articles

molecular pharmaceutics

pH-Induced Nanosegregation of Ritonavir to Lyotropic Liquid Crystal of Higher Solubility than Crystalline Polymorphs

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Received July 30, 2008; Revised Manuscript Received October 14, 2008; Accepted October 14, 2008

Abstract: Birefringent spherical vesicles of ritonavir (RTV) are formed by increasing the pH of aqueous solutions from 1 to 3 or to 7 and by addition of water to ethanol solutions at room temperature. Increasing the pH creates supersaturation levels of 30−400. Upon this change in pH, the solutions become translucent, implying that some kind of RTV assembly was formed. Small spherical vesicles of narrow size distribution are detectable only after a few hours by optical microscopy. The vesicles show similar X-ray diffraction patterns and differential scanning calorimetry (DSC) behavior to amorphous RTV prepared by melt-quenching crystalline RTV. Examination by polarized optical microscopy suggests that these are lyotropic liquid crystalline (LLC) assemblies. Small-angle X-ray scattering and synchrotron X-ray diffraction further support the presence of orientational order that is associated with a nematic structure. RTV self-organizes into various phases as a result of the supersaturation created in aqueous solutions. The LLC vesicles do not fuse but slowly transform to the polymorphs of RTV (in days), Form I and finally Form II. Amorphous RTV in aqueous suspension also undergoes a transformation to a mesophase of similar morphology. Transformation pathways are consistent with measured dissolution rates and solubilities: amorphous > LLC ≫ Form I > Form II. The dissolution and solubility of LLC is slightly lower than that of the amorphous phase and about 20 times higher than that of Form II. RTV also self-assembles at the air/water interface as indicated by the decrease in surface tension of aqueous solutions. This behavior is similar to that of amphiphilic molecules that induce LLC formation.

Keywords: Mesophases; liquid crystals; precipitation

Introduction

Approximately 50% of all pharmaceuticals are weakly acidic or basic compounds.[1] For those compounds with pK_a values in the range of physiologic pH, variations in ionization state following drug administration can impact drug delivery by influencing membrane permeability or aqueous solubility. The oral administration of high dose, low pK_a weak base drugs may be of special concern. Drugs in this class may be highly soluble when ionized in the acidic environment of the stomach but may become supersaturated in the more alkaline environment of the small intestine due to lower solubility of the un-ionized form. This raises the potential for the compound to precipitate and may limit the fraction of drug that is bioavailable. This fundamental oral delivery problem is typically overlooked and not fully understood. Knowledge of the crystallization pathways for these compounds is essential in order to optimize dissolution rate and avoid precipitation of an undesired form during GI transit.

Precipitation pathways and rates are a function not only of physiological pH but also of supersaturation. A highly

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10.1021/mp800114k CCC: $40.75
Published on Web 10/31/2008

supersaturated solution may nucleate at a fast rate, potentially resulting in nano- or submicrometer crystalline, amorphous, or soft phases with different degrees of order and mobility, whereas low supersaturation will have a slower rate of precipitation, larger particles, and more stable phases. Supersaturation is a necessary and not a sufficient condition for precipitation and in some cases may not lead to nucleation and growth in the time scale of observation. Therefore, factors such as pH which influence supersaturation will have direct effects on nucleation and crystal growth rates, crystal morphology, and precipitation pathways.[2−4]

The human immunodeficiency virus (HIV) protease inhibitors are an example of high dose, low pK_a weakly basic drugs that exhibit pH-dependent solubility. This class of structurally related peptidomimetic compounds possesses moderate molecular size and high intrinsic lipophilicity, which, in itself, presents oral formulation challenges. Two drugs in this class, ritonavir and saquinivir, have been developed as self-emulsifying or microemulsion-based lipidic formulations to overcome poor aqueous solubility and improve oral delivery.[5,6] It has been noted that only limited information is available in the literature regarding the solubility of these drugs in buffered solutions[7] or their potential for precipitation under physiologically relevant conditions.

Indinavir (Crixivan) is one HIV protease inhibitor for which the development of nephrolithiasis and crystalluria in patients has been reported, demonstrating the potential for weakly basic drugs to exhibit pH-dependent crystallization in vivo.[8] Kopp, et al.[8] found that crystals of indinavir were present in starburst forms and irregular plate forms in the urine of patients. The precipitation of indinavir was also studied at different concentrations and pH values in synthetic urine. Consistent with the pH-solubility profile of this weak base, crystallization induction times were notably shorter at

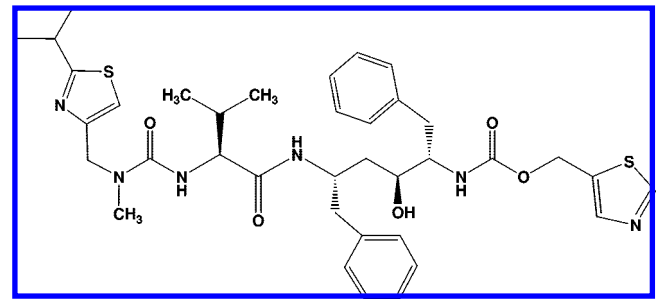

Figure 1. Chemical structure of ritonavir.

urinary pH values above 6.0 and delayed at lower pH values.[9] The potential for indinavir precipitation during gastric emptying has also been discussed in the literature. *In vitro* crystallization models were compared with clinical observations of reduced indinavir oral bioavailability in humans following elevations in gastric pH.[10,11] Indinavir precipitates when supersaturation is created by raising solution pH to 7 and crystallization induction times are shortened by the presence of formulation excipients or crystal seeds.[11]

Ritonavir (RTV), shown in Figure 1, is another weakly basic HIV protease inhibitor which exhibits a marked pH-dependent solubility profile. Polymorphic behavior of RTV was discovered following the appearance of a new, less soluble polymorph (Form II) that dictated market withdrawal of the original hydroalcoholic solution-containing capsule formulation (Norvir).[12,13] The potential for pH-induced crystallization from aqueous solutions has not been previously studied.

RTV has two thiazole groups with pK_a values of 1.8 and 2.6 and would exist as a dicationic species in the acidic environment of the stomach and as the free base at alkaline pH.[14] The aqueous solubility of RTV polymorphic Form I at pH 1.0, 37 °C is 400 μg/mL and drops to 1 μg/mL at pH 6.8.[14] The pH-solubility profile for this form is shown in

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