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Pharmaceutical Solvate Formation for the Incorporation of the Antimicrobial Agent Hydrogen Peroxide

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Abstract

Antimicrobial functionality is introduced into a pharmaceutical formulation of miconazole while improving solubility. The work leverages hydrate formation propensity in order to produce hydrogen peroxide solvates. The ubiquity of hydrate formation suggests that hydrogen peroxide can be broadly deployed in pharmaceuticals, rendering a liquid excipient suitable for solid pharmaceutical formulations.

In recent years, multicomponent crystallization, and cocrystallization in particular, has emerged as a reliable method for modifying the physical properties of active pharmaceutical ingredients (APIs).^{1,2} Generally, coformers are chosen to help improve the solubility of the API in aqueous solutions.^{3,4} However, with the exception of drug-drug cocrystals,^{5,6} the coformers are often otherwise innocent in the formulation.^{1,7} In this way, coformers often act as inert excipients- bulking media that are used in producing pills of acceptable size.⁸ Excipients may serve some purpose beyond bulking, such as binding agents, lubricants or colorants; however, these components must not inhibit the action of the API and must also be tested for their toxicity and stability.^{8,9}

One type of excipient function is to serve as an antimicrobial preservative, which is added to extend the shelf life of a formulation and reduce the chance of microbial growth in moist environments, such as in topical formulations of creams, ointments, or sprays.^{10,11} There are several common examples of antimicrobial preservatives used in pharmaceuticals, such as sodium benzoate, benzyl alcohol, and a range of parabens (the most common preservatives used in many industries).^{10,12,13} Incorporation in small amounts may not result in toxicity issues initially, but repeated exposure to parabens, for example, can result in the

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Conflicts of interest

There are no conflicts to declare.

development of allergic reactions, and parabens can exhibit estrogenic activity and cause increased cell growth.^{10,12} There is a need, therefore, in the pharmaceutical industry for the incorporation of novel antimicrobial excipients with lower toxicity and broad functionality.

Hydrogen peroxide (HP) is a commonly used antimicrobial agent in the medical and food industries and is also used as an environmentally friendly biocide.¹⁴ Commercially available solutions range in concentration from as high as 6% HP to less than 1% with proven efficacy in different applications. The low concentrations necessary for these bacteriostatic properties mean that HP can be safely used without risk of toxicity to humans.¹⁴ The molecule also easily breaks down into water and oxygen, mitigating environmental effects. The problem with incorporating HP as an antimicrobial preservative into solid pharmaceutical dosages, however, is that HP is a liquid at room temperature and thus is difficult to integrate into a solid dosage or topical formulation without decomposition. However, we hypothesize that HP could be incorporated as a solvent into crystalline forms of an API, much in the same fashion that water readily forms hydrates with many pharmaceutical compounds.^{15,16} This would enable the controlled addition of a stable liquid into a solid pharmaceutical dosage as an antimicrobial preservative for longer shelf life and reduced microbial growth.

When considering a pharmaceutical system in which to test the strategy of HP incorporation, topical medications were investigated. These types of moist formulations, subject to microbial growth,^{11,17} can rely on the presence of a solid pharmaceutical within the dosage. Common ailments that are treated with over-the-counter (OTC) topical medications include athlete's foot and vaginal yeast infections, both of which are frequently treated with azole based antifungal medications, primarily the pharmaceutical miconazole (Figure 1).^{18,19} Miconazole is typically dosed in ointments and powders as the nitrate salt but is also present as the anhydrate form in gels and tablets. This molecule also has a hemihydrate form,²⁰ which is used here to test our hypothesis that HP can be incorporated into a solvated crystal form of miconazole. HP inclusion is expected to help increase the API solubility relative to the hemihydrate form, and potentially act as an antimicrobial excipient within formulations.

