

Review

# Synthesis of Starch Nanoparticles and Their Applications for Bioactive Compound Encapsulation

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**Abstract:** In recent years, starch nanoparticles (SNPs) have attracted growing attention due to their unique properties as a sustainable alternative to common nanomaterials since they are natural, renewable and biodegradable. SNPs can be obtained by the breakdown of starch granules through different techniques which include both physical and chemical methods. The final properties of the SNPs are strongly influenced by the synthesis method used as well as the operational conditions, where a controlled and monodispersed size is crucial for certain bioapplications. SNPs are considered to be a good vehicle to improve the controlled release of many bioactive compounds in different research fields due to their high biocompatibility, potential functionalization, and high surface/volume ratio. Their applications are frequently found in medicine, cosmetics, biotechnology, or the food industry, among others. Both the encapsulation properties as well as the releasing processes of the bioactive compounds are highly influenced by the size of the SNPs. In this review, a general description of the different types of SNPs (whole and hollow) synthesis methods is provided as well as on different techniques for encapsulating bioactive compounds, including direct and indirect methods, with application in several fields. Starches from different botanical sources and different bioactive compounds are compared with respect to the efficacy *in vitro* and *in vivo*. Applications and future research trends on SNPs synthesis have been included and discussed.

**Keywords:** starch nanoparticles (SNPs); SNPs synthesis; bioactive compounds; drug delivery; encapsulation efficiency



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## 1. Introduction

Starch is a natural, renewable, and biodegradable polymer produced by many plants as a source of stored energy. It is the second most abundant biomass material in Nature. It is a carbohydrate storage product found in all chlorophyll-containing plants, such as corn, potato, rice, wheat, barley, etc. The predominant model for starch is a concentric semicrystalline multiscale structure that allows the production of new nanoelements: (i) starch nanocrystals resulting from the disruption of amorphous domains from semicrystalline granules by acid hydrolysis and (ii) starch nanoparticles produced from gelatinized starch [1]. The starch industry extracts and refines starches by wet grinding, sieving, and drying. If the starch is used as extracted from the plant it is called “native starch”, while if it undergoes one or more modifications (either enzymatic, chemical, or physical) to reach specific properties it is called “modified starch” [1]. Currently, the development and evolution of synthesis methods and instrumental techniques allow us to take advantage of

the possibilities offered by new materials, whose physical and chemical properties are on the border between those exhibited by atoms and those of matter on a larger scale. These materials are known as nanoparticles [2,3]. Today the nanoparticles field is experiencing a great expansion in scientific research thanks to its potential for application in sectors such as medicine, cosmetics, and food, among other areas.

Encapsulation is a process in which small particles or droplets are surrounded by a coating or embedded in a homogeneous or heterogeneous matrix. The adsorption of bioactive compounds on the carrier is accomplished through weak chemical interactions such as hydrogen bonding and Van der Waals forces. The choice of bioactive compound loading method depends on the size of the molecule to be encapsulated and its hydrophilic/lipophilic character [4].

At the same time, the loading method used for bioactive compounds also affects the release. The efficacy and the effect of these loading methods on the release characteristics of the loaded bioactive compounds have been previously studied to some extent [5].

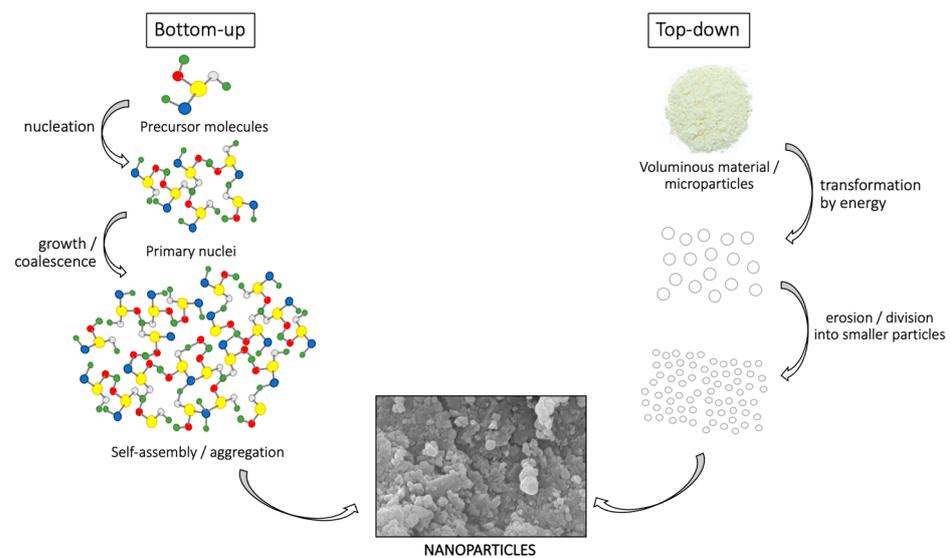
Starch-based carriers are widely used in the encapsulation and control release of bioactive compounds for applications in food and pharmaceutical formulations due to their high biocompatibility and their small size which offers a high surface/volume ratio and high surface/weight ratio when hollow SNPs are synthesized [6–8]. However, there is little research on the delivery of bioactive compounds using starch-based nanoparticles [9,10]. Moreover, even the majority of the review articles focus on the methods frequently used for fabrication of the SNPs, while reports on the encapsulation methods on SNPs are scarce. For the broader application of starch-based nanoparticles as carrier for bioactive compounds, it is important to investigate the loading and release properties of starch nanoparticles using model bioactive compounds.

In this review, different techniques for the synthesis of SNPs are described and the different size ranges obtained in each case are reviewed. Some recent novel developed techniques, such as ultrasound and high-pressure homogenization, are included. The SNPs sizes obtained were dependant on the selected synthesis method as well as the type of the starch used as the natural raw material (e.g., corn starch, banana starch, potato starch, etc.) and also the possible modifications (e.g., chemical modification, amylose/amylopectin content modification, etc.). Different types of SNPs synthesized using different parameters are reviewed. Hollow particles or coated particles prepared by novel methods, such as the sacrificial template method, are also included. Also, a revision of the advantages and disadvantages that polymeric NPs present compared to NPs synthesized with other type of materials was made (Table 1).

Moreover, special attention has been paid to the encapsulation of bioactive compounds as one of the main bioapplications of SNPs. Two main different types of encapsulation methods were described depending on whether the bioactive compounds were encapsulated (direct or indirect). The effect of the type of starch selected for the encapsulation of bioactive compounds has been revised. Finally, applications SNPs loaded with several bioactive compounds were described and their efficacy was discussed when tested *in vitro* or *in vivo*. Moreover, present and future trends on the synthesis of loaded SNPs were described.

## 2. SNP Preparation Method

SNPs are obtained from the breakdown of the starch granules using different methods, which can be classified into two processes, top-down and bottom-up depending on the material used for the synthesis [11,12] as it can be seen in Figure 1.

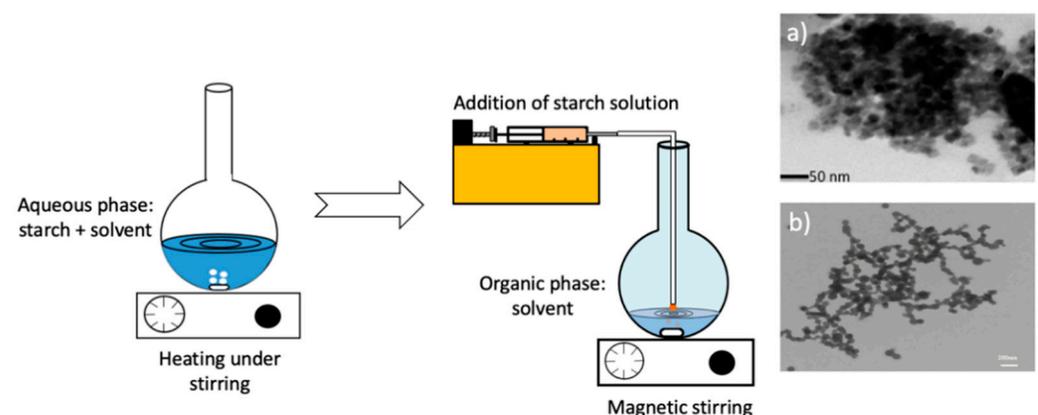


**Figure 1.** Top-down and bottom-up processes for the synthesis of the SNPs.

The top-down processes include all methods of synthesis where the size of the SNPs could be obtained from a voluminous material or microparticles that are eroding or being reduced to reach the structure of interest. The bottom-up processes cover all the synthesis procedures in which the SNPs are obtained from the assembly of molecules or macromolecules in the form of small primary nuclei that are growing. These synthesis methods are described below, and shown in Tables 2 and 3, where the type of starch employed, and the resulting particle size obtained are noted.

### 2.1. Nanoprecipitation

Nanoprecipitation process involves the successive addition of a dilute solution of polymer to a solvent which leads to the polymer precipitation on the nanoscale. This method is essentially based on the deposition or displacement of the polymer in the interface followed by the displacement of the solvent from the lipophilic solution [13]. A schematic representation of this nanoprecipitation method is shown in Figure 2.



**Figure 2.** Synthesis of SNPs through nanoprecipitation method and TEM micrographs of corn SNPs (a) [14] and (b) [15]. Reprinted from [14] with permission from Elsevier. Copyright (2015).

