(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 October 2024 (31.10.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/226778 A2

(51) International Patent Classification:
Not classified

(21) International Application Number:
PCT/US2024/026244

(22) International Filing Date:
25 April 2024 (25.04.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/498,383 26 April 2023 (26.04.2023) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,

(54) Title: 3D PRINTING ELECTROSPINNING SYSTEMS AND METHODS

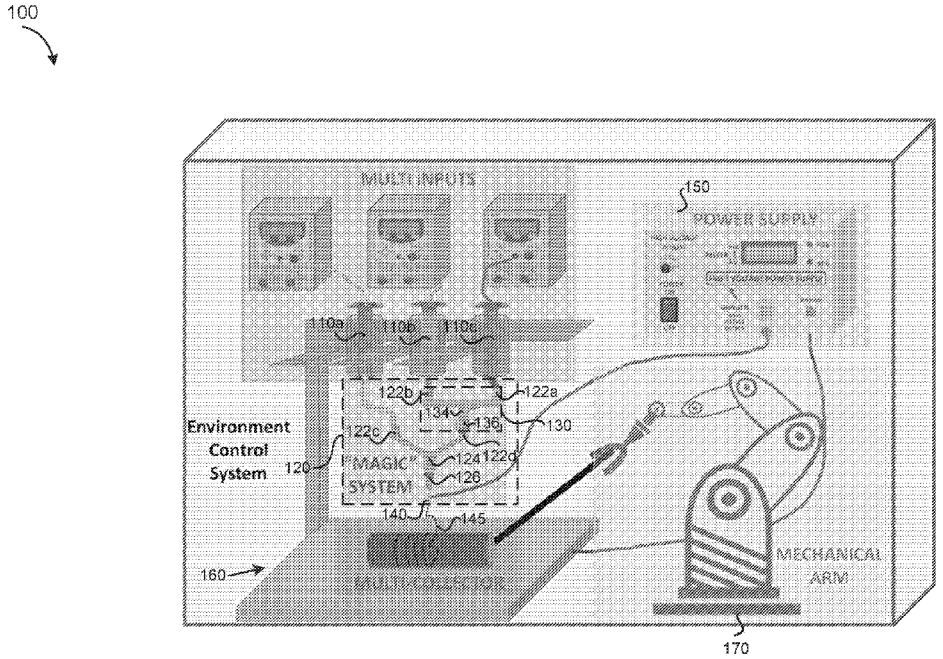

FIG. 1

(57) Abstract: Described are techniques, systems, and methods including those employing electrospinning, printheads including multiple linkers, and a mechanical arm that facilitates a continuous, safe, and efficient fiber and/or fiber mat production system. The printheads may be in fluid communication with a spinneret to form droplets suitable for electrospinning. The electrospun fibers may include complex structures. In some examples, the electrospun fibers may include multiple layers, particles (e.g., microparticles, nanoparticles, etc.) and/or different fiber components with desired structures (e.g., bi-axial fibers, tri-axial fibers, co-axial fibers, co-biaxial fibers, etc.). The electrospun fibers may be disposed in an environmental simulator to mimic the release of a disintegrated test drug in a semi-permeable membrane.

[Continued on next page]

WO 2024/226778 A2

TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

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Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

3D PRINTING ELECTROSPINNING SYSTEMS AND METHODS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of and priority to U.S. Provisional Application No. 63/498,383, filed on April 26, 2023, and titled "XSPIN – A 3D PRINTING ELECTROSPINNING SYSTEM," the content of which is herein incorporated by reference in its entirety for all purposes.

FIELD

[0002] This present disclosure is in the field of electrospinning. This present disclosure relates generally to devices, systems, and methods for electrospinning of fibers including various components.

BACKGROUND

[0003] Fibers are a form of string-like material that is characterized by a large length to width ratio. Fibers are ubiquitous in nature and are widely developed in synthetic forms. Natural fibers present in hair, fur of animals, and in bird feathers consisting mainly of proteins, provide essential functionality, such as protection, sensing, regulation, etc. Silk, produced by spiders, worms, and other species, is also a protein-based fiber with an extremely high strength-to-weight ratio. Fiber-like cellulose filaments, with diameters ranging from micrometers to nanometers, present in the cellular walls of plants serve as the base component of paper and cardboard. Plant fibers in various forms are also used in textiles and for nutrition. Man-made fibers are produced with higher uniformity and reproducibility in order to enable a more predictable manufacturing process. In particular, membranes containing fibers with nanometer diameters provide an extremely high surface area and high porosity material, presenting useful properties in terms of mechanical strength, breathability, sensitivity to external stimuli, and biomimetic environment.

[0004] Electrospinning is a fiber fabrication method combining electrospraying and conventional solution dry spinning fibers. The process uses electric force to draw charged threads of polymer solutions, or polymer melts up to form fibers. The formed fiber diameter can range from hundred nanometers to a few micrometers. Compared to other fiber fabrication processes, electrospinning avoids using coagulation chemistry or high temperature to produce solid threads from a solution. Also, this method prevents the final product containing any solvent.

SUMMARY

[0005] Fibers featuring large length-to-width ratios and strength-to-weight ratios have emerged as powerful tools in pharmaceutical science and tissue engineering. Without the involvement of

coagulation chemistry or high temperature, electrospinning is capable of fabricating fibers or mats of electrospun fibers including large and complex molecules by applying electric force to a solution droplet. Integrated with printheads and mechanical arms, a continuous, safe, and efficient fiber production system can be achieved.

[0006] In particular, electrospinning is a versatile and effective technique for producing polymer fibers with diameters ranging from several nanometers to several micrometers. This method can be useful in the pharmaceutical industry for applications relating to drug delivery and tissue engineering. In drug delivery, electrospinning can be used to create drug-loaded fibers with controlled release properties. These fibers can be used as a platform for drug delivery systems that can be implanted in the body or applied topically to the skin. Electrospun fibers have a high surface area-to-volume ratio, which can improve drug release kinetics, enhance drug bioavailability, and reduce the frequency of drug administration. General steps involved in using electrospinning for drug delivery may include: (1) polymer selection that includes choosing a suitable polymer that can be electrospun and is compatible with the drug to be delivered. The polymer should also have appropriate mechanical and chemical properties for the intended application. General steps may further include (2) drug incorporation that includes dissolving the drug in the polymer solution before electrospinning. The concentration of the drug in the polymer solution should be optimized for the desired release rate. General steps may further include (3) electrospinning that includes using an electrospinning apparatus to produce nanofibers from the polymer solution. The electrospinning parameters, such as voltage, flow rate, and distance between the spinneret and the collector, should be optimized to obtain uniform and fine nanofibers. General steps may further include (4) cross-linking including cross-linking the electrospun nanofibers to improve their stability and prevent the drug from leaching out. The cross-linking method may depend on the polymer used and the intended application. General steps may further include (5) drug release evaluation including evaluating the drug release kinetics from the electrospun nanofibers using in vitro or in vivo methods. The release rate can be adjusted by modifying the polymer composition, fiber diameter, and drug loading. General steps may further include (6) formulation development includes developing the drug delivery system based on the electrospun nanofibers, such as patches, implants, or dressings, depending on the intended application. Electrospinning is a versatile technique that can be used for drug delivery. The success of the technique may depend on the choice of polymer, drug loading, electrospinning parameters, and the intended application.

[0007] For tissue engineering, electrospinning and 3D printing are both innovative technologies that have shown great potential in the field of tissue engineering. Combining these two

technologies can offer new opportunities for the development of tissue-engineered constructs with enhanced mechanical and biological properties. One approach is to use 3D printing technology to fabricate a 3D structure or scaffold and then electrospin fibers onto the scaffold to create a hybrid structure. This can provide a more complex structure than can be achieved with electrospinning alone, allowing for more advanced tissue engineering constructs. The electrospun fibers can provide a high surface area-to-volume ratio, which can improve cell attachment, proliferation, and differentiation. Another approach is to use electrospinning to create fibers and then 3D print the fibers into a desired shape or structure. This can allow for greater control over the shape and size of the final tissue-engineered construct. Thus, combining electrospinning and 3D printing can offer new opportunities for the development of tissue-engineered constructs with enhanced mechanical and biological properties. However, further developments may be useful for optimizing these techniques and evaluate their potential in a wider range of applications.

[0008] Despite the potential benefits of electrospinning for drug delivery, there are several challenges that can be addressed to use this technique more successfully in pharmaceutical science: (1) scale-up: one challenge of electrospinning is the difficulty in scaling up the process to produce large quantities of nanofibers for drug delivery. Scaling up can be challenging due to issues such as nozzle clogging, inconsistent fiber size, and decreased electrospinning efficiency. Scaling up of electrospinning may require careful consideration of several factors including the number of nozzles, size of the collector, voltage power supply and the flow rate amongst others. The scaling up of electrospinning may benefit tremendously by using a mechanical arm. The mechanical arm can be programmed to move the spinneret over the collector in a precise pattern, and to maintain a consistent distance between the spinneret and the collector. In addition to this, the mechanical arm can help to achieve the following roles: 1) controlling the range of motions in 6 dimensions, 2) improved control on speed to ensure fiber quality is not damaged, and 3) improved control of precision.

[0009] The challenges that can be addressed to successfully use this technique in pharmaceutical science may further include (2) reproducibility: another challenge is ensuring the reproducibility of the electrospinning process, which can be affected by various factors such as environmental conditions, polymer properties, and electrospinning parameters. Reproducibility is important for the development of consistent and reliable drug delivery systems. The challenges that can be addressed to successfully use this technique in pharmaceutical science may additionally include (3) drug stability: the incorporation of drugs into electrospun nanofibers can also be challenging, as drugs may degrade or lose activity during the electrospinning process or during storage. The selection of a suitable polymer and the optimization of the drug loading and release rate may be

important to maintaining drug stability. (4) Biocompatibility: the electrospun nanofibers should be biocompatible and non-toxic to avoid adverse reactions or inflammation when used for drug delivery in vivo. Biocompatibility testing should be performed before using electrospun nanofibers for drug delivery. (5) Cost: finally, the cost of electrospinning equipment and materials can be a significant barrier to using this technique for drug delivery. The cost of materials, equipment, and labor should be carefully considered when developing electrospun drug delivery systems. While electrospinning is a promising technique for drug delivery, there are several challenges that should be addressed to ensure its successful use in pharmaceutical science. Addressing these challenges may require continued research and development to optimize electrospinning parameters, improve drug stability, ensure biocompatibility, and reduce costs. The techniques described in the present disclosure may overcome one or more of these challenges.

[0010] In addition to the challenges discussed earlier regarding the use of electrospinning in drug delivery, there are some specific challenges when using electrospinning for tissue engineering. The challenges include (1) mimicking native tissue structure: tissue engineering may employ the creation of biomimetic scaffolds that can mimic the natural structure of the target tissue. Electrospinning can produce nanofibers with high surface area-to-volume ratios, but achieving the desired three-dimensional structure can be challenging. The challenges may further include (2) mechanical properties: the mechanical properties of electrospun scaffolds, such as stiffness, strength, and elasticity, should be carefully controlled to ensure compatibility with the target tissue. Achieving the desired mechanical properties while maintaining biocompatibility and drug release can be difficult. The challenges may further include (3) cell infiltration: the electrospun scaffolds should support the growth and proliferation of cells and allow for cell infiltration and integration with the host tissue. Achieving optimal cell infiltration and integration can be challenging due to the small pore size of the nanofibers. The challenges may further include (4) long-term stability: electrospun scaffolds should maintain their structural and functional properties over an extended period to support tissue regeneration. Achieving long-term stability can be challenging due to factors such as degradation of the polymer, inflammatory response, and mechanical stress. The challenges may further include (5) standardization: standardization of the electrospinning process and scaffold properties is important for reproducibility and scalability of tissue engineering applications. Achieving standardized electrospinning protocols can be challenging due to the complex and dynamic nature of the process. Thus, even though electrospinning has great potential in tissue engineering, there are several challenges that should be addressed to achieve successful outcomes. Addressing these challenges may require continued research and development to optimize electrospinning

parameters, scaffold properties, and cell-tissue interactions. Again, the techniques described in the present disclosure may overcome one or more of these challenges.

[0011] The present disclosure provides fiber fabrication devices and electrospun fibers. In some examples, a fiber fabrication device integrates an electrospinning device, printheads containing linkers, and a mechanical arm to obtain large-scale manufacturing of fibers.

[0012] In an aspect, integrated electrospinning systems are provided. An example system includes a plurality of supply containers for preparing or storing a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API), a printhead in fluid communication with the plurality of supply containers, the printhead comprises a plurality of linkers, wherein each linker is in fluid communication with a supply outlet of one of the plurality of supply containers, a fluid mixer for mixing components from the plurality of linkers into a mixture, and one or more outlets in fluid communication with the fluid mixer, and a spinneret in fluid communication with the one or more outlets, a high-voltage power supply in electrical communication with the spinneret to form electrospun fibers, and a collector to collect the electrospun fibers. In some examples, the collector further comprises a stage, a mechanical arm, and/or a conveyor belt. In some examples, the integrated electrospinning system further comprises an environmental simulator, wherein the environmental simulator comprises a temperature chamber, refrigerated chambers, humidity chamber, vacuum chamber, freeze-thaw chambers, environmental exposure chambers, growth chamber, light control, pressure controlled, thermal cycling, hypoxia chamber, electrostatic discharge chamber, electrochemical test chambers, antistatic charge chamber, environmental exposure chamber, acceleration test chamber, biochemical test chambers, vibration chamber, stability chamber, salt spray chamber, thermal shock chamber, altitude chamber, anechoic chamber, gas-controlled chamber, air quality-controlled chambers and/or sterilization chamber. In some examples, the environmental simulator includes a plurality of environmental parameters, wherein the plurality of environmental parameters comprises temperature, humidity, gas, optical conditions, and/or stability. In some examples, the stage is adjustable in six degrees of freedom. In some examples, the stage includes a roller and/or a spinning roller. In some examples, wherein the stage is adjustable along a first axis, a second axis, and/or a third axis. In some examples, the mechanical arm is assembled with the stage. In some examples, the stage comprises a collecting surface that rotates or translates to collect the electrospun fibers. In some examples, the integrated electrospinning system includes further comprises equipment for maintaining the plurality of supply containers, the printhead, and/or the collector in a sterile environment. In some examples, the mechanical arm comprises multiple metal joints. In some examples, the mechanical arm has

six degrees of freedom. In some examples, at least one of the respective components include particles. In some examples, the particles having diameters of from 10 nm to 1100 μm. In some examples, the electrospun fibers include particles. In some examples, at least one of the respective components include live cells. In some examples, the polymer is a biodegradable polymer selected from the group consisting of poly(lactide-co-glycolide), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine). In some examples, the integrated electrospinning system further comprises a continuous belt system to deliver the electrospun fibers to a downstream process. In some examples, the integrated electrospinning system further comprises at least one pneumatic pump and/or syringe stress pump to facilitate fluidic motion of the respective component of the plurality of supply containers. In some examples, the fluid mixer comprises a coaxial arrangement of a first fluidic channel in fluid communication with a first linker and a second fluid channel in fluid communication with a second linker, wherein the first component is provided to the first linker, and wherein the second component is provided to the second linker. In some examples, the fluid mixer comprises a stirrer. In some examples, the integrated electrospinning system further comprises an electric field or a magnetic field for activating the stirrer. In some examples, the stirrer rotates about an axis parallel to a flow direction of the mixture through the one or more outlets. In some examples, the stirrer rotates about an axis perpendicular to a flow direction of the mixture through the one or more outlets. In some examples, the fluid mixer is characterized by a fluidic arrangement providing the first component and the second component in a coaxial flow configuration, a biaxial flow configuration, or a triaxial or higher axial flow configuration. In some examples, the fluid mixer comprises a mixing architecture characterized by an "S" shape or a helix shape. In some examples, the integrated electrospinning system further comprises one or more temperature sensors or temperature controllers for monitoring or controlling a temperature of mixture in the fluid mixer. In some examples, the mixture comprises or further comprises one or more of a cosolvent, a surfactant, a preservative, live cells, polymer, thickeners, diluents, biologically active polynucleotides, cellular components, an additional active ingredient, a salt, a preservative, a protein, a peptide, an amino acid, or a nucleic acid component. In some examples, the API comprises a protein, an antibody, a nucleic acid, messenger ribonucleic acid (mRNA) molecules, a lipid nanoparticle, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing endonucleases or meganucleases, a growth factor, a plasmid, a hydrophilic pharmaceutical, a lipophilic pharmaceutical, a viral particle, a virus-like

particle, a live yeast cell, a live recombinant yeast cell, a live fungus, a live bacterial cell, a live recombinant bacterial cell, a live insect cell, a live mammalian cell, or a live mesenchymal stem cell. In some examples, the polymer is a biodegradable polymer selected from the group consisting of poly(lactide-co-glycolide), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine). In some examples, the non-polymeric excipient is a hydrophilic substance, a hydrophobic substance, a non-reducing sugar, trehalose, sucrose, a polyol, mannitol, sorbitol, xylitol, an amino acid, leucine, or L-arginine. In some examples, the integrated electrospinning system further comprises one or more mixing vessels in fluid communication with the plurality of supply containers for preparing and providing components to the plurality of supply containers. In some examples, the integrated electrospinning system further comprises one or more sensors monitoring a concentration of the API. In some examples, the one or more sensors comprise a UV probe. In some examples, the plurality of linkers comprises a Luer lock, a Luer slip, or a slip tip. In some examples, the plurality of linkers is configured to couple with an outlet of an additional printhead. In some examples, the plurality of linkers is characterized by a diameter of less than or about 5.0 mm. In some examples, a first respective component comprises a first API and a second respective component comprises a second API that is different from the first API. In some examples, a first respective component has a first polarity and a second respective component has second polarity that is different from the first polarity, wherein the first respective component and the second respective component are immiscible.

[0013] In another aspect, methods of manufacturing an electrospun fiber are provided. An example method comprises providing a first component to a first linker of a plurality of linkers of a printhead, providing a second component to a second linker of the plurality of linkers of the printhead, combining the first component and the second component in a fluid mixer of the printhead, forming a mixture of the first component and the second component in the printhead, delivering the mixture to a spinneret in fluid communication with the printhead, applying voltage to the mixture at the spinneret to form fibers, and collecting the fibers by using a controlled collector. In some examples, the first component comprises a first solvent and the second component comprises a second solvent having a different polarity from the first solvent. In some examples, the first component comprises a first active pharmaceutical ingredient (API) and the second component comprises a second API that is different from the first API.

[0014] In another aspect, integrated electrospinning systems are provided. An example integrated electrospinning system comprises a plurality of supply containers for preparing or storing a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API), a plurality of spinnerets in fluid communication with the plurality of supply containers to form a plurality of electrospinning droplets, a high-voltage power supply in electrical communication with the plurality of spinnerets to form electrospun fibers from the plurality of electrospinning droplets, and a collector including a stage and a mechanical arm. In some examples, the integrated electrospinning system further comprises a printhead in fluid communication with the plurality of supply containers, wherein the printhead comprises a plurality of linkers, wherein each linker is in fluid communication with a supply outlet of one of the plurality of supply containers, a fluid mixer for mixing components from the plurality of linkers into a mixture, and one or more printhead outlets in fluid communication with the fluid mixer and the plurality of spinnerets. In some examples, the API comprises a protein, an antibody, a nucleic acid, messenger ribonucleic acid (mRNA) molecules, a lipid nanoparticle, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing endonucleases or meganucleases, a growth factor, a plasmid, a hydrophilic pharmaceutical, a lipophilic pharmaceutical, a viral particle, a virus-like particle, a live yeast cell, a live recombinant yeast cell, a live fungus, a live bacterial cell, a live recombinant bacterial cell, a live insect cell, a live mammalian cell, or a live mesenchymal stem cell. In some examples, the respective component is semi-permeable. In some examples, the polymer is a biodegradable polymer selected from the group consisting of poly(lactide-co-glycolide), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine). In some examples, the non-polymeric excipient is a hydrophilic substance, a hydrophobic substance, a non-reducing sugar, trehalose, sucrose, a polyol, mannitol, sorbitol, xylitol, an amino acid, leucine, or L-arginine.

[0015] In another aspect, electrospun fibers are provided. An example electrospun fiber comprises a first segment including a first component, and a second segment including a second component, wherein the first segment and the second segment have uniform diameters. In some examples, the second segment coaxially surrounds the first segment. In some examples, the first segment is characterized by a first axis and the second segment is characterized by a second axis. In some examples, the electrospun fiber further comprises a third segment. In some examples, the

second segment coaxially surrounds the first segment and the third segment coaxially surrounds the first segment and the second segment. In some examples, the first segment is characterized by a first axis, the second segment is characterized by a second axis, and the third segment is characterized by a third axis. In some examples, the electrospun fiber further comprises live cells. In some examples, the electrospun fiber is edible. In some examples, the first segment and the second segment are configured to overcome phase segregation and separation.

[0016] In another aspect, systems using electrospun fiber to mimic the absorption of a test drug in a simulator are provided. An example system comprises an environmental simulator including at least one simulating component, wherein the simulating component includes a simulating fluid, and a simulating fiber disposed in the environmental simulator, wherein the simulating fiber comprises an inner layer, an inter layer surrounding the inner layer, and an outer layer surrounding the inter layer and the inner layer. In some examples, the simulating fiber comprises a hydrophobic material and a hydrophilic material. In some examples, the simulating fiber is semi-permeable to only allow a selected group of materials to pass through. In some examples, the at least one simulating component comprises a temperature chamber, humidity chamber, vacuum chamber, vibration chamber, stability chamber, salt spray chamber, thermal shock chamber, altitude chamber, anechoic chamber, gas-controlled chamber, and/or sterilization chamber.

[**0017**] In an aspect, an integrated electrospinning system is provided. An example integrated electrospinning system includes a plurality of supply containers for preparing or storing a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API); a first printhead in fluid communication with the plurality of supply containers, the first printhead comprising a first linker, a second linker, and a first coil; a second printhead in fluid communication with the first printhead, the second printhead comprising a third linker, a second coil, and a connecting linker in fluid connection with the first printhead, the second coil, and a spinneret; a high-voltage power supply in electrical communication with the spinneret to form electrospun fibers; and a collector to collect the electrospun fibers. In some examples, the third linker is in fluid communication with a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API). In some examples, the first coil is configured to fabricate nanoparticles or microparticles, and wherein the second coil is configured to mix the nanoparticles or microparticles fabricated in the first coil with a respective component delivered from the third linker.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1 is a schematic illustration of an integrated electrospinning system according to some examples.

[0019] FIG. 2 provides schematic illustrations of printheads for component mixing and corresponding electrospun fibers according to some examples.

[0020] FIG. 3 provides schematic illustrations of a printhead for multi-component fabrication of an electrospun fiber according to some examples.

[0021] FIG. 4 is a schematic illustration of an integrated electrospinning system including multiple spinnerets according to some examples.

[0022] FIG. 5A is a schematic illustration of an integrated electrospinning system according to some examples.

[0023] FIG. 5B is a schematic illustration of a system including a first printhead and a second printhead according to some examples.

[0024] FIG. 5C is a schematic illustration of a system including a first printhead and a second printhead according to some examples.

[0025] FIG. 6 provides a schematic illustration of an environmental simulator according to some examples.

[0026] FIG. 7 provides scanning electron microscopy images of electrospun fiber mats according to some examples.

[0027] FIG. 8 provides a scanning electron microscopy image of electrospun fiber mats according to some examples.

[0028] FIG. 9 provides a scanning electron microscopy image of electrospun fiber mats according to some examples.

[0029] FIG. 10A provides data showing attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) of electrospun fibers according to some examples.

[0030] FIG. 10B provides data showing attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) of electrospun fibers according to some examples.

[0031] FIG. 11A provides data showing attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) of electrospun fibers according to some examples.

[0032] FIG. 11B provides data showing attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) of electrospun fibers according to some examples.

[0033] FIG. 11C provides data showing attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) of electrospun fibers according to some examples.

[0034] FIG. 12 provides a scanning electron microscopy image of electrospun fiber mats according to some examples.

[0035] FIG. 13 provides data showing thermogravimetric analysis (TGA) of raw materials and mixtures according to some examples.

[0036] FIG. 14 provides DSC thermograms of electrospun fibers according to some examples.

[0037] FIG. 15A provides a bar chart showing the hydration of electrospun fibers according to some examples.

[0038] FIG. 15B provides a bar chart showing the entrapment efficiency of CUR and RTV of electrospun fibers according to some examples.

[0039] FIG. 16A contains a graph showing the cumulative drug release of RTV from electrospun fibers according to some examples in 0.1N HCl (pH 1.2) with dialysis tube at 120 minutes.

[0040] FIG. 16B contains a graph showing the cumulative drug release of RTV from electrospun fibers according to some examples in 0.1N HCl (pH 1.2) with dialysis tube at 7200 minutes (120 hours).

[0041] FIG. 16C contains a graph showing the cumulative drug release of RTV from electrospun fibers according to some examples in 0.1N HCl (pH 1.2) with dialysis tube.

[0042] FIG. 16D contains a graph showing the cumulative drug release of RTV from electrospun fibers according to some examples in 0.1N HCl (pH 1.2) without dialysis tube.

[0043] FIG. 17A provides shear stress and viscosity as a function of shear rate for the RTV electrospinning inks according to some examples in the lamellar region of the phase diagram.

[0044] FIG. 17B provides shear stress and viscosity as a function of shear rate for the CUR electrospinning inks according to some examples in the lamellar region of the phase diagram.

