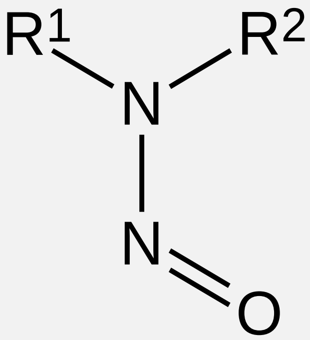
Nitrosamine Formation in Solid Drug Products

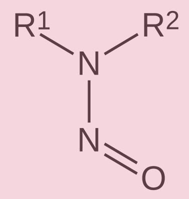
Dr Garry Scrivens

FreeThink Webinar Series 2023



Introduction

N-Nitrosamine:

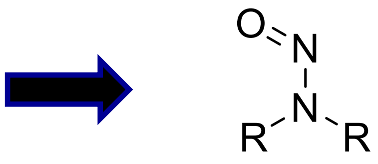


(referred to as just nitrosamine or NNO in this presentation)

- Observation of N-nitrosamines in “sartans” (July–Dec. 2018)
- Recalls of certain pharmaceutical products across industry
- Observation that nitrites are found at various levels (ppm levels) in some excipients – not on CofA

  ⇒ APIs containing a secondary amine group need to be assessed

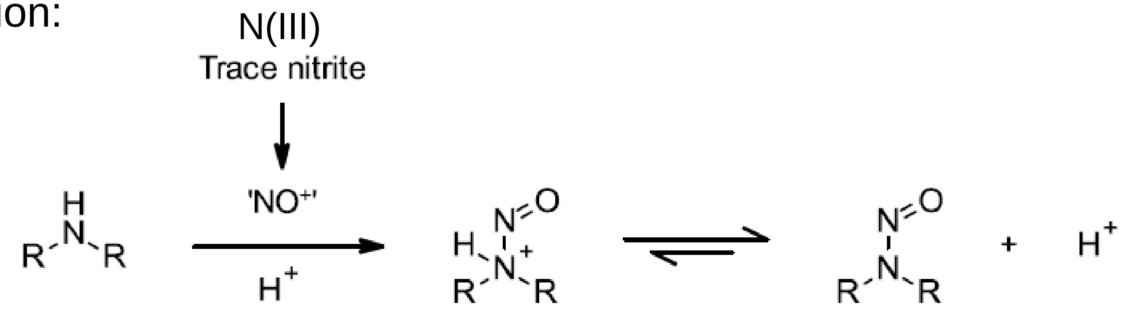
Nitrosating agent, e.g. Nitrites + “Vulnerable amine” + Favourable conditions



- Certain nitrosamines are ‘cohort of concern’ compounds
- Much of our understanding about the formation of nitrosamines comes from solution chemistry
- Much less is known about their formation in solid products – the focus of this presentation
- The aim of the work presented here is to ensure that nitrosamine risk assessments are based on the best possible underlying scientific understanding.

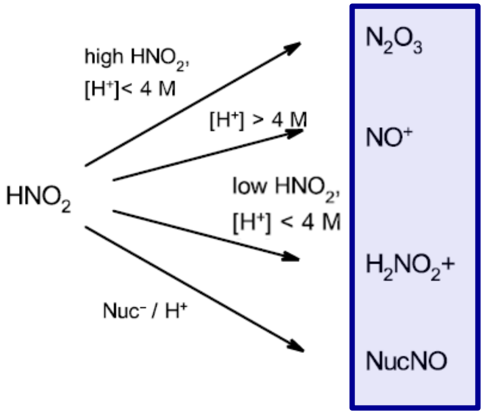
Nitrosamine Formation Mechanism In Solution

Simplified General Equation:



Ashworth, Dirat et. al
Org. Process Res. Dev. 2020, 24, 1629–1646

- Overall stoichiometry: 1 amine + 1 nitrite → 1 nitrosamine
- In aqueous solution, the actual nitrosating species is a combination of different species:



Nuc-: e.g. Cl- or other halides

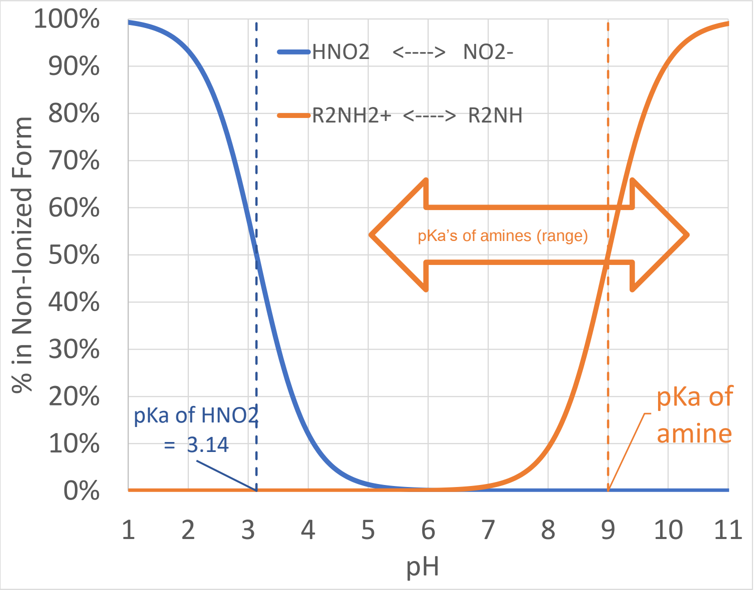
Actual Nitrosating Species

The reaction mechanism requires both the nitrite and the amine to be **non-ionised:**

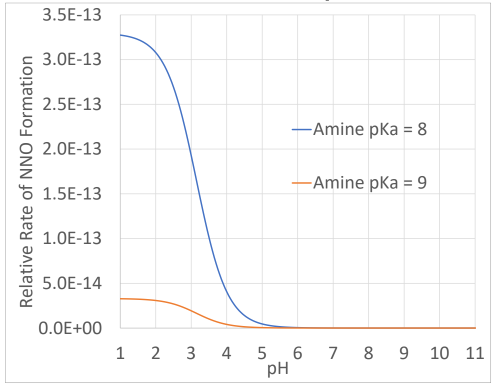
HNO_2 **R_2NH**

Therefore the reaction rate is **highly pH-dependent** and **highly dependent on the pKa of the amine**

Nitrosamine Formation in Aqueous Solution:
Dependence on Solution pH and pKa of Amine

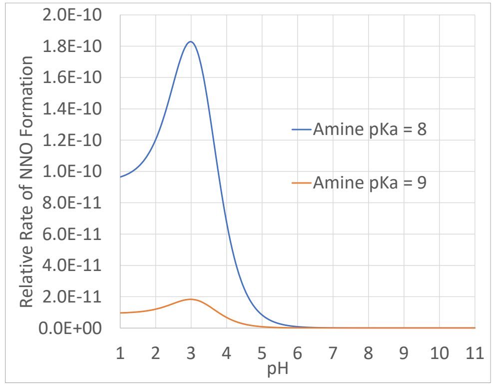


Very Dilute Nitrite Levels (most pharmaceutical scenarios)



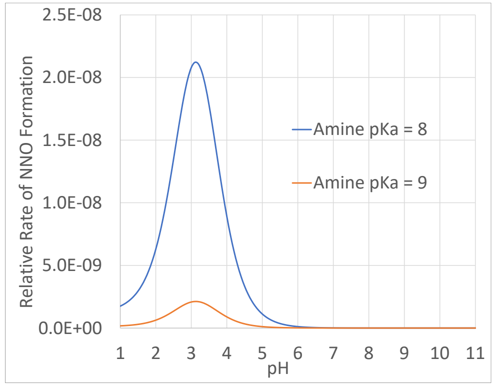
$H_2NO_2^+$ is dominant nitrosating species

Medium Nitrite Levels



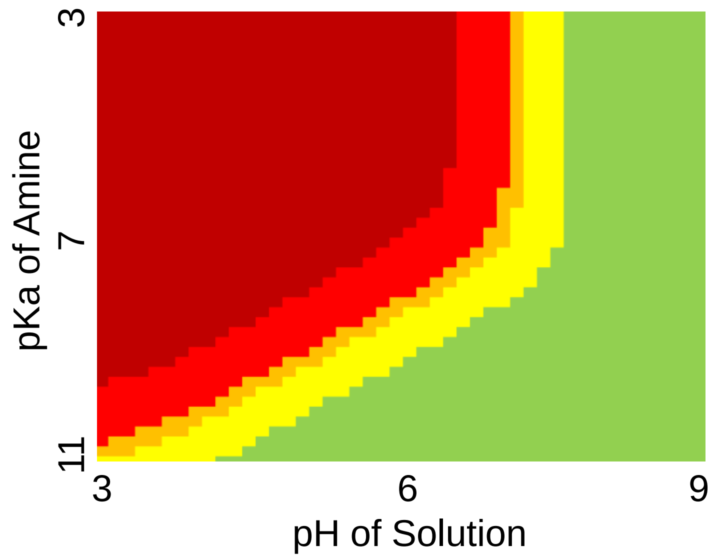
Significant contributions from both $H_2NO_2^+$ and N_2O_3

Concentrated Nitrite Levels



N_2O_3 is dominant nitrosating species

Rate of Nitrosation



Nitrites in Excipients

- Compendial specifications and CofAs for excipients do not currently include nitrite levels
- Nitrites are found at low (ppm, ppb) levels in many excipients
- Industry consortium to generate an excipient nitrite database (Lhasa)

