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(71) Applicant *(for all designated States except US)*: MOSAIC TECHNOLOGIES [US/US]; 303 Bear Hill Road, Waltham, MA 02454 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **SEWARD, George, H.** [US/US]; - (US).

(74) Agents: **HOGLE, Doreen, M.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02421 (US).

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(54) Title: CHARGE-DENSITY MEASUREMENT DEVICE (CDMD) AND METHODS OF USE THEREOF

(57) Abstract: A device for detecting a charged species in a dielectric media is provided. The device includes an insulator on a surface of the media and a probe which maintains a steady state charge on the insulator to create an electric field through the insulator and the media. The device also includes an electrical detector which measures an electrical response to the detector. Devices for detecting a biological charged species in an electrophoretic media, and methods for using the devices are also provided.

CHARGE-DENSITY MEASUREMENT DEVICE (CDMD) AND METHODS OF USE THEREOF

RELATED APPLICATION

This application claims the benefit of the United States Provisional Application entitled "Charge Density Measurement Device" filed August 9, 1999, Serial Number 60/147,793, the entire teachings of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Typically, electrophoresis is a method of separating charged components within a biological specimen utilizing the charge-to-mobility ratio of the components in solution. An electric field causes the charged components to migrate towards the oppositely charged electrode along a migration channel. The migration channel is composed primarily of water, and is supported within a porous medium such as paper or a polymeric gel. A successful electrophoretic process separates the charged components into distinct bands along the migration channel of the electrophoretic media. Each band corresponds to a specific structure or component. Upon completion of the electrophoresis process, the components are fixed in place by chemical methods to prevent further diffusion.

The bands contained within the electrophoresis media are imaged by a variety of methods. For example, large concentrations of proteins can be measured by absorption of ultraviolet light at wavelengths of circa 280 nm. Lower concentrations require more complicated methods which can be quite laborious. For example, proteins are commonly stained with organic compounds such as

Coomassie Blue which selectively attaches to the proteins. Areas of the media absent of proteins can be destained, thus creating visible bands representing concentrations of different proteins. Stained proteins are typically evaluated with an optical device.

Other chemical methods of marking are also known to those of skill in the art. Such methods typically employ a marker to the compound that alters its absorption and/or the manner in which it emits photons. These additional methods can also be quite complex and laborious (B.D. Hanes and D. Rickwood, editors, Gel Electrophoresis of Proteins: A Practical Approach, 2nd Edition, Oxford University Press 1990).

SUMMARY OF THE INVENTION

In accordance with the present invention, a charged species in a dielectric media is detected. An insulator is provided on a surface of the media and a charge is applied from a probe to the insulator to create an electric field through the insulator and the media. An electrical response to the applied charge is measured. Preferably, a steady state charge is maintained on the insulator and the electrical response is measured while maintaining the steady state charge. In a particularly advantageous application, electrophoretically distributed charge in a dielectric slab media is

Operation of the device in a constant current mode provides particular advantages. The electric potential across the device is linearly dependent upon the charge-density within the dielectric slab. This is a significant improvement over past measurement devices, which require sophisticated modeling of time-and-space dependent voltage data. In the CDMD, the applied voltage is driven to achieve a specific amount of electrical current, and the resulting voltage is linearly proportional to the charge density.

The CDMD is a very useful measurement tool for the quantification of biological components separated by the process of electrophoresis. Concentrations of the bands fixed within a migration channel can be derived easily from the linear dependence of the applied voltage. Such measurements can replace the present methods of quantification which are both complex and labor intensive.

In one embodiment, the invention is directed to a device for detecting a charged species in a dielectric media. The device includes an insulator on a surface of the media and a probe which maintains a steady state charge on the insulator to create an electric field through the insulator and the media. The device also includes an electrical detector which measures an electrical response to the detector.

In particular embodiments, the probe applies the charge with a constant current through the insulator and the media. In a preferred embodiment, the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator. In a preferred embodiment, the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

The probe can be a scanning metal probe or a scanning metal probe coated with an insulator. The device can include a plurality of probes which can be configured as an array of metal probes contained within an insulator. In a particular embodiment, the probes are in an integrated electrical circuit. The insulator can be paper, plastics or metal oxides.

In another embodiment, the invention is directed to a device for detecting a charged species in a dielectric media including an insulator on a surface of the media. The device contains both a means for maintaining a steady state charge on the insulator to create an electric field through the insulator and the media, and a means for measuring an electrical response to the applied charge while maintaining the steady state charge.

In another embodiment, the invention is directed to a device for detecting a charged species in an electrophoretic media including a dielectric slab media containing an electrophoretically distributed charged species. The device includes an insulator positioned on a longitudinal surface of the media, and a probe which applies a charge to the insulator to create an electric field through the insulator and media. The device also includes an electrical detector which measures an electrical response to the applied charge.

In another embodiment the invention is directed to a device for detecting a charged species in an electrophoretic media including a dielectric slab media containing electrophoretically distributed charged species. The device includes an insulator positioned on a longitudinal surface of the media. The device also includes both a means for maintaining a steady state charge on the insulator to create an electric field through the insulator and the media and a means for measuring an electrical response to the applied charge while maintaining the steady state charge. In particular embodiments, the dielectric slab is an electrophoretic gel formed of polyacrylamide gel, starch gel or an agarose gel.

In yet another embodiment, the invention is directed to an electrophoresis cassette including a cavity adapted to receive electrophoretic dielectric media to be subjected to a longitudinal electric field for electrophoretic distribution of charge particles longitudinally through the media, and a discontinuous electrode extending longitudinally along the cavity. In a particular embodiment, the device also includes probe electrodes extending longitudinally along the cavity in opposition to the discontinuous electrode. In a preferred embodiment, the cavity contains the electrophoretic dielectric media and the device further comprises an electrode at each end of the cavity.

In another aspect, the invention is directed to a method of detecting a charged species in a media. This method includes providing a dielectric media having the charged species therein, providing an insulator on a surface of the media, maintaining a steady state charge on the insulator to create an electric field through the insulator and the media, and measuring an electrical response to the applied charge while maintaining the steady state charge.

In another embodiment, the invention is directed to a method of detecting a charged species in an electrophoretic media. The method includes providing a dielectric slab electrophoretic media having the charged species distributed longitudinally therein, providing an insulator on a longitudinal surface of the media, applying a charge to the insulator to create an electric field through the insulator and the media, and measuring an electrical response to the applied charge.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

Figure 1 illustrates an embodiment of the CDMD including a scanning metal probe and a stratified slab-and insulator structure according to the principles of the present invention.

Figures 2A-2B illustrate the manner in which an applied electric field distorts the intrinsic potential well of a trapped carrier in an insulator.

Figures 3A-3B illustrate the influence of an electric field upon the mobility of trapped carriers of a metal at the interface of the metal and an insulator by distorting the intrinsic potential well of trapped carriers.

Figure 4 illustrates the linear relationship between charge density and change of voltage in a constant current CDMD.

Figures 5A-C illustrate the relationship between the thickness of the insulator and the applied voltage in a constant voltage CDMD.

Figure 6 illustrates yet another embodiment of the stratified slab-and-insulator structure shown in Figure 1.

Figure 7 illustrates an embodiment of the CDMD employing an insulator coated metal probe.

Figure 8 illustrates an embodiment of the CDMD employing an array of metal probes embedded within an insulator.

Figure 9 is a cross-section of an embodiment of a bottom of a CDMD for performing electrophoresis.

Figure 10 illustrates the combination of the top portion of the CDMD and the bottom portion of the CDMD shown in Figure 7.

Figure 11 illustrates an alternative embodiment for the discontinuous ground structure CDMD in which the metal probes and the electrolyte channel are repeated as an array.

DETAILED DESCRIPTION OF THE INVENTION

A charge-density measurement device (hereinafter "CDMD") is described which derives the charge-density from the voltage required for a specific amount of electrical current density that flows transversely through a stratified structure comprised of a slab and an overlying electrically insulating film. The rate of transverse electrical current density is limited by the electric field within the electrical insulator. This desirable effect can be achieved by appropriate selection of materials for both the slab and the insulator. The materials selected are poor conductors of intrinsic electrons, while also being fair conductors of extrinsic electrons. Extrinsic electrons are injected into the insulator by a metal probe in contact with the insulator. The transverse electrical current is maintained at a reference level by variation of an applied electric potential across the slab-and-insulator structure. The electric potential across the slab-and-insulator is linearly dependent upon the charge-density within the slab. The concentration of specific target molecules can be derived from the charge density, thus, the quantity of target molecule can be derived from the applied electric potential.

A description of particular embodiments of the invention follows.

Figure 1A illustrates an embodiment of a charge-density measurement device (hereinafter "CDMD") including a scanning metal probe 200 and a stratified slab-and- insulator structure 202 according to the principles of the present invention. The stratified slab-and-insulator structure 202 comprises an insulating film 204, a slab 206, and a continuous metal film 208. The metal film 208 is the ground plane for the CDMD.

The slab 206 includes a specimen for measurement. The specimen can be an electrolyte 210 of an electrophoretic process. The electrolyte 210 includes charged particles created by the electrophoretic process. The CDMD derives a quantitative measurement of the charge-density of the electrolyte 210 by measuring the applied electric potential or voltage required to provide a constant current flow transversely in direction 212 through the stratified slab-and-insulator structure 202. The voltage is applied between the metal probe 200 and the metal film 208 by a power supply 214 operating in constant current mode. The variation of the applied voltage with the constant current is sensed by a voltage detector (not shown) to derive the charge density within the slab 206 because the variation in applied voltage is directly proportional to the charge-density of the electrolyte 210.

