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(54) Title: PREPARATION OF SAMPLES FOR CONSTITUENT ANALYSIS

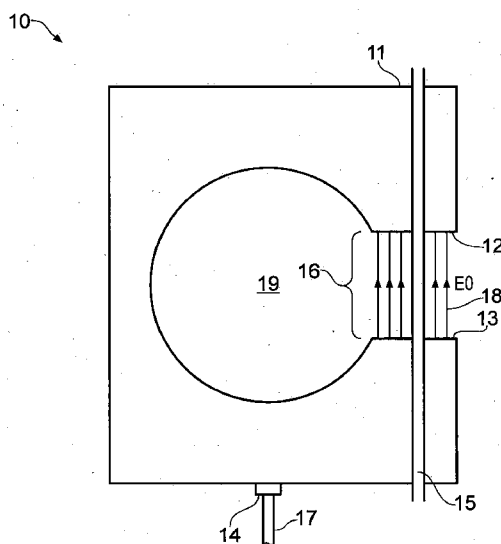


FIG. 1

(57) Abstract: A device for the preparation of samples for constituent analysis is provided. The device comprises a conductive body has a first face and a second face separated by a gap. A channel for containing a sample runs through the gap. The conductive body is arranged to produce, upon application of microwaves, parallel E-field lines extending in a first direction in a region of the gap. The channel runs in a second direction in the region and the angle between the first and second directions is not more than 20 degrees. The electric field transfers energy to the sample.

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The Preparation of Samples for Constituent Analysis

Technical field

The present invention relates to the preparation of samples for constituent analysis.

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Background

Sample analysis has applications in many areas, for example the medical industry, diagnostics, and manufacturing. Samples are analysed to determine their constituent components. Samples requiring analysis are often fluids. One context is DNA analysis.

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DNA analysis is particularly useful in medical diagnosis. For analysis of bacterial DNA, the DNA contained in bacterial cells and spores must be released through their rupture (lysis). It is known that DNA can be liberated from bacteria by mechanically vibrating a liquid sample containing the bacteria. However, this approach can take an undesirably long time.

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Summary of invention

According to a first aspect of the present invention, there is provided a device for rupturing bacteria, the device comprising: a conductive body comprising a first face and a second face, wherein the first and second faces are separated by a gap; an input for introducing microwaves to the conductive body; and a channel for containing a sample; wherein: the conductive body is arranged to produce, upon application of microwaves at the input, parallel E-field lines extending in a first direction in a region of the gap; the channel runs in a second direction in the region; and the angle between the first and second directions is not more than 45 degrees. Thus, the invention provides a device for rupturing bacteria, in which efficient transfer of energy from the microwaves to a sample under analysis is not overly impeded by polarisation of the sample. This can reduce the time required to rupture bacteria within the sample.

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In certain embodiments, the angle is not more than 10 degrees. This embodiment provides a greater efficiency of energy transfer to the sample, reducing the time required to rupture bacteria within the sample.

In certain other embodiments, the angle is substantially zero. This orientation provides a particularly efficient transfer of energy to the sample, and minimises the time required to rupture bacteria within the sample.

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There is also provided a method of rupturing bacteria using a device, the method comprising: applying microwaves to a conductive body via an input, the conductive body comprising a first face and a second face, wherein the first and second faces are separated by a gap and, due to the microwaves, parallel E-field lines extend in a first
10 direction in a region of the gap; and inserting a sample into a channel, wherein the channel runs in a second direction in the region and, the angle between the first and second directions is not more than 45 degrees. Thus, the invention provides a method for rupturing bacteria, in which efficient transfer of energy from microwaves to a sample under analysis is not overly impeded by polarisation of the sample. This can
15 reduce the time required to rupture bacteria within the sample.

Brief description of the drawings

For a better understanding of the present invention, and to show more clearly how it may be carried into effect, reference will now be made, by way of example, to the
20 following drawings, in which:

Figure 1 is a cross-sectional but schematic view of a microwave device for sample preparation;

Figure 2 is a perspective but schematic view of the microwave device shown
25 in Figure 1;

Figure 3 is a schematic view of an idealized, thin, cylindrical column of fluid positioned in, and at a first orientation to, a parallel electric field;

Figure 4 shows the same column as figure 3 but at a different orientation to the field;

30 Figure 5 shows the same column as figures 3 and 4 but at a different orientation to the field;

Figure 6 shows a schematic representation of a microwave device for sample preparation wherein the sample channel does not pierce the conductive body; and

Figure 7 shows a schematic representation of a further microwave device for sample preparation.

5

Detailed description

Figures 1 and 2 show a microwave device 10 for sample preparation. The microwave device 10 includes a conductive body 11. The body 11 may be made from an electrically conductive material that may be magnetic or non-magnetic. The material may be metal, for example copper. The conductive body 11 is shaped into a split-ring shape.

The ring-shaped body 11 has a split in one side, which forms a gap 16. A first face 12 and a second face 13 of the conductive body 11 lie on either end of the ring and sit either side of the gap. The first and second faces 12 and 13 are flat and parallel. In other examples, the faces may be uneven, and may not be parallel. The conductive body 11 surrounds a hollow central cavity 19. The body 11 has, in the cross-section shown in Figure 2, a square outer edge. However, the body 11 could conceivably be made in any other regular shape (for example it could have a circular outer edge) or, indeed, the body 11 could have an irregular shape.

