

Interactive comment on

## **“Light-induced protein nitration and degradation with HONO emission”**

by Hannah Meusel et al.

### **Anonymous Referee #1**

#### **General Comments.**

*This manuscript reports results of a study aimed at investigating photochemical formation of HONO from proteins exposed to NO<sub>2</sub>. The study employs coated wall flow tube techniques with LOPAP detection of HONO and chemiluminescence detection of NO<sub>2</sub>. The methods are appropriate for such a study and the results appear to meet the standards required by ACP. The topic is important as it addresses the byproducts associated with light-induced nitration of protein aerosols (e.g., pollen and other biological aerosols); it is novel in that it attempts to address the photochemical fate of the nitrated proteins.*

*The relevance of protein nitration to the potency of allergens has been discussed in several publications, so that is clear. However, it is not so clear that nitrated proteins will be an important component of the daytime HONO budget since proteinaceous aerosols would constitute only a minor fraction of the total aerosol surface area in the atmosphere. Furthermore, strong evidence has recently surfaced showing that the daytime HONO source is not linked to NO<sub>2</sub> (see Pusede et al. Environ. Sci. Technol. 2016). In addition, there are limited situations where the aerosol phase has proved to have an impact on atmospheric HONO concentrations. Perhaps the authors could add a more extensive discussion of settings where they predict this chemistry to be important? Regardless, it is my opinion that the chemistry presented is interesting enough to warrant publication after these issues are addressed.*

#### **Response:**

Pusede et al., 2015 (Environ. Sci. Technol., 49, 12774-12781, 2015) observed no significant weekday-weekend difference in HONO levels during daytime, while NO<sub>2</sub> levels changed significantly during weekday-weekend and concluded that HONO didn't derive from NO<sub>2</sub>. Several studies didn't find correlations with NO<sub>2</sub>, but much more publications see a correlation with NO<sub>2</sub> and an enhanced correlation with NO<sub>2</sub>\*J (e.g. Costabile et al., 2010; Spataro et al., 2013; Sörgel et al., 2011 + 2015; Su et al., 2008; Lee et al., 2016), both being in line with our observations presented in here. The absence of NO<sub>2</sub>-HONO correlation does not exclude the involvement of NO<sub>2</sub> conversion in HONO. Whatever the detailed mechanism is, there are many complex processes involved in aerosol particles and on the ground surface that could lead to a highly non-linear dependence on NO<sub>2</sub> in both concentration and time domains (HONO precursors may be stored in reservoirs, both in the physical and chemical senses). Besides heterogeneous photochemistry on aerosols also heterogeneous photoenhanced NO<sub>2</sub> conversion on ground surfaces has been proposed (Ren et al., 2011; Laufs et al., 2017). As proteins are found in both aerosol particles (coarse and fine mode) as well as on most ground surfaces (soil, leaf etc.), we think that their widespread occurrence provides reasonable justification to have a closer look into the characteristics of their HONO emissions. Indeed, we agree with the referee that pinning down their impact in individual settings is crucial, but for the time being, too uncertain to make a strong statement in here.

**Comment:**

Page 1, line 20: The authors write that “nitration degrees of about 1% were derived applying NO<sub>2</sub> concentrations...” How was the nitration degree determined?

**Response:**

The nitration degree is defined as the concentration of nitrated tyrosine divided by the concentration of all tyrosine residues. As it is written in the method part (2.1.), the nitration degrees were determined by HPLC-DAD analysis. Nitrated tyrosine residues were detected at 357 nm (and 280 nm) while tyrosine residues were detected at 280 nm only.

The respective section in the method part of the manuscript (page 4 lines 13-15) was slightly modified: “Absorbance was monitored at wavelengths of 280 (tyrosine) and 357 nm (nitrotyrosine). The sample injection volume was 10-30 µL. Each chromatographic run was repeated three times. The protein nitration degree, which is defined as the ratio of nitrated tyrosine to all tyrosine residues, was determined by the method of Selzle et al. (2013). Native and un-treated BSA did not show any degree of nitration. “

**Comment:**

1, 21: The term “Gas exchange measurements of TNM-nitrated proteins” is ambiguous.

