Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Copper(II) Mixed-Ligand Polypyridyl Complexes with Doxycycline – Structures and Biological Evaluation

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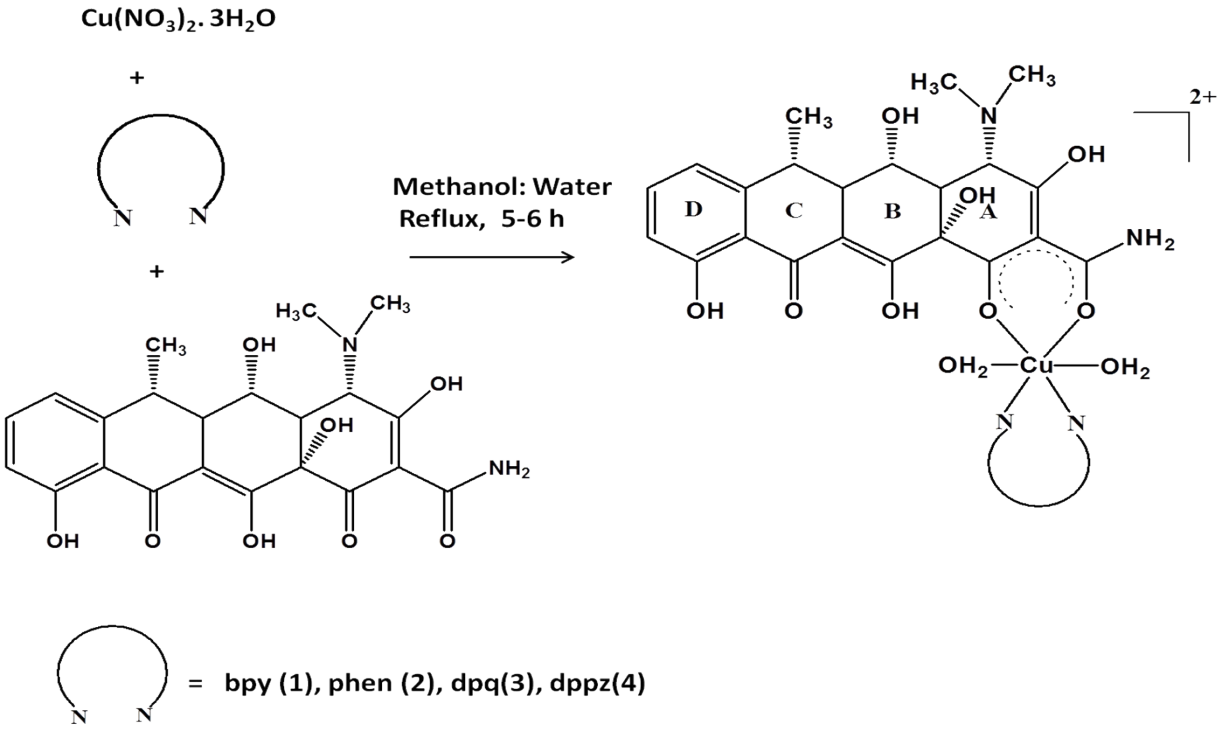
Scheme S-1: Synthetic route of complexes 1-4



