AU9514354

(12) PATENT ABRIDGMENT (11) Document No. AU-B-14354/95
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 691237

(54) Title
TRANSDERMAL DRUG DELIVERY BY ELECTROINCORPORATION OF MICROCARRIERS

International Patent Classification(s)
(51)[6] **A61N 001/30**

(21) Application No. : **14354/95** (22) Application Date : **13.12.94**

(87) PCT Publication Number : **WO95/26781**

(30) Priority Data

(31) Number	(32) Date	(33) Country
219970	30.03.94	US UNITED STATES OF AMERICA
310647	22.09.94	US UNITED STATES OF AMERICA

(43) Publication Date : **23.10.95**

(44) Publication Date of Accepted Application : **14.05.98**

(71) Applicant(s)
GENETRONICS, INC.

(72) Inventor(s)
GUNTER A. HOFMANN

(74) Attorney or Agent
MADDERNS , 1st Floor Wolf Blass House, 64 Hindmarsh Square, ADELAIDE SA 5000

(56) Prior Art Documents
US 5162042
WO 92/04937
US 5019034

(57) Claim

1. Apparatus including a source of high voltage pulses and electrodes for applying to tissue surface for trans tissue molecular delivery by electroporation characterized by:

a quantity of microcarriers for carrying molecules to be delivered across a tissue surface;

a quantity of molecules to be delivered across the tissue surface embodied in said microcarriers;

a substrate for supporting said quantity of microcarriers in contact with a selected area of the tissue surface; and

means for applying an electric field of sufficient amplitude to induce electroporation of said selected area of tissue and to enable transport of the molecules from the microcarriers into the tissue.

.../2

11. Use of electroporation for delivering molecules across a tissue surface, comprising:

selecting a quantity of selected molecules to be delivered across a tissue surface;

providing a quantity of microcarriers;

loading a quantity of said molecules to be delivered in said quantity of microcarriers;

contacting a selected area of a tissue surface with said quantity of microcarriers; and

applying an electric field of sufficient amplitude and duration to induce electroporation of said selected area of tissue and to transport the molecules from the microcarriers into the tissue.

OPI DATE 23/10/95 APPLN. ID 14354/95
AOJP DATE 07/12/95 PCT NUMBER PCT/US94/14352

AU9514354

(51) International Patent Classification 6 : A61N 1/30

A1

(11) International Publication Number: WO 95/26781

(43) International Publication Date: 12 October 1995 (12.10.95)

(21) International Application Number: PCT/US94/14352

(22) International Filing Date: 13 December 1994 (13.12.94)

(30) Priority Data:
08/219,970 30 March 1994 (30.03.94) US
08/310,647 22 September 1994 (22.09.94) US

(60) Parent Applications or Grants
(63) Related by Continuation
US 08/219,970 (CON)
Filed on 30 March 1994 (30.03.94)
US 08/310,674 (CON)
Filed on 22 September 1994 (22.09.94)

(71) Applicant *(for all designated States except US)*: GENETRONICS, INC. [US/US]; 11199-A Sorrento Valley Road, San Diego, CA 92121 (US).

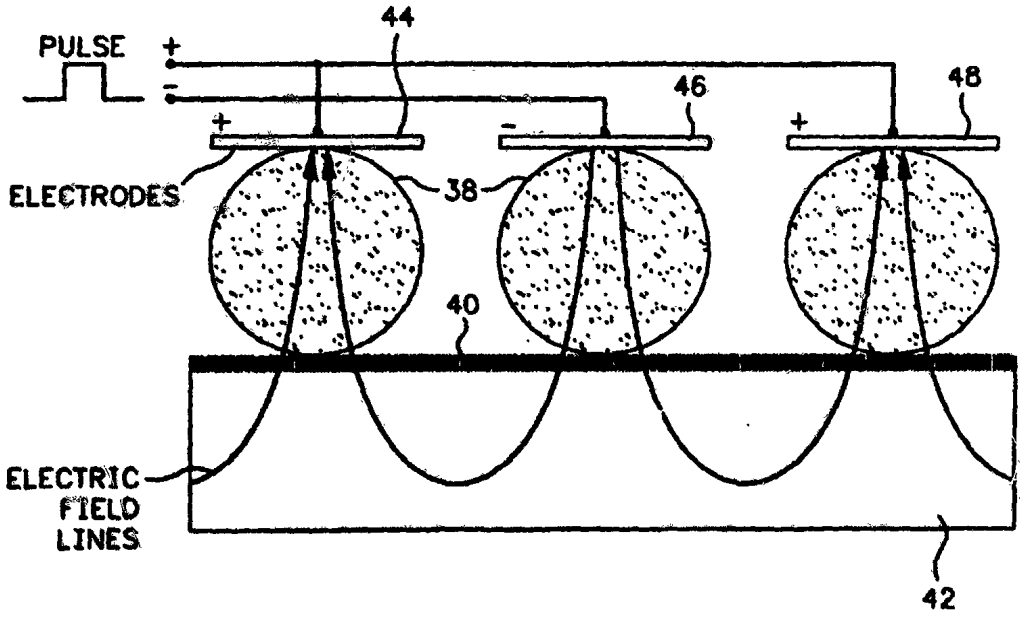
(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: HOFMANN, Gunter, A. [US/US]; 3750 Riviera Drive #6, San Diego, CA 92109 (US).

(74) Agent: BAKER, Freling, E.; Baker, Maxham, Jester & Meador, Suite 2770, 750 B Street, San Diego, CA 92101 (US).

(81) Designated States: AU, CA, CN, JP, KR, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: TRANSDERMAL DRUG DELIVERY BY ELECTROINCORPORATION OF MICROCARRIERS



(57) Abstract

A method of transdermal molecular delivery comprises the steps of encapsulating molecules to be delivered into microbubbles, contacting a selected area of a tissue surface with a solution of the microbubbles, and applying a pulsed electric field of sufficient amplitude to induce dielectric breakdown of the stratum corneum and to induce transport of the molecules through the pores in the stratum corneum into the underlying tissue to enable diffusion of molecules into the tissue. In one embodiment the microbubbles are too large to pass through the pores in the SC and are fused to the surface where pores form passages from the microbubble through the SC. In another embodiment, the microbubbles are small enough to pass through the pores in the SC and pass through the pores and broken down by enzymes in the tissue.

TRANSDERMAL DRUG DELIVERY BY ELECTROINCORPORATION OF MICROCARRIERS

TECHNICAL FIELD

The present invention relates to drug delivery and pertains particularly to a method and apparatus for the transdermal delivery of drugs and other molecules.

BACKGROUND ART

The concept of transdermal drug delivery as a means of delivering drugs and the like without the physical penetration of the tissue has been around for many years. Transdermal drug delivery has the advantage of being highly precise and consistent, similar to intravenous drip but noninvasive because there is no needle. The first skin patch that delivered controlled amounts of a drug continuously was introduced about ten years ago. The process involves the placement of a patch containing a drug on the surface of the skin so that the drug is absorbed through the skin. The drug is delivered to the bloodstream with therapeutic levels, providing convenient means to administer the drug over prolonged periods. This traditional transdermal drug delivery is a passive system and is not satisfactory for most applications.

Because of the significant barrier properties of the skin, this passive transdermal drug delivery has been limited to drugs that have a highly-potent low daily doses and can readily penetrate the skin. The skin is highly resistant to penetration by most chemicals including drugs. The outer layer, Stratum Corneum (SC) serves as a highly protective barrier against physical, most chemical and bacterial penetration.

