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(54) ELECTROPHORETIC ENRICHMENT OF ANALYTES IN DROPLETS

(57) In a method for increasing a concentration of an analyte in a sample liquid, a stream of sample droplets separated by a separating fluid is created. The droplets are sequentially transported through a microfluidic channel (21). A DC electric field is applied across the microfluidic channel to cause a concentration gradient of the analyte within each sample droplet while the sample droplet moves through the microfluidic channel. Each sample droplet is then split into at least two daughter droplets, one of the daughter droplets having a higher concentration of the analyte than the other daughter droplet. The analyte can comprise a molecular species, including a peptide, protein or nucleic acid, and the molecular species can be directly dispersed in the sample liquid without being attached to macroscopic particles.

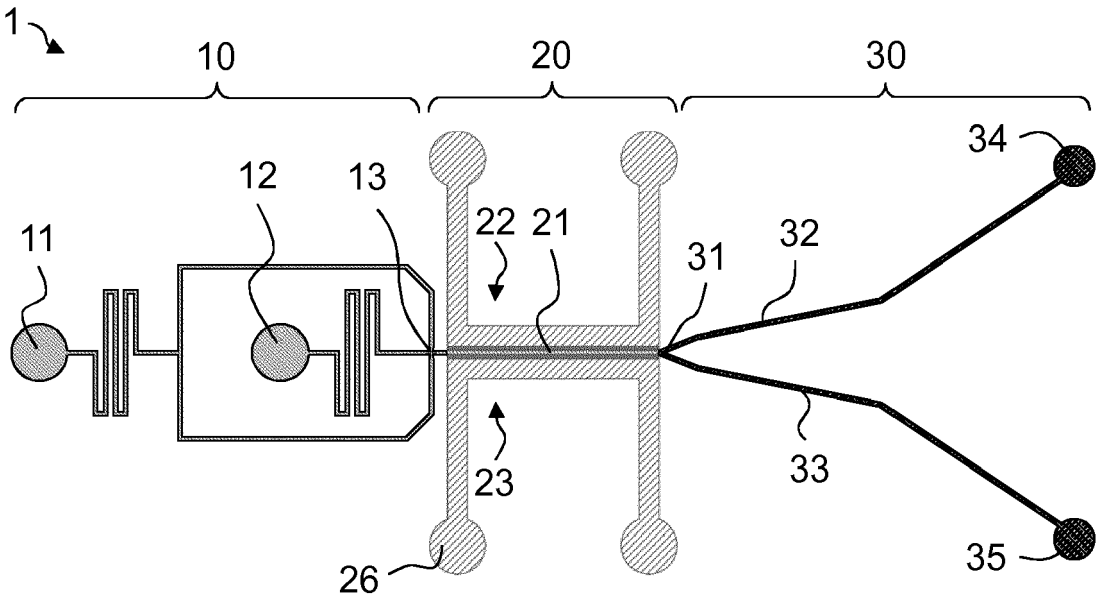

FIG. 1

EP 3 799 956 A1

Description

TECHNICAL FIELD

[0001] The present invention relates to a method for enriching an electrically charged analyte in a sample liquid and to a corresponding device.

PRIOR ART

[0002] Controlling the motion of biomolecules and colloidal particles is a fundamental component in many microfluidic bioanalytical platforms. Of special interest is the case of droplet microfluidics, where a continuous flow is segmented into discrete droplets with volumes below a few microliters surrounded by an immiscible fluid. Droplet-based microfluidics has evolved into one of the main platforms for biochemical assays and medical diagnosis. Along with the short processing time of analysis and low sample consumption that characterize microfluidic devices, droplets serve as microreactors to perform chemical and biochemical reactions in well-defined environments. Droplets can be generated at frequencies of several kHz, enabling the development of high-throughput assays for a wide spectrum of applications, including molecular assays, enzyme evolution, drug screening and single-cell analysis.

[0003] In contrast to continuous-flow microfluidics, where the flow profile is laminar and mixing is limited to molecular diffusion, a three-dimensional re-circulative flow profile develops inside droplets confined in microfluidic channels. Such patterns, caused by a biphasic nature of the flow, tend to evenly distribute particles over the whole droplet volume once a critical velocity is reached.

[0004] In recent years, various techniques have been proposed for the enrichment and separation of rather large, micrometer-scale particles inside droplets.

[0005] For instance, Hein et al. (M. Hein, M. Moskopp and R. Seemann, Lab Chip, 2015, 15, 2879-2886) exploited the sedimentation tendency of poly(methyl methacrylate) beads (8 μm in diameter) to hydrodynamically accumulate them at both sides of moving droplets. Droplets were then split into three pieces, two side-droplets containing most particles and a central droplet containing the dispersed phase. Although robust, this hydrodynamic technique was limited to low throughputs (droplet velocities around 0.75 mm/s) to avoid the re-mixing of particles in the whole droplet volume.

[0006] Han et al. (S.-I. Han, H. Soo Kim and A. Han, Biosens. Bioelectron., 2017, 97, 41-45) used negative dielectrophoresis (nDEP) for focusing polystyrene particles (5 μm) inside droplets at maximum velocities of 2 mm/s. For carrying out nDEP, a pair of planar electrodes made of Cr/Au was placed inside a flow channel. The planar electrodes were arranged in a common plane. Their longitudinal edges extended at an angle relative to the flow direction in the flow channel. A high-frequency (500 kHz) AC voltage was applied to the electrodes to create an AC electric field in the flow channel. Due to the co-planar arrangement of the angled electrode pair, the AC electric field was highly non-uniform. A Y-junction was added at the end of the microchannel to split droplets into two daughter droplets, one containing most particles.

[0007] Brouzes et al. (E. Brouzes, T. Kruse, R. Kimmerling and H. H. Strey, Lab Chip, 2015, 15, 908-919) developed a microfluidic chip that retained 98% of super-paramagnetic particles (1 μm) at a maximum velocity of 6 mm/s. The device employed a permanent magnet to focus particles in a small region inside droplets before splitting them into two pieces.

[0008] Wang et al. (Y. Wang, Y. Zhao and S. K. Cho, J. Micromechanics Microengineering, 2007, 17, 2148-2156) proposed an in-droplet magnetic particle concentration and separation method, wherein magnetic particles (size 1.5 to 10 μm) are concentrated and separated into split droplets by using permanent magnet and electrowetting-on-dielectric (EWOD) droplet manipulation.

[0009] Fornell et al. (A. Fornell, M. Ohlin, F. Garofalo, J. Nilsson and M. Tenje, Biomicrofluidics, 2017, 11, 031101) employed acoustic forces to actively control the final position of polystyrene particles (10 μm) inside droplets before splitting them into three pieces. Depending on the harmonics of the induced acoustic field, particles were focused to either the two side-droplets or the central droplet.

[0010] While these studies have made significant contributions towards the development of in-droplet separation systems, these studies were restricted to the enrichment of large (micrometer-sized) particles inside the droplets. No techniques have been reported for the enrichment and separation of small analytes (e.g., small molecules, peptides, proteins, and nucleic acids) that are directly dispersed in the sample liquid from which the droplets are formed.

[0011] It has been suggested in the literature to couple droplet microfluidics and electrophoresis. Most efforts have focused on extracting droplets into a secondary channel for electrophoretic analysis (J. Scott Edgar, Chaitanya P. Pabbati, Robert M. Lorenz, Mingyan He, Gina S. Fiorini and D. T. Chiu, Anal. Chem., 2006, 78, 6948-6954; G. T. Roman, M. Wang, K. N. Shultz, C. Jennings and R. T. Kennedy, Anal. Chem., 2008, 80, 8231-8238; M. Wang, G. T. Roman, M. L. Perry and R. T. Kennedy, Anal. Chem., 2009, 81, 9072-9078; X. Niu, F. Pereira, J. B. Edel and A. J. de Mello, Anal. Chem., 2013, 85, 8654-8660). While functional, these approaches dilute the sample into a continuous stream, limiting

the further processing of the sample.

[0012] Free-flow electrophoresis (FFE) is a variation of CE in which analytes separate in a liquid matrix flowing perpendicular to the electric field. A review of FFE is provided in P. Novo et al. (P. Novo and D. Janasek, "Current advances and challenged in microfluidic free-flow electrophoresis - A critical review", Analytica Chimica Acta 991 (2017), pp. 9-29). FFE allows for fast separation and direct sample collection; however, it is not possible to parallelize the separation conditions, i.e., only one analyte mixture can be separated at a time. This limits the applicability of FFE in high-throughput screening applications. FFE is also limited when very small amounts of sample liquid need to be handled.

SUMMARY OF THE INVENTION

[0013] In a first aspect, it is an object of the present invention to provide a method for increasing the concentration of analytes in a sample liquid, the method allowing high throughput and being applicable to small analytes that are directly dispersed in the sample liquid.

[0014] This object is achieved by a method according to claim 1. Further embodiments of the invention are laid down in the dependent claims.

[0015] A method is proposed for increasing a concentration of an electrically charged analyte in a sample liquid. The method comprises:

creating a stream of sample droplets comprising the sample liquid, the sample droplets being separated by a separating fluid that is immiscible with the sample liquid;
sequentially transporting the sample droplets through a microfluidic channel; and
applying a DC electric field across the microfluidic channel to cause a concentration gradient of the electrically charged analyte within each sample droplet while the sample droplet moves through the microfluidic channel.

[0016] The invention makes it possible that small analytes (size below 1 μm), in particular, analytes in the form of charged molecular species such as small organic molecules, peptides, proteins and nucleic acids, are directly enriched inside droplets. The analyte can have a molecular mass in a very wide range, e.g., between 400 Da and 30 MDa. The analyte can be directly dispersed in the sample liquid, i.e., the analyte does not need to be attached to macroscopic particles such as surface-functionalized beads. High enrichment performance can be achieved even at comparatively large flow velocities. No sample dilution occurs. In summary, a readily integrable, label-free platform for the enrichment of charged molecules inside droplets at high throughput is provided.

[0017] The presently proposed method is very different from prior-art methods wherein droplets are transferred into a secondary channel and diluted into a continuous stream of a carrier liquid for the electrophoretic analysis. In contrast to such prior-art methods, the proposed technique provides enrichment directly within the droplets, without dilution and at high throughput, and readily allows for subsequent further manipulation of the droplets.

[0018] The presently proposed method is also very different from free-flow electrophoresis (FFE). FFE relies on a laminar flow profile, and mixing is assumed to be limited to molecular diffusion. In contrast, in the presently proposed method the movement of the droplets in the flow channel causes strong re-circulative flows with three-dimensional vortices, which strongly affects the enrichment of analytes within the droplets. Therefore, the physical phenomena behind the enrichment of analytes in the present invention are different from the physical phenomena employed in FFE. Furthermore, FFE requires a continuous liquid matrix flowing through a relatively wide channel, typically on the order of several millimeters. It is therefore generally not possible to use an FFE device for carrying out the presently proposed method, since in the presently proposed method a stream of small droplets needs to be laterally confined while it is transported through the device.

