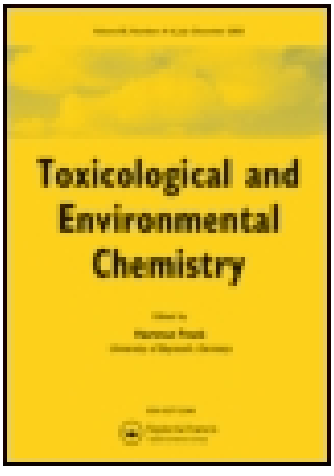

Toxicological & Environmental Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gtec20

The enhanced formation of N-nitrosamines in fulvic acid mediated environment

S. V. R. Weerasooriya [a] & C. B. Dissanayake [a]

[a] Institute of Fundamental Studies , Kandy, Sri Lanka

Published online: 20 Sep 2008.

To cite this article: S. V. R. Weerasooriya & C. B. Dissanayake (1989) The enhanced formation of N-nitrosamines in fulvic acid mediated environment , Toxicological & Environmental Chemistry, 25:1, 57-62, DOI: 10.1080/02772248909357505

To link to this article: http://dx.doi.org/10.1080/02772248909357505

Toxicological and Environmental Chemistry, Vol. 25, pp. 57–62
Reprints available directly from the publisher
Photocopying permitted by license only

THE ENHANCED FORMATION OF N-NITROSAMINES IN FULVIC ACID MEDIATED ENVIRONMENT*

S. V. R. WEERASOORIYA and C. B. DISSANAYAKE

Institute of Fundamental Studies, Kandy, Sri Lanka

(*Received in final form 17 April 1989*)

Fulvic acids leave a catalytic effect in the nitrosation process. Significant quantities of NDBA formed even at pH 5.50, in the presence of fulvic acids. The exact mechanism of fulvic acid-mediated nitrosation is not yet understood.

KEY WORDS: Nitrosation, organic matter, environment, Sri Lanka.

INTRODUCTION

This research reports evidence on the role of fulvic acids in mediating the formation of N-nitrosamines from nitrite and secondary amine precursors. The N-nitrosamines are of particular environmental and health importance, because these compounds have the potency for mutagenic,[1] teratogenic and carcinogenic activity.[2] The formation of nitrosamine depends generally on the direct nitrosation of secondary amines, although primary and tertiary amines are also potent precursors since they may be converted to secondary amines. Direct nitrosation occurs optimally at pH 3.40, the pK_a of HNO_2, but another reaction pathway catalyzed by certain carbonyl compounds (including HCHO and Cl_3CHO) can produce nitrosamines under neutral or even basic, conditions.[3] Although recent studies[4, 5] indicate that the formation of nitrosamines is enhanced in the presence of naturally occurring organic matter such as fulvic and humic substances, the exact mechanism by which fulvic acids facilitates this process is not well documented.[3]

MATERIALS AND METHODS

(*a*) *Materials*

The fulvic acid solution was prepared by dissolving protonated fulvic acids

*A contribution from The IFS Soil Vegetation and Health Study Group.

extracted and purified from soil suspensions in water and filtered through 0.45 μm membrane filter.[6,7] The standard nitrosodibutyl amine (NDBA) solution used for calibration was from Sigma Chemicals, U.S.

(*b*) *Methods*

(*1*) *The time dependence study*

For this study a 50 ml aqueous solution of 100 $\mu g\,ml^{-1}$ dibutylamine (DBA), 200 $\mu g\,ml^{-1}$ $NaNO_2$ and 1000 $\mu g\,ml^{-1}$ fulvic acid was shaken in 100 ml closed vessels on a reciprocating shaker at 100 excursion min^{-1} and 5 ml aliquots sampled at different time intervals. The final acidity of the reaction of the mixture was carefully adjusted to pH 4.0 with a buffer solution.

(*2*) *The pH dependence study*

The effect of solution pH on nitrosation process was investigated by equilibrating a 50 ml mixture of 100 $\mu g\,ml^{-1}$ DBA, 200 $\mu g\,ml^{-1}$ $NaNO_2$ and 1000 $\mu g\,ml^{-1}$ fulvic acids for 48 hrs at pH range of 1–8. The nitrosation rate was determined by monitoring the decrease in NO_2^- which is steadily consumed in the reaction at pH 6.5.