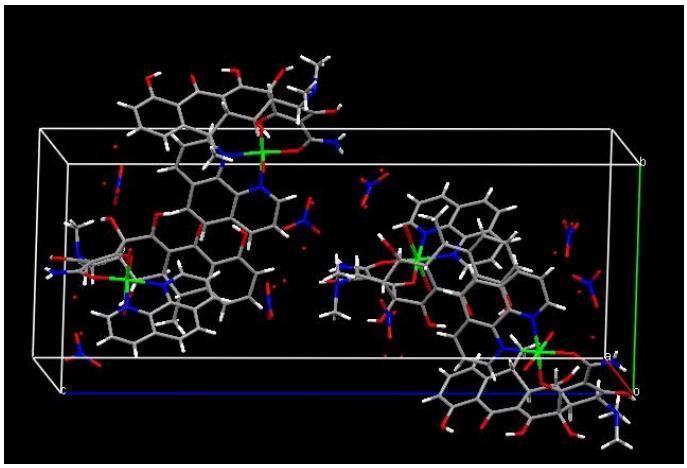


Figure S1: Packing Diagram of complex **2**

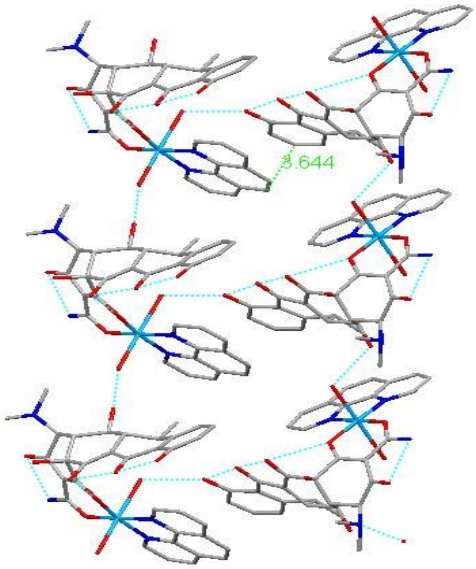


Figure S2: Intermolecular and intramolecular hydrogen bonding in **2**

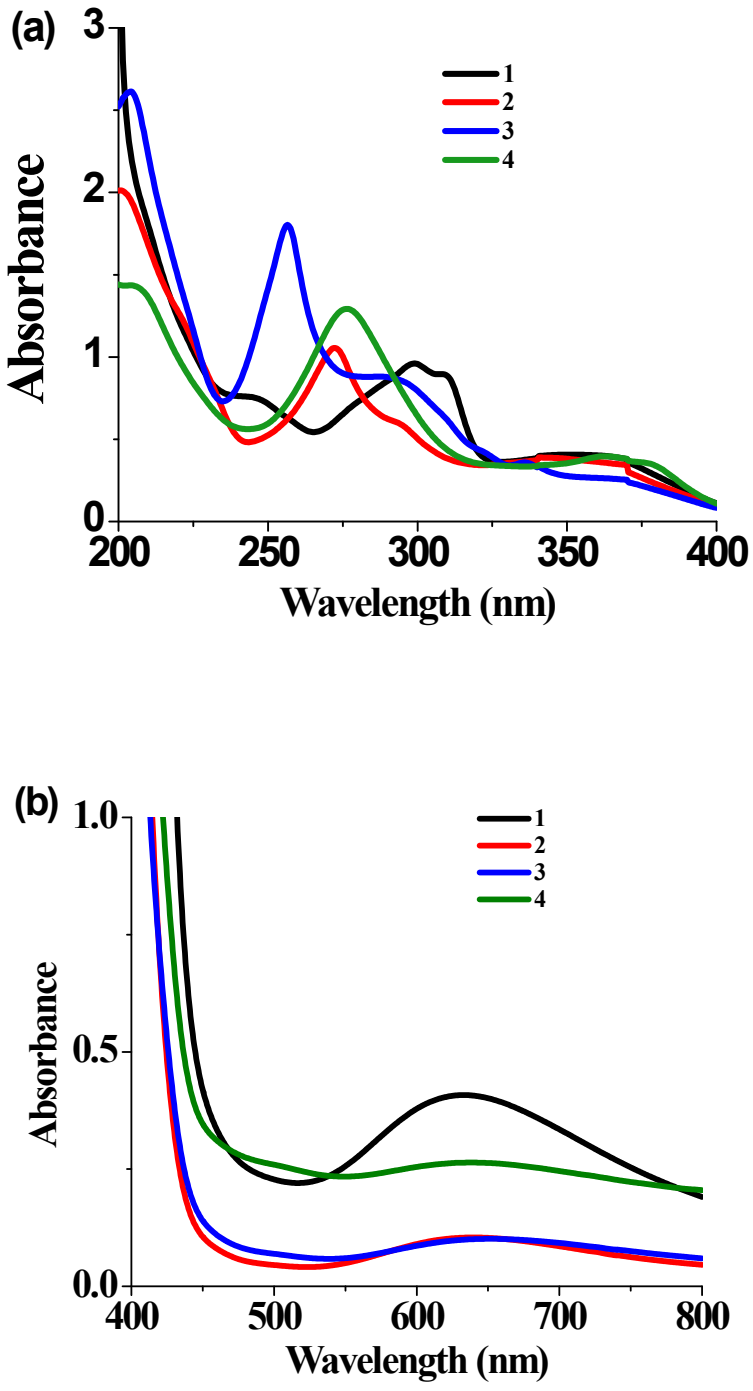


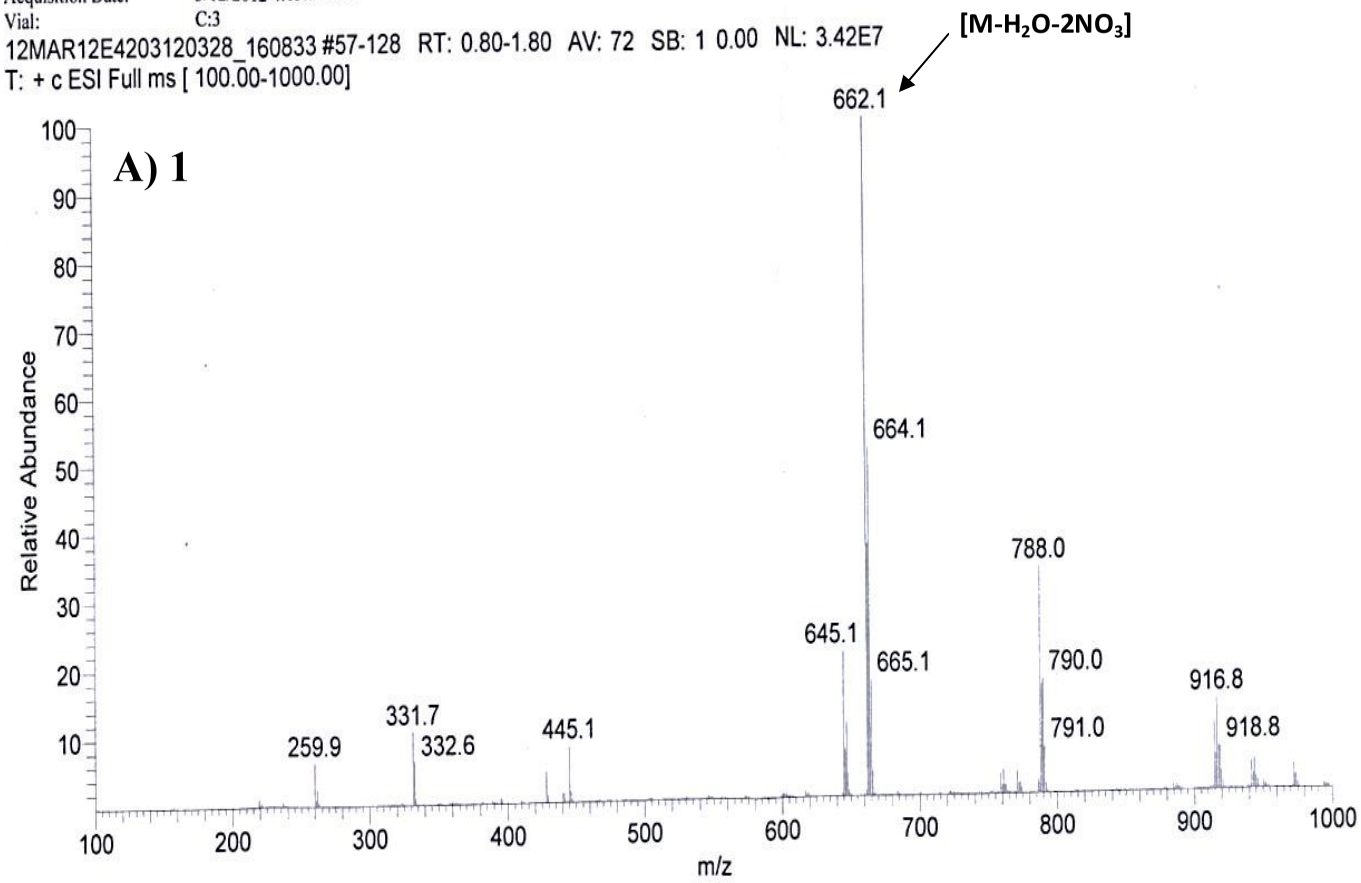
Figure S3: UV-Vis. Spectra of complex (**1-4)** a) **20 μM** b) **1 mM** in water at room temperature