The hemihydrate form of miconazole is used as the starting point for this endeavor, and it was discovered that several variables must be controlled during crystallization in order to achieve the desired HP solvated crystal in substantially pure form. HP can be purchased for consumer use at concentrations of 3 or 6% in water, but these concentrations are unable to fully displace water with HP during crystal formation. By contrast, antisolvent addition of 50% HP to an ethanolic solution results in a novel Raman spectrum (Figure S1, ESI) and novel PXRD pattern (Figure S4, ESI) when compared to the hemihydrate form; however, Karl Fisher titration shows that most of the solvent in the sample, measured as a 4.76% weight loss by thermogravimetric analysis (TGA) (Figure 2), is water. This led to the hypothesis that while HP can be incorporated into miconazole crystals, water is binding preferentially and this observation contrasts recent observations with CL-20 HP solvates.²¹ Increasing to 98% HP (Figures S2 and S5, ESI) slows crystallization significantly and this extended crystallization time allows environmental water to be absorbed (0.36% water by Karl Fisher titration, Figure 2).

To mitigate competitive incorporation of water, crystallizations were performed in a desiccator, resulting in crystals containing trace water (0.09% by Karl Fisher). The weight loss from TGA (3.94%) (Figure 2) suggests the structure contains 0.5 HP, the expected value if the structure is based on the hemihydrate form of miconazole. To confirm the presence of HP, a chemical test was performed where HP reacts with triphenylphosphine (TPP) in solution producing triphenylphosphine oxide (TPPO). By reacting a known amount of TPP with a known amount of the miconazole sample, any HP present will convert the TPP to TPPO, and the ratio of TPP to TPPO can be determined by integration of peaks in the ^{31}P NMR spectrum (Figure S8, ESI). This method further supports that miconazole samples grown in the desiccator contain 0.5 HP (see ESI for more details). TGA of the sample after sitting on the benchtop for one week shows no change in weight loss, indicating stability of the novel structure to solvent loss (Figure S10, ESI). Dynamic vapor sorption and subsequent PXRD of the hemi-HP solvate also shows no change in structure after exposure to humidities ranging from 5–95% (Figures S7 and S12, ESI). *In situ* Raman spectroscopy was also conducted on a sample of hemi-HP solvate slurried in water, showing no change in peaks from the peroxide to hydrate structure after 4 days (Figure S3, ESI). To further probe possible conversion, a sample of the hemi-HP solvate was vigorously shaken in water for 6 hours and subjected to the same chemical test described above. The ^{31}P NMR spectrum indicates negligible loss of HP (Figure S9, ESI).

Structural determination from a single crystal further verified that 1 HP molecule is incorporated for every 2 miconazole molecules in the hemi-HP solvate. The crystal structure (hydrogen atom positions normalized) shows the same hydrogen bonding pattern is as in the hemihydrate form (Figure 3); however, the crystals are not isostructural (Table S1, ESI). Both structures are comprised of racemic miconazole molecules, with the hemihydrate structure in a monoclinic space group and the hemi-HP form in a triclinic space group. Both structures feature hydrogen bonding of the solvent molecules between two miconazole imidazole nitrogens with $\text{N}\cdots\text{HO}$ hydrogen bonding distances of 1.87 and 1.83 Å in the miconazole hemihydrate and 1.75 and 1.81 Å in the hemi-HP form. Solvent oxygen atoms in both structures do not participate in classical hydrogen bonds, but do show interactions with CH groups in other miconazole molecules at distances ranging from 2.23–2.50 Å in the hemi-HP structure, to 2.26–2.47 Å in the hemihydrate structure. These data indicate that the major source of connection for these solvent molecules is through the OH hydrogen bonds and suggests that bond strengths are similar in both solvated forms. Weight loss temperatures from TGA show that water is lost at 68 °C (Figure S11, ESI) whereas HP is lost at 98 °C (Figure 2), but due to the higher boiling point for pure HP (150 °C) than water, this temperature difference is in line with expectations.

