted from [128].

Lipids of diverse characteristics and functionalities have in contemporary times remained the focus of hope for the delivery of over 90% new chemical entities in development pipeline that may encounter bioavailability challenges due to their lipophilicity [129]. Likewise, drug delivery systems based on lipids have almost taken center stage in many pharmaceutical companies due to potential profit, both financial and otherwise, accruable to lipid-based reformulation of existing medicines.

Lipids play a major role in many encapsulation processes as either membrane/shell components, core/carrier component, water-insoluble or water-soluble surfactant or as a hydrophilic cosolvent. The type of lipid used for an encapsulation process may depend on several factors that include the target application, size range required for the application, physicochemical properties of the material to be encapsulated. Lipids involved in encapsulation vary widely depending on specific application. There are also a variety of classification system available.

Homolipids: These are also known as simple lipids and are formed by an esterification action of an alcohol with fatty acids which can be short chain (less than 6 carbon atoms), medium chain (6–12 carbons) or long chain (14–24 carbon atoms). Their elemental composition is just carbon, hydrogen, and oxygen. The constituent fatty acid chain may contain a double bond which always occurs in the cis configuration. Examples are naturally occurring glycerides such as fats and oil (coconut

oil), cerides such as waxes (beeswax and carnauba wax), and sterides such as esters of fatty acids and cholesterol.

Heterolipids or compound lipids: These contain an additional nitrogen atom or phosphorus atom. They include phospholipids, sulfolipids, and glycolipids (when conjugated with a sugar moiety). Two classes of phospholipids occur in abundance naturally and include sphingolipids such as ceramide and phosphoglycerides. They abound in nature as structural components of membranes.

Complex lipids: These include overly complex lipids such as lipoprotein (when conjugated with protein and are responsible for the transport of cholesterol and other molecules) and chylomicrons.

6.2 Merits and demerits in therapeutic delivery

Challenges abound in the drug delivery terrain particularly for new chemical entities in development pipeline majority of whom are poorly soluble molecules. In addition, better understanding of the molecular basis of diseases is yielding treatment options such as large proteins that pose challenges for delivery. For instance, proteins are easily degraded when administered even parenterally necessitating frequent administrations that contribute to patient cost, side effects and compliance issues. Moreover, often they are large molecules. At the nanosized level, most nanoparticles are easily removed from the circulation by the endoplasmic reticulum. Encapsulation in lipids can solve a great number of these issues. **Figure 17** captures

