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(57) Abstract: A method of producing nanoparticles from any synthetic or biologically derived polymer that can be solvated within a liquid that is at least partially miscible with another liquid. The method comprising the steps of introducing a first fluid comprising a polymer in a first solvent to a second fluid comprising a second solvent. The first solvent is partially miscible in the second solvent and the polymer is substantially immiscible in the second solvent. The first fluid in the second fluid is subjected to shear under laminar conditions to form droplets of the first fluid in the second until an amount of the first fluid migrates into the second fluid to reduce the size of the droplets. The size of the resultant particles can be predetermined.



WO 2008/116261 A1

Production of particles

Field of the invention

This invention relates to a method of forming particles that are substantially polymer based, the particles formed and a device for forming the particles.

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Background of the invention

Recent developments in microfabrication techniques and microreaction technologies have led to new applications in nano- and micro-scale materials. In particular, the production of biopolymer microparticles using microfluidic devices has been described by many researchers, covering a large range of biologically derived and synthetic
10 polymers. Of these, alginate microparticles represent one of the most widely targeted, as a consequence of their history as suitable carriers of bioactive substances. To date, the production of Ca-alginate microbeads is still largely accomplished by macroscale single or double emulsification techniques. Unfortunately, the resulting size of the microspheres produced via these standard methods is not easily controlled and often
15 polydispersed, limiting their application.

Following the development of a number of new approaches for preparing monodisperse droplets in microfluidic devices, the preparation of solid polymer particles has been described via various two-stage microfluidic processes. In the first stage, a monomer or a liquid polymer is emulsified in a microfluidic device to produce highly monodisperse
20 droplets either at T-junctions or by flow-focusing. Monomer droplets are then solidified by means of thermally initiated or UV-initiated batch polymerization. In addition, these processes lend themselves to cross-linking synthetic polymers, where droplets of polymer solutions are hardened by either solvent evaporation, by physical or photoinitiated cross-linking or by means of chemical reactions. The particles derived
25 from this process scheme are typically a few hundred micrometres in diameter with very little deviation in size. However, similar to the limitations experienced in biopolymer microbead manufacture using microfluidic devices, the production of polymer beads via

two-step processes does not realize the full potential of continuous microfluidics-based synthesis.

Depending on the application, reductions in particle size result in improved physical properties, such as enhanced dispersion or greater mechanical strength. The synthesis
5 of particles in the of sub 1 μm size range via such techniques is thus highly desirable for numerous applications in medical, pharmaceutical, and bioengineering fields.

Summary of the invention

The invention provides in one aspect a method of producing particles in a micrometers to nano meters in size from any synthetic or biologically derived polymer that can be
10 solvated within a liquid that is at least partially miscible with another liquid.

According to one embodiment of the invention there is provided a method of producing substantially polymer particles comprising:

introducing a first fluid comprising a polymer and a first solvent into a flow path;

15 introducing a second fluid comprising a second solvent into the flow path to form droplets of the first fluid in the second fluid, the first solvent being partially miscible in the second solvent and the polymer being substantially immiscible in the second solvent; and

flowing the first fluid along the flowpath such that a sufficient amount of the first fluid migrates into the second fluid to reduce the size of the droplets.

20 According to another embodiment of this aspect, there is provided a method of producing substantially polymer particles comprising the steps of

introducing a first fluid comprising a polymer in a first solvent to a second fluid comprising a second solvent;

subjecting the first fluid in the second fluid to shear under laminar conditions to form droplets of the first fluid, the first solvent being partially miscible in the second solvent and the polymer being substantially immiscible in the second solvent, and

- 5 continuing to subject the first fluid to shear until an amount of the first fluid migrates into the second fluid to reduce the size of the droplets.

The completion of the solvent diffusion process described above in both embodiments permits the formation of a highly condensed polymer state. However according to one embodiment of the invention, the method may further include the step of fixing the size
10 of the droplets to form polymer particles of a predetermined size and/or morphology by cross linking the polymer.

When performing the invention the first fluid forms a dispersed phase and the second fluid is a continuous phase. The size of the droplets or dispersed phase is controlled by varying the flow rate of the second fluid or continuous phase.

- 15 The mutual binary solubilities of the first solvent in the second solvent should ensure sufficient interfacial tension or viscosity difference to allow the generation of droplets of the first fluid in the second fluid. The first solvent should be partially miscible in the second solvent. The solubility may be as low as 1% of the first solvent in the second solvent and no greater than 99%. This solubility is preferably 5 to 15% of the first
20 solvent in the second solvent and more preferably 5 to 10% of the first solvent in the second.

For simplicity, it is preferable that the flowpath into which the first solvent is introduced is referred to as a conduit. In the context of the invention, a conduit is a flowpath of liquid which is with or without clearly defined physical walls or boundaries. For example the
25 flowpath may be induced by the motion of the associated apparatus.

The method of the invention may be conducted in an apparatus which imparts sufficient shear on the first fluid to cause the flow of first fluid into the second fluid to form droplets

in the second fluid. This shear is equivalent to those experienced under laminar flow regimes. In a preferred form of the invention, the flowpath or conduit is a channel of a microfluidic device. The conduit is sufficiently dimensioned particularly in length to enable the required amount of the first fluid to migrate into the second fluid. The first
5 fluid and second fluid preferably flow through the conduit in co-current flow. While the first fluid and second fluids are preferably liquids, a second fluid which is a gas is within the scope of the invention.

In a given microfluidic device, the dimensions of the conduit, ie length and width are fixed. However, the method of the invention is able to vary the size of the resulting
10 droplets by varying the flow rate of the continuous phase second fluid and hence increasing the shear and rate of migration of the first solvent from the dispersed phase in the continuous phase.

The preferred step of fixing the size of the droplets and crosslinking the polymer particles, in one form involves contacting the droplets with a third fluid. The third fluid
15 preferably contains a crosslinking agent. The droplets may be contacted with the third fluid once the droplets have reached a size to form particles of the predetermined size.

In another form of the invention, the third fluid is introduced into the conduit to mix with the first fluid prior to introduction of the second fluid into the conduit. To carry out this form of the invention, the crosslinking agent is preferably miscible in the first solvent and
20 substantially immiscible in the second solvent. The first and third fluid is homogeneously mixed before introduction of the second fluid into the conduit.

Depending on the application, reductions in particle size result in improved physical properties, such as enhanced dispersion or greater mechanical strength. The synthesis of particles in the size range of sub 1 μm via such techniques is thus highly desirable for
25 numerous applications in medical, pharmaceutical, nutraceutical and bioengineering fields.

In an embodiment of this aspect of the invention, the method may further comprise the step of applying a coating to the polymer particle produced by the method above.

Additionally the cross linking step may be omitted with the polymer droplets progressing directly to the coating step.

In this embodiment, a fluid containing a coating polymer and a solvent for the coating polymer is introduced into a conduit carrying polymer droplets or particles. The polymer
5 is at least partially miscible in the solvent and miscible with the fluid (continuous phase) carrying the polymer droplets or particles to be coated. While the continuous phase will contain some of the first fluid which has diffused into the second solvent, it will nevertheless be substantially the second solvent.

When added as additional steps to the method of forming the polymer particles, a fourth
10 fluid comprising a fourth solvent and a coating polymer is introduced into the conduit after the polymer particle has been cross linked. The coating polymer is at least partially miscible in the fourth solvent and miscible in the continuous phase carrying the polymer particles in the conduit. Preferably the fourth solvent is the same or substantially the same as the second solvent and so the coating polymer will be miscible in the fourth
15 solvent.

According to this embodiment of the invention, there is provided a method of producing layered polymer particles comprising:

Introducing a first fluid comprising a polymer and a first solvent to a second fluid comprising a second solvent;

20 subjecting the first fluid in the second fluid to shear under laminar conditions to form droplets of the first fluid, the first solvent being partially miscible in the second solvent and the polymer being substantially immiscible in the second solvent, and

25 continuing to subject the first fluid to shear until an amount of the first fluid migrates into the second fluid to reduce the size of the droplets;

introducing the formed droplets to a coating fluid comprising a carrier solvent and a coating polymer, the coating polymer being at least partially miscible in the carrier solvent and miscible in the second solvent or continuous phase, and

- 5 subjecting the formed droplets in the coating fluid to shear under laminar conditions to coat the droplets with the coating fluid, and

continuing to subject the coated droplet to shear until an amount of the first fluid migrates into the coating fluid to reduce the size of the coated droplets.

- 10 In a preferred form of this embodiment, the first fluid comprising a polymer and a first solvent is introduced into a flowpath, the introduction of the second fluid comprising a second solvent to the flowpath causes the formation of droplets of the first fluid in the second fluid; and the first fluid and second fluid flows along the flowpath for a sufficient period for the amount of the first fluid to migrate into the second fluid to reduce the size of the droplets, preferably to a predetermined size.

- 15 According to this preferred embodiment the reduced sized droplets of first polymer are in a flowpath and the coating fluid is introduced into the flowpath and

the reduced size droplets of first polymer travel along the flowpath a sufficient distance for the coating polymer to coat the first polymer and form layered polymer particles.

- 20 The first polymer is preferably cross linked once the droplets have reached a predetermined size to form polymer particles of first polymer. These polymer particles continue in the continuous phase to the coating step.

- 25 In a preferred form of this aspect, the carrier solvent for the coating polymer is miscible in the second solvent and preferably is the same solvent or substantially the same solvent as the second solvent or continuous phase. The coating polymer is preferably immiscible in the first solvent.

After the first polymer have been coated with the coating polymer, further carrier solvent may be added to the conduit to prevent deposition of the coated polymer particles on the conduit wall.

5 If a multilayered polymer particle is to be produced, the coating step may be repeated a number of times as required. A micro fluidic device can be constructed to facilitate the production of coated polymer particles as a continuous process.

When multilayered polymer particles are produced, the continuous phase containing the first polymer particles or droplets or coated polymer particles may be changed to allow a coating polymer/carrier solvent combination which will allow the coating polymer to be
10 layered onto an already coated polymer particles in accordance with the invention.

According to a second aspect of the invention, there is provided a polymer particle having a size of 10-800 nm and preferably 10-100 nm. The polymer particle is preferably crosslinked and non-agglomerated.

15 In an embodiment of this aspect of the invention, there is provided a coated polymer particle comprising a core of a first polymer having a particle size in the range of 10-800nm, preferably 10-100nm, and at least one layer of a second polymer layered over the core.

According to a third aspect of the invention, there is provided a device for producing a polymer particle comprising a microfluidic device having a first inlet for a first fluid and a
20 second inlet for a second fluid and a fluidic conduit for contacting the first and second fluids, the fluidic conduit being a suitable length and width such that the resulting particles formed when a substantial amount of the first fluid is transferred from the droplet to the second fluid, are less than 800 nm and preferably in the range of 10-100nm at the exit of the device.

25 It is preferable that the fluidic device is formed from a material which can be wet by the contact of the second fluid and the conduits are less than 1000 microns.

The microfluidic device may be provided with a third inlet for the addition of a third fluid. The third inlet preferably enables the first fluid to mix with a third fluid from the third inlet prior to contacting the second fluid entering from the second inlet.

Brief description of the drawings

5 Figure 1 is a schematic view of a microfluidic device used to carry out the method of the invention,

Figure 2 is a schematic view of the mixing, section of microfluidic device of figure 1,

Figures 3(a) and 3(b) are schematic views of the first and second embodiments of the invention,

10 Figure 4 is a graphical representation of the shear viscosity against shear rate over a range of polymer concentrations,

Figure 5 is a flow diagram focusing on droplet formation in the mixing junction where droplets are formed,

15 Figure 6(a) is a schematic diagram illustrating droplet shrinkage as it progresses through the flowpaths of the microfluidic device,

Figure 6(b) and 6(c) are expanded views from figure 6(a) where indicated,

Figure 7(a) are TEM images of Na-alginate nanoparticles produced in accordance with the invention,

20 Figure 7(b) is a particle size distribution graph of alginate in DMC produced on-chip, using a very low concentrated Na-alginate solution (0.002%).

