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Review

Formation of *N*-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies

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ABSTRACT: The detection of *N*-nitrosamine impurities in medicines and the recent emergence of nitrosamine drug substance related impurities (NDSRIs) has posed a great challenge to manufacturers of drug products and regulators alike. NDSRIs are primarily associated with reactions occurring in the drug product which brings particular complexity. This paper will explore the current technical knowledge surrounding the formation of these impurities, including the risk factors, reaction conditions, and potential mitigation strategies. Scientific understanding of these areas is still evolving, and we will highlight both the scientific progress made and discuss the significant gaps in mechanistic knowledge still remaining. These gaps render accurate predictions of NDSRI formation extremely challenging. The pharmaceutical industry should continue to work on potential mitigation strategies and generation of additional scientific data to address the mechanistic gaps. Regulatory guidance and policy will continue to advance and adapt in response to further changes in scientific understanding.

KEYWORDS: *N-nitrosamine, mutagenic impurities, nitrosamine drug substance related impurities (NDSRIs)*

1. INTRODUCTION

N-Nitrosamines are a class of organic impurities that includes highly potent mutagenic substances which are classified as probable human carcinogens.[1] Isolated reports of *N*-nitrosamine impurities in medicines were documented in the past,[2] however reports on the detection of *N*-nitrosodimethylamine (NDMA) in valsartan drug substance from one manufacturer in June 2018 led to a series of regulatory actions including market recalls and an Article 31 of Directive 2001/83/EC referral procedure in the EU.[3] That scientific review was subsequently extended to other drug substances in the "sartan" class on the basis of similar structural features and synthetic processes which implied a similar risk of generating *N*-nitrosamine impurities. In addition to NDMA, *N*-nitrosodiethylamine (NDEA) was subsequently discovered in some sartan medicines. The outcome of that scientific review was the requirement for marketing authorization holders (MAHs) of sartan medicines to apply strict limits for NDMA and NDEA in their release specifications.

In September 2019, a second Article 31 referral into the safety of medicines containing ranitidine began in the EU, following reports of the presence of NDMA above acceptable levels in ranitidine tablets.[4] The referral concluded that ranitidine medicines, used to treat heartburn and stomach ulcers, should be suspended until MAHs are able to demonstrate that the levels of NDMA can be controlled to acceptable levels throughout the assigned shelf life and that no further NDMA formation occurs in the body following ingestion.[5] The root cause of NDMA formation in ranitidine was subsequently reported to be the result of an intermolecular degradation of ranitidine without the requirement for any exogenous nitrite.[6]

In light of these reports and given the potential applicability of the identified root causes to other medicines, it was decided to conduct a more holistic review of *N*-nitrosamine impurities in medicines. The EU medicines regulatory authorities therefore conducted a scientific assessment (Article 5(3) opinion[7]) of *N*-nitrosamine impurities and considered the following aspects:

- Root causes for the formation of *N*-nitrosamines and measures to mitigate them.
- Methodology for defining acceptable limits for daily intake (AIs).
- Requirements for analytical methods to detect and quantify *N*-nitrosamine impurities.
- Risk for patients exposed to medicines containing *N*-nitrosamine impurities.
- The need for MAHs to evaluate the risk of presence of *N*-nitrosamines in the medicines and the extent of products in scope.
- The need for further nonclinical and clinical studies.

The assessment arrived at a range of conclusions in terms of known and suspected root causes, analytical method requirements, methodology for setting limits, and the need for further epidemiological studies. Up until that time, the majority of *N*-nitrosamine impurities in medicines had originated in the active

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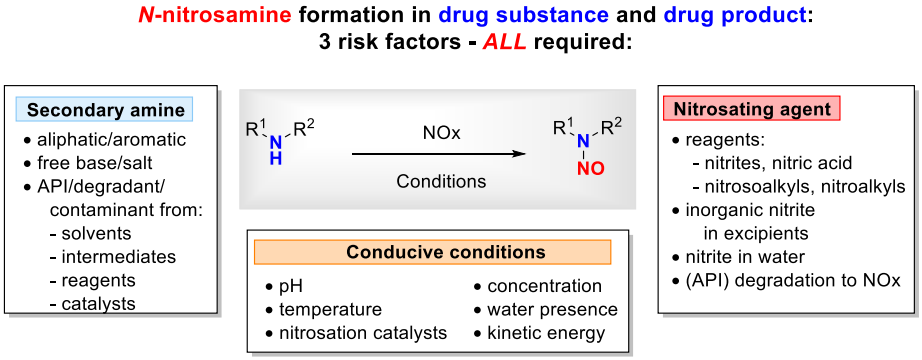
Scheme 1. Conditions for *N*-Nitrosamine Formation in Drug Substance and Drug Product



substance manufacturing process.[8] As a result, the conclusions focused on how to manage highly potent small molecule *N*-nitrosamine impurities with published limits based on well-established toxicity data such as NDMA and NDEA. Nonetheless, the possibility of *N*-nitrosamine formation in drug products was highlighted based on the potential presence of nitrosating agents in excipients, water, and packaging components.

This led to a series of recommendations for dealing with *N*-nitrosamine impurities in medicines including:

- The need to mitigate the presence of *N*-nitrosamine impurities as much as possible.
- The need to establish appropriate control strategies for both active substances and drug products.
- The need for MAHs to conduct risk evaluations for the presence of *N*-nitrosamines in their products and to perform confirmatory testing if a risk is identified.
- The need to set specification limits for detected *N*-nitrosamines in the drug product by default, in line with published limits.
- Considerations for analytical method development and sensitivity requirements.
- Control options when more than one *N*-nitrosamine is present in the same product.
- Methodology for setting limits for new *N*-nitrosamines with insufficient toxicity data including the establishment of a class-specific limit of 18 ng/day.
- Exceptions for medicines indicated for treatment of advanced cancer or where the active substance itself is genotoxic at therapeutic concentrations.
- Exceptional and temporary use of higher limits for products containing *N*-nitrosamine impurities above the acceptable intake but which still have a positive benefit/risk ratio.
- The need for further epidemiological studies.

This paper focuses on the scientific understanding related to risk factors for *N*-nitrosamine formation in medicines, including developments since the conclusion of the Article 5(3) opinion,[7] and highlights areas where further scientific investigations are required.

2. RISK FACTORS FOR *N*-NITROSAMINE FORMATION

In the most common pathway to formation of *N*-nitrosamines, three factors are required (Scheme 1):

1. Presence of a nitrosatable amine.
2. Presence of a nitrosating agent.
3. Conditions conducive to *N*-nitrosamine formation.

Removing one of these factors is sufficient to mitigate the risk of *N*-nitrosamine formation.

2.1. Mitigating the Risk in Active Substance Manufacturing Processes. There are manifold ways to accomplish this in active substance manufacturing processes depending on the source of each factor. For example, if the amine source is from a solvent (e.g., DMF, DMA, NMP), or an amine base (e.g., Et_3N, $^iPr_2NEt$), then switching to nonamine-containing solvents and/or bases removes the amine source.[9]

In the majority of cases to date, formation of *N*-nitrosamine impurities in the drug substance was due to the use of stoichiometric amounts of a nitrosating reagent in the same synthetic step as an amine source, or in steps proximal to an amine source. Using alternative reagents to accomplish the same transformation can often be envisaged. Alternatively, changing the order of synthetic steps such that nitrosating agents are used early in a process could afford more opportunity for purge of these impurities via standard purification operations should they form.[10] Since some of the *N*-nitrosamines are amphiphilic in nature, and thus, soluble to an extent in both aqueous and organic media,[11] batch data from purge studies are currently required by EU regulators to demonstrate adequate removal of a *N*-nitrosamine impurity and thus, application of the control options in ICH M7.[12]

Sodium nitrite is sometimes used as a quenching agent to remove other reactive species, e.g., azides. Conducting such operations following separation of the reactive species from the active substance or precursor intermediate is a further strategy to avoid entraining nitrosating agents or *N*-nitrosamines in the drug substance.[9]

Finally, the conditions under which a synthetic transformation is conducted could be amended to make them less favorable to *N*-nitrosamine formation. We note here that in general, mildly acidic conditions increase nitrosation rate in aqueous media.[13,14]

Overall, there are potentially multiple effective approaches to avoiding *N*-nitrosamine formation or removing generated nitrosamine impurities in the drug substance manufacturing processes, provided that the risks are considered during development and appropriately mitigated.

