Fachhochschule Nordwestschweiz
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Nitrosamine formation and scavenging in drug products

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Bachelor-Thesis, field of studies Pharmatechnology

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Research Context

Nitrosamines are considered potential mutagens in the absence of toxicological data and need to be controlled in nanogram quantities in medicinal products. In the case that a medicinal product contains a primary source of vulnerable amines, it would be desirable to have an inhibitor (nitrite scavenger) of nitrosamine formation that can be added to a formulation. Wet granulation has been identified as a critical process step in drug manufacture, this was simulated in this work. [1], [2], [3], [4]

The nitrosamine formation was monitored using two systems. On the one hand, an HPLC UV/VIS analysis was implemented to monitor the nitrite level, on the other hand the nitrosamine content was detected using a Triple Quadrupole LC/MS analysis. The rate constant of nitrosation was obtained by regression of measured nitrite concentrations.

Based on the results, the nitrite decomposition in water at different pH and temperature should be modelled in a mathematical model like MatLab.

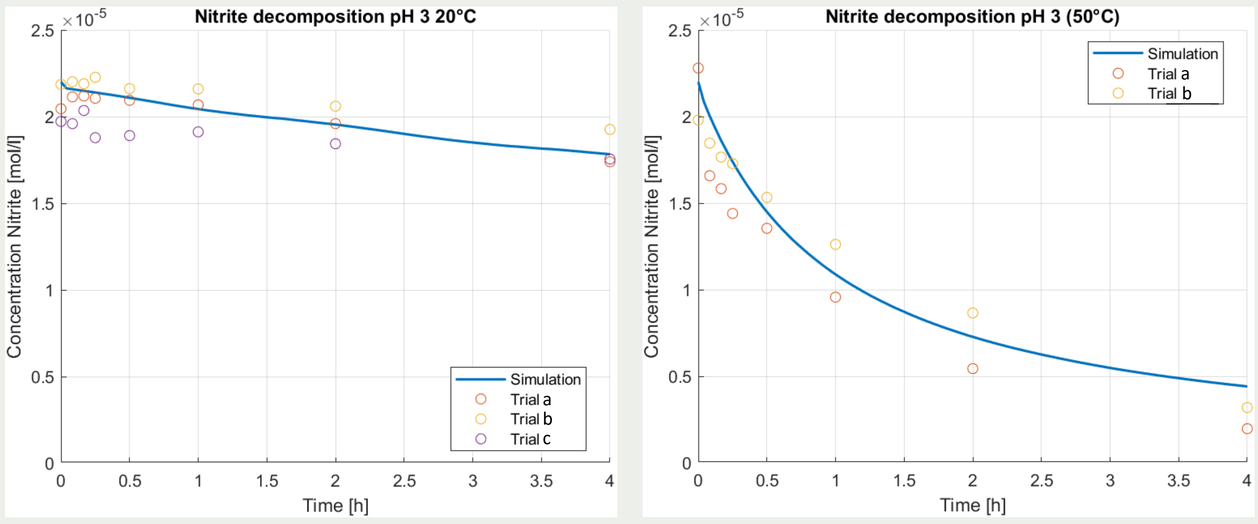
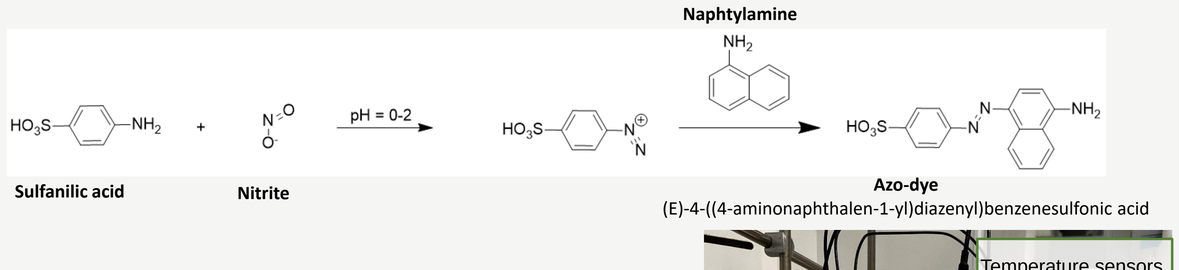


Figure 4 + 5: Mathematic model of Nitrite decomposition at pH3 and 20 °C / 50 °C, fitted by measured data and Arrhenius equation. Created by MatLab.

