Synthesis of controlled-size starch nanoparticles and superparamagnetic starch nanocomposites by microemulsion method

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Abstract

In this study, a synthesis process based on the microemulsion method (ME) was developed with the aim to produce controlled-size starch nanoparticles (SNPs). Several formulations were tested for the preparation of the W/O microemulsions varying the organic/aqueous phase ratios and co-stabilizers concentrations. SNPs were characterized in terms of size, morphology, monodispersity and crystallinity. Spherical shape particles with mean sizes 30-40 nm were prepared.

The method was then used to simultaneously synthesize SNPs and iron oxide nanoparticles with superparamagnetic properties. Starch-based nanocomposites with superparamagnetic properties and controlled size were obtained. Therefore, the microemulsion method developed could be considered an innovative technology for the design and development of novel functional nanomaterials. The starch-based nanocomposites were evaluated in terms of morphology and magnetic properties, and they are being considered as promising sustainable nanomaterials for different biomedical applications.

Keywords

Starch nanoparticles, microemulsion, size control, superparamagnetic particles, nanocomposites

Chemical compounds studied in this article

Sodium hydroxide (PubChem CID: 14798); Urea (PubChem CID: 1176); Absolute ethanol (PubChem CID: 702); CTAB (PubChem CID: 5974); 1-Butanol (PubChem CID: 263); 1-Hexanol (PubChem CID: 8103); Ammonia (PubChem CID: 222); Ferric chloride hexahydrate (PubChem CID: 16211236); Ferrous chloride tetrahydrate (PubChem CID: 16211588); Hydrochloric acid (PubChem CID: 313).

1. Introduction

Starch is a natural, renewable, biodegradable, and biocompatible polysaccharide and it is the main source of carbohydrate storage in plants (Liu, Li, Li, Zhang & Li, 2021). From the chemical point of view, it is a polymer composed by two polysaccharides, amylose and amylopectin, both made up of by glucose units. Starch nanoparticles (SNPs) are considered one of the most promising novel sustainable biomaterials for use in many different biotechnological applications. SNPs are obtained from starch granules through different physical and chemical techniques and both the synthesis method and the operating conditions influence their final properties for different further applications.

Several physicochemical methods have been reported to produce SNPs. Some of them are high-pressure nanoemulsification, crosslinking, microemulsion or nanoprecipitation, among others (Kim, Park & Lim, 2015; Le Corre, Bras & Dufresne, 2010; Saari, Fuentes, Sjöö, Rayner & Wahlgren, 2017; Chin, Azman & Pang, 2014; Chin, Pang & Tay, 2011; Najafi, Baghaie & Ashori, 2016; Morán et al, 2021). The microemulsion method is a soft chemistry technique with a growing interest is a soft chemistry alternative with a growing interest as it does not require sophisticated equipment, hazardous reagents or extreme conditions. Moreover, efficient control of the size, shape, monodispersity and composition of SNPs can be achieved (Chin et al., 2014). Microemulsions are an alternative and novel approach for the SNPs synthesis, as their composition and structure can be optimized to achieve the desired characteristics of the SNPs (Asgari, Saberi, McClements & Lin, 2019). Water-in-oil (W/O) microemulsions consist of small water droplets (dispersed or internal phase) dispersed in an oily phase (continuous, external or dispersing phase), where surfactants and co-surfactants are present to stabilize the interphase. The small water domains formed within the microemulsions can be used as nanoreactors where the starch precipitates as SNPs (Asgari et al., 2019).

Nanotechnology is opening new horizons at the biomedical field and optical, electronic, chemical and mechanical applications (Darroudi, Hakimi, Goodarzi & Kazemi Oskuee, 2014). Magnetic nanoparticles have been studied for drug delivery, enzyme immobilization (Vaghari et al., 2016) and different biotechnological purposes (Materón et al., 2021). They are composed of pure metals, metal alloys and metal oxides (Malhotra et al. 2020). Iron oxide nanoparticles (IONPs), in particular, are frequently used due their minimal toxicity and excellent physico-chemical properties such as the superparamagnetism, and their biocompatibility and stability in aqueous solutions (Medeiros et al., 2015; Soares et al., 2016; Valdiglesias et al., 2016). The feasibility of producing loaded magnetic iron oxide-impregnated SNPs by a synthesis based on an emulsion crosslinking method has been reported. These nanocomposites are attractive as possible and potential drug carriers for magnetically directed drug delivery (Likhitar & Bajpai, 2012).

Our research group has developed a microemulsion method to produce size-tuned iron oxide nanoparticles with superparamagnetic properties (Salvador et al., 2021). In the present study, the main objective was to adapt and optimize this microemulsion method to produce controlled-size SNPs, and then use it to carry out the simultaneous synthesis of both types of nanoparticles (SNPs and IONPs).

Thus, one of the most remarkable research advances of this work is to demonstrate the versatility of using the microemulsion method to synthesize sustainable nanoparticles of different nature with slight modifications in the W/O microemulsion formulation prepared for this purpose, and also the ability to nanoprecipitate them simultaneously. Therefore, the hypothesis of this study is that by optimizing the formulation of the W/O microemulsion used for the precipitation of nanoparticles, as well as the formulation of the precipitating agent, it is possible to simultaneously synthesize controlled-size starch nanoparticles and iron oxide nanoparticles with superparamagnetic properties to develop sustainable novel nanocomposites, which will demonstrate that the proposed ME method is an innovative technology for the design and development of new and novel nanomaterials.

