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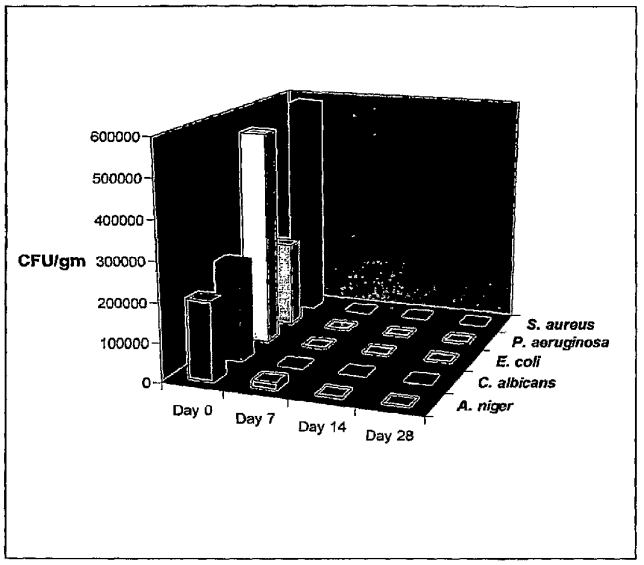
(54) **Title:** NANOSTRUCTURED COMPOSITIONS HAVING ANTIBACTERIAL, ANTI-FUNGAL, ANTI-YEAST, AND/OR ANTI-VIRAL PROPERTIES

Antibacterial Effectiveness Test for Placebo (no active agent) (as per USP)



(57) **Abstract:** The invention provides nanostructured compositions having antibacterial, anti-fungal, anti-yeast, and/or anti-viral properties. The compositions are useful as drug delivery carriers for one or more active agents or, in the absence of an active agent, in methods where such compositions are desirable.

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NANOSTRUCTURED COMPOSITIONS HAVING ANTIBACTERIAL, ANTI-FUNGAL, ANTI-YEAST, AND/OR ANTI-VIRAL PROPERTIES

FIELD OF THE INVENTION

The invention is directed to nanostructured compositions having antibacterial, anti-fungal, anti-yeast, and/or anti-viral properties. The compositions are useful as drug delivery carriers for one or more active agents or, in the absence of an active agent, in methods where such compositions are desirable.

BACKGROUND

A. Background Regarding the Use of Antibacterial Agents in Drug Dosage Forms and Consumer Use Products

Microbial preservatives are added to nonsterile dosage forms to protect them from microbiological growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. In the case of sterile articles used in multi-dose containers, antimicrobial preservatives are added to inhibit the growth of microorganisms that may be introduced by repeatedly withdrawing individual doses.

All useful antimicrobial agents are toxic substances. *See* USP 26, General Chapters, Section 51, "Antimicrobial Effectiveness Testing." For maximum protection of patients, the concentration of the preservative shown to be effective in the final prepared packaged product should be below a level that may be toxic to humans.

U.S. Food and Drug Administration guidelines require that antimicrobial effectiveness, whether inherent in the product (*i.e.*, for an antibiotic agent) or whether produced because of the addition of an antimicrobial agent, must be demonstrated for all injections packaged in multiple-dose containers or for other products containing antimicrobial preservatives. Antimicrobial effectiveness must be demonstrated for multiple-dose topical and oral dosage forms, and for other dosage forms such as ophthalmic, otic, nasal, irrigation, and dialysis fluids. *See* USP 26, General Chapters, Section 51, "Antimicrobial Effectiveness Testing."

The addition of an antimicrobial agent to a therapeutic dosage form can be undesirable, as such compounds can be toxic and they can have undesirable interactions with the primary active agent to be delivered. In addition, the use of microbiocides can promote

the generation of drug resistant bacteria, drug resistant yeast, drug resistant fungi, etc. This has been observed with the wide spread use of antibacterial lotions, soaps, cleaning products, etc.

Antibiotic resistance among bacteria has increased in recent years, and concerns have been raised that cross resistance might develop in bacteria or other microorganisms due to exposure to antibiotics or biocides. Rutala, W. A., "APIC Guideline for Selection and Use of Disinfectants," *American J., 24*:313-342 (1996); Russell et al., "Do Antiseptics and Disinfectants Select for Antibiotic Resistance?" *J. of Medicinal Microbiology, 48*:613-615 (1999). More effective disinfectants can be extremely irritant and toxic, resulting in health complications such as contact dermatitis and mucous membrane irritation among personnel. Hansen, K.S., "Occupational Dermatoses in Hospital Cleaning Women," *Contact Dermatitis, 9*:343-351 (1983); Beauchamp et al., "A Critical Review of the Toxicology of Glutaraldehyde, *Critical Reviews in Toxicology, 22*:143-174 (1992). Thus, there is a continuing need for effective and safe biocidal agents for topical and surface disinfection and microorganisms change and resistant strains develop.

B. Background Regarding Fungal, Bacterial, Yeast, and Viral Opportunistic Organisms

The USP specifies testing for effectiveness in killing *E. coli, P. aeruginosa*, and *S. aureus,* plus acting to control and limit the growth of *C. albicans*, and *A. niger,* to determine the antimicrobial effectiveness of a dosage form. *See* USP 26 Section 51, General Chapters, "Antimicrobial Effectiveness Testing." These particular microorganisms are representative of the most problematic and troublesome bacteria, fungi, and yeast.

Aspergillus niger is a fungus and one of the most common species of the genus Aspergillus. It causes black mold on certain types of fruit and vegetables, and is a common contaminant of food.

C. albicans is the major fungal pathogen of humans. Infections can be localized, such as vaginal infections and oral infections, which cause a considerable degree of discomfort. In some patient groups, whose defense system is severely compromised (prematurely born infants, leukemics and burn patients), the yeast can turn into a deadly pathogen causing systemic infections – up to 50% of the patients infected die as a result. The incidence of such infections is increasing rapidly, especially in hospitalized patients. In New Zealand, such

infections are now ten times more frequent than they were 15 years ago. Moreover, the reservoir of anti-*Candida* drugs is very limited, and these agents can have severe side effects.

E. coli is an ubiquitous bacteria, with a large number of known strains. The particularly nasty *E. coli* strain *E. coli* O157:H7 is a member of the EHEC - enterohemorrhagic *E. coli* group. Enterohemorrhagic means an intestinally-related organism which causes hemorrhaging - and therefore, loss of blood. *E. coli* O157:H7 produces a toxin, called Shiga-like toxin (SLT) or Vero toxin. The toxin is a protein which causes severe damage to intestinal epithelial cells. This condition is particularly dangerous to small children, and may be lethal, as children are too small to tolerate significant blood and fluid loss. In some cases another syndrome is involved which is called hemolytic uremic syndrome (HUS), which is characterized by kidney failure and loss of red blood cells. Approximately 5% to 10% of children progress to this stage of disease. In severe cases, the disease can cause permanent kidney damage. The presence of this bacterium can also be very dangerous to the elderly or infirm. There can be a combination of HUS and other factors which involve the blood system, which can be lethal to the elderly in 50% of the cases. There is evidence that *E. coli* O157:H7 is becoming more prevalent.

Pseudomonas aeruginosa is a Gram-negative bacterium that is noted for its environmental versatility, ability to cause disease in particularly susceptible individuals, and its resistance to antibiotics. The most serious complication of cystic fibrosis is respiratory tract infection by the ubiquitous bacterium *Pseudomonas aeruginosa*. Cancer and burn patients also commonly suffer serious infections by this organism, as do certain other individuals with immune systems deficiencies. Unlike many environmental bacteria, *P. aeruginosa* has a remarkable capacity to cause disease in susceptible hosts. It has the ability to adapt to and thrive in many ecological niches, from water and soil to plant and animal tissues. The bacterium is capable of utilizing a wide range of organic compounds as food sources, thus giving it an exceptional ability to colonize ecological niches where nutrients are limited. *P. aeruginosa* can produce a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These proteins range from potent toxins that enter and kill host cells at or near the site of colonization to degradative enzymes that permanently disrupt the cell membranes and connective tissues in various organs.

Staphylococcus aureus is a leading cause of soft tissue infections, as well as toxic shock syndrome (TSS) and scalded skin syndrome. The pathogenic effects of *Staph* are

mainly associated with the toxins it produces. Most of these toxins are produced in the stationary phase of the bacterial growth curve. In fact, it is not uncommon for an infected site to contain no viable *Staph* cells. The *S. aureus* enterotoxin causes quick onset food poisoning which can lead to cramps and severe vomiting. Infection can be traced to contaminated meats which have not been fully cooked. These microbes also secrete leukocidin, a toxin which destroys white blood cells and leads to the formation of pus and acne. Particularly, *S. aureus* has been found to be the causative agent in such ailments as pneumonia, meningitis, boils, arthritis, and osteomyelitis (chronic bone infection). Most *S. aureus* are penicillin resistant, but vancomycin and nafcillin are known to be effective against most strains.

The molds such as *Aspergillus fumigatus* are filamentous fungi. They are especially prevalent growing on the nonliving organic materials in the soil. They disperse their non-sexual spores called conidia in the air. Most Aspergilli are harmless to humans and *A. fumigatus* in particular is harmless to humans whose immune system has not been compromised by disease, drug therapy or genetic conditions. Exposure to *A. fumigatus* can cause an allergic response in sensitive individuals. More importantly, *A. fumigatus* is an opportunistic pathogen of bone marrow transplant patients, AIDS patients, and other immune compromised individuals.

Aspergillus fumigatus is the most common mold causing infection worldwide. The first infection described in man, an aspergilloma, was reported in Edinburgh in 1842 and many cases of invasive disease in non-immunocompromised patients have been reported. These cases and more recent epidemiological data emphasize that *A. fumigatus* is a primary, albeit rare, pathogen of man. Allergic disease due to *Aspergillus* was first described in London in 1952 and the first invasive (and fatal) infection in an immunocompromised patient was described in 1953 in the British Medical Journal in a patient from Gloucester.

The frequency of invasive disease has risen approximately 14-fold over the 12 years to 1992, as judged after death in unselected autopsies. Invasive aspergillosis has overtaken candidiasis as the most frequent fungal pathogen detected post mortem in tertiary care hospitals in Europe. Thus 4% of all patients dying had invasive aspergillosis, compared with about 2% with invasive candidiasis. Patients at risk based on the disease frequency include those with chronic granulomatous disease (25-40%), lung transplant recipients (17-26%), allogeneic bone marrow transplant patients (4-30%), neutropenic patients with leukemia (5-25%), heart transplant recipients (2-13%), pancreas transplant recipients (1-4%), renal transplant patients in Europe and the USA (~1%) and in India (~10%), and patients with

AIDS, multiple myeloma and severe combined immunodeficiency (~4%). Over 500,000 transplants are performed annually in the world. Acute leukemia affects about 3/100,000 of the population and on average each patient receives 3 cycles of chemotherapy, with each cycle defining a major risk period. Similar incidence is observed for high grade lymphoma patients who are also at high risk of invasive aspergillosis. In the industrialized nations alone these treatment protocols generate about 250,000 periods of major risk per year. AIDS cases are predicted to exceed 40 million by the end of the year 2000 which would result in about 1.4 million cases of invasive aspergillosis, although in developing countries most patients will not live long enough to get this disease.

