*Supporting Information*

# Formation of Dialkyl-*N*-Nitrosamines in Aqueous Solution: An Experimental Validation of a Conservative Predictive Model and a Comparison of the Rates of Dialkyl and Trialkylamine Nitrosation.

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**Derivation and Application of Equation 2**

Considering the pH dependent speciation of a poly acidic / basic compound with two p$K_a$s as described by Scheme S1. The dissociation constants $K_{a1}$ and $K_{a2}$ are defined by equations S1 and S2 respectively.

$$H_2B^{2+} \overset{K_{a1}}{\rightleftharpoons} HB^+ + H^+ \overset{K_{a2}}{\rightleftharpoons} B + H^+$$

**Scheme S1.** Ionization equilibria of a dibasic compound

$$K_{a1} = \frac{[HB^+][H^+]}{[H_2B^{2+}]} \ (S1) \ K_{a2} = \frac{[B][H^+]}{[HB^+]} \ (S2)$$

These equilibria may be linked by a mass balance expression (S3) where $[B]_T$ is the total concentration of all forms of B.

$$[B]_T = [B] + [HB^+] + [H_2B^{2+}] \ (S3)$$

By substitution from (S2) and (S1) into (S3) it is possible to obtain an expression (Equation S4) in terms of the [B], [H+] and the dissociation constants.

$$[B]_T = [B] + \frac{[B][H^+]}{K_{a2}} + \frac{[B][H^+]^2}{K_{a1}K_{a2}} \ (S4)$$

This may be rearranged to give an expression (Equation S5) for the fraction of B in the unprotonated form.

$$\frac{[B]}{[B]_T} = \frac{1}{\left(1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}}\right)} \ (S5)$$

Dividing through by $[H^+]^2/K_{a1}K_{a2}$ gives the more useful form Equation 2.

$$\frac{[B]}{[B]_T} = \frac{\frac{K_{a1}K_{a2}}{[H^+]^2}}{\left(1 + \frac{K_{a1}}{[H^+]} + \frac{K_{a1}K_{a2}}{[H^+]^2}\right)} \ (2)$$

In a similar manner expressions can be derived for the fraction of B in the mono and diprotonated forms (equations S6 and S7 respectively).

$$\frac{[HB^+]}{[B]_T} = \frac{\frac{K_{a1}}{[H^+]}}{\left(1 + \frac{K_{a1}}{[H^+]} + \frac{K_{a1}K_{a2}}{[H^+]^2}\right)} \ (S6)$$

$$\frac{[H_2B^{2+}]}{[B]_T} = \frac{1}{\left(1 + \frac{K_{a1}}{[H^+]} + \frac{K_{a1}K_{a2}}{[H^+]^2}\right)} \ (S7)$$

The general form and trend that may be seen in equations 2, S6 and S7 continues as additional p$K_a$s are added and the expansion of this approach to cover up to five p$K_a$s has been described previously.[1]

**HPLC Method 1 – HPLC Method with UV detection for Experimental Procedure 1 & 2**

Equipment

Thermo Vanquish UHPLC Systems

Column: Acquity UPLC BEH C18 1.7 µm, 3.0 x 50 mm

Chromatographic Conditions:

Flow rate: 0.7 mL/min
Injection volume: 1 µL
Column temperature: 40°C
Detector: UV, 254 nm

Gradient:

| Time (min.) | % Mobile Phase A | % Mobile Phase B |
|---|---|---|
| 0 | 95 | 5 |
| 2 | 95 | 5 |
| 7 | 30 | 70 |
| 7.5 | 30 | 70 |
| 7.6 | 95 | 5 |
| 10 | 95 | 5 |

Preparation of Reagents

Mobile Phase A: 0.1% (v/v) Formic Acid in Water (Milli-Q Filtered)

Mobile Phase B: 0.1% (v/v) Formic Acid in acetonitrile

Diluent: Acetonitrile/Water (50/50 v/v)

Quantification of analytes

For 4-phenyl piperidine, *N*-nitroso-4-phenyl piperidine and 1-methyl-4-phenyl piperidine the peak areas were used to approximate the total quantity of *N*-nitroso-4-phenyl piperidine that had formed.

For the formation of NDEA from diethylamine and triethylamine a NDEA standard was used. The standard was prepared by dissolving NDEA in acetonitrile at a dilution of 1 mg/mL.

Retention Times

| Component | Approximate Retention Time, min. |
|---|---|
| NDEA | 2.6 |
| N4PhP | 6.3 |

**HPLC Method 2 – HPLC Method with MS detection for experimental procedure 1.**

The method used was as per Method 1 with the exception that MS detection was used as described in the following section.