[0045] FIG. 18 provides schematic images of a biaxial printhead according to some examples and schematic images of the solo shape, alongside shape, and mixing shape of electrospinning bi-fibers according ot some examples.

DETAILED DESCRIPTION

[0046] Described herein are systems, methods, and techniques for electrospinning fibers, including multi-component fibers as well as fiber-based products, such as 3D-shaped fiber-based products (e.g., mats, filters, pharmaceutical dosage forms, thin films, 3D scaffolds, etc.). The disclosed systems, methods, fibers, and products are useful for a variety of applications in pharmaceutical science and tissue engineering. The disclosed systems and methods allow for continuous production of electrospun fibers of a variety of compositions and geometries, such as by using different printing nozzles, feedstocks, collection arrangements, or the like. The produced electrospun fibers and fiber-based products can include pharmaceutically loaded fibers, hydrophobic fibers, hydrophilic fibers, live cell-loaded fibers, biocompatible polymer-based fibers, mixed-component fibers, or the like, such as by employing electrospinning nozzles or spinnerets fed by or incorporating multi-component printheads. Dynamically controlled collection of the electrospun fibers, for example using a multi-dimensionally controlled collection stage, such as a collection stage that includes up to 6 degrees of freedom (e.g., X, Y, Z, Φ, θ, Ψ), can allow for creation of fiber-based products of any desirable geometry.

[0047] FIG. 1 is a schematic illustration of an integrated electrospinning system according to some examples. The integrated electrospinning system 100 includes a plurality of supply containers 110, at least one printhead 120, a spinneret 140, a power supply 150, and a collector 160. The integrated electrospinning system 100 includes the plurality of supply containers 110 (e.g., 110a, 110b, and 110c) for preparing or storing a respective component, such as a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient ("API"). The component may include, but is not limited to, poly(α-hydroxy acids) ("PAHAs"), lactic acids, polyglycolide or poly(glycolic acid) ("PGA"), poly(ε-caprolactone), poly(ethylene glycol-caprolactone), poly(lactide)("PLA"), poly(aminoacid alkyl ester) phosphazene, poly(caprolactone-co-ethyl ethylene phosphate), poly(carbonate), poly(caprolactone) ("PCL"), poly(ethylene glycol) ("PEG"), polyethyleneimine ("PEI"), poly(urethane) ("PU"), poly(vinyl alcohol) ("PVA"), poly(lactide-co-glycolide) ("PLGA"), poly(L-lactide) ("PLLA"), hydroxyapatite ("HA"), poly(propylene carbonate) ("PPC"), poly(cyclohexyl carbonate), silicate, polyphosphazenes. In some examples, the plurality of supply containers 110 (e.g., 110a, 110b, and 110c) may be connected to a pump (e.g., a pneumatic pump, a stress pump, etc.).

[0048] The printhead 120 may include a plurality of linkers (122a, 122b, 122c, and 122d) defining inlets of the printhead 120. The printhead 120 may include a fluid mixer 124 in fluid communication with the plurality of linkers 122a, 122b, 122c, 122d for mixing components from the inlets into a mixture. The fluid mixer 124 may be in fluid communication with the plurality of

linkers 122a, 122b, 122c, 122d via a plurality of fluid lumens. The printhead 120 may be connected to any components to facilitate the movement of the respective component. In some examples, the component may be a pressure pump. In some examples, the mixture may include a cosolvent, a surfactant, a preservative, live cells, polymer, thickeners, diluents, biologically active polynucleotides, cellular components, an additional active ingredient, a salt, a preservative, a protein, a peptide, an amino acid, or a nucleic acid component.

[0049] In some examples, the printhead 120 may further include sub-printhead(s) 130. The sub-printhead(s) 130 may further include linkers (e.g., 122a, 122b), sub-printhead fluid mixer 134 and sub-printhead outlet 136. In some examples, sub-printhead outlet 136 may be an outlet linker that is coupled to linker 122d. One or more of the plurality of linkers 122a, 122b, 122c, 122d may include a Luer lock, a Luer slip, a slip tip, or other couplers for establishing sealable fluid communication. As shown in FIG. 1, the plurality of linkers 122a, 122b, 122c, 122d may be various types of couplings or connectors. However, it is also contemplated that each of the plurality of linkers 122a, 122b, 122c, 122d may be the same type of coupling or connector. The linkers 122a, 122b, 122c, 122d may be characterized by a diameter of less than or about 5.0 mm, such as less than or about 4.5 mm, less than or about 4.0 mm, less than or about 3.5 mm, less than or about 3.0 mm, less than or about 2.5 mm, less than or about 2.0 mm, less than or about 1.5 mm, less than or about 1.4 mm, less than or about 1.3 mm, less than or about 1.2, mm, less than or about 1.1 mm, less than or about 1.0 mm, less than or about 0.9 mm, less than or about 0.8 mm, less than or about 0.7 mm, less than or about 0.6 mm, less than or about 0.5 mm, or less.

[0050] In some examples, the fluid mixer 124 may have a coaxial arrangement of a first fluidic channel in fluid communication with a first linker (e.g., linker 122c) and a second fluid channel in fluid communication with a second linker (e.g., linker 122d). In some examples, the fluid mixer 124 may include a stirrer. The stirrer may be activated using any means. In some examples, the stirrer may be activated by an electric field or a magnetic field. The stirrer may be disposed at an upstream location of the fluid mixer 124 or at any location along the fluid mixer 124. In some examples, the fluid mixture 124 may include a plurality of stirrers to form the mixture. The fluid mixer 124 may include a mixing architecture characterized by a “Y” shape, an “S” shape, a helix shape, or any other desirable shape to establish a desired amount of mixing of components introduced from various linkers. In some examples, the fluid mixer 124 may include a co-blender and/or a similar functional system.

[0051] The printhead 120 may include a printhead outlet 126 in fluid communication with the fluid mixer 124 via a one or more fluid lumens. The printhead outlet 126 may also be in fluid

communication with a spinneret 140 that forms droplets suitable for electrospinning via a printhead outlet 126. The spinneret 140 may be a single-needle spinneret, a multi-needle spinneret, any co-axial spinneret (e.g., bi-axial, tri-axial), or the like. In some examples, the spinneret 140 may be fluidly connected to a pump (e.g., a syringe stress pump), such as via the printhead 120. In some examples, the printhead outlet 126 may further include an outlet linker and/or a nozzle. In some examples, the outlet linker and the nozzle may be separate components. In some examples, the outlet linker and/or the nozzle may form a unitary structure with the fluid mixer 124 (e.g., no outlet linker is present, and the nozzle is joined to the fluid mixer 124). Various nozzle configurations may be used as appropriate. In some examples, the nozzle may include a plurality of nozzle ports. In some examples, the printhead outlet 126 the outlet linker (e.g., sub-printhead outlet 136), and/or any other nozzle may be characterized by a diameter of less than or about 5.0 mm, such as less than or about 4.5 mm, less than or about 4.0 mm, less than or about 3.5 mm, less than or about 3.0 mm, less than or about 2.5 mm, less than or about 2.0 mm, less than or about 1.5 mm, less than or about 1.4 mm, less than or about 1.3 mm, less than or about 1.2, mm, less than or about 1.1 mm, less than or about 1.0 mm, less than or about 0.9 mm, less than or about 0.8 mm, less than or about 0.7 mm, less than or about 0.6 mm, less than or about 0.5 mm, or less.

[0052] A droplet formed by the spinneret 140 may be subject to a high voltage (e.g., 5 to 50 kV) for electrospinning. A power supply 150 may be used to apply high voltage over the droplet. The high voltage may create a high electric field surrounding the formed droplet, thereby electrifying the droplet. When the droplet becomes highly electrified, the accumulation of charge may cause a protrusion to appear at the tip of the droplet, thereby distorting the droplet from a hemispherical shape to a conical shape. The distorted conical part of the droplet may be referred to as a "Taylor cone." As the high electric field overcomes the surface tension of the droplet, a jet of charged liquid or liquid particles is formed at the "Taylor cone" of the droplet. The jet is a very thin fiber of charged liquid or liquid particles. The very thin fiber may have a diameter ranging from micrometers to nanometers. The jet then undergoes a stretching and whipping process under the high electric field, forming a long thread of the very thin fiber 145. In some examples, the long thread of the very thin fiber 145 may be referred to as an "electrospun fiber." Additional portions of the fluid from the spinneret may be continuously ejected and join the ejected thread to create a continuous electrospun fiber.

[0053] The integrated electrospinning system 100 may use the collector 160 to collect the long thread of the very thin fiber formed by the electrospinning process. The collector 160 may include a collecting surface upon which the long thread of the very thin fiber is collected. The collector

may be a static collector or a rotating collector. In some examples, the collector 160 may include at least one stage that translates and/or rotates along at least one axis. In some examples, the at least one stage may include a roller, a spinning roller, a three-axis (e.g., x-axis, y-axis, z-axis) adjustable stage, and/or a four, five, or six degrees of freedom adjustable stage. In some examples, the collector 160 may arrange the long thread of the very thin fiber in any structure (e.g., a mat, a semi-permeable membrane including multiple layers of electrospun fibers, a 3-dimensional scaffold including electrospun fibers loaded with cells, tissues, organs, edible matter, food, etc.) as desired.

[0054] In some examples, the collector 160 may include one or more pressure sensors or pressure controllers, temperature sensors or temperature controller, position sensors, or other sensors or controllers. In some examples, the collector 160 may be connected to a mechanical arm 170 having up to six degrees of freedom ("DOF"). The DOF may include translation along the x, y, and z axes, as well as rotation around these axes. In some examples, the mechanical arm 170 may further include an assistive component to facilitate the gathering of the electrospun fiber. In some examples, the assistive component may be a static or dynamic collecting device. In some examples, the assistive component may be a cylindrical or a flat collector that is attached to an end effector of the mechanical arm 170. In some examples, the collector 160 may further include a conveyor belt or any continuous belt system that continuously transports products (e.g., electrospun fibers) of the integrated electrospinning system 100.

[0055] In some examples, it may be desirable to keep the processes under controlled conditions or sterile conditions. Optionally, the integrated electrospinning system 100 may further comprise an environmentally controlled or sterile compartment to sterilize or maintain sterility of any of the plurality of supply containers 110, the at least one printhead 120, the spinneret 140, the power supply 150, and the collector 160.

[0056] FIG. 2 provides schematic illustrations of printheads for component mixing and corresponding electrospun fibers according to some examples. Printhead 201 is a bi-axial printhead including a first linker 210 and a second linker 220. The first linker 210 may be characterized by a first axis and the second linker 220 may be characterized by a second axis that is different from the first axis. Through a first inlet 202 and a second inlet 204, the first linker 210 and the second linker 220 may be respectively connected to a first supply container and a second supply container (not shown). In some examples, the first supply container includes a first component, and the second supply container includes a second component. In some examples, the first component is different from the second component. An integrated electrospinning device

(e.g., the integrated electrospinning system 100) using the printhead 201 may fabricate an electrospun fiber 230. The electrospun fiber 230 includes a first fiber part 231 and a second fiber part 232. The first part 231 includes the first fiber component and the second part 232 includes the fiber second component.

[0057] Printhead 202 is a tri-axial printhead including a first linker 240, a second linker 250, and a third linker 260. The first linker 240 may be characterized by a first axis, the second linker 250 may be characterized by a second axis that is different from the first axis, and the third linker 230 may be characterized by a third axis that is different from the first axis and the second axis. Through a first inlet 205, a second inlet 207, and a third inlet 209, the first linker 240, the second linker 250, and the third linker 260 may be respectively connected to a first supply container, a second supply container, and a third supply container (not shown). In some examples, the first supply container includes a first component, the second supply container includes a second component that is different from the first component, and the third supply container includes a third component that is different from the first component and the second component. An integrated electrospinning device (e.g., the integrated electrospinning system 100) using the printhead 202 can fabricate an electrospun fiber 270. The electrospun fiber 270 includes a first fiber part 271, a second fiber part 272, and a third fiber part 273. The first fiber part 271 includes the first component, the second fiber part 272 includes the second component, and a third fiber part 273 includes the third component.

[0058] FIG. 3 provides a schematic illustration of a printhead for component mixing and a corresponding electrospun fiber according to some examples. Printhead 300 is a co-biaxial printhead including a first printhead 310 and a second printhead 320. The first printhead 310 is in fluid communication with the second printhead 320. The first printhead 310 further includes a first linker 311, a second linker 312, a co-axial fluid mixer 313 in fluid communication with the first linker 311 and the second linker 312, and a first outlet 314 in fluid communication with the co-axial fluid mixer 313. In some examples, the first printhead 310 may be a co-axial printhead. The co-axial fluid mixer 313 may include two capillaries that share an axis. Each of the two capillaries may respectively be in fluid communication with the first linker 311 that is in fluid communication with a first component and the second linker 312 that is in fluid communication with a second component. In an electrospinning process, the first printhead 310 allows for the injection of the second component as a core part 331 into the first component as a coat part 332, thereby forming a co-axial fiber 330 that includes the coat part 332 continuously surrounding the core part 331. The core part 331 and the coat part 332 may have any cross-sectional geometry as desired (e.g., circle, oval, rectangular, triangular, etc.).

[0059] The second printhead 320 includes a third linker 321 and a fourth linker 322. In some examples, the third linker 321 may be in fluid connection with a third component. In some examples, the third component is different from the first component and the second component. In some examples, the fourth linker 322 may be in fluid communication with the first outlet 314 to receive the co-axial fiber 330 as an input component. The second printhead 320 further includes a bi-axial fluid mixer 324. The bi-axial fluid mixer 324 includes a first channel in fluid connection with the third linker 321 and a second channel in fluid connection with the fourth linker 322. The first channel is characterized by a first axis and the second channel is characterized by a second axis that is different from the first axis. In an electrospinning process, the second printhead 320 allows for the injection of the third component as bi-axial fiber 340 that is adjacent to the co-axial fiber 330, thereby forming a co-biaxial fiber that includes the co-axial fiber 330 and the bi-axial fiber 340. The bi-axial fiber 340 and the co-biaxial fiber may have any cross-sectional geometry as desired (e.g., circle, oval, rectangular, triangular, etc.). In some examples, the co-axial fiber 330 and the bi-axial fiber 340 in the co-biaxial fiber may respectively include a first API and a second API that is different from the first API. In some examples, when the co-biaxial fiber is administered to a patient, the release rate of the first API loaded in the co-axial fiber 330 is equal to the release rate of the second API loaded in the bi-axial fiber 340.

[0060] FIG. 4 is a schematic illustration of an integrated electrospinning system 400 including multiple spinnerets according to some examples. The integrated electrospinning system 400 includes a plurality of supply containers 410 (e.g., 410a, 410b, and 410c), a plurality of spinnerets 420 (e.g., 420a, 420b, and 420c), a collector 440, and a mechanical arm 450. The plurality of supply containers 410 (e.g., 410a, 410b, and 410c) can prepare or store a respective component 412 (e.g., 412a, 412b, and 412c). In some examples, the respective component 412 may be a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient ("API"). The respective component 412 may include a lipidic material, an aqueous material, a porous material, and the like. Without limitation, each respective components 412 can be optionally substituted by a multi-component nozzle and associated supply container, such as depicted in FIG. 1. Each of the plurality of spinnerets 420 (e.g., 420a, 420b, and 420c) may be in fluid connection with its respective supply container (e.g., 410a, 410b, and 410c). The plurality of spinnerets 420 (e.g., 420a, 420b, and 420c) may form droplets of the respective component 412 (e.g., 412a, 412b, and 412c) that are suitable for electrospinning. In some examples, the integrated electrospinning system 400 may inject a first component (e.g., 412a) toward a first spinneret (e.g., 420a) to form droplets of the first component. As high-voltage 415 is applied to the droplets, the integrated electrospinning system 400 forms electrospun fibers including the first component and the

electrospun fibers may be collected at the collector 440. In some examples, the collector 440 may further include a collecting surface 442 and a rotating element 444 that revolves and translates at a controlled speed. The rotating element 444 gathers the electrospun fibers around the rotating element and aligns the electrospun fibers to form a first aligned fiber mat. In some examples, the first aligned fiber mat may be referred as an inner layer 430a. In some examples, the rotating element 444 may be coupled to an end effector 452 of the mechanical arm 450.

[0061] In some examples, the integrated electrospinning system 400 may form a multi-layer fiber that includes the inner layer 430a, an inter layer 430b surrounding the inner layer 430a, and an outer layer 430c that surrounding the inner layer 430a and the inter layer 430b. Similar to the inner layer 430a, to form the inter layer 430b, the integrated electrospinning system 400 may inject a second component (e.g., 412b) toward a second spinneret (e.g., 420b) to form droplets of the second component (e.g., 412b). As high-voltage 415 is applied to the droplets, the integrated electrospinning system 400 forms electrospun fibers including the second component and the electrospun fibers may be collected by the collector 440, thereby disposing a second aligned fiber mat, or the inter layer 430b, surrounding the inner layer 430a. In some examples, the integrated electrospinning system 400 may inject a third component (e.g., 412c) toward a third spinneret (e.g., 420c) to form droplets of the third component (e.g., 412c). As high-voltage 415 is applied to the droplets, the integrated electrospinning system 400 forms electrospun fibers including the third component and the electrospun fibers may be collected by the collector 440, thereby disposing a third aligned fiber mat, or the outer layer 430c, surrounding the inner layer 430a and the inter layer 430b. In some examples, the multi-layer fiber including the inner layer 430a, the inter layer 430b, and the outer layer 430c may be used as a semipermeable membrane, a membrane filter, etc. The inner layer 430a, the inter layer 430b, and the outer layer 430c may have any cross-sectional geometry as desired (e.g., circle, oval, rectangular, triangular, etc.).

[0062] FIG. 5A is a schematic illustration of an integrated electrospinning system according to some examples. The integrated electrospinning system 500A includes a plurality of supply containers 510 (e.g., 510a, 510b, and 510c), a first printhead including a first linker 522 and a second linker 524, a second printhead including a third linker 532 and a connecting linker 526 in fluid connection with the first printhead, a mixer 534, and a spinneret 540. The plurality of supply containers 510 (e.g., 510a, 510b, and 510c) can prepare or store a respective component 512 (e.g., 512a, 512b, and 512c). In some examples, the respective component 512 may be a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient ("API"). The respective component 512 may include a lipidic material, an aqueous material, a porous material, and the like. In some examples, the respective component 512c may include particles (e.g.,

microparticles or nanoparticles that include APIs, or cells) as desired. The particles may have any cross-sectional geometry as desired (e.g., circle, oval, rectangular, triangular, etc.). In some examples, APIs may include a protein, an antibody, a nucleic acid, messenger ribonucleic acid (mRNA) molecules, a lipid nanoparticle, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing endonucleases or mega-nucleases, a growth factor, a plasmid, a hydrophilic pharmaceutical, a lipophilic pharmaceutical, a viral particle, a virus-like particle, a live yeast cell, a live recombinant yeast cell, a live fungus, a live bacterial cell, a live recombinant bacterial cell, a live insect cell, a live mammalian cell, or a live mesenchymal stem cell. In some examples, a weight ratio of the API to the polymer or the non-polymeric excipient in the mixture may be from 1:8 to 1:15, such as from 1:8 to 1:9, from 1:9 to 1:10, from 1:10 to 1:11, from 1:11 to 1:12, from 1:12 to 1:13, from 1:13 to 1:14, or from 1:14 to 1:15.

[0063] In some examples, the particles may be a polymer, and the polymer may be a biodegradable polymer. In some examples, the biodegradable polymer may include, but is not limited to, poly(lactide-co-glycolide), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), Pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine).

[0064] In some examples, the particles may be a non-polymeric excipient, and the non-polymeric excipient may be a hydrophilic substance or a hydrophobic substance. In some examples, the non-polymeric excipient may include, but is not limited to, a non-reducing sugar, such as trehalose, or sucrose, a polyol, such as mannitol, sorbitol, xylitol, or an amino acid, such as leucine, or L-arginine.

[0065] The first linker 522 and the second linker 524 may be respectively in fluid connection with the respective component 512b and 512c. The respective component 512c may be a secondary material that can coat the particles included in the respective component 512b. In some examples, the first printhead including the first linker 522 and the second linker 524 generates a plurality of composite particles 528. The plurality of composite particles 528 may include at least one API and at least one secondary material. The plurality of composite particles 528 may flow into the second printhead via the connecting linker 526. The third linker 532 may be in fluid connection with the respective component 512a. In some examples, the respective component 512a may be base material within which the plurality of composite particles 528 can be embedded. In some examples, the integrated electrospinning system 500A mixes the plurality of composite

particles 528 and the respective component 512a at the mixer 534. The mixer 534 is in fluid communication with the spinneret 540. The spinneret 540 forms droplets including the respective component 512a and the plurality of composite particles 528 that are suitable for electrospinning. As illustrated by FIG. 5, high voltage 545 is applied to the droplets at the spinneret 540 to form electrospun fiber 550 toward the collector 560. In some examples, the electrospun fiber 550 includes a fiber body 552 including the respective component 512a as a fiber body and a plurality of embedded or loaded particles 554. In some examples, the plurality of embedded particles 554 includes the plurality of composite particles 528. The electrospun fiber 550 may have any cross-sectional geometry as desired (e.g., circle, oval, rectangular, triangular, etc.).

[0066] FIG. 5B is a schematic illustration of a system 500B including a first printhead 570 and a second printhead 580 according to some examples. The system 500B may be incorporated to the integrated electrospinning system 500A. In some examples, the first printhead 570 and the second printhead 580 may correspond to the first printhead and the second printhead in the integrated electrospinning system 500A. The first printhead 570 may perform a first process to fabricate nanoparticles and/or microparticles as described in relation to FIG. 5A. The second printhead 580 may perform a second process to mix a polymer and the nanoparticles and/or microparticles to form a mixture for a subsequent process. In some examples, the subsequent process may be an electrospinning process.

[0067] FIG. 5C is a schematic illustration of a system 500C including a first printhead 590 and a second printhead 595 according to some examples. The first printhead 590 may include a coil 590a. The second printhead may include a coil 595a. In some examples, the first printhead 590 and the second printhead 595 may correspond to the first printhead and the second printhead in the integrated electrospinning system 500A. The first printhead 590 may perform a first process to fabricate nanoparticles and/or microparticles as described in relation to FIG. 5A. In some examples, nanoparticles and/or microparticles are fabricated in coil 590a. The second printhead 595 may perform a second process to mix a polymer and the nanoparticles and/or microparticles to form a mixture for a subsequent process. In some examples, the nanoparticles and/or microparticles generated in coil 590a are mixed with one or more polymers in coil 595a to form a mixture for the subsequent process. In some examples, the subsequent process may be an electrospinning process. The first printhead or the second printhead may resemble a printhead (e.g., FIG. 9) described in WO 2024/026039, filed July 27, 2023, the entire contents of which are incorporated herein by reference for all purposes.

[0068] Each of coil 590a and coil 595a may have a number of turns. Coil 590a and coil 595a may each independently have a number of turns. Each turn may represent a full revolution. For example, the number of turns in a coil may be from 2 to 5, from 5 to 10, from 10 to 15, from 15 to 20. The inner diameter of the tube of the coil may be from 1 to 2 mm, from 2 to 3 mm, from 3 to 4 mm, from 4 to 5 mm, or over 5 mm. The length from the beginning of the coil to the end of the coil may be from 5 to 10 mm, 10 to 15 mm, 15 to 20 mm, 20 to 25 mm, 25 to 30 mm, or greater than 30 mm. The radius of the turns of the coil (based on the center of the cross section of the coil) may be from 1 to 5 mm, 5 to 10 mm, 10 to 15 mm, 15 to 20 mm, 20 to 25 mm, 25 to 30 mm, or greater than 30 mm. In some embodiments, a coil may be referred to as a helix. In some embodiments the coil may be replaced by a (repeating) S-shape. One difference with an S-shape from a coil may be that the S-shape does not appear as a circle when viewed from a certain perspective. As shown in FIG. 5C, coil 590a may be separated from coil 595a by a predominantly straight section or a section without a coil. The embodiment of FIG. 5C may help with particle generating and mixing for electrospinning. The coils in FIG. 5C may be part of a fluid mixer described herein.

[0069] FIG. 6 provides a schematic illustration of an environmental simulator according to some examples. The environmental simulator 600 includes a stomach simulator 610, an intestine simulator 620, and a simulating fiber 630. The environmental simulator 600 may control various parameters as desired. In some examples the parameters include, but are not limited to, temperature, humidity, gas, light, and stability. In some examples, the simulating fiber 630 may be the electrospun fiber 145 (FIG. 1), the electrospun fiber 230 (FIG. 2), the electrospun fiber 270 (FIG. 2), the co-biaxial fiber that includes the co-axial fiber 330 and the bi-axial fiber 340 (FIG. 3), the multi-layer fiber that includes an inner layer 430a, an inter layer 430b surrounding the inner layer 430a, and an outer layer 430c surrounding the inner layer 430a and the inter layer 430b (FIG. 4), the electrospun fiber 550 (FIG. 5) that includes a plurality of particles, or any other appropriate or suitable electrospun fiber. In some examples, the simulating fiber 630 may include a hydrophobic material and/or a hydrophilic material. In some examples, the simulating fiber 630 may be a semi-permeable membrane that includes an inner layer, an inter layer surrounding the inner layer, and an outer layer 430c surrounding the inner layer and the inter layer. Each of the inner layer, the inter layer and the outer layer may have different permeabilities to materials. In some examples, the simulating fiber 630 may only allow a selected group of materials to pass through.

