

METHODS OF MANUFACTURING CORE-SHELL MICROPARTICLES, AND MICROPARTICLES FORMED THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US provisional application No. 61/595,312 filed on 6 February 2012, the content of which is incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates to methods of manufacturing core-shell microparticles having a core comprising a hydrogel material and a shell comprising a hydrophobic polymer immiscible with the hydrogel material, as well as core-shell microparticles formed thereof, and use of the microparticles in drug delivery applications.

BACKGROUND OF THE INVENTION

[0003] Controlled drug delivery systems to achieve controlled release of therapeutic drugs have been developed. These systems are believed to allow reduction in institutional healthcare load for long term disease management, and increase patient compliance for diseases requiring extended treatment and monitoring regimes.

[0004] There is an increased research focus on the delivery of water soluble drugs, such as antibiotics and anti-cancer drugs, as well as antigens and growth factors. The drugs may be delivered *in vivo* via the use of polymeric matrices or capsules, and may be incorporated therein during fabrication. State of the art methods to encapsulate drugs within polymeric matrices or capsules include solvent evaporation, nanoprecipitation, and coacervation.

[0005] Amongst the types of polymers, biodegradable polyesters such as poly(lactide-co-glycolide) (PLGA) and poly(L-lactide) (PLLA) have been used in drug delivery applications, due to ability of the polymers to maintain release of drugs over a longer period of time *in vivo*.

[0006] However, these biodegradable polyesters are not able to load hydrophilic drugs at higher encapsulation efficiencies due to higher hydrophobicity of the polymers as compared to the drugs. Burst release of drugs from such systems is also an issue, due to localization of

drugs on particulate surfaces or pores formed within the microparticles. Furthermore, emulsion-based solvent evaporation techniques used to produce hydrophilic drug-loaded microparticles, whereby a hydrophilic drug added into the inner aqueous phase of the water-in-oil-in-water (WOW) double emulsion, exacerbate leaching of these drugs into the aqueous continuous phase during fabrication. This is because the drugs, which are loaded into the inner aqueous phase from the first primary water-in-oil emulsion, tend to escape during the synthesis process thereby reducing overall drug encapsulation efficiency.

[0007] As a result, hydrogel-based drug delivery systems, which are believed to improve loading of hydrophilic drugs, have been developed. They are also favored as a friendly environment for protein encapsulation. Examples of hydrogels include alginate and chitosan, which may be physically gelled using multivalent ions such as calcium (II) ions (Ca^{2+}). These hydrogel-based drug delivery systems however suffer from limitations, such as non-sustained release of drugs due to susceptibility of the hydrogels to swell in water when immersed in an aqueous environment.

[0008] Composite hydrogel-PLGA particulate systems have also been used in recent years to provide sustained release and increased loading of hydrophilic drugs. These particulate systems include gel particulates which are dispersed within an internal matrix or on the surface of PLGA microparticles. However, such systems require a multistep fabrication process involving prefabrication of the hydrogel component, followed by loading of drugs, and dispersion of the drug loaded hydrogel into a PLGA polymeric solution for emulsification and solvent evaporation. The increased complexity of the process translates into additional time and processing requirements during fabrication.

[0009] In view of the above, there remains a need for improved methods to prepare controlled drug release formulations and drug delivery systems that address one or more of the above-mentioned problems.

SUMMARY OF THE INVENTION

[0010] In a first aspect, the invention is directed to a method of manufacturing core-shell microparticles having a core comprising a hydrogel material and a shell comprising a hydrophobic polymer immiscible with the hydrogel material. The method comprises

a) dissolving a hydrogel-forming agent in an aqueous solution comprising an osmolyte to form a first solution;

b) dissolving a hydrophobic polymer in an organic solvent comprising a first surfactant to form a second solution;

c) dispersing the first solution into the second solution to form a first emulsion;

5 d) dispersing the first emulsion into an aqueous solution comprising a second surfactant and a reagent capable of effecting gelation of the hydrogel-forming agent to form a second emulsion; and

e) extracting the organic solvent from the second emulsion, wherein the reagent capable of effecting gelation of the hydrogel-forming agent penetrates through the second solution and effects gelation of the hydrogel-forming agent to form hydrogel microparticles,
10 and the hydrophobic polymer precipitates to form a shell around each of the hydrogel microparticles.

[0011] In a second aspect, the invention is directed to core-shell microparticles manufactured by a method according to the first aspect.

[0012] In a third aspect, the invention is directed to a core-shell microparticle having a
15 core comprising a hydrogel material and a shell comprising a hydrophobic polymer immiscible with the hydrogel material, wherein the shell is free of the hydrogel material of the core.

[0013] In a fourth aspect, the invention is directed to a pharmaceutical composition comprising core-shell microparticles manufactured by a method according to the first aspect,
20 or according to the third aspect.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Hereinafter, the present invention will be described more fully with reference to the accompanying drawings, in which exemplary embodiments of the invention are shown.
25 This invention may, however, be embodied in many different forms and should not be construed as limited to the exemplary embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. In the drawings, lengths and sizes of layers and regions may be exaggerated for clarity.

30 [0015] **FIG. 1** is a schematic diagram depicting fabrication of alginate-polymer core-shell microparticles according to an embodiment.

[0016] **FIG. 2** is a scanning electron microscopy (SEM) image of (a) cross-sectioned alginate-PLGA particle, and (b) the same microparticle after subjecting to trisodium citrate treatment.

[0017] **FIG. 3** is (a) Fourier transform infrared spectroscopy (FTIR) spectra of a typical alginate/PLGA microparticle; and (b) optical image and Raman mapping of a cross-sectioned alginate-PLGA microparticle.

[0018] **FIG. 4** is a SEM image of (a) cross-sectioned alginate-PLLA particle, and (b) the same microparticle after subjecting to trisodium citrate treatment.

[0019] **FIG. 5** is an optical image and Raman mapping of a cross sectioned alginate-PLLA particle.

[0020] **FIG. 6** is SEM image of (a) cross sectioned metoclopramide hydrochloride (HCl) (MCA) loaded alginate-PLGA particle; and (b) the same microparticle after subjecting to trisodium citrate treatment.

[0021] **FIG. 7** is an optical image and Raman mapping of a cross-sectioned alginate-PLGA microparticle loaded with metoclopramide HCl.

[0022] **FIG. 8** is a graph depicting release profile of metoclopramide HCl from alginate-PLGA MP, alginate-PLLA MP vs naked calcium alginate beads across 7 days (main plot), and a close up of the initial 24 hours (inset).

[0023] **FIG. 9** is a schematic diagram depicting principles of alginate-PLGA core shell microparticle formation.

[0024] **FIG. 10** is SEM image (a) of cross sectioned MCA loaded alginate-PLLA particle; and (b) the same microparticle after subjecting to trisodium citrate treatment.

[0025] **FIG. 11** is a SEM image of alginate-PLGA microparticles fabricated without sodium chloride (NaCl) dissolved in the internal alginate aqueous phase.

[0026] **FIG. 12** is a graph depicting metoclopramide HCl (MCA) drug release performance of fabricated core-shell alginate Poly-L-Lactide shell microparticles over a period of one week.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0027] In a first aspect, the present invention refers to a method of manufacturing core-shell microparticles having a core comprising a hydrogel material and a shell comprising a hydrophobic polymer immiscible with the hydrogel material.

[0028] Advantageously, the method of the first aspect provides a single-step method to fabricate core-shell microparticles having a core comprising a hydrogel material and a shell comprising a hydrophobic polymer that is immiscible with the hydrogel material. Positioning the hydrogel material as the core of the core-shell microparticles translates into increased loading of hydrophilic active compounds, such as hydrophilic drugs in the microparticles, while at the same time providing sustained release of the hydrophilic active compounds, since premature leaching of the active compounds to the surroundings during fabrication, for example, is prevented. The hydrophobic polymer shell may thus act as a protective layer to guard against burst release of the hydrophilic active compounds comprised within the core-shell microparticles.

[0029] As used herein, the term "microparticle" refers to a microscopic particle having a size measured in micrometres (μm). The term "core-shell microparticles" refers to a structural configuration of microparticles in which an external layer formed of a second material encompasses the inner core, thereby forming the core-shell structure.

[0030] The method includes dissolving a hydrogel-forming agent in an aqueous solution comprising an osmolyte to form a first solution.

[0031] As used herein, the term "hydrogel" refers to a broad class of polymeric materials, that may be natural or synthetic, which have an affinity for an aqueous medium, and is able to absorb large amounts of the aqueous medium, but which do not normally dissolve in the aqueous medium.

[0032] Generally, a hydrogel may be formed by using at least one or one or more types of hydrogel precursor. The term "hydrogel precursor" is also termed herein as "hydrogel-forming agent", and refers to any chemical compound that may be used to make a hydrogel. Typically, a hydrogel is formed by setting or solidifying the one or more types of hydrogel-forming agent in an aqueous solution to form a three-dimensional network, for example, by cross-linking, wherein formation of the three-dimensional network may cause the one or more types of hydrogel-forming agent to gel so as to form the hydrogel.

[0033] The hydrogel-forming agent may comprise a physically cross-linkable polymer, a chemically cross-linkable polymer, or mixtures thereof. Physically cross-linking may take place via, for example, complexation, hydrogen bonding, desolvation, van der Waals interactions, or ionic bonding. Examples of physically cross-linkable polymer that may be

used include, but are not limited to, alginate, pectin, furcellaran, carageenan, chitosan, derivatives thereof, copolymers thereof, and mixtures thereof.

[0034] Chemical crosslinking may take place via, for example, chain reaction (addition) polymerization, and step reaction (condensation) polymerization. Examples of chemically cross-linkable polymer that may be used include, but are not limited to, starch, gellan gum, dextran, hyaluronic acid, poly(ethylene oxides), polyphosphazenes, derivatives thereof, copolymers thereof, and mixtures thereof. Such polymers may be functionalized with a methacrylate group for example, and may be cross-linked *in situ* via polymerization of these groups during formation of the emulsion droplets in the fabrication process.

[0035] In various embodiments, the hydrogel-forming agent consists essentially of a physically cross-linkable polymer. In some embodiments, the hydrogel-forming agent comprises or consists essentially of sodium alginate. Sodium alginate is a water soluble form of alginate, whereby the term "alginate" refers generally to any of the conventional salts of algin, which is a polysaccharide of marine algae. Alginate may be polymerized to form a matrix for use in drug delivery and in tissue engineering due to its biocompatibility, low toxicity, relatively low cost, and simple gelation with multivalent cations such as calcium ions (Ca^{2+}) and magnesium ions (Mg^{2+}).