The device 10 has a port 14. Feed line 17 is conductively connected to the port 14 to apply microwaves to the conductive body 11. The microwaves are applied at a predetermined nominal frequency (though in practice the microwaves will have a certain bandwidth).

Upon the application of microwaves at the port 14, the conductive body 11 conducts an electrical current that oscillates when the frequency of the microwaves is at or around the natural resonant frequency of the split-ring body. The oscillating current in the body 11 creates an oscillating electric field E_0 that extends between the faces 12 and 13 in much the same way that an alternating current source connected to a capacitor will establish an oscillating electric field between the plates of the capacitor.

The electric field E_0 oscillates at the microwave resonant frequency. As shown, the port 14 is positioned on the body so as to couple energy from the microwaves from the feed line 17 into magnetic field of that is established in the cavity 19. The port 14 could be positioned differently on the body 11, however.

5

The electric field E_0 is shown by field lines 18, and the direction of the field is indicated by the direction of the arrow heads. The field lines are parallel across a region spanning the majority of the gap 16. The field lines run perpendicular to the first and second faces in that region. Although the electric field is parallel across the region, the field lines may bow out at the edges of the gap.

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The device 10 has a tube 15 running through the conductive body 11. The tube 15 passes through the gap 16 and, as shown in Figures 1 and 2, through the first and second faces 12 and 13. The tube 15 defines a channel for containing a sample.

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The tube 15 runs parallel to the direction of the field lines of the electric field E_0 . The tube 15 pierces the conductive body 11 such that the tube runs through the first face 12 and the second face 13. In Figure 1, the full length of the tube 15 in the gap 16 runs parallel to the field E_0 and perpendicular to the first and second faces 12 and 13. However, in other variants of the device 10, only part of the tube 15 may run parallel to the field E_0 (for example, as shown in Figure 6).

20

When microwaves are applied to the conductive body 11 and an electric field is established in the gap 16, there is a transfer of energy from the electric field to the sample in the part of the tube 15 that lies in the gap 16. The amount of energy transferred to the sample depends on the strength of the electric field the sample is exposed to. The size and shape of the cavity 19 dictate the resonant frequency of the conductive body. When microwaves are applied at the resonant frequency of the conductive body, standing waves are formed in electric field in the gap 16, and energy transfer to the sample is enhanced. When the frequency of the microwaves is different to the resonant frequency of the split-ring body 11, the sample will experience much lower strength of electric field, and transfer of energy to the sample may occur but will be comparatively slight.

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There are two effects seen with energy transfer from a microwave electric field to a sample.

- 5 The first effect is heating of the sample, which is caused by the presence of the electric field. Energy from the electric field transfers to the particles in the sample causing them to vibrate. Heating a sample containing DNA can denature DNA, which may not be desirable from the point of view of analysing DNA. Therefore, it is often desirable to avoid heating DNA. Limiting the amount of time the sample is exposed
10 to the electric field can constrain heating of the sample.

The second type of energy transfer is known to chemists as the "microwave effect", and is the result of the sample being exposed to an electric field which oscillates at microwave frequency causing repetitive mechanical stresses on the particles of the
15 sample. These mechanical stresses, when applied to bacteria, cause DNA to be liberated from the bacteria.

In order to liberate DNA from bacteria and yet not denature the liberated DNA through heating, the sample should be exposed to the microwave electric field in the
20 gap 16 for a minimum amount of time. To transfer energy in a minimum amount of time the power transferred to the sample should be maximised.

The absorbed power P per unit volume (W/m^3) by a sample (assuming the sample is dielectric) is given by:

25

$$P = \sigma E^2$$

Where, σ is the effective conductivity (S/m) and E is the electric field strength within material.

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Therefore the power absorbed by a sample is proportional to the square of the electric field experienced by the sample. To improve energy transfer to the sample, the strength of the electric field felt by the sample should be maximised.

In the device 10 of Figures 1 and 2, the tube 15 filled with sample runs parallel to the electric field, which as will now be explained improves the efficiency of energy transfer from the electric field 18 to the sample. An effect called the depolarisation effect affects the strength of the electric field felt by the sample. The device 10 is designed to reduce the impact of the depolarisation effect.

The depolarisation effect is seen when a dielectric fluid interacts with an electric field. In device 10, a sample is placed in an electric field of strength E_0 . Dipolar particles within the sample align with the electric field in the gap 16 and polarize the sample. At any given moment, the alignment of the particles results in an excess of negative charges at the side of sample that is closest to the one of faces 12 and 13 that happens to be positively charged due to the oscillating current in the split-ring body 11 and an excess of positive charges form at the other one of faces 12 and 13 (which happens to be negatively charged at this time). In this sense, the part of the sample that is within the gap becomes polarised. These excess charges create a second electric field, which partially counteracts the external electric field E_0 within the sample. The depolarisation reduces the strength of the overall E-field within the sample and therefore reduces the efficiency of energy transfer from the electric field to the sample. The depolarisation effect is particularly acute in aqueous samples owing to the large electric dipole moment of water, and bacterial samples to be analysed will normally be in water.

