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(54) Title: POROUS SILICA SHELL-MEDIATED ASSEMBLY OF MACROMOLECULAR CAPSULES

(57) Abstract: The present invention relates to a process of preparing macromolecular capsules. In particular, the invention relates to the use of a template with a permeable shell and an impermeable core for preparing macromolecular capsules. The process of the present invention relates to infiltrating macromolecules such as polymers into a permeable shell and then crosslinking the macromolecules to form a macromolecular network within the pores of the permeable shell. In some cases, the permeable shell and impermeable core can both be removed to yield hollow capsules. The invention involves the use of macromolecules which may be conjugated to one or more functional molecules. In some cases the macromolecules used in the invention are conjugated to biologically active molecules to form particles useful for applications including, but not limited to, drug delivery and controlled release.



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POROUS SILICA SHELL-MEDIATED ASSEMBLY
OF MACROMOLECULAR CAPSULES

FIELD OF THE INVENTION

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10 the permeable shell. In some cases, the permeable shell and impermeable core can both be removed to yield hollow capsules. The invention involves the use of macromolecules which may be conjugated to one or more functional molecules. In some cases the macromolecules used in the invention are conjugated to biologically active molecules to form particles useful for applications including, but not limited to, drug delivery and controlled release.

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BACKGROUND OF THE INVENTION

Capsules in the nanometer to micrometer size range are an important class of materials. They can be used for encapsulating and thereafter releasing various substances (e.g., drugs, cosmetics, dyes, pesticides, and inks), or in other diverse applications such as catalysis or
20 sensing. A variety of chemical and physicochemical procedures have been employed to prepare inorganic, metallic, polymeric and composite capsules, including spray pyrolysis, nozzle reactor processes, self-assembly of molecules (e.g., vesicles, dendrimers, and block copolymers), and sacrificial core template-assisted methods, such as layer-by-layer assembly and surface precipitation.

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One of the methods used to prepare well-defined polymer capsules has involved the use of layer-by-layer (LbL) assembly in tandem with colloidal templating. This method involves the alternate deposition of polymers on a colloidal surface driven by electrostatics, hydrogen bonding, covalent bonding and/or complementary base pairing, followed by subsequent
30 removal of the core by chemical or thermal means. In principle, at least two polymer components are typically required in the preparation of polymer capsules by the LbL method. In certain applications, the presence of the second polymer may limit the specific functionality of the materials. Therefore, several approaches have been used to fabricate capsules with a single polymer component, including: (i) selective cross-linking of one component in the capsule shells coupled with removal of the second polymeric component; (ii) LbL assembly of
35 one polymer with a small molecular cross-linker; and (iii) the alternate assembly of copolymers which are synthesized predominantly from one monomer but have a minor

component of a reactive moiety. Although these methods have been used in the preparation of stable capsules, they are restricted to systems in which a unique polymer or specifically modified polymer has been used. Additionally, the methods typically involve a significant number of steps to prepare the capsules, leading to a significant loss in particle number as the layer number increases (and therefore the number of washes increases), as well as a significant time expenditure in the capsule preparation. Moreover, significant aggregation of the particles can occur during the process, especially in the preparation of capsules using polymers that are weak polyelectrolytes (PE), and in the assembly of PE multilayers on small colloidal templates (<500 nm).

These limitations have greatly hindered the application of well-defined templated capsules in biological applications, particularly in delivery, where small sized capsules are preferred. Thus, there is a need for the development of a simple synthetic route toward the preparation of stable macromolecule capsules.

The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

SUMMARY OF THE INVENTION

The present invention has arisen from the inventor's discovery that macromolecules can permeate into porous structures wherein they can subsequently be reacted to form a macromolecular network. Moreover, the present inventors have also found that infiltrating macromolecules into a template with a permeable shell and an impermeable core, followed by removal of both the permeable shell and the core provides an efficient method for preparing macromolecular capsules.

Accordingly, in one embodiment, the present invention provides a process for preparing a macromolecular capsule, the process including (a) contacting a template having an impermeable core contained within a permeable shell with a solution containing macromolecules under conditions wherein a proportion of the macromolecules infiltrate into the permeable shell; (b) removing a substantial proportion of the macromolecules not infiltrated into the permeable shell; and (c) crosslinking the macromolecules infiltrated into the permeable shell.

In some embodiments, the process further includes (d) removing the template. The removal of the template in this way leads to the formation of a hollow capsule.

The template may consist of an impermeable silica core and a permeable silica shell. In some cases, the templates have an average diameter of between 50 nm and 10 μ m. In one embodiment, the templates have an average diameter of between 100 nm and 2 μ m. In another embodiment, the templates have an average diameter of between 200 nm and 1 μ m. In another specific embodiment, the templates have an average diameter of between 200 nm and 600 nm.

The permeable shell typically contains pores. In one embodiment the pores have an average pore size in the range of 2 nm to 30 nm. In another embodiment the pores have an average pore size in the range of 2 nm to 15 nm. In another embodiment the pores have an average pore size in the range of 2 nm to 10 nm. In another embodiment the pores have an average pore size in the range of 2 nm to 6 nm. The permeable shell typically has a thickness of between 10 and 800 nm. In another embodiment the permeable shell has a thickness of between 20 and 200 nm. In a specific embodiment the permeable shell has a thickness of between 20 and 80 nm.

The macromolecules used in the process of the present invention may be selected from the group consisting of chain growth polymers, step growth polymers, polyelectrolytes, polymer conjugates, proteins, polypeptides, polysaccharides, polynucleotides, deoxyribonucleic acid and ribonucleic acid and drug-polymer conjugates, or mixtures thereof. In one embodiment, the macromolecule is a polyamine. The polyamine can be selected from the group consisting of poly(allylamine hydrochloride), poly(ethyleneimine) and poly(vinylamine).

In some cases, the macromolecule is a poly(carboxylic acid), which may be selected from the group consisting of poly(methacrylic acid) or poly(acrylic acid). In other cases, the macromolecule is a polypeptide which may be selected from the group consisting of poly(glutamic acid) and poly(lysine). In yet other cases, the macromolecule is a protein. Examples of suitable proteins include proteins selected from the group consisting of lysozyme, serum albumin, insulin, ribonuclease A, myoglobin, chymotrypsin, trypsin, chymotrypsinogen, hemoglobin, hexokinase, immunoglobulin G, RNA polymerase, DNA polymerase, apolipoprotein B, glutamate dehydrogenase, lipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins and metalloproteins. In still other cases, the macromolecule is a polysaccharide. Examples of suitable polysaccharides include those selected from the group consisting of chitin, chitosan, cellulose, starch and glycogen.

In one embodiment of the invention the macromolecule is biocompatible and/or biodegradable. In another embodiment the macromolecule is conjugated to a functional molecule. The functional molecule to which the macromolecule is conjugated may be any molecule that imparts a desired functionality on the final macromolecular capsule or has functionality in its own right once released from the final macromolecular capsule in use. The functional molecule may be biologically-active or non-biologically active. For example the functional molecule may be a magnetic resonance imaging contrast agent or a biologically active species. Suitable biologically active species include drugs, antibodies, antigens, oligonucleotides, polysaccharides, proteins, enzymes, molecular recognition units and toxins. Examples of suitable drug-polymer conjugates include poly(glutamic acid)-conjugated anticancer drugs such as paclitaxel and doxorubicin. In a specific embodiment the macromolecule is conjugated to at least two biologically active species. One particularly useful form of this embodiment is one in which the macromolecule is attached to both a drug and an antibody. At least in theory this allows for the final macromolecular capsules to be used in targeted drug delivery systems.

The solution containing the macromolecule is typically aqueous, and may also contain an inorganic salt. The inorganic salt may be an alkali metal halide, and suitable alkali metal halides include sodium chloride and potassium chloride. The solution containing the macromolecule may also contain an acid or a base. The acid is typically an organic acid or an inorganic acid and may be selected from the group consisting of hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid and acetic acid. The base is typically an alkali metal hydroxide and may be selected from the group consisting of sodium hydroxide and potassium hydroxide. The solution containing the macromolecule also may contain a buffer.

In the process of the present invention after infiltration of a proportion of the macromolecules the macromolecules that have not infiltrated remain in the solution and these are removed. In one embodiment a substantial proportion of the macromolecules not infiltrated into the permeable shell are removed by filtration or centrifugation. The removal by filtration typically includes (i) passing the solution through a filter membrane whereon the templates infiltrated with macromolecules are retained while the solution containing the macromolecule passes through the membrane; (ii) rinsing the templates infiltrated with macromolecules retained on the filter membrane with a rinse solution; and (iii) dispersing the templates in the rinse solution. The removal by centrifugation typically includes (i) centrifuging the solution to provide a concentrated pellet of the templates with infiltrated macromolecule and a supernatant solution containing excess macromolecule; (ii) removing the supernatant solution

containing excess macromolecules; (iii) dispersing the concentrated pellet of templates infiltrated with macromolecules in a rinse solution; (iv) centrifuging the rinse solution containing the templates infiltrated with macromolecules to provide a concentrated pellet of templates infiltrated with macromolecules and a supernatant solution of rinse solution; (v) repeating steps (iii) and (iv) a further two times; and (vi) dispersing the templates in the rinse solution.

The rinse solution used in either the filtration or centrifugation is typically water which optionally contains any of an alkali metal halide salt, an acid such as an acid selected from the group consisting of hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid and acetic acid, a base such as a base selected from the group consisting of sodium hydroxide and potassium hydroxide, and a buffer.

The crosslinking of the macromolecules infiltrated into the templates is typically performed by the addition of a crosslinking agent, with or without the addition of an activating agent. A typical activating agent, if used, is a carbodiimide such as 1-ethyl-3-(3-methylaminopropyl) carbodiimide hydrochloride.

The crosslinking agent used to crosslink the macromolecules infiltrated into the templates may be a difunctional crosslinking agent selected from the group consisting of ethylenediamine, glutaraldehyde, 1,6-hexanediamine, 1,4-butanediamine, 1,8-octanediamine, 1,4-butanediol diacrylate, 1,6-hexanediol diacrylate, 1,8-octanediol diacrylate, bis(N-hydroxysuccinimide esters of succinic acid, adipic acid, suberic acid and sebacic acid. The difunctional crosslinking agent can optionally contain a disulfide linkage. Typical difunctional crosslinking agents containing a disulfide linkage are dimethyl 3,3'-dithiopropionimidate dihydrochloride or 2,2'-diaminodiethyl disulfide dihydrochloride.

The crosslinking agent may also be polyfunctional. Polyfunctional crosslinking agents such as poly(acrylic acid) or poly(methacrylic acid) may be used to crosslink the macromolecules infiltrated into the templates, with or without the addition of an activating agent.

The macromolecule may be modified to include moieties which react with the crosslinking agent. The moieties included in the macromolecule may react selectively with the crosslinking agent. In some embodiments, the moieties included in the macromolecule are alkyne groups and the crosslinking agent contains at least two azide groups. In other embodiments, the moieties included in the macromolecule are azide groups and the crosslinking agent contains at least two alkyne groups. In some embodiments the reaction

between the moieties included in the macromolecule and the crosslinking agent occurs in the presence of a catalyst. In one particular embodiment the catalyst is Cu(I).

