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Rapid Communication

Trace Aldehydes in Solid Oral Dosage Forms as Catalysts for Nitrosating Secondary Amines

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ABSTRACT

Nitrosamine impurities may form during drug substance manufacturing processes. Here, we focus on nitrosamine impurity level growth in oral drug products during long term stability studies. Nitrosamine growth mechanisms in oral dosage forms are typically framed as due to nitrosating agents that can be formed in solutions of nitrous acid with a required pH value of around pH 5 or below. We strive in this work to bring awareness to pharmaceutical scientists that formaldehyde, common in oral dosage form excipients, has previously been shown in solution to catalyze the reaction between secondary amines and nitrite ion to give nitrosamine products. This mechanism operates at pH ~6 and higher. We attempt to re-frame the solution work as relevant to pharmaceutical solid dosage forms. Recent examples of solid dosage form product recalls are used to demonstrate the formaldehyde catalyzed nitrosation pathway operating in the solid state.

Introduction

The FDA issued a guidance on nitrosamine impurities in February 2021.[1] The guidance requires risk assessment for nitrosamine formation during all drug substance (DS) manufacturing, as well as to assess risk of nitrosamine growth for all drug products over the expected shelf-life period. This was in response to a number of high-profile product alerts and recalls.[2] Here, we focus on potential for nitrosamine growth in drug product.[3] The pharmaceutical industry has supported the development of databases and analytical procedures to understand the nitrite ion (NO_2^-) content of common pharmaceutical excipients.[4,5] Nitrite ion is present at sub-ppm levels in most excipients and at several ppm levels in other key excipients, with a typical tablet containing about 1 ppm nitrite.[4] It is well understood that amines are not reactive directly with nitrite. To explain potential nitrosation of amines in oral dosage forms (from ambient excipient nitrite), pharmaceutical scientists will cite the well-known creation of various "nitrosating" agents that are known to form in nitrous acid solutions (the protonated nitrite ion, HNO_2, pK_a=3.1). A further protonation of HNO_2 is required to generate the nitrosating species.[6,7] The acidic nitrosation pathway is associated with pH values lower than pH 5.[6,7] There is mixed enthusiasm for always having to invoke protonation of nitrous acid in tablet "microenvironments" to explain nitrosation in our experience. This has muddled the risk assessment for growth of nitrosamines over the stability interval, and much uncertainty exists as to when the next case of a problematic nitrosamine will suddenly appear.

In this communication, we wish draw attention to the fact that aldehydes have previously been shown to be able to catalyze the "direct" reaction between a secondary amine and nitrite ion[8-11] at pH values > 6. The aldehyde and amine react to form a reactive iminium species, which then allows direct reaction with nitrite (Fig. 1). We reframe the solution work to the solid state, operating through formaldehyde and nitrite ion impurities carried by the excipients. The optimal pH range of the aldehyde catalysis is pH 6-7, higher than the nitrous acid related nitrosation pathways. Several recent examples of drug product recalls are discussed which demonstrate the formaldehyde catalysis of secondary amine nitrosation. We suggest that the known formaldehyde levels[12-14] and nitrite levels[4,5] in tablet excipients thus provide an additional, complementary nitrosation pathway to consider, if faced with having to rationalize nitrosamine growth or assess risk in drug product.

Discussion

Formaldehyde as a Catalyst for Nitrosation of Secondary Amines by Nitrite Ion

The nitrosation of secondary amines by nitrite, catalyzed by formaldehyde to yield the nitrosamine was first demonstrated by Keeper and Roller in *Science*, 1973.[8] The reaction is shown in Fig. 1. Formaldehyde reacts with the secondary amine to form the hemiaminal,