One of the most common solvents used by many authors is acetone which leads to the precipitation of the starch in the form of nanoparticles when it is added dropwise from an aqueous phase where it was previously dissolved. Najafi et al. prepared SNPs using acetylated corn starch and optimized the reaction of native starch using acetic anhydride and acetic acid with low and high degrees of substitution (DS). A size range of 221–324 nm

was obtained by studying different DS levels and water/acetone ratios [16]. In 2018, Acevedo-Guevara et al. used native and acetylated starch obtained from green bananas for the synthesis of SNPs by a nanoprecipitation method where they also used acetone as the solvent. Both systems presented similar results, obtaining a size of 135 nm when the native starch was used and 190 nm when the acetylated starch was used. In turn, to understand the size difference of the SNPs, they studied the size of the starch granules. A slight aggregation in the acetylated banana starch granules was observed which was due to the increase in the intermolecular hydrogen bonds starch granules and water which could explain the difference size observed in the nanoparticles with both starches [17]. Similar studies were carried out by Nieto-Suaza et al. who also used native and acetylated banana starches for the synthesis of the SNPs. As in the previous case, larger particle sizes (198 nm) were obtained when acetylated starch was used, while using native starch, sizes of 141 nm were obtained [18].

Another common solvent used in nanoprecipitation method is absolute ethanol. In some cases, it is added dropwise to a deionized water solution containing the dissolved starch or vice versa. In recent studies, Fu et al. synthesized NPs from corn starch and amylose by this method. They observed that most of the corn SNPs were spheres with some aggregates obtaining particles from dozens of nanometers up to 500 nm. On the other hand, the amylose NPs presented irregular shapes which were smaller than the previous ones. The diffraction patterns in both NPs presented two peaks, the smaller one had practically the same position for both, however, the position of main peak turned out to be slightly different suggesting higher sizes for the corn SNPs compared to the amylose NPs. Nevertheless, both NPs presented a V-type crystalline structure. Finally, the average size of the starch particles obtained was 350 nm while that for the amylose particles was 250 nm [19]. Nyoo Putro et al. reported a method that combined nanoprecipitation methods with acid hydrolysis resulting in SNPs with different crystallinity. The acid hydrolysis method produced SNPs with high relative crystallinity, whereas the ethanol preparation method resulted in SNPs with low crystallinity. In order to vary the hydrophilicity of the starch SNPs were also modified by using different cationic, anionic, and nonionic surfactants. The morphology observed before the modification was flaky for SNPs obtained by the hydrolysis and spherical for the nanoprecipitation ones. Significant changes were not observed after the modification with cationic and anionic surfactants for both methods. However, when nonionic surfactant was used, a significant change was observed appearing as different adsorption mechanisms on the SNPs surface in both cases. Smaller particle sizes were obtained when the acid hydrolysis method was used with particle sizes approximately between 82 and 92 nm, while sizes between 158 and 180 nm were observed when nanoprecipitation was used [20].

On the other hand, as it was aforementioned, starch can also be added in the aqueous phase in the form of short glucan chain powder dissolved into deionized water and subsequently be mixed with an ethanolic solution added dropwise leading to the starch precipitation. In the study carried out by Qiu et al., native waxy maize debranched by pullulanase was used to obtain short linear glucans to synthesize SNPs. Short glucan chains were linear and had a low degree of polymerization compared to the native starch and contributed to a more compact structure formation. Finally, particles with an average particle size of 84 nm were obtained [15]. Liu et al. also synthesized SNPs through a modified solvent displacement method using waxy corn starch which was altered into short linear glucans obtaining a particle size range between 40 and 50 nm but apparent aggregation was observed [21].

Besides, the aqueous phase, in addition to distilled water, may also contain other chemical compounds as sodium hydroxide or DMSO mixed with the starch solution allowing its precipitation by adding absolute ethanol dropwise. El-Naggar et al. synthesized crosslinked corn SNPs by a nanoprecipitation method using a mixture of distilled water and sodium hydroxide as the dispersed phase, sodium tripolyphosphate (STPP) as a crosslinking agent in the presence of Tween 80 as a surfactant. Crosslinkers can enhance

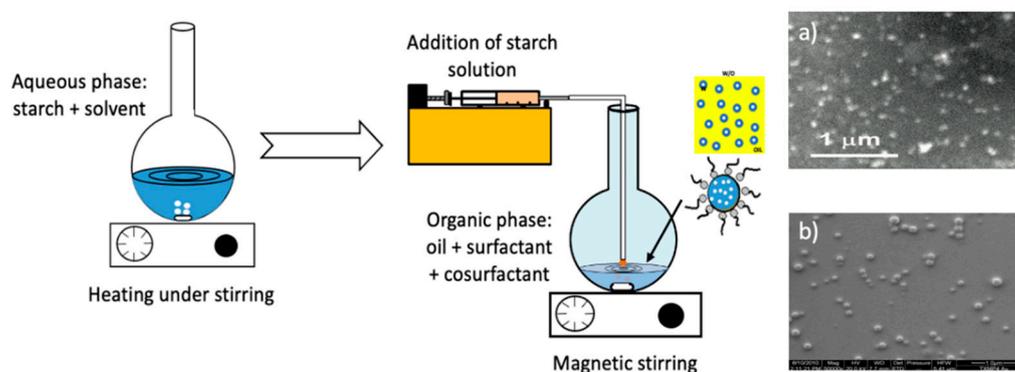
the granule stability with new covalent bonds which allows to increase the desired stability conditions [22]. Different amounts of the crosslinking agent were studied, and it was observed that the greater the amount of STPP, the greater the particle sizes. In any case, none of the experiments exceeded particle sizes of 68 nm [14]. In recent studies, Gutierrez et al. also synthesized SNPs using the nanoprecipitation method by modifying main operating parameters. Maize starch was dissolved into an aqueous phase containing sodium hydroxide in the presence or absence of urea since it was recently reported that the interactions between sodium hydroxide and urea improve the solubility of the polymers as sodium hydroxide breaks the hydrogen bonds of the starch and urea prevents self-association of the starch molecules [23]. Then, in order to precipitate the starch in the form of SNPs 1 mL of the starch solution was added into absolute ethanol as the organic phase. Also, the effect of the addition of a cationic surfactant (CTAB) into the aqueous phase was studied. As a result, SNPs from 59 nm to 118 nm were obtained, registering the higher values when surfactant was added into the aqueous phase. The result of this study showed that the total amount of SNPs obtained was greater for urea-containing formulations and fewer agglomeration were observed for an aqueous solution with 8% (*w/v*) NaOH and 10% (*w/v*) urea [24].

Abidin et al. also prepared oxidized starch nanoparticles (oxy-SNPs) modifying the native starch by liquid-phase oxidation using  $H_2O_2$  as the oxidant, followed by chemical dissolution and solvent-free precipitation. Starch was dispersed in ethanol and DMSO was added until the starch was gelatinized and dissolved in the DMSO, then the precipitation of the SNPs was carried out by mixing the starch solution with ethanol. The final size of the oxy-SNPs was confirmed in a scale range of approximately 178 nm [25].

## 2.2. Emulsion/Microemulsion

This process consists of the formation of an emulsion through two phases, an aqueous solution containing the dissolved polymer and an organic solvent that is immiscible or partially immiscible in water. Subsequently, the two phases are emulsified with intense agitation which can be carried out by means of a mechanical homogenizer such as microfluidization or ultrasonic homogenization. The presence of a surfactant is required to stabilize the emulsion. The droplets formed due to the agitation act as nanoreactors that produce the controlled precipitation of the starch in the form of nanoparticles inside. A graphic representation of this method is depicted in Figure 3.

Microemulsions can also be used to form SNPs, which typically lead to the formation of smaller and more uniform particles [26]. In this case, an intense agitation to form the microemulsions is not necessary since they form spontaneously with very little energy input.



**Figure 3.** Synthesis of SNPs through emulsion/microemulsion method and SEM micrographs of (a) corn SNPs [27] and (b) potato SNPs [28].

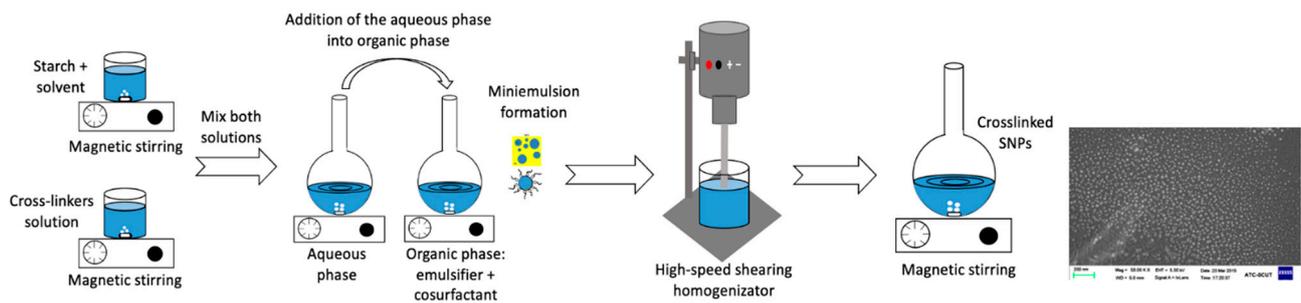
In 2017, Nor Syahida and Subash synthesized SNPs by a W/O microemulsion method where they dissolved corn starch in alkaline urea solution with different starch concentrations and further precipitated in ethanolic emulsion system using Tween 20 as surfactant

and sunflower oil by adding the starch solution dropwise into the microemulsion system. The SNPs produced this way were uniform in shape and size, due to homogeneous solution obtaining sizes of 40 nm. However, smaller sizes were obtained when the lowest starch concentrations were used [27]. Gutierrez et al. also carried out the formation of a W/O microemulsion to synthesize spherical SNPs. Maize starch was dissolved into an aqueous phase with sodium hydroxide and urea and then 1 mL of this solution was added into an organic phase under constant stirring. This organic phase was formed by oil (soybean and sunflower), a surfactant (Tween 20 and Span 60) with different concentrations, and absolute ethanol as a co-stabilizer. This method led to sizes from 35 nm to 147 nm with a higher particle formation capacity and without the presence of large agglomerates. The final SNPs size was not influenced by the type of oil used in the microemulsion formulation; however, smaller sizes were obtained by the use of very hydrophilic surfactants (Tween 20) [24].

