

Description

Title of Invention: Systems and Methods for Fabricating Nanoparticles

5 Technical Field

The present invention generally relates to the field of nanotechnology and nanoparticles. The present invention also generally relates to a system and method for the high-throughput synthesis of highly uniform nanoparticles.

10 Background Art

The field of nanotechnology is an exciting one, as certain substances exhibit new and interesting properties only on the nano-scale. These new properties, for example absorption and emission of unexpected wavelengths of electromagnetic radiation, or the propensity to accumulate in tumour tissue, are not observed when
15 the substances are in their bulk forms.

Numerous nano-compounds with useful properties have been developed, and have found their way into everyday life, for instance, the use of quantum dots in the manufacture of television displays. Nanomedicine is another area, with anticancer drugs being incorporated into nanostructures for more efficient delivery to tumour
20 sites.

A significant drawback is that many such nano-compounds and nanomaterials cannot be dissolved in water due to their high hydrophobicity. In such situations, a protective polymeric shell may be introduced to form stable encapsulated nanoparticles of various sizes for biological applications. However, the current
25 methods to transfer compounds from organic solvents to water are non-universal, resulting in nanoparticles with low levels of uniformity, throughput and reproducibility. Hence, the capability to synthesize highly-reproducible batches of precisely engineered, water-dispersible nanoparticles is advantageous for the safe

manufacture of drugs and for obtaining regulatory approval from the relevant authorities.

Bulk nanoprecipitation has been used for preparing nanoparticles in batch-type reactors. Even though such batch processing is a simple way to produce
5 nanoparticles, it has various drawbacks, such as lack of industrial scalability, batch-to-batch variance, and poor control of nanoparticle size. These problems can be ascribed to discrepancies in synthesis conditions and improper control of the mixing process during preparation, which results in the production of nanoparticles with a broad size distribution, diminishing their effectiveness and usability.

10 Recently, continuous synthesis using microfluidic 2D and 3D hydrodynamic focusing chips has garnered some attention, as the process exhibits tunable physiochemical properties and efficient mixing due to a high surface-area-to-volume interaction. However, a high level of precision and expertise is required in the fabrication of such microfluidic devices. Also, microfluidic systems exhibit
15 production scale issues due to the necessity to operate at low flow rates as a consequence of their micro-scale.

Millifluidic systems such as the confined impinging jet mixer, multi-inlet vortex mixer and co-axial jet mixer have also been used for preparing nanoparticles. However, specialized micromachining is required in fabricating these systems, and
20 these systems generally do not generate the high mixing intensity required to encapsulate large-sized molecules into polymeric shells, which is becoming increasingly important as research progresses. Therefore, a high-throughput system which can generate ultra-high-intensity mixing is needed in order to encapsulate a diverse range of hydrophobic components into polymeric shells for the synthesis of
25 a wide variety of nanoparticles with unique applications to meet the growing needs of society.

Consequently, there is a need to provide a system and method which will overcome, or at least reduce the impact of one or more of the disadvantages described above, and which addresses the need for the ability to rapidly and efficiently synthesize a
30 broad range of highly uniform nanoparticles.

Summary

According to a first aspect of the present disclosure, there is provided a system for fabricating nanoparticles, the system comprising:

- a mixing zone;
- 5 a first conduit configured to permit a flow of first solvent along a first flow direction to the mixing zone;
- a second conduit configured to permit a flow of second solvent to the mixing zone, wherein:
 - the second conduit extends coaxially into the first conduit;
 - 10 the second conduit comprises an opening substantially orthogonal to the first flow direction, and wherein said opening is configured to introduce a flow of said second solvent at the mixing zone in a second flow direction;
- wherein the second flow direction is substantially orthogonal to the first flow direction; and
- 15 wherein at least one of the first solvent or second solvent contains nanoparticle precursors.

In another aspect of the present disclosure, there is provided a method of fabricating nanoparticles, the method comprising:

- 20 (i) supplying a flow of first solvent in a first flow direction through a first conduit to a mixing zone;
- (ii) supplying a flow of second solvent through a second conduit to a mixing zone, wherein the second conduit extends coaxially into the first conduit for introducing the flow of second solvent within the flow of the first solvent,
- 25 wherein at least one of the first solvent or second solvent contains nanoparticle precursors;
- (iii) introducing the flow of second solvent within the flow of first solvent in a second flow direction, wherein the second flow direction is substantially orthogonal to the first flow direction; and
- 30 (iv) mixing the flow of first solvent and flow of second solvent to form a mixed stream comprising a nanoparticle dispersion based on the nanoparticle precursors.

In a further aspect of the present disclosure, there is provided a use of the system disclosed herein in the method disclosed herein.

In another aspect of the present disclosure, there is provided nanoparticles produced by the method disclosed herein.

5 Advantageously, the disclosed systems and methods may result in high intense mixing of first solvent and second solvent. This high intense mixing may be generated through cross-current mixing of the first and second solvents. The cross-current mixing of the first and second solvents may generate high turbulence in the system. The cross-current mixing is a result of introducing the flow of second solvent within the flow of first
10 solvent in a substantially orthogonal direction to the first flow direction. This high intense or turbulent mixing may advantageously result in the synthesis of nanoparticles with high uniformity for all size ranges.

Further advantageously, the disclosed systems and methods may result in high throughput of nanoparticles to meet industrial requirements.

15 Also, advantageously, the disclosed systems and methods may easily be optimized by adjusting solvent properties to encapsulate large molecules which are difficult to achieve with traditional methods. Due to the high intense mixing generated by the disclosed systems and methods, together with being able to adjust the properties of the solvents used, large sized conjugated polymer molecules may easily be encapsulated
20 inside polymers approved by the Food and Drug Administration of the United States of America (FDA) for fluorescence and photoacoustic imaging, photodynamic and photothermal therapy applications.

The disclosed systems and methods may also advantageously provide high versatility by allowing the fabrication of more than a hundred varieties of nanoparticles for different
25 industrial and research applications. For the production of core-shell nanoparticles, the disclosed systems and methods may advantageously be used to customize the shell and core.

The disclosed systems and methods also advantageously allow for the optimization of Reynolds number to obtain different particle sizes. This may advantageously result in
30 tight control over the size of nanoparticles for different biological applications.

The disclosed systems and methods further advantageously allow the interplay between operating parameters like Reynolds number and solvent properties which may be altered simultaneously to achieve the best operating zone for the synthesis of different varieties of nanoparticles for various applications.

- 5 The disclosed systems and methods also advantageously allow for enhanced mixing for the synthesis of polymeric nanoparticles with tailored size, high uniformity, high reproducibility and high production.

Definitions

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

As used herein, the term “anti-solvent” refers to a poor solvent for the substance in question which when added to a solution of the substance, causes the substance to precipitate or crystallize.

- 15 As used herein, the term “axial”, “axially”, or “coaxially” refers to a direction along or substantially parallel to a central axis. Furthermore, as used herein, the term “radial” or “radially” refers to a direction substantially perpendicular to the central axis.

As used herein, the terms, the terms “orthogonal” and “perpendicular” are used interchangeably and refer to an angle of 90° to a central axis.

- 20 As used herein, the terms “substantially orthogonal” and “substantially perpendicular” include absolute orthogonal/perpendicular alignment (i.e. an angle of 90° to a central axis) and deviations of 0.1° to 20° from the perpendicular alignment.

- 25 As used herein, the term “conduit” refers to any structure that is capable of conveying fluid from one point to another.

As used herein, the term “mixing zone” refers to a volume or spatial region where two solutions or fluids can be mixed. In particular, the mixing zone can include an initial mixing of at least two flows or streams (e.g., where a second stream is

initially introduced into a first stream) and any extended volume where the two streams continue to mix together.

As used herein, the terms “stream” and “flow” are used interchangeably and refer to a moving volume of fluids.

- 5 The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

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.

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, 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 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 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 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 subgeneric 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 is specifically recited herein.

Detailed Disclosure of Embodiments

- 5 In the past decades, nanotechnology research has offered solutions to a diverse range of sectors of significance to human prosperity, such as healthcare, energy, environment and electronics. Researchers have greatly advanced this field and successfully developed numerous novel compounds with superior properties, except that many compounds cannot be dissolved in water due to high hydrophobicity.
- 10 This may be addressed by introducing a protective polymeric shell to form stable and different sized nanoparticles for biological applications.

Nanoprecipitation is one of the facile techniques for the preparation of such polymeric shell nanoparticles encapsulating hydrophobic molecules. However, the current systems to transfer compounds from organic solvents to water are non-

15 universal, which lead to the production of nanoparticles with low levels of uniformity, throughput and reproducibility. The capability to synthesize highly reproducible batches of precisely engineered, water dispersible nanoparticles is one of the important parameters for the safe manufacture of drugs and for obtaining regulatory approval.

- 20 Nanoprecipitation is a solvent displacement method where the polymer and core components are dissolved in an organic solvent that is miscible with water. Swift injection of an organic solvent mixed into an antisolvent causes spontaneous formation of nanoparticles. The interplay of hydrophobic and hydrophilic forces between the core and shell aids the formation of stable micelles in the aqueous
- 25 environment. Bulk nanoprecipitation is one way to synthesize nanoparticles by using solvent displacement technique in batch type reactors. Even though batch process is a simple way to produce nanoparticles, it has various drawbacks, such as industrial scalability, batch to batch variance and poor control of nanoparticle size. These problems can be ascribed to discrepancy in synthesis conditions and

improper control of the mixing process during preparation, which result in the production of nanoparticles with broad size distribution.

In this invention, the inventors have developed an advanced and highly versatile technology which overcome the above-mentioned problems for encapsulating a large variety of hydrophobic compounds (for example, small molecule dyes, anti-cancer drugs, inorganic nanoparticles and conjugated polymers) into polymeric nanoparticles of controllable size with high throughput and high reproducibility. The high intensity in the system is generated due to its unique cross-current mixing of inner and outer solvents. This system is highly versatile, and parameters (such as Reynolds number and properties of solvent) may be adjusted for customizing the size of nanoparticles in a wide range with high uniformity.

The present invention may advantageously aid in upscaling production for a wide varieties of nanoparticles, together with tight control over the size of nanoparticles and with high uniformity and high reproducibility.

Exemplary, non-limiting embodiments of a method and system for fabricating nanoparticles according to the present invention will now be disclosed.

System for Fabricating Nanoparticles

The present invention provides a system for fabricating nanoparticles, the system comprising:

- a mixing zone;
- a first conduit configured to permit a flow of first solvent along a first flow direction to the mixing zone;
- a second conduit configured to permit a flow of second solvent to the mixing zone, wherein:
 - the second conduit extends coaxially into the first conduit;
 - the second conduit comprises an opening substantially orthogonal to the first flow direction, and wherein said opening is configured to introduce a flow of said second solvent at the mixing zone in a second flow direction;

wherein the second flow direction is substantially orthogonal to the first flow direction; and

wherein at least one of the first solvent or second solvent contains nanoparticle precursors.