The crude mortality from invasive aspergillosis is around 85% and falls to around 50% if treated. The new drugs in trial (voriconazole, etc.) may reduce the mortality slightly (~10%), but patients in trials tend to do better than those treated in clinical practice. In addition to invasive disease, *Aspergillus* causes a number of other diseases in man. These include aspergilloma ("colonization" of existing pulmonary cavities), sinusitis in normal people, allergic bronchopulmonary and sinus infections, keratitis (which usually leads to blindness in that eye and is common in the developing world) and postoperative infections in immunocompetent patients. Aspergilloma numbers are set to rise dramatically due to the increasing incidence of tuberculosis and such aspergilloma cases are notoriously difficult to treat. Cavities of 2 cm or larger after tuberculosis subsequently develop aspergillomas in 15-20% of patients (in the UK). The 5 year survival of patients with aspergillomas is about 40%. Allergic bronchopulmonary aspergillosis occurs in patients with cystic fibrosis and asthmatics (an increasing number) causing pulmonary fibrosis and death usually within 10 years of diagnosis.

Aspergillus flavus is the etiologic agent in a wide range of infections including mycotoxicoses owing to aflotoxins, hypersensitivity pneumonitis, otitis, sinusitis, and invasive disease. Some reports suggest the disease process may be potentiated by aflotoxins, particularly in the immunocompromised/neutropenic host. The organism is extremely angioinvasive with resultant necrosis and infarction.

C. Prior Art Drug Delivery Techniques

Ease of active pharmaceutical agent delivery is a key issue facing pharmaceutical companies that develop and commercialize therapeutic products. An active agent that is readily soluble in water, for example, is not difficult to formulate into a suitable dosage form.

However, formulating poorly water-soluble active agent into suitable dosage forms poses a significant challenge. This is because the human body is a water based system; thus, as a condition of producing therapeutic activity, a drug must dissolve following administration.

Some poorly water-soluble active agents are never commercialized because they cannot be effectively solubilized, and therefore fail to exhibit acceptable *in vivo* therapeutic activity. Alternatively, the quantity of poorly water-soluble active agent required to be administered to achieve an acceptable level of therapeutic activity may be too great, given the poor water solubility of the agent, and result in unacceptable toxicity. Even if an active agent is formulated into a liquid, wherein the active agent is solubilized in a solvent, such dosage forms sometimes perform sub-optimally. For example, such dosage forms may have unpredictable properties or induce undesirable side effects.

Prior art methods exist for enhancing active agent solubility. For example, the particle size of the active agent can be reduced, thereby increasing the exposed surface area of the active agent, resulting in increased dissolution rate and greater water solubility. One prior method for particle size reduction is wet milling. This method requires grinding of an active agent with beads made of hard glass, ceramic, porcelain, zirconium oxide, zirconium silicate, polymeric resin, or other suitable substance in a media in which the active agent is poorly soluble, such as water. The active agent is physically converted into much smaller particles that remain suspended in the grinding media. The resultant micron- or nanometer-sized active agent particles can then be isolated from the grinding media by methods such as filtration or centrifugation, and formulated into an appropriate dosage form. *See* U.S. Patent Nos. 5,145,684; 5,518,187; 5,862,999; and 5,718,388. The media in which the active agent is ground typically contains one or more compounds that function as a surface stabilizer for the active agent. The surface stabilizers adsorb to the surface of the active agent and act as a steric barrier to active agent particle size growth.

Conventional wet milling techniques therefore produce a "bi-phasic" system in which the stabilized active agent nanoparticles are suspended in the aqueous media. The nanoparticulate drug delivery technology commercialized by Elan Drug Delivery (King of Prussia, PA) under the trade name NanoCrystal® technology, and SkyePharma, plc's Insoluble Drug Delivery (IDD®) technology exemplify such wet milling techniques. However, wet milling of active agent has drawbacks, principally being the cost of the process. The added cost for formulating a poorly water-soluble active agent into a

nanoparticulate composition utilizing wet milling can be prohibitive. Additionally, amorphous compounds are not amenable to wet milling techniques.

Other known methods of making nanoparticulate active agent compositions include precipitation, homogenization, and super critical fluid methods. Microprecipitation is a method of preparing stable dispersions of poorly soluble active agents. Such a method comprises dissolving an active agent in a solvent followed by precipitating the active agent out of solution. Homogenization is a technique that does not use milling or grinding media. Active agent in a liquid media constitutes a process stream propelled into a process zone, which in a Microfluidizer® (Microfluidics, Inc.) is called the Interaction Chamber. The geometry of the interaction chamber produces powerful forces of sheer, impaction, and cavitation which are responsible for particle size reduction. U.S. Patent No. 5,510,118 refers to a bi-phasic process using a Microfluidizer® resulting in nanoparticulate active agent particles. Finally, supercritical fluid methods of making nanoparticulate active agent compositions comprise dissolving an active agent in a solution. The solution and a supercritical fluid are then co-introduced into a particle formation vessel. The temperature and pressure are controlled, such that dispersion and extraction of the vehicle occur substantially simultaneously by the action of the supercritical fluid. Examples of known supercritical methods of making nanoparticles include International Patent Application No. WO 97/144407 and U.S. Patent No. 6,406,718.

There is a need in the art for drug delivery dosage forms having inherent antibacterial, anti-yeast, anti-fungal, and/or anti-viral properties. In addition, there is a need for compositions having such inherent antibacterial, anti-yeast, anti-fungal, and/or anti-viral properties, and which can be used in the absence of an active agent. The present invention satisfies these needs.

SUMMARY

The invention is directed to nanostructured compositions having antimicrobial, anti-fungal, anti-yeast, and/or anti-viral properties. The compositions comprise at least one oil, at least one surfactant, at least one solvent, and water. Additionally, the compositions can comprise an active agent. The active agent can be useful, for example, as a pharmaceutical, diagnostic, or cosmetic.

Another aspect of the invention is directed to pharmaceutical compositions comprising a nanostructured composition according to the invention, as well as one or more desired pharmaceutically acceptable carriers and/or desired excipients which may include viscosity modifiers, colors, flavoring agents, fragrances, etc.

One aspect of the invention is directed to a unique active pharmaceutical ingredient nano-structured formulation, which comprises (1) a micelle component, (2) a hydro-alcoholic component, *i.e.*, a mixture of water and water-miscible solvent, (3) an oil-in-water emulsion droplet component, and optionally (4) a solid particle component of an active agent. Any or all of these components may comprise a desired active pharmaceutical, diagnostic, cosmetic, or other active ingredient. Thus the active agent may be in solution, as denoted in components 1 to 3, or it may be in precipitated suspension form, as is the case in component 4.

In one embodiment, when the composition is applied to the skin, the solubilized form travels across the skin and into deeper dermal layers, such as into the dermis. The other components, such as the micelles, oil fraction, and/or the particulate drug may typically position themselves towards the *Stratum corneum* of the skin layer. Depending on various physical and chemical properties, certain compounds may position themselves in different layers of epidermis and dermis, while others might permeate directly across the skin.

This composite formulation avoids having to incorporate chemical permeation enhancers that are otherwise necessary to induce transdermal permeation of the active pharmaceutical ingredient.

Another aspect of the invention is directed to a method for preparing the nanostructured compositions of the invention comprising: (a) combining a mixture of at least one oil, at least one solvent, and at least one surfactant (also referred to as a surface stabilizer) to form an emulsion base, (b) adding water to the emulsion base, and (c) homogenizing or vigorously stirring the mixture. If an active agent is to be utilized in the composition, an exemplary method of making the composition comprises: (a) adding the active agent to a mixture of oil, solvent, and surfactant or stabilizer to form an emulsion base, wherein the active agent is soluble in either or both of oil and solvent, but is not soluble in water, (b) adding water to the emulsion base, and (c) homogenizing or vigorously stirring the mixture.

Finally, methods of using the compositions of the invention to treat subjects in need, or as a broad spectrum antimicrobial composition for topical or surface disinfection, are also encompassed by the invention.

Both the foregoing general description and the following brief description of the drawings and detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the results of antibacterial effectiveness testing for a placebo composition (*i.e.*, lacking an active agent) of the invention conducted according to the protocol specified in USP 25, <51>, pp. 1869-1871 (testing with *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *C. albicans* (ATCC 10231), and *A. niger* (ATCC 16404)).

Figure 2 shows the results of antibacterial effectiveness testing for a placebo composition (*i.e.*, lacking an active agent) of the invention conducted according the protocol specified in USP 25, <51>, pp. 1869-1871, but with additional microorganisms (*P. aeruginosa* ATCC 13388), *P. aeruginosa* (ATCC 25619) *A. flavus*, and *A. fumigatus*).

Figure 3 shows the results of antibacterial effectiveness testing for estradiol compositions of the invention conducted according to the protocol specified in USP 25, <51>, pp. 1869-1871 (testing with *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *C. albicans* (ATCC 10231), *and A. niger* (ATCC 16404)).

Figure 4 shows the results of antibacterial effectiveness testing for testosterone compositions of the invention conducted according to the protocol specified in USP 25, <51>, pp. 1869-1871 (testing with *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *C. albicans* (ATCC 10231), *and A. niger* (ATCC 16404)).

Figure 5 shows the estradiol particle size in a nanostructured composition following homogenization.

Figure 6 shows the estradiol particle size in a nanostructured composition following the Silverson Method.

Figure 7 shows the testosterone particle size in a nanostructured composition following homogenization.

DETAILED DESCRIPTION

A. Overview of the Invention

The invention is directed to nanostructured compositions that surprisingly have antimicrobial, anti-fungal, anti-yeast, and/or anti-viral properties. The nanostructured compositions of the invention comprise at least one solvent, at least one oil, at least one surface stabilizer (also referred to as a surfactant), and aqueous medium. The compositions additionally may comprise one or more active agents, which may be dissolved or dispersed in any one of the oil, solvent, or water. The active agent can be useful, for example, as a pharmaceutical or cosmetic. No external antibacterial agent or preservative is required to be added to the compositions of the invention to impart the antimicrobial, anti-yeast, anti-fungal, and/or anti-viral properties.

The compositions of the invention meet the Antimicrobial Effectiveness Testing criteria as described in the United States Pharmacopoeia (USP – General Chapters, Section 51). This is demonstrated by the testing described in Example 4 below, in which various microbes were cultured in the presence of a composition according to the invention (testing with *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *C. albicans* (ATCC 10231), *A. niger* (ATCC 16404), *P. aeruginosa* (ATCC 13388), *P. aeruginosa* (ATCC 25619) *A. flavus*, and *A. fumigatus*). The results of the testing are also shown in Figure 1, which graphically shows the dramatic antimicrobial properties of the compositions of the invention.

The standard USP testing requires evaluation in five microorganisms: *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) *and Staphylococcus aureus* (ATCC 6538). Additional strains were used to confirm the anti-microbial effectiveness in *A. flavus, A. fumigatus, P aeruginosa* (ATCC 25619) and *P. aeruginosa* (ATCC 13388). As shown in Figure 2, which graphically shows the results of this testing, antimicrobial effectiveness in

these additional strains confirm beyond doubt that the composition vehicle itself is antimicrobial.

Moreover, the incorporation of an active agent does not compromise the antimicrobial effectiveness of the compositions of the invention. Specifically, as shown in Examples 3 and 4, compositions of the invention comprising estradiol and testosterone also exhibited significant antimicrobial effects, and satisfied the USP testing requirements for demonstrating antimicrobial activity. *See also* Figures 3 and 4.

The compositions of the invention are particularly useful in products used for topical treatment of infections, wound healing, etc., as in addition to the pharmacologic properties of the active agent in such compositions, the vehicle itself acts as a microbicidal agent. Such a composition possibly induces synergistic action and reduces the possibility of development of drug resistance microorganisms. Moreover, such a composition may enable the use of lower doses of the active agent.

