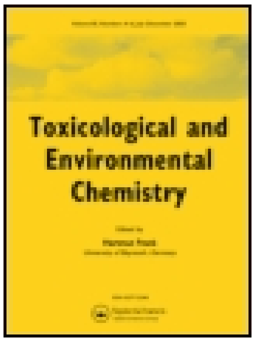

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MODELING THE NITROSATION KINETICS IN SIMULATED NATURAL ENVIRONMENTAL CONDITIONS*

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The rate of N-nitrosodibutylamine formation from dibutylamine and nitrite in aqueous solutions was studied in the presence of soil derived fulvic acids. The nitrosation rate was enhanced in fulvic acid mediated systems even at near neutral acidity (pH ~ 7.00) environments. The generalized rate law is consistent with consecutive reaction series and can be simplified as $k_1[(C_4H_9)_2NH][FA]$ at excessive fulvic acid concentrations.

KEY WORDS: Nitrosamines, Carcinogen, Dibutylamine, N-nitrosodibutylamine

INTRODUCTION

It is well established that N-nitrosamines, many of which are potent carcinogens can be produced by the interaction of nitrites and secondary or tertiary amines. Direct nitrosation occurs optimally at pH 3.40 (pK of HNO_2), but another pathway catalyzed by certain carbonyl compounds can produce nitrosamine under neutral or even basic conditions[1,2]. Although recent studies[3,4] indicate that the formation of N-nitrosamine is enhanced in the presence of naturally occurring organic substances such as humic and fulvic acids, the exact mechanism by which these organic acids facilitates this process yet remains an open question. Therefore we have examined the mechanism and kinetics of the interaction of the dibutylamine, nitrite ion with fulvic acid substrate under various laboratory conditions in order to ascertain the relevance of nitrosation in natural aquatic environments.

MATERIALS AND METHODS

(*a*) *Materials*

The fulvic acid solution was prepared by dissolving protonated fulvic acid extracted and purified according to the procedures given by the International Humic Substances Society (IHSS)[5]. The standard N-nitrosobutylamine (NDBA) and dibutylamine (DBA) used for the calibration were from Sigma Chemicals, USA.

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* A contribution from the IFS Environmental Geochemistry & Health Research Group.

(*b*) *Methods*

1) *The pH dependence study* The effect of the pH on nitrosation rate was determined by interacting a 50 ml of 50 mM DBA, 50 mM $NaNO_2$ and 1000 mg/L protonated fulvic acids (HFA) for 48 hrs at a pH range of 2–10. The exact experimental details are given in our previous paper[3].

2) *The effect of* NO_2^- *and DBA* In this study DBA was reacted with varying concentration of nitrite at three different fulvic acid levels. The effect of secondary amine on the reaction rate was determined by keeping the nitrite concentration at a fixed value while varying the concentration of DBA at each of the two different fulvic acid media. The reaction mixture was kept under relatively mild conditions viz. 50 mM nitrite, pH ~ 7.00, 25 C incubated for 48 hrs. The nitrosation rate was determined by monitoring either the nitrite or the NDBA in equilibrated reaction mixtures by extracting the NDBA/DBA with ether for gas chromatographic quantification.

3) *Analytical methods* The NDBA and DBA concentrations were determined by a Shimadzu, Model CR 9, gas chromatograph equipped with flame ionization detector (FID) and/or electron capture detector(ECD). A 3 m × 2 mm I.D. glass column packed with 25% polyethylene glycol (PEG) on chromosorb W (60–80 mesh) was used. The carrier gas was nitrogen at 60 ml/min. The initial flow rates of hydrogen and air were 42 ml/min and 240 ml/min respectively. More technical details of the columns and the operational procedures are described in Ref. 6. The nitrite determination was done by coupling to N- chloronapthylethylenediamine dihydrochloride to form a red colored azo dye for spectrometric determination at 470 nm[7].