The crosslinking of the macromolecules infiltrated into the templates may also be performed by elevating the temperature of the templates with infiltrated macromolecules. The crosslinking of the macromolecules infiltrated into the templates may also be performed by exposing the templates infiltrated with macromolecules to ionizing radiation, wherein the ionizing radiation is selected from the group consisting of X-ray radiation, gamma radiation, ultraviolet radiation and microwave radiation.

As stated above in some embodiments the template is removed following crosslinking of the macromolecules. The template is typically removed by contacting the template with a solution that degrades the template. In one embodiment the template is removed by contacting with an aqueous solution of hydrofluoric acid or an aqueous solution of an alkali metal hydroxide. In one embodiment the aqueous solution of alkali metal hydroxide is sodium hydroxide or potassium hydroxide.

The present invention also provides a method of delivering a functional molecule to its location of operation, the method including: (a) preparing a macromolecular capsule via the process of the invention, using a macromolecule that is conjugated to said functional molecule; and (b); administering an effective amount of the capsules to a subject containing the location of operation. The functional molecule may be any one or more of a drug, an antibody, an antigen, an oligonucleotide, a polysaccharide, a protein, an enzyme, a molecular recognition unit, a magnetic resonance imaging contrast agent or a toxin.

The present invention also provides a method of delivering a therapeutic agent to a living organism, the method including: (a) preparing a macromolecular capsule via the process of the invention using a macromolecules that are conjugated to a functional molecule selected from the group consisting of an antibody, a therapeutic agent and a mixture thereof; and (b) administering a therapeutically effective amount of the capsules to the organism.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic representation of the preparation of single-component of macromolecule capsules by using silica particles with a solid core and mesoporous shell (SCMS) as templates.

Figure 2 shows representative SEM (a), and TEM (inset, a) images of the SCMS template particles. Representative SEM (b), TEM (c), and ultramicrotomed TEM (d) images of PAH-

15k capsules prepared using the SCMS particles as templates after template removal. The inset in (d) shows a high magnification image of the capsule shell shown in the main figure.

Figure 3 shows TEM (a) and SEM (b) images of PAH-15k capsules loaded with ibuprofen.

Figure 4 provides TEM images of PLL (a, b) and lysozyme (c) capsules prepared with the crosslinker of GA (a) dimethyl 3,3'-dithiopropionimidate dihydrochloride (b), and EDC, respectively. The inset in Fig. 4a shows the typical morphology of PLL capsules observed under SEM. Inset in Fig. 4c is ultramicrotomed TEM image of lysozyme capsules.

Figure 5 provides TEM images of (a) PAA and (b) PMA capsules. The insets in Fig. 5a and Fig. 5b are ultramicrotomed PAA capsules and a high magnification image of the PMA capsules, respectively.

Figure 6 shows representative electron microscopy images indicating the typical morphology PGA-Dox (a-b) capsules using SCMS templates after template removal. The cross-linking agent used was cystamine. Panel (a) is a TEM image of the capsules, while panel (b) is an SEM image.

Figure 7 provides (a) *In vitro* doxorubicin release studies from PGA-Dox-SCMS particles and PGA-Dox capsules. (b) Trypan blue cell viability assay showing the percentage viability of LIM1215 colorectal tumor cells in the absence of any external agent (T1) and in the presence of PGA polymer (T2), doxorubicin (T3), PGA-Dox conjugate (T4), PGA-SCMS particle (T5), PGA capsules (T6), PGA-Dox-SCMS particles (T7) and PGA-Dox capsules (T8) after 16 h of treatment. The results shown here show the average of three separate experiments.

Figure 8 shows representative CLSM images showing the uptake of PGA-Dox-SCMS particles (a1-a4) and PGA-Dox capsules (b1-b4) in LIM1215 colorectal tumor cells. Images shown here demonstrate the fluorescent signals arising from doxorubicin (a1, b1), lysotracker blue (a2, b2), merged images of doxorubicin and lysotracker blue signals (a3, b3) and merged images of doxorubicin, lysotracker blueTM and phase contrast images (a4, b4).

DETAILED DESCRIPTION OF THE INVENTION

The process of the present invention utilizes a template having an impermeable core contained within a permeable shell. The template may be of any suitable type that provides an impermeable core contained within a permeable shell.

The permeable shell of the template may contain pores of a number of different shapes and sizes however the permeable shell is typically mesoporous. Mesoporous means that at least some pores, and generally a majority of the pores, have a pore size in the range 2 to 50 nm.

The permeable shell may be made of a number of suitable materials which allow for their subsequent removal during the template removal step. Examples include inorganic oxides such as ceria, zirconia, titania and silica, metals such as gold, and organics such as certain

polymeric material. The permeable shell of the template may or may not be made up of the same materials as the impermeable core, although in many cases the core and the shell will be made of the same material. The template is commonly a silica based material, with both a solid silica core and a permeable silica shell.

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The template may take any suitable form and may be for example in the form of, spheres, cubes, prisms, fibres, tetrahedrons or irregular particles. The shell may be a constant thickness around the outside of the core, or the shell may vary in thickness. It is typical that the template is spherical or substantially spherical.

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In one particularly useful embodiment the template is a core-shell silica particle with a permeable mesoporous silica shell and a solid impermeable silica core, in order to produce a spherical or substantially spherical hollow macromolecular particle. It will be convenient to describe the invention in terms of a spherical material, but it shall be kept in mind that the macromolecular particle produced by the process of the invention may be of any form, depending on the form of the template. Thus in general the final shape of the hollow macromolecular particles produced by the process of the invention will take the general shape or form of the template used in their synthesis. Thus for example if the template is spherical then the final product will typically be spherical. If the template is a fibre then once again the final product will typically be a fibre. There may be some fluctuation in the size of the product particle compared to the size of the template, due to shrinkage and/or swelling of the hollow macromolecular particles depending on the specific synthesis conditions.

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The surface of the template may be modified by addition of functional moieties to enhance the infiltration of the macromolecule into the permeable shell. Any of a number of functional moieties can be added onto the surface of the template with the choice of functional moiety being chosen to complement the macromolecule being infiltrated into the permeable shell. A skilled worker in the area will generally have little difficulty in choosing a functional moiety to introduce onto the surface of the template to complement the chosen macromolecule. By way of example, one method of modifying the surface of a silica template is to graft a moiety such as 3-aminopropyltriethoxysilane (APTS) onto the surface of the silica. This introduces an amine surface functionality that can interact with any carboxyl groups on the macromolecule to host the macromolecule in the permeable shell. If it was desired to host a macromolecule in the permeable shell that contains amino moieties this could similarly be carried out by attaching carboxyl moieties to the exposed surface of the template.

As stated above the permeable shell may be a mesoporous silica material. The mesoporous silica material may have a bimodal pore structure, that is, having smaller pores of about 2-3 nm and larger pores from about 4-40 nm.

5 Examples of materials that can be used as macromolecules in the present invention include but are not limited to biodegradable polymers such as polyglycolic acid (PGA), polylactic acid (PLA), poly(methacrylic acid), poly(ethacrylic acid), polyacrylic acid (PAA), poly(N-isopropylacrylamide), poly(N,N-dimethylacrylamide), polyamides, poly-2-hydroxy butyrate (PHB), gelatins, polycaprolactone (PCL), poly (lactic-co-glycolic acid) (PLGA), fluorescently
10 labelled polymers, conducting polymers, liquid crystal polymers, photoconducting polymers, photochromic polymers; poly(amino acids) including peptides and S-layer proteins; peptides, glycopeptides, peptidoglycans, glycosaminoglycans, glycolipids, lipopolysaccharides, proteins, glycoproteins, polypeptides, polycarbohydrates such as dextrans, chitosan derivatives, alginates, amyloses, pectins, glycogens, and chitins; polynucleotides such as
15 DNA, RNA and oligonucleotides; modified biopolymers such as carboxymethyl cellulose, carboxymethyl dextran and lignin sulfonates; polysilanes, polysilanol, poly(phosphazenes), polysulfazenes, polysulfide and polyphosphate. Preferred polymers include those with an amine group, for example poly(allylamine hydrochloride), poly(ethyleneimine) or poly(vinylamine hydrochloride) or a carboxylic acid group, for example poly(acrylic acid) or
20 poly(methacrylic acid). Other preferred polymers include poly(N-isopropylacrylamide), poly(N,N-dimethylacrylamide), poly(styrene-alt-maleic anhydride), poly[(poly(ethylene glycol) methyl ether methacrylate] and copolymers thereof, poly[(poly(ethylene glycol) methyl ether acrylate] and copolymers thereof, and the biocompatible polymers, such as poly(L-glutamic acid) and poly(L-lysine) and mixtures thereof.

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Another useful group of macromolecules are selected from the group consisting of poly(amino acids), peptides, glycopeptides, polypeptides, peptidoglycans, glycosaminoglycans, glycolipids, lipopolysaccharides, proteins, glycoproteins, polycarbohydrates; nucleic acid
30 polymers, nucleotides, polynucleotides, RNA and DNA. In particular, examples of preferred proteins include lysozyme, serum albumin, insulin, ribonuclease A, myoglobin, chymotrypsin, trypsin, chymotrypsinogen, hemoglobin, hexokinase, immunoglobulin G, RNA polymerase, DNA polymerase, apolipoprotein B, glutamate dehydrogenase, lipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins and metalloproteins and mixtures thereof.

35 In some cases, the macromolecule is conjugated to one or more functional molecules in order to produce a final macromolecular capsule with functional molecules attached. Any suitable functional molecule may be used with examples of functional molecules including a drug, an

antibody, an antigen, an oligonucleotide, a polysaccharide, a protein, an enzyme, a molecular recognition unit, a magnetic resonance imaging contrast agent or a toxin.

- In one embodiment the functional molecule is a biologically active species. In some cases, the conjugation is via a cleavable group which can subsequently be broken to release the biologically active species. In other cases the linkage is robust and is not able to be readily cleaved. The biologically active species may have a number of purposes, one being to act as a targeting moiety to enable the particle to be used in delivering a therapeutic agent to specific cell lines. In other embodiments, the biologically active species may be a toxin to be delivered only to specific cells. In other embodiments, there may be more than one type of biologically active species conjugated to the macromolecule infiltrated into the permeable shell. In this way it may be possible to use this approach to impart particles with both a targeting capacity and a therapeutic capacity. In addition one could employ mixtures of macromolecules containing different functional molecules in the infiltration step in order to provide a final macromolecular capsule containing more than one functionality. Thus, for example one could employ a mixture of macromolecules in which a portion of the macromolecules are conjugated to antibodies and a portion are conjugated to a therapeutic agent such as a drug such that the final macromolecular particle has both functionality.
- The macromolecule is infiltrated into the permeable structure by contacting the template with a solution of the macromolecule. The contacting of the template with macromolecule typically involves the macromolecule material being applied to the template in solution form in a suitable solvent. Generally, the macromolecule when applied in solution, will be in the form of an aqueous solution, such as an aqueous salt solution. However, this is not to exclude the possibility of using a non-aqueous solution, or an aqueous solution wherein an amount of organic solvent is also added to the solution. The macromolecule in solution typically has a concentration of from 0.001 to 100 mg mL⁻¹, more typically from about 0.1 to 30 mg mL⁻¹, even more typically from 0.5 to 10 mg mL⁻¹.
- If a solution incorporating an inorganic salt is used the salt in solution generally has a concentration of from about 0.001 to 5 M. In certain embodiments the salt concentration is from 0.05 to 5 M. In certain other embodiments the salt concentration is from 0.1 to 1 M. The macromolecule is typically applied in a salt solution as, without wishing to be bound by theory, it is thought that in the presence of a salt solution, the macromolecule can be highly coiled which assists in it infiltrating into the permeable shell of the template. In the absence of salt, the macromolecule may be restricted to the outer surface assembly of the template as it will be presented with a long chain configuration. The salt may be of any suitable type but is

typically selected from the group consisting of alkali metal halides such as potassium chloride, lithium chloride and sodium chloride. In a specific embodiment the salt is sodium chloride.

