



US 20120181178A1

(19) **United States**(12) **Patent Application Publication**
LAMAGNA et al.(10) **Pub. No.: US 2012/0181178 A1**(43) **Pub. Date: Jul. 19, 2012**(54) **FLUIDIC DEVICE FOR ELECTROPHORESIS
OF MOLECULES**(22) Filed: **Dec. 12, 2011**(30) **Foreign Application Priority Data**

Dec. 14, 2010 (AR) P-2010-01-04628

Publication Classification(51) **Int. Cl.**
C25B 7/00 (2006.01)(52) **U.S. Cl.** **204/600**(57) **ABSTRACT**

A fluidic device is disclosed for making electrophoresis of molecules. The device has channels of closed geometry and variable powers over time. Migration of molecules is contained within channel of closed geometry, whereby they can circulate again and again, thus separating the molecules at a wide migration distance. The device has an injection system which is a secondary channel which intersects the main channel. An arrangement of electrodes along this main channel connected to a variable voltage source with multiple outlets which regulates the value of the electric power in each electrode, and the frequency at which the same varies from one electrode to another. Thus it is achieved to generate a periodic arrangement of powers along the channel which set the flow of the sample optimizing the separation process. Electrodes can be added for the detection of molecules by measuring the changes in electric resistivity, current or voltage.

(75) Inventors: **Alberto LAMAGNA**, Provincia de Buenos Aires (AR); **Maximiliano Sebastián PÉREZ**, Provincia de Buenos Aires (AR); **Betiana Lerner**, Provincia de Buenos Aires (AR); **Claudio Berli**, Provincia de Buenos Aires (AR); **Andrés Felipe ORDONEZ**, Provincia de Buenos Aires (AR); **Alfredo BOSELLI**, Provincia de Buenos Aires (AR); **Francisco SACCO**, Provincia de Buenos Aires (AR); **Elda Maria SALMORAL**, Provincia de Buenos Aires (AR); **Andrés KREINER**, Provincia de Buenos Aires (AR); **Carlos Alberto RINALDI**, Provincia de Buenos Aires (AR); **Carlos LASORSA**, Provincia de Buenos Aires (AR)

(73) Assignee: **COMISION NACIONAL DE ENERGIA ATOMICA (CNEA)**, Ciudad Autonoma de Bs. As (AR)

(21) Appl. No.: **13/316,985**

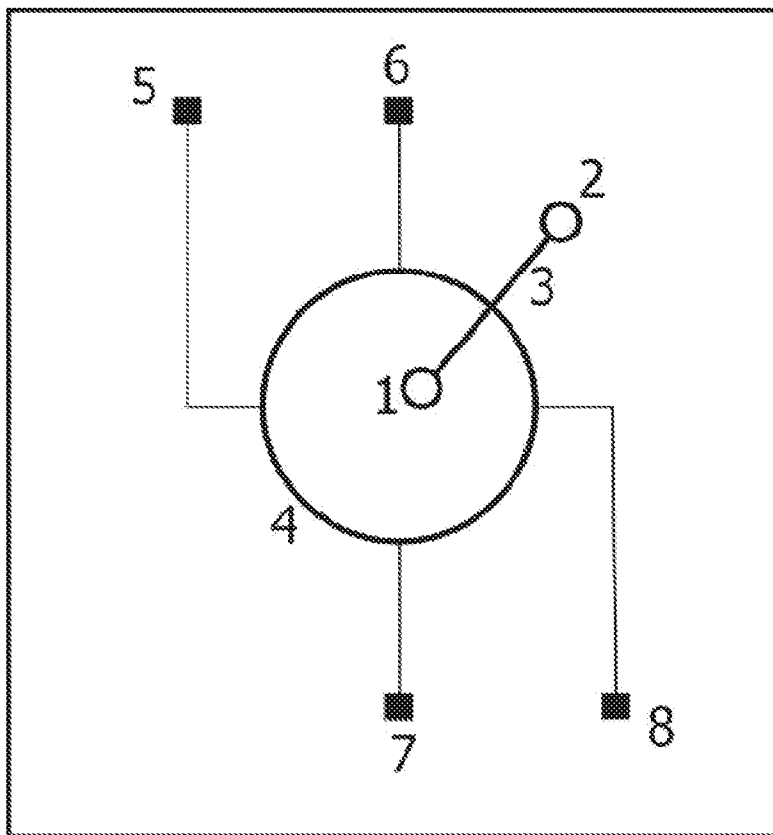


Figure N^o 1.

