

A Reversible Thermo-initiated System

Technical Field

The present invention generally relates to a reversible thermo-initiated system and a method for making the same. The disclosed thermo-initiated system may be used as a hydrogel for the sustained release of active agents.

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Background

Polymeric systems are being developed for various applications, such as hydrogels, which are particularly useful for the delivery of therapeutic biomolecules. Great efforts have been made to develop polymer systems that can exhibit desirable properties in response to changes in the temperature, pH and surrounding solution conditions.

A hydrogel, or a thermogelling copolymers system, is an aqueous polymer solution that exists as low viscosity fluids in the sol state at a low temperature and solidifies into a hydrogel at an elevated temperature (typically 37°C).

Thermogelling copolymers are amphiphilic polymers with finely tuned balance of hydrophobicity and hydrophilicity. At low concentrations, the copolymer chains self-assemble into nanostructures known as micelles. These micelles have the hydrophilic segments presented in the corona and the hydrophobic segments in the core. As the concentration of the micelles increase, the micelles are packed closer together. In order to trigger gelation of the system, a temperature change is induced. The critical concentration at which the thermogelling effect is observed is known as the critical gelation concentration (CGC). This temperature change affects the interactions present in the solution system,

namely, the polymer-polymer, polymer-solution and the solution-solution interactions. Ideally, at a critical gel point, physical cross-linking occurs in which the hydrophilic corona entangles with each other, holding the water molecules together and the hydrophobic segments act as network or crosslink points to hold the gel structure in place, presenting the macroscopic observation of a highly viscous semi-solid hydrogel.

To increase the temperature of the thermogel such that gelation is initiated, heat is applied directly to the thermogel. However, as the heat applied must be in direct contact with the thermogel, this greatly reduces the possible applications of thermogel since direct heating of the thermogel may not be possible in some applications. In addition, the direct application of heat is detrimental to heat sensitive surfaces covering the polymer solution, further limiting the potential applications possible with the thermogelling system. Further, in some cases, the thermogels are unable to revert back to the sol state, that is, the thermogels are irreversible. If the ability to alternate between the sol-gel-sol phases is essential, the irreversibility of the thermogel further limits the application of the thermogel.

There is therefore a need to provide a reversible thermo-initiated system that overcomes, or at least ameliorates, one or more of the disadvantages described above.

Summary

According to the disclosure, there is provided a reversible thermo-initiated system comprising a heat source comprising particles that act as heat initiators when excited by an electromagnetic source; and a plurality of micellar amphiphilic polymers that are capable of

aggregating when heated by said heat source and which revert back to micellar amphiphilic polymers when cooled.

According to a first aspect, there is provided a hydrogel comprising a reversible thermo-initiated system and an active agent, wherein the reversible thermo-initiated system comprises a heat source comprising particles that act as heat initiators when excited by an electromagnetic source and a plurality of micellar amphiphilic polymers that are capable of aggregating when heated by the heat source and which revert back to micellar amphiphilic polymers when cooled.

The reversible thermo-initiated system may be a reversible thermo-initiated sol-gel system. The reversible thermo-initiated system may be a reversible thermogel.

Advantageously, the particles may be used as absorbers to harvest and convert the energy delivered by the electromagnetic source to heat. This in turn heats up the system *in situ* such that the micellar amphiphilic polymers aggregates to form a gel.

Advantageously, the electromagnetic source can be applied at a distance. Hence, a heat source need not be applied directly to the system since the heat source is provided *in situ* in the form of the heat initiator particles. Accordingly, the gelation of the amphiphilic polymers can be triggered from a distance (that is remote triggered gelation), allowing the system to be applied in hard to reach areas.

Advantageously, an external heat source may not be required. The electromagnetic radiation is typically a light source which does not heat up the amphiphilic polymers on its own. The system generates heat from within based on the interactions between the electromagnetic radiation and the heat initiator particles to cause the gelation to occur.

According to the disclosure, there is provided a hydrogel comprising the thermo-initiated system as disclosed above and an active agent.

Advantageously, the hydrogel may be used as a drug
5 delivery system to deliver the active agent to a desired site *in vitro* or *in vivo*.

Due to the aqueous nature of the amphiphilic polymer solution (containing the particles therein), the solution may be administered to a target site by injection, without

requiring surgical implantation of a sustained release device. In addition, by passing through a syringe filter, the solution can be sterilized before administration. Further, the high water content of the gel matrix may
5 result in improved biocompatibility with the injection site.

Advantageously, by selecting biodegradable polymers as the amphiphilic polymers, the biodegradable polymers may be removed from a living organism when administered *in vivo* via natural excretion routes.
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Where the active agent is a peptide, the peptide/amphiphilic polymer formulations can be prepared under aqueous conditions without using peptide-denaturing organic solvents. This is advantageous for peptides
15 because formulations can be prepared in the sol state at low temperatures which reduces the denaturation rate of the peptides.

According to the disclosure, there is provided a method for forming a reversible thermo-initiated system comprising the steps of:
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- a. mixing an aqueous solution of micellar amphiphilic polymers with an aqueous solution of particles that act as heat initiators when excited by an electromagnetic radiation source; and
- 25 b. exciting the mixture with the electromagnetic radiation source to cause the particles to absorb electromagnetic radiation and generate heat, thereby causing micellar amphiphilic polymers to crosslink to form a gel.

