Investigation on the role of copper (II) on nitrosamine formation from nicotine

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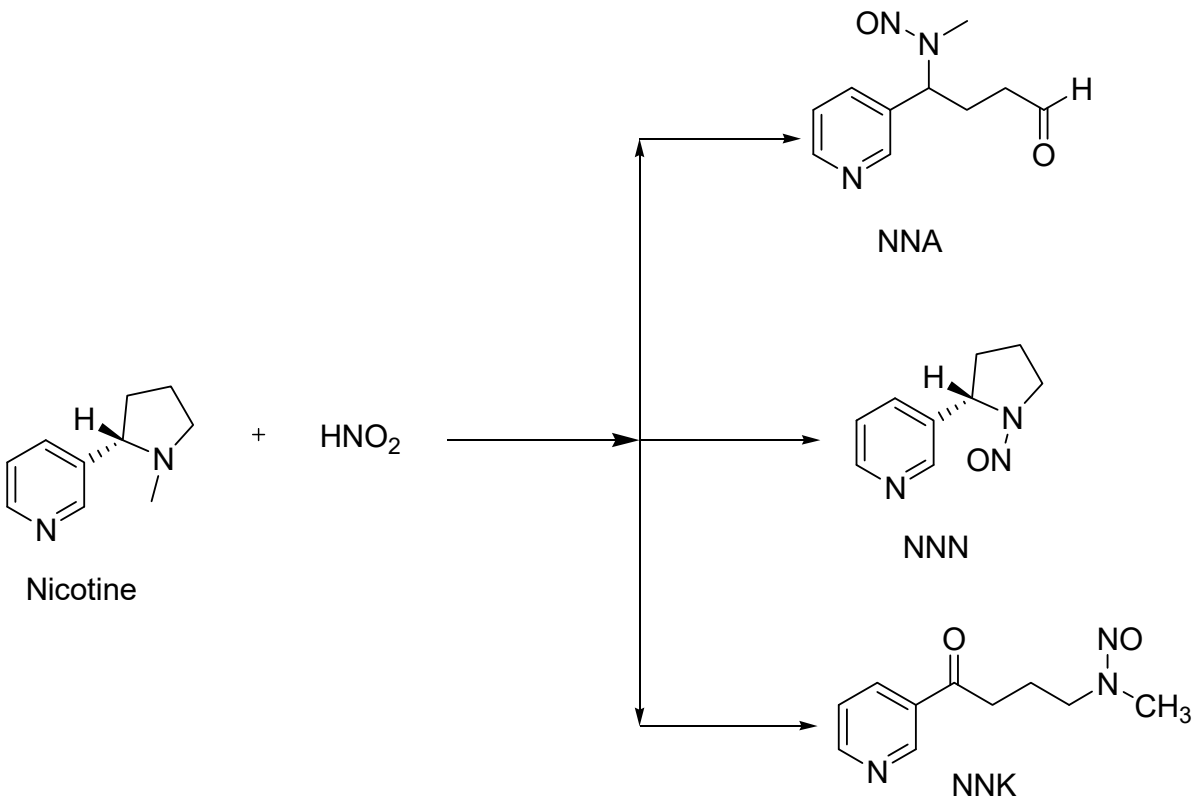
Background

- **Why copper?**
  - Copper is an essential plant nutrient
  - Copper can form complexes with basic nitrogen containing alkaloids
  - Copper can catalyse N-nitrosation reaction

Cu content in tobacco

Tobacco sample (In-house analysis)	Cu (mg/g)	Tobacco sample (Reported in literature)	Cu (mg/g)
1R6F Ground Filler	19 ± 2	Turkish Tobacco	14.1 ± 1.2
Ground Flue Cured	19 ± 1	Rothman cigarette filler	9.55
Ground Oriental	20 ± 2	Marlborow cigarette filler	10.7
Ground Burley	20 ± 3	Winston Light cigarette filler	13.1
High TSNA Ground Tobacco	16 ± 1	Marlboro Light cigarette filler	11.25
Ground Dark Fire Cured	21 ± 2	Winston cigarette filler	11.2
1R5F Ground Filler	12 ± 3	Virginia tobacco samples	16.2 ± 0.1
		Burley tobacco samples	23.9 ± 0.1

Nitrosation reaction of nicotine: formation of TSNAs



William S. Caldwell, Jackie M. Greene, David R. Plowchalk, and J. Donald DeBethizy *Chemical Research in Toxicology* **1991** *4* (5), 513-516

Main question

- Does copper play a role in the nitrosation reaction that leads to TSNA formation?