RESULTS AND DISCUSSION

Figure 1 shows the rate of nitrosation of N-nitrodibutylamine as a function of $-\log[H^+]$. Nitrosation generally occurred in acidic solution with HNO_2 or non-aqueous solutions with NOCl, N_2O_3, $NOBr_4$ or NO-3-nitrocabozole[8]. Nitrite ion, being negatively charged, encounters a barrier of electrostatic repulsive energy as it approaches any other center of negative charge, including either the electronegative nitrogen atom in secondary amines or another negatively charged, nucleophilic ion in the medium. The nitrite ion per se cannot therefore participate in the nitrosation process. On the other hand protonation of the nitrite ion in acidic medium "compensates" for these repulsive interactions by successively neutralizing, and then reversing its charge, so that the nitrite ion ultimately experiences a net electrostatic attraction towards electronegative centers[9]. As a result, efficient nitrosation is observed in acidified medium by first converting the nitrite ion to HNO_2 (pK = 3.37). This is the reason for optimal nitrosation rate in the pH range of 3–4 (curve A: Figure 1). However extremely acidic conditions are very rare in natural environmental aquatic systems. It is unlikely to expect nitrosation to occur in this pathway in neutral or near neutral conditions. As shown in curve B (Figure 1), however, the nitrosation rate increased by about 10 fold in the presence of fulvic acids. Significant nitrosation

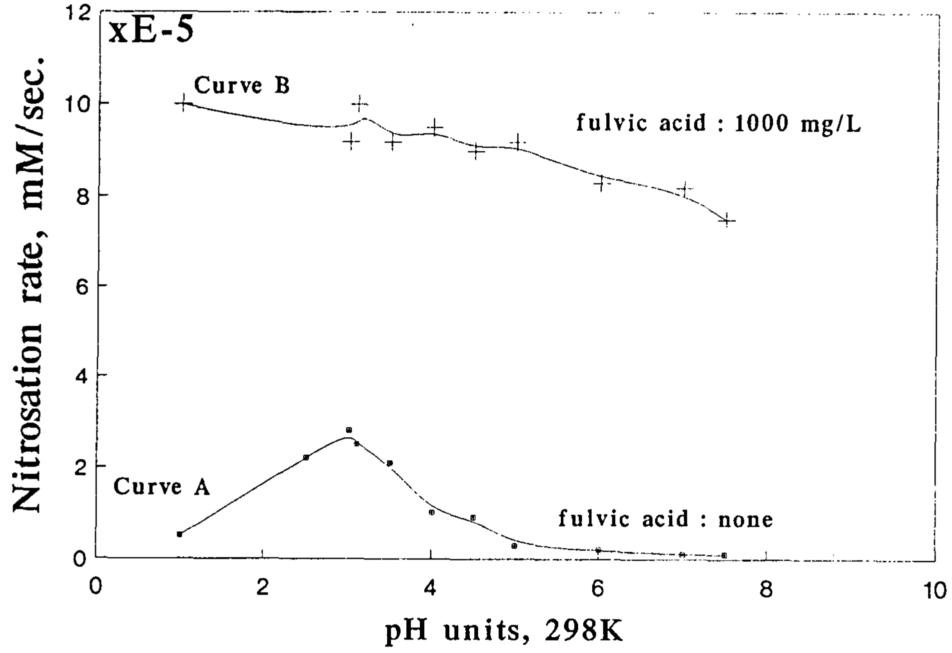

Figure 1 The variation of nitrosation with pH[3].

was also observed even at near neutral pH conditions. The other important point is that the nitrosation rate was independent of $-\log[H^+]$. These results signal an alternative pathway for nitrosation when fulvic acid is present in the reaction system.