Figures 3 to 5 show the depolarisation effect on an idealized, thin, cylindrical liquid column subject to an applied parallel electric field. The applied electric field is the same in all three figures.

In Figure 3 the channel 20 is positioned perpendicular to the direction of the electric field E_0 . The sample is fluid and is in the channel 20, indicated by shading. Dipolar particles within the fluid align with the electric field. The aligned dipoles result in an excess of positive charges at a first side 35 of the column, and an excess of negative charges at a second, opposite side 36 of the column, shown in the figures as positive charges (+) or negative charges (-). The particles therefore polarize the fluid, and

partially counteract the effect of the applied electric field. The two charge accumulations at the sides of the channel 35 and 36 create an internal electric field E_p inside the channel 20.

- 5 The overall electric field experienced by the sample in the channel 20 is the sum of the external electric field E_0 and the internal field E_p . The fields E_0 and E_p work in opposite directions. It is desirable to maximise the electric field experienced by the sample in order to maximise the efficiency of energy transfer. To do this, the internal electric field E_p should be as small as possible.

10

The strength of an electric field at a distance r from a point charge of magnitude q is given by:

$$E = \frac{kq}{r^2}$$

- 15 Where k is a dimensionless constant. Therefore, to keep E_p small, the magnitude of each of the charge accumulations should be as small as possible, and the distance between the charges should be as large as possible. The magnitude of charge in the accumulations depends on the cross-sectional area of the sample which is perpendicular to the external electric field E_0 . The larger the cross-sectional area
20 perpendicular to the field E_0 , the greater the magnitude of the charge that makes up the accumulations, and the stronger the electric field E_p is.

- Therefore, decreasing the internal field E_p can be done by decreasing the cross-sectional area of the channel perpendicular to the electric field E_0 and increasing the
25 distance between the charge accumulations.

- In Figure 3 the curved surface along the whole length l of the channel 20 (and therefore a corresponding surface of the sample) is perpendicular to the electric field E_0 . Further, the charge accumulations are separated by a relatively small distance
30 (which is, at its greatest, the width w of the tube). A large cross-sectional area and small charge separation leads to a relatively large internal electric field E_p , which deleteriously counteracts the external electric field. Therefore the efficiency of energy transfer into the sample (e.g. for DNA liberation) in Figure 3 is relatively low.

In Figure 4 the channel 20 is positioned at an acute angle α to the direction of the electric field. The angle of the channel relative to the electric field E_0 means that the cross-sectional area of the tube perpendicular to the electric field E_0 is smaller than that in Figure 3. There is also a larger distance between the opposing charge accumulations. Therefore the electric field E_p caused by the charge accumulations is smaller than that in Figure 3, so the sum of the external and internal electric field is larger (closer to the value of E_0). The sample in Figure 4 experiences a greater electric field than the sample in Figure 3, and so energy is transferred to the sample more efficiently. In the case of rupturing bacteria, this results in a reduced time required to rupture the bacteria to liberate the DNA, and a reduced chance of heating the sample and denaturing the DNA.

Figure 5 shows the channel 20 aligned parallel to the electric field. In this arrangement, the charge accumulations arise at either end of the channel. Only a small cross-sectional area of the channel is perpendicular to the electric field. Further, the distance between the charge accumulations is large. The charge accumulations here act, to an approximation, like point charges. The accumulated charges cause a very weak field in the direction opposing the electric field E_0 , and the electric field experienced by the sample in the tube is roughly the same as external electric field E_0 . The relatively high electric field experienced by the sample results in efficient energy transfer from the microwaves into sample.

Thus, the orientation of the channel in the device shown in Figures 1 and 2 effectively eliminates the impact of the depolarisation effect, such that energy transfer to a sample can be enhanced. However, improved energy transfer efficiency will be seen when the channel is positioned at any orientation other than perpendicular to the electric field. Therefore, variants of the device 10 of Figures 1 and 2 may have the channel positioned within 10° of the electric field lines, or within 20° of the electric field lines. The smaller the angle between the direction of the field lines and the direction of the channel, the higher the efficiency of energy transfer from the electric field to the sample.

Figure 6 shows a further microwave device for sample preparation 10, similar to that shown in Figure 1 with like reference numerals indicating the same features.

Only part of the tube 15 in Figure 6 is parallel to the field lines of the electric field E_0 .

5 The tube 15 does not pierce the conductive body 11, nor travel through the first face 12 and the second face 13. Instead the tube 15 travels into and out of the gap 16 from outside the conductive body 11, with a portion of the tube travelling parallel to the electric field E_0 .

10 Other conceivable configurations of the tube 15, wherein a portion of the tube runs parallel to the direction of the electric field E_0 within the gap 16, could be used. The sample in the section of the tube 15 which runs parallel to the electric field E_0 experiences the strongest electric field as it is least affected by the depolarisation effect, and therefore sees more efficient energy transfer.

15

Another microwave device for sample preparation 40 is shown in Figure 7.

The device 40 comprises a conductive body 21. The conductive body 21 is split into two parts: a first part 22 and a second part 23. The two parts fit together to form a
20 single hollow body defining a cavity 32.

