PROCESS FOR PREPARING CONCENTRATED AQUEOUS MICELLAR SOLUTIONS

The present invention relates to a process to prepare supersaturated micellar solutions that are useful to prepare novel formulations of biologically active molecules with low water insoluble.

Drug discovery programs frequently identify molecules with high biological activity and suboptimal physical properties that result in low bioavailability. Modification of physical chemical properties, mainly solubility and dissolution rate, may alter the pharmacodynamic and pharmokinetic properties of a compound. Traditionally modification of properties such as solubility, dissolution rate, hygroscopicity, stability and crystal habit was approached by forming salts of ionizable molecules with a variety of pharmaceutically acceptably counterions. More recently polymorphs and pseudopolymorphs have been screened to identify crystalline forms with improved physical chemical properties. Typically the crystal structure of different salts and polymorphs, and therefore the physical properties, differ. Co-crystals afford yet another technique to identify new crystalline materials.

While traditionally crystalline salts were sought, more recently, amorphous forms of active pharmaceutical ingredients have been investigated. Unlike crystalline solids which are comprised of regular geometric patterns or lattices, amorphous solids are comprised of randomly oriented molecules. Common examples of amorphous solids are glass and plastic. Unlike crystalline solids, amorphous solids do not have definite melting points and have a higher dissolution rate and greater solubility than crystalline forms. One difficulty in using amorphorus solids in formulations is there tendency to revert to a more stable crystalline form.

Other techniques for improving solubility and dissolution rates include modifying crystal properties by micronization and nanosizing of the crystals.

The present invention provides for a process for preparing a supersaturated aqueous solution of micelles from an amphiphilic compound whose solubility product (K_{sp}) in water is less than the critical micelle concentration (CMC) in water which process comprises the steps of:

a) dissolving an amphiphilic compound in a water miscible organic solvent;

b) adding water, and optionally a stoichiometric quantity of aqueous alkaline or alkali metal hydroxide or aqueous acid, to form a salt, to provide a homogenous mixed aqueous solvent system;

c) heating the solution under reduced pressure at a temperature which results in distillation of the organic solvent to produce a supersaturated aqueous solution of micelles and less than 0.5% of the organic solvent.

The resulting supersaturated solution of micelles can be further processed by conventional techniques such as lyophilization or freeze drying to afford a solid which can be incorporated into conventional dosage forms.

Figure 1 is a photomicrograph of a spray dried (Figure 1a) and lyophilized (Figure 1b)concentrated micellar solution of the disodium salt of I prepared as described in example 1 demonstrating different morphologies for the solid obtained from both drying techniques.

Figure 2 is an x-ray powder pattern of the solid obtained by spray drying the concentrated micellar solution of the disodium salt of I prepared as described in example 1 which establishes the compound does not have a regular crystalline structure.

Figure 3 is a photomicrograph of a spray dried concentrated micellar solution of compound II prepared as described in example 4.

Figure 4 is an x-ray powder pattern of the solid obtained by spray drying the concentrated micellar solution of compound II prepared as described in example 4.

The phrase “a” or “an” entity as used herein refers to one or more of that entity; for example, a compound refers to one or more compounds or at least one compound. As such, the terms “a” (or “an”), “one or more”, and “at least one” can be used interchangeably herein.

As used in this specification, whether in a transitional phrase or in the body of the claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising"

means that the process includes at least the recited steps, but may include additional steps. When used in the context of a compound or composition, the term "comprising" means that the compound or composition includes at least the recited features or components, but may also include additional features or components.

The term “optional” or “optionally” as used herein means that a subsequently described event or circumstance may, but need not, occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, “optionally substituted” means that the optionally substituted moiety may incorporate a hydrogen or a substituent.

The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

While the crystalline and amorphous states represent extremes in molecular order in the solid state, there can be a continuum of partially ordered liquid crystalline states that lie between these extremes. (C. L. Stevenson *et al. J. Pharm Sci.* **2005** 94(9):1861-80) These are solids with intermediate states of molecular order characterized by a partial or complete loss of positional order while retaining some degree of orientational order of the constituent molecules.

