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Review

N-nitrosamine Mitigation with Nitrite Scavengers in Oral Pharmaceutical Drug Products

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ABSTRACT

N-nitrosamines are likely human carcinogens. After N-nitrosamine contaminants were detected in pharmaceutical products in 2018, regulatory authorities set a framework for the risk assessment, testing and mitigation of N-nitrosamines in drug products. One strategy to inhibit the formation of N-nitrosamines during the manufacture and storage of drug products involves the incorporation of nitrite scavengers in the formulation. Diverse molecules have been tested in screening studies including the antioxidant vitamins ascorbic acid and α -tocopherol, amino acids, and other antioxidants used in foods or drugs, for inclusion into drug products to mitigate N-nitrosamine formation. This review article outlines key considerations for the inclusion of nitrite scavengers in oral drug product formulations.

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Introduction

N-nitrosamines are organic compounds that can be found in the environment, diet, and are also produced endogenously.^{1–4} A number of N-nitrosamines are carcinogenic and can be present as contaminants in drug products.^{5,6} They are formed by the reaction of secondary or tertiary amines together with nitrosating agents such as nitrite salts under acidic conditions.^{7,8} Secondary and tertiary amines may be present in the drug product as the active ingredient, its degradation products, or impurities from chemical synthesis or excipients. Nitrosation reactions can be mitigated in foods through the addition of substances such as ascorbic acid, α -tocopherol and sulfur dioxide.⁹ Similarly, recent work has proven that such attenuation can also be achieved in pharmaceutical products by the addition of excipients capable of nitrite scavenging.^{10–12}

N-nitrosamines are part of the so-called “cohort of concern” impurities in pharmaceutical products, and some are considered “possible” or “probable” human carcinogens by the International Agency for

Research on Cancer.^{8,13} Representative members of the N-nitrosamine class have been found to cause cancer in at least 40 different animal models.^{4,13} Even so, the carcinogenicity of most N-nitrosamines has not been tested experimentally and there remains considerable uncertainty about the exact role of N-nitrosamine formation on cancer incidence in humans.^{2,14}

N-nitrosamines require metabolic activation by oxidative reactions to impart their carcinogenic effect.¹⁵ Metabolic activation occurs either via a single α -hydroxylation resulting in fragmentation into a diazonium hydroxide and an aldehyde, or via consecutive α -hydroxylations forming unstable nitrosamides. Both these processes ultimately result in the formation of carbocations or diazonium cations that are highly reactive and capable of alkylating macromolecules and higher-level structures such as DNA or RNA.¹⁶

After N-nitrosamines were found in angiotensin II receptor blocker (ARB) drug products in 2018, a global investigation into N-nitrosamine contamination of these drug products by regulatory bodies was triggered.¹⁷ Initially, ARBs (sartans) were in the focus of the regulatory bodies. Subsequently, and after N-nitrosamine impurities were found in other classes of human medicines such as ranitidine and metformin, the focus of the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the

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Pharmaceuticals and Medical Devices Agency (PMDA) was broadened. The regulatory requirements to perform risk assessments to determine potential *N*-nitrosamine formation and propose mitigation strategies where applicable were accordingly introduced for all drug products.^{14,18} While the addition of agents to mitigate *N*-nitrosamine formation was not specifically mentioned in early guidance documents,^{8,19} the potential for inhibitors as excipients has been later put forward as a strategy to be considered in the overall management of *N*-nitrosamine contamination in drug products.²⁰

The addition of nitrite scavenging excipients to formulations was identified and recently tested as a mechanism of action to prevent *N*-nitrosamine formation in drug products.^{11,12} As part of compound screening, 19 structurally diverse compounds with an acceptable toxicological profile were evaluated by Homšak *et al.*¹¹ These included certain amino acids (L-cysteine, histidine, lysine), general antioxidants (propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT)), ammonia-related compounds (urea, ammonium sulfate and ammonium chloride), the pyrone maltol, para-aminobenzoic acid (PABA), and vitamins with antioxidant properties (ascorbic acid, α -tocopherol). Likewise, the use of ascorbic acid and α -tocopherol to inhibit *N*-nitrosamine formation in pharmaceutical products has been specifically mentioned by the FDA.²⁰ Formulations that include a nitrite scavenger could reduce the overall risk of *N*-nitrosamine formation and keep concentrations within acceptable limits. In this review, we discuss current knowledge and research gaps in the use of nitrite scavengers for *N*-nitrosamine mitigation, particularly in solid drug products.