(*3*) *The effect of fulvic acid*

The catalytic effect of fulvic acids on the nitrosation process was also determined. A 50 ml aqueous solution of 100 $\mu g\,ml^{-1}$ DBA 200 $\mu g\,ml^{-1}$ $NaNO_2$ and 0–1000 $\mu g\,ml^{-1}$ of fulvic acid was shaken for 30 min and kept for 48 hrs. The acidity of the solution was kept at 4.0 pH using a buffer.

(*4*) *Determination of NDBA and nitrites*

A polarographic method was used in analysis of NDBA. Several 10 ml filtered samples were allowed to react with 10 ml 1 M HCl followed by the addition of ammonium hydroxide and degassing for 10 min with nitrogen. The polarographic curves were obtained in differential pulse mode as described previously[8] with YANACO Model No. P1100 polarographic analyzer. The nitrite was coupled to N-C1 napthylethylenediamine dihydrochloride to form a red-coloured azo dye for photometric determination at 460 nm.[9] All measurements were made at 25 °C.

RESULTS

As depicted in Figure 1, the preliminary results show that at pH 3.5 in the medium, the formation of NDBA was rapid, and reached an apparent plateau after 24 hrs. It is also noted that the equilibrium concentration of NDBA measured

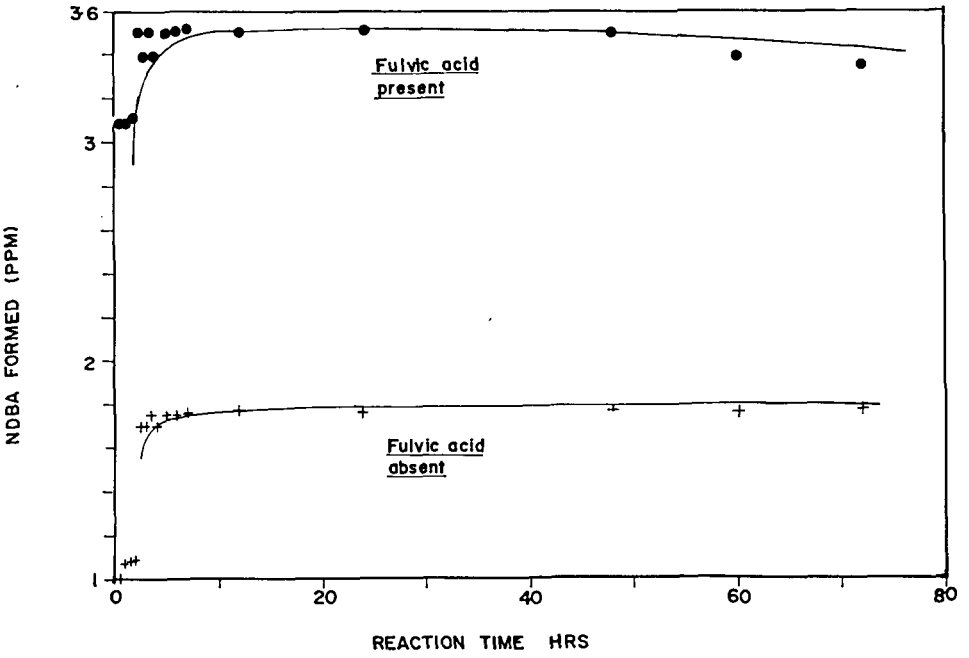

Figure 1 Effect of reaction time on nitrosation of dibutylamine (DBA) by $NaNO_2$ at 25 °C.

in the samples containing fulvic acids was about two times higher than those without fulvic acids. This observation taken together with the results shown in Figure 2 indicate that the state of equilibrium under this condition favoured nitrosamine production. This may denote a catalytic effect, because fulvic acid enhances the formation of nitrosamines without changing the final equilibrium, possibly due to a lower activation energy in the amine-nitrosamine ion complex. Fulvic acids absorb energy over a large range of the electromagnetic spectrum and they are also known to generate free radicals.[10]