Fulvic acid molecules (although chemically not defined yet) are considered as aromatic polymers with chemical properties determined largely by peripheral organic functional sites such as carbonyl, phenolic (hydroxy), carboxylic groups[10]. Therefore fulvic acid moieties may provide a permanent geochemical reservoir for various peripheral functional groups. Moreover based on our preliminary experimental observations, an H^+ independent reaction pathway can be suggested after taking into account for the following critera: (1) the formation of positively charged fulvic–amine complex upon mixing dibutylamine and fulvic acid (2) the formation of pH independent amine–fulvic–nitrite intermediate (3) the decomposition of amine–fulvic–nitrite intermediate will yield nitrosamine regenerating fulvic acids.

A possible mechanistic pathway which is consistent with the above criteria is:

$$FA + (C_4H_9)_2\ddot{N}H \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [(C_4H_9)_2N - FA]^+ \tag{1}$$

$$[(C_4H_9)_2N - FA]^+ + NO_2^- \xrightarrow{k_2} (C_4H_9)_2NFANOO \tag{2}$$

$$(C_4H_9)_2NFANOO \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} (C_4H_9)_2NNO + FA \tag{3}$$

where [FA] is fulvic acid concentration, k_1, k_2 and k_3 are forward rate constants and k_{-1} and k_{-3} backward are rate constants.

Considering the above reactions, a rate expression for the formation of N-nitrosdibutylamine can be formulated in the following way:

$$d/dt[(C_4H_9)_2NNO] = k_3[(C_4H_9)_2NFANOO] - k_{-3}[(C_4H_9)_2NNO][FA] \quad (4)$$

$$d/dt[(C_4H_9)_2NFANOO = k_2[(C_4H_9)_2NFA^+][NO_2^-] - k_3[(C_4H_9)_2NFANOO] + k_{-3}[(C_4H_9)_2NNO][FA] \quad (5)$$

$$d/dt[(C_4H_9)_2NFA)^+] = k_1[(C_4H_9)_2NH][FA] - k_{-1}[(C_4H_9)_2NFA] - k_2[(C_4H_9)_2NFAN][NO_2^-] \quad (6)$$

Furthermore it is reasonable to assume that the reaction intermediates, $[(C_4H_9)_2NFA]^+$ and $[(C_4H_9)_2NNO_2FA]$ are so reactive that it is customary to invoke pseudo steady state approximation (PSSA) and thereby assume that the rate of formation is exactly equal to the rate of disappearance i.e. $d/dt([(C_4H_9)_2NFA]^+)$ and $d/dt([(C_4H_9)_2NNOOFA]) = 0$ resulting the following expressions:

$$[(C_4H_9)_2NFA]^+ = \{k_1[(C_4H_9)_2NFA]^+[FA]\}/\{k_{-1} + k_2[NO_2^-]\} \quad (7)$$

$$k_3[(C_4H_9)_2NNOOFA] = k_2[((C_4H_9)_2NFA)^+][NO_{2-}] + k_3[NO_2^-][(C_4H_9)_2NNO] \quad (8)$$

By substituting Eq. (7) and Eq. (8) in Eq. (4) and rearranging we can obtain the overall nitrosation rate ($K_{\text{nitrosamine}}$) as:

$$d/dt\{[(C_4H_9)_2NNO]\} = \{k_1k_2[(C_4H_9)_2NH][FA][NO_2^-]\}/\{k_{-1} + k_{-2}[NO_2^-]\} \quad (9)$$

(It is interesting to note that the rate expression is independent of the N-nitrosamine concentration.)

In order to ascertain the rate constant/s of overall nitrosation and related reactions, it is necessary to simplify rate expression (Eq. 9) by choosing experimental conditions in which some of the terms become less important. The nitrosation rate ($K_{\text{nitrosamine}}$) as a function of nitrite in solution at two fulvic acid concentrations in shown in Figure 2. In the excess of fulvic acid (appr. × 1000 than other reactants) in the system, the nitrosation rate is practically independent of nitrite concentration. This situation can be explained by assuming $k_{-1} \ll k_2[NO_{2-}]$ in the rate of expression. [In other words it is safe to consider that the reaction *No.* (1) is irreversible at high fulvic acid concentrations]. The simplified reaction rate is therefore

$$d[[(C_4H_9)_2NNO]/dt = k_1[(C_4H_9)_2NH][FA] = k[(C_4H_9)_2NH] \quad (10)$$

where k_1 [FA] = k (constant) at high concentration of fulvic acids. (In fact the constant k, depends on the fulvic acid concentration[3]).