Short History of *N*-nitrosamine Formation in Drug Products

In vitro *N*-nitrosamine formation was first described in the late 1800s; inadvertently, the noxious compounds were described as “beautiful”.²¹ In 1954, after reports of liver damage in workers exposed to dimethylnitrosamine, a first study of the toxic effects of this simple member of the *N*-nitrosamine class was published.²² A landmark review published in 1970 proposed *N*-nitrosamines and their precursors, found in food, drugs, tobacco and potentially produced in the gut in situ, as ubiquitous carcinogens.³ ICH guideline M7R1 considers nitroso-compounds as part of the “cohort of concern,” containing high potency mutagenic compounds.²³

In 2018, the blood pressure medication valsartan was recalled after trace amounts of carcinogenic *N*-nitrosamines were detected. Subsequently, different series of drug products were recalled due to *N*-nitrosamine contamination, including the sartan family of angiotensin II receptor blockers, anti-diabetic drugs metformin and pioglitazone, and reflux medicine ranitidine.²⁴ In response, FDA and EMA issued guidelines to control *N*-nitrosamine contamination in drug products.^{20,25}

The use of nitrite scavengers to reduce *N*-nitrosamine formation was first studied in the 1960s, when Dahn and co-workers reported that ascorbic acid was capable of reducing nitrous acid, or more specifically the anhydride N_2O_3 to nitric oxide (NO).²⁶ Starting in the 1970s, several research groups investigated the potential for various molecules to block *N*-nitrosamine formation in vitro.²⁷ Due to interest on the dietary causes of cancer there was a focus on high-nitrite food products,²⁸ however in 1972, the reaction of ascorbic acid with nitrite, which reduced the amount available for nitrosation of the amine, was already known and being investigated as a blocking agent against the nitrosation of vulnerable amines in drug products.²⁹ This was tested in various model systems,^{29–32} notably with the aim of reducing *N*-nitrosamine formation in medicines such as tetracycline and piperazine.²⁹

The Formation of *N*-nitrosamines in Drug Products

N-nitrosamine formation requires the presence of three factors:

1) a nitrosating agent or precursor thereof, particularly nitrites.

- 2) a vulnerable secondary or tertiary amine, which may be a moiety within the drug substance.
- 3) conditions amenable for the reaction to occur such as elevated temperatures, acidic conditions, or the presence of a liquid phase.

These three factors may not be sufficient for *N*-nitrosamine formation to occur as the kinetics of *N*-nitrosamine formation can vary significantly depending on the structure and environment.^{7,16}

Nitrites do not directly nitrosate amines, rather they are precursors of nitrosating agents: nitrites are converted into nitrous anhydride (N_2O_3) already under slightly acidic conditions (Fig. 1).^{7,16} Nitrites have been found in a wide range of different excipients at varying concentrations: common examples include lactose and pre-gelatinised starch used as fillers, magnesium stearate and stearic acid used as lubricants, and the flowing aid silicon dioxide.^{33,34} In addition, other raw materials and processing conditions can be sources of nitrosating agents. Placing general limits on nitrite concentrations in drug product excipients may not be sufficient as this strategy will not effectively alleviate all risks of *N*-nitrosamine formation.^{7,33–35} Practical concerns such as wide variation in the nitrite content between excipient lots and suppliers, limitations of analytical methods and the use of risk assessments to infer nitrite content information introduce considerable uncertainties in the establishment of the nitrite content of excipients.³⁵ Furthermore, not only nitrites are sources of nitrosating agents: other impurities introduced by the excipients or the API may not be traceable (e.g. nitromethane), and thus a “nitrite-free” formulation could still form or already contain nitrosamines.¹⁶ While it may not be a sufficient measure on its own, screening for excipients with low nitrite amounts can be an important element in a holistic strategy to keep potential *N*-nitrosamine formation at a minimum level.⁷

