



(51) International Patent Classification:

A61K 8/24 (2006.01) *A61K 8/02* (2006.01)
A61Q 11/00 (2006.01)

(21) International Application Number:

PCT/SE2016/051121

(22) International Filing Date:

14 November 2016 (14.11.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1551460-7 12 November 2015 (12.11.2015) SE

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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

(54) Title: METHOD FOR MANUFACTURING OF SPHERICAL CALCIUM PHOSPHATE PARTICLES

(57) Abstract: The present invention relates to particles wherein the particle comprises 50-65 weight% of calcium, 25-40 weight% of phosphate and 5-30 weight% magnesium, and wherein the Ca/P weight ratio is in the range of 1.10 to 1.90, and wherein more than 80 % of the particles have a particle size between 200 to 600nm, and method for preparing the same.



WO 2017/082811 A1

METHOD FOR MANUFACTURING OF SPHERICAL CALCIUM PHOSPHATE PARTICLES

TECHNICAL FIELD OF THE INVENTION

5 The present invention relates to an improved method of producing spherical calcium phosphate particles containing magnesium. The invention further relates to a composition comprising said particles and its use in dental and medical applications.

BACKGROUND OF THE INVENTION

10 Calcium phosphates (CaP) and in particular hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA) are widely used in medical and dental applications due to its similarity to the mineral components of bone and teeth. It is non-toxic, biocompatible and bioactive, which means
15 that it is not harmful and not recognized as a foreign body, resulting in positive effects on bone remodeling. Hence, HA has been widely used in bone repair and as drug or gene delivery vehicle, catalyst, ion adsorption/exchange agent, photoelectric reagent, etc. Resorbable micro- or nanoparticles (i.e. particles that can be dissolved *in vivo*) are of special interest for a number of applications, such as bone void fillers, drug delivery
20 vehicles, desensitization agents of dentinal tubules, etc.

The morphology, structure, and size of CaP particles can influence their properties in above-mentioned applications. Particles with spherical shape and large pore volume are good candidates for drug delivery vehicles, protein and ion adsorption, and bone and
25 teeth fillers. Hence, they have recently attracted increased attention. CaP, such as HA, spontaneously grow like flakes, fibers or rods by wet chemical methods. Spherical CaP has previously been prepared using structure directing agents, such as ion substituents, surfactants and biomolecules. A present inventor showed in WO2011/016772 that strontium ions affected the morphology of CaP to form hollow spheres, and showed
30 further in WO2014/148997 that CaP hollow spheres can also be formed in the absence of strontium ions. But there still exists a need for a more efficient method of preparing hollow spherical CaP particles.

Not all morphologies are convenient to serve as drug delivery particles, catalyst support,
35 ion adsorption/exchange agent, etc. To make a drug delivery process efficient, high surface areas and porous structures are advantageous to adsorb as much active substance as possible. There is also the requirement of biocompatibility and proper interaction between carrier and substance. One problem for the preparation of CaP particles is to control their size distribution and shape. Often the size distribution is
40 wide and caused by the hexagonal symmetry and the lattice parameters of CaP. Most likely an orientation along the c-axis and therewith a pin-like shape occurs.

Furthermore, it is an advantage to form the hollow CaP particles without the use of techniques requiring many process steps (like sacrificing phases) or through the use of
45 additives or less biocompatible substitution ions, which might jeopardize authorial approval for biomedical use.

SUMMARY OF THE INVENTION

The object of the present invention is to provide CaP particles that are essentially spherical, and porous, and a method of preparing said particles. The method of the present invention is simpler, faster, more cost effective, and results in a higher particle yield per unit volume mixture than methods disclosed by prior art

In a first aspect the present invention relates to a spherical particle wherein the particle comprises 40-70 weight% such as 50-65 weight% of calcium, 20-40weight% such as 25-40 weight% of phosphate and 5-30 weight% magnesium, and wherein the Ca/P molar ratio is in the range of 1.10 to 1.90, and wherein the particle size is less than 1 μm , preferably less than 600nm.

In a second aspect the present invention relates to a method of preparing the particles according to the present invention wherein the method comprises:

- a) providing an aqueous buffer solution of purified water having a pH of 2 to 10 comprising sodium, potassium and phosphate ions, wherein the concentration of said ions are 100 to 400 mM for sodium, 0-10 mM for potassium and 8-25mM such as 12-25 mM for phosphate;
- b) adding calcium ions in the range of 1.5-10 mM, and magnesium ions in the range of 0.5-15 such as 1-15 mM to the buffer solution and forming a mixture;
- c) heating the mixture of step b) at 60 to 120° C for at least 2 minutes;
- d) isolating the formed particles; and
- e) optionally washing the isolated particles using a suitable solvent.

In a third aspect the present invention relates to a bleaching chewing gum comprising particles according to the present invention and a paste forming compound, and wherein the particles further comprises a bleaching agent such as a peroxide.

In a fourth aspect the present invention relates to particles obtained from the method of the present invention.

In a fifth aspect the present invention relates to a chewing gum comprising the particles or the composition according to the present invention.

In a sixth aspect the present invention relates to a composition comprising spherical particles and, a paste forming compound; wherein the particles comprises 40-70 weight% such as 50-65 weight% of calcium, 20-40weight% such as 25-40 weight% of phosphate and 5-30 weight% magnesium, and wherein the Ca/P weight ratio is in the range of 1.10 to 1.90, and wherein the particle size is less than 1 μm preferably less than 600 nm.

BRIEF DESCRIPTION OF FIGURES

Figure 1. SEM image of calcium phosphate hollow spheres.

Figure 2. SEM image of calcium phosphate hollow spheres.

Figure 3. SEM image of calcium phosphate hollow spheres.

Figure 4. SEM image of calcium phosphate particles.

Figure 5. SEM image of calcium phosphate hollow spheres.

Figure 6. SEM image of calcium phosphate hollow spheres.

Figure 7 SEM image of calcium phosphate hollow spheres.

Figure 8 SEM image of calcium phosphate hollow spheres.

Figure 9 SEM image of calcium phosphate hollow spheres.

Figure 10 SEM image of calcium phosphate hollow spheres.

Figure 11 Illustration of continuous mode process for synthesis of calcium phosphate hollow spheres.

Figure 12 SEM image of calcium phosphate hollow spheres.

Figure 13 SEM image of calcium phosphate hollow spheres.

Figure 14 SEM image of calcium phosphate hollow spheres.

DETAILED DESCRIPTION OF INVENTION

In the present invention the term “paste forming compound” relates to compounds that may be mixed with the particles of the present invention to make a paste. The paste forming compound increases the viscosity of the mixture when added to the particles. Non-limiting examples of paste forming compounds are glycerol, polyethylene glycol, polyvinyl alcohol and polysaccharides such as cellulose, hyaluronan and chitosan.

The chemical formula for stoichiometric hydroxyapatite (HA) is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, but for the purpose of this application many variations can be used. The present invention is mainly described in terms calcium phosphates (CaP), which includes but is not limited to: β -tricalcium phosphate (β -TCP) dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), tricalcium phosphate (TCP), and amorphous calcium phosphate (ACP) or any derivative thereof.

The present application discloses a method of preparing spherical particles of CaP without the use of templates or sintering steps. Instead the present invention is a method that is driven by ion concentration, time, and temperature. Compared to

previous ion doped CaP spheres, the present invention discloses a method to produce spheres that are smaller in size and at a higher yield point per unit volume of mixture. By developing a method that produces more CaP spheres per unit volume of mixture, the present inventors facilitates a production method of CaP spheres that is more cost and energy effective than what has been described in the prior art.

The particles

The particles according to the present invention have a hollow core and a shell. The core part may be described as a void not comprising any calcium phosphate material. In one embodiment the shell of the particle is porous, in another embodiment the shell is dense.

The particles of the present invention comprise 40-70 weight% of calcium (Ca), 20-40 weight% of phosphate (PO₄) and 5-30 weight% magnesium (Mg). The particles may contain other ions as well.

In one embodiment the concentration of magnesium is 3-15 weight%. In another embodiment the concentration of magnesium is 1-10 weight%. In one embodiment the particles comprise 55-65 weight% of calcium, 25-35 weight% of phosphate and 3-15 weight% of magnesium. The Ca/P molar ratio may be between 1.10 and 1.90, for example 1.10 and 1.70. In one embodiment the ratio is 1.30 to 1.70. In one embodiment the ratio is 1.40 to 1.50. The particles may contain trace amounts of potassium and/or sodium since they are used in the buffer solution as counter ions.

The mean particle size, the diameter, should be small, not more than 1µm preferably less than 600 nm. This provides a higher surface area per unit mass, which also makes it easier to fill voids for example dentinal tubules. The size should not be too small since it makes it harder to make a formulation suitable as e.g. a chewing gum. Without being bound by theory, the size is also believed to influence the particles ability to stick to the teeth. In one embodiment the particles are 600 nm or less, or 400 nm or less (mean particle size). In one embodiment the mean particle size is 200 to 600 nm. In one embodiment the particles are 10 nm or more, or 50 nm or more, or 100 nm or more, or 300 nm or more, or 450 nm or more. In one embodiment the mean particle size is between 200-500 nm such as 200-300nm or 220-280nm. Mean particle size is determined by measuring the diameter of at least three representative particles from a sample using SEM.

