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(21) International Application Number: PCT/US94/00896 (22) International Filing Date: 26 January 1994 (26.01.94) (30) Priority Data: <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">08/009,463</td> <td style="width: 40%;">27 January 1993 (27.01.93)</td> <td style="width: 30%;">US</td> </tr> <tr> <td>08/077,296</td> <td>14 June 1993 (14.06.93)</td> <td>US</td> </tr> <tr> <td>08/077,786</td> <td>14 June 1993 (14.06.93)</td> <td>US</td> </tr> <tr> <td>08/107,329</td> <td>16 August 1993 (16.08.93)</td> <td>US</td> </tr> </table> (60) Parent Applications or Grants (63) Related by Continuation <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">US</td> <td style="width: 40%;">08/077,296 (CIP)</td> <td style="width: 30%;"></td> </tr> <tr> <td>Filed on</td> <td>14 June 1993 (14.06.93)</td> <td></td> </tr> <tr> <td>US</td> <td>08/107,329 (CIP)</td> <td></td> </tr> <tr> <td>Filed on</td> <td>16 August 1993 (16.08.93)</td> <td></td> </tr> <tr> <td>US</td> <td>08/077,786 (CIP)</td> <td></td> </tr> <tr> <td>Filed on</td> <td>14 June 1993 (14.06.93)</td> <td></td> </tr> <tr> <td>US</td> <td>08/009,463 (CIP)</td> <td></td> </tr> <tr> <td>Filed on</td> <td>27 January 1993 (27.01.93)</td> <td></td> </tr> </table> (71) Applicant (for all designated States except US): AFFYMAX TECHNOLOGIES N.V. [NL/NL]; De Ruyderkade 62, Curacao (AN).	08/009,463	27 January 1993 (27.01.93)	US	08/077,296	14 June 1993 (14.06.93)	US	08/077,786	14 June 1993 (14.06.93)	US	08/107,329	16 August 1993 (16.08.93)	US	US	08/077,296 (CIP)		Filed on	14 June 1993 (14.06.93)		US	08/107,329 (CIP)		Filed on	16 August 1993 (16.08.93)		US	08/077,786 (CIP)		Filed on	14 June 1993 (14.06.93)		US	08/009,463 (CIP)		Filed on	27 January 1993 (27.01.93)		(72) Inventors; and (75) Inventors/Applicants (for US only): SELICK, Harold, E. [US/US]; 11 Somerset Court, Belmont, CA 94002 (US). OLDENBURG, Kevin, R. [US/US]; 38562 Vancouver Common, Fremont, CA 94536 (US). (74) Agents: WHITAKER, J., Steven et al.; Townsend and Townsend Khourie and Crew, 20th floor, One Market Plaza, Steuart Street Tower, San Francisco, CA 94105 (US). (81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
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COMPOSITIONS AND METHODS FOR
TRANSDERMAL DRUG DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

10 This application is a continuation-in-part of application Serial No. 08/107,329, filed August 16, 1993, which is continuation-in part of application Serial No. 08/077,786, filed June 14, 1993, which is a continuation-in-part of U.S. application Serial No. 08/009,463, filed January 27, 1993. This application is also a continuation-in-part of U.S. application Serial No. 08/077,296, filed June 14, 1993. All of the above identified applications are incorporated herein by reference for all
15 purposes.

BACKGROUND OF THE INVENTION

20 The present invention relates generally to methods of delivering pharmaceutical agents across membranes, such as the dermis or skin layer of a patient. More particularly, the invention relates to methods for iontophoretically delivering nucleotide-based pharmaceutical agents.

Heretofore, nucleotide-based drugs, such as aptamers, ribozymes, antisense compounds, and triple helix drugs have had limited success as therapeutic agents, in part, because of problems associated with their stability and
25 delivery. Nucleotide-based pharmaceutical agents contain a phosphodiester group which is sensitive to degradation by nucleases. Such degradation would be a significant impediment to the use of an oligonucleotide or nucleic acid as a pharmaceutical agent that depends upon the integrity of the sequence for its recognition specificity. Thus, naturally occurring oligonucleotides and nucleic
30 acids often must be chemically modified to render them resistant to nucleases which would degrade them *in vivo*, or even *in vitro* unless care is taken to choose appropriate conditions.

The therapeutic efficacy of pharmaceutical or therapeutic agents, including nucleotide-based pharmaceutical agents, relies on the delivery of
35 adequate doses of a pharmaceutical agent to the site of action. Many modes of delivery have been developed which include, for example, enteral (oral), parenteral (intramuscular, intravenous, subcutaneous), and topical administration. In most instances, the administration system is chosen for reliable dosage delivery and convenience.

40 Typically, parenteral administration is the most reliable means of delivering a pharmaceutical to a patient. See, Goodman *et al.*, Goodman and

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Gilman's Pharmacological Basis of Therapeutics, Pergamon Press, Elmsford, New York (1990) and Pratt *et al.* Principles of Drug Action: The Basis of Pharmacology, Churchill Livingstone, New York, New York (1990). Each parenteral mechanism insures that a prescribed dosage of the pharmaceutical agent is inserted into the fluid compartment of the body where it can be transported. The disadvantage of these modes of delivery is that they require an invasive procedure. The invasive nature of administration is inconvenient, painful, and subject to infectious contamination.

Enteral and topical administration are more convenient, generally non-painful, and do not predispose to infection; however, both are limited. The gastrointestinal and dermal surfaces present formidable barriers to transport, and therefore, some pharmaceutical agents are not absorbed across these surfaces. Another drawback to patient directed modes of administration (enteral, topical and subcutaneous) is compliance. Pharmaceutical agents which have a short half-life require multiple daily doses. As the number of doses increases, patient compliance and therapeutic efficacy decrease. Simplified and/or infrequent administration schedules would aid in optimizing patient compliance. Wilson *et al.* (1991) Harrison's Principles of Internal Medicine, 12th Ed., McGraw-Hill, Inc., New York, New York.

Efforts to develop more effective and convenient modes of pharmaceutical administration have led to the development of transdermal delivery systems. Many current transdermal pharmaceutical agent delivery systems rely upon pharmaceutical agents which are absorbed when admixed with inert carriers. See Cooper *et al.* (1987) "Penetration Enhancers", in Transdermal Delivery of Drugs, Vol. II, Kvdonieus *et al.*, Eds., CRC Press, Boca Raton, Florida. Few pharmaceutical agents fit this profile and those which do are not always predictably absorbed.

In addition, the skin is an efficient barrier to the penetration of water soluble substances, and the rate of transdermal pharmaceutical agent absorption is determined by lipid solubility and polarity. Highly polar or water soluble pharmaceutical agents are effectively blocked. Even very lipophilic pharmaceutical agents penetrate the dermis very slowly compared with the rate of penetration across cell membranes. See Pratt *et al. supra*. Various forms of chemical enhancers, such as those enhancing lipophilicity, have been developed to improve transdermal transport when physically mixed with certain therapeutic agents and provide more predictable absorption. See for example, U.S. Patents 4,645,502; 4,788,062; 4,816,258; 4,900,555; 3,472,931; 4,006,218; and 5,053,227. Carriers have also been coupled to pharmaceutical agents to enhance intracellular transport. See Ames *et al.* (1973) Proc. Natl. Acad. Sci. USA, 70:456-458 and (1988) Proc. Int. Symp. Cont. Rel. Bioact. Mater., 15:142.

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Electric gradients also have been used to enhance transdermal pharmaceutical agent delivery. See Chien *et al.* (1989) *Journal of Pharmaceutical Sciences*, 78(5):353-354 and Banga *et al.* (1988) *J. Controlled Release*, 7:1-14. This technique, known as iontophoresis, uses electrostatic forces to enhance the rate of delivery of ionized pharmaceutical agents through the skin. For iontophoretic delivery, the drug molecules must be in ionized state with either a positive or negative charge.

The rate of drug delivery in iontophoresis is directly proportional to the system current; the higher the current, the greater the driving force and pharmaceutical agent delivery. Typically, devices are used which hold a pharmaceutical agent in a reservoir near the skin, generate an electric field surrounding the pharmaceutical agent-dermal interface, and drive the agent through the skin. Ionic strength also affects the iontophoretic drug delivery rate. See Banga *supra*. Ionic strength is related to the concentration of various ions present in the solution of the pharmaceutical agent in the reservoir. The greater the ionic strength (i.e., the more ionized particles per unit volume), the higher the concentration of ions, and the greater the competition for the electric current. Other factors which may affect the delivery rate include pH, concentration, extraneous ions (e.g., from buffer solutions), conductivity, and electronic factors.

Electroporation has been used as a method of delivering nucleic acids. In this method, one or more pulses of electrical energy of sufficient voltage and duration to produce a transient increase in tissue permeability is applied to a region of a tissue. A driving force, for example an electrical, physical, or chemical force, such as provided by a temperature gradient, a pressure gradient, a concentration gradient, or an acoustic or optical pressure, is then utilized to move the molecules across the permeabilized tissue.

Because of the potential therapeutic benefits associated with nucleotide-based drugs and the difficulties associated with their delivery, there exists a significant need for improved methods for delivering these pharmaceutical agents in a controlled fashion and particularly, for delivery methods which improve the bioavailability or other pharmacological properties of these pharmaceutical agents. The present invention fulfills these needs.

SUMMARY OF THE INVENTION

The invention provides methods for delivering a nucleotide-based pharmaceutical agent in a therapeutically effective dose to a patient, the method comprising the steps of:

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contacting the skin of a patient with a therapeutically effective amount of a nucleotide-based pharmaceutical agent in an iontophoretic device, wherein the pharmaceutical agent is ionically charged; and

5 applying an electric field to the interface of the iontophoretic device and the skin, such that the pharmaceutical agent is delivered transdermally. The compositions and methods described herein can be utilized for either the systemic or local administration of a nucleotide-based pharmaceutical agent, depending on the therapeutic indication.