At present, various types of cosmetic and pharmaceutical compositions require the addition of an antimicrobial agent to retard microbial growth. Choosing the right antimicrobial agent can be challenging due to potential interactions between the active agent and the antimicrobial agent. Moreover, the antimicrobial agent can be toxic and it can induce adverse reactions in patients.

In one embodiment of the invention, the compositions comprise an active agent, and the active agent is selected from the group consisting of, but not limited to fenofibrate, estradiol, alendronic acid, acyclovir, paclitaxel, and cyclosporine.

In another embodiment, the oil is selected from the group consisting of, but not limited to, almond oil (sweet), apricot seed oil, borage oil, canola oil, coconut oil, corn oil, cotton seed oil, fish oil, jojoba bean oil, lard oil, linseed oil (boiled), Macadamia nut oil, medium chain triglycerides, mineral oil, olive oil, peanut oil, safflower oil, sesame oil, soybean oil, squalene, sunflower seed oil, tricaprylin (1,2,3-trioctanoyl glycerol), and wheat germ oil.

In one embodiment, the solvent is selected from the group consisting of, but not limited to isopropyl myristate, triacetin, N-methyl pyrrolidinone, aliphatic and aromatic alcohols, polyethylene glycols, and propylene glycol. Other examples of useful solvents are long-chain alcohols. Ethyl alcohol and benzyl alcohol are yet other examples of alcohols that may be used in the present invention.

In yet another embodiment, the stabilizer or surfactant is selected from the group consisting of, but not limited to, sorbitan esters, glycerol esters, polyethylene glycol esters, block polymers, acrylic polymers (such as Pemulen®), ethoxylated fatty esters (such as Cremophor® RH-40), ethoxylated alcohols (such as Brij®), ethoxylated fatty acids (such as Tween 20), monoglycerides, silicon based surfactants, and polysorbates. In a further embodiment, the sorbitan ester stabilizer is Span® or Arlacel®; the glycerol ester is glycerin monostearate; the polyethylene glycol ester is polyethylene glycol stearate; the block polymer is a Pluronic®; the acrylic polymer is Pemulen®; the ethoxylated fatty ester is Cremophor® RH-40; the ethoxylated alcohol is Brij®; the ethoxylated fatty acid is Tween® 20, or a combination thereof.

In another embodiment, a homogenizing step is performed via a high-pressure system at 1,000 to 40,000 psi.

In one embodiment, the active agent particles, droplets comprising active agent, or a combination thereof have a mean particle size of less than about 10 microns. In other embodiments of the invention, the active agent particles, droplets comprising active agent, or a combination thereof have a mean particle size of less than about 9 microns, less than about 8 microns, less than about 7 microns, less than about 6 microns, less than about 5 microns, less than about 4 microns, less than about 3 microns, less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2000 nm, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, or less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, less than about 50 nm, less than about 40 nm, less than about 30 nm, less than about 20 nm, or less than about 10 nm. In one embodiment, the active agent particles, droplets comprising active agent, or a combination thereof have a mean particle size of less than about 3 microns in diameter.

Three methods of making the compositions of the invention are described. The first method does not require the presence of an active agent. In this method, at least one oil, at

least one solvent, and at least one surfactant are combined to form an emulsion base, water is added to the emulsion base, and (c) the mixture is homogenized or vigorously stirred.

If an active agent is to be utilized in the composition, an exemplary method of making the composition comprises: (a) adding the active agent to a mixture of oil, solvent, and surfactant or stabilizer to form an emulsion base, wherein the active agent is soluble in either or both of oil and solvent, but is not soluble in water, (b) adding water to the emulsion base, and (c) homogenizing or vigorously stirring the mixture.

Two specific methods for making active agent compositions of the invention are described. In the first method ("Route I"), active agent is milled (*i.e.*, homogenized or vigorously stirred to reduce the particle size of the active agent) in an emulsion base. This method requires that the active agent is poorly soluble or insoluble in all phases of the oil phase/lipophilic phase and the water or buffer. In the second method ("Route II"), simultaneous milling (*i.e.*, homogenizing or vigorously stirring to reduce the particle size of the active agent) and precipitation of the active agent in an emulsion base is observed. The second method requires that the active agent is soluble or partially soluble in one or more phases of the emulsion base; *i.e.*, that the active agent is soluble in an oil, solvent, or water or buffer.

One benefit of the methods of making the compositions of the invention as compared to prior art methods, such as wet milling, is that the methods are applicable to water-soluble active agent as well as poorly water soluble active agent. Another benefit of the methods of the invention is that they do not require grinding media or specialized grinding process or equipments. The use of such grinding media can add cost and complexity to a particle size reduction process for an active agent. Yet another benefit of the methods of the invention is that it can be used to process amorphous agents.

For Route I, an active agent is first suspended in a mixture of a non-miscible liquid, such as an oil, solvent, and water or buffer to form an emulsion base, followed by homogenization or vigorous stirring of the emulsion base. Nanoparticles can be produced with reciprocating syringe instrumentation, continuous flow instrumentation, or high speed mixing equipment. High velocity homogenization or vigorous stirring, producing forces of high shear and cavitation, are preferred. High shear processes are preferred as low shear processes can result in larger active agent particle sizes. The resultant composition is a composite mixture of active agent suspended in the emulsion droplet (nano-emulsion fraction) and sterically stabilized microcrystalline and/or nanocrystalline active agent in the

media. This tri-phasic system comprises particulate drug, oil, and water or buffer. The resultant micro/nano-particulate active agent has an effective average particle size of less than about 3 microns. Smaller particulate active agent can also be obtained, as described below.

The active agent can be precipitated out from the oil droplets by adding more of the non-miscible liquid. The precipitated active agent typically has an effective average particle size of less than about 3 microns. If desired, the active agent particles can be prevented from aggregating or clumping together by incorporating a surfactant or emulsifier, *i.e.*, a "surface stabilizer."

Route II is utilized for an active agent that is soluble in at least one part of the emulsion base, such as the solvent. For Route II, an active agent is dissolved in a mixture of oil, solvent, and stabilizer to form an emulsion pre-mix. The active agent remains in soluble form if water or buffer is not added to the mixture. Upon the addition of water or buffer and the application of shear forces, the active agent is precipitated into micro/nanoparticles having an effective average particle size of less than about 3 microns. Nanoparticles can be produced with reciprocating syringe instrumentation, continuous flow instrumentation, or high speed mixing equipment. High energy input, through high velocity homogenization or vigorous stirring, is a preferred process. The high energy processes reduce the size of the emulsion droplets, thereby exposing a large surface area to the surrounding aqueous environment. High shear processes are preferred, as low shear processes can result in larger particle sizes. This is followed by precipitation of nanoparticulate active agent previously embedded in the emulsion base. The end product comprises active agent in solution and particulate suspension, both distributed between the solvent, oil, and water or buffer. In one embodiment, nanoparticulate active agent has at least one surface stabilizer associated with the surface thereof.

Examples of active agent that are poorly water soluble in water but soluble in another liquid include estradiol, which is soluble in ethanol, and fenofibrate, which is freely soluble in 1-methyl-2-pyrrolidone or N-methyl-pyrrolidinone [NMP], slightly soluble in oil and stabilizer, and insoluble in water.

If desired, the water miscible oil droplets and active agent nanoparticles prepared using Route I or Route II can be filtered through either a 0.2 or 0.45 micron filter. Larger oil droplets and/or active agent particles can be created by simply increasing the water content, decreasing the oil-stabilizer-solvent content, or changing the shear in forming the oil droplets.

For the emulsion base used as an antimicrobial composition, or used in Route I or Route II as a drug delivery vehicle, the preferred ratio of oil:stabilizer:solvent is about 23: about 5: about 4, respectively, on a weight-to-weight basis. The preferred ratio of the oil comprising phase to water or buffer is about 2: about 1, respectively. In other embodiments of the invention, the oil may be present at about 5% to about 50% (w/w); the solvent may be present at about 0.5% to about 10% (w/w); the stabilizer or surfactant may be present at about 0.5% to about 10% (w/w), the water may be present at about 20% to about 80 % (w/w), or any combination thereof.

B. Compositions of the Invention

The methods of the invention can produce several different types of compositions. A first composition comprises: (1) at least one oil; (2) at least one solvent; (3) at least one surfactant or surface stabilizer; and (4) water. This composition exhibits broad spectrum antimicrobial activity and, therefore, the composition can be used as a general purpose disinfectant.

Other types of compositions according to the invention comprise at least one active agent. For example, a second composition can comprise: (1) nanoparticulate active agent having associated with the surface thereof at least one surface stabilizer; (2) water or a buffer; (3) an emulsion pre-mix or oil phase or lipophilic phase comprising at least one oil and optionally at least one solvent; and optionally (4) microparticulate active agent. The microparticulate active agent can be the same as or different from the nanoparticulate active agent. The particulate active agent can be present in the water or buffer, oil, solvent, or a combination thereof. Such a composition is made utilizing Route I.

A third composition comprises: (1) nanoparticulate active agent having associated with the surface thereof at least one surface stabilizer; (2) water or buffer; and (3) an emulsion pre-mix or oil phase or lipophilic phase comprising at least one oil, optionally at least one solvent, and solubilized active agent. The composition may additionally comprise microparticulate active agent. The solubilized active agent can be present in the water or buffer, oil, solvent, or a combination thereof. In addition, nanoparticulate active agent can be present in the water or buffer, oil, solvent, or a combination thereof. Such a composition is made utilizing Route II. In a further embodiment of the invention, the solubilized active agent can be precipitated out from the emulsion droplets. The precipitated active agent preferably has an effective average particle size of less than about 3 microns.

The tri-phasic compositions of the invention are beneficial for several reasons. First, formulations resulting from the Route II method comprise both solid and solubilized forms of the same active agent. This enables a resultant pharmaceutical formulation to provide both immediate release and controlled release of the component active agent, providing for fast onset of activity combined with prolonged activity of the active agent.

Moreover, when formulated for topical application to the skin, in a cream or lotion for example, the solid active agent nanoparticles may provide an immediate local therapeutic effect at the skin surface, while the solubilized active agent within the emulsion base crosses the skin/cell barrier allowing the active agent to enter the body's system. That is, the solubilized active agent crosses the skin rapidly and penetrates into deeper layers, whereas the solid part does not permeate into deeper skin layers, but acts as local depot and as a reservoir for supplying drug into deeper layers. Hence, a formulation comprising both active agent nanoparticles and solubilized active agent can provide local and systemic therapeutic effects, which are particularly beneficial for transdermal dosage forms.

The different components of the two types of compositions described above can be separated and, if desired, used independently.

This invention permits many different types of active agents to be formulated into emulsion-based formulations. Examples of such active agents include, but are not limited to, acyclovir, cyclosporine, estradiol, fenofibrate, ceterizine, nicotine, naltrexone, and alendronic acid.

1. Active Agent Nanoparticles

The solid active agent nanoparticles can be separated from the aqueous suspension media and/or the emulsion globules, for instance, by filtration or centrifugation. This provides a convenient method of obtaining nanoparticles of a poorly water-soluble or water-insoluble active agent. Furthermore, when a surface stabilizer is included in the particle size reduction process, it prevents the active agent nanoparticles from aggregating and, therefore, the active agent nanoparticles are stabilized at a nanoparticulate size. If desired, the active agent nanoparticles can then be formulated into any suitable dosage form. Active agent nanoparticles can be made using food grade, USP or NF grade materials suitable for human use applications.