Thus, in this work, controlled-size SNPs were synthesized by the W/O ME method using different formulations for the microemulsions preparation and optimizing the proportion and composition of the aqueous phase, the organic phase and the amounts of stabilizer, co-stabilizer, and the type of precipitating agents used. The synthesized SNPs were characterized in terms of shape and size by dynamic light scattering (DLS) (Nanozetasizer from Malvern) and scanning electronic microscopy (SEM). X-ray powder diffraction (XRPD) was used to analyze the crystallinity of both the granules and resulting SNPs. Nanocomposites of SNPs and IONPs have been synthesized by the ME method, and their morphology and magnetic properties have been studied.

2. Materials and methods

2.1. Materials

Cetyl Trimethyl Ammonium Bromide 99 % (CTAB, $C_{19}H_{42}BrN$, M_W = 364.46 g/mol), was supplied as a white powder by Sigma-Aldrich (USA). This is a quaternary ammonium salt, with long alkyl and detergent activity. In this cationic surfactant, the hydrophilic part is positively charged, and its hydrophilic-lipophilic balance (HLB) value is 10. 1-Butanol was supplied by Sigma Aldrich (USA) and was used as a co-stabilizer in the microemulsion. 1-Hexanol, supplied by Alfa Aesar (USA), acts as the organic phase in the microemulsion as it has a longer alkyl chain than that of 1-butanol.

Milli-Q water was used to prepare the solutions to be used in the synthesis and for the subsequent washing of the nanoparticles. Absolute ethanol supplied by Sigma Aldrich (USA) was also used to wash the nanoparticles.

Maize starch with 0.25% moisture and a branching (α-1,4)/(α-1,6) ratio of 15.2 was purchased from Cerestar-AKV I/S (Denmark). It was presented as a white powder insoluble in water at room temperature.

NaOH (M_W = 39.997 g/mol) was supplied by Panreac AppliChem (Spain) and used was used as a precipitating agent for the SNPs synthesis, as well as for the formulation of the aqueous phases. Urea (M_W = 60.056 g/mol), was supplied by Serva Electrophoresis GmbH (Germany). This reagent is presented as white crystalline powder and due to its dipole moment, it is soluble in water and in alcohol. In this work it was also used for the formulation of the aqueous phases.

Ammonia 30% (NH_3, M_W = 17.03 g/mol) was used as a precipitating agent in order to alkalinize the aqueous solution containing the starch and the iron salts and precipitate them in the form of nanoparticles. It was provided by Panreac AppliChem (Spain). Ferric chloride hexahydrate ($FeCl_3 \cdot 6 H_2O$, M_W = 270.30 g/mol), a very hygroscopic yellow-orange crystalline solid, was supplied by Panreac AppliChem (Spain). Ferrous chloride tetrahydrate ($FeCl_2 \cdot 4 H_2O$, M_W = 198.81 g/mol) was supplied by J.T. Baker (USA). It is a light green solid, soluble in water and with a high tendency to oxidize to ferric chloride. Both the ferric and the ferrous chloride were used in an aqueous solution as precursors for the production of magnetite. Hydrochloric acid 38% (HCl), was supplied by Sigma-Aldrich (Germany) and it was used to prepare the aqueous solution of iron salts.

2.2. Methods

Microemulsion (ME) method was adapted from a previous works for synthesis of superparamagnetic nanoparticles (Salvador et al., 2021). The obtention of the SNPs by this ME method can achieved in two steps: (i) preparation of the microemulsion systems and (ii) synthesis of the SNPs by adding the precipitating agent.

2.2.1. Preparation of the microemulsions

Microemulsions formulated in this work were W/O type, i.e., water droplets dispersed into an oily phase. To obtain a W/O microemulsion, the oily phase contained the organic solvent, alcohol as co-stabilize and surfactant as stabilizer. The surfactant and alcohol molecules are arranged as reverse micelles, which means that the water-soluble hydrophilic heads point towards the inner part of the droplets, while the hydrophobic tails point towards the outside, where the organic solvent is found.

Subsequently, this organic phase was in contact with an aqueous phase in which the biopolymer was dissolved, and this quickly diffused towards the hydrophilic regions of the micelles, giving rise to the formation of nanometric hydrophilic regions rich in biopolymer, with diameters generally less than 100 nm. This small water regions that will later act as templates for the SNPs formation can be called nanoreactors (Asgari et al., 2019).

To obtain the microemulsions, a series of steps must be followed, as detailed below.

2.2.1.1. Preparation of the aqueous phase

First, 1% (w/v) starch solution was prepared by dissolving 0.2 g of starch into 20 mL of a solvent system. Three different solvents were tested: (i) Milli-Q water, (ii) 8% (w/v) NaOH, and (iii) 8% (w/v) NaOH + 10% (w/v) urea. This starch solution was kept under a 1000 rpm constant stirring at 80°C for 30 min until the starch was fully dissolved, obtaining a completely homogeneous solution. These synthesis parameters were selected based on a previous work (Gutiérrez et al., 2020).

2.2.1.2. Microemulsions formulation

In order to formulate the microemulsions, an organic solution consisting of a mixture of CTAB (surfactant that acts as a stabilizer), 1-butanol (co-stabilizer) and 1-hexanol (organic phase) was prepared. A 3:2 mass ratio of surfactant to co-stabilizer was kept constant in all formulations. 1-butanol is distributed mainly between the interfacial layer and the organic phase, acting as a co-stabilizer in the

interfacial layer and as a co-solvent in the organic phase (Wang, Chen, Luo, Fu, 2016).

Different microemulsion formulations were studied. In order to determine the appropriate composition for SNPs preparation, a microemulsion stability region must first be determined by the titration method in a ternary diagram (Salvador et al., 2021). Figure 1 shows the six optimal microemulsion formulations selected from previous studies for the present work (M1, M2, M3, M4, M5 and M6).