The stratum corneum (SC) consists primarily of a thin layer of dead cells bound together by certain fatty (lipid) materials. This presents a major obstacle

to the administration of drugs, immunizing agents, and genes transdermally. The stratum corneum (SC) which consists of a thin layer of dead cells with a high electrical resistance which results in high resistance to normal electroporation. This resistance to normal electroporation was recognized by the Weaver et al patent U.S. 5,019,034 entitled "Control of Transport of Molecules Across Tissue Using Electro-poration". Weaver seeks and proposes electroporation as an alternative to the traditional syringe and gun injection of medications. He describes a proposal for using high voltage, short duration electrical pulses on the tissue surface to produce electroporation of the tissue which comprises the walls of the sweat gland ducts to enable drugs and medication to pass through the sweat gland ducts iontophoretically into the tissue. He also proposes the use of synthetic tissue which has electroporation properties.

I have discovered that this layer can be perforated by the administration of short high voltage electrical field pulses, which creates as what may be appropriatedly returned to dielectric breakdown of the stratum corneum forming pores which can allow the passage of molecules However, there must be some force to move the molecules through the pores into the underlying tissue.

Another patent of interest is that of Grasso U.S. 4,955,378 entitled "Apparatus and Methods for Performing Electrofusion at Specific Anatomical Sites". He discloses a method of fusing biological particles to living tissue, preferably on corneas and in cervical areas. The tissue consists of living cells which are able to completely fuse with the biological particles, or live cells. This patent is not concerened with and does not address or solve the problem of transdermal transport of drugs, immunizing agents, and genes presented by the resistance of the stratum corneum. Also, he does not recognize the need for or suggest any means to force the drugs, immunizing agents, or genes into or across or through the tissue surface.

It is desirable that improved methods and apparatus be available for the transdermal delivery of drugs, immunizing agents, and genes.

DISCLOSURE OF INVENTION

It is the primary object of the present invention to provide an improved method and apparatus for transdermal drug delivery by electroincorporation of microcarriers. The term electroincorporation means the uptake of external materials such as drugs, proteins and antibodies by electroporation.

In accordance with the primary aspect of the present invention, drugs or genes are loaded into microcarriers, the microcarriers are brought into physical contact with the tissue surface and a pulsed electrical field is applied between the microcarriers and the tissue by means of electrodes. This forms pores at the interface of the microcarriers and the tissue, such that the microcarriers which are larger than the pores fuse with the tissue and form a channel through which drugs and genes, which are under pressure, enter through the tissue.

In another aspect of the invention, the microcarriers are smaller than the pores in the stratum corneum (SC), such that the microcarriers which carry molecules of drugs, immunizing agents, and genes, enter through the SC into the tissue where the molecules are diffused.

BRIEF DESCRIPTION OF DRAWING

The objects, advantages and features of this invention will be more readily appreciated from the following detailed description, when read in conjunction with the accompanying drawing, in which:

Fig. 1 is a perspective view of an apparatus for carrying out the process of the present invention;

Fig. 2 is an enlarged view of the head assembly of the Fig. 1 embodiment;

Fig. 3 is a diagrammatic illustration of a microcarrier loaded with molecules of drugs, immunizing agents or genes;

Fig. 4 is a diagrammatic illustration of multiple microcarriers applied to the surface of the stratum corneum;

Fig. 5 is a diagrammatic illustration of a step of applying electrodes and a pulse electrical field between the microcarriers and skin or stratum corneum;

Fig. 6 is a diagrammatic illustration of the formation of pores at the interface of microcarriers and the stratum corneum;

Fig. 7 is a diagrammatic illustration of the fusion of the microcarriers with the stratum corneum and the passage of drugs or genes through channels in the stratum corneum.

Fig. 8 is a diagrammatic illustration of the formation of pores and the passage of microcarriers through the pores in the stratum corneum; and

Fig. 9 is a diagrammatic illustration of the microcarriers below the stratum corneum and the passage of drugs, immunizing agents or genes from the vesicles into the skin below the stratum corneum;

Fig. 10 is a diagrammatic illustration of a equipotential and field line distribution around electrodes on the surface of the stratum corneum;

Fig. 11 is a diagrammatic illustration of the field lies around a microbubble and through a pore in the stratum corneum; and

Fig. 12 is a diagrammatic illustration like Fig. 11 for that of a solid microcarrier.

BEST MODES FOR CARRYING OUT THE INVENTION

The present invention takes advantage of dielectric breakdown of the stratum corneum (SC) to transfer drugs and genes contained in vesicles across the SC surface into the underlying tissue and possibly into the blood stream. When desirable, subsequent electroporation may be applied to improve the uptake of

drugs, genes, DNA or the like, into cells in the living tissue of humans and other living organism. Various techniques including electroporation is used to load molecules such as drugs and DNA into microcarriers of a size up to several μm diameters. The term microcarrier as used herein means any suitable carrier such as microbubbles, which can be liposomes, erythrocyte ghosts or other vesicles, and solid carriers. The microcarriers are then applied to the SC and electrodes are then applied over the microcarriers. Electrical field pulses are then used to create dielectric breakdown of the stratum corneum or other tissue surface forming passages through which either the drugs and other molecules or the microcarriers and the drugs or other molecules pass into the underlying tissue. The microcarriers, when larger than the pores are fused to the surface of the skin and the electric fields cause dielectric breakdown of the SC and the microbubbles so that the molecules pass from the microbubbles through the pores. When the microcarrier or bubbles are smaller than the pores they pass through the stratum corneum are then broken down and the molecules diffused into the tissue.

Electroporation involves the transient formation of pores in tissue or cell membranes utilizing a short pulse of high-voltage electric fields. Once these pores are formed in the skin or other tissue, molecules can pass the skin or tissue to desired locations in the tissue. When pores are formed in cell membranes, DNA and other molecules can enter the cells through these pores in the cell walls. Thereafter, they stay encapsulated in the cell and the cell walls reseal themselves. The DNA or other gene or drug can then act within the cell to alter the cell properties.

Referring to Fig. 1, an exemplary embodiment of an apparatus which may be utilized in carrying out the process of the present invention, is illustrated. The device comprises a manually positionable applicator designated generally by the numeral 10 which is connected to a signal generator 12 and a fluid medium source 14. The applicator 10 has a head assembly 16 which engages and applies

microcarriers with genes, immunizing agents or drugs and electrical pulses to a preselected surface tissue region of a patient. Details of the head assembly are illustrated in Fig. 2.

The head assembly comprises an electrode array 18 which is carried or mounted on a carrier or applicator such as an open pore foam elastomer 20 carried by flexible semirigid or firm dielectric planar support member 22. Adjacent parallel segments of the conductors serve as opposed electrodes for application of the electric field to the tissue surface. The electrodes are preferably small and closely spaced, such as about 0.2mm width at about 0.2mm spacing. The applicator may also be a small patch with electrodes on a surface thereof.

The applicator 10 (Fig. 1) further includes a handle portion 24 and an arm portion 26 on which is mounted the head assembly 16. The head assembly 16 is connected to a Y-shaped distal end 26a by means of a pair of pins 28. These pins enable the head to flex and conform to the curvature of the skin surface.