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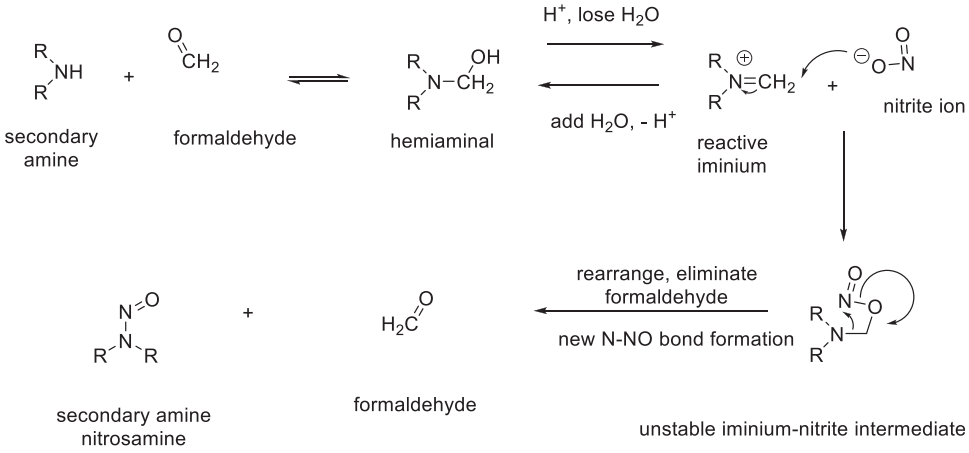

Figure 1. Formaldehyde catalysis of secondary amine nitrosation. Water and the nitrite ion compete for the reactive iminium. Formaldehyde is re-generated, while forming the new N-NO bond.[8]

which can protonate and lose water to form the iminium species. The iminium ion is susceptible to attack even by poor nucleophiles, in this case the nitrite ion. The resulting iminium-nitrite product is unstable; formaldehyde is eliminated concurrent with new N-NO bond formation (Fig. 1). In aqueous solutions, the optimal pH is in the pH 6-7 range. At room temperature, yields of nitrosamine are limited to a few percent.[8] The low yield was shown to be due to back-reaction of the iminium ion with (unit activity) water. This was demonstrated by direct preparation of a model iminium species (R= methyl in Fig. 1), followed by addition of anhydrous nitrite ion in acetonitrile. This gave over 90% yield of the nitrosamine showing the reaction of nitrite with the iminium ion, subsequent loss of formaldehyde, and new N-NO bond formation is very favorable (last steps in Fig. 1**)**. Trichloroacetaldehyde was also shown to be effective. Later efforts also found benzaldehyde provides the same catalysis.[9] Secondary amine substrates have been shown to include dimethylamine (DMA), diethylamine, and the 5 and 6 membered ring systems pyrrolidine, piperidine, and morpholine.[10] Only the very sterically encumbered diisopropylamine was found too sterically constrained to react as in Fig. 1. Many pharmaceutical secondary amines would appear to be sterically accessible. In 2009, a computational analysis of the reaction in Fig. 1 was published[11] which confirmed the iminium ion formation step as rate limiting, and suggested electron withdrawing groups on the aldehyde would enhance the catalytic properties of the aldehyde.

Keeper and Roller[8] noted the ubiquitous presence of formaldehyde in the environment, and posited that the mechanism in Fig. 1, since it operates in the pH 6-7 region could account for the known examples of environmental nitrosamine formation (occurring in foodstuffs, organic materials, combustion products, etc.). This conclusion was due to their dissatisfaction with the idea that the nitrous acid induced creation of nitrosating agents could be generally operating in typical environmental samples.

Reaction in Fig. 1 as applied to Oral dosage forms on Stability: nitrite ion, formaldehyde, low water activity, and ambient pH values in tablets

Recent focus on formation and growth of nitrosamine impurities has resulted in a fairly robust understanding of the nitrite ion impurity content of pharmaceutical excipients.[4,5] There exists some variation lot-to-lot and vendor-to-vendor. Using mean values, the excipients microcrystalline cellulose and lactose monohydrate each carry about 0.5 ppm nitrite ion, while magnesium stearate contains 2 ppm and crospovidone 8 ppm nitrite.[4,5] These latter excipients are typically used in lower % by weight in tablets. A general composition oral dosage form tablet was suggested to contain about 1 ppm nitrite ion.[4] Thus, a general source of nitrite in Fig. 1 is from tablet excipients.