CAS Number	Excipient name	Structure LUID	Common Name	LUID	Nitrite LOQ (ug/g)	Nitrite result (ug/g)	Date of manufacture	Date of test	Supplier
55589-62-3	Acesulfame potassium	38205053	Acesulfame potassium	38327451	<0.1	LLOQ	Jan-20	Nov-20	BUO
55589-62-3	Acesulfame potassium	38205053	Acesulfame potassium	38205066	<0.10	LLOQ	Jul-19	Jul-20	BUO
55589-62-3	Acesulfame potassium	38205053	Acesulfame potassium	38205065	<0.10	LLOQ	Jan-19	Jul-20	BUO
55589-62-3	Acesulfame potassium	38205053	Acesulfame potassium	38205064	<0.10	LLOQ	Apr-18	Jul-20	BUO
77-92-9	Anhydrous citric acid	40602647	Anhydrous citric acid	40602654	<0.10	LLOQ	Oct-19	Jul-20	CAX
7757-93-9	Anhydrous dibasic calcium phosphate	37350051	Anhydrous dibasic calcium phosphate	37350053	0.1	0.41	Nov-18	May-20	BXV
7757-93-9	Anhydrous dibasic calcium phosphate	37350051	Anhydrous dibasic calcium phosphate	37350052	0.1	0.17	Jun-18	May-20	BXV
63-42-3	Anhydrous lactose	37350182	Anhydrous lactose	40503551	0.02	Not detected	Nov-19	Nov-20	ZFC
63-42-3	Anhydrous lactose	37350182	Anhydrous lactose	37350192	0.5	Not detected	Aug-19	Aug-20	UXA
63-42-3	Anhydrous lactose	37350182	Anhydrous lactose	37350191	0.5	Not detected	Dec-18	Aug-20	UXA
63-42-3	Anhydrous lactose	37350182	Anhydrous lactose	37350190	0.5	Not detected	Apr-19	Aug-20	UXA
74-79-3	Arginine	40546703	Arginine	40546704	0.1	0.16	Mar-17	Nov-20	MKJ
50-81-7	Ascorbic acid	38205054	Ascorbic acid	38205068	<0.10	LLOQ	Nov-18	Aug-20	PUF
50-81-7	Ascorbic acid	38205054	Ascorbic acid	38205067	<0.10	LLOQ	May-19	Aug-20	PUF
22839-47-0	Aspartame	38205055	Aspartame	38205071	<0.10	LLOQ	Apr-20	Jul-20	HMF
22839-47-0	Aspartame	38205055	Aspartame	38205070	<0.10	LLOQ	Jul-15	Jul-20	HMF
22839-47-0	Aspartame	38205055	Aspartame	38205069	<0.10	LLOQ	Mar-13	Jul-20	HMF
9004-32-4	Carboxymethylcellulose sodium	37499018	Carboxymethylcellulose sodium	37723404	0.04	LLOQ	Jan-19	Oct-20	EZH
9004-32-4	Carboxymethylcellulose sodium	37499018	Carboxymethylcellulose sodium	37723403	0.04	LLOQ	Jan-19	Oct-20	EZH
9004-32-4	Carboxymethylcellulose sodium	37499018	Carboxymethylcellulose sodium	37499026	10	Not detected	Not specified	XXX2014	OWZ
9004-32-4	Carboxymethylcellulose sodium	37499018	Carboxymethylcellulose sodium	37499025	10	Not detected	Not specified	XXX2014	JJJ
8015-86-9	Carnauba wax	38205118	Carnauba wax	38205134	0.11	0.21	Jun-16	Oct-20	BUO
9004-35-7	Cellulose acetate	38104182	Cellulose acetate	38194559	0.1	LLOQ	XXX2016	Jun-20	QVJ
5949-29-1	Citric acid monohydrate	38205056	Citric acid monohydrate	38205073	<0.10	LLOQ	Nov-17	Aug-20	RVN
5949-29-1	Citric acid monohydrate	38205056	Citric acid monohydrate	38205072	<0.10	LLOQ	Dec-18	Aug-20	RVN
	Coating: hypromellose	38140392	Coating: hypromellose	40546707	0.05	LLOQ	Jan-20	Feb-21	VYV
	Coating: hypromellose	38140392	Coating: hypromellose	40546706	0.05	LLOQ	Apr-20	Feb-21	VYV
	Coating: hypromellose	38140392	Coating: hypromellose	40546705	0.05	LLOQ	Feb-20	Feb-21	VYV
	Coating: hypromellose	38140392	Coating: hypromellose	38205139	0.22	0.28	Oct-18	Oct-20	VYV

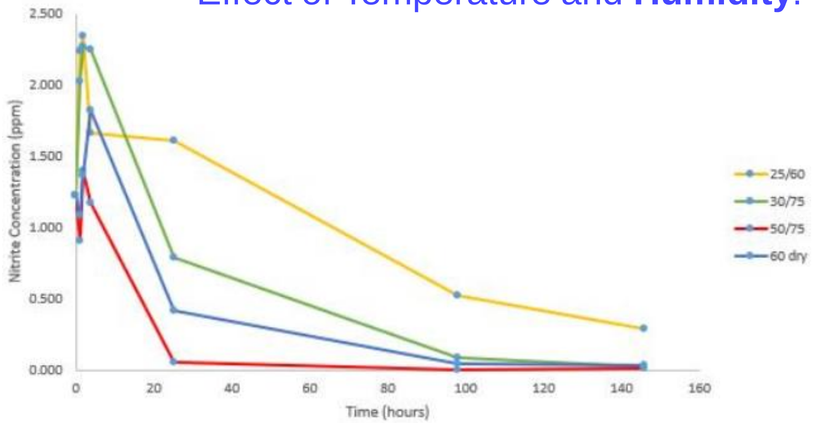
Etc….currently >400 entries in Lhasa database

“Nitrites” (NO_2^-)

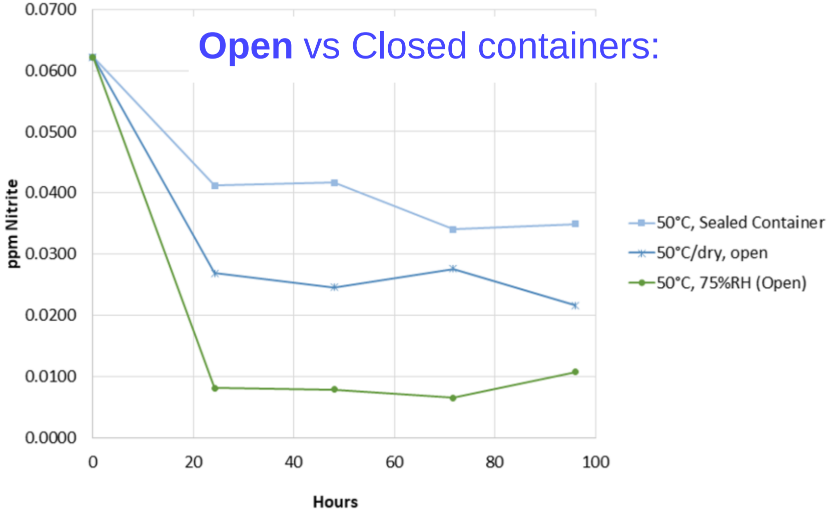
The Nature of Nitrites: Varying Nitrite levels

Lab-scale:

Effect of Temperature and **Humidity**:



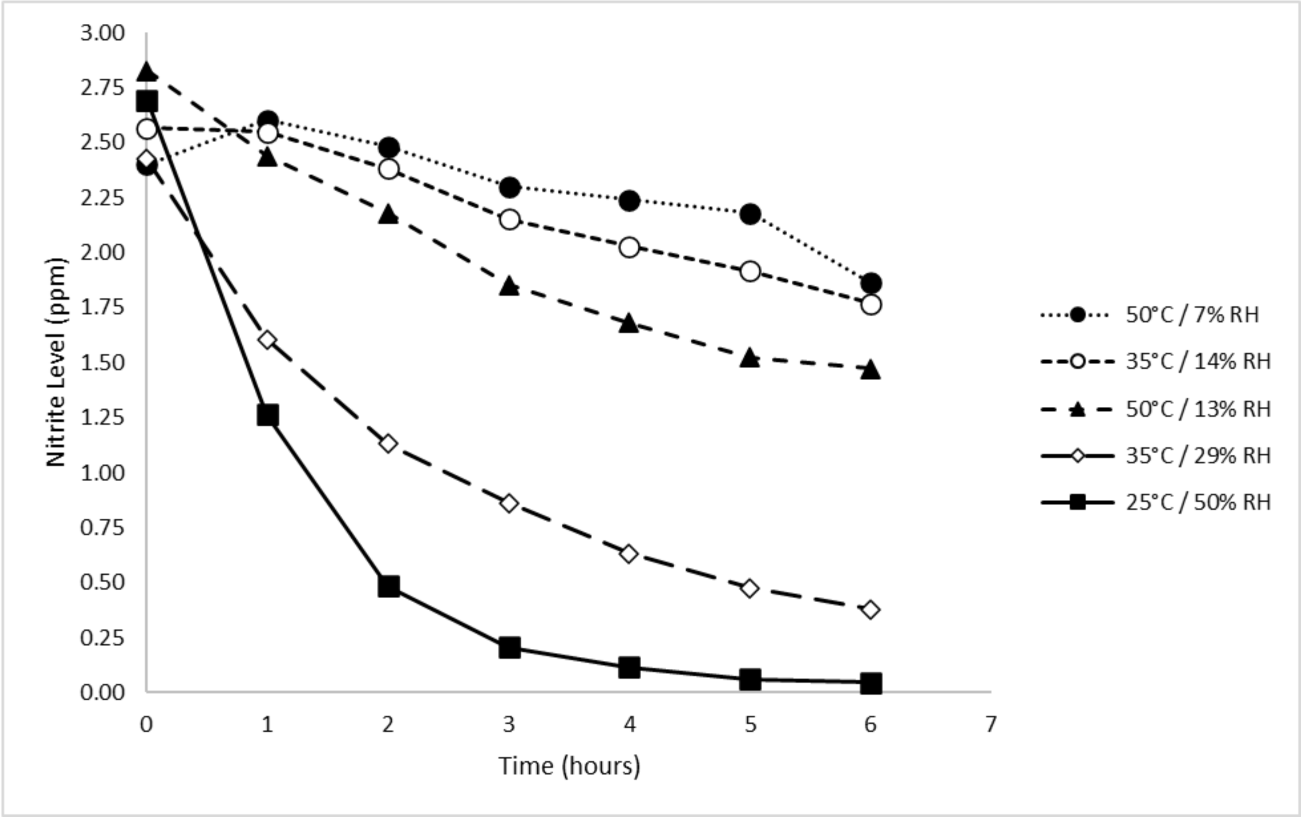
Open vs Closed containers:



Pilot-scale:

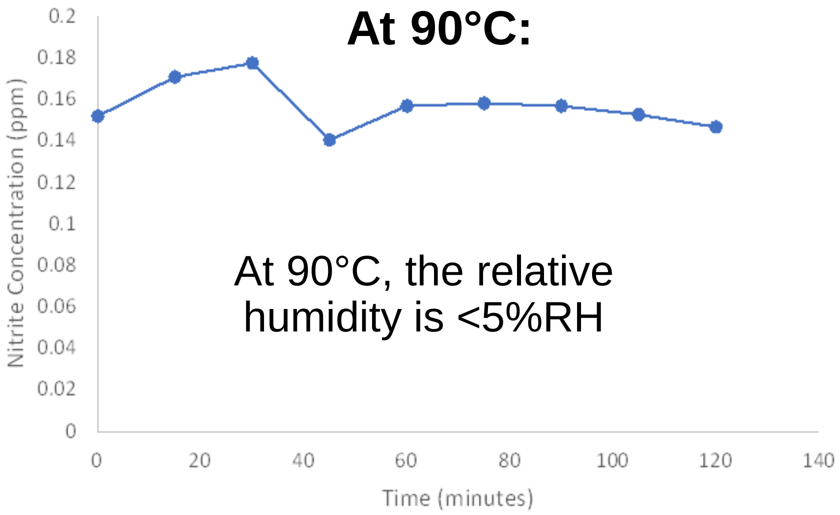
Fluid Bed Study

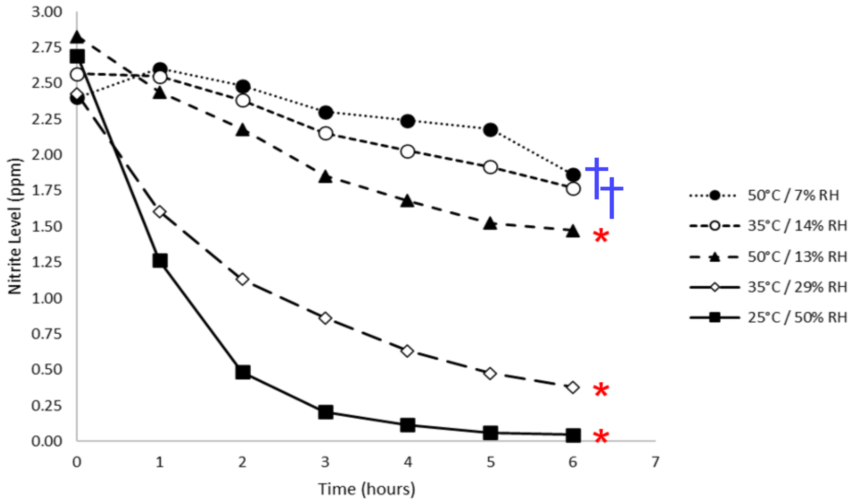
- Detailed Studies on MCC
- Similar observations with Lactose