Reactive amines can be introduced into the drug product via the API itself or its impurities, starting materials, pharmaceutical salt counterions, and excipients. A wide range of APIs are potential nitrosamine precursors.¹⁴ The total *N*-nitrosamine risk will depend on the amounts of amine and nitrosating agent present, type of *N*-nitrosamine formed, nature of processing steps used, water activity, storage conditions in the final drug product, and composition of the formulation.⁷

For the mitigation of *N*-nitrosamine formation during manufacturing, drug production processes should be evaluated to identify all potential sources. The process should be part of the risk assessment for *N*-nitrosamine formation applied to all drug products. Input from providers of raw materials including excipient manufacturers is likely required to complete the risk assessment. Tools such as a database of nitrites in excipients used in the production of drug products are valuable for estimating nitrite concentrations and variability, which are a crucial part of the risk assessment process.³³

Confirmatory testing of API raw materials, excipients and product during storage can provide evidence that risks are being managed appropriately. For example, a supplier qualification program that involves testing product lots from excipient suppliers can help to reduce the risk of *N*-nitrosamine formation.^{20,25}

In addition to risk assessment and testing, regulatory bodies of medicinal products recommend that manufacturers explore other

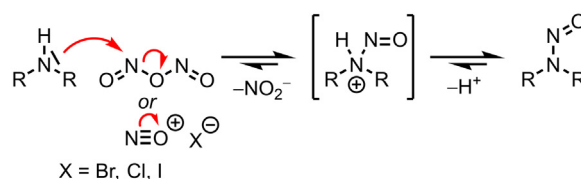


Figure 1. Proposed Mechanism for Nitrosamine Formation via an *N*-Protonated Conjugate Acid.

options to reduce the formation of *N*-nitrosamines in drug products, particularly during storage when trace amounts of nitrosating agents can cause the formation of undesirable amounts of *N*-nitrosamines.²⁰ As *N*-nitrosamine formation usually occurs at an acidic pH, one strategy is to adjust the pH of the formulation to ensure a neutral or basic pH within the shelf life of the product. However, even under neutral conditions nitrosamines can still form if certain aldehydes are present to catalyze the nitrosation reaction.³⁶ This mechanism has recently also been shown to proceed in solid oral dosage forms if traces of formaldehyde are present, which can be an impurity in certain excipients such as povidone or hydroxypropyl methylcellulose.³⁷ The addition of nitrite scavengers can also inhibit *N*-nitrosamine formation during storage.

Molecules Capable of Inhibiting or Blocking *N*-nitrosamine Formation in Drug Products

Several classes of compounds have been shown to mitigate nitrosamine formation.¹¹ Reaction mechanisms have been proposed for three main classes: the redox pathway, through the diazotization of primary amines, and nitration reactions of phenols (Fig. 2).^{12,38} Compounds capable of rapidly reducing the nitrosating agent to non-nitrosating nitric oxide (NO) can reduce or completely inhibit the formation of *N*-nitrosamine.¹⁵ This is achieved by acting as a competitive substrate for the nitrosating moiety. Both the absolute and relative concentrations of the nitrosating agent, amine and blocking agent determine the effectiveness of the blocking agent on *N*-nitrosamine formation.¹⁵

A wide range of potential nitrite scavengers are available for use as blocking agents in drug products.¹¹ Blocking agents may be water- or lipid-soluble. Water-soluble molecules include phenols, ascorbic acid, sulfite, bisulfite, and cysteine, although phenolics are not considered reliable blocking agents as they can facilitate transnitrosation.¹⁵ Fat-soluble antioxidants that are currently used in drug products and that have nitrite scavenging ability include BHA, BHT and α -tocopherol. Recent data however indicate that the effectiveness of BHA and BHT might be limited.¹¹ Ingredients that are listed as “inactive ingredients” for approved drug products by the FDA are particularly suitable for formulation into drug products.^{39,40} A summary of nitrite scavengers for potential use in pharmaceutical solid oral dosage forms is provided in Table 1.