Dandekar et al. synthesized SNPs from hydrophobic starch derivative, propyl- potato starch through an emulsion-diffusion technique. This technique differs from the conventional microemulsion in the way that a W/O emulsion is formed within a partially water-soluble solvent and subsequently water is added to the system which causes the diffusion of the solvent towards the external phase, resulting in the formation of nanoparticles [29]. It has numerous advantages such as high performance and encapsulation efficiency, reproducibility, and scalability. The authors studied different nonionic surfactants and their effect on the SNPs size and homogeneity. Best results were obtained when polyvinyl alcohol (PVA) was used as a surfactant obtaining a low particle size and the most homogeneous formulation. Finally, the lowest particle size obtained was 245.5 nm, which was achieved with a drug: starch ratio of 1:5 [30]. Alp et al. also used an emulsion-diffusion method to synthesize corn SNPs obtaining a particle size of 180 nm in the aqueous solution. Starch was dissolved in DMSO as the organic phase and a mixture of PVA and distilled water was used as the aqueous phase. The droplets obtained on the biphasic system were precipitated in the form of SNPs when DMSO was diffused by the addition of distilled water to the system [31]. Similarly, Santander-Ortega et al. synthesized SNPs with a W/O emulsion-diffusion method using two different propyl-starch derivatives with high degrees of substitution (1.05 and 1.45) and an unmodified starch polymer. The inclusion of propyl groups would allow a good solubility in low risk organic solvents and the incorporation of PVA into the formulation improved the hydrodynamic size distribution of nanoparticles. SNPs synthesized with propyl starch derivatives showed a spherical shape with a narrow size distribution with a size range of approximately 150–183 nm, where the largest size was obtained with the highest degree of substitution. However, in the case of unmodified starch, the formation of SNPs could not be observed [28].

### 2.3. Emulsion Cross-Linking

Recently, the synthesis of SNPs by an emulsion-crosslinking technique has been reported. This technique involves mixing of an aqueous phase which contains cross-linking agents with another phase that contains dissolved starch. The mixture is then added into an oily or organic phase with the presence of emulsifying agents as can be seen in Figure 4. Through this W/O emulsion, small particles are generated by cross-linking reaction within the aqueous droplets. However, the particles obtained from this emulsion cross-linking approach are relatively large and on the microscale [32,33].

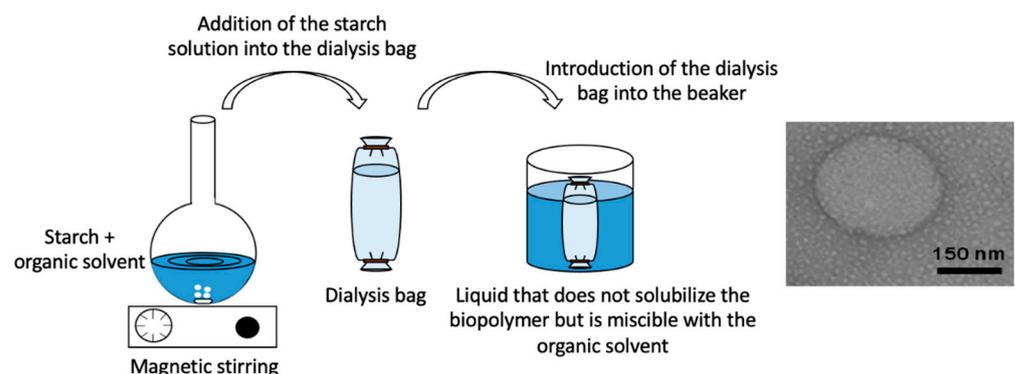


**Figure 4.** Synthesis of SNPs through emulsion cross-linking method and SEM micrograph of SNPs. Reprinted from [34] with permission from Elsevier. Copyright (2016).

To reduce the size of these particles, the emulsion should contain nanoscale droplets, which means use a miniemulsion (also called nanoemulsions) instead of an emulsion [35]. Ding et al. used retrograded starch (RS III) to synthesize SNPs through a high-speed shearing emulsification method using  $N,N'$ -methylenediacrylamide (MBAA) as the cross-linking agent and  $\text{NaHSO}_3$  as an inducing agent in the aqueous phase which contained the dissolved starch in a potassium hydroxide solution. The oil phase was formed by mixing cyclohexane with a mixture of Span 80 and Tween 80 at an 84:16 proportion. The aqueous phase was added to the oil phase in order to form the microemulsion which was homogenized by a high-shear homogenizer to break the droplets and form the nanoemulsion. A small particle size distribution was obtained, and the average size was approximately 287 nm. The stability of the particles was studied, and it was shown that these nanoparticles were stable in neutral and alkaline pH or in low NaCl concentration [36].

#### 2.4. Dialysis

The dialysis technique involves the dissolution of the biopolymer in an organic solvent. The solution is placed inside a dialysis bag, which is immersed in a liquid that is miscible with the organic solvent, but the biopolymer is insoluble in it. Due to the progressive displacement phenomenon between the organic solvent and the external liquid through the dialysis bag in which the biopolymer is not soluble, it is possible to achieve supersaturation of the biopolymer solution located inside the dialysis bag and therefore, the biopolymer will be gradually aggregated due to the change in the polarity caused by the environment where it is found [37,38]. This process is schematized in Figure 5.



**Figure 5.** Synthesis of SNPs through dialysis method and TEM micrograph of potato SNPs [39].

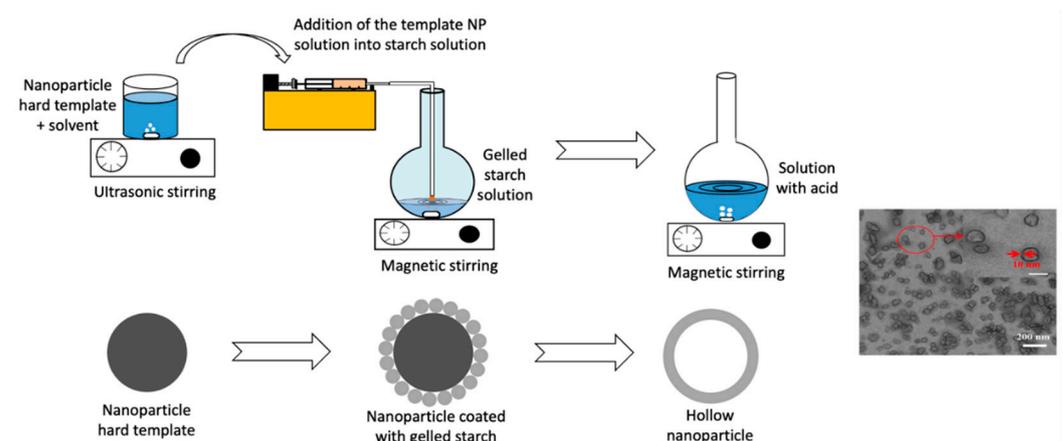
Some advantages of the dialysis method are the control of the aggregation rate, the modulation of the particle size, and the simplicity and versatility of the technology [40]. Delsarte et al. reported the synthesis of the SNPs through a dialysis method using potato starch modified with octenyl succinic anhydride (OSA) and 1,4-butane sultone (BS). The critical aggregation concentration (CAC) was initially studied to investigate the possible formation of colloidal aggregates of OSA-BS starch. The results suggested a modification on

the morphology of the polymer when its concentration increases stipulating that under the CAC of the OSA-BS starch presented an aggregate formed with a dense core and a swollen cover, which was consistent with the higher mobility of the remaining OSA. Beyond CAC, the molecular structure self-assembled in uniform aggregate with collapsed chains. TEM images revealed the presence of different sizes of nanoparticles with diameters between 5 and 200 nm [39].

Xu et al. also reported this method for the synthesis of SNPs using cholesterol-modified oxidized starch and imidazole. Most of the SNPs exhibited homogeneous sphere shapes as well as good dispersibility with mean diameters of 212 nm [41].

### 2.5. Sacrificial Template

This method is especially used for the synthesis of hollow or porous particles. Up until now, hollow nanoparticles (HNPs) have been prepared by creating a void space inside of a solid precursor through a variety of physical and chemical techniques, such as the sacrificial template method, nozzle reactor processes, emulsion polymerization, and phase separation [42]. Among these, the hard templating strategy is the simplest and most widely used method [43,44]. This approach typically consists of coating the outer surface of a material that will act as a template with a polymeric material, in this case starch, and subsequently, the material used as a template will be removed until a hollow particle is obtained [45–47] as it can be seen in Figure 6.



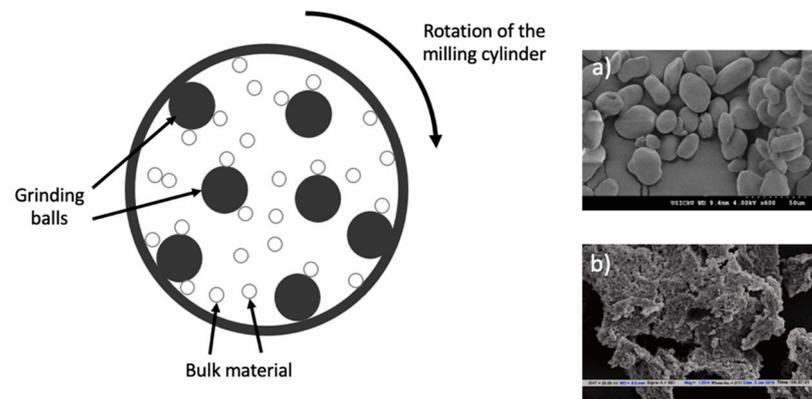
**Figure 6.** Synthesis of SNPs through sacrificial template method and TEM micrograph of pea, mung, bean, potato, and corn hollow SNPs [48].