Figure 8(a) and (b) illustrates upstream mixing of cross-linking agent and polymer solution in accordance with a second embodiment of the fixing stage of the method of the invention,

Figure 9 are TEM images of alginate particles cross linked on a chip,

Figure 10(a) illustrates DMC droplet shrinkage in alginate solution,

Figure 10(b) is a graph of relative volume variation with time of the DMC droplet in the alginate solution,

5 Figure 11 is a schematic diagram of the solvent migration in droplet shrinkage,

Figure 12 is a schematic diagram of a microfluidic device for producing layered nanoparticles,

Figures 13(a) and 13(b) are expanded views of sections A and B in figure 12,

Figure 14 is a schematic diagram of two microfluidic devices for producing multi layered
10 nanoparticles , (a) using the same continuous phase solvent throughout and (b) having provision for changing the continuous phase solvent,

Figure 15 (a) is TEM images of PLGA nanoparticles produced in accordance with the invention,

Figure 15(b) is a particle size distribution graph of PLGA produced by bulk
15 emulsification method,

Figure 16 (a) and 16(b).

Detailed description of the embodiments

The invention allows for the production of nanoparticles from any synthetic or biologically derived polymer that can be solvated within fluid that is at least partially
20 miscible with another fluid. The invention also allows the production of layered nanoparticles in which layers of synthetic or biologically derived polymer are built up over the initially formed nanoparticle or polymer droplet.

The selection of a suitable solvent may be facilitated by considering solvent-polymer interaction parameters for the polymers or polymers of interest. Thus, a solvent which affords a sufficiently low and similar interaction parameter for the selected polymer is likely to function as a solvent for that polymer. It will be appreciated that such solvent-polymer interaction parameters vary with temperature and it may be possible to improve the "solvating power" of a given solvent simply by raising its temperature. A suitable common solvent for a given polymer may also be identified through simple trial and error.

Those skilled in the art will appreciate that solvent-polymer interaction parameters may also be used as a guide in determining whether a material is likely to function as a solvent or a non-solvent for a given polymer. Thus, high interaction parameters are indicative of non-solvent properties, whereas low interaction parameters are indicative of good solvent properties.

As for the chemical nature of suitable common solvents, particular classes of various organic compounds have been found useful, including aliphatic and aromatic acids, aliphatic, aromatic and cyclic alcohols, aldehydes, primary and secondary amines, aromatic and ethoxylated amines, diamines, amides, esters and diesters, ethers, ketones and various hydrocarbons and heterocycles. Despite the diverse array of potentially suitable solvents, those skilled in the art will appreciate that the suitability of a particular solvent can be quite selective. Thus, for example, not all aromatic acids will be useful as a solvent for a given polymer and, further, not all solvents useful to dissolve a polymer such as polyethylene will necessarily be useful to dissolve a polymer such as polyvinylchloride.

Where the invention is employed to prepare nanoparticles or layered nanoparticles for use in tissue engineering applications a number of further criteria may be considered in selecting a suitable solvent or solvent/non-solvent combination. Generally, such solvents/non-solvents should have low toxicity and be capable of being thoroughly removed from the resulting polymer nanoparticles.

Examples of suitable common solvents, or non-solvents as the case may be, that may be used to prepare the porous polymer blend structures include, but are not limited to, dimethyl oxalate (DMO), ethylene carbonate (EC), N-methyl acetamide (NMA), dimethyl sulfoxide (DMSO), acetic acid (AA), 1,4-dioxane (DO), dimethyl carbonate (DMC),
5 chloroform, dichloromethane (DCM), naphthalene, sulfalene, trimethylurea, ethylene glycol or other glycols and polyglycols, N-methyl pyrrolidone (NMP), ethylene carbonate, hexane, trifluoroethanol (TFE), ethanol, acetic acid, and water, and combinations thereof.

Given the diverse array of polymers that may be used in accordance with the invention,
10 it will be appreciated that it would be impractical to provide a comprehensive list of polymers and solvent/non-solvent combinations. Nevertheless, having regard to the general guidelines set forth above for determining whether a combination of solvent/non-solvent and polymers are miscible, suitable polymers in general can be broadly classified as thermoplastic polymers. Suitable polymers will also exhibit at least
15 a limited degree of cross-linking provided that they can still be dissolved in the solvent.

Suitable polymers include, but are not limited to, low density polyethylene, high density polyethylene, polypropylene, polystyrene, polyacrylic acid and copolymers of polyacrylic acid and polystyrene, polyurethane, polyvinylchloride, polyvinylfluoride, acrylonitrile-butadiene-styrene terpolymers, styrene-acrylonitrile copolymers, styrene butadiene
20 copolymers, poly(4-methyl-pentene-1), polybutylene, polyvinylidene chloride, polyvinyl butyral, polyvinyl imidazole, chlorinated polyethylene, polyethylene oxide, ethylene-vinyl acetate copolymers, polyvinyl acetate, polyvinyl alcohol, polymethyl-methacrylate, polymethyl-acrylate, ethylene-acrylic acid copolymers, ethylene-acrylic acid metal salt copolymers, chlorosulphonate polyolefins, polyesters such as polyethylene
25 teraphthalate and polybutylene teraphthalate, polyamides such as Nylon 6, Nylon 11, Nylon 13, Nylon 66, polycarbonates and polysulfones, and polyarylene and polyalkylene oxides; agarose, cellulose, gelatin, alginate, elastin, Chitosan, hyaluronic acid, collagen, gellan, albumin, poly(lactide)s, poly(glycolide)s, poly(lactide-co-glycolide)s, poly(lactic acid)s, poly(glycolic acid)s, poly(lactic acid-co-glycolic acid)s, polycaprolactone.

polycarbonates, polyesteramides, polyanhydrides, poly(amino acids), polyorthoesters, poly(hydroxyalkanoates), polyacetyls, polycyanoacrylates, polyetheresters, poly(esters), poly(dioxanone)s, poly(alkylene alkylate)s, copolymers of polyethylene glycol and polyorthoester, poly(hydroxy acids), poly(lactones), poly(amides), poly(ester-amides),
5 poly(amino acids), poly(anhydrides), poly(ortho-esters), poly(carbonates), poly(phosphazines), poly(thioesters), polysaccharides, proteins, glycoproteins, proteoglycans, growth factors, dna, and mixtures, blends and copolymers thereof.

The method of the invention is particularly suitable for making polymer nanoparticles or layered nanoparticles that may be used in pharmaceutical, nutraceutical and tissue
10 engineering applications. Polymers used in these applications will generally be biocompatible and are preferably biodegradable. In addition to acting as adhesive substrates for cells, such polymers should also promote cell growth and allow retention of differentiated cell function, possess physical characteristics allowing for large surface to volume ratios, have sound mechanical properties and have an ability to be formed
15 into complex shapes, such as for bone or cartilage substitutes.

In pharmaceutical and nutraceutical applications, the polymers used can be conjugated with pharmaceutical and nutraceutical compounds and coated to assist delivery of the compounds to treatment or absorption sites in a patient.

A further parameter to consider when selecting a polymer for use in pharmaceutical,
20 nutraceutical or tissue engineering applications is the biodegradation kinetics of the polymer. In particular, it may be desirable that the biodegradation kinetics of the polymer match the healing rate or residence time to the absorption site associated with the specific *in vivo* application.

Examples of suitable polymers that may be used to make polymer nanoparticles or
25 layered nanoparticles for use in these applications include, but are not limited to, aliphatic or aliphatic-co-aromatic polyesters including poly(α -hydroxyesters) and copolymers thereof such as polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), PLGA-co-poly-L-Lysine (PLGA-co-PLL), and all stereo-

isomeric forms thereof; polydioxanone; polyalkanoates such as poly(hydroxy butyrate) (PHB), poly(hydroxy valerate) (PHV) and copolymers thereof (PHBV); and polyethylene oxide/polyethylene terephthalate as disclosed by Reed et al., in *Trans. Am. Soc. Artif. Intern. Organs*, page 109 (1977). Other suitable polymers include biodegradable and
5 biocompatible polycaprolactones, and copolymers of polyesters, polycarbonates, polyanhydrides, poly(ortho esters), and copolymers of polyethylene oxide/polyethylene terphthalate.

Bisphenol-A based polyphosphoesters have also been suggested for use in biodegradable porous polymer design. Such polymers include poly(bisphenol-A
10 phenylphosphate), poly(bisphenol-A ethylphosphate), poly(bisphenol-A ethylphosphonate), poly(bisphenol-A phenylphosphonate), poly[bis(2-ethoxy)hydrophosphonic terephthalate], and copolymers of bisphenol-A based poly(phosphoesters). Although these polymers have been suggested in US Pat. No. 5,686,091, the known cytotoxicity of bisphenol-A makes them less preferred candidates
15 for implantation.

Other polymers suitable for use in pharmaceutical, nutraceutical or tissue engineering applications include polymers of tyrosine-derived diphenol compounds. Methods for preparing the tyrosine-derived diphenol monomers are disclosed in US Pat. Nos. 5,587,507 and 5,670,602. The preferred diphenol monomers are des-aminotyrosyl-
20 tyrosine (DT) esters. These monomers have a free carboxylic acid group that can be used to attach a pendent chain. Usually, various alkyl ester pendent chains are employed, for example, ethyl ester, butyl ester, hexyl ester, octyl ester and benzyl ester pendant chains.

The tyrosine-derived diphenol compounds are used as monomeric starting materials for
25 polycarbonates, polyiminocarbonates, polyarylates, polyurethanes, polyethers, and the like. Polycarbonates, polyiminocarbonates and methods of their preparation are disclosed in US Pat. Nos. 5,099,060 and 5,198,507. Polyarylates and methods of their preparation are disclosed in US Pat. No. 5,216,115. Block copolymers of polycarbonates and polyarylates with poly(alkylene oxides) and methods of their
30 preparation are disclosed in US Pat. No. 5,658,995. Strictly alternating poly(alkylene

oxide ether) copolymers and methods of their preparation are disclosed in WO99/24490.

Other polymers suitable for use in pharmaceutical, nutraceutical or tissue engineering applications include the polycarbonates, polyimino-carbonates, polyarylates, polyurethanes, strictly alternating poly(alkylene oxide ethers) and poly(alkylene oxide) block copolymers polymerised from dihydroxy monomers prepared from α -, β - and γ -hydroxy acids and derivatives of tyrosine. The preparation of the dihydroxy monomers and methods of their polymerisation are disclosed in International Patent Application No. PCT/US98/036013.

- 10 Polycarbonates, polyimino carbonates, polyarylates, poly(alkylene oxide) block copolymers and polyethers of the diphenol and dihydroxy tyrosine monomers that contain iodine atoms or that contain free carboxylic acid pendent chains may also be employed. Iodine-containing polymers are radio-opaque. These polymers and methods of preparation are disclosed in WO99/24391. Polymers containing free carboxylic acid pendent chains and methods of preparation are disclosed in US patent application Ser. No. 09/56,050, filed April 7, 1998.

Examples

The invention will now be described with reference to the examples. It will be appreciated that the description of the preferred embodiment is by way of example only and is not included to limit the scope of the invention.

Materials

Alginic acid sodium salt and calcium chloride were obtained from Sigma Chemicals (USA). Anhydrous dimethyl carbonate was obtained from Aldrich Chemicals (USA).

Sodium alginate solutions with concentrations ranging from 0.1 % to 0.5 % (w/w) were prepared in deionised water (Millipore $r = 18.2 \text{ M}\Omega\cdot\text{cm}$ at 26.2°C). The cross-linker solution was prepared by dissolving calcium chloride (2N) powder in Millipore water.

Properties of polymer solutions

A video-based optical angle measuring instrument (DataPhysics OCA 15+) was used to determine the wetting properties and the values of surface and interfacial tension for all solutions. A controlled stress magnetic bearing rheometer (ARG2, TA Instruments) controlled was used for the rheological characterization of all polymer solutions with a
5 cone-plate geometry (60 mm, 2°).

Design and Fabrication of the Microfluidic Reactor

The channel network used in the present work consists of two parts: a sheath-flow junction, where droplets are formed and flow through a square spiral or serpentine channel, in which the droplets shrink and harden. A schematic of two different
10 microchannel patterns with two different total lengths for the second zone (70 cm (1a) and or 45 cm (1b)) is presented in Figure 1. Typical dimensions of the channel at the droplet formation zone are shown in Figure 2. The microchannels were either 100 μm or 200 μm deep. Masters were prepared with SU-8 photoresist (MicroChem USA) in bas-relief on silicon wafers. Microchannels were fabricated using a Dow Corning Sylgard
15 Brand 184 Silicone Elastomer with a standard soft-lithography method which allows rapid replication of the integrated microchannel prototypes.