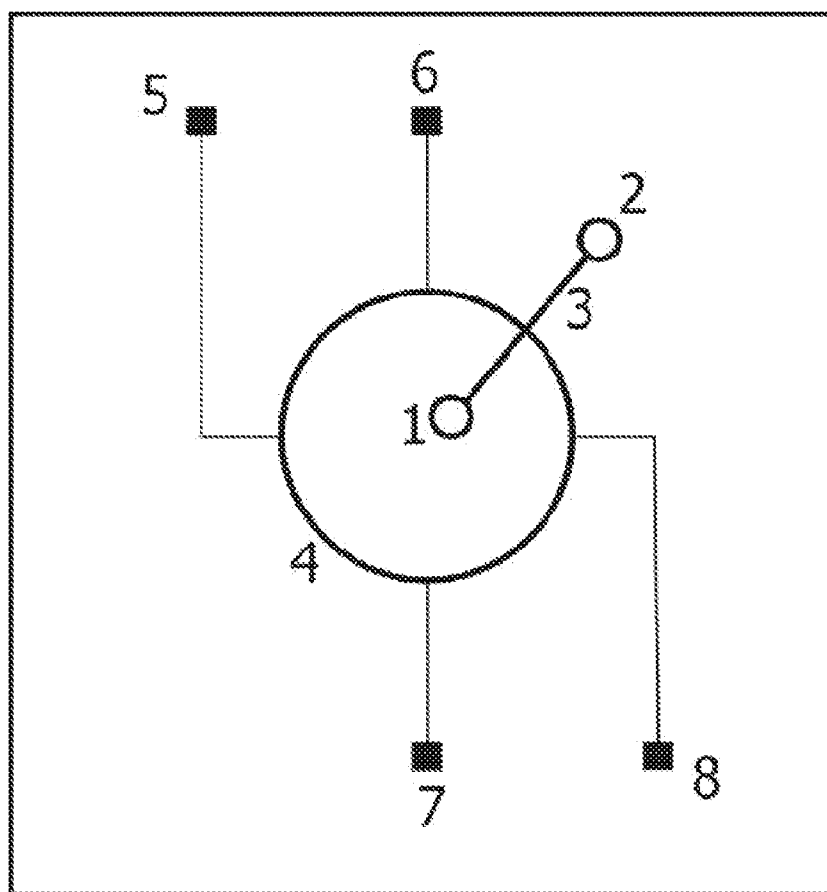


Figure N^o 2.

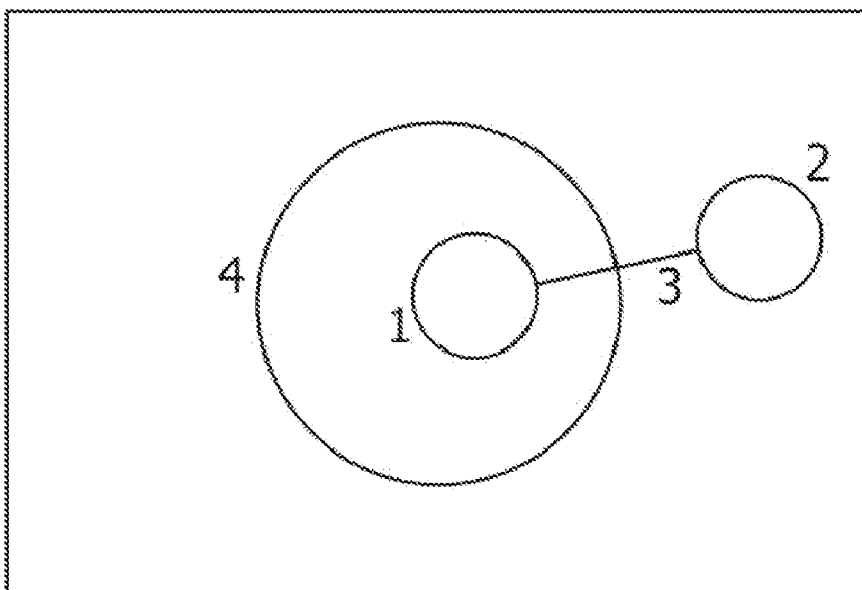


Figure N^o 3.