Experimental design

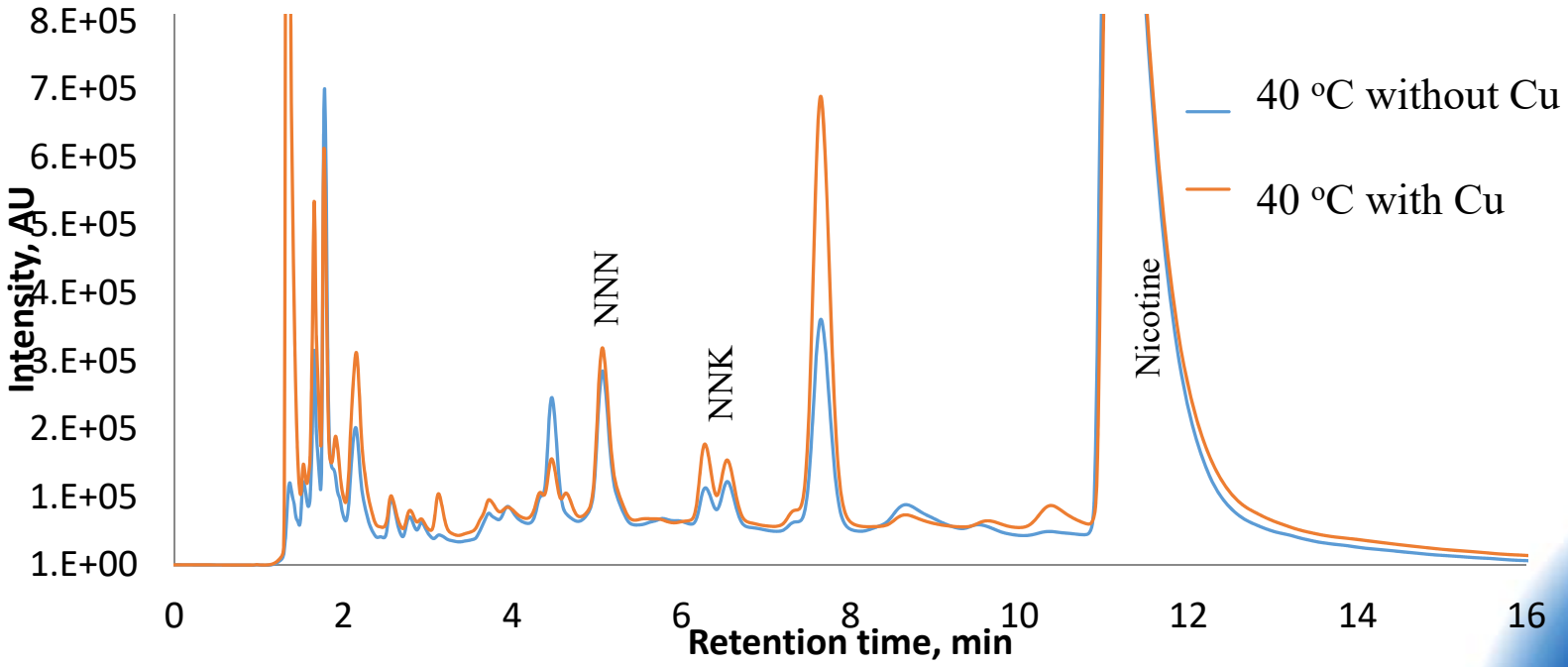
- Reaction was performed in an incubator temperature set to 40 ^{o}C for 5 days with and without presence of $CuCl_2$
- Initial concentration of the added reactants in the mixtures were 100 mM nicotine, 100 mM sodium nitrite, and 25 mM copper (II) chloride.
- Reaction matrix

