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(73) Patenthaver: **Besins Healthcare Luxembourg SARL, 2-8 rue Julien Vesque, 2668 Luxembourg, Luxembourg**

(72) Opfinder: **Malladi, Ramana, 901 Sawyer Road, Marietta, GA Georgia 30062, USA**
Miller, Jodi, 901 Sawyer Road, Marietta, GA Georgia 30062, USA

(74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**

(54) Benævnelse: **FORBEDRET TESTOSTERON-GEL OG FREMGANGSMÅDE TIL BRUG**

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DESCRIPTION

Description

BACKGROUND OF THE INVENTION

[0001] Testosterone, the major circulating androgen in men, is synthesized from cholesterol. The approximately 500 million Leydig cells in the testes secrete more than 95% of the 6-7 mg of testosterone produced per day. Two hormones produced by the pituitary gland, luteinizing hormone ("LH") and follicle stimulating hormone ("FSH"), are required for the development and maintenance of testicular function and negatively regulate testosterone production. Circulating testosterone is metabolized to various 17-keto steroids through two different pathways. Testosterone can be metabolized to dihydrotestosterone ("DHT") by the enzyme 5α-reductase or to estradiol ("E2") by an aromatase enzyme complex.

[0002] Testosterone circulates in the blood 98% bound to protein. In men, approximately 40% of the binding is to the high-affinity sex hormone binding globulin ("SHBG"). The remaining 60% is bound weakly to albumin. Thus, a number of measurements for testosterone are available from clinical laboratories. The term "free" testosterone as used herein refers to the fraction of testosterone in the blood that is not bound to protein. The term "total testosterone" or "testosterone" as used herein means the free testosterone plus protein-bound testosterone. The term "bioavailable testosterone" as used herein refers to the non-SHBG bound testosterone and includes testosterone weakly bound to albumin.

[0003] The following table from the UCLA-Harbor Medical Center summarizes the hormone concentrations in normal adult men range: -

Table 1: Hormone Levels in Normal Men

Hormone	Normal Range
Testosterone	298 to 1043 ng/dL
Free testosterone	3.5 to 17.9 ng/dL
DHT	31 to 193 ng/dL
DHT/T Ratio	0.052 to 0.33
DHT + T	372 to 1349 ng/dL
SHBG	10.8 to 46.6 nmol/L
FSH	1.0 to 6.9 mlU/mL
LH	1.0 to 8.1 mlU/mL
E_2	17.1 to 46.1 pg/mL

[0004] There is considerable variation in the half-life of testosterone reported in the literature, ranging from 10 to 100 minutes. Researchers do agree, however, that circulating testosterone has a diurnal variation in normal young men. Maximum levels occur at approximately 6:00 to 8:00 a.m. with levels declining throughout the day. Characteristic profiles have a maximum testosterone level of 720 ng/dL and a minimum level of 430 ng/dL. The physiological significance of this diurnal cycle, if any, however, is not clear.

[0005] Male hypogonadism results from a variety of patho-physiological conditions in which testosterone concentration is diminished below the normal range. The hypogonadic condition is sometimes linked with a number of physiological changes, such as diminished interest in sex, impotence, reduced lean body mass, decreased bone density, lowered mood, and decreased energy levels.

[0006] Researchers generally classify hypogonadism into one of three types. Primary hypogonadism includes the testicular failure due to congenital or acquired anorchia, XYY Syndrome, XX males, Noonan's Syndrome, gonadal dysgenesis, Leydig cell tumors, maldescended testes, varicocele, Sertoli-Cell-Only Syndrome, cryptorchidism, bilateral torsion, vanishing testis syndrome, orchiectomy, Klinefelter's Syndrome, chemotherapy, toxic damage from alcohol or heavy metals, and general disease (renal failure, liver cirrhosis, diabetes, myotonia dystrophica). Patients with primary hypogonadism show an intact feedback mechanism in that the low serum testosterone concentrations are associated with high FSH and LH concentrations. However, because of testicular or other failures, the high LH concentrations are not effective at stimulating testosterone production.

[0007] Secondary hypogonadism involves an idiopathic gonadotropin or LH-releasing hormone deficiency. This type of hypogonadism includes Kallman's Syndrome, Prader-Labhart-Willi's Syndrome, Laurence-Moon-Biedl's Syndrome, pituitary insufficiency/adenomas, Pasqualini's Syndrome, hemochromatosis, hyperprolactinemia, or pituitary-hypothalamic injury from tumors, trauma, radiation, or obesity. Because patients with secondary hypogonadism do not demonstrate an intact feedback pathway, the lower testosterone concentrations are not associated with increased LH or FSH levels. Thus, these men have low testosterone serum levels but have gonadotropins in the normal to low range.

[0008] Hypogonadism may be age-related. Men experience a slow but continuous decline in average serum testosterone after approximately age 20 to 30 years. Researchers estimate that the decline is about 1-2% per year. Cross-sectional studies in men have found that the mean testosterone value at age 80 years is approximately 75% of that at age 30 years. Because the serum concentration of SHBG increases as men age, the fall in bioavailable and free testosterone is even greater than the fall in total testosterone. Researchers have estimated that approximately 50% of healthy men between the ages of 50 and 70 have levels of bioavailable testosterone that are below the lower normal limit. Moreover, as men age, the circadian rhythm of testosterone concentration is often muted, dampened, or completely lost. The major problem with aging appears to be within the hypothalamic-pituitary unit. For example, researchers have found that with aging, LH levels do not increase despite the low testosterone levels. Regardless of the cause, these untreated testosterone deficiencies in older men may lead to a variety of physiological changes, including sexual dysfunction, decreased libido, loss of muscle mass, decreased bone density, depressed mood, and decreased cognitive function. The net result is geriatric hypogonadism, or what is commonly referred to as "male menopause." Today, hypogonadism is the most common hormone deficiency in men, affecting 5 in every 1,000 men. At present, it is estimated that only five percent of the estimated four to five million American men of all ages with hypogonadism currently receive testosterone replacement therapy.

[0009] US2005/020552 discloses a hydroalcoholic gel composition, Androgel, comprising 1 % w/w of testosterone, 0.7% w/w of isopropyl myristate, 69 % w/w of ethanol, carbopol 940 as gelling agent and water.

SUMMARY OF THE INVENTION

[0010] The present invention relates to an improved transdermal hydroalcoholic testosterone gel formulation that provides, among other things, a desirable pharmacokinetic hormone profile, for uses as defined in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

FIG. 1 is a standardized Pareto Chart demonstrating the effect of test factors testosterone, isopropyl myristate and ethyl alcohol on response variable-viscosity.

FIG. 2 is a standardized Pareto Chart demonstrating the effect of test factors testosterone, isopropyl myristate and ethyl alcohol on percent label permeated.

FIG. 3 is a standardized Pareto Chart demonstrating the effect of test factors testosterone, isopropyl myristate and ethyl alcohol on Ratio CAR.

FIG. 4 is an Estimated Response Surface Plot illustrating estimated response (Ratio CAR) for a given combination of testosterone and isopropyl myristate for an alcohol (95 % v/v) content of 74.3 wt %.

FIG. 5 is a Contour Plot illustrating the contours of the Estimated Response Surface Plot in FIG. 4 .

FIG. 6 is a graph showing the cumulated amount testosterone released as a function of time for various testosterone formulations (F57 to F59) in comparison to reference formulation (F56).

FIG. 7 is a graph showing cumulative amounts of testosterone permeated as a function of time for formulation F57.

FIG. 8 is a graph showing the cumulative amounts of testosterone permeated as a function of time for formulation F58.

FIG. 9 is a graph showing the cumulative amounts of testosterone permeated as a function of time for formulation F59.

FIG. 10 is a graph showing mean serum concentration-time profiles for observed testosterone on Day 1.

FIG. 11 is a graph showing mean serum concentration-time profiles for observed testosterone on Day 14.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention relates to an improved testosterone gel formulation for uses as defined in the claims.

[0013] In one embodiment, the present invention is directed to a hydroalcoholic gel for use in a method for percutaneous administration of testosterone. The gel comprises testosterone (or a testosterone derivative), one or more lower alcohols, chosen from ethanol or isopropanol; isopropyl myristate as a penetration enhancing agent; a thickener; and water. Additionally, the present invention may optionally include salts, emollients, stabilizers, antimicrobials, fragrances, and propellants.

[0014] The present invention also includes kits, methods, combinations, and pharmaceutical compositions for use in treating, preventing, reversing, halting or slowing the progression of hypogonadism or other low-testosterone-associated disorders in a subject once it becomes clinically evident, or treating the symptoms associated with, or related to the hypogonadism or low-testosterone-associated disorder. The subject may already have a diagnosis of hypogonadism and/or low testosterone at the time of administration, or be at risk of developing hypogonadism and/or low testosterone. The present invention preferably is for use in the treatment of adult subjects over 18 years of age. Even more preferably the present invention is for use in the treatment of adult subjects over 21 years of age.

[0015] The term "derivative" refers to a compound that is produced from another compound of similar structure by the replacement of substitution of one atom, molecule or group by another. For example, a hydrogen atom of a compound may be substituted by alkyl, acyl, amino, etc., to produce a derivative of that compound.

[0016] As used herein, the term "lower alcohol," alone or in combination, means a straight-chain or branched-chain alcohol moiety containing one to about six carbon atoms. In one embodiment, the lower alcohol contains one to about 4 carbon atoms, and in another embodiment the lower alcohol contains two to about 3 carbon atoms.

Examples of such alcohol moieties include methanol, ethanol, ethanol USP (i.e., 95% v/v), n-propanol, isopropanol, n-butanol, isobutanol, sec-butanol, and tert-butanol.

[0017] As used herein, the term "ethanol" refers to C2H5OH. It may be used as dehydrated alcohol USP, alcohol USP, or in any common form including in combination with various amounts of water.

[0018] The composition is used in a "pharmacologically effective amount". This means that the concentration of the drug administered is such that in the composition it results in a therapeutic level of drug delivered over the term that the drug is to be used. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the flux rate of the drug from the composition, for example, testosterone, from the gel, surface area of application site, etc. For testosterone, for example, the amount of testosterone necessary can be experimentally determined based on the flux rate of testosterone through the gel, and through the skin when used with and without enhancers.

[0019] In one embodiment, the present invention is directed to a hydroalcoholic gel for use in a method for percutaneous administration of testosterone in as defined in the claims. The gel comprises one or more lower alcohols, chosen from ethanol or isopropanol; isopropyl myristate as a penetration enhancing agent; a thickener; and water. In one embodiment, the gel comprises an anionic polymer thickening agent precursor neutralized with a hydroxide releasing agent, such as, e.g., sodium hydroxide. Additionally, the present invention may optionally include salts, emollients, stabilizers, antimicrobials, fragrances, and propellants.

[0020] Included in the methods and pharmaceutical compositions of the present invention are the isomeric forms and tautomers of the described compounds and the pharmaceutically-acceptable salts thereof. Illustrative pharmaceutically acceptable salts are prepared from formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, stearic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, cyclohexylaminosulfonic, algenic, b-hydroxybutyric, galactaric and galacturonic acids.

[0021] The thickening agents (aka gelling agents) suitable for use in the present invention include neutralized anionic polymers such as polyacrylic acid. Preferred are the carbomer polyacrylic acids, especially those made and sold by Noveon Inc. of Cleveland, Ohio under the trademark Carbopol®. (See information at http://www.noveon.com). Particularly preferred are Carbopols® Ultrez 10,940,941, 954,980,981, ETD 2001, EZ-2 and EZ-3. Most preferred are Carbopol® 940 and Carbopol® 980. Other suitable anionic polymers include carboxypolymethylene and carboxymethyl cellulose. Also suitable are other known polymeric thickening agents such as Pemulen® polymeric emulsifiers, and Noveon® polycarbophils. Additional thickening agents, enhancers and adjuvants may generally be found in Remington's The Science and Practice of Pharmacy, Meade Publishing Co., United States Pharmacopeia/National Formulary.

[0022] In one embodiment, the formulation is a gel, comprised of testosterone; isopropyl myristate as a penetration enhancing agent; a thickening agent, such as a neutralized carbomer; a lower alcohol, chosen from ethanol or isopropanol; and water.

[0023] In another embodiment, the formulation contains an anionic polymer thickening agent precursor such as a carbomer which has been combined with a neutralizer in an amount sufficient to form a gel in the course of forming the composition.

[0024] In another embodiment, the formulation contains an anionic polymer thickening agent precursor such as a carbomer which has been combined with a neutralizer in an amount sufficient to form a gel with a viscosity greater than 9 Pa.s (9000 cps) as measured by a Brookfield RV DVII+ Viscometer with a spindle equal to RV6, RPM (rotations per minute) equal to 10, and the temperature maintained at 20°C.

[0025] In yet a further embodiment, the formulation contains an anionic polymer thickening agent precursor such as a carbomer which has been combined with a neutralizer selected from the group consisting of sodium hydroxide, ammonium hydroxide, potassium hydroxide, arginine, aminomethyl propanol, tetrahydroxypropyl ethylenediamine, triethanolamine ("TEA"), tromethamine, PEG-15 cocamine, diisopropanolamine, and triisopropanolamine, or combinations thereof in an amount sufficient to neutralize the anionic polymer thickening agent precursor to form a gel in the course of forming the composition. Suitable neutralizing agents and their use with selected anionic polymer thickening agent precursors are disclosed in " Neutralizing Carbopol® and Pemulen® Polymers in Aqueous and Hydroalcoholic Systems," Commercial Brochure TDS-237 (October 1998) by Noveon Inc. of Cleveland, Ohio.

[0026] In yet a further embodiment, the formulation contains an anionic polymer thickening agent precursor such as a carbomer which has been combined with a neutralizer which is an aqueous solution of sodium hydroxide such as 0.1 N sodium hydroxide, or 1.5 N sodium hydroxide, or 2.0 N sodium hydroxide or any other convenient strength aqueous solution in an amount sufficient to form a gel. In one embodiment, the composition was prepared using between about 1.0% and 10.0% 0.1 N sodium hydroxide. Accordingly, embodiments employing any percentage between about 1.0% and about 10.0% 0.1N NaOH may be used, such as, e.g., 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0% or 10.0% 0.1N NaOH.