Experimental setup

Gastight microsyringes (S.G.E) were filled with the liquids and mounted on motor-driven syringe pumps (PHD 2000 Harvard, Instech), which can synchronously operate two syringes at various flow rates. The PDMS device was linked to the dispenser syringes through polyethylene tubing (I.D. 0.58 mm/O.D. 0.965 mm). Particles in the continuous phase were collected from the device in a vial containing cross-linker solution to complete the reaction, to prevent the particles from re-dissolving, and to avoid nanoparticles coalescence. Images of droplet formation and shrinkage were captured and processed using a high-speed camera (Phantom V, Vislon Research which can capture of up to 64,000 fps at 256×256 pixels resolution) attached to a Nikon inverted microscope (TE2000, Nikon) coupled with a phase contrast condenser. Frame rates used in the experiments reported here were varied from 1000 fps to 2000 fps and exposure time was approximately 130 μ s.

Production of nanoparticles

Two alternative processes have been utilized, to produce nanoparticles as shown in Figure 3. The first method Figure 3(b) is a two-step process: condensed nanoparticles are generated in the microfluidic device and are subsequently collected in a hardening solution where they cross-link. The second approach Figure 3(a) consists of performing the whole synthesis of cross-linked nanoparticles in a one step process on a single device. We will describe these two processes one after the other in order to analyse and discuss distinctly the different phenomena occurring in each processing method.

The apparatus for the processes is shown in Figures 6a, 6b, and 6c which visualize droplet shrinking through the flow, for example a 0.5 % (w/w) alginate solution. The channel design figure 6a shows polymer A being added co-currently with either a first solvent or a cross linking agent B with an organic solvent stream C being subsequently added to the flow path. As can be seen in figure 6c the channel design consists of a counter-current spiral, such that anti-clockwise particle trajectories (positions 1, 2, 3) represent flow towards the centre of the device and clock-wise particle trajectories (positions 4 and 5) represent flow towards the outside of the device and towards the exit.

Example 1: two-step process**Droplet formation at the sheath-flow junction**

As shown in figure 3(a), droplets were initially generated by shearing a stream of the dispersed phase with the flow of the continuous phase 2 via a flow-focusing geometry.

- 5 The size of the droplets is mainly governed by the properties of the polymer solution 1 and the flow rates of the continuous 2 and droplet phases (Q_c and Q_d respectively). The droplets generated at position 3 continue to shrink as the solution passes along path 4 as the first solvent diffuses from the polymer solution. The polymer droplets are then proceed to a particle collection step 5 where the droplets are collected in a solution 6
- 10 containing a cross linking agent such as calcium chloride

- The relationship between drop size and variations in the balance of viscous and interfacial forces is generally characterized by expressing the variation in droplet size as a function of a dimensionless Reynolds number (Re) and a Capillary number (Ca). The Capillary number is calculated using the average linear fluid velocity at the end of the
- 15 constriction:

$$C_a = \frac{\eta_d v_d}{\gamma_d} \quad (1)$$

- where η_d is the polymer solution viscosity (mPa), v_d is the average velocity of the dispersed phase, and γ_d is the interfacial tension between the alginate solution and dimethyl carbonate (DMC) (mN/m). In this calculation, values of η_d and γ_d were
- 20 determined to be $\eta_d = 2.74$ mPa.s (over shear rates of 10-1000 s^{-1}) and $\gamma_d = 2.23$ mN/m for the 0.1 wt. % alginate solution. The Reynold's number is calculated using:

$$Re = \frac{\rho v_c D_c}{\eta_c} \quad (2)$$

where ρ is the density of the continuous phase ($\rho_{\text{DMC}} = 1.0633 \text{ g/cm}^3$), v_c is the average velocity of the continuous phase, D_c is the equivalent diameter ($D_c = \frac{4 \times \text{cross section}}{\text{perimeter}}$) and η_0 the viscosity of the continuous phase.

As can be seen from equation (1), Ca describes the relative importance of viscosity and surface tension, which often dominate other forces. In flows where one fluid replaces another, the coherence or break-up of the interface will depend strongly on Ca , hence on the velocity of the moving interface.

As it is shown in Figure 4, the steady shear viscosities of all alginate solutions were found to be constant over a range of shear rates from 10 to 1000 s^{-1} . Therefore, we assumed that the system of alginate + water and DMC was a Newtonian/Newtonian system. The increase in viscosity observed at high shear rates for the 0.1 and 0.2 wt. % solutions is a result of the onset of Taylor vortices at high Reynolds numbers and does not represent the true viscometric properties of the fluids. Figure 5 shows the flow diagram focusing on droplet break-up at the Y-shaped junction obtained for the 0.1 wt. % alginate solution. The data points were obtained by varying Q_d and Q_c . The boundaries between the droplet formation area and the other regions with varying Reynolds number and Capillary number are shown in figure 5. Drop break-up at a constant frequency is observed within a limited area in the flow diagram (regions (A), (B) and (C)). Within this limited area, the droplet size and break-up frequency are controllable. As Ca increases (1), the drop size increases and sphere-like droplets become plug-like drops. The jet tends to stretch before it breaks at even higher values of Q_d (region (C)). As Re increases (2), the periodicity of the drop production varies whereas the drop size remains appreciably constant. This area shows a peak where Q_d is maximized. This area is surrounded by a domain where the two immiscible liquids flow together as a continuous stream (region (S)) and a domain where the break-up occurs in a random manner (region (I)). Similar flow diagrams were determined for solutions of higher alginate concentrations. When the dispersed phase is more viscous, the break-up is stable over a larger range of Ca - Re space: region (I) is shifted toward a lower value of Q_d (Ca). For the 0.3 wt. % to 0.5 wt. % alginate solutions, only plug-

shaped droplets are obtained, regardless of the values of Q_c and Q_d , (A) and (B) becoming one domain.

These results show that droplets of alginate solutions of varying concentration can be generated in microfluidic flow-focusing devices despite the low interfacial tension
5 between the DMC and the alginate solutions and the relatively high viscosities of the alginate solutions (i.e. high viscosity ratios between dispersed and continuous phases). For a given solution with a certain viscosity, the droplet size and the break-up frequency are dependent on the flow rates of the dispersed and the continuous phases.

Shrinkage of the droplets

10 After the droplets are formed, they are carried by the continuous phase along the microchannel 4. To ensure effective transport of the polymer (and potentially the reagents) contained in the droplets, the carrier fluid (continuous phase) must wet the walls of the microchannel preferentially over the aqueous polymer phase, so that the droplets remain separated from the walls by a thin layer of the continuous phase at all
15 times. This can be easily verified by measuring and comparing the contact angle values for DMC and for the different polymer solutions on a PDMS surface. The contact angle is $\sim 47^\circ$ for DMC, but ranges from 114° to 116° for alginate solutions (0.1 %, 0.3 and 0.5 % in water (w/w)). In line with these differences in contact angle, we observed that the isolated polymeric droplets were stable and did not leave any residue behind as they
20 are transported through the channels.

However, the polymeric droplets shrink when flowing downstream. The droplets formed at the sheath focusing point figure 6(b) are about $200\text{ }\mu\text{m}$ in diameter. They appeared to be approximately $10\text{ }\mu\text{m}$ before exiting the device. Once reaching this length scale, the size and shape of the particles were difficult to visualize with high accuracy with the high
25 speed camera.

Subsequent cross-linking of the alginate particles in a CaCl_2 solution

The particles thus produced in a DMC solvent phase from the device were thereafter deposited off-chip into a highly concentrated CaCl_2 solution (2N in water). The gelation has to be sufficient to prevent the particles coalescing in the DMC solution or dissolving back into the aqueous phase. The gelation behaviour of sodium alginate solution has been thoroughly investigated by others and it has been shown that after gelation for 60 minutes, full substitution is attained and the Ca-alginate particles are thereafter water-insoluble.

Figure 7 shows images of isolated Ca-alginate nanoparticles produced via the process of the invention, whose sizes is about 200nm.

10 Example 2: single-step process

In this method, the hardening of the hydrogel nanoparticles occurs solely within the microfluidic device. As shown in Figure 3a, the same design for the device is used. As shown in Figures 8(a) and 8(b), the design allows reactive mixtures to be processed in a mixing region 8 upstream of the microdroplet creation site 9. The cross-linking reaction occurs simultaneously with the droplet formation by the addition of a cross linking agent 6 with the polymer solution 1 into the flow path and further downstream at position 7 (in figure 3a, competes with the solvent exchange process between the drop phase and the continuous phase. The competing kinetics of the chemical reaction and of the water diffusing out of the droplets will determine the size and composition of the particles produced on the chip. The chemical reaction of the sodium alginate and the CaCl_2 polycations is immediate and as the gelation starts, the viscosity increases which can prevent droplet generation at the sheath-flow point. However, the length of the channel in the mixing zone can be adjusted to control the duration of the reaction prior to droplet formation, as can the relative stoichiometry of the alginate; CaCl_2 mixture.

25 Mixing Zone

Adequate mixing is a significant challenge when dealing with pressure-driven laminar microflows, as mixing occurs only through diffusion when two streams are injected into a channel at low Reynolds number (laminar). Rapid mixing of chemicals and reagents in microchannels can be difficult to achieve. The sodium alginate solution and the CaCl_2

solution are individually inserted at the same flow rate in the device and they mix within the channel to form the dispersed phase. Table 1 and figure 8(b) show the different residence times in the mixing zone, depending on the flow rate of the dispersed phase (Q_d) and also on the length of the channel in the mixing zone. This length can be adjusted. The flow rate for the dispersed phase is determined by the conditions required to form the droplets upstream. In Table 1, $m_2(1\text{mm})$ in figure 8(b) is small compared to m_1 .

Table 1

	L = 10 mm	L = 15 mm	L = 20 mm
$Q_d = 5 \mu\text{l}/\text{Hr}$	4 min 50s	7min 10s	9min 40s
$Q_d = 10 \mu\text{l}/\text{Hr}$	2 min20s	3min 40s	4min 50s
$Q_d = 20 \mu\text{l}/\text{Hr}$	1 min10s	1min 50s	2min 20s
$Q_d = 50 \mu\text{l}/\text{Hr}$	30s	40s	1min

10 Droplet Formation at the sheath-flow point

The disperse phase in this simple-step method results from a mixture of the alginate solution and the cross-linker solution. The two solutions are assumed to be homogeneously mixed together at this point and the cross-linking reaction certainly may even have occurred to a certain extent prior to droplet formation, which leads to an increase in the viscosity of the polymer dispersed phase. However, the mixing of the alginate solution with the cross-linking solution also results in a decrease in the overall polymer concentration, and therefore the viscosity. The same alginate solutions were utilized in this method as used in method 1. The viscosity of the dispersed phase was obviously low enough for the production of droplets even in the presence of cross-linkers for the time prior to formation. Similar Ca-Re diagrams as shown for method 1

can be derived for this method (not shown). The domain in which droplets are produced at a constant frequency and of uniform size covers a large range of flow rates, offering significant processing flexibility and manipulation of the total residence time for the reactive mixture within the device.

5 Shrinking/Hardening of the droplets

After the droplets are formed, they flow downstream in the microchannel, as isolated micro-reactors carried by the continuous phase. Inside each individual droplet, the cross-linking reaction consumes available calcium, which eventually leads to the hardening of the polymer droplet. However, another process is in competition with this
10 reaction: the solvent diffusion at the interface of the two phases. The water diffuses out of the polymeric droplet to the DMC, modifying the reaction conditions. Not only does the total polymer concentration now vary but so does the concentration of the calcium ions in the droplet and their diffusion into the gel network during the hardening of the particles. Further, the modification of the structure of the beads affects the diffusion of
15 solvent through the interface as the interface is becoming more solid. This prevents the particles shrinking as much as they do in the two stage process.

Collection of the cross-linked nanoparticles

The cross-linked particles are collected in a CaCl_2 solution to ensure a maximum cross-linking and to prevent coalescence of the Ca-alginate particles. Figure 9 shows TEM
20 Images of alginate particles cross-linked on chip. The particle sizes range is approximately 800 nm. The particles obtained via method 1 of figure 3a are larger than the ones obtained via method 2 from figure 3b, due to the solidification of the droplets influencing the diffusion of solvent and therefore the shrinking process.

