Electronic Supporting Information for:

μ_2-Alkyne Dicobalt(0)hexacarbonyl Complexes as Carbon Monoxide-Releasing Molecules (CO-RMs): Probing the Release Mechanism

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Contents

1. General information

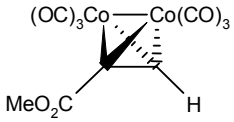
All reactions were performed in oven-dried glassware under N_2. TLC analysis was carried on Merck TLC aluminium sheets (silica gel 60 F254) and flash chromatography run on silica gel. Solution cell infra red spectroscopy was undertaken using a Thermo Nicolet Avatar 370 FT-IR spectrometer (2 cm^{-1} resolution) in the solvent indicated. Reactions were monitored using a Mettler Toledo ReactIR™ ic10 with K6 Conduit, SiComp (silicon) probe and MCT detector. Resolution 4 cm^{-1}, Range 4000-650 cm^{-1} and gain adjustment at 1×. Ultra violet-visible spectra were recorded using a JASCO V-560 (band-width = 1 nm). In certain cases the spectra in the myoglobin assay have been corrected for a better visual effect. This is achieved by translating all the data points so that all the spectra intersect at 510 nm (an isosbestic point of the Deoxy-Mb and Mb-CO curves). This is a graphical representation of how the spectra are referenced when calculating the [MbCO]. The instances of the use of this technique have been highlighted when used in this supplementary information. ^{1}H and ^{13}C NMR spectra were obtained, in the solvent indicated, using JEOL EXC400 and EX270 spectrometers at 400 and 270 MHz for ^{1}H, and 100 and 68 MHz for ^{13}C, respectively. ^{31}P NMR spectra were obtained (in the solvent as indicated in the text), using a Bruker Avance 500 spectrometer operating at 202 MHz. THF was dried over sodium-benzophenone. Octacarbonyldicobalt(0) was obtained from Strem, and the alkynes were obtained from Sigma Aldrich, which were used without further purification.

2. Synthesis of Metal-Carbonyl Complexes

Synthesis of dicobalt alkyne complexes (1a-f, general procedure):

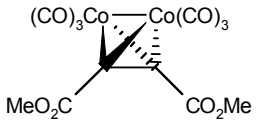
To a dried Schlenk tube under N_2 was added Octacarbonyldicobalt(0) (1 eq.). THF was added (7 mL per mmol) was added, followed by the relevant alkyne (1 eq.). The reaction mixture was stirred at 20 °C for 18 h. The solvent was removed *in vacuo* and the residue purified by chromatography on silica gel (hexane/ethyl acetate). Evaporation of the fractions corresponding to the pure product (indicated by TLC) were evaporated *in vacuo*.

μ_2-(Methyl prop-2-ynoate)hexacarbonyl dicobalt(0) (1a)[1]



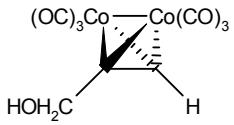
The compound was synthesised using the general procedure. Octacarbonyldicobalt(0) (1.17 mmol, 400 mg) and methyl prop-2-ynoate (1.17 mmol, 94 mg, 0.10 mL) were used to give the *title compound* (36 mg, 9%) as a red solid. Mp: 36-37 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.85 (s, 3H), 6.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 53.41 (CH_3), 73.60 (C, acetylene), 81.60 (C, acetylene), 171.11 (C); IR (CH_2Cl_2): ν = 2105 (m), 2068 (s), 2039 (s, br), 1706 (m) cm^{-1}; MS (FAB): m/z (%): 342 (14) [*M*-CO]$^+$.