The terminal ends of the conductors 18 and 19 are connected to the signal generator 12 by way of an electrical cable 30. A fluid medium carrying vesicles containing the molecules or drugs is contained within the fluid medium source 14, which may include a suitable motorized pump or pressure source, not shown. The fluid medium source 14 is coupled to the elastomer foam 20 by flexible tube 32 which extends to the applicator 10 to the foam applicator. An actuator button 33 on the handle 24 of the applicator may be depressed to activate a valve (not shown) and deliver a suitable quantity of the fluid medium to the foam elastomer 20. The elastomer 20 provides a sponge-like substrate for holding a predetermined quantity of the fluid medium in contact with the tissue surface. The signal generator 12 generates short voltage pulses which are applied to the electrodes 18 and 19 by pressing a button 34 which actuates a switch to closee the circuit.

The invention can also be carried out by a catheter type apparatus and methods disclosed in U. S patent 5, 318,514 wherein an expandable portion of the

cather carries electrodes contacts the tissue surface to apply high voltage pulses. This provides a more convenient apparatus for the delivery of drugs and genes across tissue surfaces and membranes such as in body cavities. The present invention was devised to overcome the problem presented by the resistance of the stratum corneum. However, it is applicable to the insertion of molecules such as drugs and genes across other tissue surfaces in body cavities and open wounds. Certain modifications may be necessary to the illustrated apparatus for these other applications.

Referring to Fig. 3, the process of the present invention is carried out by first encapsulating the drugs or genes 36 which are to be delivered transdermally into microcarriers 38 such as microbubbles as carriers. These microbubbles can be liposomes, erythrocyte ghosts or other vesicles. The microcarriers may also be of a matrix design where the drug or other molecules are encapsulated within the matrix. This would enable the provision of a time release function. The encapsulation of the molecules can be carried out by any one of a number of known processes, including electroporation.

The loaded microbubble, as illustrated in Fig. 4, are then brought into contact with the tissue surface or stratum corneum 40 of a skin layer 42 by suitable means and are positioned between pairs of closely spaced electrodes 44 and 46. This can be carried out by the apparatus of Fig. 1, wherein a fluid carry the microbubbles and applied by the sponge 20 would be positioned between the electrodes 18 on the surface of the applicator. It can also be carried out by a patch having a structure similar to the pad and electrodes a shown in Fig. 2.

Thereafter, a short voltage pulse is applied between the electrodes so that the electric fields of sufficient amplitude are generated to induce dielectric breakdown forming pores in the stratum corneum and in the microbubble and cause the molecules in the microbubble to pass through the pores into the underlying tissues. The microbubble can apply some of the pressure to move the molecules through the pores. As shown in Fig. 5, the electric field is applied so

that useful electric field lines are perpendicular to the tissue surface or stratum corneum surface. Typical electrical parameters for the stratum corneum are a field strength of 20 to about 60 kV/cm, which can be generated with moderate voltages of 20 to 120 volts with a pulse length of 10 microseconds (μsec) to 10 milliseconds (msec). This electric field induces a dielectric breakdown in the stratum corneum and in wall of the vesicles or microbubbles. Other tissue surfaces will typically require less field strength.

The dielectric breakdown in both the stratum corneum and the microbubbles generate or open pores 50 and 52 as illustrated in Fig. 6. These pores open up further and can join into one lumen and create a channel 54 through which the contents of the microbubbles empty through and into the dermis underlying the stratum corneum as illustrated in Fig. 7. Internal pressure within the microbubbles can act as the driving force to drive the molecules through the channel or channels. Since the stratum corneum consists essentially of dead material, the channel will not close as quickly as it would in a live tissue. This allows the drugs or genes to diffuse through the surface layer into the underlying skin tissue.

Other forms of a delivery system could be utilized, such as a small system including a patch strapped to the arm or other body part or momentarily connected, containing a rechargeable battery-powered pulse power supply with a reservoir containing microbubbles in suspension with the drug encapsulated. The applicator would have the basic components as the device in Fig. 1 such that by pushing one button, a preselected amount of microbubbles is delivered to the skin between the electrodes. The microbubbles are pressed against the skin for good mechanical contact. Activating another button or switch delivers an electrical pulse to the electrodes which fuse the microbubbles to the stratum corneum. A large number of the microbubbles are then fused to the skin and start pumping or forcing the drug through the stratum corneum.

A special patch having the basic structure of pad 20 can also be applied to the tissue surface. The microbubbles can be contained in the patch which also contains the electrode structure to create the electric field. The electrode structure can be similar to Fig. 2 and inside the patch. The electrode structure is connected to two electrodes outside the patch so that a pulse generator can be connected momentarily to these outside electrodes to provide a voltage pulse. The patch is preferably provided with an adhesive border to adhere it to the skin or tissue. It is also preferably provided with a protective cover which can be peeled off before adhering the patch to the skin or tissue.

If the drug is to be transported into the cells, a second pulse of appropriate voltage and duration after allowing appropriate diffusion time, is applied to open up pores in the cells. This allows the cells to take up the drug or molecules as in electroporation. The parameters for cell poration in the body are substantially the same as those in solution and will be known to or available to those of skilled in the art. Such parameters are also available in publications available from Genetronics Inc., of San Diego, California.

A drug delivery time profile can be created by mixing different size microbubbles. The flux can then be controlled by the pore size and the number of microbubbles delivered. The process of the present invention could also be combined with iontophoresis as an additional driving force. The iontophoresis takes advantage of ion charges to cause a migration of the ions or molecules through existing passages or pores in the tissue. The combination could use electroporation to open up channels and pores and then use ionphoresis to induce migration of the drugs or genes further into selected tissue.

The present embodiment of the invention has been demonstrated in experiments as follows:

1. Labelled calcein was placed on the skin of a nude mouse.
2. Labelled calcein was placed on the mouse skin, then electroporated.

3. Labelled calcein was encapsulated in liposomes, then placed on the mouse skin.
4. Labelled calcein was encapsulated in liposomes, then placed on the mouse skin and electrofused to the skin.

The results of this limited experiment showed that the best penetration of the skin into the underlying skin or tissue was seen in Example 4, with the liposome-encapsulated calcein which had been electrofused to the skin.

Referring to Figs. 8 and 9, an alternate embodiment is illustrated wherein like structure is identified by like numbers primed. In this embodiment, microcarriers 38' are selected to be small enough to pass through the pores 50'. At the present time I believe the maximum size to be about 9 μm or slightly larger. The dielectric breakdown in the stratum corneum allow the carriers to pass through open pores 50' as illustrated in Fig. 8. These pores open up and allow the carriers to pass through and into the dermis underlying the stratum corneum as illustrated in Fig. 9. Enzymes within the dermis act to break down walls of the carriers as soon as they enter it forming openings 52' and cause them to release the molecules into the dermis. Since the stratum corneum consists essentially of dead material, the channel will not close as quickly as it would in a live tissue. This allows the carriers containing drugs or genes to pass through the surface layer into the underlying skin tissue where the molecules are diffused into the tissue.

Other forms of a delivery system could be utilized, such as a small system strapped to the arm or other body part or momentarily connected, containing a rechargeable battery-powered pulse power supply with a reservoir containing vesicles in suspension with the drug encapsulated. The applicator would have the basic components as the device in Fig. 1 such that by pushing one button, a preselected amount of vesicles is delivered to the skin between the electrodes. The microcarriers are pressed against the skin for good mechanical contact. Activating

another button or switch delivers an electrical pulse to the electrodes which delivers the microcarriers through the stratum corneum.