Example 3

25 The reduction in size of the polymer phase when dispersed in DMC was investigated off chip in order to study the driving force for such a volume loss and the kinetics of this process. As DMC has a higher density (1.070 g/cm^3) than all of the polymer solutions

(0.993 to 0.997 g/cm³ depending on the material and the concentration), interfacial tension measurements were carried out by immersing a pendant droplet of DMC in each polymer solution. The variation of the droplet volume was measured simultaneously with the interfacial tension through a period of 10 minutes, which is
5 pertinent to the resident times of the fluids in the microdevice. Figure 10-a) shows the shrinkage of a DMC droplet immersed in an alginate solution. The volume variation with time for different alginate concentrations (0.1 wt. %, 0.3 wt. % and 0.5 wt. %) is also shown in Figure 10-b). The volume loss is smaller and slower with increasing alginate concentration. However, the interfacial tension for all alginate concentrations was very
10 close to the value for water and DMC ($\gamma_{\text{water/DMC}} = 5.31 \pm 0.05$ mN/m) and did not depend on the alginate concentration. ($\gamma_{\text{alginate/DMC}} = 4.95 \pm 0.05$ mN/m for all solutions).

In the experiments of this invention, (see Figure 11), the microdroplet 10 of alginate solution in water, once in contact with the continuous phase (DMC) 11 post creation of an interface, began to shrink. The solubility of pure water in pure DMC is 3 wt. % at
15 room temperature. Within the context of the invention, although the exchange of solvent 12 (water) from the polymer solution within the miscible interface or envelope with DMC is due to the laminar flow 13 around the droplet (diffusion controlled mixing), it is at very slow, near equilibrium, rates. This can be thought of as a pseudo equilibrium process. Due to the slow extraction of water from the droplet 10, the polymer does not effectively
20 see the nonsolvent (DMC) 11 until there is very little solvent remaining. It is at this point that the polymer may see the nonsolvent and come out of solution, which is likely to be a precipitation event. Continual exposure to the nonsolvent will eventually result in the formation of a condensed insoluble nanoparticle made from the polymer that was originally dissolved within water at low to very low concentrations. In the case of bulk
25 processes relating to condensation from supersaturated solutions, particle size is essentially a diffusion controlled process. In the case of droplets flowing in microchannels, interfacial boundaries serve to define nanoreactors that limit the size of the particles formed therein and afford a level of control which is not achieved in any macroscale batch-style reactor. Several parameters influence the resultant kinetics of
30 the reaction occurring within these polymeric drops and the ever present competition

with a thermodynamic diffusion process makes the prediction of the particle size and structure even more challenging.

Example 4

Production of PLGA nanoparticles using an emulsification technique

- 5 To further demonstrate the method of the invention off chip, the invention was performed using an emulsification technique. In this experiment, PLGA solutions were prepared in DMC, with concentrations ranging from 0.001 to 0.005 % (w/w). To create an emulsion, the polymer solution was added into water, under stirring at 1500 rpm with a motorized rotor. These stirring rates were in the laminar flow regime. The PLGA
- 10 solution was dispensed using a 30g needle, in order to create a fine thread, which was then broken up under shear to create droplets/particles. The resulting mixture is stirred for another 30 minutes at the same speed before collecting and spinning down the suspended particles. The Dynamic Light scattering data figure 15b of the TEM images shown in figure 15a show PLGA particles having a particle size varying between
- 15 0.20 μ m to 0.29 μ m. Figure 16 a and 16b is a graph of the volume loss of a pendant droplet of PLGA 0.001% (in DMC) when immersed in water as a function of time.

Layered nanoparticles

- An apparatus for the production of layered polymer particles is shown in Figure 12. In this apparatus, a microfluidic device as shown in figure 1(b) is used to shrink the
- 20 polymer droplets. In this example, the polymer droplets are not cross linked and pass directly to the layering stage shown in figure 13(b), where polymer droplets are coated and solidified to produce a layered nanoparticle.

- Alternatively, the particles are formed in accordance with Example 2 according to figure 13(a) are then passed in a continuous phase to a second device or second stage of the
- 25 same device. In this regard, figure 13(a) is the same as figure 8(a). As shown in Figure 13(b), a second polymer solution 21 of polymer in a solvent is added to the flowpath of this second stage or second microfluidic device. The solvent is preferably the same

solvent as the continuous phase. The second polymer dissolved in this solvent diffuses to the surface of the first polymer particle 20 to coat the particle. Additional solvent 22 which is the same solvent as the continuous phase or miscible in the continuous phase is preferably introduced into the conduit downstream of the coating section to prevent
5 deposition of polymer onto the conduit walls. The layered polymer droplets then pass through the serpentine path where the droplets are shrunk or solidified and collected at the exit of the second stage or second device.

In order to apply multiple coatings to the original polymer particle core, further stages or devices can be added and the coating step repeated.

10 A microfluidic apparatus for the application of multiple coating layers is shown in Figure 14 in which multiple microfluidic devices are shown adjacent each other. In the right side devices are shown adjacent each other. In the right side device, addition inlets are provided in all coating stages to allow the introduction of a second continuous phase. This second continuous phase is added after the deposition of the latest external
15 polymer layer and enables the continuous phase carrying the layered polymer particles to be changed between consecutive layer depositions. As the layering polymer needs to be miscible in the continuous phase, the ability to change the continuous phase increase the range of polymer types which can be used as a coating layer.

The applicant has successfully used the present invention to make nanoparticles from
20 alginate, agarose, gelatine and synthetic polymers block copolymers. The process of the invention will allow for the production of nanoparticles from any synthetic or biologically derived polymer that can be solvated within a liquid that is partially miscible with another liquid. The size of the nanoparticles can be explicitly controlled by the relative solvency of the two liquids, the properties of the polymer solution, including
25 polymer concentration, configuration and ionic state, and solvent conditions, processing conditions, including solution flow rates and initial droplet size, and device geometry.

Additionally, the method and apparatus of the invention enables the nanoparticles, nanodroplets or layered particles of synthetic or biologically derived polymers to be coated with single or multiple layers of polymers to produce multilayer polymer particles.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

- 5 It will also be understood that the term "comprises" (or its grammatical variants) as used in this specification is equivalent to the term "includes" and should not be taken as excluding the presence of other elements or features.

CLAIMS

1. A method of producing substantially polymer particles comprising:

introducing a first fluid comprising a polymer and a first solvent into a flowpath;

- 5 introducing a second fluid comprising a second solvent into the flowpath to form droplets of the first fluid in the second fluid, the first solvent being at least partially miscible in the second solvent and the polymer being substantially immiscible in the second solvent; and

- 10 flowing the first fluid along the conduit such that a sufficient amount of the first fluid migrates into the second fluid to reduce the size of the droplets.

2. A method of producing substantially polymer particles comprising the steps of

introducing a first fluid comprising a polymer in a first solvent to a second fluid comprising a second solvent;

- 15 subjecting the first fluid in the second fluid to shear under laminar conditions to form droplets of the first fluid, the first solvent being partially miscible in the second solvent and the polymer being substantially immiscible in the second solvent, and

continuing to subject the first fluid to shear until an amount of the first fluid migrates into the second fluid to reduce the size of the droplets.

- 20 3. The method of claim 1 or 2 further comprising the step of fixing the size of the droplets to form polymer particles of a predetermined size and/or morphology by cross linking the polymer.

4. The method of claim 1 or 2 wherein the solubility of the first solvent in the second solvent is 5 to 15% of the first solvent in the second solvent.

5. The method of claim 1 or 2 wherein the solubility of the first solvent in the second solvent is 5 to 10% of the first solvent in the second solvent.
6. The method of claim 1 wherein the flowpath is a channel of a microfluidic device and the first fluid and second fluid flow through the channel in co-current flow.
- 5 7. The method of claim 1 wherein the size of the resulting droplets is varied by changing the respective flow rates of either phase of either fluid or the geometry of the device at the site of droplet creation.
8. The method of claim 3 wherein the step of fixing the size of the droplets and cross linking the polymer particles comprises the step of contacting the droplets with a
10 third fluid which contains a cross linking agent.
9. The method of claim 8 wherein the cross linking agent is miscible in the first solvent and substantially immiscible in the second solvent and the third fluid is introduced into the flowpath to mix with the first fluid prior to introduction of the second fluid into the flowpath. To carry out this form of the invention, the crosslinking agent is
15 preferably miscible in the first solvent and substantially immiscible in the second solvent.
10. The method of claim 9 wherein the first and third fluid are homogeneously mixed before introduction of the second fluid into the flowpath.
11. The method of claim 1 wherein a fluid containing a coating polymer and a solvent
20 for the coating polymer is introduced into the flowpath carrying polymer droplets or particles, the polymer being at least partially miscible in the solvent and miscible with the fluid (continuous phase) carrying the polymer droplets or particles to be coated.
12. A method of producing layered polymer particles comprising:
- introducing a first fluid comprising a polymer and a first solvent to a
25 second fluid comprising a second solvent;

subjecting the first fluid in the second fluid to shear under laminar conditions to form droplets of the first fluid, the first solvent being partially miscible in the second solvent and the polymer being substantially immiscible in the second solvent, and

5 continuing to subject the first fluid to shear until an amount of the first fluid migrates into the second fluid to reduce the size of the droplets;

10 introducing the formed droplets to a coating fluid comprising a carrier solvent and a coating polymer, the coating polymer being at least partially miscible in the carrier solvent and miscible in the second solvent or continuous phase,

subjecting the formed droplets in the coating fluid to shear under laminar conditions to coat the droplets with the coating fluid, and

continuing to subject the coated droplet to shear until an amount of the first fluid migrates into the coating fluid to reduce the size of the coated droplets.

15 13. The method of claim 12 wherein the first fluid comprising a polymer and a first solvent is introduced into a flowpath, the introduction of the second fluid comprising a second solvent to the flowpath causes the formation of droplets of the first fluid in the second fluid; and the first fluid and second fluid flows along the flowpath for a sufficient period for the amount of the first fluid to migrate into the second fluid to reduce the size
20 of the droplets

14. The method of claim 12 wherein the carrier solvent for the coating polymer is miscible in the second solvent and is either the same solvent or substantially the same solvent as the second solvent or continuous phase.

25 15. The method of claim 12 wherein the coating polymer is immiscible in the first solvent.

16. The method of claim 12 wherein the coating step is repeated a number of times as required to produce a multilayered polymer particle.

17. A crosslinked and non-agglomerated polymer particle having a particle size of 10-800 nm.

5 18. A coated polymer particle comprising a core of a first polymer having a particle size in the range of 10-800nm, and at least one layer of a second polymer layered over the core.

10 19. A device for producing a polymer particle comprising a microfluidic device having a first inlet for a first fluid and a second inlet for a second fluid and a fluidic flowpath for contacting the first and second fluids, the resulting particles formed when a substantial amount of the first fluid is transferred from the droplet to the second fluid, are less than 800 nm.