30 According to a second aspect, there is provided a method for forming a hydrogel comprising a reversible thermo-initiated system and an active agent comprising the steps of:

- a. mixing an aqueous solution of micellar
35 amphiphilic polymers with an aqueous solution of

particles that act as heat initiators when excited by an electromagnetic radiation source; and

5 b. exciting the mixture with the electromagnetic radiation source to cause the particles to absorb electromagnetic radiation and generate heat, thereby causing micellar amphiphilic polymers to crosslink to form a gel.

Definitions

10 The following words and terms used herein shall have the meaning indicated:

The term "reversible" is defined as the ability of the amphiphilic polymers to aggregate and dissociate reversibly under suitable reaction conditions, wherein the chemical structures of the amphiphilic polymers are not
5 substantially altered before and after aggregation.

The term "aggregate" or grammatical variants thereof refers to the clustering of the micellar amphiphilic polymers, which is usually associated with a phase change of the reversible thermo-initiated system from a sol state
10 to a gel state.

The term "dissociate" then refers to the dispersion of the aggregates such that the aggregated micellar amphiphilic polymers disengage from each other, forming isolated micelles.

15 The term "sol" refers to a colloidal sol comprising one or more components that are dispersed in a continuous liquid phase.

The term "gel" refers to a non-fluid colloidal network or polymer network that is expanded throughout its
20 whole volume by a fluid. Where the gel contains a polymer network, the polymer network is formed through the physical aggregation of polymer chains that result in regions of local order acting as the network junction points. Where the regions of local order are thermally
25 reversible, the resulting swollen network is termed as a reversible thermogel.

The term "gelation temperature" refers to the temperature at which a thermogel forms. Unless otherwise indicated, the gelation temperature may be measured using
30 the vial inversion method.

The word "polymer" or "polymeric" refers to a molecule having two or more monomeric repeat units. It includes linear and branched polymer structures, and also encompasses cross-linked polymers as well as copolymers

(which may or may not be cross-linked), thus including block copolymers, alternating copolymers, random copolymers, and the like. An "amphiphilic polymer" refers to a polymer with hydrophobic and hydrophilic moieties, monomers or blocks.

The term "thermo-initiated system" refers to a polymeric system which responds to temperature changes. The thermo-initiated system may refer to a thermogel which is able to form a gel when the temperature of the system is at or above a gelation temperature and which reverts back to a sol or solution when the temperature of the system falls below the gelation temperature.

The term "photothermal effect" or "photothermal heating" refers to the change in thermal state of the system due to the absorption of electromagnetic radiation.

The term "hydrogel" is used in the conventional sense to refer to a water-swallowable polymeric system that can absorb a substantial amount of water to form an elastic gel.

The term "micellar formation" is used herein to refer to the self-assembly of amphiphilic polymeric chains into nanostructures known as micelles. These micelles have the hydrophilic segments presented in the corona and the hydrophobic segments in the core.

The term "active agent" is used herein to refer to a chemical/biological/biochemical material or compound suitable for administration to a human patient and that induces a desired beneficial effect, e.g., exhibits a desired pharmacological activity. The term includes, for example, agents that are therapeutically effective, prophylactically effective, and cosmetically (and cosmeceutically) effective. Also included are derivatives and analogs of those compounds or classes of compounds specifically mentioned which also induce the desired

beneficial effect. The active agent may be selected from the group consisting of proteins, antibodies, peptides, small-molecule drugs, nucleic acid-based drugs, nanoparticulate systems and mixtures thereof.

5 Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

10 As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value,
15 even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for
20 convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within
25 that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for
30 example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure

also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material
5 is specifically recited herein.

Detailed Disclosure of Optional Embodiments

Exemplary, non-limiting embodiments of a reversible thermo-initiated system will now be disclosed.

10 The reversible thermo-initiated system comprises a heat source comprising particles that act as heat initiators when excited by an electromagnetic source; and a plurality of micellar amphiphilic polymers that are capable of aggregating when heated by said heat source and
15 which revert back to micellar amphiphilic polymers when cooled.

The reversible thermo-initiated system may be a reversible thermo-initiated sol-gel system. The reversible thermo-initiated system may be a reversible thermogel.

20 The particles may act as heat initiators when excited by an electromagnetic source. When the particles interact with the electromagnetic radiation, the coherent collective oscillation of electrons in the conduction band induces large surface electric fields which greatly
25 enhance the radiative properties when they interact with the resonant radiation.

Advantageously, the particles may have an absorption peak that matches with the wavelength of the electromagnetic source such that the electrons oscillating
30 in the electromagnetic source collide with the surrounding lattice of atoms, rapidly dissipating the energy of the electromagnetic source. Hence, the electromagnetic radiation will be absorbed and converted into heat through the photothermal effect. The heat then dissipates into the

surrounding, and the rise in temperature causes the amphiphilic polymers to aggregate with each other, resulting in a phase change (thereby forming a gel). The strong absorption of the particles may ensure effective heating at relatively lower energies, thereby rendering the possibility of remote or non-contact heating. In addition, the particles have high photostability and do not suffer from photobleaching.