[0036] The hydrogel-forming agent may be at least substantially dissolved in an aqueous solution. The term "aqueous solution" as used herein refers to water or a solution based primarily on water such as phosphate buffered saline (PBS), or water containing a salt dissolved therein. In various embodiments, the hydrogel-forming agent is completely dissolved in the aqueous solution.

[0037] Agitation, for example, by stirring or sonication may be carried out to enhance the rate at which the hydrogel-forming agent dissolves in the aqueous solution. In some cases, heat energy may optionally be applied to the solution to increase the dissolve rate of the hydrogel-forming agent in the aqueous solution.

[0038] Concentration of the hydrogel-forming agent in the first solution may influence the size of hydrogel microparticles formed, which translates into different sizes of the hydrogel core of the core-shell microparticles formed. Generally, a larger amount of the hydrogel-forming agent with respect to the amount of hydrophobic polymer present results in formation of a larger size core of the core-shell microparticle.

[0039] In various embodiments, the amount of hydrogel-forming agent in the first solution may be between about 1 % (w/v) to about 10 % (w/v), such as between about 1 % (w/v) to about 8 % (w/v), about 1 % (w/v) to about 6 % (w/v), about 6 % (w/v) to about 10 % (w/v), or about 4 % (w/v) to about 6 % (w/v). In various embodiments, the amount of hydrogel-forming agent in the first solution is about 4.5 % (w/v).

[0040] The aqueous solution used to form the first solution may contain an osmolyte. The term "osmolyte" refers generally to compounds or substances that affect osmosis. The osmolyte may be added to provide increased osmotic pressure to mitigate out-flux of the hydrogel-forming agent while forming the core-shell microparticles. Examples of osmolyte that may be used include, but are not limited to, sodium chloride, potassium chloride, sodium bromide, sodium citrate, sodium lactate, sodium hydroxide, sodium iodide, sodium carbonate, sodium hydrogen carbonate, sodium nitrate, sodium fluoride, sodium sulfate, potassium carbonate, potassium citrate, potassium lactate, potassium hydrogen carbonate, potassium bromide, potassium hydroxide, potassium iodide, potassium nitrate, potassium sulfate, cesium chloride, rubidium chloride, lithium chloride, and mixtures thereof. In various embodiments, the osmolyte comprises or consists essentially of sodium chloride.

[0041] Concentration of osmolyte in the first solution may be between about 0.5 M to about 2 M, such as between about 0.8 M to about 1.8 M, about 1 M to about 1.6 M, or about 1.2 M to about 1.4 M. In various embodiments, the concentration of osmolyte in the first solution is about 1.35 M.

[0042] The method of the first aspect includes dissolving a hydrophobic polymer in an organic solvent comprising a first surfactant to form a second solution.

[0043] The term "hydrophobic" is generally used to describe a substance that repels water. In line with this definition, the term "hydrophobic polymer" refers to a polymer having a low affinity for aqueous solutions including water. For example, hydrophobic polymers may include polymers that do not dissolve in, be mixed with, or be wetted by water. As another example, hydrophobic polymers may also include polymers that do not absorb an appreciable amount of water.

[0044] According to embodiments of the invention, a suitable hydrophobic polymer may be one that is able to dissolve in an organic solvent, and be precipitated using a solvent extraction process. The hydrophobic polymer used in the present invention may be a natural polymer or a synthetic polymer. The term "natural polymer" as used herein refers generally

to a polymeric material that may be found in nature. Examples of a natural hydrophobic polymer include, but are not limited to, natural rubber and cellulose such as ethyl cellulose.

[0045] Examples of synthetic hydrophobic polymers include, but are not limited to, polyolefin, polystyrene, polyester, polyamide, polyether, polysulfone, polycarbonate, polyurea, polyurethane, polysiloxane, copolymers thereof, and mixtures thereof.

[0046] The hydrophobic polymer that is immiscible with the hydrogel-forming material may be biodegradable or non-biodegradable, which may depend on the intended application. For drug delivery applications, biodegradable polymers are generally used. In various embodiments, the hydrophobic polymer immiscible with the hydrogel-forming material is a biodegradable polymer or a biocompatible material.

[0047] Biodegradable polymers refer generally to natural or synthetic polymers that gradually degrade *in vivo* to produce biocompatible or non-toxic byproducts over a period of time (e.g., within days, or months, or years). Disintegration may for instance occur via hydrolysis, may be catalyzed by an enzyme and may be assisted by conditions to which the microparticles are exposed in the cell. The term "biocompatible" refers to a material that is capable of interacting with a biological system without causing cytotoxicity, undesired protein or nucleic acid modification or activation of an undesired immune response.

[0048] Examples of biodegradable polymers include, but are not limited to, polymers and oligomers of glycolide, lactide, polylactic acid, polyesters of α -hydroxy acids, including lactic acid and glycolic acid, such as the poly(α -hydroxy) acids including polyglycolic acid, poly(DL-lactic-co-glycolic acid) (PLGA), poly-L-lactic acid (PLLA), and terpolymers of DL-lactide and glycolide; ϵ -caprolactone and ϵ -caprolactone copolymerized with polyesters; polylactones and polycaprolactones including poly(caprolactone) (PCL), poly(ϵ -caprolactone), poly(valerolactone) and poly(γ -butyrolactone); polyanhydrides; polyorthoesters; polydioxanone; and other biologically degradable polymers that are non-toxic or are present as metabolites in the body.

[0049] Non-exhaustive examples of biocompatible polymers include polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terephthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes and copolymers thereof, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl

cellulose, hydroxy-propyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methylmethacrylate), poly(ethylmethacrylate), poly(butylmethacrylate), poly(isobutylmethacrylate),
5 poly(hexylmethacrylate), poly(isodecylmethacrylate), poly(laurylmethacrylate), poly(phenylmethacrylate), poly(methacrylate), poly(isopropylacrylate), poly(isobutylacrylate), poly(octadecylacrylate), polyethylene, polypropylene poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), poly(vinyl acetate) such as ethylene vinyl acetate (EVA), poly(vinyl chloride), polystyrene, polyhyaluronic acids, casein, gelatin, gluten,
10 polyanhydrides, polyacrylic acid, alginate, chitosan, any copolymers thereof, and mixtures thereof.

[0050] Choice of hydrophobic polymer to be used in a method according to the first aspect may be dependent on the end application. For example, for application in biomedical areas, polymers such as PCL, PGA, PLA and PLGA are of interest because of their biocompatibility
15 and biodegradability properties. In particular, PLGA has been FDA approved for human therapy. When degrading, PLA and PLGA chains are cleaved in the body to monomeric acids, i.e. lactic and glycolic acids that are eliminated from the organism through Krebs's cycle as CO₂ and in urine as water. A person skilled in the art is able to choose and determine the appropriate polymer type based on the area of specific application.

20 [0051] In various embodiments, the hydrophobic polymer immiscible with the hydrogel-forming material is poly-lactic-co-glycolic acid (PLGA), poly-L-lactide (PLLA), polycaprolactone (PCL), polyglycolide (PGA), derivatives thereof, copolymers thereof, or mixtures thereof.

[0052] Furthermore, depending on the hydrogel-forming agent used, different
25 hydrophobic polymers may be used, so long as they are immiscible with the hydrogel-forming agent. In this regard, the term "immiscible" as used herein is used to refer that the hydrogel-forming agent and the hydrophobic polymer do not mix, and/or that they have no or limited solubility in each other. For example, in embodiments where the hydrogel-forming agent comprises sodium alginate, the hydrophobic polymer immiscible with the hydrogel-forming material may comprise or consist essentially of poly-lactic-co-glycolic acid (PLGA)
30 or poly-L-lactide (PLLA).

[0053] The amount of hydrophobic polymer in the second solution may be any suitable amount that is able to at least substantially dissolve in the organic solvent. This may also depend on, for example, amount of hydrogel-forming agent used and thickness of the hydrophobic polymer shell to be formed. Generally, a higher concentration of hydrophobic polymer in the second solution results in formation of a thicker polymer shell on the microparticle.

[0054] In various embodiments, the amount of hydrophobic polymer in the second solution is between about 1 % (w/v) to about 30 % (w/v), such as between about 10 % (w/v) to about 30 % (w/v), about 20 % (w/v) to about 30 % (w/v), about 1 % (w/v) to about 20 % (w/v), about 5 % (w/v) to about 15 % (w/v), about 8 % (w/v) to about 12 % (w/v), about 8 % (w/v), about 10 % (w/v), or about 12 % (w/v). In various embodiments, the amount of hydrophobic polymer in the second solution is about 10 % (w/v).

[0055] The method comprises dissolving a hydrophobic polymer in an organic solvent comprising a first surfactant to form a second solution. "Organic solvent" refers to a solvent comprised of a carbon-containing chemical. By dissolving in the solvent, the hydrophobic polymer can form a homogeneous solution. Therefore, a suitable solvent is one that can dissolve the hydrophobic polymer.

[0056] In various embodiments, the solvent is one that is volatile and is immiscible in an aqueous solution. The term "volatile" as used herein refers to a compound that can be readily vaporized at ambient temperature. A measure of the volatility of a substance is its boiling point at one atmosphere. A volatile solvent may have a boiling point at one atmosphere of less than about 100 °C, such as about 75 °C or about 50 °C or about 40 °C or about 30°C. One type or more than one type of solvent may be used.

[0057] Suitable solvents that may be used in the present invention includes, but are not limited to, methylene chloride (dichloromethane or DCM), dimethylformamide (DMF), tetrahydrofuran (THF), methyl ethyl ketone (MEK), chloroform, pentane, benzene, benzyl alcohol, carbon tetrachloride, ethyl acetate (EAc), acetone, acetonitrile, dimethyl sulfoxide, propylene carbonate (PC), and mixtures thereof. In various embodiments, the organic solvent comprises or consists essentially of dichloromethane.

[0058] As in the case for the first solution, to enhance the rate at which the hydrophobic polymer dissolves in the solvent, agitation, for example, by stirring or sonication. Heat

energy may also be applied to the solution to increase the dissolve rate of polymer in the solvent.

[0059] The organic solvent may contain a first surfactant. The first surfactant may comprise or consist essentially of a hydrophobic surfactant. Hydrophobic surfactants may be used to stabilize the water-in-oil emulsion, termed herein as the first emulsion, formed from dispersing the aqueous-based first solution into the oil-based second solution. Generally, any hydrophobic surfactant that is able to stabilize the first emulsion formed using a method of the invention may be used. Examples of such surfactants include, but are not limited to, sorbitan ester, sorbitan monoester, sorbitan trioleate, sorbitan tristearate, sorbitan sesquioleate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, glycerol monooleate, glycerol monostearate, PEO/PPO copolymers, derivatives thereof, and mixtures thereof.