It is likely that free radicals in the nitrosation under neutral conditions depends on the above process which is heterolytic and more rapid under acidic conditions.[11] The effect of the pH on the rate of nitrosation is shown in Figure 3. It is seen that the initial nitrosation rate of NDBA and $NaNO_2$ is highest at 25 °C and a pH of 3.0. Our results for DBA are in agreement with those of Boyland & Walker.[12] As seen in Figure 3 the reaction rate is enhanced significantly by the presence of fulvic acids. The present results depicted in Figure 4 show a plateau until pH 6.00 and a gradual slope thereafter. However it was decided to employ pH 6.5 for the detailed kinetics study as this is the general level of acidity found in the natural aquatic environment. The fulvic acid-mediated nitrosation process at pH 6.5 followed the apparent first-order rate law (These results are not given). Although first-order kinetics are observed at pH 6.5, they are a pseudo-first order nitrosation rate which is actually dependent upon the fulvic acid concentration. This is exemplified by the experimentally derived data in Table 1 which show that the first-order rate constant k_1 varies with the fulvic acid concentration. The

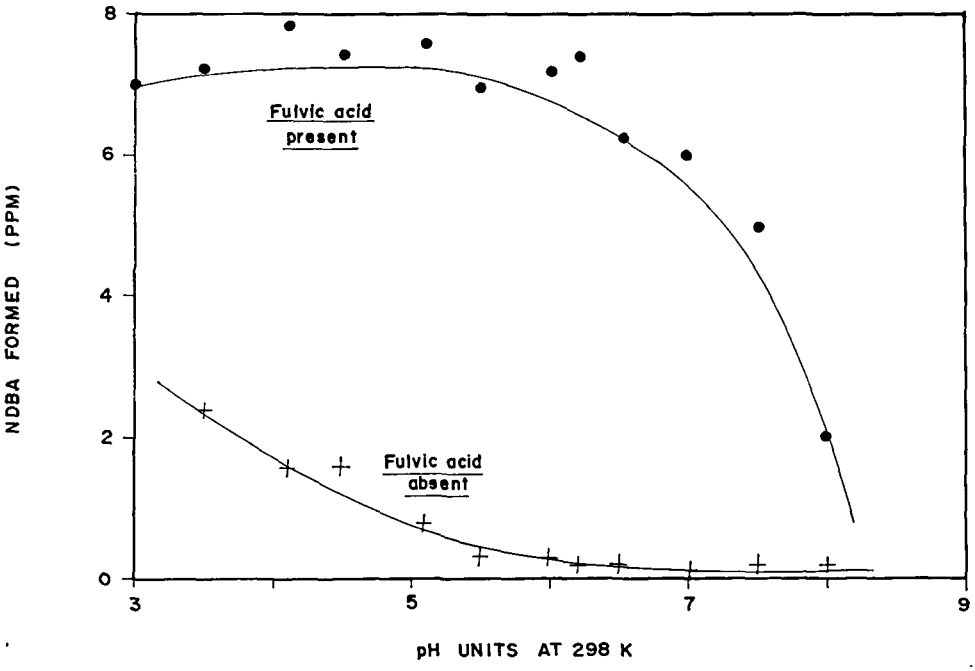

Figure 2 The dependence of pH on nitrosation of DBA by $NaNO_2$ at 25 °C.