[0019] In the presently proposed method, a DC electric field is applied across the channel. In the present context, an electric field is considered to be a DC field if it normally does not change its polarity during the passage of each single droplet through the field region. However, the proposed method does not exclude that the electric field changes its polarity from time to time, e.g., between the passage of consecutive droplets.

[0020] No electric field gradients are required in the microfluidic channel in order to carry out the method, and accordingly, the applied DC electric field can be chosen to be essentially uniform in an enrichment section of the microfluidic channel. In particular, a DC electric field is considered to be essentially uniform if it is created by a pair of parallel opposing electrodes disposed on both sides of the microfluidic channel. In more general terms, the electric field can be considered to be essentially uniform if its strength varies by less than 20% along the enrichment section of the microfluidic channel.

[0021] The presently proposed method allows the sample droplets to be transported through the microfluidic channel at considerable velocities. Preferably the velocity is between 2 and 20 mm/s, more preferably between 5 and 10 mm/s. Lower velocities are well possible, but unnecessarily reduce the throughput of the method. Higher velocities may lead to a decreased enrichment performance.

[0022] As already outlined above, enrichment of the analyte within the droplets is influenced by re-circulative flows

within the droplets. The re-circulative flows are advantageously used to stabilize the enrichment process. In order to create a stable re-circulative flow that stabilizes the enrichment process, it is advantageous if the sample droplets have a length-to-width ratio of at least 3 when they are transported through the microfluidic channel.

[0023] The width of each droplet is preferably larger than its height, preferably at least by a factor of 1.5, more preferably at least by a factor of 3. It is believed that in this manner the re-circulative flows inside the droplets are particularly stable. The sample droplets preferably have the following characteristics: volume between 1 femtoliter and 10 microliters; droplet width between 10 μm and 500 μm; droplet height between 10 μm and 150 μm.

[0024] The sample liquid is preferably an aqueous liquid, in particular, a buffer solution like TAE, TBE, HEPES or Bis-Tris, in which the analyte is molecular-dispersed or colloidally dispersed. Analyte concentration in the sample liquid is preferably between 0.001 μg/μl and 0.1 μg/μl.

[0025] In some embodiments, the method further comprises splitting each sample droplet into first and second daughter droplets when the sample droplet exits the microfluidic channel. The splitting is advantageously carried out in such a manner that the first daughter droplet receives a portion of the sample droplet that has higher concentration of the analyte than the portion received by the second daughter droplet, i.e., the first daughter droplet receives a portion in which the analyte has been enriched, while the second daughter droplet receives a portion in which the analyte has been depleted.

[0026] In another aspect, the present invention provides a microfluidic device that is configured to carry out the method of the present invention. Accordingly, a microfluidic device for increasing a concentration of a charged analyte in a sample liquid is provided. The microfluidic device defines a device plane. It comprises:

a droplet generator configured to create a stream of sample droplets comprising a sample liquid, the sample droplets being separated by a separating fluid that is immiscible with the sample liquid;
a microfluidic channel disposed downstream from the droplet generator for sequentially receiving the sample droplets separated by the separating fluid, the microfluidic channel defining a flow direction in the device plane; and
first and second electrodes for applying a DC electric field across the microfluidic channel, the first and second electrodes delimiting the microfluidic channel towards opposite lateral sides of the microfluidic channel.

[0027] Preferably the microfluidic channel is straight. Preferably it has uniform cross section. These measures contribute to uniformity of the flow of the droplets in the region of the DC electric field.

[0028] The cross section of the microfluidic channel is preferably rectangular. Preferably the first and second electrodes are parallel to one another and parallel to the flow direction. Preferably each of the first and second electrodes has a considerable height (measured perpendicular to the device plane). In particular, it is preferred if each electrode has a height that corresponds to at least 80%, preferably at least 100% of the height of the microfluidic channel. The surface of each of the first and second electrodes facing the microfluidic channel is preferably parallel to the flow direction, and these surfaces are preferably parallel to one another and perpendicular to a lateral direction in the device plane perpendicular to the flow direction. Preferably the first and second electrodes are congruent when viewed along a lateral direction. Preferably each electrode has rectangular shape when viewed in the lateral direction. These measures contribute to a uniform electric field inside the microfluidic channel.

[0029] Each electrode preferably has a length of at least 2 mm, more preferably at least 5 mm. There is no upper limit to the length of the electrodes. In practical terms, it is advantageous to keep the length of each electrode below 45 mm, which is still compatible with standard glass slides for the manufacture of the microfluidic device.

[0030] A key challenge in microfluidic devices of the presently proposed kind is the avoidance of bubble formation due to electrolysis. Bubbles negatively affect the transport of the droplets and distort the local electric field. In order to minimize bubble formation, it is advantageous if the surface of each electrode that faces the flow channel is non-metallic. More specifically, it is advantageous if the first electrode comprises a first membrane and the second electrode comprises a second membrane, the first and second membranes being made of a non-metallic material, in particular, a composite material that comprises non-metallic electrically conductive particles embedded in a polymeric matrix. Preferably each of the first and second membranes laterally delimits the microfluidic channel to one side, such that droplets that flow through the microfluidic channel can be in contact with the first and second membranes.

[0031] The electrically conductive particles are preferably carbon particles, in particular, carbon nanotubes. The polymeric matrix preferably comprises a silicon-based polymer, in particular, PDMS.

[0032] Since the first and second membranes are part of the first and second electrodes, respectively, similar considerations as for the first and second electrodes also apply for the first and second membranes. In particular, the first and second membranes are preferably parallel to one another and parallel to the flow direction. The surface of each of the first and second membranes facing the microfluidic channel is preferably parallel to the flow direction, and preferably these surfaces are parallel to one another and perpendicular to the lateral direction. Preferably the first and second membranes are congruent when viewed along the lateral direction. Preferably, each membrane has rectangular shape when viewed along the lateral direction. Each membrane preferably has a height (measured perpendicular to the device plane) that corresponds to at least 80%, more preferably at least 100% of the height of the microfluidic channel. Again,

these measures contribute to uniformity of the electric field.

[0033] The first and second membranes should neither be too thin nor too thick as measured along the lateral direction. If they are too thin, they may be difficult to manufacture and may be unstable. If they are too thick, they may impede the creation of the electric field. Preferably each of the membranes has a width between 50 and 200 μm, measured in the device plane and perpendicular to the flow direction.

[0034] Preferably, the first electrode further comprises a first electrode channel, and the second electrode comprises a second electrode channel, each of the first and second electrode channels being filled with an electrically conductive liquid, e.g., an electrolyte solution or a liquid metal. The first membrane then separates the microfluidic channel from the first electrode channel, and the second membrane separates the microfluidic channel from the second electrode channel. In the following, an electrode that comprises an electrode channel filled with an electrically conductive liquid will be called a "liquid electrode" for simplicity. A liquid electrode ensures that a uniform electric potential can be applied to each of the first and second membranes.

[0035] For the purposes of the present disclosure, the term "microfluidic channel" can be generally understood to relate to a channel whose dimensions perpendicular to the flow direction are below 1 mm. The width of the microfluidic channel, measured along the lateral direction, is preferably between 10 μm and 500 μm. The height of the microfluidic channel, measured perpendicular to the device plane, is preferably between 10 μm and 150 μm. The width of the microfluidic channel is preferably larger than its height, preferably at least by a factor of 1.5, more preferably at least by a factor of 2. The microfluidic channel is preferably formed as a groove in a layer of polymeric material attached to a substrate acting as a cover of the microfluidic channel. In other words, while the first and second electrodes delimit the microfluidic channel laterally, the channel is preferably delimited by the polymeric material towards its bottom and by the material of the substrate towards its top (or vice versa). The polymeric material and the substrate should be electrically non-conductive. The polymeric material can be, e.g., a silicon-based polymer like PDMS. In other embodiments, it can be, e.g., PMMA or a cyclic olefin copolymer (COC). The substrate can be made, e.g., of a comparatively hard, chemically inert material like silicate glass.

[0036] In some embodiments, the microfluidic device further comprises a droplet splitter disposed downstream from the microfluidic channel for splitting each sample droplet into first and second daughter droplets when the sample droplet exits the microfluidic channel. In this manner not only enrichment of the analyte in a portion of each sample droplet becomes possible, but also a separation of the portions of the sample droplets in which the analyte is enriched and depleted, respectively.

[0037] The microfluidic device can be complemented with external components to form a microfluidic system. In particular, the microfluidic system can comprise a DC voltage source connected to the first and second electrodes of the microfluidic device for creating a DC electric field across the microfluidic channel. The voltage supplied to the electrodes can be, e.g., in the range of 1 to 60 Volts.

[0038] It goes without saying that the present invention also encompasses the use of the microfluidic device of the present invention in the method of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Preferred embodiments of the invention are described in the following with reference to the drawings, which are for the purpose of illustrating the present preferred embodiments of the invention and not for the purpose of limiting the same. In the drawings:

Fig. 1 shows a schematic view of a microfluidic device for increasing the concentration of an analyte in a sample liquid.

Fig. 2 shows an enlarged view of an enrichment section of the microfluidic device in Fig. 1, where electrophoretic enrichment of analytes takes place.

Fig. 3 shows a schematic view of a complete microfluidic system for increasing the concentration of an analyte in a sample liquid.

Fig. 4 shows a sequence of steps for manufacturing the microfluidic device in Fig. 1.

Fig. 5 shows a sequence of images (left column) and schematic graphical representations (right column) as a droplet moves through the microfluidic device; (a) immediately after droplet generation; (b) at a distance of 1 mm from the inlet of the enrichment section; (c) at a distance of 3 mm from the inlet of the enrichment section; (d) at a distance of 4 mm from the inlet of the enrichment section; and (e) at the Y junction where the droplet splits. Scale bar S = 200 μm.

Fig. 6 shows a diagram illustrating the dependence of the enrichment factor on the electric field strength for four differently sized molecular species that are not nucleic acids. Droplet velocity was 7 mm/s for all experiments. The dotted lines represent the average enrichment factor as evaluated using spline functions; each point represents the average over at least 140 droplets.

Fig. 7 shows a diagram illustrating the dependence of the enrichment factor on the electric field strength for four

differently sized nucleic acids. Droplet velocity was 7 mm/s for all experiments. The dotted lines represent the average enrichment factor as evaluated using spline functions; each point represents the average over at least 140 droplets.

Fig. 8 shows a diagram illustrating the dependence of the enrichment factor for the fluorescent dye dichlorofluorescein as a function of the HPMC content. Each bar represents the average of 145 droplets.

Fig. 9 shows a diagram illustrating the dependence of the enrichment factor for dichlorofluorescein as a function of the electrode length. The dotted line represents a log-logistic function that represents the best fit to the experimental data. Each point represents the average of 175 droplets.