In one embodiment the particles have a hollow core and a shell and wherein the particle (i.e. the shell of the particle) comprises 50-65 weight% of calcium, 25-40 weight% of phosphate and 5-30 weight% magnesium, and wherein the Ca/P weight ratio is in the range of 1.10 to 1.90, preferably 1.10 to 1.70, and wherein more than 80 % of the particles have a particle size between 200 to 1000nm such as 200- 600 nm.

In another embodiment the particles comprises 55-65weight% of calcium such as 60-65weight%, 30-40weight% of phosphate such as 35-40weight% and 15-25weight% of magnesium such as 20-25weight%.

In one embodiment the particles comprises calcium, phosphate and magnesium in a total amount of at least 95 weight%, or at least 98 weight%, or at least 99weight%, or at least 99.9weight%.

The method

The synthesis is performed in an aqueous buffer solution having a pH of 2-10, preferably a pH of 6-10, or pH 6-9, preferably 6.5 to 8 or more preferably a pH of 7.0-7.5, comprising calcium, phosphate, magnesium, potassium and sodium ions. The pH value of the solutions before and after precipitation is stable.

The concentration of calcium ions may be in the range of 1.5-10 mM, the concentration of magnesium ions may be in the range of 1-15 mM, and the concentration of phosphate ions can be in the range 8-25 mM. In one embodiment the calcium ion concentration is 1.5 to less than 5 mM, or 1.5 to 3 mM, and in another embodiment the magnesium ion concentration is 1 to less than 5 mM, or 1.5 to 3 mM In one embodiment the Ca:Mg molar ratio is from 1:6 to 4:1 such as 1:3 to 4:1, for example 1:2 to 3:1 , or 1:1 to 2:1, or 1:4 to 1:6.

Without being bound by any theory it is believed that the presence of magnesium ions and the elevated temperature of the solution promotes the formation of a hollow structure.

The sodium (Na) and potassium (K) ions are believed to stabilize the buffer and acts as counter ions. These ions are not expected to be found in the formed particles or at least not in any larger amounts.

In one embodiment the concentration of sodium ions in the solution is in the range of 0.01-1420 mM and the concentration of potassium ions is in the range of 0.01-1420 mM, preferably 170-200 mM of sodium and 0.08-0.5 mM of potassium. The concentration of sodium may be 100 to 400mM and the concentration of potassium ions may be 0.08 to 10 mM, or 0.5 to 5 mM. The sodium ions may be added as NaCl or Na₂HPO₄ or as a combination, and potassium ions may be added as KCl or KH₂PO₄ or as a combination. In one embodiment the Na:K molar ratio is more than 23:1, preferably more than 30:1, more preferably more than 35:1. In one embodiment the molar ratio of sodium:potassium:phosphate is 100-400:0-10:8-25. In another embodiment the molar ratio is 100-400:0-10:12-25. In yet another embodiment the molar ratio is 170-200:0.08-0.5:12-16.

The molar ratio between Ca and P should be close to 1:10, for example 1:9.0 to 1:11, or 1:9.5 to 1:10.7, or 1:10 to 1:10.5.

The water used to prepare the aqueous buffer solution should be purified water. The water may be deionized, distilled, double distilled or ultra-pure water. For example the water may be Milli-Q®.

As mentioned above, the method of the present invention is driven by ion concentration and temperature. This means that if the ion concentrations are wrong or the temperature is too low, spheres and especially hollow spheres will not be formed or at least not formed within a reasonable period of time. The aqueous buffer solution in the present invention may be prepared and mixed at or around room temperature for subsequent heating. A preferred temperature for preparation of the buffer solution is from about 15°C to 30°C, or from about 20°C to 25°C. The reaction temperature in the present invention is at least 60°C, or at least 70°C, or at least 80°C. The reaction temperature may be 90°C or more, 100°C or more, or 120°C or less. A preferred reaction temperature range is from about 60°C to 120°C, or 70°C to 110°C, or from about 80°C to 100°C. Sphere formation is also sensitive to heating rate of the mixture. If the heating rate is too slow, spheres and especially hollow spheres will not form. In one embodiment the heating rate is at least 1.3°C per minute, or at least 1.5°C per minute, or at least 2°C per minute. In one embodiment the heating rate is 3°C per minute or more, or 6°C per minute or more. In another embodiment the heating rate is 15°C per minute or less, or 12°C per minute or less, or 10°C per minute or less, or 8°C per minute or less. A non limiting heating rate range is 1-5°C per minute such as 1.2 to 2°C per minute or 1.3 to 1.5°C per minute. The method could be a static process, stirring or shaking process, a turbulent flow process, or a hydrothermal process.

The particles may be isolated using any suitable technique for example filtering, evaporation, centrifugation or combinations thereof. The method of the present invention facilitates a very short synthesis time but it is also believed that time will influence the particle size distribution. The synthesis time may be 1 minute, but it may be a couple of hours. In one embodiment the synthesis time is at least 2 minutes, or at least 5 minutes, or at least 10 minutes, or at least 30 minutes, or at least 1 hour, or at least 2 hours, or at least 6 hours. In one embodiment the synthesis time is 20-180 minutes or 30-120 minutes. The obtained particles will have essentially a TCP structure, such as β -TCP, or magnesium substituted HA structure or a mixture thereof.

Usually when the amount of ions in the solutions is increased the particles formed are not spherical. They will instead form flakes or rods. The inventors however surprisingly found that by increasing the amount of sodium and magnesium ions in the solution, an increased amount of phosphate and calcium ions could be added and the reaction still formed spheres. The reaction time was also significantly reduced and the yield (amount of particles per unit volume of mixture) of spherical particles was increased. This in total makes the present invention a more cost and time efficient method than what has been described in prior art.

Furthermore, the present invention results in a higher degree of HA structure without the need of any further treatments. This is an advantage since HA is an approved material or structure partly due to its natural occurrence in bone and teeth. In one embodiment the particles obtained from the present method without any further treatment has at least 30%, or at least 40%, or at least 50%, or at least 75%, or at least 90% of the crystal structure is that of hydroxyapatite as determined using Rietfeld refinement from XRD spectra.

Some of the major costs for the preparation of the particles are the raw materials used to prepare the buffer solution and the energy required to heat the solution. The present inventors have therefore developed the method so that the buffer solution with the counter ions may be recycled and/or reused without compromising with the result to any major extent, Example 6.

Figure 11 discloses an illustrative set up and method of the recycling of the buffer solution. After precipitation of the particles, the buffer solution still has an adequate concentration of counter ions (sodium and potassium) which may be reused. In order to recycle the solution and/or the ions the solution is preferably first cooled down for example to room temperature (20-25C), and may then be supplemented with phosphate, calcium and magnesium ions preferably corresponding to the amount consumed in the precipitation of particles. Upon mixing and heating the solution again, new particles can be formed. The recycling step can be performed as many times as the concentration range of the ions allow formation of spherical hollow particles. The concentration of sodium ions in the recycled buffer solution may be 100 to 400 mM and the concentration of potassium ions may be 0.08 to 10mM. The concentration of phosphate ions in the supplemented recycled solution may be 8-25 mM, the concentration of calcium ions may be 1.5-10 mM, and the concentration of magnesium ions may be 1-15 mM. In one embodiment the phosphate ion concentration is from 10 to less than 25 mM, or 10 to 16 mM. In another embodiment the calcium ion concentration is 1.5 to less than 5 mM, or 1.5 to 3 mM. In another embodiment the magnesium ion concentration is 1 to less than 5 mM, or 1.5 to 3 mM. In one embodiment the Ca:Mg molar ratio is from 1:6 to 4:1 such as 1:3 to 4:1, for example 1:2 to 3:1 , or 1:1 to 2:1, or 1:4 to 1:6.

The inventors have further developed the method so that a continuous process may be used to prepare the particles, which results in significant time saving. In this process two chemical reactor vessels are used in series, where one is held at lower temperature and the other at higher temperature. The buffer solution is circulated between these reactors and supplemented with phosphate, calcium, and magnesium ions in the reactor vessel held at lower temperature. In one embodiment the supplement of phosphate, calcium and magnesium ions is made periodically, and in another embodiment the supplement of said ions is made continuously. In one embodiment the particles are circulated with the buffer solution and isolated by for example filtration at the end of the process. In another embodiment the particles are isolated continuously by for example a filtration unit placed in line between the two reactor vessels.

Applications

The particles of the present invention may be used in a bleaching agent or paste or chewing gum for teeth. A problem with prior art has been that the bleaching also degrades the enamel, resulting in increased tooth sensitivity and gingival irritation. The present invention aims at delivering a bleaching agent locally using the particles of the present invention, which will act to remineralize the tooth during and/or after the bleaching step. The preparation of a bleaching agent with the particles of the present invention comprises:

- Providing particles of the present invention and a solution of a bleaching additive, for example a peroxide solution;
- mixing the particles and the solution, for example a peroxide solution, and;
- isolating the bleaching additive containing particles.

The peroxide used may be hydrogen peroxide or carbamide peroxide or a mixture thereof. Other non-limiting examples of tooth bleaching additives include; sodium percarbonate, sodium chlorite, sodium perborate, peroxymonosulphate, peroxide plus metal catalysts and oxiredutase enzymes. The concentrations of peroxides in the solution may be 0.1-60weight% such as 1 weight% or more, or 5 weight% or more, or 10 weight% or more, or 15 weight% or more, or 20 weight% or more, or 25 weight% or more, but 55 weight% or less, or 50 weight% or less, or 45 weight% or less, or 40 weight% or less. For example the concentration may be 10-60weight% for carbamide peroxide, or 0.1-35 weight% hydrogen peroxide, such as 10-35wt% hydrogen peroxide.