[0027] In one embodiment the formulation is a gel and is obtained by combining the following substances in approximate percentages:

Table 2. Ingredients Combined to Yield Testosterone Formulations (%w/w)

T (Testosterone)	Alcohol (95% v/v)	Isopropyl Myristate	Carbopol 980	0.1N NaOH	Purified Water
1.20	73.5	1.00	1.0	7.00	16.3
1.40	73.5	1.00	1.0	7.00	16.1
1.60	73.5	1.00	1.0	7.00	15.9

[0028] In one embodiment, the composition comprises

[0029] The composition comprises from about 1.50 % to about 1.70 % (w/w) testosterone.

[0030] In one embodiment, the composition further comprises 0.6 % to 1.2 % (w/w) isopropyl myristate; 60 % to 80 % (w/w) alcohol selected from the group consisting of ethanol and isopropanol; a sufficient amount of a thickening agent to give the composition a viscosity in excess of about 9 Pa.s (9000 cps); and water.

[0031] In another embodiment, the composition further comprises 0.6 % to 1.2 % (w/w) isopropyl myristate; about 67 % to 74 % (w/w) alcohol selected from the group consisting of ethanol and isopropanol; a sufficient amount of a thickening agent to give the composition a viscosity in excess of about 9 Pa.s (9000 cps); and water.

[0032] In an embodiment, the viscosity of the composition of the present invention is about 13 Pa.s (13000 cps) to about 33 Pa.s (33000 cps) Accordingly, the viscosity of the composition of the present invention may be any amount between about 13 Pa.s (13000 cps) and 33 Pa.s (33000 cps) such as, e.g., 4, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 or 33 Pa.s.

[0033] The gel is rubbed or placed onto an area of skin of the subject and allowed to dry. The gel dries rapidly, i.e., within about 30 seconds to about 3 minutes after application. Illustratively, the gel is rubbed onto an area of skin, for example, on the upper outer thigh and/or hip once daily. Following application the subject washes his or her hands. Application of the gel results in an increased testosterone level having a desirable pharmacokinetic profile and is effective to treat or prevent hypogonadism and/or low testosterone, or the symptoms associated with, or related to hypogonadism and/or low testosterone in the subject. The composition is thus useful for treating a number of conditions or diseases.

[0034] In one embodiment, the present invention employs a packet having a polyethylene liner compatible with the components of a testosterone gel, as described below. The packet may hold a unit dose or multiple dose.

[0035] In another embodiment, the methods and compositions employ a composition that is dispensed from a rigid multi-dose container (for example, with a hand pump) having a larger foil packet, for example, of the composition inside the container. Such larger packets can also comprise a polyethylene liner as above. In one embodiment, the multi-dose container, comprises an airless pump that comprises a polyethylene lined foil pouch within a canister with a hand pump inserted. In one embodiment, the polyethylene lined foil pouch comprises 44 g or 88 g of product. In one embodiment, the pump is capable of dispensing a total amount of about 75 g of gel. In one embodiment, the pump is primed before use, such as, e.g., by fully depressing the pump three times and discarding the gel. In one embodiment, the pump contains enough product to allow for priming and a set number of precise doses. In one embodiment, each full pump depression delivers 1.25 g of testosterone gel. In this embodiment, a 3.75 g dose of gel would require 3 pump depressions. A 5 g dose of gel would require 4 pump depressions. A 7.5 g dose of gel would require 6 pump depressions. A 10 g dose of gel would require 8 depressions, and so on. Of course, each pump depression can deliver any amount of testosterone gel suitable for delivering the desired dose. The pouch size, amount dispensed and the delivery volume per depression are not limited to these embodiments and may be changed or adjusted to meet the needs of the patient population.

[0036] The methods and compositions of the present invention provide enhanced treatment options for treating, preventing, reversing, halting or slowing the progression of hypogonadism or another low-testosterone-associated disorder in a subject, for example, a man, as compared to those currently available.

[0037] In one embodiment, the pharmaceutical composition of the present invention is administered once, twice, or three times a day, or as many times necessary to achieve the desired therapeutic effect. In another embodiment the composition of the present invention is administered once, twice, or three times a day on alternate days. In another embodiment the composition of the present invention is administered once, twice, or three times a day on a weekly, biweekly, or monthly basis.

[0038] In one embodiment, a therapeutically effective dose is between about 1.0 g and 10.0 g, preferably between about 1.25 g and 6.25 g.

[0039] Besides being useful for human treatment, the present invention is also useful for veterinary treatment of mammals, reptiles, birds, exotic animals and farm animals, including mammals, rodents, and the like. In one embodiment, the mammal includes a primate, for example, a human, a monkey, or a lemur, a horse, a dog, a pig, or a cat. In another embodiment, the rodent includes a rat, a mouse, a squirrel or a guinea pig.

[0040] The composition is capable of releasing the steroid after applying the composition to the skin at a rate and duration that delivers in one embodiment of the present invention at least about 10 μg per day of the steroid to the blood serum of the subject.

[0041] In another embodiment of the present invention, the composition is capable of releasing the testosterone after applying the composition to the skin of a subject at a rate and duration that achieves a circulating serum concentration of testosterone greater than about 300 ng per dl serum.

[0042] In another embodiment of the present invention, the composition is capable of releasing the testosterone after applying the composition to the skin of a subject at a rate and duration that achieves a circulating serum concentration of testosterone greater than about 300 ng per dl serum during a time period beginning about 0.5 hours after administration and ending about 24 hours after administration.

[0043] In another embodiment of the present invention, the composition is capable of releasing the testosterone after applying the composition to the skin of a subject at a rate and duration that achieves a circulating serum concentration of the testosterone between about 298 ng testosterone per dl serum to about 1043 ng testosterone per dl serum.

[0044] In another embodiment of the present invention, after administration of the composition, the serum testosterone concentration is maintained between about 400 and 1050 ng testosterone per dl serum.

[0045] In yet another embodiment of the present invention, after administration of the composition, the serum testosterone concentration is maintained between about 200 and 1800 ng testosterone per dl serum.

[0046] In another embodiment of the present invention, after administration of the composition, an obtained C_{max} is between about 300 and 5000 ng/dl.

[0047] In another embodiment of the present invention, the composition is provided to a subject for daily administration in about a 1.25 g to about a 3.75 g dose, such as, e.g., about 1.25 g, or about 2.50 g, or about 3.75 g. Any other suitable dose may be also be administered.

[0048] In yet another embodiment of the present invention, the subject in need of treatment has a serum testosterone level before the first application (pretreatment) of the composition of the present invention of less than about 300 ng/dl.

[0049] In another embodiment of the present invention, where after at least about 30 days of daily administration of the composition of the present invention the serum testosterone concentration in a subject is at least about 300 ng/dl to about 1050 ng/dl, such as, for example, about 300 ng/dl to about 400 ng/dl, about 300 ng/dl to about 500 ng/dl, about 500 ng/dl to about 700 ng/dl, about 700 ng/dl to about 900 ng/dl, about 400 ng/dl to about 500 ng/dl, about 500 ng/dl to about 600 ng/dl, about 600 ng/dl to about 700 ng/dl, about 700 ng/dl to about 800 ng/dl, about 800 ng/dl to about 900 ng/dl, about 900 ng/dl to about 1000 ng/dl, about 1000 ng/dl to about 1100 ng/dl, about 400 ng/dl to about 1050 ng/dl, about 500 ng/dl to about 1050 ng/dl, about 600 ng/dl to about 1050 ng/dl, or about 700 ng/dl to about 1050 ng/dl.

[0050] In still another embodiment of the present invention, where after daily administration of the composition of the present invention the total testosterone concentration in a subject is greater than about 300 ng/dl. In one embodiment, the total serum testosterone concentration in the subject is greater than about 400 ng/dl, about 500 ng/dl, about 600 ng/dl or about 700 ng/dl. In one embodiment, the total testosterone concentration is measured after 24 hours of administration. In one embodiment, the total testosterone concentration is measured after more than 2 days of daily administration, such as, for example, after 10 days, 14 days, 20 days, or 30 days.

[0051] In another embodiment of the methods, kits, combinations, and compositions of the present invention, the composition of the present invention is administered once, twice, or three times daily to a subject for at least about 7 days. In one embodiment, the composition is administered once a day.

Example 1: Development of Improved Testosterone Gel(s)

Introduction

[0052] In order to develop a new testosterone gel formulation, a number of exploratory studies were conducted to prepare and test gel formulations containing different levels of testosterone, isopropyl myristate and ethyl alcohol. Preliminary studies have demonstrated that viscosity of the gel can be increased by slightly increasing the concentrations of gelling and neutralizing agents. A statistical program was used to generate a design to study the effect of 3 ingredients, testosterone, ethyl alcohol and isopropyl myristate on viscosity and *in vitro* permeation of testosterone from hydroalcoholic gels. *In vitro* permeation studies were conducted using Franz diffusion cells. The concentration of testosterone present in receptor samples was analyzed by HPLC technique or beta scintillation counter (for radiolabeled technique). Based on results from these studies three optimized formulations were prepared and tested for skin permeation using HPLC method. All three optimized formulations showed significant improvement in viscosity and *in vitro* skin permeation compared to currently marketed formulation (1% testosterone gel).

Objectives

[0053] The present disclosure summarizes studies conducted to develop testosterone gel formulation(s) with improved viscosity, reduced volume of application, and improved *in vitro* skin permeation compared to currently marketed formulation (1% testosterone gel), and potentially reduce the volume of gel application.

Procedure

a. Statistical Design

[0054] A statistical design was created (StatGraphics Plus 5.1) to study the effect of 3 ingredients, testosterone, ethyl alcohol and isopropyl myristate on viscosity and *in vitro* permeation of testosterone from hydroalcoholic gels. Concentration of two other ingredients, Carbopol 980 and 0.1N sodium hydroxide solution were kept constant. Following is the design summary:
Design class: Response Surface
Design name: Box-Behnken design
Number of experimental factors: 3 (all continuous)
Number of blocks: 1; Number of runs: 15 (randomized)
Error degrees of freedom: 5

Factors	Low(%)	High(%)
Testosterone	1.33	2.0
Isopropyl myristate	0.5	1.0
Alcohol (95% v/v)	72.5	76.1

[0055] The following table summarizes the ingredients of test formulations as created by the statistical design. These formulations were prepared at 1 kg size and packaged in to glass jars for analytical and skin permeation tests.

Table 3. Ingredients Combined to Yield Test Formulations and Control Formulation (%w/w)

F# (Formulation)	T (Testosterone)	Alcohol (95% v/v)	Isopropyl Myristate	Carbopol 980	0.1N NaOH	Purified Water
41	1.665	74.3	0.75	1.0	7.00	15.3
42	1.665	72.5	0.50	1.0	7.00	17.3
43	1.665	76.1	0.50	1.0	7.00	13.7
44	2.000	74.3	0.50	1.0	7.00	15.2
45	1.330	74.3	0.50	1.0	7.00	15.9
46	1.330	76.1	0.75	1.0	7.00	13.8
47	2.000	74.3	1.00	1.0	7.00	14.7
48	1.665	74.3	0.75	1.0	7.00	15.3
49	1.330	74.3	1.00	1.0	7.00	15.4
50	2.000	72.5	0.75	1.0	7.00	16.8

F# (Formulation)	T (Testosterone)	Alcohol (95% v/v)	Isopropyl Myristate	Carbopol 980	0.1N NaOH	Purified Water
51	1.665	76.1	1.00	1.0	7.00	13.2
52	2.000	76.1	0.75	1.0	7.00	13.2
53	1.665	72.5	1.00	1.0	7.00	16.8
54	1.330	72.5	0.75	1.0	7.00	17.4
55	1.665	74.3	0.75	1.0	7.00	15.3
56 (control)	1.000	72.5	0.50	0.9	4.75	20.4

b. Analytical Testing

[0056] All test formulations and control samples were analyzed for physical (appearance, pH and viscosity) and chemical (assays for testosterone, isopropyl myristate and alcohol) attributes.

c. *In vitro* Skin Permeation Studies

[0057] Permeation of testosterone was studied quantitatively with human skin placed on the Franz diffusion cell. The skin was mounted horizontally between the donor and receptor half. The surface area of the skin exposed to the formulation in the donor chamber was 0.64 cm^2, and the receptor volume was 5.0 mL. Temperature was maintained at 37°C with the help of a double water circulation jacket surrounding the lower part of the cell. The donor chamber was open on the top.

<u>Radiolabel method</u>

[0058] Test formulations were spiked with ^{14}C labeled testosterone. Spiked (radiolabeled) formulation (5-15 mg of gel containing 0.125-0.250 µCi) was applied over the surface of the epidermis gravimetrically. Periodic samples (0, 1, 2, 4, 6, 8, 10, 22 and 24 h) were taken from the receptor cell to measure the radioactivity/amount of drug permeated across the skin. In addition the amount of radiolabel/drug remaining on the skin, in the skin samples was also determined. Further details of these experiments and results are presented in Example 2.

<u>HPLC method</u>

[0059] Formulation (300 mg ± 5% which contains 3000 µg of the drug based on 1% gel) was applied over the surface of the epidermis gravimetrically. Aliquots were collected periodically (0, 1, 2, 4, 6, 8, 10, 22 and 24 h) and replaced with fresh buffer. Later aliquots were analyzed for testosterone content. Further details of these experiments and results are presented in Example 3.

d. Data Analysis

[0060] In addition to data reported in corresponding examples, data from both radiolabel and HPLC methods was analyzed further by statistical program (StatGraphics Plus 5.1). StatGraphics program was also used to predict optimum levels of different factors which could provide maximum response.

Results and Discussion

Analytical Data

[0061] All test formulations were clear and have pH between 5.68-5.82. The contents of testosterone, isopropyl myristate and alcohol were close to the target. The following table summarizes analytical test results after 1 month storage at 40°C/75%RH.