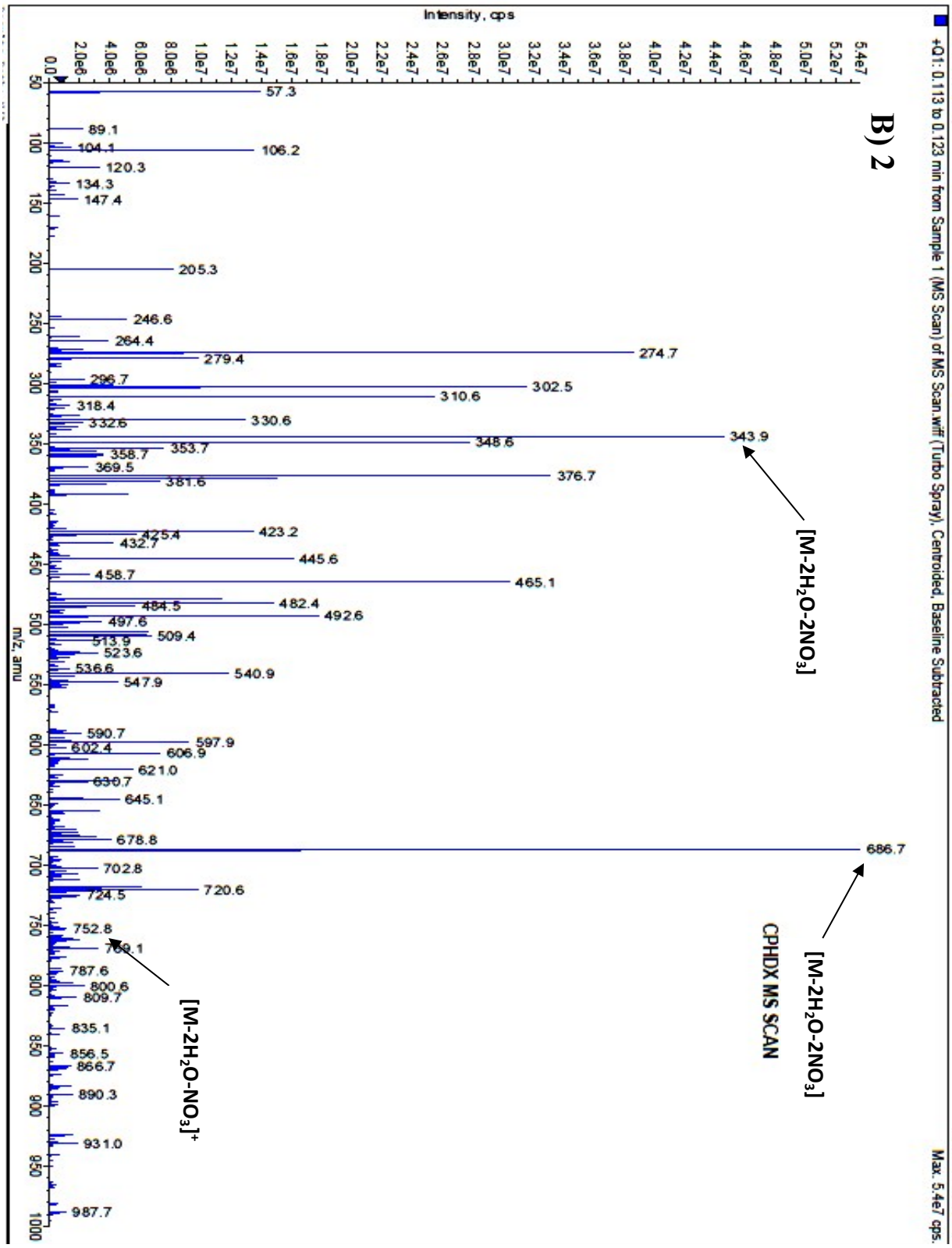
MSAIF, CDRI LUCKNOW

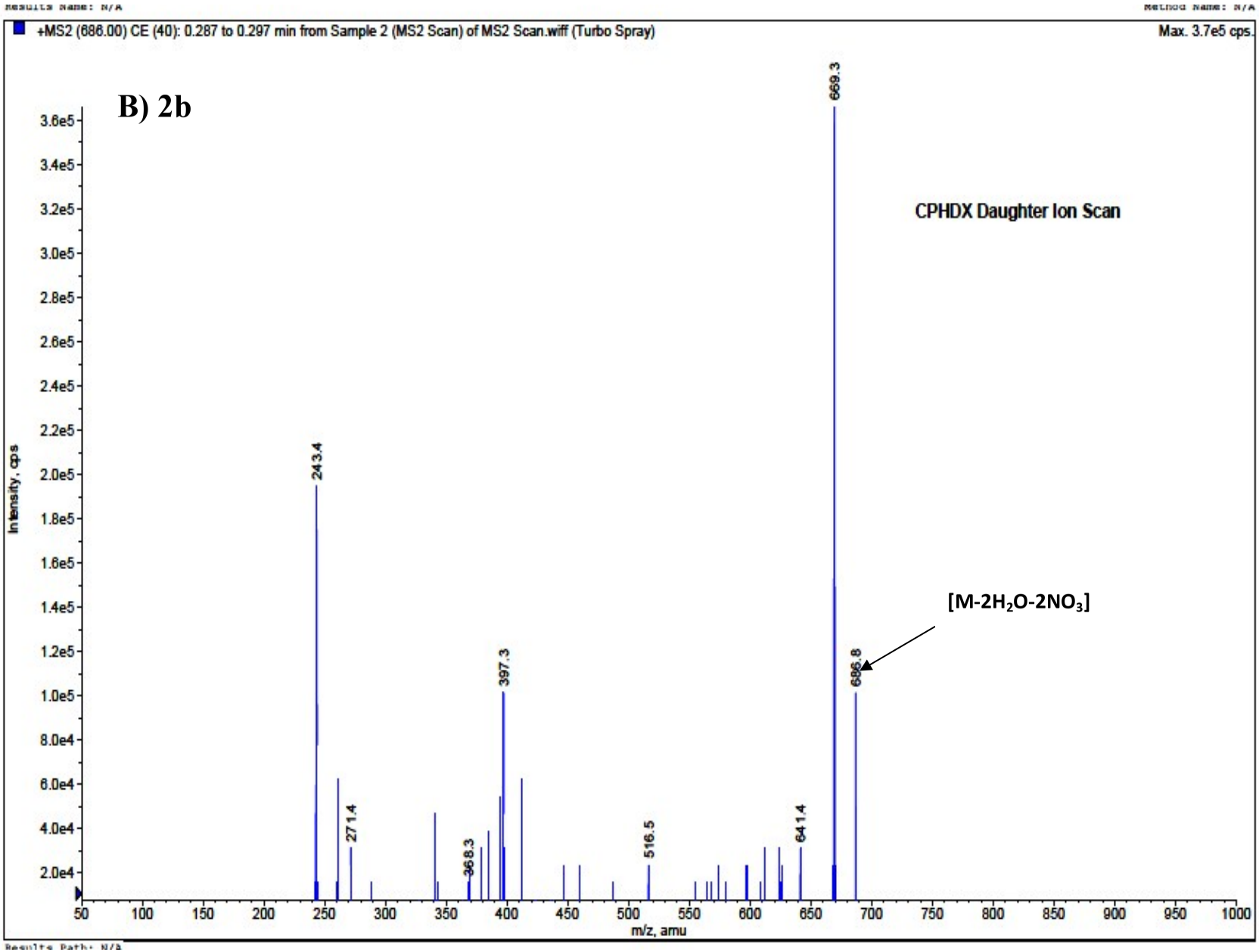
Original Data Path: 12MAR12E4203120328_160833.RAW
Current Data Path: C:\Data2012\MAR12\
Sample ID: CBDX #6778
Acquisition Date: 3/12/2012 4:13:32 PM
Vial: C:3

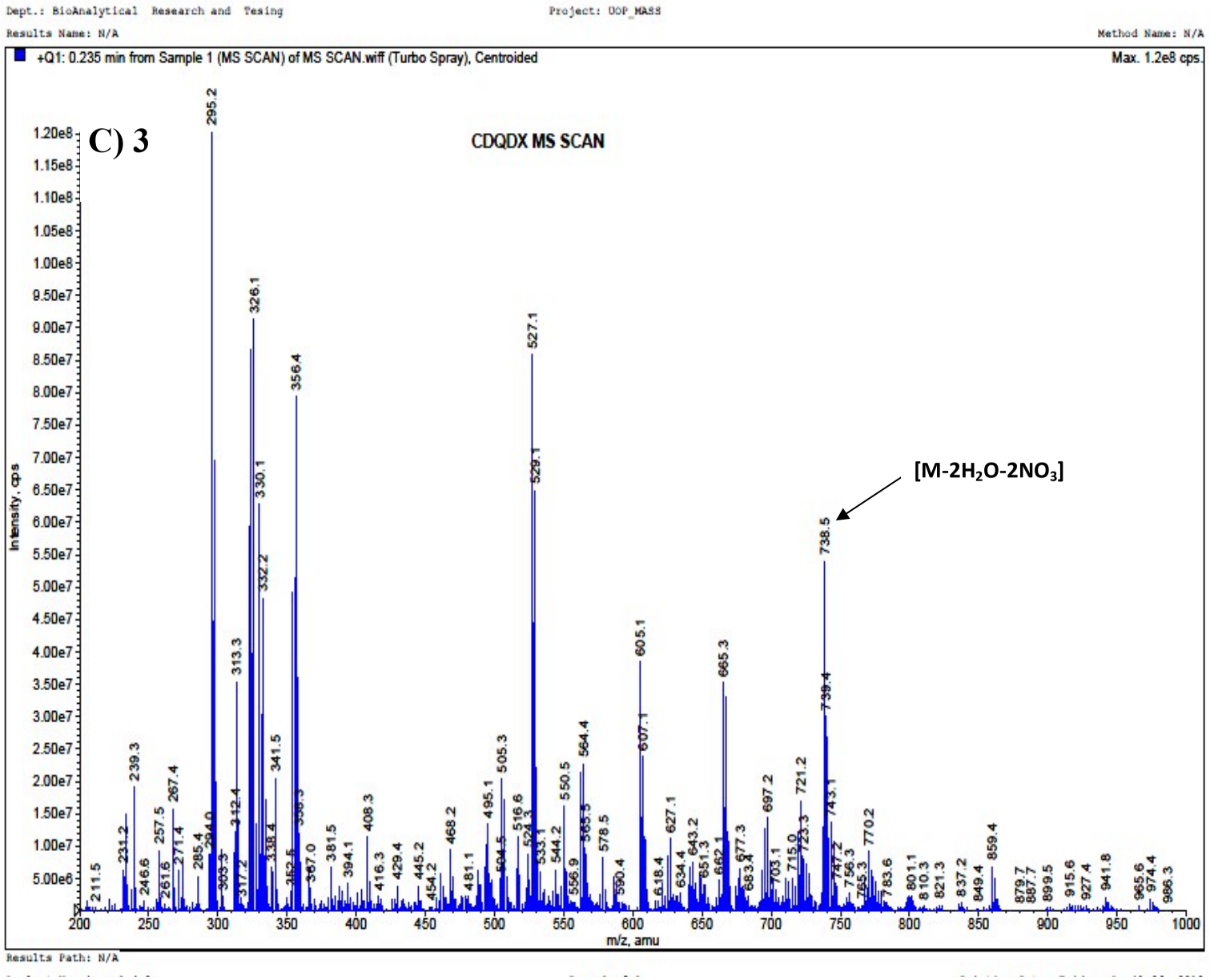
12MAR12E4203120328_160833 #57-128 RT: 0.80-1.80 AV: 72 SB: 1 0.00 NL: 3.42E7
T: + c ESI Full ms [100.00-1000.00]