EXAMPLES

Example 1:

CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at a molar ratio of 185:0.29:2:1.25:2.231:12.05 to form a phosphate buffered saline solution at 25°C. The solution was heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. During this time the CaP particles will precipitate from the solution. The particles were then filtered out of solution and dried before examination in SEM. At this ion concentration and under the described process parameters, spherical particles with a mean diameter of roughly 300 nm were produced. See Figure 1.

Example 2:

CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at a molar ratio of 170:0.08:1.5:1.5:1.5:8.1 to form a phosphate buffered saline solution at 25°C. The solution was heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. During this time the CaP particles will precipitate from the solution. The particles were then filtered out of solution and dried before examination in SEM. At this ion concentration and under the described process parameters, spherical particles with a mean diameter of roughly 560 nm were produced. See Figure 2.

Example 3:

CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at a molar ratio of 200:0.5:2.5:1.5:2.963:16 to form a phosphate buffered saline solution at 25°C. The solution was heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. During this time the CaP particles will precipitate from the solution. The particles were then filtered out of solution and dried before examination in SEM. At this ion concentration and under the

described process parameters, spherical particles with a mean diameter of roughly 230 nm were produced. See Figure 3.

Example 4:

A full factorial screening series of experiments were made according to a design of experiments (DOE) software (MODDE, Umetrics), where the variables were the molar concentrations of NaCl, KCl, CaCl₂, MgCl₂, and Na₂HPO₄. The molar concentration of KH₂PO₄ was fixed to a ratio of 1:5.4 to Na₂HPO₄.

In the screening experiments, CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at designated molar concentrations to form a phosphate buffered saline solution at 25°C. The molar concentrations varied as follows: NaCl: 100-170 mM; KCl: 0.1-5 mM; CaCl₂: 0.9-1.8 mM; MgCl₂: 0.1-1 mM; Na₂HPO₄: 8.1-16 mM; KH₂PO₄: 1.5-2.963 mM. The solution was heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. During this time the CaP particles precipitated from the solution. The particles were then filtered out of solution and dried before examination in SEM. The particles from each experiment were rated according to morphology and size, and the data was evaluated in the DOE software to identify statistically verified trends. The results of the screening revealed that certain ion concentrations, or molar ratios between different ions within the defined limitations, allowed formation of hollow and spherical CaP particles at higher concentrations or ratios than previously described in prior art. The screening also revealed regions of ion concentrations or molar ratios where spheres did not form. Figure 4 displays particles formed using a molar ratio of NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ at 100:0.1:1.8:0.1:2.963:16. With these ion concentrations, spheres did not form. By raising the concentration of NaCl and MgCl₂ along with higher concentrations of CaCl₂ and Na₂HPO₄, spheres were formed, as displayed in Figure 5. The spheres in Figure 5 were formed using a molar ratio of NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ at 170:0.1:1.8:1:2.963:16. Being able to use higher concentrations of magnesium, calcium and phosphate increases the yield (amount of particles per unit volume of mixture) of the reaction. The screening led to an extended experimental series, wherein a new window with yet higher ion concentrations was evaluated, which is further described in Example 5.

Example 5:

Following the full factorial screening experiments described in Example 4, an optimization series of experiments was made, also governed by DOE software. As previously, the variables were the molar concentrations of NaCl, KCl, CaCl₂, MgCl₂, and Na₂HPO₄. The molar concentration of KH₂PO₄ was fixed to a ratio of 1:5.4 to Na₂HPO₄.

In the optimization experiments, CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at designated molar concentrations to form a phosphate buffered saline solution at 25°C. The molar concentrations varied as follows: NaCl: 170-200 mM; KCl: 0.08-0.5 mM; CaCl₂: 1.5-2.5 mM; MgCl₂: 1-1.5 mM; Na₂HPO₄: 8.1-16 mM; KH₂PO₄: 1.5-2.963 mM. The solution was

heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. During this time the CaP particles precipitated from the solution. The particles were then filtered out of solution and dried before examination in SEM. The particles from each experiment were rated according to size and morphology, i.e. if spheres were formed or not. See Table 1. The data was evaluated in the DOE software to identify statistically verified trends. The results of the evaluation revealed that, within the ion concentration design space, spheres were formed in nearly all areas, but having different sizes. It also revealed an increase in yield of spheres, primarily according to the amount of calcium added.

Table 1

Exp. Name	[KCl] (mM)	[NaCl] (mM)	[Na ₂ HPO ₄] (mM)	[MgCl ₂] (mM)	[CaCl ₂] (mM)	Size (nm)	Spheres (Y/N)
N1	0.08	170	8.1	1	2.5	188	Y
N2	0.5	170	8.1	1	1.5	399	Y
N3	0.08	200	8.1	1	1.5	331	Y
N4	0.5	200	8.1	1	2.5	335	Y
N5	0.08	170	16	1	1.5	231	Y
N6	0.5	170	16	1	2.5	231	Y
N7	0.08	200	16	1	2.5	239	Y
N8	0.5	200	16	1	1.5	277	Y
N9	0.08	170	8.1	1.5	1.5	563	Y
N10	0.5	170	8.1	1.5	2.5	275	Y
N11	0.08	200	8.1	1.5	2.5	282	Y
N12	0.5	200	8.1	1.5	1.5	443	Y
N13	0.08	170	16	1.5	2.5	212	Y
N14	0.5	170	16	1.5	1.5	283	Y
N15	0.08	200	16	1.5	1.5	730	Y
N16	0.5	200	16	1.5	2.5	231	Y
N17	0.08	185	12.05	1.25	2	211	Y
N18	0.5	185	12.05	1.25	2	271	Y
N19	0.29	170	12.05	1.25	2	-	N
N20	0.29	200	12.05	1.25	2	264	Y
N21	0.29	185	8.1	1.25	2	451	Y
N22	0.29	185	16	1.25	2	205	Y
N23	0.29	185	12.05	1	2	-	N
N24	0.29	185	12.05	1.5	2	681	Y
N25	0.29	185	12.05	1.25	1.5	351	Y
N26	0.29	185	12.05	1.25	2.5	263	Y
N27	0.29	185	12.05	1.25	2	399	Y
N28	0.29	185	12.05	1.25	2	271	Y
N29	0.29	185	12.05	1.25	2	339	Y

Example 6:

Recycling of process water and counter ions in the solution facilitates an essential reduction of cost and energy consumption, and makes for an efficient production process of the CaP particles. A formulation retrieved from the DOE described in Example 5 was tested in a cyclic fashion, wherein the process water was re-used after the formed CaP particles had been filtered out. To this process water, after cooling down to room temperature (20-25°C), CaCl₂, MgCl₂, Na₂HPO₄, and KH₂PO₄ were added in succession to replenish the solution similar to its original composition. This solution was then heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C and held for 40 min. The particles were then filtered out of solution and dried before examination in SEM. See Figure 6 for appearance of spheres obtained from the original solution. See Figure 7 for appearance of spheres obtained after recycling the process water and counter ions. The results reveal that the process water and counter ions can be recycled to produce CaP spheres in a more cost and energy efficient way.

Example 7:

Based on experiment N7 found in Table 1, the effect of an increased amount of magnesium content was evaluated. CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at a molar ratio of 200:0.08:2.5:2.5:2.963:16 to form a phosphate buffered saline solution at 25°C. The solution was heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. The particles were then filtered out of solution and dried before examination in SEM, see Figure 8. The experiment revealed that spheres could successfully be formed also with this elevated concentration of magnesium ions.

Example 8:

The effect of a yet further increase in magnesium content was evaluated. CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at a molar ratio of 200:0.08:2.5:10:2.231:16 to form a phosphate buffered saline solution at 25°C. The solution was heated under stirring at a rate of 1.4°C per minute to a temperature of 85°C, where it was held for 40 min. The particles were then filtered out of solution and dried before examination in SEM, see Figure 9. The experiment revealed that spheres were formed also with this further elevated concentration of magnesium ions, with a significant increase in yield of particles per unit volume of mixture.

Example 9:

To evaluate the effect of reaction time, CaP particles were prepared as follows: NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄ were dissolved in water at a molar ratio of 200:0.5:2.5:1.5:2.963:16 to form a phosphate buffered saline solution at 25°C. The solution was heated under stirring at a rate of 1.4 C° per minute to a temperature of 85°C. Small samples were then taken and filtered after 0, 10, 20, 30, 40, and 45 minutes. Samples were then dried and analyzed by SEM, see Figure 10. The yield of spheres per

unit volume of mixture was also evaluated, and demonstrated similar values after the different time points. This shows that with correct ion concentrations, heating rate, pH and reaction temperature, significant savings in time can be made in the synthesis process of the CaP particles, which in turn reduces overall cost and energy consumption.