Exemplary dosage forms include, but are not limited to, liquid dispersions, oral suspensions, tablets, gels, aerosols, ointments, creams, capsules, dry powders,

multiparticulates, sprinkles, sachets, lozenges, and syrups. Moreover, the dosage forms of the invention may be solid dosage forms, liquid dosage forms, semi-liquid dosage forms, immediate release formulations, modified release formulations, controlled release formulations, fast melt formulations, lyophilized formulations, delayed release formulations, extended release formulations, pulsatile release formulations, mixed immediate release and controlled release formulations, or any combination thereof. The compositions of the invention may be formulated for delivery via any suitable method, such as for parenteral injection (*i.e.*, intravenous, intramuscular, or subcutaneous), oral administration in solid, liquid, or aerosol form, vaginal, nasal, rectal, ocular, otic, local (powders, ointments or drops), buccal, intracisternal, intraperitoneal, or topical administration, and the like.

Since the active agent nanoparticles have an effective average particle size of less than about 3 microns, the particles typically are more readily able to move across absorption barriers, such as skin, as compared to microcrystalline active agent. Similarly, the small active agent particle size enables passage through blood/tissue and blood/tumor barriers of various organs.

2. Emulsion Globules Comprising Active Agent Nanoparticles and/or Solubilized Active Agent

The emulsion globules comprising solubilized active agent, active agent nanoparticles, or a combination thereof can also be isolated, if desired, from the surrounding aqueous or buffer phase and used in therapeutic dosage forms. The emulsion globules can be made using food grade, USP or NF grade materials suitable for human use applications. Nanoparticulate oil globules comprising solubilized active agent, and methods of making the same, are described in U.S. Patent No. 5,629,021 ("the '021 patent"), which is incorporated herein by reference. The emulsion globules of the invention typically comprise: (1) at least one oil; (2) at least one solvent; (3) at least one surface stabilizer or surfactant; and optionally (4) solubilized active agent, particulate active agent, or a combination thereof. Emulsion globules comprising solubilized active agent, particulate active agent, or a combination thereof can be isolated, if desired, for example, by filtration. Emulsion globules comprising solubilized active agent are particularly suitable vehicles for transporting active agent across the skin barrier and into the blood. Hence, globules comprising solubilized active agent offer a systemic way to administer active agent to an individual.

In general, the emulsion globules comprising solubilized active agent, active agent nanoparticles, or a combination thereof have diameters of about 10 to about 1000 nm, and comprise a significant quantity of active agent, with a mean a diameter of less than about 1 micron preferred, and with the smallest globules filterable through a 0.2 micron filter, such as is typically used for microbiological purification. The range of active agent concentration in the globules can be from about 1% to about 50%. The emulsion globules can be stored at between about 20 to about 40°C. In one embodiment of the invention, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the globules in the preparation have diameters of less than about 1 micron, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, or less than about 100 nm.

By varying different parameters of Route I and Route II, the size and integrity of such globules can be modified. Hence, the stability of globules comprising dissolved active agent can be altered to enable the release of active agent, either as a solution or precipitate. This is a microreservoir-dissolution-controlled system, where the drug solids acts as depot and, as the solubilized fraction is depleted, more drug is drawn into solution form the particulate

depot. Thus, the emulsion globules comprising solubilized active agent enable controlled active agent release over time.

The small size of the emulsion globules comprising solubilized active agent, active agent nanoparticles, or a combination thereof and their compatibility with tissue render them applicable to numerous uses. For example, the emulsion globules are useful as topical drug delivery vehicles as they enable rapid dermal penetration. The globules are also exceptionally versatile in that the active agent utilized can be any active agent that is suspendable or dissolvable in any of the water or buffer, oil, or solvent. These properties allow this system to be used with active agents that are difficult to formulate for use in other delivery systems.

In addition, the emulsion globules comprising solubilized active agent, active agent nanoparticles, or a combination thereof can be diluted with aqueous solutions without stability loss. This enables the use of high active agent concentration, *i.e.* up to about 30%, products which can be diluted for use as necessary. The concentration of active agent, however, depends on the solubility of the actual drug and the amount of solvent used to dissolve it.

In one embodiment of the invention, the emulsion globules comprise as an active agent estradiol, acyclovir, or testosterone and are formulated into a dosage form for transdermal delivery.

C. Components of the Methods and Compositions of the Invention

1. Active Pharmaceutical Ingredient

a. Properties

Any suitable active agent may be employed in the compositions and methods of the invention. For an active agent to be utilized in the Route I method, the active agent must be poorly soluble, or insoluble, in all phases of the milling (*i.e.*, homogenization or vigorously stirring) system, including water and the solvent and oil to be used in the method. For an active agent to be utilized in Route II, the active agent must be poorly water soluble, or water insoluble, but soluble in at least one phase of the emulsion base, such as the oil or solvent and stabilizer or stabilizer solution.

By "poorly water-soluble" or "water insoluble" it is meant that the active agent has a solubility in water of less than about than about 20 mg/mL, less than about 10 mg/mL, less than about 1 mg/mL, less than about 0.1 mg/mL, less than about 0.01 mg/mL, or less than about 0.001 mg/mL at ambient temperature and pressure and at about pH 7.

The active agent to be used in the methods of the invention, and present in the compositions of the invention, can be amorphous, semi-amorphous, crystalline, semi-crystalline, or a mixture thereof.

b. Active Agent Particle Size

As used herein, active agent particle size is determined on the basis of the weight average particle size as measured by conventional techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, laser diffraction, or disk centrifugation.

As used herein, "nanoparticulate active agent" refers to active agent having an effective average particle size of less than about 3 microns. "microcrystalline active agent" refers to active agent having an effective average particle size of greater than about 3 microns.

As used herein, an "effective average particle size" for an active agent is the size below which about 50% of the active agent particles fall. Thus, if the effective average particle size is less than about 3 microns, at least 50% of the active agent particles in the composition have a size of less than about 3 microns. In other embodiments of the invention, the effective average particle size of the active agent particles in the compositions of the invention can be less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2000 nm, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, or less than about 100 nm.

In other embodiments of the invention, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99% of the active agent particles have a size less than the effective average, *i.e.*, less than about 3 microns, less than about 2900 nm, less than about 2800 nm, etc.

c. Exemplary Active Agents

Any suitable active agent may be used in the methods and compositions of the invention. Examples of classes of useful active agent include, but are not limited to, therapeutic and diagnostic agents, pigments, paints, inks, dyes, photographic materials, cosmetic ingredients, etc.

Amphiphile-type active agents may be incorporated into the present formulations. That is, drugs or therapeutic compounds that can be ionized and are soluble in polar or non-polar solvents may be incorporated in the formulations of the present invention. Such compounds are soluble, therefore, both in oil and aqueous environments (amphiphiles). Examples of such compounds include nicotine and ceterizine.

Hydrophilic active agents also may be incorporated into a formulation of the present invention. Such compounds include, but are not limited to naltrexone hydrochloride, alendronic acid, and ceterizine dihydrochloride.

The active agent may be a hormone, such as testosterone, progesterone, and estrogen. Other hormones include: (1) Amine-derived hormones, such as catecholamines, adrenaline (or epinephrine), dopamine, noradrenaline (or norepinephrine), tryptophan derivatives, melatonin (N-acetyl-5-methoxytryptamine), serotonin (5-HT), tyrosine derivatives, thyroxine (T4), triiodothyronine (T3); (2) peptide hormones, such as antimullerian hormone (AMH, also mullerian inhibiting factor or hormone), adiponectin (also Acrp30), adrenocorticotropic hormone (ACTH, also corticotropin), angiotensinogen and angiotensin, antidiuretic hormone (ADH, also vasopressin, arginine vasopressin, AVP), atrial-natriuretic peptide (ANP, also atriopeptin), Calcitonin, cholecystokinin (CCK), corticotropin-releasing hormone (CRH), erythropoietin (EPO), follicle-stimulating hormone (FSH), gastrin, glucagons, gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), human chorionic gonadotropin (hCG), growth hormone (GH or hGH), insulin, insulin-like growth factor (IGF, also somatomedin), leptin, luteinizing hormone (LH), melanocyte stimulating hormone (MSH or α-MSH), neuropeptide Y, oxytocin, parathyroid hormone (PTH), prolactin (PRL), relaxin, rennin, secretin, somatostatin, thrombopoietin, thyroid-stimulating hormone (TSH), thyrotropin-releasing hormone (TRH); (3) steroid hormones, such as glucocorticoids, cortisol, mineralocorticoids, aldosterone, sex steroids, androgens, testosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, dihydrotestosterone (DHT), Estrogens, estradiol, Progestagens, progesterone, Progestins, (4) sterol hormones, such as vitamin D derivatives and calcitriol,

(5) lipid and phospholipid hormones (eicosanoids) such as prostaglandins, leukotrienes, prostacyclin, and thromboxane.

In one embodiment of the invention, therefore, the active agent is estradiol, fenofibrate, acyclovir, alendronic acid, or testosterone. Specific examples of active agents that may be utilized in the methods of the invention include, but are not limited to, insulin, calcitonin, calcitonin gene regulating protein, atrial natriuretic protein, betaserori, erythropoietin, alpha interferon, beta interferon, gamma interferon, somatropin, somatotropin, somastostatin, insulin-like growth factor, luteinizing hormone releasing hormone, factor VIII, interleukins, interleukin analogues, hematological agents, anticoagulants, hematopoietic agents, hemostatics, thrombolytic agents, endocrine agents, antidiabetic agents, antithyroid agents, beta-adrenoceptor blocking agents, growth hormones, growth hormone releasing hormone, sex hormones, thyroid agents, parathyroid calcitonin, bisphosphonates, uterine-active agents, cardiovascular agents, antiarrhythmic agents, anti-anginal agents, anti-hypertensive agents, vasodilators, agents used in treatment of heart disorders, cardiac inotropic agents, renal agents, genitourinary agents, antidiuretic agents, respiratory agents, antihistamines, cough suppressants, parasympathomimetics, sympathomimetics, xanthines, central nervous system agents, analgesics, anesthetics, anti-emetic agents, anorexiants, antidepressants, anti-migraine agents, antiepileptics, dopaminergics, anticholinergics, antiparkinsonian agents, muscle relaxants, narcotic antagonists, sedatives, stimulants, treatments for attention deficit disorder, methylphenidate, fluoxamine, bisolperol, tacrolimus, sacrolimus, cyclosporine, gastrointestinal agents, systemic anti-infectives, agents used in the treatment of AIDS, anthelmintics, antimycobacterial agents, immunologic agents, vaccines, hormones; dermatological agents including, anti-inflammatory agents, elastase inhibitors, antimuscarinic agents, lipid regulating agents, blood products, blood substitutes, antineoplastic agents including, leuprolide acetate, chemotherapy agents, oncology therapies, nutrients, nutritional agents, chelating agents, interleukin-2, IL-1ra, heparin, hirudin, colony stimulating factors, tissue plasminogen activator, oxytocin, nitroglycerine, diltiazem, clonidine, nifedipine, verapamil, isosorbide-5-mononitrate, organic nitrates, diuretics, desmopressin, vasopressin, expectorants, mucolytics, fentanyl, sufentanil, butorphanol, buprenorphine, levorphanol, morphine, hydromorphone, hydrocodone, oxymorphone, methadone, lidocaine, bupivacaine, diclofenac, naproxen, paverin, scopolamine, ondansetron, domperidone, metoclopramide, sumatriptan, ergot alkaloids, benzodiazepines, phenothiozines, prostaglandins antibiotics, anti-viral agents, anti-fungals,

immunosuppressants, anti-allergic agents, astringents, corticosteroids fluorouracil, bleomycin, vincristine, or deferoxamine. The active agent may be useful in the treatment of wound-healing.