- 5 The solution of the macromolecule may also incorporate acid or base. Again, without wishing to be bound by theory, acids and bases may induce protonation and or deprotonation of certain moieties in the macromolecule, therefore reducing the intramolecular repulsions and leading the macromolecules to adopt a more coiled conformation, thereby being better able to infiltrate into the permeable shell of the template. For instance, the addition of base to a
- 10 solution of poly(allylamine hydrochloride) leads to some of the protonated amine groups to be converted back to primary amines, therefore reducing intramolecular repulsion. Likewise, the introduction of some acid into a solution of a poly(carboxylic acid) such as poly(acrylic acid) leads to protonation of the carboxylate groups, thereby reducing the intramolecular repulsion between the negative carboxylate moieties. A skilled worker in the field will generally be able
- 15 to determine from the particular macromolecule chosen whether an acid or base should be chosen in any particular instance. If an acid is used it may be selected from any organic or inorganic acid, but is typically selected from the group consisting of hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid and acetic acid. In a specific embodiment the acid is hydrochloric acid. The base can be any organic or inorganic base, but is typically selected
- 20 from the group consisting of the alkali metal hydroxides. In one specific embodiment the base is sodium hydroxide.

Because some of the macromolecules that may be used in the invention are pH sensitive, in certain embodiments it may be advantageous to use a buffer. The buffer can be any organic

25 or inorganic buffer, and the appropriate buffer for working in a particular pH range would be evident to someone skilled in the art. The buffer may be selected from the group consisting of N-morpholinoethanesulfonic acid, N-morpholinopropanesulfonic acid, sodium citrate/citric acid, sodium acetate/acetic acid, trisodium phosphate/disodium hydrogen phosphate/ sodium dihydrogen phosphate/phosphoric acid.

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Ultrasound may be used during the infiltration of the macromolecule into the permeable shell of the template to assist in allowing the macromolecule to permeate into the pores of the mesoporous template. Accordingly after the template has been contacted with a solution containing the macromolecules the mixture thus formed may be ultrasonicated. With the

35 application of ultrasound, together with agitation of the mixture of macromolecule and the template, higher molecular weight polymer material can infiltrate the pores in an efficient manner.

The temperature of the solution containing the macromolecules may also be elevated when contacting with the templates having a permeable shell. Polymers such as poly(N-isopropylacrylamide) and poly[poly(ethylene glycol) methyl ether acrylate] become more
5 coiled with elevated temperature, which may assist in infiltration of the macromolecules into the permeable shell of the templates. Other polymers become more coiled with reduction of the temperature. The appropriate conditions to use for any given macromolecule will be apparent to one skilled in the art.

10 The time period allowed for infiltration can be varied significantly, from 15 minutes to as much as 48 hours. The time period will be determined in relation to other conditions for infiltration of the macromolecules, such as the concentration of salt in the solution, the pH of the solution, the molecular weight of the macromolecule and the temperature at which the macromolecule is contacted with the template. In one embodiment the time for infiltrating the
15 macromolecule into the permeable shell of the template is less than 24 hours. In another embodiment the time is 12 hours or less.

Following the completion of infiltration excess macromolecules that have not infiltrated the pores of the template are then removed and this may be carried out using any method known
20 in the art. One method for removing the excess macromolecule which was not infiltrated into the permeable shell of the template is by using either filtration or centrifugation. Other options for removing the excess macromolecule include dialysis using a membrane with an appropriate cut-off. For larger particles sedimentation of the particles can occur, abrogating the need to use centrifugal force to separate the particles from the solution containing the
25 macromolecule. Other approaches for separation of a solution from a solid dispersion as known in the art can be employed for this step.

The macromolecules infiltrated into the template are then cross-linked and the crosslinking step may be carried out in any way known in the art. In one embodiment the macromolecules
30 are crosslinked via the addition of a chemical crosslinking agent. The crosslinking agent may be di-, tri-, tetra-, or higher in functionality. In some embodiments a polymeric crosslinking agent is used. In some embodiments, the crosslinking reaction is facilitated by the addition of an activating agent, such as a carbodiimide such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. In some embodiments, the crosslinking agent is a difunctional
35 molecule which itself contains a linkage which is biologically active, such as an aliphatic ester or a disulfide linkage. In some cases the difunctional crosslinker may contain a moiety which is selectively cut by certain biological entities such as enzymes. The exact means of

chemical cross-linking of the materials will depend upon the nature of the macromolecules chosen. In some cases, the macromolecule contains moieties which can self condense under appropriate conditions.

- 5 The macromolecule can, in some cases, be modified to include a moiety which undergoes selective reactions with certain crosslinking agents. In some embodiments, the reaction between the moiety included in the macromolecule and the crosslinking agent is a so called "click" reaction. In other embodiments, the reaction is a condensation reaction. In still other
10 embodiments, the reaction is a ring opening reaction. In other embodiments, the reaction is a cyclization reaction. In each of these instances the chemical moiety included in the macromolecule will be chosen to allow specific, selective reaction with the crosslinking agent

In some specific embodiments, the reaction between the chemical moiety included in the macromolecule and the crosslinking agent results in the formation of a heterocyclic ring
15 structure. In some embodiments, the heterocyclic ring is formed in the presence of a catalyst. The catalyst may include a metal selected from the group consisting of Au, Ag, Hg, Cd, Zr, Ru, Fe, Co, Pt, Pd, Ni, Cu, Rh and W. In some specific embodiments, the catalyst includes a metal selected from the group consisting of Ru, Pt, Ni, Cu and Pd. In one embodiment, the catalyst includes Cu(I). The heterocyclic ring formation may also occur in the absence of a
20 catalyst. In particular, the use of elevated temperature or pressurised reaction conditions or irradiation (such as by microwaves), may eliminate the need to use a catalyst.

In some embodiments, the bonds formed between the chemical moieties included in the macromolecule and the crosslinking agent result in the formation of a 1,2,3-triazole. In one
25 embodiment, this occurs by the Cu(I) catalysed variant of the Huisgen 1,3-dipolar cycloaddition.

In other cases, the macromolecule contains moieties which are able to react via the addition of an appropriate catalyst. In still other cases, the macromolecule contains moieties the
30 reaction of which is triggered by exposure to radiation, the radiation being selected from the group consisting of ultraviolet, visible, infrared, gamma, microwave, X-ray and radio waves. In further embodiments, the macromolecule is crosslinked through the generation of adventitious radicals via irradiation with ionizing radiation.

35 In one embodiment the macromolecule infiltrated into the permeable shell of the template is crosslinked by elevating the temperature. The cross-linking is generally performed by heating at a temperature of from about 100°C to 250°C. In one embodiment the crosslinking is

performed by heating at a temperature of from 140°C to 220°C. In another embodiment the crosslinking is performed by heating at a temperature of about 160°C. The amount of time taken to effect cross-linking will vary depending on the nature of the cross-linking moieties but it typically takes from 30 minutes to 12 hours.

5

Following the infiltration of the macromolecules into the permeable shell of the template and crosslinking of the same the process then optionally involves removal of the template. The template may be removed by exposure to a suitable agent that is capable of degrading the template. In general the agent will be chosen such that it is able to degrade the template but
10 such that it will not damage the crosslinked macromolecular network. An example of a suitable agent is hydrofluoric acid or sodium hydroxide. It has been found that the silicone dioxide core of the silica template is readily degraded in hydrofluoric acid, and is converted to $[\text{SiF}_6]^{2-}$ ion thereafter leaving the macromolecular structure. Typically the mixture containing the template is shaken when the template is exposed to the hydrofluoric acid. If hydrofluoric
15 acid is used it is found that the silica can be dissolved using a wide range of concentrations of acid. The acid may be of any strength although it is convenient to use an acid strength of from 1 to 10 M, more preferably about 2 M. In some cases the hydrofluoric acid is applied as a buffered solution with ammonium fluoride. Whereas hydrofluoric acid is preferred as a solvent, other suitable solvents would be well appreciated by the skilled practitioner. In
20 principle any substance that can dissolve or degrade the template may be used as the solvent.

Following removal of the template the macromolecular capsules are then isolated using processes well known in the art and may then be used in a number of applications depending
25 upon the nature of the functional molecules (if any) applied to the macromolecular capsules. A number of these applications involve the use of the macromolecular capsule to deliver the functional molecule to its location of operation typically in a subject such as a human. Applications of this type include methods of medical treatment and imaging applications for example.

30

Administration of the macromolecular capsules of the invention to humans can be by any of the accepted modes of administration well known in the art. For example they may be administered by enteral administration such as oral or rectal, or by parenteral administration such as subcutaneous, intramuscular, intravenous and intradermal routes. Injection can be
35 bolus or via constant or intermittent infusion. The macromolecular capsules are typically included in a pharmaceutically acceptable carrier or diluent and in an amount sufficient to deliver to the subject a therapeutically effective dose.

In using the macromolecular capsules of the invention they can be administered in any form or mode which makes the active agent bio-available. One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon
5 the particular characteristics of the macromolecular capsule selected, the condition to be treated, the stage of the condition to be treated and other relevant circumstances. We refer the reader to Remingtons Pharmaceutical Sciences, 19th edition, Mach Publishing Co. (1995) for further information.

10 The macromolecular capsules of the present invention can be administered alone or in the form of a pharmaceutical composition in combination with a pharmaceutically acceptable carrier, diluent or excipient.

The macromolecular capsules are, however, typically used in the form of pharmaceutical
15 compositions which are formulated depending on the desired mode of administration. The compositions are prepared in manners well known in the art.

The invention in other embodiments provides a pharmaceutical pack or kit including one or more containers filled with one or more of the ingredients of the pharmaceutical compositions.
20 In such a pack or kit can be found a container having a unit dosage of the agent(s). The kits can include a composition including an effective agent either as concentrates (including lyophilized compositions), which can be diluted further prior to use or they can be provided at the concentration of use, where the vials may include one or more dosages. Conveniently, in the kits, single dosages can be provided in sterile vials so that the physician can employ the
25 vials directly, where the vials will have the desired amount and concentration of agent(s). Associated with such container(s) can be various written materials such as instructions for use, or a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

30 The macromolecular capsules of the invention may be used or administered in combination with one or more additional drug(s) that are useful for the treatment of the disorder/diseases mentioned. The components can be administered in the same formulation or in separate formulations. If administered in separate formulations the macromolecular capsules of the
35 invention may be administered sequentially or simultaneously with the other drug(s).