For this purpose, it is necessary to add a suspension containing the particles that will act as a template to a solution in which the gelled starch is found, which will lead to the precipitation of the starch and its deposition on the template particles. Finally, a change in the medium is produced, such as a change in pH or temperature that will cause the dissolution of the material that acts as the template, thus producing the hollow SNPs. The final conditions of the hollow particles are limited by the size and shape of the starting template, while the thickness of the coating material is determined by its coating process. In 2017, Yang et al. reported this method for the synthesis of hollow SNPs using gelled starch as the shell where different concentrations of  $\text{CaCO}_3$  as hard templates were used. SNPs with diameters ranging from 30 to 300 nm and a shell thickness of 5–10 nm were obtained [48].

### 2.6. Ball Milling

Ball milling is one of the reliable techniques to produce nanoparticles without the aid of additives or the requirement of a final dehydration step to recover the nanoparticles. Over the years, the ball milling process has been explored for the nanoreduction of different polymeric materials including starch [49], cellulose [50] and protein-based polymers [51] for

different applications. This process can be conducted in dry (without solvents) or wet (with solvents) conditions. The milling occurs due to mechanical friction between the grinding media, such as balls of the same or various sizes, and the ground material. The container and the grinding balls are typically made of the same, super hard material (zirconia, corundum, or stainless steel). Mechanical energy is provided to the system by the rotary motion of the bowl, as in the case of a planetary ball mill. Wet ball milling is also considered to be a green chemistry approach, as it does not require high temperatures (energy saving) and consumes minimal quantities of solvents [52]. A schematic representation of the ball milling method is shown in Figure 7.



**Figure 7.** Synthesis of SNPs through ball milling method and SEM micrographs of (a) water chestnut SNPs [53] (reprinted with permission from Elsevier. Copyright (2019)) and (b) lotus stem, horse and water chestnut [54] (reprinted with permission from Elsevier. Copyright (2021)).

This process is a cost-efficient and environmentally friendly physical processing method that has been proven capable of changing starch properties [55,56]. In 2019, Ahmad et al. carried out a study synthesizing water chestnuts SNPs by a physical method of ball milling. SNPs were obtained by reducing the starch size with a planetary ball for 5 h and finally, the average particle size obtained after the milling process was 271 nm [53]. In recent studies, Ahmad et al. also synthesized SNPs through a ball milling method using a mixture of starches (lotus stem, horse, and water chestnut) to create nanocapsules for bioactive compounds encapsulation. SNPs were obtained in the same way as their previous study with a planetary ball for 5 h. For this study, higher hydrodynamic particle sizes were obtained, being the smallest for the horse chestnut starch with a particle size of 420 nm approximately and with a polydispersity index of 32% what indicated a narrow size distribution [54].

### 2.7. Ultrasound with or without Acid Hydrolysis

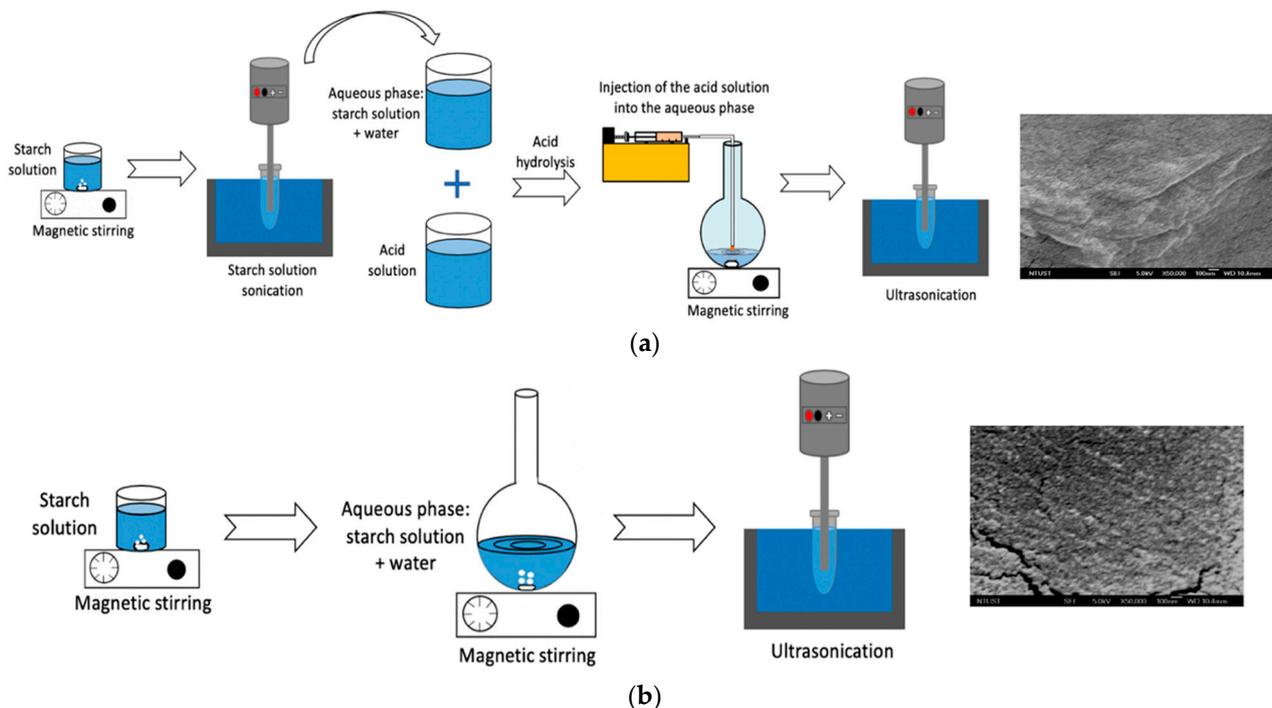
This method consists of a precipitation process that involves the addition of a solution in which the starch is diluted into an aqueous phase in the presence of ultrasound homogenization that reduces the size of the particles formed due to the breaking of the starch covalent bonds as a result of the shear forces or the mechanical effects produced by the collapse of microbubbles that form sound waves [57–59].

However, this method has been applied together with acid hydrolysis since the combination of physical and chemical processes produces nanoparticles with more desired properties [60]. This technique is simple and convenient in terms of safety, cost and can give better yield with desired particle size.

Acid hydrolysis is a process that can be conveniently divided into three stages based on the reaction rates involved: rapid stage, slow stage, and very slow stage [61]. A series of steps is typically required to produce SNPs using the acid hydrolysis process: (i) the starch is mixed with the acid; (ii) this mixture is heated for a certain time with constant stirring; (iii) the resulting suspension is washed with distilled water by successive centrifugation until

neutralization is achieved and (iv) the suspension is refrigerated for a specified time [62]. The main disadvantages of this method are the low recovery yield, slow reaction rate, and tendency for particle aggregation to occur [63].

Both processes, with and without acid hydrolysis are presented in Figure 8a,b respectively.

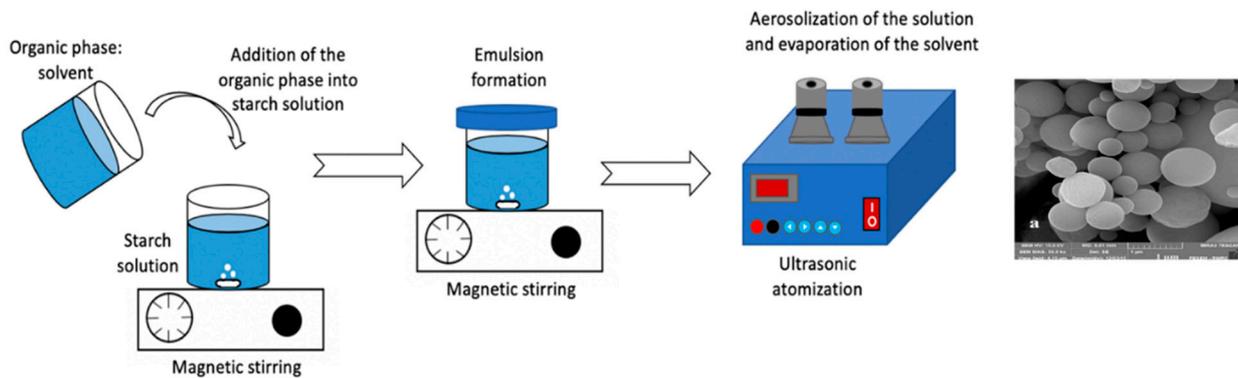


**Figure 8.** Synthesis of SNPs through ultrasound method: (a) with acid hydrolysis; (b) without acid hydrolysis and SEM micrograph of corn and potato SNPs [64].

In 2019, Shabana et al. reported the synthesis of SNP from potato starch using an ultrasound-assisted method with and without acid hydrolysis. The ultrasound-assisted acid hydrolyzed starch exfoliates the native starch and modifies the structural arrangement, in this acid treatment, the amorphous nature of the starch becomes crystalline. A solution of corn and potato starch was prepared and sonicated and subsequently treated with dilute sulfuric acid. The hydrolysis of those of the SNPs was carried out by diluting part of the starch solution in distilled water and subsequently concentrated  $H_2SO_4$  was added dropwise and sonicated until the addition of dilute sulfuric acid was completed. To neutralize this solution, a solution of NaOH and distilled water was added. Finally, particle sizes of 42 nm were obtained when the acid hydrolyzed ultrasound approach was used while sizes of 80 nm were obtained when only the ultrasound was used [64].