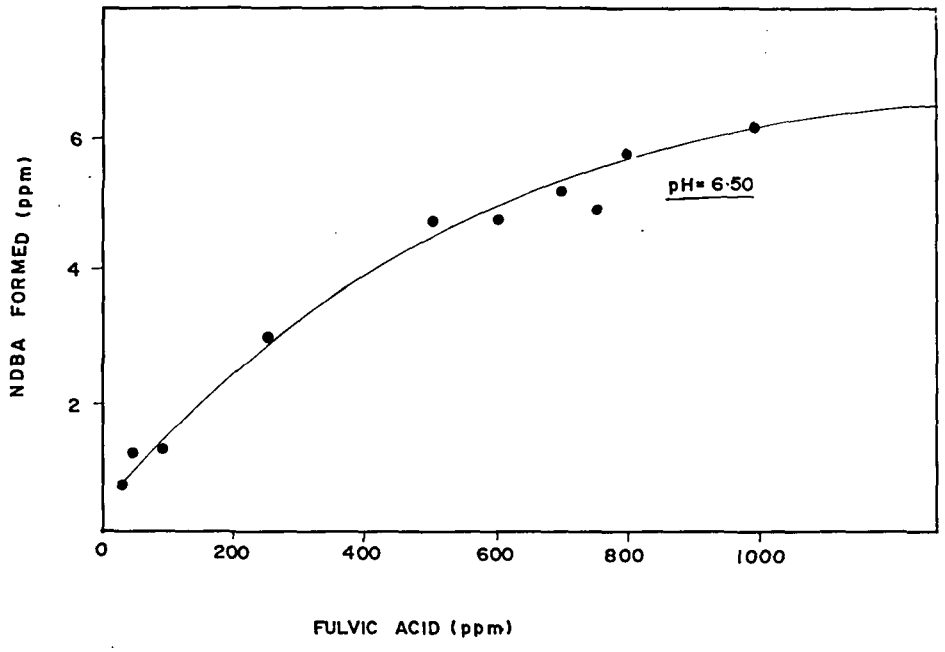

Figure 3 Effect of fulvic acid on nitrosation of DBA by $NaNO_2$ at 25 °C.

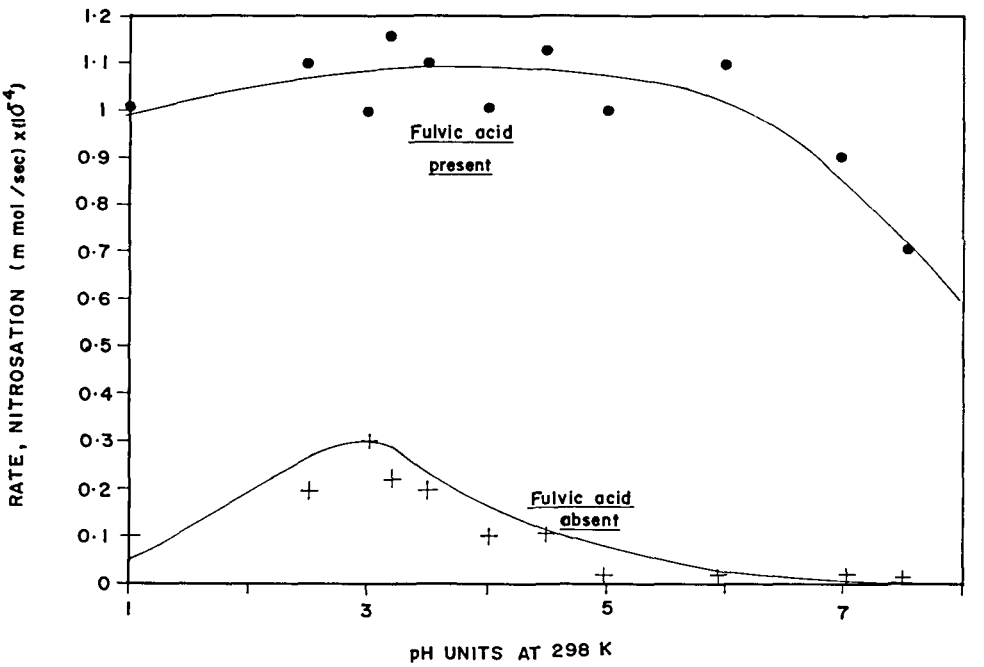

Figure 4 The variation of rate constant, k_1, with $NaNO_2$ concentration.

Table 1 Variation of rate constant, k_1, with fulvic acid concentration

Fulvic acid concentration	*Reaction rate*
mg/mL	*(mmols^{-1})*
1000	1.07×10^{-4}
2000	1.15×10^{-4}
3000	1.21×10^{-4}

degree of FA $\gg$ HNO_2 accounts for the seemingly first-order dependence of the reaction.