- 5 The opening on the second conduit may be substantially orthogonal to the first flow direction of the first solvent, wherein said opening is configured to introduce a flow of second solvent at the mixing zone in a second flow direction which is substantially orthogonal or perpendicular to the first flow direction. The second conduit may comprise an opening at a position 90° to the first flow direction. The position of the
- 10 opening may be about 90° from a central axis of the first flow direction, or may deviate about 0.1° to about 20° from this perpendicular alignment. The deviation may be about 0.1° to about 20°, about 1° to about 20°, about 2° to about 20°, about 3° to about 20°, about 4° to about 20°, about 5° to about 20°, about 10° to about 20°, about 15° to about 20°, about 0.1° to about 15°, about 0.1° to about 10°, about 0.1° to about 5°, about 0.1°
- 15 to about 4°, about 0.1° to about 3°, about 0.1° to about 2°, about 0.1° to about 1°, or about 0.1°, about 0.2°, about 0.3°, about 0.4°, about 0.5°, about 0.6°, about 0.7°, about 0.8°, about 0.9°, about 1.0°, about 1.1°, about 1.2°, about 1.3°, about 1.4°, about 1.5°, about 1.6°, about 1.7°, about 1.8°, about 1.9°, about 2.0°, about 2.1°, about 2.2°, about 2.3°, about 2.4°, about 2.5°, about 2.6°, about 2.7°, about 2.8°, about 2.9°, about 3.0°,
- 20 about 3.1°, about 3.2°, about 3.3°, about 3.4°, about 3.5°, about 3.6°, about 3.7°, about 3.8°, about 3.9°, about 4.0°, about 4.1°, about 4.2°, about 4.3°, about 4.4°, about 4.5°, about 4.6°, about 4.7°, about 4.8°, about 4.9°, about 5.0°, about 5.1°, about 5.2°, about 5.3°, about 5.4°, about 5.5°, about 5.6°, about 5.7°, about 5.8°, about 5.9°, about 6.0°, about 6.1°, about 6.2°, about 6.3°, about 6.4°, about 6.5°, about 6.6°, about 6.7°, about
- 25 6.8°, about 6.9°, about 7.0°, about 7.1°, about 7.2°, about 7.3°, about 7.4°, about 7.5°, about 7.6°, about 7.7°, about 7.8°, about 7.9°, about 8.0°, about 8.1°, about 8.2°, about 8.3°, about 8.4°, about 8.5°, about 8.6°, about 8.7°, about 8.8°, about 8.9°, about 9.0°, about 9.1°, about 9.2°, about 9.3°, about 9.4°, about 9.5°, about 9.6°, about 9.7°, about 9.8°, about 9.9°, about 10.0°, about 11.0°, about 12.0°, about 13.0°, about 14.0°, about
- 30 15.0°, about 16.0°, about 17.0°, about 18.0°, about 19.0°, about 20.0°, or any value or range therein, from the perpendicular alignment.

The system may be used to prepare or fabricate nanoparticles by introducing a flow of second solvent into or within a flow of first solvent, wherein the flow of second solvent

- is introduced into the flow of first solvent in a direction substantially orthogonal to the flow of first solvent. The second flow direction may be 90° to the first flow direction. The second flow direction may be about 90° from a central axis of the first flow direction, or may deviate about 0.1° to about 20° from this perpendicular alignment.
- 5 The deviation may be about 0.1° to about 20° , about 1° to about 20° , about 2° to about 20° , about 3° to about 20° , about 4° to about 20° , about 5° to about 20° , about 10° to about 20° , about 15° to about 20° , about 0.1° to about 15° , about 0.1° to about 10° , about 0.1° to about 5° , about 0.1° to about 4° , about 0.1° to about 3° , about 0.1° to about 2° , about 0.1° to about 1° , or about 0.1° , about 0.2° , about 0.3° , about 0.4° , about 0.5° ,
10 about 0.6° , about 0.7° , about 0.8° , about 0.9° , about 1.0° , about 1.1° , about 1.2° , about 1.3° , about 1.4° , about 1.5° , about 1.6° , about 1.7° , about 1.8° , about 1.9° , about 2.0° , about 2.1° , about 2.2° , about 2.3° , about 2.4° , about 2.5° , about 2.6° , about 2.7° , about 2.8° , about 2.9° , about 3.0° , about 3.1° , about 3.2° , about 3.3° , about 3.4° , about 3.5° , about 3.6° , about 3.7° , about 3.8° , about 3.9° , about 4.0° , about 4.1° , about 4.2° , about
15 4.3° , about 4.4° , about 4.5° , about 4.6° , about 4.7° , about 4.8° , about 4.9° , about 5.0° , about 5.1° , about 5.2° , about 5.3° , about 5.4° , about 5.5° , about 5.6° , about 5.7° , about 5.8° , about 5.9° , about 6.0° , about 6.1° , about 6.2° , about 6.3° , about 6.4° , about 6.5° , about 6.6° , about 6.7° , about 6.8° , about 6.9° , about 7.0° , about 7.1° , about 7.2° , about 7.3° , about 7.4° , about 7.5° , about 7.6° , about 7.7° , about 7.8° , about 7.9° , about 8.0° ,
20 about 8.1° , about 8.2° , about 8.3° , about 8.4° , about 8.5° , about 8.6° , about 8.7° , about 8.8° , about 8.9° , about 9.0° , about 9.1° , about 9.2° , about 9.3° , about 9.4° , about 9.5° , about 9.6° , about 9.7° , about 9.8° , about 9.9° , about 10.0° , about 11.0° , about 12.0° , about 13.0° , about 14.0° , about 15.0° , about 16.0° , about 17.0° , about 18.0° , about 19.0° , about 20.0° , or any value or range therein, from the perpendicular alignment.
- 25 At least one of the first solvent or second solvent contains nanoparticle precursors. The first solvent may be an antisolvent, and the second solvent may be an organic solvent. In another embodiment, the first solvent may be an organic solvent and the second solvent may be an antisolvent. The organic solvent may contain the nanoparticle precursors.
- When the flow of second solvent is introduced into or within the flow of first solvent in
30 a direction substantially orthogonal to the flow of first solvent, high intensity or high turbulence in the system may be generated due to cross-current mixing of second solvent (inner solvent) and first solvent (outer solvent). Nanoparticles may be generated by precipitation or crystallization. The high intensity or high turbulent mixing may

advantageously lead to the synthesis of nanoparticles with high uniformity for all size ranges.

The Reynolds number at the mixing zone may be optimized to control the size of nanoparticles synthesized. For example, by increasing the Reynolds number, the average diameters of the nanoparticles synthesized may be reduced. This may be attributed to the increase in mixing with an increase in Reynolds number, thereby resulting in rapid and uniform nanoprecipitation. A homogenous size distribution with low polydispersity index (PDI) may be obtained for all the Reynolds numbers. Tight control over the size of nanoparticles is useful for many different biological applications. The mixing zone of the system may have an average Reynolds number of between about 400 to about 12,000, about 400 to about 11,000, about 400 to about 10,000, about 400 to about 9,000, about 400 to about 8,000, about 400 to about 7,000, about 400 to about 6,000, about 400 to about 5,000, about 400 to about 4,000, about 400 to about 3,000, about 400 to about 2,000, about 400 to about 1,000, about 1,000 to about 12,000, about 2,000 to about 12,000, about 3,000 to about 12,000, about 4,000 to about 12,000, about 5,000 to about 12,000, about 6,000 to about 12,000, about 7,000 to about 12,000, about 8,000 to about 12,000, about 9,000 to about 12,000, about 10,000 to about 12,000, about 11,000 to about 12,000, about 500 to about 9,898, or about 400, about 500, about 1,000, about 2,000, about 3,000, about 4,000, about 5,000, about 6,000, about 7,000, about 8,000, about 9,000, about 9,898, about 10,000, about 11,000, about 12,000, or any range or value therein.

Advantageously, the configuration of the system of the present invention comprising a second conduit extending coaxially into the first conduit, and wherein the second conduit comprises an opening substantially orthogonal to the first flow direction for introducing a flow of second solvent into the flow of first solvent in a direction substantially orthogonal to the flow of first solvent, results in cross-current mixing between the first and second solvents results. Compared to systems wherein the mixing occurs via coaxial mixing (i.e. wherein the flow of second solvent is introduced to the flow of first solvent in a coaxial direction to the flow of first solvent), a higher turbulence is generated by the cross-current mixing achieved by the present system compared to coaxial mixing at the same Reynolds numbers. Further advantageously, high intense and high turbulent mixing may be achieved from cross-current mixing even at low Reynolds numbers. The higher turbulence generated by the present system

advantageously generates nanoparticles that have higher uniformity than nanoparticles generated through coaxial mixing.

In the disclosed system, the second conduit may be inserted into the first conduit and protrude coaxially into the first conduit such that the mixing zone occurs downstream of the first conduit. Advantageously, this because the second conduit is inserted coaxially into the first conduit, there is minimal disturbance to the flow of first solvent caused by the presence of the second conduit. As a result, the turbulence generated in this system may be solely attributed to the cross-current mixing of the second solvent flow with the first solvent flow which advantageously results in high intense or high turbulent mixing of first and second solvent and the synthesis of nanoparticles with high uniformity.

In other systems which may have a second conduit protruding perpendicularly into the first conduit to introduce a perpendicular flow of second solvent between the first solvent flow, the presence of the protruding second conduit into the first conduit disadvantageously disturbs the flow of first solvent. As a result, the turbulence generated in such a system is mainly due to the presence of the protruding second conduit, and not due to cross-current mixing, which disadvantageously results in non-uniform mixing of the first and second solvents and thus more non-uniform nanoparticles.

The outer diameter of the first conduit may be in the range of about 2.5 mm to about 3.5 mm. The outer diameter of the first conduit may be in the range of about 2.5 mm to about 3.5 mm, about 2.5 mm to about 3.5 mm, about 2.6 mm to about 3.5 mm, about 2.7 mm to about 3.5 mm, about 2.8 mm to about 3.5 mm, about 2.9 mm to about 3.5 mm, about 3.0 mm to about 3.5 mm, about 3.1 mm to about 3.5 mm, about 3.125 mm to about 3.5 mm, about 3.2 mm to about 3.5 mm, about 3.3 mm to about 3.5 mm, about 3.4 mm to about 3.5 mm, about 2.5 mm to about 3.4 mm, about 2.5 mm to about 3.3 mm, about 2.5 mm to about 3.2 mm, about 2.5 mm to about 3.125 mm, about 2.5 mm to about 3.1 mm, about 2.5 mm to about 3.0 mm, about 2.5 mm to about 2.9 mm, about 2.5 mm to about 2.8 mm, about 2.5 mm to about 2.7 mm, about 2.5 mm to about 2.6 mm, about 2.5 mm, about 2.6 mm, about 2.7 mm, about 2.8 mm, about 2.9 mm, about 3.0 mm, about 3.1 mm, about 3.125 mm, about 3.2 mm, about 3.3 mm, about 3.4 mm, about 3.5 mm, or any value or range therein.