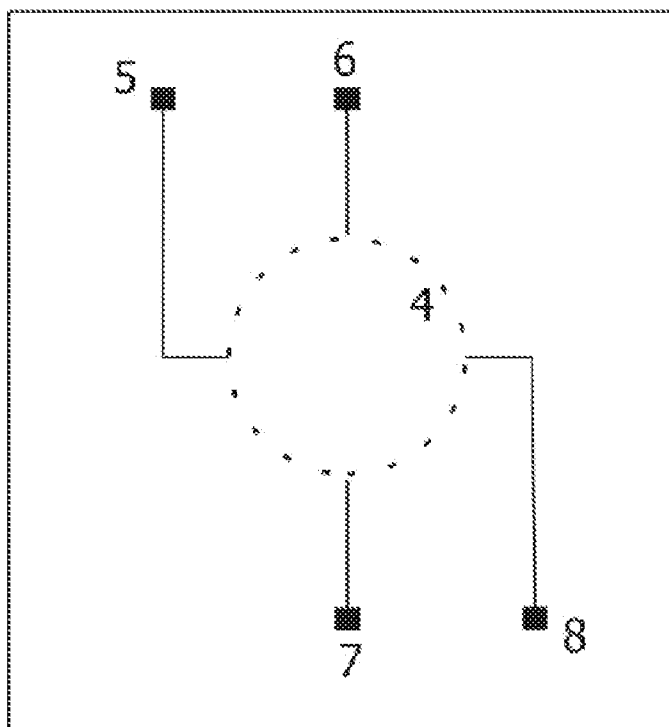
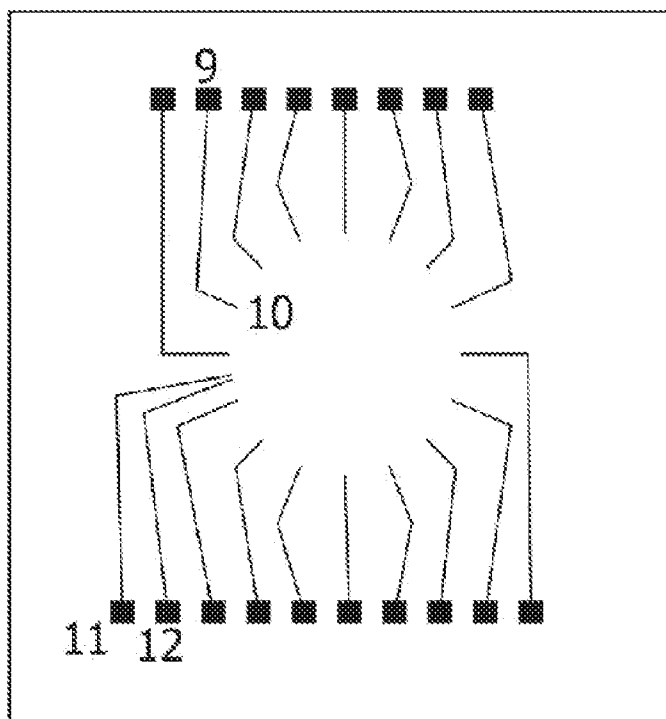


Figure N^o 4.



FLUIDIC DEVICE FOR ELECTROPHORESIS OF MOLECULES

CROSS-REFERENCE TO RELATED U.S. APPLICATIONS

[0001] Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

NAMES OF PARTIES TO A JOINT RESEARCH AGREEMENT

[0003] Not applicable.

REFERENCE TO AN APPENDIX SUBMITTED ON COMPACT DISC

[0004] Not applicable.

BACKGROUND OF THE INVENTION

[0005] 1. Field of the Invention

[0006] This invention refers to a fluidic device for electrophoresis of molecules. The device of the invention has the novel characteristic that it presents closed geometry channels (for example, circular) and variable powers over time.