A compound which has hydrophilic and hydrophobic parts within the same molecule is amphiphilic. Soaps and detergents are common examples of amphiphilic molecules. Amphiphilic molecules self assemble to form micelles when their concentration in solution exceeds their critical micelle concentration. Many amphiphilic molecules show lyotropic liquid-crystalline phase sequences depending on the volume balances between the hydrophilic part and hydrophobic part. A liquid crystalline material is lyotropic if the phases have long-range orientational order These structures are formed through the micro-phase segregation of two incompatible components on a nanometer scale. Soap is an everyday example of a lyotropic liquid crystal.

The content of water or other solvent molecules changes the self-assembled structures. At very low amphiphile concentration, the molecules will be dispersed randomly without any ordering. At slightly higher (but still low) concentration, amphiphilic molecules will spontaneously assemble into spherical micelles or vesicles. Micellar aggregates assemble to orient the hydrophilic portion of the amphiphile inside the core micelle, exposing a hydrophilic (water-soluble) surface to aqueous solution. These spherical objects do not order themselves in solution, however. At higher concentration, the still more ordered assemblies will form. A typical phase is a hexagonal columnar phase, where the amphiphiles form long cylinders (again with a hydrophilic surface) that arrange themselves into a roughly hexagonal lattice. This is called the middle soap phase. At still higher concentration, a lamellar phase (neat soap phase) may form, wherein extended sheets of amphiphiles are separated by thin layers of water. For some systems, a cubic (also called viscous isotropic) phase may exist between the hexagonal and lamellar phases, wherein spheres are formed that create a dense cubic lattice. These spheres may also be connected to one another, forming a bicontinuous cubic phase.

Removal of the solvent from concentrated solutions of micelles produces solids which, depending on the drying technique and drying conditions used, result in a liquid crystalline micellar solid or an amorphous solid. Both amorphous and lyotropic liquid crystals are sufficiently disordered that they do not produce sharp diffraction peaks in an X-ray powder diffraction pattern. These forms typical result in a halo pattern. In contrast to amorphous solids, liquid crystalline phases exhibit birefringence when viewed with a polarized light microscope.

Micellar solids afford some unique properties that can be exploited in the development of novel formulations. Specifically the molecular order provided in liquid crystals, unlike an amorphous solid, adds stability that can retard reversion to still more stable crystalline solids. However stability resulting from the aggregation is significantly less than the crystalline state resulting in increased solubility and dissolution rate typical of purely amorphous materials.

While micellar solids promise advantages to the pharmaceutical scientist, the physical properties of many pharmacologically active molecules make to difficult produce concentrated micellar solutions from which liquid crystalline solids can be recovered. One reason for this difficulty is the aqueous solubility of many molecules is sufficiently low that

the CMC cannot be attained and thus micelles do not form. The present invention provides a convenient process to produce concentrated aqueous solutions of micelles from compounds with limited aqueous solubility.

The term "amphiphile" as used herein refers to a chemical compound possessing both hydrophilic and hydrophobic properties. Such a compound is also referred to as amphiphilic or amphipathic. The hydrophilic portion of an amphiphilic molecule can be cationic, anionic or neutral. Neutral hydrophilic residues are commonly polyethers are similar residues capable of hydrogen bonding. The hydrophobic portion of an amphiphile is typically comprised of alkyl or aryl residues

The term "micelle" as used herein refers to an aggregate of amphiphilic molecules dispersed in a liquid. A typical normal phase (oil-in-water) micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions on the exterior surface in contact with surrounding aqueous phase and hydrophobic tail regions sequestered in the center of the micelle where the environment is relatively non-aqueous. Micelles in diluted solutions are approximately spherical in shape. More complex liquid crystalline phases can be formed as micellar solutions become more concentrated and the shape and size of such micelles is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH and ionic strength.

The term "liquid crystal" as used herein refers to a phase of matter that has properties between those of a amorphous solid and those of a solid crystal. A liquid crystal typically is comprised of molecules with some order but lacking the regular repeating subunits typical of a crystal lattice. When a liquid crystal is positioned between a pair of crossed polarizing filters and viewed through an optical microscope a liquid crystal appears birefringent, *i.e.* the sample appears bright against a dark (isotropic) background.

