Expediting Disulfiram Assays through a Systematic Analytical Quality by Design Approach

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Supplementary Material

Cu(DDC)$_2$ structural analysis: ^{1}H and ^{13}C RMN

The complex is paramagnetic and, because of the rapid nuclear relaxation induced by the paramagnetic properties of the Cu (II) metal ion, ^{1}H NMR signals of bound ligands (inner sphere) are broadened and/or shifted (to lower frequencies) (**Figure S1**). The large shifts and linewidths observed for the signals from the ^{1}H nuclei of CH_2 and CH_3 suggest that the ligand is coordinated to the metal ion by the S atoms of the CS_2^- group; the complex is assumed to have a metal:ligand stoichiometry of 1:2. Accordingly, the ^{13}C NMR signals of the ligands in the complex are not detected, probably because of severe broadening (**Figure S2**). Shifts, as a result of ligand binding to paramagnetic metals, may come either from through-space dipolar interactions (pseudo-contact), or from direct delocalization of unpaired electron spin-density from the metal (contact shift). The Cu (II) ($3d^9$) metal ion in the complex will have both contact shift and pseudo-contact shift contributions, resulting in very broad signals for mononuclear complexes. In contrast, binuclear copper (II) complexes show relatively sharp NMR line widths which are two orders of magnitude narrower than the mononuclear analogues. The observed broad signals support the hypothesis that the copper (II)/ligand complex is a mononuclear CuL_2 species.

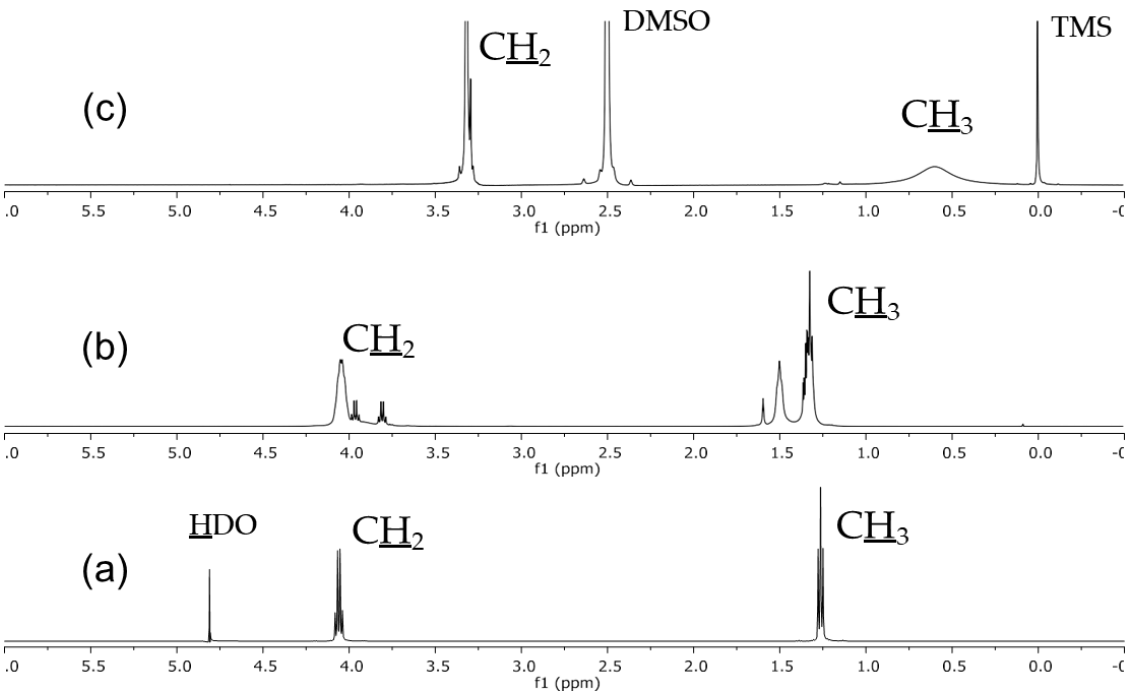

Figure S1. ^{1}H NMR spectra of **(a)** sodium diethyldithiocarbamate trihydrate in D_2O, **(b)** disulfiram in $CDCl_3$ and **(c)** copper (II) diethyldithiocarbamate in DMSO.