[0060] The hydrophobic surfactants may alternatively be identified by their commercial names. For example, Span® 85 is a commercial name for sorbitan trioleate, while Span® 80 is a commercial name for sorbitan monooleate. Both Span® 85 and Span® 80 may be used as the hydrophobic surfactant in the present invention. In various embodiments, the first surfactant comprises or consists essentially of sorbitan monooleate or otherwise known as Span® 80.

[0061] The amount of first surfactant in the second solution may be any suitable amount that is able to stabilize the first emulsion formed from dispersing the first solution into the second solution. For example, the amount of first surfactant in the second solution may be between about 0.1 % (w/v) to about 5 % (w/v), such as between about 1 % (w/v) to about 5 % (w/v), about 3 % (w/v) to about 5 % (w/v), about 0.1 % (w/v) to about 3 % (w/v), about 0.1 % (w/v) to about 1 % (w/v), about 3 % (w/v), about 2 % (w/v), or about 1 % (w/v). In various embodiments, the amount of first surfactant in the second solution is about 1 % (w/v).

[0062] In various embodiments, the organic solvent may further comprise an additive containing ions that do not effect gelation of the hydrogel-forming agent. For example, when sodium alginate is used, the additive may be a salt or an electrolyte containing sodium ions, potassium ions, or lithium ions. In various embodiments, the additive comprises sodium chloride, which dissociates in aqueous solution to form sodium ions and chloride ions. By the addition of or controlling the amount of additive present, size of the core of the core-shell microparticle formed may be controlled or varied.

[0063] Upon formation of the first solution and the second solution, the method according to the first aspect includes dispersing the first solution into the second solution to form a first emulsion.

[0064] The term "emulsion" as used herein refers to a disperse system of two or more immiscible liquids. Therefore, emulsifying of one liquid in the other can result in formation of two different phases, in which small droplets of one liquid may be dispersed, i.e. separated and distributed throughout the space, in the other liquid. The small droplets of liquid is called the dispersed phase, while the other liquid, within which the small droplets of liquid is dispersed, is called the continuous phase.

[0065] Most emulsions consist of water and oil or fat as immiscible phases. Depending on the composition and ratio of the phases two distribution options exist. In case the aqueous phase, such as water "W" is the continuous phase and the oil "O" is the dispersed phase, the result is an "O/W emulsion" or oil-in-water emulsion, whose basic character is determined by the aqueous phase. If oil "O" is the continuous phase and water "W" the dispersed phase, the result is a "W/O emulsion" or water-in-oil emulsion, wherein the basic character is determined by the oil.

[0066] As mentioned above, in the method according to the first aspect, the aqueous-based first solution is dispersed into the oil-based second solution to form the first emulsion, which is a water-in-oil (W/O) emulsion. The first surfactant that is present in the second solution functions to stabilize the first solution dispersed therein to form the water-in-oil emulsion. In various embodiments, dispersing the first solution into the second solution is carried out under continuous stirring, or any form of dispersing method that is able to emulsify two different immiscible phases.

[0067] In various embodiments, a hydrogel-forming agent is dissolved in an aqueous solution comprising an osmolyte to form a first solution. This first solution is dispersed in a second solution formed from dissolving a hydrophobic polymer in an organic solvent comprising a first surfactant, to form a water-in-oil emulsion (W1/O). This water-in-oil emulsion (W1/O) (first emulsion) is dispersed into an aqueous solution comprising a second surfactant and a reagent capable for effecting gelation of the hydrogel-forming agent to form a second emulsion. The second emulsion may be a double emulsion, in which water-in-oil droplets (W1/O) (first emulsion) are dispersed in a water continuous phase (W2) to form a water-in-oil-in-water (W1/O/W2) emulsion (second emulsion).

[0068] In accordance with this disclosure, a water-in-oil-in-water (W1/O/W2) emulsion may be used for the manufacture of a core-shell microparticle. An illustrative example of a W1/O/W2 emulsion is an emulsion formed using sodium alginate in water to which sodium chloride has been added as an osmolyte (W1), PLGA in DCM to which sorbitan monooleate has been added as a first surfactant (O), and water to which PVA has been added as a surfactant and calcium chloride has been added as a reagent capable of effecting gelation of the hydrogel-forming agent (W2).

[0069] The second surfactant may comprise or consist essentially of a hydrophilic surfactant, whereby the hydrophilic surfactant may be used to stabilize formation of water-in-oil droplets in the external aqueous phase. In other words, the hydrophilic surfactants may act as stabilizers to stabilize the W1/O/W2 emulsion. Examples of hydrophilic surfactants include, but are not limited to, an amphoteric surfactant, an anionic surfactant, a cationic surfactant, a nonionic surfactant, and mixtures thereof. Depending on the polymers used, the surfactant may influence the size of the microparticles formed.

[0070] Examples of amphoteric surfactants include, but are not limited to, dodecyl betaine, sodium 2,3-dimercaptopropanesulfonate monohydrate, dodecyl dimethylamine oxide, cocamidopropyl betaine, 3-[N,N-dimethyl(3-palmitoylaminopropyl)ammonio]-propanesulfonate, coco amphi glycinate, and mixtures thereof.

[0071] In various embodiments, addition of an anionic surfactant may render surface of the microparticles negatively charged. Examples of an anionic surfactant include, but are not limited to, sodium dodecyl sulfate (SDS), sodium pentane sulfonate, dehydrocholic acid, glycolithocholic acid ethyl ester, ammonium lauryl sulfate and other alkyl sulfate salts, sodium laureth sulfate, alkyl benzene sulfonate, soaps, fatty acid salts, and mixtures thereof.

[0072] In some embodiments, addition of a cationic surfactant may render the surface of the microparticles positively charged. Examples of a cationic surfactant include, but are not limited to, cetyl trimethylammonium bromide (CTAB), dodecylethyldimethylammonium bromide (D12EDMAB), didodecyl ammonium bromide (DMAB), cetylpyridinium chloride (CPC), polyethoxylated tallow amine (POEA), hexadecyltrimethylammonium p-toluenesulfonate, benzalkonium chloride (BAC), benzethonium chloride (BZT), and mixtures thereof.

[0073] Examples of non-ionic surfactants include, but are not limited to, poloaxamers, alkyl poly(ethylene oxide), diethylene glycol monohexyl ether, polyvinyl alcohol (PVA),

copolymers of poly(ethylene oxide) and poly(propylene oxide), hexaethylene glycol monohexadecyl ether, alkyl polyglucosides, digitonin, ethylene glycol monodecyl ether, cocamide MEA, cocamide DEA, cocamide TEA, fatty alcohols, and mixtures thereof. In various embodiments, the second surfactant comprises or consists essentially of polyvinyl alcohol (PVA). In one stated example, PVA was used to stabilize the double emulsion droplet in water.

[0074] The second surfactant may be present in any suitable amount that is able to stabilize the water-in-oil-in-water emulsion. In various embodiments, the amount of second surfactant in the aqueous solution into which the first emulsion is dispersed in is between about 0.01 % (w/v) to about 5 % (w/v), such as between about 1 % (w/v) to about 5 % (w/v), about 3 % (w/v) to about 5 % (w/v), about 0.1 % (w/v) to about 3 % (w/v), about 0.1 % (w/v) to about 1 % (w/v), about 2 % (w/v), about 1 % (w/v), or about 0.5 % (w/v). In various embodiments, the amount of second surfactant in the aqueous solution into which the first emulsion is dispersed in is about 0.5 % (w/v).

[0075] Besides the second surfactant, the aqueous solution into which the first emulsion is dispersed contains a reagent capable of effecting gelation of the hydrogel-forming agent. The reagent capable of effecting gelation of the hydrogel-forming agent may comprise or consist essentially of a salt. In various embodiments, the salt comprises a multivalent cation, such as ions of calcium, magnesium, aluminium, barium or strontium. The multivalent cations may crosslink the hydrogel-forming agent so as to effect gelation of the hydrogel-forming agent. In various embodiments, the salt comprises a divalent cation. Examples of divalent cations that may be comprised in the salt include, but are not limited to calcium and magnesium. The salt may dissolve in the aqueous solution, whereby the salt dissociates into its constituent metal cations and anions.

[0076] For example, in embodiments in which the hydrogel-forming agent comprises sodium alginate, the reagent capable of effecting gelation of the hydrogel-forming agent may comprise or consist essentially of calcium (II) chloride. When positive polyelectrolytes such as chitosan are used as the hydrogel-forming agent, the reagent capable of effecting gelation of the hydrogel-forming agent may comprise tri-polyphosphate ions.

[0077] The reagent capable of effecting gelation of the hydrogel-forming agent may be present in any amount that allows gelation of the hydrogel-forming agent. This may in turn depend on the type and amount of hydrogel-forming agent used.

[0078] For example, the concentration of reagent capable of effecting gelation of the hydrogel-forming agent in the aqueous solution into which the first emulsion is dispersed may be between about 10 mM to about 100 mM, such as between about 10 mM to about 80 mM, about 10 mM to about 50 mM, about 50 mM to about 100 mM, about 40 mM to about 60 mM, about 60 mM, about 50 mM, or about 40 mM. In various embodiments, the concentration of reagent capable of effecting gelation of the hydrogel-forming agent in the aqueous solution into which the first emulsion is dispersed is about 50 mM.

[0079] An osmolyte may also be present in the aqueous solution into which the first emulsion is dispersed. Examples of osmolyte have already been mentioned above. In various embodiments, the osmolyte comprises or consists essentially of sodium chloride. Even though in general, the same osmolyte is used in the first solution and in the aqueous solution into which the first emulsion is dispersed, it is not necessary that the two osmolytes are the same. In various embodiments, sodium chloride is used as osmolyte in both solutions.

[0080] Presence of an osmolyte in both the inner and outer water phases (W1 and W2) of the double emulsion may prevent leaching of hydrogel resulting in an inconsistent gel core formation, or formation of inhomogeneous hydrogel structures due to hydrogel partitioning effects that may be observed in confined low volumes of the hydrogel, such as the microparticles in the present case. The osmolyte may function to regulate the osmotic pressure between both aqueous phases of the double emulsion, which may at the same time, reduce hydrogel leaching. Furthermore, use of an osmolyte in the external phase is also advantageous as it may act as a non gelling ion in increasing the homogeneity of the hydrogel formed.