2.2. Risk Factors in the Drug Product. Since 2020, there has been an increased number of reports of more structurally complex *N*-nitrosamines related to the structure of the active substance itself in several drug products. These are often referred to as "nitrosamine drug substance related impurities" (NDRSIs) and are generally formed by nitrosation of an amine moiety present in the active substance. To date, secondary amine functional groups seem most vulnerable to form corresponding

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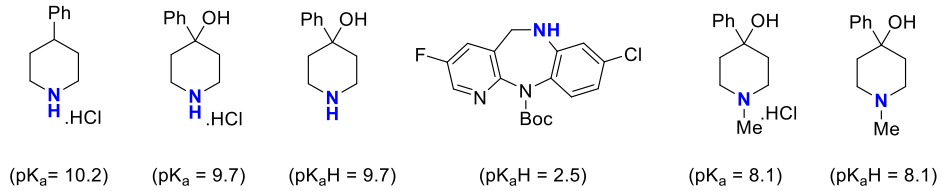
Scheme 2. Model Amines Used in the Study of NDSRI Generation during Drug Product Manufacturing[25]



(pK_a= 10.2) (pK_a = 9.7) (pK_aH = 9.7) (pK_aH = 2.5) (pK_a = 8.1) (pK_aH = 8.1)

N-nitrosamines. However, presumed dealkylative nitrosation of tertiary amines, or nitrosation of secondary amine impurities or degradants (e.g., by hydrolysis of tertiary amides) has also been observed. Investigations by the pharmaceutical industry have shown that these NDSRIs form predominantly by a reaction with trace nitrites present in excipients, but a complex picture is emerging with often multifactorial contributing factors. Increase of NDSRIs during the shelf life of the drug product has also been observed.[15−19]

Mitigating the formation of NDSRIs in drug products is challenging for a number of reasons and the options to address the problem are more limited than in the case of drug substance manufacturing:

- Since the vulnerable amine is an intrinsic (and critically important) part of the active substance, risks associated with the presence of such structural motifs are by definition impossible to address.
- The exact nature, concentration and origin of the nitrosating species during drug product manufacturing is not clear in all instances.
- The mechanisms and conditions for NDSRI generation are comparatively poorly understood.
- There is limited scope for amending manufacturing process operations without impacting the product; there is generally little conclusively known about the impact of drug product process amendments (e.g., adjusting parameters such as temperature, humidity, mixing time, etc.) on *N*-nitrosamine formation.
- The NDSRIs cannot be removed by purifying the drug product.

Finally, the risk management of NDSRIs is severely complicated by the lack of robust toxicological data on complex *N*-nitrosamines. This situation contrasts the control of *N*-nitrosamines from drug substance manufacturing processes which have generally been small molecules with simple structures (such as NDMA), with robust toxicological data from which to derive a limit. While the toxicological aspects of NDSRIs are outside the scope of this review, some *N*-nitrosamines with relatively complex structures have been shown to be mutagenic and genotoxic in a range of studies.[20] Other complex *N*-nitrosamines are considered to be highly potent mutagens/carcinogens based on (Q)SAR evaluation and read across,[21] whereas other are argued to be nonmutagenic.[22] Setting limits for NDSRIs in the absence of robust scientific information remains a tremendous challenge.[21]

3. VULNERABLE AMINES IN THE DRUG SUBSTANCE: RELEVANCE TO NDSRIS

The drug substance structural features that pose a high risk for the generation of *N*-nitrosamines during drug substance production are well-known and have been amply discussed before.[13,15,23,24] In contrast, considerably less is reported for NDSRI generation during drug product manufacture. A recent study by Moser et al. addressed these scientific gaps to a large extent, albeit the investigation focused on model compounds (Scheme 2) and formulations rather than actual drug products.[25] The main conclusions were that the trends observed in solution hold for solid-phase chemistry:

1. Aromatic (i.e., less basic) secondary amines (ArAlkNH) undergo faster nitrosation than aliphatic derivatives (Alk^1Alk^2NH).
2. Tertiary amines pose a significantly lower risk (generally 2 orders or magnitude less reactive) than secondary amines (with the exception of structures containing two small, aliphatic substituents on the amine—like R-NMe_2—which can still be problematic, particularly when R bears structural features that promote fragmentation of the R−N bond, e.g., an adjacent electron-rich heteroaromatic substituent).[6,26,27]
3. Protonated amines ($R^1R^2NH_2^+$) are significantly more reactive than amine free bases.

While the risks in (1) and (2) are intrinsically linked to the molecular structure of the drug substance, the risk in (3) could in principle mitigated by an alternative formulation. Indeed, according to studies in solution, the nitrosation of free bases with inorganic nitrite proceeds much slower compared to the corresponding protonated amines (since acidic conditions are generally required to generate an active nitrosating species, e.g., N_2O_3, from NO_2^-).[14] Furthermore, the reduced water solubility of the neutral molecule would be expected to diminish risks of nitrosation during storage of solid dosage forms produced by wet unit operations (see also discussion in Section 5). In addition, halide-mediated nitrosative pathways (e.g., via reactive nitrosyl halides), which can be operative in products with halides as counterions, would no longer be accessible.[14] On the other hand, the crystallinity of amine free bases is generally lower than that of their corresponding salts, which generally improves availability for solid state reactions. Accordingly, examples of complex *N*-nitrosamine generation in free base drug substances[28] or model amine free bases[25] have been reported in the literature.

4. EXCIPIENTS AS RISK FACTORS IN THE GENERATION OF NDSRIS

4.1. Vulnerable Amines in Excipients. Although it is a relatively infrequent situation, some excipients may introduce nitrosatable amines in the mix: e.g., L-proline, meglumine, EDTA, triethanolamine; in the latter case, the excipient is actually known to contain a nitrosamine impurity, *N*-nitrosodiethanolamine (NDELA), for which the European Pharmacopeia established the limit of 24 ppb in the trolamine monograph;[29] the EU regulatory authorities recently published an AI for NDELA of 1900 ng/day, which allows for a better control of this impurity, independent of the formulation composition or the MDD (maximum daily dose) of the medicine in question.[21] We take the opportunity here to remark

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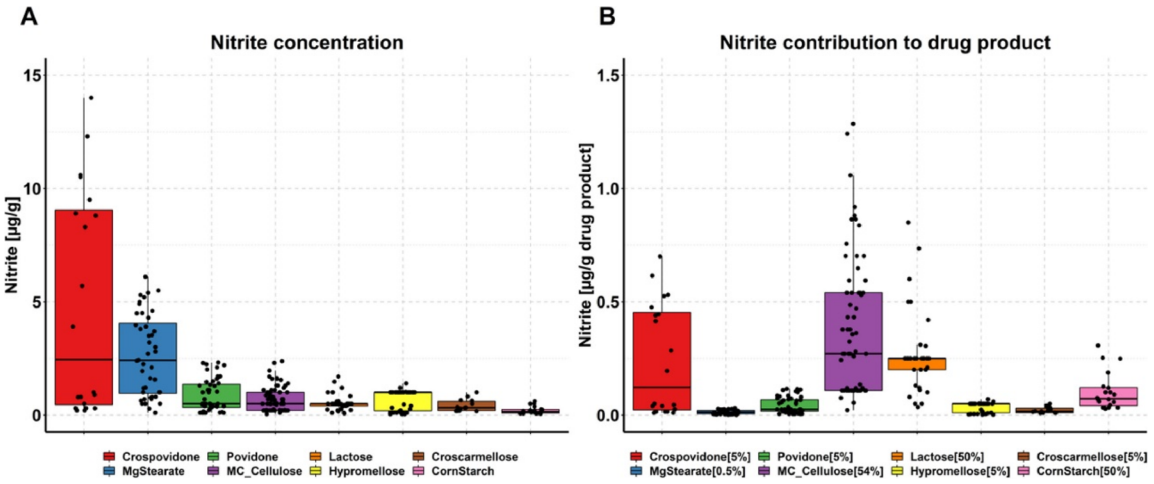