2. Oils

For both the methods of Route I and Route II and the compositions of the invention, any suitable oil can be used. Exemplary oils that can be used include, for example, vegetable oils, nut oils, fish oils, lard oil, mineral oils, squalane, tricaprylin, and mixtures thereof. Specific examples of oils that may be used include, but are not limited to, almond oil (sweet), apricot seed oil, borage oil, canola oil, coconut oil, corn oil, cotton seed oil, fish oil, jojoba bean oil, lard oil, linseed oil (boiled), Macadamia nut oil, medium chain triglycerides, mineral oil, olive oil, peanut oil, safflower oil, sesame oil, soybean oil, squalene, sunflower seed oil, tricaprylin (1,2,3-trioctanoyl glycerol), wheat germ oil, and mixtures thereof.

3. Surface Stabilizers or Surfactants

The compositions of the invention also comprise at least one surface stabilizer or surfactant. When the compositions additionally comprise a nanoparticulate active agent, the surface stabilizer used in the methods and compositions of the invention associates with, or adsorbs to, the surface of the nanoparticulate active agent, but does not covalently bind to the active agent. The surface stabilizer is preferably soluble in water. One or more surface stabilizers may be used in the compositions and methods of the invention. As used herein, the terms "stabilizer", "surface stabilizer", and "surfactant" are used interchangeably.

Any suitable nonionic or ionic surfactant may be utilized in the compositions of the invention, including anionic, cationic, and zwitterionic surfactants. Exemplary stabilizers or surfactants that may be used in the compositions of the invention lacking an active agent, and in active agent comprising compositions of the invention, include but are not limited to, non-phospholipid surfactants, such as the Tween® (polyoxyethylene derivatives of sorbitan fatty acid esters) family of surfactants (*i.e.*, Tween® 20, Tween® 60, and Tween® 80), nonphenol polyethylene glycol ethers, sorbitan esters (such as Span® and Arlacel®), glycerol esters (such as glycerin monostearate), polyethylene glycol esters (such as polyethylene glycol stearate), block polymers (such as Pluronics®), acrylic polymers (such as Pemulen®), ethoxylated fatty esters (such as Cremophor® RH-40), ethoxylated alcohols (such as Brij®), ethoxylated fatty

acids, monoglycerides, silicon based surfactants, polysorbates, and Tergitol® NP-40 (Poly(oxy-1,2-ethanediyl), α -(4-nonylphenol)-.omega.-hydroxy, branched [molecular weight average 1980]), and Tergitol® NP-70 (a mixed surfactant--AQ=70%).

4. Solvents

Any suitable solvent can be used in the methods and compositions of the invention. Exemplary solvents include, but are not limited, to isopropyl myristate, triacetin, N-methyl pyrrolidinone, long-chain alcohols, polyethylene glycols, propylene glycol, diethyleneglycol monoethyl ether, and long- and short-chain alcohols, such as ethanol. Other short chain alcohols and/or amides may be used, as well as aliphatic and aromatic alcohols, such as benzyl alchohol. Mixtures of solvents can also be used in the compositions and methods of the invention.

5. Water or Buffer

If the methods and/or compositions of the invention use or comprise water or a buffer, the aqueous solution is preferably a physiologically compatible solution such as water or phosphate buffered saline.

6. Other Ingredients

A number of other materials may be added to the compositions of the invention. Volatile oils, such as volatile flavor oils, can be used in lieu of some of the oil or can be added in addition to the primary oil. Exemplary volatile oils or fragrances that can be utilized in the invention include, but are not limited to, balm oil, bay oil, bergamot oil, cedarwood oil, cherry oil, cinnamon oil, clove oil, origanum oil, and peppermint oil. A coloring agent, such as a food coloring agent can also be used. Exemplary food colors that can be utilized in the compositions of the invention include, but are not limited to, green, yellow, red, and blue. The food colors utilized are food grade materials (McCormick), although materials from other sources can be substituted. In addition, a flavoring extract can be used in the methods and compositions of the invention. Exemplary flavored extracts include, but are not limited to, pure anise extract (73% alcohol), imitation banana extract (40% ethanol), imitation cherry extract (24% ethanol), chocolate extract (23% ethanol), pure lemon extract (84% ethanol), pure orange extract (80% ethanol), pure peppermint extract (89% ethanol), imitation

pineapple extract (42% ethanol), imitation rum extract (35% ethanol), imitation strawberry extract (30% ethanol), and pure or imitation vanilla extract (35% ethanol). Typically, the extracts utilized are food grade materials (McCormick), although materials from other sources can be substituted.

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D. Methods of Using the Compositions of the Invention

The compositions of the invention are useful as a broad spectrum antimicrobial agent. They can be used for topical or surface disinfection, for example, of biological and non-biological surfaces. The compositions can be used to disinfect surfaces likely to be exposed 10 to microbes, such as cooking surfaces, eating surfaces, any surface in a hospital, areas exposed to young children, etc. Biological surfaces include skin, hair, mucous membranes, wound surfaces, insect bites, etc.

The compositions can also be used as delivery vehicles for an active agent, such as a drug or cosmetic. The compositions avoid the requirement of adding a antimicrobial agent to 15 retard microbial growth. When formulated into a dosage form for administration to a mammal, such as a human, the compositions of the invention can be administered to a subject via any conventional means. The compositions can be useful in the formulation of active agents intended to treat wounds and would surfaces. Some of these active agents are not compatible with typical antimicrobial agents. Therefore, a vehicle that is inherently 20 antimicrobial may be an ideal delivery system.

As used herein, the term "subject" is used to mean an animal, preferably a mammal, including a human or non-human. The terms patient and subject may be used interchangeably. In addition, the compositions of the invention can be formulated into any suitable dosage form, such as liquid dispersions, oral suspensions, tablets, gels, aerosols, 25 ointments, creams, capsules, dry powders, multiparticulates, sprinkles, sachets, lozenges, and syrups. Moreover, the dosage forms of the invention may be solid dosage forms, liquid dosage forms, semi-liquid dosage forms, immediate release formulations, modified release formulations, controlled release formulations, fast melt formulations, lyophilized formulations, delayed release formulations, extended release formulations, pulsatile release 30 formulations, mixed immediate release and controlled release formulations, or any combination thereof.

The compositions of the invention may also comprise adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

Liquid dosage forms include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active agent, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

"Therapeutically effective amount" as used herein with respect to an active agent dosage shall mean that dosage that provides the specific pharmacological response for which the active agent is administered in a significant number of subjects in need of such treatment. It is emphasized that "therapeutically effective amount," administered to a particular subject in a particular instance may not be effective for 100% of patients treated for a specific disease, and will not always be effective in treating the diseases described herein, even though such dosage is deemed a "therapeutically effective amount" by those skilled in the art.

One of ordinary skill will appreciate that effective amounts of an active agent can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester, or prodrug form. Actual dosage levels of an active agent in the compositions of the invention may be varied to obtain an amount of the active agent that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the potency of the administered active agent, the desired duration of treatment, and other factors.

Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular or physiological response to be achieved; activity of the specific agent or

composition employed; the specific agents or composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts.

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

EXAMPLES

Example 1

The purpose of this example was to describe preparation of an exemplary nanostructured composition according to the invention.

Ethyl alcohol, soybean oil, and Polysorbate 80 were mixed together. Water was then added to this mixture, and the resulting composition was mixed well using a paddle stirrer. The quantities of each component are shown below in Table 1.

TABLE 1

Ingredient	Quantity
Ethyl Alcohol USP	8.8 gm
Polysorbate 80 NF	9.4 gm
Soybean oil USP	50.2 gm
Water USP	31.7 gm

The composition was then fed into a high-pressure homogenizer (APV Invensys, model APV-1000) and the pressure tuned to 10,000 psi. The mixture was run through the homogenizer for 2 passes.

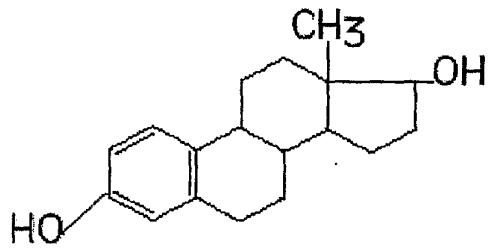
Alternatively, water can be added to the ethyl alcohol, oil, and Polysorbate 80 mixture under high-speed stirring using a rotor-stator assembly mounted on Silverson mixer (10,000 rpm for 15 minutes).

The resulting composition can be used directly as a topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, or the composition can be formulated into another suitable dosage form.

Example 2

The purpose of this example was to describe preparation of an exemplary nanostructured composition according to the invention comprising the active agent estradiol.

Estradiol is chemically described as estra-1,3,5(10)-triene-3,17beta -diol. The molecular formula of estradiol is $C_{18}H_{24}O_2$ and the structural formula is:



The molecular weight of estradiol is 272.39.

Current U.S. Food and Drug Administration (FDA) approved estradiol products include oral pills (Estinyl®, Estrace®, Gynodiol®, Ovcon 35®), transdermal patches (Climara®, Vivelle®, Estraderm®), a vaginal ring (Estring®), and a topical emulsion (Estrasorb®). The drug is used in hormone replacement therapy, to treat moderate to severe symptoms of hot flashes and night sweats associated with menopause and to prevent pregnancy.

Estradiol was dissolved in ethanol. The oil and Polysorbate 80 were added to the estradiol solution, water was added to the estradiol/ethyl alcohol/oil/Polysorbate 80 mixture, and the resulting composition was mixed well using a paddle stirrer. The quantities of each component are shown below in Table 2A.

TABLE 2A

Ingredient	Quantity
Estradiol	0.25 gm
Ethyl Alcohol USP	8.8 gm
Polysorbate 80 NF	9.4 gm
Soybean oil USP	50.2 gm
Water USP	31.7 gm

The composition was fed into a high-pressure homogenizer (APV Invensys, model APV-1000) and the pressure was tuned to 10,000 psi. The mixture was run through the homogenizer for 2 passes. The particle size of the composition is detailed in Table 2B below and is further represented in Figure 5.

Table 2B

Mean (μm)	Standard Deviation (μm)
0.0826	0.048

Alternatively, estradiol can be dissolved in ethyl alcohol, the oil and water can be added to the estradiol solution, and water can be added to the resulting composition under high-speed stirring using a rotor-stator assembly mounted on Silverson mixer (10,000 rpm for 15 minutes).

The particle size of the composition according to the Silverson method is detailed in Table 2C below and is further represented in Figure 6.

Table 2C

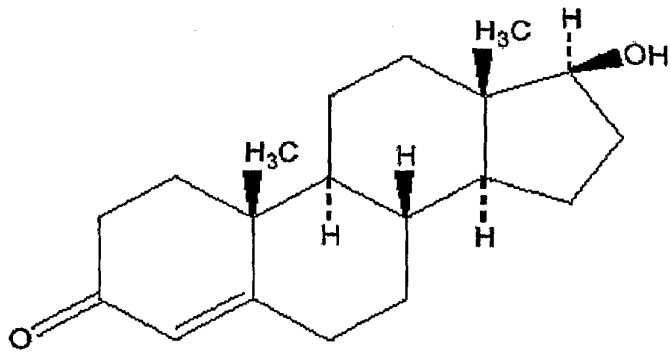
Mean (μm)	Standard Deviation (μm)
1.204	0.930

The resulting composition can be used directly as an estradiol topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, or the composition can be formulated into another suitable dosage form.

Example 3

The purpose of this example was to describe preparation of an exemplary nanostructured composition according to the invention comprising the active agent testosterone.

Testosterone USP is a white to practically white crystalline powder chemically described as 17-beta hydroxyandrost-4-en-3-one.