Ascorbic Acid

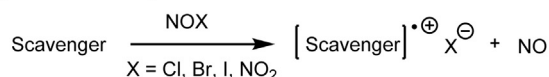
Ascorbic acid has a wide applicability for many nitrosating agents and is nontoxic at effective amounts in drug products.³² It is particularly suited to aqueous and weakly acidic conditions and can react with N_2O_3 , $H_2NO_2^+$, and NOX, converting them to NO.^{15,32} However, under aerobic conditions, NO can oxidize to NO_2 and subsequently convert back to nitrosating agents (i.e. N_2O_3 or N_2O_4). Good scavenging efficiency of redox scavengers such as ascorbic acid is thus expected under anaerobic conditions, although their efficiency is already very good in the presence of oxygen.⁴¹ A stoichiometric model can help formulate drug products with the correct amount of ascorbic acid to adequately suppress *N*-nitrosamine formation from the expected amount of nitrosating agent present under storage conditions, as was done for *N*-nitrosamine formation in aqueous solutions.⁴² However, this approach should be used with caution for solid drug forms produced via direct compression because particle size and local distribution influence inhibition performance through their effects on scavenging kinetics. Specifically, a smaller particle size increases the surface area of contact, and an even distribution ensures that the scavenger is in close proximity to the nitrosating agent throughout the drug product matrix. Wet granulation can alleviate particle size and homogeneity concerns.⁴³ The section “General considerations for the use of nitrite scavengers in solid drug forms” discusses these issues further.

Most research has focused on the prevention of nitrosation with ascorbic acid *in vivo* and in food and beverages, nevertheless the same principles can also be applied to drug products. Two research groups have investigated the use of ascorbic acid to mitigate *N*-nitrosamine formation in tablets and found it to be a potent inhibitor compared to other antioxidants.^{11,12}

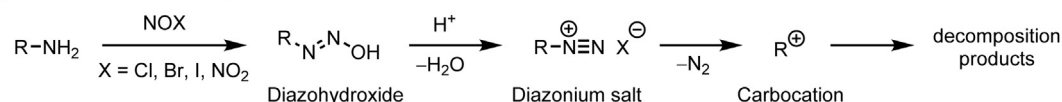
α -Tocopherol

The antioxidant capacity of α -tocopherol is responsible for preventing *N*-nitrosamine formation. Similar to ascorbic acid, α -tocopherol can reduce the nitrosating agent to a non-nitrosating compound, thus competing with susceptible amines for the nitrosating agent.³¹ While all tocopherols have a phenolic ring capable of reducing a nitrosating agent, the aromatic ring in α -tocopherol is fully substituted, therefore C-nitrosation is negligible.⁴⁴ As oxidation involves a radical formation at the hydroxyl group, only the non-esterified form of α -tocopherol can mitigate *N*-nitrosamine formation.⁴⁵ The

1) Redox Pathway



2) Diazotization of Primary Amines



3) Nitration of Phenolic Scavengers

e.g. Chlorogenic Acid

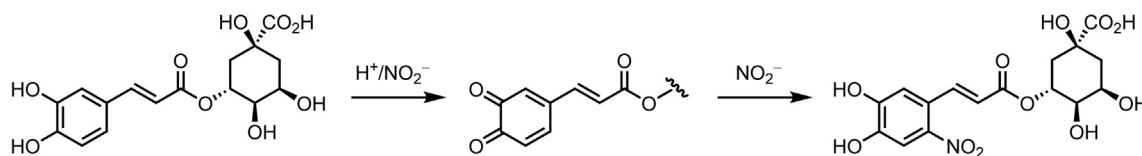


Figure 2. Proposed Mechanisms of the Main Scavenging Pathways of Nitrosating Agents.

Table 1
Nitrite Scavengers.