Example 10:

A continuous process with recycling of process water and counter ions has also been developed in pilot scale, where two 50 L reactors are placed in series, one held at 25°C and the other at 85°C. Briefly, the continuous process was carried out as follows: The first reactor was charged with NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄, in this case at a molar ratio of 200:0.5:2.5:1.5:2.963:16 to form a phosphate buffered saline solution at 25°C. The solution was then pumped through a heat exchanger and collected in the second reactor where it was continually heated at 85°C. The first reactor was then charged again with an identical buffer solution at 25°C. After holding the solution in reactor two at 85°C for 30 minutes, the solution was pumped back to reactor one at a specified flow rate through a cooling heat exchanger. Using the same flow rate, buffer solution from reactor one was also pumped through the heating heat exchanger and a steady state transfer between the two reactors was achieved. At periodic intervals, phosphate, calcium and magnesium ions were charged to reactor one to retain an estimate of the original molar ratio. Filtering of the synthesized particles was done at the end of the complete process, after several cycles. See Figure 11 for illustration of the continuous process. After filtration, a sample was drawn and appearance of the particles in SEM is shown in Figure 12. Clearly, spherical particles were formed by this method but other structures were also formed. One explanation for this is that recirculation of formed spheres offers nucleation sites for other CaP structures, which will grow on top of the originally formed spheres.

Example 11:

A pilot scale continuous process with recycling of process water and counter ions, where the precipitated particles are filtered directly in-line has also been developed. In this process, two 50 L reactors and a filter were placed in series. Briefly, the continuous process was carried out as follows: The first reactor was charged with NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄, in this case at a molar ratio of 200:0.5:2.5:1.5:2.963:16 to form a phosphate buffered saline solution at 25°C. The solution was then pumped through a heating heat exchanger and received in the second reactor where it was heated at 85°C. The first reactor was then charged again with an identical buffer solution. After holding the solution in reactor two at 85°C for 30 minutes, the solution was pumped back to reactor one at a specified flow rate through a cooling heat exchanger and a filter. Using the same flow rate, buffer solution from reactor one was also pumped through the heating heat exchanger and a steady state transfer between the two reactors was achieved. At periodic intervals, phosphate, calcium and magnesium ions were charged to reactor one to retain an estimate of the original molar ratio. Throughout the process, reactor one was held at room temperature and reactor two at 80-85°C. Upon completion of the process, the filter was emptied and a sample was drawn and prepared for SEM analysis, see Figure 13.

Example 12:

5 A semi-continuous batch process has also been developed in pilot scale, where two 50 L reactors and a filter are placed in series. Briefly, the process was carried out as follows: The first reactor was charged with NaCl, KCl, CaCl₂, MgCl₂, KH₂PO₄, and Na₂HPO₄, in this case at a molar ratio of 200:0.5:2.5:1.5:2.963:16 to form a phosphate buffered saline solution at 25°C. The solution was then pumped through a heating heat exchanger and received in the second reactor where it was heated at 85°C. The first reactor was then
10 charged again with an identical buffer solution. After holding the solution in reactor two at 85°C for 20 minutes, the solution was pumped through a cooling heat exchanger and a filter. Once emptied, reactor two was filled again from reactor one and the process repeated. Upon completion of six fillings in total, the filter was emptied and a sample was drawn and prepared for SEM analysis, see Figure 14.

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CLAIMS

1. Spherical particles wherein the particle comprises 50-65 weight% of calcium, 25-40 weight% of phosphate and 5-30 weight% magnesium, and wherein the Ca/P weight ratio is in the range of 1.10 to 1.90, and wherein more than 80 % of the particles have a particle size between 200 to 600nm.
2. The particles according to claim 1 wherein the particles comprises 5-10 weight% of magnesium
3. The particles according to claims 1 or 2 wherein the particles have a particle size of 300-400nm.
4. A method of preparing the particles according to any one of claims 1 to 3 wherein the method comprises:
 - a. Providing an aqueous buffer solution of purified water having a pH of 6 to 10 comprising sodium, potassium and phosphate ions, wherein the concentration of said ions are 100 to 400 mM for sodium, 0-10 mM for potassium and 12-25 mM for phosphate;
 - b. Adding calcium ions in the range of from 1.5 to less than 10mM, and magnesium ions in the range of 1-15 mM to the buffer solution and forming a mixture;
 - c. Heating the mixture of step b) at 60 to 120° C for at least 5 minutes;
 - d. Isolating the formed particles; and
 - e. Optionally washing the isolated particles using a suitable solvent.
5. The method according to claim 4 wherein the magnesium concentration is 10-12mM.
6. The method according to claim 4 or 5 wherein the reaction temperature is 70-90°C.
7. The method according to any one of claims 4-6 wherein the heating is done for not more than 60 minutes.
8. The method according to any one of claims 4-6 wherein the heating rate of the mixture is at least 1.3°C per minute, or 3°C per minute, or 6°C per minute.
9. The method according to any one of claims 4 to 8 wherein the buffer solution is recycled after isolation of the formed particles.
10. The method according to claim 9 wherein the recycled buffer solution is cooled, preferably to 20-30C, before addition of phosphate, calcium and magnesium ions.
11. A composition comprising the particles according to any one of claims 1 to 3 and a paste forming compound.