The inner diameter of the first conduit may be in the range of about 1.0 mm to about 2.0 mm. The inner diameter of the first conduit may in the range of about 1.0 mm to about 2.0 mm, about 1.1 mm to about 2.0 mm, about 1.2 mm to about 2.0 mm, about 1.3 mm to about 2.0 mm, about 1.4 mm to about 2.0 mm, about 1.5 mm to about 2.0 mm, about 1.6 mm to about 2.0 mm, about 1.7 mm to about 2.0 mm, about 1.8 mm to about 2.0 mm, about 1.9 mm to about 2.0 mm, about 1.0 mm to about 1.9 mm, about 1.0 mm to about 1.8 mm, about 1.0 mm to about 1.7 mm, about 1.0 mm to about 1.6 mm, about 1.0 mm to about 1.5 mm, about 1.0 mm to about 1.4 mm, about 1.0 mm to about 1.3 mm, about 1.0 mm to about 1.2 mm, about 1.0 mm to about 1.1 mm, or about 1.0 mm, about 1.1 mm, about 1.2 mm, about 1.3 mm, about 1.4 mm, about 1.5 mm, about 1.6 mm, about 1.7 mm, about 1.8 mm, about 1.9 mm, about 2.0 mm, or any value or range therein.

At the mixing zone, the first and second solvents are mixed. The mixing zone includes an area of initial mixing of first and second solvents and any extended volume or distance where the two streams continue to mix together. The extended distance may be any length 50 mm or more. The extended distance may be about 50 mm to about 100 mm, about 55 mm to about 100 mm, about 60 mm to about 100 mm, about 65 mm to about 100 mm, about 70 mm to about 100 mm, about 75 mm to about 100 mm, about 80 mm to about 100 mm, about 85 mm to about 100 mm, about 90 mm to about 100 mm, about 95 mm to about 100 mm, about 50 mm to about 95 mm, about 50 mm to about 90 mm, about 50 mm to about 85 mm, about 50 mm to about 80 mm, about 50 mm to about 75 mm, about 50 mm to about 70 mm, about 50 mm to about 65 mm, about 50 mm to about 60 mm, about 50 mm to about 55 mm, or about 50 mm, about 55 mm, about 60 mm, about 65 mm, about 70 mm, about 75 mm, about 80 mm, about 85 mm, about 90 mm, about 95 mm, about 100 mm, or any range or value therein. Advantageously, an increased mixing distance can result in more complete mixing of the first and second solvents.

The cross sectional area of the flow of first solvent may be between about 70% to about 100% the cross sectional area of the first conduit, or about 75% to about 100%, about 80% to about 100%, about 85% to about 100%, about 90% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, about 99% to about 100%, about 70% to about 99%, about 70% to about 98%, about 70% to about 97%, about 70% to about 96%, about 70% to about 95%,

about 70% to about 90%, about 70% to about 85%, about 70% to about 80%, about 70% to about 75%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, about 100%, or any value or range therein of the cross sectional area of the first conduit.

- 5 Advantageously, Reynolds number may be adjusted by adjusting the inner diameter of the first conduit. By being able to adjust the Reynolds number, optimization of Reynolds number may be done to obtain desired particle sizes for different applications, such as biological applications.

- 10 The first conduit may be any tubular element with two or more open ends. The first conduit may comprise a cylindrical tube. The first flow direction may comprise an axial direction of the tube and the second flow direction may comprise a radial direction of the cylindrical tube.

The second conduit comprises an opening for introducing a flow of second solvent within the flow of first solvent at the mixing zone.

- 15 The opening may be a space or gap located on the second conduit to permit a flow of second solvent through instead of a projecting spout or nozzle on which the opening is located. Advantageously, having the opening be flush with the second conduit means that the disruption of flow of first solvent by the presence of the second conduit is kept to a minimum. This helps to ensure that the high intensity or high turbulence generated
20 in the system is due to the cross-current mixing of the first and second solvents and is not disrupted by the presence of the second conduit.

The second conduit may have any outer diameter than is smaller than the inner diameter of the first conduit. This is so that the second conduit may be inserted into the first conduit in a coaxial direction.

- 25 The second conduit may have an inner diameter of about 0.20 mm to about 0.90 mm. The second conduit may have an inner diameter of about 0.20 mm to about 0.90 mm, about 0.25 mm to about 0.90 mm, about 0.26 mm to about 0.90 mm, about 0.30 mm to about 0.90 mm, about 0.35 mm to about 0.90 mm, about 0.40 mm to about 0.90 mm, about 0.45 mm to about 0.90 mm, about 0.50 mm to about 0.90 mm, about 0.55 mm to
30 about 0.90 mm, about 0.60 mm to about 0.90 mm, about 0.65 mm to about 0.90 mm, about 0.70 mm to about 0.90 mm, about 0.75 mm to about 0.90 mm, about 0.80 mm to

about 0.90 mm, about 0.85 mm to about 0.90 mm, about 0.20 mm to about 0.85 mm, about 0.20 mm to about 0.84 mm, about 0.20 mm to about 0.80 mm, about 0.20 mm to about 0.75 mm, about 0.20 mm to about 0.70 mm, about 0.20 mm to about 0.65 mm, about 0.20 mm to about 0.60 mm, about 0.20 mm to about 0.55 mm, about 0.20 mm to about 0.50 mm, about 0.20 mm to about 0.45 mm, about 0.20 mm to about 0.40 mm, about 0.20 mm to about 0.35 mm, about 0.20 mm to about 0.30 mm, about 0.20 mm to about 0.25 mm, about 0.26 mm to about 0.84 mm, or about 0.20 mm, about 0.25 mm, about 0.26 mm, about 0.30 mm, about 0.35 mm, about 0.40 mm, about 0.45 mm, about 0.50 mm, about 0.55 mm, about 0.60 mm, about 0.65 mm, about 0.70 mm, about 0.75 mm, about 0.80 mm, about 0.84 mm, about 0.85 mm, about 0.90 mm, or any value or range therein.

The opening on the second conduit may have a diameter that is smaller than the inner diameter of the second conduit. The opening on the second conduit may have a diameter of about 0.10 mm to about 0.40 mm. The opening on the second conduit may have a diameter of about 0.10 mm to about 0.40 mm, about 0.10 mm to about 0.35 mm, about 0.10 mm to about 0.30 mm, about 0.10 mm to about 0.25 mm, about 0.10 mm to about 0.20 mm, about 0.10 mm to about 0.15 mm, about 0.15 mm to about 0.40 mm, about 0.20 mm to about 0.40 mm, about 0.25 mm to about 0.40 mm, about 0.30 mm to about 0.40 mm, about 0.35 mm to about 0.40 mm, or about 0.10 mm, about 0.15 mm, about 0.20 mm, about 0.25 mm, about 0.30 mm, about 0.35 mm, about 0.40 mm, or any value or range therein.

The cross-sectional area of the second solvent flow may be between about 1% to about 10% of the cross-sectional area of the first solvent flow. The cross-sectional area of the second solvent flow may be about 1% to about 10%, about 1.5% to about 10%, about 2% to about 10%, about 2.5% to about 10%, about 3% to about 10%, about 3.5% to about 10%, about 4% to about 10%, about 4.5% to about 10%, about 5% to about 10%, about 5.45% to about 10%, about 5.5% to about 10%, about 6% to about 10%, about 6.5% to about 10%, about 7% to about 10%, about 7.5% to about 10%, about 8% to about 10%, about 8.5% to about 10%, about 9% to about 10%, about 9.5% to about 10%, about 1% to about 9.5%, about 1% to about 9%, about 1% to about 8.5%, about 1% to about 8%, about 1% to about 7.5%, about 1% to about 7%, about 1% to about 6.5%, about 1% to about 6%, about 1% to about 5.5%, about 1% to about 5.45%, about 1% to about 5%, about 1% to about 4.5%, about 1% to about 4%, about 1% to about

3%, about 1% to about 2.5%, about 1% to about 2%, about 1% to about 1.5%, or about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.45%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, about 10%, or any value or range therein,
5 of the cross-sectional area of the first solvent flow.

The second conduit may comprise a plurality of openings for introducing a flow of second solvent within the flow of first solvent at the mixing zone. The second conduit may have one, two, three, four, five, six or more openings for introducing a flow of second solvent within the flow of first solvent at the mixing zone. The openings may be
10 spaced circumferentially around the second fluid conduit. The openings may be spaced circumferentially and equidistant apart around the second fluid conduit.

The second conduit may have two openings for introducing a flow of second solvent within the flow of first solvent at the mixing zone. The two openings may be on opposite sides of the second conduit. The symmetry of this arrangement may
15 advantageously result in more uniform mixing of the first and second solvents which may result in more uniformly shaped and sized nanoparticles.

The second conduit may be of smaller diameter than the first conduit such that the second conduit may be inserted into the first conduit coaxially. The second conduit may protrude into the first conduit a distance of about 10 mm to about 30 mm, 15 mm to
20 about 30 mm, 20 mm to about 30 mm, 25 mm to about 30 mm, 10 mm to about 25 mm, 10 mm to about 20 mm, 10 mm to about 15 mm, or about 10 mm, about 15 mm, about 20 mm, about 25 mm, about 30 mm, or any value or range therein.

The second conduit may be any tubular element with two or more open ends. The second conduit may comprise a needle having a side-opening, wherein said side-opening faces a radial direction of the first conduit (cylindrical tube). The side opening
25 may be a space or gap located on the needle to permit a flow of solvent through instead of a projecting spout or nozzle on which the side-opening is located. Advantageously, having the opening be flush with the needle means that the disruption of flow of first solvent by the presence of the needle is kept to a minimum.

30 The needle gauge may be between 18 to 25. The gauge refers to the inner diameter of the needle. The inner diameter may be in the range of about 0.26 mm to about 0.84 mm.

The side-opening or side-hole on the needle may have a diameter of about 0.1 mm to about 0.75 mm.

The needle may be inserted into the cylindrical tube in an axial direction to the tube. The needle may protrude coaxially into the tube such that the mixing zone occurs
5 downstream of the tube. The side-opening or side-hole on the needle faces a radial direction of the tube for introducing a stream of solvent into a stream of antisolvent in a radial direction of the tube.

The disclosed system may further comprise:

- 10 a first inlet configured to permit a first stream of antisolvent;
- a second inlet configured to permit a second stream of antisolvent, wherein the flow of the first and second streams are diametric; and wherein the first conduit is configured to permit the combined flow of the first and second streams to the mixing zone.

The disclosed system may further comprise one or more pumps for controlling the rate
15 of flow of first solvent and second solvent. In one embodiment, the system comprises a pump for controlling the rate of flow of first solvent, and a pump for controlling the rate of flow of second solvent.

The flow rate of first solvent may be about 5 times to about 15 times the flow rate of second solvent. The flow rate of first solvent may be about 5 times to about 15 times,
20 about 6 times to about 15 times, about 7 times to about 15 times, about 8 times to about 15 times, about 9 times to about 15 times, about 10 times to about 15 times, about 11 times to about 15 times, about 12 times to about 15 times, about 13 times to about 15 times, about 14 times to about 15 times, about 5 times to about 14 times, about 5 times to about 13 times, about 5 times to about 12 times, about 5 times to
25 about 11 times, about 5 times to about 10 times, about 5 times to about 9 times, about 5 times to about 8 times, about 5 times to about 7 times, about 5 times to about 6 times, or about 5 times, about 6 times, about 7 times, about 8 times, about 9 times, about 10 times, about 11 times, about 12 times, about 13 times, about 14 times, about 15 times, or any value or range therein, of the flow rate of second solvent.