Formaldehyde has been known to exist in dosage form excipients for decades since observation of excipient driven cross linking of gelatin capsules. Numerous studies examining tablet excipient formaldehyde content have been detailed (see, for example, references 12-14). Polyethylene glycols, polysorbates, povidone, and hydroxypropyl methylcellulose (HPMC) can contain tens of ppm formaldehyde, while microcrystalline cellulose and lactose typically container lower formaldehyde levels (ca. 0.5 to a few ppm). Formaldehyde is the degradation product of slow autoxidative processes related to the excipients molecular structure, and thus cannot be removed. Residual formaldehyde levels are better thought of as an unwanted but characteristic trait of each excipient. Using available formaldehyde data,[12-14] a general oral tablet composition contains at *least* 1 ppm formaldehyde. The reaction of drug substance moieties with trace formaldehyde is well established.[15-18] Reference 18 highlights an oral tablet case in which excipient-based formaldehyde reacted with a drug secondary amine to form the iminium ion (as in Fig. 1), which ultimately led to the degradation product of interest. Thus, the presence of 1 ppm nitrite, at least 1 ppm formaldehyde, and conditions allowing iminium ion formation (Fig. 1) have been demonstrated to exist in oral dosage forms on stability.

Oral dosage form stability packaging is often focused around reducing the water activity in the tablet. Reduced water activity in the tablet will slow the iminium cation back-reaction with water allowing more efficient reaction with nitrite ion, as compared to the aqueous (unit water activity) yields reported by Keeper and Roller.[8] To compare the water microenvironmental pH in tablets to the optimum pH 6-7 range reported in that work, it is best to use the slurry pH values of common tablet excipients.[19,20] Common tablet excipient slurry pH values are in the pH 5-7 range, unless the excipients have acidic groups explicitly in their structures. Drug substance slurry pH values can vary widely. Many drug substances are salts of basic amines, and the salts often carry protons into the formulation (HCl, mesylate, tartrate, phosphate salts etc.). These types of drug salts with high water solubility may show “slurry” pH values near pH 5 depending on the pKa and solubility.[21] Thus, these salts in principle may access the upper limits of the pH range of the nitrous acid nitrosation route, although some undefined water activity level in the tablet might be required. The aldehyde route operates at higher pH values. Note that free base forms of basic amine drugs will have significantly higher slurry pH values than basic amine salt forms.

Solution State Aldehyde Driven Nitrosation of Pharmaceuticals: Ranitidine HCl Oral and IV Solution

Here we elected to first bridge Keeper and Roller’s solution phase work to a solution phase pharmaceutical context. King et al.[3] recently

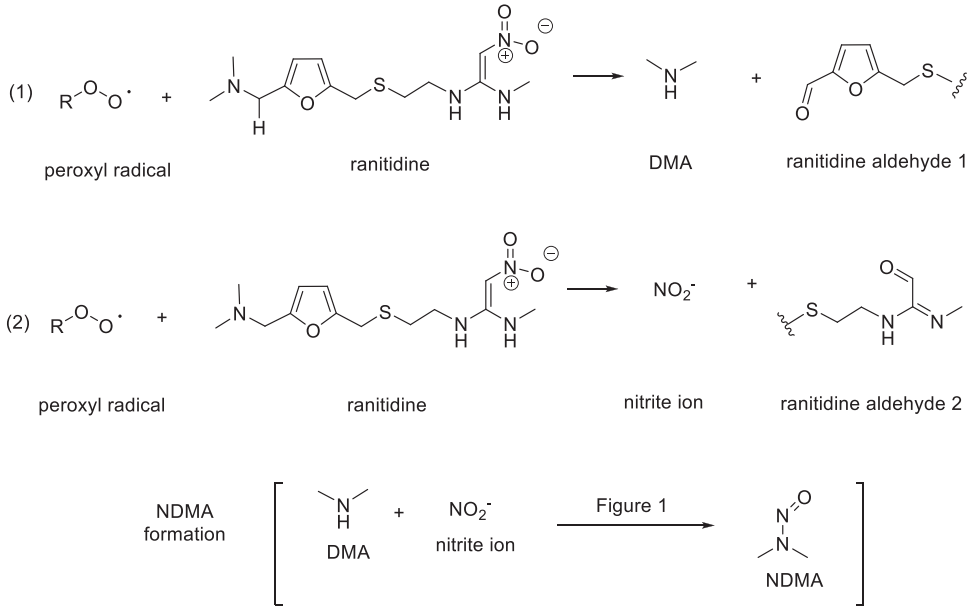

Figure 2. Peroxyl radical reactions with ranitidine to release DMA, nitrite ion, and ranitidine aldehydes (upper two reactions 1 and 2). Lower, brackets, NDMA formation from DMA and nitrite ion catalyzed by a ranitidine aldehyde in ranitidine IV and oral solutions.