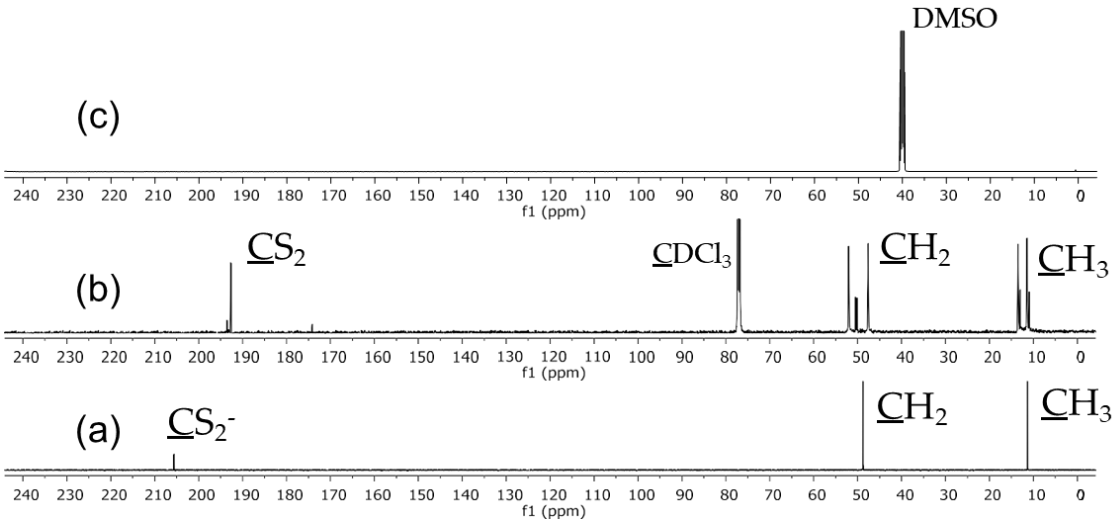

Figure S2. ^{13}C NMR spectra of **(a)** sodium diethyldithiocarbamate trihydrate in D_2O, **(b)** disulfiram in $CDCl_3$ and **(c)** copper (II) diethyldithiocarbamate in DMSO.

Table S1. ANOVA parameters for the characterization of the model fitting per CAA.

Number of Theoretical Plates (DSF)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	3	97665849	32555283	82.5452
Error	14	5521510	394393.58	**Prob > F**
C. Total	17	103187360		<.0001*
Lack Of Fit	5	1706131.7	341226	0.8049
Pure Error	9	3815378.5	423931	**Prob > F**
Total Error	14	5521510.2		0.5737
				Max RSq
				0.9630
Number of Theoretical Plates ($Cu(DDC)_2$)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	2	157315566	78657783	69.8542
Error	15	16890414	1126027.6	**Prob > F**
C. Total	17	174205980		<.0001*
Lack Of Fit	6	5364563	894094	0.6982
Pure Error	9	11525851	1280650	**Prob > F**
Total Error	15	16890414		0.6587
				Max RSq
				0.9338
Tailing Factor (DSF)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	3	0.15183087	0.050610	1070.915
Error	14	0.00066163	0.000047	**Prob > F**
C. Total	17	0.15249250		<.0001*
Lack Of Fit	5	0.00020413	0.000041	0.8031
Pure Error	9	0.00045750	0.000051	**Prob > F**
Total Error	14	0.00066163		0.5748
				Max RSq
				0.9970
Tailing Factor ($Cu(DDC)_2$)				

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.19253333	0.192533	61.3924
Error	16	0.05017778	0.003136	**Prob > F**
C. Total	17	0.24271111		<.0001*
Lack Of Fit	1	0.00694444	0.006944	2.4094
Pure Error	15	0.04323333	0.002882	**Prob > F**
Total Error	16	0.05017778		0.1414
				Max RSq
				0.8219

Resolution				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	4	197.44111	49.3603	38501.02
Error	13	0.01667	0.0013	**Prob > F**
C. Total	17	197.45778		<.0001*
Lack Of Fit	4	0.01666667	0.004167	.
Pure Error	9	0.00000000	0.000000	**Prob > F**
Total Error	13	0.01666667		.
				Max RSq
				1.0000

Retention Time (DSF)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	4	128.21791	32.0545	881.0985
Error	13	0.47294	0.0364	**Prob > F**
C. Total	17	128.69085		<.0001*
Lack Of Fit	4	0.45179167	0.112948	48.0629
Pure Error	9	0.02115000	0.002350	**Prob > F**
Total Error	13	0.47294167		<.0001*
				Max RSq
				0.9998

Retention Time ($Cu(DDC)_2$)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	4	1062.3752	265.594	918.0852
Error	13	3.7608	0.289	**Prob > F**
C. Total	17	1066.1360		<.0001*
Lack Of Fit	4	3.7544833	0.938621	1340.887
Pure Error	9	0.0063000	0.000700	**Prob > F**
Total Error	13	3.7607833		<.0001*
				Max RSq
				1.0000

Capacity Factor (DSF)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	2	99.595211	49.7976	2083.679
Error	15	0.358483	0.0239	**Prob > F**
C. Total	17	99.953694		<.0001*

Capacity Factor ($Cu(DDC)_2$)				
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	2	867.52943	433.765	2486.454
Error	15	2.61677	0.174	**Prob > F**
C. Total	17	870.14620		<.0001*