30 The flow rate of first solvent may be in the range of about 32.5 mL/min to about 650 mL/min, about 50 mL/min to about 650 mL/min, about 100 mL/min to about 650

mL/min, about 150 mL/min to about 650 mL/min, about 200 mL/min to about 650 mL/min, about 250 mL/min to about 650 mL/min, about 300 mL/min to about 650 mL/min, about 350 mL/min to about 650 mL/min, about 400 mL/min to about 650 mL/min, about 450 mL/min to about 650 mL/min, about 500 mL/min to about 650 mL/min, about 550 mL/min to about 650 mL/min, about 600 mL/min to about 650 mL/min, about 32.5 mL/min to about 600 mL/min, about 32.5 mL/min to about 550 mL/min, about 32.5 mL/min to about 500 mL/min, about 32.5 mL/min to about 450 mL/min, about 32.5 mL/min to about 400 mL/min, about 32.5 mL/min to about 350 mL/min, about 32.5 mL/min to about 300 mL/min, about 32.5 mL/min to about 250 mL/min, about 32.5 mL/min to about 200 mL/min, about 32.5 mL/min to about 150 mL/min, about 32.5 mL/min to about 100 mL/min, about 32.5 mL/min to about 50 mL/min, or about 32.5 mL/min, about 50 mL/min, about 100 mL/min, about 150 mL/min, about 200 mL/min, about 250 mL/min, about 300 mL/min, about 350 mL/min, about 400 mL/min, about 450 mL/min, about 500 mL/min, about 550 mL/min, about 600 mL/min, about 650 mL/min, or any value or range therein.

The flow rate of second solvent may be in the range of about 3.25 mL/min to about 65 mL/min, about 5 mL/min to about 65 mL/min, about 10 mL/min to about 65 mL/min, about 15 mL/min to about 65 mL/min, about 20 mL/min to about 65 mL/min, about 25 mL/min to about 65 mL/min, about 30 mL/min to about 65 mL/min, about 35 mL/min to about 65 mL/min, about 40 mL/min to about 65 mL/min, about 45 mL/min to about 65 mL/min, about 50 mL/min to about 65 mL/min, about 55 mL/min to about 65 mL/min, about 60 mL/min to about 65 mL/min, about 3.25 mL/min to about 60 mL/min, about 3.25 mL/min to about 55 mL/min, about 3.25 mL/min to about 50 mL/min, about 3.25 mL/min to about 45 mL/min, about 3.25 mL/min to about 40 mL/min, about 3.25 mL/min to about 35 mL/min, about 3.25 mL/min to about 30 mL/min, about 3.25 mL/min to about 25 mL/min, about 3.25 mL/min to about 20 mL/min, about 3.25 mL/min to about 15 mL/min, about 3.25 mL/min to about 10 mL/min, about 3.25 mL/min to about 5 mL/min, or about 3.25 mL/min, about 5 mL/min, about 10 mL/min, about 15 mL/min, about 20 mL/min, about 25 mL/min, about 30 mL/min, about 35 mL/min, about 40 mL/min, about 45 mL/min, about 50 mL/min, about 55 mL/min, about 60 mL/min, about 65 mL/min or any value or range therein.

The system may be a millifluidic system. The system may be referred to as a High Intense Millifluidic System (HIMS).

At least one parameter in the disclosed system may be controlled to control a size of the nanoparticles in the nanoparticle dispersion and/or a rate of nanoparticle fabrication of the system, wherein the at least one parameter is selected from the group consisting of Reynolds number, flow rate, ratio of the antisolvent to solvent, precursor concentration, and precursor composition.

The formation of the nanoparticles may be fully continuous. Advantageously, as the system may run in a continuous mode, the physiochemical properties of the nanoparticles synthesized may be independent of the batch size for the same parameters. Hence, the disclosed system can be used to synthesize wide varieties of nanoparticles with high production rate without conceding on the reproducibility in the system.

Method for Fabricating Nanoparticles

The present invention also provides a method of fabricating nanoparticles, the method comprising:

- (i) supplying a flow of first solvent in a first flow direction through a first conduit to a mixing zone;
- (ii) supplying a flow of second solvent through a second conduit to a mixing zone, wherein the second conduit extends coaxially into the first conduit for introducing the flow of second solvent within the flow of the first solvent, wherein at least one of the first solvent or second solvent contains nanoparticle precursors;
- (iii) introducing the flow of second solvent within the flow of first solvent in a second flow direction, wherein the second flow direction is substantially orthogonal to the first flow direction; and
- (iv) mixing the flow of first solvent and flow of second solvent to form a mixed stream comprising a nanoparticle dispersion based on the nanoparticle precursors.

The first solvent may be an antisolvent, and the second solvent may be an organic solvent. In another embodiment, the first solvent may be an organic solvent and the second solvent may be an antisolvent.

The organic solvent may contain the nanoparticle precursors. The antisolvent may be any solvent which a poor solvent for the nanoparticle precursors. The organic solvent may be any solvent where the nanoparticle precursors are completely or substantially soluble. When the flow of organic solvent is introduced within the flow of antisolvent, nanoparticles are formed through precipitation.

The antisolvent may be selected from the group consisting of water, alcohol, and mixtures thereof. The antisolvent may be selected from the group consisting of water, methanol, ethanol, and mixtures thereof.

The organic solvent may be miscible in water. The organic solvent may be completely miscible in water.

The organic solvent may be selected from the group consisting of alcohols, esters, aldehydes, ketones, amines, and nitrated hydrocarbons.

The organic solvent may be selected from the group consisting of acetaldehyde, acetic acid, acetone, acetonitrile, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 2-butoxyethanol, butyric acid, chloroform, diethanolamine, diethylenetriamine, 1,2-dimethoxy-ethane (glyme, DME), dimethyl-formamide (DMF), dimethyl sulfoxide (DMSO), 1,4-dioxane, ethanol, ethylamine, ethylene glycol, formic acid, furfuryl alcohol, glycerol, methanol, methyl diethanolamine, methyl isocyanide, N-methyl-2-pyrrolidinone (NMP), 1-propanol, 2-propanol, 1,3-propanediol, propylene glycol, pyridine, tetrahydrofuran (THF), and triethylene glycol.

The organic solvent may be selected from the group consisting of THF, acetone, methanol, chloroform or ethanol.

The ratio of antisolvent to solvent is such that the system is inside the ouzo zone. "Ouzo zone" refers to a small metastable region of a ternary phase diagram of solute/solvent/antisolvent where amphiphilic polymer forms stable and small size micelles. The reason for stability of nanoparticles in the ouzo zone is due to a minimum total energy achieved by the system, which prevents nanoparticles from further coalescing. In a composition with a ratio of high solvent to antisolvent, only a small number of solute molecules will experience supersaturation and form nuclei. Instead of encountering each other, these nuclei grow by accumulating all nearby isolated solute molecules and synthesize large aggregates through nucleation-growth mechanism.

Outside the ouzo zone, nanoprecipitation produces large aggregates above a certain solvent fraction and polymer concentration, which may result in the synthesis of highly polydisperse particles with uncontrolled sizes. Overall, there is an optimum region in terms of antisolvent, solvent and solute fraction, known as ouzo zone, which yield stable small size nanoparticles.

In an embodiment of the present invention, there is a high amount of anti-solvent compared to solvent. This is because at high amounts of solvent relative to antisolvent, the nanoparticles may dissolve in the solvent resulting in unstable nanoparticles as the system is outside the ouzo zone.

The ratio of antisolvent to organic solvent may be in the range of 5:1 to 15:1, or about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, about 10:1, about 11:1, about 12:1, about 13:1, about 14:1, about 15:1, or any value or range therein. Advantageously, at these ratios of antisolvent to organic solvent, the system lies within the ouzo zone.

Step (iv) of the disclosed method may comprise or consist of cross-current mixing.

Step (iii) of the disclosed method may occur downstream of the first conduit.

The mixed stream may have a Reynolds number of about 400 to about 12,000. The mixed stream may have an average Reynolds number of between about 400 to about 12,000, about 400 to about 11,000, about 400 to about 10,000, about 400 to about 9,000, about 400 to about 8,000, about 400 to about 7,000, about 400 to about 6,000, about 400 to about 5,000, about 400 to about 4,000, about 400 to about 3,000, about 400 to about 2,000, about 400 to about 1,000, about 1,000 to about 12,000, about 2,000 to about 12,000, about 3,000 to about 12,000, about 4,000 to about 12,000, about 5,000 to about 12,000, about 6,000 to about 12,000, about 7,000 to about 12,000, about 8,000 to about 12,000, about 9,000 to about 12,000, about 10,000 to about 12,000, about 11,000 to about 12,000, about 500 to about 9,898, or about 400, about 500, about 1,000, about 2,000, about 3,000, about 4,000, about 5,000, about 6,000, about 7,000, about 8,000, about 9,000, about 9,898, about 10,000, about 11,000, about 12,000, or any range or value therein.

The total flow rate of the mixed stream may be between about 30 mL/min to about 700 mL/min, about 35.5 mL/min to about 700 mL/min, about 50 mL/min to about 700 mL/min, about 150 mL/min to about 700 mL/min, about 200 mL/min to about 700

mL/min, about 250 mL/min to about 700 mL/min, about 300 mL/min to about 700 mL/min, about 350 mL/min to about 700 mL/min, about 400 mL/min to about 700 mL/min, about 450 mL/min to about 700 mL/min, about 500 mL/min to about 700 mL/min, about 550 mL/min to about 700 mL/min, about 600 mL/min to about 700 mL/min, about 650 mL/min to about 700 mL/min, about 30 mL/min to about 650 mL/min, about 30 mL/min to about 600 mL/min, about 30 mL/min to about 550 mL/min, about 30 mL/min to about 500 mL/min, about 30 mL/min to about 450 mL/min, about 30 mL/min to about 400 mL/min, about 30 mL/min to about 350 mL/min, about 30 mL/min to about 300 mL/min, about 30 mL/min to about 250 mL/min, about 30 mL/min to about 200 mL/min, about 30 mL/min to about 150 mL/min, about 30 mL/min to about 100 mL/min, about 30 mL/min to about 50 mL/min, about 30 mL/min to about 35.5 mL/min, or about 30 mL/min, about 35.5 mL/min, about 50 mL/min, about 100 mL/min, about 150 mL/min, about 200 mL/min, about 250 mL/min, about 300 mL/min, about 350 mL/min, about 400 mL/min, about 450 mL/min, about 500 mL/min, about 550 mL/min, about 600 mL/min, about 650 mL/min, about 700 mL/min, or any value or range therein.

The method may be performed at room temperature.

The flow of first and second solvent may be at similar temperatures. The temperature of the flow of first and second solvent may be at room temperature.