Figure 1. Nitrite content in pharmaceutical excipients. (A) Nitrite concentration by excipient. (B) Estimated nitrite contribution based on a typical maximum excipient loading. Reprinted with permission from ref 35 (Boetzel, R.; Schlingemann, J.; Hickert, S.; Korn, C.; Kocks, G.; Luck, B.; Blom, G.; Harrison, M.; François, M.; Allain, L.; Wu, Y.; Bousraf, Y. A Nitrite Excipient Database: A Useful Tool to Support *N*-Nitrosamine Risk Assessments for Drug Products. *J. Pharm. Sci.* **2022**. DOI: 10.1016/j.xphs.2022.04.016). Copyright (2022) Elsevier (article under Creative Commons Attribution-NonCommercial-No Derivatives License, https://creativecommons.org/licenses/by-nc-nd/4.0).

that, generally, ionizable or highly polar nitrosamines such as NDELA are less carcinogenic than apolar analogues (c.f. NDEA has an AI of 26.5 ng/day), likely due to their higher aqueous solubility and hence excretion rate.[30]

In cases of excipients containing nitrosatable amines, the risk assessment is rather straightforward, benefiting from the lessons learned from the initial wave of drug substance contamination with *N*-nitrosamines (and the impurity can be accordingly controlled during the manufacture of the excipient, in solution phase).[9,15,24]

4.2. Nitrosating Agents in Excipients. Most commonly, the apparent culprit in generating *N*-nitrosamines during drug product manufacturing is inorganic nitrite found as a trace impurity in excipients, and it is this specific situation that deserves the greatest attention. Understanding the presence of nitrite traces (typically ppm levels) in excipients is a complicated matter. Since the importance of the problem has only recently been acknowledged, there is little analytical data available. The excipient manufacturing industry is diverse, which translates into large variance between manufacturers.[31] In addition, relatively large batch to batch variability from individual manufacturers is often observed.[31] Notably, there are currently no regulatory requirements with respect to limiting the presence of nitrite in excipients as this was not considered to have an impact on pharmaceutical quality until recently.

Prior to the sartan cases, to the best of our knowledge, there was only one report describing drug interactions with nitrosating contaminants in excipients.[32] In the past couple of years further research has been conducted in this area. For instance, in terms of analytical developments, tools to detect very low levels (ppb) of nitrite have become available.[33] A nitrite excipient database[34] has been created by an organization called Lhasa using input from a consortium of pharmaceutical companies, and its population and utilization[35] is steadily increasing (92 excipients covered in the most recent version to date, 2023.1.0). Cooperation between some drug product manufacturers, marketing authorization holders and excipient suppliers has allowed for a better understanding of the nitrite content of various excipients.[31]

Thus, while there are still many unknowns, the situation in 2023 is much improved, particularly with the advent of the nitrite database. For example, the database allows for the identification of the most relevant pharmaceutical excipients; while there is an overwhelmingly large diversity in the excipients industry, with many different categories of excipients (each with a large number of representatives, generally very heterogeneous in structure), the number of the most commonly encountered excipients in drug product manufacturing (particularly in common solid oral dosage forms such as tablets and capsules) is only about 15−20. In terms of focal areas this allows for a significant simplification. Excipients that serve as diluents/fillers are generally the most important contributors of nitrite in solid dosage forms since they often constitute the largest component of a drug product; in fact, according to Boetzel et al., they are nearly invariably *the only* relevant contributors (Figure 1).[35] This observation was experimentally validated in a metformin case-study: at similar nitrite concentration in excipient, an HPMC-based formulation (ca. 25 wt % HPMC) generated NDMA, while HPMC-free tablets including magnesium stearate (at levels <1 wt %) did not.[36] This finding is another major simplification and a valuable lead in the development of effective mitigation strategies for the prevention of NDSRI formation.

Another important lesson provided by the Lhasa database is that there is little uniformity between the nitrite levels/ranges across different excipients, manufacturers, and even between batches. Typically the range spans more than 1 order of magnitude, and even two in the case of, e.g., hypromellose.[35] It is important to note that the majority of reported nitrite levels in excipients are, with only a few exceptions (e.g., crospovidone and magnesium stearate), not more than 1−2 ppm. Moreover, the average nitrite content in a typical solid dosage form, calculated based on usual ranges of excipients according to their function, was also found to be around the value of 1 ppm. Interestingly (and fortunately), excipients that typically feature higher concentrations of nitrite, are generally present in low percentages in the formulation (e.g., antiadherents, disintegrants, lubricants, preservatives, etc.), thus having a lower contribution to the total nitrite content; fillers and diluents typically showed relatively low nitrite levels (Figure 1).[35] One

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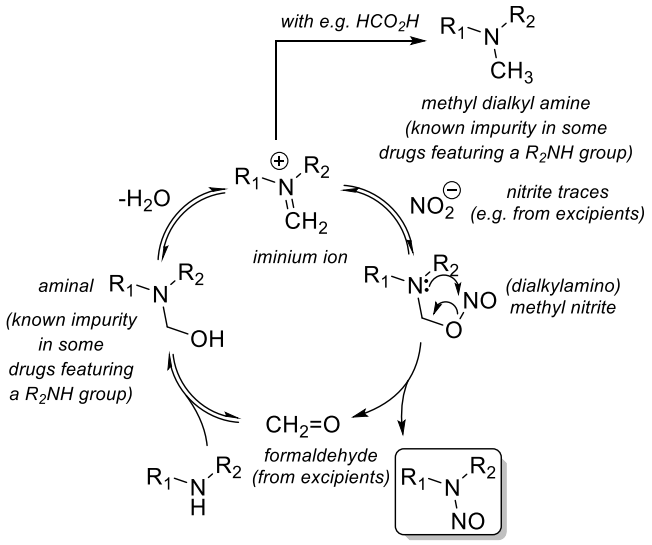
potential solution to this issue would be to set pharmacopeial limits for nitrite in excipients. However, given the dependence on the formulation type, composition, manufacturing process and drug substance structure, applying such general requirements for excipient purity would not solve the problem in all cases and would be disproportionate in others (especially if no vulnerable amine was part of the drug substance structure). A more rational approach would be to understand the root causes for a particular drug product and set nitrite limits in particular excipients on a case-by-case basis if demonstrated to be effective.

4.3. The Role of Other Impurities. *Nitrate.* While the exact origin of nitrites in excipients is still unclear, a plausible source could be the presence of higher oxidation state nitrogenous precursors such as nitrates. Indeed, according to the report by Wu et al., nitrates are also invariably present in excipients; generally in higher concentrations than nitrite, often by more than a factor of 2 (e.g., in microcrystalline cellulose, lactose, pregelatinized starch, povidone, crospovidone, magnesium stearate, stearic acid, hydroxypropyl cellulose, and silicon dioxide) and up to 10 times higher in croscarmellose sodium (CMC-Na).[32] Similarly, another recent report disclosed nitrate levels in HPMC, magnesium stearate and CMC-Na to be in the tens of ppm range;[36] data provided by the Lhasa nitrite excipient database also supports this general conclusion.[35] While in theory nitrate could undergo reduction to nitrite under certain conditions, there seems to be no correlation between the levels of these two species and most likely the role of nitrate as precursor to *N*-nitrosamines is not significant.[35]