μ_2-(Dimethyl but-2-ynedioate)hexacarbonyl dicobalt(0) (1b)[2]



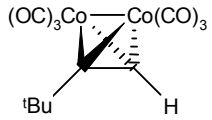
The compound was synthesised using the general procedure. Octacarbonyldicobalt(0) (1.17 mmol, 400 mg) and dimethyl but-2-ynedioate (1.17 mmol, 241 mg, 0.21 mL) were used to give the *title compound* (166 mg, 33%) as a red solid. Mp: 48-49 °C (lit 46-47 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.87 (s); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 53.38 (CH_3), 80.60 (C); IR (CH_2Cl_2): ν = 2112 (m), 2079 (s), 2050 (s, br), 1713 (m) cm^{-1}; MS (FAB): m/z (%): 428 (30) [*M*]$^+$, 400 (45) [*M*-CO]$^+$, 372 (45) [*M*-(2×CO)]$^+$, 344 (56) [*M*-(3×CO)]$^+$.

μ_2-(Prop-2-yn-1-ol)hexacarbonyl dicobalt (1c)[3]



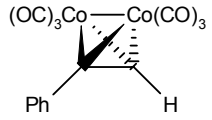
The compound was synthesised using the general procedure. Octacarbonyldicobalt(0) (1.17 mmol, 400 mg) and prop-2-yn-1-ol (1.17 mmol, 0.07 mL, 66 mg) were used to yield the *title compound* (328 mg, 82%) as a red solid. Mp: 53-55 °C (lit: 52-53 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.88 (t, 1H, *J* = 6.1 Hz), 4.80 (d, 2H, *J* = 6.1 Hz), 6.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 63.41 (CH_2), 71.25 (C, acetylene), 99.92 (C, acetylene); IR (CH_2Cl_2): ν = 2096 (m), 2056 (s), 2028 (s, br) cm^{-1}; MS (FAB): m/z (%): 314 (100) [*M*-CO]$^+$.

μ_2-(3,3-Dimethylbut-1-yne)hexacarbonyl dicobalt (1d)[4]



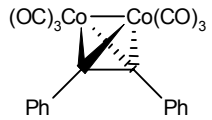
The compound was synthesised using the general procedure. Octacarbonyldicobalt(0) (1.17 mmol, 400 mg) and 3,3-dimethylbut-1-yne (1.17 mmol, 96 mg, 0.14 mL) were used to yield the *title compound* (171 mg, 40%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 9H), 6.03 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 33.40 (CH_3), 36.01 (C), 72.50 (C, acetylene), 81.26 (C, acetylene); IR (CH_2Cl_2): ν = 2090 (m), 2050 (s), 2022 (s, br) cm^{-1}; MS (FAB): m/z (%): 363 (4) [*M*-CO+Na]$^+$.

μ_2-(Phenylacetylene)hexacarbonyl dicobalt(0) (1e)[5]



The compound was synthesised using the general procedure. Octacarbonyldicobalt(0) (1.17 mmol, 400 mg) and phenyl acetylene (1.17 mmol, 119 mg, 0.13 mL) were used to yield the *title compound* (422 mg, 98%) as a dark red solid. Mp: 51-52 °C (lit: 52-53 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.37 (s, 1H), 7.33-7.34 (m, 3H), 7.53-7.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 72.57 (C, acetylene), 81.52 (C, acetylene), 128.10 (CH), 128.85 (CH), 130.20 (CH); IR (CH_2Cl_2): ν = 2093 (m), 2056 (s), 2026 (s, br) cm^{-1}; MS (FAB): m/z (%): 388 (8) [*M*]$^+$, 360 (43) [*M*-CO]$^+$, 332 (34) [*M*-(2×CO)]$^+$, 304 (39) [*M*-(3×CO)]$^+$, 276 (15) [*M*-(4×CO)]$^+$.