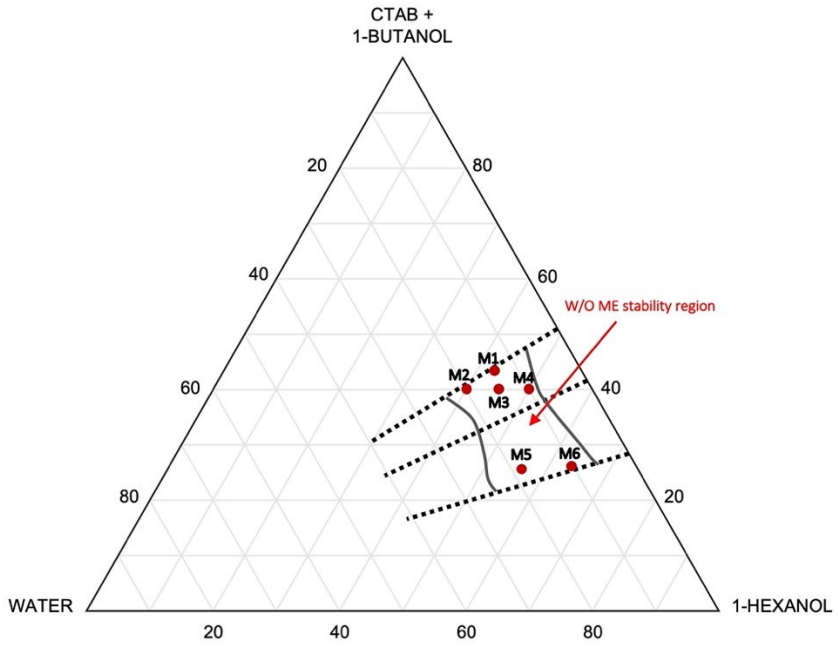

Figure 1. Ternary diagram of the CTAB-butanol-hexanol-water system with the composition of the microemulsions studied for the SNPs synthesis (M1 to M6).

Once the six best formulations for obtaining the microemulsions were identified, the surfactant and the co-stabilizer were added to the organic phase by weighing. Subsequently, this mixture was kept under high agitation for 10 min until a homogeneous solution was obtained. Afterwards, the aqueous phase prepared above was added, and the mixture was gently stirred to promote homogenization and hence microemulsion formation. Due to the small size of the aqueous droplets formed (values less than 1 micron), light can pass through them, so microemulsion formation was evident once the solution became totally translucent. Table 1 gathers the compositions of the different reagents of each microemulsion formulation.

Table 1. Microemulsion composition of the six formulations selected within the microemulsion stability region for the SNPs synthesis.

Microemulsion system	Microemulsion composition (g)			
	CTAB	1-Butanol	1-Hexanol	Aqueous phase
M1	5	4	9	3
M2	5	3	8	4
M3			9	3

M4			10	2
M5	3	2	11	4
M6			13	2

2.2.2. Synthesis of the SNPs

SNPs were obtained by adding a solution containing a precipitating agent (an organic solvent) to the previously prepared microemulsion which causes the starch to precipitate in the form of nanoparticles inside the water droplets of the microemulsion. Two precipitating agents were tested: (i) 12% (w/v) NaOH + ethanol, and (ii) pure ethanol. For the preparation of the NaOH solution, it was necessary to stir the mixture at 500 rpm for 24 h at room temperature due to the low solubility of NaOH in ethanol with respect to its solubility in water.

Once the precipitating solution was ready, it was added dropwise to the microemulsion system, keeping the mixture under constant high stirring at all times. SNPs formation was noted visually as small white aggregates appeared in the solution as drops were added. As soon as these aggregates appeared, the addition of the drops was stopped since the SNPs were formed. A scheme of this process is shown at Figure 2.

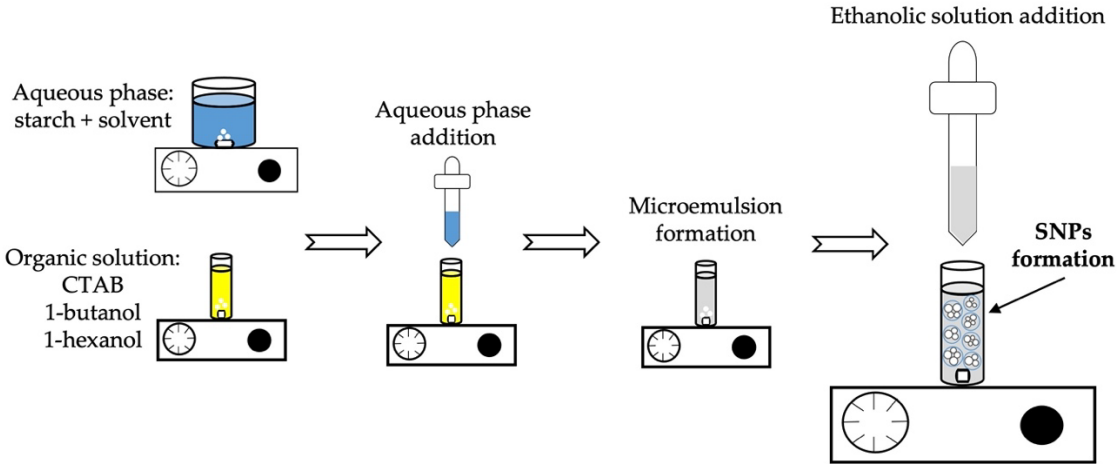

Figure 2. Scheme of the SNPs synthesis process by the microemulsion method.

Once the SNPs were obtained and prior to their characterization, samples had to be carefully washed to remove the excess of stabilizers or solvents. First, samples were centrifuged at room temperature for 10 min at 10000 rpm and the supernatant was removed to obtain the particles as pellets, which were then washed six times with alternate washes of ethanol and 50%-50% mixtures of absolute ethanol and Milli-Q water, centrifuging again under the same conditions between each washing step.