Aldehydes. While the presence of nitrite in excipients is certainly the most critical risk factor in the formation of NDSRIs during formulation, other impurities may also play a role in certain circumstances. For instance, next to the well acknowledged mechanism of nitrosation of amines under acidic conditions, it is also possible to form *N*-nitrosamines at pH > 6, under the action of certain catalysts like formaldehyde (Scheme 3).[37−39] Formaldehyde is a common impurity in many excipients, particularly polymeric materials like polyethylene glycols and polysorbates. It was also detected at levels around 10 ppm or higher in other excipients such as pregelatinized starch, crospovidone and hydroxypropyl cellulose.[32] Other carbonyl derivatives are also known impurities in excipients, for instance acetaldehyde[40] and sugar-derived heteroaromatic derivatives furfural and 5-hydroxymethyl furfural.[32] Formaldehyde is however the structure of highest concern, since it is a highly active catalyst for nitrosation due to a combination of favorable electronic and steric properties. In addition, it is often present in excipients in higher amounts than other carbonyl derivatives such as furfural. Moreover, the presence of formaldehyde can be the result of degradation reactions of certain structural motifs present in excipients (e.g., $-OCH_2CH_2O-$) and is therefore intrinsically difficult to control.[32]

Scheme 3. Nitrosation of Amines under the Catalytic Action of Aldehydes



It is not yet clear whether the contamination of excipients with formaldehyde is a real risk factor in the generation of *N*-nitrosamines during drug product manufacture although at the moment, there is no robust scientific data to exclude this possibility. Formaldehyde in excipients (typically at 5−10 ppm levels) is well-known to interact with secondary amines, producing iminium ion precursors (aminals) or products of subsequent reactions thereof (e.g., methyl amines by reduction,[41] see Scheme 3).[32] Moreover, a 2022 report by Harmon suggested that there is a link between several recent drug product recalls (e.g., nizatidine, propranolol, and metformin) and the presence of formaldehyde in excipients in solid dosage forms.[28] According to this author, NDMA in nizatidine was possibly the result of the degradation of the drug substance by peroxides into dimethylamine and nitrite, similarly to what had been previously proposed for the structurally related active substance, ranitidine.[26] Since nizatidine was formulated as free base and acidic excipients were absent, Harmon concluded that a nitrosation under neutral/basic conditions had to be operative. Indeed, formaldehyde-containing excipients had been used in the formulation (povidone); alternatively, the nitrosation catalyst could be an aldehyde drug degradation product, formed concomitantly with the release of DMA (Scheme 4 and following section on peroxides). It is important to note here that generally aldehyde impurities/degradants are invariably accompanied by the corresponding carboxylic acids (e.g., formaldehyde and formic acid)[42] which may also play a role in catalyzing NDSRI formation (i.e., by lowering the local pH which favors the generation of NOx reagents).

In the case of propranolol and metformin, drugs formulated as hydrochloride salts, where the acid-mediated nitrosation is in principle viable, Harmon observed a link between voluntary recalls of some extended-release batches by manufacturers and the presence of HPMC as excipient in those formulations. HPMC is known to contain relatively high levels of formaldehyde. As Harmon notes, there were no known voluntary recalls of batches with alternative formulations not containing HPMC (e.g., immediate-release tablets based on microcrystalline cellulose), despite the presumably similar concentration of nitrite in excipients, serving thus as negative control for the hypothesis of classical, acidic nitrosation. Formaldehyde involvement in the formation of NDSRIs thus remains a possible root cause.

While the catalytic role of formaldehyde in the formation of *N*-nitrosamines in solution is well understood and the knowledge can be directly translated to drug substance manufacturing and solution formulations, additional experimental investigations are needed to understand the potential risk in solid drug formulations. The potential impact of aldehyde-catalyzed amine nitrosation at pH > 6 is high and the topic deserves careful evaluation, particularly in the context of the development of novel formulations (e.g., drug substance

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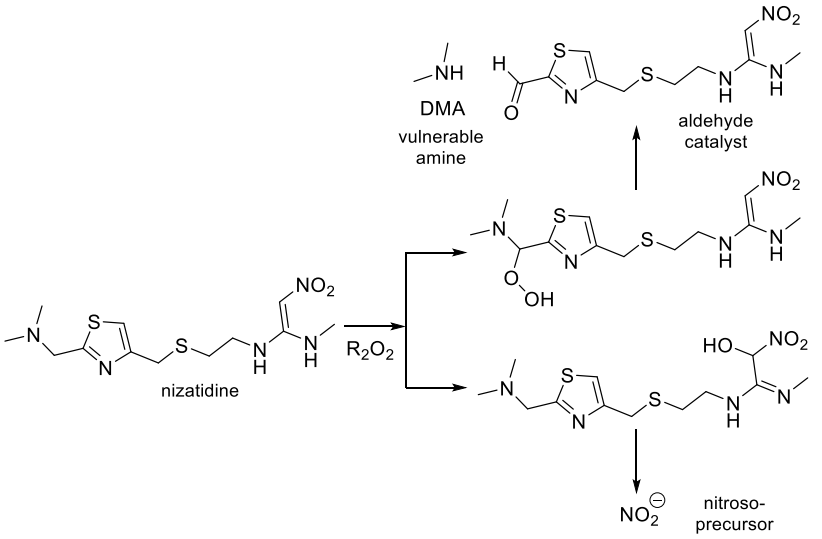
Scheme 4. Proposed Intermediates and Potential Catalysts for the Formation of NDMA from Nizatidine



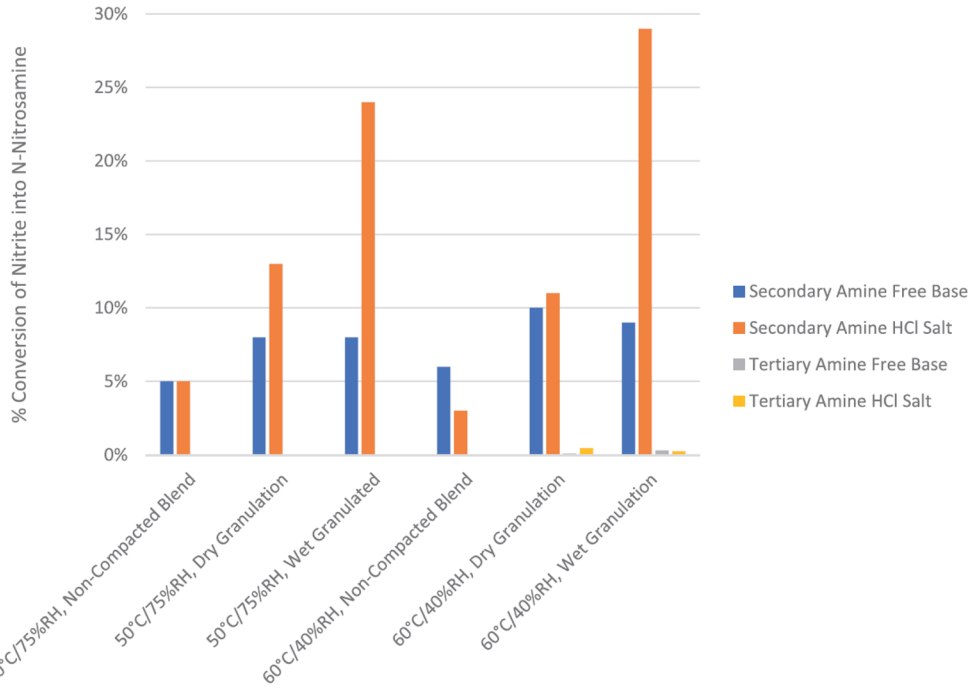


Figure 2. Comparison of unit operations during tableting of drug substance model compounds. Reprinted with permission from ref 25 (Moser, J.; Ashworth, I. W.; Harris, L.; Hillier, M. C.; Nanda, K. K.; Scrivens, G. *N*-Nitrosamine Formation in Pharmaceutical Solid Drug Products: Experimental Observations. *J. Pharm. Sci.* **2023**. DOI: 10.1016/j.xphs.2023.01.027). Copyright (2023) Elsevier.

as free base instead of salt, Na_2CO_3 as additive) to mitigate the formation of NDSRIs via the traditional acid-mediated pathways (see Section 6).