examined nitrosamine levels (NDMA, see Fig. 2 for structure) in ranitidine HCl DS and drug products. Table 1 shows the two cases of interest in the current context, the IV and oral solutions which both have pH values near pH 7.0. NDMA upper ranges were about 1 and 3 ppm for the IV and oral solutions, respectively. The IV solution contains only ranitidine and buffers. The oral solution is more complex with a variety of excipients.

In the unusual case of ranitidine, which has a vinyl nitro moiety, we have recently[22] proposed that nitrite, DMA, and ranitidine aldehydes are formed from peroxyl radical reactions of ranitidine. These reactions are represented in the upper portion of Fig. 2. Thus, ranitidine is the nitrite source in this case. NDMA results from nitrosation of DMA by the released nitrite (brackets, lower portion of Fig. 2). We propose here that in a homogenous solution at pH 7.0, the only plausible nitrosation pathway is through aldehyde catalysis. The NDMA formed in the IV solution case (no excipients) suggests at least one of the ranitidine aldehydes formed (Fig. 2) can act as the catalytic aldehyde. The pH of 7.0 is optimal for the aldehyde catalysis, but ~2 pH units higher than the upper pH range associated with the acidic nitrosation route.[6,7]

Evidence for Solid State Aldehyde Catalyzed Nitrosation in Recent Drug Product Recalls

A relatively small number of capsule lots of nizatidine were recalled in 2020, and several more recalls were initiated in early 2022, all recalls due to concerns about NDMA levels. Nizatidine is very closely related to ranitidine, except has a thiazole ring in place of the furan ring in ranitidine. We presume nizatidine can undergo the same peroxyl radical reactions as ranitidine (Fig. 2) releasing nitrite, DMA and the aldehydes. Nizatidine is provided as a free base, not as the HCl salt. The capsule composition does include povidone which is relatively high in formaldehyde levels. No acidic excipients are present. The NDMA levels found were near 0.1 ppm. Nitric acid related nitrosation is not viable in this system in our view. The 0.1 ppm levels of NDMA proceed from peroxyl radical release of nitrite, DMA, and the aldehyde catalyzed nitrosation (either from the nizatidine aldehydes or from formaldehyde associated with the povidone excipient) operating in the solid-state capsules.

The recent case of the voluntary recall of all lots of propranolol HCl extended-release (ER) capsules again demonstrates the reality of solid-state nitrosation by formaldehyde catalysis (see Fig. 3 for relevant structures). The secondary amine is on the drug substance which is the easiest possible case to get problematic nitrosamine levels since the secondary amine is at such high relative abundance. We arrive at

Table 1
Data from reference 3.

Ranitidine Drug Product	pH	NDMA range*
IV solution (1 mg/ml)	~7.0	0.3-1
Syrup (oral solution, 15 mg/ml)	~7.0	0.7-3

