

Research Article

Decomposition of S-Nitrosothiols Induced by UV and Sunlight

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Photochemical release of nitric oxide (NO) from the S-nitroso derivatives of glutathione, L-cysteine, N-acetyl-L-cysteine, L-cysteinemethylester, D,L-penicillamine, N-acetyl-D,L-penicillamine, and N-acetylcysteamine has been investigated at neutral and acidic pH. The release of NO from RSNO is one of the key reactions that could be utilized in photodynamic therapy. The UV-VIS and HPLC analyses have shown that under argon saturated conditions, disulfide (RSSR) is the major product of UV as well as sunlight induced decomposition. While in aerated conditions, nitrite—the end product of the oxidation of NO—was also observed along with disulfide. The formation of thiyl radical as the intermediate was reconfirmed by laser flash photolysis. The initial rate of formation of NO was on the order of $10^{-5} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. The quantum yields of these reactions were in the range of 0.2–0.8. The high quantum yields observed in the photo induced release of NO from RSNO using both UV and sunlight demonstrate the potential application of these reactions in photodynamic therapy.

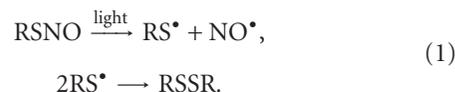
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1. Introduction

S-Nitrosothiols (RSNO) are generally believed as the bio reservoir for nitric oxide (NO) in a biological system [1] and hence their various ways of decomposition are very relevant [2–7]. The bond dissociation energy for S–N bond is between 20 and 32 kcal mol⁻¹ [8, 9]. It is reported that the absorption of 365 nm radiation by S-nitrosotoluene results in the excitation to the extent of 79 kcal mol⁻¹, which is sufficient for the S–N bond cleavage [10]. When irradiated with 355 nm laser, it was observed that S-nitroso derivatives of glutathione (GSNO) and bovine serum albumin (BSASNO) undergo homolytic cleavage to release NO [11]. It was reported that inclusion of photosensitizers resulted in a 9-fold increase in NO production [12]. This is due to the energy transfer process between the triplet state of photosensitizers and the triplet of RSNO. Controlled release of NO was observed in the case of GSNO and S-nitroso-N-acetyl-D,L-penicillamine (SNAP) [13]. In this reaction it was reported that a steady state concentration of NO could be achieved in solution for several minutes by using appropriate concentration of RSNO in different pH buffers. Report on

the photochemo-therapeutic application of GSNO is also available using visible light photolysis [14].

The reported reaction mechanism of the photo release of NO from RSNO as demonstrated in the case of GSNO and S-nitroso-N-acetyl-L-cysteine (SNAC) is



The formation of thiyl radical (GS[•]) as the intermediate radical in the case of GSNO was demonstrated using ESR technique and laser flash photolysis [11, 15, 16]. The study of the chemical and biological aspects of such photo decomposition of RSNO is thus an area of immense importance. The objective of this work was to undertake a quantitative investigation of the photodecomposition of a number of RSNOs at neutral pH and acidic pH using UV light and sunlight. This includes mainly the determination of the quantum yields of photodecomposition as well as the rate of photodecay of a number of RSNOs in aqueous medium.

2. Experimental

S-Nitroso derivatives of glutathione (GSNO), L-cysteine (CySNO), N-acetyl-L-cysteine (SNAC), L-cysteinemethyl-ester (CMESNO), D,L-penicillamine (PSNO), N-acetyl-D,L-penicillamine (SNAP), and N-acetylcysteamine (ACSNO) were synthesized using NaNO_2/HCl method [17]. Medium pressure mercury lamp was used for UV irradiation. The intensity of the UV lamp was measured using ferrioxalate actinometry and was about 1.2×10^{16} $\text{eins cc}^{-1} \text{s}^{-1}$. The Sunlight irradiations were carried out in the middle of bright sunny days during April/May at Kottayam, Kerala (latitude $9^\circ 15' \text{N}$). Ferrioxalate actinometry was carried out simultaneously with the samples to avoid the errors that may occur due to the change in light intensity. The intensity was found to be around 10^{16} $\text{eins cc}^{-1} \text{s}^{-1}$.