Table 4. Analytical Results for Test Formulations and Control Formulation*

F# (Formulation)	Appearance	pH	Viscosity (cps)	Assay-T (%)	Assay-Alcohol (95% v/v)	Assay-IPM (%)
41	Clear gel	5.79	23567	1.69	73.8	0.67
42	Clear gel	5.76	26900	1.66	71.8	0.45
43	Clear gel	5.82	23000	1.69	75.5	0.44
44	Clear gel	5.75	26700	2.00	74.1	0.44
45	Clear gel	5.76	25467	1.32	73.7	0.44
46	Clear gel	5.69	30233	1.40	74.5	0.74
47	Clear gel	5.78	24733	2.02	73.3	0.92
48	Clear gel	5.79	24767	1.66	74.3	0.68
49	Clear gel	5.76	24300	1.33	73.8	0.93
50	Clear gel	5.72	26133	2.02	71.7	0.68
51	Clear gel	5.82	20700	1.75	75.4	0.93
52	Clear gel	5.83	19733	2.00	75.5	0.68
53	Clear gel	5.72	26033	1.68	71.7	0.95
54	Clear gel	5.69	28267	1.34	71.9	0.69
55	Clear gel	5.77	23233	1.67	74.0	0.68
56 (control)	Clear gel	5.59	22033	1.01	72.4	0.44
*test results after 1 month storage at 40°C/75%RH T = testosterone, IPM = isopropyl myristate						

[0062] As one of the objectives for this study is to increase the viscosity of the gel, statistical analysis was performed to assess the effect of test factors testosterone, isopropyl myristate and ethyl alcohol on response variable-viscosity. The following is an analysis summary:

Table 5. ANOVA for Viscosity

Analysis of Variance for Viscosity					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:T	9.41613E6	1	9.41613E6	1.58	0.2488
B:IPM	3.16945E6	1	3.16945E6	0.53	0.4892
C:EtOH	1.94337E7	1	1.94337E7	3.27	0.1137
AA	1.43962E6	1	1.43962E6	0.24	0.6379
AB	3.37413E6	1	3.37413E6	0.57	0.4760
AC	3.50601E7	1	3.50601E7	5.89	0.0456
BB	1.00605E6	1	1.00605E6	0.17	0.6933
BC	1.95303E6	1	1.95303E6	0.33	0.5847

Analysis of Variance for Viscosity					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
CC	286812.0	1	286812.0	0.05	0.8325
Total *error*	4.16608E7	7	5.95155E6		
Total (corr.)	1.17733E8	16			

[0063] Referring now to FIG. 1, where in the chart A represents testosterone, B represents isopropyl myristate, C represents EtOH, and joint letters represents a combination of factors, it is clear that the combination of alcohol and testosterone (i.e., AC) has a significant negative effect on viscosity. This observation is consistent with earlier studies with alcohol; to maximize the viscosity the level of alcohol should be at the lowest level possible.

Permeation Data: Radiolabel Technique

[0064] Skin samples from two donors were used in this study. To minimize variability between skins, permeation data, % label permeated (%LP), from test formulations was normalized to control formulation tested with corresponding donor skin. Additional statistical analysis was performed on the ratio of %LP (test/control, Ratio %LP) to obtain trends and optimal concentrations of testosterone, isopropyl myristate and ethyl alcohol. Following is the analysis summary:

Table 6. Radiolabel Data Table used for Statistical Analysis *

F# (Formulation)	Donor	T	IPM	Alcohol (95% v/v)	%LP	Ratio %LP
41	8127	1.665	0.75	74.3	4.86	0.83
42	8127	1.665	0.50	72.5	3.45	0.59
43	8127	1.665	0.50	76.1	2.7	0.46
44	8127	2.000	0.50	74.3	3.8	0.65
45	8300	1.330	0.50	74.3	3.51	0.46
46	8300	1.330	0.75	76.1	6.37	0.84
47	8300	2.000	1.00	74.3	3.57	0.47
48	8300	1.665	0.75	74.3	5.38	0.71
49	8300	1.330	1.00	74.3	9.47	1.25
50	8300	2.000	0.75	72.5	5.69	0.75
51	8300	1.665	1.00	76.1	6.73	0.89
52	8300	2.000	0.75	76.1	2.22	0.29
53	8300	1.665	1.00	72.5	4.61	0.61
54	8300	1.330	0.75	72.5	4.33	0.57
55	8300	1.665	0.75	74.3	4.33	0.57
56 (control)	8127	1.000	0.50	72.5	5.87	1.00
56 (control)	8300	1.000	0.50	72.5	7.56	1.00
*%LP data from Example 2. Ratio %LP calculated from %LP values. T=testosterone, IPM=isopropyl myristate, LP= label permeated						

Table 7. ANOVA for Ratio of % Label Permeated (Ratio %LP)

Analysis of Variance for Viscosity					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:T	0.213314	1	0.213314	7.19	0.0315
B:IPM	0.107294	1	0.107294	3.62	0.0990

Analysis of Variance for Viscosity					
Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
C:EtOH	0.00430679	1	0.00430679	0.15	0.7145
AA	0.119589	1	0.119589	4.03	0.0847
AB	0.131132	1	0.131132	4.42	0.0736
AC	0.0554353	1	0.0554353	1.87	0.2140
BB	0.00724917	1	0.00724917	0.24	0.6363
BC	0.0800686	1	0.0800686	2.70	0.1445
CC	0.00941549	1	0.00941549	0.32	0.5908
Total error	0.207716	7	0.0296737		
Total (corr.)	0.947084	16			

[0065] The Pareto chart in FIG. 2 shows that the level of testosterone has significant negative effect, and the level of isopropyl myristate has a positive (not statistically significant) effect on % label permeated. Above analysis suggests that the maximum level of testosterone in gel formulation should be less than highest level studied. This analysis also suggests that the maximum level of isopropyl myristate in gel formulation should be close to the highest level studied.

Permeation Data: HPLC Technique

[0066] Same skin samples (two donors) used in radiolabel study were used in this study. To minimize variability between skins, permeation data (flux or cumulative amount released, CAR) from test formulations was normalized to control formulation tested with corresponding donor skin. Additional statistical analysis was performed on the ratio of CAR (test/control, Ratio CAR) to obtain trends and optimal concentrations of testosterone, isopropyl myristate and ethyl alcohol. Following is the analysis summary:

Table 8. HPLC Data Table used for Statistical Analysis *

F# (Formulation)	Donor	T	IPM	Alcohol (95% v/v)	CAR	Ratio CAR
41	8127	1.665	0.75	74.3	189.81	1.02
42	8127	1.665	0.50	72.5	188.21	1.01
43	8127	1.665	0.50	76.1	50.11	0.27
44	8127	2.000	0.50	74.3	151.95	0.82
45	8300	1.330	0.50	74.3	74.13	0.69
46	8300	1.330	0.75	76.1	117.87	1.09
47	8300	2.000	1.00	74.3	114.61	1.06
48	8300	1.665	0.75	74.3	208.82	1.94
49	8127	1.330	1.00	74.3	298.76	1.61
50	8127	2.000	0.75	72.5	94.71	0.51
51	8127	1.665	1.00	76.1	254.57	1.37
52	8127	2.000	0.75	76.1	113.93	0.61
53	8127	1.665	1.00	72.5	326.46	1.76
54	8127	1.330	0.75	72.5	200.45	1.08
55	8127	1.665	0.75	74.3	240.61	1.30
56 (control)	8127	1.000	0.50	72.5	185.71	1.00
56 (control)	8300	1.000	0.50	72.5	107.79	1.00

* T=testosterone, IPM=isopropyl myristate, CAR= cumulative amount released

Table 9. ANOVA for Ratio of Cumulative Amount Released (Ratio CAR)

Analysis of Variance for Viscosity					
A:T	0.452519	1	0.452519	3.27	0.1135
B:IPM	1.01713	1	1.01713	7.35	0.0302
C:EtOH	0.18431	1	0.18431	1.33	0.2864
AA	0.164241	1	0.164241	1.19	0.3120
AB	0.0291287	1	0.0291287	0.21	0.6603
AC	0.0600202	1	0.0600202	0.43	0.5312
BB	0.000234383	1	0.000234383	0.00	0.9683
BC	0.0737582	1	0.0737582	0.53	0.4891
CC	0.200542	1	0.200542	1.45	0.2678
Total error	0.968701	7	0.138386		
Total (corr.)	3.03859	16			

[0067] The results in Table 8 were subjected to a regression analysis and generated the following algorithm:

$$\text{Ratio CAR} = 5.1239 - 0.4403*T + 1.5781*IPM - 0.0607*EtOH$$

where T is an amount of testosterone % (w/w), IPM is an amount of isopropyl myristate % (w/w), and EtOH is an amount (w/w) of alcohol 95 % v/v.

[0068] In one embodiment of the invention, values of T, IPM and EtOH are selected from within the ranges given below such that the above algorithm gives a Ratio CAR value greater than 1, preferably greater than 1.1, or most preferably greater than 2. The ranges are: between 1.0 and 2.0 % (w/w) testosterone, preferably between 1.15 and 1.8 % (w/w) testosterone; between 0.2 % and 2.0 % (w/w) isopropyl myristate, preferably between 0.6 and 1.2 % (w/w) isopropyl myristate; and between about 60.0 % and 80 % (w/w) alcohol 95% v/v, preferably between about 72.5 % and 76.1 % (w/w) alcohol 95% v/v.

[0069] Referring now to the Pareto chart in FIG. 3, statistical analysis clearly shows that the level of isopropyl myristate has significant positive effect and the level of testosterone has negative (not statistically significant) effect on Ratio CAR. FIG. 3 suggests that the maximum level of testosterone in gel formulation should be less than highest level studied. This analysis also suggests that the maximum level of isopropyl myristate in gel formulation should be close to the highest level studied. Permeation results from HPLC method are qualitatively consistent with results from radiolabel method.

<u>Response Optimization</u>

[0070] Permeation results from HPLC method are qualitatively similar to those from radiolabel method. For convenience, data from HPLC study was used to predict (statistical optimization) optimum levels of testosterone, isopropyl myristate and alcohol for a given response. The statistical program produced the following combination of factor levels which maximizes the ratio of cumulative amount released (Ratio CAR).

Table 10. Optimized Factor Levels for Cumulative Amount Released (Ratio CAR)

Goal: maximize Ratio CAR		Optimum Value = 1.81748	
Factor	Low	High	Optimum
T	1.0	2.0	1.28995
IPM	0.5	1.0	1.0

Goal: maximize Ratio CAR		Optimum Value = 1.81748	
Factor	Low	High	Optimum
EtOH	72.5	76.1	73.7366

[0071] FIGS. 4 and 5 illustrate estimated response (Ratio CAR) for a given combination of testosterone and isopropyl myristate at an alcohol 95% v/v level of 74.3 % (w/w).

a. Formulation Selection

[0072] Based on response surface plots and predicted optimum factor levels, the following 3 formulations were selected for further permeation studies. Again for convenience these 3 formulations were tested by HPLC method only.

Table 11. Ingredients Combined to Yield Selected Formulations and Control Formulation (%w/w)

F# (Formulation)	T (Testosterone)	Alcohol (95% v/v)	Isopropyl Myristate	Carbopol 980	0.1N NaOH	Purified Water
57	1.20	73.5	1.00	1.0	7.00	16.3
58	1.40	73.5	1.00	1.0	7.00	16.1
59	1.60	73.5	1.00	1.0	7.00	15.9
56 (control)	1.00	72.5	0.50	0.9	4.75	20.4

[0073] The following table summarizes initial (after preparation) analytical test results for selected formulations.

Table 12. Analytical Test Results for Selected Formulations and Control Formulation

F# (Formulation)	Appearance	pH	Viscosity (cps)	Assay-T (%)	Assay-Alcohol (95% v/v)	Assay-IPM (%)
57	Clear gel	5.66	24500	1.21	73.1	0.94
58	Clear gel	5.71	25533	1.42	72.7	0.94
59	Clear gel	5.68	24800	1.61	73.3	0.94
56 (control)	Clear gel	5.57	20267	1.02	71.8	0.43

[0074] The following table and FIG. 6 summarize permeation data from 3 selected formulations (data points for FIG. 6 and the table were obtained from Example 4, Tables 17-20).

Table 13. HPLC Data Table used for Statistical Analysis*

Time (hr)	F56 (1% gel)		F57 (1.2% gel)		F58 (1.4% gel)		F59 (1.6% gel)	
	CAR	SD	CAR	SD	CAR	SD	CAR	SD
0	0	0	0.0	0	0.0	0.00	0.0	0
1	0	0	0.5	0.7	0.0	0.00	0.0	0
2	0	0	1.3	0.5	1.3	1.2	0.5	0.3
4	0.40	0.1	4.3	1.3	4.0	1.0	2.9	1.3
6	1.52	0.3	14.2	4.5	11.0	2.7	13.8	4.6
8	6.96	1.5	38.0	11.5	35.9	11.0	43.5	10.8
10	18.35	3.4	57.0	17.9	65.4	14.3	64.5	10.4
22	48.20	12.5	119.2	51.0	129.1	11.8	137.5	30.6
24	50.76	12.3	129.7	50.4	137.0	13.9	156.3	36.4

*Data points obtained from Example 4, tables 17-20.

[0075] All 3 selected formulations showed significantly improved permeation (2-3x cumulative amount released) than control. These results further support the observations from initial screening of formulations and formed the basis for selection of final formulations.

Conclusions

[0076] Statistical program was used to design the experiments based on 3 key factors i.e., testosterone, isopropyl myristate and ethyl alcohol. The program was also used to analyze the analytical and *in vitro* skin permeation data, and identify trends and optimum levels of each of the factors to maximize response (permeation).

[0077] Three selected testosterone gel formulations have higher viscosity (~ 4 Pa.s (4000 cps)) than control formulation.

[0078] Significantly improved in-vitro permeation of testosterone (2-3 times than control) through the dermatomed human skin was observed with the three selected testosterone gel formulations.

Example 2: *In vitro* Percutaneous Absorption of Experimental Testosterone Gel Formulations through Human Skin by Radiolabel Method

Materials

[0079] Formulations were prepared and supplied by Solvay Pharmaceuticals. Testosterone (^{14}C) was procured from American Radiolabeled Chemicals Inc, (St Louis, MO). All other chemicals and reagents were procured from approved vendors and were of highest quality and purity available.

Methods

Description of transdermal diffusion cell apparatus

[0080] The transdermal diffusion cell apparatus used in this study (PermeGear, Bethlehem, PA) holds up to 9 diffusion cells in series and the receptor fluid is stirred by the magnetic bead at 600 rpm. Percutaneous absorption *in vitro* was studied quantitatively with human skin placed in the Franz diffusion cell. The skin was mounted horizontally between the donor and receptor halves of the diffusion cell. The surface area of the skin exposed to the formulation in the donor chamber was 0.64 cm^2, and the receptor cell volume was 5.0 ml.

[0081] The receptor compartment was filled with phosphate buffered saline pH 7.4 (PBS) and propylene glycol (1:1) and gentamicin sulphate (50µg/ml). A double water circulation jacket (37°C) surrounds the receptor cell in order to have the skin temperature maintained at physiologic level. The donor chamber was open towards the external environment, thus exposing the surface of the skin to the surrounding air of the laboratory. The relative humidity (RH) of the experimental area (around the diffusion cell setup) was monitored for every experiment and this was found to be in the range of 35 to 45% for all the experiments.