Substance	Oral forms on FDA Inactive Ingredient list	Fat/water soluble	Reference to inhibition <i>in vitro</i>	Reference to inhibition in tablets
α -Tocopherol	Capsule, solution, tablet, suspension	Fat soluble	44	12
Ascorbic acid	Capsule, powder, solution, suspension, syrup, tablet	Water soluble	32,42	11,12
L-cysteine	Capsule, suspension, tablet	Water soluble	47,48	11
Glycine	Capsule, powder, solution, suspension, tablet	Water soluble	11	-
Arginine	Tablet	Water soluble	11	-
Lysine	Only intravenous forms available	Water soluble	11	-
Histidine	Capsule, suspension	Water soluble	11	-
Caffeic acid	-	Water soluble	49	12
Ferulic acid	-	Water soluble	49	12
BHA	Capsule, granule, solution, suspension, syrup, tablet	Fat soluble	11	-
BHT	Capsule, film, solution, tablet	Fat soluble	11	-
PABA	-	Water soluble	11	11

reaction of α -tocopherol with a nitrosating agent produces α -tocopherol quinone.⁴⁵

α -Tocopherol is soluble in the lipid phase and its poor aqueous solubility favors applications in lipophilic media, although it has also been shown to be effective in aqueous environments.^{11,45} On the other hand, both the nitrosating agents nitric anhydride (N_2O_3) and dinitrogen tetroxide (N_2O_4) are lipophilic,⁴⁶ hence it is important to consider the lipophilicity of the active substance and the nitrosating agent(s) present.

Early experiments showed that the molar ratio between α -tocopherol and nitrite affects the inhibition of *N*-nitrosamine formation, which was demonstrated in aqueous solutions.^{44,45} The reaction rate was pH-dependent and was highest at a pH between 2 and 3. For example, when α -tocopherol was tested with nitrite in spray-dried powder form, the reaction proceeded quickly at pH 2–3, with less than 25% of the nitrite remaining after one hour under the test conditions. As the pH increased to 5, the reaction speed slowed and more than 95% of the initial nitrite remained after one hour. α -Tocopherol was able to reduce the nitrite concentration more quickly than related form γ -tocopherol. *N*-nitrosamine reduction was greatest when α -tocopherol was used in combination with ascorbic acid, in a lipid-rich food matrix (bacon).⁴⁵ Inhibition of *N*-nitrosamine formation in tablet form has been demonstrated.¹²

Amino Acids

Amino acids containing primary amines or thiol groups are considered to be nitrite scavengers. The primary mechanism is a diazotization reaction with the nitrosating agent to diazo intermediates that are unstable and rapidly fragment into deamination products (e.g. alcohols)¹⁶ and nitrogen via the Van Slyke reaction, which all amino acids except proline are capable of undergoing.^{47,48}

Several amino acids have demonstrated nitrite scavenging ability in pharmaceutical drug products. L-cysteine has been previously tested in solution and in a tobacco matrix, where it suppressed the formation of tobacco-specific *N*-nitrosamine *N*-nitrosonornicotine; no effect was seen from other amino acids alanine, proline, and serine that were tested in the same system.⁴⁸ Glycine, arginine, lysine, histidine and L-cysteine were tested recently in screening studies, both in solid state and solution.¹¹ The results differed according to the temperature used. In one experiment, there was a 40–50% (glycine and lysine) to 90% (histidine) reduction in *N*-nitrosamine conversion in solution, pH 3.0 and at 60°C.¹² However, in solution at room temperature and pH 3.0, glycine, arginine, histidine and lysine showed limited nitrite scavenging ability. On the other hand there was a rapid decrease in nitrite for L-cysteine under these ambient conditions and it was selected for testing in solid-state experiments, in which it was found to be an efficient scavenger, potentially indicating that the thiol group is amplifying the scavenging effect by amino acids.¹¹

Other Compounds

Other compounds also show potential for use as *N*-nitrosamine mitigation excipients. These often show antioxidative activity such as caffeic and ferulic acid,^{12,49} resveratrol,^{50,51} butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT),⁵² para-aminobenzoic acid (PABA),⁵³ maltol,⁴⁹ and propyl gallate,^{15,54} although they have been less widely studied than ascorbic acid and α -tocopherol. In a solid dosage form, caffeic and ferulic acid inhibited the formation of *N*-nitrosamine from the model amine 4-phenylpiperidine hydrochloride by 60% at 0.1% w/w and caffeic acid completely inhibited *N*-nitrosamine formation at 1.0% w/w.