### 2.8. Ultrasonic Atomization

Ultrasonic atomization is a promising way to generate liquid microdroplets through the use of ultrasonic energy. In the droplet formation mechanism, two major hypotheses have been suggested to explain the thin vibrating liquid film disintegration during ultrasonic atomization comprising capillary waves, and cavitation mechanism [65]. Capillary waves consist of an ordered mesh-like structure with the same number of ridges and valleys per unit area [66]. In the common model of ultrasonic atomization, droplets detach from the crests of the stationary capillary waves and in the cavitation mechanism, cavitation bubbles are formed on the vibrating surface due to the application of ultrasonic waves. During the collapse of these cavities, generated hydraulic shocks initiate the segregation of the liquid film and consequently result in the ejection of the droplets [67]. This process is shown in Figure 9.

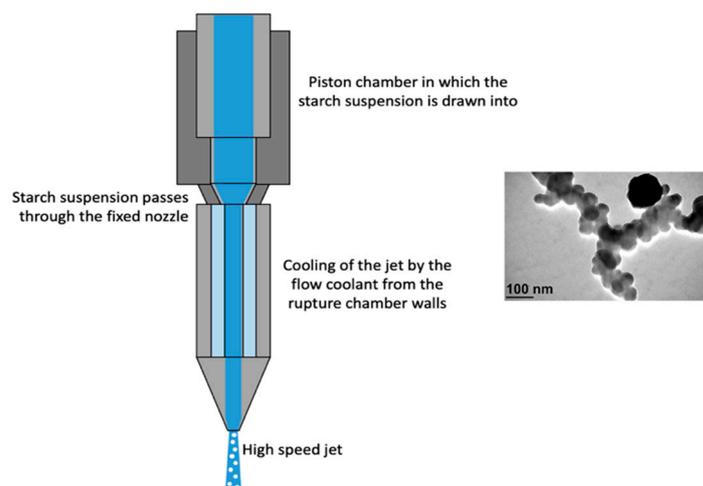


**Figure 9.** Synthesis of SNPs through ultrasonic atomization method and FE-SEM micrograph of SNPs. Reprinted from [68] with permission from Elsevier. Copyright (2019).

Recently, Shahgholian and Rajabzadeh developed a bovine serum albumin (BSA) based SNP synthesis method by an ultrasonic atomization procedure using an ultrasonic piezoelectric oscillator. An integrated apparatus was designed for the manufacturing of aerosol solutions, as well as for the drying and collection of the resulting SNPs, and finally, the formation of spherical SNPs with a uniform smooth surface and particle sizes ranging from 200 to 800 nm was resulted [68].

### 2.9. High-Speed Jet

Recently, a physical technology has been developed based on a high-speed jet treatment which is a novel combination with high pressure and speed and a model of liquid-solid impact [69]. It is basically consisted of a piston chamber in which crude suspension is drawn in through downward stroke. The suspension passes through a small, fixed nozzle that produces a high-speed jet during the upstroke. Before colliding with the target, the jet circulates through a pipe and then it is cooled by a recirculating flow of coolant from the rupture chamber walls. However, it is suggested that the high-speed jet is an intense mechanical treatment that can affect the size and the gelatinization properties of starch [70]. The schematization of this process is shown in Figure 10 above.



**Figure 10.** Synthesis of SNPs by high-speed jet method and TEM micrograph of tapioca SNPs. Reprinted from [71] with permission from Elsevier. Copyright (2020).

In a recent study Xia et al. synthesized SNPs from tapioca starch using this physical method. Before the high-speed jet treatment, the native starch was treated with a vibrating superfine mill with different micronization periods to improve the polymer solubility and after 60 min of micronization, the optimum pretreated material was achieved. Next, the

pretreated starch was mixed with distilled water and stirred at room temperature and then, the suspension was treated with a high-speed jet at different pressures followed by vacuum filtering and finally dried for 24 h. Particle size decreased after the high-speed jet treatment and an increase in the pressure produced a more uniform SNPs size achieving the smallest sizes (55.3 nm) with 240 MPa of pressure [71].

Nanoparticles have been used as vehicles for the delivery of bioactive compounds for several applications in food, cosmetic and pharmaceutical formulations. Frequently, in order to obtain more effective materials with large surface area per unit of mass, hollow nanoparticles are prepared. The most common materials used for hollow nanoparticle preparation are polymers. Starch is a natural polymer that has attracted interest for its use as raw material for covering already prepared nanoparticles of several other metal materials. However, their individual use for SNPs preparation has increased during the last decade. SNPs present both advantages and disadvantages with respect to the other type of nanoparticles which are made with other materials, such as polymers, metals, or silica, as summarized in Table 1 [72–75].

**Table 1.** Advantages and disadvantages of SNPs versus other types of nanoparticles.

Advantages	Disadvantages
- Starch nature makes it a non-toxic material with high biocompatibility and non-hazardous to human health	- Native starch present poor tolerance to a broad range of processing conditions (temperature, pressure, pH) limiting its applications in the food industry
- Starch is biodegradable, renewable, and abundant in nature	- Native starches present low solubility and retrogradation which restricts their functional properties
- Starch can be easily modified due to the presence of a large number of hydroxyl groups on its main components (amylose and amylopectin)	- Modification of starch usually requires the use of solvents that could produce a final product with undesired conditions
- SNPs present greater surface area per mass	
- Versatility: a large number of different types of starch and the physical and chemical methods used to prepare SNPs makes it possible to obtain nanoparticles with diverse crystallinity and different sizes	

### 3. Encapsulation Methods

SNPs have attracted a lot of attention due to their unique properties that are different from those of their source material (native starch granules). One of the main applications of SNPs is the encapsulation of bioactive compounds [27,76]. Encapsulation is defined as the technology by which active compounds are confined within a polymer matrix. This technique creates a microenvironment in the capsule that is capable of controlling the interactions between the interior and the exterior [77]. Encapsulation can be divided into direct and indirect encapsulation depending on how the synthesis method is performed as it will be discussed below. Table 2 summarizes all the parameters discussed in this section for the SNPs direct encapsulation.

#### 3.1. Direct Encapsulation

This type of encapsulation refers to when both the synthesis of the SNPs and the encapsulation of the active compound are carried out simultaneously.

**Table 2.** Examples of recent studies of SNPs synthesis methods and encapsulation of different compounds through direct encapsulation.

Preparation Method	Type of Starch	Particle Size (nm)	Encapsulated Compound	Evaluation Method	Applications	Tested On	EE (%)	References
Microemulsion	Corn	40	Penicillin/streptomycin	Disk diffusion	Anti-bacterial, harmful pathogenic organisms	<i>S. pyogenes</i> and <i>E. coli</i>	/	[27]
Nanoprecipitation	Acetylated corn	221–324	Ciprofloxacin	UV-vis	Medical	/	21–90	[16]
Nanoprecipitation	Native and acetylated green banana	135–190	Curcumin	UV-vis	Drug and nutraceutical delivery	SIG, SIF in vitro	85–90	[17]
Nanoprecipitation	Corn	21–68	Diclofenac sodium	UV-vis	Medical	Rat skin in vitro	73–91	[14]
Nanoprecipitation	Lotus stem, horse chestnut and chestnut	323–615	Catechin	UV-vis	Functional foods	In vitro digestion	48–57	[57]
Nanoprecipitation	Corn + pullulanase	84	Essential oils	UV	Cosmetics, food preservation, antibacterial	In vitro dialysis	72–87	[15]
Emulsion-diffusion	Corn	180	CG-1521	Crystal violet assays	Breast cancer therapy and other tumors	MCF-7 breast cancer cells in vitro	/	[31]
Nanoprecipitation	Insoluble porous corn	/	Paclitaxel	HPLC	Anti-cancer	Rats through oral administration	71–74	[78]
Nanoprecipitation	Corn	283	Triphala churna	UV-vis	Pharmaceutical	<i>S. dysenteriae</i> , <i>S. aureus</i> and <i>S. Typhi</i> in vitro	92	[79]
Nanoprecipitation	/	45	Vitamin E	UV-vis	Wound dressing	SF-PVA Aloe Vera nanofibers in vitro	92	[80]
Emulsion-diffusion	Corn + OSA	375–617	Conjugated linoleic acid	Gravimetry	Controlled release, functional foods, cosmetics	Gastrointestinal rat tract in vivo	97	[81]
Dialysis	/	212	Curcumin	UV-vis	Anti-cancer	Human lung cancer cell A549	82	[41]
Emulsion-diffusion	Corn	150–183	Testosterone, flufenamic ac., caffeine	HPLC	Transdermal delivery	Human skin undergone abdominal plastic surgery	80–95	[28]
Ultrasonic atomization	/	200–800	Curcumin	UV-vis	Bioactive encapsulation	SIG, SIF	64–92	[68]
Nanoprecipitation	Banana + Aloe Vera	141–198	Curcumin	UV-vis	Food packaging	Highly hydrophilic/lipophilic foods	82–92	[18]
Emulsion-diffusion	Potato	245.5	Docetaxel	HPLC	Anti-cancer	Caco-2 cells	82	[30]
Nanoprecipitation	Corn	250–350	Lutein	/	Food, medicine, feed	/	/	[19]
Nanoprecipitation	Quinoa	283–871	Piroxicam	UV/vis	Pharmaceutical	In vitro dialysis	84	[82]
Ultrasounds	Quinoa and maize	107–222	Rutin	Spectrophotometry	Functional foods	Simulated salivary juice	63–67	[72]

### 3.1.1. Nanoprecipitation Starch Sources

The most common synthesis method used for direct encapsulation is through nanoprecipitation method. Many authors use this method for the encapsulation of different active compounds and also, different types of starches can be used, where corn starch is the most common of them.