Methoden

Nitrite Analytics: HPLC UV/VIS (530 nm)



Lab work

- Aqueous solutions
  - Nitrite
  - Nitrite & scavenger
  - Nitrite & amine
  - Nitrite, amine & scavenger
- Nitrite concentration 1 ppm (once 10 ppm)
- Adjust pH with H_2SO_4 or NaOH
- Ascorbic acid 1:20 molar (once 1:2 molar)
- Temperature 20 °C / 50 °C
- Times to take samples [min]
  0 / 5 / 10 / 15 / 30 / 60 / 120 / 240

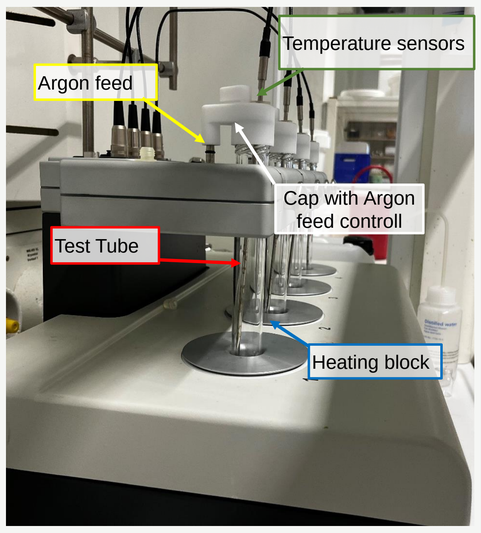


Figure 1: MYA 4 reaction station, closed system

Nitrosamine checked by Triple Quadrupole LC/MS
Mathematical model design the system by MatLab

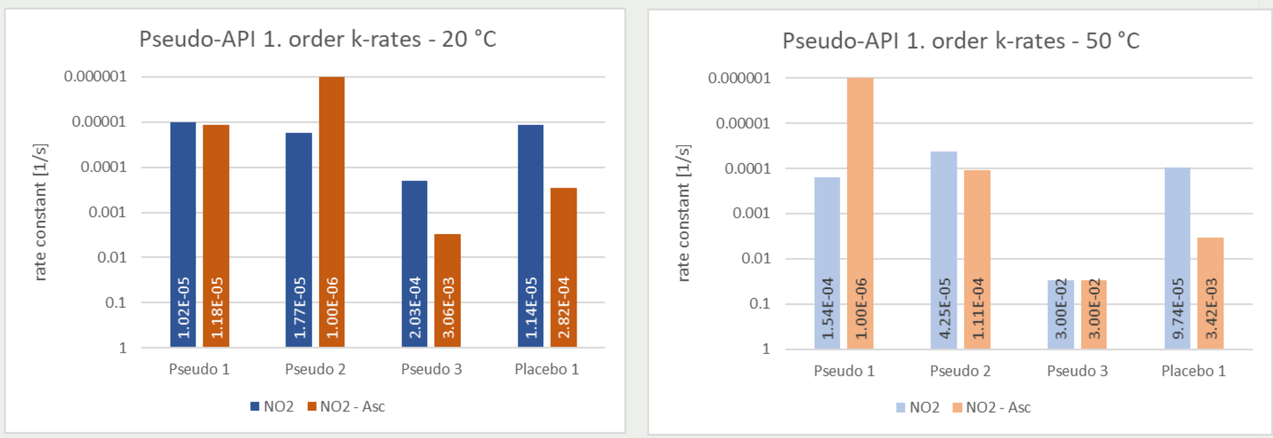


Figure 2: Pseudo-API 1. order k-rates at pH 3 and 20 °C or 50 °C; linearized in Excel from nitrite concentration measured by HPLC UV/VIS method linearized in Excel; ((*Pseudo-API 1* - (4-Methyl-piperazin-1-yl)-acetic acid; *Pseudo-API 2* - 2-(piperazin-1-yl)acetic acid; *Pseudo-API 3* - 3-Dimethylamino-benzoic acid; *Placebo* - Bezoic Acid)