In one embodiment of the present invention here is provided a process for preparing a supersaturated aqueous solution of micelles from an amphiphilic compound whose solubility product (K_{sp}) in water is less than the critical micelle concentration (CMC) in water which process comprises the steps of: (a) dissolving an amphiphilic compound in a water miscible organic solvent; (b) adding water, and optionally a stoichiometric quantity of aqueous alkaline or alkali metal hydroxide or aqueous acid to form a salt, to provide a homogenous

mixed aqueous solvent system; and (c) heating the solution under reduced pressure at a temperature which results in distillation of the organic solvent to produce a supersaturated aqueous solution of micelles and less than 0.5% of the organic solvent. One skilled in the art will appreciate that the quantities of water and the nature an quantity of the non-aqueous solvent can be varied to provide mixed aqueous solvent systems which dissolve the amphiphile and these quantities can be determined without undue experimentation.

The active pharmaceutical ingredient (API) is first dissolved in an organic solvent and a quantity of water is added to produce an homogeneous aqueous organic solution. An active pharmaceutical ingredient (API) with an acidic substituent can optionally be treated with a stoichiometric quantity of aqueous base to produce the conjugate base of the acid and the resulting anion may enhance the hydrophilicity of the API. Analogously an API with a basic residue can be treated with a stoichiometric quantity of an aqueous acid to produce the conjugate acid which can enhance the hydrophilicity of the API. Conversion of the API to a salt is an optional component of the invention and is not required if the neutral API is sufficiently amphiphilic to form micelles. The anhydrous acids or bases can also be a used to generate the salt and water added in a subsequent step. The quantities of organic solvent and water are typically adjusted produce a homogeneous solution. The organic solvent selected to be miscible with water and the boiling point of the organic should be low enough that the heat applied during the distillation not cause decomposition of the API.

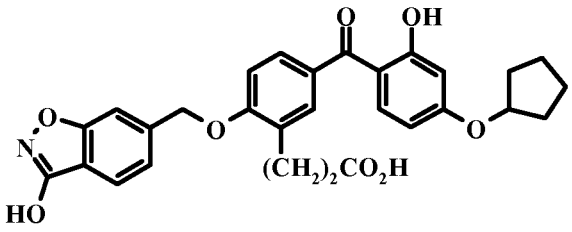
The organic solvent is distilled under a vacuum to produce a supersaturated solution of micellar API. Distillation is continued until the solvent contains less than 0.5% of the organic solvent. The concentration of the API in water can readily adjusted. In another embodiment the distillation is continued to produce a supersaturated solution containing less than 1% of the organic solvent. In still another embodiment the distillation is continued to produce a supersaturated solution containing less than 2% of the organic solvent.

In a second embodiment of the present invention there is provided a process to prepare a stable amorphous solid micelle comprising spray drying said supersaturated aqueous solution of micelles to produce a stable solid amorphous micelle which exhibits birefringence under a polarized light microscope.

In a third embodiment of the present invention there is provided a process to prepare a stable amorphous solid comprising lyophilization of said supersaturated aqueous solution of micelles to produce a stable amorphous solid.

The term "stable" as used herein refers to a physical form that is stable for at least about four weeks.

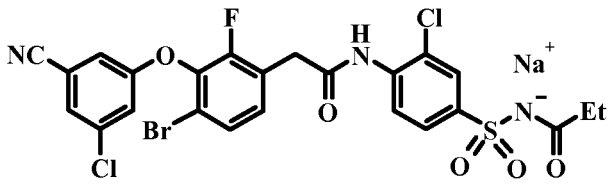
In a fourth embodiment of the present invention there is provided a process to prepare a stable amorphous solid micelle of a compound according to formula **I (S.** Hirono, *et al.,* WO 2003042150, published 5/22/2003) comprising the steps of: (a) dissolving a compound according to formula **I** in THF; (b) adding two equivalents of 1M NaOH; and (c) heating the solution under reduced pressure at a temperature which results in distillation of the organic solvent to produce a supersaturated aqueous solution of micelles and less than 0.5% of the organic solvent.