Fig. 10 shows a diagram illustrating the dependence of the enrichment factor for dichlorofluorescein as a function of the droplet velocity. The ratio of the continuous and dispersed phases was adjusted so that droplet length was approximately 3.5 times droplet width for all experiments. The dotted line represents a log-logistic function that represents the best fit to the experimental data. Each point represents the average over 235 droplets.

Fig. 11 shows a diagram illustrating the dependence of the enrichment factor for dichlorofluorescein as a function of the normalized droplet length (ratio of droplet length and droplet width). Droplet velocity was 5 mm/s for all experiments. The dotted line represents the log-logistic function that results in the best fit to the experimental data. The enrichment factor for normalized droplet lengths over 27 was adjusted based on the fluorescence decrease in the daughter droplet. Each point represents the maximum achievable enrichment, averaged over 240 droplets.

DESCRIPTION OF PREFERRED EMBODIMENTS

Microfluidic device

[0040] Figure 1 illustrates an exemplary embodiment of a microfluidic device 1 for increasing a concentration of a charged analyte in a sample liquid. The microfluidic device 1 comprises a droplet generator 10, an enrichment device 20, and a droplet splitter 30. The enrichment device 20 is shown in greater detail in Fig. 2.

[0041] The droplet generator 10 comprises a continuous-phase inlet 11 and a sample inlet 12. Microfluidic inlet channels lead from the continuous-phase inlet 11 and the sample inlet 12, respectively, to a flow-focusing intersection 13. The inlets 11, 12, the flow-focusing intersection 13 and the inlet channels are all arranged in a common device plane.

[0042] In use, a separating fluid, e.g., in the form of an inert oil, is supplied to the continuous-phase inlet, and an aqueous sample liquid is supplied to the sample inlet 12. A continuous stream of sample droplets consisting of the sample liquid, separated by plugs of the separating fluid, are formed at the intersection 13 by flow focusing, as it is well known in the art.

[0043] The enrichment device 20 is disposed downstream from the flow-focusing intersection 13. It comprises a straight microfluidic main channel 21 having rectangular cross-section with constant cross-sectional area. The main channel 21 extends in the device plane and defines a flow direction F in this plane between its inlet and its outlet. Laterally, i.e., to its sides perpendicular to the flow direction, the main channel 21 is delimited by first and second electrodes 22, 23. The construction of the electrodes is illustrated in greater detail in Figure 2. Each electrode 22, 23 comprises an electrically conductive membrane 24, 25. The membranes 24, 25 are made of a composite material comprising a polymeric matrix doped with electrically conducting carbon particles. The membranes 24, 25 are parallel to one another. Each membrane 24, 25 has rectangular cross section when viewed along the flow direction. In a projection along a lateral direction in the device plane perpendicular to the flow direction, each membrane 24, 25 also has rectangular shape, i.e., in total, each membrane 24, 25 has cuboid shape. Both membranes 24, 25 are congruent when viewed along the lateral direction. Each membrane 24, 25 separates the main channel 21 from an electrode channel 26, 27 that is filled with an electrically conductive liquid to form a liquid electrode. The electrically conductive liquid is supplied to the electrode channels 26, 27 through liquid reservoirs 28. The electrode channels 26, 27 and the liquid reservoirs 28 also extend in the device plane.

[0044] In use, the stream of sample droplets, separated by the separating fluid, is received from the droplet generator 10 at the inlet of the microfluidic channel 21 and transported through the main channel 21 from its inlet to its outlet along the flow direction F. A DC voltage is applied between the electrodes 22, 23. Thereby a DC electric field is created across the main channel 21. As will be explained in more detail below, the DC electric field causes a charged analyte in each droplet to become more concentrated (enriched) in one portion of the droplet (depending on the polarity and the charge either in a left lateral portion or in a right lateral portion with respect to the flow direction F) while being depleted in another portion of the droplet.

[0045] Immediately downstream from the enrichment device 20, adjacent the downstream end of the main channel 21, a droplet splitter 30 is disposed. The droplet splitter 30 comprises a Y junction as it is well known in the art, splitting up the main channel 21 into first and second outlet channels 32, 33 at an angle to one another. The outlet channels 32, 33 lead to first and second sample outlets 34, 35, respectively. The Y junction 31, the outlet channels 32, 33 and the sample outlets 34, 35 also extend in the device plane.

[0046] In use, each sample droplet is split by the droplet splitter 30 into first and second daughter droplets. One of the daughter droplets comprises the left half of the sample droplet, while the other daughter droplet comprises the right half of the sample droplet. Accordingly, the analyte is enriched in one of the daughter droplets while being depleted in the other daughter droplet. Like the sample droplets, also the daughter droplets are separated by plugs of the separating fluid. Two continuous outlet streams consisting of daughter droplets separated by the separating fluid are transported through the outlet channels 32, 33 to the sample outlets 34, 35, respectively.

[0047] In the present example, dimensions are as follows: width of all microfluidic channels: 0.2 mm; height of all microfluidic channels: 0.085 mm; length of each electrode channel along the flow direction: 5 mm; width of each electrode channel: 0.5 mm; height of each electrode channel: 0.085 mm; width of each membrane 24, 25 along the lateral direction: 0.1 mm; diameter of each inlet: 1.5 mm; initial opening angle between the outlet channels 32, 33 immediately downstream from the Y junction: 44°; diameter of each fluid outlet: 1 mm.

[0048] It is to be understood that the microfluidic device is shown only by the way of example, and that various modifications are possible. For instance, a different form of droplet generator can be used. Droplet generation for droplet microfluidics is generally well known in the art, and any known technique for generating a stream of droplets separated by plugs of an immiscible separating fluid can be employed. There can be one or more droplet manipulation sections in the flow path between the droplet generator and the enrichment section, for instance, for inducing mixing or chemical reactions inside the droplets. The splitting of the droplets can be carried out differently than by a simple Y junction, as it is well known in the art. Many more modifications are possible.

Microfluidic system

[0049] Figure 3 illustrates an exemplary embodiment of a complete microfluidic system that includes the microfluidic device ("microfluidic chip") 1 shown in Fig. 1. The system comprises a DC voltage source 41 connected to the first and second electrodes 22, 23, a first pump 42 in the form of a syringe pump for transporting separating fluid to the continuous-phase inlet 11, a second pump 43 in the form of a syringe pump for transporting sample fluid to the sample inlet 12, a first collecting vial 44 for receiving a first outlet stream from sample outlet 34, and a second collecting vial 45 for receiving a second outlet stream from sample outlet 35. An optical microscope 50 comprising an objective 51 and a digital camera 52 acquires images of the microfluidic device 1 and sends them to a computer 53 for display.

[0050] It is to be understood that the system is only shown by the way of example, and that various modifications are possible. For instance, the sample liquid and the separating fluid can be provided to the inlets 11, 12 in a different manner than shown. One or both of the outlet streams can be further manipulated instead of being collected in collecting vials. The microscope 50 and computer 53 are only required for visual inspection and can be left away.

Manufacture of the microfluidic device

[0051] An exemplary microfluidic device was made from PDMS (Sylgard 184, Dow Corning) and fabricated using standard soft-lithography techniques. A mold with the positive pattern of the microfluidic device was defined in a SU-8 3050 (MicroChem) photoresist layer coated on a silicon wafer. Next, a 2 mm thick layer of PDMS was cast onto the mold to produce the negative pattern of the microfluidic device. After curing at 80 °C for one hour, the resulting PDMS mold was treated with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma Aldrich) to prevent further bonding with PDMS and used as a secondary mold.

[0052] The subsequent production steps are illustrated in Fig. 4. Each part (a) to (h) of Fig. 4 symbolizes a production step. In the top of each of parts (a) to (h) of Fig. 4, a portion of the secondary mold and/or the resulting device is schematically shown in a top view; in the bottom, the same portion is schematically shown in a sectional view. It is to be understood that these views are highly schematic and not to scale.

[0053] In step (a), the secondary mold was provided. In step (b), a carbon-doped composite material (described in more detail below) was poured into the spacing between the structures that will later form the main channel 21 and electrode channels 26, 27 and set on vacuum for 10 minutes. In steps (c) and (d), the excess composite material was removed using standard adhesive tape to obtain the membranes in step (e). In step (f), a 2 mm thick layer of PDMS was cast onto the mold and cured at 80 °C for one hour. In step (g), the cured PDMS layer was peeled off and sealed in step (h) to a glass substrate by oxygen plasma. In this manner, the microfluidic device including the embedded carbon-doped membranes 24, 25 was obtained. Aquapel (Pittsburgh Glass Works) solution was injected into the microchannels for 1 min to make both the glass substrate and the PDMS microchannel walls hydrophobic.

Carbon-doped composite material fabrication

[0054] The above-described exemplary embodiment comprises membranes 24, 25 made of a carbon-doped PDMS composite to electrically interface the liquid electrodes 26, 27 and the main flow channel 21. The following steps were

followed to prepare the electrically conductive composite material: (1) Multi-walled carbon nanotubes (outer diameter: 8-18 nm, length: 0.5-2.5 μm, NanoGrafi) were suspended in isopropyl alcohol (IPA), in a ratio of 1:250 (w/w), and dispersed using a low-energy ultrasonic cleaner (45 kHz, 120 W, VWR) for 30 min. (2) Low-viscosity, methyl-terminated PDMS (M.M. 6000, VWR) was added to the carbon nanotube suspension, in a ratio of 1:2.3 (w/w) with respect to the nanotubes content, and sonicated for 15 min. The methyl-terminated PDMS adheres to the hydrophobic surface of the carbon nanotubes, creating thermodynamically stable complexes in IPA. (3) Standard PDMS pre-polymer was added to the suspension in a ratio of 1:9.2 (w/w) with respect to the nanotubes content and further sonicated for 15 min. (4) The suspension was placed on a hotplate at 60 °C and magnetically stirred at 500 rpm until all IPA evaporated. (5) Before being poured in the master mold, the PDMS curing agent was added to the mixture in a ratio of 1:10 (w/w) with respect to the pre-polymer content, manually stirred, and degassed.

Biomolecules sample preparation

[0055] Solutions containing different analytes were prepared to assess the enrichment performance as a function of size and charge. All analytes were suspended in a buffer solution containing 10 mM HEPES, 20 mM Bis-Tris and 0.1% v/v Tween 20, adjusted to a pH of 7.5 with sodium hydroxide. Nucleic acids with four different lengths were studied: (1) 10 bp, (2) 1 kbp, (3) 20 kbp and (4) 43 kbp. All DNA samples were acquired from ThermoFisher. In addition, four non-nucleic acid species were studied: (1) 2',7'-dichlorofluorescein (Alfa Aesar), (2) the (Arg)9 peptide with one trifluoroacetic acid counterion per Arg residue (Peptide Specialty Laboratories), (3) biotin (Sigma Aldrich), and (4) streptavidin (Sigma Aldrich). Table 1 introduces the size, type and fluorescent label of the analytes. The initial concentration of all species was 0.01 μg/μl, which is in the concentration range of other biochemical assays performed in microfluidics.