Testosterone

It has the chemical formula $C_{19}H_{28}O_2$ and a molecular weight of 288.42. Testosterone is commercially available, for example, in a injectable dosage forms, transdermal gel (AndroGel®, Testim®), transdermal delivery device (Androderm®, Testoderm®), and a buccal drug delivery system (Striant®). Testosterone is used, for example, in hormone replacement therapy.

Testosterone was dissolved in ethyl alcohol. Oil and Polysorbate 80 were added to the testosterone solution, and water was added to the resulting composition. The resulting composition was mixed well using a paddle stirrer. The quantities of each component are shown below in Table 3A.

TABLE 3A	
Ingredient	**Quantity**
Testosterone	3.09 gm
Ethyl Alcohol USP	8.8 gm
Polysorbate 80 NF	9.4 gm
Soybean oil USP	50.2 gm
Water USP	31.7 gm

The testosterone/ethyl alcohol/oil/Polysorbate 80/water composition was fed into a high-pressure homogenizer (APV Invensys, model APV-1000) and the pressure was tuned to 10,000 psi. The mixture was run through the homogenizer for 2 passes. The particle size of the composition is detailed in Table 3B below and is further represented in Figure 7.

Table 3B

Mean (μm)	**Standard Deviation (μm)**
1.052	0.507

Alternatively, the testosterone can be dissolved in ethyl alcohol, and oil and the Polysorbate 80 can be added to the testosterone solution. Water can be added to the testosterone/ethyl alcohol/oil/Polysorbate 80 composition under high-speed stirring using a rotor-stator assembly mounted on Silverson mixer (10,000 rpm for 15 minutes).

The resulting composition can be used directly as a testosterone topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, or the composition can be formulated into another suitable dosage form.

Example 4

The purpose of this example was to determine the effect of concentration of excipients on the antimicrobial properties of the formulation. The composition of Example 1 was diluted with water by 10-fold and tested for antimicrobial effectiveness.

The quantities of each component are shown below in Table 4

TABLE 4	
Ingredient	**Quantity**
Ethyl Alcohol USP	0.88 gm
Polysorbate 80 NF	0.94 gm
Soybean oil USP	5.02 gm
Water USP	93.16 gm

This formulation did not meet the criteria set forth when tested according to USP Antimicrobial Effectiveness Testing standards. The resulting composition cannot be used directly as a topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, nor can the composition be formulated into another suitable dosage form in order to impart antimicrobial properties.

Example 5

The purpose of this example was as yet another example to determine the effect of concentration of excipients on the antimicrobial properties of the formulation. The composition of Example 1 was diluted with water by 50-fold and tested for antimicrobial properties.

The quantities of each component are shown below in Table 4

TABLE 5	
Ingredient	**Quantity**
Ethyl Alcohol USP	0.18 gm
Polysorbate 80 NF	0.19 gm
Soybean oil USP	1.0 gm
Water USP	98.63 gm

This formulation did not meet the criteria set forth when tested according to USP Antimicrobial Effectiveness Testing standards. The resulting composition cannot be used directly as a topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, nor can the composition be formulated into another suitable dosage form in order to impart antimicrobial properties.

Example 6

The purpose of this example was to describe preparation of an exemplary nanostructured composition according to the invention.

Ethyl alcohol, soybean oil, and Pluronic F-68 were mixed together. Water was then added to this mixture, and the resulting composition was mixed well using a paddle stirrer. The quantities of each component are shown below in Table 6.

TABLE 6	
Ingredient	**Quantity**
Ethyl Alcohol USP	8.8 gm
Pluronic F-68	6.0 gm
Soybean oil USP	50.2 gm
Water USP	35.0 gm

The composition was then fed into a high-pressure homogenizer (APV Invensys, model APV-1000) and the pressure tuned to 10,000 psi. The mixture was run through the homogenizer for 2 passes.

Alternatively, water can be added to the ethyl alcohol, oil, and Pluronic F-68 mixture under high-speed stirring using a rotor-stator assembly mounted on Silverson mixer (10,000 rpm for 15 minutes).

The resulting composition can be used directly as a topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, or the composition can be formulated into another suitable dosage form.

Example 7

The purpose of this example was to describe preparation of an exemplary nanostructured composition according to the invention.

Ethyl alcohol, benzyl alcohol, isopropyl myristate, light mineral oil, and Pluronic F-68 were mixed together. Water was then added to this mixture, and the resulting composition was mixed well using a paddle stirrer. The quantities of each component are shown below in Table 7.

TABLE 7

Ingredient	Quantity
Ethyl Alcohol USP	10.6 gm
Benzyl Alcohol	5.3 gm
Pluronic F-68	7.4 gm
Isopropyl Myristate	5.3 gm
Light Mineral Oil	40.4 gm
Water USP	30.9 gm

The composition was then fed into a high-pressure homogenizer (APV Invensys, model APV-1000) and the pressure tuned to 10,000 psi. The mixture was run through the homogenizer for 2 passes.

Alternatively, water can be added to the ethyl alcohol, oil, and Pluronic F-68 mixture under high-speed stirring using a rotor-stator assembly mounted on Silverson mixer (10,000 rpm for 15 minutes).

The resulting composition can be used directly as a topical lotion or cream having antibacterial, anti-viral, anti-fungal, and/or anti-yeast properties, or the composition can be formulated into another suitable dosage form.

Example 8

The purpose of this example was to describe preparation of a nanostructured composition that included propofol, a model active pharmaceutical ingredient, and that did not meet the USP Antimicrobial Effectiveness Testing standards.

Propofol, Ethyl alcohol, soybean oil, and Polysorbate 80 were mixed together. Saline was then added to this mixture, and the resulting composition was mixed well using a paddle stirrer. The quantities of each component are shown below in Table 8.

TABLE 8

Ingredient	Quantity
Propofol	1.0 gm
Ethyl Alcohol USP	0.5 gm
Polysorbate 80 NF	0.9 gm
Soybean oil USP	4.5 gm
Saline	93.1 gm

The composition was then fed into a high-pressure homogenizer (APV Invensys, model APV-1000) and the pressure tuned to 10,000 psi. The mixture was run through the homogenizer for 2 passes.

This formulation did not meet the criteria set forth when tested according to USP Antimicrobial Effectiveness Testing standards.

Example 9

The purpose of this example was to determine the antimicrobial effects of a composition prepared as described in Example 1.

Antimicrobial testing was conducted according to USP 25, <51>, pp. 1869-1871. A quantity of the composition prepared as described in Example 1 was challenged with various bacteria, yeast, or fungus: *Aspergillus niger* (fungus), *Candida Albicans* (yeast), *Escherichia coli* (bacteria), *Pseudomonas aeruginosa* (bacteria), *Staphylococcus aureus* (bacteria), *Aspergillus fumigatus* (fungus), and *Aspergillus flavus* (fungus).

Prior to the test, the surface of a suitable volume of solid agar medium was inoculated from a recently revived stock culture of each of the organisms shown below in Table 4. The quantity of inoculum of the composition prepared as in Example 1 for each test is shown in

Table 4, below. The quantity of bacteria, fungus, or yeast was measured at the time of inoculation, at day 7, day 14, and at day 28. The culture conditions were conducted according to USP 25, <51>, pp. 1869-1871. The results of the tests are shown in Table 4. In addition, the results are graphically shown in Figure 1 and 2.

TABLE 4: Antimicrobial Effectiveness Test

Organism Time	*A. niger* ATCC* 16404	*C. albicans* ATCC 10231	*E. coli* ATCC 8739	*P. aeruginosa* ATCC 9027	*S. aureus* ATCC 6538	*A. fumigatus*	*A. flavus*	*P. aeruginosa* ATCC 25619	*P. aeruginosa* ATCC 13388
Inoculum per mL product	200,000 cfu/mL	250,000 cfu/mL	550,000 cfu/mL	220,000 cfu/mL	580,000 cfu/mL	470,000 cfu/mL	270,000 cfu/mL	777,000 cfu/mL	189,000 cfu/mL
0 hour	170,000 cfu/g	360,000 cfu/g	950,000 cfu/g	61,000 cfu/g	650,000 cfu/g	330,000 cfu/g	240,000 cfu/g	106,000 cfu/g	132,000 cfu/g
Day 7	17,000 cfu/g 1.1 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction
Day 14	2,500 cfu/g 1.9 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction
Day 28	70 cfu/g 3.5 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction	None Detected < 10 cfu/g > 4 log reduction

* ATCC = American Type Culture Collection (Manassas, VA)

The results dramatically demonstrate the surprising effectiveness of the compositions of the invention as antibacterial, antifungal, and anti-yeast agents. For all but one tested organism (*A. niger*), all organisms were eradicated after 7 days, and for *A. niger*, the growth of the organism was drastically diminished, with only 70 cfu/g measured on Day 28. Moreover, the results demonstrate that the composition meets the USP 25 requirements for Antimicrobial Effectiveness Test.

Example 10

The purpose of this example was to determine the antimicrobial effects of a composition prepared as described in Example 2, to determine if the addition of an active agent such as estradiol affects the antimicrobial, anti-yeast, anti-fungal, and/or anti-viral properties of the compositions of the invention. This example also evaluates the effect on antimicrobial activity of varying the quantity of alcohol present in the compositions of the invention.

Antimicrobial testing was conducted according to USP 25, <51>, pp. 1869-1871. A quantity of the composition prepared as in Example 2 was challenged with various bacteria, yeast, or fungus: *Aspergillus niger* (fungus), *Candida albicans* (yeast), *Escherichia coli* (bacteria), *Pseudomonas aeruginosa* (bacteria), and *Staphylococcus aureus* (bacteria).

Prior to the test, the surface of a suitable volume of solid agar medium was inoculated from a recently revived stock culture of each of the organisms shown below in Table 5. The quantity of inoculum of the composition prepared as described in Example 2 for each test is shown in Table 5, below. The quantity of bacteria, fungus, or yeast was measured at the time of inoculation, at day 14 (Table 5) and day 28 Table 6). The culture conditions were conducted according to USP 25, <51>, pp. 1869-1871. The results of the tests are shown in Tables 5 and 6. In addition, the results are graphically shown in Figure 3.

TABLE 5: Antimicrobial Effectiveness Test for Estradiol-Comprising Composition Day 14 Results					
Organism	***A. niger***	***C. albicans***	***E. coli***	***P. aeruginosa***	***S. aureus***
ATCC Strain #	16404	10231	8739	9027	6538
Inoculum per mL product	200,000 cfu/mL	250,000 cfu/mL	550,000 cfu/mL	220,000 cfu/mL	580,000 cfu/mL
% EtOH					
7.7%	30,000 cfu/g 1 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction
7.3%	17,000 cfu/g 1 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction
7.4%	17,000 cfu/g 1 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction
7.0%	13,000 cfu/g 1 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction

TABLE 6: Antimicrobial Effectiveness Test for Estradiol-Comprising Composition Day 28 Results					
Organism	***A. niger***	***C. albicans***	***E. coli***	***P. aeruginosa***	***S. aureus***
ATCC Strain #	16404	10231	8739	9027	6538
Inoculum per mL product	200,000 cfu/mL	250,000 cfu/mL	550,000 cfu/mL	220,000 cfu/mL	580,000 cfu/mL
% EtOH					
7.7%	None Detected <100 cfu/g >3 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction
7.3%	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction
7.4%	180 cfu/g 3.2 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction
7.0%	100,000 cfu/g 0.5 log reduction	None Detected <10 cfu/g > 4 log reduction	180 cfu/g 3.6 log reduction	None Detected <10 cfu/g > 4 log reduction	None Detected <10 cfu/g > 4 log reduction

Example 11

The purpose of this example was to determine the antimicrobial effects of a composition prepared as described in Example 3, to determine if the addition of an active

agent such as testosterone affects the antimicrobial, anti-yeast, anti-fungal, and/or anti-viral properties of the compositions of the invention.