2.2.3. Synthesis of the starch superparamagnetic nanocomposites

The same microemulsion method was followed for the obtention of composites SNPs-IONPs. However, in this case, the aqueous phase consisted of a mixture of solutions of starch and iron salts. A 2.5% (w/v) starch solution was prepared by dissolving 0.5 g of starch into 20 mL of Milli-Q water under 1000 rpm constant stirring at 80°C for 30 min. In turn, a 50 mL solution with a molar ratio of iron salts Fe^{2+}/Fe^{3+}

of 0.5 was prepared, containing 0.01 M of HCl to prevent further oxidation of the Fe^{2+}. When both solutions were completely dissolved, they were mixed and kept at 1000 rpm constant stirring at 80°C for 30 min. Once prepared, the final solution was allowed to cool until room temperature was reached. Finally, it was added to the CTAB-1-butanol-1-hexanol system to form the microemulsion, as for the SNPs synthesis. The same 6 microemulsion systems were also studied in this case.

The SNP-IONPs composites were achieved by adding the precipitating agent (30% (v/v) ammonia solution) dropwise to the microemulsion under high stirring. The synthesis of the nanoparticles was now visually detected by the appearance of a mixture of a black precipitate and white nanoparticles. Therefore, at that moment, the addition of ammonia was stopped. The solution was left under magnetic stirring for 2 h and then washed several times with distilled water assisted by a permanent magnet.

2.3. SNPs characterization

2.3.1. Particle size distribution

An approximate idea of the hydrodynamic size (in number) and the homogeneity (PdI) of the particles was obtained by dynamic light scattering (DLS) using a Zetasizer Nano ZS equipment (Malvern Instruments Ltd, Malvern, UK). Samples were measured with the 173° backscatter detector in low volume disposable cuvettes (Malvern Instruments Ltd, Malvern, UK).

2.3.2. Morphology and size

The final size and shape of the SNPs were analyzed using a JEOL JSM-5600 field emission scanning electron microscope at an acceleration voltage of 20 kV. Samples were dried in a stove for 24 h at 80°C. Once dehydrated, they were fractured with a spatula and deposited on a double-sided adhesive tape on a copper substrate. They were coated with a gold thin film in Balzers SCD 005 sputtering device (Bal-Tec AG, Liechtenstein) prior to the analysis to prevent the electric charge built-up under the electron beam in the microscope. The average particle size of the SNPs was determined by random measurements on the images using ImageJ software.

Furthermore, the final size and shape of the superparamagnetic SNPs were analyzed using a transmission electron microscope (TEM JEOL-2000 EX-II). An aliquot of an aqueous suspension of the samples was placed into a copper-grid supported transparent carbon foil and analyzed. The average particle size was also determined by using ImageJ software.

2.3.3. X-ray powder diffraction (XRPD) analysis

X-ray powder diffraction (XRPD) was used to determine the crystalline structure of the synthesized SNPs as well as the starch granules. The powder X-ray diffraction data for the samples were collected at RT, using CuKα1.2 radiation (λ = 1.54056 Å and 1.54439 Å) in a Bragg-Brentano reflection configuration, on a Philips Panalytical X'Pert Pro diffractometer in a 2θ range of 5-27°, with a step size of 0.08356. The estimation of the crystalline domain sizes of the SNPs was obtained using the FullProf program.

2.3.4. Magnetic characterization

An EV9 vibrating sample magnetometer (VSM) equipped with an electromagnet producing fields up to

±2.2 T was used to obtain the magnetization curves of the superparamagnetic SNPs and IONPs at room temperature (298.15 K). Powder samples were analyzed, by using 0.05 T field steps, and the results were normalized to the magnetic phase.

3. Results and discussion

3.1. Starch nanoparticles (SNPs)

3.1.1. Particle size distribution, size and morphology

As explained in sections 2.2.1 and 2.2.2, the synthesis of the SNPs was carried out under different synthesis conditions, studying different microemulsion systems (M1 to M6) and different ethanolic solutions. The purpose was to optimize the method and to achieve the precipitation of the starch in the form of nanoparticles.

Results for the morphological characterization for each system are shown at Table 2. The particle size distributions and the polydispersity indices (PdI) were obtained by DLS, and the shape of the particles (spherical or non-spherical) by SEM.

The protocol was the same for each case: first SNPs were synthesized (different compositions), after the washing steps, they were characterized by DLS and finally, they were dried and characterized by SEM. All the experiments were performed in triplicate (at least) in order to evaluate their reproducibility.

Table 2. Main sizes of SNPs obtained by the microemulsion method with the different microemulsion systems studied

Sample	Aqueous phase (%w/v)	Precipitating agent (%w/v)	Size (nm)	PdI	Spherical shape
M1-A	Starch + Milli-Q	NaOH 12% + ethanol	165±19	0.75±0.0	Yes
M1-B	Starch + Milli-Q	Ethanol	-[1]	-[1]	-[1]
M1-C	Starch + NaOH 8%	Ethanol	21±3	0.49±0.0	No
M1-D	Starch + NaOH 8% + urea	Ethanol	43±5	0.60±0.0	No
M2-A	Starch + Milli-Q	NaOH 12% + ethanol	40±3	0.81±0.1	Yes
M2-B	Starch + Milli-Q	Ethanol	-[1]	-[1]	-[1]
M2-C	Starch + NaOH 8%	Ethanol	29±4	0.27±0.0	No
M2-D	Starch + NaOH 8% + urea	Ethanol	43±10	0.55±0.0	No
M3-A	Starch + Milli-Q	NaOH 12% + ethanol	34±10	0.77±0.1	Yes
M3-B	Starch + Milli-Q	Ethanol	-[1]	-[1]	-[1]
M3-C	Starch + NaOH 8%	Ethanol	31±4	0.54±0.1	Yes
M3-D	Starch + NaOH 8% + urea	Ethanol	38±6	0.55±0.0	No
M4-A	Starch + Milli-Q	NaOH 12% + ethanol	-[1]	-[1]	-[1]
M4-B	Starch + Milli-Q	Ethanol	-[1]	-[1]	-[1]
M4-C	Starch + NaOH 8%	Ethanol	28±5	0.48±0.0	No
M4-D	Starch + NaOH 8% + urea	Ethanol	48±3	0.58±0.1	Yes
M5-A	Starch + Milli-Q	NaOH 12% + ethanol	36±7	0.98±0.0	Yes