The chemical composition of the electrolyte 210 is selected to allow transport of the macromolecules of interest along the slab 206 during electrophoresis. A preferred electrolyte 210 has a minimum of intrinsic ionic species that mask the charge of the extrinsic species. Such masking inhibits the operation of CDMD as well as the process of electrophoresis. Thus, when electrophoresis occurs, the CDMD functions at some level of sensitivity. This sensitivity improves as the masking by intrinsic ions decreases.

Electrophoresis is typically conducted using an electrolyte solution. Electrolyte solutions suitable for electrophoresis of biomolecules have been described by many authors. In electrophoresis, the electrolyte solution carries current through the medium, but also usually provides a buffer to regulate pH. For many applications, especially nondenaturing electrophoresis of proteins, it is important to carefully regulate pH, since many proteins possess titratable amino acid side chains whose charge properties depend on pH. For other applications, such as electrophoresis of nucleic acids and denaturing SDS gel electrophoresis of proteins, the macromolecules of interest all have a high negative charge, and the function of the buffer is simply to hold the pH in the neutral range, for example from about pH 7 to about pH 8.5, and to counteract changes in pH that may occur near the electrodes due to the electrolysis of water. In general, it is useful to use low concentrations of electrolyte in electrophoresis experiments, since current flow and Joule heating is proportional to the concentration of the electrolyte (at constant voltage). For this reason, it is usually preferable to use dilute solutions of buffers as the principal component of the electrolyte solution. For instance, one standard buffer used extensively for nucleic acid electrophoresis is 89 mM trishydroxymethylaminomethane (TRIS) buffered with boric acid at a pH of 8.3. Other additives to the electrolyte solutions depend on the specific application. For example, since heavy metals and divalent ions can damage nucleic acids either directly or indirectly (through the activation of contaminating nucleases), a low concentration of chelating agent such as EDTA is usually added to electrophoresis buffers for DNA or RNA analysis. Another example is the use of the charged detergent SDS, which is frequently used in experiments to determine the molecular weight of proteins by electrophoresis. Discussions of buffers and electrolytes suitable for various types of electrophoresis many be found in Sambrook, Fritch, and Maniatis; 1989, Molecular Cloning: A Laboratory Manual, 2nd Edition, Chapter 6, Cold Spring Harbor Press, Cold Spring Harbor, NY; Chrambach and Rodbard, 1971, Science 172(982):440-51; Rickwood and Hames, 1991, Gel Electrophoresis for Nucleic Acids: A Practical Approach, IRL Press, Oxford, UK. The teachings of these references are hereby incorporated by reference in their entireties.

In this embodiment, the slab 206 also includes a material with some structural integrity for supporting the electrolyte 210. The material can be paper, plastic or gel. The gel can be an electrophoretic gel, such as a polyacrylamide gel, a starch gel, an agarose gel or other suitable gel or any combination of those electrophoretic gels.

The slab 206 is transferred to the metal film 208 after the process of electrophoresis is complete, because the continuous metal film 208 (the ground electrode of the CDMD) would disturb the electric field during the electrophoresis process. The electrolyte 210 can be incorporated as part of the CDMD before the electrophoresis occurs. This requires a special ground electrode. An embodiment of such a CDMD is described in conjunction with Figure 9.

The metal film 208 is the ground electrode of the CDMD. The metal film 208 can be a metal-coated glass slide. Any metal that does not chemically react to the specimen to be tested is acceptable. The metal-coated slide can be fabricated by numerous methods. The metal can be applied to the glass slide by vacuum deposition or chemical plating. Glass slides or similar articles with optical flatness are particularly preferred. A solid block of metal can also be used as the ground electrode.

The insulating film 204 is laid over the exposed surface of the electrolyte 210. The insulating film 204 can be formed of paper, plastic or a metal oxide. An insulator with an electric permittivity range of $2\epsilon_0$ to $4\epsilon_0$ is particularly preferred for the insulating film 204. Some acceptable plastics are polyester, e.g., Mylar® (registered trademark of E.I. Dupont de Nemours, Wilmington, Delaware), polyethylene and polytetrafluoroethylene, e.g., Teflon® (registered trademark of E.I. Dupont de Nemours, Wilmington, Delaware). Acceptable metal oxides include types of glass with a refractive index below 2 which corresponds to a permittivity of $4\epsilon_0$. Many types of glass are derivatives of silicon-oxide. Other metal oxides can also be used for the insulating film 204.

The insulating film 204 may have pads of metal film 216 that determine the transverse cross-section through which current flows. A flat metal disc may be used instead of a metal probe 200 to define the region of transverse current. A flexible connection from the power supply 214 to the flat metal disc is required. The flexible connection can be provided by the use of a liquid connection. A liquid connection can be provided by an ionic solution or mercury. In order to examine the electrolyte 210, the metal probe 200 makes contact with the insulating film 204 without compressing the gel or paper in the slab 206.

To derive the charge density of the charged particles in the electrolyte 210 in the slab 206, a transverse electrical current flow in direction 212 is maintained at a reference level by the power supply 214 operating in constant current mode. Although the insulating layer 204 is an insulator, sufficient energy is provided from the power supply 214 to overcome the energy barrier of the insulator and create current flow throughout.

The parameters of the system, such as active area of the metal probe 200 and thickness of the insulator 204 can be designed to create suitable operating currents for the power supply 214. A suitable current is one that is measurable to within 0.1% of the constant current. For example, if the insulating film 204 is a polyester sheet which is 13 μm thick and there is an air gap between the metal probe 200 and the insulating film 204, conduction requires a constant current of about 1 nano amperes of current over a 1-mm by 1-mm metal probe and a voltage of 1000 volts.

The metal probe 200 is in contact with the insulating film 204 and is moved along the insulating film 204 in order to measure the charge density at different locations within the slab 206. The metal probe 200 does not have to be in intimate contact with the insulating film 204, there may be a small air gap. The metal probe 200 is about 2 to 3mm in diameter. The diameter of the metal probe 200 determines the spatial resolution.

The transverse current through the slab-and-insulator structure 202 is dominated by the transport of extrinsic electrons from the metal probe 200 through the insulating film 204 and the slab 206 to the metal film 208 in direction 212. An extrinsic electron is one that has been injected into a material, such as the insulating film 204 from an adjacent material, such as the metal probe 200. Extrinsic electrons can be easily transported through a material unlike intrinsic electrons which are ordinarily part of the material and tightly bound.

The materials selected for the slab 206 and the insulator film 204 are dielectrics. A dielectric is a poor conductor of intrinsic electrons, while also being a fair conductor of extrinsic electrons. The dielectric strength of a material refers to the material's ability to create electric dipoles when subjected to an electric field. The dielectric strength of the slab 206 for a steady-state electric field is large in comparison to that of the insulating film 204. This is typically due to the large dipoles associated with the water content of the slab 206. A dielectric is rated in terms of a dielectric constant which is a measure of the dielectric strength of the dielectric. The relative dielectric constants for the slab 206 and the insulating film 204 are approximately 10 and 3. The slab 206 including an electrolyte 210 contains water which has a very large relative dielectric constant of 80 under conditions of steady state.

As the constant current is provided to the metal probe 200 from power supply 214, the metal probe 200 injects extrinsic electrons onto the surface of the insulating film 204. The injection of electrons from the metal probe 200 into the insulating film 204 is described by one or a combination of two effects: the Poole-

Frenkel effect and the Schottky effect. The Poole-Frenkel effect applies to the surface state of an insulator, such as the insulating film 204. This occurs if there is an air gap between the metal probe 200 and the insulating film 204. The Schottky effect applies when the metal probe 200 is in intimate contact with the insulating film 204.

The Poole-Frenkel effect describes the influence of an electric field upon the mobility of a trapped electron in an insulator. The Poole-Frenkel effect is described later in conjunction with Figs 2A-2B. The Schottky effect is described later in conjunction with Figs. 3A-3B.

Figs. 2A-2B illustrate how an applied electric field distorts a potential well of a trapped electron in the insulating film 204. Figure 2A illustrates the relative energy states of an extrinsic electron 300a and an intrinsic electron 300b in one of a plurality of potential wells 310 in an insulator. The insulator can be the insulating film 204 shown in Figure 1. Potential wells 310 in weak insulators are shallow. Potential wells 310 in strong insulators are deep. Z is the transverse spatial position within the insulator. The electric field is along Z. qe is the elementary charge in coulombs. V is the electric potential in joules per coulomb. The extrinsic electron 300a in the potential well 310 has an extrinsic barrier height 302a, the intrinsic electron 300b has an intrinsic barrier height 302b. As shown, the extrinsic electron 300a in the insulating film 204 occupies a state with an extrinsic barrier height 302a which is lower than the intrinsic barrier height 302b for the intrinsic electron 300b. The barrier height 302a, 302b is the energy required by an electron to move freely within the insulating film 204. A large barrier height 302a, 302b inhibits Poole-Frenkel conduction; that is, the extrinsic electron 300a cannot escape from the potential well. A low barrier height can permit other forms of transport that nullify the beneficial aspects of Poole-Frenkel conduction; that is, the intrinsic electron 300b can escape from the potential well. If the extrinsic electron 300a escapes from the potential well 310, it is transported across the insulator through the other potential wells. A current flow including a large number of intrinsic electrons 300b masks the effect of the charge density on the extrinsic electrons 300a and inhibits the measurement of the charge density. Thus, the electric field is applied such that only the extrinsic electrons 300a can escape.

Figure 2B illustrates the Poole-Frenkel potential well after the voltage, E, has been applied. The resulting Poole-Frenkel potential well shown in Figure 3B has a lower barrier height 304 than the intrinsic potential well shown in Figure 3A because the applied electric field pulls the electron away from the charged nucleus, thus reducing the electron's binding energy. The Poole-Frenkel barrier height 306, ϕ_{PF}, is related to the extrinsic barrier height 302a, ϕ_0, by the following relation:

$$\Phi_{PF} = \Phi_0 - \beta_{PF}|\mathbf{E}|^{1/2} \tag{1}$$

in which β_{PF} is the Poole-Frenkel factor for an applied electric field, $\mathbf{E}$. The Poole-Frenkel coefficient is expressed as

$$\beta_{PF} = \left(e^3/\pi\epsilon_I\right)^{1/2} \tag{2}$$

in which: e is the elementary charge, and ϵ_I is electric permittivity of the insulator.