[0070] The stomach simulator 610 includes a stomach compartment 612 and at least one sensor 614 that is coupled to the stomach compartment 612. The stomach compartment 612 may include

fasted state simulated gastric fluid (FASSGF) with a pH value of 1.6 to mimic a stomach environment. In some examples, the at least one sensor 614 may be programmed compression probe (e.g., UV sensor probe) to monitor mechanical properties of interest (e.g., compression, stress, etc.) during the dissolution of a disintegrated test drug 608. In some examples, the environmental simulator 600 may include a feeding pump 618 to facilitate the transportation of the test drug 608 toward the intestine simulator 620. In some examples, the intestine simulator 620 may include an extraluminal compartment 622 and an intraluminal compartment 623.

[0071] The extraluminal compartment 622 may include at least one FASSGF fluid with a pH value of 6.5 to simulate intestinal conditions. In some examples, the extraluminal compartment 622 may be connected to a buffer compartment 640 that contains at least one physiologically relevant buffer as desired. In some examples, the at least one physiologically relevant buffer may be contained in a reactor tank. In some examples, the at least one physiologically relevant buffer may be a phosphate buffer having a pH value of 6.5. The at least one physiologically relevant buffer may be transported from the buffer compartment 640 into the simulating fiber 630 to adjust simulating parameters or a simulating environment as desired. In some examples, the simulating fiber 630 is hollow. The physiologically relevant buffer is transported via a buffer channel 631 that passes inside and through the hollow simulating fiber 630. The buffer channel 631 may have any length or shape as desired. In some examples, the buffer channel 631 may be in fluid communication with an intermediate compartment 624 that receives any fluid or matter flowing from the buffer compartment 640. The intermediate compartment 624 may be in fluid communication with the intraluminal compartment 623. The intermediate compartment 624 may include any component and/or device as desired. In some examples, the component and the device may include a feeding pump, a vacuum, a UV sensor probe, etc.

[0072] The buffer compartment 640 may further include any component or device as needed. In some examples, the component or device may be a feeding pump, a vacuum, an agitation system, and/or sensor (e.g., UV sensor probe). In some examples, the environmental simulator 600 may include a pump to facilitate the transportation of the test drug 608 toward the intraluminal compartment 623. The simulating fiber 630 may be disposed into the FASSGF fluid of the extraluminal compartment 622 and the test drug 608 diffuses or permeates across a part (e.g., a layer, a specific surface of the simulating fiber 630) of the simulating fiber 630. In some examples, the extraluminal compartment 622 may monitor properties related to the diffusion or permeation (e.g., diffusion rate, permeation rate, etc.) of the test drug 608 by a sensor. In some examples, the sensor may be a UV sensor probe, a dynamic light scattering ("DLS") sensor, and the like. In some examples, to minimize possible errors associated with sampling, the sensor may

be disposed in the extraluminal compartment 622 for continuous monitoring of the absorbance of the test drug 608. The simulating fiber 630 may be disposed or immersed in the extraluminal compartment 622 to allow the test drug 608 to diffuse across any part of the simulating fiber 630.

[0073] The intraluminal compartment 623 of the environmental simulator 600 may receive fluid and/or matter from the extraluminal compartment 622, the intraluminal compartment 623, and/or the intermediate compartment 624. In some examples, a mixture of the fluid and/or matter may be referred to as "waste." In some examples, a pump may be disposed between the extraluminal compartment 622 and the intraluminal compartment 623 to facilitate the transportation of fluid or any matter from the extraluminal compartment 622 toward the intraluminal compartment 623. In some examples, a pump may be disposed between the intermediate compartment 624 and the intraluminal compartment 623 to facilitate the transportation of fluid or any matter from the intermediate compartment 624 toward the intraluminal compartment 623.

[0074] In some examples, the system 600 may include a plurality of special simulating components, and each of the plurality of special simulating components may be an independent chamber. In some examples, the plurality of special simulating components may include a temperature chamber, refrigerated chambers, humidity chamber, vacuum chamber, freeze-thaw chambers, environmental exposure chambers, growth chamber, light control, pressure controlled, thermal cycling, hypoxia chamber, electrostatic discharge chamber, electrochemical test chambers, antistatic charge chamber, environmental exposure chamber, acceleration test chamber, biochemical test chambers, vibration chamber, stability chamber, salt spray chamber, thermal shock chamber, altitude chamber, anechoic chamber, gas-controlled chamber, air quality-controlled chambers and/or sterilization chamber.

EXAMPLES

[0075] ***Preparation of Biaxial Electrospun Fibers (Biaxial-NFs).*** Biaxial NFs were prepared from Eudragit® S100 (ES100) and Eudragit® EPO (EPO) to encapsulate Ritonavir (RTV) and Curcumin (CUR), respectively. Curcumin, produced by Tokyo Chemical Industry Co., Ltd. Ritonavir was purchased from Nexconn Pharmatechs Ltd. (Shenzhen, China). Methacrylic acid-Methyl methacrylate copolymer (1:2) (Eudragit® S100) and butyl methacrylate, dimethyl aminoethyl methacrylate, methyl methacrylate copolymer (Eudragit® EPO) were procured from Evonik Röhm Pharma GmbH, Darmstadt, Germany. Absolute ethanol was purchased from Fischer Scientific UK.

[0076] ***Preparation of Ritonavir NFs solution (InkA).*** 1.3 g ES 100 and 0.1 g RTV were dissolved in 10 mL absolute ethanol (InkA) using magnetic stirring (ThermoScientific, USA) for 2

hours. The produced viscous solution was subjected to ultra-sonication (Branson Ultrasonics™ CPXH, USA) for 10 minutes to remove any formed air bubbles.

[0077] ***Preparation of Curcumin NFs solution (InkB).*** 0.1 g CUR and 3.5 g EPO were dissolved in 10 mL absolute ethanol (InkB) to produce a viscous solution using magnetic stirring (ThermoScientific, USA) at 40°C for 2 hours. Subsequently, the solution was subjected to ultrasonication (Branson Ultrasonics™ CPXH, USA) for 10 minutes to discard any formed air bubbles. The solution was allowed to rest for a day in ambient conditions before electrospinning.

[0078] ***Preparation of Biaxial NFs.*** Biaxial electrospinning was performed using the electrospinning system and nozzle (shown in FIG. 18) on a setup comprising one syringe pump and a high voltage power supply. FIG. 18 shows the schematic images of the printhead which contains three parts: the two inputs, microfluid system, and one output. The input was designed as the female luer locker while the output was designed as the male luer locker. For the microfluid system, the ink flows go through a tube with the inner diameter of 3 mm, then the diameter of the tube was down to 2 mm and through the 'Janus' half tube (radius 1 mm). The Janus line finally goes through the standard 14- and 18-gauge stainless needles. NFs were collected on a collector plate covered with aluminum foil placed at 12 cm from the spinneret. All experiments were performed under ambient conditions (25 ± 2°C; 57 ± 6% relative humidity).

[0079] ***Scanning electron microscopy (SEM).*** Scanning electron microscopy (Quanta FEG 650 ESEM, FEI Company, Hillsboro, OR, USA) was employed to observe the surface of the electrospinning (NFs). The thin piece of NFS was sheared from the center using a sharp razor blade and fixed on aluminum stubs with conductive double-sided tape. The samples were coated with gold by vacuum sputtering (EMS Sputter Coater, Hatfield, PA, USA) before observation under SEM. Microscope images were captured at a working distance of ~9 mm, an accelerated voltage of 10 kV, and an emission current of 15 μÅ.

[0080] FIG. 7 provides scanning electron microscopy images of electrospun fiber mats according to some examples (701 (Sample 1, Table 1), 702 (Sample 2, Table 1), 703 (Sample 3, Table 1), and 704 (Sample 4, Table 1)). The formulation and processing conditions of an example electrospun fiber mat are summarized in Table 1.

Table 1 - Exemplary Formulation and Processing Conditions

Sample	Nozzle size (gauge)	Voltage (kV)	Material(s)	Gap distance (cm)	Flow rate (mL/hr)	Note
1 (InkB)	14	25	Eudragit PO	22	2	No dps
2 (InkB)	14	25	Eudragit PO	22	3	++ dps
3 (InkA)	14	25	Eudragit S100 (10%w/v)	22	2	+++ dps
4 (InkA)	14	25	Eudragit S100 (13%w/v)	22	2	No dps

[0081] FIG. 8 provides a scanning electron microscopy image of an electrospun fiber mat according to some examples. The electrospun fiber mat includes a plurality of bi-axial electrospun fibers. A design of experimental (DoE) modeling was utilized to optimize the electrospinning of the biaxial NFs using a custom design using I-Optimal design as an optimality criterion. DoE modeling was conducted using the custom design platform in JMP 16 software developed by the SAS institute. The processing conditions of example electrospun fibers are summarized in Table 2. As seen in Table 2, each process factor was studied at four levels while the inner needle diameter was included as numerical factor (two levels). The model parameters specified is a traditional response surface design (RSM) is listed as: applied voltage (kV) & RS, flow rate (mL/h) & RS, gap distance (cm) & RS, Nozzle diameter (gauge), applied voltage (kV)*applied voltage (kV), applied voltage (kV)*flow rate (mL/h), flow rate (mL/h)*flow rate (mL/h), applied voltage (kV)*gap distance (Cm), flow rate (mL/h)*gap distance (cm), gap distance (cm)*gap distance (cm), applied voltage (kV)*Nozzle diameter (gauge), flow rate (mL/h)*Nozzle diameter (gauge), and gap distance (cm)*Nozzle diameter (gauge). The controllable processing parameters, defined as factors included flow rate (1 to 2 mL/h), inner needle diameter (18, 14 gauge), applied voltage (15 to 25 KV), and distance between the tip of the needle and the foil surface (20 to 25 cm).

Table 2 - Exemplary Processing Conditions

Bi-NF No.	Applied voltage (kV)	Flow rate (mL/h)	Distance (cm)	Needle diameter (18, 14 gauge)
1	20	1	25	14
2	25	1.5	22.5	18
3	25	2	25	14
4	15	1	22.925	18
5	15	1.5	25	14
6	25	1.5	20	14
7	20	1.5	22.5	14
8	15	1.705	20	18
9	15	1	20	14
10	22.35	1	20	18
11	20	1.5	25	18

12	20	1.5	22.5	18
13	15	2	25	18
14	25	1	22.5	14
15	15	2	22.5	14
16	20	2	20	14
17	20	2	22.5	18
18	25	1	25	18
19	25	2	20	18
20	20	1.5	22.5	14

[0082] The SEM images presented in FIG. 9 illustrates the biaxial nanofibers (NFs) with smooth surfaces, randomly distributed, and displaying distinct features. Alongside their lengths, the NFs exhibit a bicylindrical shape. The utilization of a predetermined dissolved organic solvent (e.g., ethanol, chloroform, acetone, or combinations thereof), namely 4 wt. % EPO in ethanol, in the electrospun solution facilitated the production of NFs without any split sole bi-axial fibers.

[0083] These scanning electron microscopy images demonstrate the fabrication of nano/micro-biaxial NFs using two inks composed of different polarity organic solvents with the Xspin-biaxial electrospinning system. For instance, FIG. 9, the bi-axial fibers shown in 901, 902, and 903 were formed by combining two inks: EPO dissolved in ethanol and ES100 dissolved in chloroform, exhibiting a relative polarity difference of 0.395. Similarly, in FIG. 9, the biaxial fibers shown in 904, 905, and 906 were created by employing another set of two inks: EPO dissolved in ethanol and ES100 dissolved in acetone, with a relative polarity difference of 0.299. Notably, no split sole fibers were observed among the bi-axial fibers, confirming formation of biaxial NFs.

[0084] ***Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR).*** FIG. 10A (1001 and 1002) and FIG. 10B (1003 and 1004) provide data showing attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) of electrospun fibers according to some examples. ATR-FTIR of the electrospun fibers may be collected using a modular NicoletTM iSTM 50 FTIR system (ThermoFisher Scientific, Waltham, Massachusetts, USA). In some examples, the preparation of the electrospun fibers includes weighing 20-25 mg of sample electrospun fibers, the sample electrospun fibers may be analyzed for percentage transmittance from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and 64 scans per run. The absorbance mode was used. OMNICTM series software (Version 9.0 ThermoFisher Scientific, Waltham, MA, USA) was used to capture and analyze the spectra. The formulation and physical form of example electrospun fibers are summarized in Table 3.

Table 3 - Exemplary Formulation and Physical Form

Sample No.	Formulation	Physical form
1	Eudragit S100 (ES 100)	Powder
2	Eudragit EPO	Powder
3	Curcumin	Powder
4	Ritonavir	Powder
5	1 dissolved in ethanol	Solution
6	2 dissolved in ethanol	Solution
7	3 dissolved in ethanol	Solution
8	4 dissolved in ethanol	Solution
9	Ink A	Solution
10	Ink B	Solution
11	Bi-NF 4	Solid (electrospun fibers)
12	Bi-NF 8	Solid (electrospun fibers)
13	Bi-NF 19	Solid (electrospun fibers)
14	InkA-NF-4	Solid (electrospun fibers)
15	InkA-NF-8	Solid (electrospun fibers)
16	InkA-NF-19	Solid (electrospun fibers)
17	InkB-NF-4	Solid (electrospun fibers)
18	InkB-NF-8	Solid (electrospun fibers)
19	InkB-NF-19	Solid (electrospun fibers)
20	BI-Customized NFs by JMP	Solid (electrospun fibers)
21	NF-InkA- Customized NFs by JMP	Solid (electrospun fibers)
22	NF-InkB- Customized NFs by JMP	Solid (electrospun fibers)

[0085] In Table 3 above, Samples No. 5-8 are Samples No. 1-4 dissolved in ethanol, respectively. Sample 9 and Sample 10 represent InkA and InkB, respectively, prepared as described herein. Samples 11-22 are electrospun fibers obtained from three electrospinning processes (corresponding to No. 4, 8, 19 in Table 2) and the optimized process derived from the Design of Experiments (DoE) experiments described herein. Utilizing these four electrospinning processes (corresponding to No. 4, 8, 19 in Table 2), fibers were fabricated using InkA and InkB, respectively, through a single needle, resulting in samples 14-16 and 21 (prepared using InkA), and samples 17-19 and 22 (prepared using InkB), respectively. Subsequently, the same four process parameters were applied to produce bi-fibers via the Xspin biaxial electrospinning system, yielding samples 11-13 and 20.

[0086] The main absorption peaks correspond to the following intramolecular and intermolecular interactions of liquid ethanol: 3000-3500 cm^{-1} -OH bond; 2700-3000 cm^{-1} -valent CH bond; 1400-1000 cm^{-1} -skeleton vibrations of the C-O-H group, and CH bond; 700-1000 cm^{-1} - deformational vibrations of the CH bond.

[0087] FIG. 11 provides Fourier transform infrared spectroscopy (FT-IR) spectra of (1101) Eudragit E100 powder/dissolved in ethanol, (1102) RTV/dissolved in ethanol, (1103) CUR and

CUR dissolved in ethanol, (1104) CUR and EPO dissolved in ethanol, (1105) InkA and InkB, and (1106) RTV/ES100 and CUR@EPO electrospun bi-fibers (Samples 20 customized). Cur@EPO 16% and Cur@EPO 20%. RTV and Eudragit E100 (InkA) electrospun fibers (Sample 21 customized), CUR and EPO (InkB) electrospun fibers (Sample 22 customized).

[0088] In FIG. 11A (1101), FTIR spectrum of ES100 showed the peak at 2953.9 cm^{-1} due to presence of O–H (carboxylic acid), at 1450.7 cm^{-1} due to -CH_3 bend, and at 1731.2 cm^{-1} due to the presence of C = O (ester). RTV crystal showed strong peaks in the region of 3200 cm^{-1} to 3400 cm^{-1} (3357 cm^{-1}), which are attributed to N-H stretching of a secondary amide. Strong peaks at 1716, 1526 cm^{-1} and 1717, 1527 cm^{-1} were observed, which can be assigned to carbonyl stretch (C=O) and the N-H bending of a secondary amide. C-N stretching was confirmed by the presence of a relatively sharp peak at 1238 cm-1. The peaks of the infrared spectrum of the RTV do not shift the wavenumbers, and there is no weakening or amplification of the vibrations despite grinding and compression.

[0089] In FIG. 11A (1101), FIG. 11B(1105 and 1106), NH-O stretching vibration that appeared at 1755 cm^{-1} in anhydrous form disappears on hydration. The band at 1670 cm^{-1} that is assigned to NH-N stretching became less intense and shifted towards lower wavenumber on hydration. N=N stretching band at 1495 cm^{-1} disappeared after the drug exposure to higher humidity. O–H stretching peak observed at 1440 cm^{-1} in anhydrous form became sharp at higher humidity. This may be due to the hydrogen bonding on hydrate formation. The intensity of C=O stretching vibration at 1235 cm^{-1} was decreased after the drug exposure to higher humidity. The observed shifts may result from the increase of hydrogen contacts of –OH Group. The structural changes in the RTV sulfate exposure to higher humidity may be associated with hydrate/pseudo polymorphic transition of the drug.

[0090] In FIG. 11B (1103) the peak observed in EPO at 1210 cm^{-1} is characteristic of the C–O bond and that at 1720 cm^{-1} is characteristic of the C═O bond and is present in the physical mixture and the electrospun fibers. EPO was chosen for its positive charge, which was believed to facilitate electrostatic interactions with CUR's negative charge, in order to enhance the solubility and stability of CUR. This hypothesis was based on the aim of improving CUR's bioavailability and potential therapeutic effects. In FIG. 11B (1103 and 1104) and FIG. 11C (1105), FTIR spectra of CUR, EPO, CUR and EPO fibers (CUR-EPO) samples are depicted. The spectra of CUR-EPO samples showed some significant changes. Peak at 3492 cm^{-1}, as assigned to stretch of OH in CUR, disappeared in the spectra of all the CUR-EPO fiber samples which suggests the involvement of the phenol OH group in the interaction. Signal at 1720 cm^{-1} which was recognized

as stretch of carbonyl in the polymer shifted to lower wavelengths at 1714 cm^{-1} in the spectra of CUR-EPO. The intensity also markedly reduced in spectra of CUR-EPO than that of EPO. Hydrogen bond interaction was accountable for the observed changes. It was also notable that CUR-EPO FTIR spectra revealed two strong peaks at 1560 cm^{-1} and 1400 cm^{-1} which were assigned as the symmetric and asymmetric stretch of carboxylic group. The carboxylic group was determined as belonging to the sodium acetate which was introduced from the buffer solution during the freeze drying treatment. FTIR results also suggest that the formation of hydrogen bonding involved the carbonyl group in the EPO polymer that accepted the H atom from OH group of CUR.

[0091] In FIG. 11C (1106), FTIR spectrum shows the bi-fibers of all the identified materials including RTV, CUR, EPO, and E100. Without being limited by theory, these results suggested that hydrate formation took place when the drug is exposed to higher humidity. During processing steps like crystallization and lyophilization, wet granulation, spray drying or electrospinning pharmaceutical solids may come in contact with water. Further, the drug substances, which were subjected to stress conditions (different temperatures and relative humidity (RH) environments) during storage, lead to unexpected hydration and dehydration phenomena, which affect several drug properties such as solubility, dissolution rate, stability and bioavailability.

[0092] FIG. 12 provides SEM images of some electrospun bi-fibers listed in Table 3: (1201), (1202), and (1203) were bi-fibers for Samples No. 11, No. 12, and No. 13 of Table 3, and FIG. 12 (1204) is the customized bi-fiber (Sample No. 20) listed in Table 3. All SEM images distinctly portray a side-by-side structure with a smooth surface for the electrospun nanofibers (NFs). Notably, the ribbon-like fibers are useful for buoyant pharmaceutical dosage forms, such as capsules, tablets, or scaffolds.

[0093] ***Thermogravimetric Analysis (TGA).*** FIG. 13 provides data showing thermogravimetric analysis (TGA) of raw materials and mixtures according to some examples. In some examples, the thermal properties of the raw materials and physical mixtures may be determined through a Mettler-Toledo TGA/DSC1 analyzer (Mettler-Toledo, Schwerzenbach, Switzerland). Sample raw materials and physical mixtures may be placed in an open ceramic crucible and ramped from 35°C to 600 °C at a rate of 10 °C /min. A furnace may be purged using ultra-purified nitrogen at a flow rate of 50 mL/min. The formulation and physical form of example raw materials and mixtures are summarized in Table 4.

Table 4 - Exemplary Formulation and Physical Form

Sample No.	Formulation	Physical form
1	Eudragit S100 (ES 100)	Powder
2	Eudragit EPO	Powder
3	Curcumin	Powder
4	Ritonavir	Powder
5 (FITR 11)	Bi-NF 4	Solid (electrospun fibers)
6 (FTIR 12)	Bi-NF 8	Solid (electrospun fibers)
7 (FTIR 13)	Bi-NF 19	Solid (electrospun fibers)
8 (FITR 20)	BI-Customized NFs by JMP	Solid (electrospun fibers)

[0094] Thermogravimetric analysis (TGA) was conducted to examine the degradation patterns and thermal stabilities of EPO, S100, CUR, RTV, and Xspin bi-fibers (FIG. 13). TGA measures the change in sample mass as a function of temperature using a thermobalance. During the initial phase of TGA, a decrease in mass was observed due to the evaporation of surface-absorbed water. This resulted in a reduction of 6% for ES100 (Line No. 1), 2% for bi-fibers (Line No. 5-8), while no losses were observed for EPO (Line No. 2), CUR (Line No. 3), and RTV (Line No. 4).

[0095] In the second phase, molecular chain fracture and thermal decomposition led to significant weight reduction. ES100 exhibited an 87% weight loss between 370 and 450 °C, EPO showed a 30% weight loss between 225 and 320 °C, CUR experienced a 60% weight loss between 245 and 420 °C, RTV had a 40% weight loss between 200 and 250 °C, and the bi-fibers showed a 20-25% weight loss between 225 and 300 °C.

[0096] The TGA curve of EPO (Line No. 2, FIG. 13,) displayed two major thermal weight losses. The first occurred between 225 and 320 °C, followed by a second weight loss (~60%) between 380 and 460 °C. The TGA curve of RTV (Line No. 4, FIG. 13) demonstrated a major weight loss between 200 and 250 °C, with a second weight loss (~50%) occurring between 300 and 395 °C. Comparing the working temperature of the maximum rate of weight reduction and the terminating decomposition temperature, it was observed that the bi-fibers had higher values than EPO and RTV but lower values than ES100 and CUR. This indicated that the ES100-CUR fibers improved the thermal stability of the EPO-RTV fibers in the form of bi-fibers. These findings demonstrate the thermal decomposition behavior and thermal stability of the studied materials as characterized by TGA analysis.

[0097] ***Differential scanning calorimetry (DSC).*** Differential scanning calorimetry (DSC, DSC Q20, TA® instruments, New Castle, DE, USA) analysis was used to characterize polymer, API (RTV and CUR), and electrospun fibers (Sample 5-8) (shown in Table 4). Approximately 5 - 10 mg of samples were weighed in standard DSC aluminum pans and sealed with standard aluminum lids (DSC consumables incorporated, Austin, MN, USA) using a calibrated balance. The prepared

samples were subjected to a heat-cool-heat ramp circle heated from 20 °C to 220 °C with a ramp rate of 10 °C/min. A purge gas (Nitrogen) at a flow rate of 50 mL/min was used for all the experiments. The data were collected by TA advantage software (Q series, Version 2007 build 13029.20308) and analyzed by TA instruments Universal Analysis 2000. The results were presented as a plot of temperature (°C) versus heat flow (mW).

[0098] The differential scanning calorimeter (DSC) analysis was conducted to investigate the physical state of the bioactive compounds, as shown in FIG. 14. The DSC curves of pure CUR and RTV exhibited distinct sharp endothermic peaks at temperatures of 175 and 122 °C, respectively. These peaks corresponded to the melting of the crystalline form of CUR and RTV, indicating that the model drugs were present in a crystalline state.

[0099] The bi-fiber samples (FIG. 14, samples 5-8), the characteristic endothermal peaks corresponding to the drugs were not detected, suggesting, without being limited by theory, that CUR and RTV were extensively dispersed within the ES100/EPO polymers in an amorphous state. The absence of distinct peaks indicates that the drugs were not present in their crystalline form within the bi-fiber samples.

[0100] ***Measurement of Hydration Capacity (swell study).*** Biaxial NFs pieces (25 mg) were added to 10 cm petri dish, containing 10 mL distilled water. Then, the petri dish was kept at 37°C using a hot air Incubator (IVYX Scientific 5L Incubator, USA). The swelling behavior was observed at predetermined time intervals; 15, 30, 45, 60, 75, 90, 105, 120 min and 24 hours. At each time, samples were removed carefully from the petri dish and blotted off cautiously on tissue papers to remove the surface-adhered liquid droplets and reweighed to constant weight 30. The experiment was done in triplicate and the mean values were calculated. The water uptake percent was calculated according to the following equation:

$$Water\ uptake\ (\%) = \left[\frac{W2 - W1}{W1}\right] \times 100\%$$

where, W2 is the weight of the hydrated Biaxial NFs and W1 is the initial weight of Biaxial NFs.

[0101] The hydration and swelling of biaxial NFs contribute to their muco-adhesion to the stomach wall and subsequent drug release. The maximum hydration capacities of the samples were observed within 24 hours when immersed in distilled water. The dry biaxial NFs in all four formulations tested exhibited water absorption capability, expanding to approximately 10-11 times their original dry weight (shown in FIG. 15A and FIG. 15B).