Skin permeation study

[0082] Human skin (thigh region) dermatomed to 0.3 mm thickness was obtained from a tissue bank (US Tissue and Cell, Salt Lake City, Utah) from cadavers. The skin was collected within 8 h of donor death and frozen in 10 % w/v glycerol in normal saline. The skin was stored at -80°C until use. Skin from two different donors was used in the experiments. Each experiment was carried out with each formulation for at least 6 times using the skin from one donor. The skin permeation data of formulations was compared with that of permeation of 1% marketed gel (in 6 replicates) tested on the skin of same donor as test formulations and all data were normalized to the reference (marketed) formulation.

[0083] Radiolabeled Testosterone (^{14}C, specific activity 50-60mCi/mmol) was used for this purpose. This is supplied by American Radiolabeled Chemicals and is 99.5% pure as ascertained by HPLC.

[0084] Radioactive gels were prepared in order to apply 0.125 to 0.250 μCi in a minimum amount of the gel that spreads 0.64 cm^2 of the diffusion area of Franz cell (0.64 cm^2). The minimum quantity was at least 5.0 to 15.0 mg. An appropriate amount of radioactive testosterone (12.5 µCi per 125 µl of ethanol) was evaporated in a round bottom flask until the solvent is completely evaporated to dryness. To this flask, 500 mg of cold gel formulation was added and vortexed for 5 minutes and allowed to equilibrate over night (12 to 16 h). This gel was further vortexed for 30 minutes to obtain homogenous gels. Homogeneity of the formulation's radioactivity was determined by the counting level of 9 exactly weighted (~ 5 mg) samples (standards).

[0085] The frozen skin was thawed to room temperature by keeping the skin at ambient temperature for about 30 to 45 minutes. This was then rinsed with water to remove glycerol. The skin was then put in PBS pH 7.4 and gently agitated in a shaker (100 rpm) for 20 min to remove traces of glycerol. The washed skin was mounted on the cells approximately 30 minutes before the application of the formulations. The formulation (5 to 15 mg) was applied over the surface of the epidermis gravimetrically using a syringe (for each determination sufficient gel was dispensed to cover the test surface and the weight of the gel dispensed was determined). Periodic samples were taken from the receptor cell to measure the amount of drug transporting across the skin (1, 2, 4, 6, 8, 10, 22 and 24 h).

Washing Procedures:

[0086] At the end of the test (24 hours), the residual drug remaining at the surface of the skin was removed by washing the surface with 200 μl of different solvents according to the following protocol:

1st wash : Cetavlon™ alcoholic (10/90 v/v)

2nd wash: water

3rd wash: Cetavlon™ alcoholic (10/90 v/v)

4th wash: water

5th wash: water.

[0087] The application area was then wiped with a cotton wool stick (Q-tip). The washings, cotton stick and the donor cell were collected in 20 ml of ethanol and allowed to extract all radioactivity in to ethanol. The exposed area was collected by a biopsy punch. To account for lateral diffusion, lateral portions of skin were collected and counted for radioactivity to account for Mass balance for the experiments.

[0088] The skin of the active diffusion area as well as the lateral skin were minced into pieces with a pair of sharp point dissecting scissors (Sigma) and digested for extraction of radioactivity, with 3 ml of Soluene 350™ (PACKARD) for overnight.

[0089] The radioactivity contained in the samples obtained as previously described, was measured in the totality or in weighed aliquots using a scintillating liquid beta counter equipped with dedicated software.

[0090] The evaluation was performed for the standards (0.5 ml/5 ml picofluor40™), for the receptor fluid (1.0 ml/10 ml Picofluor 40™) and for an aliquot exactly weighed of the ethanolic solution containing the washing solvents (0.5 ml/5 ml Picofluor 40™).

[0091] For the epidermis and dermis, after digestion, 15 ml of Hionic Fluor 30™ (PACKARD) were added. The background of the count is automatically deducted from the counting rate of each sample in counts per minute (dpm).

Data analysis:

[0092] The results were expressed in quantities or in percentages of applied testosterone, found in the different compartments. Applied quantities of testosterone were determined from the counting levels of diluted standards. Each result represents the mean value of 6 experimental determinations and is associated with its standard error of mean.

1. 1. The quantity of testosterone and the % of the dose absorbed in the receptor fluid for each time were calculated as follows:
   $\% = (Qt/Qi) \times 100$
   where Qt represents absorbed amount at time t, and Qi, applied quantity at time 0,
2. 2. The total quantity and corresponding % of the dose absorbed as a function of the time (cumulated values),
3. 3. The mean flux of testosterone permeated was calculated from the slope of the linear portion of the Q versus time plot and expressed as µCi/cm2/h
4. 4. The quantity and % of the administered dose, which was found in the skin and in the washing solvents.

[0093] The validity of the test was checked by balancing the radioactivity which is found in the different samples (this summarization should be comprised, for each test, between 90% and 110% of the applied dose).

Results and Conclusion

[0094] Table 14 shows that formulations F45, F47, F52, F53, F54 and F55 permeate significantly lower amount of testosterone than F56 ($P < 0.001$). For formulations F41, 42, 43, 44, 46, 48, 50, 51 the permeated amounts appear to be lower than F56. However, the difference between these formulations and F56 were not statistically significant ($P > 0.05$). However F49 permeated higher than F56 but the difference between these two formulations were not statistically significant ($P>0.05$). The mass balance data indicate variable levels of skin retention of testosterone. Furthermore, this data also demonstrates that the total mass balance is in between 90 to 110% of the initial quantity of ^{14}C Testosterone applied (Table 14).

[0095] The flux of formulations F41, F42, 46, F49 and F50, albeit appearing to be higher than F56, the differences were not statistically significant ($P > 0.05$). The flux of the all other formulations, were although appearing to be lower than control, the differences among the formulations versus F56 were not statistically significant except for

F45 (P > 0.05).

[0096] From this study, it is clear that the rate and extent of permeation of testosterone through human skin of all the test formulations were lower (or similar in some cases) than the reference formulation and none of the test formulation demonstrated significantly higher permeation than the reference formulation (F56).

[0097] Constraints for Flux studies: The studies conducted here were based on a finite dose kinetics where the rate limiting step is amount of gel used. Due to our using a finite dose, a non-linear permeation profile was obtained for most of the formulations which made us difficult to calculate the steady state flux. Nevertheless, a 2-10 h time points were used to calculate the flux values which is an assumption of a linear progression of flux but in actuality, the steady state was not achieved in these experiments. Hence the AUC values are a better representation to compare the formulations than flux values.

Table 14: Mass Balance Studies

DONOR 8127										
	F41		**F42**		**F43**		**F44**		**F56**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% IN WASH	72.84	19.88	82.63	12.27	95.46	8.71	92.22	3.68	92.01	13.45
% LATERAL SK.	1.15	2.14	0.27	0.18	0.31	0.41	0.36	0.29	0.59	0.40
% CTRL SKIN	10.57	4.50	6.38	5.27	4.18	1.14	5.80	2.28	3.52	2.39
% PERMEATED	4.86	1.53	3.45	0.43	2.70	0.70	3.80	2.05	5.87	5.05
% TOTAL	89.42		92.73		102.65		102.18		101.99	

DONOR 8300

[0098]

	F45		**F46**		**F47**		**F48**		**F56**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% IN WASH	95.41	5.29	86.91	3.16	87.98	13.59	79.68	7.41	86.08	3.67
% LATERAL SK.	0.24	0.14	0.42	0.17	0.14	0.08	0.25	0.10	0.21	0.09
% CTRL SKIN	4.09	2.05	4.67	1.84	2.29	0.84	4.88	2.76	4.07	1.59
% PERMEATED	3.51	1.65	6.37	3.98	3.57	1.25	5.38	3.81	7.56	1.78
% TOTAL	103.25		98.37		93.98		90.19		97.92	

DONOR 8300

[0099]

	F49		**F50**		**F51**		**F52**		**F56**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% IN WASH	79.64	5.61	88.34	2.30	91.04	8.28	95.51	4.07	86.08	3.67
% LATERAL SK.	0.41	0.24	0.29	0.25	0.54	0.59	0.23	0.21	0.21	0.09
% CTRL SKIN	8.29	2.69	5.70	2.12	6.58	1.92	2.67	0.84	4.07	1.59

	F49		F50		F51		F52		F56	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% PERMEATED	9.47	2.14	5.69	1.72	6.73	2.28	2.22	0.40	7.56	1.78
% TOTAL	97.82		100.01		104.89		100.64		97.92	

DONOR 8300

[0100]

	F53		F54		F55		F56	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% IN WASH	85.33	4.58	87.39	4.37	90.02	2.64	86.08	3.67
% LATERAL SK.	0.20	0.08	0.35	0.33	0.09	0.05	0.21	0.09
% CTRL SKIN	9.14	2.93	5.88	3.69	4.37	3.29	4.07	1.59
% PERMEATED	4.61	0.62	4.33	0.68	4.33	0.68	7.56	1.78
% TOTAL	99.28		97.95		98.81		97.92	

Example 3: *In vitro* Percutaneous Absorption of Experimental Testosterone Gel Formulations through Human Skin by HPLC Method

Methods

[0101] Human Skin: Human frozen skin was supplied by U.S. Tissue and Cell (Cincinnati, OH). Skin was shipped over dry ice and once received, it was stored at -80°C until use. The average thickness of the dermatomed skin was 540 μm. Each experiment was carried out in replicates of six (n=6) using the same donor for any given formulation. Also, each donor was tested for permeation of 1% marketed gel (F56) in triplicate and all data was normalized to this measurement.

[0102] Formulations: Formulations were prepared and supplied by Solvay Pharmaceuticals. Formulations were blinded except for control/marketed product formula (F56).

[0103] Transport Studies: Percutaneous absorption *in vitro* was studied quantitatively with human skin placed on the Franz diffusion cell. The skin was mounted horizontally between the donor and receptor half. The surface area of the skin exposed to the formulation in the donor chamber was 0.64 cm^2, and the receptor volume was 5.0 mL. Temperature was maintained at 37°C with the help of a double water circulation jacket surrounding the lower part of the cell. This enabled the skin temperature to be maintained at physiological level. The donor chamber was open on the top.

[0104] The receptor compartment was filled with receptor fluid consisting of phosphate buffered saline pH 7.4 (PBS) and propylene glycol (1:1). The skin was mounted on the cells approximately 30 minutes before the application of the formulations. Formulation (300 mg ± 5% which contains 3000 µg of the drug based on 1% gel) was applied over the surface of the epidermis gravimetrically. Samples of 0.3 ml were collected periodically (0,1, 2, 4, 6, 8, 10, 22 and 24 h) and replaced with fresh buffer.

[0105] Assay: Samples were analyzed for testosterone content using HPLC assay. The conditions/details were as follows:

Mobile phase	: Acetonitrile : Water (50 : 50)
Column	: C18, 3μ, 150mm Phenomenex (Nucleosil)
Injection volume	: 30 μl
Flow rate	: 1 ml/min
UV detection	: 239 nm

Results

[0106] Referring to Table 15, the results were expressed as cumulative amounts of testosterone permeated as a function of time for the different formulations. The table shows the cumulative amount permeated relative to control/marketed product formula using the same human skin donor. The mean flux of testosterone permeated was calculated from the slope of the linear portion of the CAR (Cumulative amount released) versus time plot and expressed as µg/cm^2/h. The results were expressed as a ratio of flux of test formulation and Control formulation (test/control). The cumulative amount of drug permeated through the skin per sq. cm area was also compared with that of marketed formulation and expressed as a ratio (test/control). Therefore, each formulation was compared to the marketed product formula for its cumulative permeation and flux value and the results are compiled in Table 15. The comparison of each formulation to the marketed product formula was assessed for statistical significance using ANOVA. Mean differences with $p < 0.05$ were considered to be statistically significant. Raw data for each formulation relative to marketed product formula shows the Ratio Flux and Ratio CAR as well as the statistical conclusions.

Conclusions

[0107] Permeation of testosterone through the dermatomed human skin was observed with all the formulations and permeation ranged from 1 to 7% with various formulations.

[0108] Referring again to Table 15, it is shown that improved permeation relative to marketed product formulation as determined by a comparison of cumulative amount of drug permeated after 24 hrs and/or flux at statistically significant levels was observed for Formulations F 48, F 49 and F 53.

Relative Standard Deviation ((SD/Mean)* 100)	Ratio Flux	Ratio CAR	*Statistically better than marketed formulation?*	
			Ratio Flux	Ratio CAR
11.87	0.65	1.02	No	No
55.52	0.54	1.01	No	No
23.91	0.17	0.27	No	No
22.05	0.53	0.82	No	No
29.00	0.47	0.69	No	No
22.60	0.88	1.09	No	No
22.57	0.86	1.06	No	No
39.5	1.62	1.94	Yes	Yes
18.70	1.01	1.61	No	Yes
28.76	0.34	0.51	No	No
29.68	1.06	1.37	No	No
40.08	0.41	0.61	No	No
28.49	1.19	1.76	No	Yes
37.80	0.73	1.08	No	No
27.58	0.83	1.30	No	No
23.15	NA	NA	NA	NA
7.00	NA	NA	NA	NA

Table 15:

Formulation	Donor	Flux (µg/cm2/h)	Cum Amt Rel (CAR) (µg)
F 41(n=4)	8127	13.03	189.81
F 42(n=5)	8127	10.944	188.21
F 43(n=6)	8127	3.44	50.11
F 44(n=6)	8127	10.57	151.95
F 45(n=6)	8300	5.95	74.13
F 46(n=6)	8300	11.16	117.87
F 47(n=6)	8300	10.85	114.61
F 48(n=6)	8300	20.53	208.82
F 49(n=6)	8127	20.34	298.76
F 50(n=6)	8127	6.78	94.71
F 51(n=6)	8127	21.37	254.57
F 52(n=4)	8127	8.18	113.93
F 53(n=6)	8127	24.04	326.46
F 54(n=6)	8127	14.75	200.45
F 55(n=4)	8127	16.67	240.61
F 56(n=3)	8127	20.13	185.71
F 56(n=3)	8300	12.69	107.79

Example 4: *In vitro* Percutaneous Absorption of Three Testosterone Gel Formulations through Human Skin by HPLC Method

Methods

[0109] Human Skin: Human frozen skin was supplied by U.S. Tissue and Cell (Cincinnati, OH). Skin was shipped over dry ice and once received; it was stored at - 80°C until use. The average thickness of the dermatomed skin was about 700 µm. Each experiment was carried out in replicates of six (n=6) using the same donor for any given formulation. Permeation of marketed formula testosterone gel (1%, F56) was also carried out in replicates of six (n=6) and all data was normalized to this measurement.