#### Corn Starch

In recent studies, acetylated corn starch was formulated with high and low degree of substitution (DS) where the antibiotic-loaded SNPs were prepared by a nanoprecipitation

process by Najafi et al. Acetylated starch with different DS was dissolved and mixed with the antibiotic (ciprofloxacin) in acetone as the organic phase. Subsequently, distilled water was added into the solution containing the polymer under stirring until the acetone was completely evaporated [16]. In the same way, El-Naggar et al. synthesized diclofenac sodium (DS) loaded cross-linked corn SNPs by modified nanoprecipitation method. The aqueous phase contained corn starch dispersed in distilled water containing NaOH under continuous high-speed homogenization. Tween 80 was dissolved in distilled water containing different amounts of the drug was added dropwise to the starch solution under a high rate of homogenization and then distilled water containing different amounts of the crosslinking agent (sodium tripolyphosphate) was added to the solution. The resulting DS encapsulated crosslinked SNPs were subsequently precipitated with absolute ethanol and further purified by centrifugation and washing two times with absolute ethanol/water to remove unreacted compounds and finally with absolute ethanol [14]. Other authors reported the loading of *Triphala churna* in corn SNPs following the graft copolymerization method, which consists of loading the drug in gelatinized starch. This is accomplished by heating starch in NaOH solution. Thereafter, the gelatinized starch and the drug were mixed, and acetone is added dropwise under constant stirring for the conversion of the solubilized starch into SNPs [79]. Also, some authors used native waxy corn starch that was debranched with pullulanase to obtain short linear glucans for the synthesis of SNPs for encapsulation of essential oils (EO). The debranched starch was precipitated in presence of an excess of absolute alcohol and washed with aqueous dimethyl sulfoxide solution. The starch was dispersed in deionized water and then autoclaved. Synthesis and encapsulation were carried out by precipitating the dissolved EO in hot ethanol into the starch solution under constant stirring [15]. The loading of drugs in porous starch nanoparticles was also studied by Wang et al. in 2019. The paclitaxel used as the drug was completely dissolved in acetone and then insoluble porous corn starch (starch treated with  $\alpha$ -amylase and glucoamylase in weak acid) was added under stirring. Subsequently, the porous starch adsorbed with the paclitaxel acetone solution was added to deionized water containing hydroxypropyl methyl cellulose and then stirred. At the same time, the load of paclitaxel in porous starch was also studied as a control group [78]. Fu et al. also synthesized lutein-loaded SNPs in order to find a better dispersibility of lutein in water and its stability against chemical oxidation. Corn starch or amylose was mixed with deionized water at different concentrations. These solutions were heated under continuous stirring until the starch was gelatinized. Subsequently, half of an ethanol solution containing lutein was added dropwise. After cooling the mixture to room temperature, the same volume of the ethanol and lutein solution was added again, and the precipitate obtained was washed with ethanol. In this study, an oxidation rate of 25% for the pure lutein encapsulated in SNPs was obtained [19].

#### Banana Starch

Nieto-Suaza et al. reported the development and characterization of composite films made from starch and aloe vera gel incorporated with curcumin-loaded starch nanoparticles from native and acetylated banana starch using a nanoprecipitation method. Starch was dispersed in a curcumin-acetone solution under magnetic stirring and then water was added dropwise with constant stirring. The resulting suspension was stirred at room temperature until the acetone was completely vaporized. The obtained SNPs were washed several times with ethanol to remove any excess of curcumin, to be used in the preparation of composite films [18].

#### Mixture of Starches

A mixture of different types of starches can be also used in the synthesis of the SNPs. In recent studies, Ahmad et al. used starches from lotus stem, horse chestnut, and water chestnut for catechin nanoencapsulation. The starch mixture was then hydrolyzed in NaOH solution which was subsequently replaced with distilled water. The catechin was

dissolved in ethanol and added dropwise into the hot starch solution under constant stirring to initiate precipitation. To increase the encapsulation efficiency and reduce the size of the SNPs, the solution was subjected to an ultrasound process using a fixed probe sonicator [57].

### 3.1.2. Microemulsion Starch Sources

Another common method used for the SNPs synthesis is the microemulsion method. Also, corn starch is one of the most common starches used in this synthesis method.

#### Corn Starch

In 2017 Nor Syahida and Subash produced antibiotic-loaded SNPs by an ethanolic microemulsion system. An aqueous phase was formulated containing corn starch in an alkaline urea solution. Meanwhile, the organic phase was composed of sunflower oil, ethanol, Tween 20 as an emulsifying agent, and the antibiotic solution in different quantities (streptomycin and penicillin were used). A small volume of the aqueous phase was pumped into the solution containing the antibiotic under constant stirring. Finally, the nanoparticles were washed twice with ethanol to remove unincorporated components [27]. Alp et al. also used corn starch for the synthesis of SNPs by the microemulsion method for delivery of the histone deacetylase inhibitor CG-1521 in breast cancer. A mixture of starch and the drug dissolved in dimethyl sulfoxide (DMSO) was used as the organic phase, while polyvinyl alcohol (PVA) dissolved in distilled water was used as the aqueous phase. The starch solution was added to the aqueous phase with a homogenizer under nitrogen at room temperature. Finally, SNPs were formed when DMSO was diffused by adding distilled water followed by stirring and the SNPs loaded with the drug were washed with distilled water [31]. In addition, SNPs loaded with conjugated linoleic acid (CLA) were synthesized by an emulsion method where Yang et al. used modified octenyl succinic anhydride (OSA) waxy maize starch as the polymeric precursor of the SNPs and xanthan gum (XG) as a complex carrier to protect it. The aqueous phase consisted of the OSA starch and XG with different mass ratios kept under stirring and the organic phase contained the CLA. The emulsion was homogenized using a high shear mixer and passed twice through a high-pressure homogenizer. Finally, CLA-loaded SNPs were obtained by spray drying [81]. Also, Santander-Ortega et al. studied the capacity of SNPs as a drug delivery system using three different model drugs, flufenamic acid, testosterone, and caffeine. In this study, the organic phase was consisted of the corn starch derivative that was dissolved in ethyl acetate. This solution was added to an aqueous phase with different percentages of PVA obtaining a biphasic system that was emulsified with a high-speed homogenizer. Subsequently, highly purified water was added to force the complete diffusion of the organic solvent into the aqueous phase. The resulting SNPs were obtained when the organic solvent was completely evaporated [28].

#### Potato Starch

Dandekar et al. used potato starch for nanoparticles mediated delivery of docetaxel through the microemulsion method. The docetaxel in ethanol and the potato starch in ethyl acetate were prepared and mixed homogeneously. These two solutions were mixed to obtain the organic phase that was poured into an aqueous solution of PVA and subsequently the mixture was emulsified with the help of a high-speed homogenizer. To facilitate the complete diffusion of the organic phase in the aqueous phase, purified water was added to increase the volume. Finally, the organic phase was completely evaporated [30].

Finally, when it comes to bioactive compounds, curcumin is one of the most studied due to its chemopreventive and therapeutic properties against many tumors [83]. Shahgholian et al. studied synthesized bovine serum albumin (BSA) based SNPs for encapsulation of curcumin using an ultrasonic atomization method for the synthesis of the SNPs. BSA binary compounds including BSA/capsule starch, BSA/national starch, BSA/chitosan were applied as carriers of the model drug. Curcumin was dissolved in ethanol and this

solution was added to the aqueous biopolymer solution with stirring in a sealed beaker to form a thick emulsion. A high-frequency piezoelectric oscillator aerosolized the solution and the generated atomized droplets were blown through the hot glass drying chamber where the solvent was evaporated. The BSA SNPs loaded with curcumin were finally collected in the stainless-steel electrostatic collector [68]. Also, SNPs loaded with curcumin were synthesized using oxidized starch and simultaneously modified with cholesterol and imidazole (Cho-Imi-OS) by a simple dialysis method previously used by Xu et al. Cho-Imi-OS was dissolved in dimethyl sulfoxide (DMSO) under stirring at high temperature in an oil bath. The model drug was dissolved in DMSO and this was added dropwise to the starch solution under constant stirring. Then the mixture was transferred to a dialysis bag and dialyzed against PBS solution. Finally, the SNPs were washed with deionized water [41].

### 3.2. Indirect Encapsulation

In this case, the encapsulation is carried out just after the synthesis of the SNPs. Table 3 summarizes all the parameters discussed in this section for the indirect encapsulation of the SNPs.

**Table 3.** Examples of recent studies of SNPs synthesis methods and encapsulation of different compounds through indirect encapsulation.

Preparation Method	Type of Starch	Particle Size (nm)	Encapsulated Compound	Evaluation Method	Applications	Tested On	EE (%)	References
Dialysis	Potato + BS + OSA	5–200	Benzo[a]pyrene	Fenton reaction	Environmentally treatments	/	95	[39]
Sacrificial template	Pea, mung bean, corn and potato	30–300	Doxorubicin hydrochloride	UV-vis	Cancer therapy	AML12 and HepG2 cells in vitro	97.6	[48]
Ball milling	Water chestnut	271	Pediococcus acidolactici	Enumeration on MRS agar	Food applications	SIG, SIF	73	[53]
Microemulsion cross-linking	/	287	5-Fluorouracil	HPLC	Medical	Dialysis in a simulated digestive environment in vitro	49	[36]
Ultrasound w/ or w/o acid hydrolysis	Potato and corn	40/80	L-ascorbic acid and oxalic acid	HPLC	Food packaging, drug delivery, barrier coatings	/	/	[64]
Emulsion cross-linking	/	/	Ciprofloxacin	UV	/	Dialysis	52–94	[5]
Microemulsion cross-linking	/	94	Methylene blue	UV-vis	Medical	/	51–79	[34]
Acid hydrolysis /nanoprecipitation	Potato	82–180	Paclitaxel	UV-vis	Oral drug delivery	Mouse bone marrow cells	57–96	[20]
Microemulsion cross-linking	/	40–400	5-Aminosalicylic acid	UV-vis	Drug controlled release	Human HeLa cancer cells in vitro	/	[84]
Nanoprecipitation	Corn	140	Curcumin	UV-vis	Gastrointestinal tissue disorders	/	/	[85]
Nanoprecipitation	Corn	40–50	Polyphenols	UV-vis	Food applications	SIG, SIF	60–70	[21]
Nanoprecipitation	Corn	178	Urea	UV-vis	Dialysate regeneration system	/	95	[25]

Table 3. Cont.