The solubility of fat-soluble or poorly water-soluble nitrite scavengers such as BHA, BHT, propyl gallate and resveratrol makes them unsuitable for liquid applications. PABA was shown to be a modestly effective inhibitor of *N*-nitrosamine formation in solution and in oral tablets in one study,¹¹ while propyl gallate and maltol were ineffective in both forms.¹²

The Use of Nitrite Scavengers in Drug Products for Mitigation of *N*-nitrosamine Formation

General Considerations for the Use of Nitrite Scavengers in Solid Drug Forms

The use of nitrite scavengers that are currently listed as inactive ingredients in drug products by the FDA offers advantages from a regulatory perspective, and also in terms of the development time needed to establish *N*-nitrosamine mitigation in the drug products. In addition, excipients on this list are often available in a formulation amenable to use in solid drug forms.

The low mobility of reactants in solid-solid reactions means that the reaction rate is several orders of magnitude slower than for the same reactants in solution. Diffusion speed limits the rate of reaction. Incomplete contact between components due to, for example large crystalline particles or inhomogeneous distribution of reactants can further reduce the reaction rate. Higher reaction rates are generally seen with smaller particle sizes.^{55,56} Furthermore, the presence of high humidity and resulting water condensation can facilitate higher reaction rates due to higher mobility of one or more reactants.⁵⁷ Thus, control of process parameters such as particle size of dry components, mixing parameters, drug product packaging and storage conditions can affect the rate of the *N*-nitrosamine formation reaction.^{55,58} The better the inhibitor is distributed within the drug product, the better it can decompose any nitrite present in it.

Reformulation of existing drugs and the formulation of new pharmaceutical products needs to be carefully implemented to ensure that the product prevents the formation of *N*-nitrosamines effectively, and no unintended effects on product stability, efficacy or appearance arise. In addition, *N*-nitrosamine formation mitigation in the final product will need to be demonstrated. Bulking agents in

solid drug forms provide the highest contribution to total nitrite concentrations. Knowledge of the total potential amount of nitrites contributing to *N*-nitrosamine formation is needed for risk assessment and product lifecycle management,³³ and the dose of *N*-nitrosamine blockers should be based on the expected nitrite content in the excipients.

It is important for pharmaceutical companies to check the compatibility of nitrite scavengers with the active ingredient and other excipients for new drug product development or during the reformulation process. Some nitrite scavengers such as ascorbic acid can react with the active ingredients or excipients.⁵⁹ Miniaturized, high-throughput approaches involving factorial design may be of assistance for the rational selection of excipients that inhibit *N*-nitrosamine formation and are compatible with the formulation.⁶⁰

While antioxidants generally improve the stability of drugs sensitive to oxidation, the stability of the reformulated product will need to be confirmed. In addition, it is possible that regulators may require confirmation of API bioavailability in the reformulated product. Although biowaivers based on comparative dissolution data may be applicable for many drug products, additional studies may be needed to confirm the bioavailability of reformulated products.

Dosage rates of nitrite scavengers in the final product are likely to be low, in the range of a few milligrams per tablet for solid forms. Therefore, homogeneity of the compound(s) in the final product is important. The exact amounts required for *N*-nitrosamine mitigation will vary depending on the product and its current formulation and must be determined on a case-by-case basis. In cases when larger amounts are required, the overall manufacturing process may need adaptation.