[0110] Formulations: Formulations were prepared and supplied by Solvay Pharmaceuticals. Formulations were blinded except for control/marketed product formula (F56).

[0111] Transport Studies: Percutaneous absorption *in vitro* was studied quantitatively with human skin placed on the Franz diffusion cell. The skin was mounted horizontally between the donor and receptor half. The surface area of the skin exposed to the formulation in the donor chamber was 0.64 cm^2, and the receptor volume was 5.0 mL. Temperature was maintained at 37°C with the help of a double water circulation jacket surrounding the lower part of the cell. This enabled the skin temperature to be maintained at physiological level. The donor chamber was open on the top.

[0112] The receptor compartment was filled with receptor fluid consisting of phosphate buffered saline pH 7.4 (PBS) and propylene glycol (1:1). The skin was mounted on the cells approximately 30 minutes before the application of the formulations. Formulation (300 mg ± 5% which contains 3000 µg of the drug based on 1% gel) was applied over the surface of the epidermis gravimetrically. Samples of 0.3 ml were collected periodically (0, 1, 2, 4, 6, 8, 10, 22 and 24 h) and replaced with fresh buffer.

[0113] Assay: Samples were analyzed for testosterone content using HPLC assay. The conditions/details were as follows:

Mobile phase	: Acetonitrile : Water (50 : 50)

Column	: C18, 3μ, 150mm Phenomenex (Nucleosil)
Injection volume	: 30 μl
Flow rate	: 1 ml/min
UV detection	: 239 nm

Results

[0114] Referring to FIGS. 7-9 and Tables 17-20, the results were expressed as cumulative amounts of testosterone permeated as a function of time for the different formulations. Each table shows the cumulative amount permeated relative to control/marketed product formula using the same human skin donor. The mean flux of testosterone permeated was calculated from the slope of the linear portion of the CAR (Cumulative amount released) versus time plot and expressed as μg/cm^2/h. The result was expressed as a ratio of flux of test formulation and Control formulation (test/control). The cumulative amount of drug permeated through the skin per sq. cm area was also compared with that of marketed formulation and expressed as a ratio (test/control). Therefore, each formulation was compared to the marketed product formula for its cumulative permeation and flux value and the results are compiled in Table 16. The comparison of each formulation to the marketed product formula was assessed for statistical significance using ANOVA. Mean differences with $p < 0.05$ were considered to be statistically significant.

Conclusions

[0115] Permeation of testosterone through the dermatomed human skin was observed with the three formulations and permeation was about 3% (CAR)

[0116] Improved permeation relative to marketed product formulation as determined by a comparison of cumulative amount of drug permeated after 24 hrs and/or flux at statistically significant levels was observed for all test formulations F57, F58 and F59.

[0117] Accordingly, utilizing the teachings of the present disclosure, a hydroalcoholic gel comprising testosterone, isopropyl myristate, ethanol, water and a sufficient amount of a thickening agent to give the gel a viscosity in excess of about 9 Pa.s (9000 cps) can be prepared such that when is applied to human skin mounted in a Frantz cell in an amount of about 300 mg, after 24 hours the flux ratio is in excess of 1, or preferably in excess of 1.5 where the flux ratio is the ratio of flux of testosterone expressed in amount per unit area and per unit time which permeates the skin when the gel is so tested to the flux of testosterone which permeates the skin when a gel of similar viscosity comprising 1 wt % testosterone, 0.5 wt % isopropyl myristate and 72.5 wt % alcohol 95 % v/v is so tested. The hydroalcoholic gel has between 1.15 and 1.8% (w/w) testosterone; between 0.6 and 1.2 % (w/w) isopropyl myristate, and between about 72.0 and 78.0 % (w/w) alcohol 95% v/v.

Table 16: Summary of Testosterone Skin Permeation Data at 24 hours from Test Formulations (FS7, F58 and F59) and Control (F56)

Formulation	Donor	Flux (μg/cm2/h)	Cumulative Amount Rel (CAR, μg)	Relative Standard Deviation ((SD/Mean)* 100)	Ratio Flux	Ratio CAR	Statistically better than marketed formulation?	
							Ratio Flux	Ratio CAR
Control-F 56 (n=6)	8126	4.20	50.76	26.08	-	-	-	-
F 57 (n=6)	8126	10.69	129.66	41.77	2.55	2.55	Yes	Yes

Formulation	Donor	Flux (µg/cm2/h)	Cumulative Amount Rel (CAR, µg)	Relative Standard Deviation ((SD/Mean)* 100)	Ratio Flux	Ratio CAR	Statistically better than marketed formulation?	
							Ratio Flux	Ratio CAR
F 58 (n=6)	8126	8.70	136.98	10.88	2.07	2.70	Yes	Yes
F 59 (n=6)	8126	12.67	156.32	15.25	3.02	3.08	Yes	Yes

Table 17: Testosterone Skin Permeation Data from Control Formulation (F56)

Time(hrs)	Control (area counts)	Conc(µg/ml)	Cone X Dil	Conc*0.3	Total (5ml)	Cum. (µg)	Cum-Sqcm	SD	Flux (mcg/cm2/hr)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4.00	4137.83	0.05	0.05	0.02	0.25	0.25	0.40	0.10	0.20
6.00	15522.67	0.19	0.19	0.06	0.96	0.97	1.52	0.30	0.56
8.00	71183.00	0.88	0.88	0.26	4.38	4.46	6.96	1.49	2.72
10.00	185241.67	2.28	2.28	0.68	11.41	11.74	18.35	3.37	5.69
22.00	484302.17	5.97	5.97	1.79	29.83	30.85	48.20	12.49	2.49
24.00	481870.50	5.94	5.94	1.78	29.68	32.49	50.76	12.29	1.28

Table 18: Testosterone Skin Permeation Data from Test Formulation -F57

Time(hrs)	F 57 (area counts)	Conc(µg/ml)	Conc × Dil	Conc*0.3	Total (5ml)	Cum. (µg)	Cum-Sqcm	SD	Flux (mcg/cm2/hr)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	5171.20	0.06	0.06	0.02	0.31	0.31	0.48	0.73	0.48
2.00	13574.00	0.16	0.16	0.05	0.81	0.83	1.30	0.53	0.82
4.00	45117.33	0.54	0.54	0.16	2.71	2.77	4.33	1.29	1.52
6.00	147837.67	1.77	1.77	0.53	8.87	9.10	14.21	4.45	4.94
8.00	393201.67	4.72	4.72	1.42	23.58	24.35	38.04	11.49	11.91
10.00	571442.33	6.86	6.86	2.06	34.28	36.45	56.96	17.86	9.46
22.00	1201173.50	14.41	14.41	4.32	72.05	76.28	119.19	50.99	5.19
24.00	1240824.00	14.89	14.89	4.47	74.43	82.98	129.66	50.45	5.24

Table 19: Testosterone Skin Permeation Data from Test Formulation -F58

Time(hrs)	F 58 (area counts)	Conc(µg/ml)	Conc X Dil	Conc*0.3	Total (5ml)	Cum. (µg)	Cum-Sqcm	SD	Flux (mcg/cm2/hr)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.00	13377.80	0.16	0.16	0.05	0.80	0.80	1.25	1.23	1.25
4.00	41996.33	0.50	0.50	0.15	2.52	2.57	4.01	1.03	1.38
6.00	114316.00	1.37	1.37	0.41	6.86	7.06	11.03	2.71	3.51
8.00	372728.67	4.47	4.47	1.34	22.36	22.97	35.89	10.99	12.43
10.00	665420.00	7.98	7.98	2.39	39.91	41.86	65.41	14.32	14.76
22.00	1304913.50	15.65	15.65	4.70	78.27	82.62	129.09	11.80	5.31
24.00	1123168.83	15.72	15.72	4.72	78.62	87.67	136.98	13.89	3.95

Table 20: Testosterone Skin Permeation Data from Test Formulation -F59

Time(hrs)	F 59 (area counts)	Conc(μg/ml)	Cone X Dil	Conc*0.3	Total (5ml)	Cum. (μg)	Cum-Sqcm	SD	Flux (mcg/cm2/hr)
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.00	5610.17	0.07	0.07	0.02	0.35	0.35	0.54	0.25	0.54
4.00	30049.67	0.37	0.37	0.11	1.86	1.88	2.94	1.34	1.20
6.00	140427.33	1.74	1.74	0.52	8.69	8.82	13.79	4.64	5.42
8.00	439193.83	5.44	5.44	1.63	27.18	27.83	43.49	10.80	14.85
10.00	629849.83	7.80	7.80	2.34	38.98	41.26	64.47	10.42	10.49
22.00	1346943.00	16.67	16.67	5.00	83.36	87.98	137.47	30.64	6.08
24.00	1461065.83	18.08	18.08	5.43	90.42	100.05	156.32	36.38	9.43

Example 5: The Single and Multiple Dose Pharmacokinetics of Testosterone After Administration of 1.62% Hydro-Alcoholic Gel at Dose Levels of 1.25, 2.50, 3.75, 5.00, and 6.25 g in Hypogonadal Males

Objectives

[0118] To determine the single and multiple dose pharmacokinetics of testosterone after administration of testosterone gel 1.62% at doses of 1.25 g (20.3 mg), 2.50 g (40.5 mg), 3.75 g (60.8 mg), 5.00 g (81.0 mg), and 6.25 g (101.3 mg).

[0119] To assess the dose proportionality and accumulation of testosterone over the dose range of 1.25 g (20.3 mg) to 6.25 g (101.1 mg) of testosterone gel 1.62%.

Methods

[0120] Formulations: Formulations were prepared and supplied by Solvay Pharmaceuticals. Formulations were blinded except for control/marketed product formula.

[0121] Design: A single center, open-label, randomized, single and multiple dose, parallel group study in hypogonadal male subjects. Subjects were randomized to one of five treatment groups. Each group was to be composed of 12 subjects, for a total of 60 subjects.

[0122] Subjects who consented to participate in this study and met the inclusion/exclusion criteria were randomized to one of the following treatment groups:

Table 21: Treatment Groups

Treatment Group	Gel Dose (g)	Testosterone Dose (mg)
A	1.25	20.3
B	2.50	40.5
C	3.75	60.8
D	5.00	81.0
E	6.25	101.3

[0123] Each subject received single (Day 1) and multiple (Days 2-14) doses of testosterone gel 1.62% over a 14-day treatment period. Study drug was applied topically once daily in the morning. The total duration of the study was 17 days, not including the screening period. Subjects were confined to the clinic for the entire 17-day study period. The following table lists the ingredients combined to yield the study formulation used.

Table 22: Ingredients Combined to Yield Study Formulation (%w/w)

Component	Function	% w/w
Testosterone	Active pharmaceutical ingredient	1.62
Alcohol (95% v/v)*	Absorption enhancer	73.5
Isopropyl myristate	Absorption enhancer	1.00
Carbopol 980	Thickening agent precursor	1.00
0.1N Sodium hydroxide	Neutralizer	7.00
Purified water	Solvent	15.9
* Equivalent to about 68.1% of absolute alcohol in the formulation.		

[0124] Subjects: Fifty-six (56) hypogonadal males.

[0125] Main Criteria for Inclusion: Male subjects 18-75 years of age, inclusive; serum total testosterone < 300 ng/dL at screening as measured by the clinical site laboratory; and subjects with a Body Mass Index (BMI) of 20-35 kg/m^2, inclusive.

<u>**Procedures and Assessments**</u>

[0126] Dose Administration: Testosterone gel 1.62% was applied topically once daily in the morning on Days 1-14. The site of application was either the shoulder/upper arm area or the abdomen. The study drug was applied in 1.25 g increments until the total target dose was reached using maximum surface area possible.

[0127] Twenty (20) minutes prior to the targeted time of dose application, subjects showered and washed the application site with soap and water. Subjects were not allowed to remain in the shower for longer than 10 minutes. The designated area for gel application was thoroughly dried.

[0128] Site personnel directly involved with the dosing procedures wore gloves when handling the study gel. A fresh pair of gloves was used for each subject. Each incremental gel dose of 1.25 g +/- 0.02 g was weighed on a sheet of weighing paper on a balance. Immediately after measuring the appropriate amount of gel, the weighing paper with the measured gel dose was wiped directly onto the subject's designated site of application by the study personnel. The subject then rubbed the product into the skin of the designated application site using his hand. This process was repeated until the total target dose (1.25 g to 6.25 g) was reached.

[0129] Pharmacokinetic Sampling: Whole blood samples (10mL each) were obtained from each subject for determination of total testosterone, dihydrotestosterone, and estradiol at the following time points:

- Day -1: predose, 0.5, 1, 2, 4, 6, 8, 10, 12, and 16 hours relative to the projected time of gel application on subsequent study days;
- Day 1: predose, 0.5, 1, 2, 4, 6, 8, 10, 12, and 16 hours postdose;
- Days 2-13: predose;
- Day 14: predose, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, and 24 hours postdose.

[0130] Bioanalysis: Serum concentrations of total testosterone, dihydrotestosterone, and estradiol were determined using validated LC-MS/MS methodology.

Criteria for Evaluation

[0131] Safety: Vital signs, ECG, physical examination, clinical laboratory determinations (including PSA measurement), DRE and IPSS, safety testosterone and hematocrit measurements.

[0132] Pharmacokinetics: For this preliminary report, pharmacokinetic parameters (AUC(0-24), C_{max}, C_{avg}, C_{min}, peak to trough fluctuation, T_{min} and T_{max}) derived from both observed and baseline adjusted serum concentrations for testosterone,

[0133] Statistical methods: Descriptive statistics (n, mean, SD, CV, median, geometric mean, minimum, maximum) and graphical representations.

