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(54) **Title:** NOVEL FORMULATIONS FOR THE TREATMENT OF VAGINAL DISORDERS

(57) **Abstract:** The present disclosure provides novel formulations suitable for the intravaginal delivery of tinidazole, as well as methods of using the same.

NOVEL FORMULATIONS FOR THE TREATMENT OF VAGINAL DISORDERS

BACKGROUND OF THE INVENTION

[0001] Bacterial vaginosis (BV) is a common condition that is related to alterations in the normal vaginal flora. It is the most common cause of vaginitis in women and has a recurrence rate of approximately 20%-40% at one month after therapy. In addition to the general discomfort associated with the condition, BV has been linked to premature membrane rupture, premature delivery, low birth weight, acquisition of HIV and other STDs, development of pelvic inflammatory disease (PID), and post-operative infections following gynecological procedures.

[0002] Although the precise etiology of BV is unknown, the disease itself is associated with the decrease or absence of protective lactobacilli which are normally present in the vagina. Lactobacilli produce lactic acid from glycogen to maintain the vagina's acidic pH. This acidic environment inhibits the growth of other bacterial species typically found in the vagina, albeit at low levels. Vaginal lactobacilli also produce hydrogen peroxide (H₂O₂), which is toxic to viruses as well as to bacteria *in vitro*. When lactobacilli are sufficiently absent, bacteria such as *Gardnerella vaginalis*, *Bacteroides* spp., *Mobiluncus* spp., *Haemophilus* spp., peptostreptococci, *Mycoplasma hominis*, ureaplasma, and other anaerobes can populate the vaginal tract without difficulty.

[0003] Currently approved treatments for BV include administering metronidazole ("MTZ") 500 mg orally twice a day for 7 days; administering MTZ gel 0.75%, one full applicator (5 g) intravaginally, once a day for 5 days; administering clindamycin cream 2%, one full applicator (5 g) intravaginally at bedtime for 7 days; administering tinidazole, 2 g orally once daily for 2 days; or administering tinidazole, 1 g orally once daily for 5 days.

[0004] Although MTZ remains the primary choice for treating BV in the United States, tinidazole is particularly useful for treating MTZ-resistant *G. vaginalis*, an organism commonly associated with BV. Despite this utility, tinidazole must be dosed orally, whereas MTZ can be dose intravaginally. Common side effects associated with the oral administration of tinidazole include, but are not limited to, metallic/bitter taste, nausea, anorexia, dyspepsia/cramps/epigastric discomfort, vomiting, constipation, tongue discoloration, stomatitis, diarrhea, decreased appetite, and flatulence.

[0005] Thus, there exists a need for novel tinidazole formulations suitable for the treatment of BV and other diseases of the vaginal cavity that respond to or are susceptible to tinidazole (*e.g.* trichomoniasis) that reduce or alleviate many of the side effects commonly associated with the oral form of the drug. The present disclosure provides such formulations.

BRIEF SUMMARY OF THE INVENTION

[0006] The present disclosure provides formulations suitable for the intravaginal delivery of tinidazole to a subject in need thereof, the formulations comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives.

[0007] In some embodiments, the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block. In certain embodiments, the hydrophobic block is poly(propylene oxide). In some embodiments, the hydrophilic block is poly(ethylene oxide).

[0008] In some embodiments, the thermoreversible gelling agent is poloxamer 407.

[0009] In some embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols comprise a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol. In some embodiments, the first and second pharmaceutically acceptable C₁-C₇ alcohols are independently selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.

[0010] In some embodiments, the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.

[0011] In certain embodiments, the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol.

[0012] In some embodiments, the isopropanol is a 60% (w/w) solution in water.

[0013] In some embodiments, the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide. In some embodiments, the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.

[0014] In certain embodiments, the water comprises a mixture of sterile deionized water and a buffered aqueous solution. In certain embodiments, the buffered aqueous solution is citrate buffer.

- [0015] In some embodiments, the formulation has a buffered pH selected from the group consisting of about 3 to about 5, about 3.5 to about 4.5, and about 4 to about 4.5.
- [0016] In certain embodiments, the tinidazole comprises from about 0.1 to about 2% (w/w) of the formulations and in certain embodiments, the tinidazole comprises about 1%, about 1.25%, or about 1.5% (w/w) of the formulations.
- [0017] In some embodiments, the formulations comprise about 20% poloxamer 407 (w/w), about 49.6% citrate buffer (w/w), about 3.1% benzyl alcohol (w/w), about 12% (w/w) of a 60% (w/w) solution of isopropanol in water, about 13.8% dimethylisorbide (w/w), and about 1.5% tinidazole.
- [0018] In other embodiments, the formulations comprise about 19% poloxamer 407 (w/w), about 53% citrate buffer (w/w), about 2.9% benzyl alcohol (w/w), about 11% (w/w) of a 60% (w/w) solution of isopropanol in water, about 12.7% dimethylisorbide (w/w), and about 1.25% tinidazole.
- [0019] In still further embodiments, the formulations comprise about 18% poloxamer 407 (w/w), about 57% citrate buffer (w/w), about 2.5% benzyl alcohol (w/w), about 10% of a 60% (w/w) solution of isopropanol in water, about 11.5% dimethylisorbide (w/w), and about 1% tinidazole.
- [0020] In other embodiments, the present disclosure provides methods for treating bacterial vaginosis, trichomoniasis, or other diseases of the vaginal cavity susceptible to tinidazole, comprising intravaginally administering to a subject in need thereof a formulation comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives.
- [0021] In certain embodiments of the methods described herein, the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block. In certain embodiments of the methods described herein, the hydrophobic block is poly(propylene oxide). In some embodiments of the methods described herein, the hydrophilic block is poly(ethylene oxide). In certain embodiments of the methods described herein, the thermoreversible gelling agent is poloxamer 407.
- [0022] In certain embodiments of the methods described herein, the one or more pharmaceutically acceptable C₁-C₇ alcohols comprises a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol. In some embodiments of the methods described herein, the first and second pharmaceutically acceptable C₁-C₇

alcohols are selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof. In other embodiments of the methods described herein, the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol. In some embodiments of the methods described herein, the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol. In certain embodiments of the methods described herein, the isopropanol is a 60% (w/w) solution in water.

[0023] In some embodiments of the methods described herein, the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide. In some embodiments, the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.

[0024] In certain embodiments of the methods described herein, the water comprises a mixture of sterile deionized water and a buffered aqueous solution. In certain embodiments of the methods described herein, the buffered aqueous solution is citrate buffer.

[0025] In some of the embodiments of the methods disclosed herein, the formulation has a buffered pH selected from the group consisting of about 3 to about 5, about 3.5 to about 4.5, and about 4.

[0026] In certain embodiments of the methods described herein, the tinidazole comprises from about 0.1% to about 2% (w/w) of the formulation. In other embodiments of the methods described herein, the tinidazole comprises about 1%, about 1.25%, or about 1.5% (w/w) of the formulation.

[0027] In certain embodiments of the methods described herein, the subject is a human.

[0028] The present disclosure also relates to a pharmaceutical composition comprising: about 18-20% poloxamer 407 (w/w), about 49.6-57 % citrate buffer (w/w), about 2.5-3.1% benzyl alcohol (w/w), about 10-12% (w/w) of a 60% (w/w) solution of isopropanol in water, about 11.5%-13.8% dimethylisosorbide (w/w), and about 1-1.5% tinidazole (w/w) (and/or an active metabolite thereof), for treating bacterial vaginosis, trichomoniasis, or other disease(s) of the vaginal cavity susceptible to tinidazole.

[0029] In certain embodiments the pharmaceutical composition exists as a viscous liquid at room temperature and gels at or close to body temperature.

[0030] The present disclosure further relates to the use of the formulations as described herein for treating bacterial vaginosis, trichomoniasis, or other disease(s) of the vaginal cavity susceptible to tinidazole.

- [0031] The present disclosure relates also to the use of the formulations as described herein for the manufacture of a medicament for the treatment of bacterial vaginosis, trichomoniasis, or other disease of the vaginal cavity susceptible to tinidazole.
- [0032] An additional aspect of the present disclosure relates to a method for treating a vaginal infection comprising intravaginally administering to the subject a formulation containing about 0.1-2% tinidazole, or an active metabolite thereof.
- [0033] An additional aspect of the present disclosure relates to a method for administering tinidazole to a human subject, comprising obtaining a formulation containing about 0.1-2% tinidazole, or an active metabolite thereof, wherein said formulation exists as a viscous liquid at room temperature; and intravaginally administering the formulation to a human subject, wherein the formulation gels at or close to body temperature.
- [0034] In a further embodiment, the present disclosure provides a formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the formulation comprising tinidazole, wherein the formulation has a Franz cell flux of from about 2 to about 10 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$.
- [0035] In some embodiments, the Franz cell flux is from about 6 to about 9 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$.
- [0036] In another embodiment, the present disclosure provides a formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the formulation comprising tinidazole, wherein the formulation has a mean flux rate in EpiVaginal™ tissue of from about 15 $\text{nmol}/\text{cm}^2/\text{min}$ to about 30 $\text{nmol}/\text{cm}^2/\text{min}$.
- [0037] In certain embodiments, the mean flux rate in EpiVaginal™ tissue is from about 18 $\text{nmol}/\text{cm}^2/\text{min}$ to about 22.5 $\text{nmol}/\text{cm}^2/\text{min}$.
- [0038] The present disclosure also provides a formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the formulations comprising tinidazole, wherein the formulation has a mean tinidazole receiver fluid concentration at 1 hour of from about 0.5 mM to about 2.5 mM.
- [0039] In some embodiments, the formulation has a mean tinidazole receiver fluid concentration at 1 hour of from about 1.14 mM to about 1.74 mM.
- [0040] The present disclosure also provides a tinidazole gel formulation suitable for the intravaginal delivery of tinidazole to a subject in need thereof, the formulation comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable

C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives, the tinidazole gel formulation prepared by the process of a) dissolving the thermoreversible gelling agent in the water to form a first solution; b) combining the one or more pharmaceutically acceptable C₁-C₇ alcohols, the solubility enhancer, and the tinidazole to form a second solution; and c) adding the second solution to the first solution.

- [0041] In certain embodiments, the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block.
- [0042] In certain embodiments, the hydrophobic block is poly(propylene oxide).
- [0043] In certain embodiments, the hydrophilic block is poly(ethylene oxide).
- [0044] In certain embodiments, the thermoreversible gelling agent is poloxamer 407.
- [0045] In some embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols comprise a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol.
- [0046] In some embodiments, the first and second pharmaceutically acceptable C₁-C₇ alcohols are independently selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.
- [0047] In certain embodiments, the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.
- [0048] In some embodiments, the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol. In certain embodiments, the isopropanol is a 60% (w/w) solution in water.
- [0049] In some embodiments, the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide. In certain embodiments, the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.
- [0050] In some embodiments, the water comprises a mixture of deionized water and a buffered aqueous solution. In particular embodiments, the buffered aqueous solution is citrate buffer.
- [0051] In certain embodiments, the formulation has a pH of from about 3 to about 5. In other embodiments, the formulation has a pH of from about 3.5 to about 4.5.
- [0052] The present disclosure further provides a process for the preparation of a tinidazole gel formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the process comprising a) dissolving a thermoreversible gelling agent

in water to form a first solution; b) combining one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and tinidazole to form a second solution; and c) adding the second solution to the first solution.