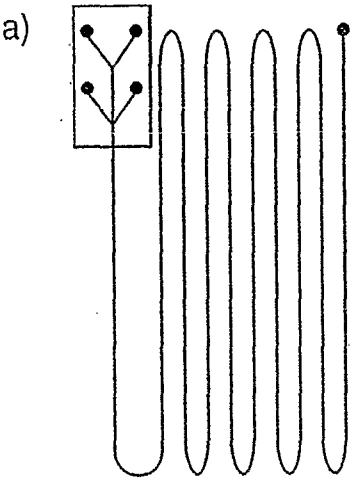


FIGURE 1(a)

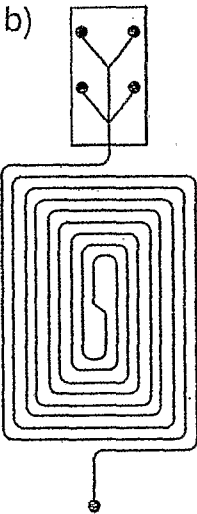


FIGURE 1(b)

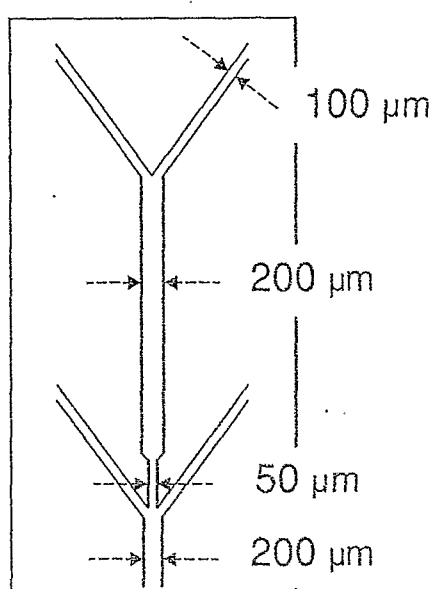
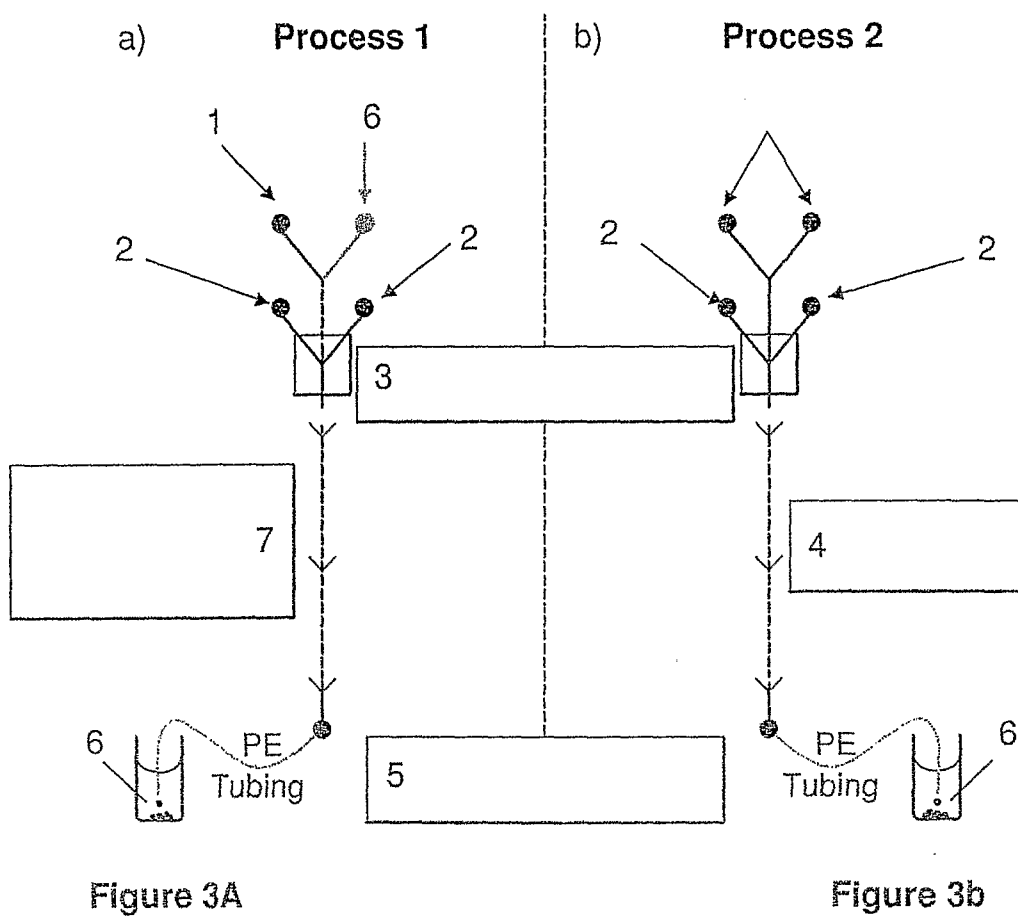


FIGURE 2

**FIGURE 3**

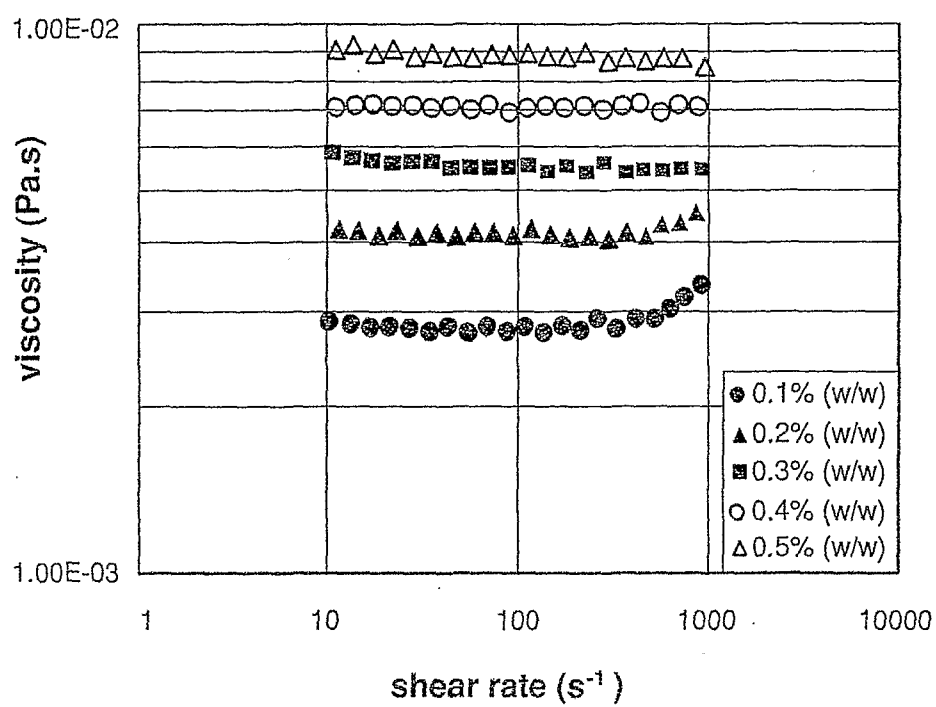


FIGURE 4

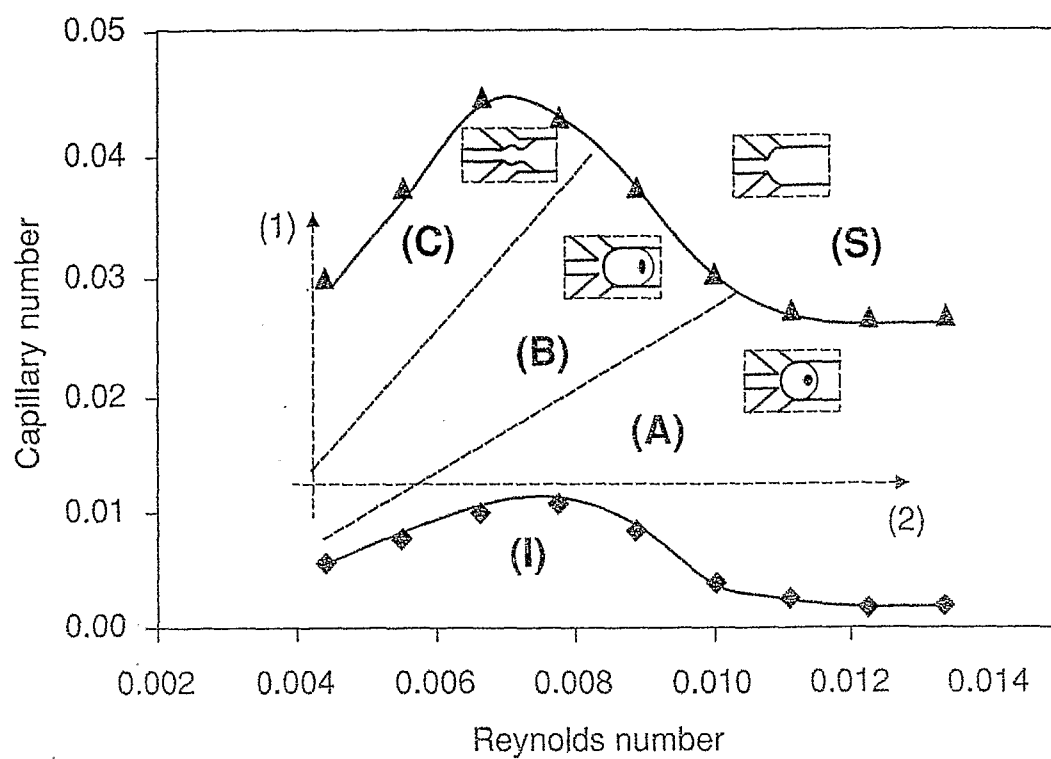
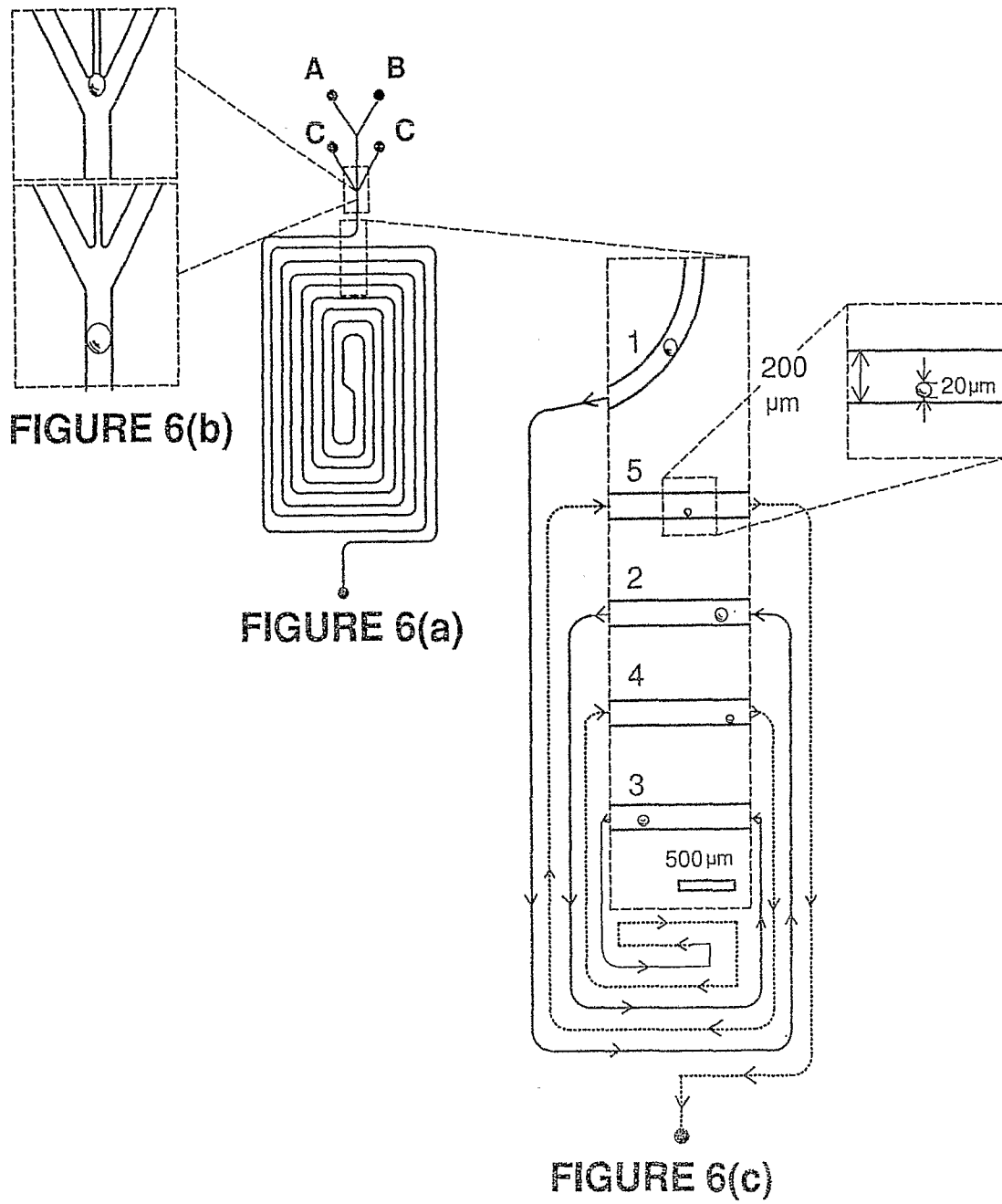