The concentrations of RSNO were generally kept as 1 mM. All the irradiation experiments were carried out in the presence of 0.1 mM EDTA to prevent catalytic decomposition due to traces of metal ions. RSNOs were found to be stable up to about 3 hours when kept in dark in neutral and acidic pH (data not shown). The decay as well as the decay products of RSNO was monitored using UV-VIS spectrophotometer and HPLC (Shimadzu LC-10AS) coupled with a UV-VIS detector (Shimadzu SPD10A) using a 25 cm, Nucleosil, 5C-18 column. 25% of methanol containing 0.05% of trifluoro acetic acid (TFA) was used as mobile phase with a flow rate of 0.75 mL/min.

The laser flash photolysis experiments were carried out using a SP-Quanta ray GCR-2(10) Nd-YAG laser (355 nm [third harmonic out put] with a pulse width of 8 ns and energy of 60 mJ/pulse) connected with an optical absorption detection setup.

3. Results and Discussion

The decomposition reaction of a number of RSNOs induced by UV and sunlight was investigated at neutral and acidic pH under Ar saturated or aerated conditions. The decay of the irradiated solutions was monitored using UV-VIS spectrophotometry. The UV-VIS spectra in the case of the decay of S-nitroso-L-cysteine methyl ester (CMESNO) using both UV and sunlight (Figure 1) have shown that it undergoes a time dependent decay as can be seen from the decrease in absorbance at the absorption maximum at 334 nm. The decay of the spectrum was also associated with a red shift around 250–270 nm (Figure 1).

Very similar decay patterns were obtained at neutral as well as at acidic pH. The irradiated solutions were then analyzed using HPLC. It was found that disulfide was the major product in argon saturated solutions, in agreement with earlier reports [5–9]. Meanwhile, in aerated solutions, nitrite was also formed along with disulfide. A typical chromatogram obtained in the case of CMESNO in Ar saturated and aerated conditions is shown in Figure 2. All the selected RSNOs showed a similar decay and product patterns at both pH. A reduction in the pH of the neutral solution after irradiation was also observed in the case of aerated solutions. No such change in pH was observed in

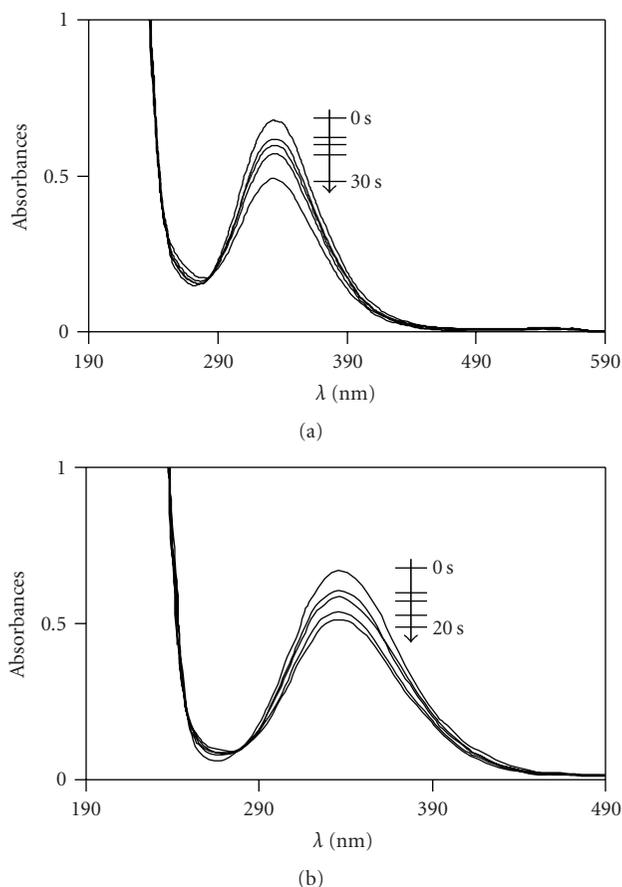


FIGURE 1: (a) UV-VIS spectra of argon saturated CMESNO (1mM) solutions containing EDTA (0.1 mM) (pH 7) irradiated by UV light at 0, 10, 15, 20 and 30 seconds (b) Irradiated by sunlight at 0, 5, 10, 15 and 20 seconds.