- [0053] In certain embodiments, the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block.
- [0054] In some embodiments, the hydrophobic block is poly(propylene oxide).
- [0055] In some embodiments, the hydrophilic block is poly(ethylene oxide).
- [0056] In some embodiments, the thermoreversible gelling agent is poloxamer 407.
- [0057] In some embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols comprise a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol.
- [0058] In some embodiments, the first and second pharmaceutically acceptable C₁-C₇ alcohols are independently selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.
- [0059] In some embodiments, the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.
- [0060] In certain embodiments, the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol. In some embodiments, the isopropanol is a 60% (w/w) solution in water.
- [0061] In certain embodiments, the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide. In some embodiments, the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.
- [0062] In some embodiments, the water comprises a mixture of deionized water and a buffered aqueous solution. In certain embodiments, the buffered aqueous solution is citrate buffer.
- [0063] In some embodiments, the formulation has a mean tinidazole receiver fluid concentration at 2 hours of from about 1.5 mM to about 3.5 mM.
- [0064] In other embodiments, the formulation has a mean tinidazole receiver fluid concentration at 4 hours of from about 3 mM to about 6 mM.
- [0065] In other embodiments, the formulation has a mean tinidazole receiver fluid concentration at 2 hours of from about 2.3 mM to about 2.6 mM.

[0066] In some embodiments, the formulation has a mean tinidazole receiver fluid concentration at 4 hours of from about 4 mM to about 5.5 mM.

[0067] The present disclosure further provides a tinidazole gel formulation prepared by any of the processes described herein.

[0068] The present disclosure further provides a tinidazole gel formulation, wherein the formulation does not inhibit or only minimally inhibits lactobacillus strains at a tinidazole concentration sufficient to inhibit *Gardnerella vaginalis* strains associated with bacterial vaginosis.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0069] The foregoing summary, as well as the following detailed description of the embodiments, will be better understood when read in conjunction with the appended drawings. For the purpose of illustration, the drawings may describe the use of specific embodiments. It should be understood, however, that the formulations described herein are not limited to the precise embodiments discussed or described in the figures.

[0070] **Figure 1** describes the standard for visually evaluating rabbit vaginal tissue exposed to the formulations described herein.

[0071] **Figure 2** describes the observed effects of vehicle formulation V1 on external rabbit vaginal tissue.

[0072] **Figure 3** describes the observed effects of vehicle formulation V2 on external rabbit vaginal tissue.

[0073] **Figure 4** describes the observed effects of vehicle formulation V3 on external rabbit vaginal tissue.

[0074] **Figure 5** describes the observed effects of formulation F1 on external rabbit vaginal tissue.

[0075] **Figure 6** describes the observed effects of formulation F2 on external rabbit vaginal tissue.

[0076] **Figure 7** describes the observed effects of formulation F3 on external rabbit vaginal tissue.

[0077] **Figure 8** describes the evaluation standard for histopathological analysis of explanted rabbit vaginal tissue.

- [0078] **Figures 9a and 9b** describe the observation and results of histopathological evaluation of rabbit vaginal explants previously treated with vehicle formulation V1.
- [0079] **Figures 10a and 10b** describe the observation and results of histopathological evaluation of rabbit vaginal explants previously treated with vehicle formulation V2.
- [0080] **Figures 11a and 11b** describe the observation and results of histopathological evaluation of rabbit vaginal explants previously treated with vehicle formulation V3.
- [0081] **Figures 12a and 12b** describe the observation and results of histopathological evaluation of rabbit vaginal explants previously treated with formulation F1.
- [0082] **Figures 13a and 13b** describe the observation and results of histopathological evaluation of rabbit vaginal explants previously treated with formulation F2.
- [0083] **Figures 14a and 14b** describe the observation and results of histopathological evaluation of rabbit vaginal explants previously treated with formulation F3.
- [0084] **Figure 15** is a graphical representation of the mean \pm SD of tinidazole released from formulations F1, F2, and F3 in Franz cell studies.
- [0085] **Figure 16a** is a graphical representation of a culture well and insert used in the EpiVaginal™ tissue studies described herein.
- [0086] **Figure 16b** is an example of a multi-well culture plate representative of the types of multi-well culture plates that can be used in the EpiVaginal™ tissue studies described herein.

DETAILED DESCRIPTION OF THE INVENTION

- [0087] The articles "a," "an," and "the" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.
- [0088] The phrase "substantially free of" as used herein means less than 5%, less than 2.5%, less than 1%, less than 0.1%, less than 0.01%, or less than 0.001% of a given component in the formulations described herein.
- [0089] As used herein, "administration" or "administering" to a subject includes, but is not limited to, the act of a physician or other medical professional prescribing a pharmaceutical composition of the invention for a subject.
- [0090] Throughout the specification, the present disclosure provides certain ranges and certain sub-ranges within those ranges. The identification of certain sub-ranges

notwithstanding, the present disclosure should be read to include and disclose all sub-ranges within a given range (or sub-range). For example, if a range of 1 to 20 is described herein and a sub-range of 5 to 15 is also described, the present disclosure should be understood to include and disclose all other sub-ranges within the originally defined range and sub-range, e.g., 1 to 5, 7 to 12, 15 to 20, 2 to 18, etc.

[0091] The present disclosure provides novel aqueous tinidazole formulations suitable for intravaginal administration to a subject in need thereof. In particular embodiments, the formulations can comprise water, tinidazole or an active metabolite thereof, a thermoreversible gelling agent, one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives. In certain embodiments, the formulations can further include a polyol, such as, but not limited to polyethylene glycol, an optional penetration enhancer, or an optional excipient such as HPMC. In particular embodiments, the formulations can be completely or substantially free of boric acid and/or ethylenediaminetetraacetic acid (EDTA). In certain embodiments, the formulations can be free or substantially free of a second active ingredient, such that tinidazole is the only active ingredient in the formulations.

[0092] An important characteristic of the formulations described herein are their thermoreversible nature. In particular embodiments, the formulations exist as a viscous liquid at room temperature (i.e. about 20°C to about 23°C). At higher temperatures, though, the formulations can exist as a gel. In particular embodiments, the formulations can be a gel at or close to body temperature (i.e. about 35°C to about 37°C). Without wishing to be bound to any particular theory, it is believed that gel formation at higher temperature is a useful property because it allows the formulations to flow into the vaginal cavity and to reach the smallest irregularities of the mucosal surface where they subsequently gel and adhere to biological surfaces such as the vaginal epithelia.

[0093] Although using thermoreversible gels for the prevention of STDs was previously disclosed, *see, e.g.* WO 97/42962 and WO 99/53897 (both of which are hereby incorporated by reference in their entirety), neither publication teaches the use of tinidazole. Moreover it has now been unexpectedly discovered that the percentage of tinidazole in a formulation including a thermoreversible gelling agent can be significantly increased using the formulations disclosed herein.

[0094] In certain embodiments, tinidazole can be used in the formulations described herein at any suitable concentration. In other embodiments, the concentration of tinidazole can be about 0.1% to about 2% (w/w), about 0.5% to about 2% (w/w), or about 0.75% to about 2% (w/w). In particular embodiments, the concentration of tinidazole can be about 0.75 to about 1.5% (w/w). In specific embodiments, the concentration of tinidazole can be about 1%, about 1.25%, or about 1.5% (w/w). The formulations can also include an active metabolite of tinidazole at any suitable concentration. Known active metabolites of tinidazole include, but are not limited to 1-(2-(ethylsulfonyl)ethyl)-2-methyl-4-nitro-1*H*-imidazol-5-ol and 2-hydroxymethyl tinidazole.

[0095] In certain embodiments, the thermoreversible gelling agent can be present in the formulations described herein at any suitable concentration. In certain embodiments, the thermoreversible gelling agent can be at a concentration of about 5% to about 50% (w/w) and in certain embodiments, at a concentration of about 15% to about 35% (w/w). In particular embodiments, the thermoreversible gelling agent can be present at a concentration of about 15% to about 25% (w/w). In certain embodiments, the thermoreversible gelling agent can be present in an amount ranging from about 15% to about 25% (w/w); in an amount ranging from about 17% to about 22% (w/w); or in an amount ranging from about 18% to about 20% (w/w). In certain embodiments, the thermoreversible gelling agent can be present at a concentration of about 18% (w/w), about 19% (w/w), or about 20% (w/w). Exemplary thermoreversible gelling agents include, but are not limited to, triblock copolymers having a central hydrophobic block flanked on each side with a hydrophilic block. In certain embodiments the hydrophobic block can be a block of poly(propylene oxide). In certain embodiments, the hydrophilic blocks can be blocks of poly(ethylene oxide). In particular embodiments, the thermoreversible gelling agent can be selected from the group consisting of poloxamer 407 (CAS 9003-11-6), poloxamer 124, poloxamer 188, poloxamer 237, poloxamer 338. In specific embodiments, the thermoreversible gelling agent is poloxamer 407.

[0096] The one or more pharmaceutically acceptable C₁-C₇ alcohols can be present in the formulations at any suitable concentration and can include any appropriate straight chain, branched, cyclic, or aromatic alcohol having the designated number of carbons. Exemplary pharmaceutically acceptable C₁-C₇ alcohols include, but are not limited to, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, and benzyl alcohol. In certain

embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols can be present at a concentration of about 2% to about 20% (w/w). In other embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols can be present at a concentration of about 5% to about 15% (w/w). In particular embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols can be present from about 8% to about 11% (w/w).

[0097] In certain embodiments, the one or more pharmaceutically acceptable C₁-C₇ alcohols can include one to ten, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 pharmaceutically acceptable C₁-C₇ alcohols. In certain embodiments, the formulations can include a first C₁-C₇ alcohol and a second C₁-C₇ alcohol. In certain embodiments the first C₁-C₇ alcohol can be present at a concentration of about 2.25% to about 3.25% (w/w). In certain embodiments, the first C₁-C₇ alcohol can be present in an amount of about 2.5%, about 2.9%, or about 3.2% (w/w). In other embodiments, the second C₁-C₇ alcohol can be present at a concentration of about 10% to about 12% (w/w), and in certain embodiments can be about 6%, about 6.5%, or about 7.2% (w/w) of the formulation. In certain embodiments, the first C₁-C₇ alcohol can be benzyl alcohol. In certain embodiments, the second C₁-C₇ alcohol can be isopropyl alcohol. In certain embodiments, the isopropyl alcohol can be a 60% solution (w/w) in water.