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of micro-organisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminium monostearate and gelatin.

If desired, and for more effective distribution, the macromolecular capsules can be incorporated into slow release or targeted delivery systems such as polymer matrices, liposomes, and microspheres.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the macromolecular capsules is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

- 5 The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding
10 compositions which can be used include polymeric substances and waxes.

If desired, and for more effective distribution, the macromolecular capsules can be incorporated into slow release or targeted delivery systems such as polymer matrices, liposomes, and microspheres.

15

The macromolecular capsules can also be in microencapsulated form, if appropriate, with one or more of the above-mentioned excipients.

20

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the macromolecular capsules, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene
25 glycols and fatty acid esters of sorbitan, and mixtures thereof.

30

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavouring, and perfuming agents.

35

Suspensions, in addition to the macromolecular capsules, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the macromolecular capsules of this invention with suitable non-irritating

excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the macromolecular capsules

- 5 Dosage forms for topical administration of macromolecular capsules of this invention include powders, patches, sprays, ointments and inhalants. The macromolecular capsules are mixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives, buffers, or propellants which may be required.
- 10 The amount of macromolecular capsule administered will preferably treat and reduce or alleviate the condition. A therapeutically effective amount can be readily determined by an attending diagnostician by the use of conventional techniques and by observing results obtained under analogous circumstances. In determining the therapeutically effective amount a number of factors are to be considered including but not limited to, the species of animal, its
- 15 size, age and general health, the specific condition involved, the severity of the condition, the response of the subject to treatment, the particular macromolecular capsule administered, the mode of administration, the bioavailability of the preparation administered, the dose regime selected, the use of other medications and other relevant circumstances.
- 20 A preferred dosage will be a range from about 0.01 to 300 mg per kilogram of body weight per day. A more preferred dosage will be in the range from 0.1 to 100 mg per kilogram of body weight per day, more preferably from 0.2 to 80 mg per kilogram of body weight per day, even more preferably 0.2 to 50 mg per kilogram of body weight per day. A suitable dose can be administered in multiple sub-doses per day.

25

Examples of materials and methods for use with the process of the present invention will now be provided. In providing these examples, it is to be understood that the specific nature of the following description is not to limit the generality of the above description.

30

EXAMPLES

Example 1 – Template Particles

- The SCMS template particles were prepared by the method reported by Unger and co-workers (*Adv. Mater.* **1998**, *10*, 1036). Specifically, absolute ethanol, deionized water, and
- 35 aqueous ammonia (32 wt%) were mixed in a flask and heated to 303 K. Tetraethoxysilane (TEOS) was then added quickly to the mixture. The solution was mixed for 5 s by shaking to ensure homogeneity. After 1 h, a mixture of TEOS and n-octadecyltrimethoxysilane was

added dropwise over a period of 20 min while stirring. After the mixture was added, the stirring was stopped and the solution was kept at ambient temperature for 1 h. The solvent was then removed and the resulting white powder was dried. To remove the porogen, the powder was calcined in air for a period of 6 h at 823 K (heating rate: 1 K/min). Scanning electron microscopy (SEM) reveals that these particles are relatively homogeneous with a diameter of ca. 420 nm (Fig. 2a). The particles have a solid core and a mesoporous shell with a thickness of ~60 nm, as shown by the transmission electron microscopy (TEM) image (inset of Fig. 2a). Nitrogen sorption data indicated that the SCMS particles have a surface area of 390 m² g⁻¹ and an average pore size of 4.5 nm with a pore volume of 0.28 mL g⁻¹.

Example 2 – Polymer Infiltration into Template Particles

The weak polyelectrolyte, poly(allylamine hydrochloride) (PAH), was first employed as a model macromolecule. Specifically, PAH with a molecular weight of either 5 000, 15 000, or 70 000 g mol⁻¹ was used (denoted hereafter as PAH-5k, PAH-15k, and PAH-70k, respectively). Stock solutions of PAH with a concentration of 5 mg mL⁻¹ in 0.2 M NaCl were used. The solutions were prepared by dissolving PAH in deionized water, and adjusting the solution pH to 8.5 with 0.1 M NaOH. Without wishing to be bound by theory, it is understood that under these conditions PAH adopts a coiled conformation allowing it to infiltrate into the nanoporous shell of the particles. Typically, the infiltration experiments involved mixing 2 mL of PAH stock solution with 10 mg of SCMS particles, and then shaking the mixture at room temperature for 12h. Excess PAH was then removed by three cycles of centrifugation and washing with water. Microelectrophoresis measurement showed a surface charge which reversed from -32 mV (the original SCMS particles) to +43 mV after PAH loading, indicating successful deposition of PAH in the template particles.

Example 3 – Characterization of Polymer Infiltrated into Template Particles

The deposition of PAH in the mesoporous shells was further evidenced by nitrogen adsorption measurements. The surface areas and porosities of the MS particles were measured by a Micromeritics Tristar surface area and porosity analyzer at -196 °C using nitrogen as the adsorption gas. Polymer infiltrated particles prepared in Example 2 were dried and used in the analysis. The surface areas of the SCMS particles (390 m² g⁻¹) loaded with 5k, 15k, and 70k PAH decreased to 160, 230, and 290 m² g⁻¹, respectively. This is most likely caused by different amounts of PAH filling the mesoporous shells (the shells constitute the majority of the surface area of the material). The different loadings were further confirmed by thermogravimetric analysis measurement, which showed PAH loadings of about 110, 65, and 35 mg g⁻¹ for the PAH-5k, PAH-15k, and PAH-70k samples, respectively. These data

clearly suggest that significantly more PE molecules can be loaded in the mesoporous shells when the PAH molecule weight is decreased from 70k to 5k.

Example 4 – Polymer Loading on Non-Mesoporous Templates (Comparative Example)

5 Thermogravimetric analysis was also used to analyse the loading of PAH onto solid silica particles which did not have a mesoporous shell. An identical procedure was used for this determination as for the silica templates which did have a mesoporous shell. Specifically, stock solutions of PAH with a concentration of 5 mg mL^{-1} in 0.2 M NaCl were employed. As for the experiments utilizing SCMS template particles, the solutions were prepared by
10 dissolving PAH in deionized water, and the pH of the solution was adjusted to 8.5 with 0.1 M NaOH. The solid silica particles were exposed to the PAH solution by mixing 2 mL of PAH stock solution with 10 mg of solid silica particles, and then shaking the mixture at room temperature for 12h. Excess PAH was then removed by three cycles of centrifugation and washing with water. Thermogravimetric analysis of the polymer-exposed particles revealed a
15 loading of 7 mg g^{-1} . This result is a factor of five lower than the minimum loading that was achieved by using SCMS, and demonstrates the usefulness of the SCMS template particles for achieving significantly higher loadings than using conventional solid templates which do not have a mesoporous shell.

Example 5 – Crosslinking of Polymer Infiltrated into Mesoporous Shell and Template Removal

To stabilize the PAH assembled in the mesoporous shells, PAH-loaded SCMS particles (as prepared in Example 2) were dispersed in an aqueous solution of glutaraldehyde (GA, 5 mg L^{-1}) for 20 min. The covalent reaction between amines and aldehydes in aqueous solution at
25 room temperature is very efficient. After cross-linking and removal of glutaraldehyde, the SCMS particles were removed by exposure to a solution of 2M hydrofluoric acid / 8M ammonium fluoride buffer (pH 5) to obtain the polymer capsules. The capsules had a ξ -potential of +35mV, suggesting a considerable density of amine groups in the capsule shells. When visualized under an optical microscope, the resulting capsules were well-dispersed in
30 water and resembled the shape and size of the SCMS particles.

SEM revealed individual capsules from PAH-15k with a diameter of $\sim 370 \text{ nm}$ (Fig. 2b), which is $\sim 12\%$ smaller than the template particles used. The capsules preserve their structural integrity, and show significantly less folds and creases than those typically found in
35 conventional polymeric capsules (like a deflated balloons) prepared by the LbL procedure. This is likely caused by the small size of the capsules and the thick capsule shells.

Additionally, cross-linking the shell provides extra mechanical strength and enhances the stability of the capsules prepared.

TEM showed that the capsules have a homogeneous size distribution with a diameter of ~370 nm (Fig. 2c). The fine structures of the capsules were examined by TEM analysis of ultramicrotomed samples (~ 90 nm thin slices) of the capsules (Fig. 2d). The apparent heterogeneity of size (100-500 nm) is due to the slicing of the capsules through random sections. In addition, a slight increase in capsule size (~25%) is observed, which is likely caused by the swelling of the capsules during ultramicrotoming. It was also noted that the hollow capsules were permeable, as the resin used to set the hollow capsules before slicing permeated the capsules (Fig. 2d). At higher magnification, a uniform shell is clearly seen (Fig. 2d inset), confirming the structural integrity of the capsule shells. The PAH-15k capsule shell assembled by a one-step polymer infiltration into the permeable shell has a thickness of ~27 nm, which is about 12 times thicker than the average increment of a single PAH layer adsorbed on a planar or colloidal surface (~2.2 nm) deposited on nonporous particles using the typical LbL assembly method. The thickness of the capsule shells increases as PAH molecular weight decreases. For instance, the prepared capsules have a shell thickness of ~45 nm and ~16 nm when PAH with molecular weights of 5 000 Da and 70 000 Da were used, respectively. This is likely due to more efficient infiltration of the smaller species of PAH into the mesoporous shells.

Example 6 – Loading SCMS-Templated Polymer Capsules with Drugs

The robust capsules with thick shells make it possible to effectively load substances by two different modes: loading in the hollow cores and/or loading in the thick shells. To achieve high loading of drugs in the hollow cores, a high concentration of drug solution is required in the loading process. In this work, ibuprofen, a hydrophobic drug, dissolved in hexane (concentration of 60 mg mL⁻¹) is used as an example. TEM image of the ibuprofen-loaded capsule (Fig. 3a) clearly showed efficient filling of the core of the capsules. In addition, SEM (Fig. 3b) showed that the capsules were more spherical, which is in stark difference to the collapsed capsules showed in Figure 2b. The capsules also show an excellent capacity to sequester drugs from low concentration solutions because of their thick and porous shells, which provide high amount of active sites for affinity adsorption of drugs. For instance, vancomycin could be accumulated to a concentration of ~40 mg mL⁻¹ in the PAH-15k capsules, when the capsules were incubated in a diluted vancomycin aqueous solution with a concentration of 0.2 mg mL⁻¹. In addition, the high amount of functional groups (i.e., amine groups) available in the shells could provide abundant sites for further functionalization of the

capsules (e.g., to conjugate with drug molecules and to attach targeting ligands in the thick polymer shells).

Example 7 – SCMS-Templated Polypeptide Capsules

Capsules comprising of a single component of peptides were also prepared by the same procedure as outlined for PAH in Examples 2 and 5. Positively charged poly(L-lysine) (PLL, M_w 1000 - 4000 g mol⁻¹) was used in this work as an example. PLL capsules with a homogeneous size distribution were prepared by a one-step PLL assembly in the SCMS template, followed by GA cross-linking and removal of the silica template. TEM image of the PLL capsule showed a ring-like morphology, with a diameter of ~270 nm, representing ~36% shrinkage from the original template particles (Fig. 4a). The integrity of the capsules was confirmed by SEM, which showed a donut-like morphology (Fig. 4a, inset). The donut-like morphology is likely caused by the thicker capsule shells (~50 nm) of the PLL capsules compared with that of the PAH-15k capsules (~27 nm).