The barrier height 302a, 302b shown in Figure 2A also effects the Poole-Frenkel co-efficient. The lower barrier height created by the Poole-Frenkel effect influences the current density, J, in the following manner:

$$\begin{aligned} J &= J_0 \exp\left(-\frac{\Phi_{PF}}{kT}\right) \\ &= J_0 \exp\left(-\frac{\Phi_0}{kT}\right) \exp\left(\frac{\beta_{PF}|\mathbf{E}|^{1/2}}{kT}\right) . \end{aligned} \tag{3}$$

in which:

K is Boltzmann's constant; T is temperature; J_0 is current density representing intrinsic qualities of the trapped states and the current density, J, is the electrical current per area across the direction of current expressed in Amps per area.

Equation 3 shows how the injected current density J increases as the exponential of the square root of the electric field for Poole-Frenkel injection because of the lowering of the barrier height.

Poole-Frenkel conduction through a material involves extrinsic carriers originating outside the material. Thus, the material selected for the insulating film 204 is selected such that the barrier height allows Poole-Frenkel conduction of extrinsic electrons through the material. The resistivity of the selected material should be large enough to insure that injection of extrinsic carriers is the dominant form of conduction through the material. Any insulator with a resistivity of 10^{12} Ω-cm or greater can be used for the insulating film 204. Resistivity of thin-film polyester, e.g., Mylar® can be researched in Lilly and McDowell *JAP 39*, 141 (1968) or similar references, the teachings of which are incorporated herein by reference in their entirety. Intrinsic barrier heights 300a of two surface states of polyester are 0.55eV and 0.85ev respectively. Intrinsic barrier heights are described in "Model for electrostatic imaging of forensic evidence via discharge through Mylar–paper path," by G. H. Seward in *J. Appl. Phys.* 83(3):1450–1456 (1998), the entire teachings of which are incorporated herein by reference in their entirety.

The Shottky effect describes the influence of an electric field upon the mobility of trapped carriers of a metal at the interface of the metal and an insulator. Figures 3A-B illustrate the influence of an electric field upon the mobility of trapped carriers of a metal probe 200 at the interface of the metal probe 200 and an insulating film 204 by distorting the intrinsic potential well of trapped carriers.

Figure 3A illustrates an insulator-to-metal potential well. Figure 3B illustrates the Schottky potential barrier after the potential has been applied. The insulator-to-metal potential well has a barrier height 402. The barrier height 402 is the energy required by an electron in the metal to move freely within the insulator. The resulting Schottky potential well in Figure 3B has a lower barrier height 404 than the barrier height 402 in the insulator-to-metal potential well shown in Figure

3A. The Shottky barrier height 406, ϕ_S, is related to the barrier height, ϕ_0, by the following relationship:

$$\phi_S = \phi_0 - \beta_S |\mathbf{E}|^{1/2} \tag{4}$$

in which β_S is the Poole-Frenkel factor for an applied electric field, $\mathbf{E}$. The Shottky coefficient is expressed as

$$\beta_S = \left(e^3/4\pi\varepsilon_I\right)^{1/2} = \beta_{PF}/2 \ . \tag{5}$$

The lower barrier height created by the Schottky effect influences the current density, J, in the following manner:

$$\begin{aligned} J &= J_0 \exp\left(-\frac{\phi_S}{kT}\right) \\ &= J_0 \exp\left(-\frac{\phi_0}{kT}\right) \exp\left(\frac{\beta_S |\mathbf{E}|^{1/2}}{kT}\right) \ . \end{aligned} \tag{6}$$

Equation 6 shows how the injected current density increases as the exponential of the square root of the electric field for Schottky injection.

If the intrinsic conduction dominates, then the current density can become linear.

$$J = \sigma_I \frac{(V_I - V_S)}{d_I} \ . \tag{7}$$

in which V_S is voltage across the specimen and σ_I is the conductance of the insulator. The voltage across the specimen is

$$V_s = J \frac{d_s}{\sigma_s} + V_{pert}$$

in which σ_S is the conductance of the specimen. Combining these equations creates

$$J = \left(\frac{d_I}{\sigma_I} + \frac{d_S}{\sigma_S} \right)^{-1} (V_I - V_{pert}) \ . \tag{9}$$

This configuration requires that the current be limited by ohmic transport in both the polyester and the specimen. Even so, the intrinsic conduction can be too small to measure with precision. When the intrinsic conduction is large enough to measure, then there must also be a large concentration of mobile electrons in the specimen. This mobile intrinsic population of electrons can tend to cancel the potential created by an extrinsic charge.

If the specimen is a poor conductor of intrinsic electrons while the insulator is a good conductor of intrinsic electrons, then

$$J = \sigma_I \frac{(V_I - V_{pert})}{d_I} \frac{c_{MS}}{c_M} \ . \tag{10}$$

Ideally, materials with extremely low values of conductance are desired for the specimen in the slab 206. A low conductance corresponds to a large resistivity. The resistivity of the specimen preferably is in the range 10^{12} Ω-cm or greater.

The transverse current density within the insulator of the CDMD can be expressed as:

$$J = J_0 \exp\left(-\frac{\Phi_0}{kT} \right) \exp\left(\frac{\beta_I \left| \mathbf{E}_I \right|^{1/2}}{kT} \right) \ . \tag{11}$$

in which: β_I is equal to either the Poole-Frenkel coefficient or the Shottky coefficient, and $\mathbf{E}_I$ is the electric field vector within the insulator.

The scalar electric field of the insulator can be expressed as:

$$E_I = \frac{c_{IS}}{\epsilon_I}\left(V_I - V_{pert}\right) \tag{12}$$

in which c_{IS} is the capacitance per area of the insulator-slab structure. This area capacitance can be expressed as:

$$c_{IS} = \left(\frac{d_S}{\epsilon_S} + \frac{d_I}{\epsilon_I}\right)^{-1} . \tag{13}$$

in which: d_S is the thickness of the slab, ϵ_S the permittivity of the slab, and d_I is the thickness of the insulator.

The permittivity of a material is a material's ability to create dipoles when subjected to an electric field. It corresponds to the dielectric strength of the material. In the constant current mode, the electric field within the insulator must be maintained as constant.

The electric permittivity of an insulator is dependent on the insulator's dielectric constant. The electric permittivity of the insulator has an effect upon the Poole-Frenkel (PF) coefficient. An extremely large permittivity, such as $10\epsilon_0$ will adversely affect the PF conduction by greatly reducing the PF coefficient. A smaller PF coefficient requires a larger electric field for creation of a measurable current. The electric permittivity of the insulator should be as small as possible. Therefore, the following relation must be true:

$$\left(V_I - V_{pert}\right) = V_0 \tag{14}$$

in which V_0 is an arbitrary reference voltage. This reference voltage is selected based upon a desired current density. Current density can only be estimated from the voltage vs time data of existing work. Absolute current is very dependent upon configuration, such as the area of the probe. Rearrangement of the previous relation creates following linear expression:

$$V_{\mathrm{pert}} = V_I - V_0 \ . \tag{15}$$

Incorporation of the expression for the perturbation potential of a volume-charge leads to:

$$\frac{1}{2}\frac{\rho_s}{\epsilon_s} d_s^2 = V_I - V_0 \ . \tag{16}$$

where: ρ_s is the charge density of the specimen; $\in_s$ is the permittivity of the specimen; d_s is the thickness of the specimen; V_I is the voltage from the top of the insulator with respect to ground and V_0 is the voltage required for constant current without perturbations. Equation 17 can be rearranged as follows:

$$\rho_s = \frac{2\,\epsilon_s}{d_s^2}\left(V_I - V_0\right) \ . \tag{17}$$

This expression for the charge-density of the slab 206 is linearly dependent on the applied potential of the insulator. The reference potential actually refers to the background voltage of the slab 206 which does not contain charge density of interest. This background voltage includes all the electric perturbations of the slab 206 which can include bound charge of its own. In such a situation, the change in applied potential relative to the background voltage is still proportional to the charge-density which is not present in the background voltage. Thus, the above relationship remains valid even if there is some intrinsic charge within the slab 206. Therefore, the electric charge-density of the proteins within the slab 206 is linearly proportional to the applied voltage. The slope of this relationship is described by the intrinsic parameters of slab 206.

Figure 4 illustrates the linear relationship between charge density and change of voltage in a constant current CDMD. The current density J is

$$J = Jo \exp(-A(V_{app} - V_{pert}))$$

The CDMD is operated in a constant current mode to order to quantify the charge density of the electrolyte 210. The rate of injection of extrinsic electrons is controlled mainly by the electric field within the insulating film 204. The electric field of the insulating film 204 is linearly proportional to the voltage across the insulating film 204. The voltage across the insulating film 204 is linearly dependent upon the perturbation potential of the slab 206. The perturbation potential is the electric potential created by a perturbation to the slab 206. This perturbation potential is the extrinsic charge density of the slab 206. The perturbation potential of the slab 206 is proportional to the density of charge within the slab 206. The voltage from the metal probe 200 to the metal-film 208 is linearly dependent upon the charge density within the slab 206. The charge density is the net charge per volume. Thus, the CDMD measures the charge density of the slab 206 by measuring an applied voltage that is linearly proportional to the charge density within the slab 206.

As shown in Figure 4, in the mode of constant current, the shift in the applied voltage is exactly equal to the perturbation potential V_{pert}. An increment of -100 volts in V-Pert 450 creates a change of -100 volts in the applied voltage for any constant current. For example the shift in voltage while at 24 nano Amperes is from 1700 to 1600 volts. Another increment of -100 volts in V-Pert 452 creates another change of -100 volts in the applied voltage.