10 According to a preferred embodiment of this invention, an electric field between about 0.1 and 0.5 mAmp/cm² is applied to deliver a nucleotide-based pharmaceutical agent in an iontophoresis device. This embodiment results in the delivery of between about 0.1 and 20 mg, and preferably 0.1 and 10 mg of the pharmaceutical agent during a 24 hour period for an iontophoresis device having about a 20 cm² donor reservoir.

15 The nucleotide-based pharmaceutical agents that can be delivered using the methods described herein will generally comprise oligonucleotides and nucleic acids, having between about 2 and 100 nucleotides, preferably between about 2 and 50 nucleotides, and more preferably between about 10 and 40 nucleotides. Representative pharmaceutical agents include aptamers, ribozymes, 20 antisense compounds, and triple helix drugs. According to some embodiments, the nucleotide-based pharmaceutical agent is contacted with a liposomal formulation, such as Lipofectin™ prior to delivery of the pharmaceutical agent.

25 BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 is a graphic depiction of the effect of various concentrations of sodium chloride on flux (nanograms (ng)/cm²·hr) as a function of time (hours).

Fig. 2 is a graphic depiction of the effect of oligonucleotide size on flux (ng/cm²·hr) as a function of time (hours).

30 Fig. 3 is a graphic depiction of the effect of oligonucleotide concentration on flux (ng/cm²·hr) as a function of time (hours).

Fig. 4 is a graphic depiction of the effect of Lipofectin™ concentration on flux (ng/cm²·hr) as a function of time (hours).

35 Fig. 5 is an illustration of the structure of the thrombin aptamer d(GGTTGGTGTGGTTGG).

Fig. 6 is a graphic depiction of the effect of oligonucleotide conformation on flux (ng/cm²·hr) as a function of time (hours).

DETAILED DESCRIPTION OF THE INVENTION

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I. Terminology

The following terms are intended to have the following general meanings:

30 "Pharmaceutical agent or drug" refers to any chemical or biological material, compound, or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Some drugs are sold in an inactive form that is converted *in vivo* into a metabolite with pharmaceutical activity. For purposes of the present invention, the terms "pharmaceutical agent" and "drug" encompass both the inactive drug and the active metabolite.

35 "Transdermal delivery" refers to the transport of substance across the epidermis and dermis, such as the skin or mucous membranes, where the substance can contact and be absorbed into the capillaries. In certain instances, the delivery will be enhanced across other membranes.

40 "Enhanced transdermal delivery" refers both to the facilitation of transdermal delivery and an absolute increase in the molar volume transported per unit time through a constant surface area utilizing an equimolar pool of transported material as compared to unenhanced transdermal delivery.

"Iontophoresis" or "iontophoretic" refers to the introduction of an ionizable chemical through skin or mucous membranes by the application of an

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electric field to the interface between the ionizable chemical compound and the skin or mucous membrane.

"Permeability" refers to the ability of an agent or substance to penetrate, pervade, or diffuse through a barrier, membrane, or a skin layer.

5 "Pharmaceutically or therapeutically effective dose or amount" refers to a dosage level sufficient to induce a desired biological result. That result can be transdermal delivery of a pharmaceutical agent, alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system.

10 "Nucleotide" refers to a phosphoric acid ester of a N-glycoside of a heterocyclic nitrogenous base and is meant to encompass both non-cyclic and cyclic derivatives. The phosphate can be present on position 2', 3', and/or 5'. Generally, the glycoside component will be a pentose, however, in some embodiments, hexoses will be employed. The nitrogenous base typically will be selected from the group consisting of adenine, guanine, hypoxanthine, uracil, cytosine, and thymine, and analogs or chemical modifications thereof.

"Nucleotide-based pharmaceutical agent" or "nucleotide-based drug" refer to a pharmaceutical agent or drug comprising an oligonucleotide or nucleic acid.

20 "Oligonucleotide" generally refers to linear sequences of nucleotides, joined by phosphodiester bonds, typically prepared by synthetic means. Position 3' of each nucleotide unit is linked via a phosphate group to position 5' of the next unit. In the terminal units, the respective 3' and 5' positions can be free (i.e., free hydroxyl groups) or phosphorylated. Those oligonucleotides employed in the present invention will vary widely in length, generally from 2 to 100 nucleotides. Oligonucleotides and nucleic acids can be described in terms of their length. For example, an oligonucleotide comprising 20 nucleotides is termed a "20-mer", whereas an oligonucleotide having 30 nucleotides is a "30-mer".

25 Suitable oligonucleotides can be prepared by the phosphoramidite method described by Beaucage and Carruthers, Tetra. Letts. 22:1859-1862 (1981), or by the triester method according to Matteucci, *et al.*, J. Am. Chem. Soc., 103:3185 (1981), or by other methods, such as commercial automated oligonucleotide synthesizers. Oligonucleotide also is meant to include chemical modifications of the naturally occurring oligonucleotide skeleton. Such modifications include, but are not limited to, modifications at cytosine exocyclic amines, substitution of 5-bromouracil, backbone modifications, base analogs, methylations, and the like. *Infra.*

30 "Nucleic acid" refers to either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), whether single-stranded or double-stranded, and any chemical modifications thereof. Such modifications include, but are not limited to,

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modifications at cytosine exocyclic amines, substitution of 5-bromouracil, backbone modifications, base analogs, methylations, unusual base-pairing combinations, and the like. *Infra*.

5 "Phosphate ester" refers to a compound having the general formula $RO(PO)(OR')(OR'')$, where R, R' and R'' are independently selected from hydrogen, alkyl, aryl, arylalkyl, and heteroaryl.

10 "Phosphodiester" refers to a phosphate ester in which two hydroxyl groups of the phosphoric acid are esterified with organic residues: $R'O-PO_2H-OR''$ where R' and R'' are independently selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, or heteroaryl. Oligonucleotides and nucleic acids are typically phosphodiesters in which the 3' and 5' positions of neighboring pentose units are linked by esterification with a phosphate residue.

15 "Phosphoramidate" refers to a phosphodiester in which one or more of the hydroxyl groups is replaced with an amino group.

II. Iontophoretic Delivery of Nucleotide-Based Drugs

A. Overview

20 In accordance with one aspect of the present invention, novel methods for delivering nucleotide-based pharmaceutical agents across membranes, such as skin, in a controlled fashion are provided, such methods providing enhanced transdermal transport of nucleotide-based pharmaceutical agents, such as aptamers, ribozymes, antisense compounds, and triple helix drugs, through the use of iontophoresis.

25 Nucleotides are phosphate esters of glycosides of heterocyclic bases and are the structural units of both oligonucleotides and nucleic acids. Each phosphate functionality found in a nucleotide, oligonucleotide, or nucleic acid can impart a negative charge to the molecule. For example, a single-stranded DNA of 20 nucleotides will have a molecular weight of approximately 6500 and a net charge of -21 or approximately 1 negative charge per 325 daltons.

30 Nucleotide-based pharmaceutical agents suitable for transdermal electrotransport are those which are effective at low concentrations, for example, less than 50 milligrams per day, or are topically administered. Marked improvements in pharmaceutical agent bioavailability can be expected for those pharmaceutical agents which are poorly absorbed enterally or undergo extensive first pass hepatic inactivation.

35 Exemplary nucleotide-based pharmaceutical agents which can be delivered by the system of the present invention include analgesics, anesthetics, antifungals, antibiotics, antiinflammatories, anthelmintics, antidotes, antiemetics, 40 antihistamines, antihypertensives, antimalarials, antimicrobials, antipsychotics,

antipyretics, antiseptics, antiarthritics, antituberculotics, antitussives, antivirals, cardioactive drugs, cathartics, chemotherapeutic agents, antidepressants, depressants, diagnostic aids, diuretics, expectorants, hypnotics, parasympathomimetics, sedatives, stimulants, sympathomimetics, tranquilizers, urinary antiinfectives, vasoconstrictors, vasodilators, and the like.

The nucleotide-based drugs of the present invention include aptamers, ribozymes, antisense compounds, and triple helix drugs. The nucleotide-based drugs typically will have a molecular weight greater than about 350 and may range from between about 2 up to about 100 nucleotides, preferably, from between about 2 to 50 nucleotides, and more preferably from between about 10 and 40 nucleotides. Using the methods described herein, the electrotransport flux rate of DNAs has been found to be dependent upon the molecular weight of the DNA, with higher flux rates occurring with lower molecular weight DNAs. However, it should be noted that a subpopulation of the larger molecular weight oligonucleotide-based drugs having a specific conformation may exist that can be transported with efficiencies comparable to those found with the smaller molecular weight drugs.

Examples of smaller nucleotide-based drugs include, but are not limited to di- and trinucleotides, such as GS 375, a dinucleotide analog with potential therapeutic activity against the influenza virus (Gilead Sciences, Inc., Foster City, CA). In a particularly preferred embodiment, the nucleotide-based pharmaceutical agent will comprise an aptamer, a ribozyme, an antisense compound, or a triple helix drug.

B. Aptamers

Aptamers (or nucleic acid antibody) are single- or double-stranded DNA or single-stranded RNA molecules, typically of about 10-50 and preferably from about 15-30 nucleotides in length. Examples of aptamers include Gilead's antithrombin inhibitor GS 522 and its derivatives (Gilead Science, Foster City, CA). See also Macaya *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:3745-9; Bock *et al.* (1992) Nature (London) 355:564-566 and Wang *et al.* (1993) Biochem. 32:1899-904.

Aptamers bind specific molecular targets and are useful for "protein epitope targeting", especially as receptor antagonists. Generally, aptamers function by inhibiting the actions of the molecular target by binding to the pool of the target circulating in the blood. Typically, the target will comprise a protein and the aptamer will disrupt normal protein-protein and protein-receptor interactions through competitive binding. As antagonists to cell surface receptors, aptamers can exert their effects without having to penetrate the cell membrane.