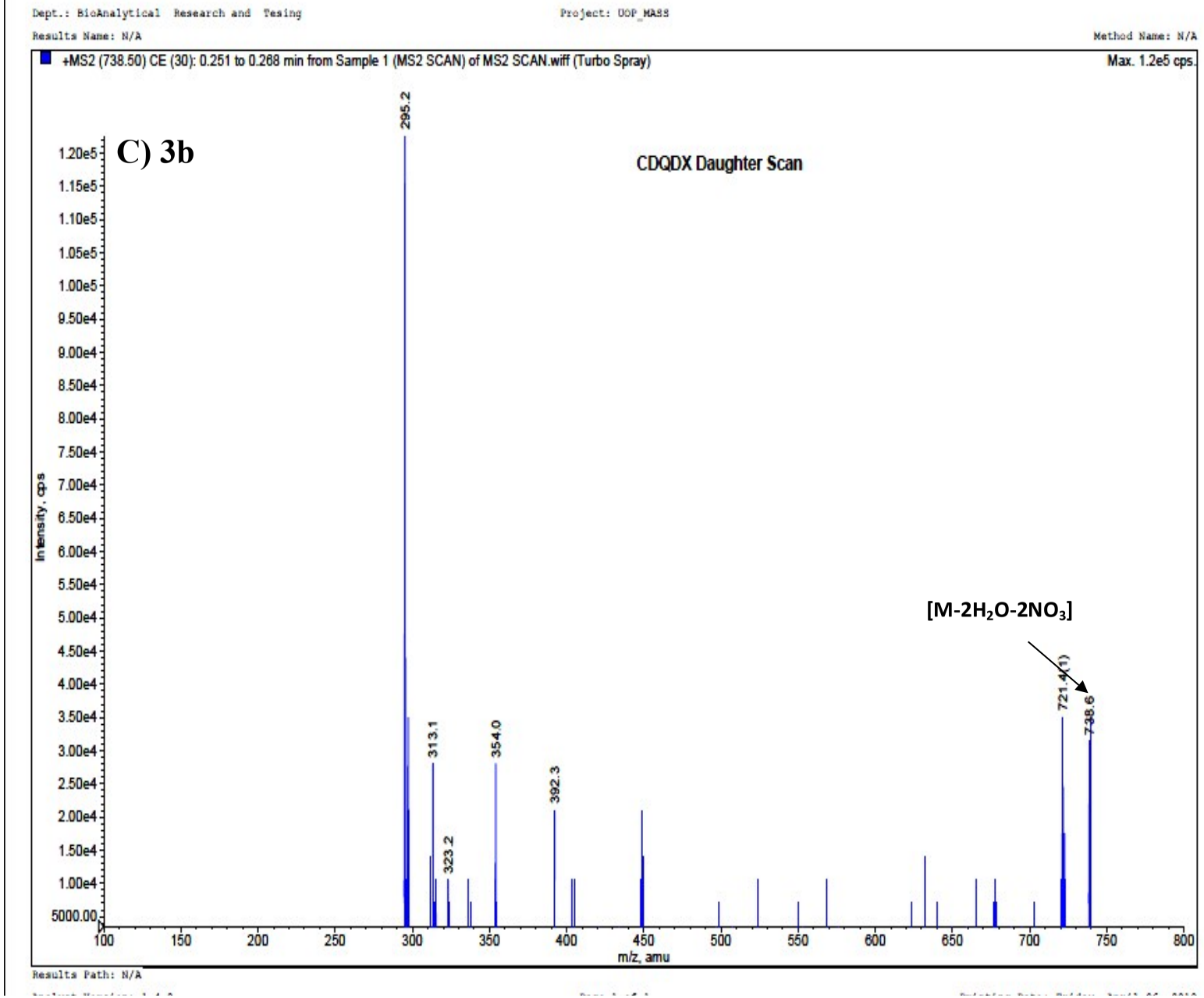
A) 1




B) 2
+Q1: 0.113 to 0.123 min from Sample 1 (MS Scan) of MS Scan.wiff (Turbo Spray), Centroided, Baseline Subtracted
Max. 5.4e7 cps.
Intensity, cps
m/z, amu
[M-2H2O-2NO3]
[M-2H2O-2NO3]
[M-2H2O-NO3]+
CPHDX MS SCAN


+MS2 (686.00) CE (40): 0.287 to 0.297 min from Sample 2 (MS2 Scan) of MS2 Scan.wiff (Turbo Spray)
Max. 3.7e5 cps.
B) 2b
CPHDX Daughter Ion Scan
[M-2H2O-2NO3]
Intensity, cps
m/z, amu
669.3
243.4
397.3
686.8
271.4
368.3
516.5
641.4


Dept.: BioAnalytical Research and Tesing
Project: DOP_MASS
Results Name: N/A
Method Name: N/A
+Q1: 0.235 min from Sample 1 (MS SCAN) of MS SCAN.wiff (Turbo Spray), Centroided
Max. 1.2e8 cps.
C) 3
CDQDX MS SCAN
[M-2H2O-2NO3]
Intensity, cps
m/z, amu
Results Path: N/A


Dept.: BioAnalytical Research and Tesing
Project: DOP_MASS
Results Name: N/A
Method Name: N/A
+MS2 (738.50) CE (30): 0.251 to 0.288 min from Sample 1 (MS2 SCAN) of MS2 SCAN.wiff (Turbo Spray)
Max. 1.2e5 cps.
C) 3b
CDQDX Daughter Scan
295.2
313.1
323.2
354.0
392.3
721.4(1)
738.6
[M-2H2O-2NO3]
Intensity, cps
m/z, amu
Results Path: N/A