The Use of Ascorbic Acid and α -Tocopherol in Solid Drug Products

The inhibition of *N*-nitrosamine formation by ascorbic acid and α -tocopherol in oral solid dosage forms was recently tested.^{11,12} In the experiment conducted by Nanda and co-workers, 100 mg tablets were made with 10% w/w of the model amine 4-phenylpiperidine hydrochloride. Tablets were made following standard formulations using an excipient known to contain nitrite impurities. The tablets were spiked with inhibitors ascorbic acid, sodium ascorbate, α -tocopherol, caffeic acid or ferulic acid at 0.1% and 1.0% w/w. The tablets were stored under accelerated *N*-nitrosamine formation conditions (50 °C and 75% relative humidity) for one month. Ascorbic acid and α -tocopherol showed strong inhibition of *N*-nitrosamine formation at the 1% w/w dosing level.¹² The results demonstrate an appropriate method for drug product manufacturers to show mitigation of the formation of *N*-nitrosamine via the redox pathway.¹²

A second experiment tested the effectiveness of ascorbic acid and other nitrite scavengers on *N*-nitrosamine formation in tablets and in solution.¹¹ A compressed tablet formulation containing 0.82% w/w of different scavengers including ascorbic acid and sodium ascorbate was tested against two different nitrosatable model amines, *N*-methylaniline (MA) and *N*-phenylpiperazine (PP). The filler microcrystalline cellulose was spiked with sodium nitrite to a 2 ppm concentration, and compared to non-spiked tablets. After stress testing at 50°C and 75% relative humidity for 28 days under atmospheric conditions, the formation of *N*-methyl-*N*-nitrosoaniline (NMA) from *N*-methylaniline and *N*-nitroso-*N'*-phenylpiperazine (NPP) from *N*-phenylpiperazine was measured. All NMA formed from MA was degraded or lost to evaporation. For PP, ascorbic acid completely inhibited NPP formation in the non-spiked tablets and halved *N*-nitrosamine formation in the spiked tablets after stress testing.¹¹

Degradation of Ascorbic Acid

Ascorbic acid is generally stable in an acidic environment but will oxidize to dehydroascorbic acid under weak acidic, neutral or basic

conditions, and in the presence of oxygen and moisture. Dehydroascorbic acid will be further oxidized to diketogulonic acid irreversibly, dependent on temperature, oxygen and moisture.⁶¹

The type of primary and secondary packaging will affect degradation of ascorbic acid and thus its ability to mitigate *N*-nitrosamine formation during storage and after the primary packaging has been opened. Water-insoluble polymers such as ethyl cellulose are widely used in controlled-release tablets usually as a tablet coating and can have the added advantage of protecting the active substance from moisture. For other formulations where it is not advisable to apply a coating, oxygen scavenging packaging may be appropriate.

Inhibition of Non-Enzymatic Browning Caused by Ascorbic Acid

Ascorbic acid is structurally a reducing sugar. The decomposition of ascorbic acid can result in discoloration and off-flavors in drug products via non-enzymatic browning (Maillard reaction). Ascorbic acid can be unstable under typical storage conditions for drug products, such as at room temperature with atmospheric oxygen and at a neutral or basic pH.⁶² Discoloration also occurs due to protein ascorbylation, which is the covalent binding of ascorbic acid or its degradation products to proteins.⁶³ Drug product manufacturers might need to take steps to avoid the formation of decomposition products when adding ascorbic acid to existing product formulations.

Slowing or inhibition of the Maillard reaction can be achieved through five main mitigation methods that might be appropriate in drug products: keep at low temperatures, add sulfur dioxide (as such, or in the form of a salt), adjust the moisture content, lower the pH, and reduce or minimize amines/amino acids and carbonyl compounds such as reducing sugars.⁶⁴

Regulatory Aspects of the Use of Ascorbic Acid and α -Tocopherol in Drug Products

Due to the complex, global nature of the pharmaceutical manufacturing and supply chain, and widespread potential for *N*-nitrosamine contamination, the FDA, EMA, European Directorate for the Quality of Medicines and Healthcare (EDQM), Health Canada, Therapeutic Goods Administration (Australia), Ministry of Health, Labour and Welfare/PMDA (Japan), Health Sciences Authority (Singapore), Swissmedic (Switzerland), and regulatory authorities of other countries have been cooperating to mitigate *N*-nitrosamines in medicinal products.^{8,65}