The wavelength of the absorption peak of the particles may be tuned by altering their size, shape, composition and surface coating. The particles may have an absorption peak in the range selected from the group consisting of about 100 nm to about 1,000 nm, about 200 nm to about 1,000 nm, about 300 nm to about 1,000 nm, about 100 nm to about 900 nm, about 200 nm to about 900 nm, about 300 nm to about 900 nm, about 300 nm to about 800 nm, about 400 nm to about 1,000 nm and about 400 nm to about 800 nm. The absorption peak of the nanoparticles may be preferably in the range of about 400 nm to about 800 nm.

The size of the particles may be in the micron-range or in the nano-range. Where the size of the particle is in the micron-range, the particle is termed as a microparticle and where the size of the particle is in the nano-range, the particle is termed as a nanoparticle.

Where the particle is a nanoparticle, the size of the nanoparticle may be in the range of about 1 nm to about 100 nm, about 10 nm to about 100 nm, about 20 nm to about 100 nm, about 30 nm to about 100 nm, about 40 nm to about 100 nm, about 50 nm to about 100 nm, about 10 nm to about 90 nm, about 10 nm to about 80 nm, about 20 nm to about 80 nm or about 30 nm to about 80 nm. The size of the nanoparticles may be in the range of about 30 nm to about 60 nm.

The shape of the particle is not limited and may include rods, reefs, boxes, fibers, tubes, cups and spheres. Where the particle is a nanoparticle, the shape of the nanoparticle may be selected from the group
5 consisting of metal nanorods, nanoreefs, nanoboxes, nanofibres, nanotubes, nanocups and nanospheres.

The particles may be selected from the group consisting of metal particles and carbon particles.

The metal of the metal particles may be a noble
10 metal. The noble metal may be selected from the group consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, mercury, rhenium, copper and alloys thereof. Here, noble metallic nanoparticles are chosen because: 1) preparation of the well dispersible
15 metallic nanoparticles is relatively simple; 2) the small size of metallic nanoparticles offers great potential for *in vitro* and *in vivo* applications; 3) the noble metallic nanoparticles are resistant to oxidation under ambient condition, which allows the direct contact of the metallic
20 surface with surrounding media. Through mechanisms such as chemisorptions (e.g. Au-S bonding), electrostatic attraction or hydrophobic interaction, the particle surface can be modified with desired molecules in a controlled manner; 4) noble metallic nanoparticles have
25 unusual optical properties (especially surface plasmon resonances (SPR)), wavelength of the absorption maximum of the nanocrystals can be easily tuned by altering their sizes, shapes, composition and surface coating; and 5) the biosafety of metallic nanoparticles such as gold
30 nanoparticles is well known and their medical usage in the human body has been approved by the health regulatory agencies such as the US Food and Drug Administration (FDA).

The carbon particles may be selected from the group consisting of carbon tubes, graphene particles and graphene oxide particles.

Where the particle is a silver nanoparticle, the
5 absorption maximum of the nanoparticle may be in the range of about 400 nm to about 420 nm. Where the particle is a gold nanoparticle, the tunable absorption maximum of the nanoparticle may be in the range of about 510 nm to about 600 nm. Where the particle is a gold-silver alloyed
10 nanoparticle, the tunable absorption maximum of the nanoparticle may be in the range of about 410 nm to about 510 nm. Where the particle is a gold nanorod, the transverse plasmonic peak may be in the range of about 500 nm to about 540 nm and with tunable longitudinal plasmonic
15 peak in the range of about 600 nm to about 800 nm. Where the particle is a gold nanoshell or gold nanocage, the absorption maximum may be that of near-infrared wavelength, or about 750 nm to about 1400 nm. Where the particle is a carbonaceous species, the particle may have
20 a broad band absorption. Hence, by selecting the desired absorption maximum via selecting the type of particle and the shape of the particle, the reversible thermo-initiated system may be able to respond to a wide range of electromagnetic radiation wavelengths. For example, by
25 alloying silver into gold nanocrystal, the absorption maximum can be shifted into the blue region while increasing the size or changing the shape of the gold nanocrystals shift the absorption maximum into the red region. As such, by embedding the particles into the
30 thermogelling polymer solutions, the formed nanocrystal-polymer composites are enabled to respond to low power laser with a broad range of wavelengths.

It was advantageously found that the rate of aggregating (and hence gelation) varies with the

concentration of the particles. The higher the concentration of the particles, the shorter the time required for gelation due to the greater amount of heat energy produced by the particles upon excitation by the
5 electromagnetic source.

The concentration of the particles may be selected from the range of about 0.1 ppm to about 100 ppm, about 0.5 ppm to about 100 ppm, about 1 ppm to about 100 ppm, about 5 ppm to about 100 ppm, about 10 ppm to about 100
10 ppm, 10 ppm to about 80 ppm, 10 ppm to about 60 ppm, about 20 ppm to about 100 ppm, about 30 ppm to about 100 ppm, about 20 ppm to about 80 ppm, about 15 ppm to about 60 ppm or about 1 ppm to about 50 ppm. The concentration of the nanoparticles may be selected from the range of about 15
15 ppm to about 60 ppm.

Upon excitation by an electromagnetic source, the particles absorb the electromagnetic radiation from the electromagnetic source and convert the electromagnetic radiation to heat. The heat produced is then transferred in
20 situ from the particles to the amphiphilic polymers, thereby leading to a temperature increase in the entire system, causing the temperature-responsive micellar amphiphilic polymers to aggregate and form a gel. When the electromagnetic source is removed, the temperature of the
25 system decreases and the entire system cools, resulting in a phase change from the gel back to sol. Advantageously, the presence of the particles in the system allows the heating of the thermo-initiated system via remote means, thereby circumventing the need for direct heating. In
30 addition, the gelation may be reversible when the temperature of the system is decreased.