A special patch can also be applied to the tissue surface. The microcarriers can be contained in the patch which also contains the electrode structure to create the electric field. The electrode structure can be similar to Fig. 2 and inside or on a surface of the patch. The electrode structure is connected to two electrodes outside the patch so that a pulse generator can be connected momentarily to these outside electrodes to provide a voltage pulse. The patch is preferably provided with an adhesive border to adhere it to the skin or tissue. It is also preferably provided with a protective cover which can be peeled off before adhering the patch to the skin or tissue.

If the drug is to be transported into the cells, a second pulse after allowing appropriate diffusion time, is applied to open up pores in the cells. This allows the cells to take up the drug or molecules by electroporation.

A drug delivery time profile can be created by mixing different size microcarriers. The flux can then be controlled by the pore size and the number of vesicles delivered. The process of the present invention could also be combined with iontophoresis as an additional driving force. The iontophoresis takes advantage of ion charges to cause a migration of the ions or molecules through existing passages or pores in the tissue. The combination could use electroincorporation to deliver vesicles through the SC and then use iontophoresis to induce migration of the drugs, immunizing agents, or genes further into selected tissue.

The present embodiment of the invention has been demonstrated in experiments as follows:

1. Labelled calcein was loaded into small liposomes of about 300nm in diameter, as well as large liposomes of 9 μm diameter. These were placed on the skin of hairless mice and electrodes placed on top of the liposomes in order to

create electric fields with components perpendicular to the skin. A pulse of about 60 V and 1.2 msec pulse length was applied.

2. Examination by fluorescent microscopy disclosed that calcium was present in the epidermis and dermis after the pulse, not just in the hair follicles but also in between. Further examination by transmission electromicroscopy "TEM" revealed that whole liposomes were present after the pulse below the SC. This indicates that the liposomes which average in size about 300 nm or 9 μm had crossed the stratum corneum during the pulse.

3. Further study and examination through TEM disclosed that liposomes decomposed and released their contents into the tissue in the dermis. Further tests showed that calcein was entering the blood stream within minutes after the pulse. Further analysis revealed that starting with an amount of calcein on the skin of 25 μg the amount found in the blood was about 300 ng per ml. Assuming a total amount of blood of about 5 ml, the total amount of calcein in the blood was about 1.5 μg. This calculates to an efficiency of 1.5 per 25 which equals about 6%.

In plotting this over a period of time, the plot revealed that the concentration of calcein in the blood rose dramatically during the first five minutes, peaking at 15 minutes and dropping off gradually along an almost constant slope at 90 minutes.

Referring to Fig. 10, an equipotential and electric field line distribution around electrodes of about 0.2 mm in width spaced about 0.2 mm is illustrated. The electrodes 44, 46 and 48 are illustrated on the surface of the SC. The stratum corneum is intact with a high resistivity. The equal potential lines are concentrated in the stratum corneum, leading to a high field strength. The stratum corneum 40 shields the underlying epidermis 42 from the field.

Referring to Fig. 11 a field plot around a liposome 58 of about 300 μm in diameter at the entrance to a hole 60 in the SC is shown. Charged liposomes will

experience a Coulomb force (force on charged particles by an electric field) and can be drawn into the SC and epidermis after break-down of the SC. Uncharged liposomes, such as small liposomes used in my experiments, do not experience a Coulomb force in a homogenous electric field. They are polarized in the electric field and a subjected to a force caused by the inhomogeneous field in the pores of the SC. This "dielectrophoretic" force is proportional to the product of the field strength and the gradient of the field.

Referring to Fig. 12, a similar field plot around a solid particle 62 is illustrated at the entrance to a hole or pore 64 in the SC. This field distribution around a liposome and solid particle in proximity to a pore in the SC is substantially the same.

The following simple model describes the uncharged liposome movement through a pore driving by the electrophoretic force:

1. *Dielectric breakdown of SC:* $E \geq 20\ kV/cm$

2. *Dielectrophoretic force F_D on neutral particles:*

$$F_D = \frac{a\,V}{2} \nabla |E|^2$$

a = *Polarizability*
V = *Volume*
E = *Field Strength*

3. *Stokes force F_S determines velocity v:*

$$F_S = 6\pi r v \eta$$

r = *radius of particle*
v = *velocity*
η = *viscosity of medium*

$$v = \frac{a}{9\eta} r^2 \nabla |E|^2$$

4. *Pulse duration determines penetration depth d:*

$$d = VTN$$

T = *pulse length*
N = *number of pulses*

5. example: *2r = 9 um*
E = 36 kV/cm
3 pulses at 1 msec each

Penetration depth d = 129 um

A more accurate estimate would require knowledge of the shape of the electric field in pores in the SC. Electroincorporation is expected to work well with solid vesicles as well as with vesicles with a membrane.

Dielectrophoresis as well as electrophoresis as a driving force through the SC do not require a vesicle with a membrane. This is different from the electrofusion mechanism where a membrane is essential. It is expected that electroincorporation can be applied to a wide variety of vesicles or microspheres which contain drugs in a matrix. It will be appreciated that the vesicles must be small enough to pass through pores or openings formed in the SC and skin. At the present time I believe this to be about 9 μm or slightly larger.

The following study has been conducted:

Chemical Delivered: Calcein (MW 623)

Animal Model: Shaved Mouse

Analysis: Fluorescence Microscopy

Picture shows tissue to a depth of about 1,500 μm

Stratum Corneum at the top.

Experimental Conditions:

1. Topical Calcein

2. Topical Calcein plus electroporation
3. Liposome calcein (24 hours incubation)
4. Liposomal calcein plus electrofusion (5 minutes incubation after electroporation)

Conclusions:

1. Little, if any, penetration
2. Minor penetration near surface
3. Major penetration into hair shafts, no uptake into the blood
4. Major penetration into tissue between hair shafts, detectable in the blood in less than 15 minutes.

The results of this limited experiment showed that the best penetration of the skin into the underlying skin or tissue was seen in Example 4, with the liposome-encapsulated calcein and electric pulses.

I have illustrated and described my invention by means of specific embodiments, it is to be understood that numerous changes and modifications may be made therein without departing from the scope of the invention as defined in the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Apparatus including a source of high voltage pulses and electrodes for applying to tissue surface for trans tissue molecular delivery by electroporation characterized by:

a quantity of microcarriers for carrying molecules to be delivered across a tissue surface;

a quantity of molecules to be delivered across the tissue surface embodied in said microcarriers;

a substrate for supporting said quantity of microcarriers in contact with a selected area of the tissue surface; and

means for applying an electric field of sufficient amplitude to induce electroporation of said selected area of tissue and to enable transport of the molecules from the microcarriers into the tissue.

2. The apparatus of claim 1 wherein said means for applying an electric field to the selected area of tissue has means for applying a field strength of from about 10 to about 60 kV/cm with a pulse length of from 10 μsec to 10 msec.

3. The apparatus of claim 1 wherein said microcarriers are of a size to inhibit passage through pores induced by said electroporation.

4. The apparatus of claim 1 wherein said microcarriers are of a size to pass through pores induced by said electroporation.

5. The apparatus of claim 1 wherein said microcarriers are of a matrix construction and the molecules are encapsulated within the matrix.