[0007] The method of electrophoresis consists in the separation of similar molecules, but with a different electric charge and size, due to the migration of the latter from a point A, to a point B. For this purpose a power difference is set between such points, which generates an electroosmotic flow of the biomolecular sample, in response to the electrostatic force exerted on the constituent molecules. This force depends on the electric charge thereof, hence, migration of molecules is not like the motion of a whole, but gradually fragments and separates in groups of molecules with the same electric charge, assuming that there is enough room for that separation to take place.

[0008] Some examples of separation of molecules can be deoxyribonucleic acid (DNA), proteins, sugars, lipids, amino acids, ions, polymers, etc.

[0009] Electrophoresis process is widely used in laboratories worldwide, and its execution contributes to other applications such as for example DNA sequencers, detection of DNA polymorphisms, determination of DNA and protein molecular weight, etc.

[0010] 2. Description of Related Art Including Information Disclosed Under 37 CFR 1.97 and 37 CFR 1.98.

[0011] Nowadays there are devices that are used to perform the electrophoresis technique separating DNA and various molecules. The main characteristic of these devices is that the channel does not have a closed geometry, generally being of a straight line shape, and for the separation of the sample only two electrodes are used at each end of the channel, separation length being limited to the length thereof, which is usually in the order of a few centimeters.

[0012] Among microfluidic devices (Koch et al (Koch et al., 2000; Tabeling, 2005), analysis microsystems are conceived to integrate several laboratory operations: sample and reagent injection, mixing, incubation, reaction, separation, detection, isolation, etc. These microsystems are identified as lab-on-a-chip (LOC), or micro-total-analysis-systems (μ TAS) in international literature (Lion et al., 2004; Walt,

2005; Erickson and Li, 2004; Whitesides, 2006; Dittrich et al., 2006; Chin et al., 2007). They basically consist of a microchannel network which incorporates various sections, chambers, columns and reservoirs. All the components are integrated into a glass or polymeric material plate, in the form of a laboratory slide or compact disc. Flow through the microchannels is set by applying pressure or, more conveniently, electric fields. This last mechanism is the most widely used in analysis microsystems, since it allows to obtain microchannel and electrode chips, without mobile mechanical parts, which uses electric power and can be efficiently controlled (Li, 2004; Stone et al., 2004; Berli, 2008).

[0013] Electrophoresis method of molecules such as DNA and proteins, used in biology and biotechnology laboratories for the separation and analysis of such molecules, was originally implemented in electrophoretic tanks which require a large sample and reagent volume. The main driving force of electrophoresis evolution were the ambitious programs of genetic (gene and protein) engineering programs and thus capillary electrophoresis was born, which allowed to develop sequencers which require a very small sample volume and allow to run several parallel essays. Then, miniaturized capillary electrophoresis in silicon chips (Harrison et al, 2003) led to a considerable time and yield gain (Jacobson et al, 1998; Freemantle, 1999).

[0014] Finally, with nanotechnology boom and for the purpose of integrating all routine molecular biology assays into one single device (Thorsen et al, 2002; Haeberle and Zengerle, 2007), new materials started to be used, such as poly-dimethylsiloxane, PDMS (Duffy et al, 1998). Nowadays there are several microelectrophoretic devices reported in literature (Wu et al, 2008), and several commercially available (Agilent®, Micralyne®, Micronit®, Fluidigm®).

[0015] In current electrophoretic devices, the separation process is limited to channel length, this usually being about 2 to 3 cm. The characteristics of these devices may be appreciated in US patents such as: U.S. Pat. No. 6,010,607, U.S. Pat. No. 7,740,747 B2, U.S. Pat. No. 6,337,740 B1. This is an intrinsic limitation that the device imposes on electrophoresis, narrowing the power offered by the method for making the separation of more complex configurations of molecules. One of the advantages of the device of the invention is solving this limitation. The migration of molecules is contained in the channel of closed geometry, along which they can circulate again and again, thereby separating the molecules in a rotational flow in principle infinite, established by the powers set along the channel. The device of the invention incorporates a voltage source which regulates the value of the electric power in each electrode, and the frequency at which the same varies from one electrode to another. Thus it is achieved to generate a periodic arrangement that establishes a stable rotational flow of the sample optimizing the separation process.