Table 1. *Type, size and fluorescent label of the studied analytes. The reported size includes the size contribution of the fluorescent dye.*

Analyte	Type	Size	Label
Dichlorofluorescein	Molecule	401 Da	Self-fluorescent
Biotin	Vitamin	1.4 kDa	Atto 488
(Arg)9	Peptide	1.99 kDa	Alexa 488
Streptavidin	Protein	53.6 kDa	Atto 565
DNA (10 bp)	Nucleic acid	6.5 kDa	SYBR Safe
DNA (1 kbp)	Nucleic acid	650 kDa	SYBR Safe
DNA (20 kbp)	Nucleic acid	13 MDa	SYBR Safe
DNA (43 kbp)	Nucleic acid	28 MDa	SYBR Safe

Experimental procedure

[0056] Experiments started with a clean main channel 21 that was filled with Fluorinert FC-40 oil (3M), supplied with a 0.5% of 008-FluoroSurfactant (Ran Biotechnologies). This oil-surfactant combination served as the continuous phase to discretize the analyte samples. High-precision syringe pumps (neMESYS, Cetoni) were used to deliver both the dispersed phase (sample liquid) and continuous phase (separating liquid) through the respective channel inlets. A 3 M potassium chloride solution was injected in the electrode channels 26, 27 to serve as liquid electrodes. Platinum wire electrodes were then placed at the electrode channel reservoirs 28, and a DC electric voltage was applied using a source meter (2612A, Keithley). The electrical current flowing through the microfluidic device was monitored and recorded by the source meter. An EMCCD camera (iXon DV887, Andor) was used to capture fluorescent microscopy images and videos.

Fluorescence measurements

[0057] The fluorescent signals of the sample droplets before splitting and of the daughter droplets were tracked to assess the enrichment performance of the proposed technique. The average fluorescence intensity of each droplet was determined using a home-made code developed in Fiji, which included a binary image segmentation process coupled with the particle detection tool. In this manner, the fluorescence intensity was measured only in the regions within the droplets and the background signal was easily distinguished, producing reproducible measurements. The post-process-

ing of the fluorescent signals was performed in R. Calibration curves (average fluorescence vs. concentration) were constructed for all analytes to estimate their concentration in the daughter droplets as a function of the induced electric field. The exposure time (0.005 ms) and gain (ranging between 0 and 200) of the EMCCD camera were selected to achieve a linear calibration curve.

[0058] An enrichment factor (E) was defined to assess the enrichment performance of the proposed technique. Such factor computed the difference in concentration in the daughter droplet where analytes were enriched to before (C_O) and after (C_E) the application of the electric potential, normalized by the initial concentration, C_O:

$$E = \frac{C_E - C_0}{C_0} \times 100\%. \qquad (1)$$

[0059] Therefore, the enrichment factor is relative to the case where no electric field is induced, and ranges from 0% (both daughter droplets had the same concentration) to 100% (all analytes were recovered in one daughter droplet). In contrast, the total fraction of analytes in the daughter droplet (F) can be estimated as:

$$F = \frac{C_E}{2\,C_0} \times 100\%, \qquad (2)$$

where F ranges from 0% (no analytes are present in the daughter droplet) to 100% (all analytes were recovered in the daughter droplet).

Working mechanism

[0060] Figure 5 illustrates what happens when droplets 62 separated by plugs of separating liquid 61 are transported through the microfluidic device 1. The left column shows images of droplets containing dichlorofluorescein at various positions in the device; the right column shows schematic graphical representations at these positions. Immediately after droplet generation, when the sample droplets 62, laterally confined by channel walls 63, leave the droplet generator 10, analytes are uniformly distributed in the whole droplet volume, as shown schematically in Figure 5 (a). When sample droplets arrive in the enrichment device 20 between the parallel electrodes, a uniform electric field is established inside them. Charged analytes are transported inside the droplets by the resulting electrophoretic force. Negatively charged analytes are attracted towards the anode, and are progressively enriched in the right half 64 of droplets as they move into this region, while the left half 65 becomes depleted of analyte. The evolution of this enrichment process is shown in Figures 5 (b) to (d) for different positions within the enrichment device 20. Positively-charged analytes, in contrast, would be transported towards the cathode, in the opposite direction, while neutral species would remain unaffected in the whole droplet volume. The sample droplets are then split in two pieces: one daughter droplet 66 contains the (negatively charged) enriched analytes, and the other daughter droplet 67 contains the depleted portion of the sample droplet, as illustrated in Fig. 5 (e).

Enrichment performance of the system

[0061] The electrophoretic force exerted on the analytes is a function of their size (hydrodynamic radius) and charge, the applied electric potential, and the properties of the surrounding buffer. The enrichment factor for all analytes as a function of the induced electric field is shown in Figure 6 (for the non-nucleic acid species) and Figure 7 (for the nucleic acids). The profile of the enrichment factor follows a similar concave behavior for all analytes: the enrichment performance increases with the electric field and reaches a maximum value before decreasing. This decrease always occurred shortly before large electrolytic bubbles were observed. Thus, it may be caused by the formation of small, non-observable bubbles that affect the transport of analytes and distort the local electric field. Electrothermally-induced convective flows, caused by the heating of the suspending medium, may also have a contribution on the observed decrease in performance. Both effects, however, can be decreased by fine-tuning the electrical conductivity of the suspending buffer for a given separation, as commonly done in electrophoretic studies.

[0062] The proposed in-droplet technique is able to enrich all the analytes tested, from small molecules to large DNA strands. The maximum enrichment and required electric field, however, vary for the different species, as illustrated by the height and position of the peaks in Figures 6 and 7. Dichlorofluorescein, the smallest studied analyte, shows the highest enrichment factor, with an average value of over 96% at an applied electric field of 27 V/mm. The enrichment of dichlorofluorescein is followed by that of biotin, which shows an average enrichment of 63% when an electric field of 25 V/mm is induced. Although both dichlorofluorescein and biotin have a similar charge of -1, the larger size of biotin

seems to be responsible for the observed decrease in the enrichment factor. The (Arg)9 polypeptide, whose size and charge magnitude are similar to those of biotin, exhibits a similar enrichment performance (60%) for an electric field of 30 V/mm. A different behavior is observed for streptavidin, a protein significantly larger than the former analytes. Streptavidin requires only half the electric field (17 V/mm) to achieve its maximum enrichment, partially driven by a higher surface charge (calculated as -1.5). The maximum enrichment is, however, limited to 50%, which is significantly lower than the enrichment of smaller analytes. These results evidence the role of analytes size on their recovery. In general, the maximum enrichment seems to decrease with increasing analyte size, while both analyte size and charge have an impact on the electric field required to achieve the maximum enrichment performance.

[0063] The profile of the enrichment factor for the nucleic acids (Fig. 7) also emphasizes the interplay between size and charge. In average, 60% of the shortest strand (10 base pairs) can be recovered when 26 V/mm are applied to the device. Both the maximum enrichment factor and the required electric field are similar to those of biotin and (Arg)9, which are on the same size scale as this nucleic acid. An increase in size to 1,000 base pairs, which is accompanied with an increase in the surface charge of the molecule, results in a higher enrichment (98%) for a similar electric field (28 V/mm). For DNA molecules larger than 400 base pairs, the electrophoretic mobility is independent of size. An increase in size to 20,000 base pairs has little effect in the enrichment performance (94%). The increase in size, however, scales the required field to 32 V/mm. This behavior is more drastic when size is further increased to 43,000 base pairs, which scales the required field to 42 V/mm for a similar enrichment performance (94%) to take place.

Dynamics of the enrichment process

[0064] The observed size dependence on the enrichment factor is believed to arise mainly from a drag force induced by the re-circulative flow profile inside droplets, which is a function of the dynamic viscosity of the dispersed medium and the in-droplet flow velocity. To demonstrate the impact of this force on the enrichment performance, hydroxypropyl methylcellulose (HPMC), an inert viscoelastic polymer, was added to the analytes suspensions to increase its viscosity. The addition of 0.05% w/v HPMC, which increases the viscosity by 11%, decreases the average enrichment factor from 96% to 68% (Figure 8). The change in the enrichment factor is believed to be caused by a larger drag force and a deformation of the in-droplet flow profile, which prevents analytes migration towards one half of each droplet. At higher concentrations of HPMC (0.10% and 0.25% w/v), the viscosity increase (25% and 76%) results in a significant decrease in the enrichment process (40% and 39%), as shown in Figure 8.

[0065] As previously described, once an electric field is stablished inside droplets, (negatively-charged) analytes are transported to one half of each droplet (Figure 5). At low velocities, the internal flow profile is believed to have two main re-circulation regions, corresponding to the two halves of each droplet. The enrichment process is thus highly dynamic: analytes trapped in the re-circulative flow of one half are forced to escape into the other half by the action of electrophoresis, and are retained in the other half by a combination of the re-circulative flow in that half and the electrophoretic force. In other words, analytes are progressively enriched in one half of each droplet as they move through the enrichment region.

[0066] Figure 9 shows the evolution of the enrichment process as a function of the length of the electrodes 22, 23. The enrichment process dynamics can be described by the cumulative distribution function of a log-logistic distribution, which allows the characterization of the start and stabilization of the enrichment process as a function of the electrodes length. As noticed from Figure 9, only 0.75 mm of electrode length are needed for the analytes to start migrating into one half of each droplet. The initial transport rate of analytes is rather high, and 81% of the analytes can be enriched after 1.7 mm. Beyond this point, the enrichment process reaches a semi-stationary phase, where the enrichment factor asymptotically approaches the final value of 96%. Doubling the electrode length from 2.5 to 5.0 mm only increases the enrichment by 7%. Therefore, these results demonstrate the importance of a careful selection of the electrode length, and seem to indicate that other analytes (e.g., streptavidin) require longer electrodes for the enrichment process to reach the stationary phase.

Effect of droplet speed and size

[0067] The movement of droplets in microchannels can generate complex circulation flows and three-dimensional vortices that affect the enrichment and extraction of analytes. The dynamics of the internal flow is believed to be strongly dominated by the velocity of moving droplets, the viscosity of the continuous phase, and the interfacial tension, as represented by the capillary number.

[0068] Figure 10 illustrates the enrichment factor of dichlorofluorescein for several droplet velocities, where three main regimes can be observed. At the lower velocities (below 10 mm/s), the symmetry of the microchannel cross-section is believed to be replicated in the flow profile. Four long vortices are believed to arise, spanning most of the droplet length. Analytes are then transported in closed loops next to the droplets' rim, facilitating their enrichment in one half of the droplet and resulting in high enrichment performances (an average of 96%). Above 10 mm/s, however, the enrichment performance decays rapidly with droplet velocity. This decay is a transition regime believed to be caused by flow reversal

starting to form at the front and rear of droplets, creating new vortices and a more complex re-circulative flow field. The decay model indicates that 50% of the analytes can be enriched at a velocity of 20 mm/s. At velocities close to 50 mm/s, the flow reversal is believed to be fully developed and the enrichment approaches a minimum of 38%. Therefore, analytes tend to be uniformly distributed over the whole droplet volume at high droplet velocities.