Example 8 – Crosslinking SCMS-Templated Capsules with Various Crosslinkers

This technique can be used to prepare capsules with various crosslinking means, which made the capsule properties largely tunable for different applications. For instance, Dimethyl pimelimidate dihydrochloride and Dimethyl 3,3'-dithiopropionimidate dihydrochloride are widely used biocompatible agents to crosslink proteins and peptides through the formation of amidine bonds with the amine groups. The formed amidine bond retains net charge character of the proteins and peptide to be crosslinked, and the Dimethyl 3,3'-dithiopropionimidate dihydrochloride can provide a cleavable disulfide bond in the capsule shells. Figure 4b is a TEM image of the PLL (M_w 40 000-60 000 Da) capsules with the use of Dimethyl 3,3'-dithiopropionimidate dihydrochloride as the crosslinker. The intact capsules showed a ring-like morphology with a diameter of ~400 nm and a shell thickness of ~40 nm.

Example 9 – SCMS-Templated Protein Capsules

To further demonstrate the general applicability of the process, protein capsules were also prepared. The first step of the preparation involved immobilizing positively charged proteins (i.e., lysozyme, 14 600 g mol⁻¹, 3-4.5 nm, pI 11) in the SCMS particles by physical adsorption. Then, low molecular weight poly(acrylic acid) (PAA, M_w 2000 g mol⁻¹) was infiltrated into the protein-loaded mesoporous shells, "bridging" the proteins by cross-linking the carboxyl groups (in PAA) and -NH₂ moieties (in lysozyme) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as we previously reported. Lysozyme capsules with a homogeneous size distribution were obtained after removal of the silica template. The lysozyme capsules had a diameter of ~330 nm (Fig. 4c), which

represents ~20% shrinkage from the original template particles. The capsules have homogeneous shells with thickness of ~40 nm, as evidenced by TEM analysis of ultramicrotomed samples (Fig. 4c, inset).

5 **Example 10 – SCMS-Templated Capsules Derived from Negatively-Charged Macromolecules**

The technique can also be extended to prepare capsules composed of only negatively charged macromolecules. To assemble negatively charged macromolecules in the mesoporous shells, the SCMS particles were modified with 3-aminopropyltriethoxysilane (APTS) to reverse their charge from negative to positive. This modification has previously been proven to be effective for the LbL infiltration of PEs in bimodal mesoporous silica particles in the preparation of nanoporous polyelectrolyte spheres. The APTS-modified SCMS particles had a ξ -potential of +49 mV (in Milli-Q water). The negatively charged polymers such as poly(L-glutamic acid) (PGA, M_w 1500 - 3000 g mol⁻¹), poly(methacrylic acid) (PMA, M_w 15 000 g mol⁻¹), and PAA (M_w 15 000 g mol⁻¹) were used as examples. The polymer was loaded in the mesoporous shells through incubation with APTS-modified SCMS particles dispersed in respective polymer solutions containing 0.1 M salt at pH 4.5 for 12 h. After removal of the excess polymers, 0.2 mL of aqueous 2,2'-diaminodiethyl disulfide dihydrochloride (cystamine) (5 mg mL⁻¹) and 0.2 mL of EDC solution (60 mg mL⁻¹) were separately added to the pellet and incubated at room temperature for 2 h. Cystamine is a small molecule (M_w 225 g mol⁻¹) with two amine groups at each end that can be used to associate with the carboxyl groups distributed along different polymer chains. The stability of the polymers loaded in the mesoporous shells can be enhanced via formation of stable amide bonds between the -COOH groups (in PGA, PMA, or PAA) and the two NH₂- end groups in cystamine in the presence of EDC. The advantage of using cystamine as "bridging" molecules comes from the cleavable disulfide bond in the middle of cystamine, which can be used to trigger the disassembly of the capsule shells (and hence release their cargo materials) in response to triggering agents (e.g., dithiothreitol, a thiol-disulfide exchange reagent) and/or protein-assisted thiol-disulfide exchange encountered within the reducing environment of cells.

After removal of the silica template, negatively charged capsules with a homogeneous size distribution were obtained. Capsules were prepared successfully from poly(glutamic acid) (PGA) crosslinked with cystamine, with a thick shell evident in the TEM measurements. The capsules also preserve their morphology upon drying, which is indicative of a relatively thick shell. PAA capsules (Fig. 5a) have a diameter of ~300 nm, which is ~30% smaller than the original template particles. The PAA capsules have a homogeneous shell with a thickness of

~35 nm, as determined by TEM analysis of ultramicrotomed samples (Fig. 5a, inset). Less shrinkage was observed with the PMA and PGA capsules, with the PMA capsules having a diameter of ~340 nm as observed by TEM (Fig. 5b). At higher magnification, it was also observed that the PMA and PGA capsules had less shell density and were less aggregated (after drying) than the PAA capsules.

Example 11 – SCMS-Templated PGA Capsules Incorporating a PGA-Drug Conjugate

Doxorubicin-conjugated PGA (PGA-Dox) was used to prepare macromolecular capsules incorporating a drug-conjugated polypeptide. Doxorubicin (Dox - Mw 580 g mol⁻¹), a model anticancer drug, was conjugated to the γ -COOH group of the PGA side chains using carbodiimide chemistry. The presence of a large number of γ -COOH groups in the PGA chains provides a suitable site on which to conjugate the drug, via the -NH_2 group present in Dox. The conjugation was performed in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), via the formation of an amide bond. Moreover, the drug loading can be controlled by adding a different amount of Dox to the PGA polymer chains. In this study, the results shown pertain to PGA with 10 % of the γ -COOH groups functionalized with Dox.

PGA-Dox conjugates were loaded into the mesoporous shells by incubating the conjugates with APTS-modified SCMS particles. Thereafter, the PGA-Dox conjugates were cross-linked using 2,2'-diaminodiethyl disulfide dihydrochloride (cystamine) in the presence of EDC. After removal of the SCMS template, negatively charged PGA/PGA-Dox nanocapsules with a homogeneous size distribution were obtained. Little shrinkage was observed with the PGA and PGA-Dox capsules, as is evident from the TEM (Fig. 6a) and SEM (inset, Fig. 6b) images of PGA-Dox capsules. The capsules are thick walled (~ 55 nm) and have an average diameter of ~370 nm. As visualized using fluorescence microscopy, the resulting capsules were well-dispersed in PBS (pH 7.4) and resemble the shape and size of the SCMS template particles.

Example 12 – Degradation of SCMS-Templated PGA Capsules Incorporating a PGA-Drug Conjugate

Because Dox-loaded PGA particles and capsules may have potential for drug delivery applications, preliminary degradation and release studies were then undertaken. PGA-Dox SCMS particles (hereafter referred to as PGA-Dox particles) and PGA-Dox capsules were extensively washed in PBS (pH 7.4), and then subjected to *in vitro* degradation and Dox release studies at conditions close to those within living cells. The physiological pH of the blood stream is approx. 7.4, while subcellular lysosomal compartments (endosomes and

lysosomes) of the tumor cells have reducing environments and possess several hydrolytic enzymes at highly acidic pH (pH ≤ 5.8). For a drug-delivery vehicle to be highly effective, it is desirable that the drug-delivery vehicles should not degrade readily in the blood stream; however they should be easily degraded and release their cargo after reaching the lysosomal compartments of the tumor cells. The PGA-Dox particles and capsules were therefore exposed to 10 mM carboxypeptidase at 37°C in 100 mM PBS (pH 5.8), under which conditions the carboxypeptidase should cleave the amide bonds in PGA-Dox, thereby releasing the Dox molecules into the solution. The time-dependent Dox release profile, when monitored for 48 h using fluorescence spectroscopy, suggests that the PGA-Dox capsules are degraded rapidly in the presence of carboxypeptidase, and therefore release Dox in a narrow time window (> 70 % Dox release in 16 h). However, significantly less Dox is released from PGA-Dox particles even after 48 h (< 20 % Dox release in 48 h) (Fig. 7a). The slow release profile of Dox from the particles can be attributed to the physical confinement of Dox molecules in the mesoporous silica pores, which restricts the access of the carboxypeptidase to the PGA-Dox conjugates. Further, insignificant amounts of Dox were released when both particles and capsules were exposed to 100 mM PBS at pH 7.4 for 48 h.

Example 13 – Application of SCMS-Templated PGA Capsules Incorporating a PGA-Drug Conjugate in Drug Delivery

The ability of PGA-Dox particles and capsules to cause death of LIM1215 colorectal tumor cells was investigated using the trypan blue cell viability assay. The cells were incubated with particles, capsules and several controls for 16 h (Fig. 7b). When equivalent amounts of PGA polymer (T2, Fig. 7b), PGA particles (T5, Fig. 7b) or PGA capsules (T6, Fig. 7b), were incubated with LIM1215 tumor cells, there was no significant impact on cell viability, suggesting that PGA is biocompatible in free polymer form as well as in the form of particles or capsules. Conversely, treatment of LIM1215 colorectal tumor cells with PGA-Dox particles (T7, Fig. 7b) and PGA-Dox capsules (T8, Fig. 7b) resulted in a significant decrease in the number of viable tumor cells. The PGA-Dox capsules were found to be more effective in eradicating the tumor cells (> 85 % cell death) than PGA-Dox particles (< 40 % cell death), which correlates well with the *in vitro* Dox-release studies. This suggests that the PGA-Dox conjugates confined in SCMS particles are not fully accessible to the subcellular hydrolytic enzymes, and this renders the release of Dox difficult. Conversely, the PGA-Dox chains in the capsules are readily accessible to subcellular enzymes, and therefore the release of Dox is facilitated by the action of hydrolytic enzymes present in the tumor cells. Interestingly, when LIM1215 tumor cells were treated with PGA-Dox polymer conjugates, insignificant tumor cell death was observed (T4, Fig. 7b). We speculate that the extremely high negative charge of the small PGA-Dox polymer chains restricts their uptake by the negatively charged cell

membranes (and hence reduced cell death). However, the PGA-Dox particles and capsules can be internalized into the tumor cells via endocytosis, due to their larger sizes. Although the equivalent or higher amount of free Dox (T3, Fig. 3b) is as efficient as PGA-Dox capsules (T8, Fig. 7b) in causing tumor cell death, Dox is known to cause high systemic toxicity when administered into animals in free form. The PGA-Dox capsules shown here provide an added advantage of controlled release, wherein the Dox molecules will be released only after the capsules reach the target tumor site, and therefore without causing any systemic toxicity. In addition, the remaining free -COOH groups of PGA-Dox capsules can be easily conjugated to targeting moieties, thereby enabling the PGA-Dox capsules to be targeted to various tumors.