[0102] Without being limited by theory, the water absorption capacity of the NFs can be attributed to the presence of water-soluble groups within the ES 100 and EPO polymers. These groups, such as -COOH and aminoalkyl groups, respectively, form hydrogen bonds with the surrounding aqueous medium. Consequently, the hydration of these functional groups facilitates the entry of water molecules into the polymer chain.

[0103] ***In-vitro floating (buoyancy) test.*** In vitro floating and buoyancy studies were carried out for all selected four nanofibrous mats based on the technique of Abd El Hady, W. E.; Soliman, O. A. E.-A.; El Sabbagh, H. M.; Mohamed, E. A. Glutaraldehyde Crosslinked Chitosan-Polyethylene Oxide Nanofibers as a Potential Gastroretentive Delivery System of Nizatidine for Augmented Gastroprotective Activity. Drug Deliv 2021, 28 (1), 1795–1809, incorporated herein by reference. Nanofibrous mats (1×1 cm^2) were placed in flask containing 100 ml of 0.1 N HCl (pH 1.2) at 37 ◦C. The time necessary for test samples to go from the bottom to the surface of dissolution medium was called floating lag time (FLT). Additionally, the time duration of mats continuously floated on the surface of dissolution media was considered as the "Total Floating Time" (TFT). All measurements were carried out three times.

[0104] All four selected nanofibrous mats exhibited immediate floating behavior, with a floating time (FLT) of less than 1 minute upon contact with the dissolution medium at pH 1.2. Specifically, sample 8, customized, remained floating on the surface for the subsequent 24 hours. However, within the triplicate of sample 4 and 19, one mat sank to the bottom after 1 hour and the other after 2.5 hours, respectively.

[0105] Visual observation revealed a rapid decrease in mat density, attributed to the fast solubility of Eudragit EPO in 0.1 N HCl. Additionally, the yellow color of CUR was quickly dispersed throughout the 0.1 N HCl solution, indicating the release and dispersion of the loaded CUR.

[0106] In contrast, the ES 100 NFs loaded with RTV continued to float on the surface for the following 24 hours, displaying sustained floating behavior. All four nanofibrous scaffolds demonstrated immediate floating upon contact with the dissolution medium at pH 1.2.

[0107] ***Determination of entrapment efficiency (EE%).*** Twenty-five mg of the freeze-dried Biaxial NFs (samples no. 4,8,19, and customized) were cut and dissolved in 45 mL methanol using a magnetic stirrer (Heidolph, USA) for 5 min until complete dissolution of the NFs. The drug content was analyzed spectrophotometrically using UV/VIS spectrophotometer microplate reader (BioTek Synergy H1) at 238 and 420 nm for RTV and CUR, respectively. The experiment was

done in triplicate and the mean values were calculated. The entrapment efficiency percent (EE%) was calculated according to the following equation:

$$EE\ (\%) = \left[\frac{Weigth\ of\ the\ drug\ in\ Biaxial\ NFs}{Theoretical\ weight\ of\ the\ drug\ in\ Biaxial\ NFs}\right] \times 100\%$$

[0108] The evaluation of drug-loaded (NFs) includes the measurement of entrapment efficiency (EE%). In FIG. 15A, in the case of EPO NFs containing CUR, the EE% exceeded 87% (w/w) across all the prepared biaxial NF samples. Similarly, in ES 100 NFs containing RTV, the EE% was above 84% in all four samples. Without being limited by theory, these observed entrapment efficiencies may be due to the substantial surface area of the biaxial NFs and the incorporation of the drugs into the polymeric solution, which subsequently solidifies during the electrospinning process.

[0109] ***In-vitro release study.*** In-vitro studies of the prepared biaxial NFs containing CUR and RTV, samples no. 4, 8, 19, and customized, (55.5 mg) were carried out and using an Excella E24R Inc/Ref shaker (New Brunswik, Eppendorf, Hamburg, Germany) for the release.

[0110] In 20 mL pH 1.2 media representing stomach media, at 37 ± 0.5°C and shaking rate 100 rpm, a 10k Da dialysis bag was used for measuring the release of RTV. While no dialysis bag was needed in the determination of the CUR release. A 200 µL of the dissolution media were taken at regular time interval till 120 min for CUR and 5 days for RTV. An equal volume of the respective dissolution medium was added to maintain the sink conditions throughout the experiment. The collected aliquots were analyzed spectrophotometrically using UV/VIS spectrophotometer microplate reader (BioTek Synergy H1) at 238 and 420 nm for RTV and CUR, respectively. A blank biaxial NFs were used to eradicate any possible interference. All measurements were done in triplicate from three independent samples and the mean values were calculated.

[0111] The data of the two in-vitro drug release from the prepared biaxial NFs were analyzed mathematically using the following models zero-order and first-order and Higuchi's model equations shown below. The model with the highest correlation coefficient (r^2) is the predominant one.

Zero-order equation:

$$Q_t = Kt$$

First-order equation:

$$M_t/M_\infty = 1 - e^{k0t}$$

Higuchi equation:

$$M_t/M_\infty = k_1 t^{1/2}$$

where, Qt is the amount of the drug release at time t and $Mt/M\infty$ is the amount of the drug released at time t. "k, k_0, and k_1" are the zero-order, first-order, and Higuchi release constants, respectively.

[0112] The Korsmeyer-Peppas model was used for further analysis (equation 6), whereby it was found that the release mechanism is governed by the value of the exponent (n) 32; it was calculated from the first 60% of release data. Values of (n) equal to or less than 0.5 were distinguishable for Fickian diffusion, whereas (n) values in the range of 0.5–1 were characteristic of anomalous mechanisms for drug release (non-Fickian). A unity value (n) indicated zero-order release.

$$M_t/M_\infty = k_p t^n$$

where, M_t/M_∞ is the amount of the drug released at time t, k_p is the Korsmeyer-Peppas constant and n is a diffusion exponent.

[0113] ***Ritonavir release from biaxial NFs with dialysis tube.*** In FIG. 16A and FIG. 16B, the release profile of Ritonavir from the four biaxial nanofiber (NF) samples demonstrated a gradual and sustained pattern, with approximately 30% of the drug released within the initial 5-hour period for all four samples, gradually increasing but remaining below 50% over the subsequent five days. Without being limited by theory, this sustained-release behavior is attributed to the prolonged diffusion of the drug from the acidic and insoluble Eudragit S100 nanofibers. The developed bi-axial NFs improved aqueous solubility and sustained RTV release, reaching around 30% within the first 2 hours, compared to less than 10% from ritonavir tablets, showcasing enhanced solubility and dissolution properties through the electrospinning technique's amorphous drug preparation. Despite other methods like lyophilized milkbased solid dispersion achieving 33 to 99% w/w dissolution within the first 2 hours, and polymer-based matricial nanostructures achieving over 60% dissolution, the biaxial nanofibers, with a higher surface area to volume ratio due to electrospinning, offer sustained Ritonavir release over an extended period, demonstrating their suitability for controlled drug delivery applications.

[0114] ***Curcumin release from bi-fibers with/without dialysis tube.*** FIG. 16C and FIG. 16D illustrate the in vitro release profiles of Curcumin from various biaxial nanofiber (NF) formulations. In 0.1 N HCl, a rapid burst release occurred, with over 65% released within 20 minutes for all formulations, attributed to the swift disintegration of EPO nanofibers due to EPO's high solubility in acidic environments. Despite the solubilization of Curcumin-loaded EPO NFs, no Curcumin release was detected outside the dialysis bag, as revealed by Fourier Transform

Infrared (FTIR) analysis, indicating an interaction between Curcumin and EPO, eliminating the need for the dialysis bag in measuring Curcumin release.

[0115] Kinetic analysis of the release of Curcumin and Ritonavir appear in the tables below.

Table 5. Kinetic analysis of the release of Curcumin.

Formula	**Coefficient of determination (R^2)**			**Korsmeyer-Peppas**		**Main Transport Mechanism**
	Zero-order	**First-order**	**Higuchi model**	**(R^2)**	**Diffusional exponent (n)**	
Sample 4	0.2049	0.0804	0.3433	0.09137	0.07766 ±0.04399	Fickian
Sample 8	0.1246	0.0053	0.2834	0.01329	0.01442 ±0.02232	Fickian
Sample 19	0.4334	0.4303	0.6452	0.6305	0.1858 ±0.02555	Fickian
Customized Sample	0.2694	0.2312	0.4966	0.4457	0.1054 ±0.02112	Fickian

Table 6. Kinetic analysis of the release of Ritonavir.

Formula	**Coefficient of determination (R^2)**		
	Zero-order	**First-order**	**Higuchi model**
Sample 4	0.6609	0.5800	0.7862
Sample 8	0.6346	0.5854	0.7485
Sample 19	0.4582	0.4397	0.5404
Customized Sample	0.6434	0.6389	0.7198

[0116] The drug release kinetics of electrospun CUR bi-fiber scaffolds were investigated by applying various mathematical models. The dissolution data was analyzed using the zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to understand the underlying mechanism of drug release. In Table 5, among the models, the Higuchi model equation provided the best fit for the release of CUR in pH 1.2 solution from sample 4, sample 8, sample 19, and customized samples. The R^2 values obtained for each respective sample were 0.3433, 0.2834, 0.6305, and 0.4457, indicating a good fit with the Higuchi model. The choice of models was based on selecting the model with the highest R^2 value. Additionally, the adjusted correlation coefficients for the Korsmeyer-Peppas model closely resembled those of the Higuchi model, further supporting the applicability of the Higuchi model in describing the CUR release profile.

[0117] In Table 6, according to the Higuchi model, the active ingredient is uniformly dispersed, and the release from the selected samples occurs primarily through diffusion, without significant matrix erosion or swelling. The evaluation of the in vitro drug release properties of the CUR combined with EPO (CUR-EPO) bi-fibers drug delivery system revealed the drug release profile of loaded CUR within the composite NFs (FIG. 16B). Notably, the burst effect was substantially reduced in the CUR/EPO NFs with dialysis tube compared to the CUR/EPO electrospun NFs without a dialysis tube. The values of (n) equal to or less than 0.5 indicate Fickian diffusion, while (n) values ranging from 0.5 to 1 suggest anomalous mechanisms for drug release (non-Fickian). Without being limited by theory, the release of CUR/EPO closely adhered to a Fickian diffusion mechanism.

[0118] The release profiles of RTV in pH 1.2 solution from sample 4, sample 8, sample 19, and customized samples exhibited a good fit with the Higuchi model equation. This was evident from the relatively higher "r" values compared to the zero-order and first-order regression coefficient values. The Higuchi model, which describes drug release through diffusion, was found to be the appropriate model for these formulations. These findings support the Higuchi model as the most suitable for characterizing the release of RTV from floating dosage forms. Furthermore, the exponent 'n' values for all the formulations were observed to be between 0.5 and 1, indicating a non-Fickian mode of drug release. The results indicate that the release of RTV from the electrospun bi-fiber scaffolds follows a Higuchi diffusion mechanism and exhibits a non-Fickian release behavior.

[0119] ***Viscosity and Rheology.*** A Discovery series hybrid rheometer (DHR) (model HR-2, TA instruments) under strain-controlled mode and fitted with an 8 mm sand-blasted smart-swap upper

geometry was used for analysis of the relationship between the stress (MPa), shear rate (1/s), and the viscosity of the materials i.e. ES 100, EPO, ES 100 and EPO to encapsulate RTV and CUR.

[0120] The rheological characteristics of the inks were determined prior to the electrospinning process. FIG. 17A and FIG. 17B show the results obtained from rheological characterization of the inks. The graphs show the viscosity of the inks that are exposed to various shear rates ranging from 0.01 s^{-1} to 300 s^{-1}. The prepared InkA and InkB tend to exhibit non-Newtonian behavior, viscosity increases with increasing shear rate (shear thickening) shown in FIG. 17A and FIG. 17B. Viscosity data collected for two ink samples shown in FIG. 17A and FIG. 17B. These flow curves would indicate that both InkA and InkB that increase at higher rates of shear velocity rate, stress. Without being limited by theory, this is the phenomenon of shear thickening and usually due to structure rearrangements as a result of the applied shear.

[0121] The high viscosity of the solution decreased significantly as the solution entered the syringe needle owing to the collapse of the network between the polymers because of shear force. The shear force that the solution undergoes can be approximated by the following equation:

$$\gamma = \frac{4Q}{\pi R^3}$$

where γ is the shear rate at the needle wall, Q is the volumetric flow rate, and R is the radius of the needle. The shear rate was estimated to be 30 s^{-1} for our experimental setup. The corresponding shear stress region is highlighted in FIG. 17A and FIG. 17B. At this region, both InkA and InkB solutions exhibited stable viscosity, implying, without being limited by theory, that the high viscosity attained by the addition of the ink solution can be lowered inside the syringe needle owing to the API, which is beneficial for electrospinning.

REFERENCES

[0122] U.S. Patent 9,476,149.

[0123] Chinese Patent Publication CN104862787.

STATEMENTS REGARDING INCORPORATION BY REFERENCE AND VARIATIONS

[0124] All references throughout this application, for example, patent documents including issued or granted patents or equivalents; patent application publications; and non-patent literature documents or other source material; are hereby incorporated by reference herein in their entireties, as though individually incorporated by reference.

[0125] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the present disclosure pertains. References cited herein are

incorporated by reference herein in their entirety to indicate the state of the art, in some cases as of their filing date, and it is intended that this information can be employed herein, if needed, to exclude (for example, to disclaim) specific embodiments that are in the prior art.

[0126] When a group of substituents is disclosed herein, it is understood that all individual members of those groups and all subgroups and classes that can be formed using the substituents are disclosed separately. When a Markush group or other grouping is used herein, all individual members of the group and all combinations and sub-combinations possible of the group are intended to be individually included in the disclosure. As used herein, "and/or" means that one, all, or any combination of items in a list separated by "and/or" are included in the list; for example, "1, 2, and/or 3" is equivalent to "1, 2, 3, 1 and 2, 1 and 3, 2 and 3, or 1, 2 and 3".

[0127] Every formulation or combination of components described or exemplified can be used to practice the present disclosure unless otherwise stated. Specific names of materials are intended to be exemplary, as it is known that one of ordinary skill in the art can name the same material differently. It will be appreciated that methods, device elements, starting materials, and synthetic methods other than those specifically exemplified can be employed in the practice of the present disclosure without resorting to undue experimentation. All art-known functional equivalents, of any such methods, device elements, starting materials, and synthetic methods are intended to be included in this present disclosure. Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges, and subranges, as well as all individual values included in the ranges given are intended to be included in the disclosure.

[0128] As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of elements of a device, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements. The present disclosure illustratively described herein suitably may be practiced in the absence of any element, elements, limitation, or limitations which is not specifically disclosed herein.

[0129] The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art and that such modifications and variations are considered to be within the scope of this present disclosure as defined by the appended claims.

WHAT IS CLAIMED IS:

1. An integrated electrospinning system comprising:

a plurality of supply containers for preparing or storing a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API);

a printhead in fluid communication with the plurality of supply containers, the printhead comprising:

a plurality of linkers, wherein each linker is in fluid communication with a supply outlet of one of the plurality of supply containers;

a fluid mixer for mixing components from the plurality of linkers into a mixture; and

one or more outlets in fluid communication with the fluid mixer; and

a spinneret in fluid communication with the one or more outlets;

a high-voltage power supply in electrical communication with the spinneret to form electrospun fibers; and

a collector to collect the electrospun fibers.

2. The integrated electrospinning system of claim 1, wherein the collector further comprises a stage, a mechanical arm, and/or a conveyor belt.

3. The integrated electrospinning system of claim 1, further comprising an environmental simulator, wherein the environmental simulator comprises a temperature chamber, refrigerated chambers, humidity chamber, vacuum chamber, freeze-thaw chambers, environmental exposure chambers, growth chamber, light control, pressure controlled, thermal cycling, hypoxia chamber, electrostatic discharge chamber, electrochemical test chambers, antistatic charge chamber, environmental exposure chamber, acceleration test chamber, biochemical test chambers, vibration chamber, stability chamber, salt spray chamber, thermal shock chamber, altitude chamber, anechoic chamber, gas-controlled chamber, air quality-controlled chambers and/or sterilization chamber.

4. The integrated electrospinning system of claim 3, wherein the environmental simulator includes a plurality of environmental parameters, wherein the plurality of environmental parameters comprises temperature, humidity, gas, optical conditions, and/or stability.

5. The integrated electrospinning system of claim 2, wherein the stage is adjustable in six degrees of freedom.

6. The integrated electrospinning system of claim 2, wherein the stage includes a roller and/or a spinning roller.

7. The integrated electrospinning system of claim 2, wherein the stage is adjustable along a first axis, a second axis, and/or a third axis.

8. The integrated electrospinning system of claim 2, wherein the mechanical arm is assembled with the stage.

9. The integrated electrospinning system of claim 2, wherein the stage comprises a collecting surface that rotates or translates to collect the electrospun fibers.

10. The integrated electrospinning system of claim 1, further comprising equipment for maintaining the plurality of supply containers, the printhead, and/or the collector in a sterile environment.

11. The integrated electrospinning system of claim 2, wherein the mechanical arm comprises multiple metal joints.

12. The integrated electrospinning system of claim 2, wherein the mechanical arm has six degrees of freedom.

13. The integrated electrospinning system of claim 1, wherein at least one of the respective components include particles.

14. The integrated electrospinning system of claim 13, wherein the particles having diameters of from 10 nm to 1100 μm.

15. The integrated electrospinning system of claim 1, wherein the electrospun fibers include particles.

16. The integrated electrospinning system of claim 1, wherein at least one of the respective components include live cells.

17. The integrated electrospinning system of claim 1, wherein the polymer is a biodegradable polymer selected from the group consisting of poly(lactide-co-glycolide),

polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine).

18. The integrated electrospinning system of claim 1, further comprising a continuous belt system to deliver the electrospun fibers to a downstream process.

19. The integrated electrospinning system of claim 1, further comprising at least one pneumatic pump and/or syringe stress pump to facilitate fluidic motion of the respective component of the plurality of supply containers.

20. The integrated electrospinning system of claim 1, wherein the fluid mixer comprises a coaxial arrangement of a first fluidic channel in fluid communication with a first linker and a second fluid channel in fluid communication with a second linker, wherein a first component is provided to the first linker, and wherein a second component is provided to the second linker.

21. The integrated electrospinning system of claim 1, wherein the fluid mixer comprises a stirrer.

22. The integrated electrospinning system of claim 21, further comprising an electric field or a magnetic field for activating the stirrer.

23. The integrated electrospinning system of 21, wherein the stirrer rotates about an axis parallel to a flow direction of the mixture through the one or more outlets.

24. The integrated electrospinning system of claim 21, wherein the stirrer rotates about an axis perpendicular to a flow direction of the mixture through the one or more outlets.

25. The integrated electrospinning system of claim 20, wherein the fluid mixer is characterized by a fluidic arrangement providing the first component and the second component in a coaxial flow configuration, a biaxial flow configuration, or a triaxial or higher axial flow configuration.

26. The integrated electrospinning system of claim 1, wherein the fluid mixer comprises a mixing architecture characterized by an “S” shape or a helix shape.

27. The integrated electrospinning system of claim 1, further comprising one or more temperature sensors or temperature controllers for monitoring or controlling a temperature of mixture in the fluid mixer.

28. The integrated electrospinning system of claim 1, wherein the mixture comprises or further comprises one or more of a cosolvent, a surfactant, a preservative, live cells, polymer, thickeners, diluents, biologically active polynucleotides, cellular components, an additional active ingredient, a salt, a preservative, a protein, a peptide, an amino acid, or a nucleic acid component.

29. The integrated electrospinning system of claim 1, wherein the API comprises a protein, an antibody, a nucleic acid, messenger ribonucleic acid (mRNA) molecules, a lipid nanoparticle, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing endonucleases or meganucleases, a growth factor, a plasmid, a hydrophilic pharmaceutical, a lipophilic pharmaceutical, a viral particle, a virus-like particle, a live yeast cell, a live recombinant yeast cell, a live fungus, a live bacterial cell, a live recombinant bacterial cell, a live insect cell, a live mammalian cell, or a live mesenchymal stem cell.

30. The integrated electrospinning system of claim 1, wherein the polymer is a biodegradable polymer selected from the group consisting of poly(lactide-co-glycolide), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine).

31. The integrated electrospinning system of claim 1, wherein the non-polymeric excipient is a hydrophilic substance, a hydrophobic substance, a non-reducing sugar, trehalose, sucrose, a polyol, mannitol, sorbitol, xylitol, an amino acid, leucine, or L-arginine.

32. The integrated electrospinning system claim 1, further comprising one or more mixing vessels in fluid communication with the plurality of supply containers for preparing and providing components to the plurality of supply containers.

33. The integrated electrospinning system claim 1, further comprising one or more sensors monitoring a concentration of the API.

34. The integrated electrospinning system claim 33, wherein the one or more sensors comprise a UV probe.

35. The integrated electrospinning system of claim 1, wherein the plurality of linkers comprises a Luer lock, a Luer slip, or a slip tip.

36. The integrated electrospinning system of claim 1, wherein the plurality of linkers is configured to couple with an outlet of an additional printhead.

37. The integrated electrospinning system of claim 1, wherein the plurality of linkers is characterized by a diameter of less than or about 5.0 mm.

38. The integrated electrospinning system of claim 1, wherein a first respective component comprises a first API and a second respective component comprises a second API that is different from the first API.

39. The integrated electrospinning system of claim 1, wherein a first respective component has a first polarity and a second respective component has second polarity that is different from the first polarity, wherein the first respective component and the second respective component are immiscible.

40. A method comprising:

providing a first component to a first linker of a plurality of linkers of a printhead;

providing a second component to a second linker of the plurality of linkers of the printhead;

combining the first component and the second component in a fluid mixer of the printhead;

forming a mixture of the first component and the second component in the printhead;

delivering the mixture to a spinneret in fluid communication with the printhead;

applying voltage to the mixture at the spinneret to form fibers; and

collecting the fibers by using a controlled collector.

41. The method of claim 40, wherein the first component comprises a first solvent and the second component comprises a second solvent having a different polarity from the first solvent.

42. The method of claim 40, wherein the first component comprises a first active pharmaceutical ingredient (API) and the second component comprises a second API that is different from the first API.

43. An integrated electrospinning system comprising:

a plurality of supply containers for preparing or storing a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API);

a plurality of spinnerets in fluid communication with the plurality of supply containers to form a plurality of electrospinning droplets;

a high-voltage power supply in electrical communication with the plurality of spinnerets to form electrospun fibers from the plurality of electrospinning droplets; and

a collector including a stage and a mechanical arm.

44. The integrated electrospinning system of claim 43, further comprising a printhead in fluid communication with the plurality of supply containers, wherein the printhead comprises:

a plurality of linkers, wherein each linker is in fluid communication with a supply outlet of one of the plurality of supply containers;

a fluid mixer for mixing components from the plurality of linkers into a mixture; and

one or more printhead outlets in fluid communication with the fluid mixer and the plurality of spinnerets.

45. The integrated electrospinning system of claim 43, wherein the API comprises a protein, an antibody, a nucleic acid, messenger ribonucleic acid (mRNA) molecules, a lipid nanoparticle, clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), homing endonucleases or meganucleases, a growth factor, a plasmid, a hydrophilic pharmaceutical, a lipophilic pharmaceutical, a viral particle, a virus-like particle, a live yeast cell, a live recombinant yeast cell, a live fungus, a live bacterial cell, a live recombinant bacterial cell, a live insect cell, a live mammalian cell, or a live mesenchymal stem cell.

46. The integrated electrospinning system of claim 43, wherein the respective component is semi-permeable.

47. The integrated electrospinning system of claim 43, wherein the polymer is a biodegradable polymer selected from the group consisting of poly(lactide-co-glycolide), polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), pluronic F127, sodium alginate, hyaluronic acid, chitosan, cyclodextrin, dextran, agarose, gelatin, albumin, collagen, lipids, a polyethylene glycol (PEG) derivative, a pharmaceutical grade polymer, poly(hydroxy butyrate), poly(β-malic acid), or poly(L-lysine).

48. The integrated electrospinning system of claim 43, wherein the non-polymeric excipient is a hydrophilic substance, a hydrophobic substance, a non-reducing sugar, trehalose, sucrose, a polyol, mannitol, sorbitol, xylitol, an amino acid, leucine, or L-arginine.

49. An electrospun fiber comprising:
a first segment including a first component; and
a second segment including a second component,
wherein the first segment and the second segment have uniform diameters.

50. The electrospun fiber of claim 49, wherein the second segment coaxially surrounds the first segment.

51. The electrospun fiber of claim 49, wherein the first segment is characterized by a first axis and the second segment is characterized by a second axis.

52. The electrospun fiber of claim 49, further comprising a third segment.

53. The electrospun fiber of claim 52, wherein the second segment coaxially surrounds the first segment, wherein the third segment coaxially surrounds the first segment and the second segment.

54. The electrospun fiber of claim 52, wherein the first segment is characterized by a first axis, the second segment is characterized by a second axis, and the third segment is characterized by a third axis.

55. The electrospun fiber of claim 49, further comprising live cells.

56. The electrospun fiber of claim 49, wherein the electrospun fiber is edible.

57. The electrospun fiber of claim 50, wherein the first segment and the second segment are configured to overcome phase segregation and separation.

58. A system comprising:

an environmental simulator including at least one simulating component, wherein the simulating component includes a simulating fluid; and

a simulating fiber disposed in the environmental simulator, wherein the simulating fiber comprises an inner layer, an inter layer surrounding the inner layer, and an outer layer surrounding the inter layer and the inner layer.