μ_2-(1,2-Diphenylethyne)hexacarbonyl dicobalt(0) (1f)[2]



The compound was synthesised using the general procedure. Octacarbonyldicobalt(0) (2.00 mmol, 684 mg) and diphenyl acetylene (2.00 mmol, 356 mg) were used to yield the *title compound* (881 mg, 95%) as a black solid. Mp: 109-111 °C (lit: 108-109 °C), ^{1}H NMR(400 MHz, $CDCl_3$): δ = 7.34-7.41 (m, 3H), 7.53-7.62 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 91.95 (C, acetylene), 127.87 (CH), 128.95 (CH), 129.21 (CH), 138.29 (C); IR (CH_2Cl_2): ν = 2090 (m), 2055 (s), 2026 (s, br) cm^{-1}; MS (FAB): m/z (%): 464 (93) [*M*]$^+$, 436 (100) [*M*-CO]$^+$, 408 (42) [*M*-(2×CO)]$^+$, 380 (69) [*M*-(3×CO)]$^+$, 352 (37) [*M*-(4×CO)]$^+$, 324 (22) [*M*-(5×CO)]$^+$.

$[Co(CO)_4][NEt_4]$[6]

The *title compound* was prepared according to existing literature. In a Schlenk tube, tetraethyl ammonium bromide (1.5 mmol, 315 mg) and sodium borohydride (10.0 mmol, 380 mg) were added to water (50 mL). This was added to a solution of octacarbonyldicobalt(0) (1.0 mmol, 342 mg) in DCM (70 mL). The reaction was stirred at 20 °C for 1 h. and reaction progress was monitored by IR. The organic layer was washed with water, dried over magnesium sulphate and concentrated *in vacuo* to give the *title compound* as a grey powder (208 mg, 98%). Mp 147-148 °C (lit: 145-146.5 °C); IR (CH_2Cl_2): ν =1889 (s) cm^{-1}.

$[Co(PPh_3)_3(CO)_2][Co(CO)_4]$[7]

The *title compound* was prepared according to existing literature. A solution of octacarbonyldicobalt(0) (0.88 mmol, 300 mg) in THF (50 mL) was added triphenylphosphine (7.0 mmol, 1.84 g). The reaction mixture was stirred at 20 ˚C for 30 min. The solution was cooled to 0 ˚C and filtered *via* cannular into dry hexane. The resulting precipitate was washed with three portions of hexane and dried in vacuo to yield the title compound as a brown, crystalline solid (553 mg, 75%). Mp 135-140 (dec); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.46 (br m, 12H), 7.67 (br m, 18H); ^{31}P NMR (202.5 MHz, $CDCl_3$): δ = 54.8; IR (CH_2Cl_2): ν = 2009 (sbr), 1889 (sbr) cm^{-1}; IR (nujol): ν = 2000 (sbr), 1885 (sbr), 1870 (sbr) cm^{-1}; MS (ESI-MS-MS): m/z (%): 667 (100) $[Co(CO)_3(PPh_3)_2]^+$, 611 (17) $[Co(CO)(PPh_3)_2]^+$, 583 (25) $[Co(PPh_3)_2]^+$, 408 (42) $[CoPPh_3]^+$.

3. Assay Procedures and Results

3.1. Myoglobin Assay Procedure[8]

The release of CO from metal carbonyl compounds was studied spectrophotometrically by measuring the conversion of deoxy-myoglobin (deoxy-Mb) to carbonmonoxy myoglobin (Mb-CO). The amount of Mb-CO formed was quantified by measuring the absorbance at 540 nm. A stock solution of myoglobin (lyophilised horse heart) (Sigma) (66 μM final concentration) was prepared fresh by dissolving the protein in Phosphate buffered saline (PBS) (0.01M, pH = 7.4) (Sigma). Sodium dithionite (0.1%) (Alfa Aesar) was added to convert the myoglobin stock to deoxy-Mb. A 1 mL quantity of this was measured to obtain a deoxy-Mb curve and then bubbled with CO to get a Mb-CO curve. CO-RMs were dissolved in solvent (DMSO or EtOH)(4, 8, 12 mM) and added to deoxy-Mb in the cuvette (to give a final concentration of 20, 40, 60 μM), mixed using a pipette and then overlaid with 500 μL light mineral oil (Sigma) to prevent CO escaping or the myoglobin being oxygenated. This is the standard procedure; other experiments have been undertaken using different concentrations of myoglobin and different concentrations of DMSO. All solutions were homogeneous unless otherwise stated.

