The Use of *N*-Methylpyrrolidone as a Cosolvent and Oxidant in Pharmaceutical Stress Testing

DAN W. REYNOLDS,[1] MARINA GALVANI,[2] SIMON R. HICKS,[3] BIREN J. JOSHI,[1] SONYA A. KENNEDY-GABB,[4] MARK H. KLEINMAN,[4] PARESH Z. PARMAR[3]

[1]Active Pharmaceutical Ingredient Chemistry and Analysis, GlaxoSmithKline, Research Triangle Park, North Carolina 27709, USA

[2]Pharmaceutical Development, Aptuit Verona, Verona 37135, Italy

[3]Active Pharmaceutical Ingredient Chemistry and Analysis, GlaxoSmithKline, Stevenage, Hertfordshire SG12, United Kingdom

[4]Active Pharmaceutical Ingredient Chemistry and Analysis, GlaxoSmithKline, King of Prussia, Pennsylvania 19406

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ABSTRACT: The use of *N*-methylpyrrolidone (NMP) as an oxidant and cosolvent in pharmaceutical stress testing (forced degradation) is examined. Various active pharmaceutical ingredients were heated in NMP–water solutions under nitrogen, air, and oxygen and then analyzed by high-performance liquid chromatography, usually with ultraviolet diode array detection and mass spectrometry detection. In some cases, degradation products were isolated and characterized by nuclear magnetic resonance. The NMP–water–air–heat system provided oxidative and hydrolytic degradation products. The observed oxidation products were consistent with products expected from free radical autoxidation, reactions with hydroperoxides, and possibly singlet oxygen. Oxidative and hydrolytic pathways could be distinguished by comparison of the reactions carried out under air/oxygen and nitrogen. In many cases, the oxidation products observed during stress testing were also observed during formal stability studies of drug products. The NMP–water–air–heat stress condition facilitates various oxidative degradation pathways, which are often relevant to drug product on stability. This approach facilitates stability-indicating method development and helps elucidate degradation pathways. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:761–776, 2012

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INTRODUCTION

Stress testing or forced degradation of pharmaceuticals is performed to help elucidate degradation pathways, facilitate development and validation of stability-indicating methods, and determine the intrinsic stability of an active pharmaceutical ingredient (API).[1,2] Stress testing is a regulatory requirement.[3] Stress studies can help guide formulation development, facilitate screening for genotoxin formation, lead to better product packaging, and provide the knowledge required to solve drug substance and product stability problems.[4] In a stress study, a drug is typically subjected to hydrolysis over a broad pH range, oxidation, heat, heat with high relative humidity (RH), and light.[2] Much has been written about stress testing in recent years with the first book dedicated to the subject being published in 2005.[5] There are many experimental approaches to stress testing in use throughout industry.[6] Perhaps this diversity is best exemplified by the variety of conditions used for oxidation. Some of the conditions and reagents recommended for oxidative stress testing include oxygen headspace,[7] peroxides,[8] free radical initiators,[9] metals,[10] electrooxidation,[11] potassium permanganate ($KMnO_4$),[12] Tween 80/Fe(III),[13] glyme/Fe(II),[14] and singlet oxygen.[15] This diversity stems in part from the variety of mechanisms[16] by which a drug molecule may oxidize in a formulation: free radical autoxidation, electron transfer, photooxidation, reactions with singlet oxygen, reactions

Abbreviations used: NMP, *N*-methylpyrrolidone; MS, mass spectrometry; HPLC, high-performance liquid chromatography; API, active pharmaceutical ingredient; NMR, nuclear magnetic resonance.

Correspondence to: Dan W. Reynolds (Telephone: +919-483-5379; Fax: +919-483-6053; E-mail: dan.w.reynolds@gsk.com)

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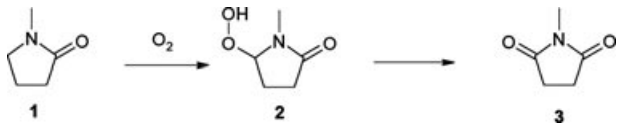

Figure 1. Oxidation of *N*-methylpyrrolidone.