Table 23: Subject Demographics (Mean (range))

Treatment Group	N	Age (years)	BMI (kg/m^2)
A (1.25 g)	11	50 (27-69)	30.4 (25.6-33.6)
B (2.50 g)	11	50 (31-66)	31.0 (25.9-36.1)
C (3.75 g)	11	52 (38-65)	29.3 (21.8-35.3)
D (5.00 g)	12	55 (37-68)	29.6 (22.6-34.0)
E (6.25 g)	11	48 (27-68)	30.2 (27.3-32.7)
All Groups	56	51 (27-69)	30.1 (21.8-36.1)

Table 24: Ethnicity of the Subjects (N (%))

Treatment Group	N	White Not Hispanic or Latino	White Hispanic or Latino	Black	Asian
A	11	5	5	0	1
B	11	5	6	0	0
C	11	5	5	1	0
D	12	5	7	0	0
E	11	2	9	0	0
All Groups	56	22 (39.3%)	32 (57.1%)	1 (1.8%)	1 (1.8%)

Screening Testosterone Baseline Values

[0134] All subjects at screening had testosterone concentrations < 300 ng/dL, confirming the hypogonadal status of all subjects prior to exposure to study drug. The local clinical laboratory used chemiluminescence methodology for these evaluation. Mean screening baseline serum total testosterone concentrations ranged from 215 to 232 ng/dL for the five individual dose groups. Table 25 provides the screening baseline mean (range) by treatment group.

Table 25: Mean Screening Testosterone Baseline Values

Treatment Group	N	Mean Baseline Testosterone (ng/dL)	Range
A	11	215	73-286
B	11	231	132-293
C	11	230	93-295
D	12	232	132-293

Treatment Group	N	Mean Baseline Testosterone (ng/dL)	Range
E	11	225	158-282
All	56	227	23-295

Testosterone Concentration-Time Data

[0135] The mean concentration-time profiles for observed testosterone on Day 1 and Day 14 are provided in FIGS. 10 and 11, respectively.

[0136] Referring to FIG. 10, on Day 1, a continuous increase in testosterone concentrations occurred in all treatments for approximately 8 hours postdose. Testosterone concentrations then remained consistent over the remainder of the 24-hour dosing interval. Based on the mean concentration-time profiles, all treatments provided sufficient testosterone exposure to increase levels above the lower limit of the eugonadal range (> 300 ng/dL) after a single dose on Day 1.

[0137] Consistent testosterone levels were observed throughout the majority of the 24-hour concentration-time profiles after multiple dosing of testosterone gel 1.62%. The exception to this is Treatment D, 5.00 g, where a significant peak was observed at 6 hours postdose. This increase in the mean profile was due to the results of one subject who had a reported testosterone concentration of 4980 ng/dL at 6 hours postdose.

[0138] Referring now to FIG. 11, the mean profiles on Day 14 demonstrate testosterone concentrations remain above the lower limit of the eugonadal range (>300 ng/dL) over the 24-hour dose interval for all five doses. An increase in testosterone exposure was observed with increased dose over the 1.25 g to 6.25 g range, with the exception of the second profile peak in Treatment D, 5.00 g.

Testosterone Pharmacokinetic Results

[0139] The pharmacokinetic results for observed and baseline adjusted testosterone are provided in Table 26 below after subjects received a single dose of 1.62% testosterone gel on Day 1.

Table 26: Single Dose Pharmacokinetic Parameters for Testosterone Gel 1.62% on Day 1

Parameter	Treatment Group	Gel Dose (g)	Testosterone Dose (mg)	Arithmetic Mean (SD)			
				N	Observed	N	Baseline Adjusted
AUC(0-24) (ng*h/dl)	A	1.25	20.3	11	7376 (1465)	11	1934 (1005)
	B	2.50	40.5	11	9112 (3354)	9	3168-(1845)
	C	3.75	60.8	11	8719 (2831)	10	3330 (1875)
	D	5.00	81	12	11933 (3188)	12	5329 (3296)
	E	6.25	101.3	11	11137 (3024)	11	5573 (2602)
C_{avg} (ng/dl)	A	1.25	20.3	11	307 (61)	11	81(42)
	B	2.50	40.5	11	380 (140)	9	132 (77)
	C	3.75	60.8	11	363 (118)	10	139 (78)
	D	5.00	81	12	497 (133)	12	222 (137)

Parameter	Treatment Group	Gel Dose (g)	Testosterone Dose (mg)	Arithmetic Mean (SD)			
				N	Observed	N	Baseline Adjusted
	E	6.25	101.3	11	464 (265)	11	232 (108)
C_{max} (ng/dL)	A	1.25	20.3	11	387 (80)	11	159 (57)
	B	2.50	40.5	11	479 (161)	9	234 (102)
	C	3.75	60.8	11	533 (211)	10	305 (178)
	D	5.00	81	12	698 (231)	12	424 (200)
	E	6.25	101.3	11	657 (215)	11	424 (210)
T_{max}[a] (h)	A	1.25	20.3	11	12 (4 - 24)	11	12(4 - 24)
	B	2.50	40.5	11	12 (4 - 24)	9	16(8 - 24)
	C	3.75	60.8	11	16 (8 - 24)	10	16(8 - 24)
	D	5.00	81	12	12 (2 - 24)	12	12 (6 - 24)
	E	6.25	101.3	11	12 (8 - 24)	11	12 (8 - 24)
[a] median (range).							

[0140] Observed mean C_{avg} on Day 1 was in the eugonadal range of 300 - 1000 ng/dL for all dose levels. Mean AUC and C_{avg} generally increased over the 1.25 g to 6.25 g dose range, with similar values for Treatments B and C, and Treatments D and E, respectively. Mean C_{max} increased with dose from 1.25 g to 5.00 g, then leveled off. Median T_{max} for all groups, except 5.00 g, was 12 hours and ranged from 2 to 24 hours.

[0141] Observed C_{max} values on Day 1 for 1.25 g, 2.50 g, and 3.75 g remained below the upper limit of the eugonadal range (<1000 ng/dL). In treatment D, 5.00 g, one subject had a C_{max} value of 1070 ng/dL. In treatment E, 6.25 g, one subject had a C_{max} value of 1020 ng/dL. All other Day 1 C_{max} values were <1000 ng/dL in Treatment groups D and E.

[0142] Baseline adjusted mean AUC and C_{avg} parameter values increased with dose over all five treatment levels. Baseline adjusted mean C_{avg} indicates endogenous testosterone concentrations increased from 81 to 232 ng/dL over the 1.25 g to 6.25 g dose range after single dose administration of testosterone gel 1.62%.

[0143] The multiple dose pharmacokinetic results for observed and baseline adjusted testosterone are provided in Table 27 below for testosterone gel 1.62% on Day 14.

Table 27: Multiple Dose Pharmacokinetic Parameters for Testosterone Gel 1.62% on Day 14

Parameter	Treatment Group	Gel Dose (g)	Testosterone Dose (mg)	Arithmetic Mean (SD)			
				N	Observed	N	Baseline Adjusted
AUC(0-24) (ng*h/dl)	A	1.25	20.3	11	7731 (2914)	9	3149 (2909)
	B	2.50	40.5	11	9232 (4146)	11	3174-(2628)
	C	3/75	60.8	11	11132 (2950)	11	5346 (3834)
	D	5.00	81	8	16115(11345)	8	9646 (12002)
	E	6.25	101.3	10	15250 (4123)	10	10005 (4474)
C_{avg} (ng/dl)	A	1.25	20.3	11	322 (121)	9	131 (121)
	B	2.50	40.5	11	385 (173)	11	132 (110)
	C	3.75	60.8	11	464 (123)	11	223 (160)

Parameter	Treatment Group	Gel Dose (g)	Testosterone Dose (mg)	Arithmetic Mean (SD)			
				N	Observed	N	Baseline Adjusted
	D	5.00	81	8	671 (473)	8	402 (500)
	E	6.25	101.3	9	634 (182)	9	413 (197)
C_{max} (ng/dL)	A	1.25	20.3	11	464 (158)	9	293 (170)
	B	2.50	40.5	11	506 (195)	11	266 (119)
	C	3.75	60.8	11	750 (221)	11	523 (241)
	D	5.00	81	8	1422 (1450)	8	1145 (1466)
	E	6.25	101.3	10	1179 (520)	10	965 (527)
T_{max}[a] (h)	A	1.25	20.3	11	8 (0 - 16)	9	4 (0.5 - 16)
	B	2.50	40.5	11	4 (0 - 16)	11	8. (0.5 - 24)
	C	3.75	60.8	11	8 (1 - 12)	11	1 (1 - 12)
	D	5.00	81	8	1.5 (0.5 - 24)	8	1.5 (0.5 - 24)
	E	6.25	101.3	10	6 (0.5 - 24)	10	6 (0.5 - 24)
C_{min} (ng/dl)	A	1.25	20.3	11	209 (91)	9	46 (77)
	B	2.50	40.5	11	263 (138)	11	29 (70)
	C	3.75	60.8	11	310 (106)	11	89 (152)
	D	5.00	81	8	414 (184)	8	154 (203)
	E	6.25	101.3	10	351 (43)	10	102 (74)
Fluctuation	A	1.25	20.3	11	81 (20)	9	271 (166)
	B	2.50	40.5	11	66 (18)	11	262 (188)
	C	3.75	60.8	11	97 (43)	11	252 (149)
	D	5.00	81	8	129 (66)	8	355 (306)
	E	6.25	101.3	9	117 (52)	9	201 (69)
[a] median (range).							

[0144] Observed AUC, C_{avg}, and C_{max} parameter values on Day 14 increased across the dose range of 1.25 g to 5.00 g, with an apparent leveling off with the 6.25 g dose. In the 5 g dose level group, one subject (#25791) on Day 14 had C_{max} and C_{avg} values of 4980 ng/dL and 1801 ng/dL, respectively. These values were approximately 4-fold higher than the other subjects in this same treatment group. The cause of elevated levels in this subject is unknown. When this subject's values are removed from the group mean presented in the table above, the pharmacokinetic parameters for the 5.00 g dose group are reduced from 1422 to 914 ng/dL for C_{max} and 671 to 510 ng/dL fro C_{avg}, respectively. Using these revised mean values, a trend of increased C_{avg} and C_{max} values over the entire dose range of 1.25 g to 6.25 g is observed.

[0145] Observed mean C_{min} values remained above the lower limit of the eugonadal range (>300ng/dL) with multiple dosing at the 3.75 g, 5.00 g, and 6.25 dose levels. Observed mean C_{avg} for all dose levels ranged from 322 to 671 ng/dL and were in the eugonadal testosterone range of 300 to 1000 ng/dL.

[0146] Observed C_{max} values on Day 14 for the 1.25 g dose level remained below the upper limit of the eugonadal range (<1000 ng/dL). In the other dose groups, a total of 12 subjects had observed C_{max} values above 1000ng/dL. At the 2.50 dose level, one subject had a C_{max} value of 1010 ng/dL. At the 3.75 dose level, one subject had a C_{max} value of 1070 ng/dL. At the 5.00 g dose level, four subjects had C_{max} values >1000 ng/dL ranging from 1050 to 4980 ng/dL. At the 6.25 dose level, six subjects had C_{max} values >1000 ng/dL ranging from 1110 to 2080 ng/dL. These observations are based on the bioanalytical results from the LC-MS/MS assay. These values were not identified during the predose testosterone safety testing conducted at the clinical site.

[0147] Baseline adjusted mean C_{avg} values increased with dose across the entire dose range. Baseline adjusted mean C_{avg} indicates endogenous testosterone concentrations increased from 131 to 413 ng/dL over the 1.25 g to 6.25 g dose range after fourteen days of multiple dose administration of testosterone gel 1.62%.

Conclusions

[0148] Based on the preliminary review of adverse event data, safety testosterone and hematocrit laboratory measures, and application site evaluation, testosterone gel 1.62% was safe and well tolerated at dose levels ranging from 1.25 to 6.25 g of gel (20.3 to 101.1 mg of testosterone). After single and multiple dose administration of testosterone gel 1.62% at dose levels ranging from 1.25 g to 6.25 g (20.3 to 101.1 mg of testosterone), mean C_{avg} values in the eugonadal range of 300 - 1000 ng/dL are obtained.

[0149] At the highest dose levels of 5.00 g and 6.25 g (81.0 and 101.3 mg of testosterone, respectively) a greater incidence of C_{max} values exceeding the upper limit of normal for eugonadal men was observed. Appropriate monitoring in Phase 3 clinical development is indicated.

[0150] The use of individual numerical values are stated as approximations as though the values were preceded by the word "about" or "approximately." Similarly, the numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word "about" or "approximately." In this manner, variations above and below the stated ranges can be used to achieve substantially the same results as values within the ranges. As used herein, the terms "about" and "approximately" when referring to a numerical value shall have their plain and ordinary meanings to a person of ordinary skill in the art to which the particular subject matter is most closely related or the art relevant to the range or element at issue. The amount of broadening from the strict numerical boundary depends upon many factors. For example, some of the factors which may be considered include the criticality of the element and/or the effect a given amount of variation will have on the performance of the claimed subject matter, as well as other considerations known to those of skill in the art. As used herein, the use of differing amounts of significant digits for different numerical values is not meant to limit how the use of the words "about" or "approximately" will serve to broaden a particular numerical value. Thus, as a general matter, "about" or "approximately" broaden the numerical value. Also, the disclosure of ranges is intended as a continuous range including every value between the minimum and maximum values plus the broadening of the range afforded by the use of the term "about" or "approximately." Thus, recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it there individually recited herein.

[0151] Use of the phrase 'the invention' or 'the present invention' is not meant to limit the claims in any manner and no conclusion should be drawn that any description or argument associated with a particular use of the phrase 'the invention' or 'the present invention' applies to each and every claim. The use of the phrase 'the invention' or 'the present invention' has been used solely for linguistic or grammatical convenience and not to effect a limitation of any nature on any of the claims.

[0152] It is to be understood that any ranges, ratios and ranges of ratios that can be formed by, or derived from, any of the data disclosed herein represent further embodiments of the present disclosure and are included as part of the disclosure as though they were explicitly set forth. This includes ranges that can be formed that do or do not include a finite upper and/or lower boundary. Accordingly, a person of ordinary skill in the art most closely related to a particular range, ratio or range of ratios will appreciate that such values are unambiguously derivable from the data presented herein.

[0153] The use of the terms "a" and "an" and "the" and similar referents in the context of this disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., such as, preferred, preferably) provided herein, is intended merely to further illustrate the content of the disclosure and does not pose a limitation on the scope of the claims. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the claimed invention.