**Response:**

Now corrected in the manuscript (page 1, lines 21-22) to: “Measurements of gas exchange on TNM-nitrated proteins...”

**Comment:**

1, 23: The term “fumigation” is not appropriate here. Please replace.

**Response:**

Now corrected to “NO<sub>2</sub> exposure...”

**Comment:**

3, 22-24: I note that nitrated ovalbumin (OVA) was used in only one experiment in this study (section 3.2.1) while bovine serum albumin (BSA) was used for everything else. Ideally, one would use one protein for all the studies to facilitate comparison of results. Please explain why one protein was not used for everything.

**Response:**

Unfortunately, only nitrated OVA but no nitrated BSA was available from our partner.

**Comment:**

3, 32: The methods section indicates that tetranitromethane is used to nitrate the OVA samples. This is a highly toxic and explosive reagent. Appropriate warnings should be included in this section to bring awareness of the dangers of using this reagent to anyone wishing to repeat these experiments.

**Response:**

Although in most other publications safety notes/warnings of toxic chemicals are not mentioned, we acknowledge the advice and now added a respective note in the manuscript (page 3, line 34-35):

“Please note that TNM is toxic if swallowed, can cause skin, eye and respiration irritation, is suspected to cause cancer and causes fire or explosion.”

**Comment:**

9, 33 (and other places in the text, e.g. 10, 14): The term “catalytic converter” is an engineering term and is not appropriate in this context. I would replace with “catalytic surface”.

**Response:**

Now corrected in the text according to the referee’s suggestion.

**Comment:**

10, 6: It is not clear what ND refers to in this line. Please clarify.

**Response:**

ND refers for nitration degree as in the whole manuscript. This abbreviation was introduced when first mentioned in the manuscript on page 5 lines 19/20 (original manuscript). The nitration degree is defined as the concentration of nitrated tyrosine divided by the concentration of all tyrosine residues (see also comment above).

**Comment:**

10, 27: It seems to me the term  $[HONO]1 + [HONO]2$  is incorrect. Instead of indicating concentrations, should one not be using rates (i.e.,  $d[HONO]1/dt + d[HONO]2/dt$ )?

**Response:**

Thank you very much for noting, indeed that’s the case. Now corrected in the text as suggested by the referee and moved to a new supplement (see comment below).

**Comment:**

*Kinetics studies section: The derivation of some of the indicated terms is not so clear. I question the need to go into the level of detail displayed in eq. 1-5. Please check over the derivation of  $k_{eff}$ . Also, perhaps I missed this explanation, but why are the reversible reactions in Figure 9 not included?*

**Response:**

To simplify the calculations, the reversible processes of  $NO_2$  were neglected ( $k_1$  would be the effective rate constant for the adsorption; including adsorption and desorption). In addition, the adsorption of HONO to the protein surface is supposed to be very small in relation to the desorption, as proteins are slightly acidic (similar to  $k_1$   $k_3$  would be the effective rate constant including desorption and adsorption). Details are now displayed and discussed in a new supplement.

**Comment:**

Figure 1: Ozone is included above the arrow in the first step. However, there is no indication that ozone was used in this study. Please clarify or correct.

**Response:**

True, in our study only  $N_2$  was applied as a carrier gas (no  $O_3$ ). Fig. 1 is meant to give a complete overview of possible nitration mechanisms and refers to another study on nitration of proteins with  $O_3$  and  $NO_2$  (Shiraiwa et al., 2012 as indicated in the caption).