FIGURES

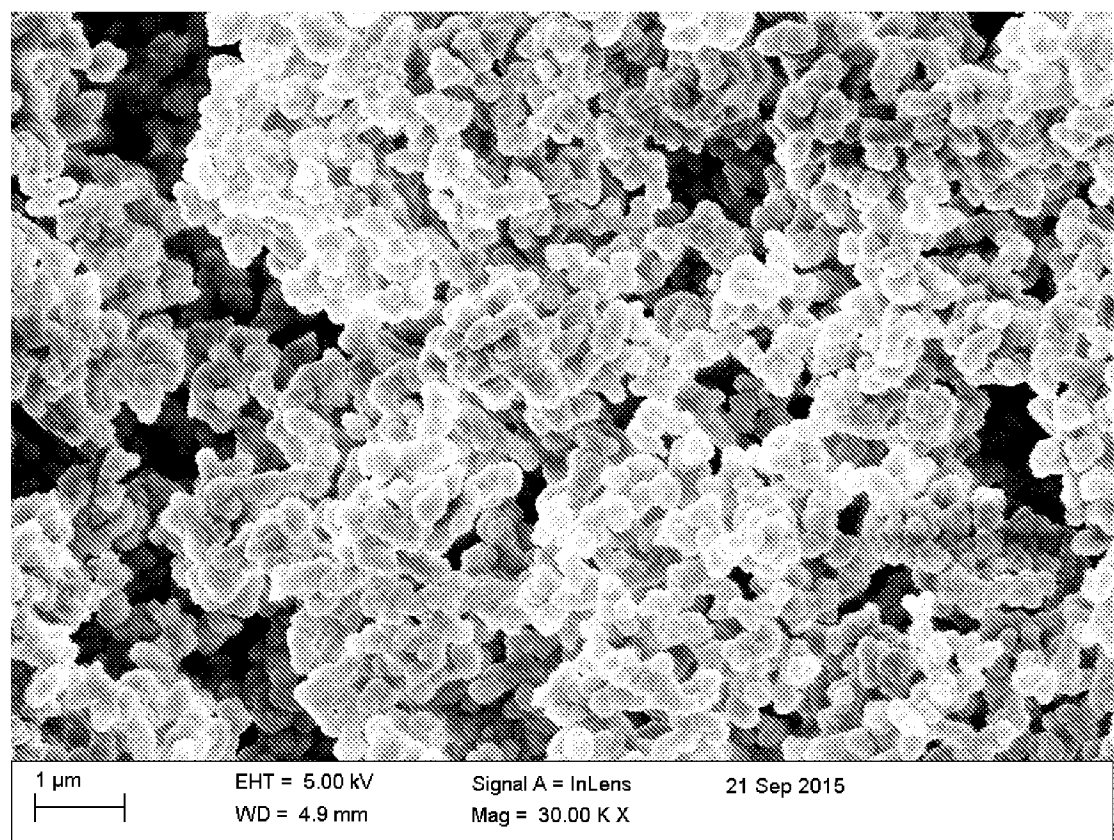


Figure 1

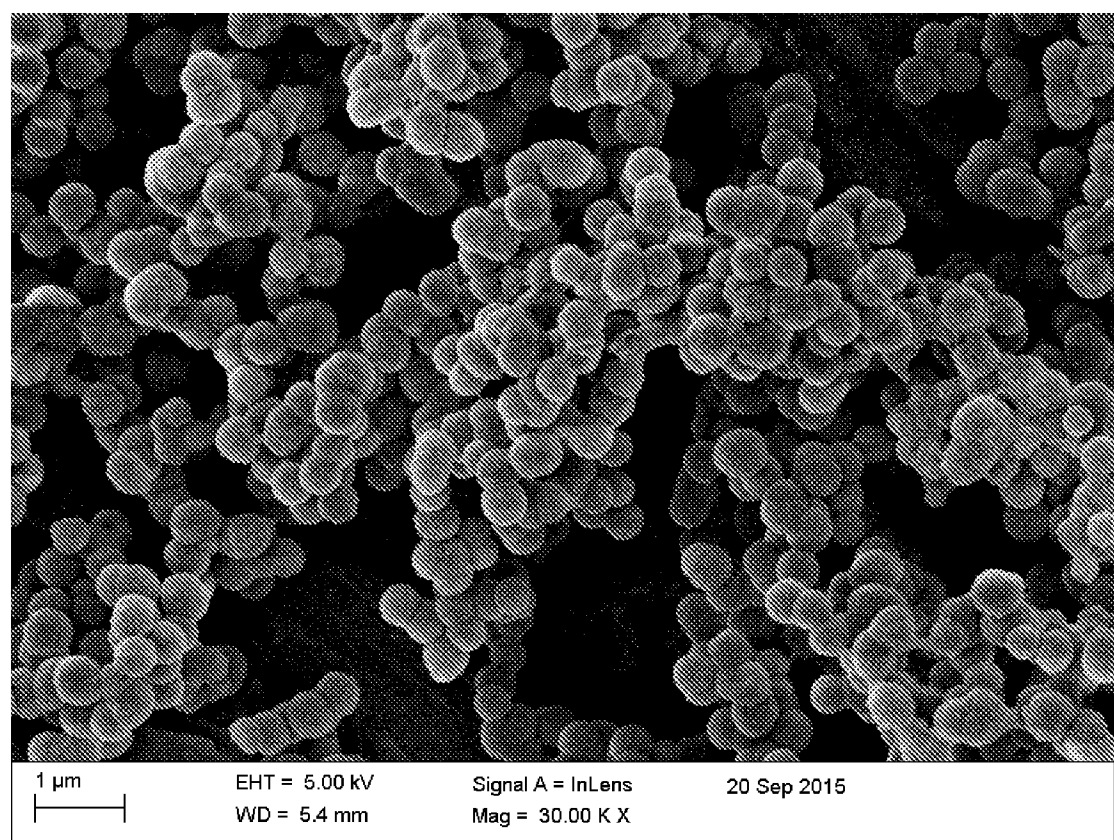
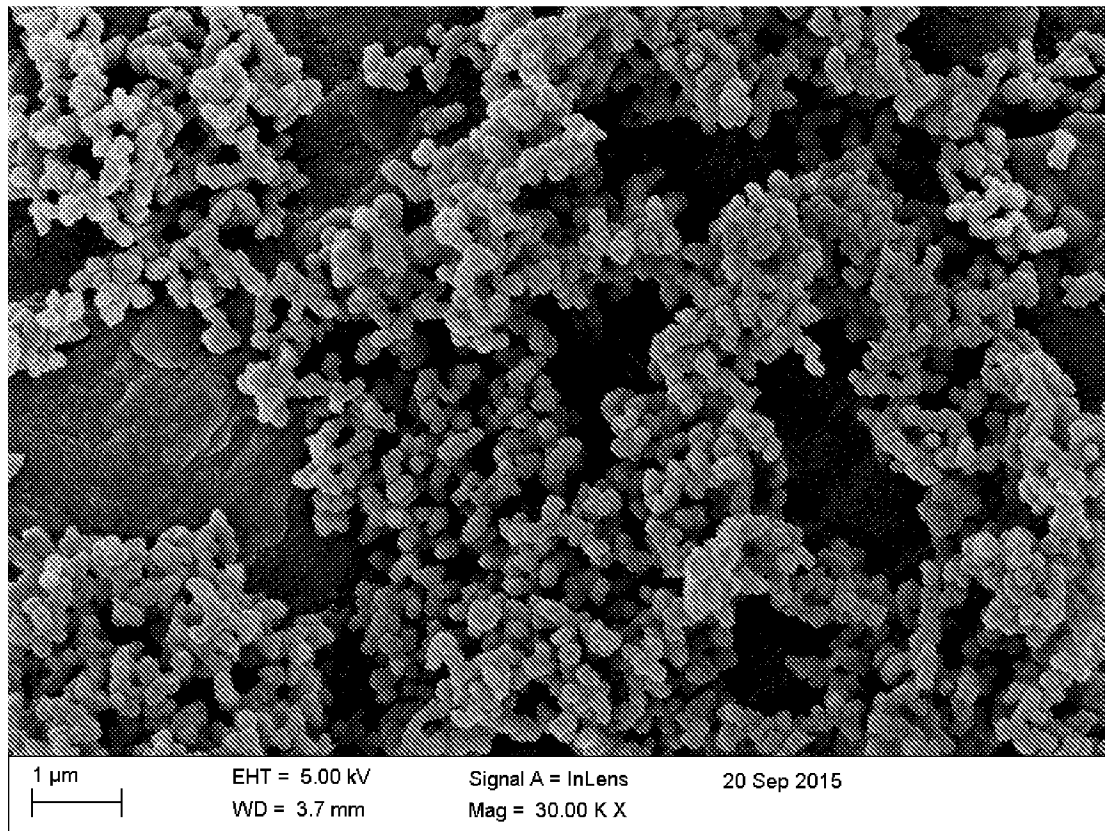