This correlation is independent of operating current and charge density. This linear dependency of the applied voltage upon the V-pert in the constant current mode is extremely desirable, because the incremental applied voltage is directly proportional to the extrinsic charge density of the dielectric slab.

The CDMD can be operated in a constant voltage mode instead of a constant current mode. In the mode of constant voltage, the current changes nonlinearly with perturbation potential V_{pert}. As shown in the figure below, an increment of -100 volts in V-Pert 450 creates a change in current from 24 to 66 nano Amperes (nA) at a constant applied voltage of 1700 volts. This is an increase of 42 nA. An additional increment of -100 volts in V-Pert 452 creates a change from 66 to 170 nA, an increase of 104 nA. This second increment of current is about 2.5 times that of the previous increment.

This non-linearity of the constant voltage mode can be successfully employed to extract the charge density, but it is far more difficult than manipulation of the linearity of the constant current mode. In the constant voltage mode, the exact shape of the I-V curve must be known. Conversely, in the constant current mode, the shape of the curve is irrelevant. Thus the constant current mode is far superior to the constant voltage mode.

The CDMD provides a significant improvement over past measurement devices, which required sophisticated modeling of time-and-space dependent voltage data. The applied voltage is, once again, linearly proportional to the charge density.

The CDMD is particularly useful as a measurement tool for determining the quantity of a target molecule contained in the slab, for example, for determining the quantity of a biological target molecule in an electrophoretic slab gel after electrophoresis. Concentrations of a biological target molecule within a migration channel can be readily derived from the linear dependence of an applied voltage. The CDMD can replace the labor intensive quantification process.

Due to the longitudinal metal film 208 in the CDMD shown in Figure 1, electrophoresis cannot be conducted within the CDMD because the metal film longitudinal plane disrupts the electric field of the migration channel. Thus, for the embodiment shown in Figure 1, the migration channel must be transferred from the electrophoretic apparatus to the CDMD. The migration channel can be easily moved, if the migration channel is comprised of paper or gel. Returning to Figure 1, the migration channel is placed on top of the metal film 208. The insulating film

204 is placed on top of the migration channel. Thus, the migration channel is the slab 206 between the metal film 208 and the insulating film 204. A voltage is applied to the slab-and-insulator structure 202 and the charge density is determined by observing the change in applied voltage required to maintain a constant transverse current flow through the slab-and-insulator structure 202. Thus, the CDMD provides quantification of charge density which can also be converted to a molecular concentration.

The CDMD can be operated in the opposite polarity; that is, by applying a negative potential instead of a positive potential. This is beneficial for detection of a mobile negative charge. If the applied negative potential can drive the ions to the ground plane, then the perturbation potential associated with that charge goes to zero and the charge is not detected. If a positive potential is applied, then the mobile negative charges are drawn to the insulator at which location the perturbation potential is maximized.

During application of a positive potential, the Poole-Frenkel conduction occurs at surface states at the interface between the insulating film 204 and the electrolyte 210. The mathematical expressions for current density remain unchanged because they are dependent upon the magnitude of the electric field and not the polarity. The direction of current flow is reversed.

A second insulating film can be added between the metal film 208 and the slab 206 plane. This second insulating film prevents mobile ions from being driven to the metal film 208 where their perturbation potential falls to zero. In the stratified slab-and-insulator structure 218, the CDMD is symmetric. It can be operated in either polarity of applied voltage for detecting either polarity of charge with current flowing transversely through the structure towards the metal film 208 in direction 212 or towards the insulating layer 204 in direction 216.

In the situation of mobile ions residing at the interface between the slab 206 and the insulator 204, the surface charge density becomes the relevant parameter. The surface charge density is the charge per area. The perturbation potential for a surface charge is:

$$(V_I - V_O) = \frac{\sigma d s}{\varepsilon s}$$

The relative thicknesses of the slab 206 and the insulating film 204 are not crucial. They should be kept to a ratio in which one is not more than ten times larger than the other, preferably with the slab 206 being thicker than the insulating film 204, but it is not necessary that the slab 206 be thicker than the insulating film. The absolute magnitude of the thicknesses determines the magnitude of the operating voltage. Figures 5A-C illustrate the relationship between the thickness of an insulator 500 and the thickness of the slab 502 in a constant voltage CDMD. Fig. 5A illustrates a slab 206 and an insulating film where the thickness of the slab 502 is approximately 20 times thicker than the thickness of the insulator 500. Figure 5C illustrates a constant voltage CDMD in which the thickness of the insulator 500 is thinner than that shown in Fig. 5A. As shown in Fig. 5B, the extrinsic current has a steeper slope resulting in a greater extrinsic current with a lower applied potential than for the embodiment shown in Figure 5A. Figure 5C illustrates an embodiment in which no insulator is provided resulting in no measurable extrinsic current. Thus, the preferred embodiment is shown in Figure 5C with a thin insulator.

Figure 6 illustrates yet another embodiment of the stratified slab-and-insulator structure shown in Figure 1. The CDMD shown in Figure 1 may be inverted for the purpose of easier fabrication. Inverting the stratified slab-and-insulator structure also allows for a thinner insulating film 204 than provided in the embodiment shown in Figure 1. The inverted stratified slab-and-insulator structure may also be useful for drawing ions to the surface of the slab 206. In this slab-and-insulator structure 220 the insulating film 204 is located on top of the metal film 208. The slab 206 is then placed on top of the insulating film 204. The metal probe 200 may be electrically connected to the gel in the slab 206 with ionic solution. This gentle connection to the slab 206 does not compress the electrolyte 210. Furthermore, very thin insulating films 204 are possible. Thus, a lower operating voltage is possible with the metal probe 200.

The electrolyte 210 must be a fair conductor of extrinsic electrons. If not, the electrolyte 210 will charge up like a capacitor, and the CDMD will not function effectively. While a specific measure for extrinsic mobility is not available, the electrolyte 210 should contain a species that can accept an extrinsic electron and easily transport it across the electrolyte 210. The transport can be via a single molecule or by transfer from one molecule to another.

In addition, the intrinsic electrons of the electrolyte 210 must be bound to molecules in order to minimize their electric mobility. Extrinsic electrons may possess much larger mobility. Therefore, the electric mobility of the intrinsic ions of the electrolyte 210 should be kept to a minimum to minimize the masking of the extrinsic ions by the intrinsic ions.

The mobile ions in the electrolyte 210 can reduce the perturbation potential associated with the extrinsic charge. The mobile ions are drawn to the extrinsic charge in a manner that nullifies the electric field created by the extrinsic charge. This effect is commonly known as masking. This sort of current created by an electric field is a drift current. Fortunately, the mobile ions also experience diffusion currents due to concentration gradients. These diffusion currents can effectively counter the drift current.

The drift current is dependent upon the charge-to-mass ratio while the diffusion current is not. The drift current is expressed as:

$$J = \mu E. \tag{20}$$

in which μ the electric mobility of the ion or electron. The mobility is defined as

$$\mu = \frac{e\tau}{m}, \tag{21}$$

where τ is the mean free-time between collisions and m is the mass of the ion. The charge-to-mass ratio of a typical ion is 10^{-5} that of electron. Further reduction is possible by deliberate design. Consequently, masking of the extrinsic charge by an ionic species is much less effective than masking by electrons. Typically, the diffusion current of ions can successfully overcome the drift current.

Figure 7 illustrates another embodiment of the CDMD employing an insulator coated metal probe 500. The coated metal probe 500 is a metal probe 504 coated with an insulator coating 502. The coated metal probe 500 is about 2 to 3mm in diameter. The diameter of the coated metal probe 500 determines the spatial resolution. The coated metal probe 500 has several desirable features. The interface between the metal probe 504 and the insulator coating 502 is carefully controlled and can be easily reproduced. In the embodiment with a separate metal probe 200 and insulating film 204 described in conjunction with Figure 1, the insulator, a polyester sheet, *e.g.*, Mylar® can be 10 μm in thickness. Poole-Frenkel conduction across such a polyester sheet requires an applied voltage in the range of about 1000 - 5000 volts for a nano ampere of current over a 1-mm by 1-mm probe. The insulator coating 502 can be thinner than a polyester sheet. The insulator coating 502 can be 1 μm thick. Consequently, a much smaller voltage is required for the same electric field and corresponding current density. The voltage required for a 1 μm thick insulator is in the range of 100 - 500V much less than the 1000 - 5000V required for the insulator layer described in conjunction with the embodiment shown in Figure 1. Reduction of the voltage reduces the cost of the CDMD and increases the safety of the CDMD. The insulator coating 502 can be provided through vacuum deposition or by chemical plating of a metal oxide on the metal probe 504. The coated metal probe 500 must make contact with the slab without compressing the slab. A liquid connection is possible similar to the embodiments described in conjunction with Figs. 1A-1C).

Figure 8 illustrates an embodiment of the CDMD employing a plurality of probes 604 embedded within an insulating material 602. The probes are 10 to 100 microns in dimension a. The probes 604 are completely surrounded by insulating material 602 except for the electrical connections to an external circuit or an integrated circuit 600. The integrated circuit 600 supplies power. The plurality of probes 604 can be fabricated as part of integrated circuit. This integrated circuit can be manufactured by methods which are well-known in the semiconductor industry. (J. Millman, Microelectronics, McGraw-Hill, New York (1979). Both pure-metals and metal-oxides of common usage are sufficient for the probes 604 and insulator material 602 respectively.

This integrated device can serve as an examination platform for the slab 206 gel used in electrophoresis. The gel is transferred to the integrated CDMD, then the electronic output of the CDMD is a one-dimensional scan of the charge-density of the migration channel. This provides a rapid and accurate quantification of the charge-density of a migration channel.