(I)

In a fifth embodiment of the present invention there is provided a process to a stable amorphous solid micelle containing a compound according to formula **I** comprising spray drying the supersaturated aqueous solution of micelles produced in the fourth embodiment (*supra*).

In a sixth embodiment of the present invention there is provided a process to prepare a stable amorphous solid of a compound according to formula **II** comprising the steps of: (a) dissolving a compound according to formula **II** in *iso*-propanol; and (c) heating the solution under reduced pressure at a temperature which results in distillation of the organic solvent to produce a supersaturated aqueous solution of micelles and less than 0.5% of the organic solvent.



(II)

In a seventh embodiment of the present invention there is provided a process to a stable amorphous solid containing a compound according to formula **II** comprising spray drying the supersaturated aqueous solution of micelles produced in the sixth embodiment (*supra*).

The following examples illustrate the preparation and biological evaluation of compounds within the scope of the invention. These examples and preparations which follow are provided to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Example 1

Preparation of an 35-45% aqueous micellar solution of disodium salt of compound **I**

A 12 L round bottom flask fitted with a mechanical stirrer and maintained under a N_2 atmosphere was charged with **I (**601.5 g, 1.163 mol) and THF (*ca.* 4.8 L). The suspension was agitated at moderate speed and heated to approximately 60° C to produce a homogeneous solution. The solution was cooled to room temperature (RT) and a 1M NaOH solution (2320 mL of a solution of 120g NaOH diluted to 3 L with sterile water for irrigation which assayed as 0.985 M by titrametric analysis) was added with stirring. The temperature of the solution rose from 22° C to 30.8° C. This solution was vacuum transferred to a Buchi Rotovap (12L flask). The THF was removed *in vacuo* (25-27 Torr). The water bath temperature was maintained at 50-55° C, the vapor temperature was *ca.* 21° C and the flask was rotated at 67-72 revolutions per minute. As the volume decreased, the temperature of the vapor increased to 35-36° C. The temperature of the water bath was raised to 60° C to maintain the distillation rate. When the vapor temperature had reached about 35° C, the solution became cloudy and the solution was aged at about 60° C for 1 h to re-clarify the solution while slowly bleeding N_2 into the rotary evaporator to prevent foaming and "bumping. When the temperature of the vapor remained constant at 36° C a sample was removed and the pH determined to be 8.5. The solution was diluted with sterile water (*ca.* 500 mL) and concentration of the solution was continued until the final volume reached *ca.*

1.5 L. Distillation was discontinued and the residual THF concentration was determined to be 0.015%, the concentration of the disodium salt of **I** was determined to be 44% (wt/vol) and the pH was *ca.* 8.5. The resulting micellar solution (1.726 kg) was stored in sterile bottles.

Example 2

Spray Drying of the micellar solution from example 1

The pH of the solution from example 1 was adjusted to pH 9 with 1N NaOH and transferred to a Buchi B-290 spray drier and the operating parameters were adjusted as follows:
inlet temperature - 175° C (resulting outlet temperature *ca.* 106° C);
air pressure of the spray nozzle - *ca.* 30 psi;
spray pump speed - 18 – 20%;
aspirator - *ca.* 80%.

Spray drying was begun when the inlet temperature reaches to 175 C. Typically the exhaust pressure is about 15 psi at 80% aspirator speed. After the solution was run through the spray drier the inlet heater pump and air inlet are turned off and the aspirator reduced to *ca.* 50%). When the exhaust temperature drops to 60-70° C the aspirator is turned off and the powder is collected from the chambers. The resulting powder can be dried in an oven to reach the desired moisture content.

Example 3

Lyophilization of the micellar solution from example 1

A round-bottom flask was charged with a solution from example 1 and immersed and swirled in a dry ice/isopropanol slurry to freeze the solution. The flask containing the frozen solution was attached to a lyophilizer to remove the water. Complete drying required 8-20 h. The vacuum is broken and the flask removed and the resulting powder collected. Any lumps can be broken with light pressure with a spatula or in a mortar and pestle.