REFERENCES CITED IN THE DESCRIPTION

Cited references

Patent documents cited in the description

- US2005020552A [0009]

Non-patent literature cited in the description

- Remington's The Science and Practice of PharmacyMeade Publishing Co. [0021]
- Neutralizing Carbopol® and Pemulen® Polymers in Aqueous and Hydroalcoholic SystemsCommercial Brochure TDS-237Noveon Inc.19981000 [0025]

Patentkrav

1. Hydroalkoholisk farmaceutisk gelsammensætning omfattende:
i. ca. 1,50 % til ca. 1,70 % (vægt/vægt) testosteron;
ii. ca. 0,6 % til ca. 1,2 % (vægt/vægt) isopropylmyristat;
iii. ca. 60 % til ca. 80 % (vægt/vægt) af en alkohol valgt fra gruppen bestående af ethanol og isopropanol;
iv. en tilstrækkelig mængde af et fortykkelsesmiddel til at give sammensætningen en viskositet på over ca. 9 Pa.s (9000 cps); og
v. vand;
til anvendelse i en fremgangsmåde til behandling af hypogonadisme hos et hankønsindivid, hvilken fremgangsmåde omfatter indgivelse af en terapeutisk effektiv dosis af præparatet til et hudområde af individet; hvor sammensætningen efter påføring af sammensætningen på individets hud tilvejebringer tilstrækkelig testosteron til at give patienten en blodserumkoncentration af testosteron på mellem ca. 298 og 1043 ng testosteron pr. dl serum, hvor den terapeutisk effektive dosis er ca. 2,5 g af sammensætningen, og hvor den terapeutisk effektive dosis indgives én gang dagligt i mindst 7 dage.

2. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor sammensætningen omfatter ca. 67,0 % til ca. 74,0 % (vægt/vægt) af en alkohol valgt fra gruppen bestående af ethanol og isopropanol.

3. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor den totale testosteronkoncentration i individet efter daglig indgivelse af sammensætningen er større end 300 ng/dl.

4. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor efter mindst 30 dages daglig indgivelse af sammensætningen er serumtestosteronkoncentrationen i individet ca. 400 ng/dl til ca. 1050 ng/dl.

5. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor sammensætningen omfatter ca. 1,62 % (vægt/vægt) testosteron.

6. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor sammensætningen omfatter ca. 0,6 % til ca. 1,4 % (vægt/vægt) af et fortykkelsesmiddel.

7. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor fortykkelsesmidlet er dannet ved at kombinere en forløber-polyacrylsyre med en neutralisator under dannelsen af sammensætningen.

8. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 7, hvor fortykkelsesmidlet er neutraliseret carbopol® 980.

9. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 7, hvor fortykkelsesmidlet er neutraliseret carbopol® 940.

10. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 8 eller 9, hvor neutralisatoren er valgt fra gruppen bestående af natriumhydroxid, ammoniumhydroxid, kaliumhydroxid, arginin, aminomethylpropanol, tetrahydroxypropylethylendiamin, triethanolamin, tromethamin, PEG-15-cocalamin, diisopropanolamin, diisopropanolamin, triisopropanolamin og kombinationer deraf.

11. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 7, hvor neutralisatoren er mellem ca. 6,5 % og 7,5 % (vægt/vægt) 0,1N NaOH.

12. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor sammensætningen har en viskositet på ca. 13 Pa.s til ca. 33 Pa.s (ca. 13.000 cps til ca. 33.000 cps).

13. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor individet har en forbehandlingsserumtestosteronkoncentration på mindre end ca. 300 ng/dl.

14. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor sammensætningen er i stand til at frigive testosteron efter påføring af sammensætningen på huden af et individ med en hastighed og varighed, der opnår en cirkulerende serumkoncentration af testosteron på mere end ca. 300 ng pr. dl serum.

15. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 14, hvor serumkoncentrationen af testosteron er større end ca. 300 ng pr. dl serum i løbet af en tidsperiode, der begynder ca. 0,5 timer efter indgivelse og slutter ca. 24 timer efter indgivelse.

16. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 15, hvor serumtestosteronkoncentrationen holdes mellem ca. 400 og 1050 ng testosteron pr. dl serum.

17. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor en opnået C_{max} er mellem omkring 300 og 5000 ng/dl.

18. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 1, hvor den hydroalkoholiske farmaceutiske gelsammensætning omfatter resultatet af at kombinere følgende ingredienser:
a. ca. 1,5 % til ca. 1,7 % (vægt/vægt) testosteron;
b. ca. 67,0 % til ca. 74,0 % (vægt/vægt) ethanol;
c. ca. 0,6 % til ca. 1,4 % (vægt/vægt) isopropylmyristat;
d. ca. 0,6 % til ca. 1,4 % (vægt/vægt) carbomer;

e. ca. 6,5 % til ca. 7,5 % (vægt/vægt) 0,1N NaOH; og
f. ekstra vand.

19. Hydroalkoholisk farmaceutisk gelsammensætning, der omfatter resultatet af at kombinere følgende ingredienser:
i. ca. 1,50 % til ca. 1,70 % (vægt/vægt) testosteron;
ii. ca. 0,6 % til ca. 1,4 % (vægt/vægt) isopropylmyristat;
iii. ca. 67,0 % til ca. 74,0 % (vægt/vægt) ethanol;
iv. ca. 0,6 % til ca. 1,4 % (vægt/vægt) carbomer;
v. ca. 6,5 % til ca. 7,5 % (vægt/vægt) 0,1N NaOH; og
vi. ekstra vand;
til anvendelse i en fremgangsmåde til behandling af hypogonadisme hos et hankønsindivid, hvilken fremgangsmåde omfatter indgivelse af en terapeutisk effektiv dosis af præparatet til et hudområde af individet; hvor sammensætningen efter påføring af sammensætningen på individets hud tilvejebringer tilstrækkelig testosteron til at give patienten en blodserumkoncentration af testosteron på mellem ca. 298 og 1043 ng testosteron pr. dl serum, og hvor sammensætningen indgives til skulderen/overarmen af hankønsindividet.

20. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 19, hvor sammensætningen har en viskositet mellem ca. 13 Pa.s til ca. 33 Pa.s (ca. 13.000 cps og 33.000 cps).

21. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 19, hvor sammensætningen omfatter ca. 1,62 % (vægt/vægt) testosteron.

22. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 19, hvor den terapeutisk effektive dosis er ca. 2,50 g af sammensætningen dagligt.

23. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 22, hvor den terapeutisk effektive dosis indgives dagligt.

24. Hydroalkoholisk farmaceutisk gelsammensætning til anvendelse ifølge krav 22, hvor den terapeutisk effektive dosis indgives én gang dagligt i mindst 14 dage.

DRAWINGS

Drawing

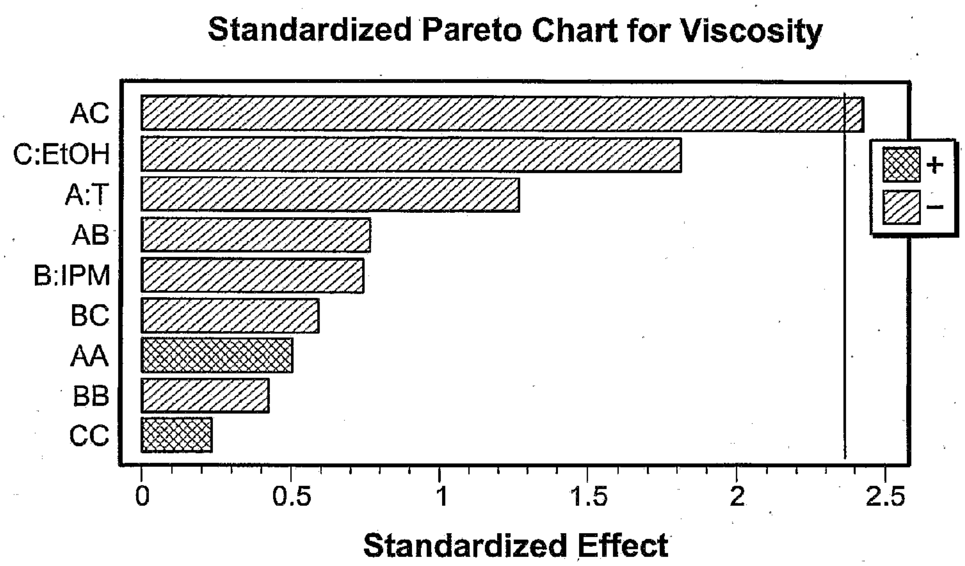

FIG. 1

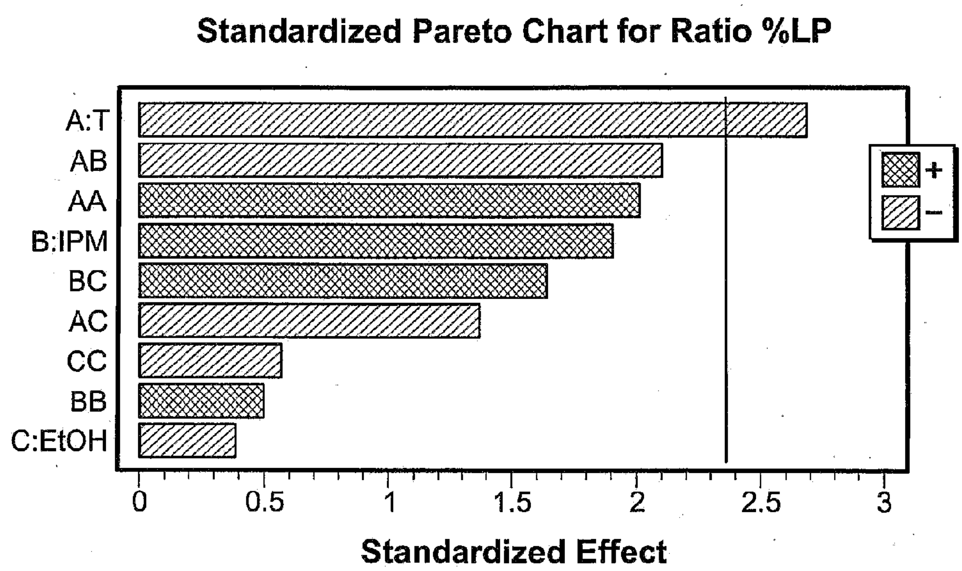

FIG. 2

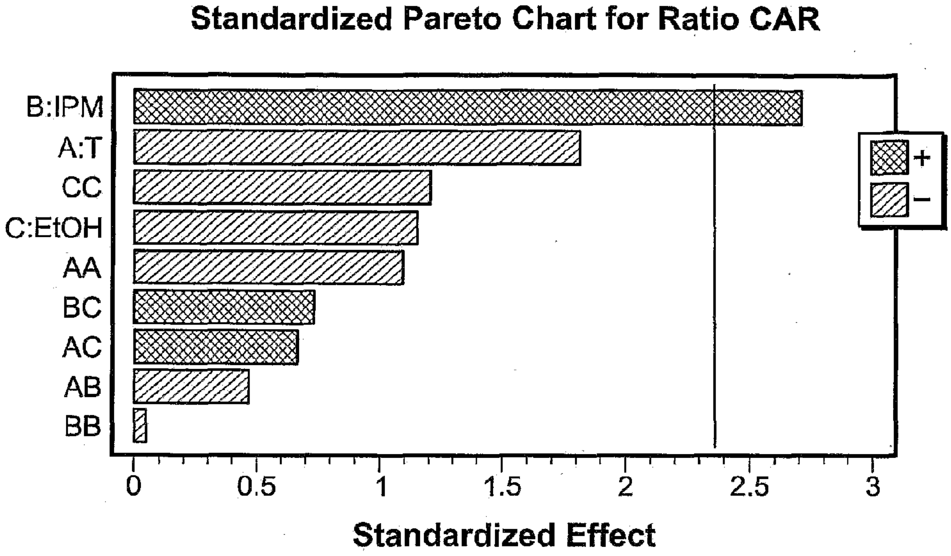
Standardized Pareto Chart for Ratio CAR
B:IPM
A:T
CC
C:EtOH
AA
BC
AC
AB
BB
+
−
0
0.5
1
1.5
2
2.5
3
Standardized Effect

FIG. 3

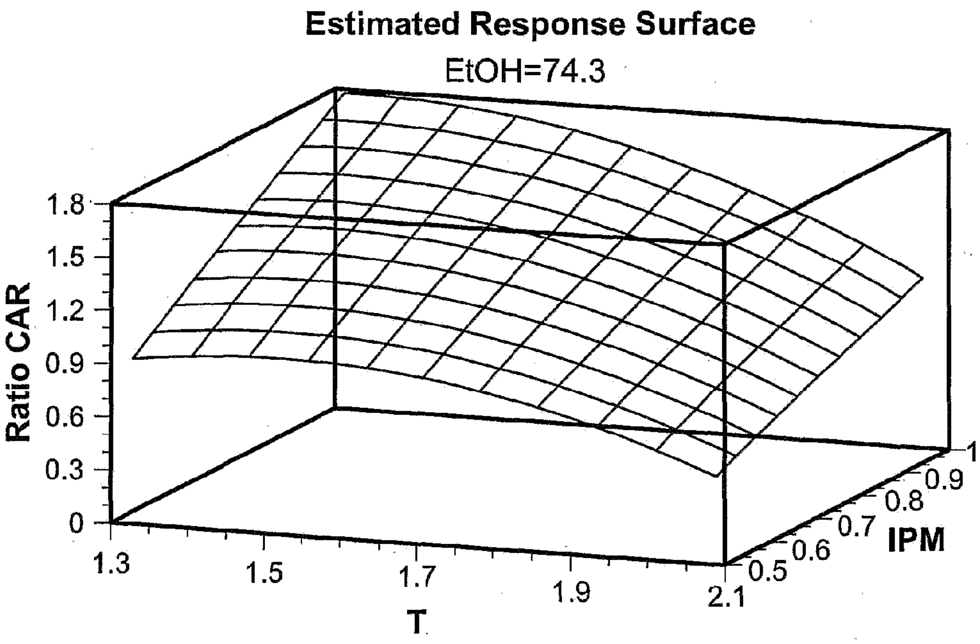
Estimated Response Surface
EtOH=74.3
Ratio CAR
1.8
1.5
1.2
0.9
0.6
0.3
0
1.3
1.5
1.7
1.9
2.1
T
0.5
0.6
0.7
0.8
0.9
1
IPM