Antimicrobial testing was conducted according to USP 25, <51>, pp. 1869-1871. A quantity of the composition prepared as in Example 2 was challenged with various bacteria, yeast, or fungus: *Aspergillus niger* (fungus), *Candida albicans* (yeast), *Escherichia coli* (bacteria), *Pseudomonas aeruginosa* (bacteria), and *Staphylococcus aureus* (bacteria).

Prior to the test, the surface of a suitable volume of solid agar medium was inoculated from a recently revived stock culture of each of *A. niger*, *C. albicans*, *E. coli*, *P. aeruginosa*, and *S. aureus*. The quantity of bacteria, fungus, or yeast was measured at the time of inoculation and at day 7, 14, and 28. The culture conditions were conducted according to USP 25, <51>, pp. 1869-1871. The results, shown in Figure 4, dramatically demonstrate the surprising effectiveness of the compositions of the invention as antibacterial, anti-fungal, and anti-yeast agents when the compositions are used as drug delivery vehicles. Surprisingly, the presence of an active agent does not negate the antimicrobial effects of the compositions of the invention. Moreover, the results demonstrate that the composition meets the USP 25 requirements for Antimicrobial Effectiveness Test.

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

WE CLAIM:

1. An antimicrobial composition comprising an emulsion of:
   (a) at least one solvent;
   (b) at least one surfactant;
   (c) at least one oil; and
   (d) water;
   wherein the composition meets the United States Pharmacopeia (USP) testing requirements for antimicrobial effectiveness.

2. The composition of claim 1, having antimicrobial effectiveness against one or more of *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), *C. albicans* (ATCC 10231), and *A. niger* (ATCC 16404).

3. The composition of claim 2 having antimicrobial effectiveness against one or more of *P. aeruginosa* ATCC 13388), *P. aeruginosa* (ATCC 25619) *A. flavus*, and *A. fumigatus*.

4. The composition of claim 1, comprising a ratio of oil:stabilizer:solvent of about 23: about 5: about 4, respectively, on a weight-to-weight basis.

5. The composition of claim 1, comprising a ratio of the oil comprising phase to water or buffer of about 2: to about 1, respectively.

6. The composition of claim 1, comprising:
   (a) the oil in about 10% to about 30% (w/w);
   (b) the solvent in about 0.5% to about 10% (w/w);
   (c) the surfactant in about 1% to about 8% (w/w);
   (d) the water in about 20% to about 80 % (w/w); or
   (e) any combination thereof.

7. The composition of claim 1, further comprising: (a) at least one active agent dissolved in the solvent; (b) at least one active agent dissolved in the oil; (c) at least one active agent dissolved in the water; (d) particles of at least one active agent present in the solvent; (e) particles of at least one active agent present in the oil; (f) particles of at least one active agent present in the water; (g) or a combination thereof.

8. The composition of claim 7, wherein the active agent is acyclovir, cyclosporine, naltrexone, alendronic acid, ceterizine, nicotine, testosterone, progesterone, or estradiol.

9. The composition of claim 7, wherein the composition comprises globules of oil comprising dissolved active agent.

10. The composition of claim 9, wherein the oil globules have a particle size selected from the group consisting of less than about 1 micron, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, and less than about 100 nm in diameter.

11. The composition of claim 7, wherein the active agent particles, oil droplets comprising solubilized active agent, water droplets comprising solubilized agent, or a combination thereof, have a mean particle size of less than about 10 microns in diameter.

12. The composition of claim 11, wherein the active agent particles, oil droplets comprising solubilized active agent, water droplets comprising solubilized agent, or a combination thereof, have a mean particle size selected from the group consisting of less than about 9 microns, less than about 8 microns, less than about 7 microns, less than about 6 microns, less than about 5 microns, less than about 4 microns, and about 3 microns or greater in diameter.

13. The composition of claim 7, wherein the active agent particles, oil droplets comprising solubilized active agent, water droplets comprising solubilized agent, or a combination thereof, have a mean particle size of less than about 3 microns in diameter.

14. The composition of claim 13, wherein the active agent particles, oil droplets comprising solubilized active agent, water droplets comprising solubilized agent, or a combination thereof, have a mean particle size selected from the group consisting of less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2 microns, less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1 micron, less than about 900 nm, less than about 800 nm,

less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, less than about 50 nm, less than about 40 nm, less than about 30 nm, less than about 20 nm, and less than about 10 nm.

15. The composition of claim 1, wherein the solvent is selected from the group consisting of isopropyl myristate, triacetin, N-methyl pyrrolidinone, aliphatic and aromatic alcohols, ethanol dimethyl sulfoxide, dimethyl acetamide, ethoxydiglycol, polyethylene glycols, and propylene glycol.

16. The method of claim 1, wherein the oil is oil is selected from the group consisting of almond oil (sweet), apricot seed oil, borage oil, canola oil, coconut oil, corn oil, cotton seed oil, fish oil, jojoba bean oil, lard oil, linseed oil (boiled), Macadamia nut oil, medium chain triglycerides, mineral oil, olive oil, peanut oil, safflower oil, sesame oil, soybean oil, squalene, sunflower seed oil, tricaprylin (1,2,3-trioctanoyl glycerol), and wheat germ oil.

17. The method of claim 1, wherein the surfactant is selected from the group consisting of sorbitan esters, glycerol esters, polyethylene glycol esters, block polymers, acrylic polymers (such as Pemulen), ethoxylated fatty esters (such as Cremophor RH-40), ethoxylated alcohols (such as Brij), ethoxylated fatty acids (such as Tween 20), monoglycerides, silicon based surfactants, and polysorbates.

18. The method of claim 17, wherein the sorbitan ester surfactant is Span and Arlacel, wherein the glycerol ester is glycerin monostearate, wherein the polyethylene glycol ester is polyethylene glycol stearate, wherein the block polymer is a Pluronic, wherein the acrylic polymer is Pemulen, wherein the ethoxylated fatty ester is Cremophor RH-40, wherein the ethoxylated alcohol is Brij, and wherein the ethoxylated fatty acid is Tween 20.

19. A method of disinfecting a biological or non-biological surface comprising exposing the surface to a composition comprising:

(a) at least one solvent;

(b) at least one surfactant;

(c) at least one oil; and

(d) water;

wherein the composition meets the United States Pharmacopeia (USP) testing requirements for antimicrobial effectiveness.

FIGURE 1

Antibacterial Effectiveness Test for Placebo (no active agent) (as per USP)

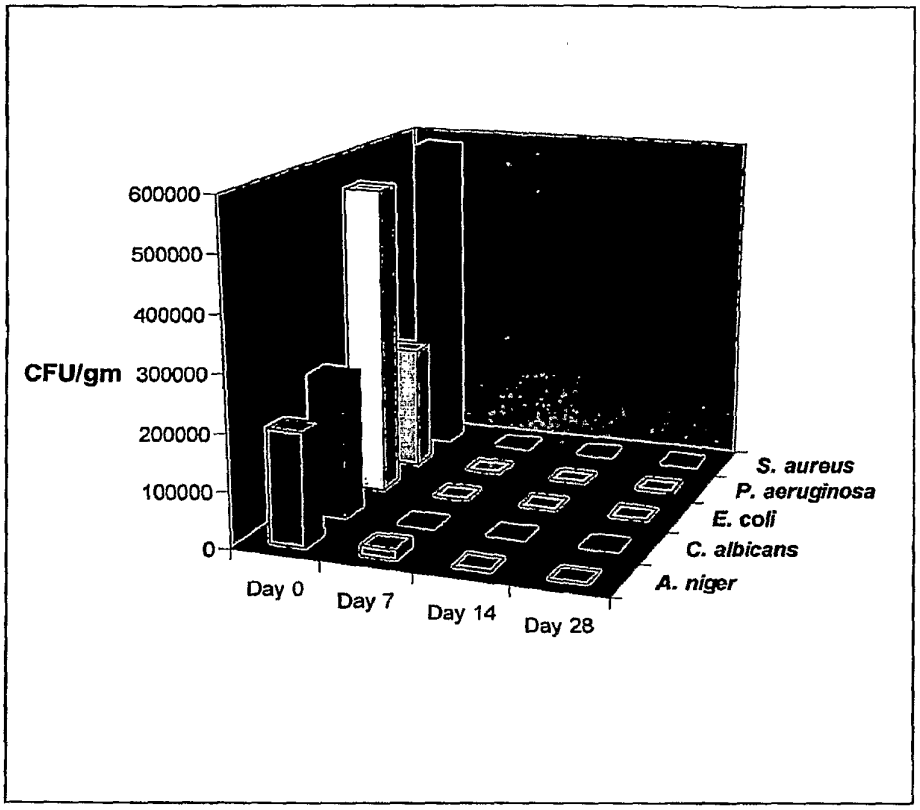


FIGURE 2

Antibacterial Effectiveness Test for Placebo (no active agent)
(additional microbes – modified USP)

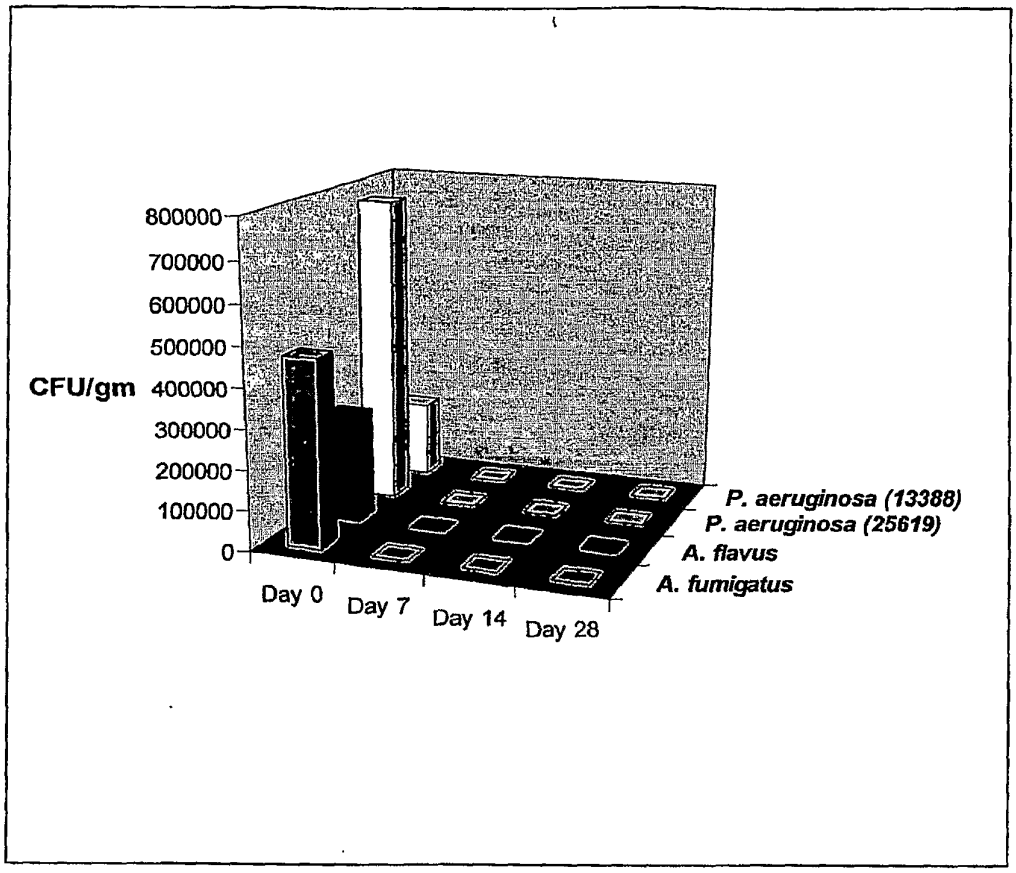


FIGURE 3

Antibacterial Effectiveness Test for Estradiol Containing Composition (as per USP)