Preparation Method	Type of Starch	Particle Size (nm)	Encapsulated Compound	Evaluation Method	Applications	Tested On	EE (%)	References
High-speed jet	Tapioca	55.3–120	Myricetin	/	Food formulations	SIG, SIF in vitro	/	[71]
Ultrasounds and crystallization	Maize	351–468	Tangeretin	UV-vis	Food, pharmaceutical and cosmetic industries	Human microenvironments in vitro	74–83	[86]
Ball milling	Lotus stem, horse chestnut and water chestnut	419–797	Resveratrol	Spectrophotometry	Food and pharmaceutical sector	SIG, SIF digestion	76–81	[54]
Ultrasound and nanoprecipitation	Maize	65–390	Ardisia compressa anthocyanins	Differential pH method	Biodegradable food packaging or antioxidant additives	/	45–52	[87]

The most common methods used for the synthesis of SNPs before the encapsulation in the indirect encapsulation method, similar to the direct encapsulation method, are based on nanoprecipitation and also microemulsion crosslinking. At the same time, in addition to the SNPs synthesis method, the phase where the model drug is dissolved before the encapsulation may vary.

### 3.2.1. Bioactive Compound Dissolved in an Aqueous Solution

One of the most common procedures followed by many authors is to dissolve the drug in an aqueous solution. Liu et al. carried out a study on SNPs as carriers for polyphenols where the SNPs were synthesized through a simple nanoprecipitation method. Four types of polyphenols were used: catechin, epicatechin, epigallocatechin-3-gallate, and proanthocyanidins, dissolved in water. The loaded SNPs were prepared by adsorption experiments and incubated in tubes containing different volumes of polyphenol for different periods of time under constant shaking. Once these times were exceeded, the SNPs were washed three times with water [21]. Also, in 2018, Ding et al. prepared retrograded starch (RS III) nanoparticles for application as a wall material for colon-specific drug delivery by high-speed shearing emulsification using MBAA as the crosslinking agent. The retrograde SNPs were added to 5-fluorouracil (5-FU) aqueous solutions which is one of the common drugs for the treatment of colon cancer and finally, the 5-FU concentration was determined using high-performance liquid chromatography (HPLC) in order to obtain the loading capacity and the encapsulation efficiency [36]. In addition, Shi et al. studied two different methods for drug loading: coating and adsorption method. Also, ciprofloxacin was used as the model drug since it is a commonly used antibiotic. The loading of the model drug through the coating method was carried out at the same time as the SNPs synthesis (direct encapsulation) by adding the ciprofloxacin into the emulsion aqueous phase containing the starch and the crosslinking agent. Nevertheless, through the adsorption method SNPs were dispersed into an aqueous solution containing the drug and the adsorption was allowed for 12 h. At last, in both cases, the concentration of ciprofloxacin was determined using UV spectrophotometry. Finally, compared to the coating method, the release of ciprofloxacin from the SNPs was faster when the adsorption method was used [5]. 5-Aminosalicylic acid (5-ASA), which is an anti-inflammatory agent used primarily to treat inflammatory bowel diseases, was used as a model drug to investigate drug loading and controlled release behaviour of crosslinked SNPs synthesized by a microemulsion crosslinking method. SNPs were dispersed in boiled aqueous solution containing the drug with shaking for several days. Subsequently, they were centrifuged and lyophilized to obtain the drug-loaded SNPs in order to determine the drug loading content [84]. Delsarte et al. prepared SNPs from octenyl succinic anhydride (OSA) starch and 1,4-butane sultone (OSA-BS SNPs) by using

the water dialyzed method for its use to solubilize benzo[a]pyrene (BaP) as a result of the degradation by Fenton process. The drug was introduced in dichloromethane solution and after the solvent evaporation, the starch aqueous solution was added at different concentrations. The results showed that the BaP was almost completely degraded, after the encapsulation and Fenton oxidation, which is a very promising result for developing an environmentally friendly treatment [39]. In a recent study Shabana et al. synthesized SNPs from modified potato starch using ultrasound-assisted and acid hydrolyzed ultrasound-assisted methods. Next, modified SNPs were coated with antioxidants since they improve food properties during its shelf-life. Two types of antioxidants (L-ascorbic acid and oxalic acid) were added to the SNPs solution and sonicated for half an hour. The antioxidant-laden starch pellet was collected and dried in an incubator [64]. Also, Wang et al. prepared SNPs based on the W/O microemulsion method using epichlorohydrin as the crosslinking agent to investigate their drug loading and release properties with methylene blue (MB) as a model drug. SNPs were suspended in a glucose aqueous solution with different amounts of MB and the suspensions were then shaken at different temperatures and for different periods of time. Subsequently, the solutions were centrifuged, and 1 mL of each supernatant was extracted and diluted to a certain volume to determine the drug loading extent and encapsulation efficiency [34].

### 3.2.2. Bioactive Compound Dissolved in Phosphate Buffer Solution

Another procedure followed by some authors is to dissolve the model drug into a phosphate buffer solution (PBS) since it maintains the required pH in the solution. Yang et al. encapsulated doxorubicin hydrochloride (DOX-HCl) into hollow SNPs that were synthesized through a hard template sacrifice process using gelled pea, mung bean, potato, and corn starches as the coat. A mixture of SNPs and DOX was dissolved in PBS and the solution was stirred for one day in the dark to fully charge the model drug. The suspension was centrifuged and washed with PBS to rule out DOX-HCl adsorbed on the surfaces of the SNPs [48]. In addition, oxidized starch nanoparticles (oxy-SNPs) were synthesized for urea adsorption in a recent study by Abidin et al. Urea solutions with different concentrations in PBS were prepared to maintain a neutral pH. SNPs were added to a flask containing the urea solution and the mixture was stirred for one day under ambient conditions. This study confirmed the hypothesis that the oxy-SNPs presented significantly more active sites for the urea union than the typical oxy-starch [25].

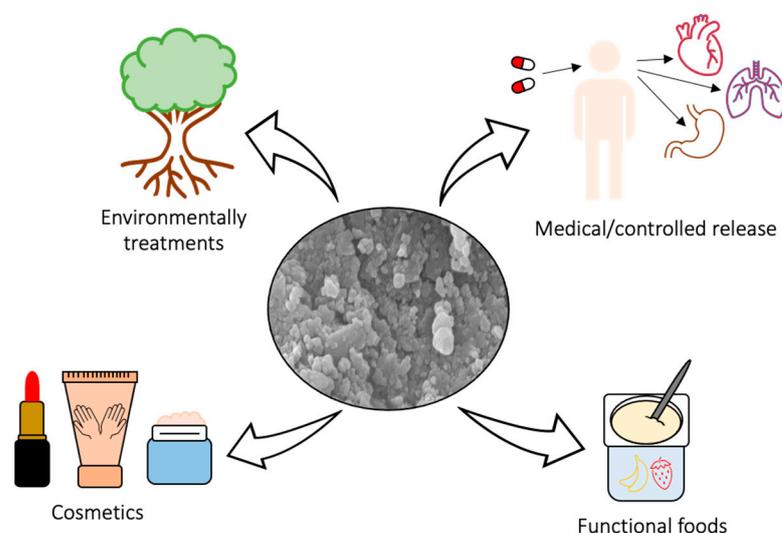
### 3.2.3. Bioactive Compound Dissolved in an Organic Solution

The drug can also be dissolved into an organic phase. It could be both, into an organic solvent and also into an emulsion or an oily phase which will form an emulsion. In a recent study, Nyoo Putro et al. compared the drug loading capacity of SNPs synthesized through nanoprecipitation and acid hydrolysis, in order to know which method may result in a higher loading capacity. Also, different types of surfactants (CTAB, SDS, and Tween 20) were used to modify the SNPs and obtain the highest drug loading. The drug used in this study was paclitaxel, which was dissolved in ethanol at different concentrations, and mixed with the SNPs under stirring. The concentration of unbound paclitaxel was analyzed using UV-Vis spectrophotometry and the characterization of paclitaxel concentration was carried out by mixing drug solution with a mixture of PBS and ethanol [20]. Sufi-Maragheh et al. reported the use of amphiphilic crosslinked SNPs as a stabilizer for Pickering emulsion formulation which was then used for curcumin encapsulation. SNPs were prepared through the alkali freezing method followed by crosslinking by citric acid. The crosslinked SNPs were then dispersed in water at different contents and sonicated followed by the addition of an oil phase containing the curcumin dissolved at room temperature to the aqueous phase in order to form the solid particle-stabilized oil-in-water emulsions [85]. Ahmad et al. carried out a comparative study with native starch and SNPs synthesized through a ball milling mechanical method for the encapsulation of probiotic bacterial cells (*Pediococcus acidolactici*). A phase containing the starch dispersed in Milli-Q water was

prepared and subsequently emulsified with the addition of vegetable oil and surfactant. This mixture was homogenized and the cell culture containing the bacteria was added under slow agitation. The study concluded that SNPs were not the optimal distribution vehicles for probiotics, making native starch a better alternative for this application [53].

#### 4. Applications

SNPs are considered a good vehicle to improve the controlled release of many bioactives. Encapsulated bioactives have numerous applications in different fields such as medicine, cosmetics, biotechnology, or the food industry among others. In addition to Tables 2 and 3 where different applications of encapsulated bioactive compounds are summarized, a general schematic representation of different applications is shown below in Figure 11.