[0098] The formulations can also include a solubility enhancer, useful for enhancing the solubility of tinidazole in the formulation. Exemplary solubility enhancers include, but are not limited to, mono and di- alkyl ethers of isosorbide. In particular embodiments, the solubility enhancer is dimethylisosorbide ("DMI"). The solubility enhancer can be present in the formulations at any suitable concentration; however, in certain embodiments it can be present at a concentration of about 10% to about 15% (w/w). In other embodiments, the solubility enhancer can be present at a concentration of about 11.5%, about 12.7%, or about 14% (w/w).

[0099] The formulations can further include an optional penetration enhancer. The optional penetration enhancer can be used in the formulations in any suitable concentration; however in certain embodiments, it can be present in the formulations in a range of about 0.01% to 15% (w/w). Exemplary penetration enhancers include, but are not limited to terpenes and terpenoids (such as menthol and the like); pyrrolidones (such as N-methyl-2-pyrrolidone, 1-dodecylazacycloheptan-2-one, and the like); sulfoxides (such as DMSO and the like); phospholipids; cyclodextrins such as, but not limited to, β -cyclodextrin; dodecyl-N,N-dimethylamino acetate; clofibric acid; and amino acid derivatives such as, but not

limited to, dodecyl *N,N*-dimethylamino isopropionate. While in certain embodiments the optional penetration enhancer can be present, in other embodiments, the penetration enhancer can be completely or substantially absent.

[0100] The formulations can optionally include one or more preservatives in a pharmaceutically acceptable amount. Suitable exemplary preservatives include, but are not limited to, methylparaben, propylparaben, BHA, BHT, and combinations of the foregoing. In certain embodiments, the formulations can comprise less than about 1% (w/w), less than about 0.5% (w/w), or less than about 0.05% (w/w) of the formulation.

[0101] In typical embodiments, the remainder of the formulations comprises water. As used herein "water" refers to sterile deionized water, buffered aqueous solutions comprising sterile deionized water, or any combination of the foregoing. The amount of water in the formulations can be at least about 10%, 20%, 30%, 40%, 45%, 47%, 50%, 55%, 58%, 60%, 61%, 65% or at least about 70% (w/w). In certain embodiments, the amount of water in the formulations can range from about 40% (w/w) to about 70% (w/w), from about 50% to about 70% (w/w), or from about 50% to about 65% (w/w). In particular embodiments, the amount of water in the formulations can be about 54.4% (w/w), about 57.6% (w/w), or about 61% (w/w).

[0102] Buffered aqueous solutions can contain one or more pharmaceutically acceptable buffering agents suitable for maintaining the pH of the formulations in a range of about 3 to about 5, and in certain embodiments, about 3.5 to about 4.5. Suitable buffering agents and systems are well known to those of ordinary skill in the art; however in certain embodiments, the buffering agent is a citrate buffer. Other possible buffering agents include, but are not limited to, veronal acetate buffer, and acetate buffer. In particular embodiments, the pH of the formulations is buffered to a pH of about 4 to about 4.5.

[0103] The viscosity of the formulations can range from about 1 cP to about 100,000 cP, about 1 cP to about 50,000 cP, about 1 cP to about 10,000 cP, or about 1 cP to about 1,000 cP in its ungelled state when measured using a Brookfield DV-II+ Pro Extra LV™ viscometer with spindle type 63 at 100 RPM at about 21°C to about 22°C. In particular embodiments, the ungelled formulations can have a viscosity of about 1 to about 900 cP. In certain embodiments, the ungelled formulations can have a viscosity of about 200 cP to about 750 cP, and in other embodiments, the ungelled formulations can have a viscosity of about 300 cP to about 550 cP. In particular embodiments, the composition can have a viscosity of about

315 cP to about 345 cP. In other embodiments, the ungelled formulations can have a viscosity of about 420 cP to about 460 cP. In still other embodiments, the formulations can have a viscosity in the ungelled state of about 480 cP to about 550 cP.

[0104] In certain embodiments, the formulations can have a temperature of gelation (T_{gel}) of about 18 to about 37°C. In other embodiments, the formulations can have a T_{gel} of about 22 to about 35°C. In still further embodiments, the formulations can have a T_{gel} of about 30°C to about 35°C. And in still other embodiments, the formulations can have a T_{gel} of about 32°C.

[0105] In addition to providing the formulations discussed above, the present disclosure also provides methods for treating BV, trichomoniasis, or other disease or disorder suitable for treatment via the intravaginal administration of a tinidazole. Suitable methods for treating BV include intravaginally administering the formulations to a subject in need thereof. Exemplary subjects include mammals, preferably humans. In certain embodiments, the formulations can be administered intravaginally once a day for a period of up to about 30 days. However, in other embodiments, the formulations can be administered chronically over a period of months or years.

[0106] In certain embodiments, the formulations can be administered intravaginally twice a day for a period of up to about 30 days. In specific embodiments, the formulations can be administered intravaginally once or twice a day for a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days. In some embodiments, the formulations can be administered intravaginally once or twice a day every other day, once or twice a day every third day, once or twice a day every fourth day, once or twice a day every fifth day, once or twice a day every sixth day, once or twice a day once a week, once or twice a day one time every other week, once or twice a day one time every third week, once or twice a day once a month. In certain embodiments, the formulations can be administered intravaginally in a single application, *i.e.*, in the substantial absence of further dosing until such time as symptoms recur or until the subject is directed by a physician to resume treatment.

[0107] The present formulations also have surprising stability at low temperature. For example, in certain embodiments, the formulations will show little or no tinidazole crystallization when chilled to as low as about 6°C for 24 hours and then warmed to 22 C for 24 hours. In other embodiments, the formulations will show little or no tinidazole

crystallization when chilled to as low as about 14°C for 24 hours and then warmed to 22°C for 24 hours.

- [0108] The present formulations similarly exhibit unexpected long-term stability at 22°C, showing little or no tinidazole crystallization at 1, 2, and in certain embodiments 2.6 or 2.8 months. In other embodiments the formulations can be stable for at least about 10 months, at least about 12 months, and in other embodiments, at least about 18 months.
- [0109] The present formulations have similarly shown a surprising lack of irritation in a rabbit vaginal model, as described in detail below.
- [0110] In addition to the properties noted above, the formulations described herein can exhibit a "Franz cell flux." As used herein, a "Franz cell flux" refers to the rate at which tinidazole in the formulations described herein crosses a polycarbonate membrane having 0.40 μm pores and an area of 1.77 cm^2 in a Franz cell diffusion study at 37°C wherein the receiver fluid is 0.05 M citrate buffer (pH 3.3). In some embodiments, Franz cell flux can range from about 2 to about 15 ($\mu\text{mol}/\text{cm}^2$)/ \sqrt{t} , where t is measured in hours (" $\sqrt{t}(\text{h})$ "). In other embodiments, the Franz cell flux can range from about 4 to about 10 ($\mu\text{mol}/\text{cm}^2$)/ \sqrt{t} . In still further embodiments, the Franz cell flux can range from about 6 to about 9 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$. In particular embodiments, the Franz cell flux can be about 6, about 7.5, or about 8.7 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$. In particular embodiments, the formulations can be any of formulations F1, F2, or F3 described elsewhere herein.
- [0111] In other embodiments, Franz cell flux can range from 2 to 15 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$. In other embodiments, the Franz cell flux can range from 4 to 10 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$. In still further embodiments, the Franz cell flux can range from 6 to 9 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$. In particular embodiments, the Franz cell flux can be 6, 7.5, or 8.7 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t}(\text{h})$. In particular embodiments, the formulations can be any of formulations F1, F2, or F3 described elsewhere herein.
- [0112] The formulations described herein can further exhibit a mean flux rate in EpiVaginal™ tissue culture. The "mean flux rate in EpiVaginal™ tissue culture" refers to the rate at which tinidazole in a 100 μL aliquot of the formulations described herein at 37°C crosses a full thickness tissue culture produced from normal human-derived vaginal-ectocervical epithelial cells (VEC-100-FT EpiVaginal™ tissue available from MatTek Corporation, Ashland, MA), mounted on polycarbonate membranes having 0.4 μm pores,

wherein the culture medium (alternatively referred to as the receiver fluid) is 0.5 ml VEC-100-ASY available from MatTek Corporation (Ashland, MA).

- [0113] In particular embodiments, the mean flux rate (nmol/cm²/min) can range from about 15 nmol/cm²/min to about 30 nmol/cm²/min. In other embodiments, the mean flux rate can range from about 15 nmol/cm²/min to about 25 nmol/cm²/min. In other embodiments, the mean flux rate can range from about 17 nmol/cm²/min to about 25 nmol/cm²/min. In other embodiments, the mean flux rate can range from about 18 nmol/cm²/min to about 22.5 nmol/cm²/min. In particular embodiments, the mean flux rate can be about 18, about 19, or about 22.5 nmol/cm²/min.
- [0114] In other embodiments, the mean flux rate (nmol/cm²/min) can range from 15 nmol/cm²/min to 30 nmol/cm²/min; from 15 nmol/cm²/min to 25 nmol/cm²/min; from 17 nmol/cm²/min to 25 nmol/cm²/min; or from 18 nmol/cm²/min to 22.5 nmol/cm²/min. In particular embodiments, the mean flux rate can be 18, 19, or 22.5 nmol/cm²/min.
- [0115] The formulations described herein can also have a mean tinidazole receiver fluid concentration. The "mean tinidazole receiver fluid concentration" is the concentration of tinidazole in receiver fluid at a given time point after application of a 100 μ L aliquot of the formulations described herein at 37°C to full thickness tissue culture produced from normal human-derived vaginal-ectocervical epithelial cells (VEC-100-FT EpiVaginal™ tissue available from MatTek Corporation, Ashland, MA), mounted on polycarbonate membranes having 0.4 μ m pores, wherein the culture medium/receiver fluid is VEC-100-ASY available from MatTek Corporation (Ashland, MA).
- [0116] In particular embodiments, the mean tinidazole receiver fluid concentration after 1 hour can be about 0.5 mM to about 2.5 mM. In other embodiments, the mean tinidazole receiver fluid concentration after 1 hour can be about 1 mM to about 2 mM. In still other embodiments, the mean tinidazole receiver fluid concentration after 1 hour can be about 1.14 mM to about 1.74 mM. In particular embodiments, the mean tinidazole receiver fluid concentration after 1 hour can be about 1.14 mM, about 1.5 mM, or about 1.74 mM.
- [0117] In other embodiments, the mean tinidazole receiver fluid concentration after 1 hour can range from 0.5 mM to 2.5 mM; from 1 mM to 2 mM; or from 1.14 mM to 1.74 mM. In particular embodiments, the mean tinidazole receiver fluid concentration after 1 hour can be 1.14 mM, 1.5 mM, or 1.74 mM.