Peroxides. Another class of commonly encountered excipient impurities with relevance to the formation of NDSRIs are peroxides. Peroxides are particularly prevalent in polymeric excipients synthesized by radical processes (e.g., povidone and crospovidone, ca. 30−70 ppm; they are also present in, e.g., hydroxypropyl cellulose, ca. 10 ppm[32]). Peroxides could be involved in the generation nitrosating agents and/or vulnerable amines and/or acidic degradants that catalyze amine nitrosation. In certain situations, peroxides may directly lead to the *N*-nitrosamine impurity. In addition, as discussed previously, peroxides may also favor *N*-nitrosation at atypical pH values (i.e., 6−10) by producing catalytically active aldehydes.

Peroxide was recently shown to lead to the formation of NDMA in metformin film coated tablets in the absence of an explicit nitrosating agent, in a model study by Dousa et al.[43] The mechanism consists of a multistep oxidation of dimethyl amine to hydroxyl amine, which condenses with another molecule of DMA to form 1,1-dimethyl hydrazine and ultimately NDMA by oxidation. However, this pathway seems to be minor, not producing more than a tenth of the total NDMA levels observed. On the other hand, peroxyl-mediated oxidation has been linked to the release of nitrite from drugs bearing the 2-nitroethene-1,1-diamino group (e.g., ranitidine, nizatidine), as discussed above, and represents a significant risk factor in these cases.[26] In

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Table 1. List of Reported Nitrite Scavengers: Structure, Safety, and Mechanism of Action[a]

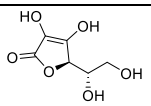
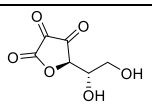
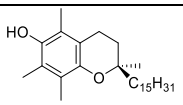
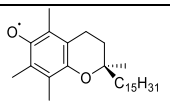
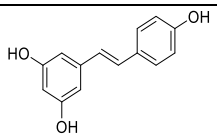
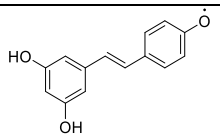
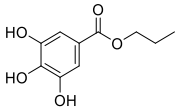
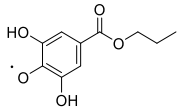
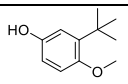
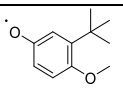
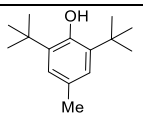
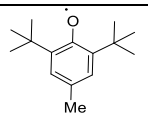
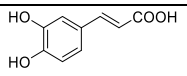
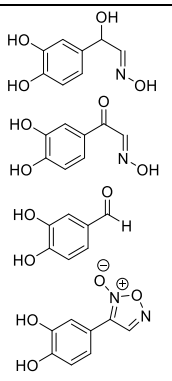
No	Name	Structure	NDSRI formation inhibition mechanism	By-products
1[55]	ascorbic acid (ascorbate)		redox (antioxidant)	 NO
2[73]	α-tocopherol		redox (antioxidant)	 NO
3[74]	maltol		redox (antioxidant)	 NO
4[75]	resveratrol		redox (antioxidant)	 NO
5[76]	propyl gallate		redox (antioxidant)	 NO
6[77]	BHA		redox (antioxidant)	 NO
7[77]	BHT		redox (antioxidant)	 NO
8[60]	caffeic acid		NOx capture and redox	

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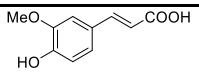
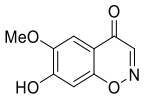
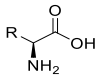
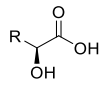
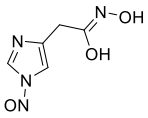
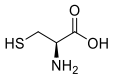
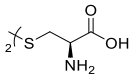
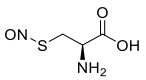
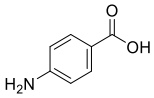
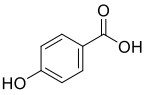
Table 1. continued

No	Name	Structure	NDSRI formation inhibition mechanism	By-products
9[59]	ferulic acid		NOx capture	
10[78]	α-aminoacids (glycine, lysine, histidine*)		NOx capture (diazotation)	N_2  
11[78,79]	L-cysteine		redox (antioxidant) or NOx capture	 NO 
12[80]	*p*-aminobenzoic acid (PABA)		NOx capture (diazotation)	 N_2
13[65]	urea		NOx capture (diazotation)	N_2, CO_2
14[65]	sodium sulfite	$NaHSO_3$	redox (antioxidant)	NO, $NaHSO_4$
15[64,81]	ammonium chloride	NH_4Cl	NOx capture (diazotation)	N_2
16[36]	sodium carbonate	Na_2CO_3	pH modulator	$NaHCO_3$

[a]For an overview of most of the structures in the table, see refs 70, 72. *The proposed structure of the byproduct of nitrite scavenging with histidine is shown; this byproduct was found to be mutagenic.[69]

addition, peroxides can promote the degradation of drug substances with the release of nitrosatable secondary amines. We refer again to the demonstrated examples of ranitidine and nizatidine. However, tertiary amines with at least one (hetero)-benzylic-type[24]/allylic[44] substituent might also be at risk. While the evidence so far relates to ranitidine and nizatidine, a more general possibility is the peroxide-promoted hydrolysis of tertiary amide bonds,[45] a structural motif that is very common in drug substances,[46] to afford secondary amines. A related mechanism may operate in the case of metformin, in which the guanidine structure's lability in the presence of peroxides was confirmed.[36] While it is demonstrated that the NDSRI formation due to secondary amine degradants is considerably less problematic than for secondary amine drug substances due to the lower secondary amine concentration,[25] information provided to EU regulators suggests that active substances containing tertiary amides are still at risk of *N*-nitrosamine formation, particularly when the amine degradant is a low-molecular weight, volatile, and hence highly mobile compound.[44]

Finally, peroxides could be directly involved in the formation of *N*-nitrosamines by oxidative processes in drug (degradant) molecules that bear a hydrazine or hydrazone moiety (e.g., gliclazide, rifampicin).[47,48]

5. SOLID DOSAGE FORMS: RISKS ASSOCIATED WITH UNIT OPERATIONS DURING MANUFACTURING

As already mentioned, the risks associated with the formation of *N*-nitrosamines in solution,[13,14] particularly in the context of drug substance manufacturing, are generally well understood

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and the implementation of control measures and corrective actions is relatively straightforward. This is in contrast to the generation of NDSRIs during formulation of the drug substance into solid dosage forms where reactions are occurring between different components of solid or heterogeneous mixtures. Work has begun to investigate risk factors and mechanistic aspects.[49]

A recent study investigated the impact of various manufacturing operations including wet granulation, dry granulation and noncompacted blending on nitrosation of model compounds containing secondary amines as either free bases or hydrochloride salts (see Scheme 2 in Section 3 for structures).[25] The extent of nitrosation of secondary amine free bases did not differ much between the three manufacturing techniques. However, for the HCl salts, the amount of *N*-nitrosamine formed was at least 5 times higher for wet granulation processes compared to noncompacted blending (see Figure 2). This indicates that the acidic formulation promotes nitrosation, more so in the presence of water.

Water seems to play a role in NDSRI formation, not just during the manufacturing process, but also during storage, as NDSRI levels generally and consistently increased in correlation with increased humidity as used in accelerated stability studies.[25] The influence of water is likely multifold. During manufacturing, water can enhance the homogeneous distribution of the drug substance and impurities (especially nitrite) by the solubilization of these components, ultimately translating into an increased accessibility of nitrite for reaction with a proximal secondary amine. Partial solubilization of the drug substance may also alter its physicochemical properties such as increasing amorphous content, altering morphology and particle size as well as increasing surface area. All these factors can increase the rate of nitrosation reactions in the solid phase.[25] Finally, the water content in the solid dosage form increases the mobility of reactants, according to the so-called "saturated solution layer" model.[50] Thus, the presence of water during drug product manufacturing and storage is an apparent risk factor for NDSRI formation. However, it is also important to note that elevated water presence during storage can lead to a slowing down of the *N*-nitrosamine formation rate due to the dilution of its precursors, as demonstrated by You, Song et al., who showed that increasing the water content in a metformin formulation from 1.5-% to 4 wt % produced about 3 times less NDMA.[36] The observed differences with respect to the impact of water in these reactions makes the predictive utility of water's role in formulations challenging.