FIGURE 5



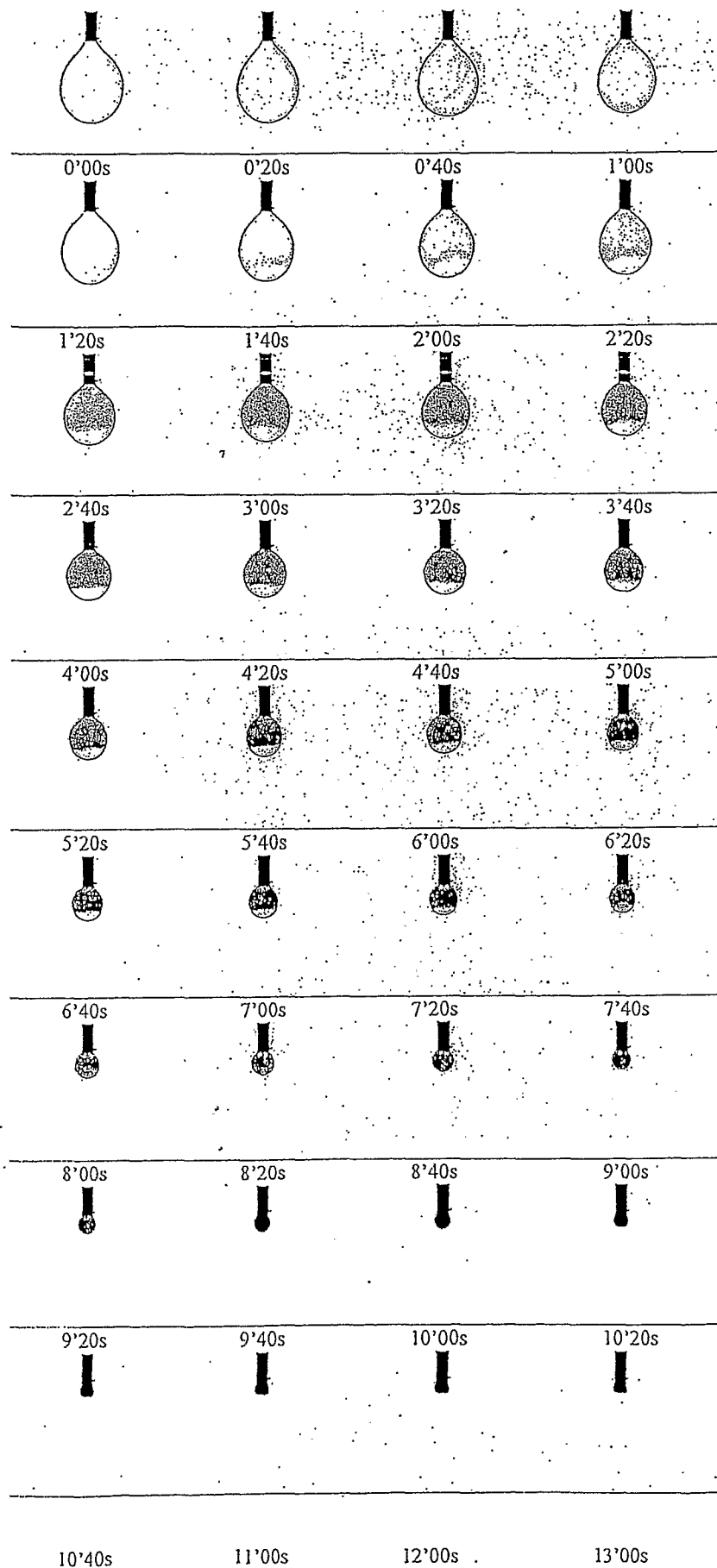


FIGURE 6(d)

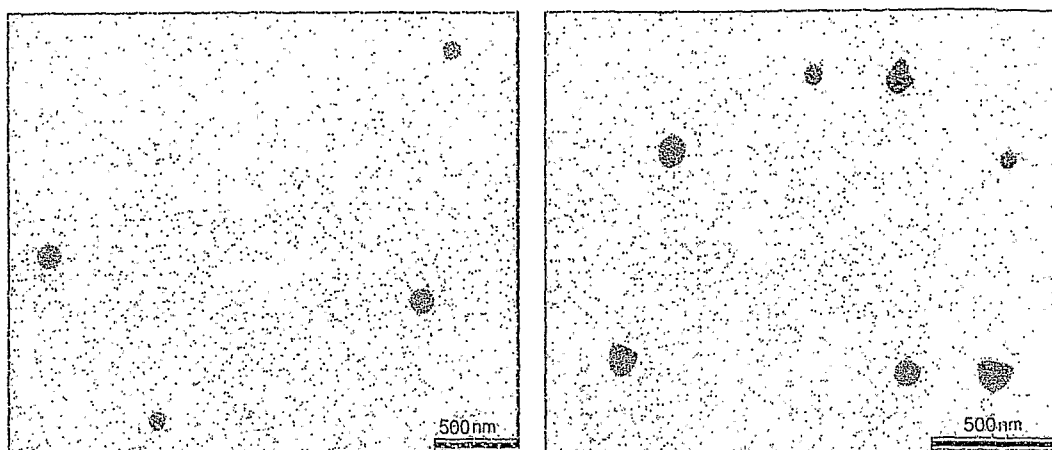


FIGURE 7(a)

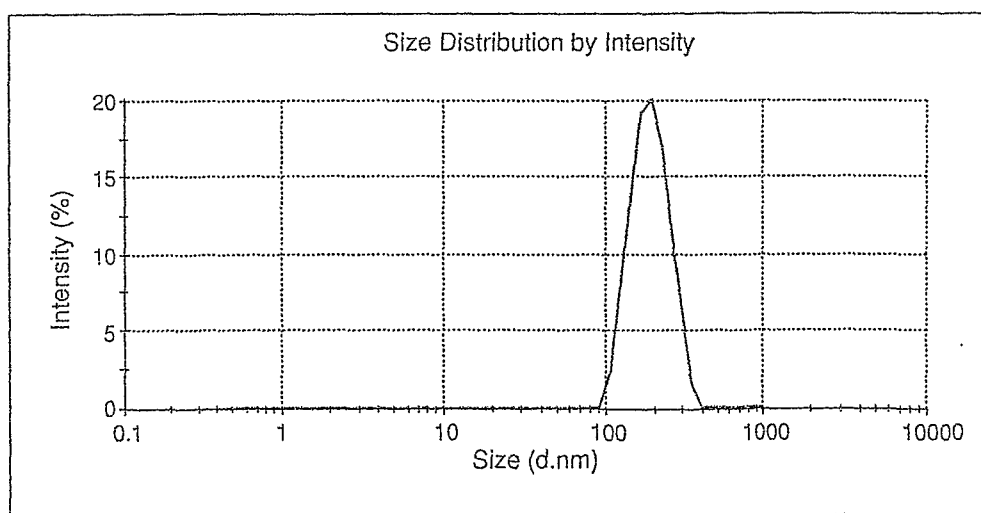


FIGURE 7(b)

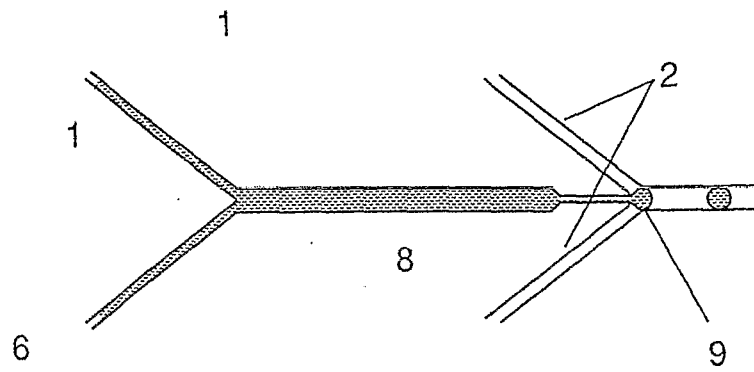


FIGURE 8(a)

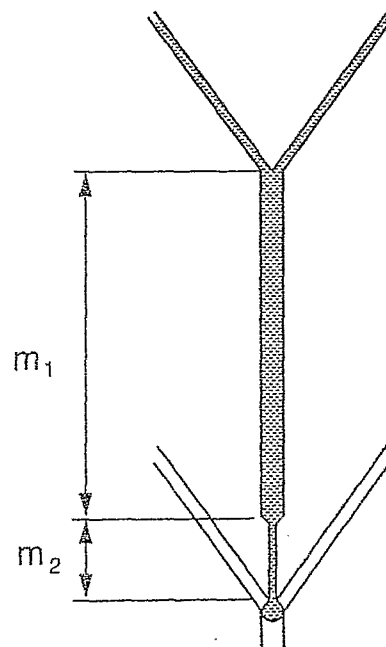


FIGURE 8(b)

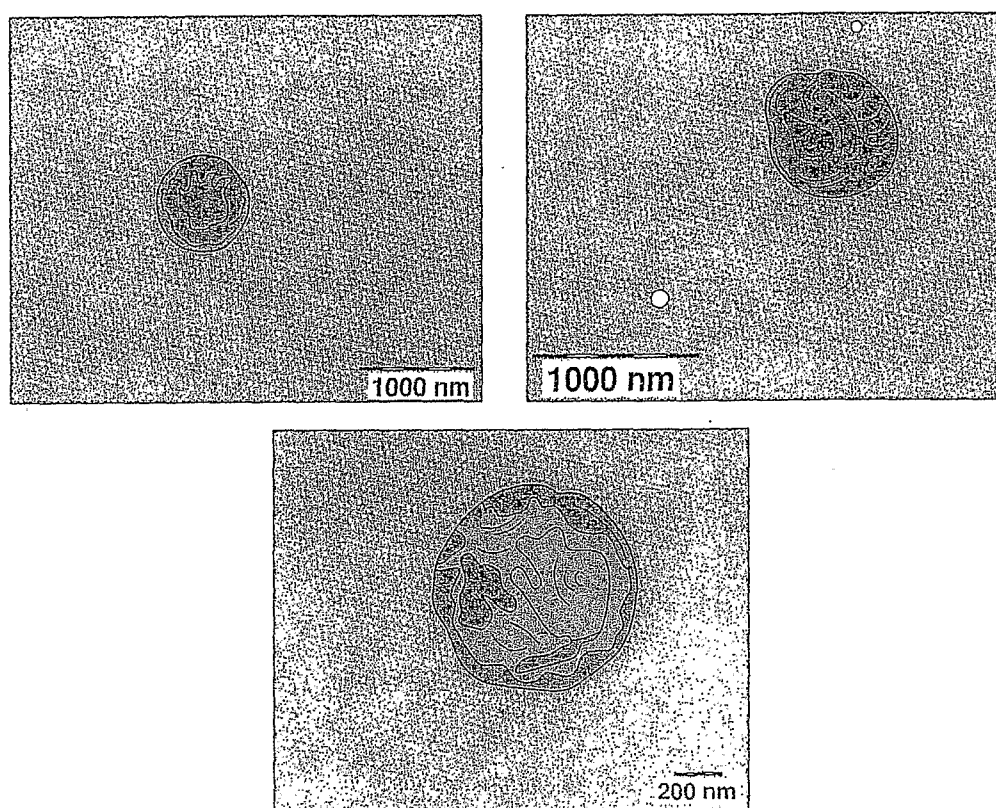
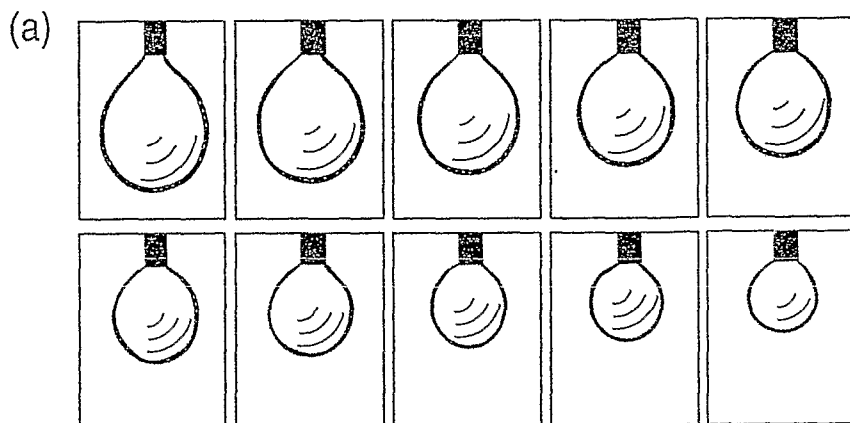
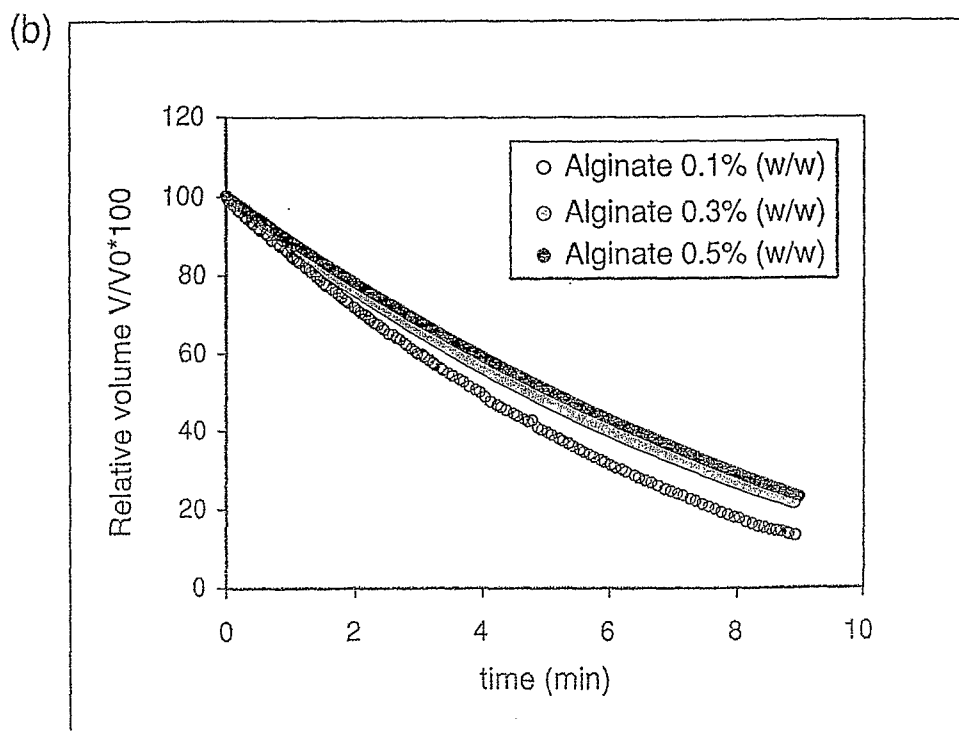