The concentration of the amphiphilic polymer may be selected from a range about 0.5 wt% to about 30 wt%, about 1 wt% to about 30 wt%, about 2 wt% to about 30 wt%, about

3 wt% to about 30 wt%, about 5 wt% to about 30 wt%, about 7 wt% to about 30 wt%, about 0.5 wt% to about 20 wt%, about 0.5 wt% to about 15 wt%, about 0.5 wt% to about 10 wt%, about 1 wt% to about 20 wt% or about 10 wt% to about 30 wt%. The concentration of the amphiphilic polymer may be selected from a range of about 1 wt% to about 20 wt%.

The amphiphilic polymer may be selected from the group consisting of block copolymers, graft copolymers and homopolymers. The amphiphilic polymer may be biodegradable or non-biodegradable.

The amphiphilic polymer may comprise monomers selected from the group consisting of diols, urethanes, esters, hydroxyesters, carboxylic acids, lactones, carbonates, siloxanes, alkylenes, amides, oxazolines, imines, alcohols, lactams, aminos and combinations thereof. The amphiphilic polymers may be selected from the group consisting of polyols, polyethylene glycol/poly(lactic-co-glycolic) acid, PLURONIC® and TETRONIC® from BASF. The structure of the polymers may be selected from the group consisting of poly(PEG/PPG/X) urethane, X-PEG-X, PEG-X-PEG, X-PEG, X-g-PEG and PEG-g-X, where g refers to graft and X may be selected from the group of poly(hydroxybutyrate), poly(lactic acid), poly(caprolactone), poly(ester)s, poly(carbonate)s, poly(siloxane), poly(ethylene butylene), poly(amide)s, poly(propylene glycol), poly(butylene glycol), poly(ethylene butylene), poly(2-ethyl-2-oxazoline), polyethylenimine, poly(methacrylic acid), poly(vinyl alcohol), polyvinylpyrrolidone, polyallylamine, and combinations thereof.

The molecular weight of the polymers may be selected from the group consisting of about 1000 g/mol to about 1,000,000 g/mol, about 1000 g/mol to about 5000 g/mol, about 1000 g/mol to about 10,000 g/mol, about 1000 g/mol

to about 50,000 g/mol, about 1000 g/mol to about 100,000 g/mol, about 1000 g/mol to about 500,000 g/mol, about 5000 g/mol to about 1,000,000 g/mol, about 10,000 g/mol to about 1,000,000 g/mol, about 50,000 g/mol to about 1,000,000 g/mol, about 100,000 g/mol to about 1,000,000 g/mol or about 500,000 g/mol to about 1,000,000 g/mol,

The electromagnetic source may have a wavelength that matches an absorption peak of the nanoparticles so that excitation may occur. The electromagnetic source may have a wavelength selected from the group consisting of about 100 nm to about 1,000 nm, about 200 nm to about 1,000 nm, about 300 nm to about 1,000 nm, about 100 nm to about 900 nm, about 200 nm to about 900 nm, about 300 nm to about 900, about 300 nm to about 800 nm, about 400 nm to about 1,000 nm and about 400 nm to about 800 nm. The wavelength of the electromagnetic source may be in the visible light region, or in the range of about 400 nm to about 800 nm or about 390 nm to about 700 nm.

The heating of the thermo-initiated system may be carried out via remote means, and the distance of the electromagnetic source from the system may not be particularly limited and may be selected from the group consisting of about 1 cm, about 2 cm, about 3 cm, about 4 cm, about 5 cm, about 7 cm, about 9 cm, about 10 cm, about 12 cm, about 14 cm, about 16 cm, about 18 cm, about 20 cm, about 25 cm, about 30 cm, about 35 cm, and about 40 cm.

An advantage of the reversible thermo-initiated system is that it may allow for active agents to be incorporated into the system, which allows for the controlled incorporation and release of a variety of different active agents from the thermo-initiated system.

As the temperature of the thermo-initiated system decreases, the aggregated micellar amphiphilic polymers dissociate or revert back to the (isolated) micellar

amphiphilic polymers when cooled. When this occurs, the gel phase reverts to the sol (or solution) phase.

Exemplary, non-limiting embodiments of a hydrogel comprising the reversible thermo-initiated system and an
5 active agent will now be disclosed.

The active agent may be selected from the group consisting of DNA, proteins, antibodies, peptides, small-molecule drugs, nucleic acid-based drugs, nanoparticulate systems and mixtures thereof.

10 Advantageously, the reversible thermo-initiated system may not require the use of organic solvents that may have a denaturing effect on one or more of the active agents incorporated into the system.

The hydrogel may also include conventional additives
15 such as fillers, preservatives, pH regulators, softeners, thickeners, pigments, dyes, refractive particles, stabilizers, toughening agents, detackifiers, pharmaceutical agents, and permeation enhancers.