6. USE OF NITRITE SCAVENGERS AND OTHER ADDITIVES TO PREVENT NDSRI FORMATION

An important recent development to control NDSRI formation is the addition of nitrite scavengers/nitrosation inhibitors to the formulation. While the inhibitory action of certain additives has been known for a long time and has been investigated in the context of food products,[51] cosmetics,[52] and even medicines,[53] to the best of our knowledge, the incorporation of specific nitrosation inhibitors in the formulation of pharmaceuticals has not yet been explicitly reported (although, as will be detailed in the following, many common excipients may serve as such).[54]

Based on literature reports, two main categories of additives can be identified to facilitate the control of (complex) *N*-nitrosamines in drug products: (1) nitrite scavengers (antioxidants, amino acids, etc.) and (2) pH modulators (inorganic bases).

Nitrosating agents (generally inorganic nitrite) can be chemically inactivated via a number of different strategies, namely redox reactions and quenching by nucleophiles. Natural antioxidants such as ascorbic acid[55] or polyphenols[56] are known to reduce nitrite (NO_2^-) to nitric oxide (NO), a gaseous small-molecule no longer able to affect amine nitrosation;[57] notably, NO is a well-known signaling molecule in the human body, involved in a number of biological processes.[58] NOx is known to be captured by C-nucleophiles such as ferulic acid, caffeic acid and tocopherols.[59−61] Typically, the primary products of these reactions are reactive species that undergo subsequent molecular rearrangements and fragmentations, leading to aldehydes, nitro-compounds and N−O-containing 5-membered and 6-membered heterocycles *inter alia* (Table 1).

Another effective and well-known class of nitrite scavengers are primary amines which includes naturally occurring compounds such as amino acids. In this case, nitrosation leads to a short-lived diazonium salt that is trapped by nucleophiles in close proximity, for instance water. All proteinogenic amino acids undergo this reaction, most of them cleanly, affording nontoxic α-hydroxy acids as products (also known as the van Slyke reaction).[62] The reaction goes through diazonium and α-lactone intermediates. A similar process occurs in the case of aromatic amino acids, such as *p*-aminobenzoic acid (PABA); lactone formation cannot occur in this case and the final product is phenolic.[63] Naturally occurring amino acids are endogenous substances and therefore present few safety concerns if used as excipients. The broad scope of this reaction provides flexibility in the choice of the most compatible scavenger, as will be exemplified later on in this section. Other amine derivatives (including ammonia[64]) also undergo the van Slyke reaction; even less nucleophilic amines such as urea[65] and sulfamic acid[66] are known to scavenge nitrite, albeit with generally slower kinetics, unless the pH is below 2.[67]

Finally, an alternative option to mitigate NDSRI formation in drug products is to reduce the acidity of the formulation by using basic excipients (e.g., Na_2CO_3).[36,43] This approach is generally effective, as the nitrosation rate decreases strongly at higher pH.[13,14,23] However, there are cases where conditions favor *N*-nitrosamine formation at neutral and alkaline pH, typically under the action of a catalyst (e.g., a carbonyl compound such as formaldehyde, as detailed in Section 4.3). The main limitation of this approach is that relevant pharmacological properties of the active substance such as stability, dissolution, bioavailability or palatability may be altered by changing the formulation pH.

In contrast to pH modulators which inhibit nitrosation activity (i.e., act on the *conducive conditions* risk factor), the action of antioxidants/scavengers consists of a kinetic competition with the nitrosatable amine, therefore addressing the *NOx* element. Consequently, the formulation process may require tuning of parameters to effectively impede NDSRI formation, depending on the vulnerability of the secondary amino group in the drug substance (or contaminant/degradant thereof). For example, drug substances containing highly nucleophilic/less basic secondary amino-groups (e.g., aromatic amines) feature fast nitrosation kinetics which render the direct nitrite-scavenging approach more challenging. For instance, in a recent example reported to the EU regulatory authorities, a molecule containing a sterically encumbered secondary amine reacted extensively to form the related *N*-nitrosamine, despite the presence of a primary amine in the structure, which might have been expected to react preferentially and thus scavenge the nitrite.

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Accordingly, the selection of a scavenger with adequate reactivity relative to the API-amine is critical, allied to the inhibitor content and formulation pH. The scavenger could be included directly in the drug product formulation or premixed with the active substance. Alternatively, an indirect strategy based on the scavenging of the nitrite in a separate step, prior the addition of the drug substance (e.g., pretreatment of excipients, process water, etc.) could be considered.

When incorporating a scavenger into a formulation, several other factors should be considered in additional to the inhibitory effect:

- The absence of undesired drug/excipient interactions.
- The newly incorporated additive should not hamper the formulation process.
- The additives should be toxicologically acceptable.
- The byproducts of the nitrite deactivation process should be well understood and safe.
- The absence of compatibility/storage issues such as color change of tablets.

First, the nitrite scavenger should selectively react with nitrite or nitrite-derived species, leaving the active substance intact. For instance, the drug substance itself should not be susceptible for reduction by the antioxidant or should not undergo conjugate addition to, e.g., ferulic acid, etc.

Second, the formulation conditions may require adjustment to accommodate an additional component. For example, most of the structures in Table 1 are acidic and the pH during the formulation might be detrimentally altered; identifying the compatible form of the additive (i.e., neutral molecule/salt) might require investigation on a case-by-case basis.

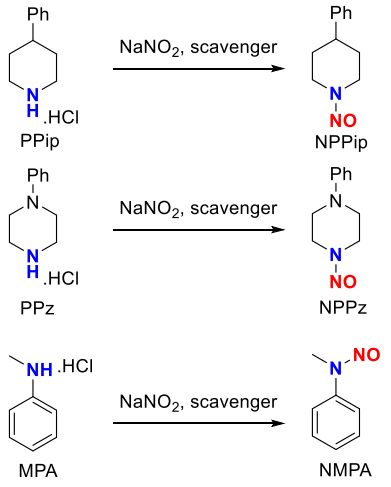
Next, the safety of the additive is of paramount importance. Since the proposed candidates are generally natural compounds found in food and have previously been used as pharmaceutical excipients,[68] safety of the additive has been investigated in many cases.

It is equally critical to understand that the nitrite scavenging reactions result in innocuous byproducts. It should however be noted that not all the proposed scavengers react cleanly toward a single product, which can be a complication (Table 1). For example, the reaction between nitrite and histidine leads to 5 different products, where the major product was experimentally shown to be mutagenic.[69] Similarly, the reaction between caffeic acid and nitrite produces a plethora of structures, depending on conditions (the most important ones are shown in Table 1);[60] notably, the outcome of caffeic acid's nitrosation translates to either a suppressive or a stimulating effect on the *N*-nitrosamine formation in gastric juice, suggesting that some of the resulting structures (like, e.g., the furoxan, formed at low caffeic acid concentration) can serve as NOx donors.

Finally, drug product properties such as color stability upon storage cannot be neglected in the quest for mitigating NDSRI risks with nitrite scavenging solutions. The pioneering studies presented herein highlight potential incompatibilities between some additives[70] (e.g., ascorbate,[71] PABA) and other components of the matrix with respect to the optical properties of the drug product. These findings can serve as guidance for future development efforts by drug product manufacturers.