- [0118] In particular embodiments, the mean tinidazole receiver fluid concentration after 2 hours can be about 1.5 mM to about 3.5 mM. In other embodiments, the mean tinidazole receiver fluid concentration after 2 hours can be about 2 mM to about 3 mM. In still other embodiments, the mean tinidazole receiver fluid concentration after 2 hours can be about 2.3 mM to about 2.6 mM. In particular embodiments, the mean tinidazole receiver fluid concentration after 2 hours can be about 2.3 mM, about 2.9 mM, or about 2.6 mM.
- [0119] In other embodiments, the mean tinidazole receiver fluid concentration after 2 hours can range from 1.5 mM to 3.5 mM; from 2 mM to 3 mM; or from 2.3 mM to 2.6 mM. In particular embodiments, the mean tinidazole receiver fluid concentration after 2 hours can be 2.3 mM, 2.9 mM, or 2.6 mM.
- [0120] In other embodiments, the mean tinidazole receiver fluid concentration after 4 hours can be about 3.5 mM to about 6 mM. In other embodiments, the mean tinidazole receiver fluid concentration after 4 hours can be about 4 mM to about 5.5 mM. In particular embodiments, the mean tinidazole receiver fluid concentration after 4 hours can be about 4.4 mM, about 5.6 mM, or about 5.1 mM.
- [0121] In other embodiments, the mean tinidazole receiver fluid concentration after 4 hours can range from 3.5 mM to 6 mM or from 4 mM to 5.5 mM. In particular embodiments, the mean tinidazole receiver fluid concentration after 4 hours can be 4.4 mM, 5.6 mM, or 5.1 mM.
- [0122] The formulations described herein can also have a concentration dependent mean peak tinidazole concentration in EpiVaginal™ tissue ("mean peak concentration"). The mean peak concentration is the average maximum concentration of tinidazole in full thickness tissue culture produced from normal human-derived vaginal-ectocervical epithelial cells (VEC-100-FT EpiVaginal™ tissue available from MatTek Corporation, Ashland, MA) after application of a 100 μ L aliquot of the formulations described herein at 37°C for a period of time sufficient to achieve the maximum tinidazole concentration in the tissue, wherein the tissue is mounted on polycarbonate membranes having 0.4 μ m pores, wherein the culture medium/receiver fluid is VEC-100-ASY available from MatTek Corporation (Ashland, MA).
- [0123] In particular embodiments, the mean peak concentration can range from about 1500 ng tinidazole/mg tissue to about 4500 ng tinidazole/mg tissue. In other embodiments, the mean peak concentration can range from about 1800 to about 4200 ng tinidazole/mg tissue. In still further embodiments, the mean peak concentration can range from about 2000

ng tinidazole/mg tissue to about 4000 ng tinidazole/mg tissue. In certain embodiments, the mean peak concentration can be about 2000 ng tinidazole/mg tissue, about 2700 ng tinidazole/mg tissue, or about 3400 ng tinidazole/mg tissue.

[0124] In other embodiments, the mean peak concentration can range from 1500 ng tinidazole/mg tissue to 4500 ng tinidazole/mg tissue. In other embodiments, the mean peak concentration can range from 1800 to 4200 ng tinidazole/mg tissue. In still further embodiments, the mean peak concentration can range from 2000 ng tinidazole/mg tissue to 4000 ng tinidazole/mg tissue. In certain embodiments, the mean peak concentration can be 2000 ng tinidazole/mg tissue, 2700 ng tinidazole/mg tissue, or 3400 ng tinidazole/mg tissue.

[0125] In a further embodiment, the formulation can have a mean peak tinidazole concentration that satisfies the formula:

$$y = 2834x - 804.83$$

over a range of tinidazole concentrations of from about 0.8 % (w/w) to about 1.8 % (w/w), wherein y is the mean peak concentration of tinidazole measured in the culture and x is the concentration of tinidazole in the formulation.

Examples

[0126] The formulations and methods are now further detailed with reference to the following examples. These examples are provided for the purpose of illustration only and the formulations and methods described herein should in no way be construed as being limited to these examples. Rather, the formulations should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

[0127] The 60% IPA solution (w/w) described below was prepared by adding 600 g of IPA USP and 400 g of water USP to a 1.0 L vessel and mixing the combined solution well.

[0128] The 0.05 M citric acid buffer solution used and described in the examples below was prepared as follows. 21.016 g of citric acid monohydrate was added to a 2.0 L vessel with 2000 mL of water ("solution 1"). In a separate 2 L vessel, 25.806 g of sodium citrate (anhydrous) was mixed with 2000 mL of water until the material was dissolved ("solution 2"). Finally, 550 mL of solution 1 and 450 mL of solution 2 were combined and mixed in an appropriately sized vessel. The pH of the resulting solution was measured at 3.30 using a standard pH electrode.

Example 1: Tinidazole Gel Formulations

[0129] Formulations having 1%, 1.25%, and 1.5% tinidazole as shown in Table 1, below, were prepared according to the following general procedure.

[0130] An appropriate quantity of poloxamer 407 and citrate buffer were combined in an appropriate vessel to give a suspension. The vessel was then placed in an ice-water bath and the suspension was mixed at 400 rpm for 6 hours to dissolve all of the poloxamer. Upon complete dissolution, the clear solution was warmed to room temperature (22°C) and this temperature was maintained with a water bath.

[0131] Next, the appropriate quantities of 60% IPA, benzyl alcohol, DMI and tinidazole were combined in an appropriately sized vessel. The resulting mixture was then heated at 37°C for approximately 30 minutes until all of the tinidazole was dissolved. Once dissolved, the solution was then cooled to room temperature and added slowly and with agitation to the poloxamer solution. The resulting mixture was then stirred at low RPM until uniform. After stirring, the mixing apparatus was removed and the resulting material was allowed to settle for 24 hours to allow trapped air to bubble to the surface.

TABLE 1

Formulation #	Poloxamer 407 (g)/(% w/w)	Citrate Buffer (g)/(% w/w)	Benzyl Alcohol (g)/(% w/w)	60% IPA (g)/(% w/w)	DMI (g)/(%w/w)	Tinidazole (g)/ (% w/w)
F1	10.00 (20.00)	24.79 (49.58)	1.56 (3.12)	6.00 (12.00)	6.90 (13.80)	0.750 (1.50)
F2	9.50 (19.00)	26.62 (53.24)	1.43 (2.86)	5.50 (11.00)	6.33 (12.6)	0.625 (1.25)
F3	9.00	28.50	1.25 (2.50)	5.00	5.75	0.500

	(18.00)	(57.00)		(10.00)	(11.50)	(1.00)
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Example 2

[0132] Formulations 4 and 5, described in Table 2, below, were prepared according to the following general procedure.

[0133] An appropriate quantity of poloxamer 407 and citrate buffer were combined in an appropriate vessel to give a suspension. The vessel was then sealed and placed on a shaker rotating at ~160 rpm in a cold room at 4°C and was left shaking overnight until complete dissolution of the poloxamer was observed. The resulting clear solution was allowed to stand at 4°C for a few hours to remove air bubbles present in the solution. The poloxamer solution was then placed in an incubator at 18°C for approximately 30 min.

[0134] Next, the appropriate quantities of DMI, 70% IPA, benzyl alcohol, and tinidazole were combined in an appropriately sized vessel. The resulting mixture was heated at 37°C for approximately 10 min until complete dissolution of the tinidazole was observed. The clear solution was then cooled to room temperature (22°C) and was added slowly with agitation (shaking) to the poloxamer solution at room temperature (22°C) until a uniform clear solution was obtained. The final formulations were allowed to stand at room temperature (22°C) to remove air bubbles.

TABLE 2

Formulation #	26% Poloxamer Gel Soln (g)	DMI (ml)	70% IPA (ml)	Benzyl Alcohol (ml)	Citrate Buffer (ml)	Tinidazole (mg)	Tinidazole %
F4	14.23	2.2	2.4	0.55	0.37	250	1.25
F5	14.23	2.0	2.2	0.50	0.87	200	1.00

[0135] The cold temperature stability of formulations 4 and 5 was tested by cooling the formulations to a specified temperature for 24 hours and then warming the formulations to room temperature (22°C) where they were allowed to stand for a further 24 hours. During

the temperature challenge, the formulations were visually evaluated for the formation of crystals either at low temperature or upon subsequent warming. The results are shown in Table 3.

TABLE 3

Formulation #	Temp (°C)			
	18	14	10	6
F4	No crystals	No crystals	Crystals	Crystals
F5	No crystals	No crystals	No crystals	No crystals

[0136] This data demonstrates that the formulations can be subjected to relatively cool temperatures and maintain tinidazole in solution, even at high tinidazole concentrations.

Example 3: *In Vitro* Drug Release

[0137] To determine whether tinidazole would be released upon intravaginal administration, an *in vitro* test (membrane-free model) simulating formulation erosion in the vaginal cavity was conducted. Two different tinidazole 1.25% gel formulations were prepared (F6 = 18.6% gel, 1.75% hydroxypropyl methylcellulose (HPMC), $T_{melt} = 31^{\circ}\text{C}$; F7 = 19.01% gel, no HPMC, $T_m = 29^{\circ}\text{C}$). Specifically, 1 g of each formulation was added to a pre-weighed vial equilibrated at 37°C . Next, 0.5 ml of deionized water was slowly added to the top of the vial and the vial was allowed to stand for 2 hours, 6 hours, 24 hours, or 4 days. After the appropriate amount of time passed, the liquid supernatant sitting on top of the formulation was carefully removed, weighed, and analyzed for its tinidazole concentration using standard HPLC methodology. All studies were conducted in triplicate to ensure quality data. This membrane-free system is a suitable choice to mimic natural gel erosion in the vaginal cavity.

[0138] These studies showed that the formulations described herein are susceptible to gradual erosion/dissolution with minimal or no agitation.

Example 4: Vaginal Irritation in Rabbits

[0139] Formulations F1, F2, and F3 (1 mL) (as described in Example 1) and Vehicle Formulations V1, V2, and V3 (as described below in Table 4) were each administered into the vaginal vaults of 3 female test rabbits (New Zealand White strain) for five consecutive days. The formulations were administered with a blunt tipped animal-feeding needle attached to a 3 cc syringe. Control animals were dosed with 1 mL of a 0.9% saline solution under identical conditions.

TABLE 4 - Vehicle Formulations

	Poloxamer (g)/ (% w/w)	Citrate Buffer (g)/ (% w/w)	Benzyl Alcohol (g)/ (% w/w)	60% IPA (g)/ (% w/w)	DMI (g)/ (% w/w)	Tinidazole (g)/(% w/w)
V1	10.00 (20.00)	40.00 (80.00)	N/A	N/A	N/A	N/A
V2	10.00 (20.00)	25.54 (51.10)	1.56 (3.12)	6.00 (12.00)	6.90 (13.80)	N/A
V3	8.75 (17.50)	30.45 (60.90)	1.13 (2.26)	4.50 (9.00)	5.18 (10.36)	N/A

[0140] Prior to the first dose of the control, vehicle, or active formulations, and before each daily dose thereafter, the external vaginal tissues of each animal was observed for erythema, exudate, and edema according to the scoring system shown in Figure 1. The results of the daily monitoring of the external vaginal tissue are shown in Figures 2 (V1), 3 (V2), 4 (V3), 5 (Formulation 1), 6 (Formulation 2), and 7 (Formulation 3). As can be seen in the Figures, each of the formulations caused minimal or no visual changes in the external vaginal tissue of the subject animals.