The molecular target can comprise either an exogenous or endogenous substance. Typically, for embodiments employing an exogenous

molecular target, a non-covalently associated complex of the aptamer and the molecular target will be produced and the complex will be delivered iontophoretically according to the methods described herein. The dissociation constants for these non-covalently associated complexes typically will be between about 10^{-3} and 10^{-15} , preferably between about 10^{-6} and 10^{-12} , and more preferably between about 10^{-8} and 10^{-10} molar, under physiological conditions of salt, divalent ion concentration, and temperature. Techniques for measuring dissociation constants are well known in the art. See, e.g., PCT Publication No. WO 91/19813.

Aptamers that are specific for a given biomolecule and that can be delivered using the methods described herein can be identified by using techniques known in the art. See, e.g., Toole *et al.* (1992) PCT Publication No. WO 92/14843; Tuerk and Gold (1991) PCT Publication No. WO 91/19813; Weintraub and Hutchinson (1992) PCT Publication No. 92/05285; and Ellington and Szostak (1990) *Nature* 346:818. Briefly, these techniques typically involve the complexation of the molecular target with a random mixture of oligonucleotides. The aptamer-molecular target complex is separated from the uncomplexed oligonucleotides. The aptamer is recovered from the separated complex and amplified. This cycle is repeated to identify those aptamer sequences with the highest affinity for the molecular target.

C. Ribozymes

Ribozymes are structured RNA molecules that are capable of catalyzing a chemical reaction, such as the cleavage of a phosphodiester bond. See Symons (1992) *Ann. Rev. Biochem.* 71:641-671; Kruger *et al.* (1982) *Cell* 31:147-157; Zaug and Cech (1986) *Science* 231:470; Cech *et al.* (1982) *Cell* 31:147-157; Altman, in "Ribonuclease P: An Enzyme with a Catalytic RNA Subunit" in *Adv. Enzymol.* 62, 1-36 (1989). More specifically, ribozymes can comprise bifunctional RNA molecules containing a catalytic RNase activity as well as antisense sequences that can direct the molecule to the appropriate target mRNA. Ribozymes are typically 30-50 nucleotides in length.

Ribozymes can be readily altered or synthesized to cleave any desired single-stranded DNA sequence, and thus can be used to target virtually any RNA transcript or to characterize DNA molecules with only few limitations on the sites that are recognized. See, e.g., Kim *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:8788-8792; Haseloff *et al.* (1988) *Nature* 334:585-591; Cech (1988) *JAMA* 260:3030-3034; Jeffries *et al.* (1989) *Nucleic Acids Research* 17:1371-1377. These ribozyme "derivatives" include any RNA molecule which has the active site of any known ribozyme which has a deoxyribonuclease activity. This active site may be altered to specifically cleave a desired single-stranded DNA sequence. Such an

RNA molecule need only contain those essential portions of the ribozyme necessary for the deoxyribonuclease activity. Such ribozymes can be readily designed by those of ordinary skill in the art by use of any number of standard techniques and no undue experimentation is required to determine which of those ribozymes are active. See, e.g., Cech *et al.* PCT/US887/03161 and WO 88/04300; Lambowitz (1989) *Cell* 56:323; and Van der Veen (1986) *Cell* 44:225; and Murphy and Cech (1990) *Proc. Natl. Acad. Sci. USA* 86:9218-9222. For example, it is well known in the art that a ribozyme can be constructed by the interaction of two separate oligoribonucleotides, one of which is cleaved at a particular phosphodiester bond when incubated under known, appropriate conditions. See, e.g., Uhlenbeck (1987) *Nature* 328:590-600; Haseloff and Gerlach (1988) *Nature* 334:585-591.

D. Antisense Compounds

For diseases that result from the inappropriate expression of genes, specific prevention or reduction of the expression of such genes represents an ideal therapy. In principle, production of a particular gene product may be inhibited, reduced or shut off by hybridization of a single-stranded deoxynucleotide or ribodeoxynucleotide complementary to an accessible sequence in the mRNA, or a sequence within the transcript which is essential for pre-mRNA processing, or to a sequence within the gene itself. This paradigm for genetic control is often referred to as antisense or antigene inhibition.

Antisense compounds are oligonucleotides that are designed to bind and disable or prevent the production of the mRNA responsible for generating a particular protein. Antisense compounds include antisense RNA or DNA, single or double stranded, oligonucleotides, or their analogs, that can hybridize specifically to individual mRNA species and prevent transcription and/or RNA processing of the mRNA species and/or translation of the encoded polypeptide and thereby effect a reduction in the amount of the respective encoded polypeptide. Ching *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 86:10006-10010 (1989); Broder *et al.* *Ann. Int. Med.* 113:604-618 (1990); Loreau *et al.* *FEBS Letters* 274:53-56 (1990); Holcenberg *et al.* WO91/11535; U.S.S.N. 07/530,165 ("New human CRIPTO gene"); WO91/09865; WO91/04753; WO90/13641; WO 91/13080, WO 91/06629, and EP 386563).

Antisense compounds can provide a therapeutic function by inhibiting *in vivo* the formation of one or more proteins that cause or are involved with disease. Antisense compounds complementary to certain gene messenger RNA or viral sequences have been reported to inhibit the spread of disease related to viral and retroviral infectious agents (See, for example,

Matsukura *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:7706, and references cited therein).

Antisense compounds of various lengths can be delivered using the methods described herein, although such antisense compounds typically comprise a sequence of at least about 15 consecutive nucleotides. Examples of antisense compounds include G 1128 (Genta, Inc., San Diego, CA), OL(1)p53 (Lynx Pharmaceuticals), Ampligen (Hemm Pharmaceuticals), Isis 1082 and Isis 2105 (Isis Pharmaceuticals, Carlsbad, CA).

E. Triple Helix Drugs

Oligonucleotides also can bind to duplex DNA via triple helix formation and inhibit transcription and/or DNA synthesis. *See, e.g.,* Maher *et al.* (1989) *Science* 245:725-730. Triple helix drugs (also referred to as triple strand drugs) are oligonucleotides that bind to sequences of double-stranded DNA and are intended to inhibit selectively the transcription of disease-causing genes, such as viral genes, e.g., HIV and herpes simplex virus, and oncogenes, i.e., they stop protein production at the cell nucleus. These drugs bind directly to the double stranded DNA in the cell's genome to form a triple helix and thus, prevents the cell from making a target protein. *See, e.g.,* PCT publications Nos. WO 92/10590, WO 92/09705, WO91/06626, and U.S. Patent No. 5,176,996. Typically, the triple helix drug will comprise a DNA oligonucleotide in the range of about 20 to 40 nucleotides.

F. Analogs and Derivatives

The site specificity of oligonucleotides (e.g., aptamers, ribozymes, antisense compounds, and triple helix drugs) is not significantly affected by modification of the phosphodiester linkage or by chemical modification of the oligonucleotide terminus. Consequently, these oligonucleotides can be chemically modified; enhancing the overall binding stability, increasing the stability with respect to chemical degradation, increasing the rate at which the oligonucleotides are transported into cells, and conferring chemical reactivity to the molecules. The general approach to constructing various oligonucleotides useful in antisense therapy has been reviewed by vander Krol *et al.* (1988) *Biotechniques* 6:958-976 and Stein *et al.* (1988) *Cancer Res.* 48:2659-2668.

Accordingly, aptamers, ribozymes, antisense compounds, and triple helix drugs also can include nucleotide substitutions, additions, deletions, or transpositions, so long as specific hybridization to or association with the relevant target sequence is retained as a functional property of the oligonucleotide. For example, some embodiments will employ phosphorothioate analogs which are more resistant to degradation by nucleases than their naturally occurring

phosphate diester counterparts and are thus expected to have a higher persistence *in vivo* and greater potency (see, e.g., Campbell *et al.* (1990) J. Biochem. Biophys. Methods 20:259-267). Phosphoramidate derivatives of oligonucleotides also are known to bind to complementary polynucleotides and have the additional capability of accommodating covalently attached ligand species and will be amenable to the methods of the present invention. See, for example, Froehler *et al.* (1988) Nucleic Acids Res. 16(11):4831.

In some embodiments the nucleotide-based pharmaceutical agent will comprise O-methylribonucleotides (EP Publication No. 360609). Chimeric oligonucleotides may also be used (Dagle *et al.* (1990) Nucleic Acids Res. 18: 4751). For some applications, the pharmaceutical agent will comprise polyamide nucleic acids (Nielsen *et al.* (1991) Science 254:1497 and PCT publication No. WO 90/15065) or other cationic derivatives (Letsinger *et al.* (1988) J. Am. Chem. Soc. 110:4470-4471). Other applications may utilize oligonucleotides wherein one or more of the phosphodiester linkages has been substituted with an isosteric group, such as a 2-4 atom long internucleoside linkage as described in PCT publication Nos. WO 92/05186 and 91/06556, or a formacetal group (Matteucci *et al.* (1991) J. Am. Chem. Soc. 113:7767-7768) or an amide group (Nielsen *et al.* (1991) Science 254:1497-1500).

In addition, nucleotide analogs, for example wherein the sugar or base is chemically modified, can be employed in the present invention. "Analogous" forms of purines and pyrimidines are those generally known in the art, many of which are used as chemotherapeutic agents. An exemplary but not exhaustive list includes 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl- 2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N⁶-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N⁶-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, β -D-mannosylqueosine, 5-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N⁶-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine. In addition, the conventional bases by halogenated bases. Furthermore, the 2'-furanose position on the base can have a non-charged bulky group substitution. Examples of non-charged bulky groups include branched alkyls, sugars and branched sugars.