[0081] The concentration of osmolyte in the aqueous solution into which the first emulsion is dispersed may be between about 0.2 M to about 2 M, such as between about 0.8 M to about 1.8 M, about 0.4 M to about 1 M, or about 0.2 M to about 0.8 M. In various embodiments, the concentration of osmolyte in the aqueous solution is about 0.6 M.

[0082] After the second emulsion is formed, the method of the first aspect includes extracting the organic solvent from the second emulsion. Extracting the organic solvent is carried out in a way such that the reagent capable of effecting gelation of the hydrogel-forming agent penetrates through the second solution and effects gelation of the hydrogel-forming agent to form hydrogel microparticles.

[0083] In various embodiments, gelation of the hydrogel-forming agent to form hydrogel microparticles is carried out via ionic gelation.

[0084] Depending on the type of hydrogel-forming agent used, for example, the hydrogel-forming agent comprised in the W1 phase of the second emulsion may be physically or chemically crosslinked by the reagent capable of effecting gelation of the hydrogel-forming agent to form the hydrogel material. For example, when sodium alginate is used as the hydrogel-forming agent, the reagent capable of effecting gelation of the hydrogel-forming agent may comprise a multivalent salt, such as those mentioned above. In various embodiments, the multivalent cation is selected from the group consisting of calcium, magnesium, aluminium, barium and strontium. An illustrative example of a salt containing calcium ions is calcium (II) chloride. The calcium chloride may dissociate in the external aqueous phase (W2) into calcium ions and chloride ions. The calcium ions may penetrate through the second solution present as the O phase comprised in the W1/O droplets into the dissolved alginate in the first solution present as the W1 phase in the W1/O droplets, and effect gelation of the dissolved alginate to form the hydrogel microparticles.

[0085] The mean diameter of the hydrogel microparticles formed, which constitute a component or core of the resulting core-shell microparticles, may be between about 100 μm to about 800 μm , such as between about 500 μm to about 800 μm , about 400 μm to about 600 μm , or about 250 μm to about 750 μm . In various embodiments, the hydrogel microparticles formed are essentially monodisperse.

[0086] Besides forming the hydrogel microparticles, extracting the organic solvent from the second emulsion is also carried out such that the hydrophobic polymer precipitates to form a shell around each of the hydrogel microparticles. In various embodiments, extracting of the organic solvent from the second emulsion takes place by solvent evaporation. Formation of the hydrogel microparticles and precipitation of the hydrophobic polymer shell around each of the hydrogel microparticles may take place concurrently. The rate at which the hydrogel microparticles and the polymer shell are formed may be the same or different. In some embodiments, formation of the hydrogel microparticles takes place at a faster rate than precipitation of the hydrophobic polymer shell around each of the hydrogel microparticles.

[0087] When the organic solvent is removed from the emulsion droplets dispersed in the aqueous solution, the concentration of hydrophobic polymer in the emulsion droplets increases until a point at which the polymer and organic solvent may phase separate, i.e.

coacervate forming a first coacervate phase within the emulsion droplet. The first coacervate phase comprising the hydrophobic polymer may coacervate to form a layer on the hydrogel microparticles. The polymer precipitation rate may take place at an appropriate speed that is sufficient to allow time for the coacervate droplets to move into and coalesce with their
5 respective phases. The process may continue until residual solvent is removed, after which the core-shell microparticles are formed.

[0088] By forming the core-shell microparticles using a method of the first aspect, there is improved control over the homogeneity of hydrogel localized in each particle, since emulsification of the first solution containing a hydrogel-forming agent into the second
10 solution comprising a hydrophobic polymer is not subjected to any gravitational sinking effects that may be formed from other processes involving formation of the hydrogel microparticles prior to coating of a polymer layer on the microparticles. In embodiments whereby the microparticles are used for drug delivery applications, for example, this in turn translates into improved consistency in drug loading in each particle.

[0089] The thickness of the hydrophobic polymer shell formed may be in the range of between about 50 μm to about 200 μm , such as between about 50 μm to about 150 μm , about
15 50 μm to about 100 μm , about 75 μm to about 150 μm , about 100 μm to about 200 μm , or about 125 μm to about 150 μm .

[0090] Dispersing of the first emulsion into the aqueous solution comprising the second
20 surfactant and the reagent capable of effecting gelation of the hydrogel-forming agent may be carried out under any suitable dispersing method that is able to result in formation of a double emulsion. Examples of dispersing methods include, but are not limited to, continuous stirring, ultrasonic emulsification and homogenization using a homogenizer.

[0091] In various embodiments, dispersing of the first emulsion into the aqueous solution
25 comprising the second surfactant and the reagent capable of effecting gelation of the hydrogel-forming agent is carried out under continuous stirring. Advantageously, use of continuous stirring allows size of microparticles formed to be controlled simply by varying the speed of stirring. Generally, a lower stirring speed results in a larger emulsion droplet size, and may translate into an increase in size of the microparticles formed. On the other hand, a
30 higher stirring speed may result in a smaller microparticle. This may in turn translate into formation of smaller sized core-shell microparticles. Accordingly, speed of stirring may be used to affect the size of microparticles formed. Stirring speed as used herein may have a

range of between about 150 rpm to about 2000 rpm, such as between about 500 rpm to about 2000 rpm, about 1000 rpm to about 2000 rpm, about 150 rpm to about 1500 rpm, about 150 rpm to about 1000 rpm, about 200 rpm to about 800 rpm, about 300 rpm to about 500 rpm, about 300 rpm, about 400 rpm, or about 500 rpm. In various embodiments, continuous stirring is carried out at a speed of about 300 rpm to about 2000 rpm. In some embodiments, continuous stirring is carried out at a speed of about 400 rpm. Generally, depending on the type of materials used to form the first solution and the second solution, for example, a lower stirring speed than 150 rpm may result in an insufficient shear force for forming the emulsion droplets.

[0092] Continuous stirring may be carried out for any suitable amount of time that is necessary to form the core-shell microparticles. For example, the continuous stirring may be carried out for a time period of between about 1 hour to about 12 hours, such as between about 1 hour to about 8 hours, about 1 hour to about 6 hours, about 6 hours to about 12 hours, about 3 hours to about 6 hours, about 5 hours, about 4 hours or about 3 hours. In various embodiments, the continuous stirring is carried out for about 3 hours.

[0093] The method according to the first aspect may further comprise adding a hydrophilic active compound to be encapsulated to the hydrogel-forming agent.

[0094] The term “hydrophilic”, in contrast to the term “hydrophobic”, is generally used to describe a substance that has a high affinity for water. For example, a hydrophilic material may be one that is able to be dissolved in, be mixed with or be wetted by water. The term “hydrophilic active compound” as used herein refers to a hydrophilic compound which is intended to be delivered or released. The hydrophilic active compound may be encapsulated in the core-shell microparticles.

[0095] In various embodiments, the hydrophilic active compound is added to the hydrogel-forming agent, in which it dissolves and is thereby encapsulated within the core of the microparticles. Advantageously, this allows incorporating hydrophilic active compound into the hydrogel-forming agent in a single step process, which translates into processing simplicity and efficiency.

[0096] One or more different types of hydrophilic active compound may be added. The type of interaction between the hydrophilic active compound and the hydrogel-forming agent may be physical or chemical in nature. In various embodiments, one or more hydrophilic active compounds may be loaded in the hydrogel-forming agent via physical bonding, for

example, by any one of hydrophobic forces, hydrogen bonding, van der Waals interaction, or electrostatic forces. In various embodiments, one or more hydrophilic active compounds may be loaded in the hydrogel-forming agent via chemical bonding, for example, by covalent bonding, ionic bonding, or affinity interactions (e.g. ligand/receptor interactions, antibody/antigen interactions, etc.). Therefore, one or more types of hydrophilic active compound may be loaded and localized in the core of the microparticle by choosing the appropriate type of interaction between the hydrophilic active compound and the hydrogel-forming agent. This may in turn be exploited for controlled release of the hydrophilic active compound in application.

[0097] Examples of hydrophilic active compound that may be encapsulated include, but are not limited to, a drug, a protein, an enzyme, an antibody, a peptide, a growth factor, an organic molecule, a nucleic acid, a cell, a pesticide, a dye, a chemical indicator and a fertilizer.

[0098] The term "drug" refers to a substance useful for the treatment of or the prevention of a human or an animal disorder or in the regulation of a human or animal physiological condition or metabolic state. Examples of drug include, but are not limited to, antihistamines, e.g. diphenhydramine and chlorpheniramine, and drugs affecting the cardiovascular, renal, hepatic and immune systems, such as antihypertensives, beta blockers, and cholesterol lowering agents; sympathomimetic drugs, such as the catecholamines, e.g. epinephrines; noncatecholamines, e.g. phenylephrine and pseudoephedrine; anti-infective agents, including antibacterial, antiviral and antifungal agents, such as the aminoglycosides, e.g., streptomycin, gentamicin, kanamycin; anti-arthritis drugs, such as narcotic pain relievers; anti-inflammatory agents, e.g. indomethacin, dexamethasone and triamcinolone; and antitumor agents, e.g. 5-fluorouracil and methotrexate; tranquilizers, such as diazepam.

[0099] In various embodiments, the hydrophilic active compound comprises or consists essentially of a drug. For example, the drug may be a vaccine, a protein, an inorganic molecule, or mixtures thereof. Examples of some common pharmaceuticals/drugs include, but are not limited to, atorvastatin, clopidogrel, enoxaparin, celecoxib, omeprazole, esomeprazole, fexofenadine, quetiapine, metoprolol and budesonide. Encapsulation of vaccines, such as group B Streptococcus vaccine (GBS), tetanus toxoid (TT), Japanese encephalitis virus (JEV), diphtheria toxoid (DT), vibrio cholerae (VC), SPF 66 malaria vaccine, multivalent vaccines of Haemophilus influenza type b (Hib), pertussis toxin (PT),

Rotavirus to name only a few, is also possible. In various embodiments, the drug comprises or consists essentially of metoclopramide HCl.