FIG. 4

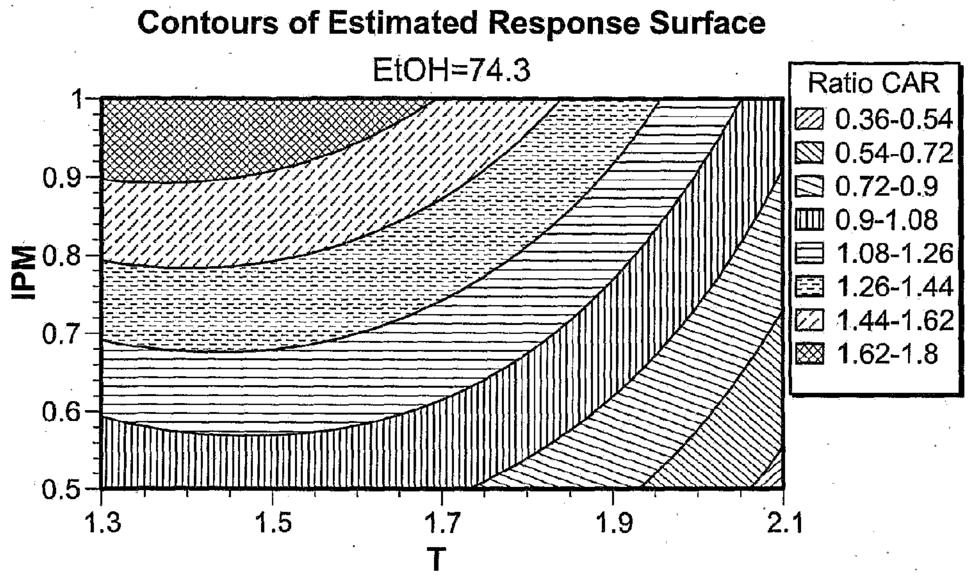
Contours of Estimated Response Surface
EtOH=74.3
Ratio CAR
0.36-0.54
0.54-0.72
0.72-0.9
0.9-1.08
1.08-1.26
1.26-1.44
1.44-1.62
1.62-1.8
IPM
1
0.9
0.8
0.7
0.6
0.5
1.3
1.5
1.7
1.9
2.1
T

FIG. 5

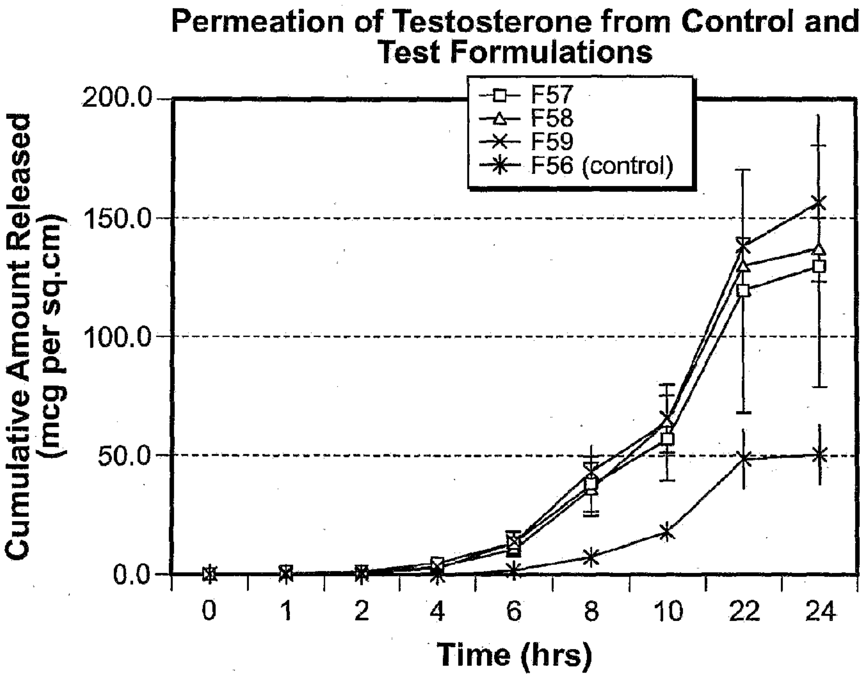
Permeation of Testosterone from Control and Test Formulations
F57
F58
F59
F56 (control)
Cumulative Amount Released (mcg per sq.cm)
200.0
150.0
100.0
50.0
0.0
0
1
2
4
6
8
10
22
24
Time (hrs)

FIG. 6

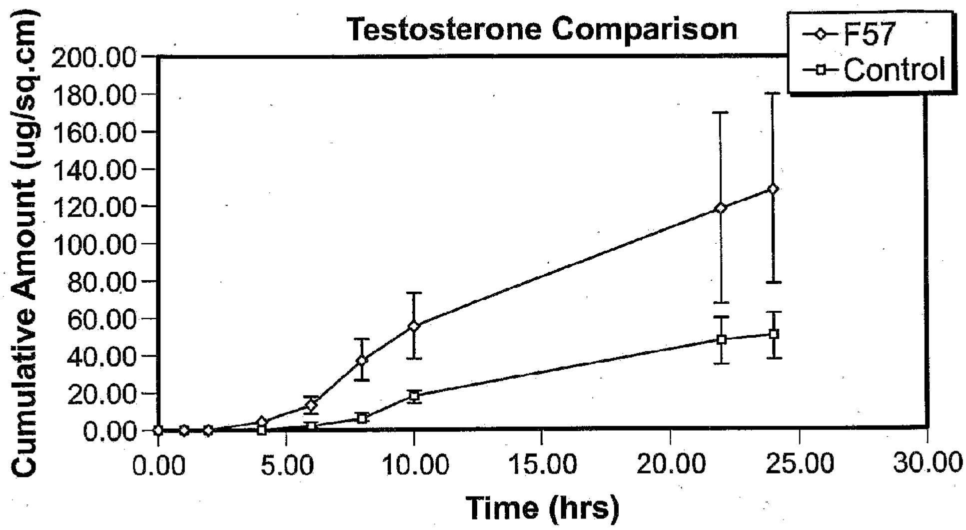
Testosterone Comparison
F57
Control
Cumulative Amount (ug/sq.cm)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Time (hrs)

FIG. 7

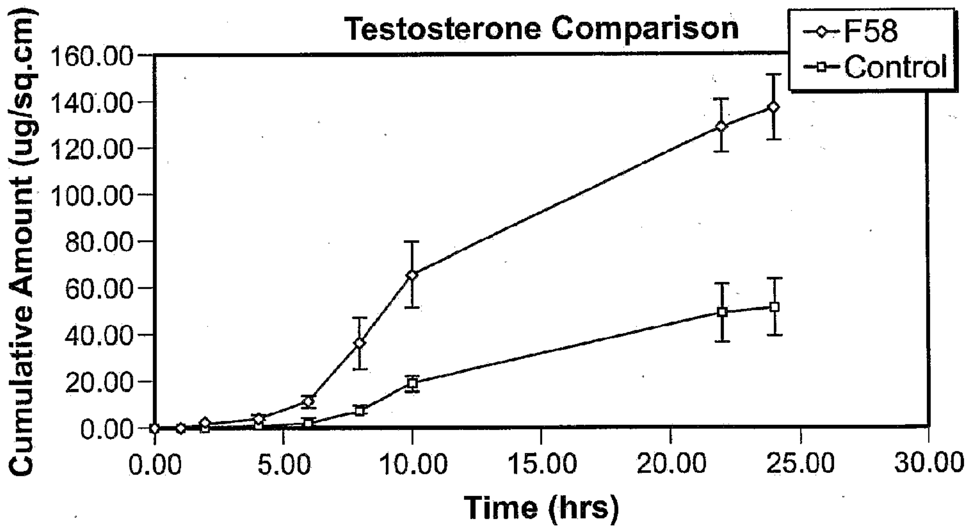
Testosterone Comparison
F58
Control
Cumulative Amount (ug/sq.cm)
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Time (hrs)

FIG. 8

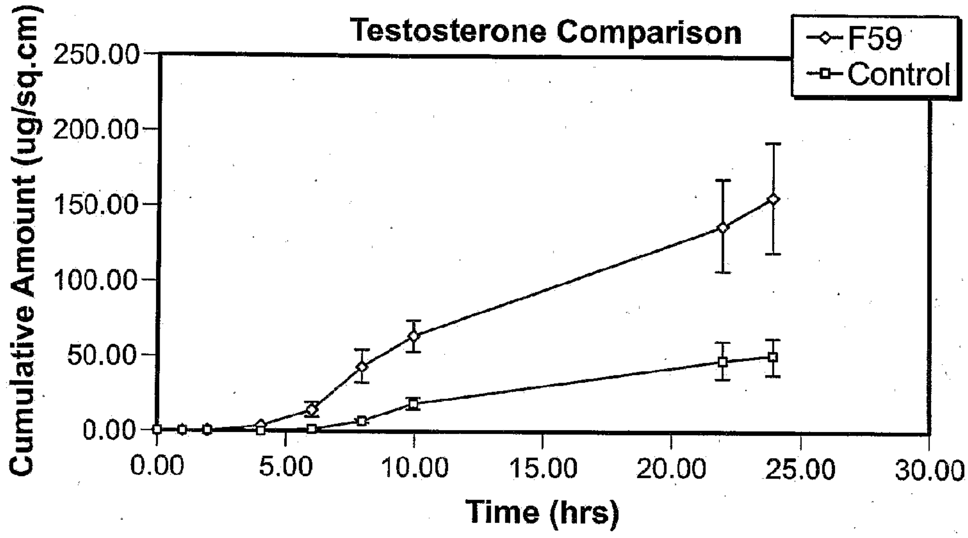
Testosterone Comparison
F59
Control
Cumulative Amount (ug/sq.cm)
250.00
200.00
150.00
100.00
50.00
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Time (hrs)

FIG. 9

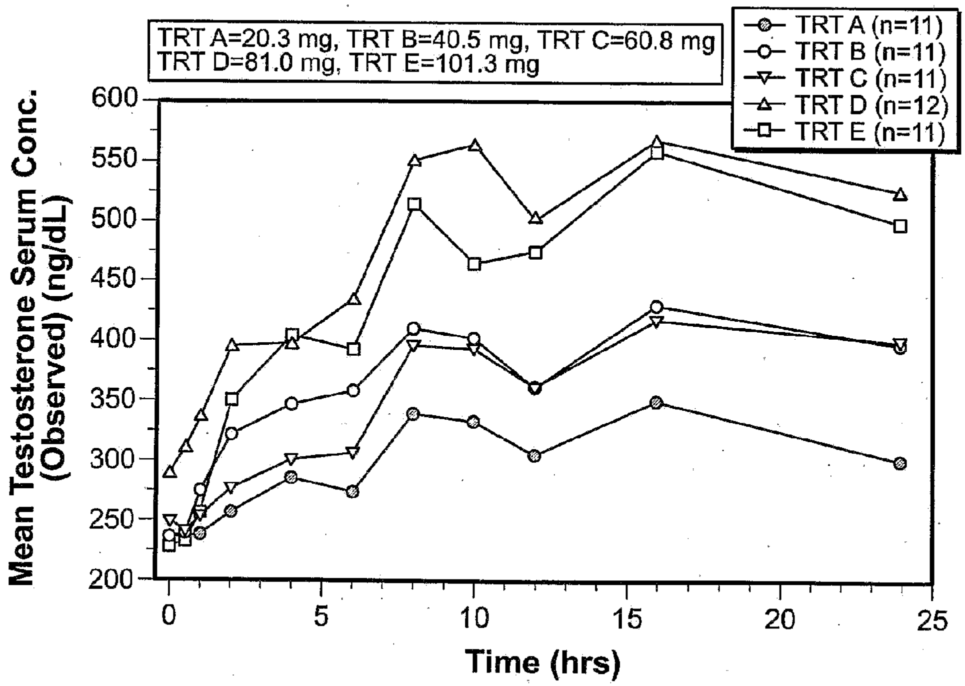
TRT A=20.3 mg, TRT B=40.5 mg, TRT C=60.8 mg
TRT D=81.0 mg, TRT E=101.3 mg
TRT A (n=11)
TRT B (n=11)
TRT C (n=11)
TRT D (n=12)
TRT E (n=11)
Mean Testosterone Serum Conc. (Observed) (ng/dL)
600
550
500
450
400
350
300
250
200
0
5
10
15
20
25
Time (hrs)

FIG. 10

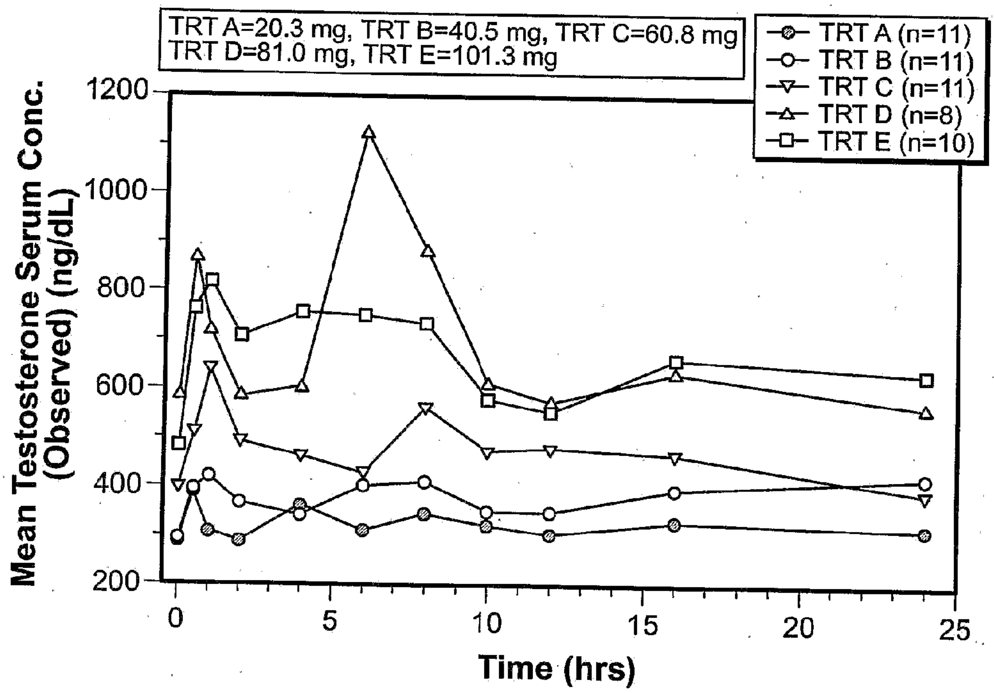
TRT A=20.3 mg, TRT B=40.5 mg, TRT C=60.8 mg
TRT D=81.0 mg, TRT E=101.3 mg
TRT A (n=11)
TRT B (n=11)
TRT C (n=11)
TRT D (n=8)
TRT E (n=10)
Mean Testosterone Serum Conc. (Observed) (ng/dL)
1200
1000
800
600
400
200
0
5
10
15
20
25
Time (hrs)

FIG. 11