6. The apparatus of claim 1 wherein the microcarriers have an electrical charge.

7. The apparatus of any one of claims 1-4 and 6 wherein said microcarriers have a membrane forming microbubbles and the molecules are encapsulated within the microbubbles.

8. The apparatus of claim 7 wherein said means for applying the electric field comprises a plurality of closely spaced electrodes applied to the surface of the tissue and said field is applied as pulses of from 10 to several hundred volts with a pulse length of between 100 μsec to 100 msec.

9. The apparatus of claim 8 wherein the microcarriers are constructed to have a time release characteristic.

10. The apparatus of claim 1 or 9 wherein the electrodes are parallel strips conductively mounted on the substrate.

11. Use of electroporation for delivering molecules across a tissue surface, comprising:

selecting a quantity of selected molecules to be delivered across a tissue surface;

providing a quantity of microcarriers;

loading a quantity of said molecules to be delivered in said quantity of microcarriers;

contacting a selected area of a tissue surface with said quantity of microcarriers; and

applying an electric field of sufficient amplitude and duration to induce electroporation of said selected area of tissue and to transport the molecules from the microcarriers into the tissue.

12. The use of claim 11 wherein said microcarriers are microbubbles and are of a size to inhibit passage through pores induced by said electroporation.

13. The use of claim 11 wherein said microcarriers are of a size to pass through pores induced by said electroporation.

14. The use of claim 11 wherein said microcarriers are of a matrix construction and the molecules are encapsulated within the matrix.

15. The use of any one of claims 11 to 14 wherein said microcarriers have a membrane forming microbubbles and the molecules are encapsulated within the microbubbles.

16. The use of claim 12 wherein the microcarriers are constructed to have a time release characteristic.

17. The use of claim 13 wherein said microcarriers have an electrical charge.

18. The use of claim 11 wherein said step of applying the electric field comprises providing a plurality of closely spaced electrodes, applying said electrodes to the surface of the stratum corneum and applying said field as pulses of from 10 to several hundred volts with a pulse length of between 100 μsec to 100 msec.

19. The use of claim 11 wherein said step of applying an electric field includes providing field generating means for applying a field strength of from about 10 to about 60 kV/cm with a pulse length of from 10 μsec to 10 msec and operating said field generating means for said duration.

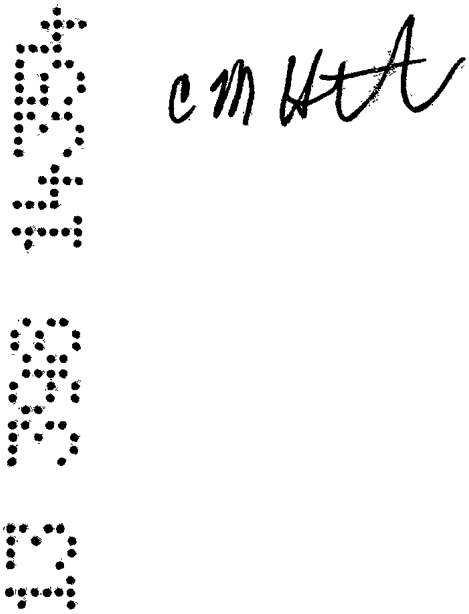
20. Apparatus according to any one of claims 1 to 10, substantially as described herein and with reference to any one of the accompanying drawings.

21. The use of electroporation for delivering molecules across a tissue surface, according to any one of claims 11 to 19, substantially as described herein and with reference to any one of the accompanying drawings.

Dated this 13th day of March 1998.