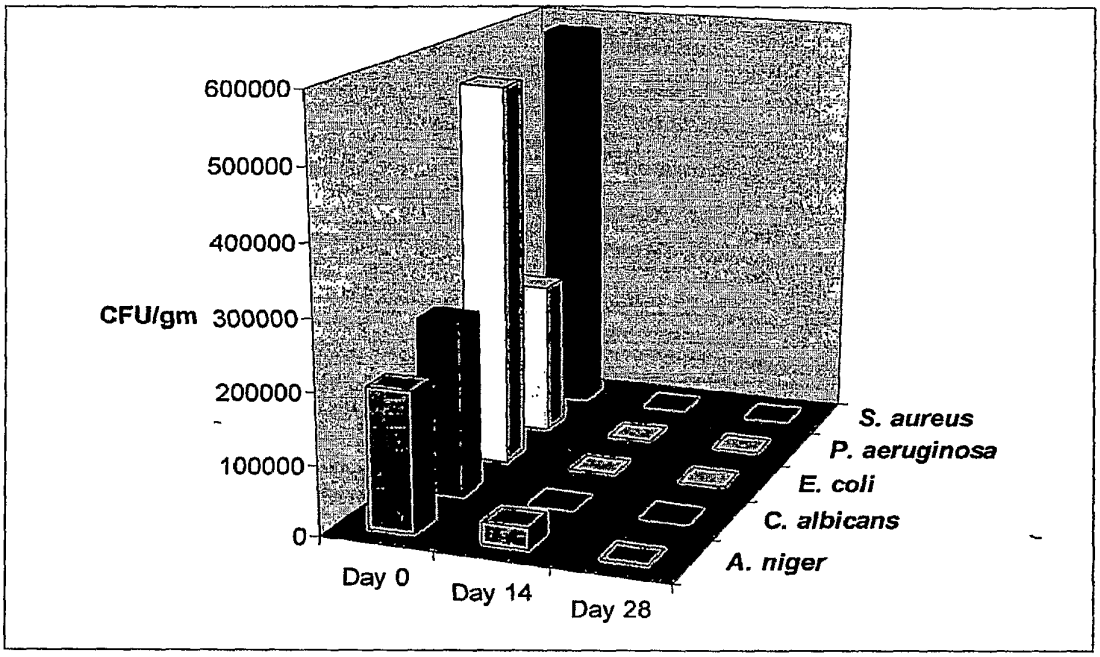


FIGURE 4

Antibacterial Effectiveness Test for Testosterone Containing Composition (as per USP)

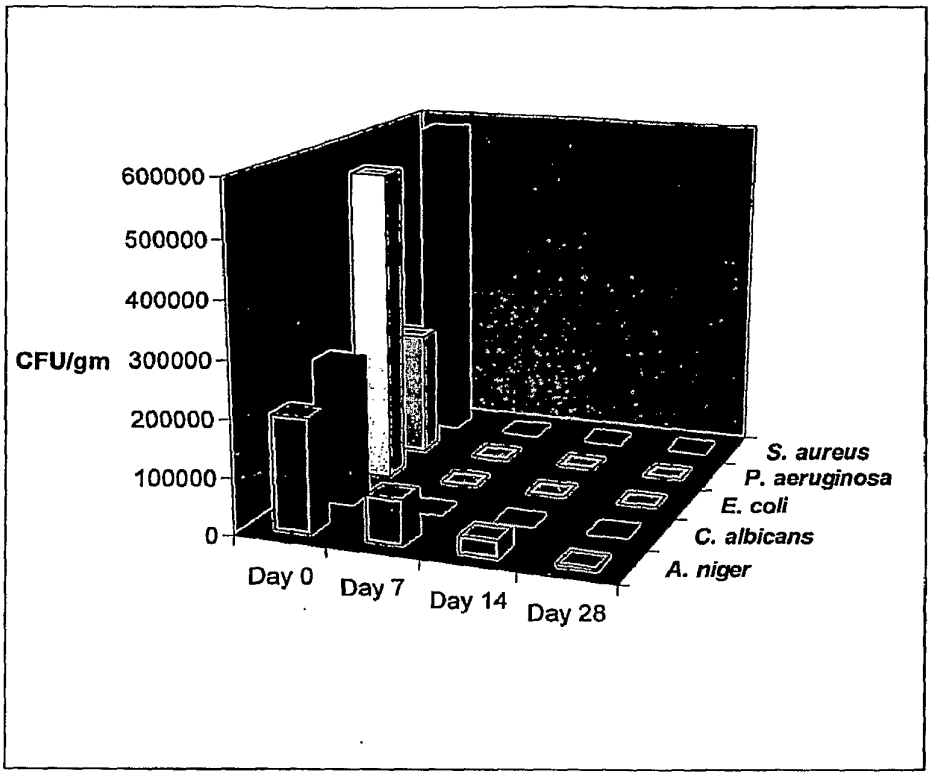
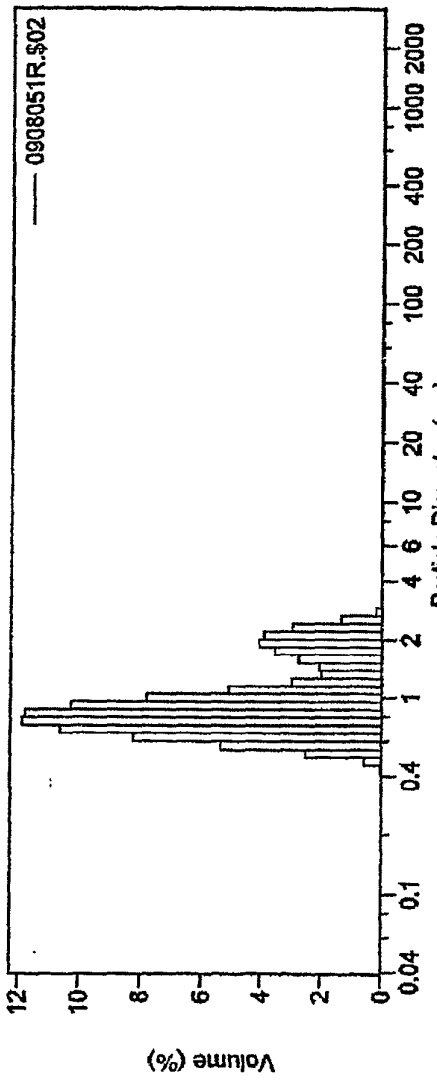


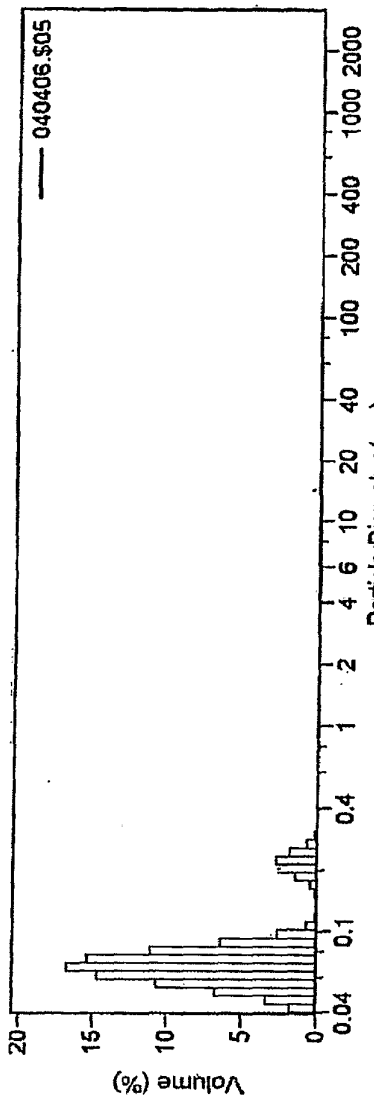
Figure 5



Volume:	100%					
Mean:	1.052 µm	S.D.:		0.507 µm		
Median:	0.862 µm	C.V.:		48.2%		
D(3,2):	0.886 µm	Skewness:		1.365 Right skewed		
Mode:	0.755 µm	Kurtosis:		0.851 Leptokurtic		
% <	10	25	50	75	90	
µm	0.609	0.706	0.862	1.177	1.939	

Testosterone containing nano-structured formulation (prepared using Silverson method)

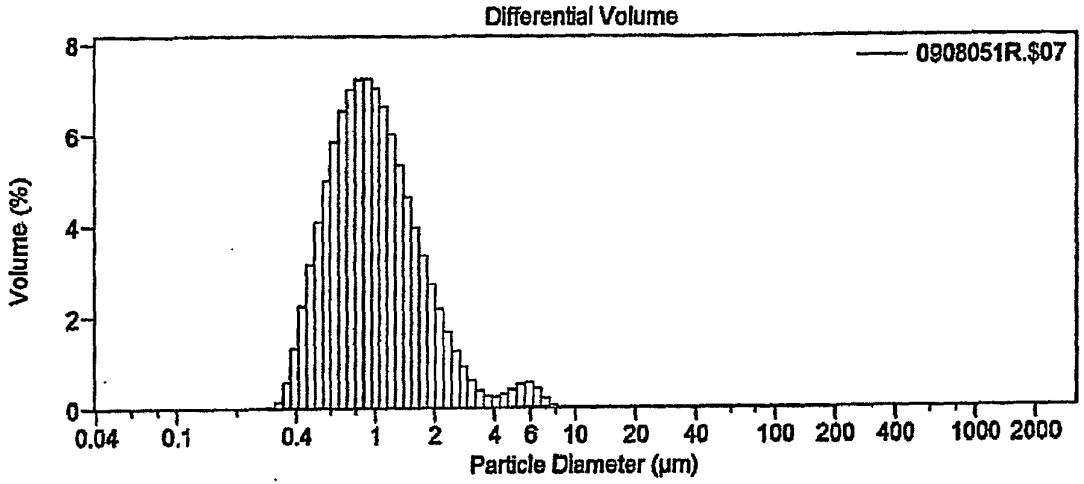
Figure 6



Volume:	100%				
Mean:	0.0826 µm	S.D.:	0.048 µm		
Median:	0.0685 µm	C.V.:	58.0%		
D(3,2):	0.0699 µm	Skewness:	2.534 Right-skewed		
Mode:	0.0668 µm	Kurtosis:	5.499 Leptokurtic		
d_{50}:	0.0685 µm				
d_{90}:	0.112 µm				
% <	10	25	50	75	90
µm	0.0516	0.0590	0.0685	0.0807	0.112

Estradiol containing nano-structured formulation (prepared using APV method)

Figure 7



Volume:		100%			
Mean:		1.204 µm	S.D.:		0.930 µm
Median:		0.950 µm	C.V.:		77.2%
D(3,2):		0.884 µm	Skewness:		3.347 Right skewed
Mode:		0.910 µm	Kurtosis:		14.30 Leptokurtic
% <	10	25	50	75	90
µm	0.527	0.683	0.950	1.378	2.012

Estradiol containing nano-structured formulation
(prepared using Silverson method)

The AET results for Placebo and Estradiol containing formulations was generated using this method of manufacture