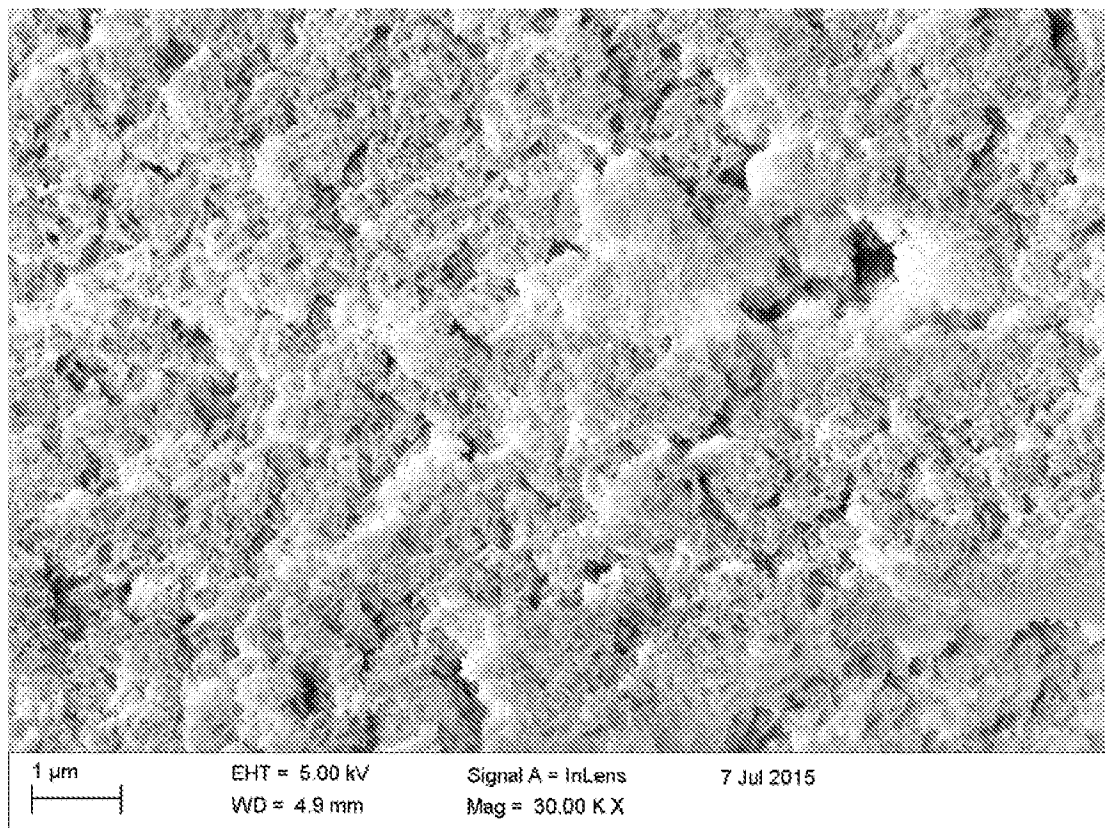
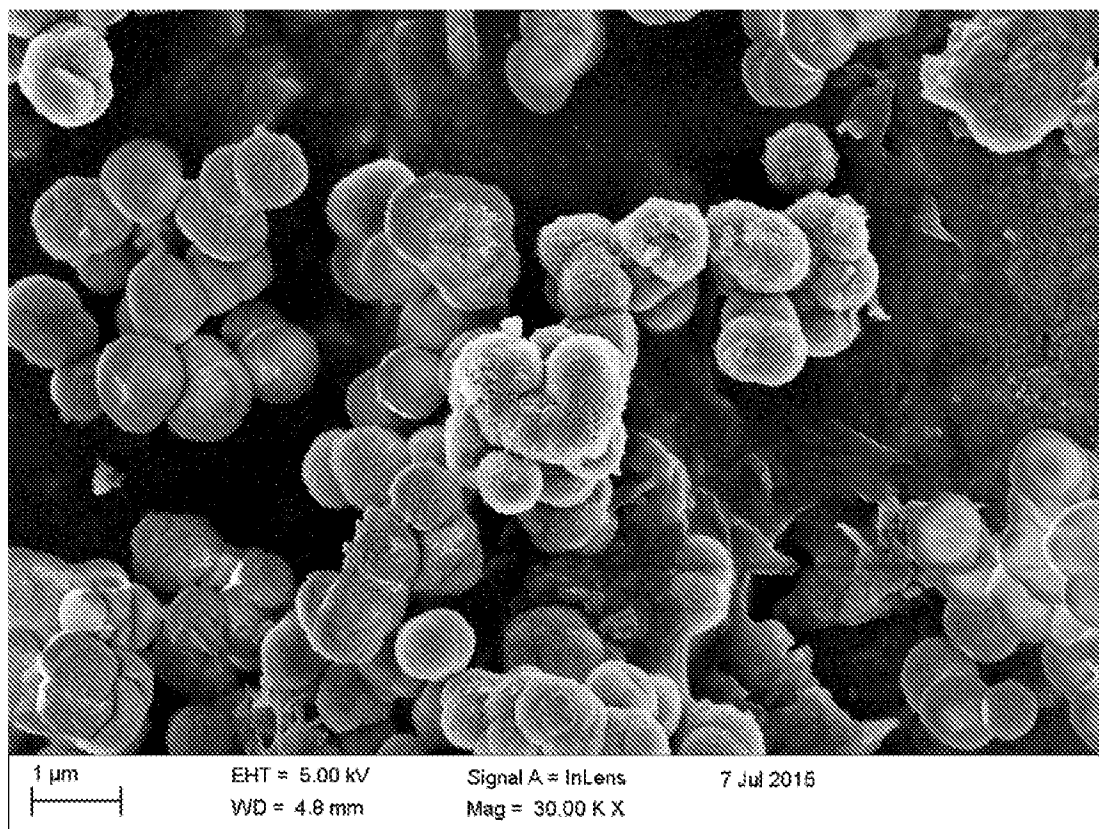
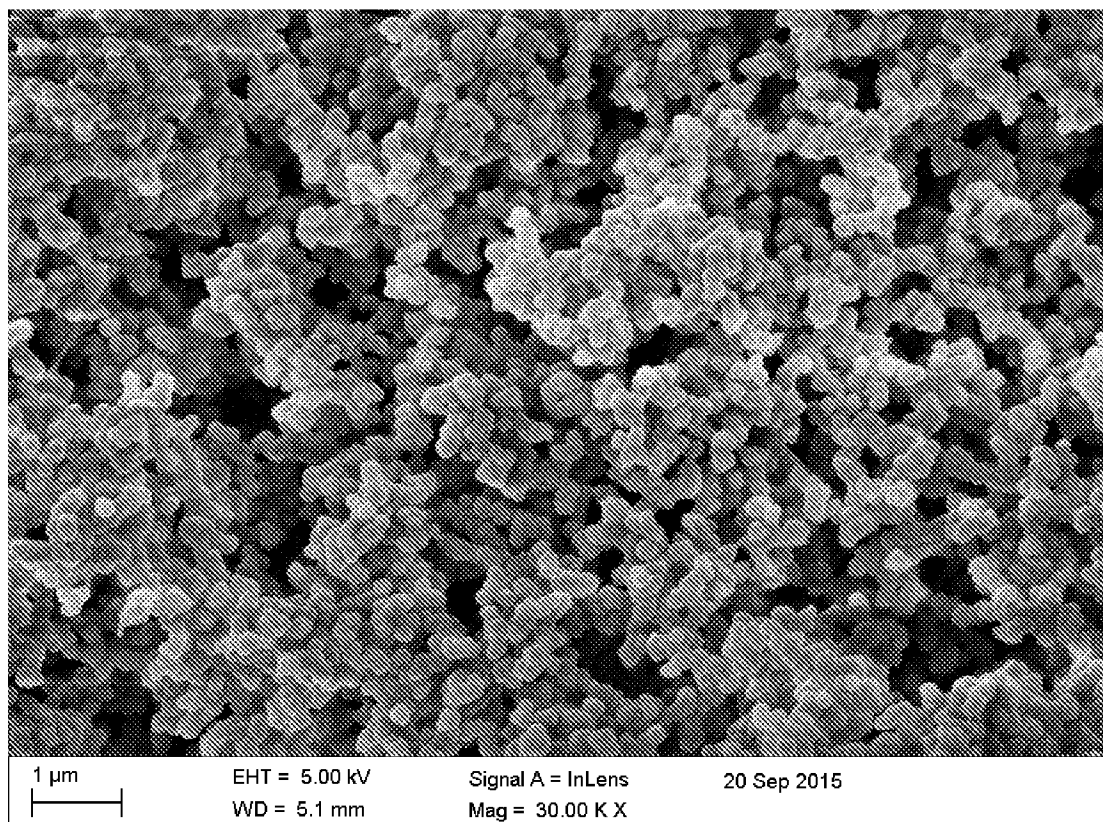
