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"Dark" Singlet Oxygen and Electron Paramagnetic Resonance Spin Trapping as Convenient Tools to Assess Photolytic Drug Degradation

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ABSTRACT

Forced degradation studies are an important tool for a systematic assessment of decomposition pathways and identification of reactive sites in active pharmaceutical ingredients (APIs). Two methodologies have been combined in order to provide a deeper understanding of singlet oxygen-related degradation pathways of APIs under light irradiation. First, we report that a "dark" singlet oxygen test enables the investigation of drug reactivity toward singlet oxygen independently of photolytic irradiation processes. Second, the photosensitizing properties of the API producing the singlet oxygen was proven and quantified by spin trapping and electron paramagnetic resonance analysis. A combination of these techniques is an interesting addition to the forced degradation portfolio as it can be used for (1) revealing unexpected degradation pathways of APIs due to singlet oxygen, (2) clarifying photolytic drug-drug interactions in fixed-dose combinations, and (3) synthesizing larger quantities of hardly accessible oxidative drug degradants.

Introduction

Active pharmaceutical ingredients (APIs) can easily be exposed to light anytime during manufacturing, storage, and in-use conditions. Hence, a thorough evaluation of photolytic reactivity and degradation pathways at an early stage is a prerequisite for the development of a safe drug. An adequate and reliable shelf-life as well as product safety precautions and protective actions to be taken during manufacturing, packaging, transport, and storage depend on those studies. Consequently, the assessment of photolytic drug stability is required by means of regulatory guidelines such as ICH Q1B.[1]

Because in most cases photolytic degradation takes place in the presence of oxygen, a large number of photodegradation processes is oxidative in nature. These reactions can generally be classified as either type I or type II reactions (Scheme 1).[2] In a simplified picture, the API gets converted from its ground state (S_0) to a singlet excited state (S_1) upon light irradiation with an appropriate wavelength. Although relaxation by fluorescence or radiationless processes leads to de-excitation, intersystem crossing allows the formation of a triplet state (T_1). In its excited states, the photosensitized API can subsequently degrade via free radical or redox reactions, classically labeled as type I reactions. Alternatively, the API in its triplet state (T_1) can also interact with triplet ground state molecular oxygen ($^3O_2$) to generate singlet oxygen ($^1O_2$) via energy transfer. The latter process liberates the API in its ground state (S_0) again. In this case, the API acts as a photosensitizer and its chemical structure stays unchanged throughout this process as these transitions represent only changes of electron configurations within the API. Degradation will only occur when the API subsequently reacts with the generated singlet oxygen, a transformation referred to as a type II reaction. Due to its particularly high reactivity, singlet oxygen can react with a broad range of organic scaffolds. Many of those, for example, heterocycles undergoing cycloadditions, are often present in small molecule APIs.[3] Although many sources of singlet oxygen are known,[4] photosensitization by the API is the most relevant in the context of drug degradation. Parameters like concentration and solvent have a strong impact on experimentally measured quantum yields for formation of singlet oxygen,[5] but the fact that these values can be quite high for some APIs (e.g., 0.62 for tiaprofenic acid and 0.39 for ketoprofen[6]) further illustrates the importance of understanding singlet oxygen-induced type II degradation processes.

Attempts to apply light irradiation as a synthetic tool to prepare sufficient amounts of type II degradant for further structure or

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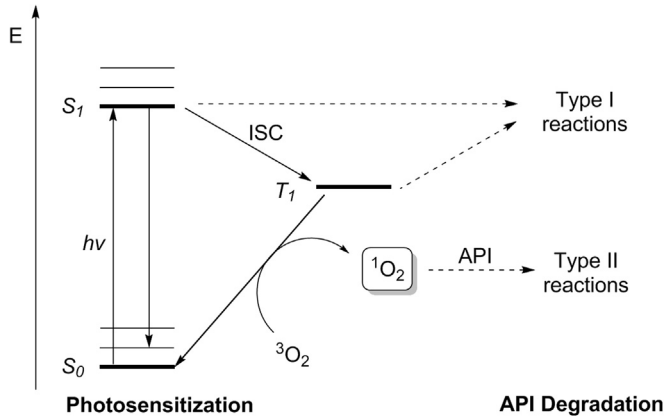


Scheme 1. Photolytic drug degradation (simplified).

toxicological evaluation are often significantly hampered by concurrent complex type I degradation processes. One approach to overcome this issue includes the addition of very potent photosensitizers like, for example, rose bengal to boost the light-induced singlet oxygen generation and hence favor type II reactions.[7] Unfortunately, this procedure does not always suppress alternative type I degradation pathways. The use of irradiation-free generated singlet oxygen—so called "dark" singlet oxygen—represents a valuable approach in order to synthesize the targeted degradant.