Figure 1 caption was modified: “**Overview on possible reaction mechanism...**”

## Reference:

- Costabile, F., Amoroso, A., and Wang, F.: Sub-micrometre particle size distributions in a suburban Mediterranean area. Aerosol populations and their possible relationship with HONO mixing ratios, *Atmospheric Environment*, 44, 5258-5268, 10.1016/j.atmosenv.2010.08.018, 2010.
- Laufs, S., Cazaunau, M., Stella, P., Kurtenbach, R., Cellier, P., Mellouki, A., Loubet, B., and Kleffmann, J.: Diurnal fluxes of HONO above a crop rotation, *Atmos. Chem. Phys.*, 17, 6907-6923, 10.5194/acp-17-6907-2017, 2017.
- Lee, J. D., Whalley, L. K., Heard, D. E., Stone, D., Dunmore, R. E., Hamilton, J. F., Young, D. E., Allan, J. D., Laufs, S., and Kleffmann, J.: Detailed budget analysis of HONO in central London reveals a missing daytime source, *Atmos. Chem. Phys.*, 16, 2747-2764, 10.5194/acp-16-2747-2016, 2016.
- Pusede, S. E., VandenBoer, T. C., Murphy, J. G., Markovic, M. Z., Young, C. J., Veres, P. R., Roberts, J. M., Washenfelder, R. A., Brown, S. S., Ren, X., Tsai, C., Stutz, J., Brune, W. H., Browne, E. C., Wooldridge, P. J., Graham, A. R., Weber, R., Goldstein, A. H., Dusanter, S., Griffith, S. M., Stevens, P. S., Lefer, B. L., and Cohen, R. C.: An Atmospheric Constraint on the NO<sub>2</sub> Dependence of Daytime Near-Surface Nitrous Acid (HONO), *Environmental Science & Technology*, 49, 12774-12781, 10.1021/acs.est.5b02511, 2015.
- Ren, X., Sanders, J. E., Rajendran, A., Weber, R. J., Goldstein, A. H., Pusede, S. E., Browne, E. C., Min, K. E., and Cohen, R. C.: A relaxed eddy accumulation system for measuring vertical fluxes of nitrous acid, *Atmospheric Measurement Techniques*, 4, 2093-2103, 10.5194/amt-4-2093-2011, 2011.
- Selzle, K., Ackaert, C., Kampf, C. J., Kunert, A. T., Duschl, A., Oostingh, G. J., and Pöschl, U.: Determination of nitration degrees for the birch pollen allergen Bet v 1, *Analytical and Bioanalytical Chemistry*, 405, 8945-8949, 10.1007/s00216-013-7324-0, 2013.
- Shiraiwa, M., Selzle, K., Yang, H., Sosedova, Y., Ammann, M., and Pöschl, U.: Multiphase Chemical Kinetics of the Nitration of Aerosolized Protein by Ozone and Nitrogen Dioxide, *Environmental Science & Technology*, 46, 6672-6680, 10.1021/es300871b, 2012.
- Sörgel, M., Regelin, E., Bozem, H., Diesch, J. M., Drewnick, F., Fischer, H., Harder, H., Held, A., Hosaynali-Beygi, Z., Martinez, M., and Zetzsch, C.: Quantification of the unknown HONO daytime source and its relation to NO<sub>2</sub>, *Atmospheric Chemistry and Physics*, 11, 10433-10447, 10.5194/acp-11-10433-2011, 2011.
- Sörgel, M., Trebs, I., Wu, D., and Held, A.: A comparison of measured HONO uptake and release with calculated source strengths in a heterogeneous forest environment, *Atmos. Chem. Phys.*, 15, 9237-9251, 10.5194/acp-15-9237-2015, 2015.
- Spataro, F., Ianniello, A., Esposito, G., Allegrini, I., Zhu, T., and Hu, M.: Occurrence of atmospheric nitrous acid in the urban area of Beijing (China), *The Science of the total environment*, 447, 210-224, 10.1016/j.scitotenv.2012.12.065, 2013.
- Su, H., Cheng, Y. F., Shao, M., Gao, D. F., Yu, Z. Y., Zeng, L. M., Slanina, J., Zhang, Y. H., and Wiedensohler, A.: Nitrous acid (HONO) and its daytime sources at a rural site during the 2004 PRIDE-PRD experiment in China, *Journal of Geophysical Research-Atmospheres*, 113, 10.1029/2007jd009060, 2008.