Terminal modification also provides a useful procedure to modify cell type specificity, pharmacokinetics, nuclear permeability, and absolute cell uptake rate for oligonucleotide pharmaceutical agents. For example, substitutions at the 5' and 3' ends include reactive groups which allow covalent crosslinking of the nucleotide-based pharmaceutical agent to other species and bulky groups which improve cellular uptake. See, e.g., Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression, (1989) Cohen, Ed., CRC Press; Prospects for Antisense Nucleic Acid Therapeutics for Cancer and AIDS, (1991), Wickstrom, Ed., Wiley-Liss; Gene Regulation: Biology of Antisense RNA and DNA, (1992) Erickson and Izant, Eds., Raven Press; and Antisense RNA and DNA, (1992), Murray, Ed., Wiley-Liss. For general methods relating to antisense compounds, see Antisense RNA and DNA, (1988), D.A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

1.5 G. Secondary Structure of DNA

Polynucleotide chains contain several bonds about which there is free rotation. In the absence of any intrastrand interactions each monomer would be free to rotate with respect to its adjacent monomers. The three-dimensional configuration of such a chain is called a random coil; it is a somewhat compact and globular structure that changes shape continually. However, few nucleic acid molecules exist as random coils due to the many interactions (e.g., hydrogen bonds, hydrophobic interactions, ionic bonds, van der Waals interactions, and disulfide bonds) between the elements of the chain.

DNA structure is very rich in variety. Although the double-stranded B helix is the predominant form, additional helical and other forms exist, including the A helix, the Z helix, circular, and superhelical DNA. Additionally, tRNA is composed of single strands of ribonucleic acid containing segments that have a high degree of base complementarity with downstream segments which allows intrastrand base pairing and the formation of loops. Consequently, tRNA contains a high degree of secondary structure consisting of base-paired double-helical segments, resembling a clover leaf, in which the four stems represent double-stranded helical segments composed of complementary base sequences and hydrogen-bonded bases.

Although not wishing to be bound by the following theory, by analogy with polyacrylamide and agarose gel electrophoresis, the conformation of the oligonucleotide-based pharmaceutical agent might be expected to have an effect on the electrotransport rate of the oligonucleotide, with the more compact oligonucleotide more easily traversing the skin as compared with the "floppy" conformation of an extended conformation. Conversely, extended conformations might be "snaking" their way through channels in the stratum corneum, while

the transport of the compact or conformational restricted oligonucleotides may be retarded due to their relatively larger cross-sectional areas.

This conformation effect could be important, since aptamers have been suggested to adopt tightly-folded structures (*see* Wang *et al.* (1993) Biochemistry 32:1899-1904); whereas ribozymes typically will consist of both self-complementary hairpin catalytic as well as unstructured, single-stranded antisense domain (*see* Zaug *et al.* (1986) Nature 324:429-433); and in contrast, individual antisense compounds typically will possess no inherent secondary structure. Thus, these nucleotide-based pharmaceutical agents conceivably could exhibit different electrotransport behavior depending on their conformation. Surprisingly, we have found that the conformation of the oligonucleotide did not seem to have any appreciable effect on electrotransport flux rate. These results are shown in Fig. 6.

15 III. Compositions of Nucleotide-based Pharmaceuticals

As applied to the iontophoretic delivery of nucleotide-based pharmaceutical agent, the invention provides nucleotide-based pharmaceutical agents with a charge-to-mass ratio that allows the pharmaceutical agent to be delivered in therapeutically effective amounts. Typically, the charge-to-mass ratio of such a composition will exceed one charge per 5000 daltons, and more typically, one charge per 2500 daltons. Preferably, the charge-to-mass ratio will be equal to or exceed one charge per 1000 daltons, more preferably, one charge per 500 daltons.

The nucleotide-based pharmaceutical agent can be admixed with an acceptable physiological carrier solution, such as water, aqueous alcohols, propylene glycol, and dimethylsulfoxide, to make a composition suitable for dermal contact and iontophoretic delivery. "Acceptable physiological carrier" includes those solutions which do not interfere with the effectiveness or the biological activity of the active ingredients and which are not toxic to the hosts to which it is administered. Well known techniques for choosing appropriate carriers and formulating the proper mixtures are exemplified in Banga *et al. supra*; Lattin *et al.* (1991) *Ann. N.Y. Acad. Sci.*, 618:450; and Remington's Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pennsylvania. (1980), which are incorporated herein by reference.

Typically, the carrier solution will also contain other ionic species, in addition to the oligonucleotides. For example, these ionic species can arise from buffer solutions that may be present to maintain the pH of the solution. As expected from a coulombic mechanism of electrotransport, to achieve the highest transport efficiency, the concentration of all ionic species, save the oligonucleotide or DNA itself, should be minimized. More specifically, we have found that

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increasing the salt concentration in the carrier solution results in a net decrease in the electrotransport rate of the DNA. These results are shown in Fig. 1.

According to some embodiments, LipofectinTM (available from BRL, Gaithersburg, Md., see Felgner *et al.* (1987) Proc. Natl. Acad. Sci. USA 84:7413); DOTMA (N-[1-(2,3-dioleoyloxy)propyl] -N,N,N-trimethylammonium chloride) will be utilized in combination with the nucleotide-based pharmaceutical agent. LipofectinTM is a liposome formulation of a cationic and a neutral lipid that interacts with DNA to form a lipid-DNA complex. See, e.g., Felgner *et al.* (1989) Nature 337:387. The fusion of the lipid-DNA complex with cells results apparently in efficient transfer of the DNA into the cell and is commonly used to increase the transfection efficiency of mammalian cells in culture. Surprisingly, we have found that the addition of small amounts of LipofectinTM to DNAs enhanced the electrotransport of the DNAs slightly. These results are shown in Fig. 4.

According to other embodiments, other liposomal formulations, for example, those comprising 3- β -[N-(N', N'-dimethyl-aminoethane)-carbamoyl] cholesterol in combination with dioleoylphosphatidylethanolamine (see Gao and Huang (1991) Biochem. Biophys. Res. Commun. 179:280-5); or lipopolylysines (i.e., low molecular weight (Mr approximately 3000) poly(L-lysine) (PLL) conjugated to N-glutarylphosphatidylethanolamine and containing an average of two phospholipid groups per molecule of PLL, see Zhou *et al.* (1991) Biochim. Biophys. Acta 1065:8-14) will be used. The liposomal formulations also can comprise pH-sensitive liposomes (e.g., composed of cholesteryl hemisuccinate and dioleoylphosphatidylethanolamine at a 2:1 molar ratio) or non-pH-sensitive liposomes.

When LipofectinTM or another liposomal formulation is used in combination with the compositions and methods described herein, the additive will typically be present in the donor reservoir in an amount between about 1 and 100 $\mu\text{g/ml}$, preferably, between about 2 and 50 $\mu\text{g/ml}$, and more preferably, between about 2 and 25 $\mu\text{g/ml}$.

In addition to the nucleotide-based pharmaceutical agents, the composition can contain other materials such as dyes, pigments, inert fillers, or other permeation enhancers, excipients, and conventional components of pharmaceutical products and transdermal therapeutic systems known in the art. Thus, according to some embodiments of this invention, chemical enhancers (i.e., penetration or permeation enhancers) will be incorporated into the donor reservoir of the iontophoretic device and utilized to alter the iontophoretic transport rate. For example, the coapplication of oleic acid to the skin causes a large decrease in the skin impedance or resistance which is inversely related to permeability or transport. See Potts *et al.* (1992) Solid State Ionics 53-56:165-169. Thus, instead of the current passing primarily through the shunt pathways (e.g.,

the follicles and sweat ducts), the ions (e.g., the nucleotide-based pharmaceutical agent) constituting the current can more uniformly permeate the lipid milieu of the stratum corneum at a lower current density. Alternatively, the use of chemical enhancers will allow for an increased rate of iontophoretic transport of the nucleotide-based pharmaceutical agent as compared to the transport rate found at the same current density in the absence of the chemical enhancer.

Using the compositions and methods described herein, the electrotransport flux rate of the nucleotide-based pharmaceutical agents has been found to increase in a concentration-dependent manner. These results are shown in Fig. 3. Generally, the nucleotide-based pharmaceutical agent will be present in the reservoir of the iontophoresis device in an amount ranging from between about 1 to about 500 mg/ml, and preferably from between about 5 to about 200 mg/ml and the device will be capable of delivering between about 0.1 and 20 mg, and preferably between about 0.1 and 10 mg, of the nucleotide-based pharmaceutical agent during a 24 hour period for an iontophoresis device having about a 20 cm² donor reservoir.

IV. In Vitro Testing of Nucleotide-Based Pharmaceutical Agents

The *in vitro* skin permeation rate of a pharmaceutical agent can be measured using diffusion cells. Human, mouse or porcine skin is placed on the lower half of the diffusion cell with the stratum corneum facing the donor compartment. The donor compartment contains a solution of the pharmaceutical agent and the cathode. The receiver compartment contains a buffer solution and the anode. An electric current is applied and the amount of transported drug can be calculated ($\mu\text{g}/\text{cm}^2\cdot\text{hr}$). Alternatively, an iontophoresis device containing the pharmaceutical agent to be tested can be placed on the stratum corneum. The receiver compartment again would contain a buffer solution. The device is activated and the amount of transported drug can be calculated ($\mu\text{g}/\text{cm}^2\cdot\text{hr}$).

Conventional flow-through diffusion cells can also be used to measure the *in vitro* skin permeation rate of pharmaceutical agents. Typically these cells will have an active area of 1 cm² and a receiving volume of 3 ml. The receptor fluid, generally isotonic saline or buffer solution, is pumped into and through the cells, by a peristaltic pump. Samples can be collected in glass vials arranged in an automatic fraction collector. The amount of drug permeating across the skin ($\mu\text{g}/\text{cm}^2\cdot\text{hr}$) is calculated from the cumulative release.

The electrotransport behavior of a pharmaceutical agent can also be assessed using the conventional analytical techniques and gel or capillary electrophoresis. Preparation measurements may also be performed on excised skin in conventional diffusion cell tests. See e.g., Lattin *et al. supra*.

V. In Vivo Delivery of Nucleotide-Based Pharmaceutical Agents

A. Therapeutic Indications

5 The compositions and methods described herein will find use in the treatment of a variety of diseases, including but not limited to those described below. The compositions and methods described herein can be utilized for either the systemic or local administration of the nucleotide-based pharmaceutical agent, depending on the therapeutic indication.

