

A Novel Route to Peroxynitrite Anion

J. Ramón Leis*, M. Elena Peña and Ana Ríos

Departamento de Química Física, Facultad de Química, Universidad de Santiago, 15706 Santiago de Compostela, Spain

HOO^- is nitrosated efficiently and directly by alkyl nitrites in basic media to yield quantitative amounts of peroxynitrite.

Whereas peroxynitrous acid HOONO is an elusive species, peroxynitrite anion ONOO^- is relatively stable in alkaline solutions.¹ The growing interest in the chemistry and biochemistry of nitric oxide since the discovery of its crucial role in several biological processes,² has prompted an increasing amount of research on ONOO^- , which can form *in vivo* from $\cdot\text{NO}$.³ The strong oxidising power of ONOO^- —presumably owing to release of highly reactive hydroxyl radicals^{4,5}—makes it a highly cytotoxic species, able to initiate lipid peroxidation and protein sulfhydryl oxidation^{3,6} and to effect several crucial processes, such as Na^+ uptake across epithelial cells.⁷

Additionally, ONOO^- is involved in several photochemical processes, including the controversial problem of the photolysis of nitrates⁸ and the system $\text{HOONO}/\text{ONOO}^-$ plays a key role in several important reactions in solution, such as nitrosation of H_2O_2 ,⁹ autoxidation of hydroxylamine and chloramine,¹⁰ *etc.* and is probably involved in environmental processes.^{11,12}

Despite the obvious relevance of ONOO^- in such diverse aspects of chemistry, producing it in solution remains problematic. The available method is based upon the fast nitrosation of H_2O_2 in acid medium to yield solutions of the unstable HOONO , to which alkali is added immediately to generate the peroxynitrite anion. However, the great instability of HOONO ($t_{1/2}$ ca. 1 s) makes the 'timing' of both processes (nitrosation and addition of alkali) the key point for the success of the procedure. It is recognized¹ that the method can give, at its best, yields of peroxynitrite between 45 and 50%. A more sophisticated improvement of the method, usually followed in the biological studies, requires the use of a quenched-flow reactor.¹³ The solutions obtained through these methods contain an excess of H_2O_2 , which must be destroyed—usually with MnO_2 . This last step results in a further loss of ONOO^- .^{6a} Another shortcoming of the

method is the unavoidable presence of nitrite and nitrate ions in the final solutions.¹

During the course of a study of the reactivity of nucleophiles towards alkyl nitrites, we discovered a new method, which yields stable solutions of peroxynitrite, in a clean, simple and fast way.

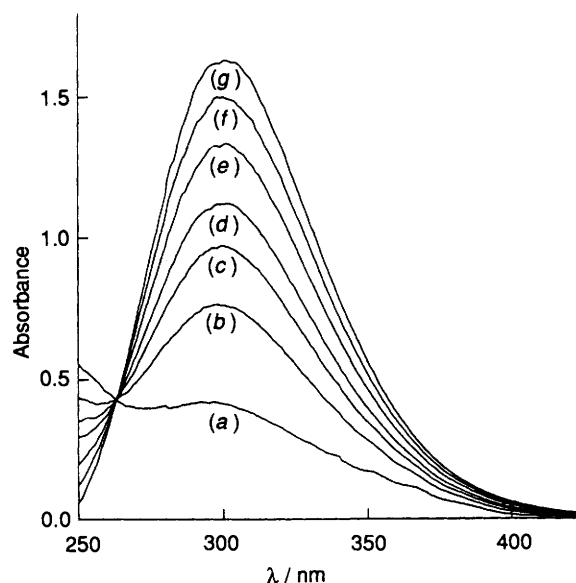


Fig. 1 Typical reaction spectrum showing formation of peroxynitrite from HOO^- ($6 \times 10^{-3} \text{ mol dm}^{-3}$) and 2-ethoxy ethyl nitrite ($1.03 \times 10^{-3} \text{ mol dm}^{-3}$, added last to the reaction mixture) at 0.2 mol dm^{-3} NaOH . $T = 25^\circ\text{C}$. *t*: (a) 6 s, (b) 31 s, (c) 56 s, (d) 81 s, (e) 131 s, (f) 206 s, (g) 486 s.

The strategy relies on the formation of ONOO⁻ directly in basic media by making use of the strong nucleophilicity of HOO⁻ towards the nitroso group of alkyl nitrites [eqn. (1)]. We used a moderately reactive alkyl nitrite, such as 2-ethoxyethyl nitrite.



Fig. 1 shows a typical reaction spectrum. As the reaction proceeds, a new band centred on 300–302 nm, corresponding to the peroxy nitrite anion,¹ is formed. The reaction spectrum displays a clean isosbestic point at 263 nm, which is indicative of a kinetically simple process, *i.e.* without any interference from ONOO⁻ decomposition. In fact, the last spectrum shows the spectral characteristics corresponding to quantitative (>97%) formation of ONOO⁻ ($\lambda_{\text{max}} = 302 \text{ nm}$, $\epsilon = 1670 \pm 50 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). Owing to the instability of alkyl nitrites in acid or neutral aqueous media,¹⁴ alkyl nitrite must be added to the reaction medium either in its pure form, in basic solution (*ca.* $10^{-2} \text{ mol dm}^{-3} \text{ NaOH}$) or as a small volume of a stock solution prepared in an inert solvent (dioxane; acetonitrile, *etc.*). For preparative purposes, the reaction can be carried out with equimolar amounts of H₂O₂ and alkyl nitrite in alkaline solution (0.2–1 mol dm⁻³ NaOH). In this way, the presence of H₂O₂ in the final solution is avoided. For example, a typical procedure to prepare 100 ml of a solution of ONOO⁻ *ca.* 0.016 mol dm⁻³ is as follows: 15 ml of a 0.109 mol dm⁻³ H₂O₂ aqueous solution are mixed with 15 ml of 2 mol dm⁻³ NaOH and 70 ml of water, 0.2 ml (0.195 g) of 2-ethoxyethyl nitrite are added to the mixture. After 5 min a yellow solution containing 0.015 mol dm⁻³ (spectrophotometrically determined) peroxy nitrite anion is obtained.