Where the active agent is a biologically active agent
20 or a therapeutic agent, the agent/polymer formulation can be prepared by mixing the copolymer solution with the agent in a sol state. This is followed by a syringe injection of the formulation to a target site to induce the formation of a hydrogel upon exposure to body
25 temperature. When formed, the *in situ* formed hydrogel acts as a sustained drug delivery depot. A typical target site can be the subcutaneous layer or muscle. If the active agent is a cell, the cell may also be incorporated into the gel system. When the cells proliferate at the target
30 site, they can be manipulated into a three dimensional tissue in the shape of the *in situ* formed hydrogel.

Exemplary, non-limiting embodiments of a method for forming a reversible thermo-initiated system will now be disclosed. The method comprises the steps of:

a. mixing an aqueous solution of micellar amphiphilic polymers with an aqueous solution of particles that act as heat initiators when excited by an electromagnetic radiation source; and

5 b. exciting the mixture with the electromagnetic radiation source to cause the particles to absorb electromagnetic radiation and generate heat, thereby causing micellar amphiphilic polymers to crosslink to form a gel.

10 A laser source may be used as an electromagnetic source to excite the mixture. This occurs when the laser source, which has a wavelength that matches an absorption peak of the nanoparticles, is applied to the mixture. The power of the laser source may be controlled to vary the
15 temperature of the resultant solution, thereby controlling the rate of aggregating (and hence gel formation). Advantageously, the time taken for gelation decreases as the power of the laser source increases.

20 The type of laser used may be selected from the group consisting of diode laser, portable laser, optical fiber and coupled laser.

25 The power of the laser source may be selected from a range of about 100 mW to about 5 W, about 500 mW to 4 W, about 100 mW to about 5 W, about 100 mW to about 4 W, about 100 mW to about 3 W, about 100 mW to about 2 W, about 100 mW to about 1 W, about 500 mW to 5 W, about 1 W to about 5 W, about 2 W to about 5 W, about 3 W to about 5 W or about 4 W to about 5 W. The power of the laser source may be in the range of about 500 mW to 4 W.

30 The time taken for aggregating (or gelation) is typically from about 1 to about 60 minutes. Suitable time taken for aggregating (or gelation) may be a value selected from the group consisting of: 1 minute, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes,

30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes and 60 minutes.

The gelation may be carried out at a temperature of about 27°C to about 40°C. The temperature for gelation may be selected from the group consisting of: about 27°C, about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, and about 40°C.

The concentration of the aqueous solution of particles may be selected to thereby control the rate of aggregating (or gelation).

The method may further comprise the step of cooling the gel to reverse the aggregating and thereby obtain the aqueous mixture (or sol).

Brief Description Of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1 is a process flowchart showing the formation of a thermogel.

Fig. 2 is a transmission electron microscopy (TEM) image at a scale bar of 50 nm of gold nanoparticles that are formed in accordance with Example 1.

Fig. 3 is a graph showing the absorption spectra of gold nanoparticles, gold nanoparticles in sol and gold nanoparticles in gel.

Fig. 4(A) is a photograph of transparent and flowable sol of Pluronic polymer and gold nanoparticles before laser irradiation. Fig. 4(B) is a photograph of a semi-

solid gel of Pluronic polymer and gold nanoparticles formed after laser irradiation.

Fig. 5(A) is a photograph showing the Pluronic polymer and gold nanoparticles sol in a bottle. Fig. 5(B) is a photograph showing the flowability of the Pluronic polymer and gold nanoparticles sol when the bottle was turned 180°. Fig. 5(C) is a photograph showing the Pluronic polymer and gold nanoparticles gel after laser irradiation in the up-right bottle.

Fig. 6 is a graph showing the relationship between gold nanoparticles concentration and the time for the polymer sol to form a gel under laser irradiation at a power of 500 mW.

Fig. 7 is a graph showing the relationship between laser irradiation power and the time for the polymer sol containing 60 ppm of 50 nm gold nanoparticles to form a gel.

Fig. 8 is a graph showing the representative dynamic rheological analysis of poly(PEG/PPG/PHB urethane) with gold nanoparticles, 5 wt% aqueous solution as a function of temperature.

Fig. 9(a) is a graph showing the representative dynamic rheological analysis of poly(PEG/PPG/PHB urethane), 3 wt% aqueous solution as a function of frequency at temperature of 10°C. Fig. 9(b) is a graph showing the representative dynamic rheological analysis of poly(PEG/PPG/PHB urethane), 3 wt% aqueous solution as a function of frequency at temperature of 37°C. The respective pictures above the graphs serve to confirm the rheological measurements in which the sample at 10°C exhibits liquid properties while the sample at 37°C behaves like a gel.

Detailed Description of Drawings

Referring to Fig. 1, there is shown a process flowchart 100 showing the formation of a thermogel from a blend 6 of amphiphilic polymer 2 and heat initiators 4.

5 The blend 6 is prepared as a solution of the amphiphilic polymer 2 and heat initiators 4 in the form of colloidal particles. The blend 6 is then exposed to a heat source 8. After a period of exposure, the blend 6 starts to heat up to form a heated blend 12, which then undergoes
10 almost instantaneous gelation to form a gel 14. In order to revert to the liquid phase, the temperature of the gel 14 can be reduced until the gel 14 forms the blend 6.

Examples

15 Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

20 Example 1 - Synthesis of Thermogel

The process of Fig. 1 was used here to form the thermogel.