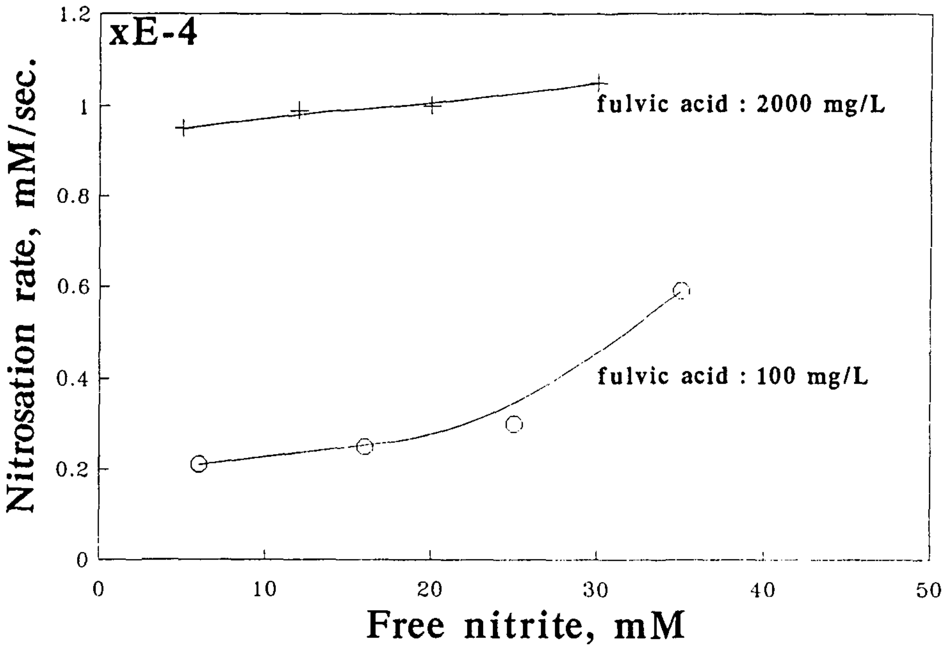

Figure 2 The variation of nitrosation rate with free nitrite concentration. pH of the solution ~7.00 temperature = 298 K.