In the context of NDSRIs, the role of nitrite scavengers incorporated in drug product formulation has only recently started to be evaluated. A proof-of-concept study investigated the inhibition of *N*-nitrosamine formation in oral solid dosage forms by a range of inhibitors, using 4-phenylpiperidine (PPip) hydrochloride as model structure for a vulnerable aliphatic secondary amine (Scheme 5).[82] Two categories of scavengers were investigated, namely antioxidants (ascorbic acid, α-tocopherol, ferulic acid and caffeic acid) and amino acids (glycine, lysine and histidine). Remarkably, accelerated stability studies (50 °C, 75% RH, 1 month) of the tablets spiked with 1 wt % of antioxidant indicated that all the inhibitors reduced the level of 1-nitroso-4-phenylpiperidine (NPPip) by more than 80%. In the absence of nitrite scavenger and under the same conditions, levels of NPPip tripled from ca. 150 ppb to ca. 500 ppb over the same time period. Second, the potential inhibitory action of amino acids was tested, in solution, as model study for pharmaceuticals formulated as solutions/suspensions. In a stress test (API:nitrite:scavenger 1:4:20, 60 °C, pH = 3, 23 h), the NDSRI formation was inhibited in by 44%, 57% and 91% for glycine, lysine and histidine respectively. This appears to be a promising lead given that the experiments were performed in conditions that greatly favor *N*-nitrosamine formation, (aqueous solution, highly concentrated nitrosating agent and optimal nitrosation pH).[67] However, it should be reiterated that one of the byproducts of the reaction of histidine with nitrite is a known mutagen.[69]

Scheme 5. Model Studies for the Inhibition of NDSRI Generation in Drug Products by the Use of Nitrite Scavengers[70,82]



A subsequent study reports the screening of 19 structurally and functionally diverse nitrite scavengers, initially in solution and then in solid phase, using 4-phenylpiperazine (PPz) and *N*-methyl phenylamine (MPA) as model compounds for secondary aliphatic and aromatic amine derivatives, respectively.[70] Control experiments shortlisted six candidate scavengers, namely ascorbic acid, sodium ascorbate, maltol, propyl gallate, *p*-aminobenzoic acid (PABA) and L-cysteine, agents that completely removed the nitrite from solution under the experimental conditions (20 °C, pH 3, 24 h). According to the authors, the nitrite scavenging ability cannot be predicted in a straightforward manner based on literature precedents. For instance, amino acids glycine, lysine, arginine and histidine were highly ineffective, in contrast to earlier reports discussed above.[82] The only effective amino acid was L-cysteine, suggesting the involvement of the thiol group in the nitrite scavenging mechanism (capture of NOx and/or reduction to nitric oxide[79]).

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Regarding the *N*-nitrosamine formation risk in solution (conditions: 2 mM amine, API:nitrite:scavenger 1:1.1:22, 25 °C, pH 3, phosphate buffer), a great difference in reactivity was demonstrated in the case of the two model compounds, PPz and MPA, with the former being only 5% converted in 24 h, whereas the latter was fully nitrosated within 30 min. As suggested by previous reports,[23] less basic amines are generally more prone to forming *N*-nitrosamines, an important observation to keep in mind when assessing risk factors for NDSRI presence. Of note, the secondary aliphatic amine motif is much more prevalent in drug substances than (hetero)aryl alkyl amines.[23] Nonetheless, even in the case of the highly nitrosatable MPA, the *N*-nitrosamine formation could be nearly completely suppressed by PABA, a result consistent with the structural similarity between the scavenger and the model drug substance. In addition, ascorbic acid and L-cysteine also showed significant levels of impurity reduction (75−90%). In the case of the less reactive PPz, the efficiency of all these three additives was more evident (>98%); moreover, propyl gallate also inhibited NPPz with >80% effectiveness.

The solid-state experiments (50 °C, 75% RH, 1 month, 100 mg tablets) confirmed the higher risk for NDSRI presence in the case of aromatic MPA compared to aliphatic PPz, with ca. 20 ppm of *N*-nitrosamine being formed during the granulation and tableting processes. Nitrite in the excipients was confirmed as a risk factor by spiking experiments; spiking the main excipient, microcrystalline cellulose, with 2 ppm nitrite led to approximately double the initial *N*-nitrosamine levels. For the aliphatic amine, the initial *N*-nitrosamine levels were up to 2 ppm, but in most cases, including nitrite spiking experiments, the values were in fact below the LoQ of 10 ppb. Remarkably, under these stressed conditions, despite its rapid generation, nitroso-MPA could no longer be detected at the end of the experiments: most likely decomposition occurred. The authors postulated that the nitroso group may migrate to the aromatic ring (the Fischer−Hepp rearrangement[83]) or decompose by denitrosation.[84] The instability of *N*-nitroso-methyl phenylamine was probed further in a preliminary investigation of several parameters, like concentration, temperature and time, confirming that the molecule is indeed very labile, with significant decomposition (70−90%) taking place already after 8 days, at a reduced temperature of 40 °C. This observation is important in the context of *N*-nitrosamine impurities in medicines. Additional research would be required to confirm this result and understand the scope and conditions that favor *N*-denitrosation and the potential for reduction in nitrosamine content over time.

The study of the inhibition of NDSRI formation in the case of aliphatic PPz showed that ascorbic acid was the most effective scavenger[82] whereas PABA and L-cysteine were also promising. These experiments generated some intriguing results, however: for instance, some scavengers actually led to an increase in the levels of the *N*-nitrosamine impurity. This phenomenon has been reported previously.[53,73] In particular, sodium ascorbate showed roughly a doubling of *N*-nitrosamine levels in the case of nonspiked tablets and a 4-fold increase in the nitrite-spiking experiments. This change in activity compared to ascorbic acid is peculiar, especially since ascorbate is expected to be a superior antioxidant[85] based on experience from tableting of MPA.[70] According to model studies in solution by Kamm et al., the efficiency of nitrite removal by ascorbate decreases with increasing pH.[86] However, extrapolation of its behavior in solution into solid-state chemistry is not straightforward because factors such as particle crystallinity, size, distribution and water content etc. could influence the outcome.[25,36] More work is required to validate these model studies and translate them to real-life scenarios in the formulation of medicines, including addressing issues such as tablet color preservation during storage, which is particularly challenging for Maillard reaction-prone additives such as vitamin C.[71]

Recent investigations into the effectiveness of nitrite scavengers in the drug product formulation indicate that this is a promising strategy, with significant reduction in *N*-nitrosamine formation observed in some model studies. The complexity of solid phase drug product matrices and subtle differences in formulation conditions currently make predicting the effectiveness of scavengers very difficult. While some learnings from the studies are transferable, there is likely no universal effective solution and the effectiveness of scavengers requires a case-by-case evaluation. Furthermore, the nature and toxicity of byproducts from scavenging reactions need to be better understood. Finally, a change in composition would require altering the manufacturing process and re-evaluation of the properties of the formulated product, including its impurity profile, stability and bioavailability. Depending on the extent of the changes and the inherent properties of the active substance (solubility, permeability), this could be achieved by *in vitro* studies or may require clinical demonstration of bioequivalence. The use of scavengers may be easier to accommodate during development of new products, compared with altering the formulation of an already marketed product.

7. MITIGATION STRATEGIES

7.1. Control of Nitrite Introduced during Formulation.

The concentration of nitrite in the formulation is a critical risk factor in the generation of NDSRIs,[87,88] and accordingly an opportune area for implementing corrective and preventative actions (CAPAs). Current understanding is that nitrite is the limiting reagent in the formation of NDSRIs and thus its concentration determines the maximum amount of *N*-nitrosamine that can form in the drug product.[35] It is important to note however that only a fraction of the total nitrite effectively leads to *N*-nitrosamine impurities under usual formulation and storage conditions, with the levels depending on the reactivity of the vulnerable secondary amine: typically a few percent for aliphatic amines, whereas the extent of nitrosation of aromatic derivatives can be much higher (e.g., 25% nitrosation of *N*-methyl phenylamine).[70]

In contrast to solution nitrosation where nearly complete conversions can be attained,[14] in the solid phase, the formation of NDSRIs seems to plateau at levels that are generally well below 100% of nitrite consumed (the maximal nitrite conversion reported to date, for a secondary aliphatic amine, was 38%—a value obtained during accelerated stability studies at 60 °C[25]). It is plausible that this is due to the restricted mobility of the two reacting species (i.e., nitrite and vulnerable amine) in the solid phase and the heterogeneous nature of the system where reactants may be immobilized in highly crystalline phases or in the core of large particles. Other factors that may play a role here are the limited stability of nitrite[89] and/or some *N*-nitrosamines.[25,70] Data in this area are scarce, and more research is necessary to clarify these aspects.