59. The system of claim 58, wherein the simulating fiber comprises a hydrophobic material and a hydrophilic material.

60. The system of claim 58, wherein the simulating fiber is semi-permeable to only allow a selected group of materials to pass through.

61. The system of claim 58, wherein the at least one simulating component comprises a temperature chamber, humidity chamber, vacuum chamber, vibration chamber, stability chamber, salt spray chamber, thermal shock chamber, altitude chamber, anechoic chamber, gas-controlled chamber, and/or sterilization chamber.

62. An integrated electrospinning system comprising:

a plurality of supply containers for preparing or storing a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API);

a first printhead in fluid communication with the plurality of supply containers, the first printhead comprising a first linker, a second linker, and a first coil, the first linker and the second linker in fluid communication with the plurality of supply containers;

a second printhead in fluid communication with the first printhead, the second printhead comprising a third linker, a second coil, and a connecting linker in fluid connection with the first printhead, the second coil, and a spinneret;

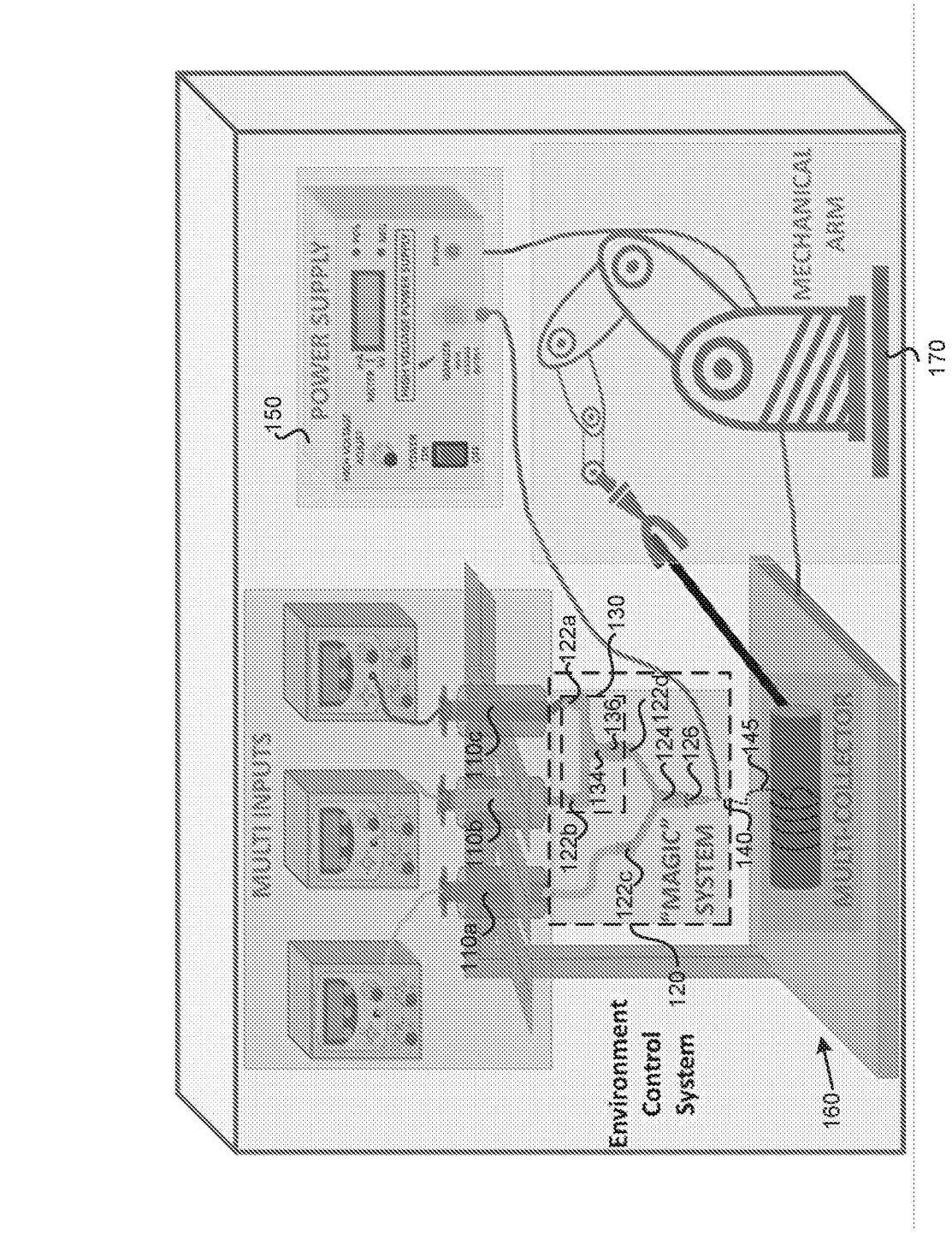
a high-voltage power supply in electrical communication with the spinneret to form electrospun fibers; and

a collector to collect the electrospun fibers.

63. The integrated electrospinning system of claim 62, wherein the third linker is in fluid communication with a respective component comprising a polymer or a non-polymeric excipient, a solvent, or an active pharmaceutical ingredient (API).

64. The integrated electrospinning system of claim 62, wherein the first coil is configured to fabricate nanoparticles or microparticles, and wherein the second coil is configured to mix the nanoparticles or microparticles fabricated in the first coil with a respective component delivered from the third linker.

5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150
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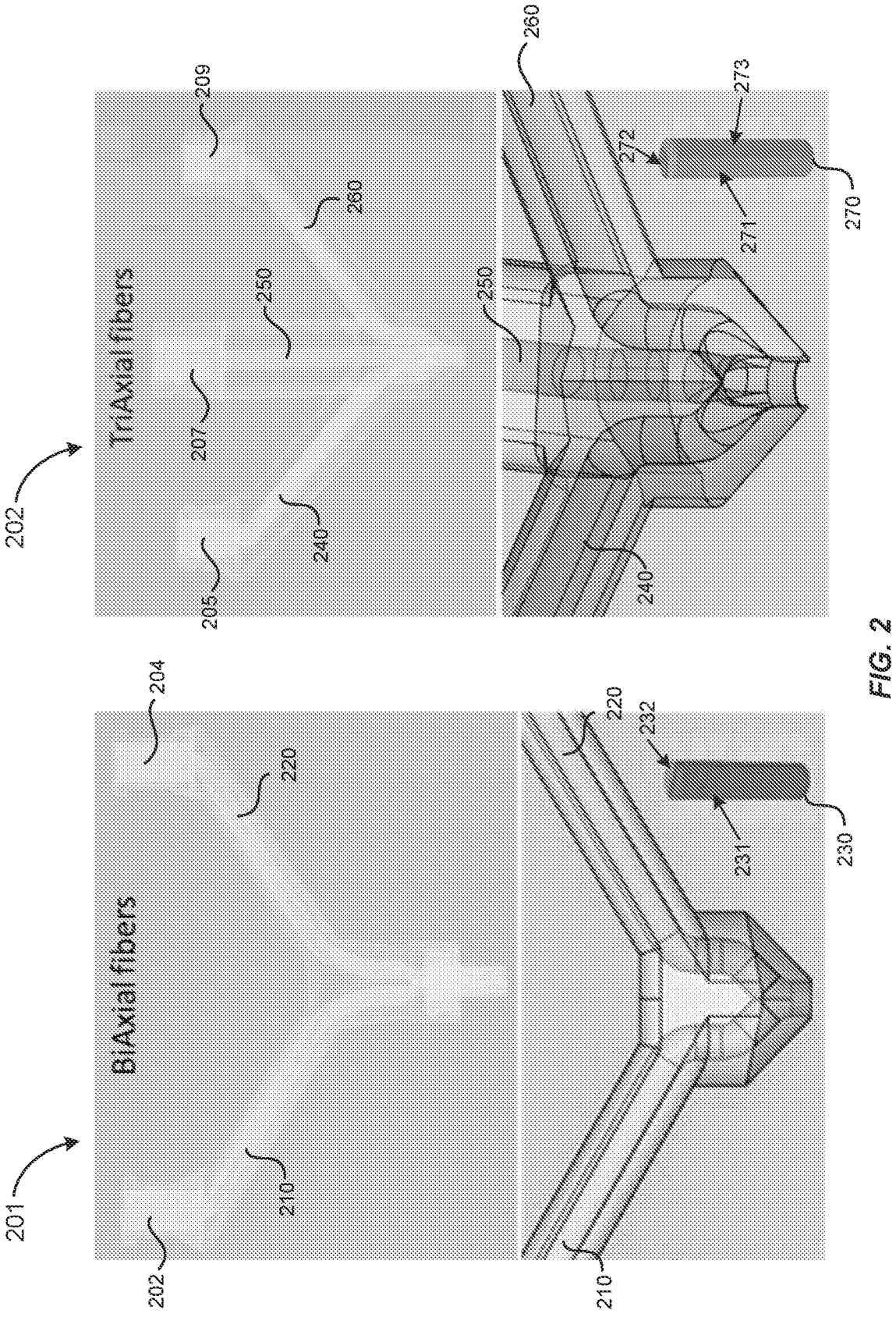
MECHANICAL ARM
170
MULTI INPUTS
110a
110b
110c
122a
130
136
134
124
122d
126
122c
120
140
145
Environment Control System
"MAGIC" SYSTEM
160

FIG. 1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220
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202
232
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TriAxial fibers
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271
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240

FIG. 2

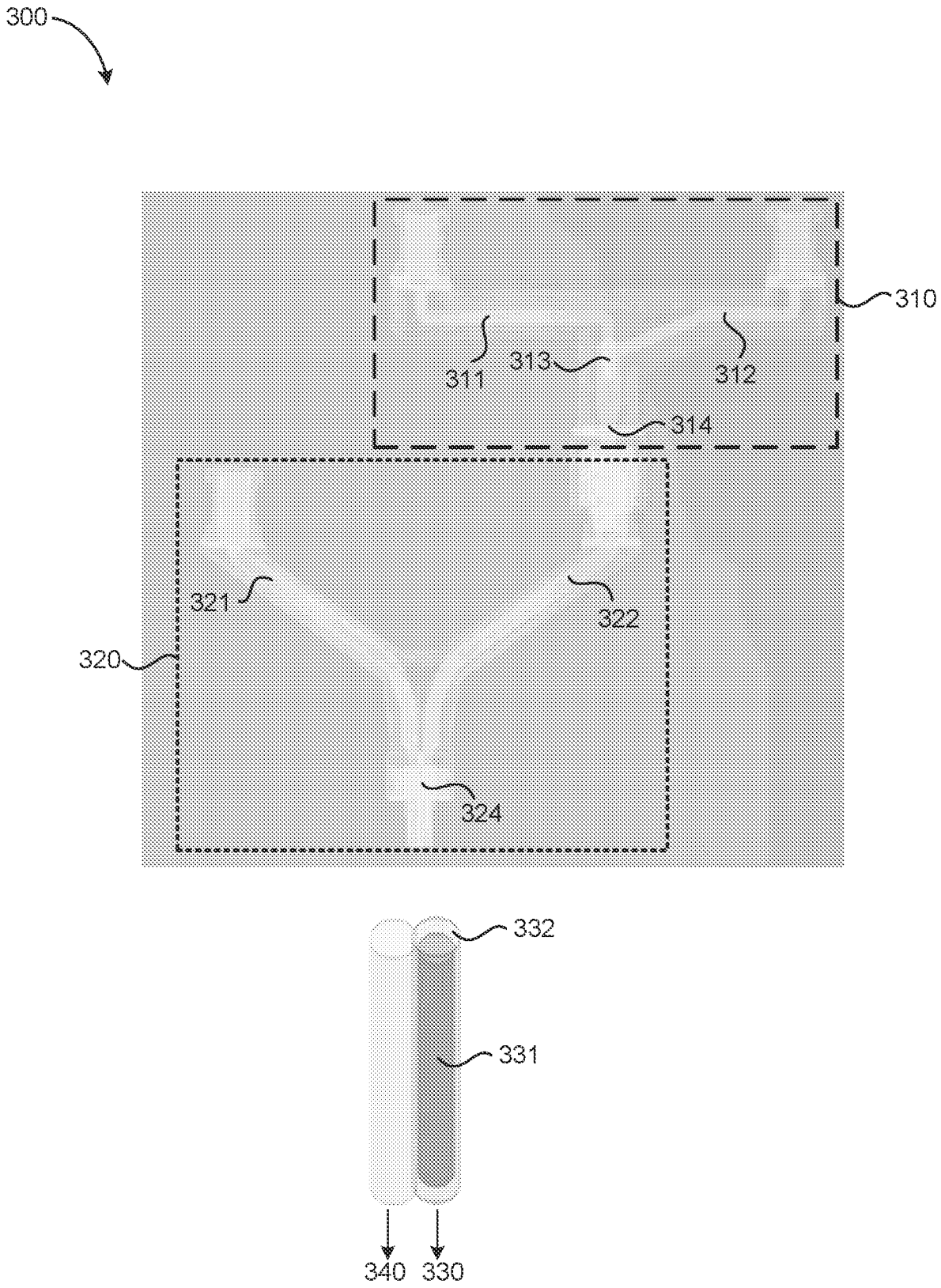
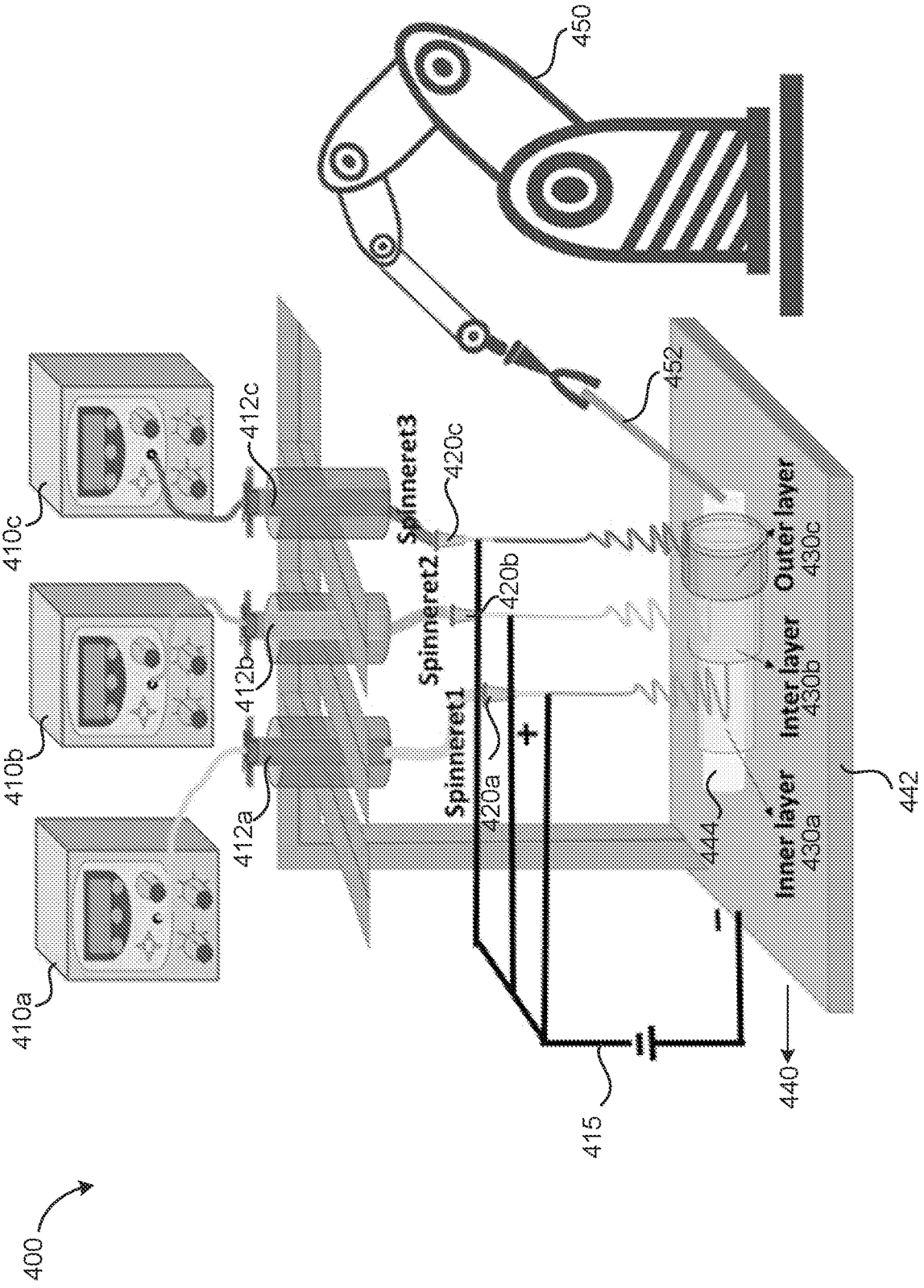


FIG. 3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410a
410b
410c
412a
412b
412c
Spinneret1
Spinneret2
Spinneret3
420a
420b
420c
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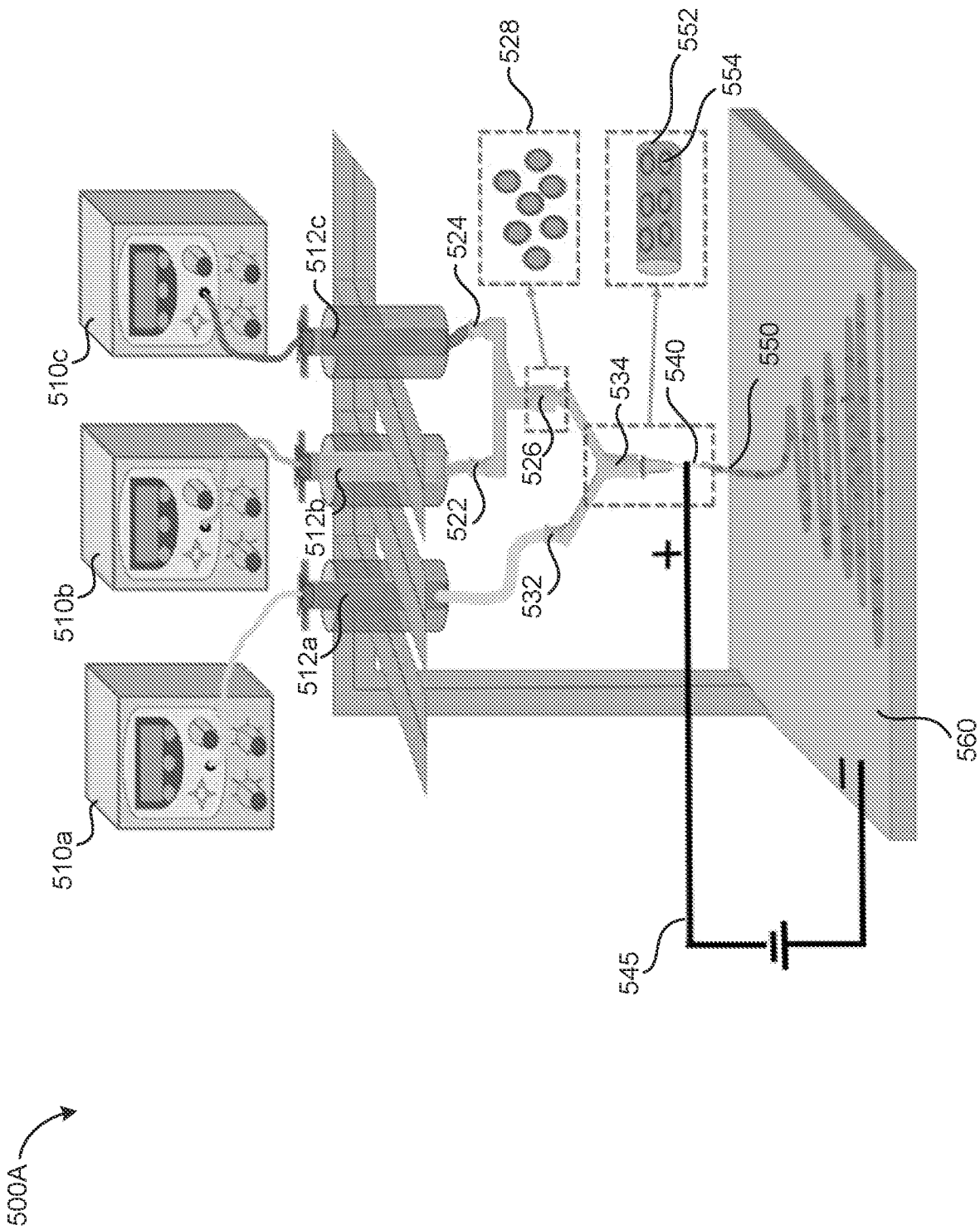
452
Inner layer
430a
Inter layer
430b
Outer layer
430c
442
444
440
415
+
−

FIG. 4


500A
510a
510b
510c
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512b
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528
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534
540
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552
554
560
+
−

FIG. 5A

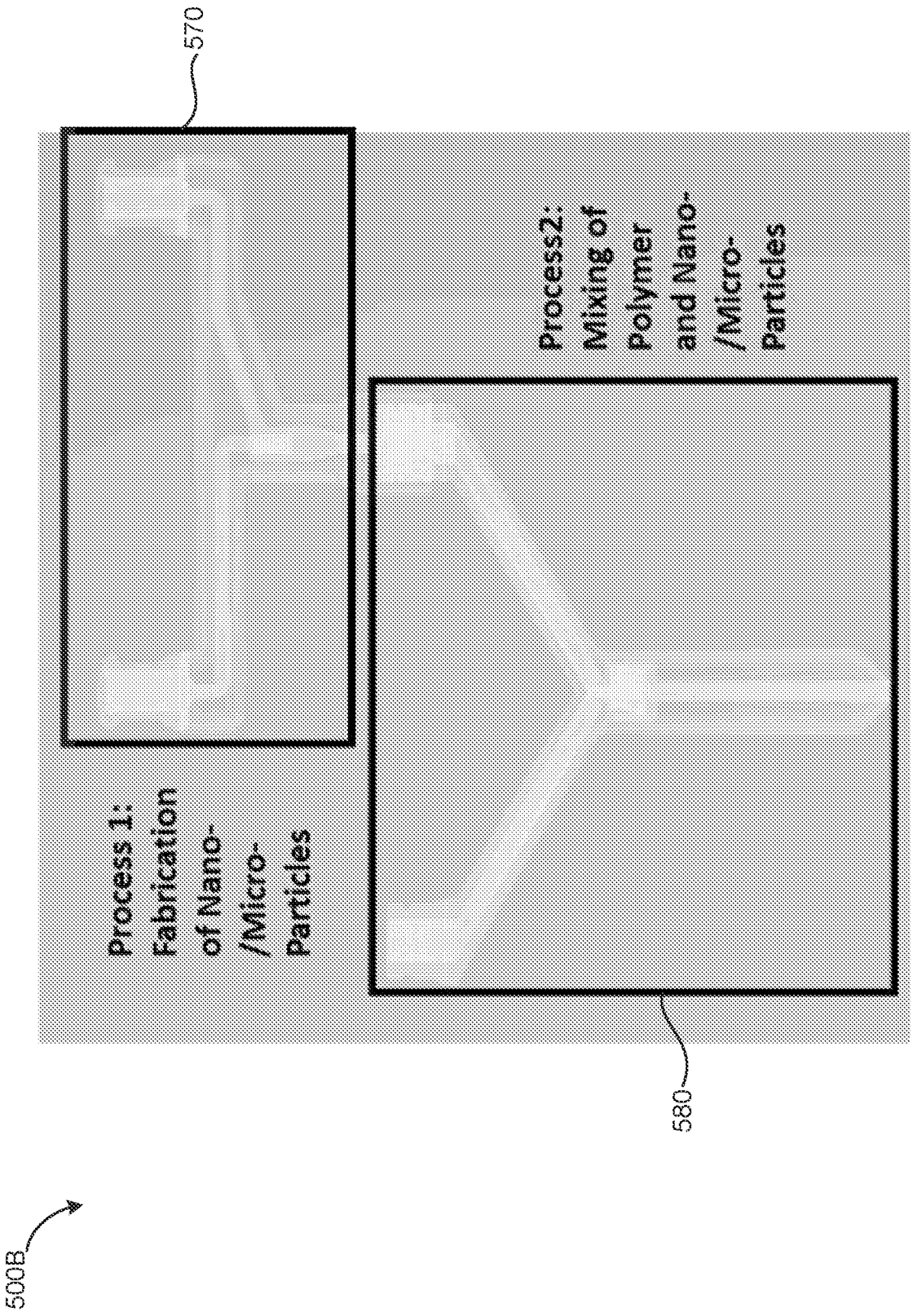

500B
570
Process 1: Fabrication of Nano- /Micro- Particles
Process2: Mixing of Polymer and Nano- /Micro- Particles
580