The two parts 22 and 23 could fit together in a number of ways. The two parts 22 and 23 could clip together. Alternatively, the second part 23 may rest on top of the first part 22, or the two parts may be bolted together. Other conceivable methods of
25 attaching the first part 22 to the second part 23 may be used.

When the first part 22 and the second part 23 are fitted together to form a single body, the parts are conductively connected. Therefore a current can flow between the first and second parts 22 and 23 without interruption.

30

The first part 22 of the body has a protrusion 25 which extends into the cavity 32. The protrusion forms a neck 24 in the cavity 32. In other words, the cavity 32 is a re-entrant cavity. The sides of the cavity facing each other across the neck 24 can be

regarded as a first face 26 and second face 27 opposing each other across a gap. Of course, the protrusion 25 could equally well be provided on part 23 in order to define the neck 24. Also, it is of course possible to provide protrusions on both of parts 22 and 23, the protrusions extending towards each other to define the neck in the cavity
5 32.

In the device 40, the protrusion 25, the conductive body 21 and the cavity 32 are all cylindrical, and all share a common axis. However, the protrusion 24, the conductive body 21 and the cavity 32 may be other shapes, and may not share a common axis.
10

The device 40 has a port 29. A feed line 30 is attached to the port to apply microwaves to the conductive body 21. To ensure that there is adequate coupling between the feed line and the conductive body, the feed line extends a small way into the conductive body 21.
15

In Figure 7 the port 29 is positioned on the second part 23. Since the first and second parts 22 and 23 are joined together to form a single conductive body 21, they are conductively connected and the microwaves applied to the second part at the port 29 travel to the first part. Therefore the port can be positioned on either the first part 22
20 or the second part 23.

Upon application of microwaves to the conductive body 21, an alternating current, oscillating at the frequency of the applied microwaves, circulates within the body 21. Due to this alternating current, an electric field E_0 is established between the faces 26
25 and 27 and across the neck 24. Over the majority of face 26 at the extremity of the protrusion 25, the electric field E_0 extends perpendicularly between the faces 26 and 27. At the periphery of the protrusion 25 - its circumferential edge, recalling that it is cylindrical - the field lines of the electric field E_0 will diverge somewhat. The microwaves can be applied at a resonant frequency of the body 21 at which a standing
30 wave is established in the electric field E_0 between the faces 26 and 27. When this condition is met, the transfer of the energy from the electric field E_0 to the sample (e.g. to achieve lysis) is enhanced. At the same time, a resonant magnetic field is

established in the cavity 32, and the port 29 is positioned to couple energy from the applied microwaves into this magnetic field.

5 The device 40 also includes a tube 31. The tube pierces the first part 22 and the second part 23 of the conductive body and traverses the neck 24. In a region of the neck 24 where the electric field lines are parallel, the tube 31 runs substantially parallel to the direction of the field lines. The tube 31 defines a channel in which a sample can flow.

10 The tube 31 could be positioned differently in the conductive body 21 of Figure 7, such that it does not pierce the first and second faces 22 and 23 of the conductive body. In a similar arrangement to that shown in Figure 6, the tube 31 could have sections at different angles within the gap, such that only a portion of the channel is parallel to the electric field, but another portion is positioned at a different angle. In a
15 further variant of the device of Figure 7, the tube 31 could be oriented so that it is not perpendicular to the faces 26 and 27. However, the further the tube 31 departs from the orientation where the tube is perpendicular to the faces 26 and 27, the more deleterious the polarisation effect will become.

20 The conductive bodies illustrated in Figures 1, 2, 5 and 6 are straightforward and inexpensive to manufacture, requiring the shaping of a solid block of suitable material.

The microwave devices for sample preparation described above may be used to expose a large fluid sample to an electric field. In this case the fluid sample is moved
25 through the tube, for example by pumping or through capillary action. As a volume of the sample moves through the tube, it is exposed to the electric field. At this time, energy is transferred to that volume of the sample. The volume is then moved further along the tube and out of the electric field. The sample is moved through the tube until all of the sample has been exposed to the electric field. The microwaves are
30 pulsed constantly for the duration of moving fluid through the tube, which provides a pulsed electric field for the duration of the process.

The microwave devices for sample preparation described above each have a single port for the application of microwaves to cause lysis. It is possible to add a second port to which a frequency sensitive detector can be coupled. That way, microwaves can be injected into one of the ports and extracted through the other port. Such an arrangement allows the resonant frequency of the device to be determined, e.g. by sweeping the frequency of the microwaves that are applied at one port and looking for a resonance in the microwaves that are extracted through the other port, e.g. by using a spectrum analyser as the frequency sensitive detector. The frequency of the microwaves that are applied to the device can then be tuned not to a nominal resonant frequency but to the actual, measured resonant frequency so as to better ensure an efficient transfer of energy to the sample that is to undergo lysis.

It must also be remembered that the drawings are schematic. For example, in practice, the internal and external diameters of tube 15 are typically 1 millimetre and 2 millimetres respectively and the perpendicular distance between faces 12 and 13 is typically 3-4 millimetres. Likewise, the internal and external diameters of tube 15 are typically 1 millimetre and 2 millimetres respectively and the perpendicular distance between faces 26 and 27 is also typically 4 millimetres.