1. Pure citrate-phosphate buffer pH 3.5 or
2. 50/50 mixture of buffer and tobacco matrix

- Aliquots were withdrawn periodically from the reaction mixture and analyzed in HPLC

Chromatograms of the reaction mixture in presence and absence of Cu^{2+}

HPLC-UV chromatogram of the reaction mixture after 48 hours



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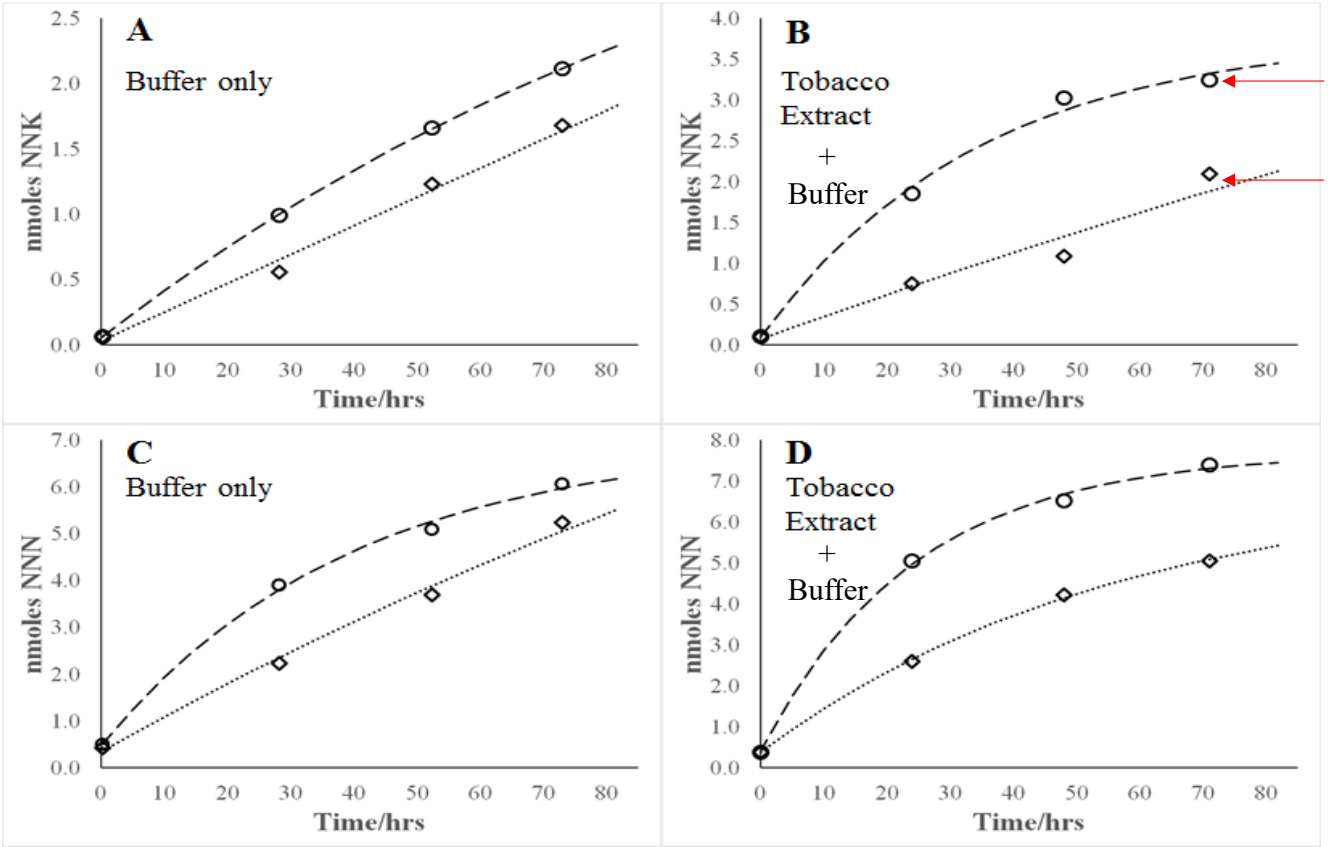
Comparison of nitrosation kinetics in different media