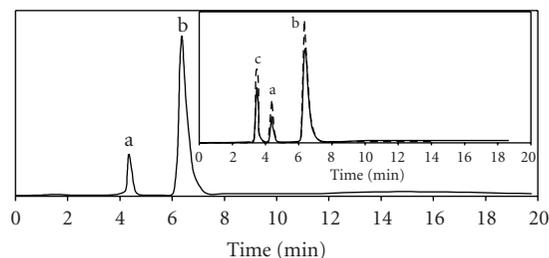


FIGURE 2: HPLC chromatogram of UV-irradiated argon saturated solution of CMESNO at pH 7, (a) disulfide and (b) CMESNO, Inset: HPLC chromatogram (—) under aerated conditions and control data (---) for (a) disulfide, (b) CMESNO, and (c) nitrite.

the case of the argon saturated solutions. This change in pH is attributed to the formation of nitrite in aerated solutions. The formation of nitrite in aerated solution is a result of the reaction of NO with oxygen, (2) [18]. Very similar results were obtained at pH 3 also.

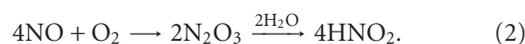


TABLE 1: Quantum yields of the decay of RSNO solutions under UV irradiation (values in brackets are obtained for sunlight irradiation).

RSNO	Aerated		Ar saturated	
	pH 3	pH 7	pH 3	pH 7
SNAP	0.54 (0.78)	0.74 (0.81)	0.33 (0.40)	0.33 (0.49)
CMESNO	0.38 (0.48)	0.83 (0.84)	0.26 (0.32)	0.62 (0.52)
ACSNO	0.58 (0.65)	0.62 (0.76)	0.51 (0.59)	0.54 (0.73)
PSNO	0.23	0.48	0.22	0.36
SNAC	0.39	0.42	0.26	0.34
GSNO	0.41	0.49	0.28	0.34
CySNO	0.54	0.69	0.42	0.49

The red shift around 250–270 nm in the UV-VIS spectra (Figure 1) is an indication of the formation of disulfide as this has absorbance in the same region [19]. The products from HPLC eluent were also separately collected in the case of CySNO and analyzed by MS which reconfirmed the formation of RSSR. Very similar results were obtained for the degradation of RSNO induced by sunlight at pH 3 and 7. As shown in Figure 1, the UV-VIS spectra showed a time dependent decomposition of RSNO. The disappearance of RSNO per second was determined from (Figure 1) the absorbances at 334 nm, and the quantum yields (φ) of the decay were calculated from these values (Table 1). These values are in the range of 0.2 and 0.8. The sunlight irradiation experiments were also carried out with SNAP, CMESNO and ACSNO. Nearly similar quantum yields were obtained for these compounds (Table 1).

The decay of the absorption maximum at 334 nm can be assigned to the homolytic breakage of the S–N bond (see Figure 1). In order to reconfirm the formation of RS^{*} as an intermediate in the photodecomposition of RSNO, we have carried out laser flash photolysis studies of GSNO under nitrogen saturated conditions at neutral pH. The time resolved absorption spectrum obtained under nitrogen saturation (Figure 3) has shown a bleaching around 300–380 nm region and around 500–550 nm region which agrees well with the earlier report [11]. This transient absorption spectrum looks like the mirror image of the ground state absorption spectrum of GSNO indicating a bleaching of the absorption maxima at the excited state. It can be seen from the time resolved absorption spectra that the bleaching in the region 300–380 nm continues from 1.4 μ s to 40.4 μ s while the bleaching in the region 500–550 nm is complete in 1.4 μ s. When the difference in the absorption spectra between 1.4 μ s and 40.4 μ s is plotted, an absorption spectrum (Figure 3) similar to the reported thiyl radical spectrum has been obtained [20]. It is known that thiyl radical has a relatively weak absorption spectrum in the region 300–380 nm, while it has no absorption in the region 500–550 nm and hence the bleaching is complete in 1.4 μ s. The decrease in the absorbance observed in the region 300–380 nm is attributed to the loss of GS^{*} (corresponding to the formation of GSSR). Thus the formation of GS^{*} is reconfirmed.

Since RSNO undergoes S–N bond cleavage on irradiation, the formation of NO can be directly equated to the