The ability of PGA-Dox particles and capsules to internalize into LIM1215 colorectal tumor cells was further investigated using confocal laser scanning microscopy (CLSM) (Fig. 8). Confocal microscopy images of LIM1215 colorectal tumor cells incubated with PGA-Dox particles (Fig. 8a) and capsules (Fig. 8b) for 16 h show internalization of both particles and capsules, with nearly all the cells containing at least a few particles and capsules. Most of the internalized particles and capsules are taken up by the lysosomes, as is evident from the merged confocal images (Fig. 8a3 and b3), showing the co-localization of fluorescence signals arising from Dox (Fig. 8a1 and b1) and lysotracker blue, a lysosome staining dye (Fig. 8a2 and b2). The uptake of the PGA-Dox particles and capsules by lysosome would facilitate the drug-release, as was observed in the previous section.

Finally, it will be appreciated that various modifications and variations of the methods and compositions of the invention described herein will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are apparent to those skilled in the art are intended to be within the scope of the present invention.

CLAIMS

1. A process for preparing a macromolecular capsule, the process including:
 - a) contacting a template having an impermeable core contained within a permeable shell with a solution containing macromolecules under conditions wherein a proportion of the macromolecules infiltrate into the permeable shell;
 - b) removing a substantial proportion of the macromolecules not infiltrated into the permeable shell; and
 - c) crosslinking the macromolecules infiltrated into the permeable shell.
2. A process according to claim 1, further including the step of removing the template.
3. A process according to claim 1 or claim 2, wherein the template consists of an impermeable silica core and a permeable silica shell.
4. A process according to any one of claims 1 to 3, wherein the permeable shell contains pores.
5. A process according to claim 4, wherein the pores have an average pore size in the range of 2 nm to 30 nm.
6. A process according to claim 5, wherein the pores have an average pore size in the range of 2 nm to 15 nm.
7. A process according to claim 6, wherein the pores have an average pore size in the range of 2 nm to 10 nm.
8. A process according to claim 7, wherein the pores have an average pore size in the range of 2 nm to 6 nm.
9. A process according to any one of claims 1 to 8, wherein the templates have an average diameter of between 50 nm and 10 μ m.
10. A process according to claim 9, wherein the templates have an average diameter of between 100 nm and 2 μ m.

11. A process according to claim 10, wherein the templates have an average diameter of between 200 nm and 1 μ m.
- 5 12. A process according to claim 11, wherein the templates have an average diameter of between 200 nm and 600 nm.
13. A process according to any one of claims 1 to 12, wherein the permeable shell of the template has a thickness of between 10 and 800 nm.
- 10 14. A process according to claim 13, wherein the permeable shell of the template has a thickness of between 20 and 200 nm.
- 15 15. A process according to claim 14, wherein the permeable shell has a thickness of between 20 and 80 nm.
16. A process according to any one of claims 1 to 15 wherein the macromolecules are selected from the group consisting of chain growth polymers, step growth polymers, polyelectrolytes, proteins, polypeptides, polysaccharides, polynucleotides, deoxyribonucleic acid and ribonucleic acid, or a mixture thereof.
- 20 17. A process according to any one of claims 1 to 16 wherein the macromolecule is a polyamine.
- 25 18. A process according to claim 17 wherein the polyamine is selected from the group consisting of poly(allylamine hydrochloride), poly(ethyleneimine) and poly(vinylamine).
19. A process according to any one of claims 1 to 16 wherein the macromolecule is a poly(carboxylic acid).
- 30 20. A process according to claim 19 wherein the poly(carboxylic acid) is poly(methacrylic acid) or poly(acrylic acid).
21. A process according to any one of claims 1 to 16, wherein the macromolecule is a polypeptide.
- 35 22. A process according to claim 21, wherein the polypeptide is selected from the group consisting of poly(glutamic acid) and poly(lysine).

23. A process according to any one of claims 1 to 16, wherein the macromolecule is a protein.
- 5 24. A process according to claim 23 wherein the protein is selected from the group consisting of lysozyme, serum albumin, insulin, ribonuclease A, myoglobin, chymotrypsin, trypsin, chymotrypsinogen, hemoglobin, hexokinase, immunoglobulin G, RNA polymerase, DNA polymerase, apolipoprotein B, glutamate dehydrogenase, lipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins and
10 metalloproteins.
25. A process according to any one of claims 1 to 16, wherein the macromolecule is a polysaccharide.
- 15 26. A process according to claim 25 wherein the polysaccharide is selected from the group consisting of chitin, chitosan, cellulose, starch and glycogen.
27. A process according to any one of claims 1 to 26, wherein the macromolecule is biocompatible.
20
28. A process according to any one of claims 1 to 27, wherein the macromolecule is biodegradable.
29. A process according to any one of claims 1 to 28, wherein the macromolecule is
25 conjugated to a functional molecule.
30. A process according to claim 29, wherein the functional molecule is selected from the group consisting of magnetic resonance imaging contrast agents and biologically active species.
30
31. A process according to claim 30, wherein the biologically active species is selected from the group consisting of a drug, an antibody, an antigen, an oligonucleotide, a polysaccharide, a protein, an enzyme, a molecular recognition unit and a toxin.
- 35 32. A process according to claim 31, wherein the biologically active species is a drug.
33. A process according to claim 32, wherein the drug is doxorubicin.

34. A process according to any one of claims 1 to 33 wherein the solution containing the macromolecule is aqueous.
- 5 35. A process according to any one of claims 1 to 34 wherein the solution containing the macromolecule also contains an inorganic salt.
36. A process according to claim 35 wherein the inorganic salt is an alkali metal halide.
- 10 37. A process according to claim 36 wherein the alkali metal halide is selected from the group consisting of sodium chloride and potassium chloride.
38. A process according to any one of claims 1 to 37 wherein the solution containing the macromolecule also contains an acid.
- 15 39. A process according to claim 38 wherein the acid is an organic acid or an inorganic acid.
- 20 40. A process according to claim 39 wherein the acid is selected from the group consisting of hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid and acetic acid.
41. A process according to any one of claims 1 to 37 wherein the solution containing the macromolecule also contains a base.
- 25 42. A process according to claim 41 wherein the base is an alkali metal hydroxide.
43. A process according to claim 42 wherein the base is selected from the group consisting of sodium hydroxide and potassium hydroxide.
- 30 44. A process according to any one of claims 1 to 43, wherein the solution containing the macromolecule also contains a buffer.
- 35 45. A process according to any one of claims 1 to 44 wherein the substantial proportion of the macromolecules not infiltrated into the permeable shell are removed by filtration.
46. A process according to claim 45 wherein the removal by filtration includes:

- 5 a) passing the solution through a filter membrane whereon the templates infiltrated with macromolecules are retained while the solution containing the macromolecule passes through the membrane;
- b) rinsing the templates infiltrated with macromolecules retained on the filter membrane with a rinse solution;
- c) dispersing the templates in the rinse solution.
- 10 47. A process according to any one of claims 1 to 44 wherein the substantial proportion of the macromolecules not infiltrated into the permeable shell are removed by centrifugation.
- 15 48. A process according to claim 47 wherein the removal by centrifugation includes:
- a) centrifuging the solution to provide a concentrated pellet of the templates with infiltrated macromolecule and a supernatant solution containing excess macromolecule;
- b) removing the supernatant solution containing excess macromolecules;
- c) dispersing the concentrated pellet of templates infiltrated with macromolecules in a rinse solution;
- 20 d) centrifuging the rinse solution containing the templates infiltrated with macromolecules to provide a concentrated pellet of templates infiltrated with macromolecules and a supernatant solution of rinse solution;
- e) repeating steps (c) and (d) a further two times;
- f) dispersing the templates in the rinse solution.
- 25 49. A process according to any one of claims 45 to 48 wherein the rinse solution is water which optionally contains any of:
- a) an alkali metal halide salt;
- b) an acid selected from the group consisting of hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid and acetic acid;
- 30 c) a base selected from the group consisting of sodium hydroxide and potassium hydroxide; and/or
- d) a buffer.
- 35 50. A process according to any one of claims 1 to 49 wherein the crosslinking of the macromolecules infiltrated into the templates is performed by contacting the macromolecules infiltrated into the templates with a crosslinking agent.

51. A process according to any one of claims 1 to 49 wherein the crosslinking of the macromolecules infiltrated into the templates is performed by contacting the macromolecules infiltrated into the templates with crosslinking agent and an activating agent.
52. A process according to claim 51 wherein the activating agent is a carbodiimide.
53. A process according to claim 51 or claim 52 wherein the activating agent is 1-ethyl-3-(3-methylaminopropyl) carbodiimide hydrochloride.
54. A process according to any one of claims 50 to 53 wherein the crosslinking of the macromolecules infiltrated into the templates is achieved using a difunctional crosslinking agent.
55. A process according to claim 54 wherein the difunctional crosslinking agent is selected from the group consisting of ethylenediamine, glutaraldehyde, 1,6-hexanediamine, 1,4-butanediamine, 1,8-octanediamine, 1,4-butanediol diacrylate, 1,6-hexanediol diacrylate, 1,8-octanediol diacrylate, bis(N-hydroxysuccinimide esters of succinic acid, adipic acid, suberic acid and sebacic acid, succinoyl chloride, adipoyl chloride, suberoyl chloride and sebacoyl chloride.
56. A process according to claim 54 wherein the difunctional crosslinking agent contains a disulfide linkage.
57. A process according to claim 56 wherein the difunctional crosslinking agent containing a disulfide linkage is dimethyl 3,3'-dithiopropionimidate dihydrochloride or 2,2'-diaminodiethyl disulfide dihydrochloride.
58. A process according to any one of claims 50 to 53 wherein the crosslinking agent is polyfunctional.
59. A process according to claim 58 wherein the crosslinking agent is poly(acrylic acid) or poly(methacrylic acid).
60. A process according to any one of claims 50 to 59, wherein the macromolecule is modified to include moieties which react with the crosslinking agent.

61. A process according to claim 60, wherein the moieties included in the macromolecule react selectively with the crosslinking agent.
- 5 62. A process according to claim 61, wherein the moieties included in the macromolecule are alkyne groups and the crosslinking agent contains at least two azide groups.
63. A process according to claim 61, wherein the moieties included in the macromolecule are azide groups and the crosslinking agent contains at least two alkyne groups.
- 10 64. A process according to any one of claims 60 to 63, wherein the reaction between the moieties included in the macromolecule and the crosslinking agent occurs in the presence of a catalyst.
- 15 65. A process according to claim 64 wherein the catalyst is Cu(I).
66. A process according to any one of claims 1 to 49 wherein the crosslinking of the macromolecules infiltrated into the templates is performed by elevating the temperature of the templates with infiltrated macromolecules.
- 20 67. A process according to any one of claims 1 to 49 wherein the crosslinking of the macromolecules infiltrated into the templates is performed by exposing the templates infiltrated with macromolecules to ionizing radiation.
- 25 68. A process according to claim 67 wherein the ionizing radiation is selected from the group consisting of X-ray radiation, gamma radiation, ultraviolet radiation and microwave radiation.
- 30 69. A process according to any one of claims 1 to 68, wherein the template is removed by contacting the template with a solution that degrades the template.
70. A process according to claim 69, wherein the solution is an aqueous solution of hydrofluoric acid.
- 35 71. A process according to claim 70, wherein the solution is an aqueous solution of an alkali metal hydroxide.