1st order kinetics plots using HPLC



With Cu^{2+}

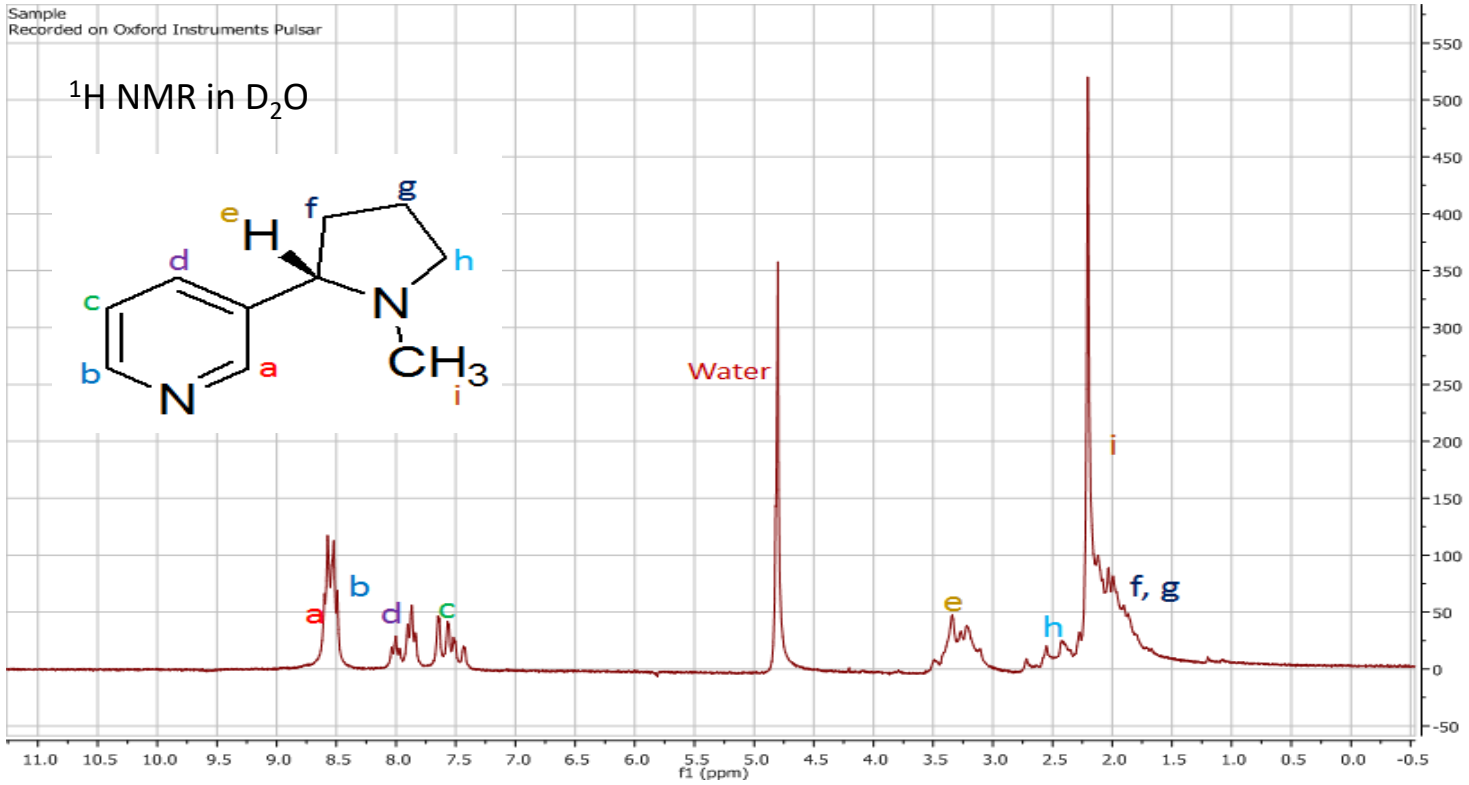
Without Cu^{2+}

Comparison of rate constants: 1st order kinetics of nitrosation of nicotine at 40°C