Mass Spectrometry Conditions (For Limited Nitrite Experiment):

| **MS Parameter** | **Value** |
|---|---|
| Instruments | Thermo Orbitrap ID-X (High Res MS) |
| Ion Source | H-ESI |
| Scan Mode | SIM (m/z 191.12, isolation window m/z 0.4) |
| Ion Mode | Positive |
| Vaporizer Temp (°C). | 250 |
| Ion Transfer Temp (°C) | 300 |
| Orbitrap Resolution | 120000 |
| Divert Valve (min) | 0-6 ; 7-10 |

**HPLC Method 3 – HPLC Method with MS Detection for Experimental Procedure 3**

Equipment

Thermo Scientific Ultimate 3000 Liquid Chromatography System

Column

X-Select HSS T3 3.5 µm, 100 x 4.6 mm

Chromatographic Conditions:

Flow rate: 0.5 mL/min
Injection volume: 5 µL
Column temperature: 40°C
Detector: UV, PDA 200 to 400 nm

Gradient:

| Time (min.) | % Mobile Phase A | % Mobile Phase B |
|---|---|---|
| 0 | 70 | 30 |
| 3 | 60 | 40 |
| 6 | 45 | 55 |
| 8 | 10 | 90 |
| 12 | 10 | 90 |
| 12.1 | 70 | 30 |
| 18 | 70 | 30 |

Preparation of Reagents

Mobile Phase A: 0.1% (v/v) Formic Acid in Water (Milli-Q Filtered)

Mobile Phase B: 100% Methanol

Diluent: Methanol/Water (50/50 v/v)

Quantification of analytes

For the detection and quantification of *N*-nitroso-4-phenylpiperidine, a *N*-nitroso-4-phenylpiperidine standard was used. The standard was prepared by dissolving *N*-nitroso-4-phenylpiperidine in acetonitrile at a dilution of 0.5 mg/mL. Using this standard, the linear range of the method was established from 1 ppb to 50 ppb.

Retention Times

| Component | Approximate Retention Time, min. |
|---|---|
| *N*-Nitroso-4-phenylpiperidine | 11.7 |

Mass Spectrometry Conditions (For Limited Nitrite Experiment):

| **MS Parameter** | **Value** |
|---|---|
| Instruments | Thermo Orbitrap QExtractive (High Res MS) |
| Ion Source | H-ESI |

| | |
|---|---|
| Scan Mode | SIM |
| Ion Mode | Positive |
| Capillary Temp | 275 deg C |
| Auxiliary Gas Temp | 400 deg C |
| Sheathe Gas Flow Rate | 55 |
| Auxiliary Gas Flow Rate | 15 |
| Sweep Gas Flow Rate | 3 |
| Spray Voltage | 3.50 |
| Maximum IT | 200 ms |
| Mass Range | 70 to 300 m/z |
| Orbitrap Resolution | 70000 |
| Divert Valve (min) | 0 – 9.5 min |

## Published Berkeley Madonna Model[2] with Temperature Dependence

Berkeley Madonna model used to simulate 4-phenyl piperidine and diethylamine nitrosation. Temperature set at 25 °C (ARR = 1) changing the ARR term to 10, 100 and 1000 varies the temperature to 35, 45 and 55 °C respectively.

```
METHOD RK4

STARTTIME = 0
STOPTIME=86400        {86400 s is equivalent to 24 hours}
DT = 100
DTOUT = 0             {Output time interval (0 = store every step) can be used to limit number of output time points in long simulations}

; I Ashworth, December 2019
; Model for rate of nitrosamine formation by N2O3, ClNO & H2NO2+ in aqueous media at 25°C

INIT R2NH = 0.1
INIT NO2 = 0.2
CL = 0

INIT NITROSAM = 0

KNA = 7.079E-4              {Ka of nitrous acid at 25°C}
KECLNO = 1.1E-3             {association constant of CLNO, M-2}
KRCLNO = 3.1E7              {approximate rate constant for secondary amine nitrosation by CLNO, M-1s-1}
KEN2O3 = 3E-3         {association constant of N2O3, M-1}
KRN2O3 = 1.2E8              {typical rate constant for secondary amine nitrosation by N2O3, M-1s-1}
KRNO = 7000                 {approximate rate constant for NO+ nitrosation, M-2s-1}
PKA = 10.5                  {pKa of secondary amine, 4-phenyl piperidine}
KA=10^(-PKA)
PH = 3.15             {pH of reaction 3.15 is the maximum for N2O3 based nitrosation}
H=10^(-PH)
ARR = 1               {temperature term – 1 = 25C, 10 = 35C, 100 = 45C, 1000 = 55C (higher may be used but the model will become extremely conservative}

; pH speciation models

fH = H/(H+KNA)        {f HNO2 in protonated form}
fN = KA/(H+KA)        {f R2NH in free base form}

; Kinetic model

RXN1 = ARR*KRCLNO*KECLNO*H*R2NH*NO2*CL*fH*fN        {Nitrosation by ClNO}
RXN2 = ARR*KRN2O3*KEN2O3*R2NH*NO2*NO2*fH*fH*fN      {Nitrosation by N2O3}
RXN3 = ARR*KRNO*H*R2NH*NO2*fH*fN                    {Nitrosation by H2NO2+}

D/DT(R2NH) = -RXN1-RXN2-RXN3
D/DT(NO2) = -RXN1-RXN2-RXN3
D/DT(NITROSAM) = RXN1+RXN2+RXN3
```