[0016] Another problem presented by the use of current electrophoretic devices, is compromise relationship that there is between the voltages used in the electrodes, the sample to be separated, and the separation time. The sample flows into an aqueous medium, to which a matrix serving as a molecular sieve can be added in order to optimize the process of separation by size, is usually used as an agarose or polyacrylamide matrix. The same is spread over the whole channel of the device, this medium is in turn in contact with the electrodes responsible for setting the electrostatic force. It can be seen that the higher the voltage in the electrodes, the higher is the acceleration experienced by the constituting molecules and

therefore, a reduction in the time that the process lasts is achieved. But when rising voltage, in conventional devices we can see generation of undesirable reactions in the aqueous medium such as electrolysis and heating effects (Joule effect), which generate undesirable bubbles which alter the process. The device of the invention solves this problem of the state of the art by nullifying these undesirable effects, even using high voltages in the electrodes. This is achieved with the process of variation of active electrodes. The channel of closed geometry allows to establish a high voltage between, for example, two consecutive electrodes, and turning them off before heating effects occur, but immediately activating the neighbor electrodes, so that there is always a voltage in the channel that allows to separate the sample. In general the number of electrodes used is over 3, and it can be so large as permitted by the manufacturing process.

BRIEF SUMMARY OF THE INVENTION

[0017] The development of the invention is applied in electrophoresis of molecules on a channel of closed geometry. By closed geometry it is meant any shape of the channel that can offer a way in principle indefinite to any molecule which can run along the same, for example, a channel of a circular, elliptic, triangular, rectangular shape. In general, any channel of a closed loop shape, since it has neither a starting point nor an end point, falls into a category which we call closed geometry, and is a part of the aforementioned invention.

[0018] The device integrates the channel, together with a distribution of several electrodes placed along the channel geometry. By a multiple outlet variable voltage source, it is achieved alternate the value of the voltage in each individual electrode, generating a plurality of configurations of voltage values along the channel which enable to achieve a novel form of the electrophoresis technique therein.

[0019] The development of the invention can be applied to separate any type of molecules of different sizes and electric charges, from a small liquid sample which in principle can contain them all together in a disorderly and non-separated way. This technique is for purposes widely used in biochemistry, biology, molecular biology and nanotechnology laboratories, among others.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0020] In order to improve understanding of this invention, the description of an embodiment of a preferred example of the device is made, based on the following figures:

[0021] FIG. 1 shows a top view of one embodiment of the whole device. In the same, the main channel (4) has a circular shape. Minor circles (1) and (2), joined by the straight injection channel (3), are a part of a starting injection system of the sample to be separated. The arrangement of 4 electrodes (5), (6), (7) and (8) can be seen. The square ends are connected to the external voltage source that controls the value of the electric power of each electrode, as well as the frequency at which each of them is activated, and the time that they last activated. The thin ends of the electrodes end around the main channel (4).

[0022] FIG. 2 shows a top view of an embodiment of the channel. In it, the main channel (4) has a circular shape. Minor circles (1) and (2), joined by straight injection channel (3), are a part of a starting injection system of the sample to be separated.

[0023] FIG. 3 shows a top view of an arrangement of 4 electrodes, (5), (6), (7) and (8). The square ends are connected to the external voltage source which controls the electric power value of each electrode, as well as the frequency at which each of them is activated, and the time that they last activated. The thin ends of the electrodes end around main channel (4).

[0024] FIG. 4 shows a more complex alternative in the number of electrodes. The same as in FIG. 3, ends (9) and (10) of each electrode are connected to the external source and to the contour of the channel respectively. Electrodes (11) and (12) can also be added which are useful for detection of molecules. As examples of detection with electrodes (11) and (12), an amperometric detection can be made, achieving changes in the values of electric current which runs along electrodes (11) and (12) when they pass between the molecules separated. Another example of detection with electrodes (11) and (12) consists of placing carbon nanotubes between them and measuring the changes in their resistance when they interact with the molecules separated that run within the channel. The thin ends of these detection electrodes are closer than those of the remaining ones, so that changes in electric resistivity, current or voltage within the channel when the samples pass between them, helping to monitor molecule flow into it. Electrodes (11) and (12) are connected to an external device able to detect the aforementioned parameters.

DETAILED DESCRIPTION OF THE INVENTION

[0025] Detection of molecules in the channel can be made by using other known elements and methods, such as: fluorescence spectroscopy, nanotube-based nanosensor systems, system of detection of passage of molecules through nanopores placed at specific points of the channel.