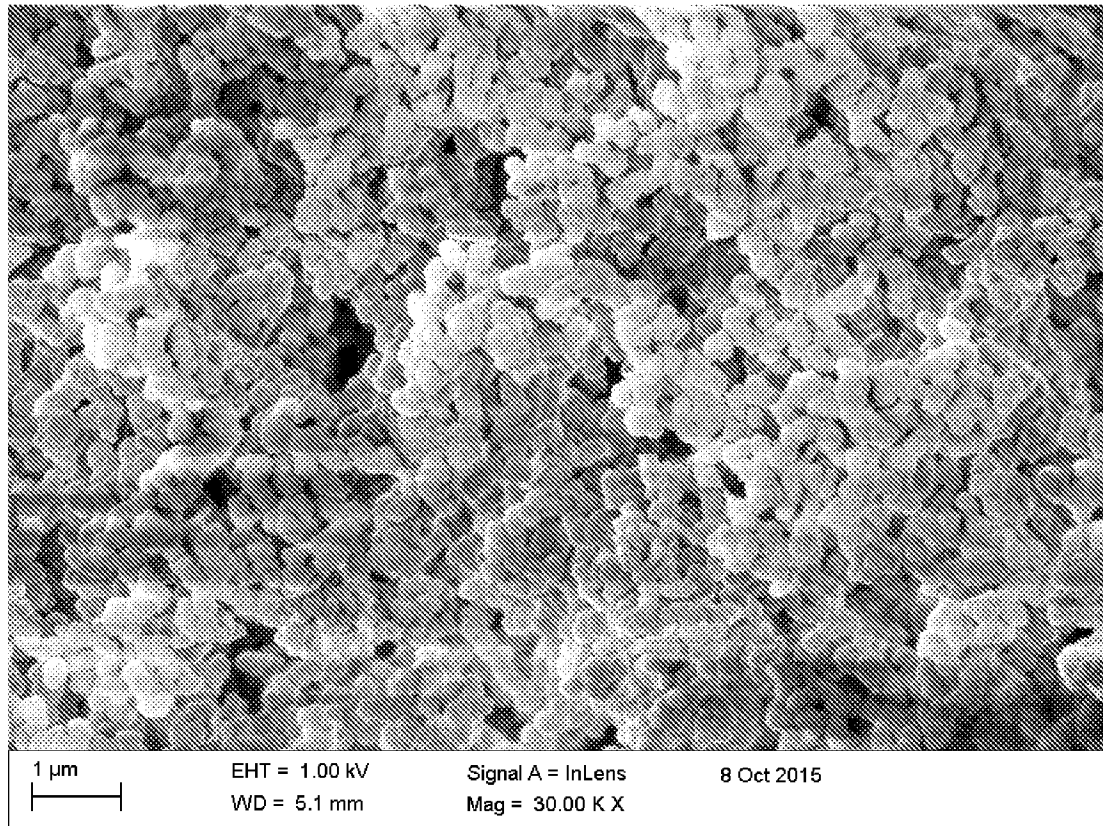
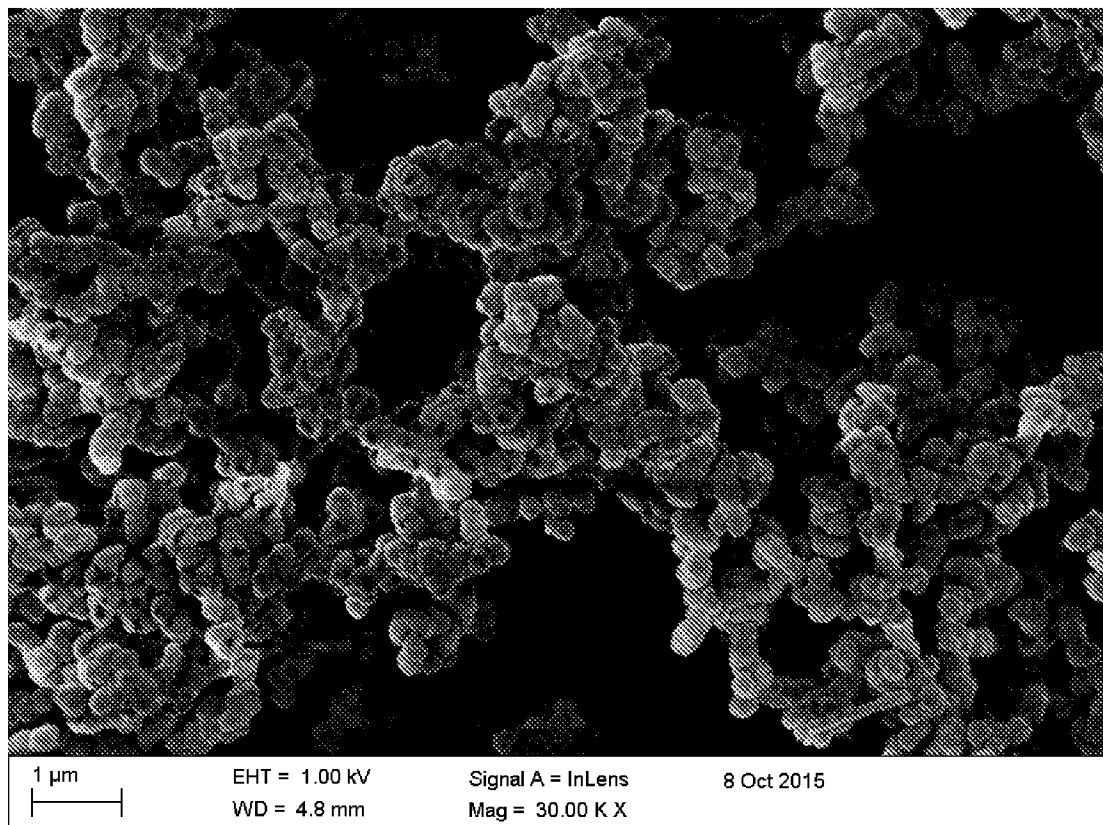
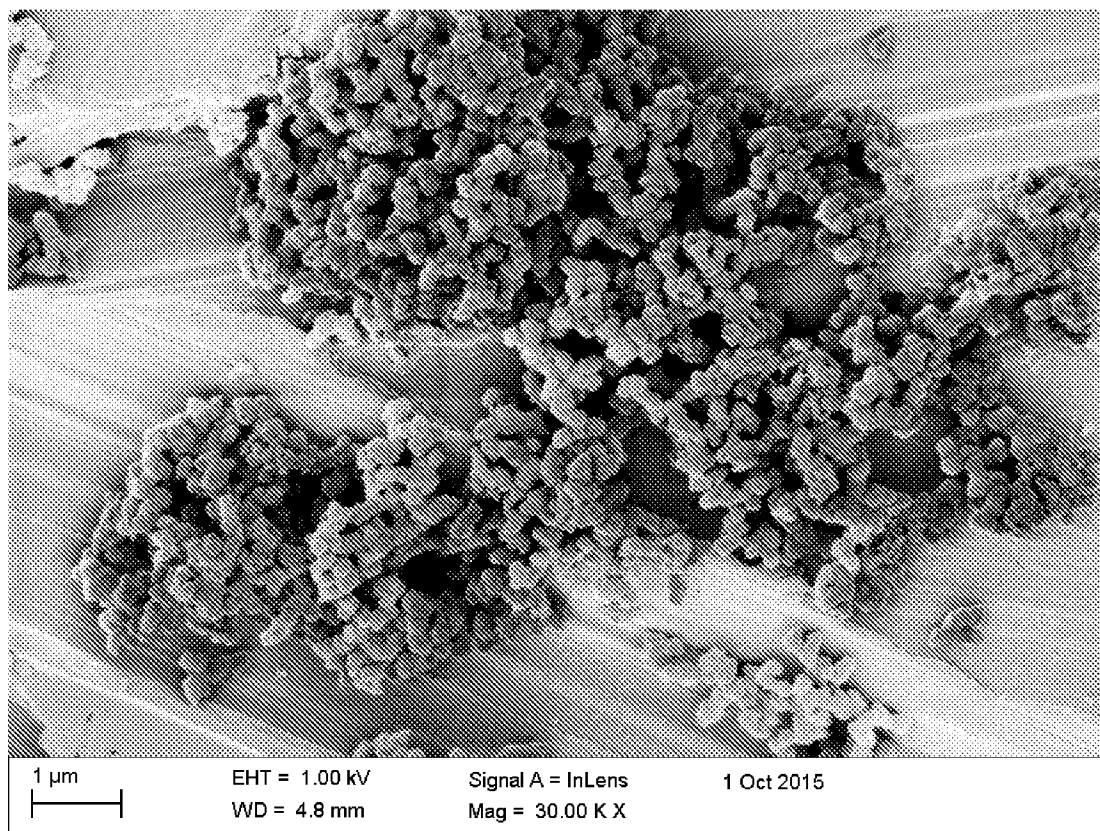