In the microwave devices for sample preparation described above, a sample is placed in, and orientated relative to, an electric field so as to decrease the impact of the depolarisation effect. Thus there are provided devices for sample preparation which achieve efficient energy transfer from an electric field. The devices provide a way of liberating DNA from bacteria in a relatively short amount of time without damaging the DNA through heating.

Those skilled in the art will appreciate that various amendments and alterations can be made to the embodiments described above without departing from the scope of the invention as defined in the claims appended hereto.

Claims

1. A device for rupturing bacteria, the device comprising:
 - a conductive body comprising a first face and a second face, wherein the
 - 5 first and second faces are separated by a gap;
 - an input for introducing a microwave signal to the conductive body; and
 - a channel for containing a sample;
 - wherein:
 - the conductive body is arranged to produce, upon application of the
 - 10 microwave signal at the input, parallel E-field lines extending in a first direction in a region of the gap;
 - the channel runs in a second direction in the region; and
 - the angle between the first and second directions is not more than 45 degrees.
- 15 2. A device according to claim 1, wherein the angle is not more than 10 degrees.
3. A device according to claim 1 or claim 2, wherein the angle is substantially zero.
- 20 4. A device according to any of the preceding claims, wherein the microwaves are applied at a resonant frequency of the device.
5. A device according to any of the proceeding claims, wherein the conductive body is a conductive ring that is split to provide said gap.
- 25 6. A device according to any of claims 1 to 4, wherein the conductive body is hollow and encloses a space, and the body protrudes into the space to provide the gap.
7. A device according to claim 6, wherein the body comprises first and second
- 30 parts that can be releasably assembled to define the space.
8. A device according to claim 7 wherein the first part carries a protrusion that extends into the space to define the gap.

9. A device according to any of the preceding claims wherein the channel extends into the body to reach said region.
- 5 10. A device according to any of the preceding claims, wherein at least a portion of the part of the channel within said region runs in a third direction that is not parallel to the second direction.
11. A device according to any of the preceding claims, wherein the channel is
10 defined by a tube.
12. A device according to claim 11, wherein the sample is a fluid and flows through the tube.
- 15 13. A device according to any of the proceeding claims, wherein the channel is a microfluidic channel.
14. A method of rupturing bacteria using a device, the method comprising:
applying a microwave signal to a conductive body via an input, the conductive
20 body comprising a first face and a second face, wherein the first and second faces are separated by a gap;
wherein, due to the microwave signal, parallel E-field lines extend in a first direction in a region of the gap;
the method further comprises inserting a sample into a channel, wherein the
25 channel runs in a second direction in the region and the angle between the first and second directions is not more than 20 degrees.
15. A method according to claim 14, wherein the angle is not more than 10 degrees.
- 30 16. A method according to claim 14 or claim 15, wherein the angle is substantially zero.

17. A method according to any of claims 14 to 16, wherein the microwaves are applied at a resonant frequency of the device.
18. A method according to any of claims 14 to 17, wherein the conductive body is a
5 conductive ring that is split to provide said gap.
19. A method according to any of claims 14 to 17, wherein the conductive body is hollow and encloses a space, and the body protrudes into the space to provide the gap.
- 10 20. A method according to claim 19, wherein the body comprises first and second parts that can be releasably assembled to define the space.
21. A method according to claim 20 wherein the first part carries a protrusion that extends into the space to define the gap.
- 15 22. A method according to any of claims 14 to 21, wherein the channel extends into the body to reach said region.
23. A method according to any of claims 14 to 22, wherein at least a portion of the
20 part of the channel within said region runs in a third direction that is not parallel to the second direction.
24. A method according to any of claims 14 to 23, wherein the sample is fluid and wherein inserting the sample into a channel comprises moving the sample through the
25 channel.
25. A method according to any of claims 14 to 24, comprising pumping the sample through the channel until the volume of the sample has passed through the channel and been exposed to the E-field.
- 30 26. A method according to any of claims 14 to 25, wherein the channel is defined by a tube.