[0069] The flow-reversal transition regime is believed to be dependent on droplet size, where smaller droplets tend to be more prone to fall into the complete-mixing regime. The influence of droplet length (normalized by its width, i.e., length-to-width ratio) on the enrichment factor is illustrated in Figure 11. Elongated droplets (those larger than approximately 3 times their width) show high enrichment. As droplets become shorter (below 3 times their width), there is a sharp decrease in the enrichment of analytes. When droplets with a length equal to their width are employed, only 66% of the analytes can be enriched. Below this point, droplets do not make full contact with the conductive membranes 24, 25, and the enrichment approaches zero. Therefore, droplets whose length is above approximately 3 times their width should be preferred for the enrichment of analytes using this approach.

Conclusions

[0070] In this disclosure, the direct in-droplet enrichment and extraction of small analytes (e.g., molecules, peptides, proteins and nucleic acids) in microfluidic devices is demonstrated. Electric fields are induced inside droplets by three-dimensional liquid electrodes coupled to the flow channel by conductive PDMS-based membranes. Analytes with sizes ranging from small molecules to large nucleic acids were enriched inside moving droplets. Droplets were then split into two daughter droplets, one containing the enriched analytes. The fluorescent signals of the mother and daughter droplets were analyzed to assess the enrichment performance of the proposed technique. Experimental results demonstrate that the size and charge of analytes have a strong influence on their enrichment. In general, smaller analytes and highly charged species tend to be more easily enriched and extracted.

[0071] The observed size dependency is believed to arise from a drag force exerted on the analytes by the surrounding flow, which increases with both the viscosity of the continuous phase and droplet velocity. High enrichment factors (over 95%) were observed for the small dichlorofluorescein molecule and for nucleic acids larger than 1,000 base pairs at droplet velocities as high as 10 mm/s. Therefore, this technique ranks among the in-droplet methods with highest throughput. The impact of the length of the parallel electrodes was assessed, showing that some species require longer electrodes to achieve a complete enrichment. It was also found that the proposed technique performs better in droplets whose length is more than approximately 3 times their width. Since the electrophoretic mobility of analytes scales with their surface charge, this technique has the potential to achieve higher throughputs by fine-tuning the experimental setup.

Modifications

[0072] As has already been discussed above, various modifications are possible. In particular, the device can be manufactured based on different materials than PDMS. For instance, the device can be manufactured from a cyclic olefin copolymer (COC) or PMMA. Instead of using an electrolyte solution like a potassium chloride solution for the liquid electrodes, a liquid metal can be used, e.g., an alloy of indium and gallium. The dimensions of the device can be varied according to need. While the separating fluid is advantageously an oil, other separating fluids are conceivable, including gases. Many other modifications are possible.

Claims

1. A method for increasing a concentration of an electrically charged analyte in a sample liquid, the method comprising:

   creating a stream of sample droplets (62) comprising the sample liquid, the sample droplets (62) being separated by a separating fluid (61) that is immiscible with the sample liquid;
   sequentially transporting the sample droplets (62) through a microfluidic channel (21); and
   applying a DC electric field across the microfluidic channel (21) to cause a concentration gradient of the electrically charged analyte within each sample droplet (62) while the sample droplet (62) moves through the microfluidic channel (21).

2. The method of claim 1, wherein the analyte comprises a charged molecular species, in particular, at least one peptide, protein and/or nucleic acid, the molecular species being directly dispersed in the sample liquid without being attached to macroscopic particles, the molecular species preferably having a molecular mass between 400 Da and 30 MDa.

3. The method of claim 1 or 2, wherein the applied DC electric field is essentially uniform inside an enrichment section of the microfluidic channel (21).

4. The method of any one of the preceding claims, wherein the sample droplets have a length-to-width ratio of at least 3 when they are transported through the microfluidic channel (21).

5. The method of any one of the preceding claims, wherein the sample droplets (62) are transported through the microfluidic channel (21) at a velocity between 2 and 20 mm/s, preferably between 5 and 10 mm/s.

6. The method of any one of the preceding claims, further comprising splitting each sample droplet (62) into first and second daughter droplets (66, 67) when the sample droplet (62) exits the microfluidic channel (21) in such a manner that the first daughter droplet (66) has a higher concentration of the analyte than the second daughter droplet (67).

7. A microfluidic device for increasing a concentration of a charged analyte in a sample liquid, the microfluidic device defining a device plane, the microfluidic device comprising:

a droplet generator (10) configured to create a stream of sample droplets (62) comprising a sample liquid, the sample droplets (62) being separated by a separating fluid (61) that is immiscible with the sample liquid;
a microfluidic channel (21) disposed downstream from the droplet generator (10) for sequentially receiving the sample droplets (62) separated by the separating fluid (61), the microfluidic channel (21) defining a flow direction (F) in the device plane; and
first and second electrodes (22, 23) for applying a DC electric field across the microfluidic channel (21), the first and second electrodes (22, 23) delimiting the microfluidic channel towards opposite lateral sides of the microfluidic channel (21).

8. The microfluidic device of claim 7, wherein the first and second electrodes (22, 23) have surfaces towards the microfluidic channel that are parallel to one another and parallel to the flow direction, and/or wherein the first and second electrodes (22, 23) are congruent when viewed in the device plane perpendicular to the flow direction (F).

9. The microfluidic device of claim 7 or 8, wherein the first electrode (22) comprises a first membrane (24) and the second electrode (23) comprises a second membrane (25), each of the first and second membranes (24, 25) being made of a composite material that is non-metallic and preferably comprises non-metallic electrically conductive particles embedded in a polymeric matrix.

10. The microfluidic device of claim 9, wherein the electrically conductive particles are carbon particles, in particular, carbon nanotubes, and/or wherein the polymeric matrix comprises a silicon-based polymer, in particular, PDMS.

11. The microfluidic device of claim 9 or 10, wherein each of the first and second membranes (24, 25) has a width between 50 and 200 micrometers, measured in the device plane and perpendicular to the flow direction (F).

12. The microfluidic device of any one of claims 9 to 11, wherein the first electrode (22) comprises a first electrode channel (26), wherein the first membrane (24) separates the microfluidic channel (21) from the first electrode channel (26), wherein the second electrode (23) comprises a second electrode channel (27), wherein the second membrane (25) separates the microfluidic channel (21) from the second electrode channel (27), and wherein the first and second electrode channels (26, 27) are filled with an electrically conductive liquid.

13. The microfluidic device of any one of claims 7 to 12, further comprising a droplet splitter (30) disposed downstream from the microfluidic channel (21) for splitting each sample droplet (62) into first and second daughter droplets (66, 67) when the sample droplet (62) exits the microfluidic channel (21).

14. A microfluidic system comprising:

the microfluidic device (1) of any one of claims 7 to 13, and
a DC voltage source (41) connected to the first and second electrodes (22, 23) of the microfluidic device (1) for creating a DC electric field across the microfluidic channel (21).

15. Use of the microfluidic device of any one of claims 7 to 13 in a method for increasing a concentration of an analyte in a sample liquid according to any one of claims 1 to 6.

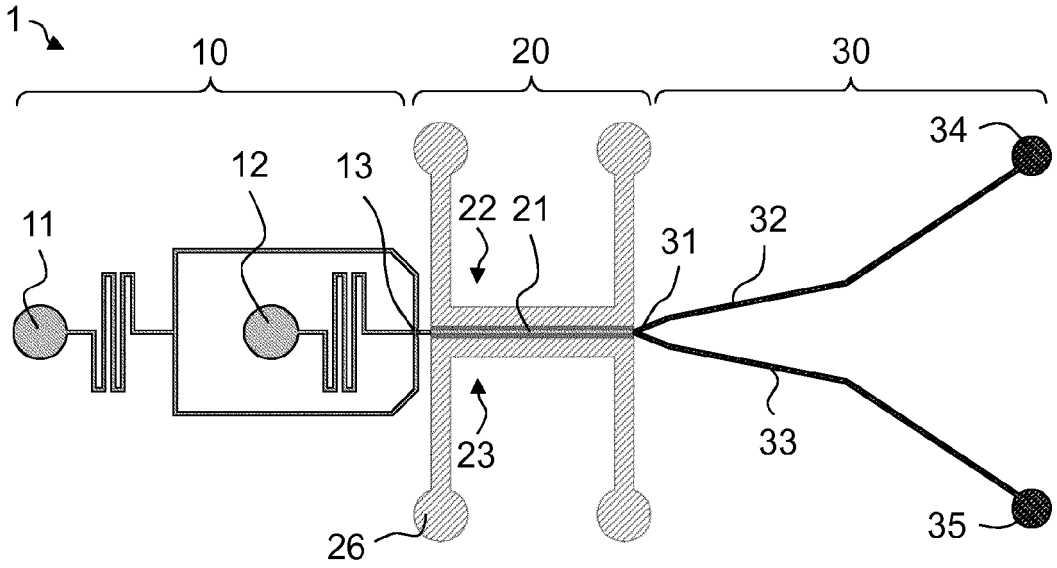
1
10
20
30
11
12
13
22
21
31
32
34
23
33
26
35

FIG. 1

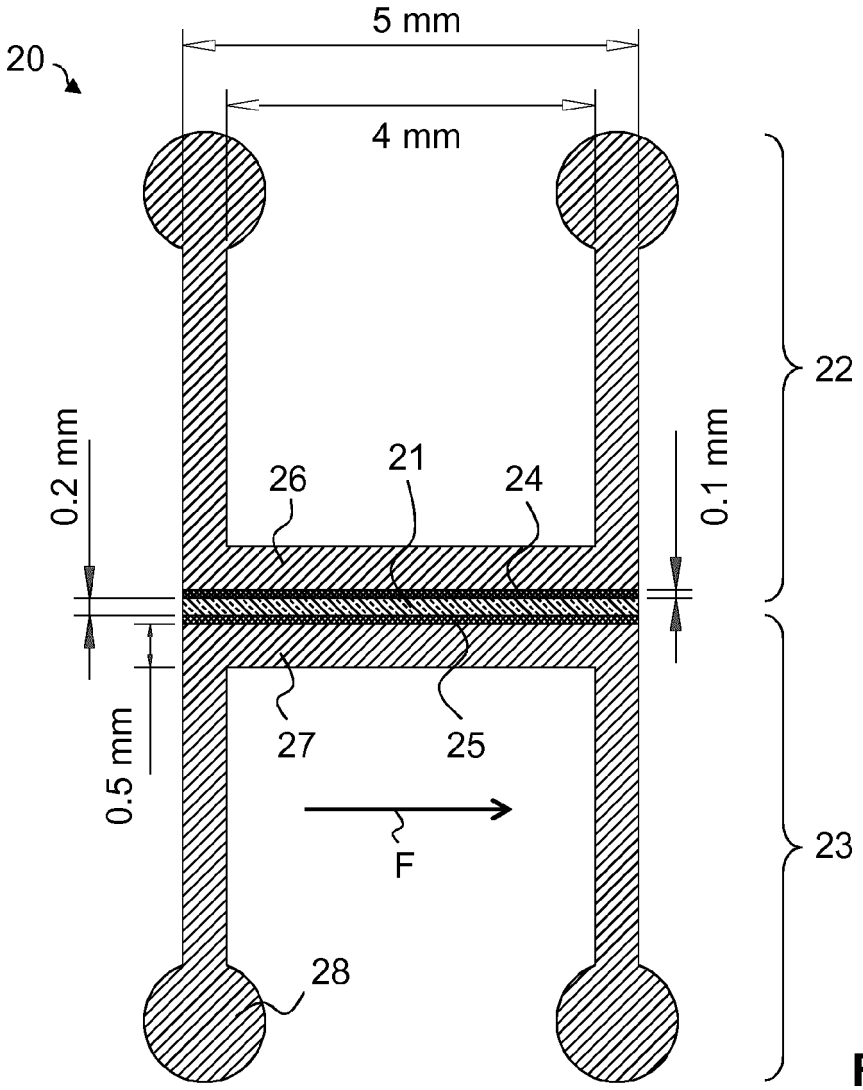
20
5 mm
4 mm
22
0.2 mm
26
21
24
0.1 mm
0.5 mm
27
25
23
F
28