Solubility studies were conducted in phosphate-citrate buffer (PCB) at pH 4, a physiologically relevant pH for medications used vaginally. Solubility measurements were determined using a Pion Rainbow system with *in situ* UV-Vis probes to monitor absorbance over time until equilibrium solubility was reached for each sample. The results in Table 1 show that, on average, the hemi-HP solvate of miconazole does display increased solubility in comparison to the hemihydrate form, consistent with dissolution of HP driving API solubilization by a mechanism now widely accepted in cocrystals.^{4,7} The hemi-HP solvate also displays similar solubility to the anhydrate form of miconazole. Determination of

equilibrium solubility of the nitrate salt is not possible via this method due to supersaturation and reprecipitation of miconazole hemihydrate before equilibrium is reached (See Section S8 in ESI).

In order to determine if the incorporation of HP shows any adverse effects on the miconazole formulation, fungal assays were performed. Traditional fungal susceptibility testing methods involve dosing the drug as a solution of known concentration, but such approaches are not suited for probing differences arising from the solid state. There is no standard method for using solid forms in fungal susceptibility testing,¹⁹ therefore a method is devised here (See ESI for more details). Inspiration for the outlined method comes from classical agar diffusion testing. Instead of dosing the medication on a disc soaked in solutions of the forms, direct dosage of the solid medication onto the plate surface is carried out. Dosage of the miconazole solid forms directly onto the plate allows for slow diffusion and release of the medication across the growth surface.

The fungal strain chosen for this study is *Candida glabrata*, a common yeast strain in vaginal yeast infections that has shown resistance to miconazole formulations in the past.^{22–24} Solid forms were dosed with talc powder as a filler, allowing for dilution of the samples to control the percentage of miconazole tested at or near commercially available products. Talc powder was chosen due to its use in other OTC miconazole products, indicating that it does not interfere with the drug. Results show that at a commercially relevant concentration of 2% drug in talc, the hemi-HP solvate of miconazole indeed shows a zone with inhibition of growth of *C. glabrata* around the area of dose application, when compared to the control of pure talc powder and 2% miconazole hemihydrate in talc, at both 24 and 48 hours (Figure 4).

Through modification of crystallization parameters, including antisolvent and environment, a novel hemi-HP solvate of miconazole was discovered. Characterization by Karl Fisher titration, thermogravimetric analysis, and chemical reactivity were used to verify the solvent content of the crystals, while single crystal X-ray diffraction indicates a similar, but not isostructural relationship between the hemi-HP solvate and the hemihydrate forms of miconazole. Antifungal susceptibility tests show that the hemi-HP solvate of miconazole does not have adverse effects on API action. This proof of concept study supports the hypothesis that HP can be incorporated into a pharmaceutical with a known hydrate crystal structure, producing a solid drug form with an antimicrobial preservative built in to the structure. This finding paves the way for use of HP as an excipient in pharmaceutical solid forms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Chemical structure of miconazole.

Figure 2.

Thermogravimetric analysis of miconazole samples grown with 50% HP, 98% HP (open atmosphere), and 98% HP (in desiccator) showing weight loss from solvent inclusion in each sample. Inset shows calculated amounts of water present in each sample from Karl Fisher titration and remaining weight loss %, corresponding to HP in the structures.

Figure 3.
Asymmetric units from crystal structures of a) miconazole hemi-HP solvate and b)
miconazole hemihydrate.

Figure 4.

Agar plates with wild type *C. glabrata* yeast strain grown in the presence of talc dosages (white powder) in a line down the center of the plates. Talc control (middle) as well as 2% miconazole hemi-HP solvate in talc (left) and 2% miconazole hemihydrate in talc (right) are shown at 24 hours (top) and 48 hours (bottom) of incubation. Inhibition of yeast growth can be seen surrounding the miconazole containing dosages.

Table 1.

Average Solubility of Miconazole Forms in Phosphate-Citrate Buffer (PCB), pH 4.

Miconazole Form	Solubility ($\mu\text{g/mL}$)
Hemi-HP solvate	231 ± 32
Hemihydrate	159 ± 18
Anhydrate	202 ± 29