Synthesis of gold nanoparticles

25 Here, gold nanoparticles were used as the metal initiators 4.

50 nm gold nanoparticles (obtained from Sigma-Aldrich of Missouri of the United States of America) were prepared with a conventional seed growth method. First, uniform
30 gold nanoparticles of about 13 nm in diameter were prepared by rapidly injecting 5 mL of 38.8 mM sodium citrate solution into 50 mL 1 mM boiling HAuCl_4 aqueous solution (sodium citrate and HAuCl_4 solutions were obtained from Sigma-Aldrich of Missouri of the United States of

America) with vigorous stirring. After 15 minutes, the heating mantle was removed, allowing the solution to cool to room temperature. The gold nanoparticles of about 13 nm diameter were used as seeds for the seeded growth of gold nanoparticles of 50 nm in diameter.

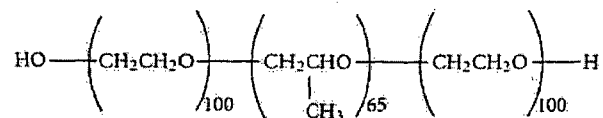
In a typical preparation of 50 nm gold nanoparticles, 250 mL of 0.3 mM freshly prepared HAuCl_4 aqueous solution was first heated to boil, followed by sequential injection of 2.25 mL as-prepared seed solutions (13 nm gold nanoparticles) and 1.12 mL of 38.8 mM sodium citrate solution with vigorous magnetic stirring. The whole solution was then boiled for 30 minutes. 10 mL of 38.8 mM sodium citrate solution was the injected as an extra stabilizer into the above boiling solution, and boiled for another hour.

The obtained gold nanoparticles were characterized by using a Philip CM300 transmission electron microscope (TEM) (from Philips of the Netherlands) operating at an accelerating voltage of 300 kV. Fig. 2 shows the TEM image of the gold nanoparticles, showing that the diameter of the gold nanoparticles is about 50 nm.

The UV-vis absorption spectrum of the colloidal gold nanoparticles was recorded using a Shimadzu UV-1800 spectrometer (from Shimadzu Corporation of Japan). The UV-vis absorption spectrum of the colloidal gold nanoparticles is shown in Fig. 3.

Preparation of Blend

A blend 6 was prepared using a mixture of gold nanoparticles and Pluronic® F127 as the amphiphilic polymer 2. Pluronic® F127 is a block copolymer based on ethylene oxide and propylene oxide and has the following structure



20 mg of Pluronic[®] F127 polymer was dissolved in 1 mL as-prepared 50 nm colloidal gold nanoparticles (60 ppm) to form a sol. The concentration of the gold nanoparticles (60 ppm) was calculated based on the sol, that is, after the gold nanoparticles were mixed into the polymer solution. The UV-vis absorption spectrum of this sol was also measured using the same spectrometer as mentioned above, with the spectrum as shown in Fig. 3. Based on the absorption spectra of the 50 nm gold nanoparticles, it was found that a suitable wavelength to match the absorption peak falls at 532±10 nm. Hence, green light at a wavelength of 532 nm (continuous wave, power tunable 500 mW to 4W) with a beam size of about 3 mm was selected as the light source.

The above composite blend was cooled to 7°C and subjected to 500 mW 532 nm laser irradiation. The whole solution became a gel after 3 minutes of irradiation, with an increase in temperature until 30°C. The UV-vis absorption spectrum of this gel was also measured using the same spectrometer as mentioned above, with the spectrum as shown in Fig. 3. As shown in Fig. 3, the absorption spectrum of the nanoparticles was not affected by the formation of the gel.

The heating effect on light absorption in gold nanoparticles is a result of electron dynamics in their metallic lattices. When gold nanoparticles are under the irradiation of light, some effects will occur: 1) surface scattering of electron, 2) electron-phonon coupling, and 3) electron-electron thermalization. As the electron density in gold is very high, the electron-electron

interactions are strong enough to thermalize the electron gas during the laser irradiation. When the gold nanoparticles are irradiated by light, the electrons absorb the photon energy, leading to a nonequilibrium
5 temperature difference between the electron gas and the metallic lattices. The electron-photon collisions then cause the energy exchange between the electron subsystem and the lattice, enabling the thermal equilibrium. Since the thermal conductivity of gold itself is very high, heat
10 generated under the laser irradiation diffuses to surrounding media leading to the heating up of the whole solution. When gold nanoparticles are blended with the thermal-responsive polymer sol, once irradiated by laser, the gold nanoparticles will convert the absorbed light to
15 heat, thus increasing the system temperature, enabling the gelation.

Fig. 4 and Fig. 5 are photographs showing the sol before laser irradiation and gel after laser irradiation. As shown in Fig. 4(A), the sol is transparent and flowable
20 while in Fig. 4(B), the sol became a semi-solid gel after laser irradiation, and does not flow when the bottle is tilted at an angle. In order to reverse the gelation, the gel was simply cooled to room temperature for 2 minutes to revert back to the sol state (Fig. 4(A)).

25 Fig. 5(A) is a photograph of the sol in the bottle before laser irradiation, which flows when the bottle is rotated 180° as shown in Fig. 5(B). After laser irradiation, the bottle is rotated upright and as can be seen, the gel is not able to flow to the bottom of the
30 bottle.

This Example illustrates that the gold nanoparticles are the key to convert the absorbed light into heat to trigger the gelation.

Example 2 - Concentration of Gold Nanoparticles

The effect of changing the concentration of the gold nanoparticles was investigated in this example.