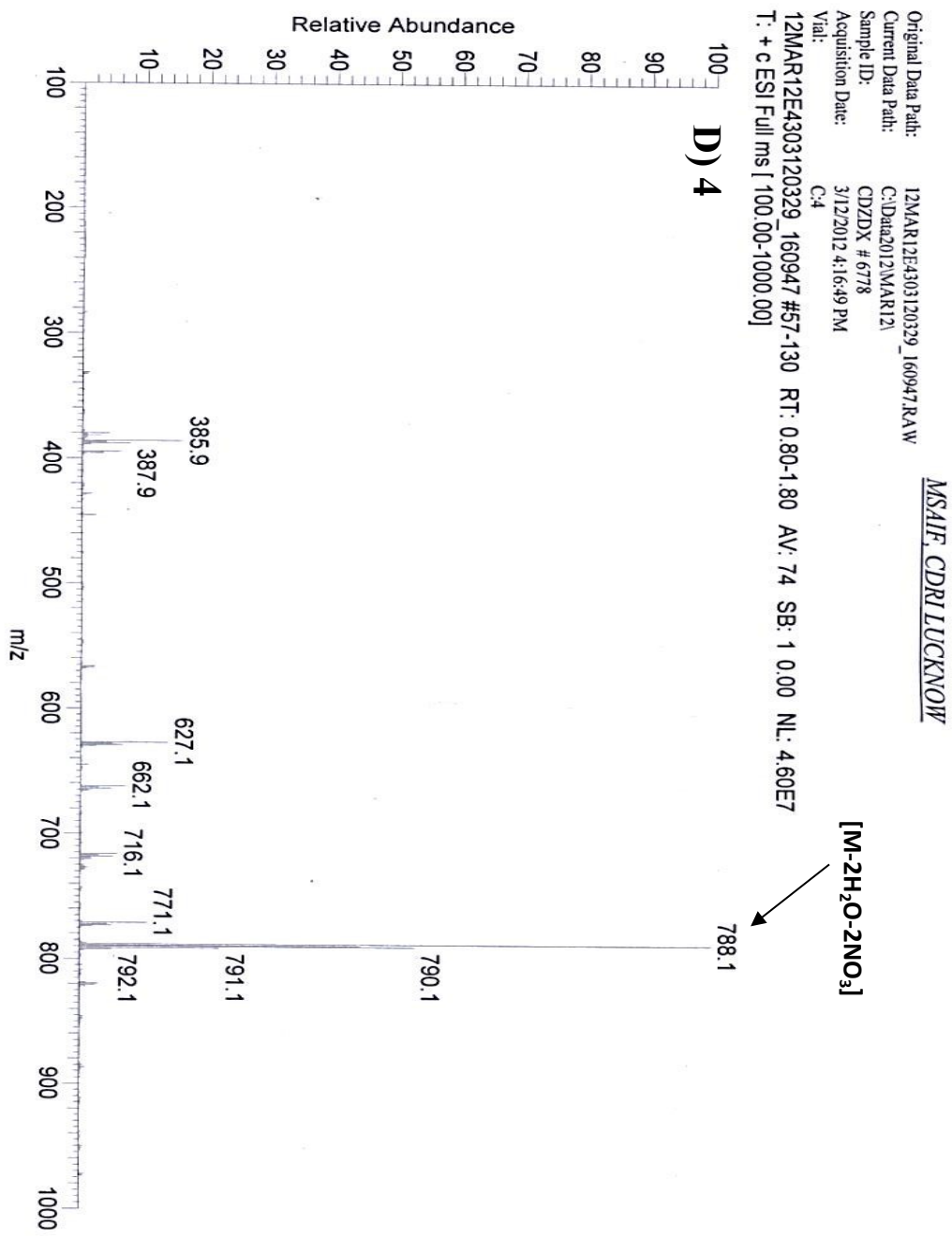


Figure S4.ESI-MS of A) **1,** B) **2, 2b** C) **3, 3b**, D) **4** in methanol

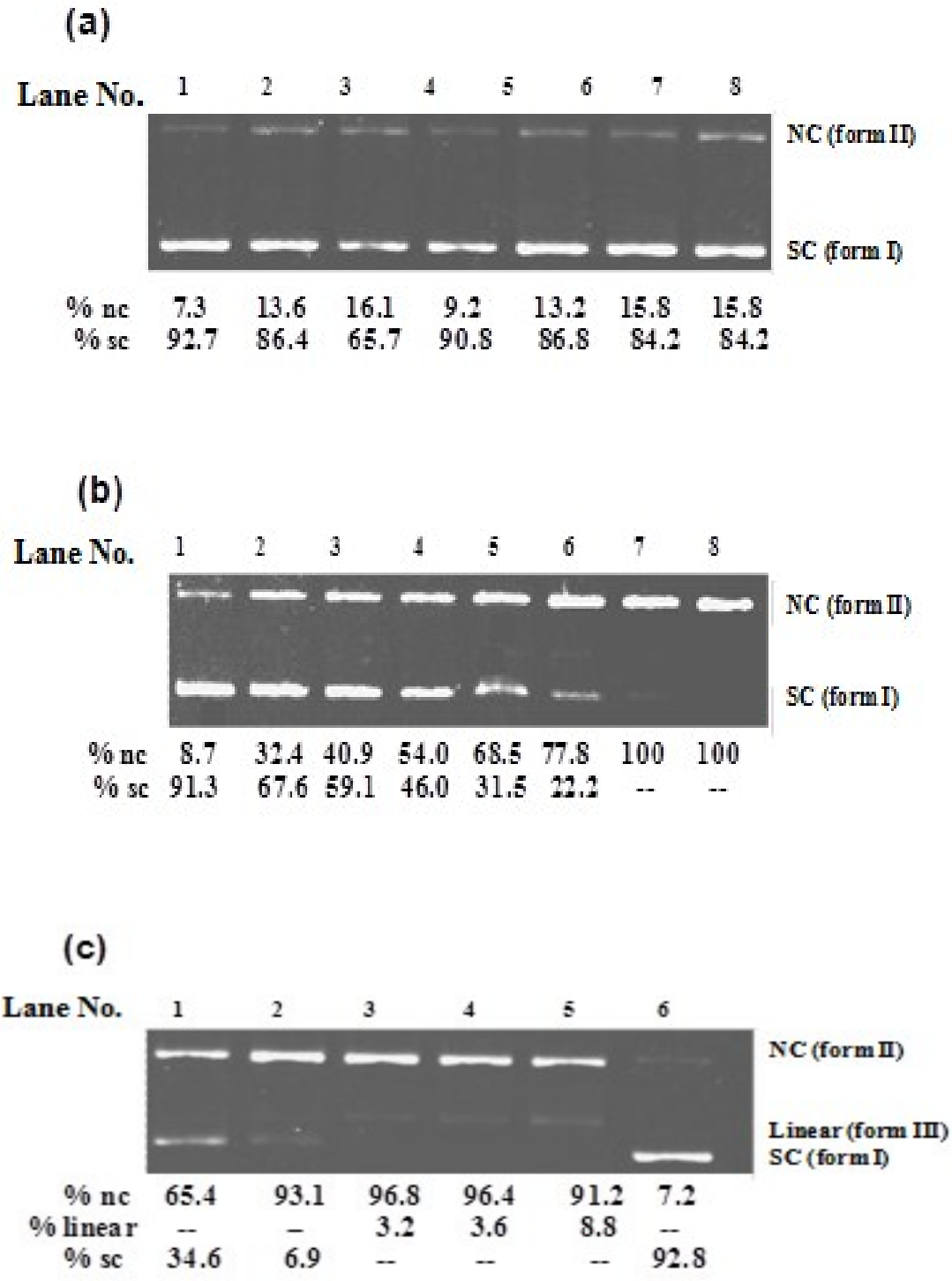


Figure S5: Photographs of 1% Agarose gel showing cleavage of plasmid *pBR*322 DNA by **1-3** incubated at 37°C for 20 min. [DNA] = 300 ng. (a) **1**; Lane 1, DNA Control; Lanes 2-7, DNA + 10, 20, 30, 40, 50, 100 μM (b) **2**; Lane 1, DNA Control; Lanes 2-7, DNA + 5, 10, 20, 30, 40, 50,100 μM (c) **3**; Lanes 1-5, DNA + 10, 20, 30, 40, 50 μM; Lane 6, DNA Control.

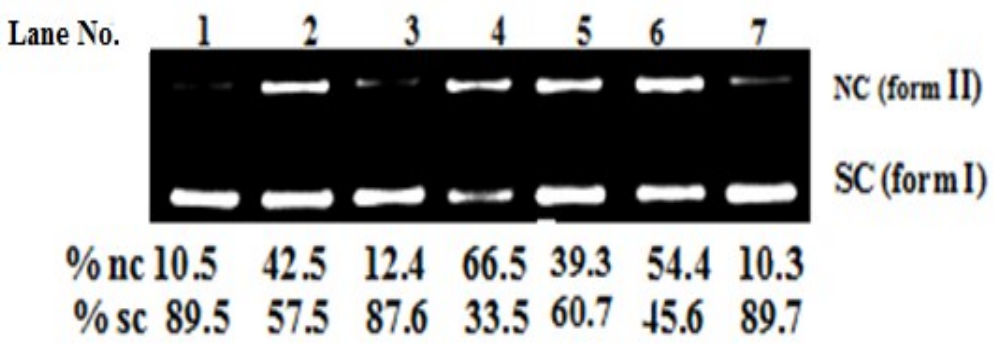


Figure S6: 1% agarose gel showing the effect of inhibitors on cleavage of *pBR*322 plasmid DNA by **2** on incubation for 20 min at 37°C. [DNA] = 300 ng, [**2**] =30 μM. Lane 1 DNA control; lane 2, DNA + [**2**]; lane 3, DNA + **2** + DMSO; lane 4, DNA + **2** + mannitol (50 mM); lane 5, DNA + **2** + DABCO (10 mM); lane 6, DNA + **2** + NaN_3 (20 mM); lane 7, DNA + **2** +SOD (15 units).

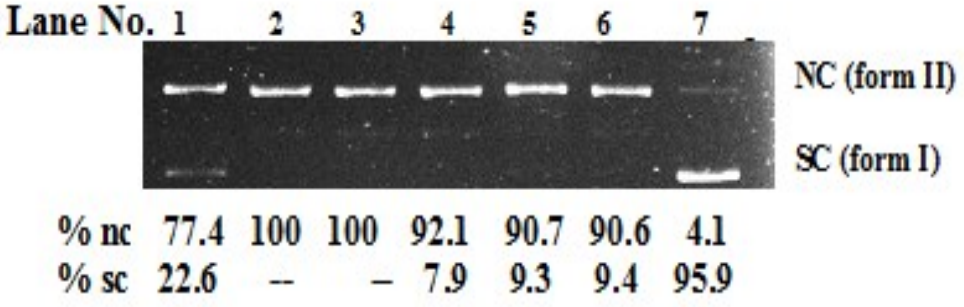


Figure S7: 1% agarose gel showing the effect of inhibitors on cleavage of *pBR*322 plasmid DNA by **3** on **i**ncubation for 20 min at 37°C. [DNA] = 300 ng, [**3**] = 30 μM; Lane 1, DNA + **3** + DMSO; lane 2, DNA + **3** + mannitol (50 mM); lane 3, DNA + **3** + DABCO (10 mM); lane 4, DNA + **3** + NaN_3 (20 mM) lane 5, DNA + **3** + SOD (15 units); lane6 DNA + **3**; lane 7, DNA control

Synthesis of binary complex [Cu(Hdoxycycline)$_2$](NO$_3$)$_2$.0.121 g (0.5 mM) $CuNO_3.3H_2O$ and 0.513 g (1 mM) doxycycline hyclate were stirred at room temperature in 10 ml methanol for five hr. The resulting green solution was allowed to evaporate slowly at room temperature. Green crystalline solid formed after one week was filtered and washed with water and dried in vacuum desiccator over calcium chloride. Yield: 0.3525 g, 74%; FT-IR (KBR pellet, ν cm^{-1}): 3431, 3390, 3292, 30.55, 2879, 2845, 2708, 1610, 1570, 1454, 1381, 1325, 1244, 1215, 1170, 1128, 1060, 1039 1001, 935, 864, 711, 661, 584, 511, 466 ESI-MS (*m/z* positive mode): $[M-(2NO_3)+H)]^+$, 950.2; $[M-NO_3]^+$, 1014.1 and $[M-(NO_3)+Na]^+$,1036.1.

Table 1: Antimalarial activity of complexes against CQ-sensitive *P.* falciparum NF54 strain

Compound	IC_{50} (μM)	Relative activity to doxycycline[a]	Relative activity to CQDP[b]
1	12.11	1.61	0.004
2	2.00	9.75	0.026
3	0.35	55.71	0.15
4	1.10	17.72	0.047
$[Cu(dox)_2]^{2+}$ [d]	20.46	0.95	0.003
Doxycycline hyclate	19.50	-	0.003
Chloroquine Diphosphate(CQDP)	0.052	375.00	-

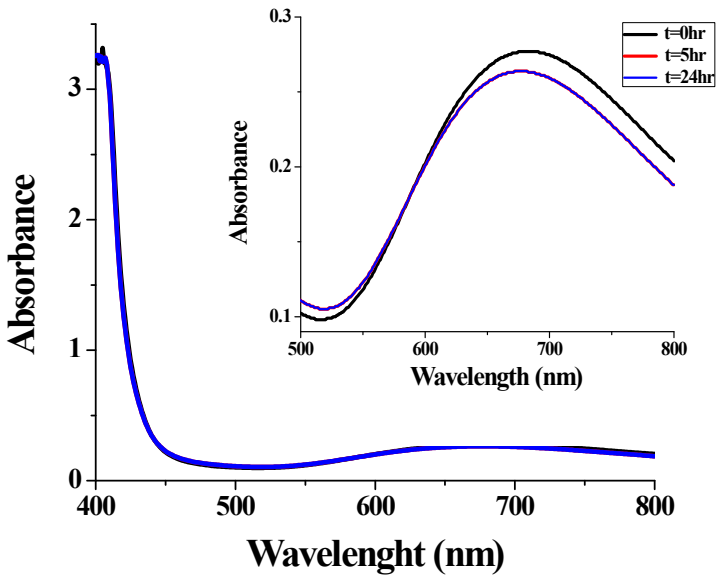
[a] Relative activity = IC_{50} of doxycycline/ IC_{50} of complex, [b] Relative activity = IC_{50} of CQDP/ IC_{50} of complex, [c] dox= doxycycline