[00100] Proteins that may be used include, but not limited to, pharmaceutically active ingredients such as hormones, insulins, enzymes, antibodies, and growth factors. The term "growth factors" refers to factors affecting the function of cells such as osteogenic cells, fibroblasts, neural cells, endothelial cells, epithelial cells, keratinocytes, chondrocytes, myocytes, cells from joint ligaments, and cells from the nucleus pulposus. The term "nucleic acid" refers to any nucleic acid in any possible configuration, such as single stranded, double stranded or a combination thereof. Nucleic acids include for instance DNA molecules, RNA molecules, locked nucleic acid molecules (LNA), PNA molecules, analogues of the DNA or RNA generated using nucleotide analogues or using nucleic acid chemistry, DNA-RNA hybrid molecules and tecto-RNA molecules

[00101] Other hydrophilic active compounds include, for example other pharmaceutical substances, such as a fertilizer, an insecticide or a pesticide, a chemical indicator (such as a pH indicator) or a dye, such as an azo dye or leuco dye, may be encapsulated in the microparticle.

[00102] Insecticides can be selected from the group of chlorinated hydrocarbons, such as Endosulfan and Aldrin, organophosphates, such as Acephate and Malthion, carbamates, such as Aldicarb and 2-(1-Methylpropyl)phenyl methylcarbamate, phenothiazine, pyrethroids, such as Allethrin and Tralomethrin, neonicotinoids, such as Acetamiprid and Nithiazine, plant derived compounds, such as caffeine, Anabasine, Linalool and Pyrethrum.

[00103] Fertilizer are chemical compounds given to plants to promote growth and can include inorganic fertilizer, such as sodium nitrate, or organic fertilizer, such as manure and sewage.

[00104] As an illustrative example, a drug such as metoclopramide HCl (MCA) may be loaded to a core-shell PLGA/calcium alginate microparticle. During the fabrication process, the hydrophilic nature of MCA allows localization of the drug in the aqueous inner phase (W1) of the double emulsion, and which forms the core of the core-shell microparticle.

[00105] The method of the first aspect may further comprise at least one centrifugation and at least one washing step after extracting the organic solvent. In various embodiments, the method of the first aspect may further comprise lyophilizing the core-shell microparticles formed in the method.

[00106] Lyophilization also increases the storage stability of active compounds, such as proteins. Freeze drying itself exposes the active compound to destabilizing stresses, therefore suitable excipients and stabilizing additives are included in formulation for stability during freeze drying. Lyoprotectants such as dextran, glycols, glycerol and cyclodextrins may be used to minimize instability in some freeze-dried formulations.

[00107] The microparticles formed assume a core-shell structure with a hydrophobic polymer shell surrounding the hydrogel microparticles. In various embodiments, the core-shell microparticles are at least substantially spherical in shape. The core-shell microparticles may have a mean diameter of between about 150 μm to about 1000 μm , such as about 150 μm to about 800 μm , about 300 μm to about 500 μm , about 600 μm to about 1000 μm , or about 500 μm to about 800 μm .

[00108] In a second aspect, the invention is directed to core-shell microparticles manufactured by a method according to the first aspect.

[00109] For a core-shell microparticle comprising a hydrophilic active compound, the core-shell microparticle may be degraded at a site of intended usage to release the hydrophilic active compound encapsulated therein. The term "degrade" as used herein refers to breaking down of the microparticle to smaller molecules. Different parts of the microparticle may degrade at different rates or at substantially uniform rates. This may depend on the environment the microparticles are placed in, and the conditions at which the microparticles are subjected to. For example, the shell of the microparticles may degrade at a faster rate compared to the core due to its proximity with the external environment. Degradation may take place under certain conditions, such as temperature, abrasion, pH, ionic strength, electrical voltage, current effects, radiation and biological means. In some embodiments, degradation of the microparticle takes place over a time period ranging from a few seconds to a few days or months. The time period required for the microparticle to degrade may be dependent on a few parameters, for example, constituent of the microparticles, such as type of polymer and hydrophilic active compound used, size of the microparticles, temperature, pH and pressure. In various embodiments, the core-shell microparticles formed using a method of the first aspect are used for sustained release of a hydrophilic active compound encapsulated therein.

[00110] Using a method of the first aspect, the core-shell microparticles prepared may enhance loading efficiency and control release kinetics of hydrophilic and amphiphilic drugs,

which would otherwise be difficult to achieve for a purely hydrophobic polymer based drug delivery system, such as PLGA or PLLA particulate drug delivery system.

[00111] Examples of applications in which the core-shell microparticles may be used include, but are not limited to, a drug delivery system (DDS) for delivery of drugs, proteins, peptides, DNA, cells, dyes and other biomedical-applied systems, and therapeutic agents.

[00112] In a third aspect, the invention refers to a core-shell microparticle having a core comprising a hydrogel material, and a shell comprising a hydrophobic polymer immiscible with the hydrogel material, wherein the shell is free of the hydrogel material of the core.

[00113] The core-shell microparticle according to the third aspect has a core that may comprise, or consist essentially of a hydrogel material. The core may be in the form of a single hydrogel microparticle. Examples of the hydrogel material that may be comprised in the core of the core-shell microparticle have already been described above. In various embodiments, the core comprises or consists essentially of calcium alginate or magnesium alginate.

[00114] The diameter of the core may be between about 100 μm to about 800 μm , such as between about 500 μm to about 800 μm , about 400 μm to about 600 μm , or about 250 μm to about 750 μm .

[00115] The core of the core-shell microparticle is encapsulated by a hydrophobic polymer, which forms the shell of the core-shell microparticle. The shell of the core-shell microparticle is free of the hydrogel material of the core. In other words, the hydrogel material that is contained in the core is not present in the shell of the core-shell microparticle. Advantageously, this allows customization of the core-shell microparticles, for example, in varying the thickness of the hydrophobic polymer shell or the size of the hydrogel material, to achieve different release profiles and release rates in applications such as drug delivery. In particular, such a core-shell microparticle configuration, in which the shell of the core-shell microparticle is free of the hydrogel material of the core, translates into improved control over drug release, as compared to a non core-shell microparticle or a core-shell microparticle having a shell that contains a hydrogel material.

[00116] In various embodiments, the shell consists of the hydrophobic polymer. In some embodiments, the core and the shell of the core-shell microparticle are formed of distinctly different material, in that the core is formed entirely of a hydrogel material and the shell is formed entirely of a hydrophobic polymer. Examples of suitable hydrophobic polymers have

already been described above. In various embodiments, the hydrophobic polymer may comprise or consist essentially of poly-lactic-co-glycolic acid (PLGA), poly-L-lactide (PLLA), poly-caprolactone (PCL), polyglycolide (PGA), derivatives thereof, copolymers thereof, and mixtures thereof.

5 [00117] The choice of hydrophobic polymer may depend on the intended application. For example, in drug delivery applications, the hydrophobic polymer may be a biodegradable polymer or a biocompatible polymer.

[00118] The core-shell microparticle may further comprise a hydrophilic active compound, which may be present in the core of the microparticle. Examples of suitable hydrophilic
10 active compounds that may be used have already been described above. In various embodiments, the hydrophilic active compound comprises or consists essentially of a drug. For example, the drug may be a vaccine, a protein, an inorganic molecule, or mixtures thereof. In one embodiment, the drug comprises or consists essentially of metoclopramide HCl.

[00119] In a fourth aspect, the invention refers to a pharmaceutical composition comprising
15 core-shell microparticles manufactured by a method according to the first aspect, or according to the third aspect.

[00120] In various embodiments, the composition may be poured or injected into a mold having a desired shape, and then hardened to form a matrix having microparticles dispersed therein. The core comprising the hydrogel material and the shell comprising the hydrophobic
20 polymer immiscible with the hydrogel material may degrade, leaving only the active compound. Therefore, a sustained release of the target substance such as fertilizer or pesticide may be achieved along with degradation of the microparticles. In some embodiments, the composition is adapted to be deliverable to a site, such as a defect site, in an animal or a human body. The composition may be injected directly into a site, such as a defect site, in a
25 patient, where the polymer may harden into a matrix having microparticles dispersed therein. The polymer may be biodegradable. Therefore, a sustained release of the active compound, such as drugs, may be achieved along with degradation of the polymer and the hydrogel material.

[00121] The terminology used herein is for the purpose of describing particular
30 embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises"

and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

5 [00122] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed
10 herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may
15 be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00123] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or
20 negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00124] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of
25 any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[00125] **Example 1: Materials**

[00126] Poly(DL-lactide/glycolide) 50:50 (Intrinsic viscosity (IV) 1.03), Poly(L-Lactide)
30 (IV 2.4) were obtained from Purac Biomaterials; Alginic acid sodium salt, from brown algae; Span® 80, Poly(vinyl alcohol) (PVA) (molecular weight 30-70 kDa); trisodium citrate; trifluoroacetic acid; calcium chloride and metoclopramide HCl (MCA) were obtained from

Sigma-Aldrich. High performance liquid chromatography (HPLC) grade dichloromethane (DCM) and acetonitrile were from Tedia Co Ltd. Sodium chloride (NaCl) was obtained from J.T.Baker Ltd. Phosphate Buffer Saline (PBS) (pH 7.4) was obtained from OHME Scientific Pte Ltd Singapore. All items were used as received.

5 [00127] **Example 2: Fabrication of microparticles**

[00128] To fabricate Alg-PLGA MP with a core-shell structure, a double emulsion based solvent evaporation method was used. Microparticles of different types were synthesized by preparing polymer solutions, as tabulated in **Table 1**. Essentially, two different polymer solutions were separately prepared, one of which was a 10 % (w/v) PLGA solution prepared
10 by dissolving 400 mg of PLGA in DCM. Span 80 (1 % (w/v)) was also added in the PLGA/DCM solution to stabilize the primary water-in-oil emulsion. The other polymer solution prepared was a 4.5 % (w/v) sodium alginate (NaAlg) aqueous solution, made by dissolving 45 mg of sodium alginate in water. Sodium chloride (1.35M) was also added as an osmolyte for the double emulsion. For drug-loaded microparticles, MCA was dissolved
15 within the internal water phase for a theoretical drug loading of 20 % (w/w).

[00129] An overview schematic of the fabrication process according to an embodiment is shown in **FIG. 1**. The alginate solution was first emulsified in the PLGA/DCM solution under magnetic stirring for the formation of the primary water-in-oil (W/O) emulsion. This W/O emulsion was then further dispersed into a 100 ml aqueous solution of 0.5 % (w/v)
20 PVA, 50 mM CaCl_2 and 0.6M NaCl to form a double water-oil-water (W/O/W) emulsion, with an overhead stirrer (Calframo BDC1850-220). The stirrer was operated at 400 rpm for 3 hours to concurrently initiate the extraction of DCM and ionotropic gelation of sodium alginate. The resultant microparticles were then recovered via centrifugation, rinsed with deionised water, lyophilized and then stored in a dessicator for characterization.