GENETRONICS, INC

By its Patent Attorneys
MADDERNS



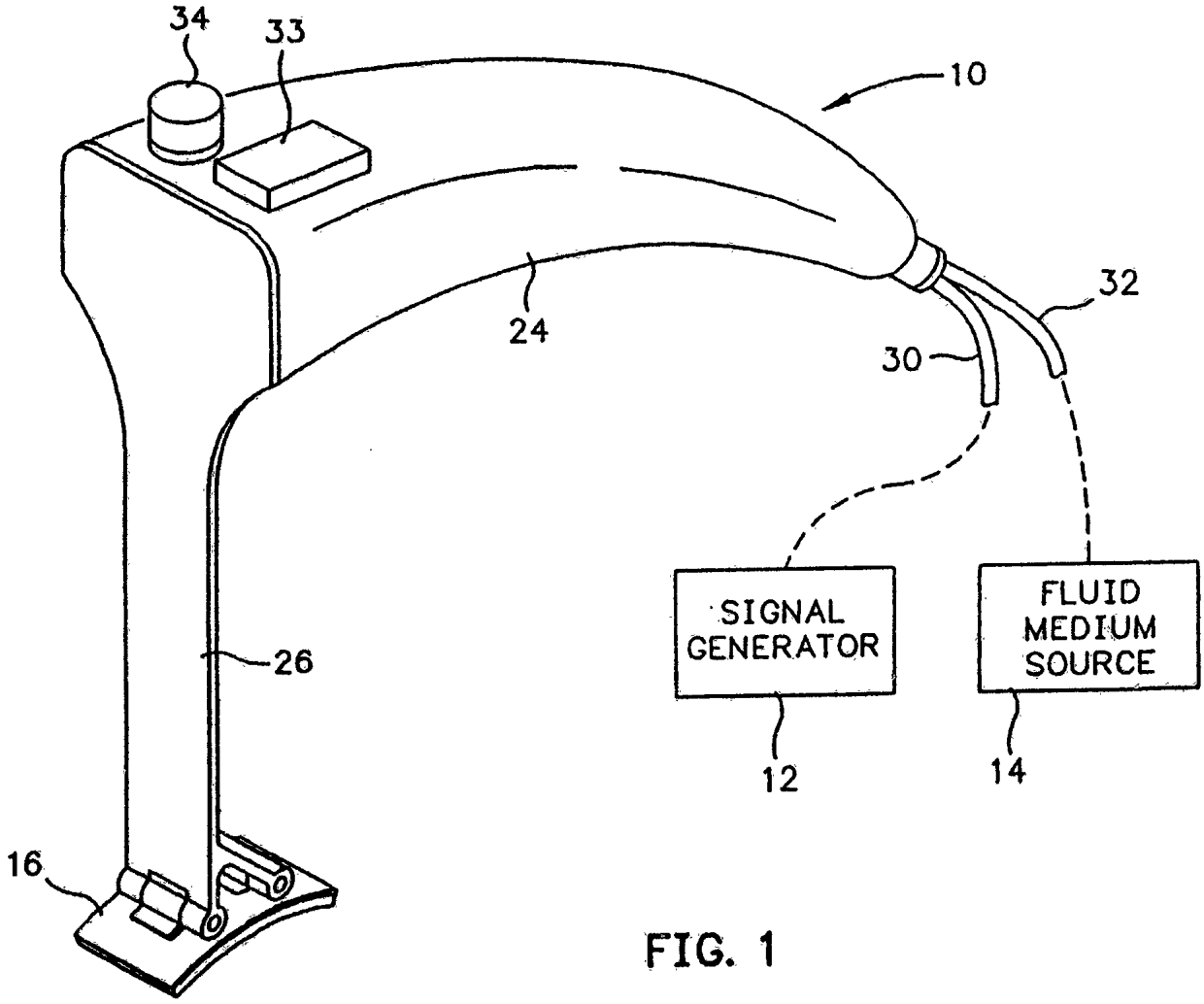


FIG. 1

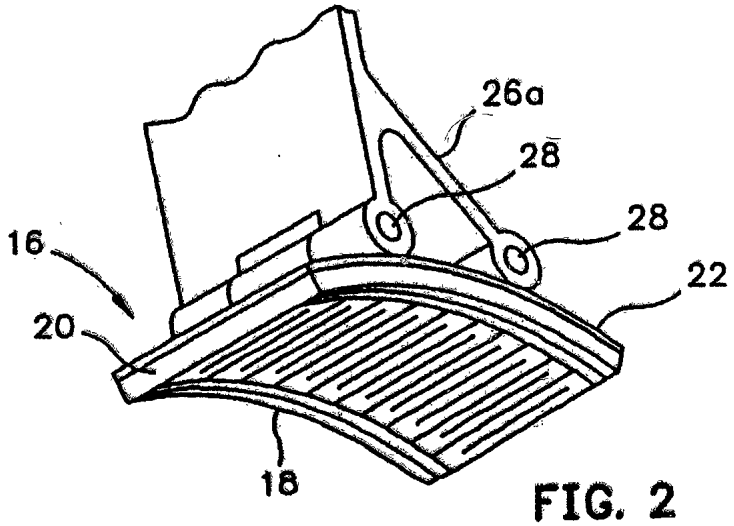


FIG. 2

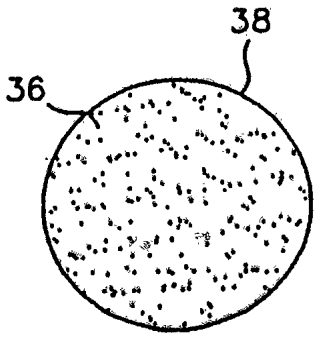


FIG. 3

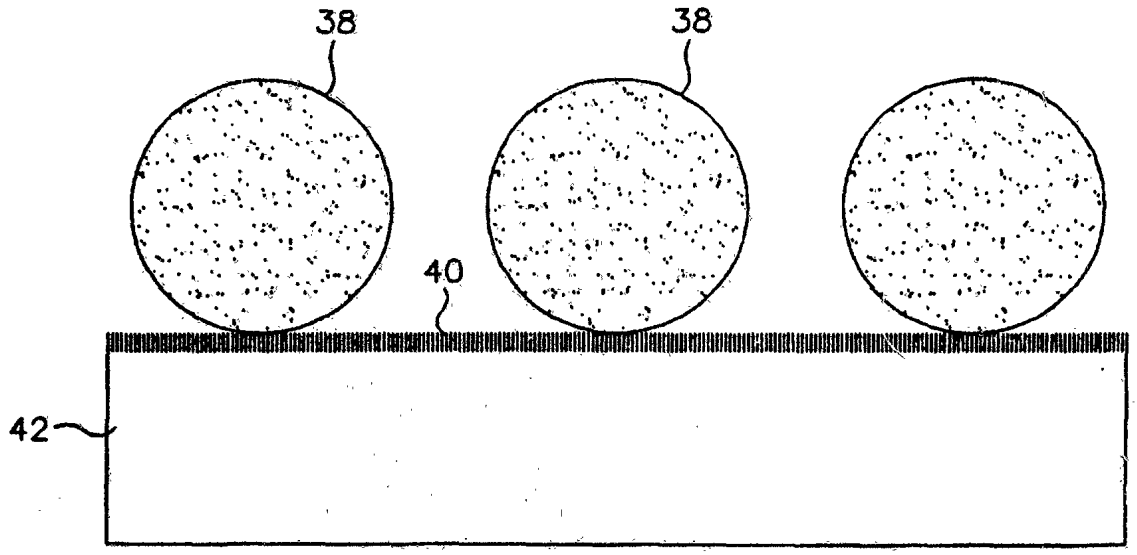

38
38
40
42

FIG. 4

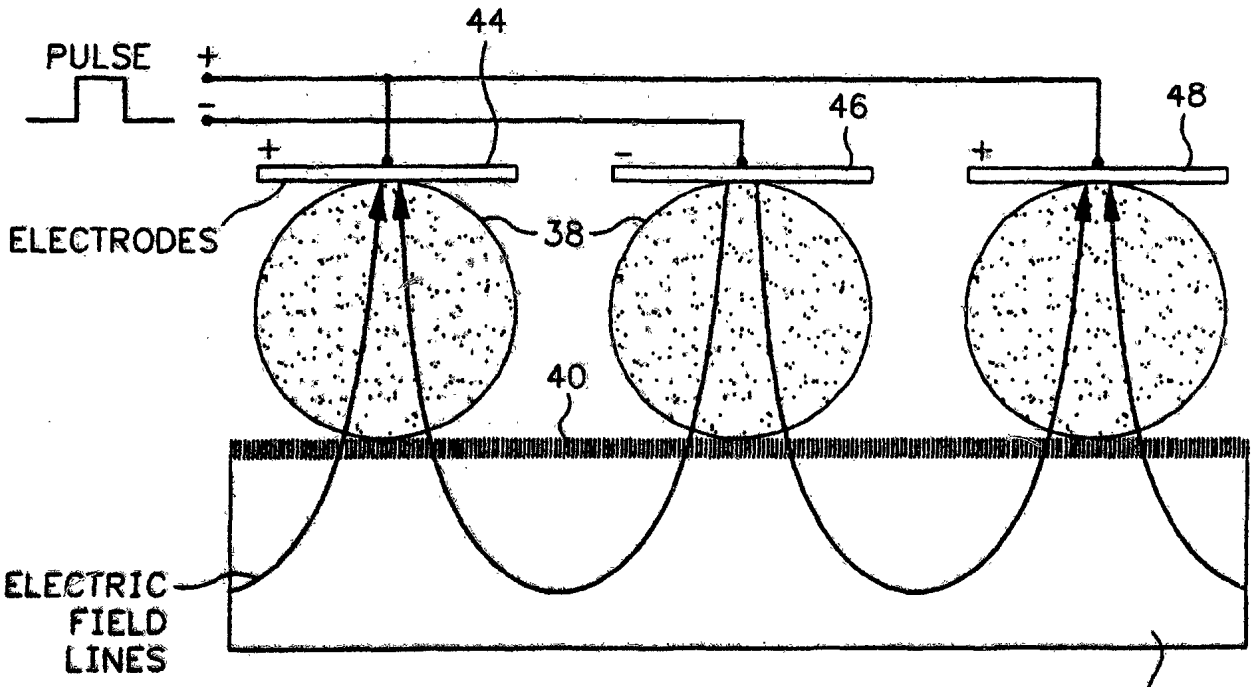

PULSE
+
-
44
46
48
+
-
+
ELECTRODES
38
40
ELECTRIC FIELD LINES
42

FIG. 5

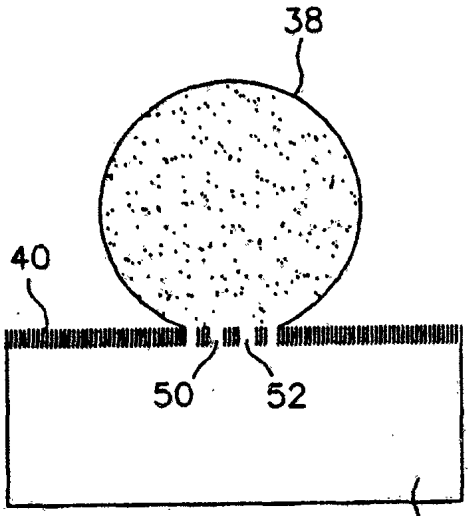


FIG. 6

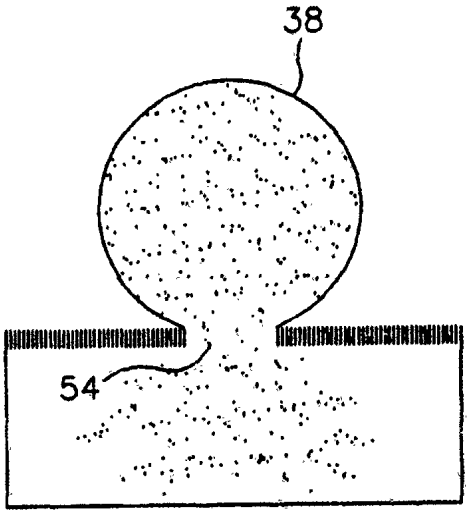


FIG. 7

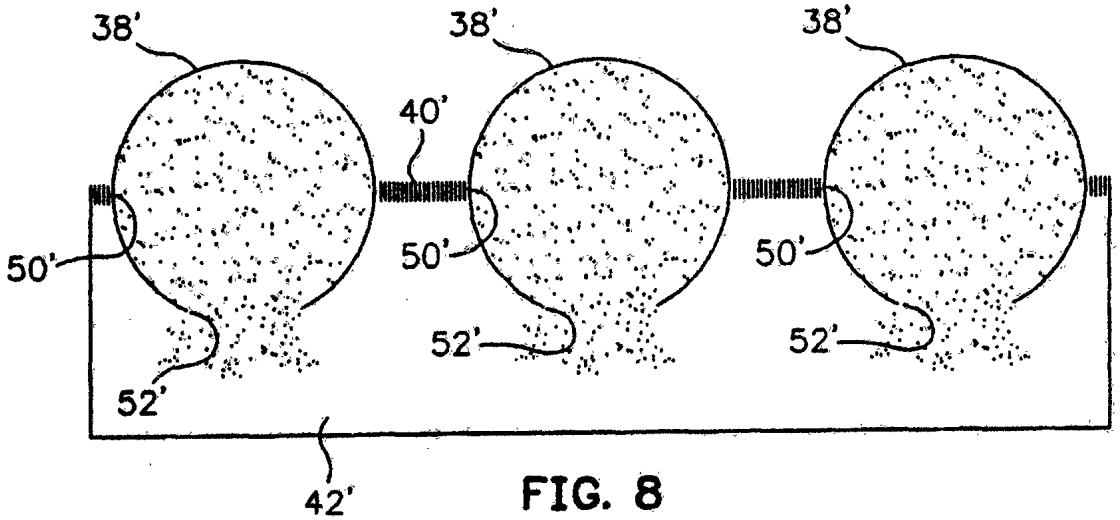


FIG. 8

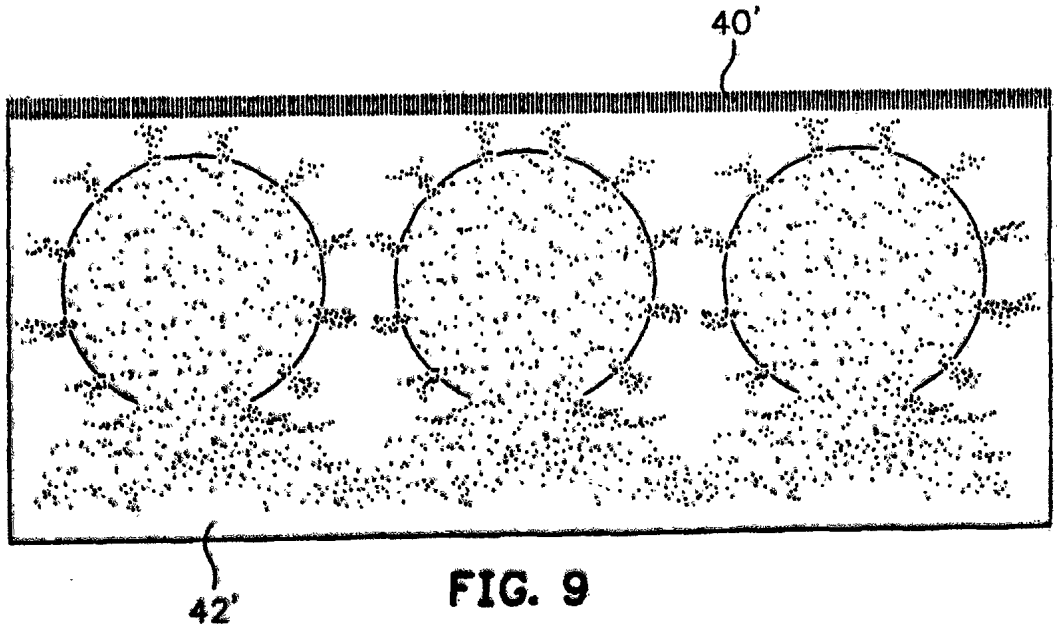


FIG. 9

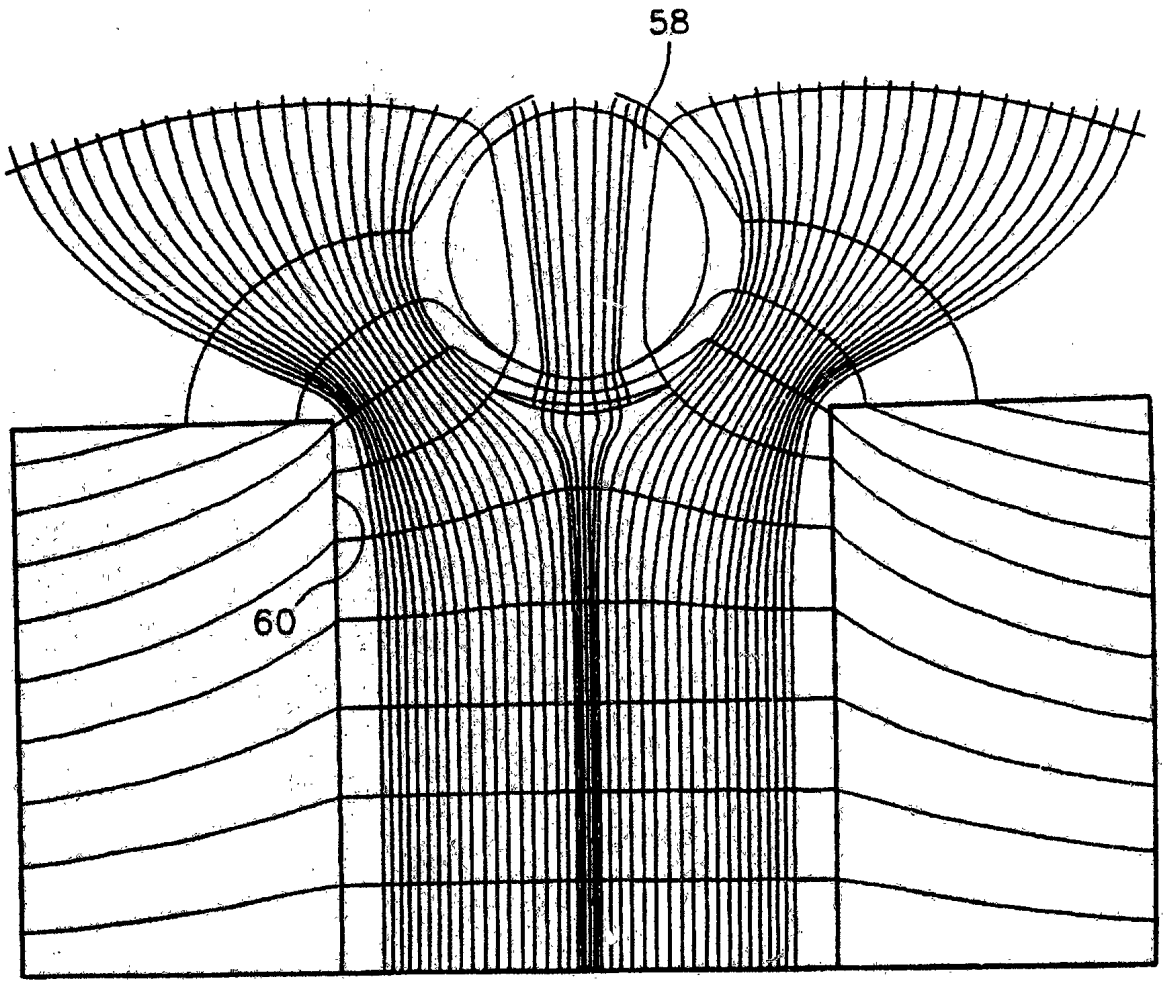