The temperature of the flow of first solvent may be between about 20 °C to about 35 °C, about 21 °C to about 35 °C, about 22 °C to about 35 °C, about 23 °C to about 35 °C, about 24 °C to about 35 °C, about 25 °C to about 35 °C, about 6 °C to about 35 °C, about 27 °C to about 35 °C, about 28 °C to about 35 °C, about 29 °C to about 35 °C, about 30 °C to about 35 °C, about 31 °C to about 35 °C, about 32 °C to about 35 °C, about 33 °C to about 35 °C, about 34 °C to about 35 °C, about 20 °C to about 34 °C, about 20 °C to about 33 °C, about 20 °C to about 32 °C, about 20 °C to about 31 °C, about 20 °C to about 30 °C, about 20 °C to about 29 °C, about 20 °C to about 28 °C, about 20 °C to about 27 °C, about 20 °C to about 26 °C, about 20 °C to about 25 °C, about 20 °C to about 24 °C, about 20 °C to about 23 °C, about 20 °C to about 22 °C, about 20 °C to about 21 °C, or about 20 °C, about 21 °C, about 22 °C, about 23 °C, about 24 °C, about 25 °C, about 26 °C, about 27 °C, about 28 °C, about 29 °C, about 30

°C, about 31 °C, about 32 °C, about 33 °C, about 34 °C, about 35 °C, or any value or range therein.

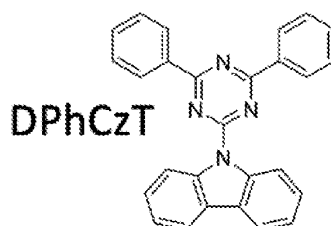
The temperature of the flow of second solvent may be between about 20 °C to about 35 °C, about 21 °C to about 35 °C, about 22 °C to about 35 °C, about 23 °C to about 35 °C, about 24 °C to about 35 °C, about 25 °C to about 35 °C, about 26 °C to about 35 °C, about 27 °C to about 35 °C, about 28 °C to about 35 °C, about 29 °C to about 35 °C, about 30 °C to about 35 °C, about 31 °C to about 35 °C, about 32 °C to about 35 °C, about 33 °C to about 35 °C, about 34 °C to about 35 °C, about 20 °C to about 34 °C, about 20 °C to about 33 °C, about 20 °C to about 32 °C, about 20 °C to about 31 °C, about 20 °C to about 30 °C, about 20 °C to about 29 °C, about 20 °C to about 28 °C, about 20 °C to about 27 °C, about 20 °C to about 26 °C, about 20 °C to about 25 °C, about 20 °C to about 24 °C, about 20 °C to about 23 °C, about 20 °C to about 22 °C, about 20 °C to about 21 °C, or about 20 °C, about 21 °C, about 22 °C, about 23 °C, about 24 °C, about 25 °C, about 26 °C, about 27 °C, about 28 °C, about 29 °C, about 30 °C, about 31 °C, about 32 °C, about 33 °C, about 34 °C, about 35 °C, or any value or range therein.

Advantageously, the present method is not affected or minimally affected by differing temperatures between the first and second solvents.

The size of the nanoparticles fabricated by the disclosed method may substantially uniform. The nanoparticles may have a polydispersity index (PDI) of about ≤ 0.15 . The nanoparticles may have a polydispersity index (PDI) of about ≤ 0.15 , about ≤ 0.14 , about ≤ 0.13 , about ≤ 0.12 , about ≤ 0.11 , about ≤ 0.10 , about ≤ 0.09 , about ≤ 0.08 , or about ≤ 0.07 . The nanoparticles may have a polydispersity index (PDI) in the range of about 0.07 to about 0.15, about 0.08 to about 0.15, about 0.09 to about 0.15, about 0.10 to about 0.15, about 0.11 to about 0.15, about 0.11 to about 0.15, about 0.12 to about 0.15, about 0.13 to about 0.15, about 0.14 to about 0.15, about 0.07 to about 0.14, about 0.07 to about 0.13, about 0.07 to about 0.12, about 0.07 to about 0.11, about 0.07 to about 0.10, about 0.07 to about 0.09, about 0.07 to about 0.08, or about 0.07, about 0.08, about 0.09, about 0.10, about 0.11, about 0.12, about 0.13, about 0.14, about 0.15, or any value or range therein.

The nanoparticle precursors may be selected from the group consisting of organic molecules, drug molecules, metal oxides, dyes, polymers, conjugated polymers, proteins, biological molecules, fluorescent molecules, metal oxides, and lipid vesicles.

The nanoparticle precursors may be selected from the group consisting of 4,7-bis[4-
 5 (1,2,2-triphenylvinyl)phenyl]benzo-2,1,3- thiadiazole (BTPEBT), 1,2-Distearoyl-*sn*-
 glycerol-3-phosphoethanolamine-polyethylene glycol (DSPE-PEG), 1,2-distearoyl-*sn*-
 glycerol-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG-NH₂),
 1,2-distearoyl-*sn*-glycerol-3-phosphoethanolamine-polyethylene glycol-carboxylic acid
 (DSPE-PEG-COOH), 1,2-distearoyl-*sn*-glycerol-3-phosphoethanolamine-N-
 10 [maleimide(polyethylene glycol)] (DSPE-PEG-MAL), poly(lactide-co-glycolic acid)
 (PLGA), polystyrene (PS), tamoxifen (TAM), doxorubicin (DOX), camptothecin
 (CPT), conjugated polymer (CP), iron oxide (IO), poly(diketopyrrolopyrrole-
 terthiophene) (PDPP3T), and 4,6-diphenyl-2-carbazolyl-1,3,5-triazine (DPhCzT) .



15 The nanoparticles may be core-shell nanoparticles, wherein the core is selected
 from the group consisting of 4,7-bis[4-(1,2,2-triphenylvinyl)phenyl]benzo-2,1,3-
 thiadiazole (BTPEBT); tamoxifen (TAM); doxorubicin (DOX), camptothecin (CPT);
 conjugated polymer (CP); iron oxide (IO) and poly(diketopyrrolopyrrole-terthiophene)
 (PDPP3T); and 4,6-diphenyl-2-carbazolyl-1,3,5-triazine (DPhCzT), and

20 the shell is selected from the group consisting of 1,2-Distearoyl-*sn*-glycerol-3-
 phosphoethanolamine-polyethylene glycol (DSPE-PEG); 1,2-distearoyl-*sn*-glycerol-3-
 phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG-NH₂); 1,2-
 distearoyl-*sn*-glycerol-3-phosphoethanolamine-polyethylene glycol-carboxylic acid
 (DSPE-PEG-COOH); 1,2-distearoyl-*sn*-glycerol-3-phosphoethanolamine-N-
 25 [maleimide(polyethylene glycol)] (DSPE-PEG-MAL); poly(lactide-co-glycolic acid)
 (PLGA); and polystyrene (PS).

The amount of shell precursor may be more than the amount of core precursor. The ratio of shell nanoparticle precursor to core nanoparticle precursor may be about 1:1 to about 4:1. The ratio of shell nanoparticle precursor to core nanoparticle precursor may be about 1:1, about 2:1, about 3:1, about 4:1, or any value or range therein.

- 5 The nanoparticles fabricated by the disclosed method may be selected from the group consisting of:

- (i) 4,7-bis[4-(1,2,2-triphenylvinyl)phenyl]benzo-2,1,3-thiadiazole (BTPEBT) encapsulated by 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-polyethylene glycol (DSPE-PEG);
- 10 (ii) BTPEBT encapsulated by 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG-NH₂);
- (iii) BTPEBT encapsulated by 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-polyethylene glycol-carboxylic acid (DSPE-PEG-COOH);
- 15 (iv) BTPEBT encapsulated by 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)] (DSPE-PEG-MAL);
- (v) BTPEBT encapsulated by poly(lactide-co-glycolic acid) (PLGA);
- (vi) BTPEBT encapsulated by polystyrene (PS);
- (vii) tamoxifen (TAM) encapsulated by DSPE-PEG;
- 20 (viii) TAM encapsulated by PLGA;
- (ix) TAM encapsulated by PS;
- (x) doxorubicin (DOX) encapsulated by DSPE-PEG
- (xi) DOX encapsulated by PLGA;
- (xii) DOX encapsulated by PS;
- 25 (xiii) camptothecin (CPT) encapsulated by DSPE-PEG;
- (xiv) CPT encapsulated by PLGA;
- (xv) poly(diketopyrrolopyrrole-terthiophene) (PDPP3T) encapsulated by DSPE-PEG;
- (xvi) CP encapsulated by PLGA;
- 30 (xvii) iron oxide (IO) and PDPP3T encapsulated by DSPE-PEG;
- (xviii) IO and PDPP3T encapsulated by PLGA; and
- (xix) DPhCzT encapsulated by DSPE-PEG.

- The size of the nanoparticles fabricated by the disclosed method may be between about 10 nm to about 400 nm, or about 50 nm to about 400 nm, about 100 nm to about 400 nm, about 150 nm to about 400 nm, about 200 nm to about 400 nm, about 250 nm to about 400 nm, about 300 nm to about 400 nm, about 350 nm to about 400 nm, about 10 nm to about 350 nm, about 10 nm to about 300 nm, about 10 nm to about 250 nm, about 10 nm to about 200 nm, about 10 nm to about 150 nm, about 10 nm to about 100 nm, about 10 nm to about 50 nm, or about 10 nm, about 50 nm, about 100 nm, about 150 nm, about 200 nm, about 250 nm, about 300 nm, about 350 nm, about 400 nm, or any value or range therein.
- 10 The disclosed method may advantageously be used to fabricate a high volume of nanoparticles. The amount of nanoparticles fabricated may depend on the concentration of nanoparticle precursors in the organic solvent. The nanoparticle precursor concentration may be about 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, 15 about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 45 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 55 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 65 mg/mL to about 100 mg/mL, about 70 mg/mL to about 100 mg/mL, about 75 20 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 85 mg/mL to about 100 mg/mL, about 90 mg/mL to about 100 mg/mL, about 95 mg/mL to about 100 mg/mL, about 2 mg/mL to about 95 mg/mL, about 2 mg/mL to about 90 mg/mL, about 2 mg/mL to about 85 mg/mL, about 2 mg/mL to about 80 mg/mL, about 2 mg/mL to about 75 mg/mL, about 2 mg/mL to about 70 mg/mL, about 2 mg/mL to about 65 25 mg/mL, about 2 mg/mL to about 60 mg/mL, about 2 mg/mL to about 55 mg/mL, about 2 mg/mL to about 50 mg/mL, about 2 mg/mL to about 45 mg/mL, about 2 mg/mL to about 40 mg/mL, about 2 mg/mL to about 35 mg/mL, about 2 mg/mL to about 30 mg/mL, about 2 mg/mL to about 25 mg/mL, about 2 mg/mL to about 20 mg/mL, about 2 mg/mL to about 15 mg/mL, about 2 mg/mL to about 10 mg/mL, about 2 mg/mL to about 5 mg/mL, about 2 mg/mL, about 5 mg/mL, about 10 mg/mL, about 15 mg/mL, 30 about 20 mg/mL, about 25 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about

65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, about 100 mg/mL, or any value or range therein.