The process to form the sol was the same as that described in Example 1, except that the concentrations of the gold nanoparticles used were 45 ppm, 30 ppm and 15 ppm. The various sols were then subjected to the same 500 mW 532 nm laser irradiation as in Example 1 and the gelation times were measured. Table 1 below and Fig. 6 show the gelation times for the various sols as a result of the various gold nanoparticles concentrations.

Table 1: Gelation times as a result of differing gold nanoparticles concentrations

Sol	Concentration of gold nanoparticles (ppm)	Gelation time (minutes)
1	60	3
2	45	5
3	30	8
4	15	10

From Table 1 and Fig. 6, it was observed that the higher the concentration of the gold nanoparticles, the faster the gelation.

Example 3 - Laser Power

The effect of changing the laser power was investigated in this example.

The process to form the sol was the same as that described in Example 1, except that the laser power used during irradiation were 1 W and 2 W, and the gelation times were measured. Table 2 below and Fig. 7 shows the gelation times for the various sols.

Table 2: Gelation times as a result of differing laser power

Sol	Laser power (mW)	Gelation time (minutes)	Final temperature (°C)
1	500	3	30
5	1000	2	31
6	2000	1.5	32

From Table 2 and Fig. 7, it was observed that increasing the laser power results in a faster gelation of the sol.

Example 4 - Synthesis of Thermogel using poly(poly(ethyleneglycol)/(polypropylene glycol)/polyhydroxybutyrate urethane)

Poly(PEG/PPG/PHB urethane)s were synthesized from polyhydroxybutyrate (PHB)-diol, poly(ethyleneglycol (PEG), and polypropylene glycol (PPG) with molar ratios of PEG/PPG fixed at 2:1 and PHB content ranging from 5 to 20 mol % (calculated from the M_n of PHB-diol) using hexamethylene diisocyanate (HDI) as a coupling reagent. The amount of HDI added was equivalent to the reactive hydroxyl groups in the solution. Typically, 0.064 g of PHB-diol (M_n 1070, 6.0×10^{-5} mol), 1.44 g of PEG (M_n 1890, 7.6×10^{-4} mol), and 0.82 g of PPG (M_n 2180, 3.8×10^{-4} mol) were dried in a 250-mL two-neck flask at 50°C under high vacuum overnight. Then, 20 mL of anhydrous 1,2-dichloroethane was added to the flask, and any trace of water in the system was removed through azeotropic distillation with only 1 mL of 1,2-dichloroethane being left in the flask. When the flask was cooled down to 75 °C, 0.20 g of HDI (1.2×10^{-3} mol) and two drops of dibutyltin dilaurate (8×10^{-3} g) were added sequentially. The reaction mixture was stirred at 75°C under a nitrogen atmosphere for 48 hours. The resultant copolymer was

precipitated from diethyl ether and further purified by redissolving into 1,2-dichloroethane followed by precipitation in a mixture of methanol and diethyl ether to remove remaining dibutyltin dilaurate. A series of
5 poly(PEG/PPG/PHB urethane)s with different compositions of PHB were prepared. The yield was 80% and above after isolation and purification.

To form the gel, the process of Example 1 was used here, but with the polymer being poly(PEG/PPG/PHB
10 urethane)s instead of Pluronic® F127 polymer.

The obtained gels formed from the thermogelling copolymers were subjected to rheological studies to determine the physical properties of the gels (see Fig. 8 and Fig. 9).

15 First, the rheological profile of the gels as a function of temperature was determined. In Fig. 8, the rheological profile of the thermogel as a function of temperature is presented.

At the start of the experiment at low temperatures,
20 the solution was not very viscous and existed in a liquid-like state. Here, the loss modulus, G'' was higher than the storage modulus, G' . When the temperature was raised, both the values of the storage modulus and the loss modulus began to rise. At a certain temperature, the storage
25 modulus became higher than the loss modulus. The crossover temperature was noted and this was denoted as the sol-gel transition temperature.

Fig. 9 shows the sol-gel-sol transition process of the copolymers as the temperature increased. At 10°C, the
30 polymer solution existed as a low-viscosity sol that flowed freely in the vial. At 37°C, a non-flowable gel was obtained. This material did not flow even when the sample vial was inverted.

Fig. 9 shows the dynamic rheology of the thermogel at 10 and 37°C. At 10°C, the sample behaved like a liquid, the frequency sweep of the sample revealed that the G'' was much greater than G' and both moduli were strongly dependent on frequency. When the temperature was raised to 37°C, solid-like behavior was observed with G' greater than G'' , and both moduli being independent of frequency. This gel behaved as a permanent gel, it did not flow even when the sample vial is inverted. Thus, dynamic rheology confirmed the thermogelling transition in this sample.

The gel can be reversed by again cooling the temperature of the gel to reinstate the sol state.

Applications

Due to the presence of the particles in the reversible thermo-initiated system, remote triggering of the gelation using an electromagnetic source is possible such that the reversible thermo-initiated system can be applied to any location, even into a body, as long as the system can be triggered remotely by the electromagnetic radiation. Hence, heat induced damage as a result of direct heat application is negated, increasing the flexibility in the use of such a system.

The reversible thermo-initiated system may be used in temperature-responsive biodegradable systems for drug delivery, tissue engineering, wound healing or injectable tissue engineering scaffolds to promote tissue repair and/or regeneration. The reversible thermo-initiated system may be useful in other materials in related applications where controlled release of multiple active agents is desired, for example, in biosensors, detoxification, DNA delivery, etc.