With respect to the *N*-nitrosamine formation rate, since the amine nitrosation rate under conditions most pertinent to drug product manufacturing is generally proportional to the square of $[NO_2^-]$,[23,67] a 10-fold reduction in nitrite concentration would theoretically lead to a 100-fold reduction in nitrosation rate.

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Several potential actions could be envisaged to reduce the nitrite content of excipients. Since different excipient manufacturers use different manufacturing, isolation and drying processes,[31] identifying an excipient source with a lower nitrite content in some cases could be a solution, although its implementation faces a number of challenges. For e.g., while the Lhasa Nitrite Excipient Database is undoubtedly an excellent source of relevant information, the anonymized character of the data impedes its use in the direct selection of the best supplier or grade. Another possibility would be the identification of a replacement for an excipient that is known to contain high levels of nitrite with one containing lower levels (e.g., substitution of the disintegrant sodium starch glycolate with povidone[35]). A further option would be to implement a purification process for the excipient in question in order to reduce nitrite content.[87] For instance, aqueous washings might have a positive effect in lowering the nitrite content in water-insoluble excipients such as magnesium stearate or crospovidone, provided that drying steps do not reintroduce nitrosating agents. Previously and in response to the pharmaceutical industry's needs, several excipients have become available in the past decades with improved purity grades, for example polyethylene glycols (PEGs) and polysorbates with lower levels of aldehydes and peroxides, and povidone and crospovidone with lower peroxide content.[32] A similar option for excipients with problematic nitrite levels for certain products could follow. It is important to reiterate here that most likely it is sufficiently impactful to implement such CAPAs for excipients used in high proportions only, such as fillers and diluents.

An elusive piece in the puzzle remains the source of nitrite in excipients. In contrast to other reactive impurities, whose mechanism of generation is usually well-understood,[32] there is great ambiguity around the presence of inorganic nitrite in the highly structurally heterogeneous set of excipients commonly used in medicines. Moreover, it is difficult to ascertain whether there is a common source or there are various root causes, depending on the excipient type and the manufacturing process. It has been speculated that drying unit operations may play a role,[32] by generating nitrogen oxides that could be trapped into the bulk excipient material by interaction with water (leading to HNO_2) or basic inorganic constituents/impurities (forming nitrite salts). In this respect, a recent study demonstrated that methamphetamine undergoes nitrosation in the presence of (very dilute) gaseous HNO_2 (a common indoor air pollutant).[90] It is equally interesting to note that consistently high nitrite levels seem to correlate with the use of (superstoichiometric) sodium hydroxide in the excipient manufacturing process: magnesium stearate, hypromellose, sodium starch glycolate are generally high in nitrite. Notable exceptions to this observation would be croscarmellose sodium (relatively low nitrite content despite a NaOH-based manufacturing process) and crospovidone (rich in nitrite but not produced using NaOH). Alternatively, in excipients manufactured by late-stage treatment with ammonia (e.g., microcrystalline cellulose), the most likely nitrite species would be the ammonium salt.[64] The nature of the cation is of crucial relevance for the safety of the drug product, since the decomposition of NH_4NO_2 into N_2 and H_2O would reduce the NOx concentration over time and may thus limit the formation of NDSRIs in such cases.[64]

Identifying the source (and exact speciation) of nitrosating agent in excipients is of critical importance as it would enable the rational implementation of corrective measures. For example, if nitrogen oxides formed during hot-air drying unit operations are indeed involved, their concentration could theoretically be reduced by the use of lower temperatures and/or nitrogen-enriched gas stream instead of air.[91] Alternatively, acidic NOx species could potentially be trapped by scrubbing prior the actual drying of excipients. Another option would be to redesign the drying process, for example, using vacuum drying. Technical solutions for addressing NOx species in air as *N*-nitrosamine precursors are in fact already available in the brewing industry where investigations into NDMA formation during malt kilning were concluded several decades ago.[92]

7.2. Control of *N*-Nitrosamine Formation during Formulation. Alternative to the control of nitrite in excipients, modification of the formulation conditions could also contribute to the reduction in the risk of NDSRI generation. For instance, nitrite scavengers could be introduced in the formulation; the current candidate list is generous and the science in the field is advancing. Other regulators have made statements regarding this strategy.[93] Furthermore, the formulation pH could be adjusted, either by a simple approach such as adding basic additives or if applicable, by the more laborious substitution of salt formulations of amine-containing APIs with their corresponding free bases. A complementary area of intervention could be the physical properties of the drug substance such as the polymorph or particle size; these are demonstrated factors that influence solid-state NDSRI formation.[25,36] Amending manufacturing processes by, for example, adjusting the water content in the formulation might be an impactful measure. Finally, avoiding high risk unit operations (e.g., fluid-bed drying, wet granulation) or adjusting manufacturing process parameters could also reduce the extent of NDSRI formation during drug product formulation.

8. CONCLUSIONS

Focus has shifted over the past 2 years from highly potent small molecule *N*-nitrosamine impurities formed during manufacture of drug substances, to NDSRIs predominantly formed during formulation/storage of the drug product. Effective strategies to avoid or mitigate the presence of *N*-nitrosamine impurities originating during drug substance manufacturing processes are well-known and have been documented, but the control of NDSRIs in drug products is presently a significant challenge.

Vulnerable amines (or their precursors) are common and important structural elements of many drug substances, generally essential for the *in vivo* activity but also important in modulating pharmacokinetic and physicochemical properties. As such, their elimination from medicines is unlikely, and alternative strategies need to be employed to avoid generation of NDSRIs at unacceptable levels. While much progress has been made on understanding the various risk factors associated with NDSRI formation in drug products, there are still significant gaps in mechanistic knowledge. This currently makes it almost impossible to predict accurately whether, and to what extent, an API bearing a vulnerable amine will result in an NDSRI considering the formulation, manufacturing process, and storage conditions. MAHs and API manufacturers should bear in mind not only whether the structure of the API bears a risk of formation of NDSRIs, but additionally whether nitrosatable impurities or degradants may pose a risk of *N*-nitrosamine formation during drug product manufacture. The mitigating measures discussed at length in this article should be considered where an NDSRI is identified in a medicinal product above the acceptable intake. MAHs, drug product manufacturers, drug substance manufacturers, as well as excipient and packaging

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manufacturers are encouraged to collaborate further to expand the understanding of risk factors for *N*-nitrosamine formation in medicines. Further collaboration and research will hopefully enable appropriate reductions in NDSRI levels when this is required, allow for more accurate risk assessments, and reduce the need for specific testing in the future. Regulatory guidance and policy have been periodically updated as scientific understanding has evolved.[21] Further guidance updates can be anticipated as more knowledge is gained.

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Notes

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ABBREVIATIONS

AI, acceptable intake; API, active pharmaceutical ingredient; CAPA, corrective and preventive action; CMC-Na, croscarmellose sodium; DMA, dimethylamine; DMF, dimethylformamide; EDTA, ethylenediaminetetraacetic acid; EU, European Union; HPMC, hydroxypropyl methylcellulose; LoQ, limit of quantitation; MAH, market authorization holder; MDD, maximum daily dose; MPA, methylphenylamine; NA, *N*-nitrosamine; NDEA, *N*-nitrosodiethylamine; NDELA, *N*-nitrosodiethanolamine; NDMA, *N*-nitroso dimethylamine; NDSRI, nitrosamine drug substance related impurity; NMP, *N*-methyl pyrrolidone; NMPA, nitroso methyl phenylamine; *N*-NPPip, 1-nitroso 4-phenylpiperidine; *N*-NPPz, 1-nitroso 4-phenylpiperazine; ppb, parts per billion (ng/g); PABA, *p*-aminobenzoic acid; PEG, polyethylene glycol; PPip, 4-phenylpiperidine; ppm, parts per million (μg/g); PPz, 4-phenylpiperazine; RH, relative humidity.

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