[0141] Following completion of the protocol, the animals were sacrificed with sodium pentobarbital and the vaginal tissue of each animal was carefully removed. The tissue was then observed macroscopically and scored for irritation and injury to the epithelial layer. Following macroscopic observation, the tissue was sectioned to provide the cervical, central, and caudal portions. The samples were then individually placed in 10% neutral buffered formalin and submitted for histopathological evaluation. Samples were evaluated based on the criteria shown in Figure 8.

[0142] Specific results, corresponding to formulations V1, V2, V3, F1, F2, and F3 are shown in Figures 9a and 9b through Figures 14a and 14b, respectively. Overall, each of the vehicle and drug-containing formulations showed minimal observable effects, suggesting that the formulations are suitable for intravaginal administration despite the propensity for the individual components of the formulations to cause irritation.

Example 5: Isolation And Identification Of Bacterial Strains Associated With Bacterial Vaginosis

[0143] To obtain clinical isolates from women suspected of having bacterial vaginosis, 192 microscope slides from 192 vaginal swabs (obtained from women consulting in the Quebec City area) submitted for Nugent scoring were analyzed. Based on microscopic observations of bacterial cell morphology and Nugent score, 46 swabs were selected for bacterial isolation.

[0144] Bacterial isolates were identified by polymerase chain reaction (PCR) and DNA sequencing of the 16S rRNA gene. A total of 30 swabs were positive for at least one isolate of *Bacteroides* spp., *Gardnerella vaginalis*, *Peptostreptococcus* spp., *Prevotella* spp., *Lactobacillus* spp., or *Mobiluncus* spp. Isolates from all bacterial strains of interest were identified, resulting in 39 strains in total. Isolates were frozen and stored for later use.

Example 6: Antimicrobial Susceptibility Testing

[0145] Reference type strains (8 strains total) were thawed and tested against the formulations described herein. After initial culture of the selected frozen preserved bacteria from Example 5, and 2 additional passages (sub-cultures) - (total = 3 passages) on blood agar and colony suspension in saline, the saline suspension was used to inoculate the surface of Brucella Agar with 5% Sheep Blood. Small (6 mm) discs with 10 μ L of formulations F1, F2, or F3 and control gel formulation were centered on the agar plates and incubated at 35°C

under anaerobic conditions. The diameter of the inhibition zone in mm was measured after 48 to 96 hours of incubation, depending on bacterial strains. Testing was conducted in triplicate.

[0146] Testing showed that a specific *Lactobacillus jensenii* strain and *Mobiluncus curtisii* subsp. *Holmesii* strain were not affected by the presence of formulations F1, F2, and F3. But *B. fragilis*, *G. vaginalis*, *L. crispatus*, *M. mulieris*, *P. anaerobius*, and *P. bivia* were each inhibited by the formulations described herein, with inhibition zones varying from about 20 to about 60 mm. No inhibition was observed using control formulations F1, F2, and F3 prepared without tinidazole.

Example 7: MIC Determination

[0147] Minimum inhibitory concentrations were determined for 39 clinical isolates and 8 reference strains as described below.

[0148] A hemin stock solution (5 mg/mL) was prepared by dissolving 0.1 g of hemin in 2 mL of 1.0 N NaOH. Next, the volume was adjusted to 20 mL with distilled water and the mixture was sterilized at 121°C for 15 minutes.

[0149] A stock vitamin K solution (10 mg/mL) was prepared by combining 0.2 mL of vitamin K₁ with 20 ml of 95% ethanol. A working solution (1 mg/ml) was prepared by combining 1 ml of the stock vitamin K solution with 9 ml sterile deionized water.

[0150] Laked sheep blood was prepared by freezing defibrinated sheep blood at -80°C. The blood was then thawed slowly at 2 to 8°C overnight. The thawed blood was mixed thoroughly before use and warmed in a 48 to 50°C water bath immediately before adding to a molten brucella agar base.

[0151] Serial dilutions of formulations F1 (1.5% tinidazole), F2 (1.25% tinidazole), and F3 (1.0% tinidazole) were prepared at the following concentrations – F3: 1,000 µg/ml; 500 µg/ml; 250 µg/ml; 125 µg/ml; 62.5 µg/ml; 31.25 µg/ml; and 15.63 µg/ml; F2: 1,250 µg/ml; 625 µg/ml; 312.5 µg/ml; 156.25 µg/ml; 78.125 µg/ml; 39.06 µg/ml; and 19.53 µg/ml; F1: 1,500 µg/ml; 750 µg/ml; 375 µg/ml; 187.5 µg/ml; 93.75 µg/ml; 46.875 µg/ml; 23.44 µg/ml; and 11.72 µg/ml.

[0152] Brucella laked blood agar with vitamin K₁ and hemin was prepared as follows. 28 g of brucella broth powder was combined with 15 g of agar and 1 ml each of the hemin stock solution and vitamin K working solution in 1 L distilled water. The solution was boiled to dissolve the agar and subsequently autoclaved at 121 °C for 15 minutes. The resulting agar solution was then cooled to about 50 °C and 50 ml of sterile laked sheep blood was added.

Next, 2 ml of each of a given serial dilution of formulation F1, F2, or F3 (as described above) was combined with 18 ml of the laked sheep blood containing agar solution. The resulting solution was then added to a 15 x 100 mm Petri dish and allowed to solidify and prereduce in an anaerobic chamber for at least 24 hours. This gave tinidazole infused agar plates having tinidazole concentrations of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, and 1.563 µg/ml (F3); 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.63 µg/ml, 7.81 µg/ml, 3.91 µg/ml, and 1.95 µg/ml (F2); and 150 µg/ml, 75 µg/ml, 37.5 µg/ml, 18.75 µg/ml, 9.37 µg/ml, 4.69 µg/ml, and 2.34 µg/ml (F1).

[0153] As in Example 6, cultures of the frozen preserved bacteria from Example 5, were grown on blood agar for 24 to 96 hours. Colonies of similar morphology were selected and suspended in prereduced brucella broth to achieve a turbidity equivalent to 0.5 McFarland.

[0154] The resulting suspension was then used to inoculate the tinidazole infused and control agar plates in triplicate. MIC was determined by comparing control plates (not infused with tinidazole) against each of the inoculated tinidazole-infused plates. MIC was read as the minimum concentration at which a marked reduction in bacterial growth was observed. The data is reported in Table 5.

TABLE 5

Bacteria	MIC (µg/ml)
<i>Bacteroides fragilis</i>	Less than about 5, and in certain instances, less than about 1.6
<i>Gardnerella vaginalis</i>	From less than about 2 to about 150; about 2 to about 125; or about 2 to about 100
<i>Lactobacillus crispatus</i>	Less than about 50, and in certain instances, less than about 30, or less than about 25 (reference strain)
<i>Mobiluncus mulieris</i>	From about 15 to about 25
<i>Peptostreptococcus anaerobius, Prevotella</i>	Less than about 2

<i>bivia, Prevotella disiens, and Prevotella timonensis</i>	
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[0155] The MICs for each identified strain of *Lactobacillus gasseri*, *Lactobacillus iners*, *Lactobacillus jensenii*, *Lactobacillus vaginalis*, and *Mobiluncus curtisii* were each greater than 150 µg/ml. The non-reference strain of *Lactobacillus crispatus* was similarly unaffected by tinidazole at the highest concentration tested. This data demonstrates that these strains, and in particular the *Lactobacillus* strains which are important for vaginal health, are not inhibited by tinidazole at concentrations sufficient to inhibit bacterial strains associated with bacterial vaginosis.

[0156] In addition to testing the formulations including tinidazole as noted above, formulations equivalent to formulations F1, F2, and F3 were prepared without tinidazole to determine the anti-microbial activity attributable to the components of the F1, F2, and F3 formulations. These tinidazole-free formulations were appropriately diluted and tested against the various identified bacterial strains. No inhibition of growth for any bacterial isolate was observed using the tinidazole free formulations.

Example 8: Franz Cell Diffusion Testing

[0157] Formulations F1, F2, and F3 were subjected to Franz cell diffusion testing. In the Franz cell (PermeGear, Inc. Hellertown, PA), a 0.40 µm pore polycarbonate membrane (Fisher Scientific, Pittsburgh, PA) was used. The membrane area was 1.77 cm². A 480 µL sample of each test formulation was loaded in the donor cell. The receptor cell contained 8 mL of 0.05 M citrate buffer, pH 3.3 ("receiver fluid"). At 0.5, 1, 2, 3, 4, and 6 hours (n = 6 replicates), a 500 µl sample was removed from the receptor cell and replaced with 500 µL of fresh buffer. The study was conducted at 37°C. Concentration of tinidazole in the receiver fluid removed from the Franz cell was determined by LC-MS/MS.

[0158] Drug concentration per unit membrane area was plotted against the square root of time in hours. Figure 15 shows the mean ± standard deviation of tinidazole released (µmol)/cm² versus square root of time in hours.

[0159] There was no gel left in any of the chambers at the end of 6 hours. Due to tinidazole saturation in the receiver chambers occurring around 180 minutes, all in vitro release rates (slopes) were calculated using only the linear portion of the curve ($R^2 \geq 0.9$, i.e.,

samples up to and including 180 minutes). Rate and recovery data are shown in Table 6, below.

TABLE 6

Formulation	Rate^a ([$\mu\text{mol}/\text{cm}^2/\sqrt{t(\text{h})}$])	% Recovery^a (at 6 hours)
F3	6.04 ± 0.893	98.5 ± 5.34
F2	7.56 ± 1.32	96.5 ± 4.24
F1	8.70 ± 0.964	89.6 ± 4.51
a = Mean ± Standard Deviation		

[0160] As can be seen in Table 6, release rate increased as tinidazole concentration increased. Based on 90% confidence intervals computed for the ratios of the median *in vitro* release rate for pairs of formulations F1, F2, and F3 (e.g. F1 and F2; F1 and F3; F2 and F3), the F1 and F2 comparison showed these formulations can be considered to have equivalent release rates (default "no difference" 90% confidence intervals limits were 75% to 133.33%). The statistical equivalence of release rates of the F2 and F3 formulations was not determined by the present comparison, while the difference in release rate between F1 and F3 was statistically significant.

Example 9: Flux and Tissue Association Using EpiVaginal™ Tissue

[0161] EpiVaginal™ tissue (produced from normal human-derived vaginal-ectocervical epithelial cells) full thickness tissues (VEC-100-FT) on polycarbonate membranes (NUNC® single well tissue culture plate, pore size 0.4 μm) and culture medium (VEC-100-ASY) were purchased from MatTek Corporation (Ashland, MA).