10 For example, nucleotide-based pharmaceutical agents can be designed to prevent expression of diverse potential target genes, including oncogenes, fungal genes, and any other gene known to be activated specifically in the skin. These pharmaceutical agents can then be delivered directly to melanomas, Kaposi sarcomas, psoriasis lesions, and fungal infected skin, and the like. The compositions and methods described herein will find use in the treatment of viral, 15 fungal, and bacterial infections of the skin and mucous membranes, including genital warts caused by the human papilloma virus and infections caused by Herpes viruses.

In addition, the present invention provides for the delivery of therapeutic compositions of nucleotide-based pharmaceutical agents directly to 20 block mediators of inflammation, including cytokines, growth factors, cell adhesion molecules or their ligands and receptors thereof, as well as key enzymes in pathways leading to inflammation. More specifically, these blocking actions include preventing the expression of cytokines (such as IL-1), growth factors (such as TGF- α and EGF), or cell adhesion molecules (such as ELAM and ICAM); or the 25 receptors for cytokines (such as IL-1), growth factors, or cell adhesion molecules. Key enzymes whose expression may be blocked include protein kinase C and phospholipase A or C. Thus, according to one aspect of this invention, topical or transdermal formulations of the complexes described herein are applied directly to knees or other joints to alleviate inflammation and the like.

30 In yet another aspect, the compositions and methods described herein can be used to treat conditions in which improper immune or inflammatory responses have been implicated such as psoriasis (e.g., by blocking the expression of IL-1, TGF- α , amphiregulin, or IL-6); atopic dermatitis and eczema (e.g., by blocking the overexpression of IgE); rheumatoid arthritis; allergic rhinitis (e.g., by 35 blocking the expression of IL-4); and the like. In an additional aspect, the compositions and methods described herein can be used to treat certain cancers of the skin and mucous membranes, such as melanoma, mycosis fungoides, and squamous cell carcinoma (including of the cervix), for example, by blocking the expression of certain factors which promote cell growth and/or adhesion and 40 which are believed to be involved in metastasis.

The compositions and methods described herein also can be employed for "transdermal genoculation". See Watanabe *et al.* (1993) Proc. Natl. Acad. Sci. 90:4523-4527 and Bioworld Today, "'Genoculation' Induces Immune Responses in Mice", May 17, 1993. More specifically, a host suffering from advanced melanoma have been innoculated with an incompatible human major histocompatibility complex (MHC) gene that has been incorporated within a liposome envelope. In BALB/C mice with induced forms of colon adenocarcinoma or fibrosarcoma, pretreating the animals with the MHC gene a few days before inducing tumors was found to double survival time. This "genoculation" is thought to induce a cytotoxic T cell response to the MHC tag, and more importantly, to tumor antigens that the immune system normally does not see and that prevent the body's immune response from recognizing and destroying the malignant tissue. Thus, one aspect of the present invention provides for the iontophoretic delivery of MHC genes to illicit an immune response.

B. Dosages

According to this invention, a therapeutically or pharmaceutically effective amount of a nucleotide-based pharmaceutical agent is delivered iontophoretically to a patient in need of such an agent. The compositions and methods described herein can be employed for the prophylactic and/or therapeutic treatments. In therapeutic applications, the compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective amount or dose." Amounts effective for this use will depend on the severity and course of the disease, previous therapy, the patient's health status and response to the drugs, and the judgment of the treating physician. For standard dosages of conventional nucleotide-based pharmaceutical agents, see, e.g., Physicians Desk Reference (1992 Edition); and American Medical Association (1992) Drug Evaluations Subscriptions.

In prophylactic applications, the nucleotide-based pharmaceutical agent is administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective amount or dose." In this use, the precise amounts again depend on the patient's state of health, weight, and the like.

Once improvement of the patient's conditions has occurred, a maintenance dose can be administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the

symptoms have been alleviated to the desired level, treatment can cease. Patients can, however, require intermittent treatment on a long-term basis upon any recurrence of the disease symptoms.

In general, a suitable effective dose of the nucleotide-based pharmaceutical agent which can be delivered iontophoretically according to the methods described herein will be in an amount ranging from between about 0.1 to about 10 milligram (mg) per recipient per day using an iontophoresis device having a 20 cm² donor reservoir and a current of less than about 0.5 mAmps/cm², preferably in the range of between about 0.5 to about 5 mg per day, and most preferably in an amount of about 0.5 to about 1 mg.

C. Iontophoretic Delivery

The nucleotide-based pharmaceutical agents described herein can be administered transdermally using iontophoresis. This form of administration typically involves the delivery of a pharmaceutical agent for percutaneous passage of the drug into the systemic circulation of the patient. However, the nucleotide-based pharmaceutical agents also can be delivered directly to pathological or diseased tissue using iontophoresis for the local administration of the analog. The skin sites include anatomic regions for transdermally administering the drug as represented by the forearm, abdomen, chest, back, buttock, mastoidal area and the like.

The therapeutic composition can be delivered by a standard iontophoretic device. In general, iontophoresis is an introduction, by means of electric current, of ions of soluble salts into the tissues of the body. More specifically, iontophoresis is a process and technique which involves the transfer of ionic (charged) species into a tissue (for example through the skin of a patient) by the passage of a electric current through an electrolyte solution containing ionic molecules to be delivered (or precursors for those ions), upon application of an appropriate electrode polarity. That is, ions are transferred into the tissue, from an electrolyte reservoir, by application of electromotive force to the electrolyte reservoir. In iontophoretic systems, the rate of release is primarily controlled by the voltage or current.

A wide variety of iontophoresis devices are presently known. See, e.g., Phipps *et al.* U.S. Patent No. 4,744,788; Phipps *et al.* U.S. Patent No. 4,747,819; Tapper *et al.* European Patent Application Publication No. 0318776; Jacobsen *et al.* European Patent Application Publication No. 0299631; Petelenz *et al.* U.S. Patent No. 4,752,285; Sanderson *et al.* U.S. Patent No. 4,722,726; Phipps *et al.* U.S. Patent No. 5,125,894; and Parsi U.S. Patent No. 4,731,049. Badzinski *et al.* (1993) U.S. Patent No. 5,207,752; Gyory *et al.* (1993) U.S. Patent No. 5,203,768; Gyory *et al.* (1992) U.S. Patent No. 5,162,042; Phipps (1992) PCT Publication No. WO 92/17239; Landrau *et*

et al. (1992) PCT Publication No. WO 92/15365; Gyory *et al.* (1992) Canadian Patent Publication 2,042,994; Gyory *et al.* (1992) U.S. Patent No. 5,158,537; Gyory *et al.* (1992) PCT Publication No. WO 92/07618; Myers *et al.* (1992) U.S. Patent No. 5,147,297; Gyory *et al.* (1992) U.S. Patent No. 5,147,297; Gyory *et al.* (1991) Canadian Patent Publication No. 2,015,597; Gyory *et al.* (1992) U.S. Patent No. 5,084,006; Gyory *et al.* U.S. Patent No. 5,162,043; Haak *et al.* (1992) U.S. Patent No. 5,167,616; Gyory *et al.* (1990) PCT Publication No. 90/09413; Theeuwes *et al.* (1992) U.S. Patent No. 5,080,646; Theeuwes *et al.* (1992) U.S. Patent No. 5,147,296; Theeuwes *et al.* (1992) U.S. Patent No. 5,169,382; Theeuwes *et al.* (1992) U.S. Patent No. 5,169,383; Theeuwes (1990) U.S. Patent No. 4,978,337; Moodie *et al.* (1992) U.S. Patent No. 5,125,894; Haak *et al.* (1990) U.S. Patent No. 4,927,408; the full disclosures of each which are incorporated herein by reference.

In typical, conventional, electrotransport or iontophoresis devices, two electrodes are generally used. Both electrodes are disposed so as to be in intimate electrical contact with some portion (typically skin) of the subject (human or animal) typically by means of two remote electrolyte-containing reservoirs, between which current passes as it moves between the skin and the electrodes. Generally the active electrode includes the therapeutic species as a charged ion, or a precursor for the charged ion, and the transport occurs through application of the electromotive force to the charged therapeutic species. An appropriate potential is initiated between two electrode systems (anode and cathode) in electrical contact with the skin. If a positively charged drug is to be delivered through the skin, an appropriate electromotive force can be generated by orienting the positively charged drug species at a reservoir associated with the anode. Similarly, if the ion to be transferred across the skin is negatively charged, appropriate electromotive force can be generated by positioning the drug in a reservoir at the cathode. Of course, a single system can be utilized to transfer both positively charged and negatively charged drugs into a patient at a given time; and, more than one cathodic drug and/or more than one anodic drug may be delivered from a single system during a selected operation.

In conjunction with the patient's skin in electrical communication with the electrodes, the circuit is completed by connection of the two electrodes to a source of electrical energy as a direct current; for example, a battery or a source of appropriately modified alternating current. For general discussions of iontophoresis, *see, e.g.*, Tyle (1989) J. Pharm. Sci. 75:318; Burnette, *Iontophoresis* (Chapter 11) in Transdermal Drug Delivery Hadgraft and Guy (eds.) Marcel Dekker, Inc.: New York, NY; Phipps *et al.* (1988) Solid State Ionics 28-30:1778-1783; Phipps *et al.* (1989) J. Pharm. Sciences 78:365-369; and Chien *et al.* (1988) J. Controlled Release 7:1-24, the full disclosures of which are incorporated herein by reference.

"Electrode" refers to an electrically conductive member, through which a current passes during operation. A variety of electrode materials, ranging from platinum to silver-silver chloride, are available for these devices. The primary difference in these materials is not in their ability to generate an electric potential across the skin, but rather in certain nuances associated with their performance of this function. For example, platinum electrodes hydrolyze water, thus liberating hydrogen ions and subsequently, changes in pH. Obviously, changes in pH can influence the ionization state of therapeutic agents and their resulting rate of iontophoretic transport. Silver-silver chloride electrodes, on the other hand, do not hydrolyze water. However, these electrodes require the presence of chloride ion which may compete for current-induced transport.