The Nature of Nitrites: Volatility, Effect of Temperature and Humidity

Initially, it was believed that higher temperatures would drive off the volatile species…





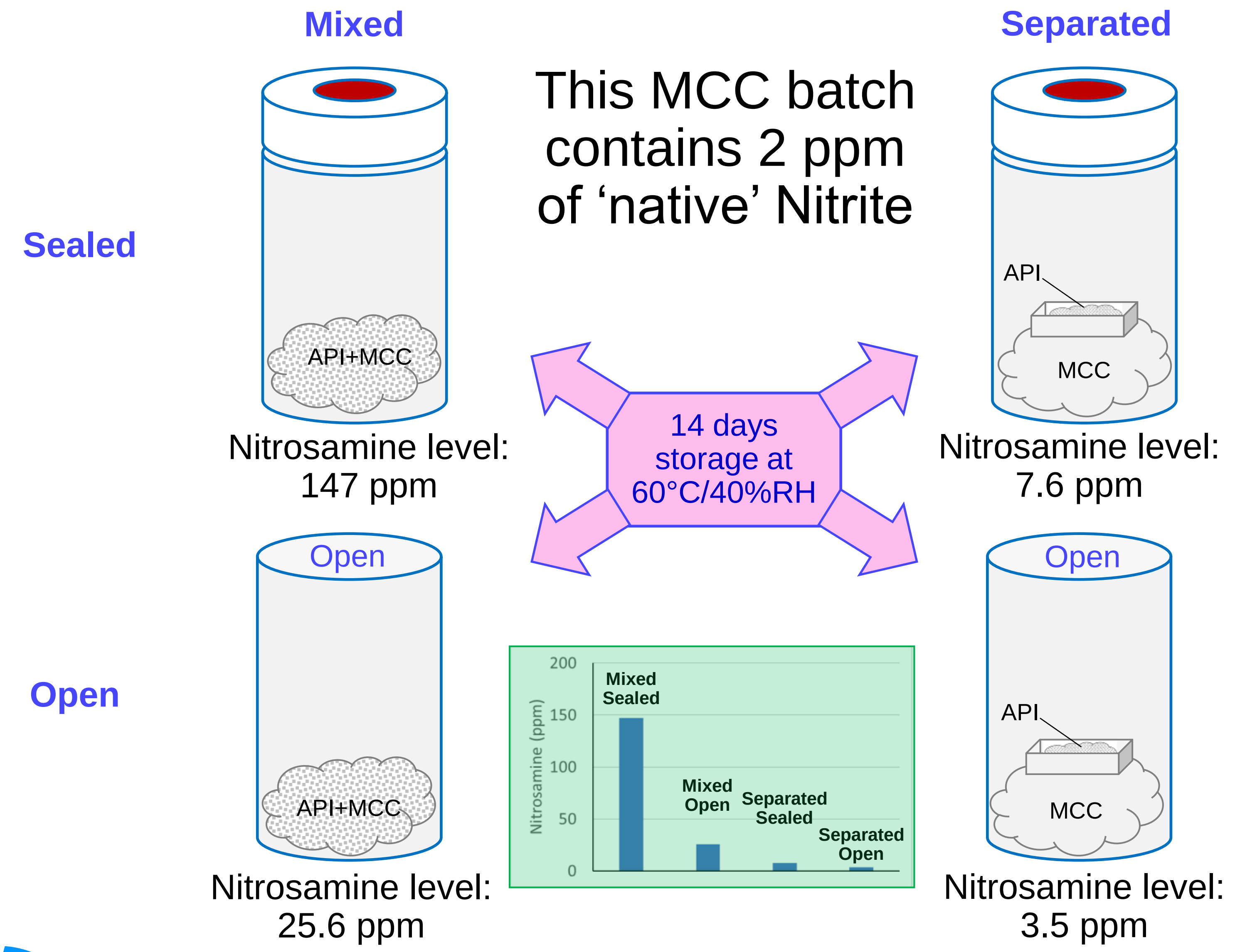
* Conditions have same absolute humidity of 11.6 mg water per litre of air

† Absolute humidity = ~0.6 mg/L

Conclusion: Relative Humidity is Key

Temperature is of secondary importance

The Nature of Nitrites: Nitrosation of API via Volatile Species

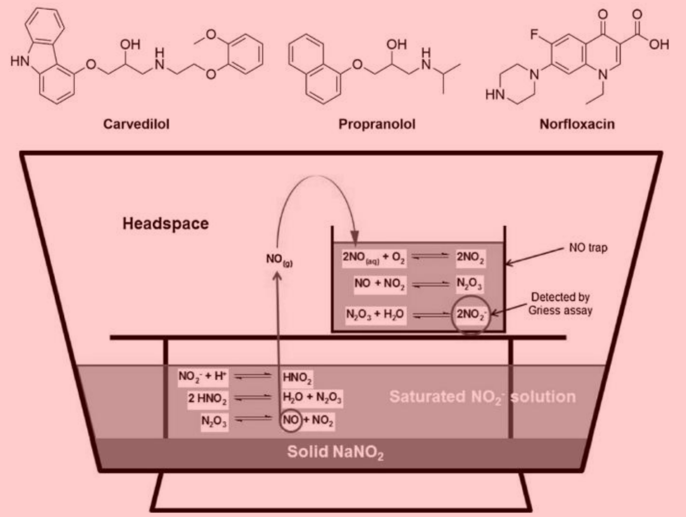


See Also: Journal of Pharmaceutical and Biomedical Analysis, 149 (2018), 206-213

Short communication

Artifactual degradation of secondary amine-containing drugs during accelerated stability testing when saturated sodium nitrite solutions are used for humidity control

Gregory W. Sluggett[a,*], Todd Zelesky[a], Evan M. Hetrick[b], Yelizaveta Babayan[b], Steven W. Baertschi[c]

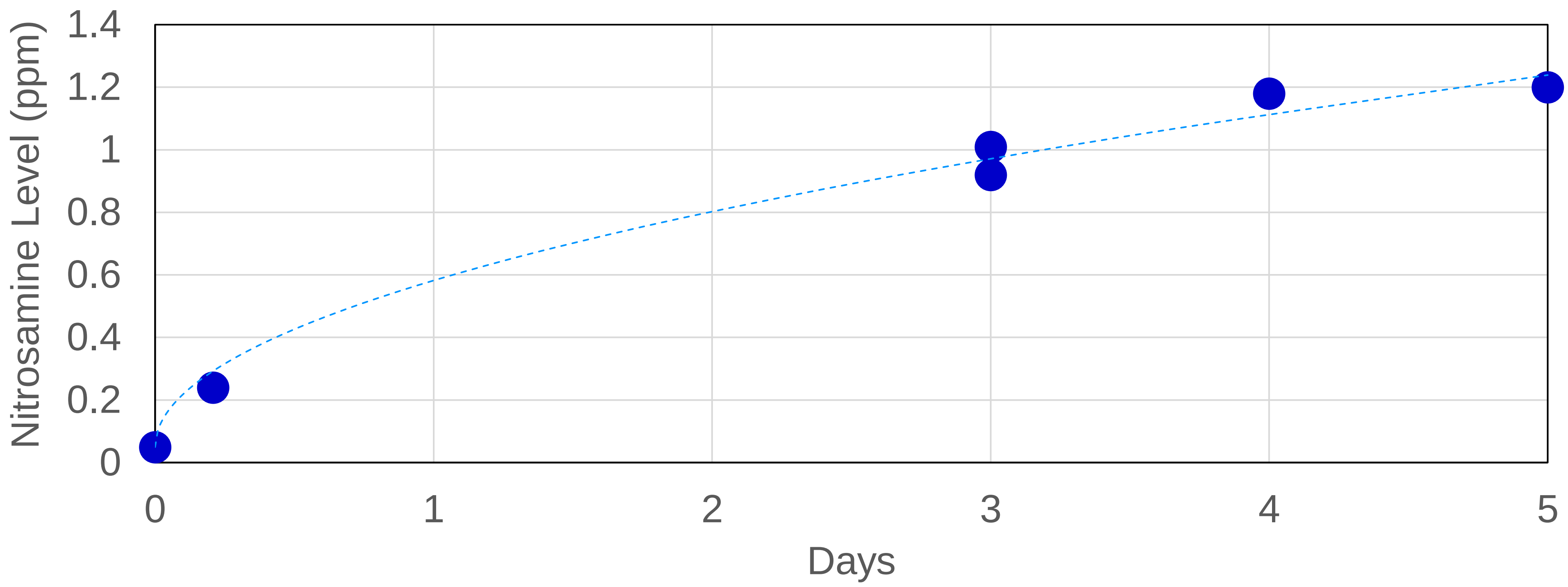


Also: Examples of API forming nitrosamine when dried in an oven also containing an excipient with high nitrite levels

The Nature of Nitrites: Volatile Nitrosating Species: Air Quality (NO_X)

A reactive secondary amine API on open bench

(Chennai Study):