[0026] In general, the manufacturing process of one of the embodiments of the device of the invention, by using as the manufacturing material of the channel, the PDMS polymer consisting of the following steps:

[0027] a) Creating masks: A design software is used for designing the masks which shape the geometry of the channel, and the arrangement of the electrodes.

[0028] b) Lithography on the substrate: in this process the shapes of the channel and the arrangement of the electrodes are created, on the respective manufacturing substrates. In the case of the channel, one example of lithography is that made on a silicon wafer on which the channel mask which keeps its shape is printed with photosensitive resin. After an exposure to ultraviolet radiation and subsequent developing, a high relief of the channel of the desired shape and thickness is printed on silicon. In the case of the electrodes, one example of lithography is that made on a glass sheet, also by using a photosensitive resin, the form of distribution of the electrodes to be used is achieved to be printed on the glass.

[0029] c) Deposition of metal for shaping the electrodes: with a metal evaporation process on the substrate containing photoresin which keeps the form of distribution of the electrodes, making thereof on a conductive material is achieved, optimal for setting the variable powers along the same.

[0030] d) Manufacturing the channel on PDMS: on the silicon substrate with photoresin which keeps the high relief of the channel, the deposition of PDMS polymer having the property of copying the shape of the mold on which it is deposited, is made. After a curing and drying process, the PDMS is separated from the wafer and a three-dimensional negative of the channel designed ready to be used, is obtained.

[0031] e) Joining the electrodes to the channel: by a process of junction by oxygen plasma, the glass substrate having the electrodes printed in a conductive material is bonded, the three-dimensional being manufactured in PDMS, this forms the sealed final device which offers a hollow three-dimensional channel, of a desired shape, which rests on the glass substrate which has on its contour the electrodes which follow the channel geometry.

[0032] Having described and determined the nature and scope of this invention and the way in which the same shall be practiced, the following is declared to be claimed as the invention and of exclusive property.

We claim:

1. A fluidic device for electrophoresis of molecules, having an injection system of molecules to a main channel wherein the electrophoresis process is carried out, comprising such main channel having a closed geometry, with 3 or more electrodes arranged along the channel, connected to a variable voltage source of multiple outlets.

2. A fluidic device for electrophoresis of molecules, as claimed in claim 1, further comprising 2 detection electrodes for detecting the molecules, by measuring some physical variable.

3. A fluidic device for electrophoresis of molecules, as claimed in claim 1, wherein such main channel of a closed geometry has a circular shape and such variable voltage source with multiple outlets, regulates the value of electric power in each electrode and the frequency at which the same varies from one electrode to another.

4. A fluidic device for electrophoresis of molecules, as claimed in claim 3, further comprising 2 detection electrodes for detecting molecules, by measuring some physical variable.

5. A fluidic device for electrophoresis of molecules, as claimed in claim 4, wherein such detection electrodes make the detection of molecules, by detecting changes in electric resistivity, current or voltage.

6. A fluidic device for electrophoresis of molecules, as claimed in claim 1, wherein such molecule injection system is an injection channel intersecting such main channel wherein the electrophoresis process is carried out, having a supply at one end and a discharge at the other end.

7. A fluidic device for electrophoresis of molecules, as claimed in claim 4, wherein such molecule injection system is an injection channel which intersects such main channel wherein such electrophoresis process is carried out, having a supply at one end and a discharge at the other end.

8. A fluidic device for electrophoresis of molecules, as claimed in claim 5, wherein such molecule injection system is an injection channel which intersects such main channel wherein the electrophoresis process is carried out, having a supply at one end and a discharge at the other end.

9. A fluidic device for electrophoresis of molecules, as claimed in claim 3, wherein such main channel of a circular shape is made in polydimethylsiloxane (PDMS).

10. A fluidic device for electrophoresis of molecules, as claimed in claim 3, wherein such main channel of circular shape is made in acrylic.

11. A fluidic device for electrophoresis of molecules, as claimed in claim 3, wherein such main channel of circular shape is made in glass.

12. A fluidic device for electrophoresis of molecules, as claimed in claim 3, wherein such main channel of circular shape is made in silicon.

* * * * *