*ppm relative to ranitidine free base

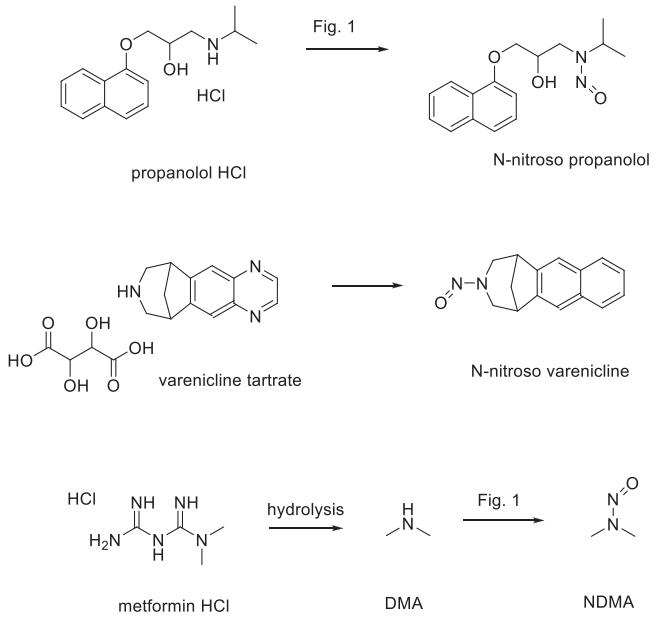

Figure 3. Structures of propranolol HCl, varenicline tartrate, and metformin HCl and their associated nitrosamine impurities of concern.

formaldehyde catalyzed nitrosation (Fig. 1) as the nitrosation pathway in this case by comparison of the immediate release (IR) propranolol HCl tablet and propranolol HCl (ER) capsule compositions, and by noting the IR tablets have never been recalled for worrisome nitrosamine levels. The only germane compositional difference in this context is the presence of significant HPMC in the ER capsules which is not present in the IR tablets. HPMC has about the same nitrite content as the microcrystalline cellulose it displaces in the IR tablet,[4] thus no significant nitrite ion differences derive from the use of HPMC. However, HPMC has a much higher formaldehyde content (3-10-fold) than microcrystalline cellulose[12-14] due to the oxidizability of C-H bonds adjacent to the ether linkages of the hydroxy-propyl and methoxy groups added to the cellulose. Acidic pathways to nitrosation are apparently not significant in the IR product, and thus, the same excipients and DS in the ER product cannot manifest significant acidic nitrosation pathways. On current evidence, this leaves only the HPMC derived formaldehyde catalysis (Fig. 1) as a reasonable explanation of the problematic nitrosation.

Another recent example of nitrosation of a drug substance secondary amine that resulted in voluntary recall of all lots is varenicline tartrate 0.5 and 1.0 mg tablets (see **Fig. 3** for relevant structures). The excipients used do not appear to be unusually high in formaldehyde or nitrite content. It is included here as an example not because we suspect involvement of the formaldehyde pathway, but rather because we do not. Given the high-water solubility of this tartrate salt and the presence of the excipient calcium monohydrogen phosphate anhydrous, which is known to have acidic surfaces and a rather low slurry pH of 5.2,[19] the acidic route of nitrosation appears a more plausible route to new N-NO bond formation in this case.

A final example highlights the formaldehyde catalysis pathway nitrosating a drug substance impurity/degradate. Numerous recalls of metformin ER dosage forms (made by multiple different vendors) occurred throughout 2021, often involving recalls of all currently disturbed ER lots. The nitrosamine of concern was NDMA, the relevant structures are shown at the bottom of **Fig. 3.** DMA is a known impurity in metformin DS. The same logic sequence described above for propranolol HCl IR and ER applies in this case. The IR metformin HCl products have not shown problematic levels of NDMA, and have not been recalled (and thus acidic nitrosation routes not operating). The only excipient present at significant levels in the core tablets in the ER cases (which is not present in the IR cases) is again HPMC (sometimes accompanied by carboxymethyl cellulose depending on the type of extended release technology being used). The result is formaldehyde driven nitrosation of the impurity/degradate DMA which approached 0.1-0.2 ppm NDMA (with respect to metformin). Given the large daily doses of 500-1000 mg metformin, this was enough to cause concerns.

Summary and Conclusions

We have re-cast Keeper and Roller's work[8] to the solid state in which nitrite ion and formaldehyde are brought together by tablet excipients to affect the new N-NO bond formation. Recent recalls of nizatidine capsules, propranolol HCl ER capsules and metformin ER tablets are cited as examples of this formaldehyde pathway (Fig. 1) operating in the solid state. Thus, when assessing risk for nitrosamine formation, or when faced with nitrosamine growth post-manufacture, we believe formaldehyde catalysis outlined here must be considered along with the nitrous acid derived nitrosating agents. This provides two different potential mechanisms and pH regions which can be explored, each leading to somewhat different mitigation strategies, or at least, different perspectives on how standard control strategies might have their impact.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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