The concentration of synthesized nanoparticles may be about 0.002 mg/mL to about 2.00 mg/mL, about 0.005 mg/mL to about 2.00 mg/mL, about 0.010 mg/mL to about 2.00 mg/mL, about 0.050 mg/mL to about 2.00 mg/mL, about 0.100 mg/mL to about 2.00 mg/mL, about 0.200 mg/mL to about 2.00 mg/mL, about 0.400 mg/mL to about 2.00 mg/mL, about 0.600 mg/mL to about 2.00 mg/mL, about 0.800 mg/mL to about 2.00 mg/mL, about 1.00 mg/mL to about 2.00 mg/mL, about 1.20 mg/mL to about 2.00 mg/mL, about 1.40 mg/mL to about 2.00 mg/mL, about 1.60 mg/mL to about 2.00 mg/mL, about 1.80 mg/mL to about 2.00 mg/mL, about 0.002 mg/mL to about 1.80 mg/mL, about 0.002 mg/mL to about 1.60 mg/mL, about 0.002 mg/mL to about 1.40 mg/mL, about 0.002 mg/mL to about 1.20 mg/mL, about 0.002 mg/mL to about 1.00 mg/mL, about 0.002 mg/mL to about 0.800 mg/mL, about 0.002 mg/mL to about 0.600 mg/mL, about 0.002 mg/mL to about 0.400 mg/mL, about 0.002 mg/mL to about 0.200 mg/mL, about 0.002 mg/mL to about 0.100 mg/mL, about 0.002 mg/mL to about 0.050 mg/mL, about 0.002 mg/mL to about 0.010 mg/mL, about 0.002 mg/mL to about 0.005 mg/mL, or about 0.002 mg/mL, about 0.005 mg/mL, about 0.010 mg/mL, about 0.050 mg/mL, about 0.100 mg/mL, about 0.200 mg/mL, about 0.400 mg/mL, about 0.600 mg/mL, about 0.800 mg/mL, about 1.00 mg/mL, about 1.20 mg/mL, about 1.40 mg/mL, about 1.60 mg/mL, about 1.80 mg/mL, about 2.00 mg/mL, or any value or range therein.

The disclosed method may fabricate more than 1 kg/day of nanoparticles. The disclosed method may fabricate about 1 kg/day to about 10 kg/day of nanoparticles, or about 1 kg/day to about 9 kg/day, about 1 kg/day to about 8 kg/day, about 1 kg/day to about 7 kg/day, about 1 kg/day to about 6 kg/day, about 1 kg/day to about 5 kg/day, about 1 kg/day to about 4 kg/day, about 1 kg/day to about 3 kg/day, about 1 kg/day to about 2 kg/day, about 2 kg/day to about 10 kg/day, about 3 kg/day to about 10 kg/day, about 4 kg/day to about 10 kg/day, about 5 kg/day to about 10 kg/day, about 6 kg/day to about 10 kg/day, about 7 kg/day to about 10 kg/day, about 8 kg/day to about 10 kg/day, about 9 kg/day to about 10 kg/day, about 1 kg/day, about 2 kg/day, about 3 kg/day, about 4 kg/day, about 5 kg/day, about 6 kg/day, about 7 kg/day, about 8 kg/day, about 9 kg/day, about 10 kg/day, or any value or range therein.

The present invention also relates to a use of the system of the present invention in the method of the present invention.

The present invention further relates to nanoparticles produced by the method of the present invention.

- 5 The system of the present invention is a versatile platform and advantageously may be used for high throughput synthesis (> 1 kg/day) of polymeric nanoparticles with controllable sizes and high reproducibility. The system of the present invention may advantageously allow for the fabrication of a wide variety of nanoparticles for various applications by customizing shell (for example, FDA-approved polymers, DSPE-PEG, PLGA and PS) and core (for example, molecules, nanoparticles, drugs, proteins) in it. An increase in Reynolds number may advantageously reduce mixing time scale and enable homogeneous nucleation. Smaller nanoparticles may be formed at high Reynolds number (for example, 9898). Using the system and method of the present invention, the behaviour of many drugs and dyes can be characterized, and a high throughput of nanoparticle production can be obtained. Overall, the system and method of the present invention may be a versatile and high throughput platform to synthesize dye, drug or inorganic nanoparticles loaded polymeric NPs with uniform size distribution (for example, $PDI < 0.13$) and desirable size in a reproducible manner for different applications.
- 10
15
20

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.
- 25

[Fig. 1A] is a schematic diagram of an embodiment of a system of the present invention. [Fig. 1B] is a schematic diagram showing an embodiment of a system of the present invention depicting fluid path. [Fig. 1C] is the schematic diagram of Fig. 1B showing the dimensions (in mm) of the system.

[Fig. 2A] is a graph showing the effect of Reynolds number (Re) on the number average diameter of BTPEBT loaded DSPE-mPEG nanoparticles synthesized by a method of the present invention by changing Re from 500 to 9898. [Fig. 2B] shows transmission electron microscopy (TEM) images of BTPEBT-DSPE-mPEG₂₀₀₀ nanoparticles synthesized by a method of the present invention of different size and homogeneous size distribution synthesized at Re 500 and 9898 (scale bar 200 nm).

[Fig. 3A] is a graph showing the number average diameter and polydispersity index (PDI) of TAM loaded DSPE-PEG nanoparticles synthesized by a method of the present invention by varying Re from 500 to 9898. [Fig. 3B] shows the effect of Re on the number average diameter and PDI of TAM loaded PLGA nanoparticles synthesized by a method of the present invention.

[Fig. 4A] is a graph showing the effect of Re on the number average diameter and PDI of conjugated polymer loaded DSPE-PEG nanoparticles synthesized by a method of the present invention. [Fig. 4B] shows TEM images of CP loaded DSPE-PEG nanoparticles synthesized at Re 9898 (Scale: 200 nm).

[Fig. 5A] is a graph showing the effect of Re on the number average diameter and PDI of IO-CP loaded DSPE-PEG nanoparticles synthesized by a method of the present invention. [Fig. 5B] shows TEM images of IO-CP loaded DSPE-PEG NPs synthesized at Re 9898.

[Fig. 6A] is a UV-Vis absorption spectrum comparing absorbance of DPhCzT nanocrystals obtained from a sonification method versus a method of the present invention. [Fig. 6B] is a Photoluminescence (PL) spectra comparing PL intensity of DPhCzT nanocrystals obtained from a sonification method versus a method of the present invention. [Fig. 6C] is a TEM image of DPhCzT nanocrystals synthesized using a sonification method. [Fig. 6D] is a TEM image of DPhCzT nanocrystals synthesized using a method of the present invention at Re 500. [Fig. 6E] is a TEM image of DPhCzT nanocrystals synthesized using a method of the present invention at Re 1000. [Fig. 6F] is a TEM image of DPhCzT nanocrystals synthesized using a method of the present invention at Re 1500. [Fig. 6G] is a TEM image of DPhCzT nanocrystals synthesized using a method of the present invention at Re 2500. [Fig. 6H] is a TEM

image of DPhCzT nanocrystals encapsulated with polymer DSPE-PEG synthesized using a method of the present invention at Re 2500.

[Fig. 7] is a graph showing the number average diameter of nanoparticles synthesized at 5 different runs and using same Re 9898 to verify the reproducibility of a method of the present invention.

[Fig. 8] is a diagram showing a comparison between the turbulence kinetic energy achieved between coaxial and co-current mixing captured using computational fluid dynamics (CFD).

Detailed Description of Drawings

Referring to Fig. 1A, antisolvent (4) and organic solvent containing nanoparticle precursors (5) are flowed via conduits (8a, 8b) into a system (1) of the present invention. Pump A (2) is used to control the flow rate of anti-solvent and Pump B (3) is used to control the flow rate of organic solvent containing nanoparticle precursors. The antisolvent (4) and organic solvent (5) are mixed in system (1) to form a mixed stream (6) comprising a nanoparticle dispersion based on the nanoparticle precursors, which is collected in a container (7).

Referring to Fig. 1B, a schematic diagram of a system (1) of the present invention is shown. The system comprises a mixing tee (9) with three inlets (10a, 10b, 10c). Each inlet is linked with Teflon tubing in which flow is controlled by Pump A (2) (not shown), and Pump B (3) (not shown). Pump A (2) (not shown) is used to control the flow rate of antisolvent (4) for two inlets (10a, 10b) through Conduit A (12). Arrows A1, A2, A3, A4 depict the flow path of the antisolvent (4). Pump B (3) (not shown) controls the flow rate of organic solvent (5) for one inlet (10c) through Conduit B (13). Arrows B1 and B2 depict the flow path of the organic solvent (5). Conduit (B) (13) is inserted into Conduit (A) (12) and protrudes coaxially into Conduit A (12) such that the mixing zone (11) occurs downstream of Conduit B (13). Conduit B (13) comprises a side-hole (14) which faces substantially orthogonal to the flow path of antisolvent (4) (Arrows A3, A4). Organic solvent (5) is introduced or injected within the flow of antisolvent (5) (Arrows A3, A4) at the mixing zone (11) in a substantially orthogonal direction (Arrow B2). In the mixing zone (11), organic solvent containing nanoparticle precursors (5) is rapidly mixed

with antisolvent (4), resulting in a mixed stream (6) comprising a nanoparticle dispersion through a self-assembly process, known as nanoprecipitation.

Examples

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

Example 1 - Fabrication of High Intense Millifluidic System (HIMS)

Reference is made to Figs. 1A and 1B. The system (1) or platform is composed of a
10 mixing tee with three inlets (10a, 10b, 10c) (Fig. 1B). Each inlet (10a, 10b, 10c) is linked with a Teflon tubing in which flow rate is controlled by programmable peristaltic pumps. Pump A (Masterflex™ peristaltic pump) (2) is used to control the flow of anti-solvent (4) (for example, water) for two inlets (10a, 10b) (Figs. 1A and 1B), whereas Pump B (Masterflex™ peristaltic pump) (3) controls the flow of organic solvent (for
15 example, THF, acetone, methanol, chloroform or ethanol) which contains nanoparticle precursors (for example, molecules, nanoparticles, drugs, proteins, FDA approved polymer) (5).

In the mixing zone (11), the organic solvent is rapidly mixed with anti-solvent (4), resulting in nanoparticles through a self-assembly process, known as nanoprecipitation.
20 Using this system (1), monodispersed nanoparticles of different sizes were synthesized by varying total flow rate from 35.5 to 700 mL min⁻¹ (Re 500 to 9898).

The tee mixer (9) was assembled using Teflon tee, tubings and fittings (Chemikalie Pte Ltd.). A stainless-steel syringe needle (13) of 21 gauge with a side hole (14) (perpendicular to the direction of flow) is fitted into the tee (9). Tubing of 1.5 mm inner
25 diameter was used and fitted into the tee (9) using fittings. Organic solvent (5) was injected into the mixer through the stainless-steel syringe (13). Antisolvent (for example, water) was injected into the two tee inlets (10a, 10b) through flexible polydimethylsiloxane (PDMS) tubing.

Table 1 below shows the operating parameters of the system and method of the present invention and their range of values.