FIG. 2

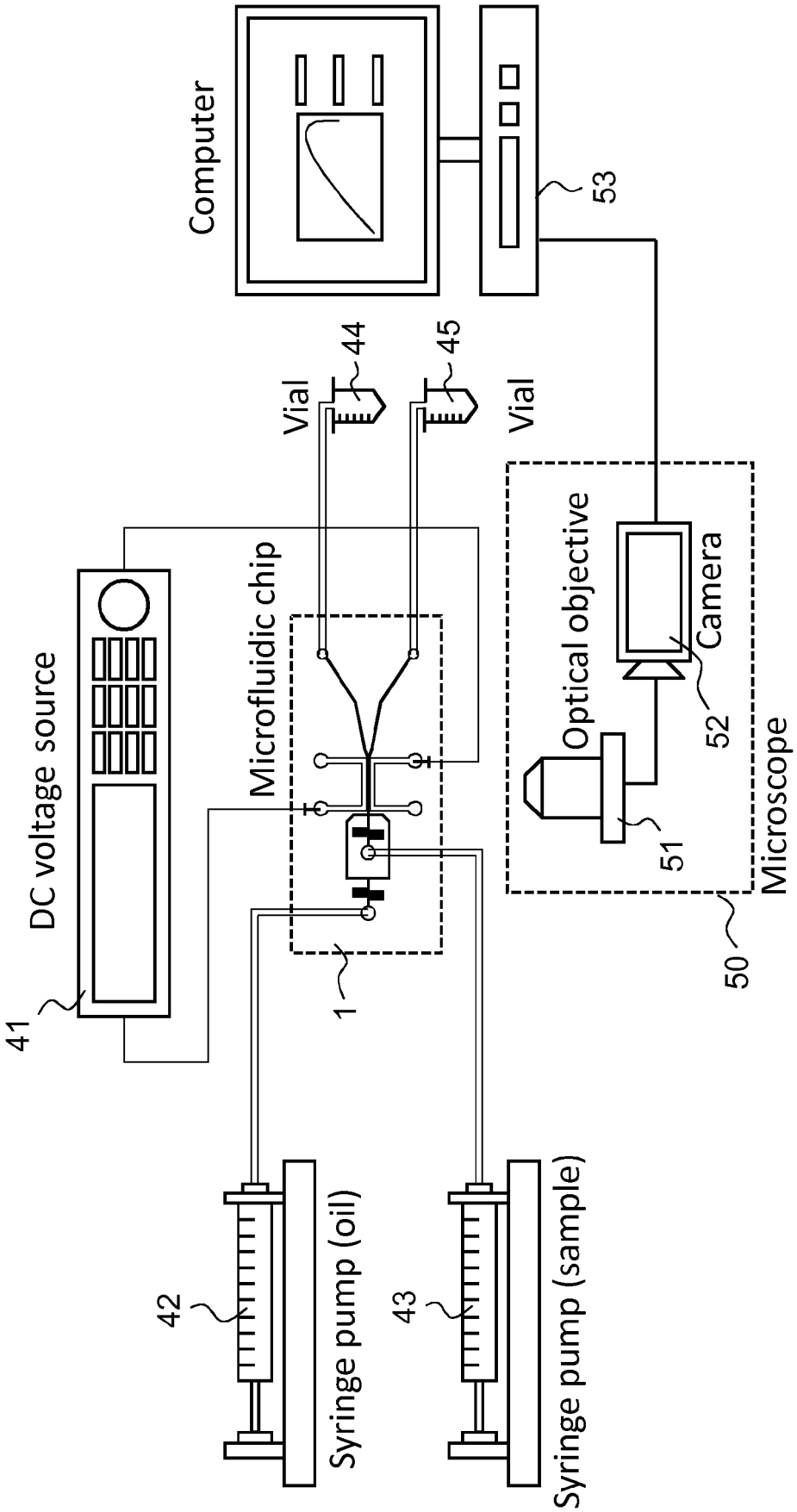
Computer
53
Vial
44
45
Vial
Optical objective
Camera
52
Microscope
50
51
Microfluidic chip
1
DC voltage source
41
42
Syringe pump (oil)
43
Syringe pump (sample)

FIG. 3

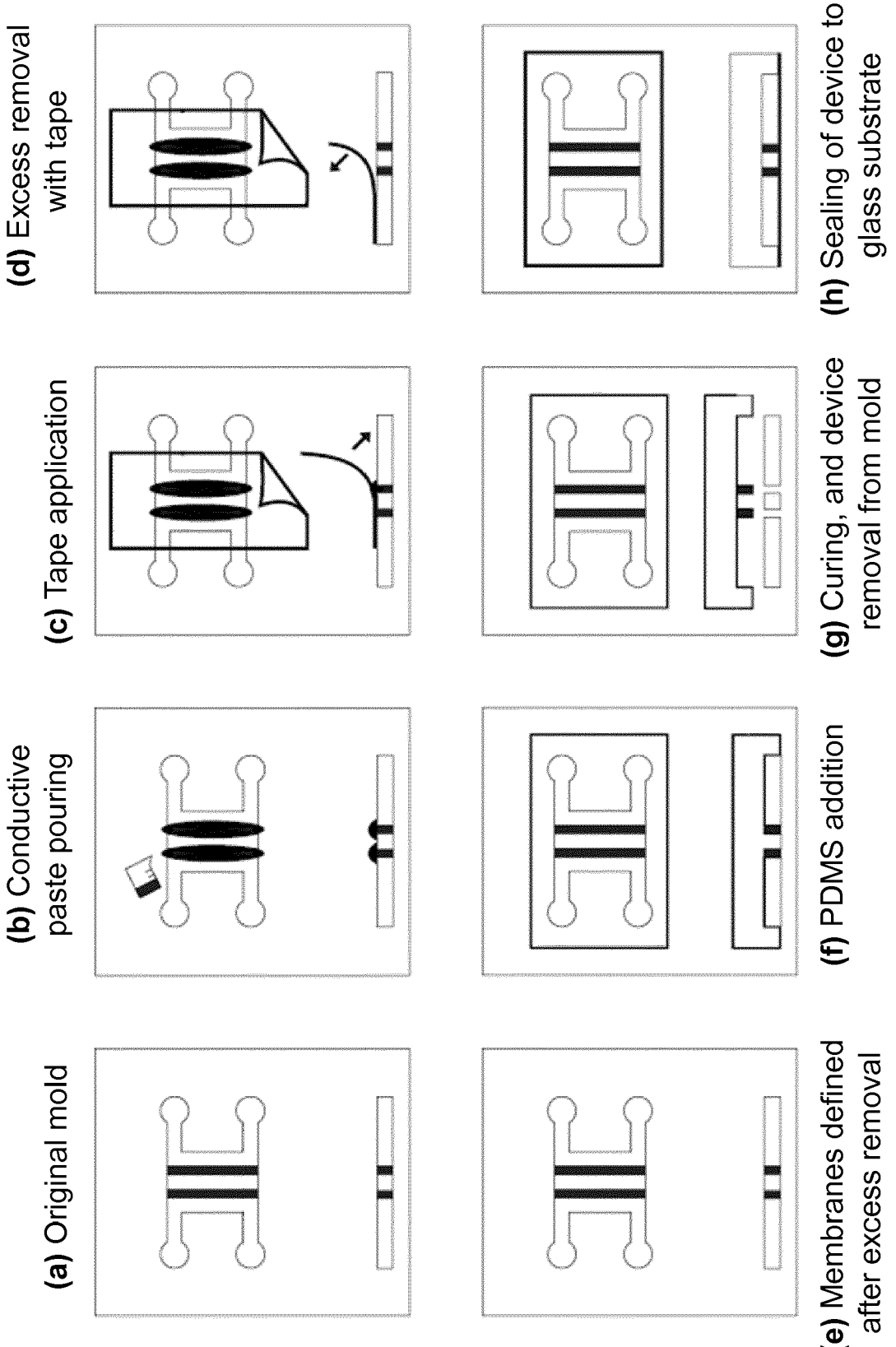
(a) Original mold
(b) Conductive paste pouring
(c) Tape application
(d) Excess removal with tape
(e) Membranes defined after excess removal
(f) PDMS addition
(g) Curing, and device removal from mold
(h) Sealing of device to glass substrate