The maximal absorption peak of deoxy-Mb at 560 nm is converted to the two maximal absorption peaks of Mb-CO at 540 and 578 nm.
The concentration of myoglobin in the stock solution was calculated from the maximal absorption peak of the Mb-CO solution at 540 nm (equation 1).

Equation 1. Equation for calculating total myoglobin concentration in a saturated solution of Mb-CO. ε = extinction coefficient of Mb-CO = 15.4 $mM^{-1}cm^{-1}$, OD_{540} = absorbance of Mb-CO solution at 540 nm.

$$\text{Mb-CO}_{max} = (OD_{540} / \varepsilon) \times 1000$$

Intermediate concentrations of Mb-CO are calculated from the OD_{540}. A new extinction coefficient (ε_2) must be calculated to take into account the change in absorbance at 540 nm (ΔOD_{540}). To aid in the accuracy of this calculation, another wavelength is used as a constant reference point. The deoxy-Mb and Mb-CO spectra share four isosbestic (OD_{iso}) points (510, 550, 570, 585 nm). The value at 510 nm (OD_{iso510}) was used in this set of experiments. The new extinction coefficient was calculated (equation 2).

Equation 2. Equation needed to calculate unknown Mb-CO extinction coefficient. ΔOD_{iso510} = change in absorbance at the isosbestic point, ΔOD_{540} = change in absorbance at 540 nm, Mb-CO_{max} = maximum concentration of myoglobin. ε_2 = new extinction coefficient.

$$\varepsilon_2 = (\Delta OD_{540} - \Delta OD_{iso510} \times 1000) / \text{Mb-}CO_{max}$$

From the new extinction coefficient and the change in absorbance at 540 and 510 nm will give the concentration of myoglobin in any unknown sample. (equation 3)

Equation 3. Equation to calculate the Mb-CO concentration in samples. ΔOD_{540} = change in absorbance at 540 nm, ΔOD_{iso510} = change in absorbance at the isosbestic point, ε_2 = calculated absorption coefficient.

$$\text{Mb-CO} = 1000 \times (\Delta OD_{540} - \Delta OD_{iso510}) / \varepsilon_2$$

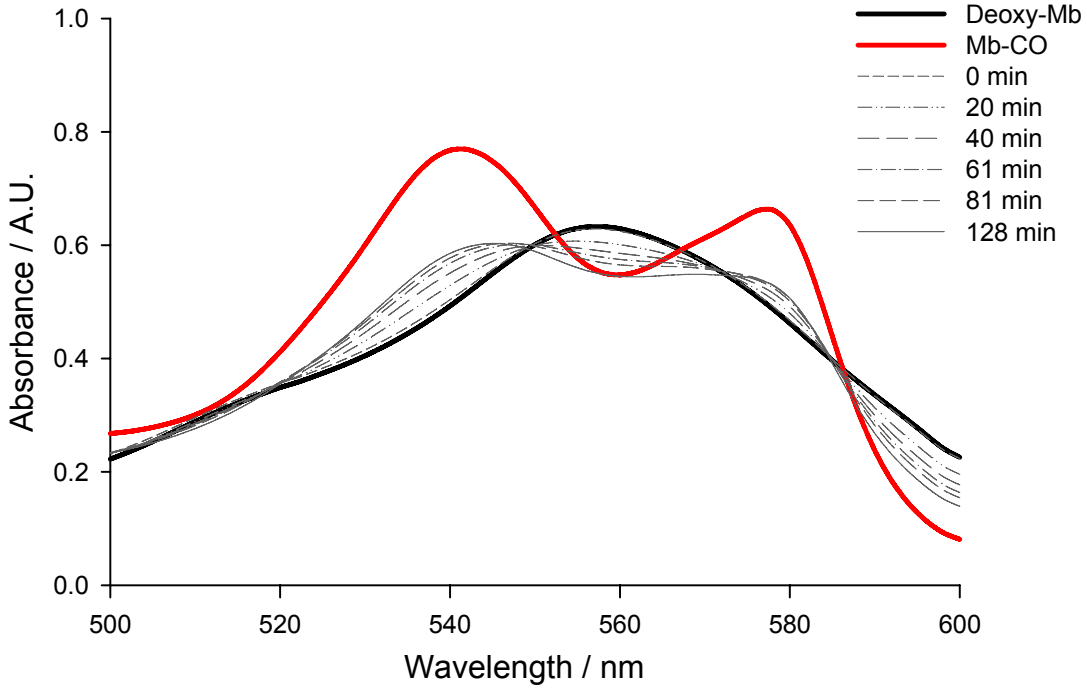
$t_{1/2}$ times are reported as the time taken for the concentration of Mb-CO formed to be equal to half the initial concentration of CO-RM. In theory this number should be the same for all concentrations of CO-RM; however, discrepancies occur due to the complex release mechanisms involved.