[0162] The culture medium (VEC-100-ASY) was pre-warmed at 37°C. Under sterile conditions and using sterile forceps, the EpiVaginal™ tissues were transferred into 12-well plates containing the pre-warmed culture medium. Then, the 12-well plates containing the EpiVaginal™ tissue samples were placed in a humidified incubator (37 ± 2 °C, 5 ± 1% CO₂)

for overnight recovery. The next morning, the culture medium in the plates was aspirated, and the EpiVaginal™ tissues were transferred into new 24-well plates.

[0163] An aliquot (100 µL) of one of formulation F1, F2, or F3 was added to the apical (AP) side of EpiVaginal™ tissue in the culture insert (*see* Figure 16), and 500 µL of the culture medium was added to the basolateral (BL) side of the insert. For a control group, an aliquot (100 µL) of culture medium was added to the AP side of the insert, and 500 µL of culture medium was added to the BL side (the "Medium" in Figure 16A). All dosing was performed under sterile conditions in a laminar flow hood.

[0164] The plates containing the tissue were incubated in a humidified incubator (37 ± 1 °C, $5 \pm 1\%$ CO₂) during the study and removed only for assays at 1, 2, 4, 6, 8, 24, or 48 hours. Four replicates were studied at each time point, for a total of 28 samples per formulation. The noted studies were run in parallel such that plates assayed at a given time point were not assayed at any other time point.

[0165] At the completion of each incubation time point, the 500 µL of culture medium on the BL side was analyzed for tinidazole content using LC-MS/MS. The tinidazole concentrations recorded at the 1, 2, and 4 hour time points were used to calculate the mean flux rate in EpiVaginal™ tissue culture.

[0166] Simultaneously, the EpiVaginal™ tissue culture was removed from the reactor and the contents in both the AP and BL sides were carefully aspirated and the tissues were washed twice with assay media (300 µL) to remove residual formulations. The rinses were discarded and the tissue was gently blotted on a Kim wipe and weighed. The tissues were stored at -80°C until further analysis, which comprised homogenization with the resulting tissue lysates analyzed by LC-MS/MS to determine the tissue tinidazole concentration at each time point.

TABLE 7

Formulation	Mean Flux Rate (nmol/cm ² /min)	Mean Peak Tinidazole Concentration in EpiVaginal™ Tissue at 1 Hour (ng/mg) ^b	Mean ^a Tinidazole Receiver Fluid Concentration (nM)		
			1-Hour	2-Hour	4-Hour
F3	18 ± 0.993	2,017 ± 161	1,144,500 ± 149,089	2,290,000 ± 303,425	4,400,000 ± 199,499
F2	22.5 ± 2.31	2,762 ± 533	1,517,500 ± 207,746	2,855,000 ± 245,289	5,567,500 ± 279,687
F1	19.0 ± 3.52	3,434 ± 498	1,735,000 ± 270,000	2,627,500 ± 257,472	5,105,000 ± 713,559
^a n = 4 replicates per treatment at 1-, 2-, and 4-hour time point.					
^b Mean peak EpiVaginal™ tissue concentrations occurred at the 1-hour time point for all treatments (4 replicates).					

[0167] As can be seen in Table 7, the mean tinidazole tissue flux rates differed by less than 20% between formulations F1, F2, and F3, implying that flux rates were not

concentration dependent. Even though flux rates were not concentration dependent, concentration-dependent increases in the mean maximal EpiVaginal™ tissue-associated tinidazole concentrations were observed at the 1 hour incubation time point. All of the maximal tissue-associated tinidazole concentrations exceeded 2,000 ng/mg of tissue across the 3 formulations evaluated. Additionally, for each of formulations F1, F2, and F3, the receiver fluid mean tinidazole concentrations increased approximately proportionally with incubation times of up to 4 hours duration.

[0168] It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0169] The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A formulation suitable for the intravaginal delivery of tinidazole to a subject in need thereof, the formulation comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives.
2. The formulation of claim 1, wherein the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block.
3. The formulation of claim 2, wherein the hydrophobic block is poly(propylene oxide).
4. The formulation of claim 3, wherein the hydrophilic block is poly(ethylene oxide).
5. The formulation of any one of claims 1-4, wherein the thermoreversible gelling agent is poloxamer 407.
6. The formulation of any one of claims 1-5, wherein the one or more pharmaceutically acceptable C₁-C₇ alcohols comprise a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol.
7. The formulation of claim 6, wherein the first and second pharmaceutically acceptable C₁-C₇ alcohols are independently selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.
8. The formulation of any one of claims 6 or 7, wherein the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.

9. The formulation of claims 6-8, wherein the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol.
10. The formulation of claims 7 or 9, wherein the isopropanol is a 60% (w/w) solution in water.
11. The formulation of any one of claims 1-10, wherein the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide.
12. The formulation of claim 11, wherein the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.
13. The formulation of any one of claims 1-10, the water comprises a mixture of deionized water and a buffered aqueous solution.
14. The formulation of claim 13, wherein the buffered aqueous solution is citrate buffer.
15. The formulation of any one of claims 1-14, wherein the formulation has a buffered pH selected from the group consisting of about 3 to about 5, about 3.5 to about 4.5, and about 4 to about 4.5.
16. The formulation of any one of claims 1-15, wherein the tinidazole comprises from about 0.1 to about 2% (w/w) of the formulation.
17. The formulation of any one of claims 1-16, wherein the tinidazole comprises about 1%, about 1.25%, or about 1.5% (w/w) of the formulation.
18. The formulation of claim 1, wherein the formulation comprises about 20% poloxamer 407 (w/w), about 49.6% citrate buffer (w/w), about 3.1% benzyl alcohol (w/w), about 12% (w/w) of a 60% (w/w) solution of isopropanol in water, about 13.8% dimethylisosorbide (w/w), and about 1.5% tinidazole (w/w).

19. The formulation of claim 1, wherein the formulation comprises about 19% poloxamer 407 (w/w), about 53% citrate buffer (w/w), about 2.9% benzyl alcohol (w/w), about 11% (w/w) of a 60% (w/w) solution of isopropanol in water, about 12.7% dimethylisorbide (w/w), and about 1.25% tinidazole (w/w).
20. The formulation of claim 1, wherein the formulation comprises about 18% poloxamer 407 (w/w), about 57% citrate buffer (w/w), about 2.5% benzyl alcohol (w/w), about 10% of a 60% (w/w) solution of isopropanol in water, about 11.5% dimethylisorbide (w/w), and about 1% tinidazole (w/w).
21. A method for treating bacterial vaginosis, trichomoniasis, or other disease of the vaginal cavity susceptible to tinidazole, comprising intravaginally administering to a subject in need thereof a formulation comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives.
22. The method of claim 21, wherein the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block.
23. The method of claim 22, wherein the hydrophobic block is poly(propylene oxide).
24. The method of claim 23, wherein the hydrophilic block is poly(ethylene oxide).
25. The method of claim 22, wherein the thermoreversible gelling agent is poloxamer 407.
26. The method of any one of claims 21-25, wherein the one or more pharmaceutically acceptable C₁-C₇ alcohols comprises a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol.

27. The method of claim 26, wherein the first and second pharmaceutically acceptable C₁-C₇ alcohols are selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.
28. The method of claims 26 or 27, wherein the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.
29. The method of any one of claims 26-28, wherein the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol.
30. The method of claims 27 or 29, wherein the isopropanol is a 60% (w/w) solution in water.
31. The method of any one of claims 21-30, wherein the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide.
32. The method of claim 31, wherein the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.
33. The method of any one of claims 21-32, wherein the water comprises a mixture of sterile deionized water and a buffered aqueous solution.
34. The method of claim 33, wherein the buffered aqueous solution is citrate buffer.
35. The method of any one of claims 21-34, wherein the formulation has a buffered pH selected from the group consisting of about 3 to about 5, about 3.5 to about 4.5, and about 4.
36. The method of any one of claims 21-35 wherein the tinidazole comprises from about 0.1% to about 2% (w/w) of the formulation.

37. The method of any one of claims 21-36, wherein the tinidazole comprises about 1%, about 1.25%, or about 1.5% (w/w) of the formulation.
38. The method of any one of claims 21-27, wherein the subject is a human.
39. A pharmaceutical composition comprising: about 18-20% poloxamer 407 (w/w), about 49.6-57% citrate buffer (w/w), about 2.5-3.1% benzyl alcohol (w/w), about 10-12% (w/w) of a 60% (w/w) solution of isopropanol in water, about 11.5%-13.8% dimethylisosorbide (w/w), and about 1-1.5% tinidazole (w/w) or an active metabolite thereof, for treating bacterial vaginosis, trichomoniasis, or other disease of the vaginal cavity susceptible to tinidazole.
40. A pharmaceutical composition consisting of: about 18-20% poloxamer 407 (w/w), about 49.6-57% citrate buffer (w/w), about 2.5-3.1% benzyl alcohol (w/w), about 10-12% (w/w) of a 60% (w/w) solution of isopropanol in water, about 11.5%-13.8% dimethylisosorbide (w/w), and about 1-1.5% tinidazole (w/w) or an active metabolite thereof, for treating bacterial vaginosis, trichomoniasis, or other disease(s) of the vaginal cavity susceptible to tinidazole.
41. The pharmaceutical composition of claim 39 or 40, wherein said pharmaceutical composition exists as a viscous liquid at room temperature and gels at or close to body temperature.
42. Use of the formulation of any one of claims 1-20 for treating bacterial vaginosis, trichomoniasis, or other disease(s) of the vaginal cavity susceptible to tinidazole.
43. Use of the formulation of any one of claims 1-20 for the manufacture of a medicament for the treatment of bacterial vaginosis, trichomoniasis, or other disease of the vaginal cavity susceptible to tinidazole.

44. A method for treating a vaginal disorder comprising intravaginally administering to the subject a formulation containing about 0.1-2% tinidazole, or an active metabolite thereof.
45. A method for administering tinidazole to a human subject, comprising:
 - obtaining a formulation containing about 0.1-2% tinidazole, or an active metabolite thereof, wherein said formulation exists as a viscous liquid at room temperature; and
 - intravaginally administering the formulation to a human subject, wherein the formulation gels at or close to body temperature.
46. A formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the formulation comprising tinidazole, wherein the formulation has a Franz cell flux of from about 2 to about 10 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t(\text{h})}$.
47. The formulation of claim 46, wherein the Franz cell flux is from about 6 to about 9 ($\mu\text{mol}/\text{cm}^2$)/ $\sqrt{t(\text{h})}$.
48. A formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the formulation comprising tinidazole, wherein the formulation has a mean flux rate in EpiVaginal™ tissue of from about 15 $\text{nmol}/\text{cm}^2/\text{min}$ to about 30 $\text{nmol}/\text{cm}^2/\text{min}$.
49. The formulation of claim 48, wherein the mean flux rate in EpiVaginal™ tissue is from about 18 $\text{nmol}/\text{cm}^2/\text{min}$ to about 22.5 $\text{nmol}/\text{cm}^2/\text{min}$.
50. A formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the formulations comprising tinidazole, wherein the formulation has a mean tinidazole receiver fluid concentration at 1 hour of from about 0.5 mM to about 2.5 mM.
51. The formulation of claim 50, wherein the formulation has a mean tinidazole receiver fluid concentration at 1 hour of from about 1.14 mM to about 1.74 mM.