In this embodiment, the insulating layer is about 0.1 micron and the operating potential is in the range of about 10 to 50V. A further reduction in thickness can create an operating range of about 1 to 5V.

The buried array can be fabricated using well-known semiconductor processes. The electrode pattern is created by deposition of the desired material on the substrate, followed by selective removal of the material. The selective removal can be achieved by the use of photoresist. The integrated device is mounted on a glass substrate. A different voltage is applied to each probe dependent on the charge density at that point. The shape of the electrode can be rectangular. The number of probes are implementation dependent.

Electrophoresis is not performed simultaneously with measurement in the embodiments of the CDMD shown and described in conjunction with Figures 1A-3A, 4 and 5. The required orientation of the electric field in electrophoresis is orthogonal to the electric field required for the CDMD. Thus, the continuous ground plane of the embodiments of the CDMD shown in Figures 1A-3A, 4 and 5 are likely to disrupt the electric field of the electrophoresis. The free electrons in the metal move until the electric field becomes perpendicular to the surface of the metal, in which state the boundary of the metal prevents further motion by the electrons. This effect can be eliminated by disrupting the continuity of the ground plane along the desired direction of current for electrophoresis, thus, providing a CDMD that is compatible with electrophoresis. This ground structure is not only continuous along the desired flow of current for the CDMD, but is also discontinuous along the desired direction of current for electrophoresis.

Figure 9 is a cross-section of an embodiment of a bottom of a CDMD 700 for performing electrophoresis. The embodiments described in conjunction with Figures 1, 6, 7 and 8 all include a continuous ground plane; that is the metal film 208. The continuous ground plane does not allow electrophoresis to be performed because electrophoresis requires an electrical field. In a continuous ground field, the free electrons go to the ends of the continuous ground plane as the voltage is applied and thus the electric field goes to zero. The discontinuous ground structure 700 includes a discontinuous ground structure 702 and an electrolyte 704. The discontinuous ground structure 702 includes an array of metal strips 706 and insulator strips 708. Two outer metal strips 730 are provided for supplying the voltage for electrophoresis. The electric field for the discontinuous ground structure 702 is represented by continuous fine lines running between the metal strips 706 and the outer metal strips 730.

The metal strips 706a in the discontinuous ground structure 700 are separated by an insulator. The electrolyte 704 is placed on top of the discontinuous ground structure 702. The electrolyte 704 cannot penetrate the discontinuous ground structure 702. For the purpose of chemical isolation from the electrolyte 704, a thin metal oxide (not shown) can be applied to the top of the discontinuous ground structure 702.

The field lines 710 represent both the magnitude and the direction of the electric field. The magnitude is inversely proportional to the spacing between the field lines 706. The direction is tangent to the field line. Current tends to flow along the field lines 710. Three types of electric fields are present: a uniform electric field 716, an irregular electric field 718 and a segmented electric field 720.

Two integrated circuits 712 and 714 provide two modes of operation. During electrophoresis, the voltage supply 722 is connected only to the outermost metal strips 730 by closing switch 724 and opening switches 726 to disconnect the inner metal strips 706 from each other. Current flows through switch 724 to the outermost metal strips 706a. During charge density measurement, the inner metal strips 706 are connected to each other by closing switches 726, while the outer strips 730 are disconnected from voltage supply 722 by opening switch 724. Current flows through switches 726 to the innermost metal strips 706.

During electrophoresis, the discontinuous ground structure 702 disrupts the electric field only within proximity of the discontinuous ground structure 702. The extent of this disruption is on the order of three times the spacing of the metal strips 706. At larger distances from the discontinuous ground plane 702, the electric field is essentially undisturbed by the discontinuous ground structure 702. In such regions, the electrophoresis proceeds as if the discontinuous ground structure 702 were not present.

The bulk of the electrolyte 704 can function normally if the spacing between the metal strips 706 is much smaller than the depth of the electrolyte 704. The metal strips 706 can be made extremely thin by fabrication methods used for integrated circuits which are well known in the art. For example, for a strip spacing of 10 microns, the corresponding electrolyte is on the order of 100 microns.

The construction of the discontinuous ground structure is similar to the construction of the metal probes in the top portion of the CDMD 728. The substrate is an insulator in order to maintain the desired electrical discontinuity. Parts of the discontinuous ground structure 702 may be external to the integrated ground structure, such as the switches 726, 724 and the voltage supply 722. The outer metal strips 730 may also be external to the discontinuous ground structure 702 because the positioning of the outer metal strips 706 is not critical.

Figure 10 illustrates the combination of the top portion of the CDMD 728 and the bottom portion of the CDMD 700 shown in Figure 6. After completion of electrophoresis on the bottom portion of the CDMD 700, the top portion of the CDMD 728 is placed on top of the bottom portion of the CDMD 700 as shown in Figure 8. The bottom portion of the CDMD 700 has been described in conjunction with Figure 6. The top portion of the CDMD 728 includes an integrated probe circuit 600, an array of metal probes 604 and an insulator 602. The top portion of the CDMD 728 has already been described in conjunction with the embodiment shown in Figure 5. After combining the top portion of the CDMD 728 and the bottom portion of the CDMD 700, the charge density of the electrolyte in the electrolyte channel 704 can be measured as has been described in conjunction with Figure 7. The bottom portion 700 is recyclable by replacement of the electrolyte in the electrolyte channel 704.

Figure 11 illustrates an alternative embodiment for the discontinuous ground structure CDMD in which the metal probes 604 and the electrolyte channel 704 are repeated as an array.

The array of electrolyte channels 704 can be fabricated in two steps. First, an array of slots (not shown) is created within a thin sheet of an electrical insulator. The electric insulator is bonded to the ground structure. Second, the slots are filled with the desired electrolyte which can be gel or liquid. At this point, the bottom portion of the CDMD 700 is ready for electrophoresis.

If the metal probes 604 are created with a discontinuity similar to that of the ground structure, then the top portion of the CDMD 728 may be applied to the bottom portion of the CDMD 700 before electrophoresis occurs. Such capability facilitates management of a liquid electrolyte. The array can be a one-dimensional array of metal strips or a two dimensional array of metal pads. The embodiment shown in Figure 11 may be reusable.

The features and other details of the invention will now be more particularly described and pointed out in the example. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

EXAMPLE

Analysis of Restriction Fragments Produced from Plasmid pRBR322 Using the CDMD

A sample of 0.5 µg of pBR322 plasmid DNA (New England Biolabs #N3033S, NEB 2000-2001 catalog, Beverly, MA) is digested with the restriction enzyme Msp I (NEB #R0106S) for one hour according to the enzyme manufacturer's instructions. After digestion, glycerol is added to 25% final concentration (volume/volume) and SDS (sodium dodecyl sulfate) is added to 0.5% (weight/volume).

A vertical 5% polyacrylamide gel (29:1 ratio monomer:bis) is prepared in a commercially available electrophoresis device (Bio Rad Mini Protean II Apparatus, Bio-Rad, Richmond, CA), using the device manufacturer's instructions. The gel is approximately 10 cm wide by 10 cm long by 0.8 mm thick when cast between the glass plates of the assembled electrophoresis cassette. The electrophoresis buffer used in the gel and the electrode compartments is 89 mM Tris-borate buffer pH 8.3, 2mM EDTA.

The sample containing the restriction digest containing 0.5 μg of pBR322 DNA (final volume 20 μl) is loaded into the sample well at the top of the gel and electrophoresed through the gel for one hour at room temperature at a constant voltage of 150 V. After electrophoresis, the gel cassette is removed from the buffer reservoir, and the plates are carefully disassembled taking care not to tear the gel. The gel is carefully transferred to the lower electrode plate of the CDMD device. The gel is covered with plastic food wrap, and gently rubbed to remove air bubbles and excess buffer that may be trapped between the electrode and the lower surface of the gel. The plastic wrap is removed from the gel and excess liquid is blotted off with absorbent tissue. The gel is now covered with an insulating sheet of Mylar® (DuPont, Wilmington, DE) which has a thickness of 25 microns.

The probe is a silver electrode with a 1 mm circular cross section at the tip that is scanned across the surface of the insulating sheet. The probe is connected to the negative side of a Bertan 105 power supply (Bertan High Voltage Corp., Hickville, Ny). The positive side of the power supply is connected to the lower electrode plate on which the gel rests.

The probe is then placed in contact with the surface of the Mylar®-covered gel and scanned over the gel with the power supply operating in constant current mode to maintain a steady state current of 100 nanoamps. The probe is positioned at the top of the gel over the lane in which the DNA sample was loaded, and scanned down the gel in the direction of electrophoretic migration. In this way, the probe will pass over each of the discrete DNA bands produced by the restriction digest. As the probe is scanned at constant speed over the gel, the voltage is recorded as a function of the probe position. The positions which produce high voltage readings will correspond to the positions of the negatively charged DNA restriction fragments from the sample. The distance traveled in the gel will be proportional to the molecular weight of the fragment, as described (see Sambrook, Fritch, and Maniatis; 1989, Molecular Cloning: A Laboratory Manual, 2nd Edition, Chapter 6, Cold Spring Harbor Press, Cold Spring Harbor, NY, the entire contents of which are hereby incorporated by reference).

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A device for detecting a charged species in a dielectric media comprising:

   an insulator on a surface of the media;

   a probe which maintains a steady state charge on the insulator to create an electric field through the insulator and the media; and

   an electrical detector which measures an electrical response to the detector.

2. The device of Claim 1, wherein the probe applies the charge with a constant current through the insulator and the media.

3. The device of Claim 2, wherein the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator.

4. The device of Claim 2, wherein the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

5. The device of Claim 1, wherein the probe comprises a scanning metal probe.

6. The device of Claim 1, wherein the probe comprises a scanning metal probe coated with an insulator.

7. The device of Claim 1 further comprising a plurality of probes.

8. The device of Claim 6, wherein the plurality of probes are configured as an array of metal probes contained within an insulator.