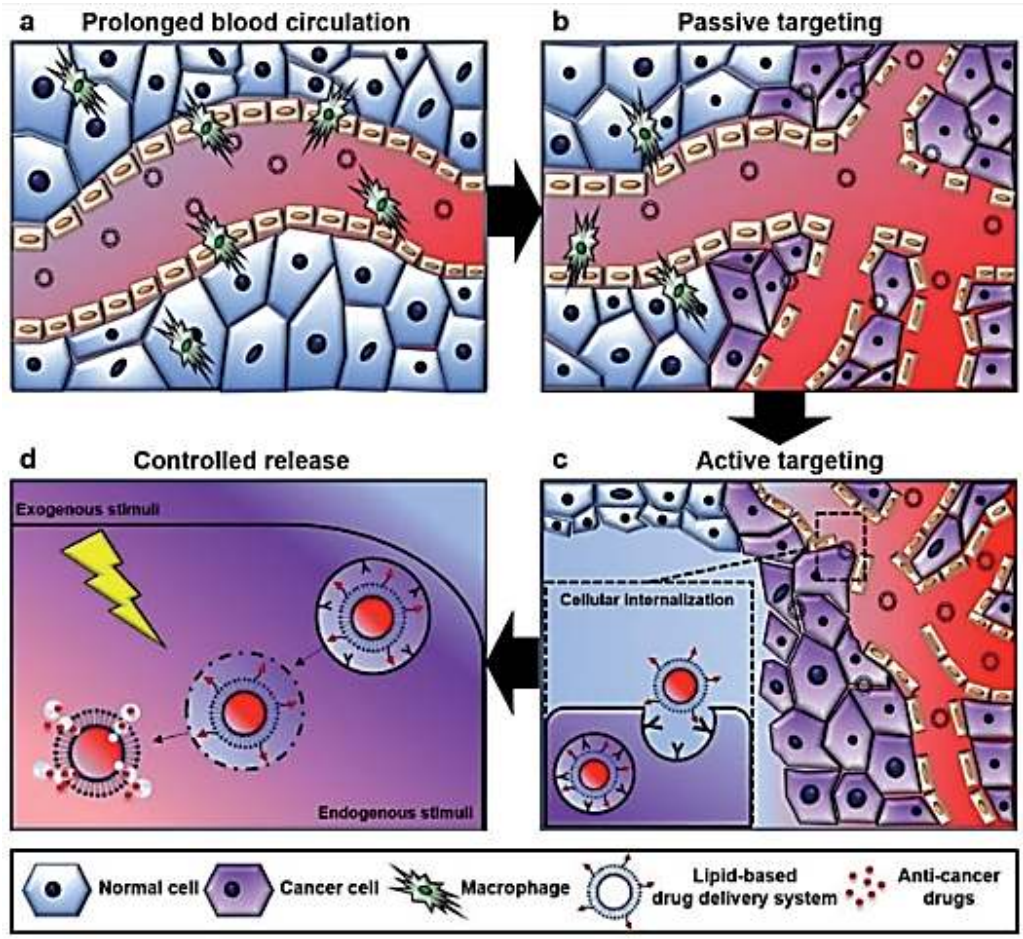

Figure 17.
Some factors contributing to efficiency of lipid encapsulation to therapeutic drug delivery [130].

some of the benefits of lipid encapsulation to drug delivery. The merits of a lipid-based system for drug delivery may vary slightly depending on the type of lipid system and the route of administration.

Modulation of bioavailability: Irrespective of the route of administration or lipid system involved, lipid-based systems have been employed to modulate rate and extent of absorption of active ingredient. They bring about an increase in surface area available for dissolution thereby increasing absorption. In oral delivery, lipid systems have predominantly been used to improve solubilization of poorly soluble solids thereby increasing bioavailability. Solubilization in lipid systems also greatly diminishes intra and inter subject variability enabling caregivers to better adjust dosing to individual needs. Lipid based delivery also reduces the hepatic first pass metabolism for susceptible drugs. The overall improvement may lead to a reduction in the required dose and a proportional decrease in the accompanying side effects and toxicities which may translate to better compliance. An example is the formulation of amphotericin B initially as fungizone with high toxicity as compared to the lipid particle formulation, Abelcet [131]. In addition, existence of areas of opposite polarity within the same systems opens the possibility of delivering 2 physically different compounds through one system.

Lipid based systems in the form of micro and nano particulate systems modulate biodistribution [132]. They are usually used to sustain drug release and target drugs to specific sites. Lipids have been used to deliver large protein macromolecules to specific sites through lipid-drug conjugates. Encapsulation in lipid bilayer membrane spares the drug the attack of the reticuloendothelial system or shield a drug from detection by the immune cells since they have similar membrane. Lipid systems improve or maintain the chemical and physical stability of the included API. They also effectively mask taste, and odor. Formulation efforts are also targeted towards stabilizing the API both during storage and from endogenous enzymes and chemicals until it arrives its site of action. Physicochemical properties of vesicles such as size, charge or surface functionalization with specific ligands can be modulated for specific tasks.

Most lipids used for encapsulation are relatively cheap, biocompatible, biodegradable and exhibit low toxicity and allergenicity. The use of organic solvents is limited in their preparation. Ease of formulation, ease of characterization, sterilization and scale up, and amenability to delivery by various routes all contribute to their versatility.

In spite of the plethora of advantages, lipid-based system has a few challenges.

Digestibility of lipids and drug leakage: Most lipids used for encapsulation and other lipid-based delivery systems are natural. Lipids therefore have a natural propensity to be digested and degraded in the body by enzymes [133]. Digestion of one or more components will break up the membrane or shell which may result in supersaturation, precipitation or dilution depending on the route of administration and may result in toxicity or loss of efficacy.

Drug loading: Passive drug loading may lead to low efficiency, but active drug loading ensures high efficiency depending on the API and the other excipients.

Uncontrolled precipitation and aggregation: many of the lipid systems are prone to physical destabilization of their membranes requiring extra effort to stabilize them. The nanocapsules are prone to aggregation which may lead to non-uniformity of doses.