For such an irradiation-free procedure, different chemical methods to generate singlet oxygen can be considered. Classical approaches based on reagents like hypochlorite,[8] ozone,[9] or periodate[10] are mainly limited to aqueous systems and are considered to be too harsh to be applicable for selective drug degradation processes. The use of a sodium molybdate/hydrogen peroxide microemulsion system of water in dichloromethane together with a surfactant and *n*-butanol appears to be a reasonable solution to circumvent these drawbacks.[11,12] In such a system, singlet oxygen is produced by the reaction of hydrogen peroxide and sodium molybdate within aqueous micelles. After butanol-induced diffusion into the organic phase, singlet oxygen can then react with the API. The advantage of such a system is that the presence of peroxides is mainly restricted to the aqueous micelles, making the protocol applicable to APIs that are sensitive to peroxides.

As the corresponding analytical tool, electron paramagnetic resonance (EPR) in combination with spin trapping has been shown to be a useful technique to follow singlet oxygen generation through photosensitization.[13] When singlet oxygen is formed in the presence of the spin trap 4-hydroxy-2,2,6,6-tetramethylpiperidine (4-OH-TMP), a persistent TEMPOL radical is formed, which can easily be detected by EPR spectroscopy (Scheme 2).[14] It has been shown that 4-OH-TMP is a superior reagent compared to other sterically hindered amines for this purpose in terms of specificity and selectivity.[15] Because TEMPOL is a stable and commercially available compound, it can be used as a calibration standard, allowing quantification of the signal.

To our knowledge, by combining the 2 above-mentioned protocols, it is the first time that the photosensitizing potency of an API and its singlet oxygen-induced degradation can be studied independently from each other. First, drug candidate TMC647055[16] was used to exemplify the general suitability of this methodology. Subsequently, our protocol was used to reveal photolytic incompatibility of 2 APIs—VU0409551/JNJ-46778212-AAA[17] and JNJ-54119936-AAA.[18] By means of the latter model study it was shown that the investigation of photolytic API-API interactions in fixed-dose combinations (FDC) is a useful application of this 2-fold approach. Despite the rising importance of FDCs, in the vast number of studies stress testing is conducted on single compounds and examples using mixtures of APIs are scarce.[19] That disregard can be explained by the lack of uniform guidelines,[20] but also by the fact that in most cases the single components of an FDC have already been marketed as monotherapies and their individual degradation pathways are already well studied. The herein described procedure to separately address photosensitizing potency (by EPR spin trapping) and reactivity toward singlet oxygen (by dark singlet oxygen) conveniently allows the identification of API couples in which one is a potent photosensitizer while the other one is very sensitive toward reaction with singlet oxygen. This approach allows foreseeing potential API-API incompatibilities already by performing stress testing of the single components only.

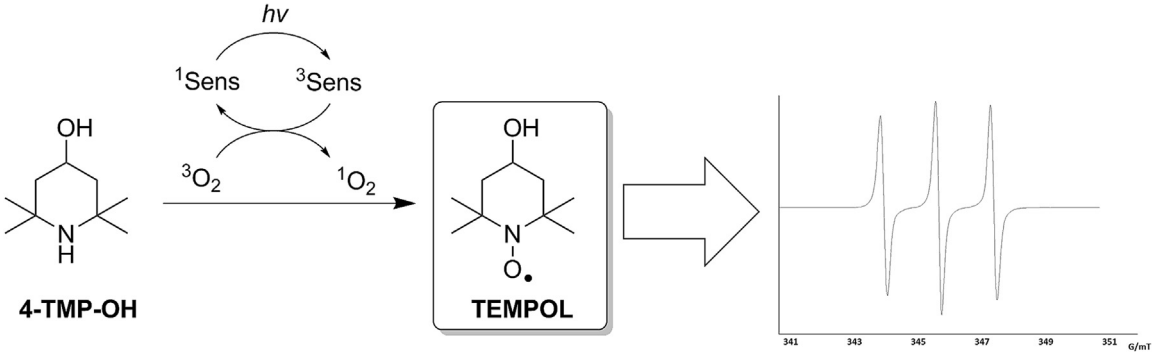
Experimental

Materials

TMC647055 (**1**), VU0409551/JNJ-46778212-AAA (**4**), and JNJ-54119936-AAA (**5**) were obtained from Janssen Pharmaceutica N.V. Other reagents were obtained from Sigma-Aldrich and used without further purification.

Light Irradiation

For light irradiation, each compound [VU0409551/JNJ-46778212-AAA (**4**) or JNJ-54119936-AAA (**5**)] was dissolved in a mixture of tetrahydrofuran (THF)/H_2O (1:1) to give a final concentration of 5 mM. For the detection of singlet oxygen, spin trap 4-OH-TMP ($c = 100$ mM) was also added. Solutions were placed in 10 mL screw cap vials (quartz) and irradiated at 300 W/m^2 at a constant ambient temperature in a Suntest CPS apparatus equipped



Scheme 2. Formation of TEMPOL by a reaction of 4-OH-TMP with singlet oxygen and its EPR spectrum.