FIG. 11

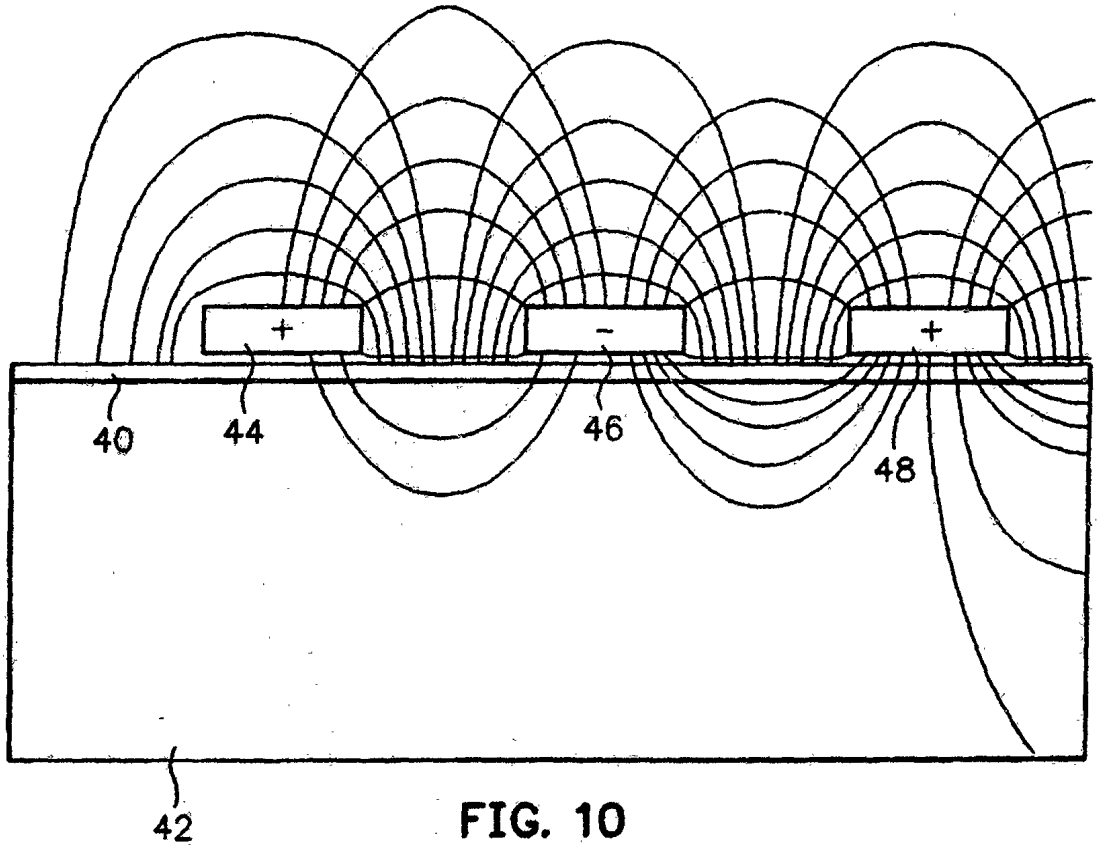


FIG. 10

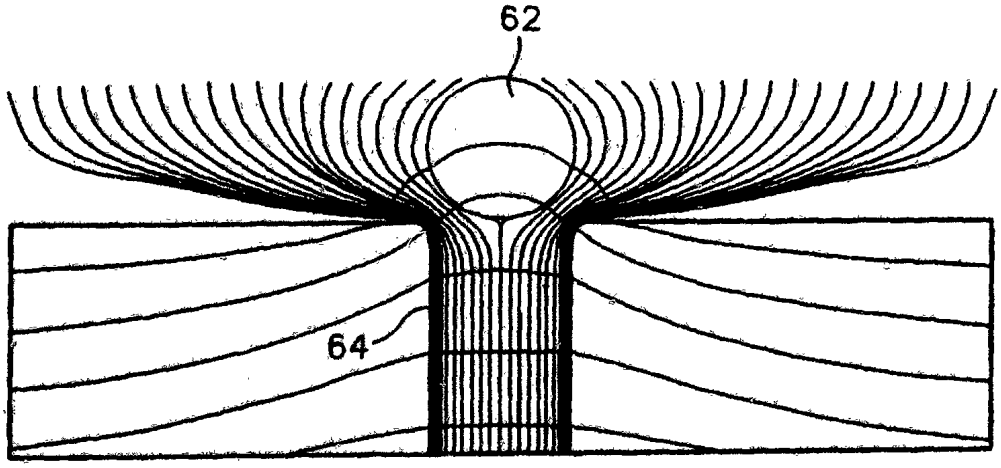


FIG. 12

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 94/14352

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61N1/30

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 6 A61N

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 942 883 (NEWMAN) 24 July 1990 see column 2, line 26 - column 5, line 18; figures	1-20
A	WO,A,92 04937 (HENLEY) 2 April 1992 see page 7, line 3 - page 12, line 13; figures	1-20
A	US,A,5 162 042 (GYORY ET AL.) 10 November 1992 see column 4, line 50 - column 5, line 22; figures	1-20
P,A	US,A,5 318 514 (HOFMANN) 7 June 1994 cited in the application see the whole document	1-6,9, 14,20

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 April 1995	17.05.95

Name and mailing address of the ISA	Authorized officer
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	Rakotondrajaona, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter ual Application No
PCT/US 94/14352

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4942883	24-07-90	NONE	
WO-A-9204937	02-04-92	US-A- 5160316	03-11-92
US-A-5162042	10-11-92	CA-A- 2042934	18-04-92
US-A-5318514	07-06-94	NONE	