6.3 Case studies/applications

6.3.1 Encapsulation of small molecules

Small molecules are low molecular weight compounds that include drugs, xenobiotics, lipids, metabolites, metal ions, monosaccharides, second messenger, etcetera. Encapsulation of small molecules using lipids predominantly aims to solubilize poorly soluble molecules, target or control release of the medicament. Lipids

being major constituents of the cell membranes can ferry included cargo through the tightly controlled formidable barrier and through various ports of entry such as the stratum corneum, the ocular cornea, parenteral or oral route.

Cantón and colleagues [134] recently reported the preparation of SN-38-β-cyclodextrin complex in solid lipid nanoparticles. The aim was to develop a delivery system that will deliver, stabilize, and protect the FDA approved drug for colorectal cancer. This was necessary since SN-38 is highly insoluble and unstable at physiological pH and easily converts to the carboxylate form that has higher binding affinity to serum and is more stable at the basic pH of the GIT. Initial attempt to include SN-38 -cyclodextrin inclusion complex to a liposome lead to the disassembly of the liposome and the formation of solid lipid nanoparticles. The lipids used were hydrogenated L-α-phosphatidylcholine, 1,2-distearoyl-*sn*-glycero-3- phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] (DSPE-PEG-biotin), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N- [folate(polyethylene glycol)-5000] (DSPE-PEG-folate) 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (DHPE-TR). Evaluation of the stability by determining the presence of the inactive form was undertaken using the size exclusion chromatography. Ultimately, stable, solid lipid particles containing the SN-38 cyclodextrin complex was prepared even though the concentration of the encapsulated drug was narrow.

Photodynamic therapy (PDT) is a treatment modality for cancers that involve the use of reactive oxygen species, photosensitizers, and light for destruction of cancer cells. However, PDT is limited by the inability of high energy light used in PDT to penetrate tissues, and the ability of the body to disperse used photosensitizers systemically [135]. Unfortunately, too, the internal tumor environment is hypoxic, the low oxygen content limiting the efficacy of PDT and being responsible for angiogenesis and subsequent metastasis within cancer cells. Therefore, to improve the outcomes of PDT, particularly for solid tumors, there is a need for the presence of oxygen generator within the tumor cells and the presence of the photosensitizer near the targeted tumor cell. Liu and colleagues [136] developed a calcium peroxide/methylene blue-loaded liposome as an oxygen generating species which targets a photosensitizer, methylene blue in PDT therapy. Many of the oxygen generated previously studied were encapsulated in hydrophobic polymers that had limited capacity for hydrophilic cargo and delayed the generation of oxygen. The use of liposomes provided a hydrophobic shell that served to carry the photosensitizer and a means to penetrate the cell membrane while carrying a hydrophilic cargo in its core. On irradiation, the phospholipid bilayer is easily disrupted causing the release of calcium peroxide which reacts with water rapidly to generate oxygen. On subsequent irradiation, the generated oxygen potentiates the effect of PDT on tumor hypoxia. The first step involved the preparation of calcium peroxide nanoparticles and further encapsulation of the nanoparticles in a pegylated liposome. Composed of DSPE-PEG, DPPC-egg lecithin, and cholesterol. In-vivo tests in a mouse model of mammary cell carcinoma demonstrated the efficacy of the system to limit hypoxia in treated animals when compared to untreated animals.

Kenechukwu and colleagues [137] prepared a lipid matrix made up of sun seed oil: Softisan® in the ratio 1:9 and PEG 4000 by a melt homogenization process for the intravaginal delivery of a poorly soluble drug, Miconazole. The concentration of PEG was varied giving rise to different formulations. The PEG content consequently affected the particle sizes, the encapsulation efficiency, and the loading capacity. The optimum concentration of PEG 4000 according to their study was 40% w/w.

Stella and colleagues [138] investigated the possibility of delivering a doxorubicin pro-drug, squalenoyl-derivative through entrapment in solid lipid nanoparticles. The highly reduced cardiotoxicity of liposomal doxorubicin catapulted the search for other lipid-based carrier systems that will also help in mitigating the resistance to doxorubicin. Squalenoyl derivative is highly lipophilic derivative that has shown capacity to form

very stable self-assembles in water. Their absorption in the body had been shown to be mediated by endogenous low-density lipoproteins. Therefore, the researchers initially prepared squalenoyl derivative self-assembly in water using the nanoprecipitation technique. The solid lipid particles were prepared by complex coacervation using fatty acids that were precipitated by acidification and stabilized with poly vinyl alcohol.