Table 2: Antimalarial activity of complexes against CQ-resistant *P. falciparum* Dd2 strain

Compound	IC_{50} (μM)	Relative activity to doxycycline[a]	Relative activity to CQDP[b]	Resistance Index[c]
1	4.86	2.23	23.17	0.40
2	0.85	12.7	132.51	0.42
3	0.62	17.53	181.66	1.78
4	0.59	18.42	190.90	0.54
$[Cu(dox)_2]^{2+}$ [d]	6.41	1.70	17.57	0.31
Doxycycline hyclate	10.87	-	10.36	0.56
Chloroquine Diphosphate(CQDP)	112.63	0.10	-	2165.96
Artesunate	9.23	1.17	12.20	-

[a] Relative activity = IC_{50} of doxycycline/ IC_{50} of complex, [b] Relative activity = IC_{50} of CQDP/ IC_{50} of complex, [c] Resistance Index= IC_{50} towards Dd2/ IC_{50} towards NF54, [d] dox= doxycycline.

a)


Absorbance
Wavelenght (nm)
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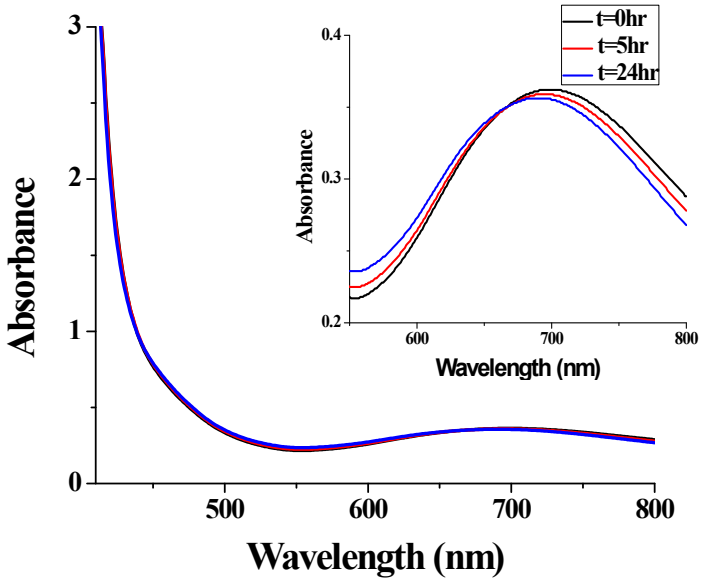
t=5hr
t=24hr
Absorbance
Wavelength (nm)

b)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Wavelength (nm)
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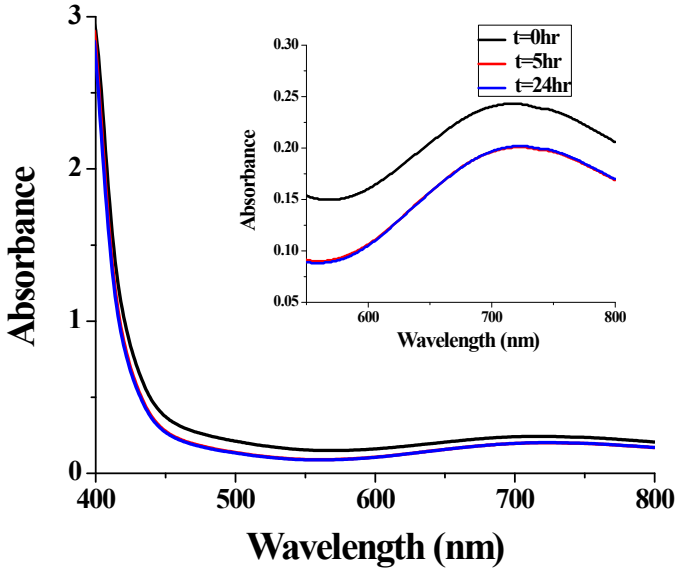
t=24hr
Absorbance
Wavelength (nm)

c)



d)

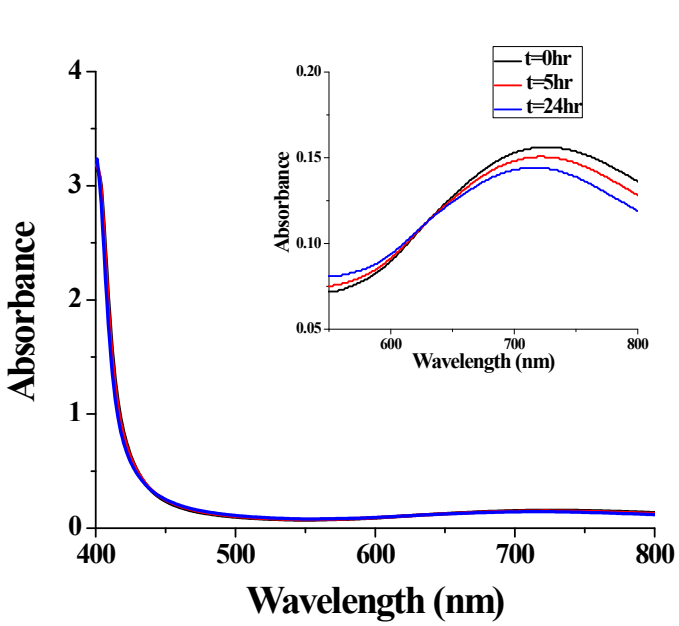
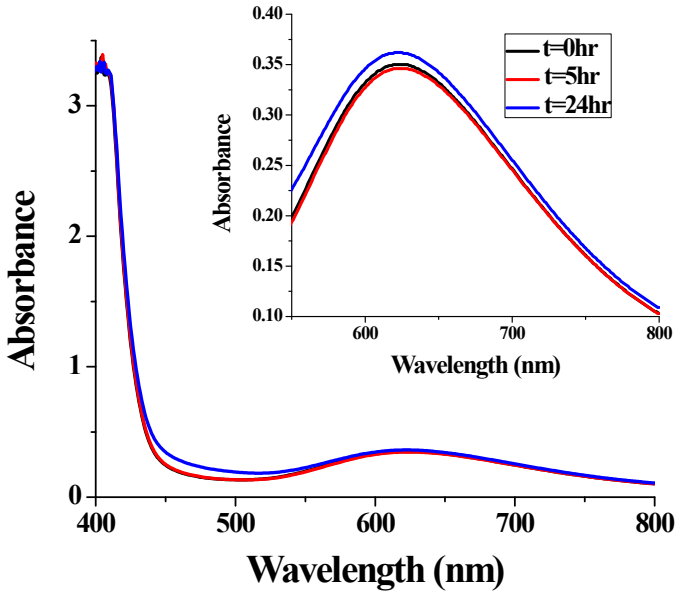


Figure S8: Absorption spectra of a)**1,** b) **2,** c)**3 and** d) **4** in DMSO at 0 h, after 5 and 24 h, room temperature

a)


Absorbance
Wavelength (nm)
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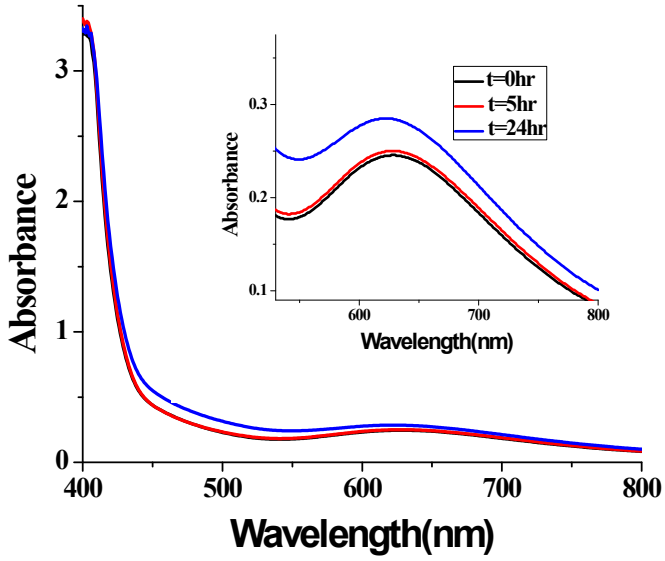
b)