**Figure 11.** Schematic representation of different fields in which SNPs can be used.

##### 4.1. Medical Applications

One of the main medical applications in which SNPs could be used is cancer therapy. In a recent study, Alp et al. demonstrated the advantages offered by the encapsulation of antitumor agents in polymeric nanoparticles for improving the solubility of the drug while protecting against rapid systemic metabolism to increase the time of exposure and reducing the toxicity. To measure the effectiveness of the encapsulated drug, the authors conducted tests with MCF-7 breast cancer cells. The data demonstrated that the encapsulation of CG-1521 in the SNPs improved the therapeutic efficacy against MCF-7 cells and suggested that this encapsulation technology may be useful for other hormone-dependent cancers [31].

Yang et al. also performed *in vitro* experiments with synthesized and antibiotic-loaded SNPs. They evaluated normal liver cells (AML12) and liver hepatocellular cells (HepG2) using MTT assays where it was revealed that SNPs had excellent biocompatibility for normal cells and hepatocellular cells. However, the free drug showed greater antitumor efficacy than particles charged to HepG2 cells due to direct contact with cancer cells, although loaded SNPs showed to release the drug more slowly. On the other hand, they also demonstrated that the viability of AML12 cells was greater than that of HepG2 cells for the different concentrations of the antibiotics studied [48].

Wang et al. also demonstrated the efficacy of SNPs as drug delivery systems. Experiments were carried out in rats by oral administration of the drug or the drug-loaded SNPs as well as the drug directly on the starch and blood samples were collected for the analysis. The mean values obtained for the residence time of the particles were not significantly different from those of the raw paclitaxel. It was concluded that the preparation method of SNPs had a very significant effect on the bioavailability of paclitaxel and that the improving

effect for SNPs was better than when the drug directly loaded on starch. Therefore, the preparation of SNPs is an effective way to improve the bioavailability of paclitaxel [78].

Recently, Xu et al. evaluated the cellular absorption capacity of SNPs using a human lung cancer cell line A549 using curcumin as a control group. Cytotoxicity tests were carried out using free curcumin, which showed limited efficacy against cancer cells. Compared to free curcumin, the SNPs showed an inhibitory effect on cancer cells and the results indicated that these particles as drug-bearing material were not cytotoxic at the required concentrations and could be a promising drug delivery system for the lung cancer cells, acting as an effective vehicle to target and administer medications to the tumor site [41].

Dandekar et al. also demonstrated the safety and efficacy of drug-loaded SNPs as an effective but safe anticancer treatment through cellular cytotoxicity assays using Caco-2 cells. The efficacy of the free drug was studied, and both were analyzed at equimolar concentration. The authors performed the tests during the exponential growth phase of the cells in order to provide an unequivocal indication of any type of cellular toxicity. The effect of the drug on cancer cells was, in turn, compared to that of non-cancer cells using the same test conditions. Finally, the possibility and efficacy of propyl starch to encapsulate and control the release of hydrophobic anticancer agents such as docetaxel in nanocarriers were confirmed [30].

In a most general medical field of application, some authors also carried out tests to determine the functioning of SNPs loaded with drugs to verify their functionality as drug delivery vehicles. El-Naggar et al. conducted a histological pathology study on rat skin to confirm whether the formulation was tolerated without symptoms of skin irritation. They coated the skin of rats with the gel having diclofenac sodium as the drug which was loaded in the cross-linked SNPs and the results were compared with control where the skin of the rats was not coated. Finally, the study revealed that the SNPs loaded with the drug did not show any harmful effect on the skin of the rats and that therefore they could be considered as a good vehicle for treat rheumatoid disorders and other chronic inflammatory diseases [14].

#### 4.2. Food Applications

Among food applications, Ahmad et al. recently studied the release of catechin from SNPs to retain the properties of the bioactive compound throughout simulated gastric and intestinal conditions. The SNPs protected catechin against the hostile gastric environment and helped to retain its bioactive properties during the *in vitro* digestion process [57]. Ahmad et al. also performed *in vitro* studies on the viability of probiotic-loaded SNPs. The study was carried out under simulated gastrointestinal and processing conditions, using both gastric juice and simulated intestinal fluid. However, in this case, it was concluded that SNPs are not good vehicles for probiotics since it was shown that the particles were too small to encapsulate the probiotic cells within them and, therefore, they were immediately released into the simulated gastric or intestinal solutions that lead to cell death due to the harsh conditions of these environments [53]. In another study carried out by Nieto-Suaza et al. recently, different food simulators were used to evaluate curcumin release profiles, where some intended to simulate highly hydrophilic foods and others intended to simulate highly lipophilic foods. The authors concluded that the released curcumin was favored in lipophilic substances and can be controlled by reducing the polarity of the SNPs [18]. Also, Liu et al. investigated the *in vitro* release of SNPs loaded with polyphenols in simulated gastric and intestinal juices. This study showed sustained release profiles of polyphenols from SNPs and suggested that SNPs may be promising and effective nanocarriers to protect bioactive compounds against sensitive environments and control their release [21].

### 5. Future Research Perspectives

Among the methods mentioned in previous sections, there are other types of preparation methods for NPs that have been explored recently from which promising results were obtained, such as enzymatic hydrolysis, which has been used to produce SNPs

for application as stabilizers of Pickering emulsions [88,89]. Recrystallization is another physical method to prepare SNPs with a clear environmental advantage since it does not use solvents during the preparation process, it is based on a molecular reassociation during a cooling or drying process to produce starch gel [90]. In addition, ultrasound as a unique technique, has been used to produce SNPs combined with other conventional techniques [91]. Plasma had also been used as a preparation technique for SNPs providing a promising method to develop SNPs with low crystallinity [92].

During the last years, SNPs preparation methods have been focused on the use of green technology which could reduce or even eliminate the use of organic solvents that could have negative effects on further SNPs applications or even reduce their potential applications. Moreover, the reduction of the use of solvents will have a positive effect from an environmental point of view.

Moreover, in recent years, the interest in SNPs has been increased since apart from the mentioned applications such as Pickering emulsions preparation, other applications are being investigated with some preliminary promising results [6]. The treatment of tumor cells by targeted delivery is one major potential for the SNPs since they present a large area/volume ratio which makes them suitable for the delivery of bioactive compounds as well as drugs. Moreover, the molecular structure of the main compounds of starch allows to easily attach many types of proteins, bioactive compounds, or antibodies which will be a promising approach for specific delivery and avoiding systemic effects [93]. Hence, starch not only presents a promising alternative as nanostructured systems but also present clear advantages as therapeutic systems [94]. Also, another promising application of SNPs is in DNA precipitation where SNPs interact with DNA and result in precipitation in an ethanol solution at room temperature [95].

Furthermore, the emerging applications of SNPs include their use for sensor development. Filter paper has been coated with pyrene modified SNPs and tested as a fluorescence quenching sensor platform. Nitroaromatic compounds were detected with good limits of detection and selectivity over non-nitrated aromatics, amines, and aromatic ketones [96]. Also, in recent studies, nanocomposites prepared with potato starch and ZnS quantum dots were used as a sensor for  $Pb^{2+}$  and  $Cu^{2+}$  ions. The detection was based on the decreased emission intensity of the photoluminescent spectral bands [97].

Even with the numerous advantages and applications of SNPs, their production is poorly industrialized on a large scale. As it is the case of the other types of NPs, for which the techno-economic production analysis has been described in other works [98], the first step will be the collection of information from the laboratory experiments to identify the main streams and processes. Further pilot plant experiments should be designed in order to have data related to the scale-up production, energy requirements, and raw material required. Further major unit operations are needed to be defined and designed, as well as main process streams.

## 6. Conclusions

Currently, the field of nanoparticles is experiencing a great expansion in terms of scientific research due to its great potentials as nanocarriers in different bioapplications in fields as medicine, cosmetics, or food. SNPs offer a clear advantage compared to the other types of nanoparticles due to their high biocompatibility and versatility since there is a wide range of potential synthesis methods that allow the controlling of the final shape properties such as and size and size distribution.

SNPs have been synthesized through different synthesis methods using starches from several sources. Usually, both the synthesis method and the type of starch will affect the final properties of the nanoparticles, as well as their capacity for encapsulation. Maize starch is one of the most commonly used for SNPs synthesis both individually or in combination with other starches, such as potato, pea, or mung bean, which has shown to led to small particle sizes. Both nanoprecipitation and microemulsion methods allow obtaining SNPs with target small sizes.

SNPs have been used to encapsulate bioactive compounds for controlled release in different biomedical applications such as drug administration, enzyme inhibition process, and even DNA precipitation.

The encapsulation efficiency of the bioactive compounds did not vary significantly when the encapsulation was performed directly or indirectly and therefore, it was concluded that the encapsulation method does not influence the amount of bioactive compound encapsulated in the SNPs. Nevertheless, the type of starch selected has shown to have a major influence since higher encapsulation efficiencies were obtained when maize starch was used for the SNPs synthesis with both encapsulation methods.

It has been demonstrated through in vivo and in vitro experiments that SNPs are a good vehicle for the controlled release of bioactive compounds and the use of these loaded SNPs are promising agents to encapsulate drugs and antitumor agents that could be used in therapy. However, there is still not enough research performed on the characterization of the SNPs for in vivo drug delivery formulations which is necessary to study the possible interactions with the human body and their consequences in order to determine how SNPs will adversely affect human health in short and long terms.

The main disadvantage of SNPs is that their synthesis process is not scaled up yet. Even some pilot plant processes have been designed for SNPs preparation, there is still further research necessary to develop more novel methods that will allow obtaining large-scale production of SNPs with control on the final particle size. Moreover, the studies found regarding the preparation of SNPs containing encapsulated bioactive compounds have not yet been industrialized and future research is necessary in order to obtain satisfactory bioactive or drug-loaded SNPs on a large scale.

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