25 [00130] **Table 1** Contents dissolved in different phases for the different microparticles fabricated

Fabricated microparticles	Oil Phase	Internal Aqueous Phase (W1)		External Aqueous Phase (W2)
	Polymer Type	NaCl	CaCl_2	NaCl
Alg-PLGA MP	PLGA	1.35 M	50 mM	0.6 M
Alg-PLLA MP	PLLA	1.35 M	50 mM	0.6 M

MCA-loaded Alg-PLGA MP	PLGA	-	50 mM	-
MCA-loaded Alg-PLLA MP	PLLA	-	50 mM	-
MCA-loaded neat-PLGA MP	PLGA	-	-	-
MCA-loaded neat-PLLA MP	PLLA	-	-	-

[00131] Monolithic MCA-loaded neat PLGA and PLLA microparticles were also fabricated as a reference to MCA-loaded Alg-Polymer core-shell microparticles. Sodium alginate was not included in the preparation of the internal aqueous phase.

5 [00132] **Example 3: Formation of Calcium Alginate (CaAlg) beads**

[00133] CaAlg beads were fabricated as a control sample for comparison with Alg-PLGA MP. The beads were formed by extruding 4 ml of 4.5 % (w/v) NaAlg solution through a syringe needle into a 5 ml cross-linking bath of 50 mM CaCl₂ and 0.6 M NaCl (i.e. same concentration for salts used in the external phase when fabricating neat Alg-PLGA MP).

10 Beads were then left to gel in the solution for 3 hours before being lyophilized and used for Fourier Transform Infra-red (FT-IR) characterization. MCA-loaded CaAlg beads were similarly formed, with a 4 ml 4.5 % (w/v) alginate solution dissolved with 90 mg MCA prior to extrusion into a 5 ml 50 mM CaCl₂ cross linking solution. Drug-loaded beads were stored in deionised water at 4 °C.

15 [00134] **Example 4: Characterization**

[00135] **Example 4.1: Scanning Electron Microscopy (SEM)**

[00136] The cross-sectional morphologies of microparticles fabricated were analyzed with a JEOL 6360A scanning electron microscope. Microparticles prior to imaging were mounted on carbon tape then immersed into liquid nitrogen for a brief moment, before incisions were made to cross-section the microparticles with a surgical blade. For the purpose of visually distinguishing the CaAlg core from the external shell, microparticles imaged after cross-sectioning were immersed in 0.1 M trisodium citrate overnight in order to dissolve the alginate core. This was achieved by the sequestering of calcium ions by citrate ions, resulting in hydrogel dissolution. The microparticles were then rinsed with deionised water, dried in air
25 before being imaged again using the SEM.

[00137] Example 4.2: Fourier Transform Infra-red (FT-IR) Microscopy

[00138] To facilitate visual identification of the different chemical components in the Alg-PLGA MP formed, a Thermo Electron Nicolet Continuum FT-IR microscope was used. The core and shell of the cross-sectioned particle were separated from each other using tweezers and placed on a gold coated glass slide. FT- IR spectra were then collected using the FTIR microscope in reflectance mode, from 4000 cm^{-1} to 650 cm^{-1} . FT-IR spectra of CaAlg beads and raw PLGA were also captured for comparison with the spectra of the microparticle core and shell.

[00139] Example 4.3: Raman Mapping

[00140] Raman mapping was used to determine the polymer and drug distribution within the microparticles. Microparticles were first cross-sectioned as described above for SEM imaging, after which is then placed under a microscope objective of a laser power of up to approximately 20 mW. Raman measurements were performed on an area of 400 x 200 μm with a step size interval of 5 μm to form a grid map, using an In-Via Reflex, Renishaw Raman microscope equipped with a near infrared enhanced deep depleted thermoelectrically Peltier-cooled CCD detector array (576 x 38 g pixels) and a high grade Leica microscope. The sample was irradiated with a 785 nm near infrared diode laser, and the back scattered light was collected by a 20 x objective. Measurement scans were collected through a static 1800- groove/mm dispersive grating from 300-1900 cm^{-1} and each spectrum acquisition time for each was approximately 35 s.

[00141] Spectral pre-processing, including the removal of spikes due to cosmic rays, was carried out before the collected Raman spectra were subjected to the band target entropy minimization (BTEM) algorithm analysis. The BTEM algorithm was used to reconstruct pure component spectral estimates. When the entire normalized pure component spectra of underlying constituents had been reconstructed, the relative contributions of each measured point of these signals was calculated by projecting them back onto the baseline corrected and normalized data set. The colour-coded scale represents the intensities of each component recovered as a score image, in which the summation of the intensities (colour-coded scale) of all components at each particular grid pixel is equal to unity. These score images are then used to show the spatial distribution and the semi-quantitative content for all observed component in the microparticles.

[00142] Example 4.4: Encapsulation Efficiency

[00143] Actual drug loading and encapsulation efficiency of the microparticles fabricated were defined below:

[00144] Actual Drug Loading % (w/w) = 100 % x (Mass of drug loaded / Total polymer mass)

5 [00145] Encapsulation Efficiency (%) = 100 % x (Actual Drug Loading / Theoretical Drug Loading)

[00146] To calculate the actual drug loading of the microparticles, approximately 5 mg of microparticles were weighed and digested in 1 M NaOH by ultrasonication, for 10 min in an 80 °C water bath. The resultant solution was then neutralized with 1M HCl and filtered. The
10 drug concentration of the solution was measured using a reverse phase high performance liquid chromatography (HPLC) method, using the Agilent 1100 HPLC system with a XDB-C18 column,. The analysis was done in gradient mode, with a varied proportion of 0.1 % (v/v) trifluoroacetic acid and acetonitrile as the mobile phase. The amount of MCA was quantified using a detection wavelength of 309 nm at room temperature.

15 [00147] Example 4.5: Drug release study

[00148] Microparticles (5 mg) were immersed in vials of 5 ml PBS each in triplicate, placed in a shaking incubator operating at 37 °C. At pre-determined time intervals, 1 ml of the release medium was extracted from the vial and analyzed for concentration of MCA released. A UV-VIS Spectrophotometer (Shimadzu UV- 2501) was used to measure the
20 MCA concentration, at a detection wavelength of 309 nm. The vials were then replenished with fresh PBS of the same volume.

[00149] Example 5: Results for Non drug-loaded Alg-PLGA MP and Alg-PLLA MP

[00150] The SEM cross-sectional view of an Alg-PLGA MP is shown in **FIG. 2A**. Cross-sectioned microparticles revealed a core and a shell within a particle. After immersion in trisodium citrate, the same cross-sectioned particle was again viewed under the SEM,
25 whereby this time the core portion was shown to have dissolved (**FIG. 2B**). This suggests that the layer removed was calcium alginate, as the citrate ion is known to be a chelating agent that can sequester Ca^{2+} ions from calcium alginate gel, causing the dissolution of the gel structure.

30 [00151] **FIG. 3A** shows the respective IR spectra of the microparticle shell and core, with the representative spectra of pure PLGA and CaAlg for comparison. The spectrum of the core was different from the shell, with the presence of a strong broad peak at around 3500-3000

cm⁻¹ arising from the core. This is characteristic of the O-H stretching of repeat -COOH and -OH group units on the alginate polymer chain. In addition, a COO⁻ stretching absorption peak was also observed at around 1608 cm⁻¹ for the alginate core. The doublet peak observed between 1790-1720 cm⁻¹ for the shell, on the other hand, is representative of the C=O stretch of the lactide and glycolide groups arising from PLGA. In essence, the IR spectra of the core and shell of the microparticle matches with the reference spectra of alginate and PLGA, respectively. In **FIG. 3B**, Raman mapping of a cross-sectioned microparticle and its associated pure component BTEM spectra estimates are shown, indicating the localization of each polymeric component. The core of the cross-sectioned microparticle again proved consistent to be alginate, which can be visually distinguished from the surrounding PLGA shell. This is in agreement with the IR analysis and citrate dissolution test, concluding that the formed microparticle has a core-shell morphology of alginate and PLGA, respectively.

[00152] Similar to Alg-PLGA MP, Alg-PLLA MP exhibited a core-shell structure, as shown in **FIG. 4A**. Similarly, the alginate core was removed when cross-sectioned particles were immersed in citrate solution (**FIG. 4B**). This is in concordance to the Raman mapping analysis in **FIG. 5**, whereby the core and shell were verified to be alginate and PLLA, respectively. Carbon was also detected, as the particles were mounted on carbon tape during Raman mapping.

[00153] **Example 6: Results for MCA-loaded Alg-PLGA MP and Alg-PLLA MP**

[00154] To fabricate MCA-loaded microparticles, MCA was dissolved in the inner aqueous alginate phase prior to emulsification during the fabrication of drug-loaded Alg-PLGA MP. Similarly, a core-shell structure was obtained (**FIG. 6A**), and Raman mapping showed a strong MCA signal from the core and PLGA in the shell (**FIG. 7**). This time, the Raman spectrum of alginate was however not recovered due to a greater Raman scattering from the MCA component, hence resulting in a relatively stronger signal that may have overshadowed any alginate present. Citrate treatment of this set of MCA-loaded microparticles further confirmed that the core was CaAlg (**FIG. 6B**). This same core-shell structure was also reproduced when PLGA was replaced with PLLA to give MCA-loaded Alg-PLLA MP (see **FIG. 10**).

[00155] **Table 2** Comparison of Encapsulation Efficiencies (%) of MCA loaded microparticles of various polymer types and configuration

MCA Encapsulation Efficiency (%)		
Shell Polymer / Microparticle Type	Monolithic matrix	Alginate core + Polymeric shell
PLGA	30.1 ± 2.40	59.96 ± 13.82
PLLA	32.26 ± 1.99	66.54 ± 2.23

[00156] **Table 2** compares the encapsulation efficiency of MCA in PLGA and PLLA microparticles, fabricated with and without the inclusion of alginate. The encapsulation efficiency of MCA in Alg-PLGA MP and Alg-PLLA MP was almost double of neat PLGA and PLLA microparticles. This indicates that the incorporation of a hydrophilic polymer can significantly improve loading and encapsulation of water soluble drugs.

[00157] Release profile for the Alg-PLGA MP, Alg-PLLA MP and CaAlg beads are shown in **FIG. 8**. Pure CaAlg beads showed a complete drug release within 6 hours, while both Alg-PLGA MP and Alg-PLLA MP exhibited a suppression of the initial burst release across the first 24 hours, followed by a sustained release of MCA up to 4 and 7 days, respectively. In addition, it was observed that Alg-PLLA MP exhibited a more sustained release as compared to Alg-PLGA MP.