Electrotransport devices generally require a reservoir as a source of the species (or a precursor of such species) which is to be moved or introduced into the body. The reservoir typically will comprise a pool of electrolyte solution, for example an aqueous electrolyte solution or a hydrophilic, electrolyte-containing, gel or gel matrix, semi-solid, foam, or absorbent material. Such pharmaceutical agent reservoirs, when electrically connected to the anode or the cathode of an iontophoresis device, provide a source of one or more ionic species for electrotransport.

Many iontophoresis devices employ a selectively permeable membrane. The composition of this membrane will vary with the particular needs of the system and will depend upon the composition of the electrolyte reservoir, i.e., the nature of the pharmaceutical agent, the transference of current out of the reservoir, and the desired selectivity to transport of particular types of charged and uncharged species. A microporous polymer or hydrogel such as is known in the art can be utilized. *See, e.g.,* U.S. Patent No. 4,927,408.

Suitable permeable membrane materials can be selected based on the desired degree of permeability, the nature of the complex, and the mechanical considerations related to constructing the device. Exemplary permeable membrane materials include a wide variety of natural and synthetic polymers, such as polydimethylsiloxanes (silicone rubbers), ethylenevinylacetate copolymer (EVA), polyurethanes, polyurethane-polyether copolymers, polyethylenes, polyamides, polyvinylchlorides (PVC), polypropylenes, polycarbonates, polytetrafluoroethylenes (PTFE), cellulosic materials, e.g., cellulose triacetate and cellulose nitrate/acetate, and hydrogels, e.g., 2-hydroxyethylmethacrylate (HEMA).

Generally, buffers will also be incorporated into the reservoir to maintain the reservoir environment at the same charge as the electrode. Typically, to minimize competition for the electric current, a buffer having the opposite charge to the drug will be employed. In some situations, for example, when the appropriate salt is used, the drug may act as its own buffer. Other

variables which may effect the rate of transport include drug concentration, buffer concentration, ionic strength, nonaqueous cosolvents, and any other constituents in the formulation. However, as discussed above, to achieve the highest transport efficiency, the concentration of all ionic species, save the pharmaceutical agent
5 itself, is minimized.

The backing or enclosure of the drug delivery system is intended primarily as a mechanical support for the reservoir or matrix. In the simplest case, the matrix is exposed directly to the skin or membrane of the host, and the backing is a strip or patch capable of being secured to the skin, typically with the matrix
10 acting as an adhesive. In such constructions, the backing will usually be impermeable to the complex. This impermeability inhibits the loss of the complex. Suitable backing materials will generally be thin, flexible films or fabrics such as woven and non-woven fabrics and polymeric films, such as polyethylene, polypropylene, and silicone rubber; metal films and foils; and the like.

The delivery device can be held in place with the adhesive of the
15 matrix, with an adhesive along the perimeter of the matrix, with tape or elastic, or any other means, so long as the device allows the pharmaceutical agent to be transported through the skin. The device can be placed on any portion of the skin or dermal surface, such as the arm, abdomen, thigh, and the like. Furthermore,
20 the device can be in various shapes and can consist of one or more complexes and/or transport areas. Other items can be contained in the device, such as other conventional components of therapeutic products, depending upon the desired device characteristics.

In the conventional topical treatment by iontophoresis, the direct
25 current is applied through moist pad-type electrodes with size corresponding to that of the skin region to be treated. The interposition of a moist pad between the electrode plate and the skin is necessary for making a perfect contact, preventing any skin burns, overcoming skin resistance, and protecting the skin from absorbing any caustic metal compounds formed on the metal plate surface.

The drug is administered through an electrode having the same
30 charge as the drug, and a return electrode opposite in charge to the drug is placed at a neutral site on the body surface. The operator then selects a current intensity below the pain threshold level of the patient and allows the current to flow for an appropriate length of time. Ions transferred through the skin are taken up by the
35 micro-circulation at the dermal-epidermal junction, while the current proceeds through the skin tissues to the return electrode. The current intensity should be increased slowly, maintained for the length of time of the treatment, and then decreased slowly at the end of the treatment. The current must be within comfortable toleration of the patient, with a current density which is generally less
40 than 0.5 mAmp/cm² of the electrode surface.

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The therapeutic composition can be delivered by a standard iontophoretic device. Owing to differences in available iontophoretic devices the procedure for use can vary. The manufacturer's instructions should be followed for appropriate pharmaceutical agent delivery. Body fluid or blood levels of the uncomplexed pharmaceutical agent will be determined to measure the effectiveness of the transport and bioconversion.

One aspect of this invention provides for the delivery of therapeutic compositions of nucleotide-based pharmaceutical agents directly to pathological or diseased tissue for the local administration of the nucleotide-based pharmaceutical agent. Nucleotide-based pharmaceutical agents can be designed to prevent expression of diverse potential target genes, including oncogenes, fungal genes, and any other gene known to be activated specifically in the skin. These pharmaceutical agents can then be delivered directly to melanomas, Kaposi sarcomas, psoriasis lesions, and fungal infected skin, and the like using the methods described herein. In addition, the present invention further provides for the delivery of therapeutic compositions of nucleotide-based pharmaceutical agents directly to knees or other joints to alleviate inflammation and the like.

The invention will be more fully described and understood with reference to the following examples. These examples are provided by way of illustration only and not by way of limitation. Those skilled in the art will readily appreciate a variety of noncritical parameters which could be changed or modified to yield essentially similar results.

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EXPERIMENTALMATERIALS

Oligonucleotides were obtained commercially or synthesized using a commercially-available oligonucleotide synthesizer (e.g., Applied Biosystems Model 394 Oligonucleotide Synthesizer) and cyanoethyl phosphoramidite chemistry. The DNAs were end-labeled with ^{32}P and T4 polynucleotide kinase and rigorously purified from unincorporated ATP by reverse phase chromatography.

In addition, collections of radioactively-labeled, single-stranded oligonucleotides and nucleic acids of random sizes can be generated using techniques well known in the art. For example, multiple rounds of DNA synthesis from a DNA template using Taq DNA polymerase, dideoxynucleotide triphosphates, and either ^{32}P -labeled oligonucleotide primers or ^{32}P , ^{33}P , or ^{35}S -labeled deoxynucleosides can be performed. See Promega Protocols and Applications Guide, 2nd Ed., Promega Corp., Madison, WI (1991).

In addition, any of a variety of other methodologies can be used, including Bal 31 nuclease digestion of DNA followed by radioactive labeling, "nick translation" or "random primer synthesis", which uses Dnase 1 or random oligonucleotide primers, respectively, to create primer-template junctions for the incorporation of radioactively-labeled deoxynucleosides by DNA polymerases, etc. The labeled DNA's should be in sufficient molar excess over their templates, as well as devoid of detectable secondary structures (unless engineered into the template sequence), to ensure that no higher order, macromolecular structures are formed. The size distribution of a sample of a mixture of labeled fragments can be assessed by electrophoresis using a standard DNA sequencing gel and autoradiography. See, e.g., Sambrook *et al.* Molecular Cloning. Typically, a distribution of uniformly labeled fragments extending from approximately 5-200 nucleotides is created.

Synthetic membrane, hairless guinea pig, hairless mouse, or human (either living or cadaverous) skin can be prepared by techniques known in the art. Although the experiments described herein employed custom-made Teflon diffusion cells, one of skill in the art will appreciate that the methods can be easily modified for use with conventional, commercially available diffusion cells.

A random 20-mer was shown to be resistant to degradation by either the dermal or epidermal surfaces of freshly-prepared hairless mouse skin *in vitro* during a 6 hour incubation in PBS at room temperature. A random 20-mer that had been iontophoresed through hairless mouse skin *in vitro* was shown to be intact as judged by polyacrylamide gel electrophoresis followed by autoradiography and comparison with control DNA standards.

25

EXAMPLE 1GENERAL METHODS

Hairless mouse skin was inserted into custom-made Teflon cells possessing 0.2 ml donor and receptor volumes, at 0.3 mAmps constant current/cm² hairless mouse skin at room temperature. The compound to be tested was placed in an appropriate buffer in a "donor" chamber on the exterior side of the skin or membrane. A "counter" chamber containing suitable buffer is placed on the interior side of the skin or membrane. The donor chamber contained a silver chloride (AgCl) cathode and the receptor chamber contained a silver (Ag) anode. An experiment was performed in which DNA delivery from the anode was attempted. However, no measurable flux rate was found. Likewise, a passive transport experiment in which no current was applied was performed. Again, no measurable flux of DNA was found.

Following application of electric current, a sample is withdrawn from the counter chamber and analyzed. Sampling was performed by first mixing the buffer within the desired cell, removing 0.025 ml of solution, and immediately replacing it with 0.025 ml of fresh buffer. Except where noted, all donor cells contained 30 millimolar (mM) sodium chloride (NaCl), 50 mM MOPS, pH 6., and 50 mg/ml of the ³²P-labeled oligonucleotide and the receptors contained the same, minus the oligonucleotide.

The extent of DNA transport was determined following scintillation counting using the values of specific activity calculated following the kinase end-labeling of the oligonucleotides. All flux experiments were performed in triplicate and the average values were plotted. Transport can also be assessed using an antibody-mediated reaction, an activity assay, or by radioactively prelabeling the test compound, either enzymatically or metabolically, and monitoring the radioactivity.

EXAMPLE 2EFFECT OF SALT CONCENTRATION

The electrotransport of random 20-mer DNAs at three different NaCl (6 mM, 30 mM, and 150 mM) concentrations was tested. The donor and receptor cells contained equivalent amounts of NaCl. As expected from a coulombic mechanism of electrotransport, in which the DNA itself is transported by virtue of its actually carrying the current (as opposed to a passive electroosmotic mechanism in which the DNA is carried along with the flow of solvent), increasing the salt concentration and thereby decreasing the fractional contribution of the DNA to the current conductance resulted in a net decrease in DNA flux. At the lowest NaCl concentration tested (6 mM), the DNA flux dropped dramatically following the 4

26

hour timepoint, presumably due to ion depletion in the receptor cell. These results are shown in Fig. 1.