with peroxides, and so on. In this paper, the use of yet another reagent for oxidative stress testing will be presented. The reagent, *N*-methylpyrrolidone (NMP), is a water-soluble dipolar aprotic solvent, which when heated in the presence of oxygen forms a hydroperoxide[17,18] via a free radical autoxidation mechanism.[19,20] Hence, stress testing in the presence of NMP provides both free radicals and peroxides. There is also evidence for the formation of thermally generated singlet oxygen from NMP (see below). NMP is also a good surrogate for several common excipients including polyene glycols, tweens, polyvinylpyrrolidone (PVP), and crospovidone. Typical oxidative stress conditions involve an API dissolved in a NMP–water mixture heated at 60°C–80°C in the presence and absence of oxygen. These conditions allow oxidative and hydrolytic processes to proceed simultaneously while facilitating discernment between mechanisms.

Properties of NMP

N-methylpyrrolidone is a cyclic amide that is completely miscible in water, ethanol, and diethyl ether. It is highly soluble in lower alcohols, lower ketones, ethyl acetate, chloroform, and benzene, and moderately soluble in aliphatic hydrocarbons.[21] With a boiling point (b.p.) of 202°C–204°C, it is essentially nonvolatile at temperatures used in stress testing ($\leq$80°C). The ultraviolet (UV) cutoff is 280 nm. NMP, like most alkyl amides, is relatively hydrolytically stable but will hydrolyze in base.[22] It has relatively low toxicity but can irritate the skin, eyes, and respiratory tract. There is indirect evidence that NMP may be harmful to a developing fetus.[23]

Oxidation of NMP

N-methylpyrrolidone (1) is known to oxidize in the presence of oxygen and heat to the corresponding hydroperoxide (2) and imide (3)[17] as shown in Figure 1.

The hydroperoxide (2) and imide (3) have been isolated and characterized by nuclear magnetic resonance (NMR). With pure NMP, the formation of the hydroperoxide (2) follows a free radical autoxidation mechanism under oxygen with an induction period of 24 h at 75°C. A steady state ([ROOH] ~1.3 M) is reached after 72 h. A smaller [ROOH] is observed in the presence of water. The reactivity of the ROOH derived from NMP is similar to that of *t*-butyl hydroperoxide.[17] It is the production of hydroperoxy radicals and peroxides during the autoxidation of NMP that makes NMP useful for oxidative stress testing of pharmaceuticals.

This paper explores the use of NMP for oxidative stress testing. Topics addressed include the oxidation of NMP, the scope of API functional group transformations, a brief comparison with other approaches, examples of the predictive value of NMP oxidative stress conditions, and some limitations and problems with the approach. Overall, the NMP procedure is presented as a simple, powerful, and predictive approach for oxidative stress testing of pharmaceuticals.

MATERIALS AND METHODS

N-methylpyrrolidone from various manufacturers was used. The water was Milli-Q quality. Stress testing was usually carried out in flint glass serum vials (Wheaton, Millville, NJ) with rubber septum closures in thermostatted ovens. High-performance liquid chromatography (HPLC) analyses were usually carried out on a reverse-phase column with a gradient with mass-spectrometry (MS)-compatible mobile phases using UV diode array detection and MS detection. Reactions were stopped when the extent of reaction was 10%–20%. Some compounds were isolated using preparative HPLC and then assigned unequivocal structures using NMR.

General Procedure

For best results, we used a fresh bottle of NMP that came stored under nitrogen. These are available from Sigma (St. Louis, MO) in 100 mL bottles with a septum closure.

Typically, a solution of API (1–10 mg/mL) in an NMP–water (usually 1:1) mixture was prepared. Then, 2–3 mL of this solution were placed into seven separate 30 mL clear serum vials (Wheaton) that were then sealed with rubber septa held in place with aluminum (Al) flange collars. The headspace of one of the vials was then purged with nitrogen or argon by use of hypodermic syringe needles (Fig. 2). Another vial was then purged with pure oxygen in the same way. There were now seven vials with three different headspaces: one with nitrogen, five with air, and one with oxygen (The serum vials were designed for autoclaving. The rubber septum/Al flange collar closure was air tight, even after multiple penetrations with syringe needles. This facilitated mass balance determinations as well as headspace analysis). The vials were then heated to the desired temperature (usually 60°C–80°C). After a period of time, one of the