52. A tinidazole gel formulation suitable for the intravaginal delivery of tinidazole to a subject in need thereof, the formulation comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and, optionally, one or more preservatives, the tinidazole gel formulation prepared by the process of:
- dissolving the thermoreversible gelling agent in the water to form a first solution;
 - combining the one or more pharmaceutically acceptable C₁-C₇ alcohols, the solubility enhancer, and the tinidazole to form a second solution; and
 - adding the second solution to the first solution.
53. The tinidazole gel formulation of claim 52, wherein the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block.
54. The tinidazole gel formulation of claim 53, wherein the hydrophobic block is poly(propylene oxide).
55. The tinidazole gel formulation of claim 53, wherein the hydrophilic block is poly(ethylene oxide).
56. The tinidazole gel formulation of any one of claims 52-55, wherein the thermoreversible gelling agent is poloxamer 407.
57. The tinidazole gel formulation of any one of claims 52-55, wherein the one or more pharmaceutically acceptable C₁-C₇ alcohols comprise a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol.
58. The formulation of claim 57, wherein the first and second pharmaceutically acceptable C₁-C₇ alcohols are independently selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.

59. The tinidazole gel formulation of any one of claims 57 or 58, wherein the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.
60. The tinidazole gel formulation of claims 57-59, wherein the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol.
61. The tinidazole gel formulation of claims 58 or 60, wherein the isopropanol is a 60% (w/w) solution in water.
62. The tinidazole gel formulation of any one of claims 52-61, wherein the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide.
63. The tinidazole gel formulation of claim 62, wherein the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.
64. The tinidazole gel formulation of any one of claims 52-63, wherein the water comprises a mixture of deionized water and a buffered aqueous solution.
65. The tinidazole gel formulation of claim 64, wherein the buffered aqueous solution is citrate buffer.
66. The tinidazole gel formulation of any one of claims 46-65, wherein the formulation has a pH of from about 3 to about 5.
67. The tinidazole gel formulation of claim 66, wherein the formulation has a pH of from about 3.5 to about 4.5.
68. A process for the preparation of a tinidazole gel formulation suitable for the intravaginal delivery of tinidazole to a human subject in need thereof, the process comprising:
 - dissolving a thermoreversible gelling agent in water to form a first solution;

combining one or more pharmaceutically acceptable C₁-C₇ alcohols, a solubility enhancer, and tinidazole to form a second solution; and
adding the second solution to the first solution.

69. The process of claim 68, wherein the thermoreversible gelling agent is triblock copolymer having a central hydrophobic block flanked on each side with a hydrophilic block.
70. The process of claim 69, wherein the hydrophobic block is poly(propylene oxide).
71. The process of claim 69, wherein the hydrophilic block is poly(ethylene oxide).
72. The process of any of claims 68-71, wherein the thermoreversible gelling agent is poloxamer 407.
73. The process of any of claims 68-72, wherein the one or more pharmaceutically acceptable C₁-C₇ alcohols comprise a first pharmaceutically acceptable C₁-C₇ alcohol and a second pharmaceutically acceptable C₁-C₇ alcohol.
74. The process of claim 73, wherein the first and second pharmaceutically acceptable C₁-C₇ alcohols are independently selected from the group consisting of methanol, ethanol, isopropanol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, benzyl alcohol, and combinations thereof.
75. The process of claim 73 or 74, wherein the first pharmaceutically acceptable C₁-C₇ alcohol is benzyl alcohol.
76. The process of claims 73-75, wherein the second pharmaceutically acceptable C₁-C₇ alcohol is isopropanol.
77. The process of claim 74 or 76, wherein the isopropanol is a 60% (w/w) solution in water.

78. The process of any of claims 64-77, wherein the solubility enhancer is selected from the group consisting of mono- and di-alkyl ethers of isosorbide.
79. The process of claim 78, wherein the mono- or di-alkyl ether of isosorbide is dimethyl isosorbide.
80. The process of any of claims 64-79, wherein the water comprises a mixture of deionized water and a buffered aqueous solution.
81. The process of claim 80, wherein the buffered aqueous solution is citrate buffer.
82. The formulation of claim 50, wherein the formulation has a mean tinidazole receiver fluid concentration at 2 hours of from about 1.5 mM to about 3.5 mM.
83. The formulation of claim 82, wherein the formulation has a mean tinidazole receiver fluid concentration at 4 hours of from about 3 mM to about 6 mM.
84. The formulation of claim 82, wherein the formulation has a mean tinidazole receiver fluid concentration at 2 hours of from about 2.3 mM to about 2.6 mM.
85. The formulation of claim 83, wherein the formulation has a mean tinidazole receiver fluid concentration at 4 hours of from about 4 mM to about 5.5 mM.
86. A tinidazole gel formulation prepared by the process of any one of claims 68-81.
87. The tinidazole gel formulation of claims 1-20, wherein the formulation does not inhibit or only minimally inhibits lactobacillus strains at a tinidazole concentration sufficient to inhibit *Gardnerella vaginalis* strains associated with bacterial vaginosis.

Figure 1

Observations	Numerical Grading
Erythema	
No Redness	0
Slight Redness	1
Redness with Distinct blood vessels	2
Deep redness accompanied with numerous engorged blood vessels	3
Exudate	
No pus	0
Few small globules of pus	1
Large globules of pus	2
Large amounts of pus and or sloughed tissue exuding from the vulva	3
Edema	
No swelling of the vulva	0
Slight swelling of the vulva to less than twice its size	1
Swelling of the vulva to twice its size	2
Swelling of the vulva to greater than twice its size	3

Figure 2

Animal #	Group	Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Total	Irritation Score	CII
		ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED			
30428	Test	1	0	0	1	0	1	2	0	1	2	0	1	2	0	1	2	0	1	14	0.9	
30439	Test	1	0	0	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	15	1.0	1.0
30440	Test	1	0	0	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	15	1.0	
30424	Control	1	0	0	2	0	1	2	0	1	1	0	1	1	0	1	2	0	1	13	0.9	
30425	Control	0	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	2	11	0.7	0.9
30426	Control	0	0	0	2	0	1	2	0	1	2	0	1	2	0	1	2	0	2	16	1.1	
Difference (test – control)																				0.1		

ER = erythema
 EX = exudate
 ED = edema
 CII = Cumulative Irritation Index

Figure 3

Animal #	Group	Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Total	Irritation Score	CII
		ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED			
30231	Test	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	6	0.4	
30233	Test	1	0	0	1	0	0	1	0	0	1	0	0	2	0	0	2	0	0	7	0.5	0.6
30251	Test	1	0	0	2	0	1	2	0	1	1	0	1	2	0	1	2	0	1	14	0.9	
30427	Control	1	0	0	1	0	0	2	0	0	1	0	0	1	0	0	1	0	0	6	0.4	
30086	Control	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	3	0	1	8	0.5	0.4
30093	Control	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	6	0.4	
Difference (test – control)																			0.2			

ER = erythema
 EX = exudate
 ED = edema
 CII = Cumulative Irritation Index

Figure 4

Animal #	Group	Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Total	Irritation Score	CII	
		ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED				
30535	Test	1	0	0	2	0	1	2	0	1	2	0	2	0	1	2	0	1	2	1	2	18	1.2
30536	Test	0	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	4	0.3
30537	Test	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	5	0.3
30545	Control	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.1
30544	Control	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	5	0.3
30539	Control	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	1	1	9	0.6
																			Difference (test - control)			0.3	

ER = erythema
 EX = exudate
 ED = edema
 CII = Cumulative Irritation Index

Figure 5

Animal #	Group	Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Total	Irritation Score	CII
		ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED			
30565	Test	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	4	0.3	
30564	Test	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	9	0.6	0.5
30568	Test	0	0	0	1	0	0	1	0	1	1	0	1	1	0	1	1	0	1	9	0.6	
30559	Control	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	3	0.2	
30561	Control	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	1	0	1	6	0.4	0.4
30562	Control	1	0	0	1	0	0	1	0	1	1	0	1	1	0	1	2	0	2	11	0.7	
Difference (test – control)																			0.1			

ER = erythema
 EX = exudate
 ED = edema
 CII = Cumulative Irritation Index

Figure 6

Animal #	Group	Day 0			Day 1			Day 2			Day 3			Day 4			Day 5			Total	Irritation Score	CII
		ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED	ER	EX	ED			
30555	Test	1	0	0	1	0	0	2	0	0	1	0	0	1	0	0	1	0	0	6	0.4	
30557	Test	0	0	0	0	0	0	2	0	0	3	0	3	0	3	0	3	0	2	18	1.2	0.6
30556	Test	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	5	0.3	
30550	Control	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	3	0.2	
30553	Control	1	0	0	1	0	0	1	0	0	1	0	0	1	1	1	1	1	2	10	0.7	0.5
30554	Control	1	0	0	1	0	1	1	0	1	1	0	1	1	0	1	2	0	1	11	0.7	
Difference (test – control)																					0.1	

ER = erythema
 EX = exudate
 ED = edema
 CII = Cumulative Irritation Index

Figure 7

Animal #	Group	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Total	Irritation Score	CII	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED				
30547	Test	0	0	2	0	1	2	0	1	2	0	2	0	2	18	1.2	
30548	Test	0	0	1	0	0	1	0	1	2	0	2	0	1	13	0.9	0.8
30549	Test	0	0	0	0	0	0	0	0	1	0	1	0	0	5	0.3	
30519	Control	0	0	1	0	0	2	0	1	2	0	1	2	0	14	0.9	
30546	Control	0	0	0	0	0	0	0	1	0	1	2	0	2	9	0.6	0.7
30542	Control	1	0	1	0	0	1	0	0	1	0	1	0	0	7	0.5	
Difference (test – control)															0.1		

ER = erythema
 EX = exudate
 ED = edema
 CII = Cumulative Irritation Index

Figure 8

Scoring Criteria		Score	Leukocytes (per HPF*)	Score	Vascular Congestion	Score	Edema	Score
Intact Epithelium	0	Absent	0	Absent	0	Absent	0	
Cell degeneration or flattening	1	Minimal (<25)	1	Minimal	1	Minimal	1	
Metaplasia	2	Mild (26-50)	2	Mild	2	Mild	2	
Focal erosion	3	Moderate (51-100)	3	Moderate	3	Moderate	3	
Generalized erosion	4	Marked (>100)	4	Marked	4	Marked	4	

*HPF = high power field (40X)

Figure 9a

ANIMAL #:	TEST ARTICLE			CONTROL ARTICLE		
	30428	30439	30440	30424	30425	30426
Epithelium	0	0	0	0	0	0
Leukocytes	1	2	2	1	1	2
Vascular Congestion	1	1	1	2	1	1
Edema	0	0	0	0	0	0
Total	2	3	3	3	2	3
Group Mean*	3			3		