FIG. 4

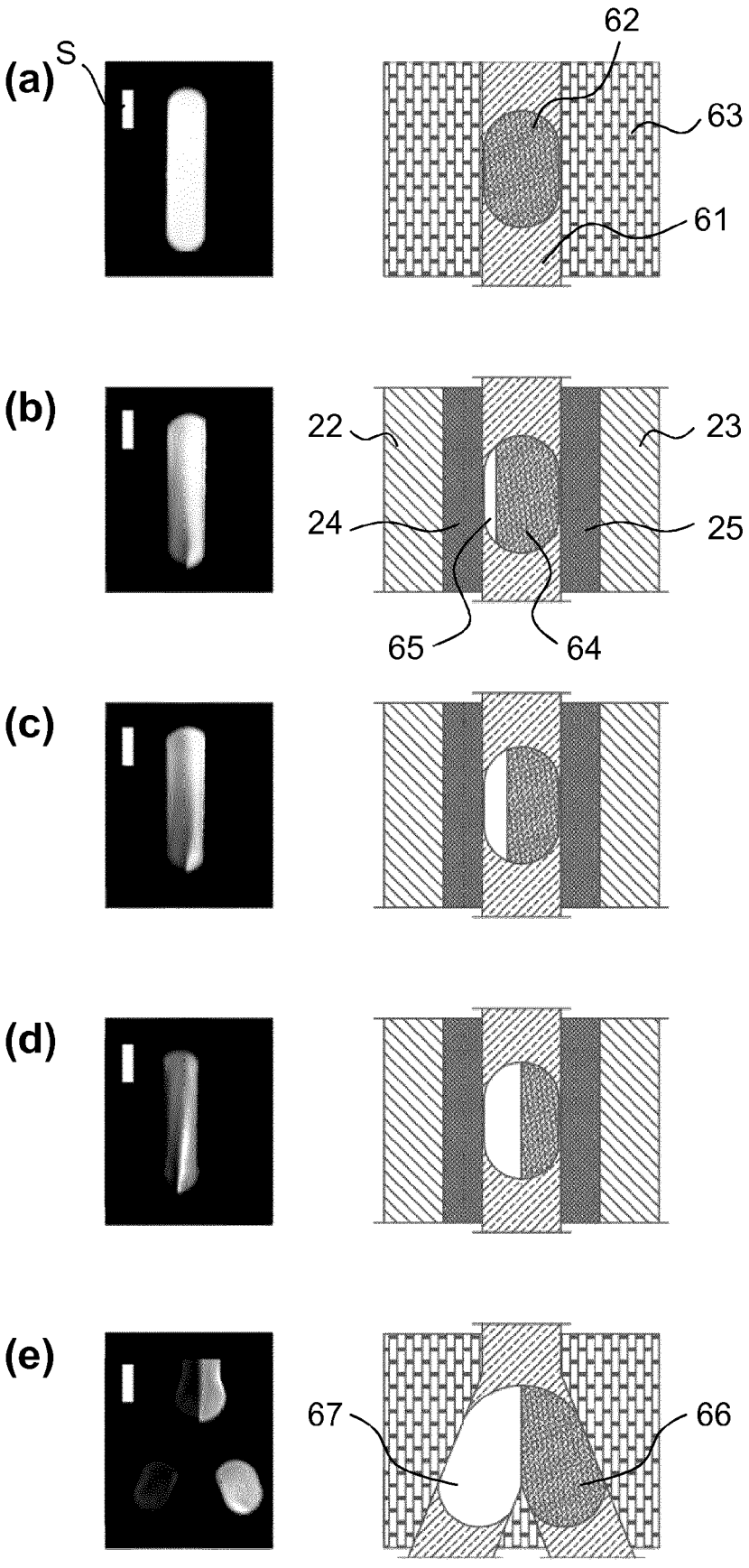

FIG. 5

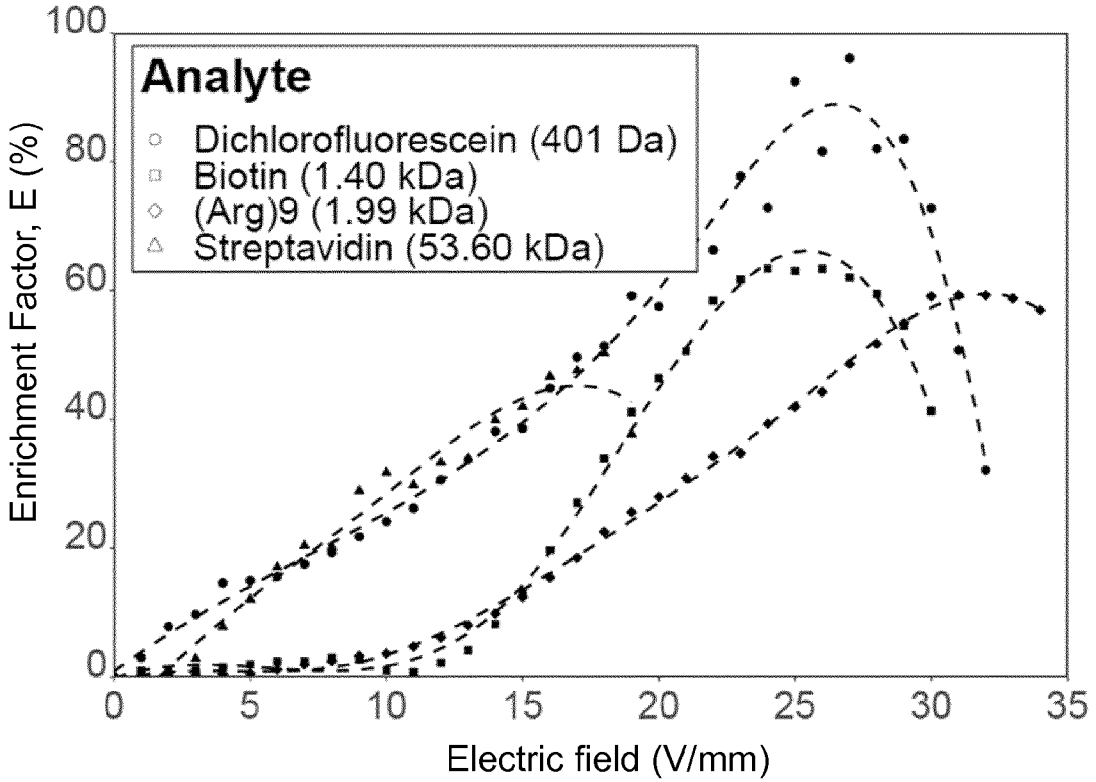
Analyte
Dichlorofluorescein (401 Da)
Biotin (1.40 kDa)
(Arg)9 (1.99 kDa)
Streptavidin (53.60 kDa)
Enrichment Factor, E (%)
Electric field (V/mm)
0
20
40
60
80
100
0
5
10
15
20
25
30
35

FIG. 6

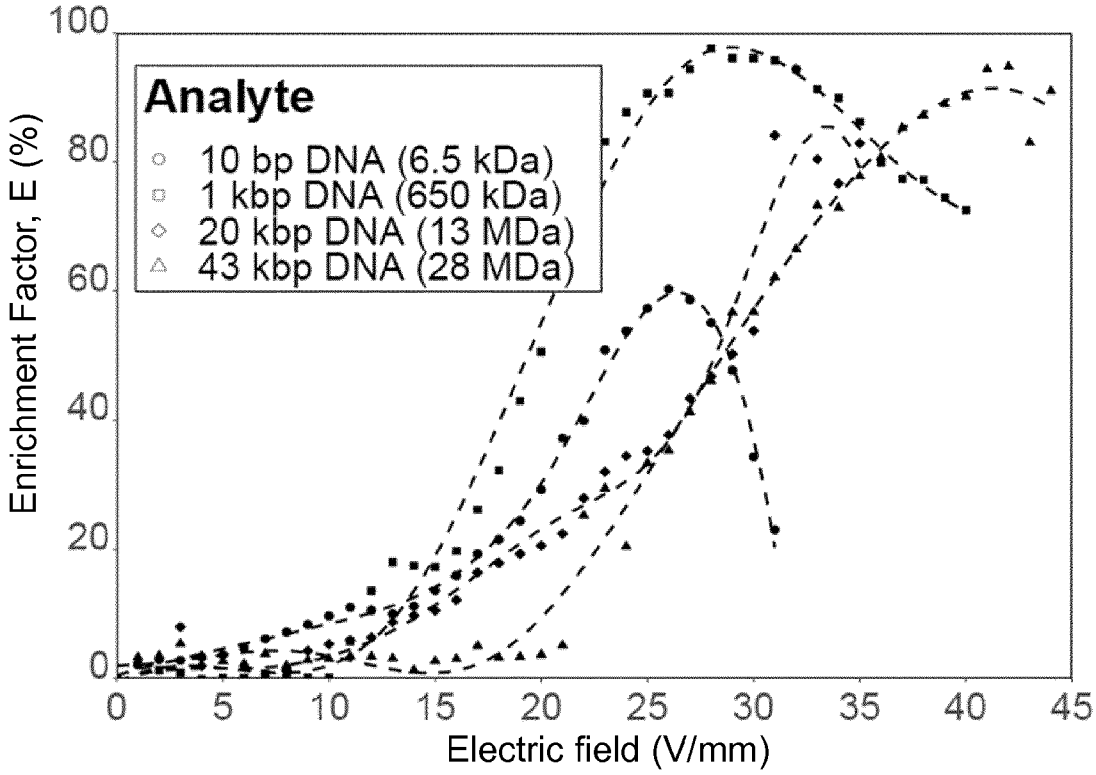
Analyte
10 bp DNA (6.5 kDa)
1 kbp DNA (650 kDa)
20 kbp DNA (13 MDa)
43 kbp DNA (28 MDa)
Enrichment Factor, E (%)
Electric field (V/mm)
0
20
40
60
80
100
0
5
10
15
20
25
30
35
40
45

FIG. 7

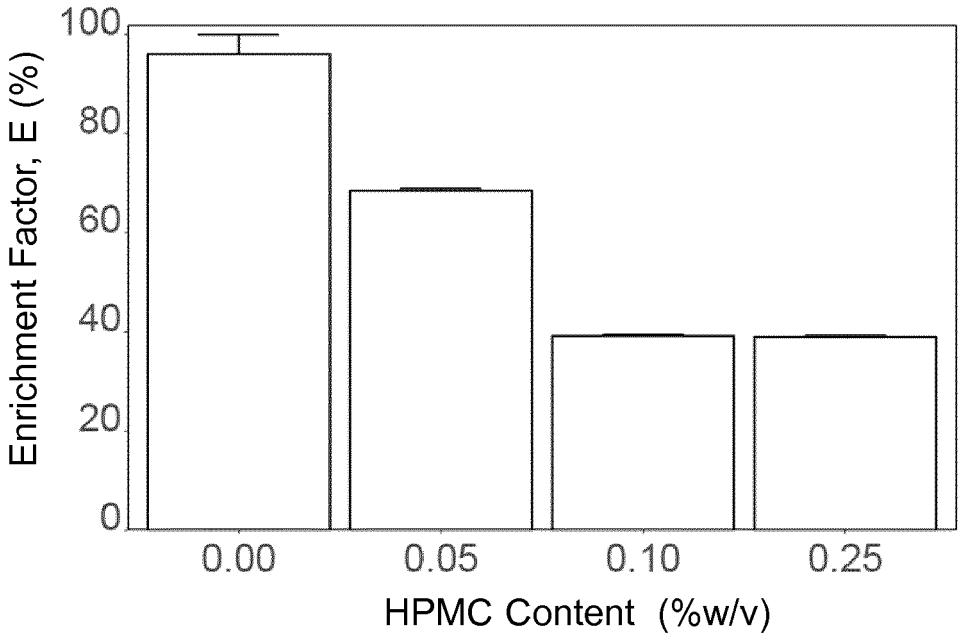
100
80
60
40
20
0
Enrichment Factor, E (%)
0.00
0.05
0.10
0.25
HPMC Content (%w/v)