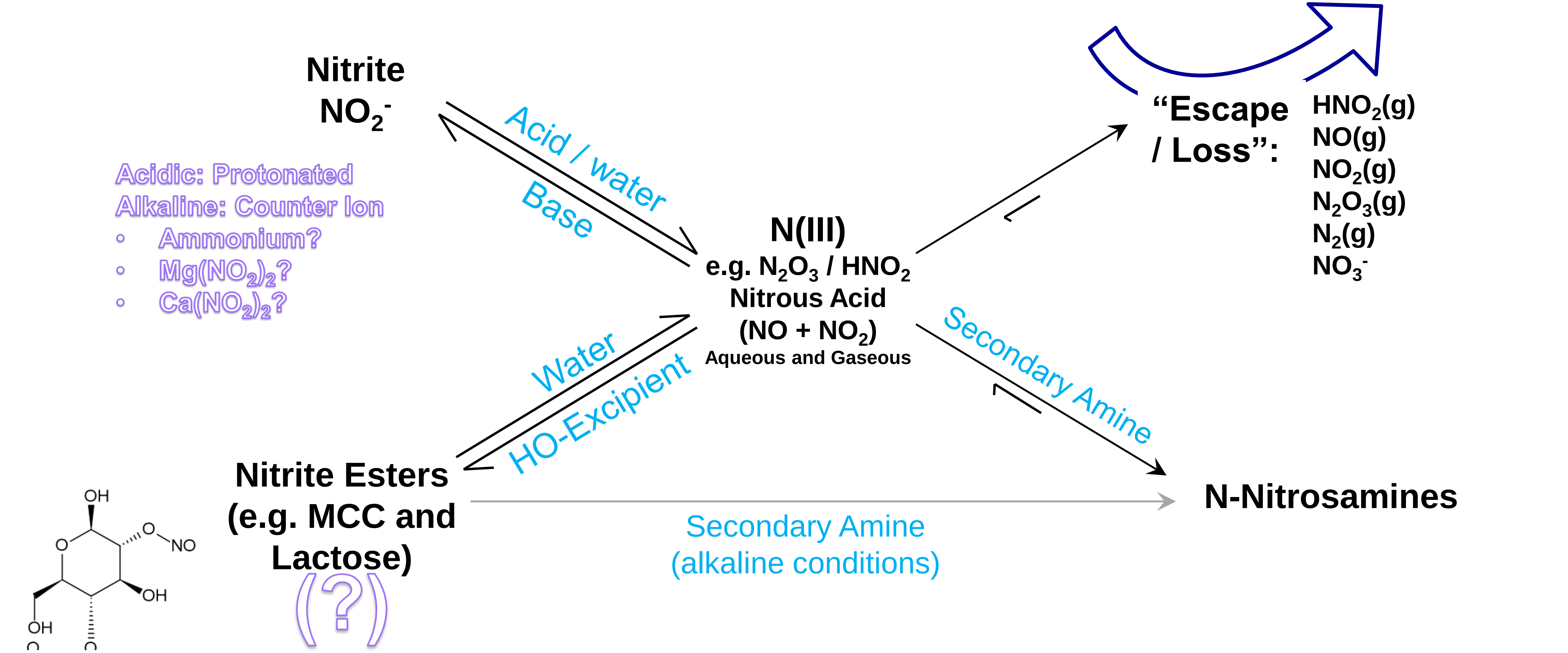
Nitrosamine Formation in Solid State Drug Products

- Mechanisms
- Factors affecting rate and extent of formation (T & RH)

Excipient Environment

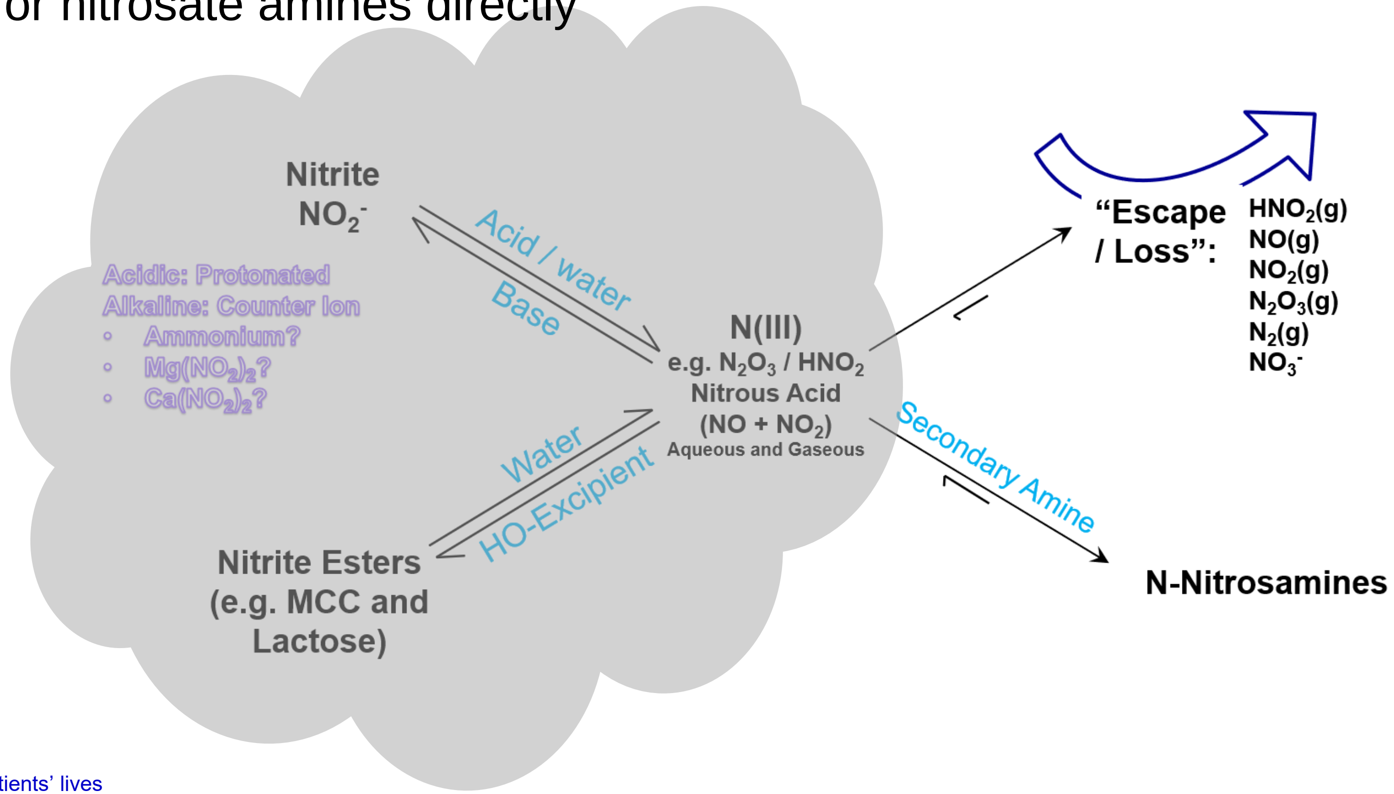
Reactive and Volatile Species

Fate: A Competition Between Loss and Reaction



Nitrite Levels (Analytical Results) vs Nitrosation Potential

- Is all nitrite 'available' to react?
- The "Nitrite" species interconvert:
  - In the aqueous environments of the analytical method, all nitrosating species are probably quantified together as "nitrite"
  - If the "nitrite" is present as (e.g.) nitrite esters in excipient, these could rapidly convert to reactive nitrosating species or nitrosate amines directly



Competing Reactions…and Plateauing Nitrosamine Formation

Reactive and Volatile Nitrosating Species
(“HNO_2”)

→ **“Escape / Loss”**

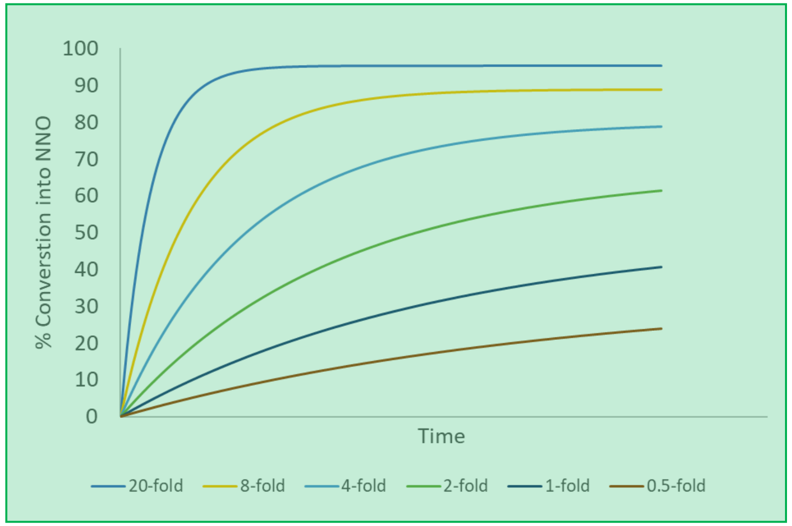
- Airflow: open containers / well ventilated environment

→ *Amine* → **N-Nitrosamines**

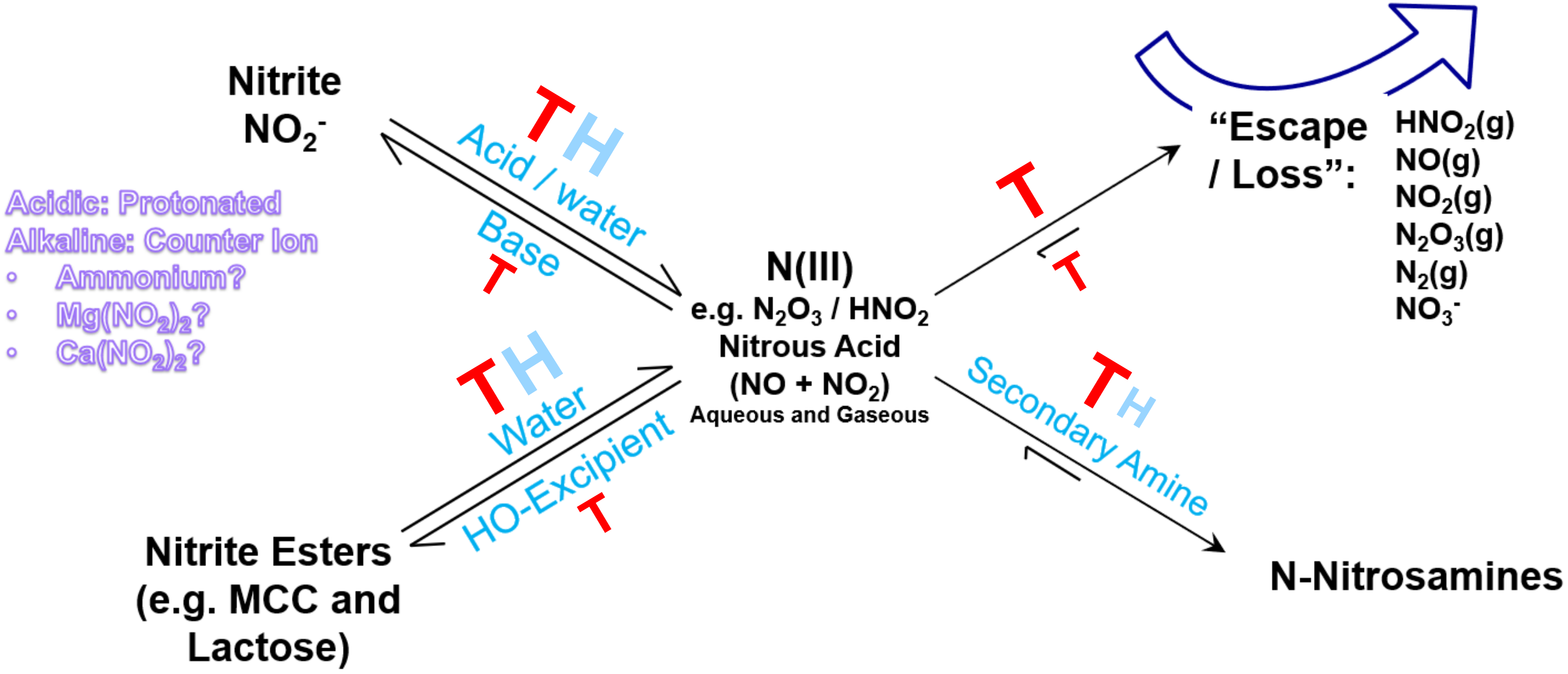
- Sealed containers
- Proximity of amine
- Reactivity of amine
- Concentration of Amine / Drug Load