Figure 9b

Average Test Article Score	3
(-) Average Control Score	3
Irritant Rank Score	0
Interpretation of the Irritant Rank Score:	Non-irritant

Figure 10a

ANIMAL #:	TEST ARTICLE			CONTROL ARTICLE		
	30231	30233	30251	30086	30093	30427
Epithelium	0	0	0	0	0	0
Leukocytes	1	1	1	1	1	1
Vascular Congestion	1	1	2	1	1	1
Edema	0	0	0	0	0	0
Total	2	2	3	2	2	2
Group Mean*	2					

Figure 10b

Average Test Article Score	2
(-) Average Control Score	2
Irritant Rank Score	0
Interpretation of the Irritant Rank Score:	Non-irritant

Figure 11a

ANIMAL #:	TEST ARTICLE			CONTROL ARTICLE		
	30535	30536	30537	30539	30544	30545
Epithelium	0	0	0	0	0	0
Leukocytes	2	1	2	1	1	1
Vascular Congestion	1	1	1	1	1	1
Edema	0	0	0	0	0	0
Total	3	2	3	2	2	2
Group Mean*		3			2	

Figure 11b

Average Test Article Score	3
(-) Average Control Score	2
Irritant Rank Score	1
Interpretation of the Irritant Rank Score:	Minimal Irritant

Figure 12a

ANIMAL #:	CONTROL			TEST ARTICLE		
	30559	30561	30562	30564	30565	30568
Epithelium	0	0	0	1	3	0
Leukocytes	2	1	2	1	2	1
Vascular Congestion	1	0	1	1	2	1
Edema	0	1	0	0	1	0
Total	3	2	3	3	8	2
Group Mean*		3			4	

Figure 12b

Average Test Article Score	4
(-) Average Control Score	3
Irritant Rank Score	1
Interpretation of the Irritant Rank Score:	Minimal Irritant

Figure 13a

ANIMAL #:	TEST ARTICLE			CONTROL ARTICLE		
	30555	30556	30557	30550	30553	30554
Epithelium	0	0	0	0	0	0
Leukocytes	1	2	1	2	1	1
Vascular Congestion	2	1	1	1	1	2
Edema	0	0	0	0	0	0
Total	3	3	2	3	2	3
Group Mean*	3			3		

Figure 13b

Average Test Article Score	3
(-) Average Control Score	3
Irritant Rank Score	0
Interpretation of the Irritant Rank Score:	Non-irritant

Figure 14a

ANIMAL #:	TEST ARTICLE			CONTROL ARTICLE		
	30547	30548	30549	30519	30542	30546
Epithelium	0	0	0	0	0	0
Leukocytes	1	2	2	1	2	1
Vascular Congestion	2	1	2	2	2	2
Edema	0	0	0	0	0	0
Total	3	3	4	3	4	3
Group Mean*	3			3		

Figure 14b

Average Test Article Score	3
(-) Average Control Score	3
Irritant Rank Score	0
Interpretation of the Irritant Rank Score:	Non-irritant

Figure 15

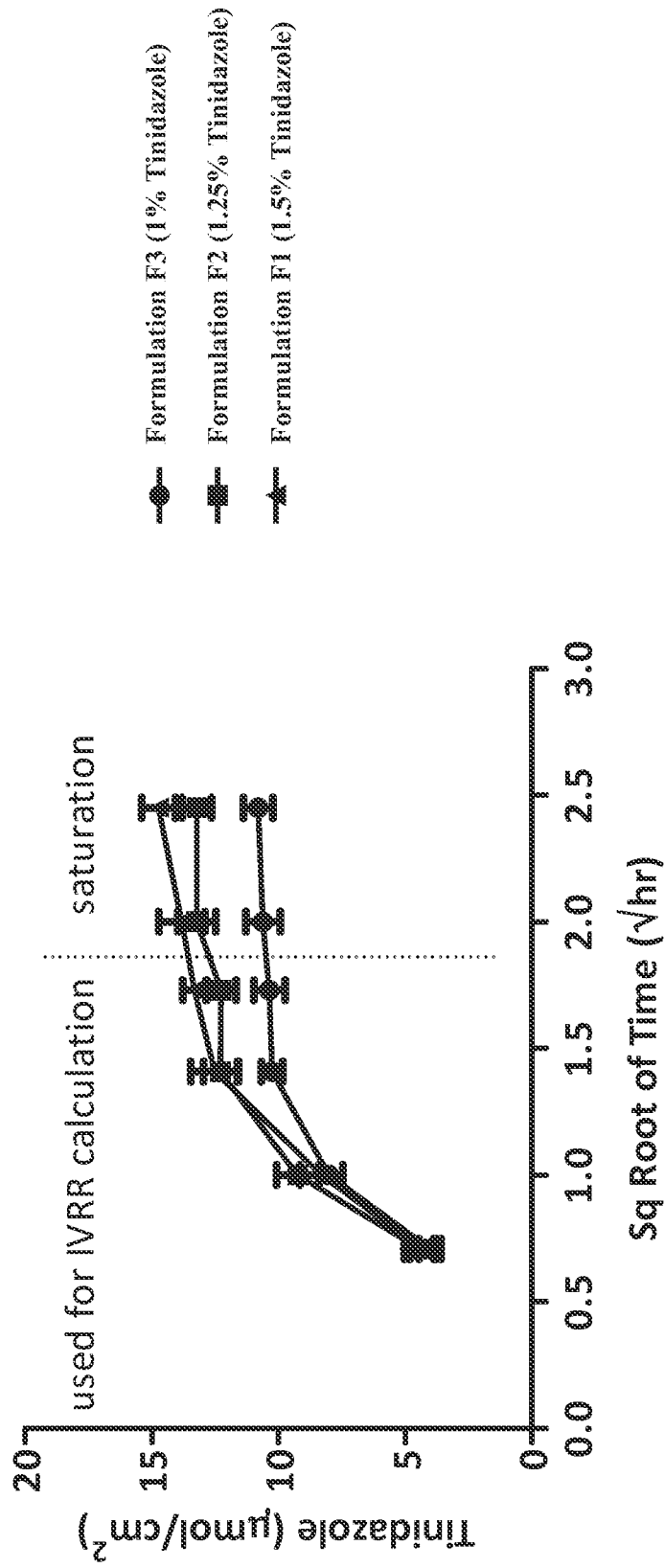
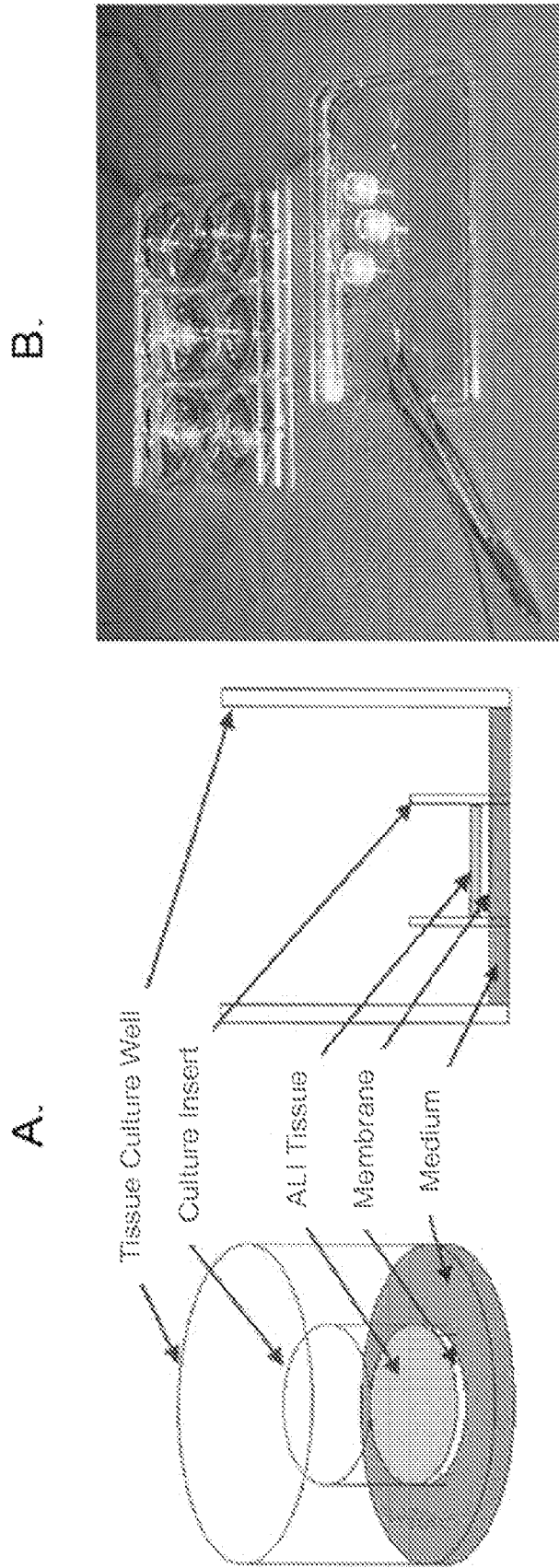


Figure 16



A. Diagram of culture well with insert containing full-thickness EpiVaginal tissue.
B. Example of a multiwell culture plate with culture inserts.

INTERNATIONAL SEARCH REPORT

International application No PCT/US2014/044097
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A. CLASSIFICATION OF SUBJECT MATTER INV. A61K47/10 A61K9/06 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/087270 A1 (EDKO TRADING REPRESENTATION [TR]; EMBIL KORAL [TR]; BAKTIR GUL [TR]; F) 22 September 2005 (2005-09-22) example 4 <p style="text-align: center;">-----</p>	1-43, 52-81, 86,87
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search	Date of mailing of the international search report	
9 September 2014	24/11/2014	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Vázquez Lantes, M	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/044097

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-43, 52-81, 86, 87

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-43, 52-81, 86, 87

A formulation comprising water, tinidazole, a thermoreversible gelling agent, one or more pharmaceutically acceptable C1-C7alcohols, a solubility enhancer, and, optionally, one or more preservatives.

2. claims: 44, 45

A formulation containing about 0.1-2% tinidazole, or an active metabolite thereof.

3. claims: 46, 47

A formulation comprising tinidazole, wherein the formulation has a Franz cell flux of...

4. claims: 48, 49

A formulation comprising tinidazole, wherein the formulation has a mean flux rate in EpiVaginal tissue of...

5. claims: 50, 51, 82-85

A formulation comprising tinidazole, wherein the formulation has a mean tinidazole receiver fluid concentration at 1 hour of...

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/044097

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005087270	A1	22-09-2005	
		BR PI0418623 A	29-05-2007
		CN 1960763 A	09-05-2007
		EA 200601647 A1	27-02-2007
		TR 200606411 T1	22-01-2007
		WO 2005087270 A1	22-09-2005