M5-B	Starch + Milli-Q	Ethanol	-[1]	-[1]	-[1]
M5-C	Starch + NaOH 8%	Ethanol	66±13	0.47±0.0	Yes
M5-D	Starch + NaOH 8% + urea	Ethanol	47±4	0.61±0.0	Yes
M6-A	Starch + Milli-Q	NaOH 12% + ethanol	40±4	0.64±0.1	Yes
M6-B	Starch + Milli-Q	Ethanol	-[1]	-[1]	-[1]
M6-C	Starch + NaOH 8%	Ethanol	24±3	0.42±0.0	Yes
M6-D	Starch + NaOH 8% + urea	Ethanol	58±17	0.52±0.0	No

[1] No DLS available.

Looking at the DLS size results in Table 2, a first conclusion can be reached: the smallest particle sizes were reached when NaOH was present in the aqueous phase, obtaining relatively small sizes both in presence and absence of urea (samples C and D of each system) in most of the cases studied. For each formulation tested, particle sizes were smaller in the absence of urea, except for the M5 microemulsion system where the smallest sizes were obtained when both NaOH and urea were present in the aqueous phase with the starch. However, when samples were analyzed by SEM, only five of them presented a spherical shape (M3-C, M4-D, M5-C, M5-D and M6-C) but most of them shown agglomerates.

When NaOH acted as the precipitating agent (sample A of each system), small particle sizes were obtained by DLS (M2-A, M3-A, M5-A and M6-A), except for samples M1-A and M4-A which presented large particles and no particles, respectively. It is important to point out that M1, M4 and M6 represent the formulations with lower water content compared to the other systems. When M2-A, M3-A, M5-A and M6-A samples were analyzed by SEM, all of them showed a spherical shape, although some agglomerates were observed in M2-A sample. However, the size was also measured with ImageJ software for all formulations tested. A size range between 12 nm and 47 nm was obtained for M2-A sample. For M3-A sample a size range between 27 nm and 74 nm was obtained. In spite of the higher sizes than the previous ones, the particles presented less aggregates and a greater number of particles was obtained. The same effect was observed with sample M5-A, where the amount of particles obtained was also higher, and the size range varied from 24 nm to 51 nm. Finally, large spherical particles were obtained for sample M6-A, in contrast with the small sizes expected with the DLS results: the size range for the SNPs varied from 64 nm to 180 nm and some particle aggregates were observed, also indicating in this case that the formulations that correspond to the stability region with less water content indicate is not suitable to form uniform SNPs.

Taking into account these sizes obtained, it can be concluded that the best results were achieved when an aqueous phase containing 1% (w/v) starch dissolved in Milli-Q water was used in combination with a precipitant solution containing NaOH (12% (w/v) NaOH in ethanol). Low aspect ratio particles were thus obtained (samples A). NaOH breaks the hydrogen bonds between water and starch, resulting in the disruption of the existing molecular orders within the starch granules (Neelam, Vijay & Lalit, 2021), thus improving starch solubility in water (Han & Lim, 2004).

At the same time, with these synthesis parameters, microemulsion systems M2, M3 and M5 showed the smallest particle sizes. The size obtained by DLS was around 40 nm for the sample M2-A, with a PdI of 0.81. This high value of the polydispersity index can be explained by the fact that particle size varied over a fairly wide range as mentioned above (from 12 nm to 47 nm). This explains that the sample is not monodisperse, but it rather presents great variety of sizes. The same happened with the other two samples: size obtained by DLS was around 34 nm for sample M3-A, with a PdI of 0.77 but ranging from 27 nm to 74 nm; for sample M5-A, size obtained with DLS was around 36 nm with a PdI of 0.98 in an interval from 24 nm to 51 nm. The micrographs in Figure 3 show that small particles predominated in samples M3-A and M5-A, which is consistent with the data obtained by DLS where slightly smaller particles were observed compared to sample M2-A.