9. The device of Claim 7, wherein the probes are in an integrated electrical circuit.

10. The device of Claim 1, wherein the insulator is selected from the group consisting of paper, plastics and metal oxides.

11. A device for detecting a charged species in a dielectric media comprising:

an insulator on a surface of the media;

means for maintaining a steady state charge on the insulator to create an electric field through the insulator and the media; and

means for measuring an electrical response to the applied charge while maintaining the steady state charge.

12. The device of Claim 11, wherein the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator.

13. The device of Claim 11, wherein the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

14. The device of Claim 11, wherein the probe comprises a scanning metal probe coated with an insulator.

15. The device of Claim 11 further comprising a plurality of probes.

16. The device of Claim 15, wherein the plurality of probes are configured as an array of metal probes contained within an insulator.

17. The device of Claim 16, wherein the probes are in an integrated electrical circuit.

18. The device of Claim 11, wherein the insulator is selected from the group consisting of paper, plastics and metal oxides.

19. A device for detecting a charged species in an electrophoretic media comprising:

a dielectric slab media having electrophoretically distributed charged species therein;

an insulator positioned on a longitudinal surface of the media;

a probe which applies a charge to the insulator to create an electric field through the insulator and media; and

an electrical detector which measures an electrical response to the applied charge.

20. The device of Claim 19, wherein the probe maintains a steady state charge on the insulator to create an electric field through the insulator and the media.

21. The device of Claim 19, wherein the probe applies a charge in a constant current through the insulator and the media.

22. The device of Claim 19, wherein the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator.

23. The device of Claim 19, wherein the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

24. The device of Claim 19, wherein the probe consists of a scanning metal probe.

25. The device of Claim 19, wherein the probe consists of a scanning metal probe coated with an insulator.

26. The device of Claim 23 further comprising a plurality of probes.

27. The device of Claim 24, wherein the plurality of probes are configured as an array of metal probes contained within an insulator.

28. The device of Claim 25, wherein the probes are in an integrated electrical circuit.

29. The device of Claim 19, wherein the insulator is selected from the group consisting of paper, plastics and metal oxides.

30. The device of Claim 19, wherein the dielectric slab is formed from an electrophoretic gel selected from the group consisting of polyacrylamide gel, starch gel and agarose gel.

31. A device for detecting a charged species in an electrophoretic media comprising:

a dielectric slab media having electrophoretically distributed charged species therein;

an insulator positioned on a longitudinal surface of the media;

means for maintaining a steady state charge on the insulator to create an electric field through the insulator and the media; and

means for measuring an electrical response to the applied charge while maintaining the steady state charge.

32. The device of Claim 31, wherein the probe applies the charge with a constant current through the insulator and the media.

33. The device of Claim 31, wherein the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator.

34. The device of Claim 31, wherein the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

35. The device of Claim 31, wherein the probe comprises a scanning metal probe.

36. The device of Claim 31, wherein the probe comprises a scanning metal probe coated with an insulator.

37. The device of Claim 31 further comprising a plurality of probes.

38. The device of Claim 37, wherein the plurality of probes are configured as an array of metal probes contained within an insulator.

39. The device of Claim 38, wherein the probes are in an integrated electrical circuit.

40. The device of Claim 31, wherein the insulator is selected from the group consisting of paper, plastics and metal oxides.

41. The device of Claim 31, wherein the dielectric slab is formed from an electrophoretic gel selected from the group consisting of polyacrylamide gel, starch gel and agarose gel.

42. An electrophoresis cassette comprising:

a cavity adapted to receive electrophoretic dielectric media to be subjected to a longitudinal electric field for electrophoretic distribution of charge particles longitudinally through the media; and

a discontinuous electrode extending longitudinally along the cavity.

43. The device of Claim 42, further comprising probe electrodes extending longitudinally along the cavity in opposition to the discontinuous electrode.

44. The device of Claim 42, wherein the cavity contains the electrophoretic dielectric media and the device further comprises an electrode at each end of the cavity.

45. A method of detecting a charged species in a media including the following steps:

providing a dielectric media having the charged species therein;

providing an insulator on a surface of the media;

maintaining a steady state charge on the insulator to create an electric field through the insulator and the media; and

measuring an electrical response to the applied charge while maintaining the steady state charge.

46. The method of Claim 45, wherein the charge is applied by a constant current applied through the insulator and media.

47. The device of Claim 45, wherein the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator.

48. The device of Claim 45, wherein the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

49. The method of Claim 45, wherein the probe comprises a scanning metal probe.

50. The method of Claim 45, wherein the probe comprises a scanning metal probe coated with an insulator.

51. The method of Claim 45 further comprising a plurality of probes.

52. The method of Claim 45, wherein the plurality of probes are configured as an array of metal probes contained within an insulator.

53. The method of Claim 45, wherein the probes are in an integrated electrical circuit.

54. The method of Claim 45, wherein the insulator is selected from the group consisting of paper, plastics and metal oxides.

55. A method of detecting a charged species in an electrophoretic media including the following steps:

providing a dielectric slab electrophoretic media having the charged species distributed longitudinally therein;

providing an insulator on a longitudinal surface of the media;

applying a charge to the insulator to create an electric field through the insulator and the media; and

measuring an electrical response to the applied charge.

56. The method of Claim 55, wherein the charge is maintained in a steady state as the electrical response is measured.

57. The method of Claim 55, wherein the charge is applied by a constant current applied through the insulator and media.

58. The method of Claim 55, wherein the resistivity of the insulator is sufficient for extrinsic electrons to dominate conduction of electricity through the insulator.

59. The method of Claim 55, wherein the energy state of extrinsic electrons is substantially higher than the energy state of intrinsic electors in the insulator and the media.

60. The method of Claim 55, wherein the probe comprises a scanning metal probe.

61. The method of Claim 55, wherein the probe comprises a scanning metal probe coated with an insulator.

62. The method of Claim 55 further comprising a plurality of probes.

63. The method of Claim 62, wherein the plurality of probes are configured as an array of metal probes contained within an insulator.

64. The method of Claim 63, wherein the probes are in an integrated electrical circuit.

65. The method of Claim 55, wherein the insulator is selected from the group consisting of paper, plastics and metal oxides.

66. The method of Claim 55, wherein the dielectric slab is formed from an electrophoretic gel selected from the group consisting of polyacrylamide gel, starch gel and agarose gel.