FIG. 5B

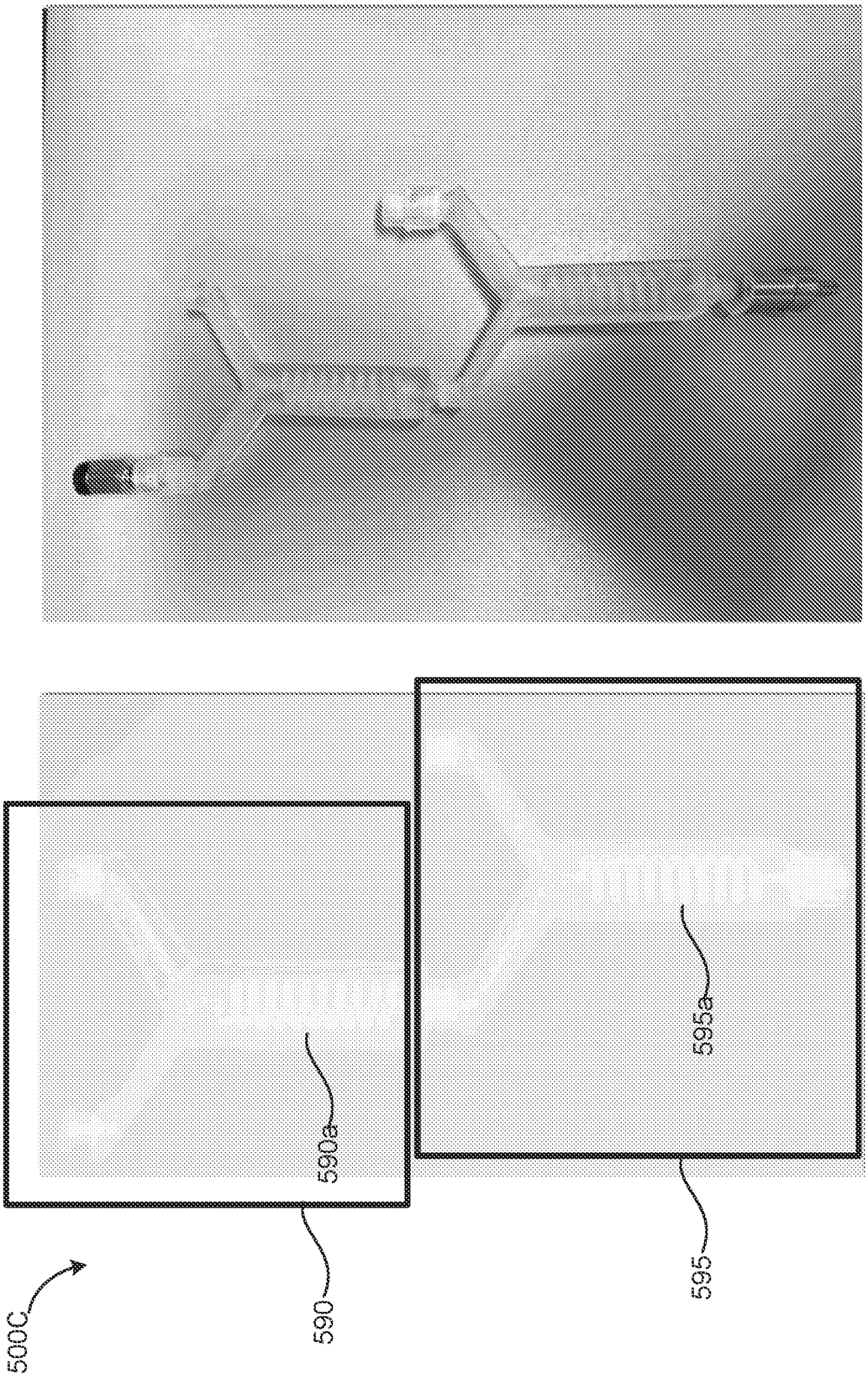
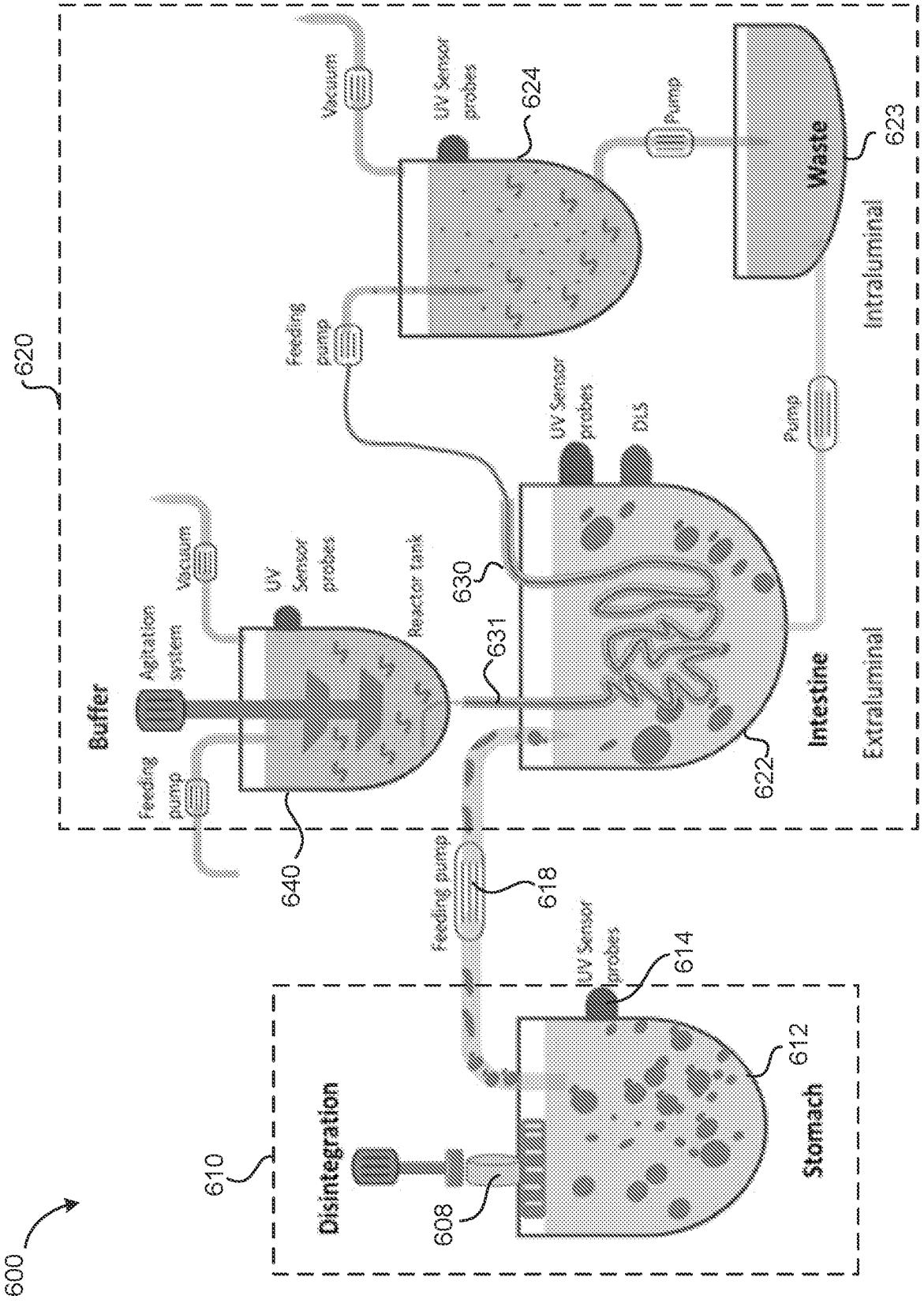


FIG. 5C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Disintegration
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612
Stomach
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UV Sensor probes
618
Feeding pump
620
Buffer
Agitation system
Vacuum
Feeding pump
640
UV Sensor probes
Reactor tank
630
631
UV Sensor probes
DLS
622
Intestine
Extraluminal
Pump
Feeding pump
Vacuum
624
UV Sensor probes
Pump
Waste
623
Intraluminal

FIG. 6

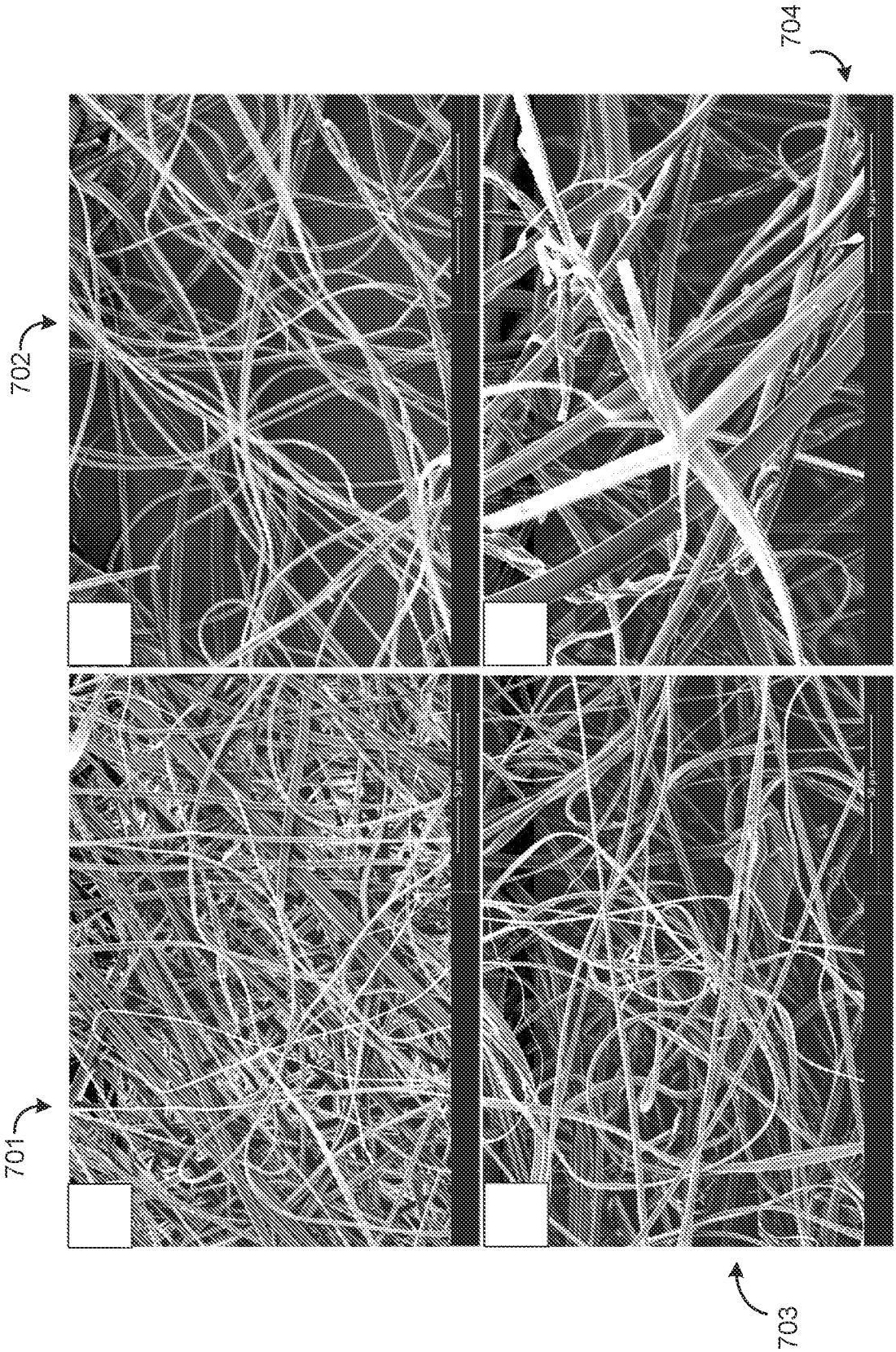

FIG. 7

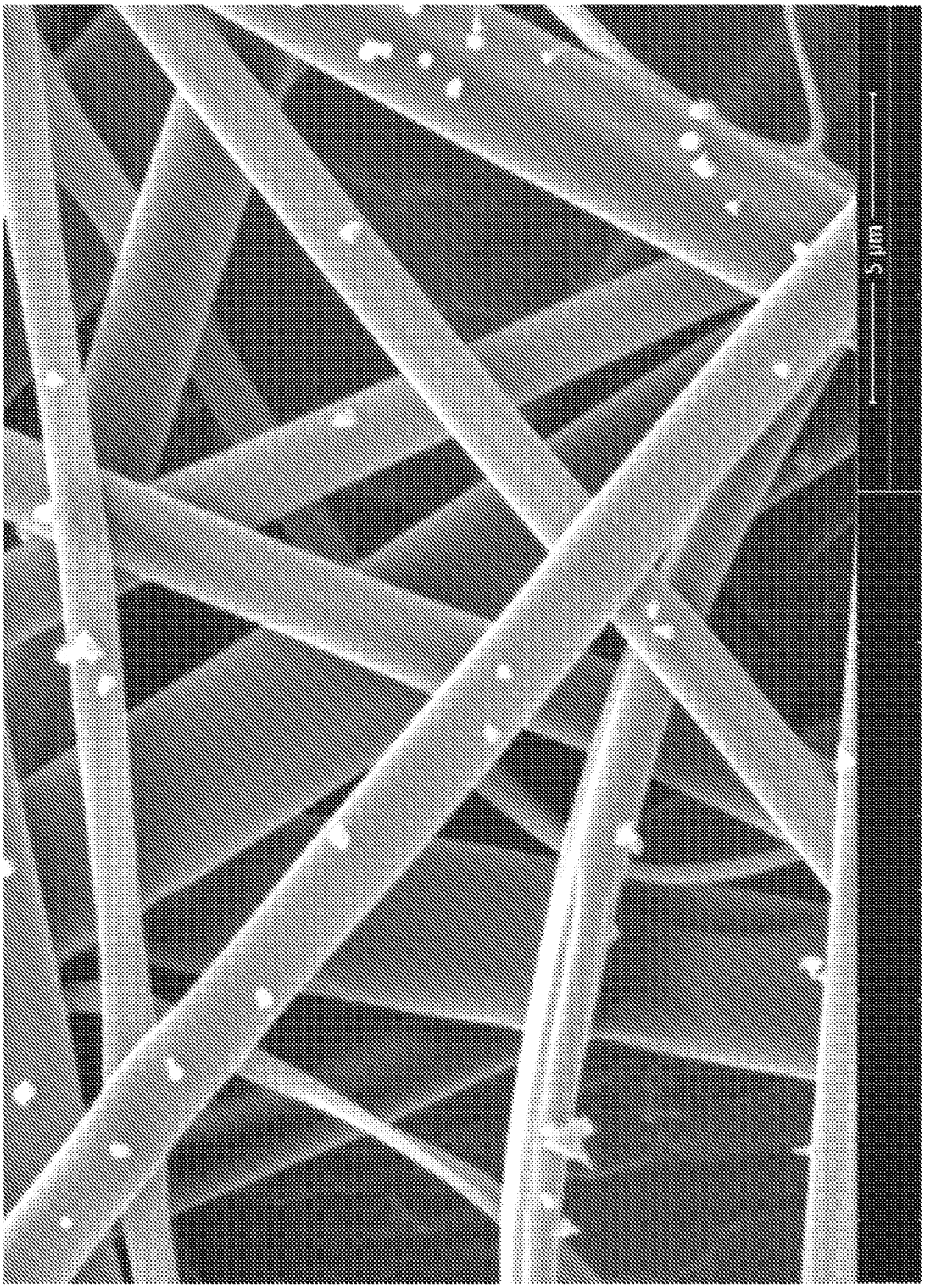

FIG. 8

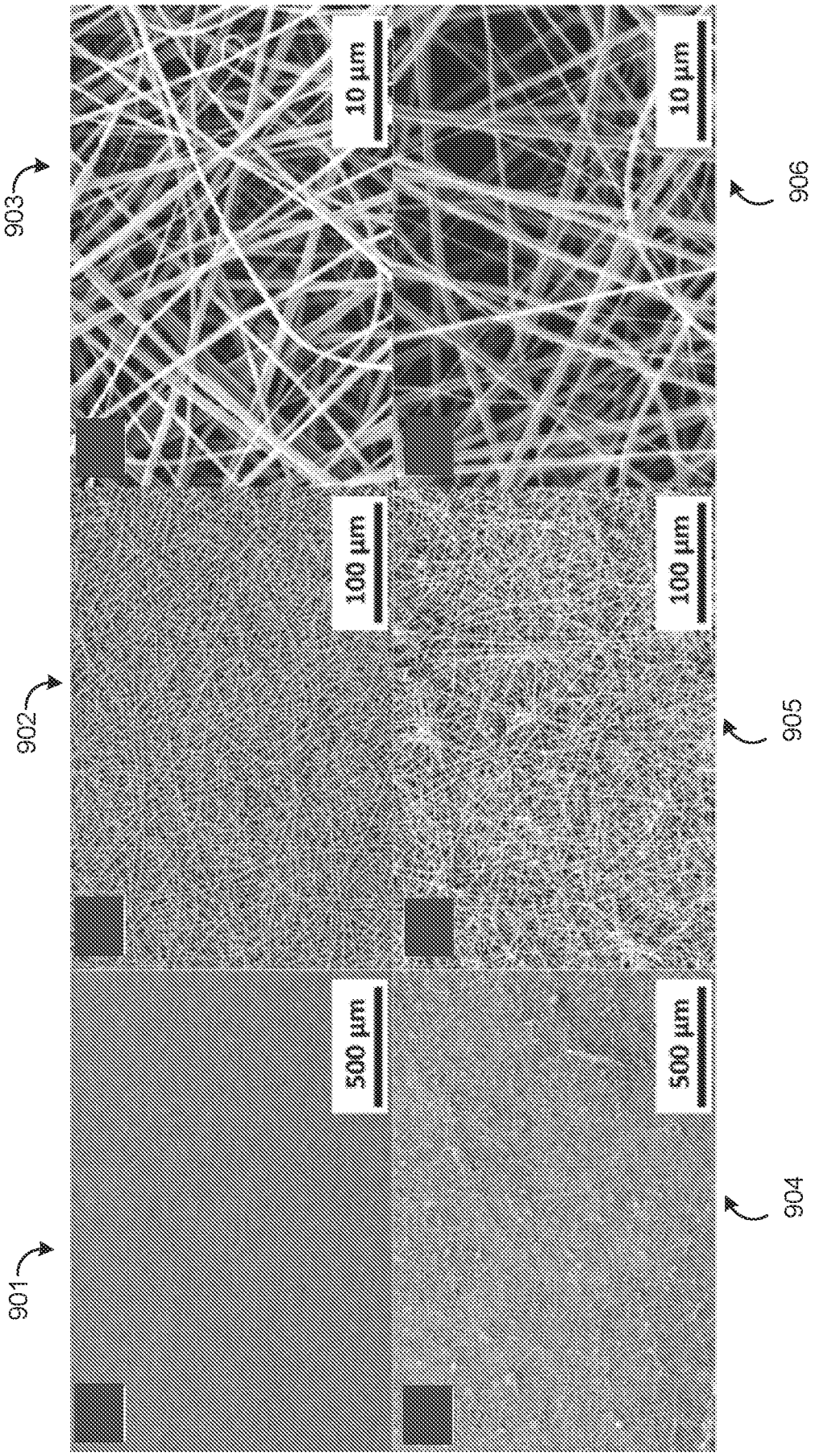

FIG. 9

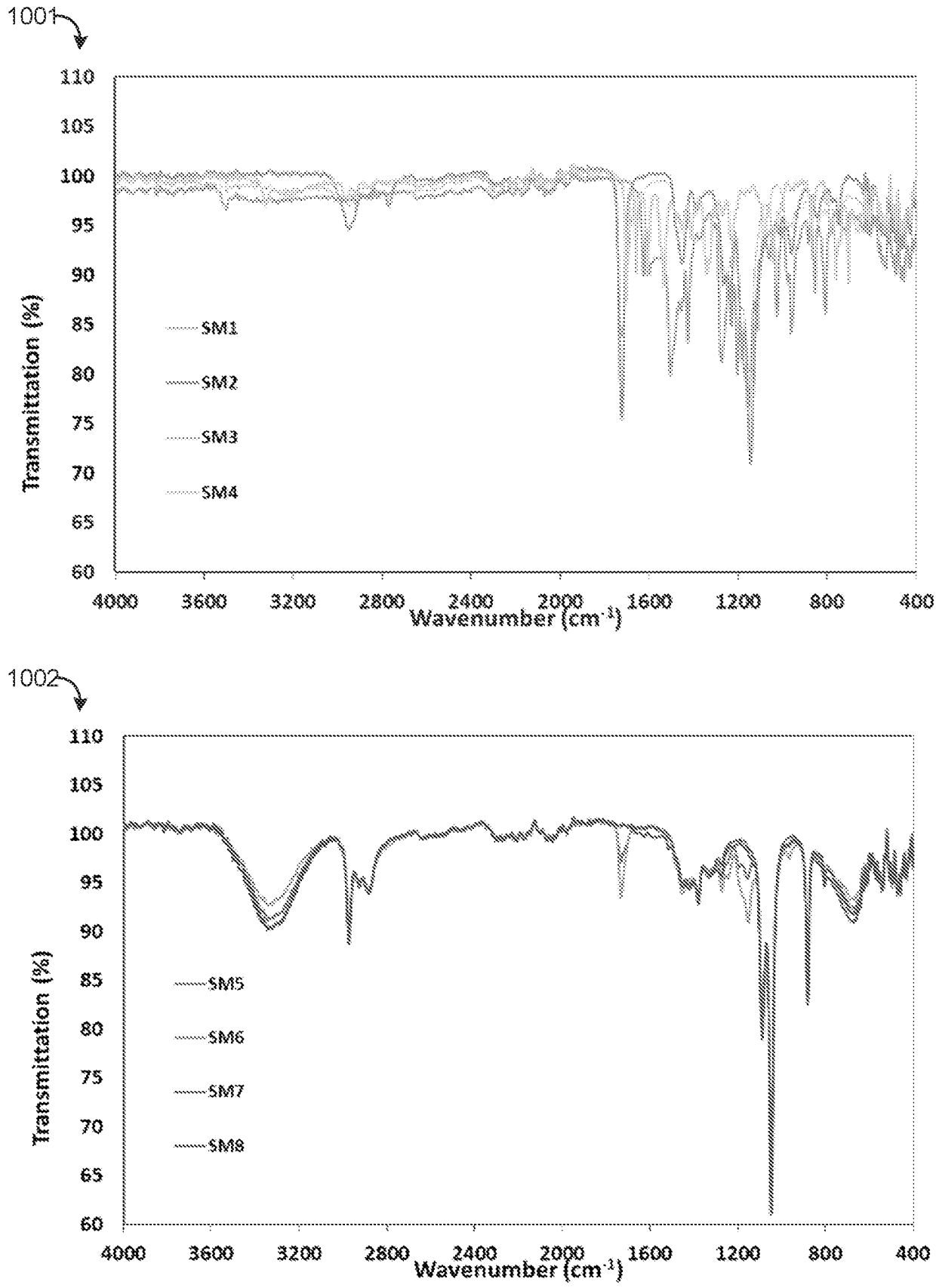
1001
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM1
SM2
SM3
SM4
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm-1)
1002
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM5
SM6
SM7
SM8
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm-1)

FIG. 10A

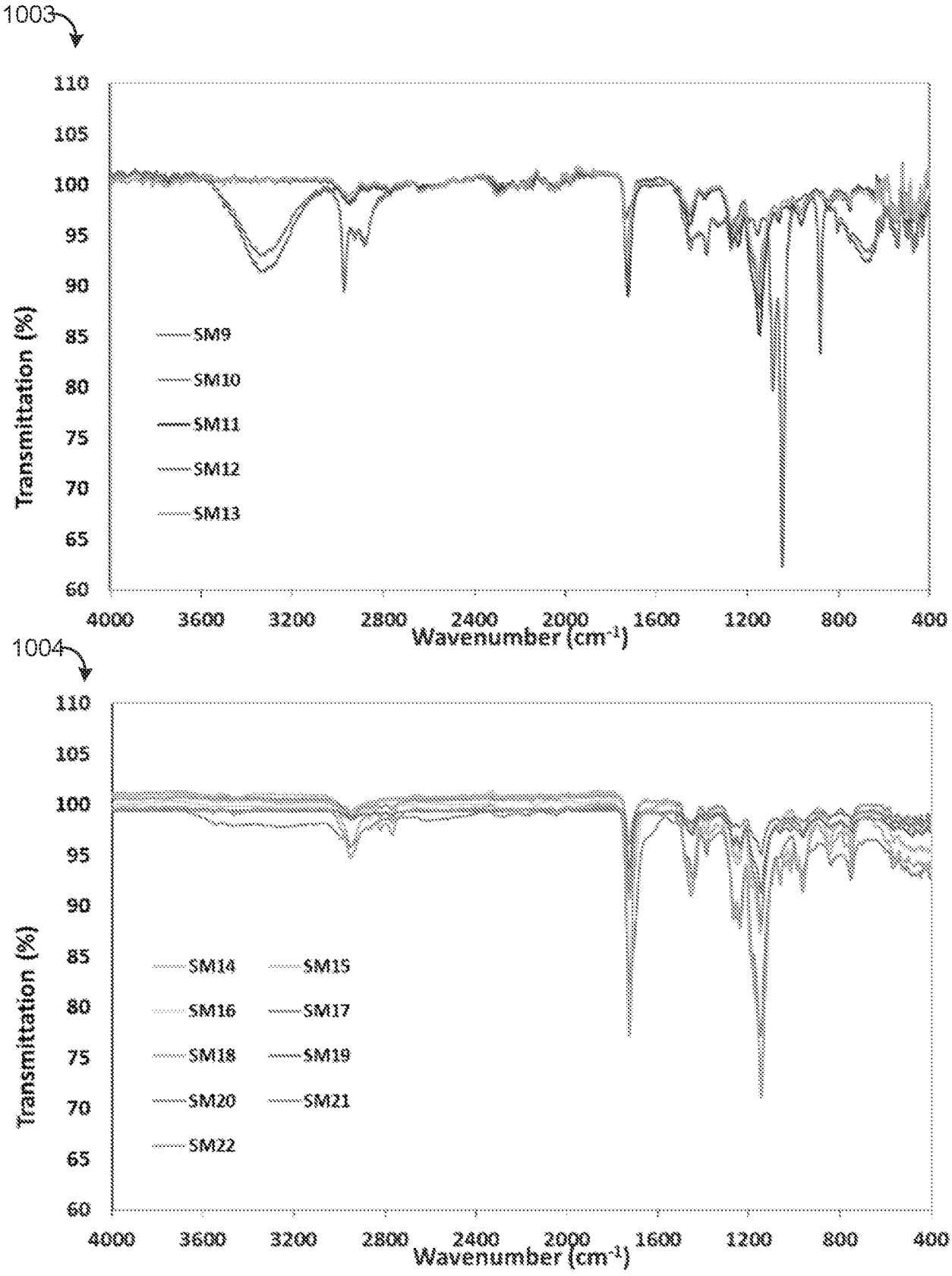
1003
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM9
SM10
SM11
SM12
SM13
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm-1)
1004
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM14
SM15
SM16
SM17
SM18
SM19
SM20
SM21
SM22
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm-1)