**Modified Berkeley Madonna model for an amine with 2 p$K_a$s**

Berkeley Madonna model that may be used to simulate 4-(piperidin-2-yl)pyridine nitrosation

```
METHOD RK4

STARTTIME = 0
STOPTIME=86400    {86400 s is equivalent to 24 hours}
DT = 100
DTOUT = 0         {Output time interval (0 = store every step) can be used to limit
number of output time points in long simulations}

; I Ashworth, January 2023
; Model for rate of nitrosamine formation by N2O3, ClNO & H2NO2+ in aqueous media at
25°C for a dibasic amine where the unprotonated (free base) form is reactive

INIT R2NH = 0.1
INIT NO2 = 0.2
CL = 0

INIT NITROSAM = 0

KNA = 7.079E-4            {Ka of nitrous acid at 25°C}
KECLNO = 1.1E-3           {association constant of CLNO, M-2}
KRCLNO = 3.1E7            {approximate rate constant for secondary amine nitrosation by
CLNO, M-1s-1}
KEN2O3 = 3E-3             {association constant of N2O3, M-1}
KRN2O3 = 1.2E8            {typical rate constant for secondary amine nitrosation by
N2O3, M-1s-1}
KRNO = 7000               {approximate rate constant for NO+ nitrosation, M-2s-1}
PKAAM1 = 4.6              {pKa1 of amine}
PKAAM2 = 8.6              {pKa2 of amine}
KAAM1=10^(-PKAAM1)
KAAM2=10^(-PKAAM2)
PH = 3.15         {pH of reaction 3.15 is the maximum for N2O3 based nitrosation}
H=10^(-PH)
ARR = 1           {temperature term – 1 = 25C, 10 = 35C, 100 = 45C, 1000 = 55C
(higher may be used but the model will become extremely conservative}

; pH speciation models

fH = H/(H+KNA)            {f HNO2 in protonated form}

A1 = KAAM1/H
A2 = KAAM1*KAAM2/(H^2)
DENOMINATOR = 1+A1+A2
FH2AM = 1/DENOMINATOR     {f amine in most (di) protonated form - unreactive}
FHAM = A1/DENOMINATOR     {f amine in mono protonated form – unreactive}
FAM = A2/DENOMINATOR      {f amine in unprotonated form – reactive}

; Kinetic model

RXN1 = ARR*KRCLNO*KECLNO*H*R2NH*NO2*CL*fH*FAM      {Nitrosation by ClNO}
RXN2 = ARR*KRN2O3*KEN2O3*R2NH*NO2*NO2*fH*fH*FAM    {Nitrosation by N2O3}
RXN3 = ARR*KRNO*H*R2NH*NO2*fH*FAM                  {Nitrosation by H2NO2+}
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D/DT(R2NH) = -RXN1-RXN2-RXN3
D/DT(NO2) = -RXN1-RXN2-RXN3
D/DT(NITROSAM) = RXN1+RXN2+RXN3

**References**


1. Ashworth, I. W.; Meadows, R. E. A General Liquid-Liquid Partitioning Equation and Its Consequences: Learning from the pH Dependent Extraction of a Pharmaceutical Intermediate. *J. Org. Chem.* **2018**, *83*, 4270-4274. See ESI Page S9.
2. Ashworth, I. W.; Dirat, O.; Teasdale, A.; Whiting, M. P. Potential for the Formation of *N*-Nitrosamines During the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. *Org. Process Res. Dev.*, **2020**, *24*, 1629-1646.