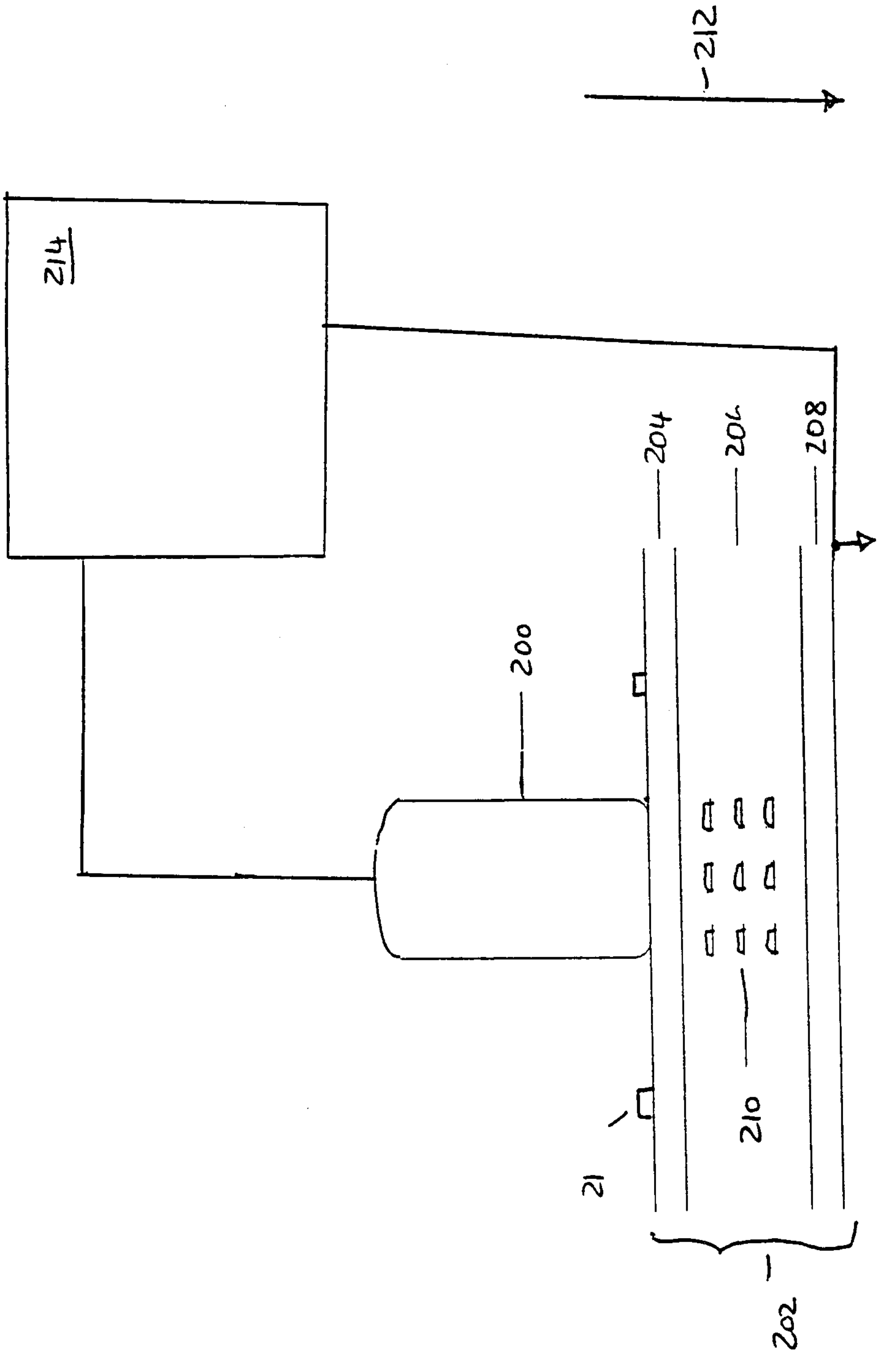

FIG. 1

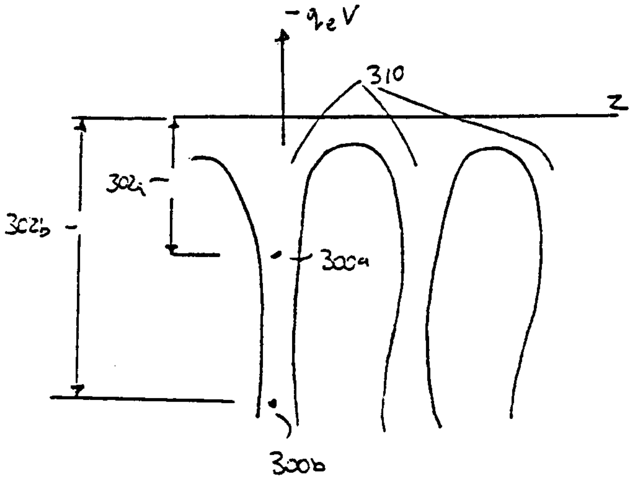

FIG. 2A

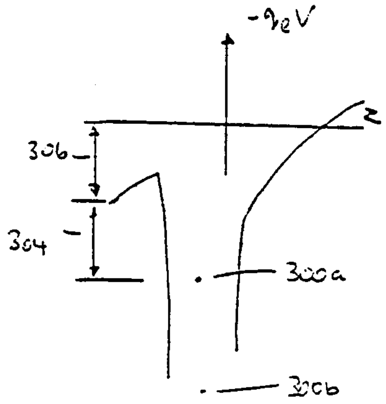

FIG 2B

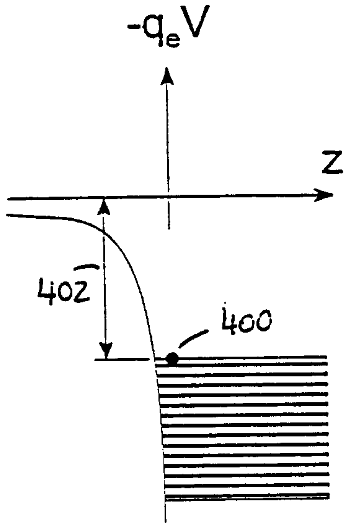

FIG 3A

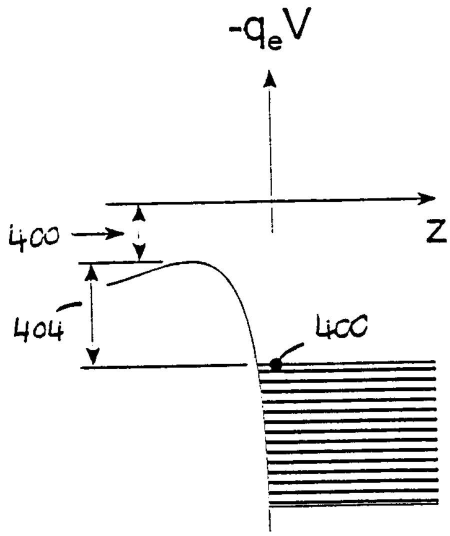

FIG. 3B

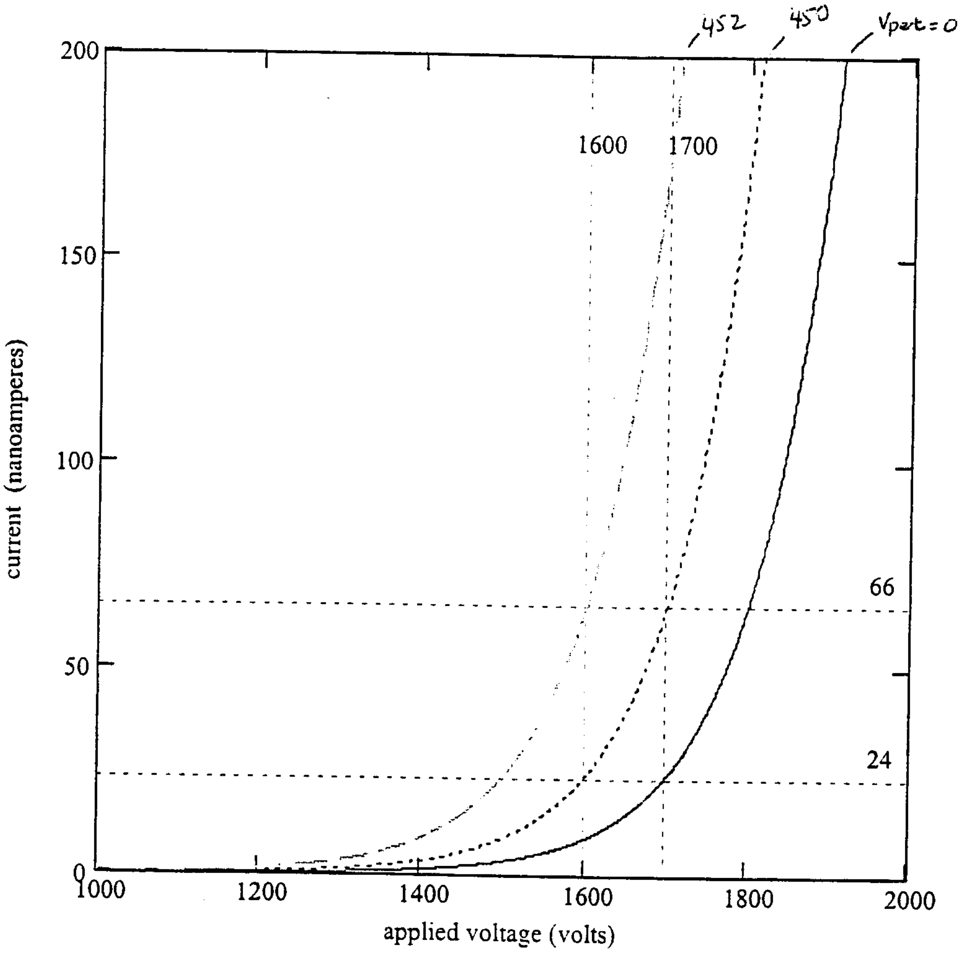
452
450
Vpart=0
200
150
100
50
0
1600
1700
66
24
1000
1200
1400
1600
1800
2000
current (nanoamperes)
applied voltage (volts)