Targeting cytotoxic drugs to the tumor environment has always been both desirable and a challenge due to severe side effects to normal cells and the peculiarities of the tumor microenvironment. For instance, multi drug resistance to drugs like doxorubicin has been associated to the hypoxia encountered in tumors. In this study, Xie and colleagues [139] aimed to use methotrexate conjugated with a polymer-lipid hybrid through an imine linkage, as both a targeting moiety and as the drug targeted to the cancer cells, through the nanoplatform of self-assembled lipid micelles also incorporating curcumin. In addition, pH responsiveness and prodrug status were built into the platform using an imine crosslinker. The intent was that at a particular pH unique to the tumor environment, the acid responsive imine aldehyde linkage will be disrupted leading to the release of the active methotrexate. The methotrexate with its strong resemblance to folate will be used as a target to the folate receptors on the tumor. Curcumin, a well-known naturally occurring polyphenol with strong anti-inflammatory and antiproliferative properties was assembled into the hydrophobic core of the resulting lipid-polymer hybrid micelles to forestall drug resistance. In their study, lipid-polymers such as DSPE-PEG and DSPE-Mpeg were used. The first step was the formation of the prodrug complex, DSPE-MPEG-imine- methotrexate, by the conjugation of methotrexate to DSPE-PEG through a Schiff base reaction between the aldehyde group of the polymer and the aromatic group of methotrexate. The resulting prodrug complex was later self-assembled in the presence of the poorly soluble and unstable curcumin. One pot ultra-sonification with solvent evaporation was the method of micelle formation for both drug-loaded and unloaded micelles. The animal studies carried out on HeLA tumor bearing BALB/c nude mice demonstrated the workability of the concept and confirmed the ability of the lipid carrier system to effectively transport the curcumin and methotrexate to the tumor site.

6.3.2 Encapsulation of biologics

Biologics are large high molecular weight proteins, nucleic acids, monoclonal antibodies, vaccines, and enzymes. The delivery of macromolecular proteins is particularly challenging.

Synthetic small interfering RNA, siRNA are nucleic acid fragments that can modify the activities of mRNA when they enter the cell. Many diseases are due to certain abnormality or malefaction at the genetic level and hence the silencing of the specific mRNA can translate to cure. The problem of delivering such proteins are multiple and varied. One of such is the rapid clearance of such protein on systemic administration due to nuclease activity and renal filtration and the induction of immunogenic reactions. In addition, siRNA, and its like are rarely able to diffuse into the cell, hence requiring the complex generation of multiply functionalized systems. Patisiran is the first FDA approved siRNA formulation prepared using lipid nanoparticulate platform for delivery to hepatocytes [140]. It is a double stranded siRNA which degrades 3'untranslated region of the wild type transthyretin by RNA interference [141]. Hereditary transthyretin mediated amyloidosis is a disorder resulting from deposition of abnormal form of the protein produced in liver cells. The entrapment of nucleic acids in lipid nanoparticles require the presence of a cationic lipid to trap the negatively charged nucleic acid. Secondly, the pKa of the lipid should be such that at physiological pH, there is a net neutral charge. The lipid should also display a positive charge when in the endosome environment and finally,

it must display the hexagon shape. For proteins, there must be rational design to take into cognizance; linkers, acyl chains and the ionizable groups required.

Vascular endothelial growth factor, VEGF, has been implicated in tumor progression and metastasis. It has been shown that in cancer and some other diseases such as age-related macular degeneration, the expression for VEGF is usually upregulated to promote angiogenesis. The possibility of using RNA interference to silence genes through the interference of siRNA will be a welcome option in the battle against tumors. Important barriers to the use of siRNA is the availability of vectors, low transfection efficiency and stability. Polymeric lipids have been in the lead as a choice for siRNA delivery material. Cationic lipids are of importance due to the need for a positive charge to stabilize the negatively charged nucleic acid and for internalization. Cationic lipids however pose a problem of toxicity and may offer no protection to the nucleic acid. In Chen and co-workers' study [142], polyethyleneimine (PEI) was used for protonation of the polycation liposome while 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), was used as a destabilizer to promote the escape of siRNA into the cytosol. siRNA was conjugated to calcium phosphate nanoparticles which has shown low toxicity, biocompatibility and biodegradability in previous studies involving the delivery of DNA and siRNA. siRNA-calcium phosphate nanoparticles were first prepared and introduced as an aqueous solution into a polycation liposome prepared by the film dispersion method using equimolar concentration of DOPE and PEI-Cholesterol. The in vitro gene silencing assay was performed using human breast adenocarcinoma cell lines while animal studies were done in tumor infected BALB/C-nu female mice. The results obtained showed the superior silencing effect of the siRNA delivered through the core-shell polycationic liposome.