Europe

After *N*-nitrosamine contaminants were found in several medicinal products, the executive director of the European Medicines Agency requested a review on *N*-nitrosamine impurities in chemically synthesized APIs in September 2019, and this was extended to cover all human medicinal products in 2020. The responsibility for the risk assessment of drug products lies with the manufacturer or Marketing authorisation Holders (MAHs).⁷ A three-step approach was set out for MAHs to mitigate *N*-nitrosamine risks in drug products. In the first step, the risk of *N*-nitrosamine contamination in APIs and Finished Products is assessed. If a risk is identified, the product must be tested to confirm or refute the presence of *N*-nitrosamines and the outcome should be reported as soon as possible. As a third step, effective risk mitigation measures need to be undertaken and the results of confirmatory tests submitted to the Regulatory Authorities. Thus, if *N*-nitrosamine formation is a possibility in a drug product and is identified in confirmatory testing, the effect of changes to the formulation such as the addition of nitrite scavengers to block *N*-nitrosamine formation will need to be shown, usually by testing the final product.

USA

The FDA released a Guidance for Industry document in September 2020 in response to unexpected finding of *N*-nitrosamine impurities of a number of widely used pharmaceutical products.⁸ The background and possible mitigation strategies were outlined in the report. The updated guidance matches the EMA's three-step procedures and includes dates for drug manufacturers to complete each step for approved and pending drug applications. In addition, a document listing possible *N*-nitrosamine mitigation strategies specifically mentions ascorbic acid and α -tocopherol as common antioxidants that significantly inhibit *N*-nitrosamines in drug products.²⁰

Further Research

Due to concerns about the health effects of *N*-nitrosamines from the diet and environment, there has been considerable research conducted in aqueous solutions, simulated gastric conditions and various food matrices. However, there is a lack of studies conducted into drug product formulations. In particular, the effects of nitrite scavengers on the formation of *N*-nitrosamines in general during storage has not been adequately addressed in the current literature. The effect of factors such as storage time, pH, temperature, humidity and the presence of oxygen are likely to affect the rate of *N*-nitrosamine formation and need to be examined in more detail to assist in the formulation and reformulation of drug products to keep *N*-nitrosamine amounts within established ranges. Assessment of the physical form (e.g. crystalline or amorphous) of the vulnerable amine is an important factor to characterize. As in any other solid-state reaction, the mobility or availability of the reactant is important to the rate of reaction and thus the degree of nitrosation risk. The study of *N*-nitrosamine content during different steps of processing will help isolate the dominant step contributing to *N*-nitrosamine formation risk and the associated physicochemical properties of the drug product driving conditions for *N*-nitrosamine formation. In addition, the formation of many types of *N*-nitrosamines is possible in drug products, however a comprehensive study of the effect of nitrite scavengers on the formation of a wide range of *N*-nitrosamines is lacking.

Conclusions

A number of compounds have potential as nitrite scavengers in drug products manufacturing to limit *N*-nitrosamine formation, including some amino acids, antioxidative molecules and vitamins. Ascorbic acid, α -tocopherol and L-cysteine have been shown to block *N*-nitrosamine formation in solution and in solid drug forms.^{11,12,20,29,31} Approved inactive substances in drug products offer a potential option for drug product manufacturers to explore with respect to new *N*-nitrosamine mitigation regulations. Considerations of dose, potential reactivity with the API or excipients in combination with stability testing is advisable, thus anticipating regulatory obligations and ensuring a safe and effective (re)formulated drug product.

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Submission Declaration

This work has not been published previously, it is not under consideration for publication elsewhere, it has been approved by all authors and has been tacitly or explicitly approved by the responsible authorities where the work was carried out.

Declaration of Interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A.-C.V.B, Z.M., R.T.S., M.W., M.F. and A.B. are employees of DSM Nutritional Products Ltd., Kaiseraugst, Switzerland. J.K.B is a consultant for and former employee of DSM Nutritional Products Ltd. Kaiseraugst, Switzerland.

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