Reaction matrix	K_{NNN}, With Cu^{2+} ($x10^{-5}$ hr^{-1})	K_{NNN}, Without Cu^{2+} ($x10^{-5}$ hr^{-1})	K_{NNk}, With Cu^{2+} ($x10^{-5}$ hr^{-1})	K_{NNk}, Without Cu^{2+} ($x10^{-5}$ hr^{-1})
Clean buffer only	1125 ± 281	693.8 ± 94.6	830.2 ± 66.4	105.1 ± 0.118
Tobacco extract + buffer (1:1)	2454 ± 992	840.4 ± 25.6	1281 ± 78.9	623.2 ± 79.08

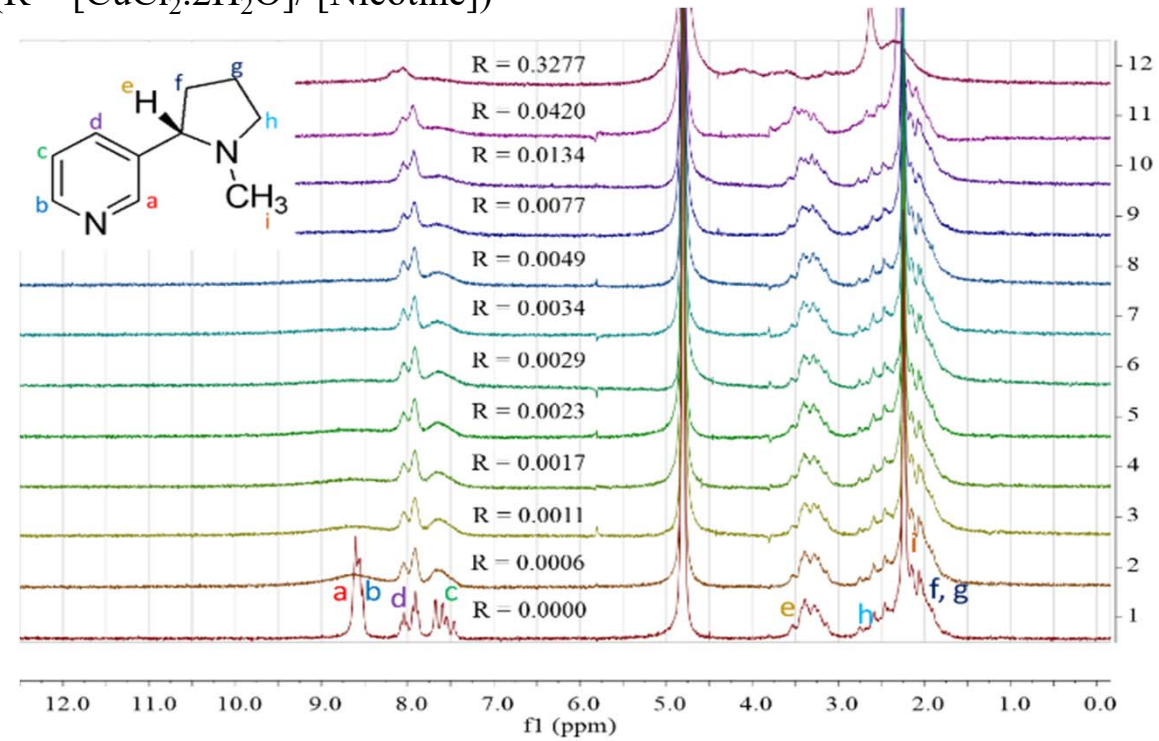
Nicotine-Copper complexation: ^{1}H NMR experiments