DISCUSSION AND SUMMARY

A thorough comprehension of the role of nitrosation catalysts is central to any attempts to understand and predict the environmental distribution of N-nitroso compounds. In this study it was observed that chemically mediated formation of N-nitrosamines from precursor secondary amines and nitrite in aqueous solutions is enhanced by the presence of fulvic acids. Although the catalytic role of fulvic acids in nitrosation is not clear, it is worthy of note that they contain a wide variety of oxygenated functional groups such as hydroxyl, carboxyl, quinones or peroxides.[13,14] In one study Stevenson and Swaby[15] postulated that phenolic

compounds react with excess HNO_2 to produce N_2 and N_2O. This fact was confirmed by Walker *et al.*[16] who have found that the formation of nitrodiethylamine from HNO_2 and diethylamine was enhanced in the presence of phenol. Moreover Bogovski *et al.*[17] have noted that tannins inhibited nitrosation by a competitive reaction with HNO_2.

Acknowledgements

Our thanks are due to Professor Cyril Ponnamperuma, The Director, Institute of Fundamental Studies, Sri Lanka for his keen interest in this project. We are indebted to Mr D. G. A. Perera for editing the manuscript. Our thanks are also due to Ganga Weerasooriya and Manel Samarawickrema for the assistance in preparing the manuscript.

References

1. P. N. Magee and J. M. Barnes, *Advances in Cancer Research* **10,** 163 (1967).
2. W. Lijinsky, L. Keefer, E. Conrad and R. Van De Bogart, *Nature* **239,** 165 (1972).
3. L. K. Keefer and P. P. Roller, *Science* **181,** 1245 (1973).
4. A. L. Mills and M. Alexander, *Journal of Environmental Quality* **5,** 437 (1976).
5. A. Ayanaba and M. Alexander, *Journal of Environmental Quality* **3,** 83 (1974).
6. M. E. Melcer and J. P. Hassett, *Toxicological and Environmental Chemistry* **11,** 147 (1986).
7. W. E. Baker, *Geochimia et Cosmochimica Acta* **37,** 269 (1980).
8. H. Lundi, *Acta Chemica Scandinavia* **11,** 990 (1957).
9. V. Madera, H. E. Allen and R. A. Minea, In: *Examination of Water for Pollution Control* (M. J. Suess, ed) Pergamon, UK (W.H.O., 1982), Vol. 2, p. 169.
10. W. Flaig, H. Beutelspalscher and E. Reitz, In: *Soil Components* (J. E. Gieseking, ed.) (Sprint-Veerlag, 1975), Vol. 1, p. 1.
11. A. J. Buglass, B. C. Challis and M. R. Osborne, In: *N-nitroso Compounds in the Environment* (P. Bogovski, E. A. Walker and W. Davis, eds) International Agency for Research on Cancer, Lyon (I.A.R.C. Publication, 1974), pp. 94–100.
12. E. Boyland and S. A. Walker, In: *N-nitroso Compounds in the Environment* (P. Bogovski and E. A. Walker, eds) (I.A.R.C. Publication No. 9, Lyon, 1974), pp. 132–136.
13. J. A. Marinsky and J. Ephraum, *Environmental Science and Technology* **20,** 349 (1986).
14. G. C. Choudry, In: *Environmental Geochemistry, Reactions and Processes* (O. Hutzinger, ed) (Springer-Verlag, 1982), Vol. 2, Part B, pp. 103–127.
15. F. J. Stevenson and R. J. Swaby, *Soil Science Society of America Proceedings* **28,** 773 (1964).
16. E. A. Walker, B. Pignatelli and M. Castegnaro, *Journal of Agriculture and Food Chemistry* **27,** 393 (1979).
17. P. Bogovski, M. Categnaro, B. Pinatelli and E. A. Walker, In: *N-nitroso Compounds Analyses and Formation* (P. Bogovski, R. Preussman and E. A. Walker, eds) (I.A.R.C. Scientific Publication No. 3, Lyon, 1972), p. 127.