FIG. 10B

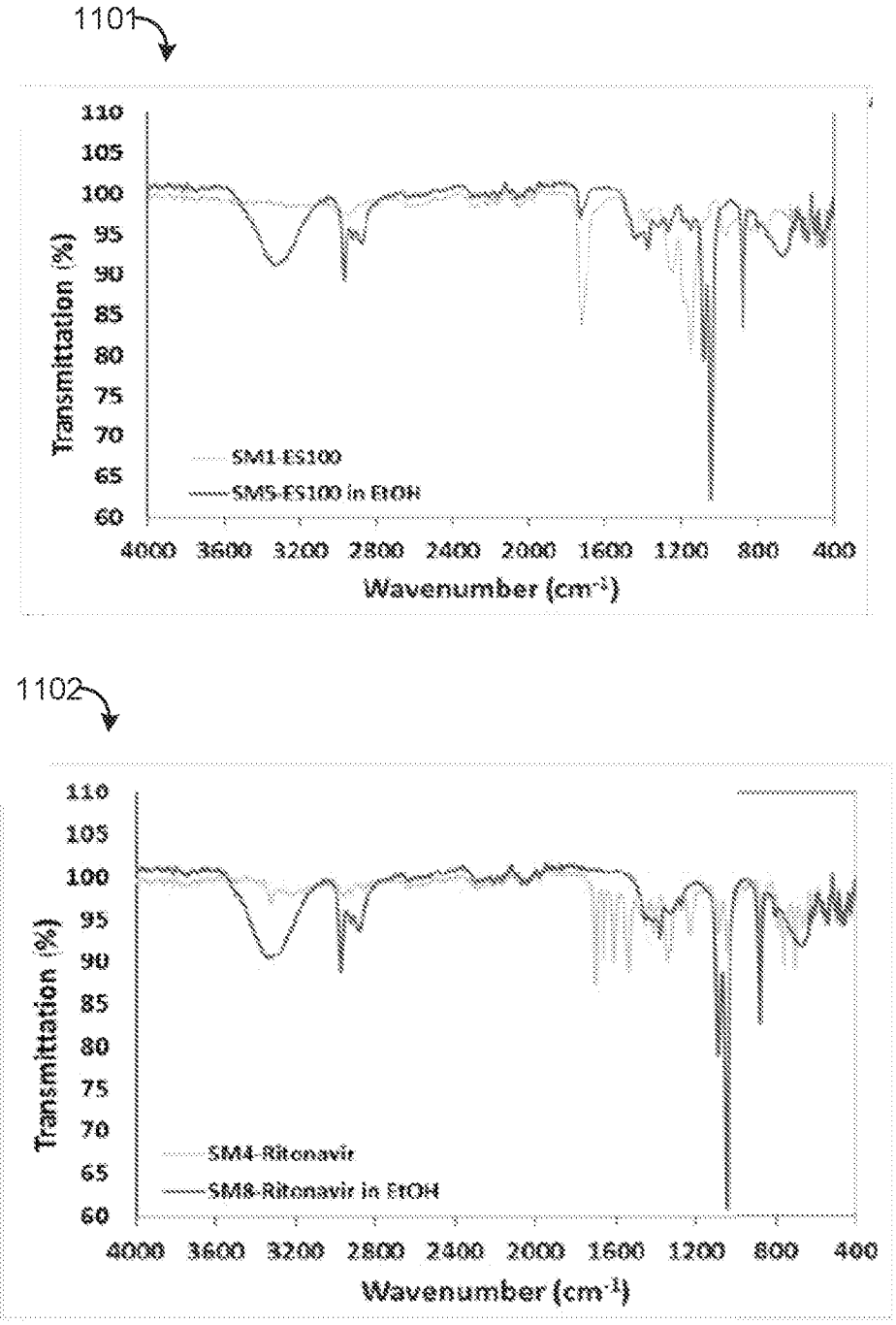
1101
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM1-ES100
SM5-ES100 in EtOH
4000 3600 3200 2800 2400 2000 1600 1200 800 400
Wavenumber (cm-1)
1102
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM4-Ritonavir
SM8-Ritonavir in EtOH
4000 3600 3200 2800 2400 2000 1600 1200 800 400
Wavenumber (cm-1)

FIG. 11A

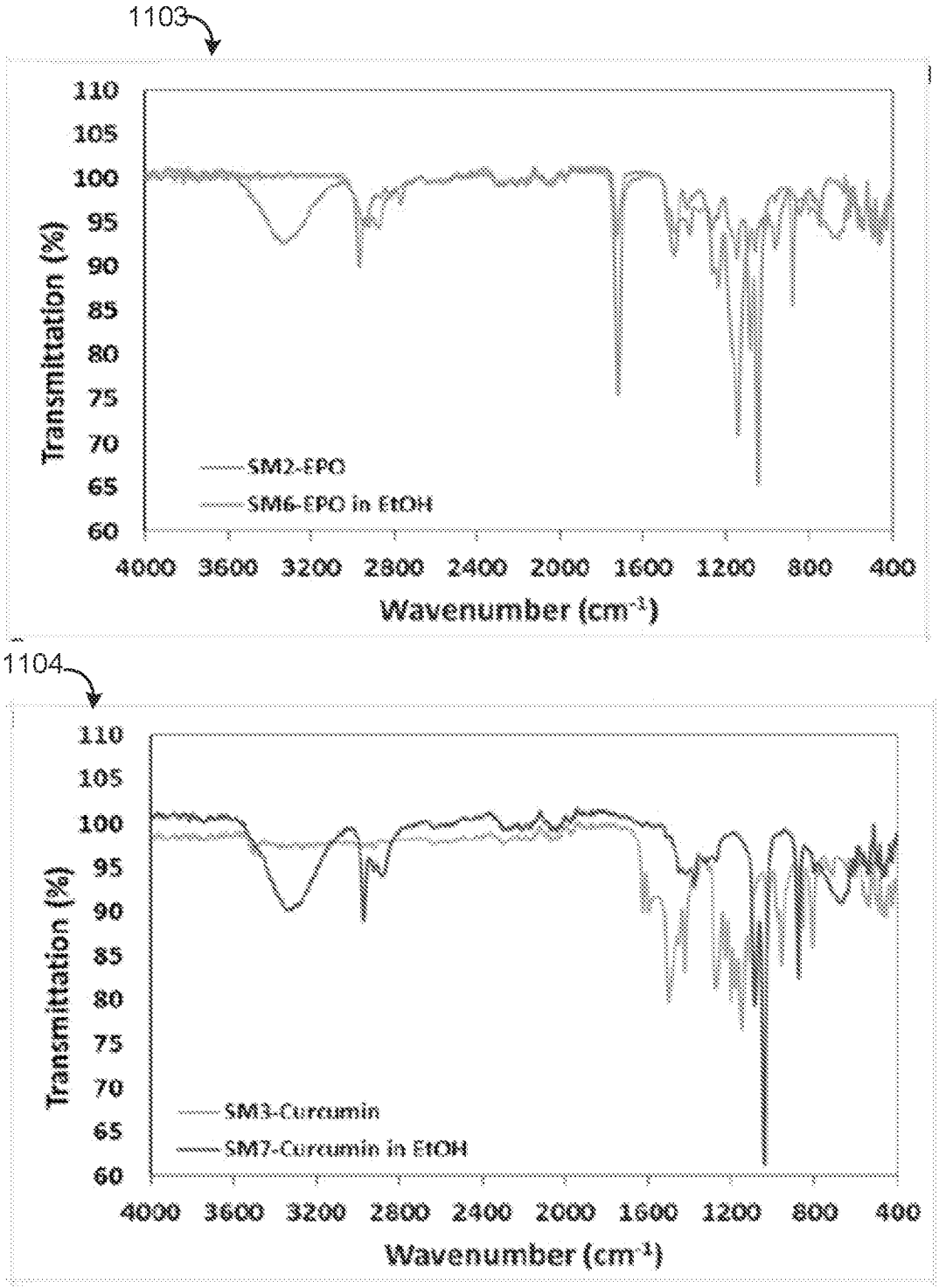
1103
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM2-EPO
SM6-EPO in EtOH
4000 3600 3200 2800 2400 2000 1600 1200 800 400
Wavenumber (cm-1)
1104
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM3-Curcumin
SM7-Curcumin in EtOH
4000 3600 3200 2800 2400 2000 1600 1200 800 400
Wavenumber (cm-1)

FIG. 11B

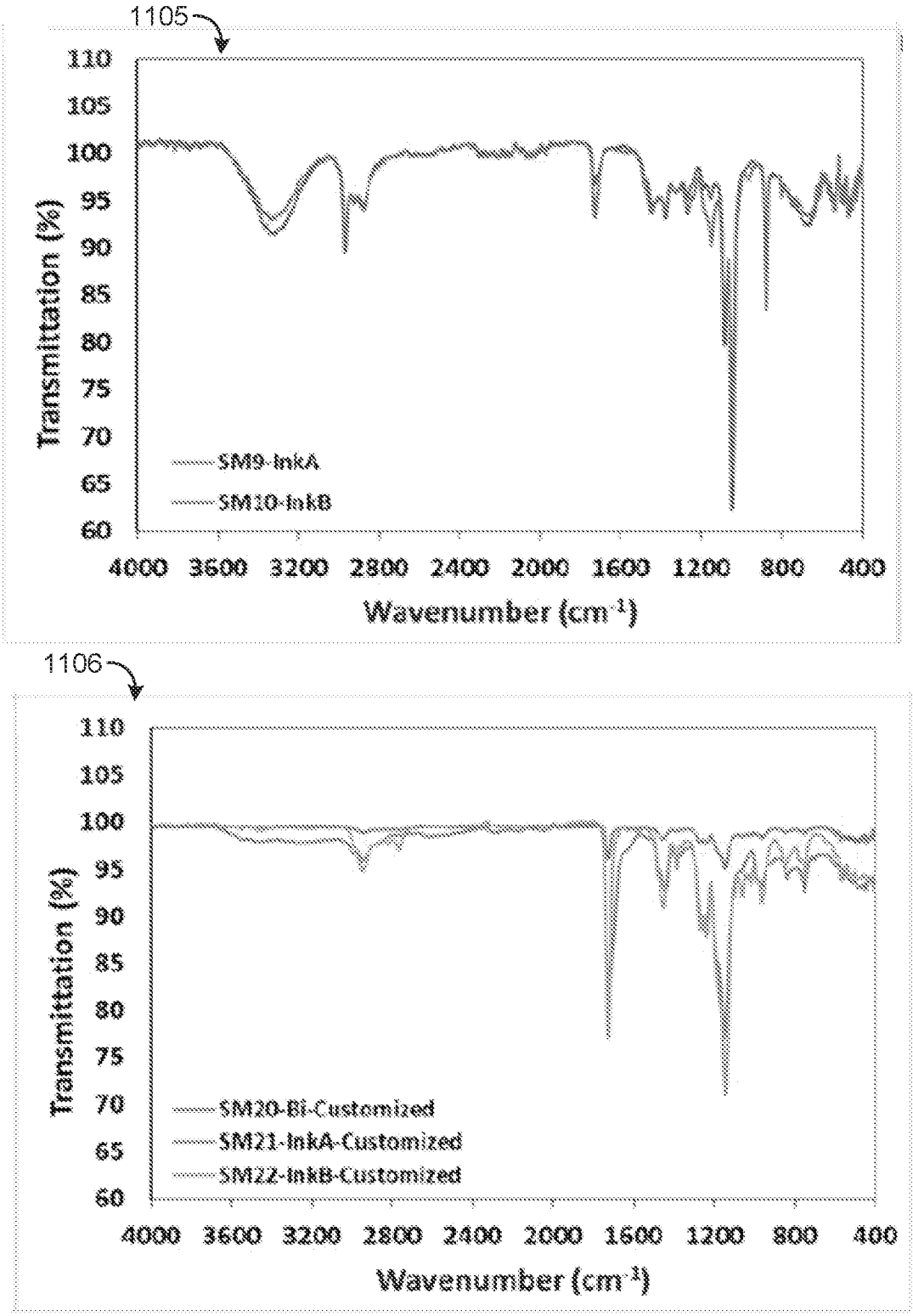
1105
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM9-InkA
SM10-InkB
4000 3600 3200 2800 2400 2000 1600 1200 800 400
Wavenumber (cm-1)
1106
110
105
100
95
90
85
80
75
70
65
60
Transmittation (%)
SM20-Bi-Customized
SM21-InkA-Customized
SM22-InkB-Customized
4000 3600 3200 2800 2400 2000 1600 1200 800 400
Wavenumber (cm-1)

FIG. 11C

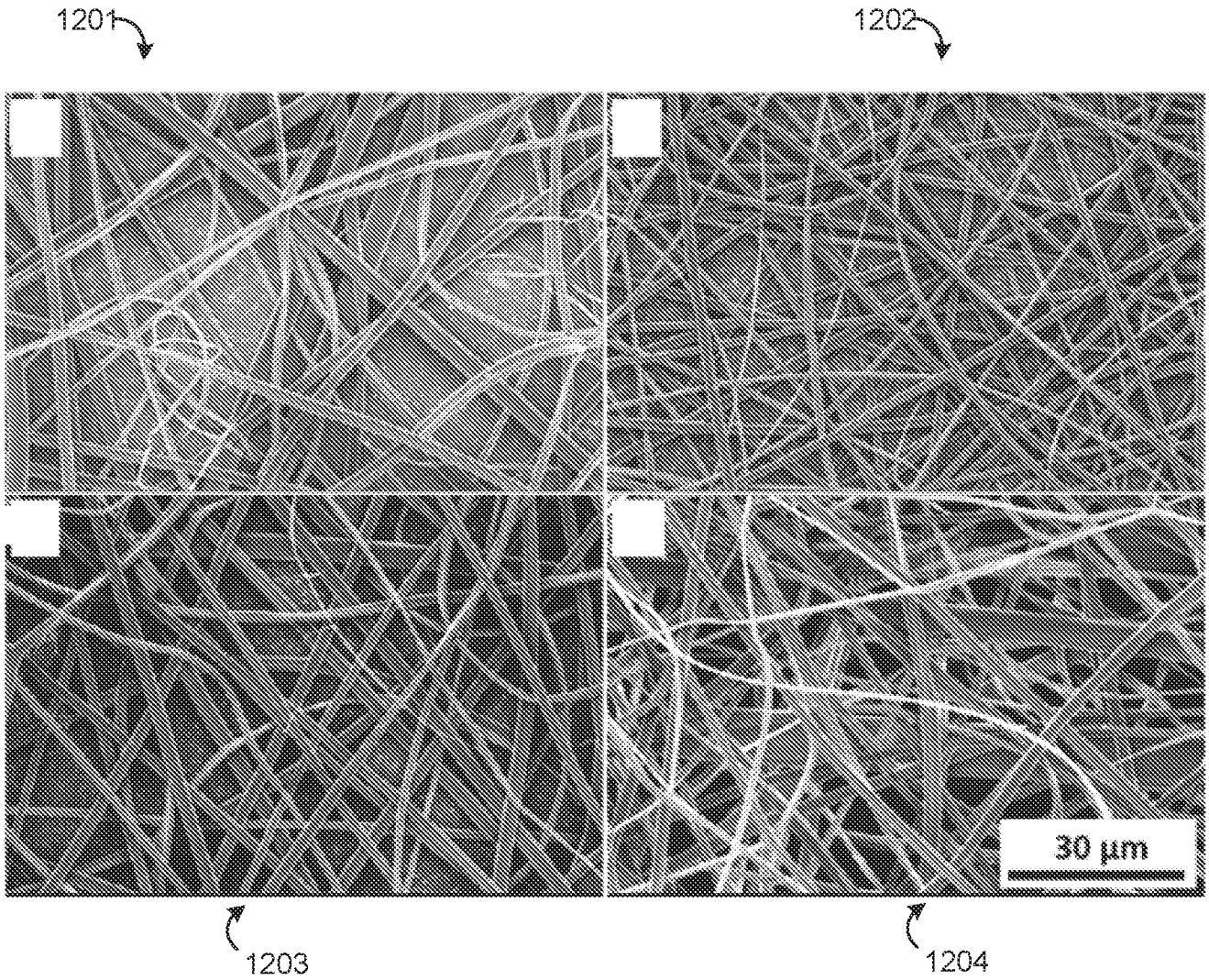

FIG. 12

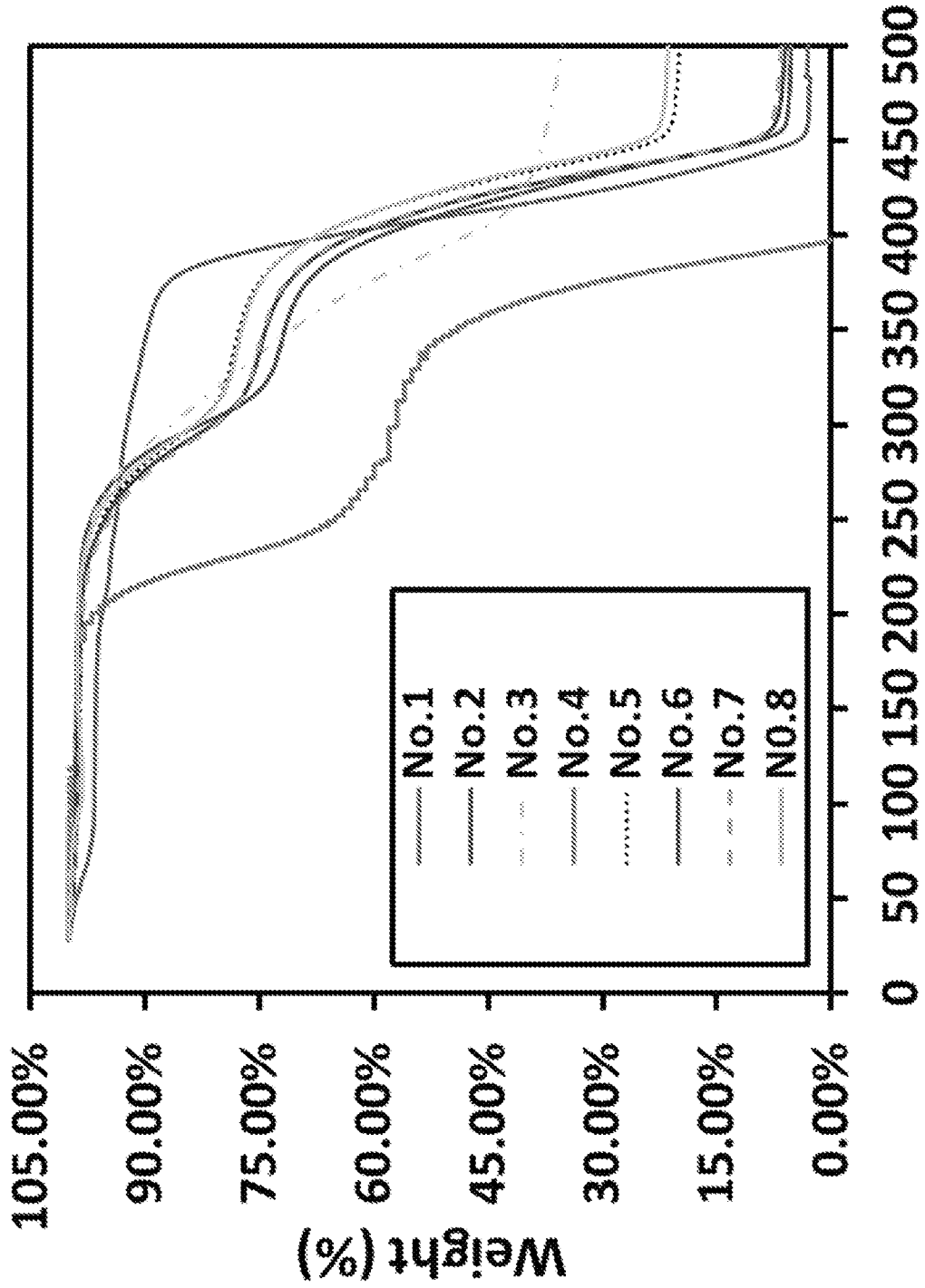
105.00%
90.00%
75.00%
60.00%
45.00%
30.00%
15.00%
0.00%
Weight (%)
0
50
100
150
200
250
300
350
400
450
500
Temperature (°C)
No.1
No.2
No.3
No.4
No.5
No.6
No.7
No.8

FIG. 13

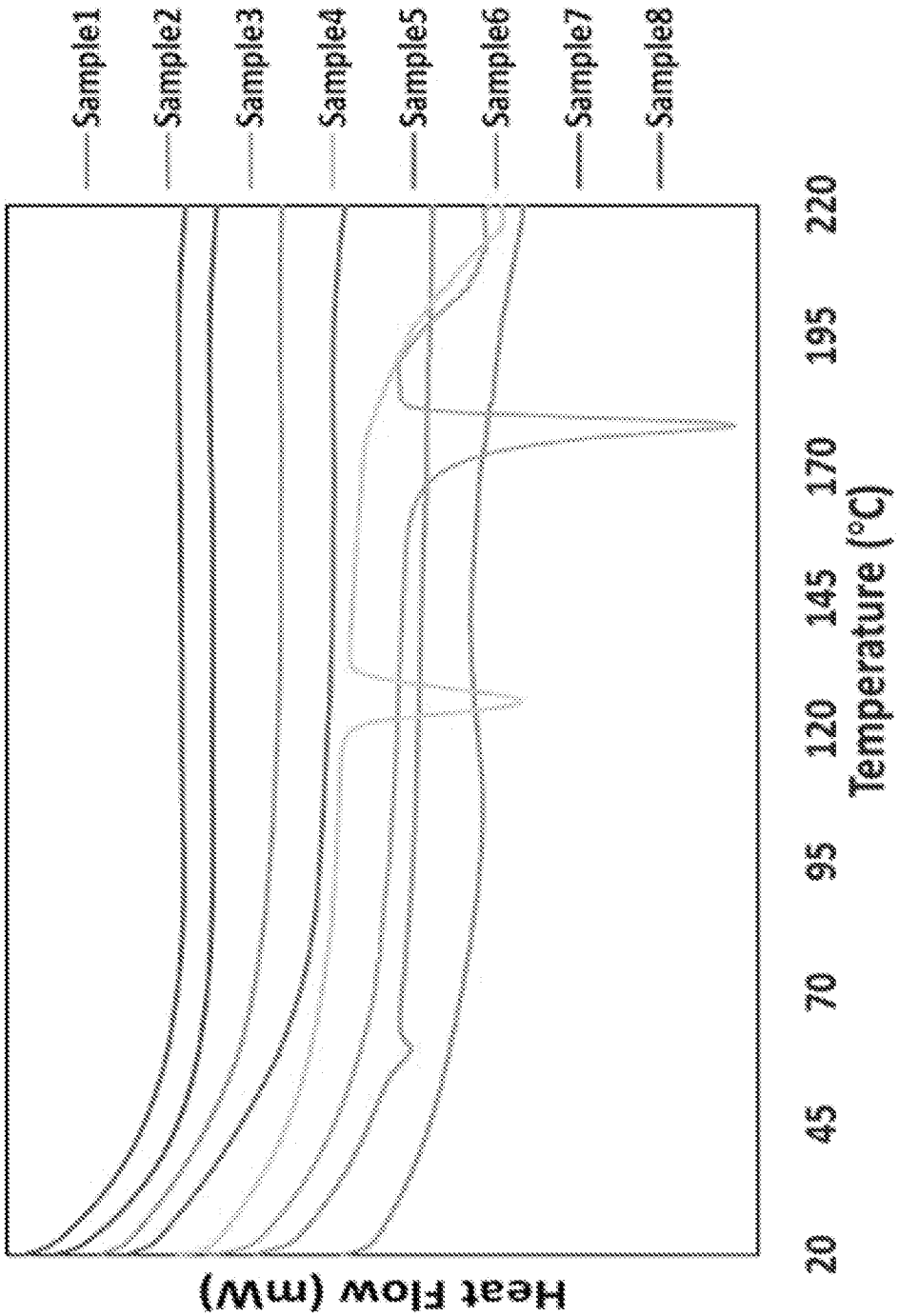
Sample1
Sample2
Sample3
Sample4
Sample5
Sample6
Sample7
Sample8
Heat Flow (mW)
20
45
70
95
120
145
170
195
220
Temperature (°C)