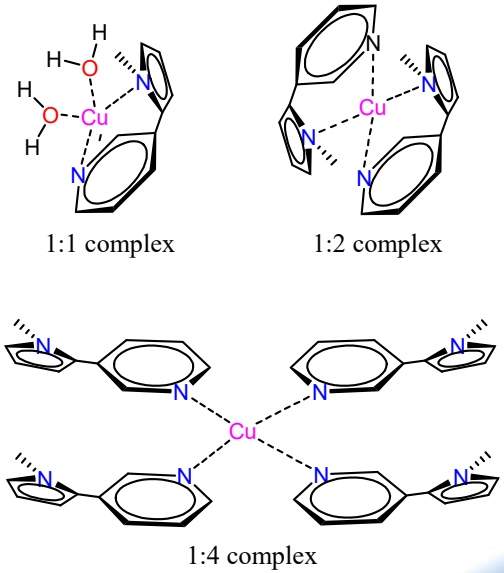
^{1}H NMR characterization spectrum of Nicotine in D_2O



Nicotine copper complexation ^{1}H NMR titration in D_2O

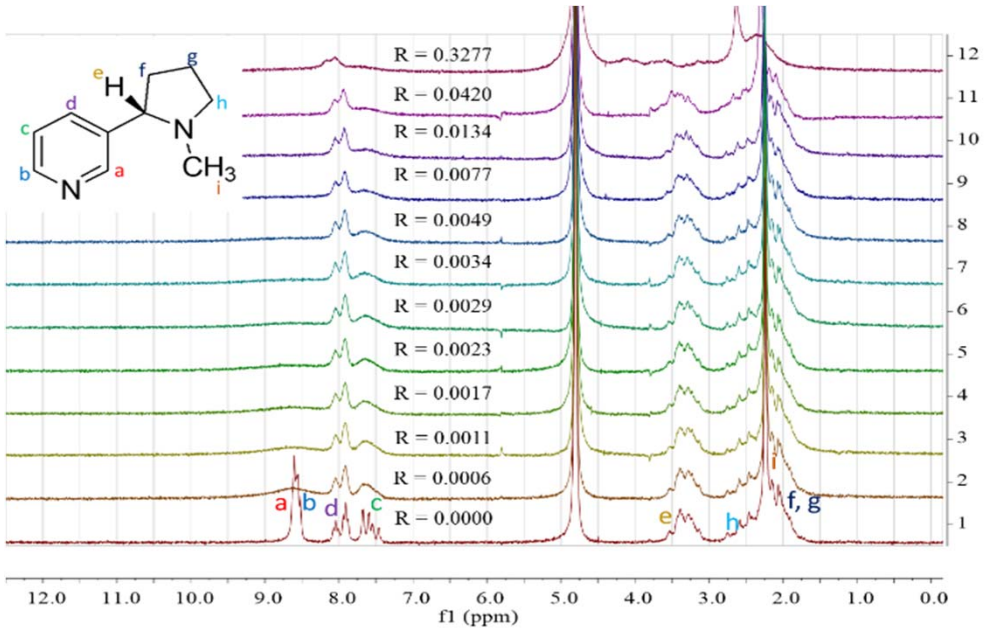
^{1}H NMR titration spectra of nicotine upon gradual addition of $CuCl_2.2H_2O$ (R = [$CuCl_2.2H_2O$]/ [Nicotine])