Figure 2

**Figure 3****Figure 4**

**Figure 5****Figure 6**

**Figure 7****Figure 8**

**Figure 9**

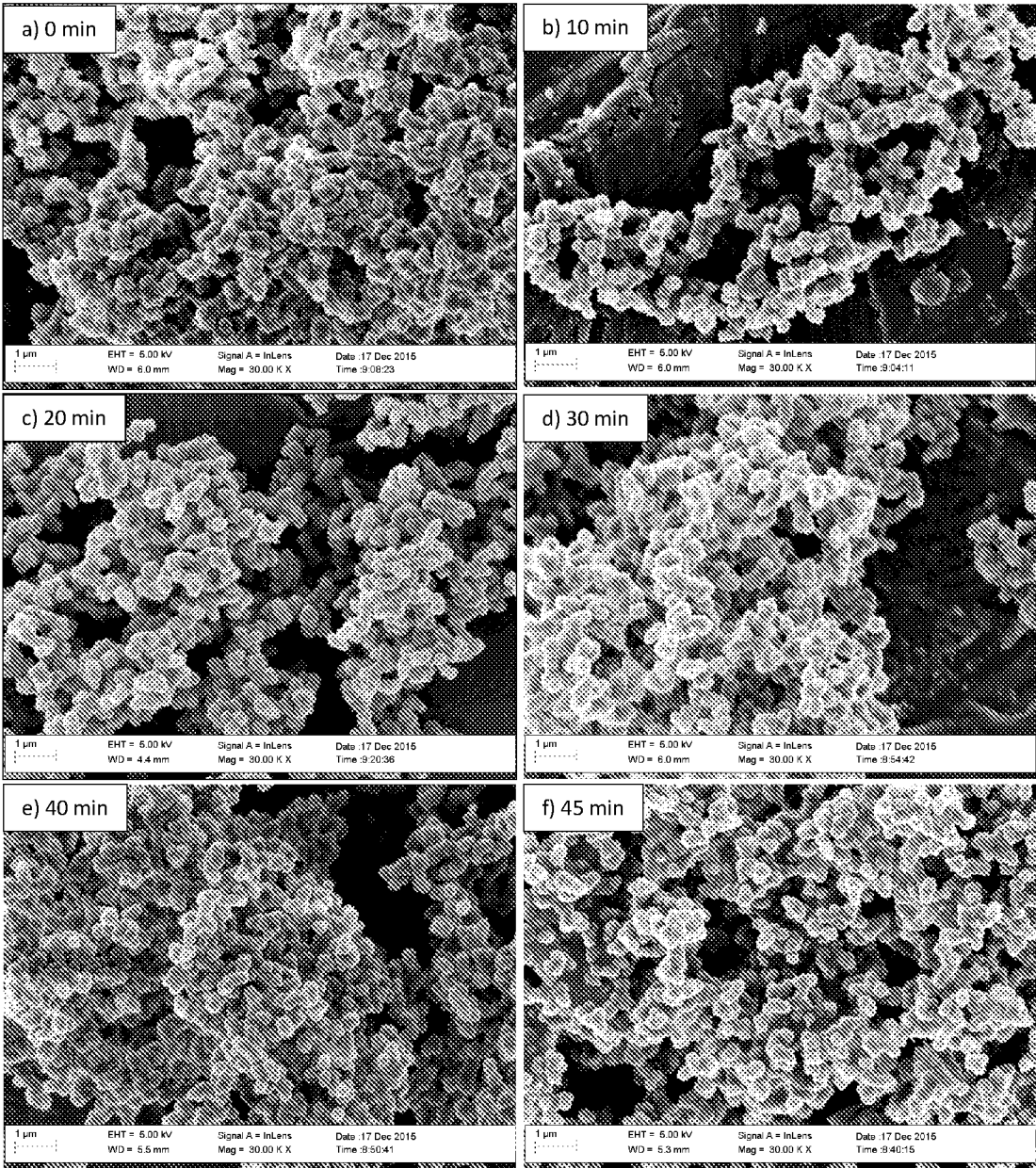


Figure 10

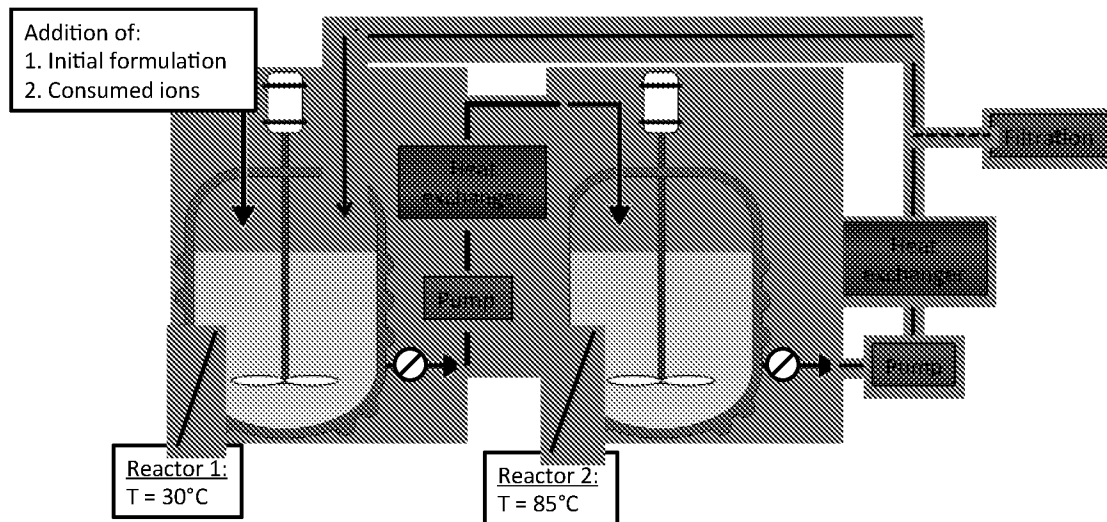


Figure 11

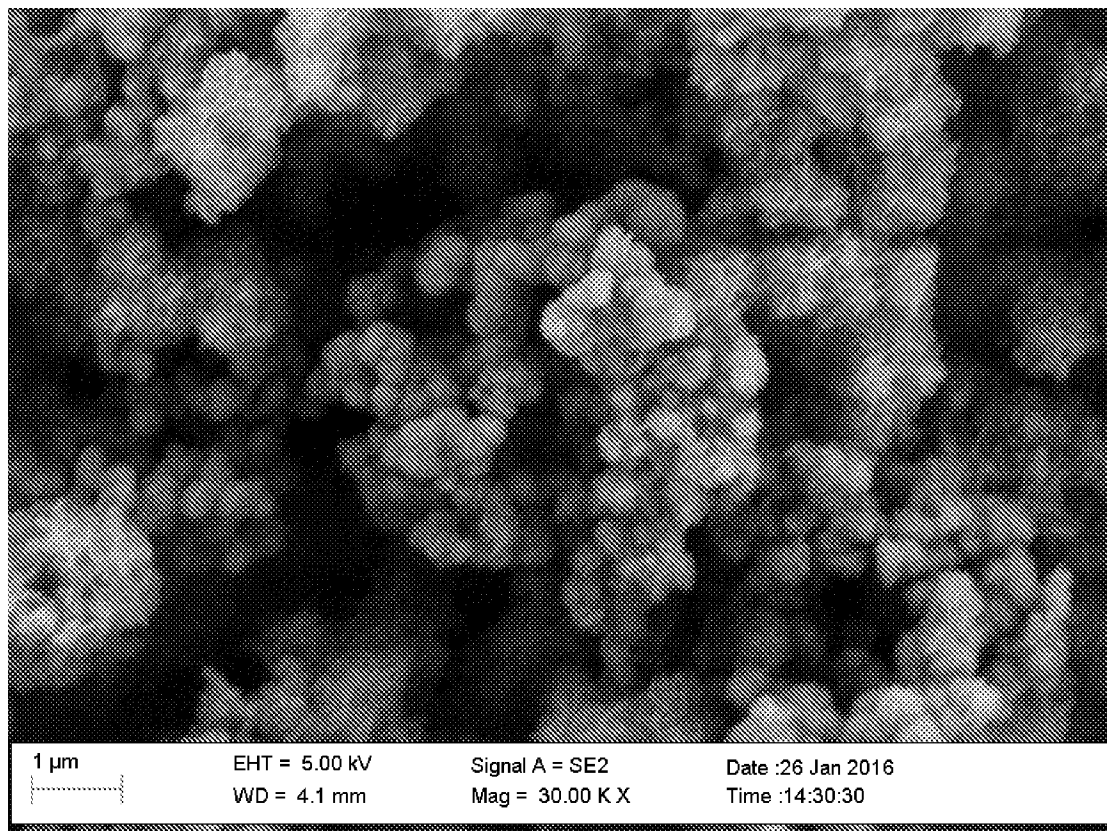
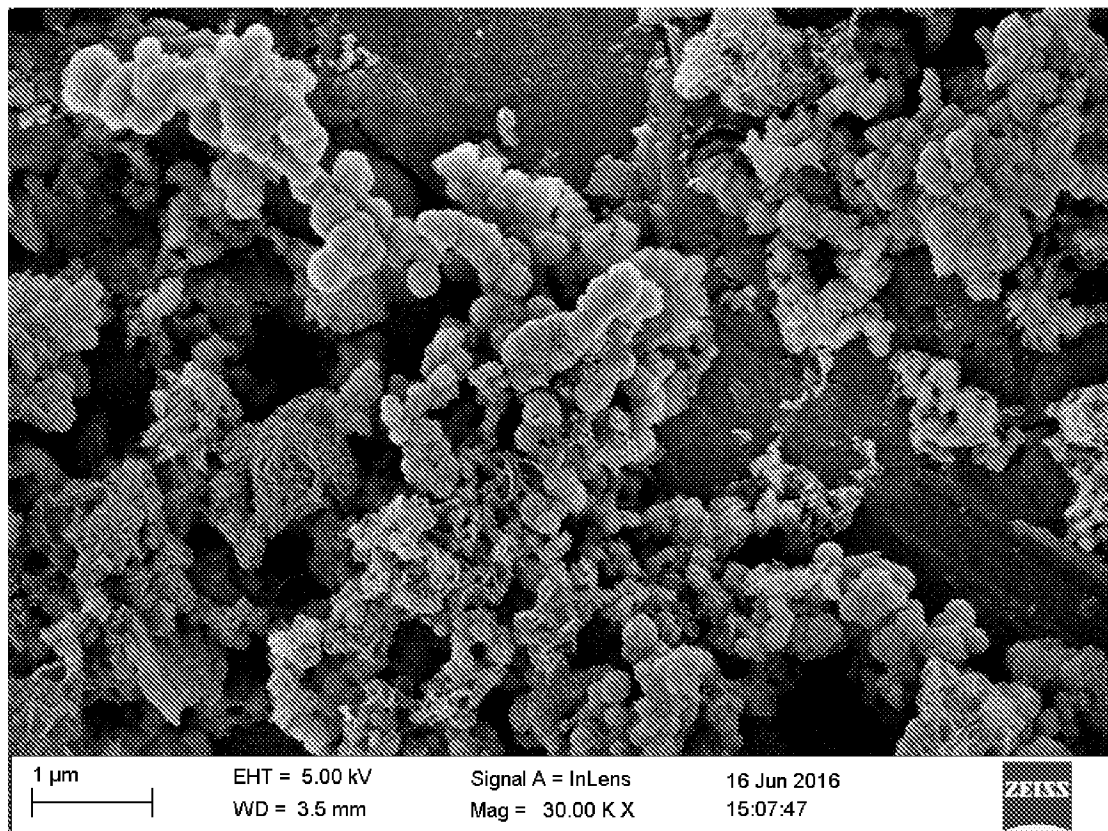
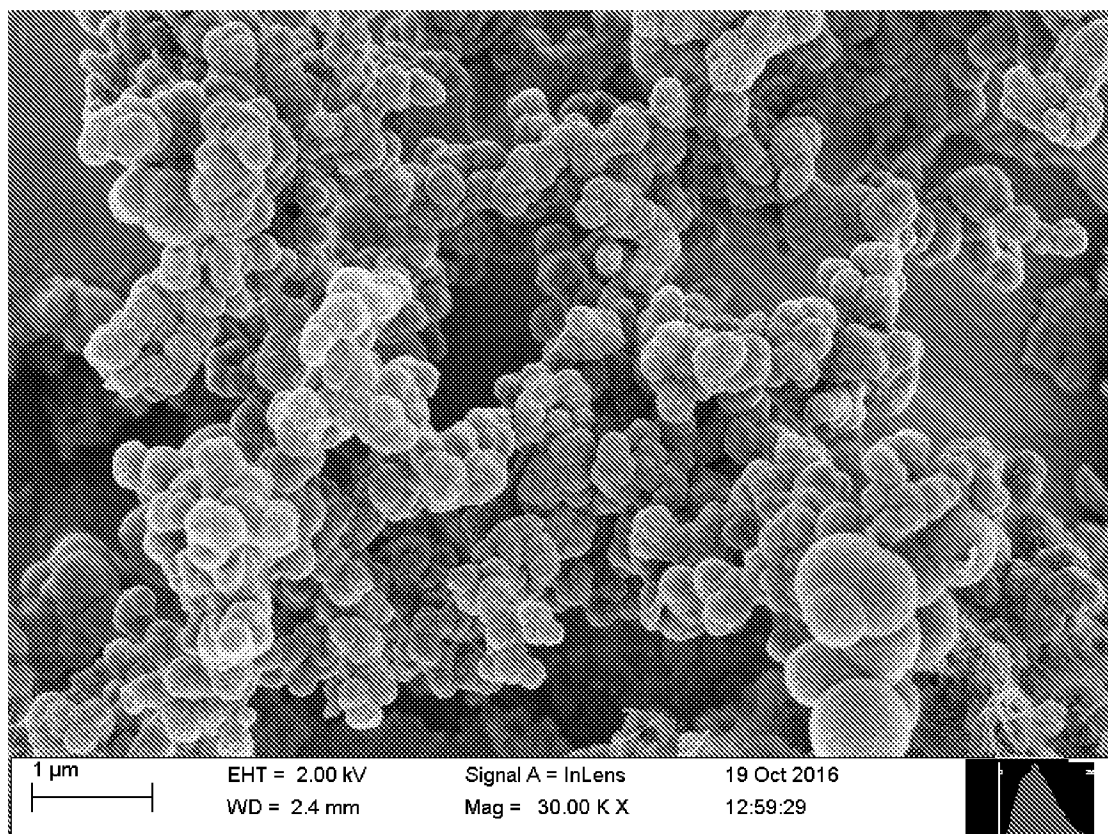


Figure 12

**Figure 13****Figure 14**

INTERNATIONAL SEARCH REPORT

International application No
PCT/SE2016/051121

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/24 A61Q11/00 A61K8/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/148997 A1 (BIOMATCELL AB [SE]) 25 September 2014 (2014-09-25) cited in the application	1-8,11
Y	page 3, line 8 - line 11 page 8, line 6 - line 8 claims 1,10	9,10
Y	----- WO 2005/087661 A1 (RAO ABBURI VISWESWARA [IN]) 22 September 2005 (2005-09-22) claims 1-7 -----	9,10



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

12 January 2017

Date of mailing of the international search report

20/01/2017

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Lenzen, Achim

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/SE2016/051121

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 2976111 A1	27-01-2016
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WO 2005087661 A1	22-09-2005	NONE	