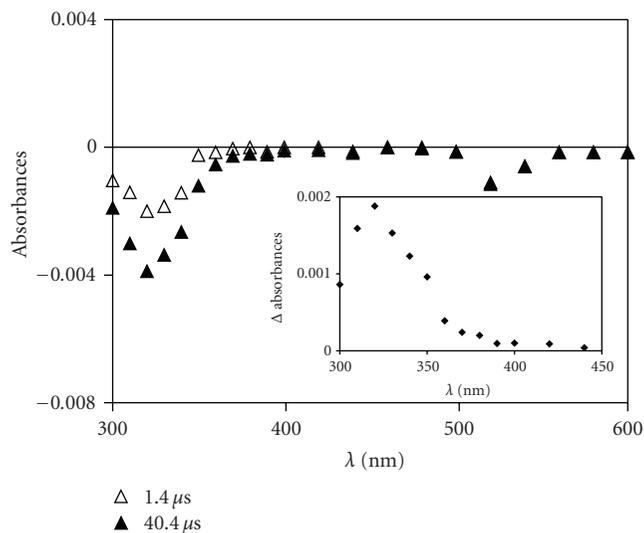


FIGURE 3: The transient absorption spectra of GSNO solution (argon saturated) obtained at 1.4 and 40.4 μ s after the laser pulse (pH 7). Inset: Difference in absorption of GSNO solution at 1.4 μ s after laser pulse of argon saturated and aerated conditions at neutral pH.

decay of RSNO. The concentration of NO released at a time t can be thus calculated using (3), according to Beer's law,

$$[\text{NO}]_t = [\text{RSNO}]_0 - [\text{RSNO}]_t, \quad (3)$$

where $[\text{RSNO}]_0$ and $[\text{RSNO}]_t$ are the concentrations of RSNO at initial and after time t , respectively. The initial rates of NO formation from RSNO are calculated from the slope of the concentration of NO versus time plot (Figure 4) and are in the order of $10^{-5} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. Very similar rates of formation of NO were obtained from the sunlight induced degradation.

Thus it can be seen that the high quantum yields of NO release from all the selected RSNO using UV light and sunlight are high (0.2–0.8). In an earlier report, the quantum yield of photo release of NO from SNAC was about 0.65, and the values obtained in the present study match well with the reported value [20].

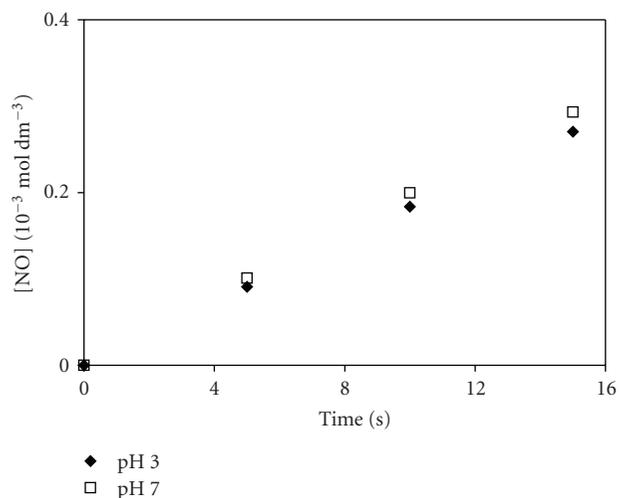


FIGURE 4: Formation of NO (calculated from (3)) after UV irradiation of CMESNO under argon saturated conditions.

4. Conclusions

The high quantum yields of NO release from all the selected RSNOs using UV light and sunlight (0.2–0.8) support the general notion that photo release of NO can be very useful in photodynamic therapy. It is known that the light with wavelength of 600 nm can be easily penetrated through the skin membrane, and in this context the sunlight induced decomposition of RSNO is very important as the absorption spectrum has the second maximum at 550 nm. Further works need to be done in vivo conditions to investigate the stability of RSNOs as their decay can be influenced by a number of other factors such as the presence of metal ions and other reducing agents.