EXAMPLE 3

EFFECT OF OLIGONUCLEOTIDE SIZE

5 The electrotransport flux rates for three different sizes of random DNA (20-mer, 30-mer, and 40-mer) were determined. While the charge densities for each of the three size classes of DNA were virtually identical, the DNAs ranged in molecular weight from 6500 to 13,000 daltons. The flux rate was found to
10 decrease rapidly with increase in molecular weight, with the 40-mer transporting at approximately one-fifth the rate of the 20-mer. These results are shown in Fig. 2.

EXAMPLE 4

EFFECT OF OLIGONUCLEOTIDE CONCENTRATION

15 The electrotransport flux rates of random 20-mers at three different donor DNA concentrations (5 mg/ml, 50 mg/ml, and 200 mg/ml) were measured. The flux rate was found to increase in a concentration-dependent manner with the highest concentration tested (200 mg/ml) appearing to be sub-saturating. This
20 experiment was done at 150 mM NaCl concentration. If corrected to account for the 30 mM NaCl concentration, flux rates in excess of 1 mg/cm²hr would be expected for a donor concentration of 200 mg/ml of random 20-mer. These results are shown in Fig. 3.

EXAMPLE 5

EFFECT OF LIPOFECTIN™ CONCENTRATION

25 [1-(2,3,-dioleyloxy)propyl]-N,N,N-triethylammonium chloride, which is commercially available as Lipofectin™, is a liposome formulation of a cationic and a neutral lipid that interacts with DNA to form a lipid-DNA complex. See, e.g.,
30 Felgner *et al.* (1989) *Nature* 337:387. Much as liposomes have been used as delivery vehicles for passive transdermal drug delivery, we believed that complexation of the DNA with the Lipofectin™ might render the complex more accessible to hydrophobic pathways in the stratum corneum, thus resulting in an enhancement of flux. Alternatively, the Lipofectin™ may be acting as a
35 permeability enhancer directly.

Lipofectin™ was added to a random DNA 20-mer in the donor cell (0 mg/ml, 2 mg/ml, and 20 mg/ml). It was found that Lipofectin™ enhanced the electrotransport of DNA slightly. These results are shown in Fig. 4.

EXAMPLE 6
EFFECT OF DNA CONFORMATION

5 The electrotransport flux rate of the 15-nucleotide thrombin aptamer
d(GGTTGGTGTGGTTGG) SEQ ID NO:1 (*see* Bock *et al.* (1992) Nature 355:564-566)
was compared to that of a random DNA 20-mer. The structure of the aptamer,
consisting of a pair of stacked G tetrads and 3 loops, has been deduced by NMR
analysis and is shown in Fig. 5. The conformation of the DNA did not seem to
have any appreciable effect on electrotransport flux rate. These results are shown
10 in Fig. 6.

It is to be understood that the above description is intended to be
illustrative and not restrictive. Many embodiments will be apparent to those of
skill in the art upon reviewing the above description. The scope of the invention
should, therefore, be determined not with reference to the above description, but
15 should instead be determined with reference to the appended claims, along with
the full scope of equivalents to which such claims are entitled.

The invention has been described above in some detail for the
purposes of clarity and understanding. The disclosures of all articles and
references, including patent publications, are incorporated herein by reference.
20 Changes and modifications can be practiced within the scope of the appended
claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Affymax Technologies N.V.
- (ii) TITLE OF INVENTION: Compositions and Methods for Transdermal Drug Delivery
- (iii) NUMBER OF SEQUENCES: 1
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Affymax Technologies N.V., Administrative Offices
 - (B) STREET: 4001 Miranda Avenue
 - (C) CITY: Palo Alto
 - (D) STATE: California
 - (E) COUNTRY: USA
 - (F) ZIP: 94304
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/107,329
 - (B) FILING DATE: 16-AUG-1993
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/077,786
 - (B) FILING DATE: 14-JUN-1993
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/009,463
 - (B) FILING DATE: 27-JAN-1993
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/077,296
 - (B) FILING DATE: 14-JUN-1993
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Stevens, Lauren L.
 - (B) REGISTRATION NUMBER: 36,691
 - (C) REFERENCE/DOCKET NUMBER: 11509-78-3/1031.2

- (ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (oligonucleotide)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTTGGTGTG GTTGG

15

WHAT IS CLAIMED IS:

1. A method for the delivery of a pharmaceutical agent in a therapeutically effective dose to a patient, said method comprising the steps of:
5 contacting the skin of the patient with a therapeutically effective amount of a pharmaceutical agent in an iontophoresis device, wherein the pharmaceutical agent comprises an oligonucleotide or a nucleic acid and the pharmaceutical agent is ionically charged; and
10 applying an electric field to the interface of the device and the skin, such that the electric field transdermally delivers the pharmaceutical agent.
2. The method of Claim 1 wherein the pharmaceutical agent in an iontophoretic device is applied directly to pathological tissue or an area of localized
15 inflammation.
3. The method of Claim 1 wherein the pharmaceutical agent comprises an oligonucleotide or nucleic acid having between 2 and 100 nucleotides
- 20 4. The method of Claim 3 wherein the pharmaceutical agent comprises an oligonucleotide or nucleic acid having between 15 and 50 nucleotides.
5. The method of Claim 3 wherein the pharmaceutical agent comprises an aptamer, a ribozyme, an antisense compound or a triple helix drug.
25
6. The method of Claim 5 wherein the pharmaceutical agent comprises the aptamer d(GGTGGTGTGGTTGG).
7. The method of Claim 1 wherein the pharmaceutical agent in the iontophoresis device further comprises Lipofectin™, wherein the Lipofectin™ is
30 present in an amount sufficient to enhance the rate of transport of the pharmaceutical agent as compared to the rate of transport of the pharmaceutical agent in the absence of the Lipofectin™.
- 35 8. The method of Claim 1 wherein an electric field of between about 0.1 and 0.5 mAmp/cm² is applied.
9. The method of Claim 1 wherein the pharmaceutical agent is transdermally delivered in an amount ranging from between 0.1 and 10 mg per 24
40 hours using a iontophoresis device having a 20 cm² donor reservoir.

10. A system for delivering a nucleotide-based pharmaceutical agent comprising:

- 5 (a) a source of the nucleotide-based pharmaceutical agent to be delivered through a selected intact area of skin or mucosal tissue; and
(b) an iontophoretic device containing said source.

11. The system of Claim 10 wherein the pharmaceutical agent comprises an oligonucleotide or nucleic acid having between 2 and 100 nucleotides

10 12. The system of Claim 11 wherein the pharmaceutical agent comprises an oligonucleotide or nucleic acid having between 15 and 50 nucleotides.

13. The system of Claim 10 wherein the pharmaceutical agent comprises
15 an aptamer, a ribozyme, an antisense compound or a triple helix drug.

14. The system of Claim 13 wherein the pharmaceutical agent comprises the aptamer d(GGTTGGTGTGGTTGG).

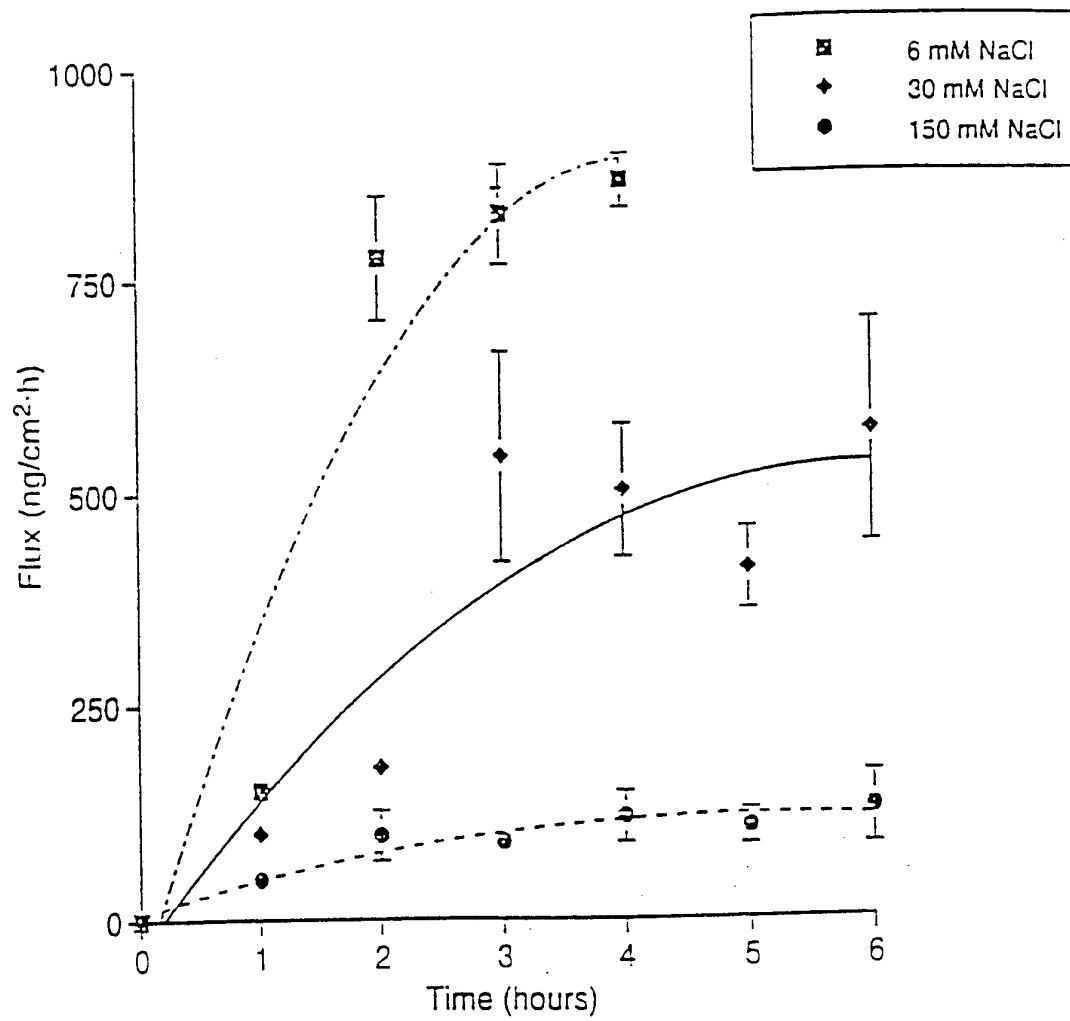
20 15. The system of Claim 10 wherein the system further comprises Lipofectin™, wherein the Lipofectin™ is present in an amount sufficient to enhance the rate of transport of the pharmaceutical agent as compared to the rate of transport of the pharmaceutical agent in the absence of the Lipofectin™.