72. A process according to claim 71, wherein the solution is an aqueous solution of sodium hydroxide or potassium hydroxide.
73. A macromolecular capsule prepared by the process of any one of claims 1 to 72.
- 5 74. A method of delivering a functional molecule to its location of operation, the method including: (a) preparing a macromolecular capsule by a process according to any one of claims 1 to 72, using macromolecules that are conjugated to said functional molecule; and (b); administering an effective amount of the capsules to a subject
- 10 containing the location of operation.
75. A method according to claim 74, wherein the functional molecule is selected from the group consisting of drugs, antibodies, antigens, oligonucleotides, polysaccharides, proteins, enzymes, molecular recognition units, magnetic resonance imaging contrast
- 15 agents and toxins.
76. A method of delivering a therapeutic agent to a living organism, the method including: (a) preparing a macromolecular capsule via the by a process according to any one of claims 1 to 72 using macromolecules that are conjugated to a functional molecule
- 20 selected from the group consisting of an antibody, a therapeutic agent and a mixture thereof; and (b); administering a therapeutically effective amount of the capsules to a the organism.
77. A process for preparing a macromolecular capsule according to claim 1 substantially
- 25 as hereinbefore described with reference to any one of the examples.

Figure 1

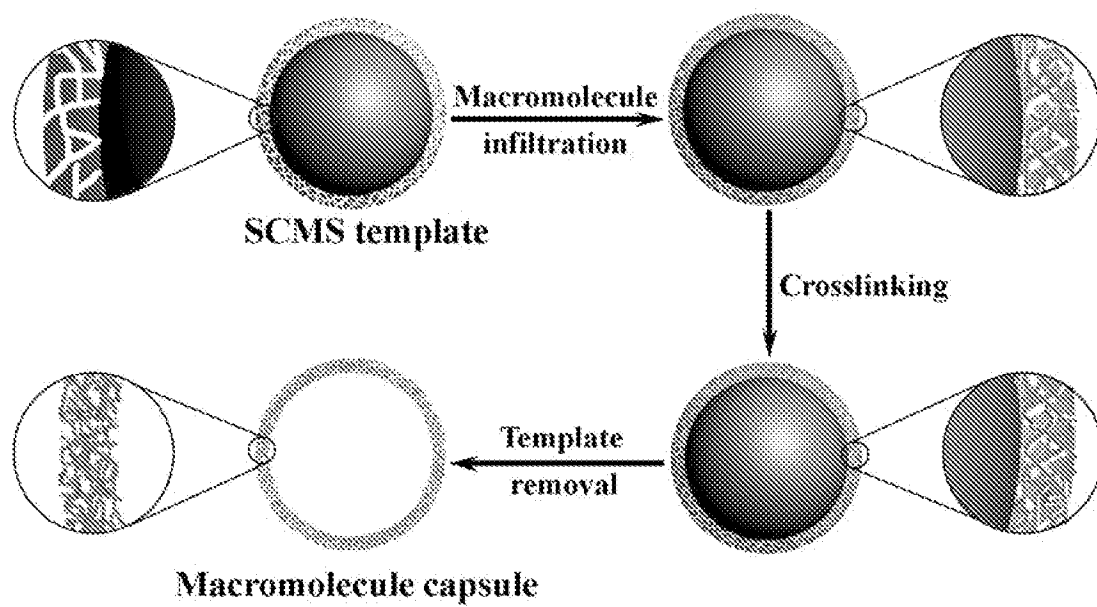


Figure 2

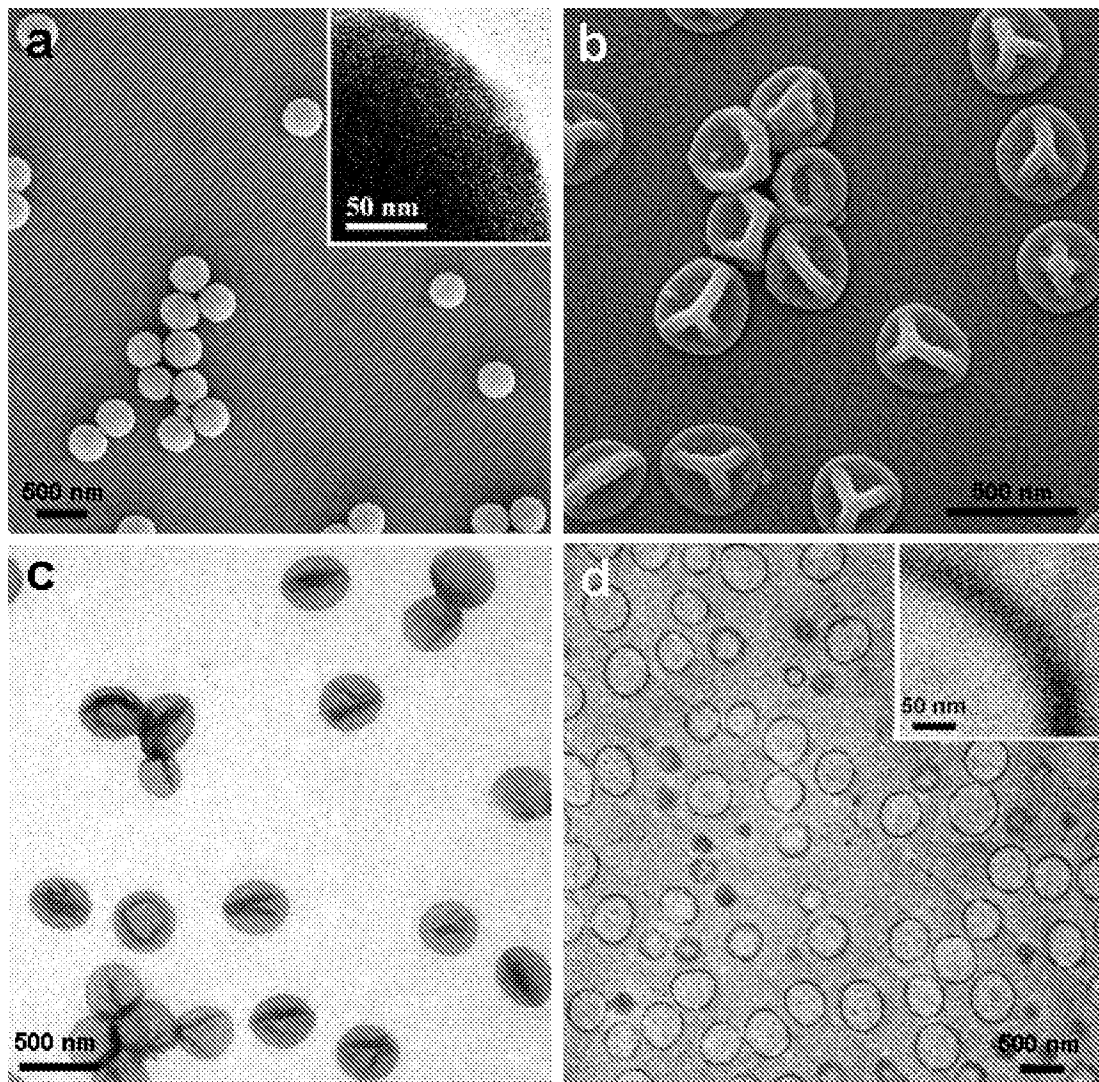


Figure 3

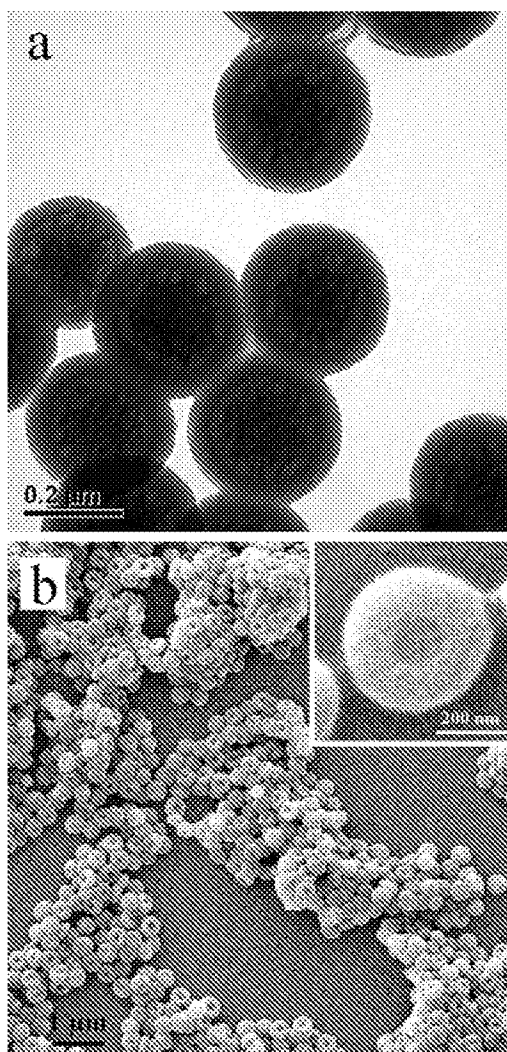


Figure 4

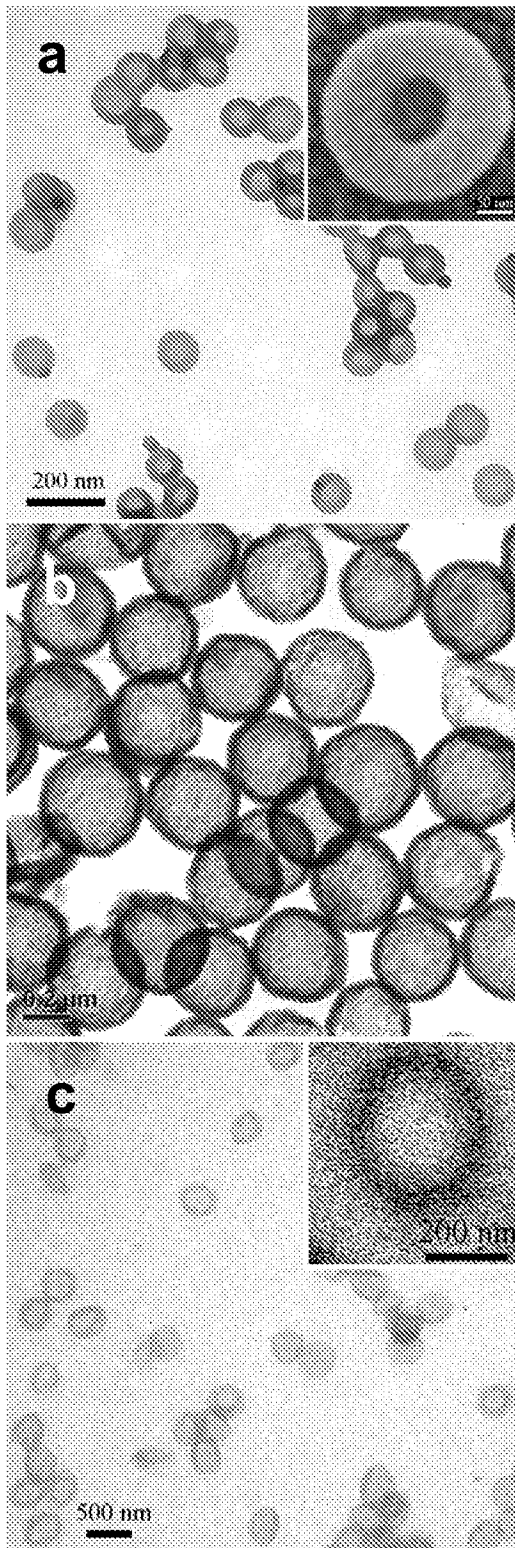


Figure 5

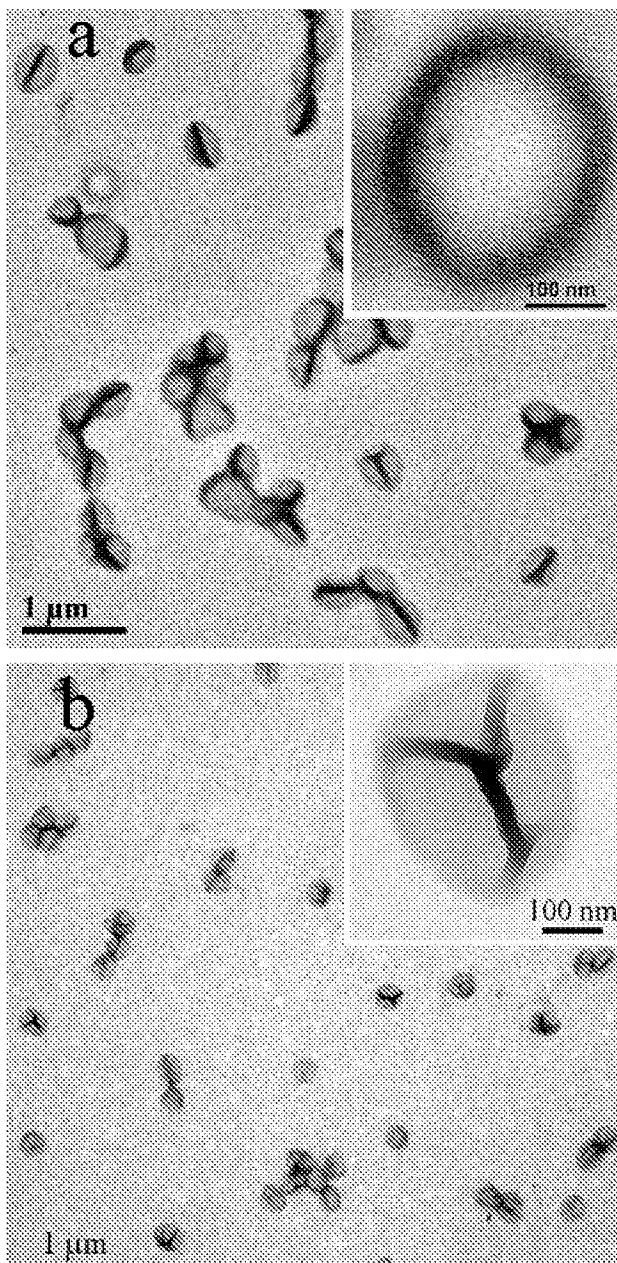


Figure 6

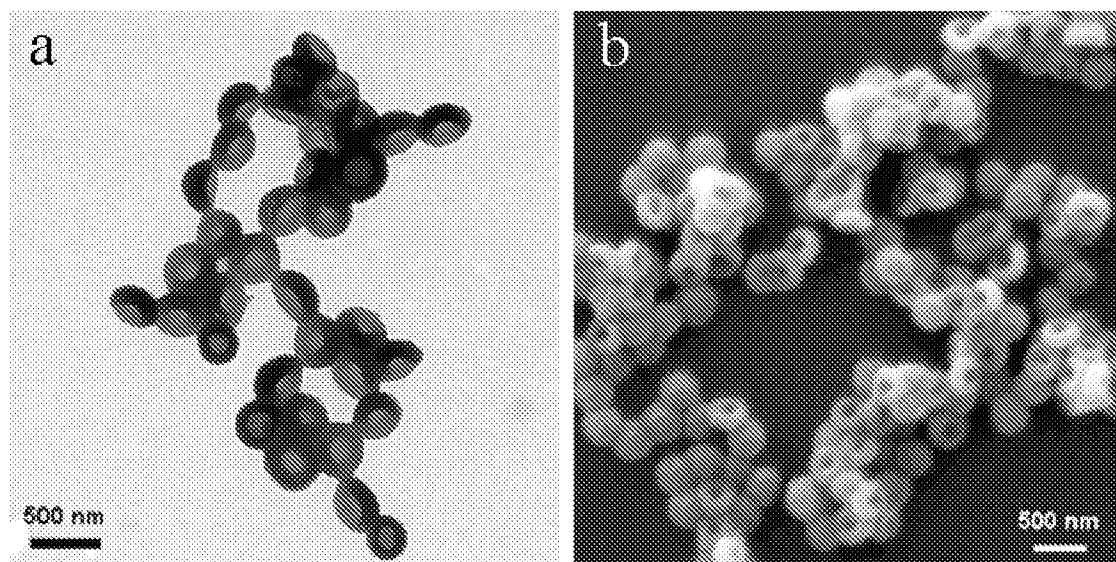


Figure 7

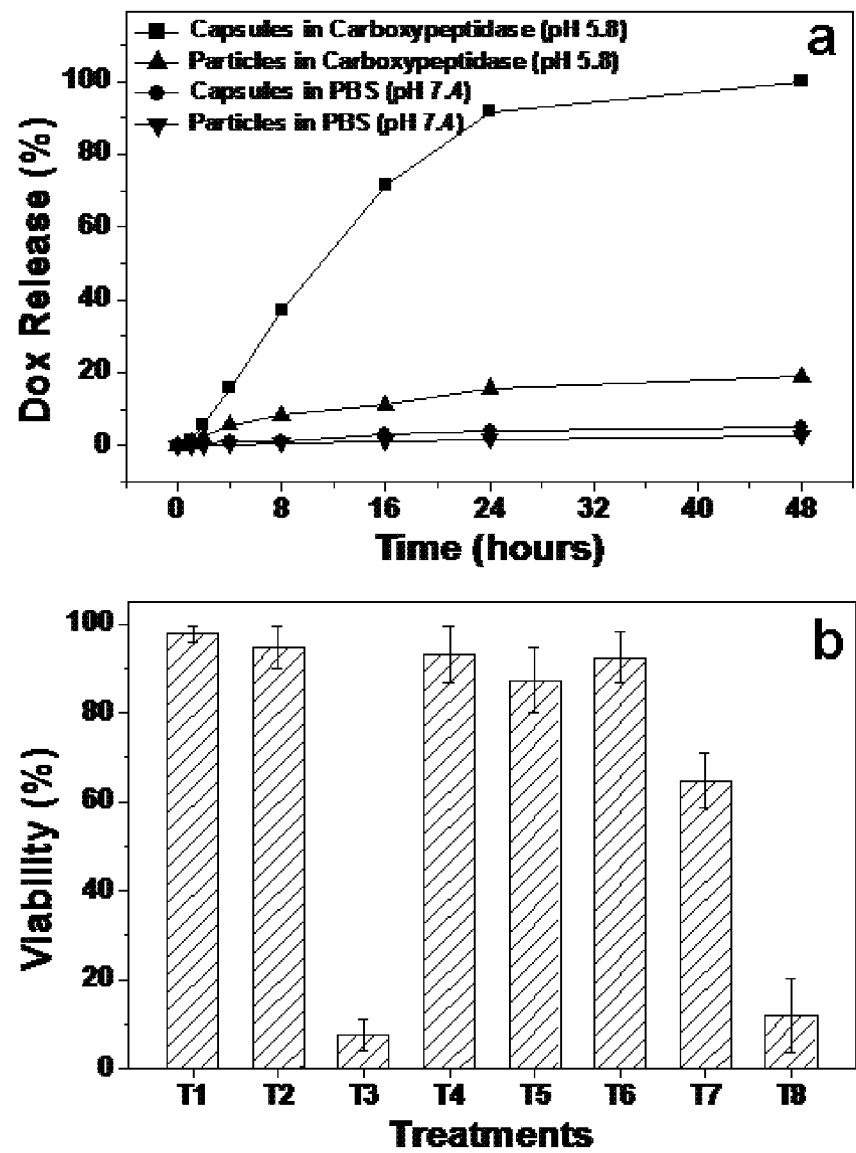
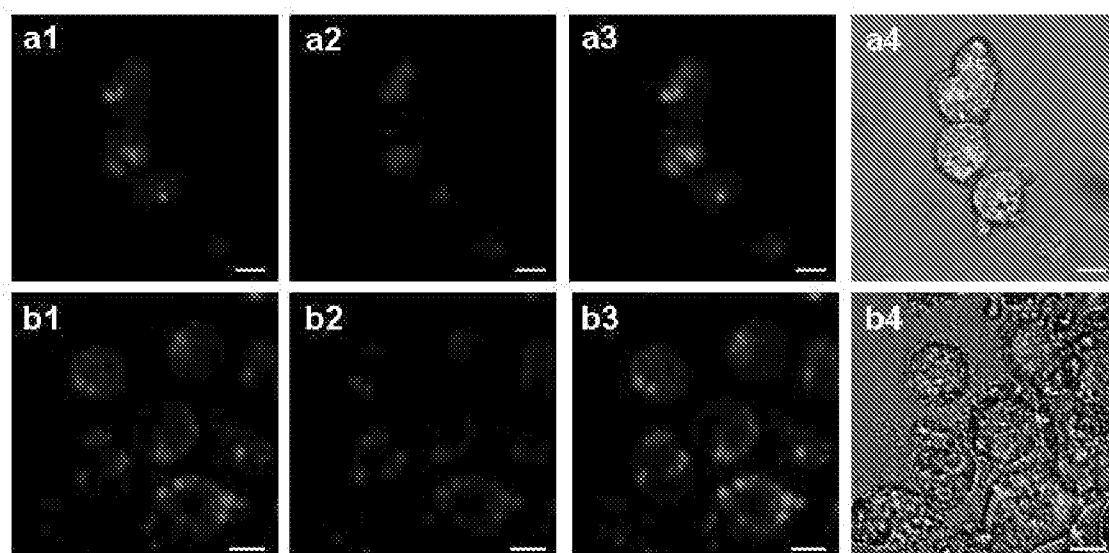


Figure 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/001862

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C08J 5/00 (2006.01)

A61K 9/51 (2006.01)

C08F 6/00 (2006.01)

A61K 9/50 (2006.01)

B82B 1/00 (2006.01)

C08J 9/00 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

WPI, EPODOC : capsule, template, core/shell, nano or micro and other related keywords

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,881,804 B1 (SELLERGREN et al) 19 April 2005 Whole document	1-77
A	US 6,602,932 B2 (FELDHEIM et al) 5 August 2003 Whole document	1-77



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 February 2009

Date of mailing of the international search report

19 FEB 2009

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2008/001862

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
US	6881804	AU	13204/01	EP	1268626	EP	1776949
		US	7393909	US	2005189291	WO	0132760
US	6602932	US	2002115747				
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							