Absorbance
Wavelength(nm)
t=0hr
t=5hr
t=24hr

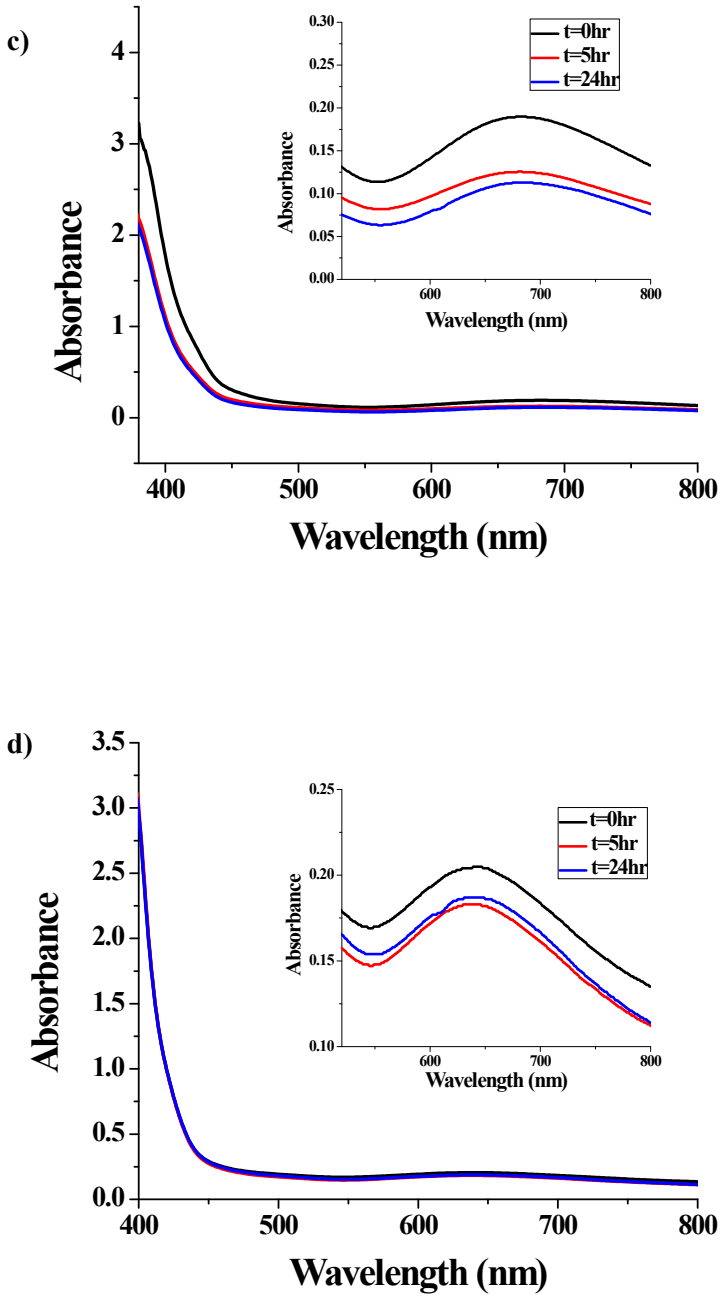
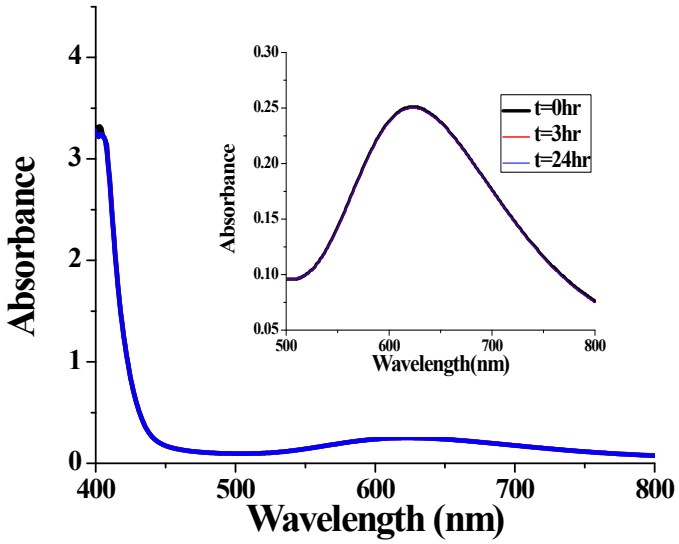


Figure S9: Absorption spectra of a)**1,** b) **2,** c)**3 and** d) **4** in water at 0 h, after 5 and 24 h, room temperature

a)


Absorbance
Wavelength (nm)
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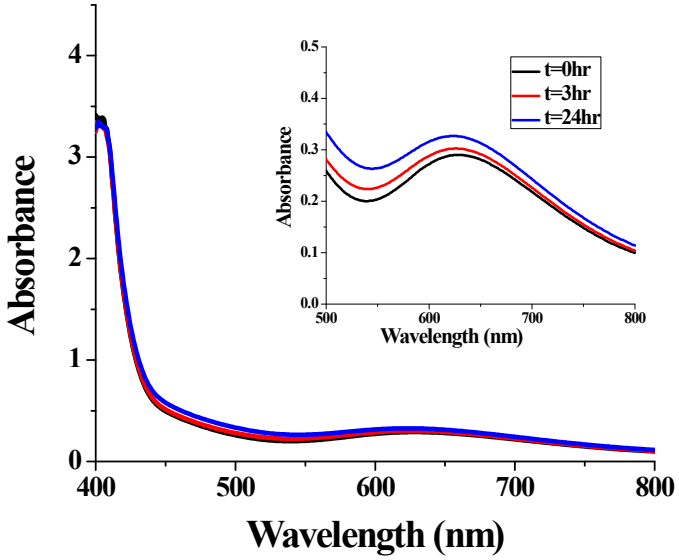
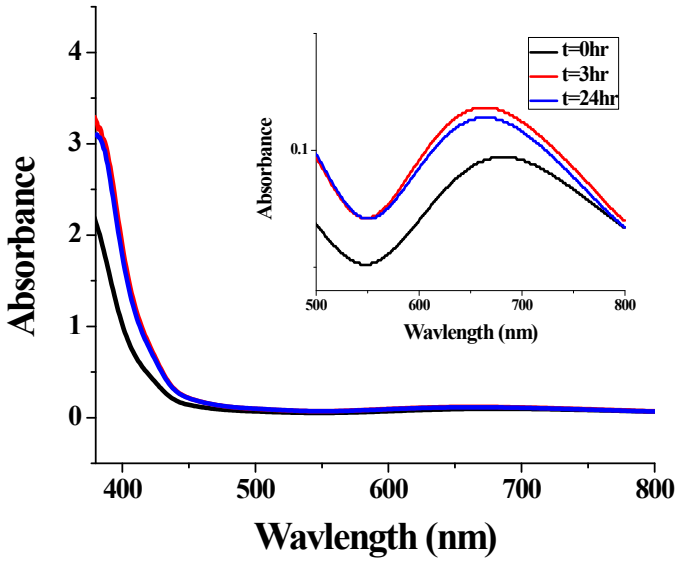
t=3hr
t=24hr
Absorbance
Wavelength(nm)

b)


Absorbance
Wavelength (nm)
t=0hr
t=3hr
t=24hr
Absorbance
Wavelength (nm)

c)



d)

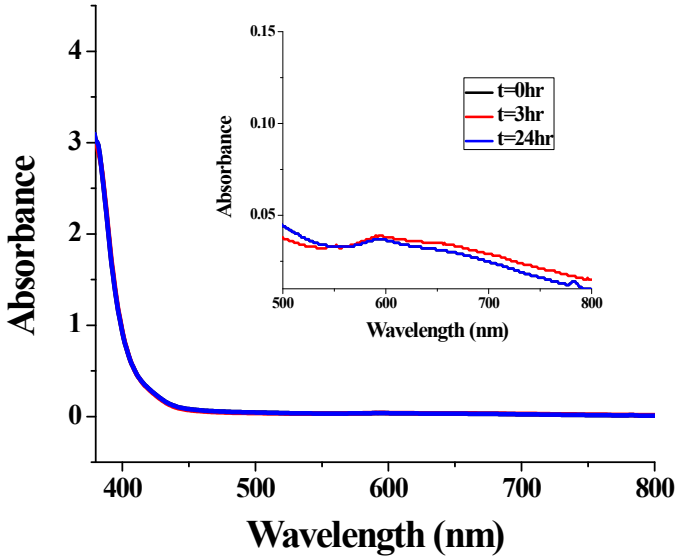


Figure S10: Absorption spectra of a)**1,** b) **2,** c)**3 and** d) **4** in 1mM NaCl Solution at 0 h, after 5 and 24 h, room temperature