[00158] **Example 7: Discussion**

[00159] Alg-PLGA MP was fabricated through a W/O/W double emulsion solvent evaporation based technique according to an embodiment, as shown in **FIG. 1**. This involves the formation of a primary W/O emulsion, obtained by emulsifying an aqueous solution containing NaAlg and NaCl into an oil phase (i.e. PLGA dissolved in DCM).

[00160] The W/O emulsion was then subsequently dispersed into an external water phase with PVA, CaCl_2 and NaCl dissolved. To achieve a stable W/O/W double emulsion, two different surfactants were used to disperse the primary W/O emulsion and the secondary double emulsion droplets in the external water phase. In this case, a hydrophobic Span® 80 surfactant and hydrophilic PVA surfactant was selected respectively to achieve this purpose. This subsequently forms the secondary double emulsion, as depicted in **FIG. 9**, and initiates two concurrent processes resulting in the formation of the core-shell microparticle. One is the extraction of DCM solvent from the double emulsion droplet, which resulted in the precipitation of PLGA, and leading to the formation of PLGA shell. The second concurrent process is the ionotropic gelation of the aqueous NaAlg phase within the inner aqueous droplet. Alginate can be gelled, i.e. physically cross-linked in the presence of divalent ions

such as Ca^{2+} to form CaAlg hydrogels. The influx of Ca^{2+} ions from the external water phase into the internal alginate phase in this process causes the gelation of the dissolved alginate. Hence, microparticles can be formed with an alginate-PLGA core-shell structure, whereby water insoluble CaAlg core is gelled in situ within the hardened PLGA shell via this single
5 step synthesis technique.

[00161] The presence of large osmotic pressure differences between the two aqueous phases in the double emulsion has an effect on the stability of the W/O/W emulsion, which can effect a large movement of water between both phases. This can rupture the DCM/PLGA oil layer, causing the double emulsion droplets to break. Preliminary studies showed that
10 microparticles fabricated without NaCl in the internal aqueous phase resulted in a large number of ruptured microparticles such as that shown in **FIG. 11**. At the same time, the contents of the internal aqueous phase (i.e. alginate and MCA) can also leach into the external phase due to molecular migration. Leaching of alginate may result in an inconsistent gel core formation or formation of inhomogeneous alginate gel structures due to alginate
15 partitioning effects seen in confined low volumes of alginate. To overcome these issues, NaCl, as an osmolyte, was therefore dissolved in both inner and outer water phase of the double emulsion. The intent was to regulate the osmotic pressure between both aqueous phases of the double emulsion, while at the same time, reduce alginate leaching. The use of NaCl in the external phase also acts as a non gelling ion in increasing the homogeneity of the
20 gel formed.

[00162] Interestingly, when MCA was loaded into the microparticles, NaCl was no longer required in the formulation. Given that alginate can form strong complexes with poly-cations such as chitosan and poly-L-lysine, it is deduced that the dissolved MCA in drug-loaded formulations could also form a similar complex with alginate in the same way poly-cations
25 do, possibly due to the presence of amine/amide groups in the drug. Such interactions could possibly slow down and disrupt the coupled diffusion of sodium alginate polymer to the gelling front, resulting in a reduced partitioning rate of alginate and leading to the formation of a more homogeneous gel. At the same time, the hydration of MCA in the internal water phase may also have resulted in an increased osmotic pressure in the double emulsion. As
30 such, this would reduce the alginate leaching and increase the retention of alginate within the double emulsion droplet. A drug like MCA can therefore replace NaCl, as an osmotic agent, in achieving drug-loaded Alg-PLGA MP.

[00163] In terms of drug loading efficiency, an overall increased loading of MCA was observed for the core-shell MP as compared to the monolithic polymer particles. Given that the encapsulation efficiency is affected by the drug loss to the external phase in emulsion solvent evaporation methods, the presence of a hydrophilic polymer like alginate can better
5 associate with water soluble drugs, as compared to a more hydrophobic PLGA. This would subsequently increase retention of drugs within the emulsion droplets and reduce out-flux of water soluble drugs during fabrication.

[00164] Compared to naked CaAlg beads that exhibited complete burst release within one day, both Alg-PLGA MP and Alg-PLLA MP demonstrated sustained release over 4 and 7
10 days respectively (**FIG. 8**). This reduced burst was due to the shell of the microparticles serving as a protective encapsulating envelope, which acts as a membrane to regulate the release of drugs. While hydrogels are known to release its contents rapidly, the enveloping PLGA or PLLA shell limits the rate of water influx and act as a rate-limiting membrane for drug diffusion. The use of a more crystalline and hydrophobic polymer such as PLLA as a
15 shell can further reduce the release rate, by inhibiting movement of drugs through this polymer to a greater extent, as compared to amorphous PLGA. Hence, it is envisaged that release kinetics of these core-shell microparticles can be regulated and tailored through appropriate selection of polymer as the shell material.

[00165] With improved drug loading and the reduced burst release from core-shell alginate-
20 PLGA/PLLA microparticles as compared to CaAlg, these findings would pave a way towards protein encapsulation using the same particulate architecture.

[00166] **Example 8: Sustained Drug Release Capability of APMS**

[00167] In terms of controlled release of a model water soluble drug, Metoclopramide HCl (MCA), fabricated core-shell alginate Poly-L-Lactide shell microparticles showed sustained
25 release of the encapsulated MCA over a period of one week, as shown in the plot in **FIG. 12**. This is in contrast to naked calcium alginate beads where release of the same drug was complete within a single day.

[00168] This demonstrates the advantages of a hydrophobic polymer shell encapsulating an inner hydrophilic polymer core, allowing control over the sustained release of a water soluble
30 drug over time.

[00169] **Table 3** summarizes the results from comparison of encapsulation efficiencies (%) of MCA loaded microparticles of various polymer types and configuration.

MCA Encapsulation Efficiency		
Shell Polymer / Microparticle Type	Monolithic matrix	Alginate core + Polymeric shell
PLGA	30.1 $\pm$ 2.40	59.96 $\pm$ 13.82
PLLA	32.26 $\pm$ 1.99	66.54 $\pm$ 2.23

[00170] As may be seen from the table, incorporation of alginate within PLGA or PLLA microparticles showed an increase in encapsulation efficiency of Metoclopramide HCl as compared to naked PLGA or PLLA microparticles.

5 [00171] It has been shown here that a hydrophobic encapsulating material such as PLGA or PLLA may be used to control the release of drugs encapsulated, thereby acting as a membrane to retard the release of Metoclopramide, as compared to naked unprotected alginate beads.

10 [00172] In addition, it is also shown that PLLA, which is more hydrophobic than PLGA, shows reduced release of MCA as compared to PLLA. This demonstrates that the shell properties can be tailored via the choice of polymers used to encapsulate the drug loaded alginate core.

15 [00173] Future work would entail investigation into the release behaviour and bioactivity of proteins from such microparticles. As such, this new particulate drug delivery platform would be beneficial in areas such as vaccines or peptide delivery.

[00174] In this study, a novel microparticulate drug delivery system was fabricated with an alginate hydrogel core and encapsulated by a biodegradable hydrophobic polymeric shell. These microparticles were fabricated through a single step method, via a concurrent emulsion solvent evaporation and ionotropic gelation processes. The incorporation of alginate within PLGA or PLLA were shown to increase encapsulation efficiency when MCA, a model hydrophilic drug was loaded in the particles and compared to PLGA and PLLA microparticles fabricated without alginate. The shell formed was able to serve as a physical barrier between the MCA-loaded hydrogel core and the PBS medium during release. Thus, these gel-core hydrophobic-shell microparticles would allow for the improved loading and release of water soluble drugs, and potentially for protein loading.

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[00175] While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

CLAIMS

1. A method of manufacturing core-shell microparticles having a core comprising a hydrogel material and a shell comprising a hydrophobic polymer immiscible with the hydrogel material, the method comprising:-
 - a) dissolving a hydrogel-forming agent in an aqueous solution comprising an osmolyte to form a first solution;
 - b) dissolving a hydrophobic polymer in an organic solvent comprising a first surfactant to form a second solution;
 - c) dispersing the first solution into the second solution to form a first emulsion;
 - d) dispersing the first emulsion into an aqueous solution comprising a second surfactant and a reagent capable of effecting gelation of the hydrogel-forming agent to form a second emulsion; and
 - e) extracting the organic solvent from the second emulsion, wherein the reagent capable of effecting gelation of the hydrogel-forming agent penetrates through the second solution and effects gelation of the hydrogel-forming agent to form hydrogel microparticles, and the hydrophobic polymer precipitates to form a shell around each of the hydrogel microparticles.
2. The method according to claim 1, wherein the hydrogel-forming agent comprises a physically cross-linkable polymer, a chemically cross-linkable polymer, or mixtures thereof.
3. The method according to claim 2, wherein the physically cross-linkable polymer is selected from the group consisting of alginate, pectin, furcellaran, carageenan, chitosan, derivatives thereof, copolymers thereof, and mixtures thereof.
4. The method according to claim 2, wherein the chemically cross-linkable polymer is selected from the group consisting of starch, gellan gum, dextran, hyaluronic acid, poly(ethylene oxides), polyphosphazenes, derivatives thereof, copolymers thereof, and mixtures thereof.

5. The method according to any one of claims 1 to 4, wherein the hydrogel-forming agent comprises or consists essentially of sodium alginate.
- 5 6. The method according to any one of claims 1 to 5, wherein the osmolyte is selected from the group consisting of sodium chloride, potassium chloride, sodium bromide, sodium citrate, sodium lactate, sodium hydroxide, sodium iodide, sodium carbonate, sodium hydrogen carbonate, sodium nitrate, sodium fluoride, sodium sulfate, potassium carbonate, potassium citrate, potassium lactate, potassium hydrogen
10 carbonate, potassium bromide, potassium hydroxide, potassium iodide, potassium nitrate, potassium sulfate, cesium chloride, rubidium chloride, lithium chloride, and mixtures thereof.
7. The method according to any one of claims 1 to 6, wherein the osmolyte comprises or
15 consists essentially of sodium chloride.
8. The method according to any one of claims 1 to 7, wherein the amount of hydrogel-forming agent in the first solution is between about 1 % (w/v) to about 10 % (w/v).
- 20 9. The method according to any one of claims 1 to 8, wherein the amount of hydrogel-forming agent in the first solution is about 4.5 % (w/v).
10. The method according to any one of claims 1 to 9, wherein the concentration of osmolyte in the first solution is between about 0.5 M to about 2 M.
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11. The method according to any one of claims 1 to 10, wherein the concentration of osmolyte in the first solution is about 1.35 M.
12. The method according to claim 1, wherein the hydrophobic polymer immiscible with
30 the hydrogel-forming material is selected from the group consisting of poly-lactic-co-glycolic acid (PLGA), poly-L-lactide (PLLA), poly-caprolactone (PCL), polyglycolide (PGA), derivatives thereof, copolymers thereof, and mixtures thereof.