We carried out a kinetic study on this reaction at 25 °C. This study was carried out under pseudo-first-order conditions, with the concentration of HOO⁻ in a large excess with respect to that of the alkyl nitrite. pH control was achieved by using the buffering ability of the system H₂O₂/HOO⁻ ($\text{p}K_{\text{a}} 11.6$) in alkaline media. Other experimental details are similar to those described for the reactions between amines and alkyl nitrites in basic media.¹⁵ Kinetic results confirmed that HOO⁻ was the reactive species, the bimolecular rate constant corresponding to reaction between this anion and 2-ethoxyethyl nitrite being measured as $2.2 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$. It is worth mentioning that the high nucleophilicity of HOO⁻ would facilitate the production of peroxy nitrite in basic media without interference from the competitive process of alkaline hydrolysis of the alkyl nitrite. We measured the bimolecular rate constant for reaction between OH⁻ and 2-ethoxyethyl nitrite and found it to be *ca.* $8 \times 10^{-4} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, that is, about 3000 times lower than the value found with HOO⁻. The

enhanced reactivity of HOO⁻ with respect to OH⁻ is undoubtedly a consequence of the sensitivity of the reaction to α -effect nucleophiles.¹⁵

Finally, the proposed method can also be of use for obtaining nitrosyl species that are usually formed, in acidic solution, only with difficulty. For example, we have followed a similar procedure to prepare solutions of nitrosyl thiosulfate (S₂O₃NO⁻) in neutral or basic media. The traditional method of preparing such solutions involves nitrosation of thiosulfate with sodium nitrite in acidic media,¹⁶ the instability of thiosulfate in acidic solution being the major limitation of this procedure.

Financial support from the Dirección General de Investigación Científica y Técnica (PB 90-0767) is gratefully acknowledged. A. R. thanks the Xunta de Galicia for a grant.

Received, 22nd April 1993; Com. 3/02326E

References

- 1 M. N. Hughes and H. G. Nicklin, *J. Chem. Soc. (A)*, 1968, 450.
- 2 R. M. J. Palmer, D. S. Ashton and S. Moncada, *Nature*, 1988, **333**, 664; S. Moncada, A. G. Herman and P. M. Vanhoutte, *Trend. Pharmacol. Sci.*, 1987, **8**, 365; S. Moncada, R. M. J. Palmer and E. A. Higgs, *Biochem. Pharmacol.*, 1989, **38**, 1709.
- 3 R. Radi, J. S. Beckman, K. M. Bush and B. A. Freeman, *J. Biol. Chem.*, 1991, **266**, 4244.
- 4 L. R. Mahoney, *J. Am. Chem. Soc.*, 1970, **92**, 5262.
- 5 J. S. Beckman, T. W. Beckman, J. Chen, P. A. Marshall and B. A. Freeman, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 1620.
- 6 (a) J. J. Moreno and W. A. Pryor, *Chem. Res. Toxicol.*, 1992, **5**, 425; (b) R. Radi, J. S. Beckman, K. M. Bush and B. A. Freeman, *Arch. Biochem. Biophys.*, 1991, **288**, 481.
- 7 M. L. Bauer, J. S. Beckman, K. J. Bridges, C. M. Fuller and S. Matalon, *Biochim. Biophys. Acta*, 1992, **1104**, 87.
- 8 R. C. Plumb and J. O. Edwards, *J. Phys. Chem.*, 1992, **96**, 3245, and references cited therein.
- 9 M. Anbar and H. Taube, *J. Am. Chem. Soc.*, 1954, **76**, 6243.
- 10 M. Anbar and G. Yagil, *J. Am. Chem. Soc.*, 1962, **84**, 1790; G. Yagil and M. Anbar, *J. Inorg. Nucl. Chem.*, 1964, **26**, 453.
- 11 N. V. Blough and O. C. Zafiriou, *Inorg. Chem.*, 1985, **24**, 3504.
- 12 J. S. Robertshaw and I. W. M. Smith, *J. Phys. Chem.*, 1982, **86**, 785; B. M. Cheng, J. W. Lee, Y. P. Lee, *J. Phys. Chem.*, 1991, **95**, 2814.
- 13 J. W. Reed, H. H. Ho and W. L. Jolly, *J. Am. Chem. Soc.*, 1974, **96**, 1248.
- 14 E. Iglesias, L. García-Río, J. R. Leis, M. E. Peña and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1673.
- 15 L. García-Río, E. Iglesias, J. R. Leis, M. E. Peña and A. Ríos, *J. Chem. Soc., Perkin Trans. 2*, 1993, 29.
- 16 M. S. Garley and G. Stedman, *J. Inorg. Nucl. Chem.*, 1981, **43**, 2863.