FIG. 8

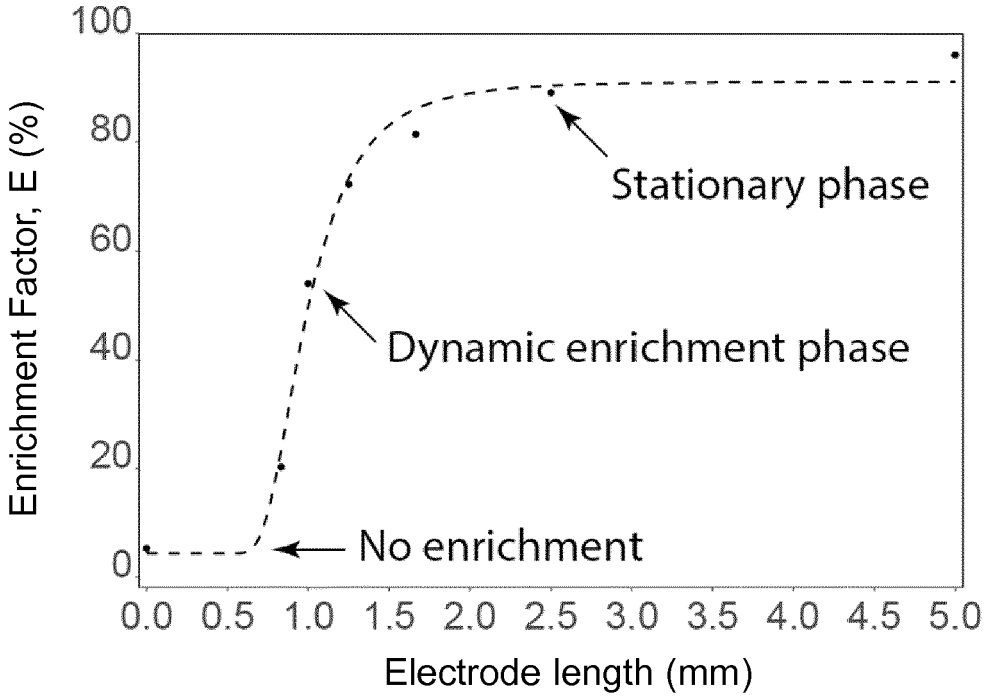
100
80
60
40
20
0
Enrichment Factor, E (%)
Stationary phase
Dynamic enrichment phase
No enrichment
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
Electrode length (mm)

FIG. 9

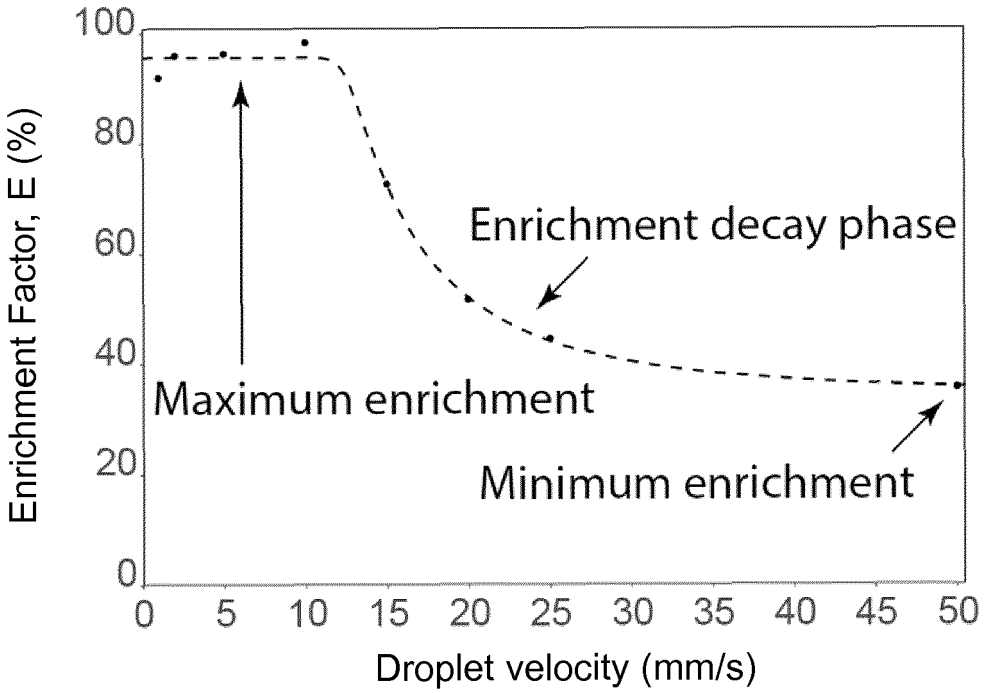
Enrichment Factor, E (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
Droplet velocity (mm/s)
Enrichment decay phase
Maximum enrichment
Minimum enrichment

FIG. 10

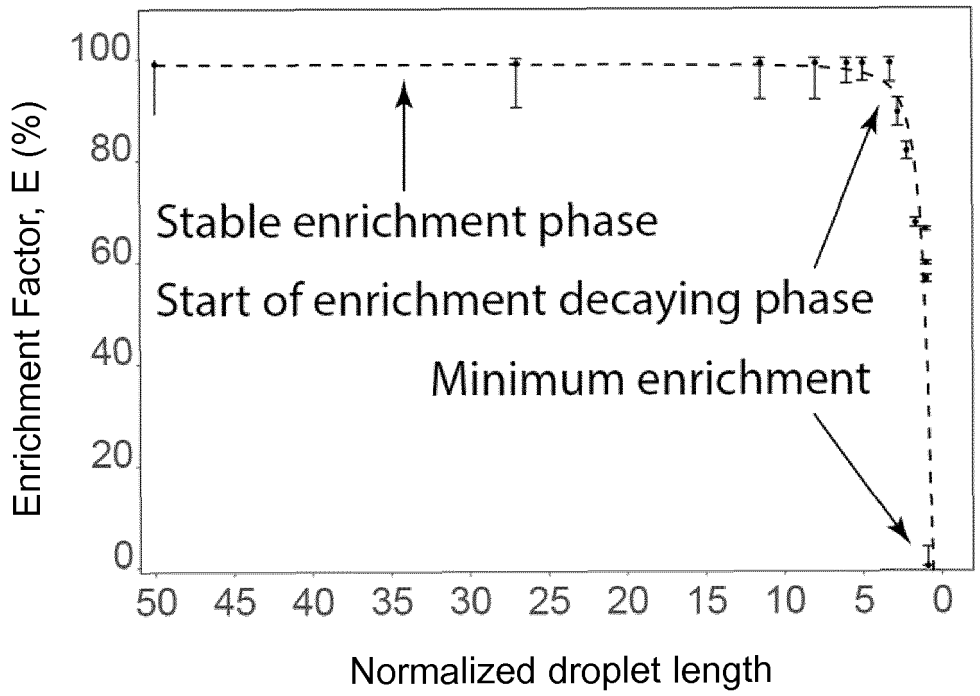
Enrichment Factor, E (%)
100
80
60
40
20
0
50
45
40
35
30
25
20
15
10
5
0
Normalized droplet length
Stable enrichment phase
Start of enrichment decaying phase
Minimum enrichment

FIG. 11

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 19 20 1393

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	US 2019/262834 A1 (MERTEN CHRISTOPH A [DE] ET AL) 29 August 2019 (2019-08-29) * paragraphs [0018] - [0024], [0031] - [0037], [0059], [0072] - [0073], [0092], [0099] - [0105]; figures 1, 7 *	1,2,4-8, 13-15	INV. B01L3/00
X	US 2012/196288 A1 (BEER NEIL REGINALD [US]) 2 August 2012 (2012-08-02) * paragraphs [0022] - [0025], [0035] - [0036], [0041]; figures 1, 5A *	7-13	
X	US 2010/022414 A1 (LINK DARREN [US] ET AL) 28 January 2010 (2010-01-28) * paragraphs [0114], [0136] - [0140], [0145], [0202]; figures 1, 3 *	1-6,15	
A	US 2017/173580 A1 (LOWE JR RANDALL D [US] ET AL) 22 June 2017 (2017-06-22) * paragraphs [0130], [0150]; figure 1C *	9-12	

TECHNICAL FIELDS SEARCHED (IPC)

B01L

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	24 February 2020	Ruiz-Echarri Rueda

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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24-02-2020

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2019262834 A1	29-08-2019	EP 3515598 A1	31-07-2019
		US 2019262834 A1	29-08-2019
		WO 2018054975 A1	29-03-2018
US 2012196288 A1	02-08-2012	US 2012196288 A1	02-08-2012
		US 2014255946 A1	11-09-2014
US 2010022414 A1	28-01-2010	EP 2315629 A1	04-05-2011
		US 2010022414 A1	28-01-2010
		US 2017028365 A1	02-02-2017
		US 2018353913 A1	13-12-2018
		WO 2010009365 A1	21-01-2010
US 2017173580 A1	22-06-2017	NONE	

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description

- **M. HEIN ; M. MOSKOPP ; R. SEEMANN.** *Lab Chip,* 2015, vol. 15, 2879-2886 **[0005]**
- **S.-I. HAN ; H. SOO KIM ; A. HAN.** *Biosens. Bioelectron.,* 2017, vol. 97, 41-45 **[0006]**
- **E. BROUZES ; T. KRUSE ; R. KIMMERLING ; H. H. STREY.** *Lab Chip,* 2015, vol. 15, 908-919 **[0007]**
- **Y. WANG ; Y. ZHAO ; S. K. CHO.** *J. Micromechanics Microengineering,* 2007, vol. 17, 2148-2156 **[0008]**
- **A. FORNELL ; M. OHLIN ; F. GAROFALO ; J. NILSSON ; M. TENJE.** *Biomicrofluidics,* 2017, vol. 11, 031101 **[0009]**
- **J. SCOTT EDGAR ; CHAITANYA P. PABBATI ; ROBERT M. LORENZ ; MINGYAN HE ; GINA S. FIORINI ; D. T. CHIU.** *Anal. Chem.,* 2006, vol. 78, 6948-6954 **[0011]**
- **G. T. ROMAN ; M. WANG ; K. N. SHULTZ ; C. JENNINGS ; R. T. KENNEDY.** *Anal. Chem.,* 2008, vol. 80, 8231-8238 **[0011]**
- **M. WANG ; G. T. ROMAN ; M. L. PERRY ; R. T. KENNEDY.** *Anal. Chem.,* 2009, vol. 81, 9072-9078 **[0011]**
- **X. NIU ; F. PEREIRA ; J. B. EDEL ; A. J. DE MELLO.** *Anal. Chem.,* 2013, vol. 85, 8654-8660 **[0011]**
- **P. NOVO ; D. JANASEK.** Current advances and challenged in microfluidic free-flow electrophoresis - A critical review. *Analytica Chimica Acta,* 2017, vol. 991, 9-29 **[0012]**