25 16. The system of Claim 10 wherein the iontophoretic device is capable of applying an electric field of between about 0.1 and 0.5 mAmp/cm².

30 17. The system of Claim 10 wherein the pharmaceutical agent is transdermally delivered in an amount ranging from between 0.1 and 10 mg per 24 hours using a iontophoresis device having a 20 cm² donor reservoir.

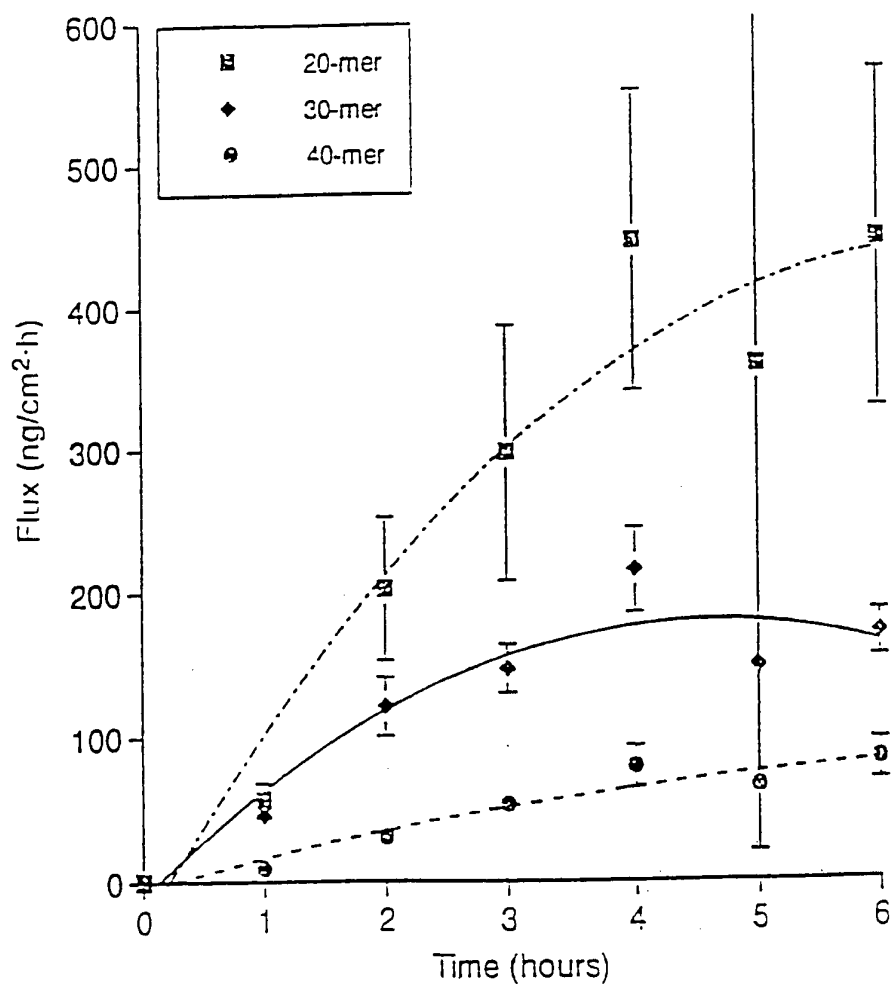
1 / 6

Fig. 1



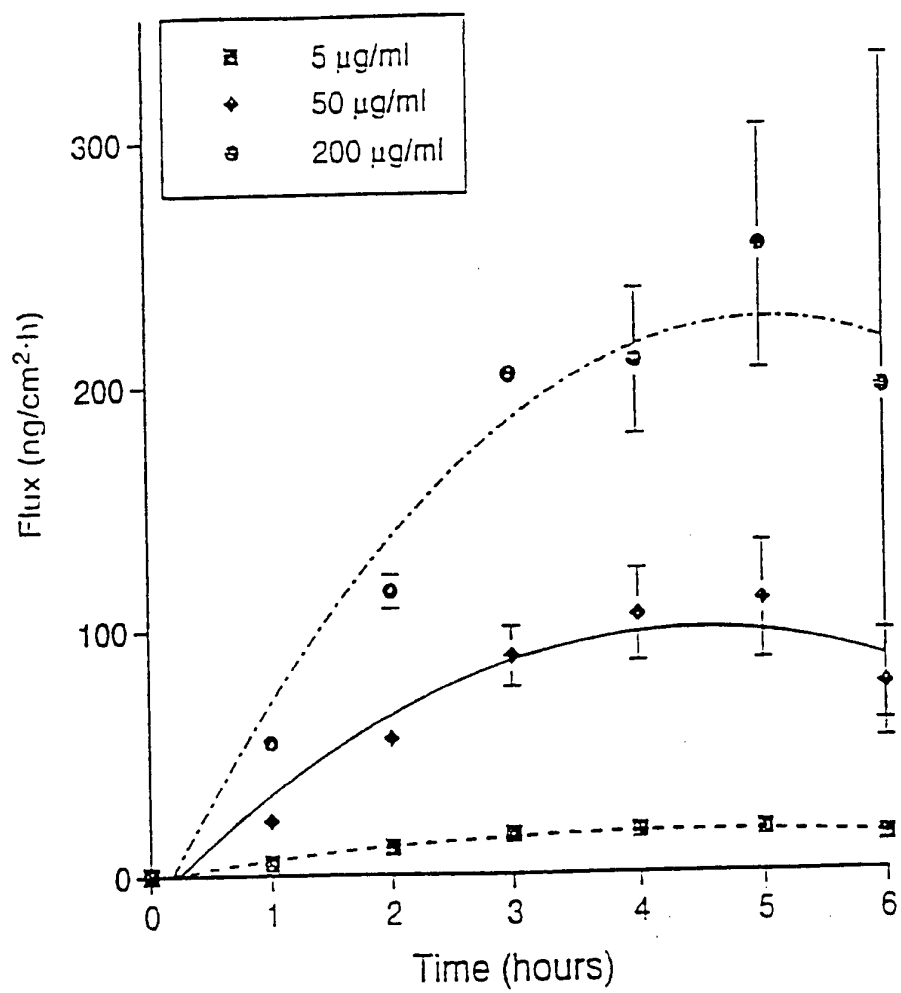
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Fig. 2



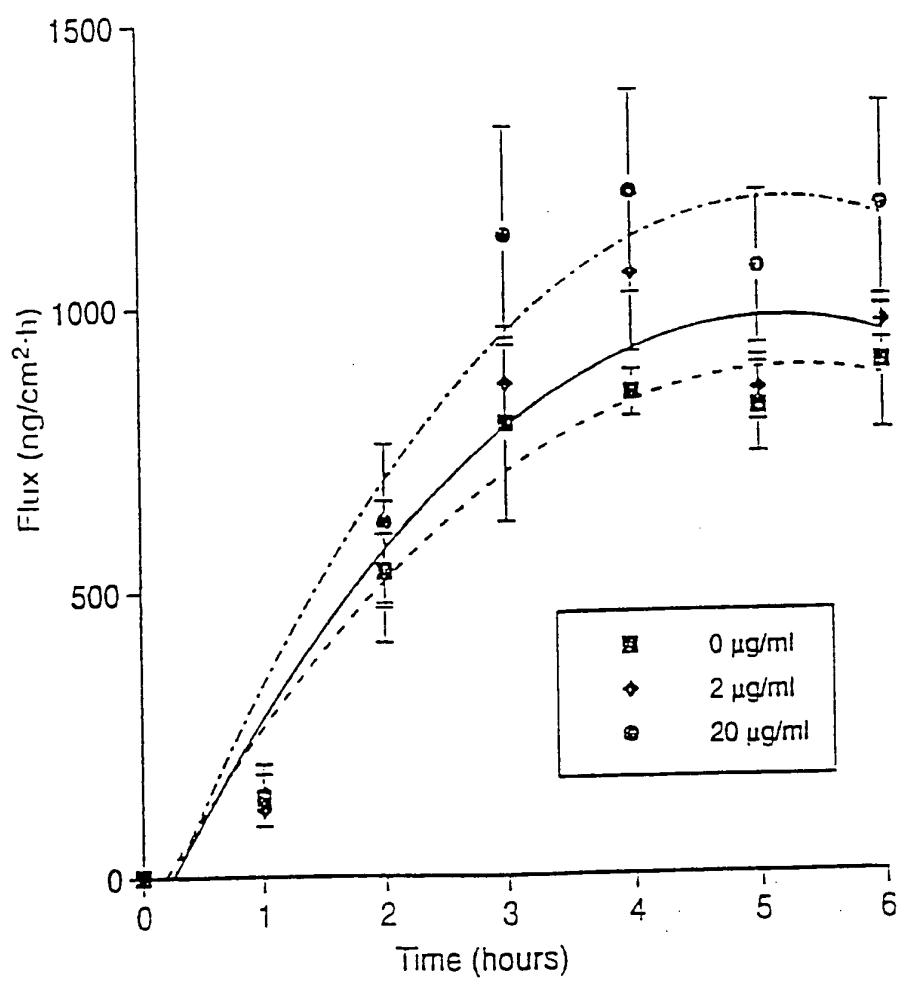
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Fig. 3



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Fig. 4



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Fig. 6