Antibodies can be generated as inhibitory agents to many diseases causing proteins in the cytosol. Unfortunately, this has not been effectively utilized in treatment modalities due to inability of the large antibody proteins to transverse the cytosol. Wang and coworkers [143] developed a lipid carrier for immunoglobulin G, IgG, by first conjugating it with anionic polypeptides and subsequently complexed them (through electrostatic interactions) with cationic lipids previously used for the delivery of nucleic acids. They initially fused the polypeptide to a photoreactive antibody binding domain and subsequently to a chain of IgG without disturbing the IgG binding site and enabling the easy exchange of cargo functionalization of of-the-shelf IgG. The functionalized IgG was subsequently complexed with diverse types of cationic lipids and evaluated comparatively with cell penetrating peptides for cytosolic delivery of about 500 nM of IgG. The lipid complexed IgG was functional and also capable of inhibiting the drug efflux pump MRP I (responsible for multi drug resistance) and the transcription factor NFκB. Results also showed the supremacy of this method over traditional cell penetrating peptide method (delivering small proteins) in terms of delivering very large proteins.

Kose and coworkers [144] developed a lipid nanoparticle encapsulated mRNA encoding the antibody against chikungunya virus. The lipid nanoparticle system was prepared by the ethanol drop nanoprecipitation using ionizable lipid, lipid-polymer hybrid, cholesterol and DSPC in a microfluidic mixer. The lipid nanoparticle system provided approximately 90% encapsulation with particle sizes in the range of 80–100 nm. The protective ability of the developed system was tested in AG129 mice. The study showed that treatment lipid encapsulated mRNA protected the mice in a dose dependent manner.

6.3.3 Encapsulation of diagnostics

Certain factors can trigger responses in lipid particles of vesicles and include pH, reactive oxygen species, redox agents' presence of biomolecules, as well as certain environmental stimuli such as temperature, and light. Biosensors constitute a receptor

that will interact with the stimuli to be detected and a transducer that will translate the analyte/stimuli -receptor information to measurable signal. Treatment and survival for many terminal and chronic diseases depend on early detection and diagnosis. Fortunately, many biosensing and bioimaging materials are being developed for possible use in diagnosis and treatment. There is a need to transport these nanoplatforms to the targeted site in a non-invasive and hidden manner to avoid destruction by the bodyguards of the body. One way this has been mitigated is by enclosure into vesicles that have a close resemblance to the body's own cells, the liposomes. Encapsulation based on lipid systems is driving the development of bioimaging and biosensing devices towards picogram detection thereby aiding both treatment such as fluorescence guided surgeries and survival. Some of the challenges being mitigated by lipid based or encapsulated devices include targeting, sustained release, and circulation.

Photoacoustic tomography (PAT) that makes use of light and sound has been considered a viable alternative to overcoming some of the limitations of conventional imaging systems such as computed tomography (CT) and magnetic resonance imaging (MRI), in early detection of atypical liver cancers that are less than 10 mm diameter. In addition, surgical resection remains about the most viable treatment option. Gold nanorods, due to their easy effusion into solid tumors, biocompatibility, and low toxicity [135] is usually considered good for PAT. It is also possible to move its absorption peak from red to near infrared (NIR) due to its anisotropic shape and enhancing its photoacoustic signal with large absorption cross section. Exploiting these factors, Guan and coworkers [145] developed dual PAT-NIR probe to aid early liver cancer detection and for guided surgical resection. They tapped a sort of synergistic effect of both gold nano rods and indocyanine green, an FDA approved photoacoustic NIR fluorescent dye that has dominated clinical practice for a while, and played down on some limitations of indocyanine green such as aggregation, rapid clearance, low energy conversion efficiency as a dual photoacoustic and fluorescence dye, and fluorescence quenching. In a relatively facile process, indocyanine green liposomes were prepared with phosphatidylcholine and cholesterol using the thin film hydration method. Pegylated gold nanorods were subsequently encapsulated with the indocyanine green liposomes by ultrasonication overnight. The dual system was used for resection surgery in tumor infected laboratory animals and proved successful.