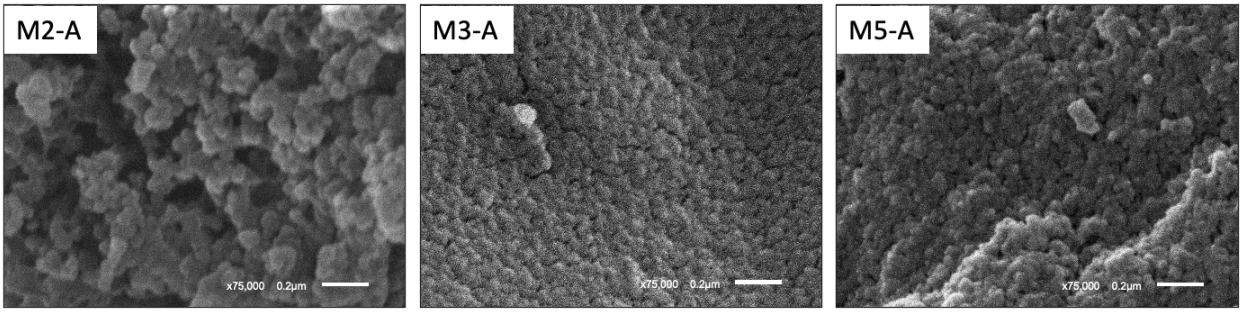

Figure 3. SEM micrographs for M2-A, M3-A and M5-A systems

From Figure 3, it could be estimated that both the M2-A system, as well as the M3-A and M5-A systems were optimal the formulations to obtain small, spherical and homogeneous SNPs by the microemulsion method. Similar results were reported by other authors (Zhou, Luo & Fu, 2014) who suggested the use of the ME method to obtain SNPs with good sphericity, small size and a narrow particle size distribution to be used as drug delivery systems.

3.1.2. SNPs production yield

Once the synthesis conditions and the optimal microemulsion systems were selected, SNPs production yield was determined. For this purpose, the total amount of SNPs per amount of starch added into the aqueous phase was calculated. Samples were dried in a stove, in the same way as for the SEM characterization, for 24 h at 80ºC in small flat-bottomed glass tubes.

The experiments were carried out in triplicate and finally, for each 0.2 g of starch added to the aqueous phase, 0.032 g of particles were obtained for the M2 microemulsion system, 0.020 g for the M3 system and 0.029 g for the M5 system. The production yield of SNPs obtained was 16% for the M2 system, 10% for the M3 system and 15% for the M5 system.

3.1.3. XRPD analysis

XRPD was applied to determine the crystalline structure of starch granules as well as that of the SNPs of the optimal formulations. The spectra are shown in Figure 4. Starch granules showed peaks at Bragg angles (2θ) at 15°, 17°, 18° and 23° corresponding to A-type X-ray diffraction patterns, which is in good

agreement with results from previous studies (Lin et al, 2020).

Nevertheless, these starch characteristic peaks did not appear in the spectra obtained for the SNPs. This may indicate that the synthesis process affected the crystalline structure of the starch granules, and finally all synthesized SNPs showed an amorphous crystalline structure, which is in agreement with the results obtained by other authors (Ding, Lin & Kan, 2018).

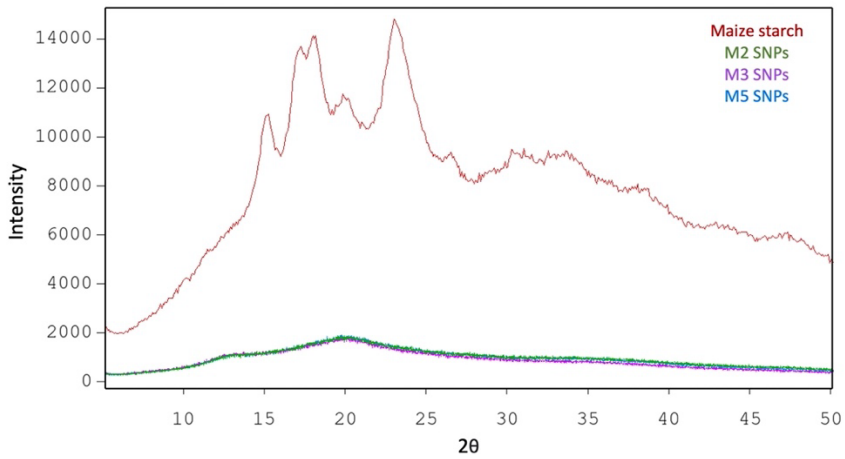

Figure 4. XRPD spectra of starch granules and the resulting SNPs synthesized with the optimal formulations.

Dufresne et al summarized the polymer nanocomposite trend as a function of the nature (crystalline or amorphous) of the matrix and its interaction with nanostructured fillers where several nanocomposites presented amorphous structure with good interaction for different fillers. (Dufresne, Medeiros, & William, 2009)

3.2. Starch superparamagnetic nanocomposites (SNP-IONPs)

Formulations M3 and M5 were used to simultaneously synthetized SNPs and IONPs and produce size-tuned starch-based superparamagnetic nanocomposites susceptible to be used for further bioapplications. It has been demonstrated in previous studies that starch-based magnetic nanocapsules have a major potential for the targeted delivery of hydrophilic bioactives through a magnetic field-generated (Sousa et al, 2021). Similar trend was observed in another study where an effectively delivery of the antitumor drug cisplatin from superparamagnetic nanoparticles of crosslinked starch impregnated was reported in the presence and absence of magnetic field via diffusion-controlled pathway (Likhitar & Bajpai, 2012).

Best results for the synthesized nanoparticles were obtained for these M3 and M5 systems with a size range that varied between sizes from 28 nm to 55 nm for system M3, and between 23 nm y 73 nm for system M5. The average diameters varied between 44 nm and 43 nm respectively. TEM micrographs shown in Figure 5 revealed the formation of low aspect ratio particles despite of certain agglomeration degree and some irregular shapes. In both micrographs, the SNPs with IONPs can be easily identified. In turn, the properties of these SNP-IONPs composited can be compared with those of the iron salts in absence of starch (IONPs M3 and IONPs M5).