SAIF, IIT, BOMBAY
Field Set
Scan Range 3000 G
Scan Time hrs 4 min Sec
Time Constant
Modulation Frequency Hz
Modulation Amplitude X 1 G
Temperature RT K
Receiver Gain 10 X 10 X 10
Microwave Frequency 9.1 Ghz
Microwave mW 5
Operator
Date 19/12/15
Remarks Cu bpydox
(a)
EPR CHART A
Sample
Spectrum No.
SCAN RANGE
-5
-4
-3
-2
-1
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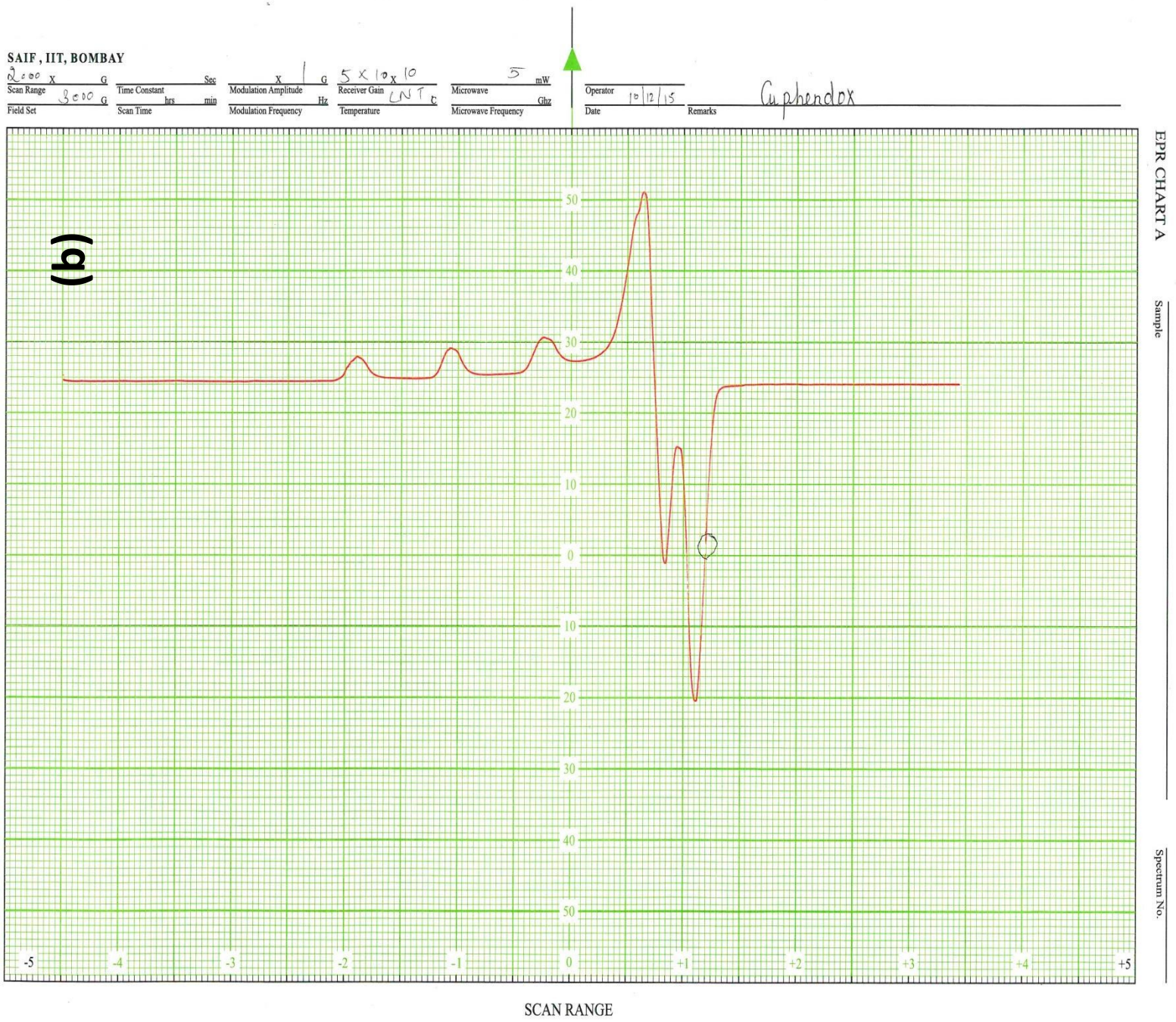
+1
+2
+3
+4
+5
50
40
30
20
10
0
10
20
40
50


SAIF , IIT, BOMBAY
2000 X G
Scan Range
3000 G
Field Set
Sec
Time Constant
hrs min
Scan Time
X 1 G
Modulation Amplitude
Hz
Modulation Frequency
5 X 10 X 10
Receiver Gain
LNT C
Temperature
5 mW
Microwave
Ghz
Microwave Frequency
Operator
10/12/15
Date
Cuphendox
Remarks
(b)
50
40
30
20
10
0
10
20
30
40
50
-5
-4
-3
-2
-1
0
+1
+2
+3
+4
+5
SCAN RANGE
EPR CHART A
Sample
Spectrum No.

SAIF, IIT, BOMBAY
Field Set
Scan Range
Scan Time
Time Constant
Modulation Amplitude
Modulation Frequency
Receiver Gain
Temperature
Microwave
Microwave Frequency
Operator
Date
Remarks
EPR CHART A
Sample
Spectrum No.
SCAN RANGE
(c)

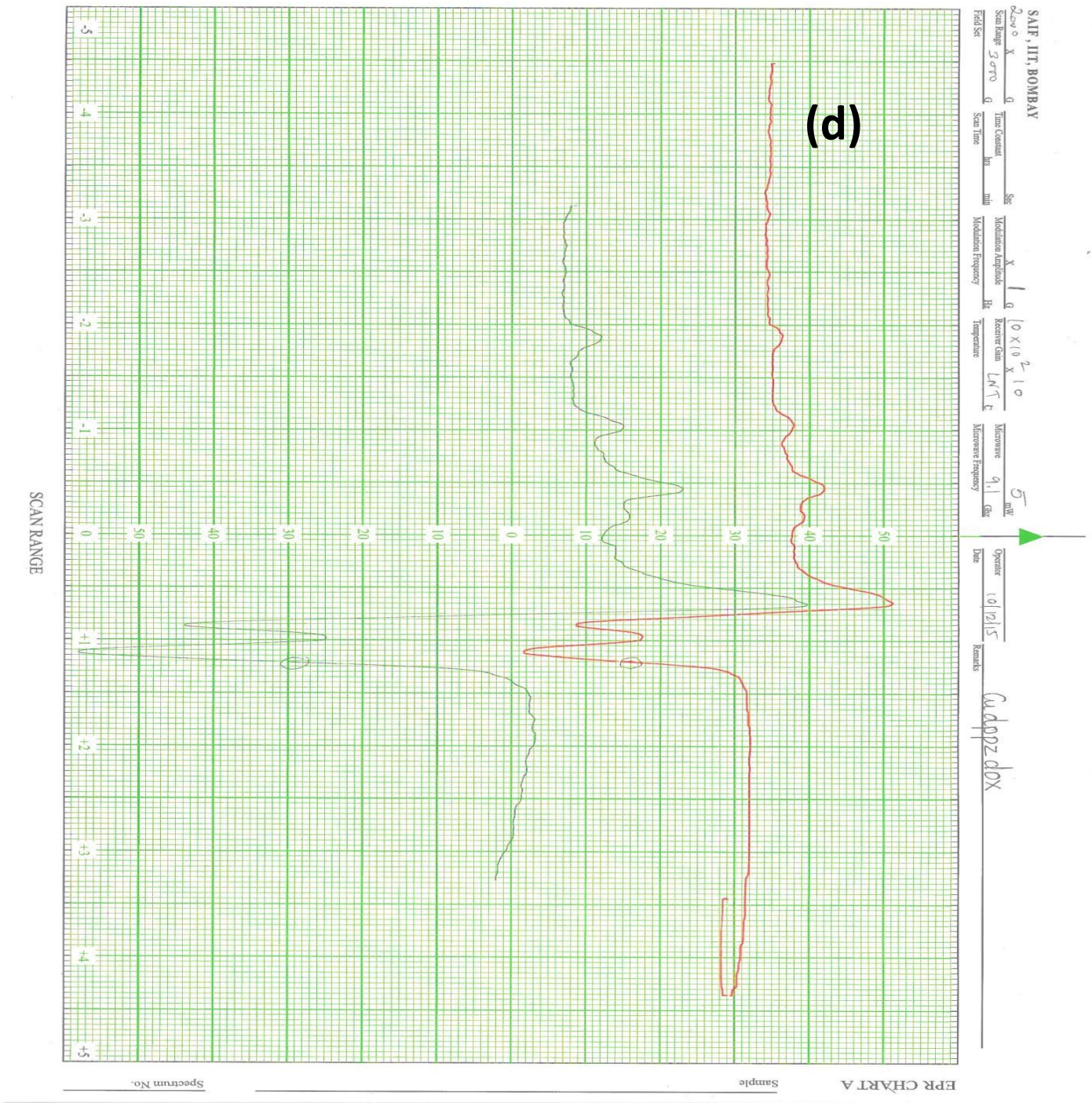


Figure S11: EPR spectra of complex **1** a), **2** b),**3** c), **4** d) in frozen DMSO