FIG. 4

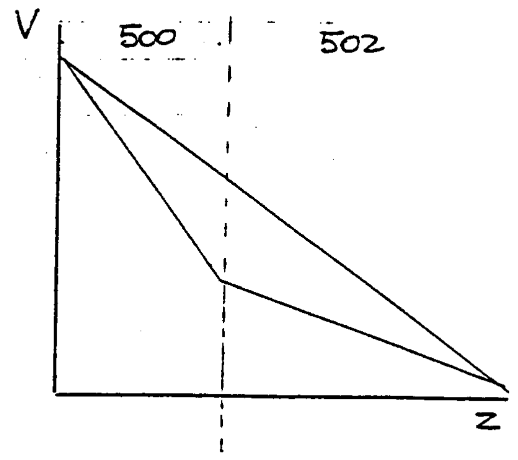

FIG. 5A

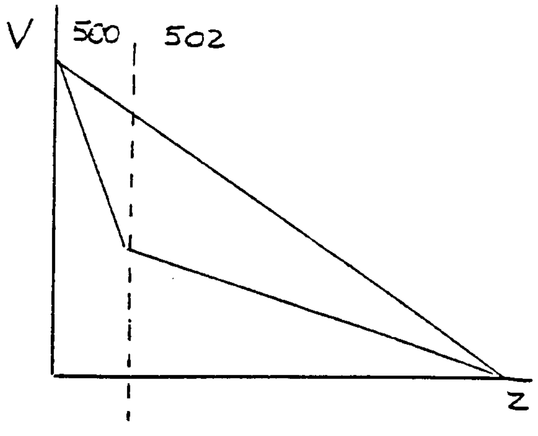

FIG. 5B

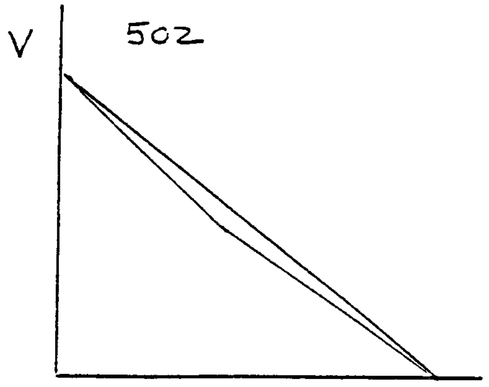

FIG. 5C

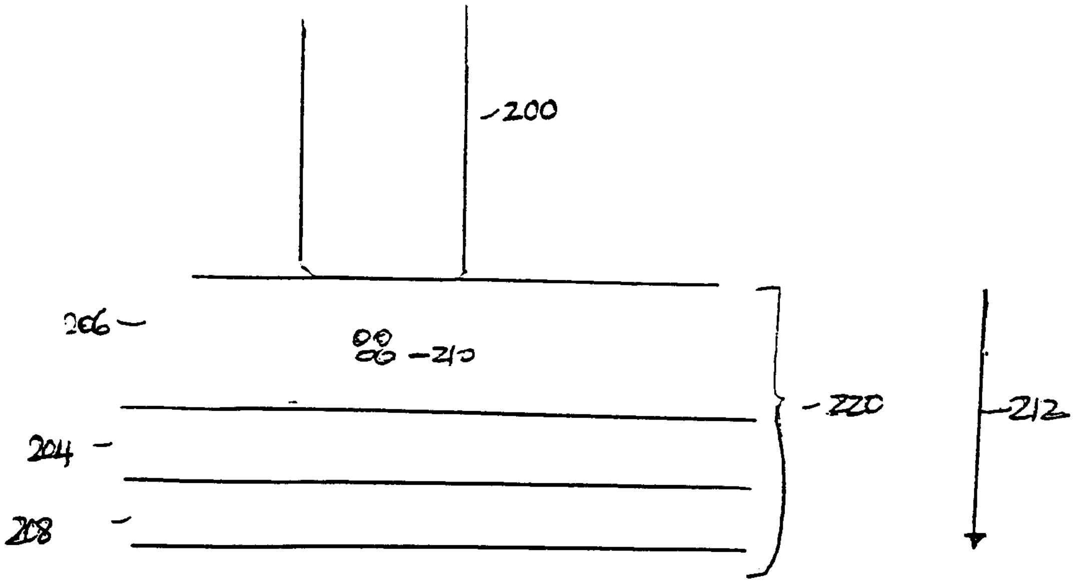

FIG. 6

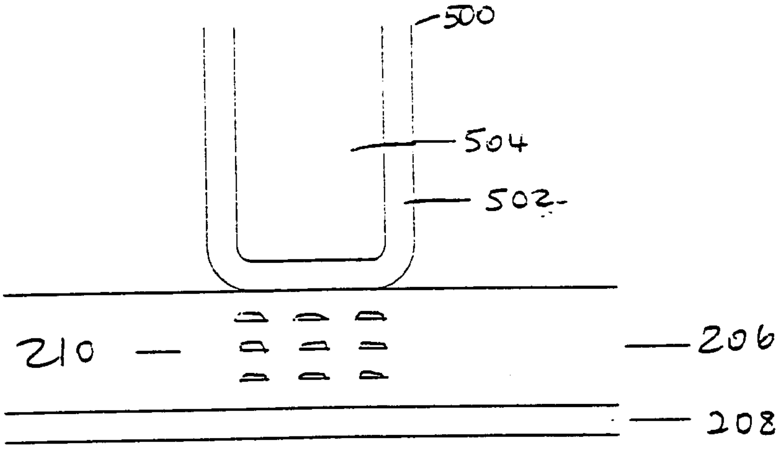

FIG. 7

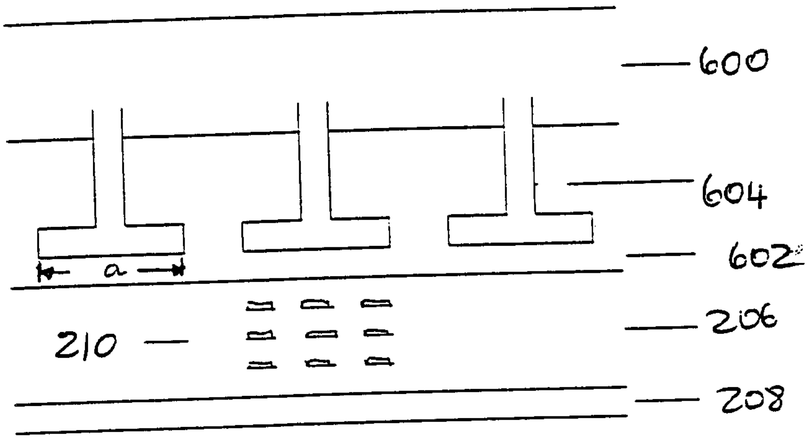

FIG. 8

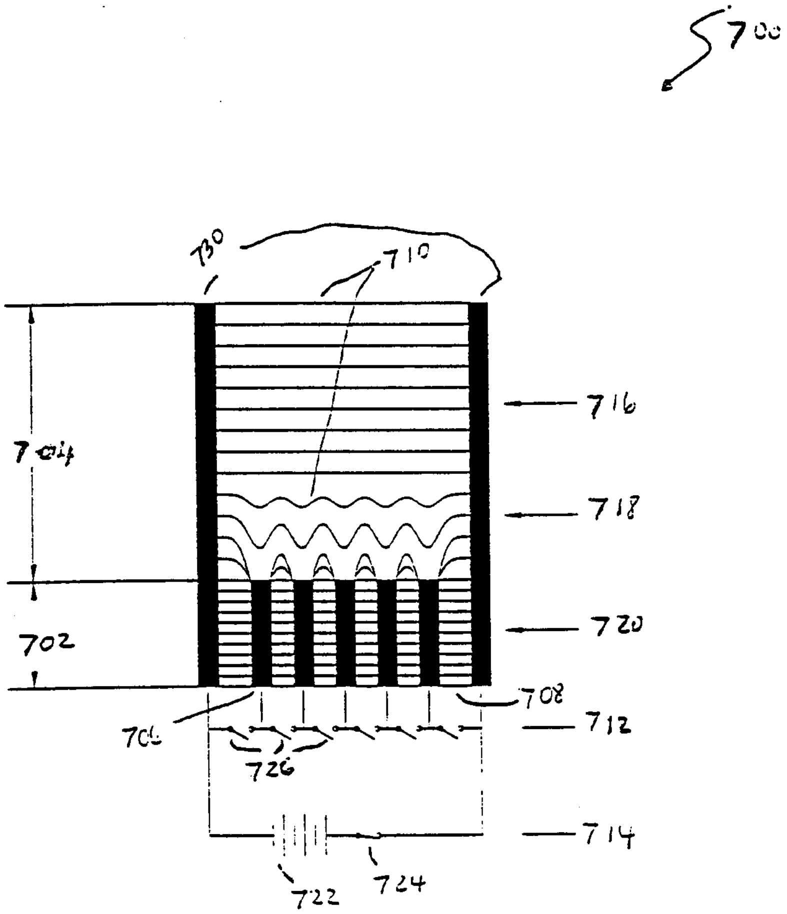

FIG. 9

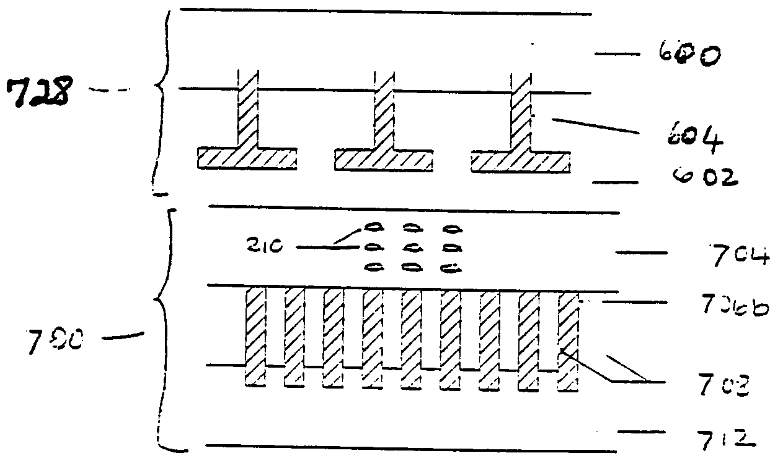

FIG. 10

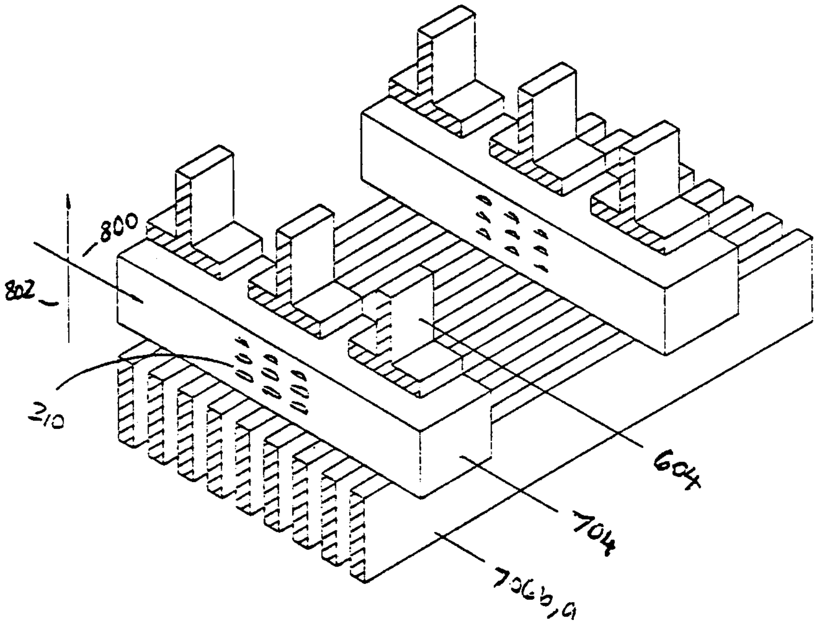

FIG. 11

INTERNATIONAL SEARCH REPORT

Inte, ,onal Application No
PCT/US 00/21691

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/447 G01N27/60

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)
IPC 7 G01N

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 practical, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 125 805 A (NAGAMATSU KAZUO ET AL) 14 November 1978 (1978-11-14) column 5, line 56 -column 6, line 39; figure 3A ---	1,11,19, 31,42, 45,55
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search
3 January 2001

Date of mailing of the international search report
15/01/2001

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Duchatellier, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	G. H. SEWARD: "model for electrostatic imaging of forensic evidence via discharge through mylar film path" J. APPL. PHYS., vol. 83, no. 3, 1 February 1998 (1998-02-01), pages 1450-1456, XP000955244 cited in the application the whole document	1

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