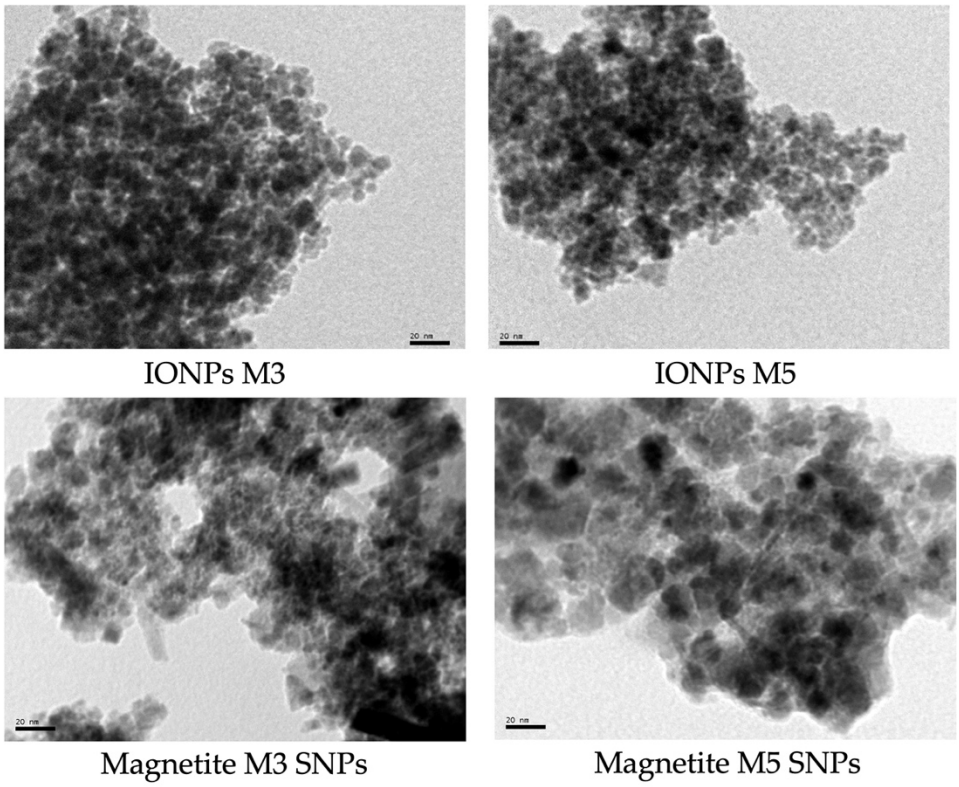

Figure 5. TEM micrographs of the superparamagnetic SNPs and IONPs obtained with M3 and M5 microemulsion systems.

In addition, Figure 6 shows the SEM micrographs where the different particle sizes for each system can be seen.

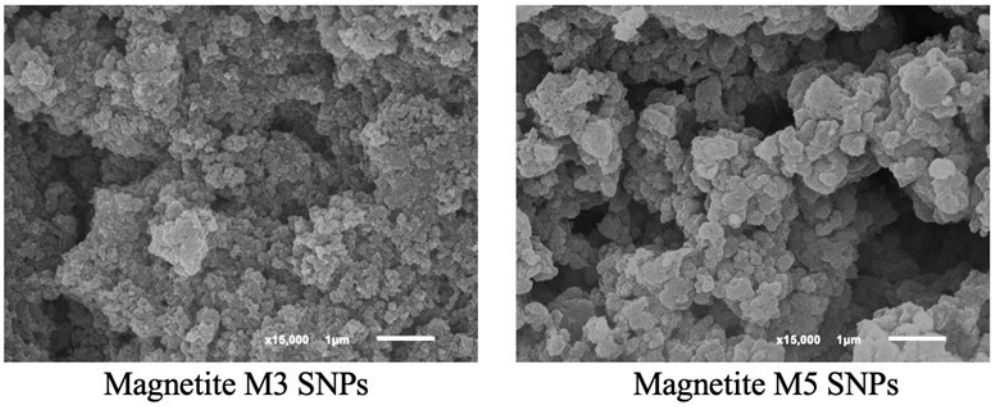

Figure 6. SEM micrographs of the superparamagnetic SNPs (SNP-IONPs composites) obtained with M3 and M5 microemulsion systems.

At the same time, magnetization studies with iron oxide SNPs were performed and compared with IONPs synthesized in absence of starch (named as magnetite samples M3 and M5). Rebodos & Vikesland, reported that, based on magnetisation loops, magnetite nanoparticles exhibited superparamagnetic-like behaviour, which was expected for particles within a small size range (Rebodos & Vikesland, 2010).

Figure 7 shows the magnetization (M) versus the applied magnetic field (H) curve obtained at room temperature curves for SNPs-IONPs composites (M3 magnetite + starch and M5 magnetite + starch samples) and the corresponding bare IONPs in absence of starch (M3 magnetite and M5 magnetite samples). The absence of hysteresis loop confirms the superparamagnetic behaviour of all samples analysed. The saturation magnetization of magnetite nanoparticles has been determined by setting the experimental data to the law of approach to saturation (Zhang, Zeng & Liu, 2010) and their values were 49.5 emu/g for M3 sample and 58.5 emu/g for M5 sample, being similar to those reported in a previous study where IONPs were synthesized by the ME method (Salvador et al., 2021). Similar results were also reported by Likhitar & Bajpai who obtained a saturation magnetization of about 58 emu/g (Likhitar & Bajpai, 2012). However, it was noticed that, for both formulations, the iron oxide impregnated SNPs showed lower magnetization and superparamagnetic behaviour properties than their corresponding bare IONPs. This can be explained by the fact that IONPs are impregnated in a starch matrix consisting of SNPs.