27. A method according to any of claims 14 to 26, wherein the channel is a microfluidic channel.

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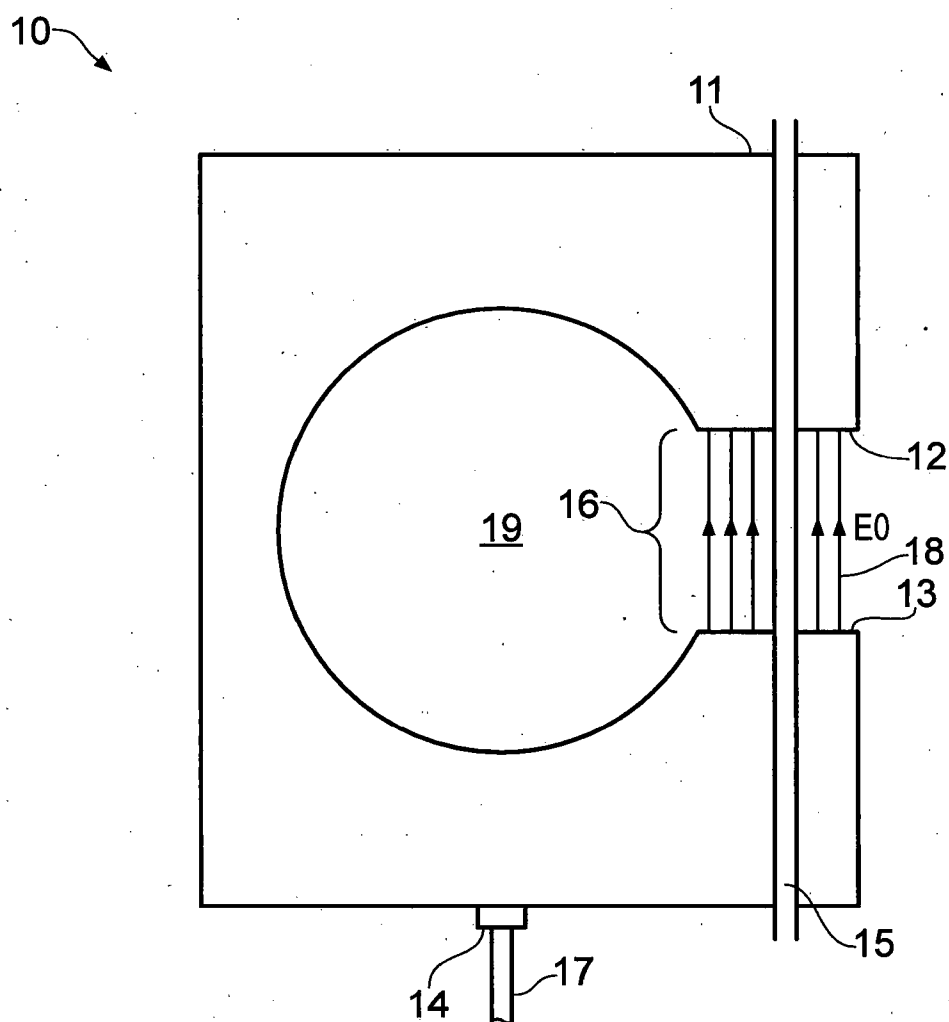


FIG. 1

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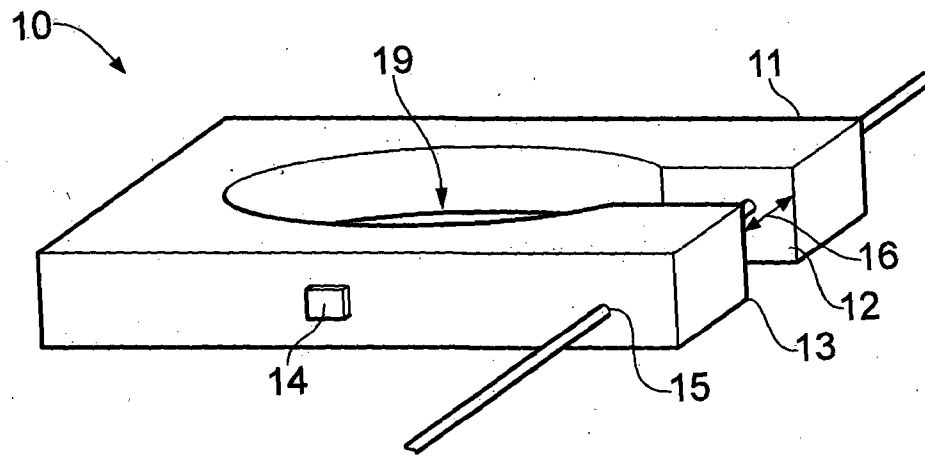


FIG. 2

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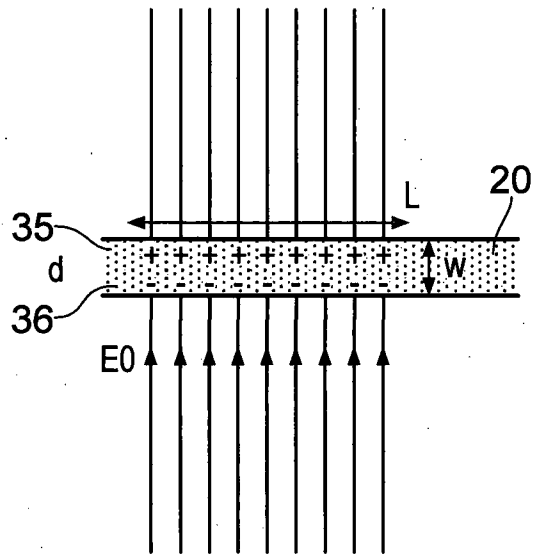