Plateauing Behaviour is Observed:

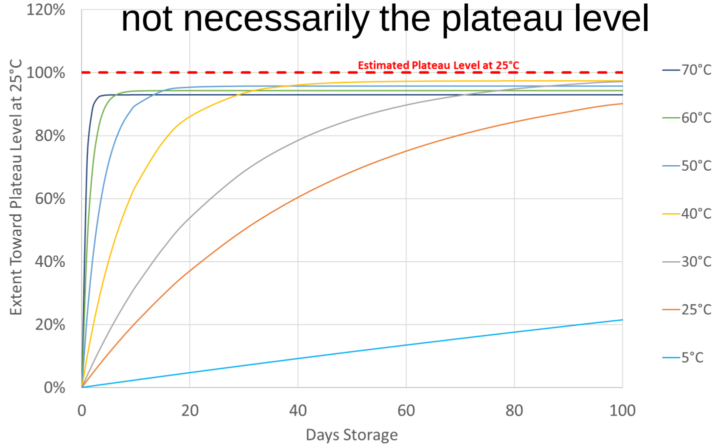
- This is to be expected: finite source of reactive limiting reagent (nitrite) available to the amine
- In the systems we studied, **Plateau Level (PL)** is more important than ‘**rate’** for assessing drug product nitrosamine risk (because PL will typically be reached within <12 months at 25°C/60%RH)
- The observed plateau level is linked to the amount of reactive limiting reagent (nitrite) and the relative rates of “Loss” vs reaction with amine



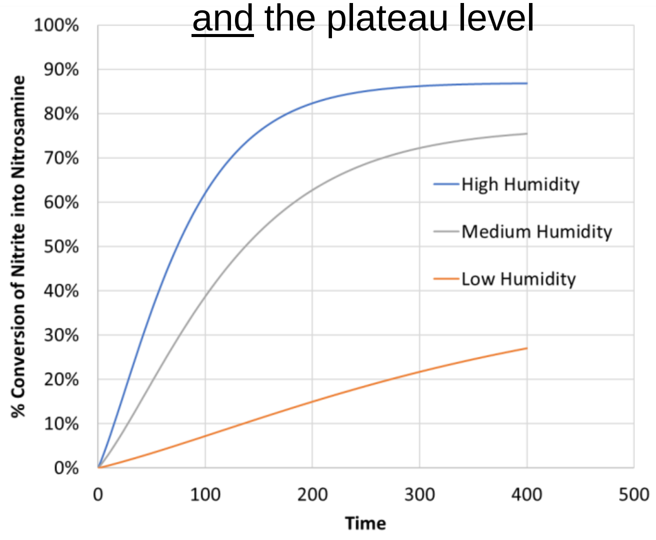
Effect of Temperature and Humidity on Nitrosamine Formation in Solid State



Temperature accelerates rate, but not necessarily the plateau level



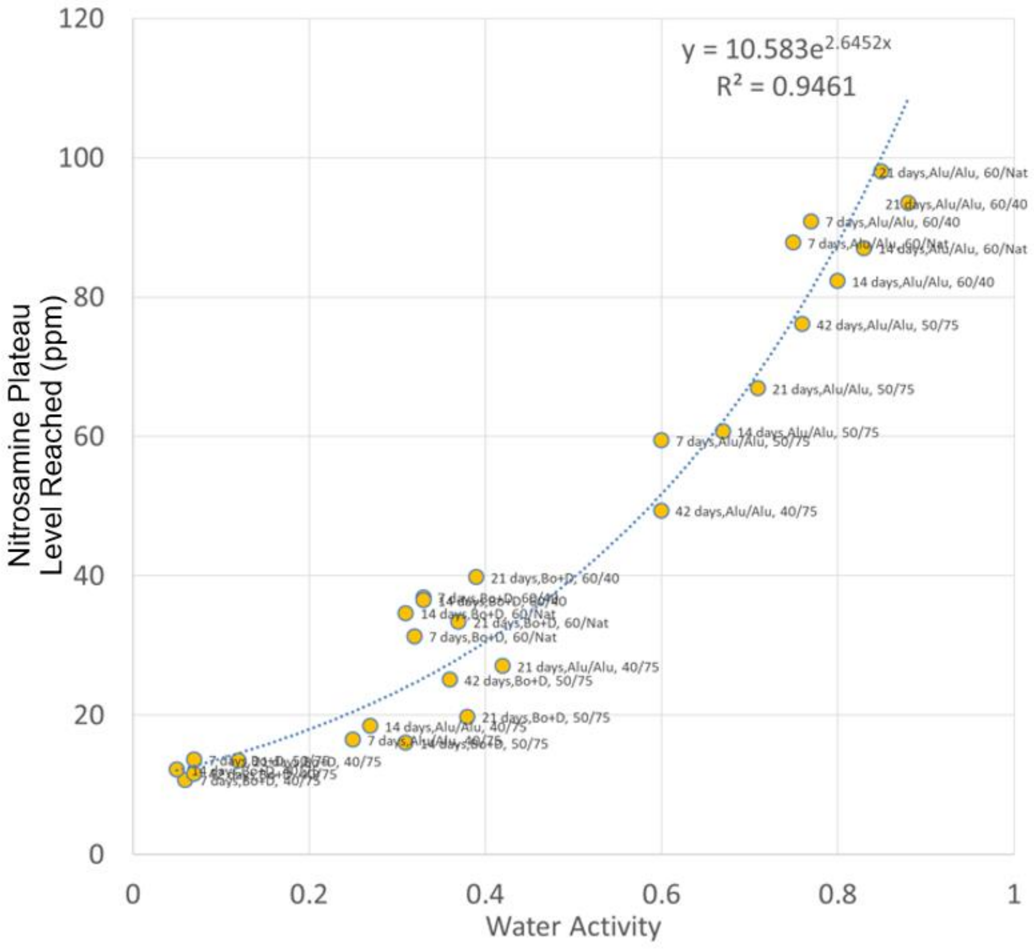
Humidity could affect the rate and the plateau level



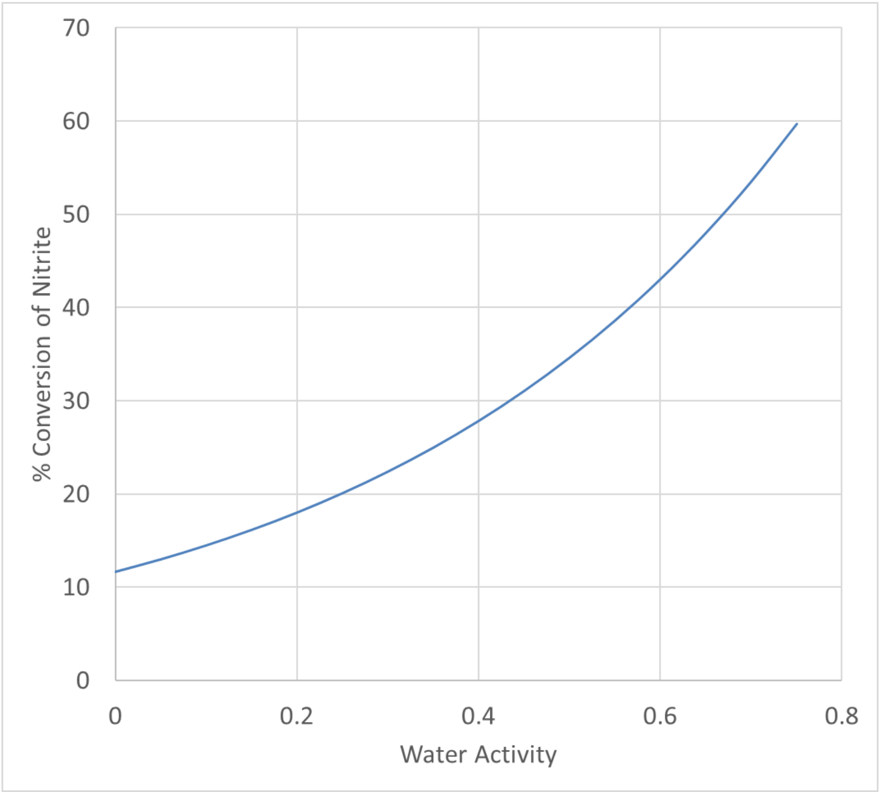
Refrigeration will slow down nitrosamine formation

Dry conditions and the use of desiccants may be a **useful strategy** for inhibiting nitrosamine formation

Nitrosamine - Water Activity (a_w) Trend



Nitrite Levels in Formulation



The observed ‘exponential’ trend between nitrosamine and water activity is reminiscent of the ‘ASAP’ humidity-modified Arrhenius relationship and consistent with the high-level model for humidity (previous slide)

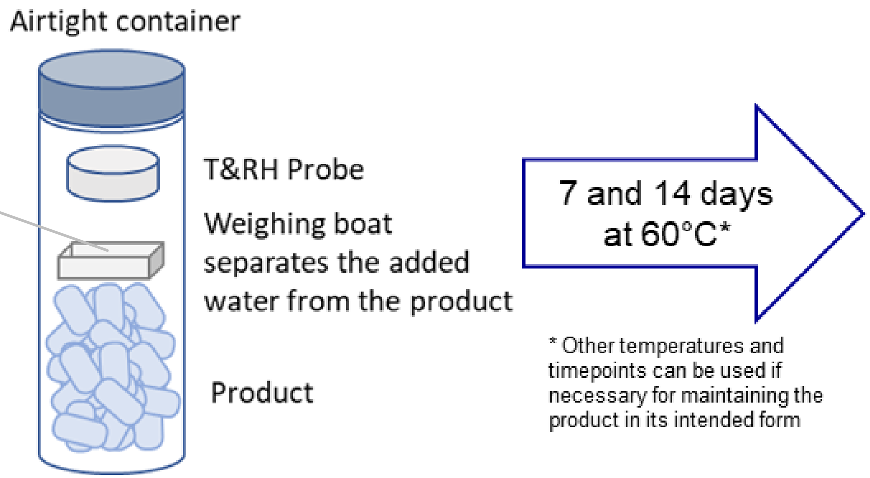
Nitrosamine ("ASAP")

(Accelerated Stability Assessment Protocols)

ASAP Studies

- ASAP studies have been an essential tool for providing a rapid estimation of long-term nitrosamine levels
  - At room temperature, it takes >6 months for the plateau level to be measured/estimated (API Dependent)
- ‘Standard’ ASAP protocols **may not be applicable**
  - The volatility of the reactants mean ‘open’ containers inside humidity-controlled ovens would lead to falsely-low results (as discussed above).
  - The use of saturated salt solutions inside airtight containers to control humidity also may not be suitable (if the salt solution acts as a ‘sink’ for the volatile species – e.g. alkaline salt solutions).
- Sample Handling can affect results
  - Prior storage, ventilation and container material can affect the nitrosamine levels; this extends to manufacturing processes such as film coating

