Development of an Analytical Method for Nitric Oxide Radical Determination in Natural Waters

Emmanuel F. Olasehinde Kazuhiko Takeda and Hiroshi Sakugawa\*.

## Abstract

The measurement of photochemically generated nitric oxide radicals (NO) in natural waters has long been an arduous task because of a lack of simple analytical techniques, even though the environmental significance of this radical is paramount. We have developed a simple analytical method for the determination of photochemically generated NO in natural waters using 4,5-diaminofluorescein (DAF-2) as a probe compound. This method is based on the reaction of photoformed NO with DAF-2 in airsaturated solution to produce a highly fluorescent triazolofluorescein (DAF-2T) product. DAF-2T was determined by using reversed-phase HPLC with fluorescence detection, with excitation and emission wavelengths of 495 and 515 nm, respectively. Under optimum conditions, the calibration curve exhibited linearity in the range of 0.025–10 nM DAF-2T. The coefficients of variance for the measurements of the signal intensities of DAF-2T (from the photolysis of 0.5  $\mu$ M and 5  $\mu$ M NO2- with DAF-2) were less than 5% and 3%, respectively. For a total irradiation time of 30 min, the detection limit of the photoformation rate of NO was  $1.65 \times 10-13$  M s-1, defined as  $3\sigma$  of the lowest measured DAF-2T concentration (0.025 nM). The proposed method is relatively unaffected by potential interferents in seawater. The method was employed to determine the photoformation rate of NO in the Seto Inland Sea and the Kurose River in Hiroshima Prefecture, Japan. The measured NO photoformation rates in seawater and river water samples ranged from  $(5.3-32) \times 10-12$  M s-1 and  $(9.4-300) \times 10-12$  M s-1, respectively.

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