FIG. 3

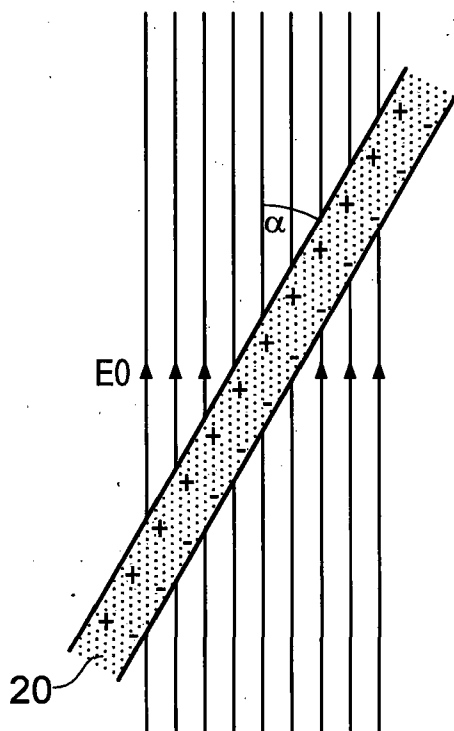


FIG. 4

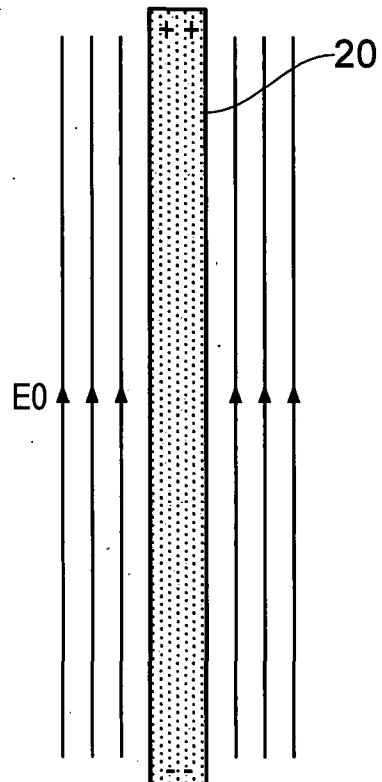


FIG. 5

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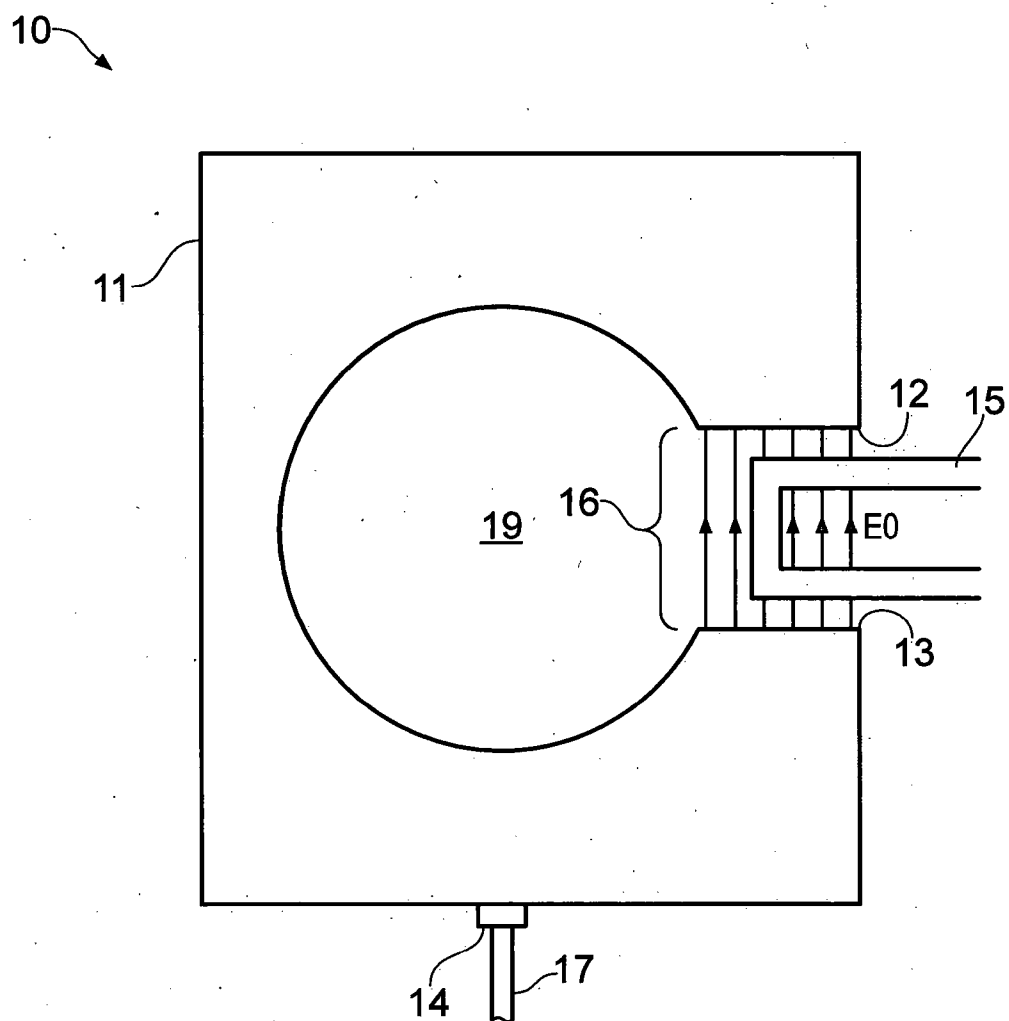