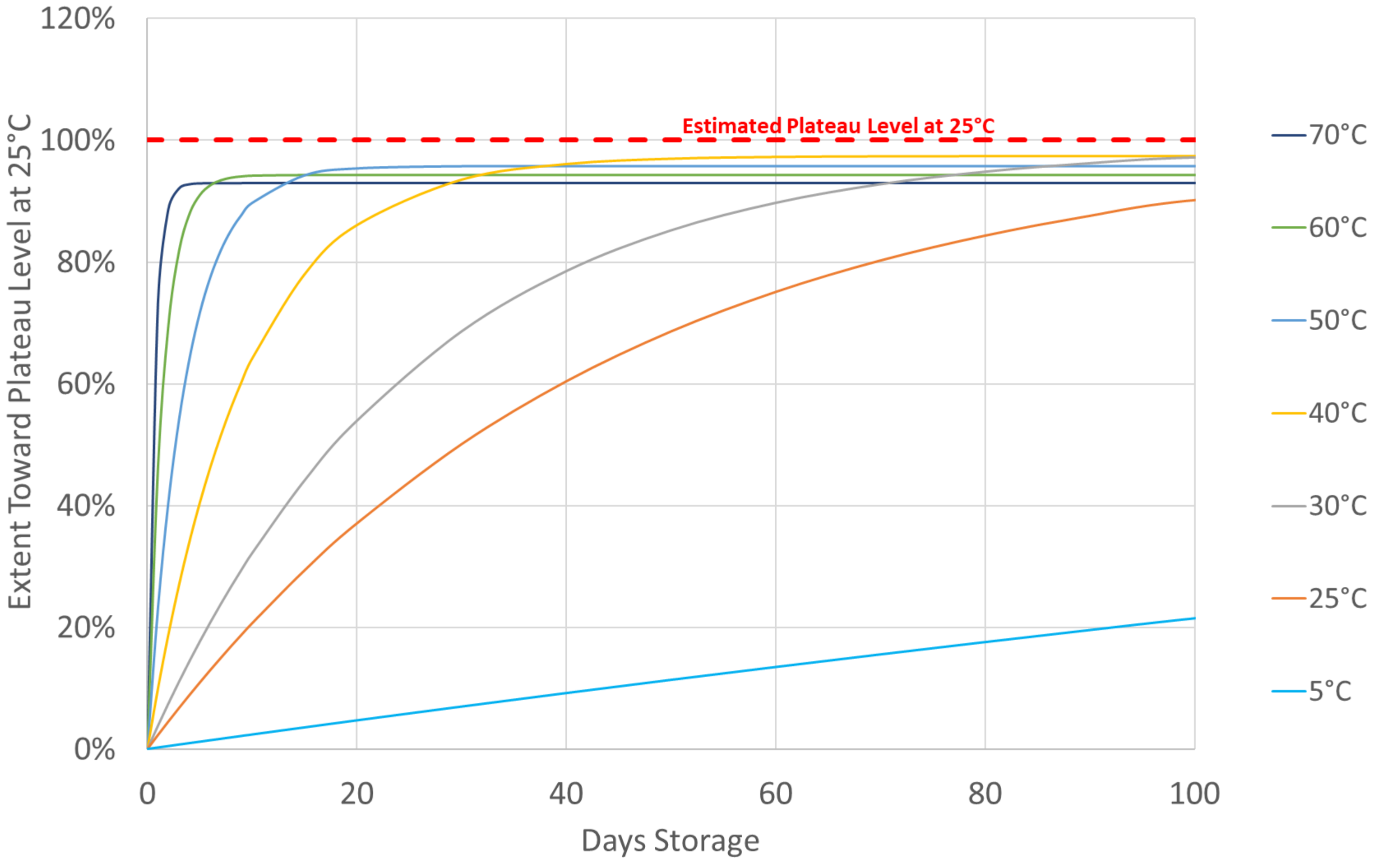
RH in vial can be adjusted by adding very small amounts of water to the weighting boat via a microsyringe. The amount of water added is calculated from the product’s moisture sorption isotherm. The water rapidly disperses amongst product and the resulting RH in vial is verified by an RH probe.



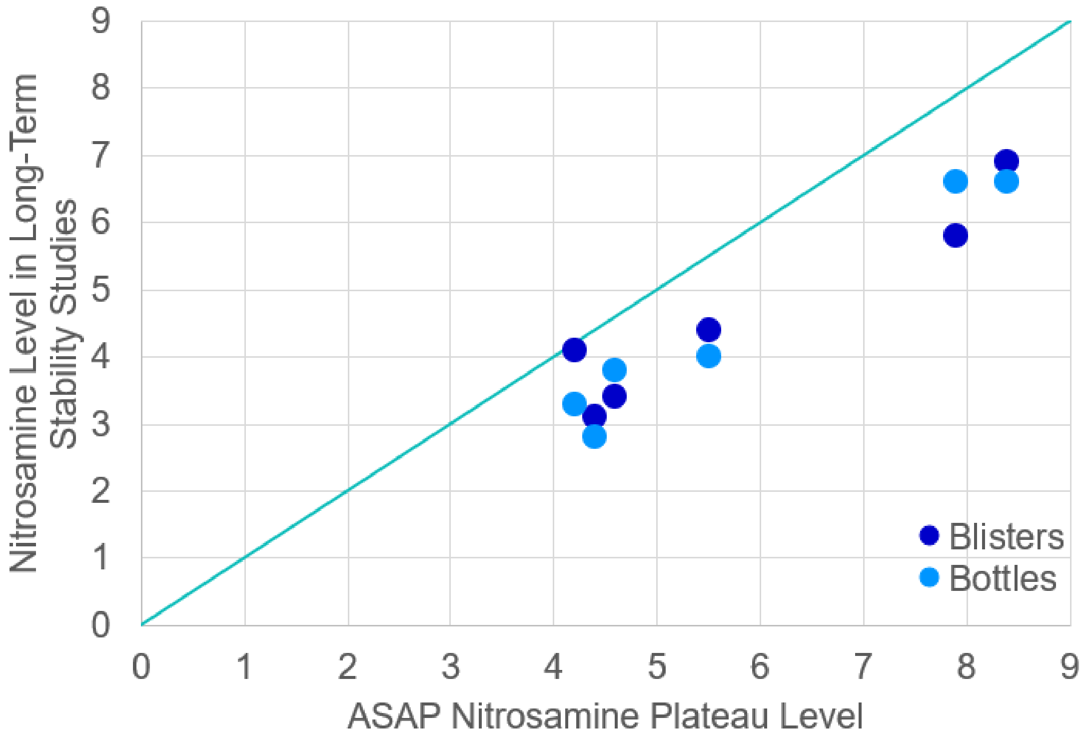
ASAP Studies

Experimental data so far indicate elevated temperatures:

- Accelerate nitrosamine formation
- Do not significantly affect plateau level



ASAP Studies: Comparisons with Long-Term Data:



6 batches:

- All contain antioxidant
- 2 strengths
- Different levels of nitrite across the lots
- 2 types of packaging

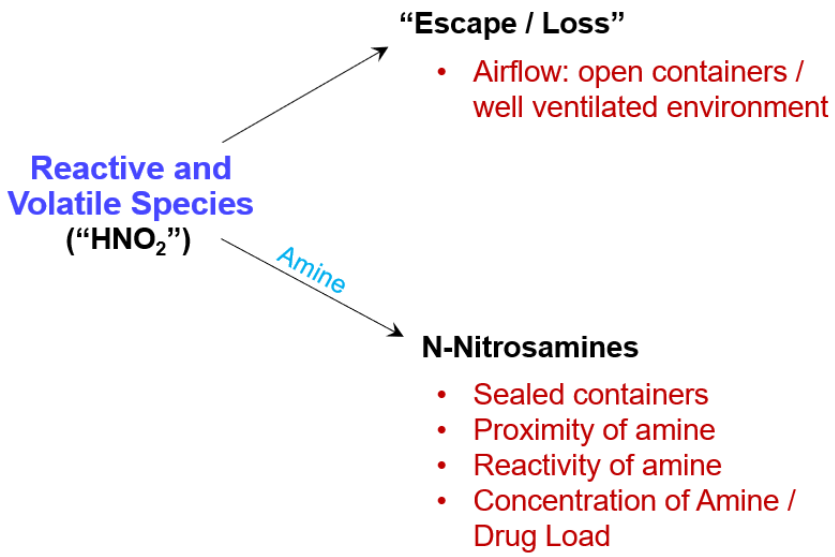
- **ASAP data correlate well with long-term data**
- **ASAP data are consistently 1-2 ppm higher than the long-term data**
  - ⇒ **Provides realistic worst case.**
  - ⇒ **Long-term data may not have fully reached plateau level**
  - ⇒ **The use of a less permeable container in ASAP may prevent loss of volatile nitrosating species**

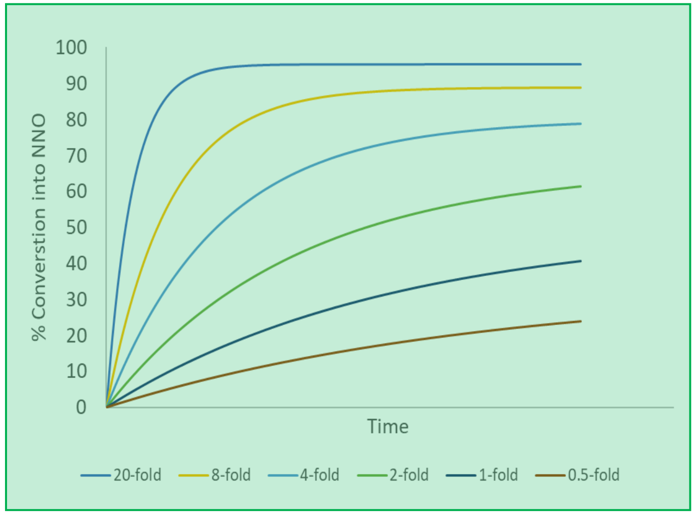
API Reactivity

- Chemical Reactivity
  - pKa
  - Aryl vs Alkyl amines
- API Reactivity Screening
- Salt Form / Solid Form

API Reactivity

- In Solids:
  - API reactivity will affect both rate and extent of nitrosamine formation
  - Additional considerations such as particle size, surface area and degree of disorder are also likely to be important





API Reactivity

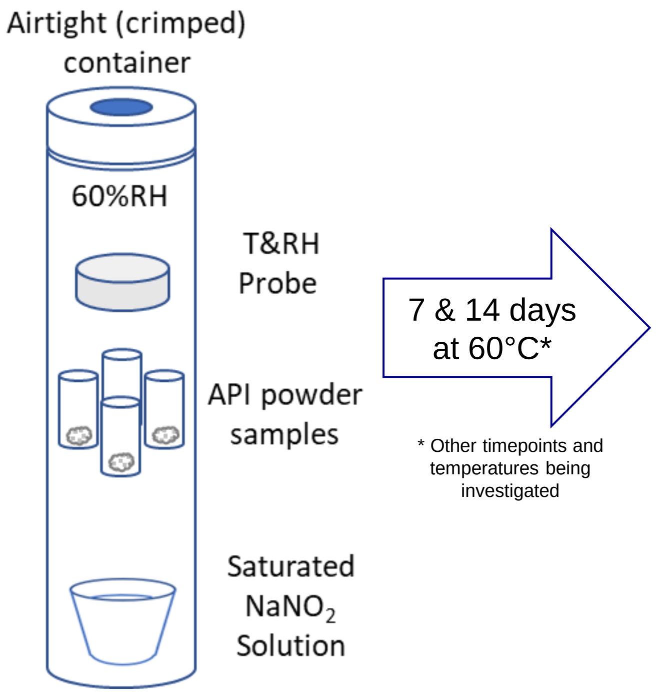
- "NAP" test and "IQ conditions" can be used to assess API potential to form nitrosamines: these are solution-based conditions (rate influenced by pKa, pH, temperature, structure of amine etc.)
- In solids, additional considerations such as **solid form**, **choice of salt / free base**, **particle size**, **surface area** and **degree of disorder** are also **<u>key</u>** in determining API reactivity – these factors are invisible to the solution-based tests.