Nucleolin is a nuclear and cytoplasmic protein also expressed on the cell surface and partly responsible for angiogenesis and by extension metastasis and tumor progression. Unlike other markers of tumor progression, that become less prominent as the tumor size increases, nucleolin is detected even in big tumors. It could therefore be mapped as a means of tracking metastasis. In current practice, Nucleolin is tracked using biopsy which is invasive and cannot indicate the full extent of metastasis or spread. Zhang and colleagues [146] developed a nucleolin targeted ultrasound contrast agent for detecting the presence of nucleolin in cells. The contrast agent is microbubble which is an encapsulated air commonly generated by sonicating a polymer solution in the presence of air or by compressing air into a polymer solution and then releasing it through specialized nozzles. In this study, Zhang and colleagues [146] synthesized an F3 peptide that has been shown to target nucleolin and conjugated it to the surface of generated microbubbles. Subsequently, they evaluated the ability of the F3 conjugated nucleolin targeting microbubble to detect the presence of nucleolin non-invasively. They initially synthesized the F3 peptides, lipo-peg peptides. Subsequently the microbubble which was encapsulated in a liposomal shell was synthesized using disteroylphosphatidylcholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG2K), and lipo-peg-peptide earlier synthesized. The liposomal shell was prepared using the thin film hydration method and the microbubble generated by shaking the liposomal solution in air and used within 2 hours of purification. Two batches were made, one batch without the targeting

peptide contained just the lipids DSPC: DSPE-PEG2K in the ratio of 90:10 while the targeted batch had I % of the lipo-peg-peptide replacing part of the DSPE-PEG2K. Evaluation of the contrast media in tumor infected female FVB mice in vivo and nucleolin expressing breast cancer cell line in vitro demonstrated that the accumulation of the contrast media facilitated the detection of murine breast tumors.

Deng and colleagues [147] recently developed a multicomponent system for diagnosis and treatment of cancers. They combined near infrared luminescence of quantum dots and thermo-sensitivity of magnetic liposomes to encapsulate and control drug localization and release. They prepared thermosensitive liposomes that were eventually loaded with drug, paclitaxel, magnetic nanoparticles and NIR luminescent quantum dots. The liposomes were prepared by the thin film hydration method using dipalmitoylphophatidylcholine, DPPC, 1,2-diaccyl-sn-glycero-3-phosphoethanolamine-N-(methoxy [polyethylene glycol]-2000 (DSPE–MPEG-2000), 1,2-diaccyl-sn-glycero-3-phosphoglycerol sodium (DSPG-Na). Effect of the developed systems were studied on cancer cell MCF-7 and SKOV-3 cell lines and uptake of the drug followed in real time by confocal scanning microscope. **Tables 2** and **3**

Micro-encapsulating materials	Active compounds	Micro-encapsulation technique	Diagnostic applications	EE %	Ref.
DPPC, DSPE–MPEG-2000 and DSPG-Na.	Paclitaxel	Liposomes	NIR imaging	86.46 ± 1.43%.	[147]
DPPC, CHOLESTEROL, DSPE-PEG-MALEIMIDE, DSPE-PEG-PDP.	Indocyanine green liposomes/ Perfluorobutane	Microbubble/ liposomes	Fluorescence and ultrasound imaging	—	[148]
DSPC, DSPE-PEG2K	Gas	microbubble	Ultrasound imaging	Not applicable	[146]
Lipids	perfluoropropane	microbubble	Ultrasound imaging	Not applicable	[149]
DSPC, PEG40 stearate, DSPE-PEG3400-maleimide	Perfluorocarbon	Emulsion/ microbubble decorated with antibody	Tumor cell isolation	—	[150]
DMPA, DPPC, DPPA, Cholesterol	Perfluoropentane	Emulsion containing perfluoropentane loaded liposome	Ultrasound guided tumor destruction.	—	[151]
DSPC	Perfluorobutane	Gene loaded microbubble	Ultrasound guided tumor destruction	—	[152]
Cholesterol, DDSPC, DSPE-PEG and DOPC	Magnetic iron oxide nanoparticles	Liposome	Magnetic resonance imaging contrast agent	—	[153]

Note: 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid: DMPA, 1,2 Distearoyl-sn-glycero-3-phosphocholine: DSPC, Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000: DSPE-MPEG2000, 1,2 dipalmitoyl-sn-glycero-3-phosphate: DPPA, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine: DPPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000]): DSPE-PEG-COOH, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[PDP(polyethylene glycol)-2000]: DSPE-PEG-PDP, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine: DOPC; EE: Encapsulation Efficiency.