FIG. 6

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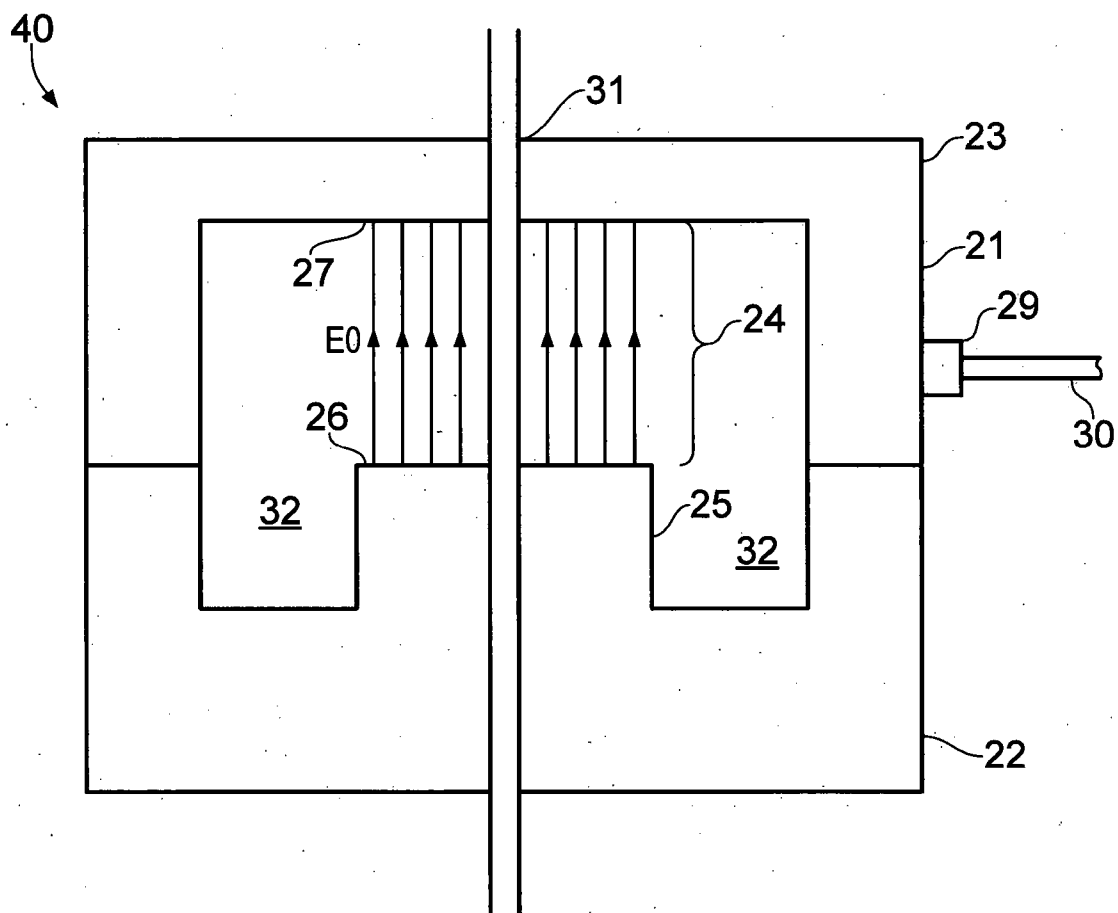


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/053420

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N1/06 C12N13/00
ADD.

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)
C12N

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)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/312174 A1 (GEDDES CHRIS D [US]) 27 October 2016 (2016-10-27) column 1 - column 4; figures 1,9,10,13,16,17 -----	1-27
X	WO 01/19963 A2 (MOTOROLA INC [US]; NAIR VIJAY K [US]; GORONKIN HERBERT [US]) 22 March 2001 (2001-03-22) page 3 - page 4; figure 1 page 8 - page 9 -----	1-27
A	WO 2016/026829 A1 (IFP PRIV INST PRODUKTQUALITAET [DE]) 25 February 2016 (2016-02-25) the whole document ----- -/-	14



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

25 January 2018

Date of mailing of the international search report

07/02/2018

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Authorized officer

Oderwald, Harald

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2017/053420

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MENON P K ET AL: "COMPARISON OF RAPID METHOD OF DNA EXTRACTION USING MICROWAVE IRRADIATION WITH CONVENTIONAL PHENOL CHLOROFORM TECHNIQUE FOR USE IN MULTIPLEX PCR FOR mec A AND fem B GENES TO IDENTIFY GENOTYPES OF MRSA FROM CULTURES.", MEDICAL JOURNAL, ARMED FORCES INDIA JUL 2001, vol. 57, no. 3, July 2001 (2001-07), pages 194-196, XP002777613, ISSN: 0377-1237 the whole document -----	14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2017/053420

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2016312174	A1	27-10-2016	NONE

WO 0119963	A2	22-03-2001	AU 1961301 A 17-04-2001
			CA 2384990 A1 22-03-2001
			EP 1214867 A2 19-06-2002
			JP 2003509822 A 11-03-2003
			US 6623945 B1 23-09-2003
			US 2002115201 A1 22-08-2002
			WO 0119963 A2 22-03-2001

WO 2016026829	A1	25-02-2016	EP 3183344 A1 28-06-2017
			WO 2016026829 A1 25-02-2016