Table 1

Parameters	Range of Values
Output Flow (mL/min)	35.5 to 700
Average Reynolds number	500 to 9898
Antisolvent to Organic Solvent Ratio	10
Shell nanoparticle precursor to Core nanoparticle precursor ratio	2

5 Example 2 - Calculation of Reynolds number (Re) and flow velocity

The total flow rate in the system was varied from 35.5 mL min⁻¹ to 700 mL min⁻¹. The relation between flow velocity (u) and volumetric flow rate (Q) is defined by Equation 1.

$$u = \frac{4Q}{\pi D_i} \quad (1)$$

where, D_i is the inner diameter of the pipe (1.5 mm).

Re in the system was varied on the basis of total flow rate using the following Equation 2.

$$Re = \frac{\rho u L}{\mu} \quad (2)$$

where, μ represents the viscosity of fluid, ρ represents the density of the fluid and L is a characteristic linear dimension (m). For a circular pipe, the characteristic linear dimension is the same as hydraulic diameter (inner diameter of pipe). Equation 3 represents the relation between Re and flow rate.

$$Re = \frac{4\rho Q}{\pi\mu}$$

(3)

Table 2 shows the variation of Re with respect to flow rate using a system and method of the present invention.

5 Table 2

Total Flow Rate (mL min⁻¹)	35.5	177	700
Reynolds No	500	2500	9898

Example 3 – Preparation of various nanoparticles

Example 3a - AIE encapsulated PLGA and DSPE-PEG Nanoparticles for Bioimaging

- 10 Aggregation induced emission (AIE) dyes generally have rotor like structures, which are known for their high fluorescence emission in aggregate state contrary to other organic dyes which show aggregation caused quenching. They have been successfully applied for cell tracking, vascular imaging, organelle specific imaging and as turn-on probes. High quantum yield AIE dyes possess remarkable photostability and
- 15 biocompatibility. AIE dyes are typically small molecules (300-800 kDa) that spontaneously aggregate in aqueous media like most small hydrophobic drug molecules. Therefore, encapsulating it through nanoprecipitation is a suitable technique.

- An AIE dye, 4,7-bis[4-(1,2,2-triphenylvinyl)phenyl]benzo-2,1,3- thiadiazole (BTPEBT), was encapsulated into different types of phospholipid-polymers, 1,2-
- 20 Distearoyl-*sn*-glycero-3-phosphoethanolamine-polyethylene glycol (DSPE-PEG), FDA approved biocompatible amphiphilic polymers poly(lactide-co-glycolic acid) (PLGA) and polystyrene (PS) through nanoprecipitation.

The hydrophobic AIE dye, BTPEBT and amphiphilic polymeric matrix DSPE-PEG were dissolved in organic solvent tetrahydrofuran (THF). The organic phase was then

mixed with a large amount of water in a tubing of small cross-sectional area. THF is water soluble and its diffusion causes nucleation of the hydrophobic dye.

According to the classical nucleation theory, the process of nanoprecipitation occurs when spontaneous nucleation of the hydrophobic molecule occurs due to supersaturation. Supersaturation has been described as the main driving force during the synthesis of nanoparticles. High and uniform supersaturation throughout the mixing zone gives small size nanoparticles with high homogeneity. If mixing is incomplete or slow during aggregation, non-uniform and large nanoparticles would form. Slow mixing of liquid phases results in non-uniform and low level of local supersaturation, which results in the synthesis of non-uniform and large size nanoparticles. However, if mixing is fast and complete before the aggregation of nanoparticles, then uniform and high level of supersaturation is experienced by the precursors, which gives rise to nanoparticles with smaller diameter and narrower size distribution. There is hence, a need to control the mixing to ensure swift diffusion of solvent into water and the polymer-dye interaction for the synthesis of uniform nanoparticles. In the present method, the mixing is controlled by optimizing one of the most critical flow parameters, Reynolds number (Re).

For the synthesis of BTPEBT encapsulated DSPE-mPEG₂₀₀₀ nanoparticles, the Re was changed from 500 to 9898 at a polymer to dye ratio (PDR) 2 and water to solvent ratio (WSR) 10. By changing the Re from 500 to 9898, the average diameters of BTPEBT-DSPE-PEG NPs reduced from 95 nm to 15 nm (Fig. 2A). This can be attributed to the increase in mixing with an increase in Re, thereby resulting in rapid and uniform nanoprecipitation. The homogeneous size distribution with low PDI was obtained for all the Re numbers. The number average diameter of NPs acquired from Dynamic Light Scattering (DLS) analysis (Fig. 2A) is in good agreement with TEM images (Fig. 2B) for all Re.

Through the method of the present invention, BTPEBT was also encapsulated into DSPE-PEG-NH₂, DSPE-PEG-COOH, DSPE-PEG-MAL, PLGA and PS by varying RE from 500 to 9898 at PDR 2 and WSR 10 (Table 3). The change in the pattern of size of nanoparticles were the same for all polymer matrixes. Due to enhanced mixing, homogeneous size distribution of nanoparticles with low PDI was noticed for all polymer matrixes at different Re. This shows that HIMS is a high throughput platform

for synthesizing AIE dye loaded polymeric nanoparticles with uniform size distribution and desirable size for bioimaging applications.

Table 3. Synthesis of uniform and tailored size AIE (BTPEBT) nanoparticles with different polymeric shell.

Polymers (Shell)	Size Range (min-max, nm)	Polydispersity (PDI)
DSPE-PEG-NH ₂	21-98	0.08-0.11
DSPE-PEG-COOH	18-95	0.07-0.10
DSPE-PEG-MAL	17-91	0.08-0.12
PLGA	20-110	0.08-0.13
PS	72-186	0.10-0.13

5

Example 3b - Drug encapsulated PLGA and DSPE-PEG Nanoparticles for Chemotherapy

To check the versatility of the method of the present invention, several drug encapsulated polymeric nanoparticles of different sizes were synthesized by varying Re (Table 4). For example, Tamoxifen (TAM) loaded DSPE-PEG NPs with different functionalities (methoxy, amine, carboxylic and maleimide) and TAM loaded PLGA nanoparticles were synthesized. By increasing Re from 500 to 9898, the average diameter of TAM loaded DSPE-PEG NPs decreased from 270 to 132 nm with PDI in the range of 0.08 to 0.10 (Fig. 3A). In the case of TAM loaded PLGA nanoparticles, the size of nanoparticles reduced from 255 to 105 nm with increase of Re from 500 to 9898 and PDI in the range of 0.075 to 0.11 (Fig. 3B). Chemotherapy drugs, Doxorubicin (DOX) and Camptothecin (CPT), were also encapsulated into polymeric nanoparticles with tailored sizes and uniform size distribution through the method of the present invention (Table 4). As compared to previously reported bulk synthesis and controllable microfluidic production, the present platform advantageously produced smaller and narrower distributed drug encapsulated polymeric nanoparticles with a tunable size range.

20

Table 4. Synthesis of uniform and tailored size anti-cancer drugs nanoparticles with different core and shell.

Anti-Cancer Drug (Core)	Polymer (Shell)	Size Range (min- max, nm)	Polydispersity (PDI)
Tamoxifen (TAM)	DSPE-PEG	132-270	0.08-0.10
	PLGA	105-255	0.07-0.11
	PS	173-290	0.07-0.10
Doxorubicin (DOX)	DSPE-PEG	17-91	0.08-0.12
	PLGA	20-110	0.08-0.13
	PS	173-290	0.07-0.10
Camptothecin (CPT)	DSPE-PEG	75-180	0.09-0.11
	PLGA	90-210	0.07-0.10

Example 3c - Conjugated Polymer Encapsulated Polymeric Nanoparticles for 5 Photothermal Therapy

Conjugated polymers (CPs) have been successfully used for many biomedical applications such as fluorescence and photoacoustic imaging, photodynamic and photothermal therapy (PTT). They have alternating double-(sp^2) and single-(sp^1) bonds and π -electrons delocalized among their backbones. This assists them through molecular
10 engineering to achieve large optical absorptivity, tuneable absorption wavelength, as well as exciton dissipation pathways including non-radiative decay, fluorescence and phosphorescence emission and as well as reaction with surround molecules to generate reactive oxygen species.

PTT is a promising non-invasive method for cancer therapy, in which light absorbers
15 convert near-infrared light (NIR) energy into heat energy to increase the localized temperature and thereby induce tumor cell necrosis and apoptosis. Owing to the spatiotemporal controlling manner and negligible drug resistance of PTT, nanoparticles for PTT of diverse tumor types were widely applied *in vitro* and *in vivo*. For example, a)

Porphyrin CP containing alternating porphyrin donor (D) and benzothiadiazole (BT) acceptor (A) through ethynylene bridge has been developed for photothermal therapy; b) CP containing alternating 4H-dithieno[3,2-b:2,3-d] pyrrole (DTP) and diketopyrrolopyrrole (DPP) has been effectively used for photoacoustic imaging and photothermal therapy; c) Poly(1,2-bis(4-((6-bromohexyl)oxy)phenyl)-1,2-diphenylethene-co-alt-9,10-anthraquinone) (PTPEAQ) has been successfully applied for photodynamic therapy with image guided ablation via AIE emission.

Through the method of the present invention, CP loaded DSPE-PEG and CP loaded PLGA NPs were synthesized with uniform size distribution by increasing Re from 500 to 9898. The average diameter of CP loaded DSPE-PEG NPs decreased from 135 to 42 nm with PDI in the range of 0.08 to 0.11 (Fig. 4A). In case of CP loaded PLGA NPs, the size of NPs reduced from 148 to 53 nm with increase of Re from 500 to 9898 and PDI in the range of 0.07 to 0.10.

15 **Example 3d - Inorganic Iron Oxide Nanoparticles and Conjugated Polymer Encapsulated Polymeric Nanoparticles**

Nanoparticle-based diagnostic agents for non-invasive biomedical imaging are emerging as a promising paradigm towards the early diagnosis of cancer. While conventional biomedical imaging techniques have played a key role in preclinical and clinical cancer diagnosis, valuable information provided by a single imaging modality is usually limited and insufficient. An integration of different imaging modalities into a single nano-formulation thus holds great promise to obtain complementary information to enhance the precision, accuracy and efficacy of tumor diagnosis.

Inorganic iron oxide (IO) nanoparticles and conjugated polymers (CP) were integrated into one single nanocomposite using DSPE-PEG or PLGA as the polymer matrix through the method of the present invention. Inorganic IO nanoparticles have been studied extensively during the past decades as a biocompatible negative MRI contrast agent with high transversal relaxivity. CP based nanoparticles with intrinsically long wavelength absorption and large extinction coefficient have attracted increasing attention for photoacoustic (PA) imaging due to their high photostability, superior PA signal intensity and good biocompatibility. Various engineering strategies have been

developed to yield IO-based or CP-based nanoparticles with superior imaging performance for brain vascular imaging, targeted tumor imaging, reactive oxygen species imaging, etc. Through the encapsulation of these two types of nanomaterials into one single nanoformulation, a more comprehensive imaging performance using multimodal tumor imaging techniques may be achieved.