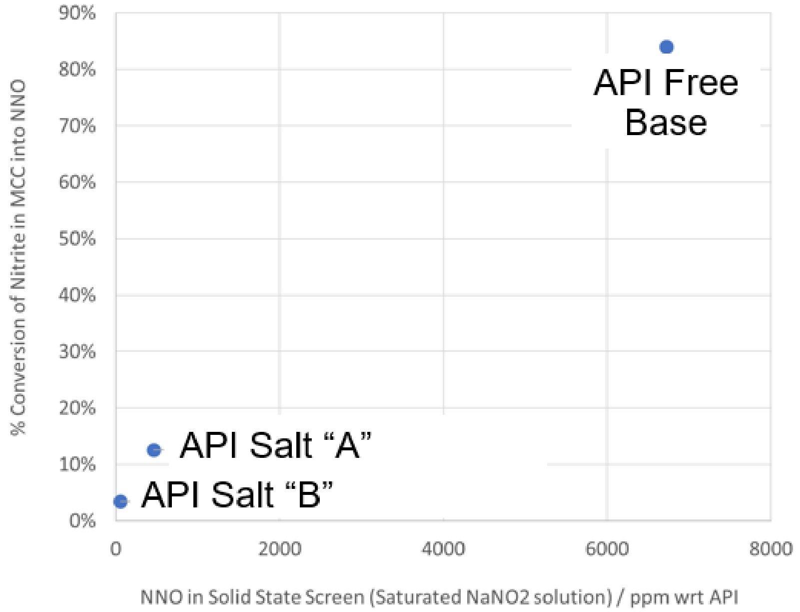
Solid State API Reactivity Screen

Designed to be:

- Simple
- Rapid
- Consistent
- Representative of 'Real World'
- Relevant to <u>Solid State</u> Products

The saturated $NaNO_2$ solution serves two purposes:
- A source of gaseous nitrosating species
- Maintains a constant and standardized relative humidity of 60%RH.



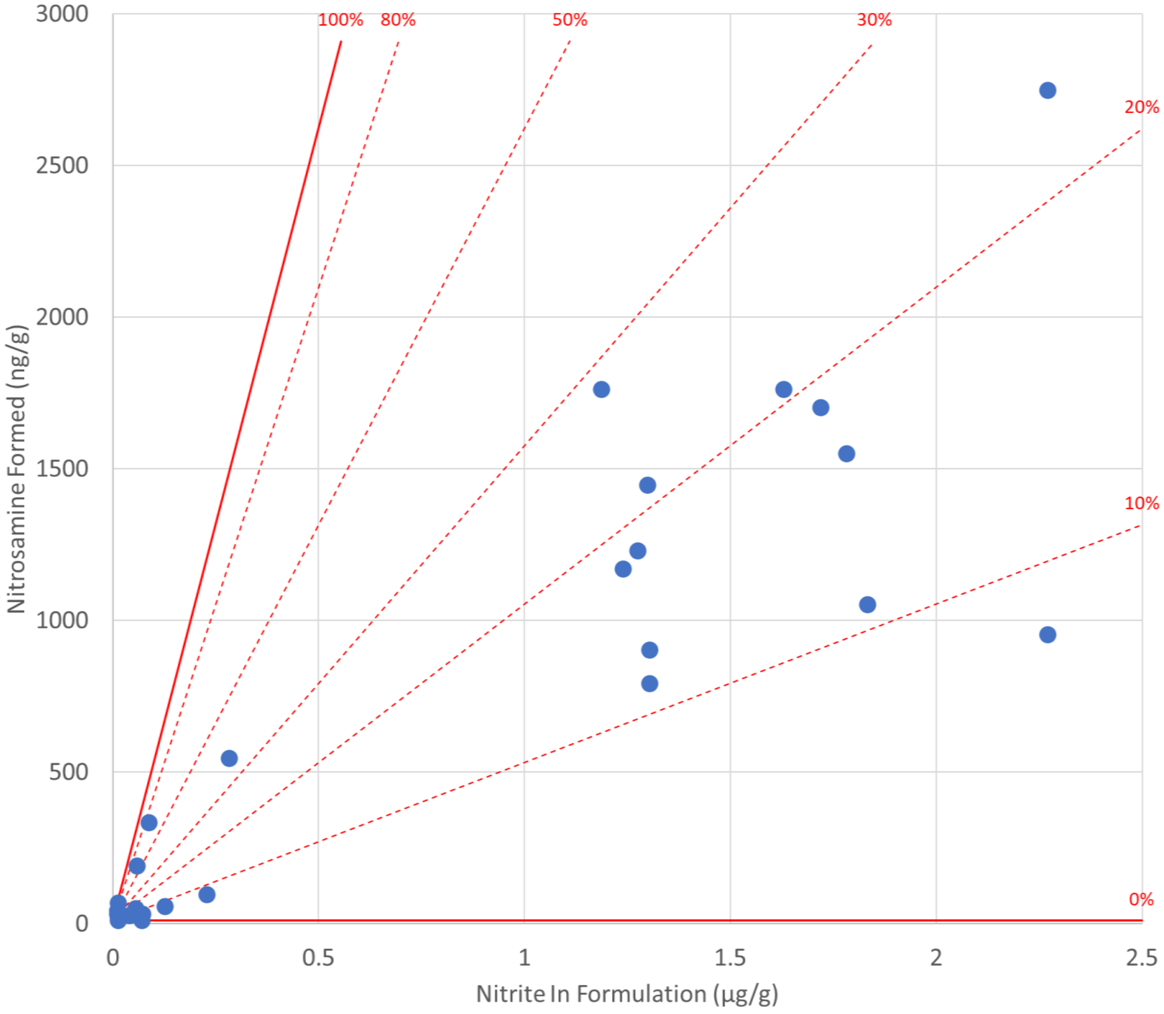


Breakthroughs that change patients' lives

Formulation Investigations

- Nitrites in excipients → nitrosamine levels
- Comparison of API:Excipient binary mixtures with multicomposite formulations
- “Activating” excipients
- (Effect of drug load)

Nitrosation by Individual Excipients (Binary mixtures)



Each datapoint is a different excipient

Nitrosamine Level (y-axis) is the maximum level reached (i.e. plateau level) obtained from accelerated studies

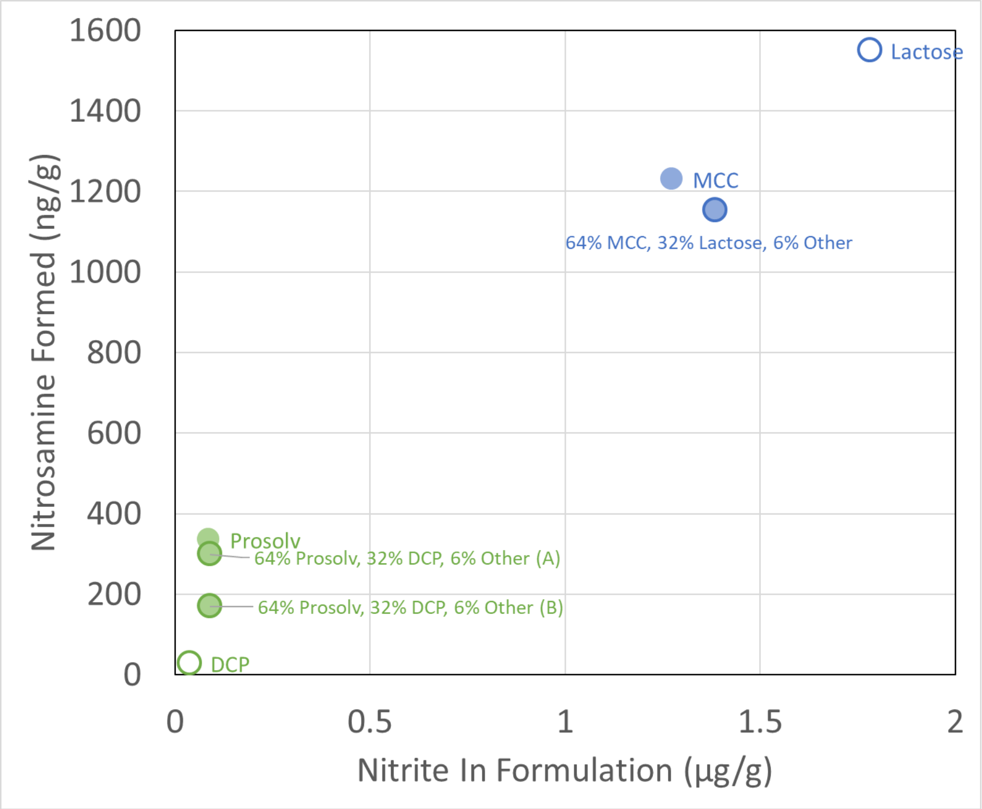
Correlation between ingoing excipient nitrite level and resulting nitrosamine level (R^2) = 0.79

<100% Conversion of nitrite – helpful to know for nitrosamine risk assessments based on nitrite levels

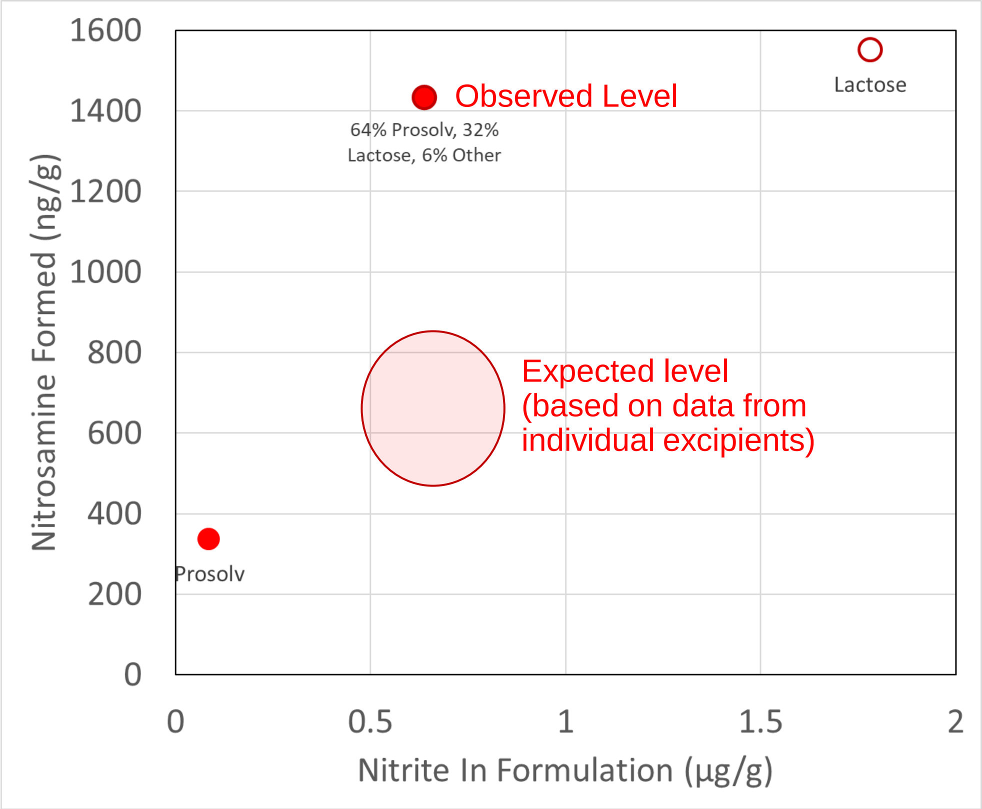
Wide range of nitrite→nitrosamine conversion levels observed