FIGURE 9

**FIGURE 10(a)****FIGURE 10(b)**

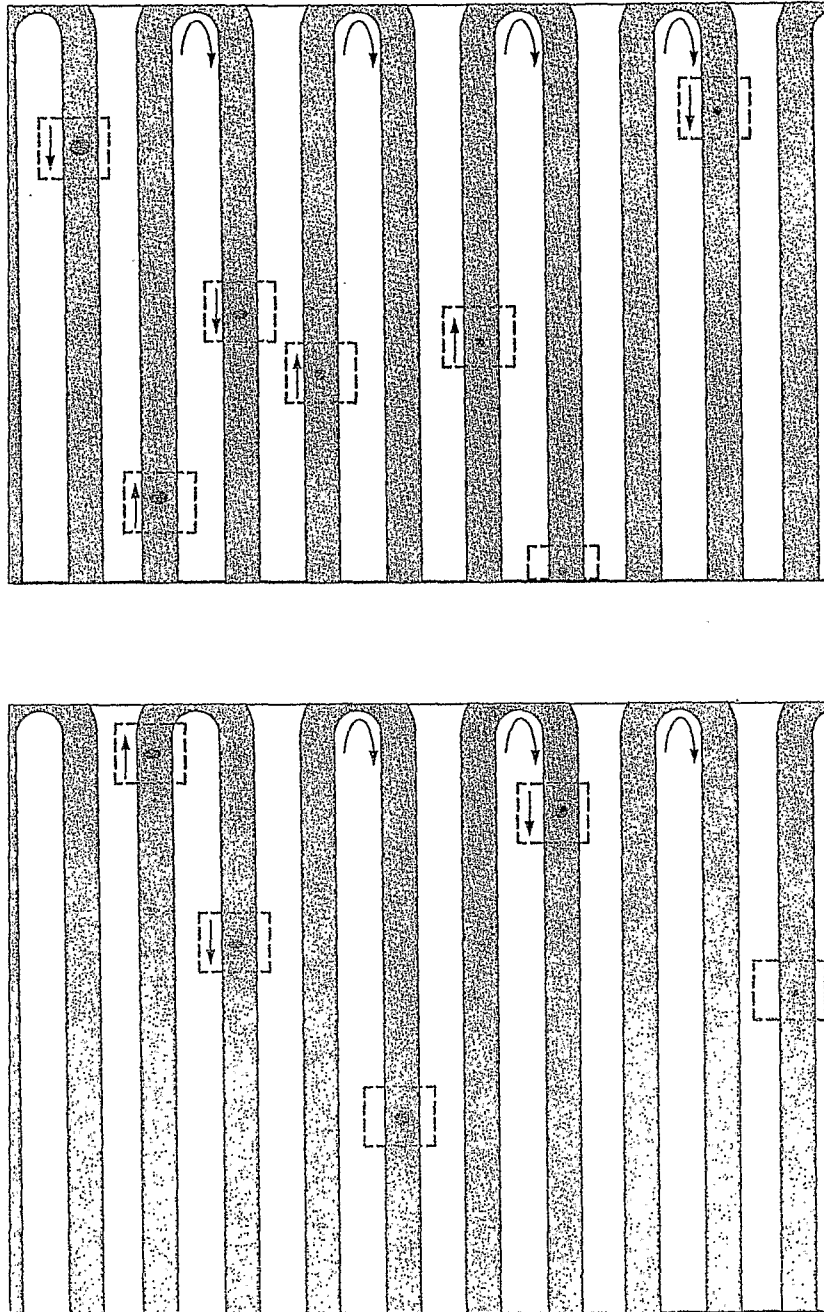


FIGURE 10(c)

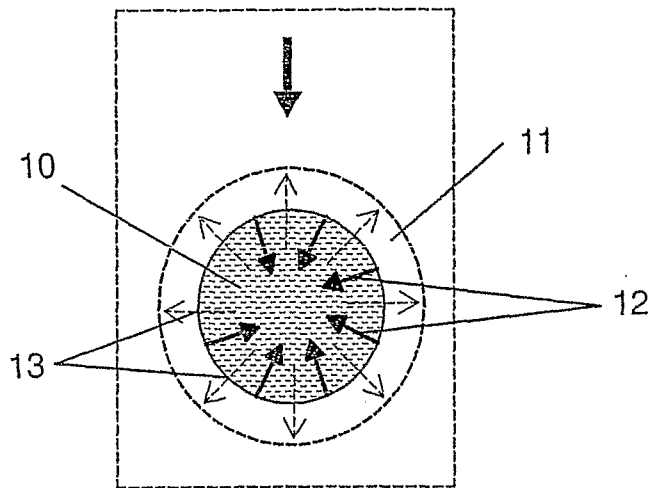


FIGURE 11

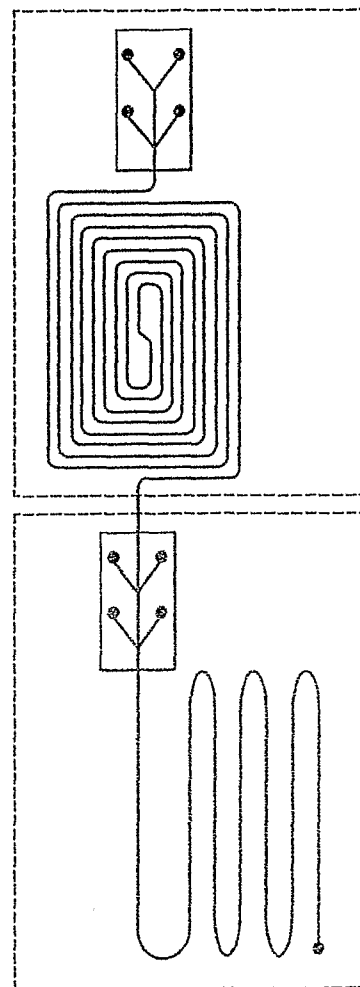


FIGURE 12

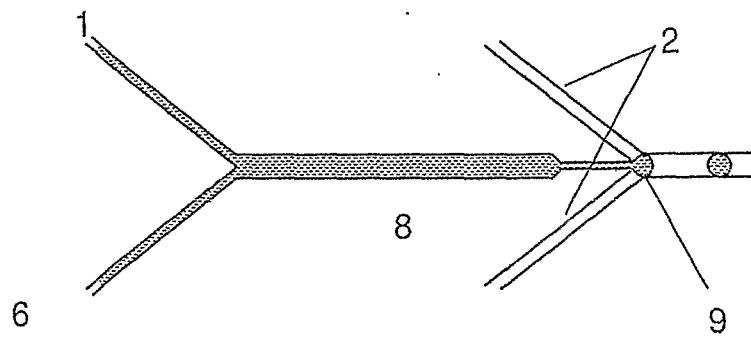


FIGURE 13(a)

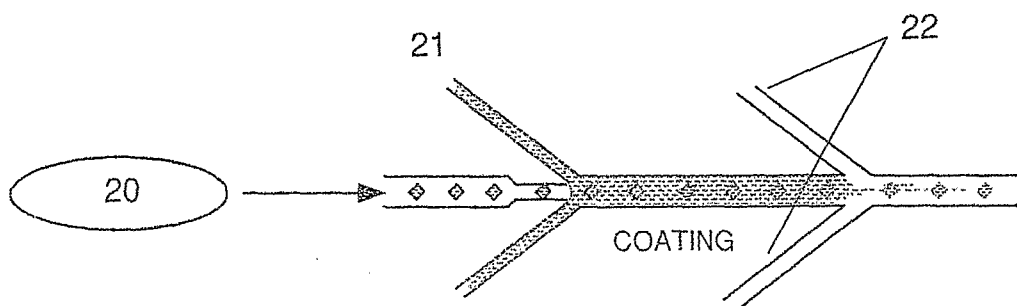


FIGURE 13(b)