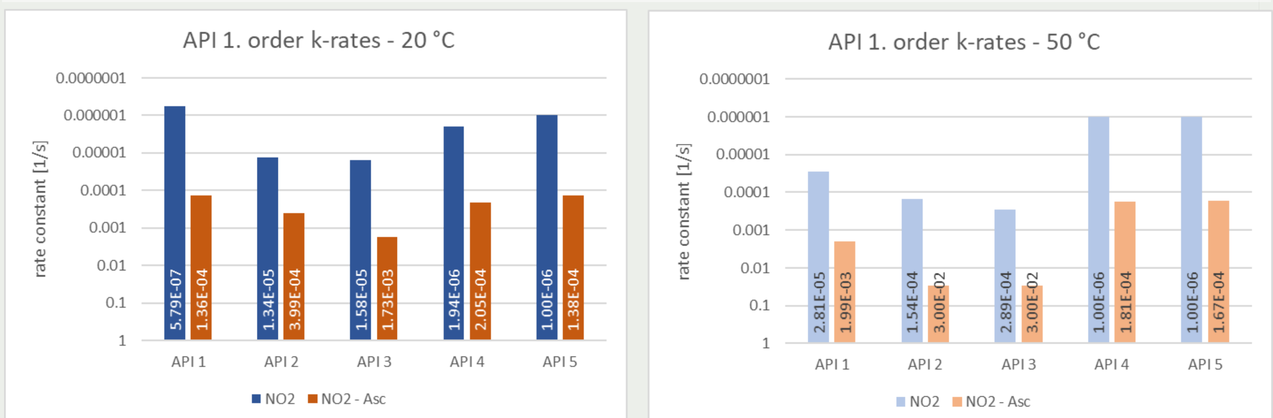


Figure 3: API 1.order k-rates without adjusted pH and 20 °C or 50 °C; linearized in Excel from nitrite concentration measured by HPLC UV/VIS method linearized in excel; (*API 1* – Lapatinib; *API 2* – Terbinafine; *API 3* – Cetirizine; *API 4* – Tamsulosin; *API 5* – Salbutamol)

Results & Conclusion

The HPLC UV/VIS method was chosen for the quantitative determination of the nitrite content. The linear trend line is in the range of 2 - 0.0006 ppm nitrite. A dilution series showed a variance of 99.99 % after 3 days, which is a very good value. Thus, one has sufficient time to analyze the samples after preparation of the dye. The HPLC UV/VIS method has been developed for the detection of nitrite, which is simple, sensitive, robust, rapid and inexpensive.

For Lapatinib (API 1) and Cetirizine (API 3), ascorbic acid is a suitable scavenger, this could be shown with both kinetics and nitrosamine measurements. For Tamsulosin (API 4), 2-piperazin-1-yl acetic acid (Pseudo-API 2) and 3-dimethylamino-benzoic acid (Pseudo-API 3), ascorbic acid is not a suitable scavenger, at least at this concentration. Salbutamol (API 5), Terbinafine (API 2) and 4-methyl-piperazin-1-yl-acetic acid (Pseudo API 1) did not form nitrosamines during the studies. This means that nitrosamine formation is very slow in these structures. The scavenging effect could therefore not be proven. Nevertheless, it can be assumed that the addition of ascorbic acid does not form more nitrosamines than without it, and therefore the addition of ascorbic acid can be considered.

In addition, the pH of the solution is a decisive factor for nitrosation. For example, it has been shown that 2-piperazin-1-yl acetic acid (Pseudo-API 2) formed up to 80 ppm of nitrosamine at pH 3, while no nitrosamine was formed without adjusting the pH (7.23).

In general, ascorbic acid can be a suitable scavenger, but it is not suitable for all amines. If ascorbic acid is not a suitable scavenger, others can be tested. For example, L-cysteine, which has a much faster scavenging effect, but also has disadvantages, such as the unpleasant odor (rotten eggs).

The model for the decomposition of nitrite was successfully implemented. The rate constant was fitted with the measured k-rate. On this basis, the values at 50 °C could also be successfully simulated using the Arrhenius equation. In MatLab, the modeling of the scavenging and nitrosation still need to be completed. Modeling in other mathematical modelling Systems can now be started.

In conclusion, a suggestion / idea for a simple test to quickly determine if ascorbic acid is suitable as a scavenger. In an early development stage of the formulation, it can be checked if ascorbic acid is suitable. Two formulations are prepared, one with an excess of ascorbic acid and one without scavenger. Using a suitable LC/MS method, it can then be checked whether nitrosamines have formed and, if so, whether ascorbic acid has influenced their formation.

Literature

[1] Bundesinstitut für Arzneimittel und Medizinprodukte (2018); https://www.bfarm.de/SharedDocs/Pressemitteilungen/DE/2018/pm5-2018.html?nn=933556, zuletzt geprüft am 15.08.2022.
[2] Swissmedic (2020): Kampf gegen Nitrosamine; https://www.swissmedic.ch/swissmedic/de/home/ueber-uns/publikationen/visible/swissmedic-visible-june-2020.spa.v1.app/de/kampf-gegen-nitrosamine.htm
[3] Snodin, David J.; Elder, David P. (2019): Short commentary on NDMA (N-nitrosodimethylamine) contamination of valsartan products; DOI: 10.1016/j.yrtph.2019.01.007.
[4] IPEC Federation (March 2022): The Role of Excipients in Determining N-Nitrosamine Risks for Drug Products. IPEC Position Paper. Brussels, Belgium.

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