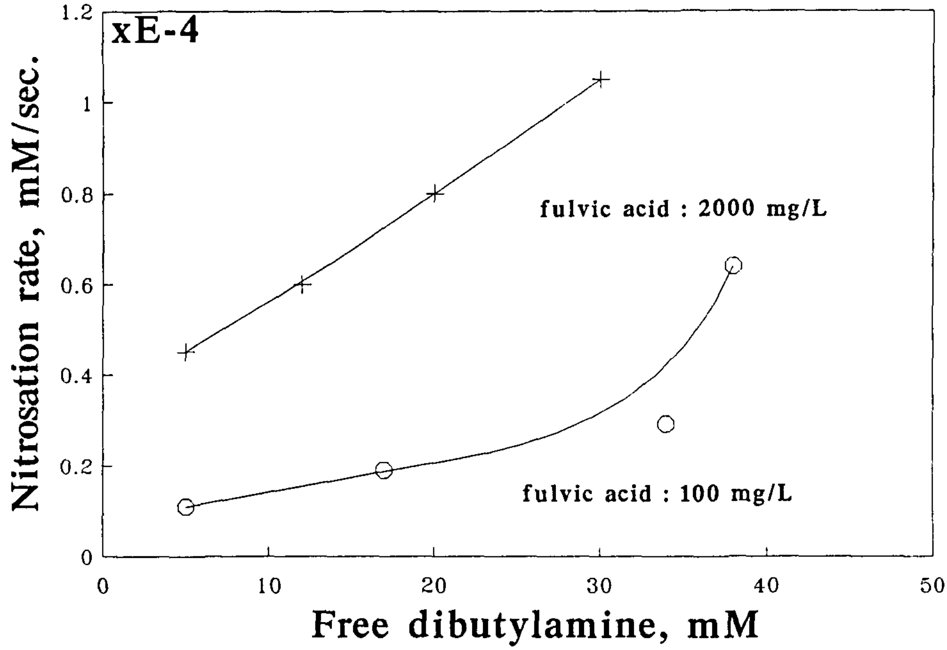

Figure 3 The variation of nitrosation rate with free dibutylamine concentration. pH of the solution ~7.00, temperature = 298 K.

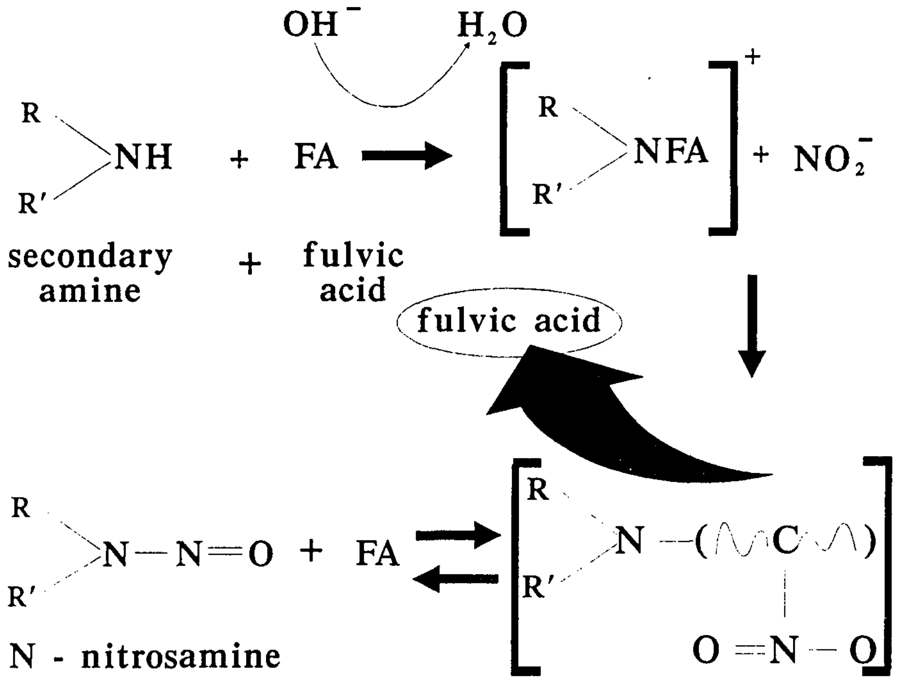

Figure 4 Suggested mechanism for nitrosation in fulvic acid mediated reaction system.

Figure 3 shows the variation of nitrosation rate with the free $(C_4H_9)_2NH$ in solution at various fulvic acid mediated reaction conditions. As expected the nitrosation rate shows a linear relationship with the free $(C_4H_9)_2NH$ when the fulvic acid levels are greater than 1000 mg/L. The shape of the graph is exponential at low fulvic acid (e.g. 100 mg/L) levels, indicating the influence of other ions in the rate expression. In conclusion, as shown in Figure 4 an overall mechanism can be suggested for nitrosation of secondary amines in the presence of fulvic acids. The suggested scheme of mechanism is similar to that outlined in Ref. 10 to explain nitrosation in the presence of formaldehyde[10].

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