The temperature responsive nature of the amphiphilic polymer may be tuned to induce an *in situ* gelation at

physiological temperature to provide controlled drug release. In controlled release drug delivery, the reversible thermo-initiated system may be combined with a therapeutic agent in the solution state and injected into
5 the tissue target.

The system may be reversible leading to the recyclability and reuse of the system as desired.

The system may also be used in face masks in sun tan machines for facial treatments, in diagnostics, in
10 optical-controlled valves/switches, high viscosity formulation under the effect of sun rays, remote actuators (for example, gelation induces the formation of a semi solid form which prevents flow), coatings for warming effect without using heat or in military applications.

15 It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such
20 modifications and adaptations come within the scope of the appended claims.

CLAIMS

1. A hydrogel comprising a reversible thermo-initiated system and an active agent, wherein said reversible
5 thermo-initiated system comprises:

a heat source comprising particles that act as heat initiators when excited by an electromagnetic source; and

10 a plurality of micellar amphiphilic polymers that are capable of aggregating when heated by said heat source and which revert back to micellar amphiphilic polymers when cooled.

2. The hydrogel of claim 1, wherein said particles
15 is selected from the group consisting of metal particles and carbon particles.

3. The hydrogel of claim 2, wherein the metal of said metal particles is selected from the group
20 consisting of ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, gold, mercury, rhenium, copper and alloys thereof.

4. The hydrogel of claim 2, wherein said carbon
25 particles are selected from the group consisting of carbon tubes, graphene particles and graphene oxide particles.

5. The hydrogel of any one of the preceding
30 claims, wherein said particles have an absorption peak in the range selected from the group consisting of 100 nm to 1,000 nm, 200 nm to 1,000 nm, 300 nm to 900, 400 nm to 900 nm and 400 nm to 800 nm.

35 6. The hydrogel of any one of the preceding claims, wherein said particles have a concentration in the range selected from the group consisting of 0.1 ppm to

100 ppm, 0.5 ppm to 100 ppm, 1 ppm to 100 ppm, 5 ppm to 100 ppm, 10 ppm to 100 ppm, 20 ppm to 100 ppm, 30 ppm to 100 ppm, 1 ppm to 50 ppm and 15 ppm to 60 ppm.

5

7. The hydrogel of any one of the preceding claims, wherein said particles have a size in the micron-sized range or in the nano-sized range.

10 8. The hydrogel of claim 7, wherein said particles are nanoparticles and the size of said nanoparticles is in the range selected from the group consisting of 1 nm to 100 nm, 10 nm to 100 nm, 20 nm to 100 nm, 30 nm to 100 nm, 40 nm to 100 nm, 50 nm to 100 nm, 10 nm to 90 nm, 10
15 nm to 80 nm, 20 nm to 80 nm and 30 nm to 80 nm.

9. The hydrogel of any one of the preceding claims, wherein said amphiphilic polymer is selected from the group consisting of block copolymers, graft
20 copolymers and homopolymers.

10. The hydrogel of any one of the preceding claims, wherein said amphiphilic polymers comprise monomers selected from the group consisting of diols,
25 urethanes, esters, hydroxyesters, carboxylic acids, lactones, carbonates, siloxanes, alkylenes, amides, oxazolines, imines, alcohols, lactams, aminos and combinations thereof.

30 11. The hydrogel of any one of the preceding claims, wherein the concentration of said amphiphilic polymer is in the range selected from the group consisting of 0.5 wt% to 30 wt%, 1 wt% to 30 wt%, 5 wt% to 30 wt%, 10 wt% to 30 wt% and 0.5 wt% to 20 wt%.

35

12. The hydrogel of any one of the preceding claims, wherein the electromagnetic source has a wavelength that matches an absorption peak of said nanoparticles.

5

13. The hydrogel of claim 1, wherein said active agent is selected from the group consisting of DNA, protein, antibody, peptide, small-molecule drug, nucleic acid-based drug, enzyme inhibitor, antibiotic, chemotherapeutic agent, anti-inflammatory agent, analgesic and mixtures thereof.

14. A method for forming a hydrogel comprising a reversible thermo-initiated system and an active agent comprising the steps of:

a. mixing an aqueous solution of micellar amphiphilic polymers with an aqueous solution of particles that act as heat initiators when excited by an electromagnetic radiation source; and

b. exciting said mixture with said electromagnetic radiation source to cause said particles to absorb electromagnetic radiation and generate heat, thereby causing micellar amphiphilic polymers to crosslink to form the hydrogel.

15. The method of claim 14, comprising the step of using a laser source to excite said mixture.

16. The method of claim 15, comprising the step of selecting the power of said laser source to thereby control the aggregating rate.

17. The method of claim 14 or 15, comprising the step of selecting the wavelength of said laser source to match an absorption peak of said particles.

5 18. The method of any one of claims 14 to 17, comprising the step of selecting the concentration of said particles to thereby control the aggregating rate.

10 19. The method of any one of claims 14 to 18, wherein said exciting step (b) is carried out for 1 to 60 minutes.

15 20. The method of any one of claims 14 to 18, comprising the step of cooling said hydrogel to reverse said aggregating and thereby obtain said aqueous mixture.