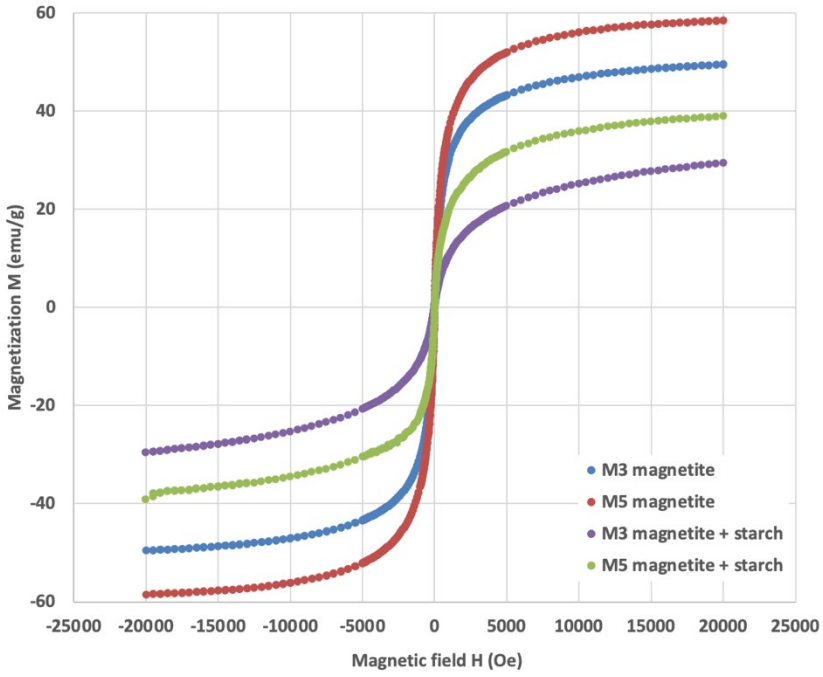

Figure 7. Magnetization curves for SNPs-IONPs composites and the corresponding bare IONPs in absence of starch.

Finally, XRPD diagrams were collected to compare the obtained nanocrystalline phases with the magnetite reference structure (Nakagiri, Manghnani, Ming & Kimura, 1986) since XRPD analysis is an important tool for determination of crystallinity of materials (Gupta & Bajpai, 2010) and has also been used for previous authors to demonstrate the presence of iron oxide nanoparticles in superparamagnetic nanoparticles of crosslinked starch impregnated designed for drug delivery purposes (Likhitar & Bajpai, 2012). The spectra are shown in Figure 8. In all the XRPD diagrams the corresponding peaks match the spectral pattern of the pure magnetite structure, named in the diagram as magnetite sample, which means

that pure crystalline phases have been obtained in this work.

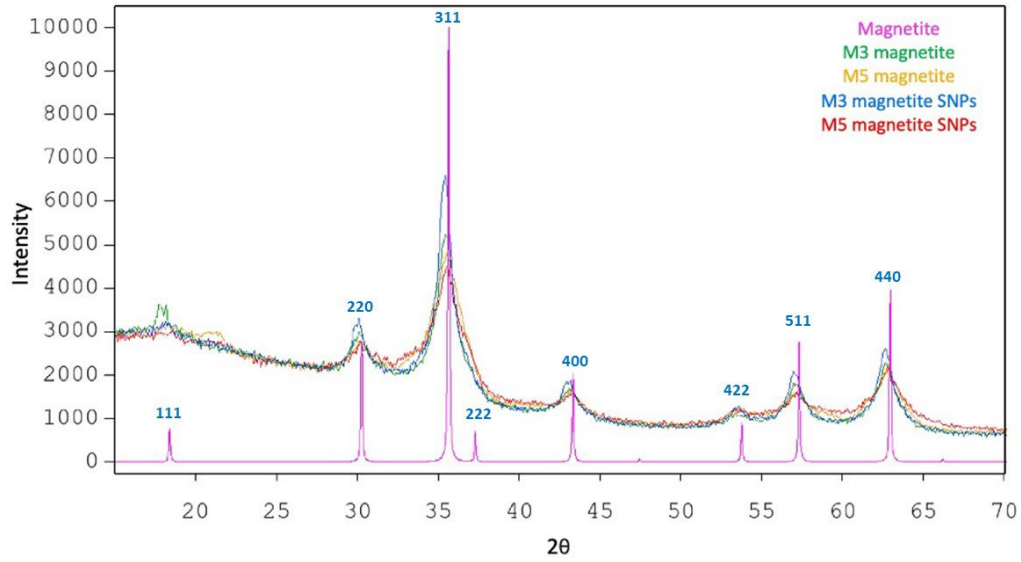

Figure 8. XRPD spectra of IONPs (M3 and M5 magnetite) and SNPs-IONPs (M3 magnetite SNPs and M5 magnetite SNPs) for M3 and M5 systems.

4. Conclusions

The synthesis of size-tuned SNPs (30-40 nm) was achieved through the use of a microemulsion method. This was possible by controlling the ratio and composition of the reagents used in the microemulsion system (aqueous and organic phases, stabilizer and co-stabilizer), as well as the precipitating agents used.

Moreover, the versatility of the developed ME method has also been demonstrated, as iron oxide SNPs with controlled size and superparamagnetic properties were also synthesized simultaneously using this method what validates the hypothesis stated and confirms the potential of this method as an innovative technology for the design and development of new functional nanomaterials.

The synthesized size-tuned magnetic-polymeric nanocomposites are promising materials that are likely to be used for different biomedical applications, such as drug delivery nanocarriers or potential markers for theragnostic purposes.

CRediT authorship contribution statement

D. Morán: Conceptualization, Validation, Formal analysis, Resources, Data curation, Writing - original draft. **G. Gutiérrez:** Conceptualization, Validation, Writing - review & editing, Supervision, Project administration. **M. Rayner:** Conceptualization, Validation. **C. Blanco-López:** Conceptualization, Validation, Funding acquisition. **R. Mendoza:** Conceptualization, Validation, Formal analysis. **M. Matos:** Conceptualization, Validation, Writing - review & editing, Supervision, Project administration.

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