A diketopyrrolopyrrole (DPP) based conjugated polymer poly(diketopyrrolopyrrole-terthiophene) (PDPP3T) was chosen as the PA active component due to its high PA brightness and biologically inert nature. The molecular weight of the CP used was 31,000 with a polydispersity of approximately 3.0, so as to facilitate its good solubility in tetrahydrofuran (THF). Iron oxide nanoparticles coated with a layer of hydrophobic oleic acid on the surface were prepared by thermal decomposition method and used as the MRI active component. The CP-IO nanocomposites encapsulated polymeric nanoparticles was fabricated through the HIMS system by controlling Re. DSPE-PEG or PLGA were experimented as the polymer matrix, through which the hydrophilic PEG group endows the as-prepared CP-IO nanocomposites with excellent water dispersibility and biocompatibility, such that it could be further developed for different biological applications. By increasing Re from 500 to 9898, the average diameter of CP-IO loaded DSPE-PEG NPs decreased from 153 to 49 nm with PDI in the range of 0.08 to 0.12 (Fig. 5A). In case of CP-IO loaded PLGA NPs, the size of the nanoparticles reduced from 165 to 55 nm with increase of Re from 500 to 9898 and PDI in the range of 0.09 to 0.12. The number average diameter of nanoparticles acquired from DLS analysis (Fig. 5A) is in good agreement with TEM images (Fig. 5B).

Example 3e - Fabrication of DPhCzT Nanocrystals and DPhCzT nanocrystals encapsulated by DSPE-PEG

In the last decade, bioimaging through room-temperature phosphorescence (RTP) has gradually become an active area of research due to their persistent lifetime imaging, which can range from several milliseconds to few seconds. This unique property of long and persistent emission provides for a wide range of applications, such as anti-counterfeiting labeling for advanced data security, molecular sensing, and time resolved phosphorescence biological imaging.

Most of the phosphorescent compounds possess high intermolecular and intramolecular motions (non-radiative process), which restrains their RTP behavior. Through the transformation from amorphous to crystalline state of these molecules, significant enhancement in the RTP has been observed, as it locks down the intramolecular motions and diminishes the vibrational relaxation pathways of excited molecules.

However, until now, there exists no simple and robust method available for the synthesis of small and uniform nanocrystals with size around 100 nm. Currently available methods involve the transformation of phosphorescent compounds from amorphous to crystalline state through the method of ultra-sonication, which disadvantageously results in the formation of micron-size and non-uniform nanocrystals.

The methods of the present invention overcome this limitation. As evidence of this, the method of the present invention was used in the synthesis of 4,6-diphenyl-2-carbazolyl-1,3,5-triazine (DPhCzT) phosphorescent nanocrystals and the results were compared with the sonication technique. The results are shown in Figs. 6A to 6H.

In the sonication method, anti-solvent (water) to organic solvent volume ratio was maintained as 10 for the crystallization of DPhCzT through ultrasonication. The DPhCzT dye was dissolved in tetrahydrofuran (THF) at a concentration of 2 mg/mL and mixed with water through probe sonication for 1 minute. After sonication, the mixture was kept for solvent evaporation. As per Transmission Electron Microscopy (TEM) analysis, large aggregates of DPhCzT were formed with large microns size and high polydispersity (Fig. 6C).

In the method of the present invention, a DPhCzT solvent mixture was mixed with water by using the present HIMS system at different Re. Through HIMS, significant reduction in the size of DPhCzT nanocrystals (from a few microns to 100 nm) can be seen with an increase in Re from 500 to 2500 (Figs. 6D-6G). Table 5 below shows the difference in particle sizes obtained using the sonification method verses the method of the present invention at different Re:

Table 5

Method	Particle size (nm)
Sonification Method (Fig. 6C)	1429 nm
HIMS (Re 500) (Fig. 6D)	921 nm
HIMS (Re 1000) (Fig. 6E)	329 nm
HIMS (Re 1500) (Fig. 6F)	184 nm
HIMS (Re 2500) (Fig. 6G)	62 nm

Additionally, with reference to Fig. 6A, it can be seen that the DPhCzT molecules can be excited in a short range of wavelength (around 310 nm) in crystalline form. With reference to Fig. 6B, it can be seen that the DPhCzT molecules after crystallization show multiple emission peaks at 448, 476 and 506 nm, indicate the formation of crystalline DPhCzT from amorphous state. In addition, strong emission can be captured at peak points during biological applications.

As the DPhCzT dye is hydrophobic in nature, the synthesized nanocrystals are prone to aggregation over time through the process of Ostwald Ripening. Therefore, by using HIMS, DPhCzT nanocrystals with a DSPE-mPEG shell may also be synthesized for biological applications. Using the method of the present invention, DSPE-PEG and DPhCzT were dissolved together in THF at PDR 4 with dye concentration of 2 mg/mL in solvent and mixed with water through HIMS by maintaining WSR 10. As shown in Fig. 6H, highly uniform and small size polymer (51 nm) encapsulated DPhCzT nanocrystals were synthesized by using HIMS.

Example 4 - Reproducibility and Production

To check the reliability of the system of the present invention, nanoparticles were synthesized using the same flow parameters (Re 9898 and WSR 10) for 5 runs of the experiment for different varieties of nanoparticles (Fig. 7). The number average mean diameters of NPs were reproducible with particle average size $\pm 3\text{nm}$ in different runs, indicating the high reproducibility of this platform in terms of size. For all the runs, particles were synthesized with uniform size distribution with PDI less than 0.13. Such a high degree of reproducibility in terms of NP size and its distribution makes the system of the present invention a simple, versatile and reproducible platform for synthesizing polymeric nanoparticles.

As the present system operates at high flow rate, it provides high production (1.01 kg day^{-1}) of nanoparticles during the operation (Table 6). Typically, flow rate in the present system may range from 35.4 mL min^{-1} to 700 mL min^{-1} with Re varying from 500 to 9898 (Table 1). It is assumed that all the nanoparticle precursors pumped into the system were converted into nanoparticles. The maximum concentration of precursors in the organic solvent used to synthesize nanoparticles was 10 mg mL^{-1} , which resulted in the production of nanoparticles with a concentration of 1 mg mL^{-1} after evaporation of solvent at WSR 10. Therefore, at high Re (9898) or flow rate (700 mL min^{-1}), the preparation of nanoparticles can reach 1.01 kg day^{-1} (Table 6). The anticipated nanoparticle synthesis rate for clinical studies is in the order of 0.1 kg day^{-1} and 1 kg day^{-1} for industrial-scale requirement, which can be easily met in the system of the present invention by changing the flow parameters and polymer concentrations. As the system runs in a continuous mode using peristaltic pump, the physiochemical properties of nanoparticles are also independent of the batch size for the same parameters. Hence, the system of the present invention can be used to synthesize wide varieties of nanoparticles with high production rate without conceding the reproducibility in the system.

Table 6

Reynolds number	500	9898
Total flow rate (mL min ⁻¹)	35.5	700
Production rate a* (kg day ⁻¹)	0.009	0.20
Production rate b* (kg day ⁻¹)	0.045	1.01

* Rate a and rate b are with a precursor concentration of 2 mg mL⁻¹ and 10 mg mL⁻¹.

Comparative Examples

5 Comparative Example 1 – Comparison of turbulence between coaxial and cross-current mixing

Computational fluid dynamics (CFD) was used to capture the mixing of organic solvent (for example, THF) into antisolvent (for example, water) by comparing the turbulence achieved for both coaxial (or co-current) and cross-current systems. At high Re number, mixing can be computed by the amount of turbulence achieved in the system (Fig. 8). Turbulence kinetic energy (m²/s²) of the system represents the mean kinetic energy per unit mass associated with the eddies present in the flow. More turbulence kinetic energy dissipated in the system means more eddies resulting in rapid mixing and uniformity in self-assembly of the nanoparticle precursors. As per the CFD simulation, at Re 2500, the turbulence kinetic energy dissipated in cross-current mixing is much higher than coaxial-current mixing (Fig. 8).

To conduct CFD study, ANSYS FLUENT 18.2 software was used to solve laminar model for low Re and k-epsilon (2 equation) model for high Re (transition to turbulence region). Along with momentum equations models, we included the species transport model for diffusion and convection of a diluted species. Contours of mass fraction of organic solvent with smooth shading and lines were captured to study the mixing process for coaxial and cross-current mixing at different Re and water to solvent ratio

(WSR). For high Re, the contour of turbulence kinetic energy was also obtained to compare the eddies generated in coaxial and cross-current mixing (Fig. 8).

In Fig. 8, the contours of turbulence kinetic energy are outlined and labelled according to the key located at the bottom of the figure (labelled 1 to 25 for the range between 10^{-6} m^2/s^2 to $9 \times 10^{-2} \text{ m}^2/\text{s}^2$). For coaxial mixing, it is clearly shown that much lower turbulence kinetic energy (m^2/s^2) was achieved, with the highest turbulence kinetic energy only being about 7 to 8. In comparison, for cross-current mixing, a much higher turbulence kinetic energy (m^2/s^2) was achieved, with the highest turbulence kinetic energy being about 21 to 25. Furthermore, the highest turbulence zone from co-current mixing is much larger in area compared to the highest turbulence zone from coaxial mixing. As discussed above, higher turbulent flow advantageously results in a higher degree of mixing which results in the generation of highly uniform nanoparticles. Hence, it is shown that co-current mixing for nanoparticle fabrication is superior to coaxial mixing for nanoparticle fabrication.

Industrial Applicability

The disclosed systems and methods may be used in fabricating nanoparticles. The disclosed systems and methods may result in high intense mixing of first solvent and second solvent. This high intense mixing may be generated through cross-current mixing of the first and second solvents. This high intense mixing may advantageously result in the synthesis of nanoparticles with high uniformity for all size ranges.

Further advantageously, the disclosed systems and methods may result in high throughput of nanoparticles to meet industrial requirements.

Also advantageously, the disclosed systems and methods may easily be optimized by adjusting solvent properties to encapsulate large molecules which are difficult to achieve with traditional methods. Due to the high intense mixing generated by the disclosed systems and methods, together with being able to adjust the properties of the solvents used, large sized conjugated polymer molecules may easily be encapsulated inside polymers approved by the Food and Drug Administration of the

United States of America (FDA) for fluorescence and photoacoustic imaging, photodynamic and photothermal therapy applications.

The disclosed systems and methods may also advantageously provide high versatility by allowing the fabrication of more than a hundred varieties of nanoparticles for different industrial and research applications. For the production of core-shell nanoparticles, the disclosed systems and methods may advantageously be used to customize the shell and core.

The disclosed systems and methods also advantageously allow for the optimization of Reynolds number to obtain different particle sizes. This may advantageously result in tight control over the size of nanoparticles for different biological applications.

The disclosed systems and methods further advantageously allow the interplay between operating parameters like Reynolds number and solvent properties which may be altered simultaneously to achieve the best operating zone for the synthesis of different varieties of nanoparticles for various applications.

The disclosed systems and methods also advantageously allow for enhanced mixing for the synthesis of polymeric nanoparticles with tailored size, high uniformity, high reproducibility and high production.

The disclosed systems and methods aid in upscaling production for a wide variety of nanoparticles while being able to tightly control the size of nanoparticles with high uniformity and high reproducibility.

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 modifications and adaptations come within the scope of the appended claims.