Table 2.
Plant and animal lipid-based microencapsulating carriers for diagnostic applications.

Nano-encapsulating materials	Active compounds	Nano-encapsulation technique	Diagnostic applications	EE %	Ref.
DPPC, Stearic acid	Sulfur hexafluoride	Nanobubble	Ultrasound imaging	Not Applicable	[154]
Lipoid S75–3	oil	Phase inversion	O_2 sensor for MRI	—	[155]
DPPC, DSPC, DSPE-PEG 2000	Topotecan	liposomes	MRI guided focused ultrasound	—	[156]
DSPE-PEG2000-amine, DPPC	perfluoro-15-crown-5-ether	Pressure extrusion	MRI	—	[157]
DSPC, DSPE-PEG	camptothecin-floxuridine	Nanoprecipitation/ microbubble	Focused ultrasound	56.7 ± 2.3	[158]
DSPC, DSPE-PEG3400-maleimide, DSPE-PEG-1000	Perfluorohexane (antibody ligated)	Emulsion	Isolation of Circulating Tumor Cells.	—	[159]
Phosphatidylcholine, cholesterol.	Indocyanine green and gold nanorods	Liposomes	Photoacoustic and fluorescence imaging	97% (implied)	[145]
DPPC, DPPA, DPPE, DSPE-PEG-COOH	Pentafluoroctane	Antibody conjugated nanobubble	Contrast agent for ultrasound imaging for ovarian cancer diagnosis	Not applicable	[160]
DPPC, DSPE-MPEG, DPPA, DPPE	pentafluoroctane	Nanobubble	Contrast agent for ultrasound imaging	Not applicable	[161]
DSPC, DPPE, DSPE-PEG2000-Biotin.	octafluoropropane	Nanobubble	Ultrasound imaging for cancer detection	Not applicable	[162]

Note: 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid: DMPA, 1,2 Distearoyl-sn-glycero-3-phosphocholine: DSPC, Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000: DSPE-MPEG2000, 1,2 dipalmitoyl-sn-glycero-3-phosphate: DPPA, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine: DPPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000]): DSPE-PEG-COOH, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[PDP(polyethylene glycol)-2000]: DSPE-PEG-PDP, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine: DOPC; EE: Encapsulation Efficiency.

Table 3.
Plant and animal lipids-based nano-encapsulating carriers for diagnostic applications.

indicate diagnostic applications based on micro- and nano-encapsulation utilizing animal/plant lipids as encapsulating materials.

7. Conclusions and future trends

Application of natural polymers and their semi-synthetic polymer derivatives provide the basis for many commercially available DDSs that range from the traditional macroscale DDSs to microscale, nanoscale, targeted, and stimuli- responsive DDSs. The effective encapsulation technique and the achieved encapsulation efficiency depend on the physical and chemical properties of the selected natural polymer (e.g. polysaccharides, proteins, lipids) such as solubility, thermal stability, and its ability to form stable colloidal particles in a specific system. Therefore, various encapsulation methods including chemical, physical, physicochemical, mechanical and thermal encapsulation were discussed in details, **Table 1**.

Lipid vesicles nanocarriers have been discovered in 1960s, and later became known as "liposomes". In 1995, the first nano-drug based on Pegylated liposomal doxorubicin "DOXIL" was approved by FDA for cancer treatment. Polymer-drug conjugates and liposomes represent most of the marketed NP therapeutics and continue to be investigated extensively. Nanoencapsulation using lipid nanoparticles offers a practical approach to increase the solubility of water-insoluble drugs or poorly water soluble agents. Lipids have shown to be effective natural material for micro- and nanoencapsulation of bioactive small molecules and biologics for therapeutic and diagnostic drug delivery applications. Compared to other encapsulating materials, lipid-based encapsulation systems offer advantages such as low-cost and easy to scale-up and sterilize high biocompatibility, higher encapsulation efficiency, higher drug loading, and feasibility of carrying both hydrophilic and hydrophobic drugs.

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