Example 4

Preparation of an Aqueous Micellar Solution of Compound **II**

Compound **II** (2 g) was slowly dispersed in 70% IPA (20 mL) and water (14 mL) was added. (The solubility of the **II** is about 47 mg/mL in 70% IPA/H_2O, 4 mg/mL in water and 0.08 mg/mL in IPA.) The dispersion was sonicated to dissolve all the dispersed solid to produce a

pale yellow solution. The IPA was distilled using rotary evaporator to achieve an aqueous solution containing *ca.*400 mg/mL of **II**. The concentrated aqueous solution was spray dried using a Buchi B-290 spray drier and the operating parameters were adjusted as follows:
inlet temperature - 180° C (resulting outlet temperature *ca.* 100° C);
air pressure of the spray nozzle - *ca.* 30 psi;
spray pump speed - 12%;
aspirator - *ca.* 90%.

Example 5

Stability study of micellar solid from spray drying of **I**

A small amount of the solid (about 10 mg from example 1) was weighed into a weighing bottle and placed in a chamber with controlled relative humidity for 4 weeks and the percentage of water absorbed was calculated form the weight gain. The sample was also assayed by HPLC against an external standard on a Waters 2690 HPLC at 276 nm. The data was processed using Waters Millennium software version 3.2. The thermal stability also was determined at 60° C and at 40° C/75 % relative humidity. The purity of the sample was determined by assaying weighed aliquots by HPLC against an external standard. The experiments suggest that the micelles are not hygroscopic and are thermally stable at 40 and 60° C over the duration of the assay.

CONDITIONS	Area %		
	T_o	4 weeks	5 weeks
40°C/75%RH (open)	99.81		99.26
40°C/75%RH (closed)	99.88	99.91	
25°C/60%RH (closed)	99.88	99.88	
60°C (closed)	99.81		99.72

The foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive.

The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.

Claims

1. A process for preparing a supersaturated aqueous solution of micelles from an amphiphilic compound whose solubility product (K_{sp}) in water is less than the critical micelle concentration (CMC) in water which process comprises the steps of:

   (a) dissolving an amphiphilic compound in a water miscible organic solvent;

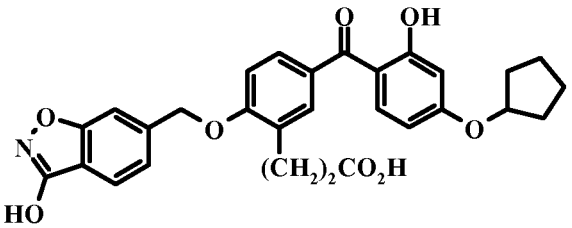
   (b) adding water, and optionally a stoichiometric quantity of aqueous alkaline or alkali metal hydroxide or aqueous acid to form a salt, to provide a homogenous mixed aqueous solvent system; and,

   (c) heating the solution under reduced pressure at a temperature which results in distillation of the organic solvent to produce a supersaturated aqueous solution of micelles and less than 0.5% of the organic solvent.

2. A process according to claim 1 further comprising the step of spray drying the resulting solution to afford a stable amorphous solid micelle.

3. A process according to claim 1 further comprising the step of lyophilizing the resulting solution to afford a stable amorphous solid.

4. A process according to claim 1 wherein:
   said compound is a compound of formula **I**:



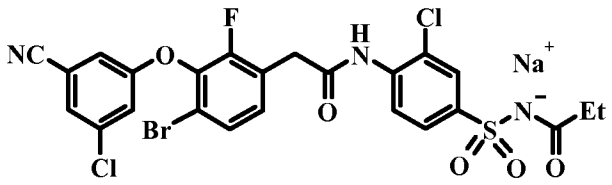
(I)

   said organic solvent is tetrahydrofuran (THF);
   two equivalents of 1M NaOH are added.

5. A process according to claim 4 further comprising the step of spray drying the aqueous micelle solution to afford a stable amorphous micellar solid.

6. A process according to claim 1 wherein:

said compound is a compound of formula **II**; and,



(II)

said solvent is *iso*-propanol (IPA)

7. A process according to claim 6 further comprising the step of spray drying the aqueous micelle solution to afford a stable amorphous micellar solid.