13. The method according to any one of claims 1 to 12, wherein the hydrophobic polymer immiscible with the hydrogel-forming material is a biodegradable polymer or a biocompatible polymer.

14. The method according to any one of claims 1 to 13, wherein the organic solvent is selected from the group consisting of dichloromethane (DCM), dimethylformamide (DMF), tetrahydrofuran (THF), methyl ethyl ketone (MEK), chloroform, pentane, benzene, benzyl alcohol, carbon tetrachloride, ethyl acetate, acetone, acetonitrile, dimethyl sulfoxide, propylene carbonate, and mixtures thereof.

15. The method according to any one of claims 1 to 14, wherein the organic solvent comprises or consists essentially of dichloromethane.

16. The method according to any one of claims 1 to 15, wherein the first surfactant comprises or consists essentially of a hydrophobic surfactant.

17. The method according to claim 16, wherein the hydrophobic surfactant is selected from the group consisting of sorbitan ester, sorbitan monoester, sorbitan trioleate, sorbitan tristearate, sorbitan sesquioleate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, glycerol monooleate, glycerol monostearate, PEO/PPO copolymers, derivatives thereof, and mixtures thereof.

18. The method according to any one of claims 1 to 17, wherein the first surfactant comprises or consists essentially of sorbitan monooleate.

19. The method according to any one of claims 1 to 18, wherein the amount of hydrophobic polymer in the second solution is between about 1 % (w/v) to about 30 % (w/v).

20. The method according to any one of claims 1 to 19, wherein the amount of hydrophobic polymer in the second solution is about 10 % (w/v).
21. The method according to any one of claims 1 to 20, wherein the amount of first surfactant in the second solution is between about 0.1 % (w/v) to about 5 % (w/v).
22. The method according to any one of claims 1 to 21, wherein the amount of first surfactant in the second solution is about 1 % (w/v).
23. The method according to any one of claims 1 to 22, wherein dispersing the first solution into the second solution is carried out under continuous stirring.
24. The method according to any one of claims 1 to 23, wherein the second surfactant comprises or consists essentially of a hydrophilic surfactant.
25. The method according to claim 24, wherein the hydrophilic surfactant is selected from the group consisting of amphoteric surfactant, anionic surfactant, cationic surfactant, nonionic surfactant, and mixtures thereof.
26. The method according to claim 25, wherein the amphoteric surfactant is selected from the group consisting of dodecyl betaine, sodium 2,3-dimercaptopropanesulfonate monohydrate, dodecyl dimethylamine oxide, cocamidopropyl betaine, 3-[N,N-dimethyl(3-palmitoylaminopropyl)ammonio]-propanesulfonate, coco amphi glycinate, and mixtures thereof.
27. The method according to claim 25, wherein the anionic surfactant is selected from the group consisting of sodium dodecyl sulfate (SDS), sodium pentane sulfonate, dehydrocholic acid, glycolithocholic acid ethyl ester, ammonium lauryl sulfate and other alkyl sulfate salts, sodium laureth sulfate, alkyl benzene sulfonate, soaps, fatty acid salts, and mixtures thereof.

28. The method according to claim 25, wherein the cationic surfactant is selected from the group consisting of cetyl trimethylammonium bromide (CTAB), dodecylethyldimethylammonium bromide (D12EDMAB), didodecyl ammonium bromide (DMAB), cetylpyridinium chloride (CPC), polyethoxylated tallow amine (POEA), hexadecyltrimethylammonium p-toluenesulfonate, benzalkonium chloride (BAC), benzethonium chloride (BZT), and mixtures thereof.
29. The method according to claim 25, wherein the nonionic surfactant is selected from the group consisting of poloaxamers, alkyl poly(ethylene oxide), diethylene glycol monohexyl ether, polyvinyl alcohol (PVA), copolymers of poly(ethylene oxide) and poly(propylene oxide), hexaethylene glycol monohexadecyl ether, alkyl polyglucosides, digitonin, ethylene glycol monodecyl ether, cocamide MEA, cocamide DEA, cocamide TEA, fatty alcohols, and mixtures thereof.
30. The method according to any one of claims 1 to 29, wherein the second surfactant comprises or consists essentially of polyvinyl alcohol (PVA).
31. The method according to any one of claims 1 to 30, wherein the reagent capable of effecting gelation of the hydrogel-forming agent comprises or consists essentially of a salt.
32. The method according to claim 31, wherein the salt comprises a multivalent cation.
33. The method according to claim 32, wherein the multivalent cation is selected from the group consisting of calcium, magnesium, aluminum, barium, and strontium.
34. The method according to any one of claims 1 to 33, wherein the reagent capable of effecting gelation of the hydrogel-forming agent comprises or consists essentially of calcium (II) chloride.
35. The method according to any one of claims 1 to 34, wherein the aqueous solution in step (d) further comprises an osmolyte.

36. The method according to claim 35, wherein the osmolyte comprises or consists essentially of sodium chloride.
- 5 37. The method according to claim 35 or 36, wherein the concentration of osmolyte in the aqueous solution in step (d) is between about 0.2 M to about 2 M.
38. The method according to any one of claims 35 to 37, wherein the concentration of osmolyte in the aqueous solution in step (d) is about 0.6 M.
- 10 39. The method according to any one of claims 1 to 38, wherein the amount of second surfactant in the aqueous solution in step (d) is between about 0.01 % (w/v) to about 5 % (w/v).
- 15 40. The method according to any one of claims 1 to 39, wherein the amount of second surfactant in the aqueous solution in step (d) is about 0.5 % (w/v).
41. The method according to any one of claims 1 to 40, wherein the concentration of reagent capable of effecting gelation of the hydrogel-forming agent in the aqueous solution in step (d) is between about 10 mM to about 100 mM.
- 20 42. The method according to any one of claims 1 to 41, wherein the concentration of reagent capable of effecting gelation of the hydrogel-forming agent in the aqueous solution in step (d) is about 50 mM.
- 25 43. The method according to any one of claims 1 to 42, wherein dispersing the first emulsion into the aqueous solution is carried out under continuous stirring.
44. The method according to claim 43, wherein the continuous stirring is carried out at a speed of between about 300 rpm to about 2000 rpm.
- 30

45. The method according to claim 43 or 44, wherein the continuous stirring is carried out for a time period of between about 1 hour to about 12 hours.
46. The method according to any one of claims 43 to 45, wherein the continuous stirring
5 is carried out for a time period of about 3 hours.
47. The method according to any one of claims 43 to 46, wherein gelation of the hydrogel-forming agent to form hydrogel microparticles is carried out via ionic gelation.
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48. The method according to any one of claims 1 to 47, wherein the mean diameter of the hydrogel microparticles formed is between about 100 μm to about 800 μm .
49. The method according to any one of claims 1 to 48, wherein the hydrogel microparticles are essentially monodisperse.
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50. The method according to any one of claims 1 to 49, further comprising adding a hydrophilic active compound to be encapsulated to the hydrogel-forming agent in step (a).
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51. The method according to claim 50, wherein the hydrophilic active compound is selected from the group consisting of a drug, a protein, an enzyme, an antibody, a peptide, a growth factor, an organic molecule, a nucleic acid, a cell, a pesticide, a dye, a chemical indicator, and a fertilizer.
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52. The method according to claim 51, wherein the hydrophilic active compound comprises or consists essentially of a drug.
53. The method according to claim 51 or 52, wherein the drug is selected from the group
30 consisting of a vaccine, a protein, an inorganic molecule and mixtures thereof.

54. The method according to any one of claims 51 to 52, wherein the drug comprises or consists essentially of metoclopramide HCl.
55. The method according to any one of claims 1 to 54, further comprising at least one centrifugation and at least one washing step after extracting the organic solvent.
56. The method according to any one of claims 1 to 55, further comprising lyophilizing the core-shell microparticles formed in the method.
57. The method according to any one of claims 1 to 56, wherein the core-shell microparticles have a mean diameter of between about 150 μm to about 1000 μm .
58. Core-shell microparticles manufactured by a method according to any one of claims 1 to 57.
59. Core-shell microparticles according to claim 58 for sustained release of a hydrophilic active compound encapsulated therein.
60. A core-shell microparticle having a core comprising a hydrogel material and a shell comprising a hydrophobic polymer immiscible with the hydrogel material, wherein the shell is free of the hydrogel material of the core.
61. The core-shell microparticle according to claim 60, wherein the shell consists of the hydrophobic polymer.
62. The core-shell microparticle according to claim 60 or 61, wherein the core comprises or consists essentially of calcium alginate or magnesium alginate.
63. The core-shell microparticle according to any one of claims 60 to 62, wherein diameter of the core is between about 100 μm to about 800 μm .

64. The core-shell microparticle according to any one of claims 60 to 63, wherein the hydrophobic polymer is selected from the group consisting of poly-lactic-co-glycolic acid (PLGA), poly-L-lactide (PLLA), poly-caprolactone (PCL), polyglycolide (PGA), derivatives thereof, copolymers thereof, and mixtures thereof.
- 5 65. The core-shell microparticle according to any one of claims 60 to 64, wherein the hydrophobic polymer is a biodegradable polymer or a biocompatible polymer.
66. The core-shell microparticle according to any one of claims 60 to 65, wherein the core
10 further comprises a hydrophilic active compound.
67. The core-shell microparticle according to claim 66, wherein the hydrophilic active compound is selected from the group consisting of a drug, a protein, an enzyme, an antibody, a peptide, a growth factor, an organic molecule, a nucleic acid, a cell, a
15 pesticide, a dye, a chemical indicator, and a fertilizer.
68. The core-shell microparticle according to claim 66 or 67, wherein the hydrophilic active compound comprises or consists essentially of a drug.
- 20 69. The core-shell microparticle according to claim 68, wherein the drug is selected from the group consisting of a vaccine, a protein, an inorganic molecule and mixtures thereof.
70. The core-shell microparticle according to any one of claims 67 to 69, wherein the
25 drug comprises or consists essentially of metoclopramide HCl.
71. A pharmaceutical composition comprising core-shell microparticles manufactured by a method according to any one of claims 1 to 57 or according to any one of claims 58 to 70.