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References

- [1] M. Keshive, S. Singh, J. S. Wishnok, S. R. Tannenbaum, and W. M. Deen, "Kinetics of S-nitrosation of thiols in nitric oxide solutions," *Chemical Research in Toxicology*, vol. 9, no. 6, pp. 988–993, 1996.
- [2] V. M. Manoj and C. T. Aravindakumar, "Reaction of hydroxyl radicals with S-nitrosothiols: determination of rate constants and end product analysis," *Organic and Biomolecular Chemistry*, vol. 1, no. 7, pp. 1171–1175, 2003.
- [3] V. M. Manoj and C. T. Aravindakumar, "Hydroxyl radical induced decomposition of S-nitrosoglutathione," *Chemical Communications*, no. 23, pp. 2361–2362, 2000.
- [4] L. Heikal, G. P. Martin, and L. A. Dailey, "Characterisation of the decomposition behaviour of S-nitrosoglutathione and a new class of analogues: S-nitrosophytochelatin," *Nitric Oxide—Biology and Chemistry*, vol. 20, no. 3, pp. 157–165, 2009.
- [5] L. A. Peterson, T. Wagener, H. Sies, and W. Stahl, "Decomposition of S-nitrosocysteine via S- To N-transnitrosation," *Chemical Research in Toxicology*, vol. 20, no. 5, pp. 721–723, 2007.
- [6] S. Hwang and M. E. Meyerhoff, "Organoditelluride-mediated catalytic S-nitrosothiol decomposition," *Journal of Materials Chemistry*, vol. 17, no. 15, pp. 1462–1465, 2007.
- [7] V. M. Manoj, H. Mohan, U. K. Aravind, and C. T. Aravindakumar, "One-electron reduction of S-nitrosothiols in aqueous medium," *Free Radical Biology and Medicine*, vol. 41, no. 8, pp. 1240–1246, 2006.
- [8] M. D. Bartberger, J. D. Mannion, S. C. Powell, J. S. Stamler, K. N. Houk, and E. J. Toone, "S-N dissociation energies of S-nitrosothiols: on the origins of nitrosothiol decomposition rates," *Journal of the American Chemical Society*, vol. 123, no. 36, pp. 8868–8869, 2001.
- [9] J.-M. Lü, J. M. Wittbrodt, K. Wang, et al., "NO affinities of S-nitrosothiols: a direct experimental and computational investigation of RS-NO bond dissociation energies," *Journal of the American Chemical Society*, vol. 123, no. 12, pp. 2903–2904, 2001.
- [10] J. Barrett, L. J. Fitzgibbons, J. Glauser, R. H. Still, and P. N. W. Young, "Photochemistry of the S-nitroso derivatives of hexane-1-thiol and hexane-1,6-dithiol," *Nature*, vol. 211, no. 5051, p. 848, 1966.
- [11] P. D. Wood, B. Mutus, and R. W. Redmond, "The mechanism of photochemical release of nitric oxide from S-nitrosoglutathione," *Photochemistry and Photobiology*, vol. 64, no. 3, pp. 518–524, 1996.
- [12] R. J. Singh, N. Hogg, J. Joseph, and B. Kalyanaraman, "Photosensitized decomposition of S-nitrosothiols and 2-methyl-2-nitrosopropane possible use for site-directed nitric oxide production," *FEBS Letters*, vol. 360, no. 1, pp. 47–51, 1995.
- [13] V. R. Zhelyaskov, K. R. Gee, and D. W. Godwin, "Control of NO concentration in solutions of nitrosothiol compounds by light," *Photochemistry and Photobiology*, vol. 67, no. 3, pp. 282–288, 1998.
- [14] D. J. Sexton, A. Muruganandam, D. J. McKenney, and B. Mutus, "Visible light photochemical release of nitric oxide from S-nitrosoglutathione: potential photochemotherapeutic applications," *Photochemistry and Photobiology*, vol. 59, no. 4, pp. 463–467, 1994.
- [15] S. P. Singh, J. S. Wishnok, M. Keshive, W. M. Deen, and S. R. Tannenbaum, "The chemistry of the S-nitrosoglutathione/glutathione system," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 25, pp. 14428–14433, 1996.
- [16] R. J. Singh, N. Hogg, J. Joseph, and B. Kalyanaraman, "Mechanism of nitric oxide release from S-nitrosothiols," *Journal of Biological Chemistry*, vol. 271, no. 31, pp. 18596–18603, 1996.
- [17] T. W. Hart, "Some observations concerning the S-nitroso and S-phenylsulphonyl derivatives of L-cysteine and glutathione," *Tetrahedron Letters*, vol. 26, no. 16, pp. 2013–2016, 1985.
- [18] B. S. Wolfenden and R. L. Willson, "Radical-cations as reference chromogens in kinetic studies of one-electron transfer reactions: pulse radiolysis studies of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate)," *Journal of the Chemical Society, Perkin Transactions 2*, no. 7, pp. 805–812, 1982.

- [19] M. Quintiliani, R. Badiello, M. Tamba, A. Esfandi, and G. Gorin, "Radiolysis of glutathione in oxygen-containing solutions of pH7," *International Journal of Radiation Biology*, vol. 32, no. 2, pp. 195–202, 1977.
- [20] S. M. Shishido and M. G. de Oliveira, "Polyethylene glycol matrix reduces the rates of photochemical and thermal release of nitric oxide from S-nitroso-N-acetylcysteine," *Photochemistry and Photobiology*, vol. 71, no. 3, pp. 273–280, 2000.



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