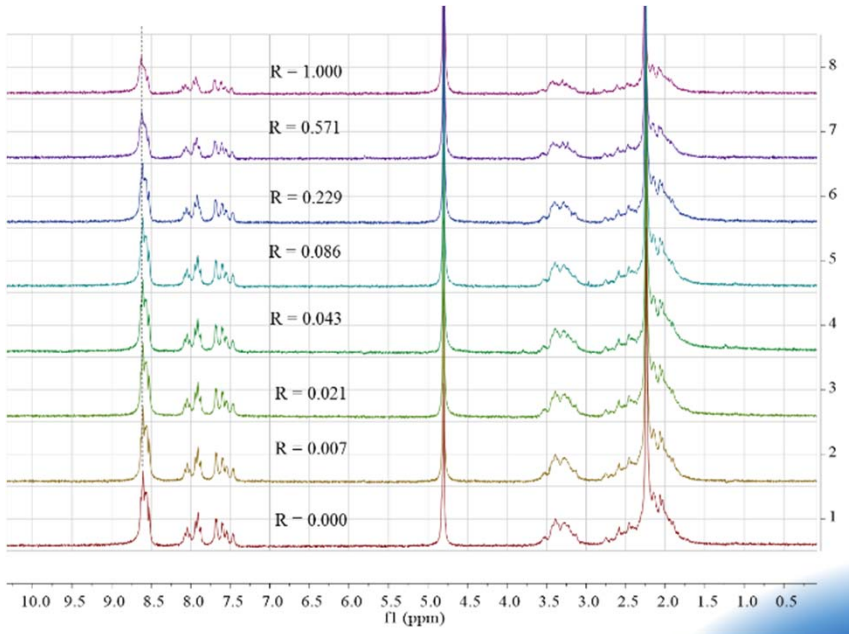
Shen, L.; Zhang, H.-Y.; Ji, H.-F. Computational note on the SOD-like antioxidant potential of nicotine–copper(II) complexes. *Journal of Molecular Structure: THEOCHEM* **2007**, *817*, 161-162.

Catalytic effect of Cu^{2+}: ^1H NMR titration of nicotine with Cu^{2+}



Nicotine - Copper chloride titration in D_2O

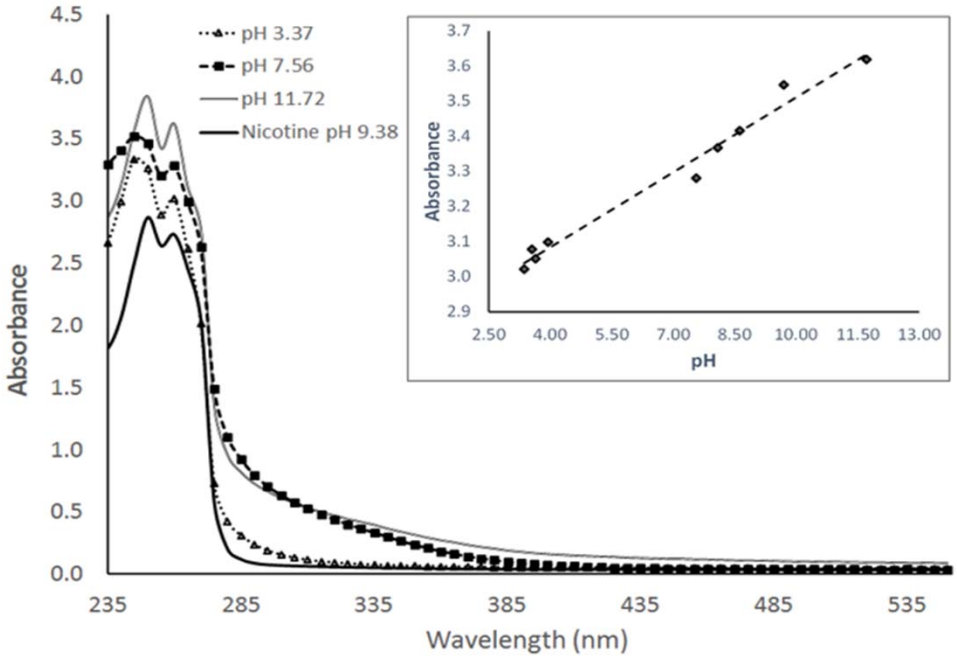
$R = [CuCl_2.2H_2O]/ [Nicotine]$



$NaNO_2$ - Copper chloride titration

$R = [NaNO_2]/ [Nicotine]$

pH dependent UV-vis titration



- The complexation was favored at neutral to basic pH

Conclusions

- Our study strongly suggests that copper potentially catalyzes the TSNA formation
- Copper binds more favorably to the pyridine moiety of nicotine
- Copper complexation of nicotine is pH dependent

Acknowledgement

- FDA
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- University of Kentucky