From Binary Mixtures to Multicomponent Mixtures

Multicomponent mixtures may behave 'as expected' based on the ingoing individual excipients (i.e. a linear combination of the amounts expected from binary mixtures):



Nitrosamine levels in multicomponent mixtures are sometimes different from the levels that may be expected from the binary mixtures of ingoing individual excipients



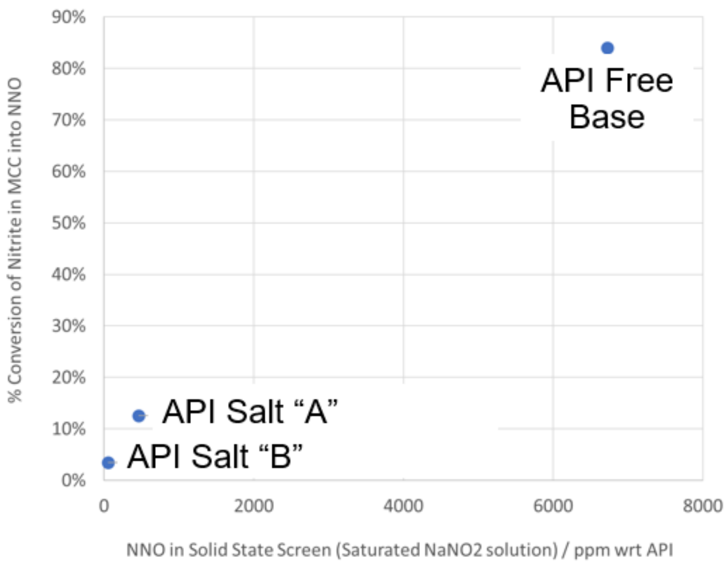
Why?

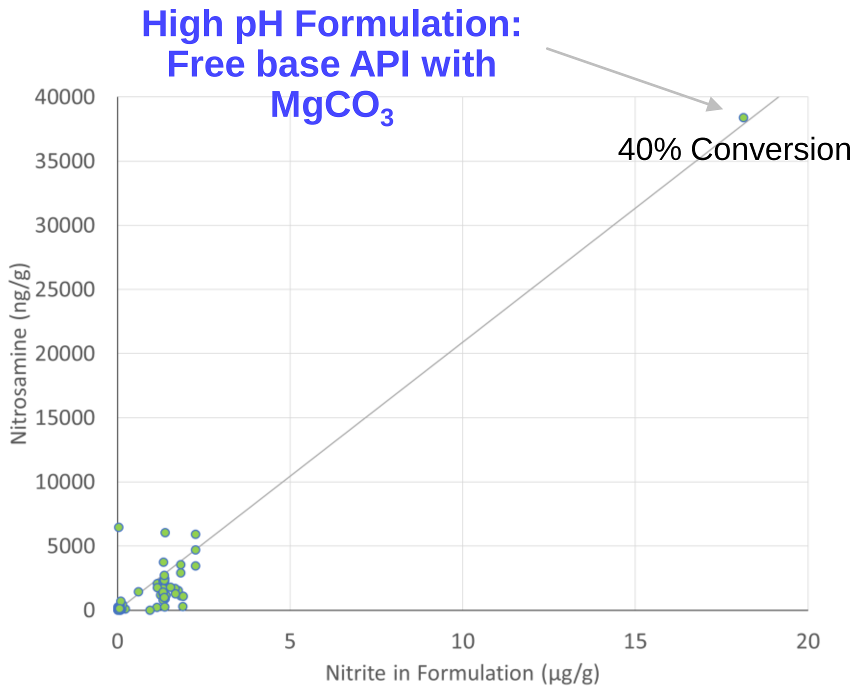
- Localized pH? E.g. acidity of Prosolv (SiO_2) activates nitrites in lactose?
- Water activity differences?
- Minor components ("other") may be ionic (=> interact with API salt)?
- Excipients with different particle sizes, surface areas and nitrite "availabilities"?

High pH formulations for controlling nitrosamines

May be effective for solution-phase products

For solid dosage forms…?





- ‘Alkaline’ excipients may have high nitrite levels (may trap ‘NOx’ as nitrite)
- Non-protonated amines (i.e. free base) more readily form nitrosamines than salts
- Mixing ‘alkaline’ excipients with API-salts could lead to salt disproportionation

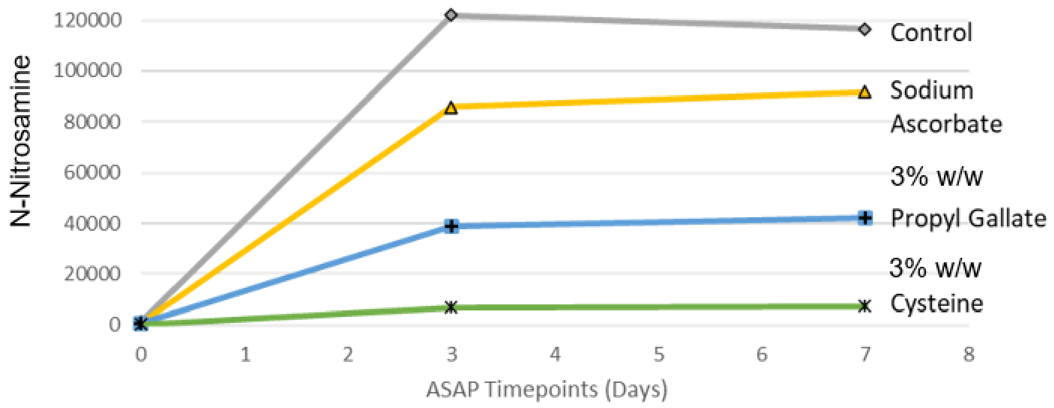
Summary: Reducing Potential for Nitrosamine Formation in Drug Products

- Antioxidants
- Low Nitrite Excipients and Suppliers
- Desiccants
- API Salt Selection
- Other Strategies

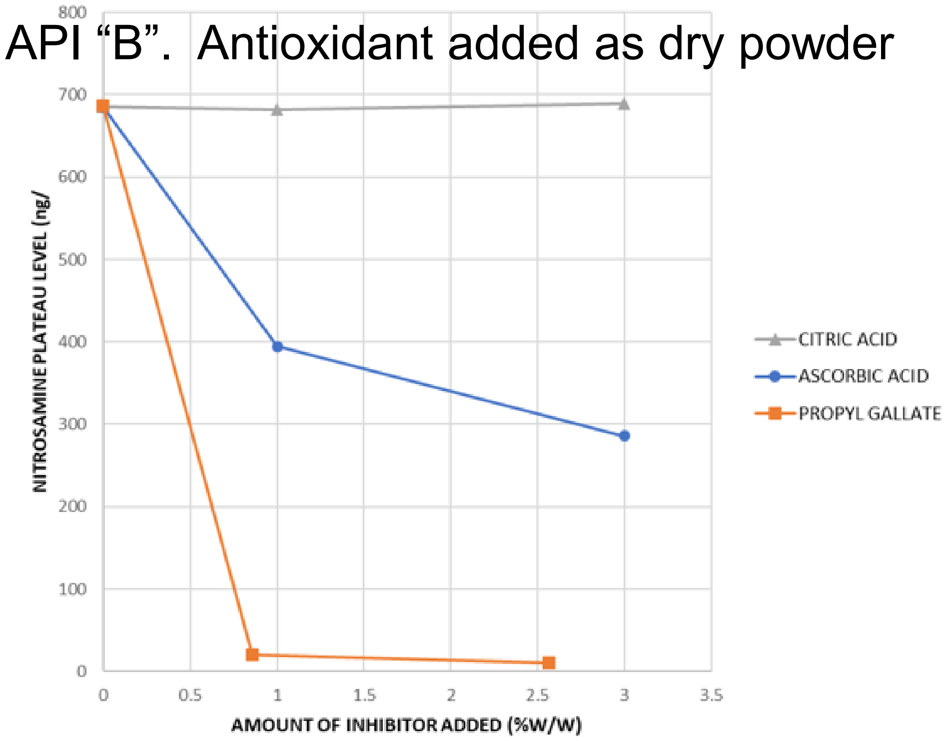
Antioxidants / Inhibitors

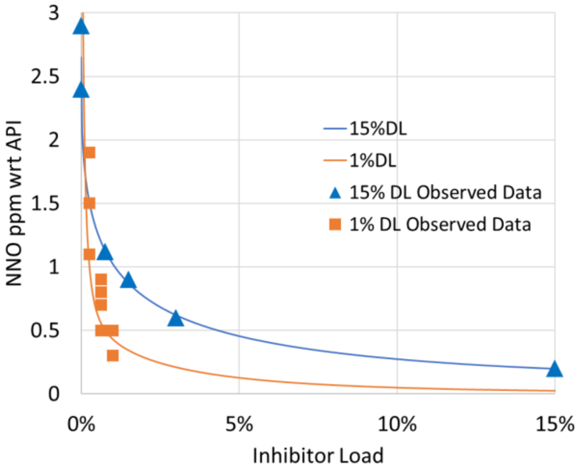
- The effectiveness of the antioxidant is API dependent
- Caution: discolouration may be an issue with antioxidants

API "A". Antioxidant added as dry powder



API "B". Antioxidant added as dry powder





API "B": nitrosamine levels at different drug loads and propyl gallate loads are consistent with a 'proximity' model

Summary

Progress in Understanding Mechanisms:

- Relationship between nitrite and nitrosamine levels
- Not all amines nitrosate. Nitrosation tests have important role in understanding reactivity.
- Volatility, mobility and reactivity of nitrites and nitrosating species
- Factors that affect %conversion of nitrite into nitrosamines: API reactivity, drug load, humidity, temperature, activating excipients
- Differences between solid and solution-phase dosage forms

Nitrosamine Reduction:

- Low nitrite drug product platforms: low nitrite excipients
- Desiccants
- Antioxidants
- API salt and solid form selection (evaluated by solid-state nitrosation test)
- Processes for reducing nitrite levels in excipients

Thank You For Your Attention

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