FIG. 14

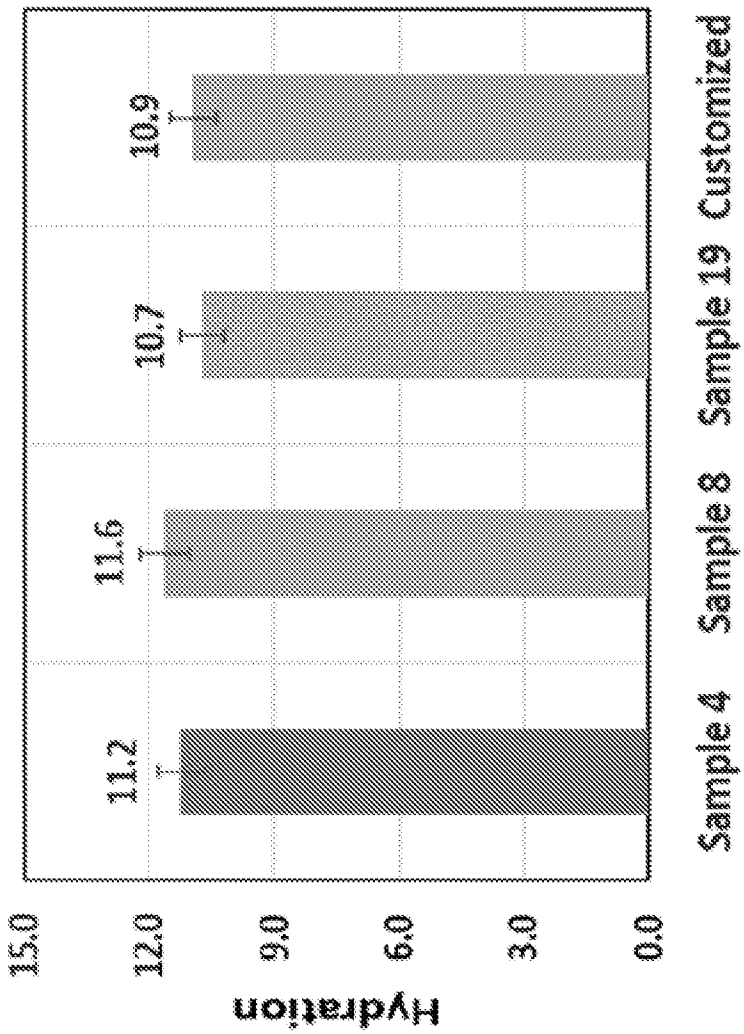
Hydration
15.0
12.0
9.0
6.0
3.0
0.0
11.2
11.6
10.7
10.9
Sample 4
Sample 8
Sample 19
Customized

FIG. 15A

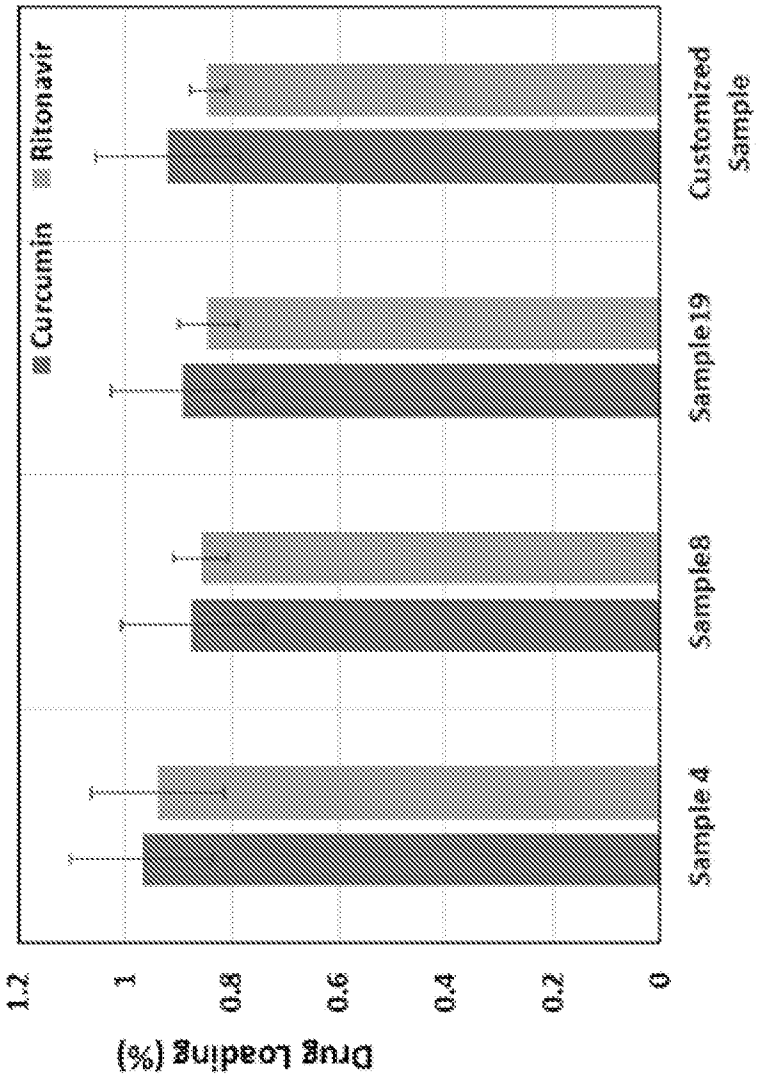
Curcumin
Ritonavir
Drug loading (%)
1.2
1
0.8
0.6
0.4
0.2
0
Sample 4
Sample8
Sample19
Customized Sample

FIG. 15B

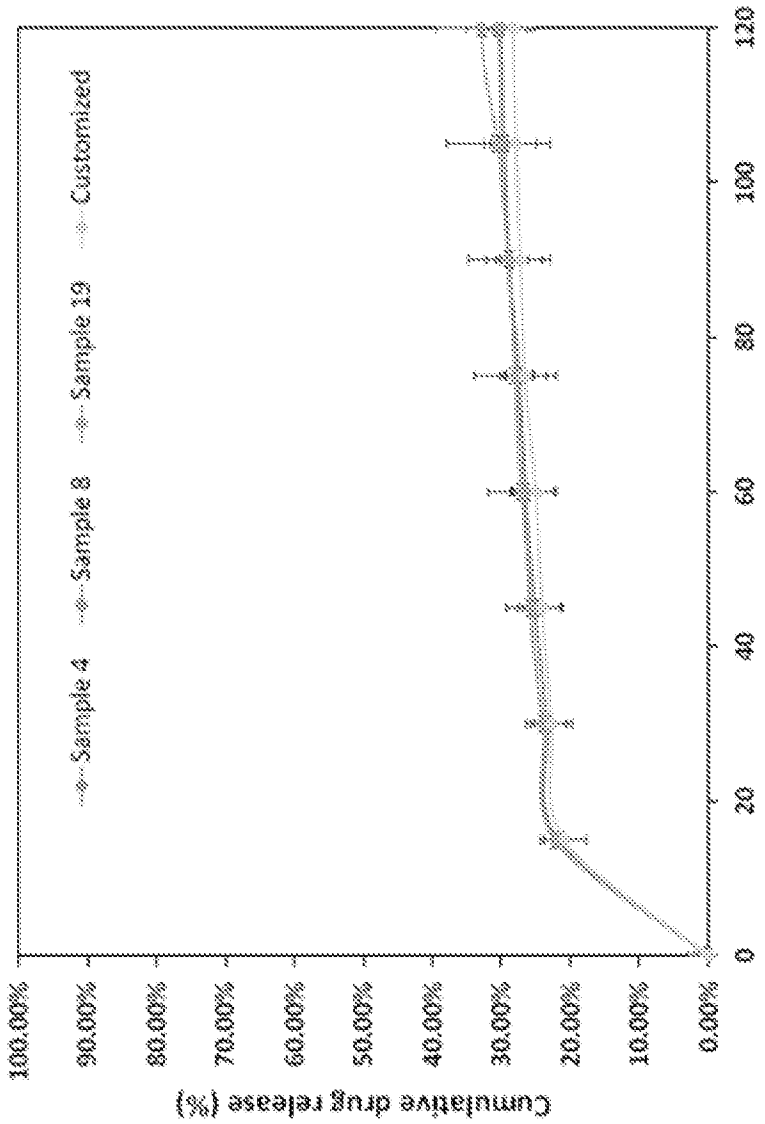
Cumulative drug release (%)
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
0
20
40
60
80
100
120
Time (minutes)
Sample 4
Sample 8
Sample 19
Customized

FIG. 16A

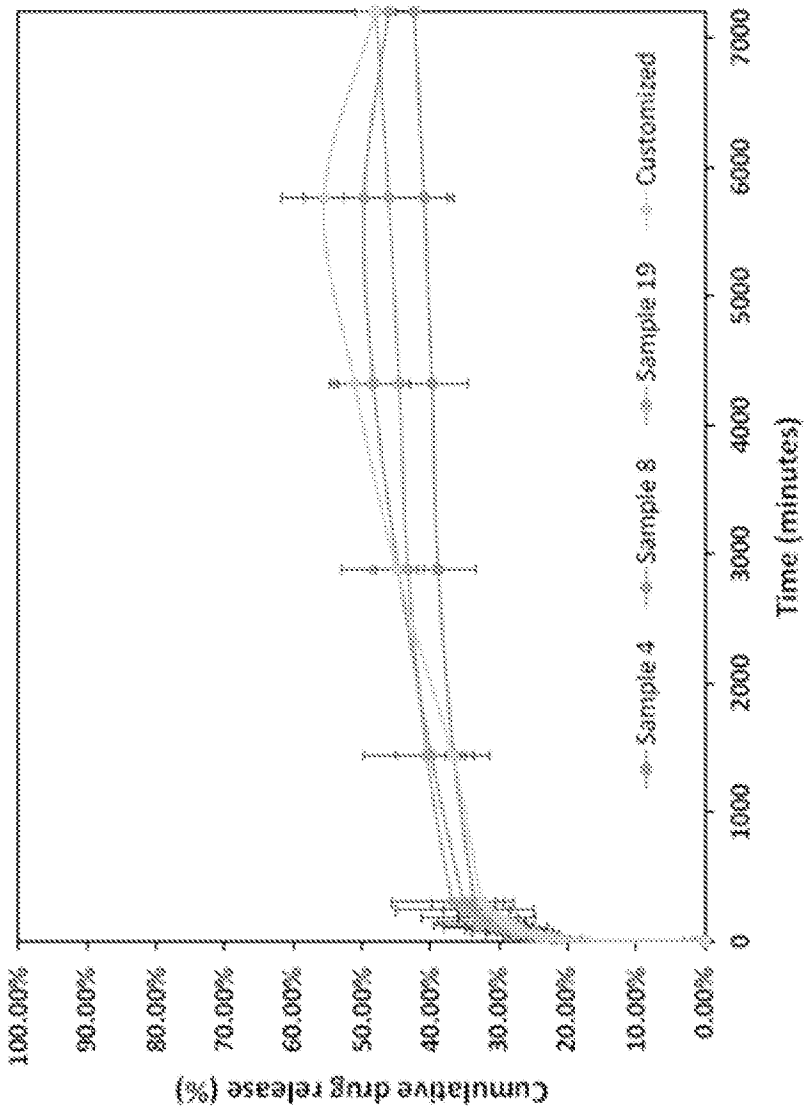
Cumulative drug release (%)
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
0
1000
2000
3000
4000
5000
6000
7000
Time (minutes)
Sample 4
Sample 8
Sample 16
Customized

FIG. 16B

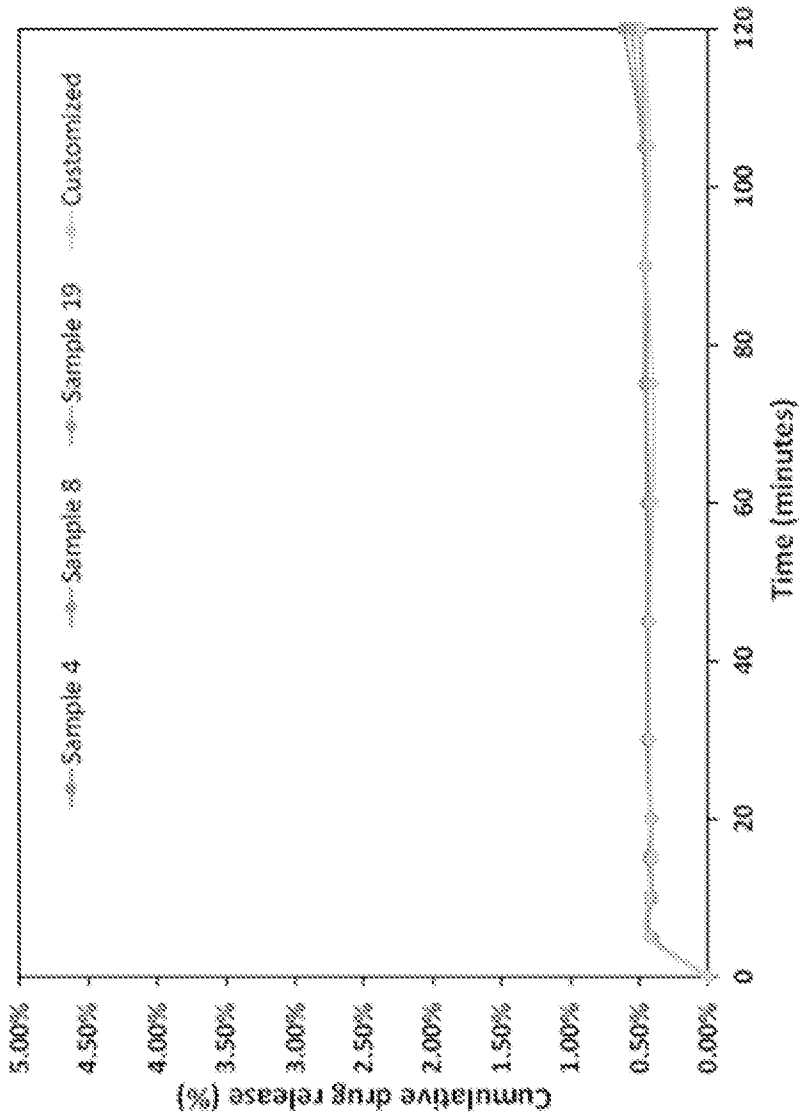
Cumulative drug release (%)
5.00%
4.50%
4.00%
3.50%
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
0
20
40
60
80
100
120
Time (minutes)
Sample 4
Sample 8
Sample 19
Customized

FIG. 16C

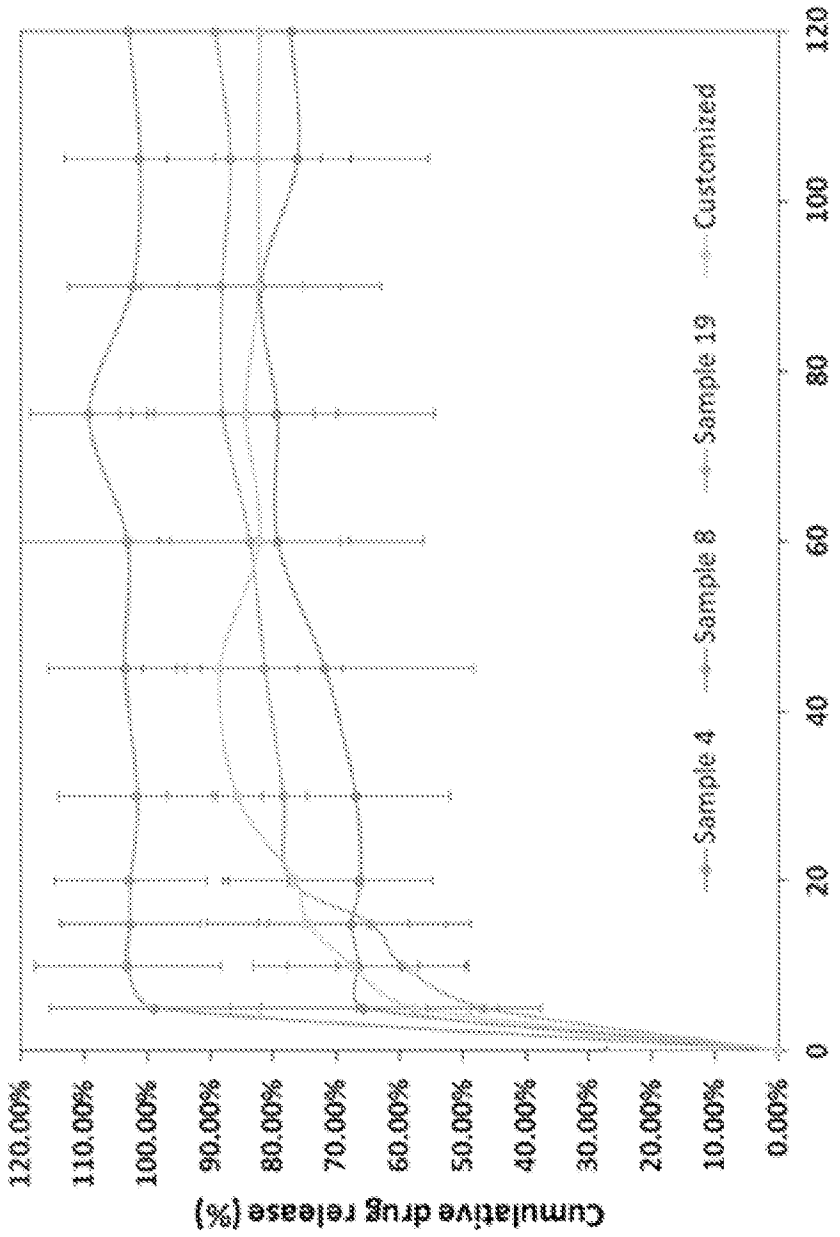
Cumulative drug release (%)
120.00%
110.00%
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
0
20
40
60
80
100
120
Time (minutes)
Sample 4
Sample 8
Sample 19
Customized

FIG. 16D

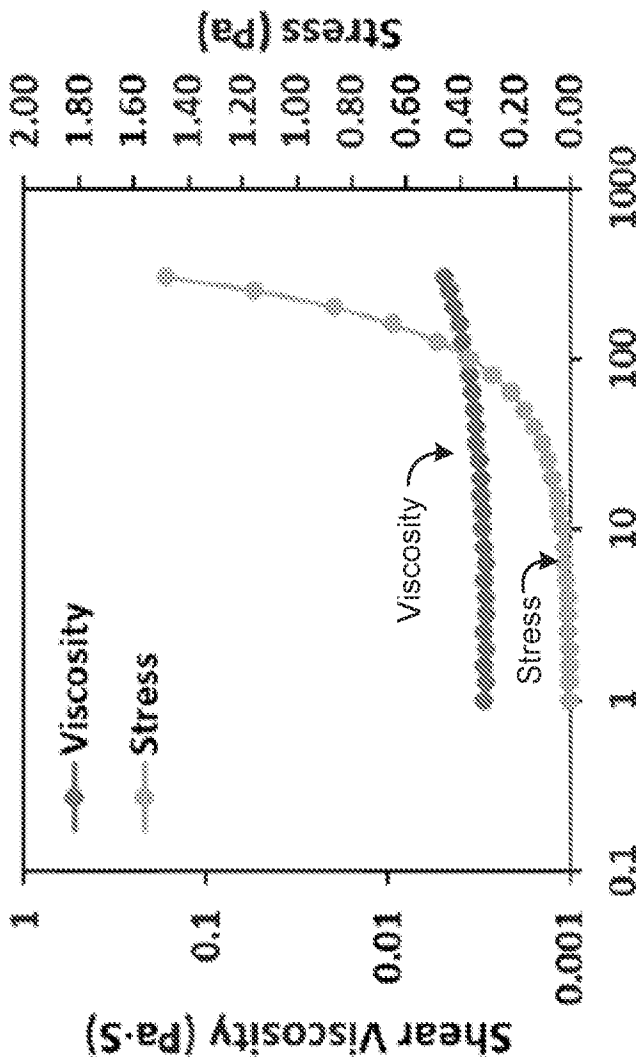
Shear Viscosity (Pa·S)
1
0.1
0.01
0.001
0.1
1
10
100
1000
Stress (Pa)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Viscosity
Stress
Viscosity
Stress

FIG. 17A

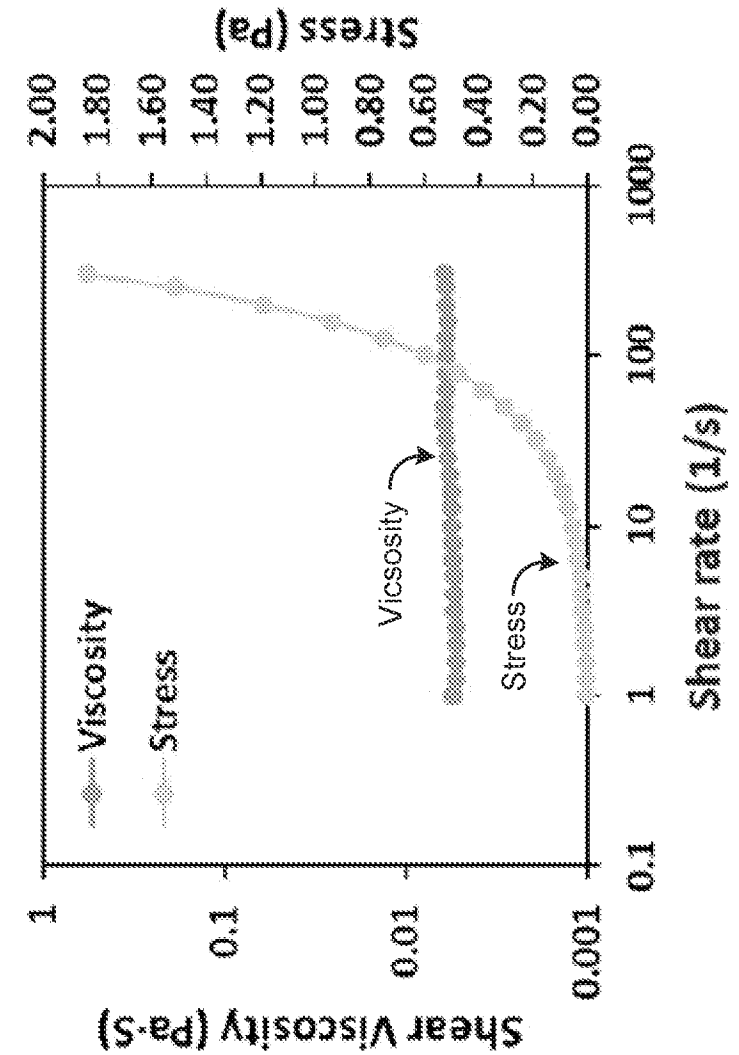
Shear Viscosity (Pa·S)
1
0.1
0.01
0.001
Stress (Pa)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.1
1
10
100
1000
Shear rate (1/s)
Viscosity
Stress
Vicsosity
Stress

FIG. 17B

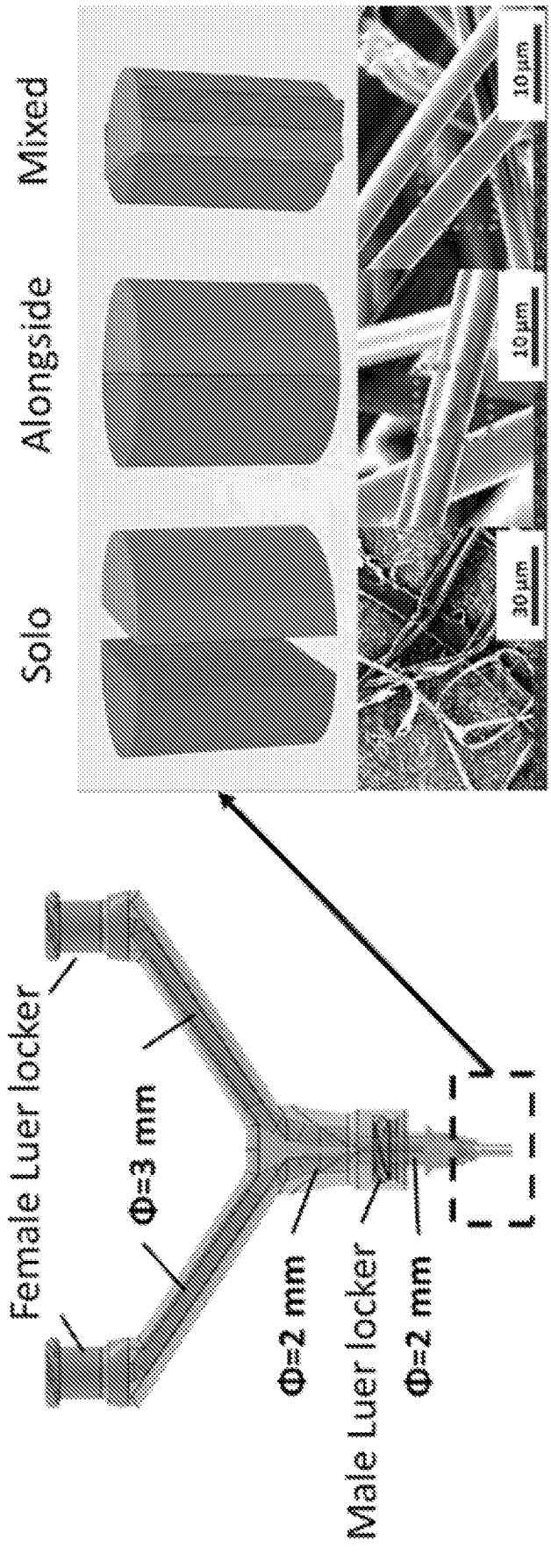
Solo
Alongside
Mixed
30 µm
10 µm
10 µm
Female Luer locker
Φ=3 mm
Φ=2 mm
Male Luer locker
Φ=2 mm
L

FIG. 18