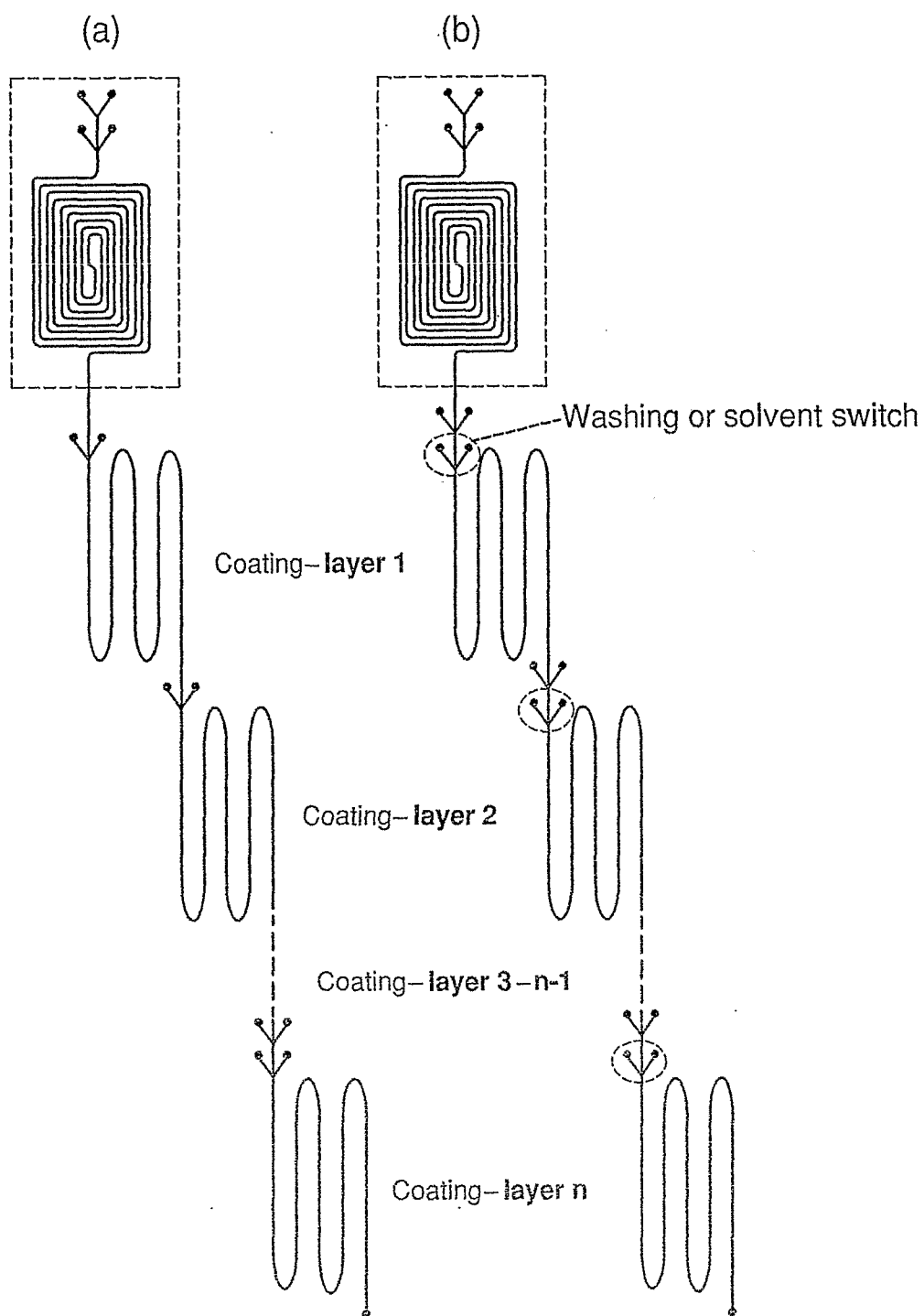


FIGURE 14

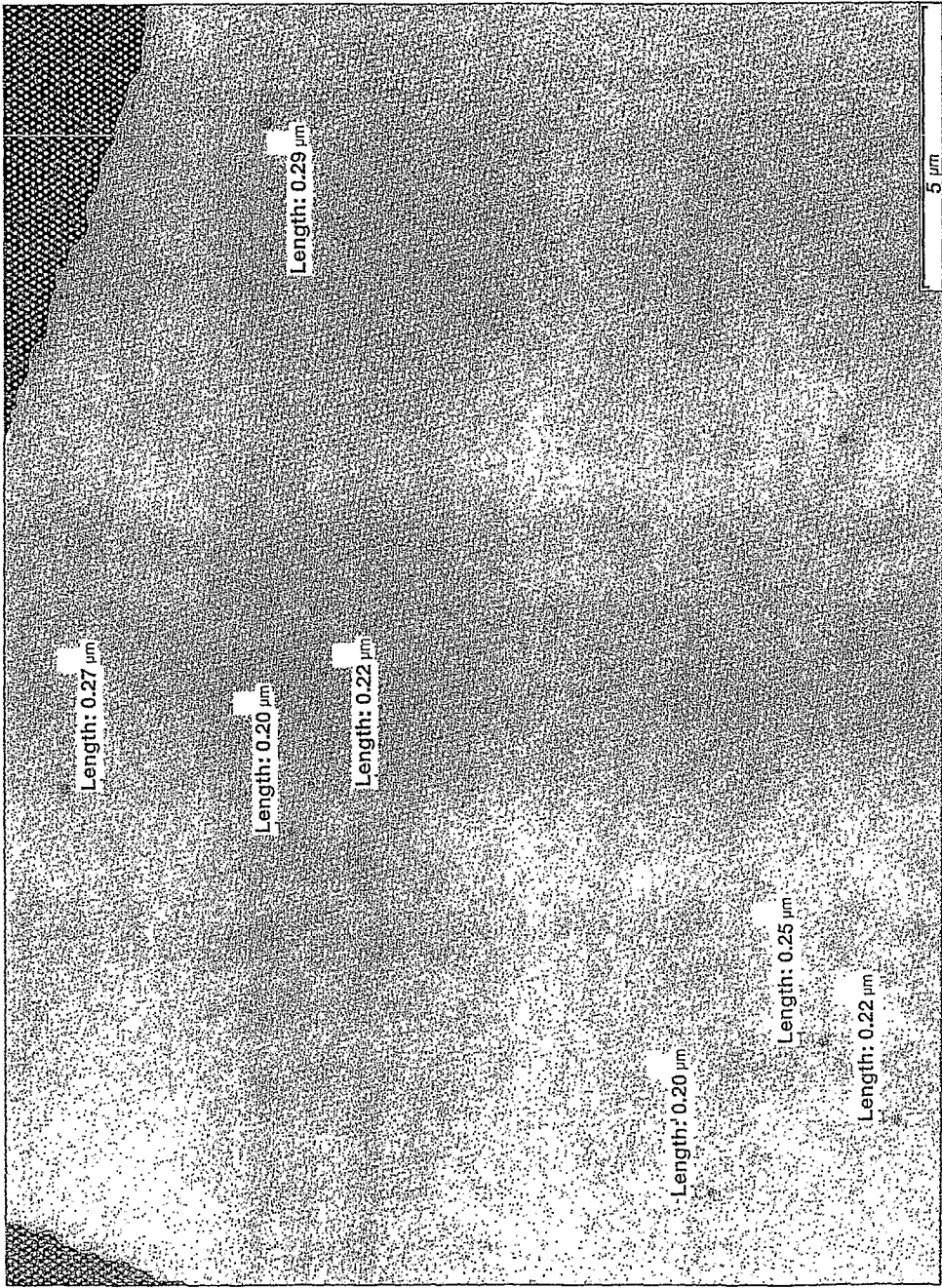


FIGURE 15

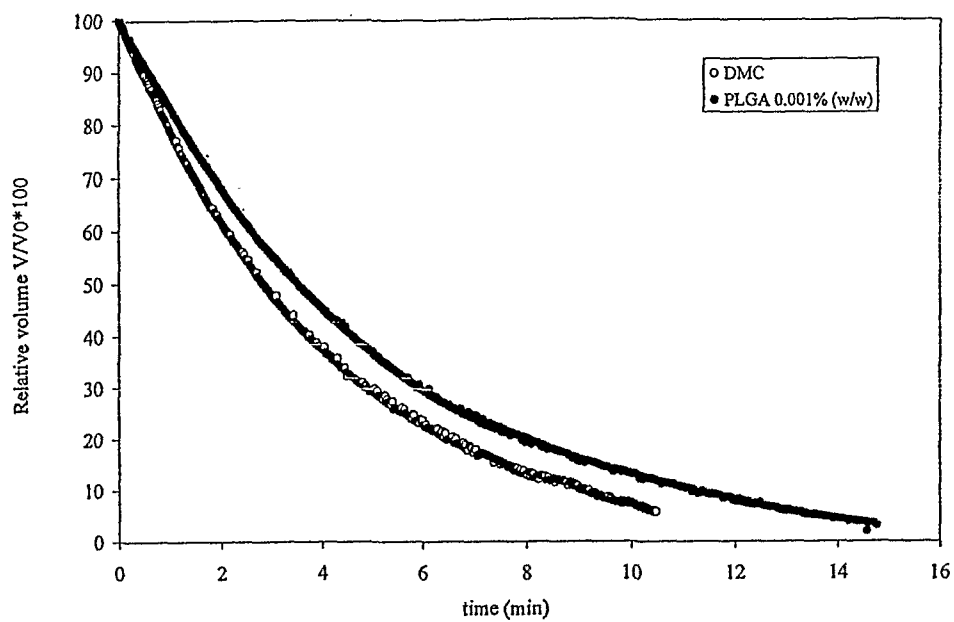


FIGURE 16(a)

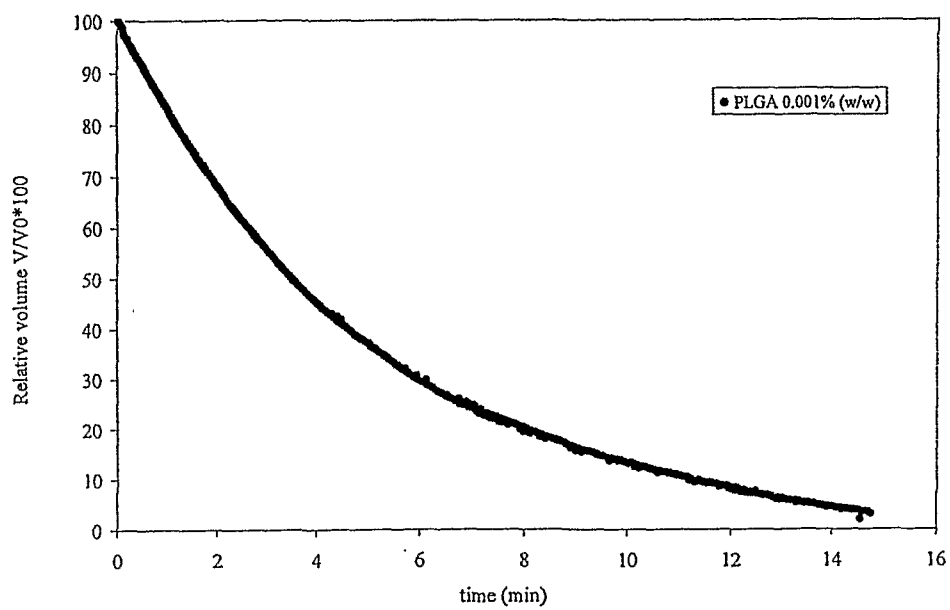


FIGURE 16(B)

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/AU2008/000423

A. CLASSIFICATION OF SUBJECT MATTER

 Int. Cl. **C08J 3/00** (2006.01) **C08G 63/88** (2006.01) **C08F 6/06** (2006.01) **C08J 3/24** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

WPIDS, JAPIO, CAPLUS, Esp@cenet & USPTO: ?polymer? and (size or nano or micro), (solvent or fluid or liquid or xylene), (flow or laminar or shear or stream), ?miscible, (drop? Or particle), (first(w)solvent or second(w)solvent), (move or migrate or transfer), (polyester or polyglycolic or polylactic or PLGA), (biocompat? Or biopoly?), (dispers? Or continuous), microfluidic device, microreaction and like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/0228882 A1 (QIU et al.) 18 November 2004 Abstract; paragraph [0010]; paragraphs [0032]-[0033]; paragraphs [0053]-[0055]; paragraph [0090]; paragraph [0097]; claim 1	1-2, 4-5, 7, 19
A	US 5091296 A (BAGCHI et al.) 25 February 1992 Claims 1-2, 4, 10, 12-14	1-19
A	EP 0863161 A2 (AMCOL INTERNATIONAL CORPORATION) 9 September 1998 Abstract, Claim 1	1-2, 19

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

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

 Date of the actual completion of the international search
 19 May 2008

 Date of mailing of the international search report
 02 JUN 2008

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2008/000423

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4444961 (TIMM) 24 April 1984 see whole document	1-2, 16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2008/000423

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-16, 19**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box

(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. In coming to this conclusion the International Searching Authority has found that there are two inventions:

- Group I: Claims 1-16 and 19 are directed to a method of producing polymer particles comprising introducing a first fluid comprising a polymer and a first solvent into a flowpath; introducing a second fluid comprising a second solvent into the flowpath to form droplets of the first fluid in the second fluid. It is considered that the method of producing polymer particles as defined in claim 1 comprises a first distinguishing feature.
- Group II: Claims 17-18 are directed to a crosslinked polymer particle having a particle size of 10-800 nm. It is considered that the polymer particle per se defined in claims 17-18 comprises a second distinguishing feature.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

The only feature common to all of the claims is polymer particles. However this concept is not novel in the light of:

Citation D1: EP 1500670 A1 (JAPAN SCIENCE AND TECHNOLOGY AGENCY et al.) 26 January 2005; claims 1-2, page 15

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art.

Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention *a posteriori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2008/000423

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

Patent Document Cited in Search Report				Patent Family Member			
US	2004228882	CA	2526965	CN	1822893	EP	1633463
		EP	1875959	US	7307104	US	2004234566
		WO	2004103539				
US	5091296	JP	4233538	US	5279931		
EP	0863161	CA	2214920	CA	2217755	CA	2228807
		EP	0709406	EP	0830398	EP	0863159
		EP	0863160	JP	8208719	JP	10244152
		JP	10244153	JP	10245409	JP	2008031488
		US	5608005	US	5618877	US	5677407
		US	5712358	US	5773546	US	5777054
		US	5830960	US	5830967	US	5834577
		US	5837790	US	5955552	US	6107429
		US	6248849	US	6387995	WO	9640809
US	4444961	AU	76569/81	BR	8107167	CA	1166413
		EP	0051210	ES	8303480	HK	27186
		JP	5194610	JP	5194611	JP	57102905
		MX	159468	US	4666673	YU	257881
		ZA	8107188				
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							