3.2. Myoglobin Assay Results

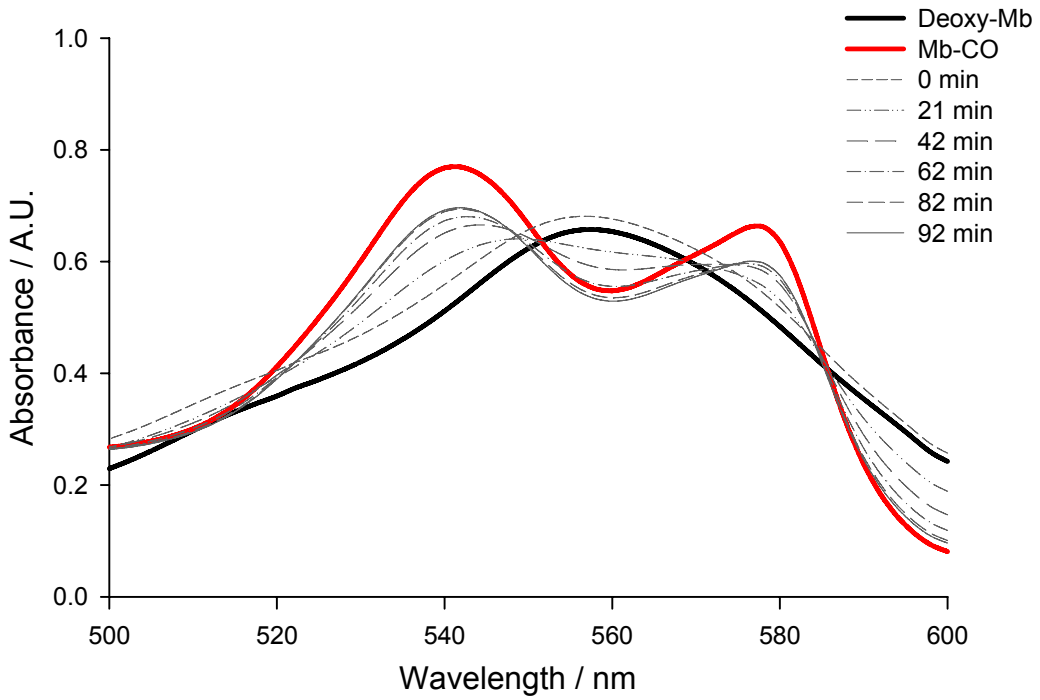
Table showing the CO-release rates of CO-RMs

CO-RM	Addition Solvent	$t_{1/2}$ times (min)		
		20 μM	40 μM	60 μM
1a	DMSO	32.7	32.6	32.5
1b	DMSO	1.1	1.8	1.8
1b	EtOH	-	-	5.3
1c	DMSO	-	-	67.8 [a]
$[Co(CO)_4][NEt_4]$	DMSO	-	-	21.6

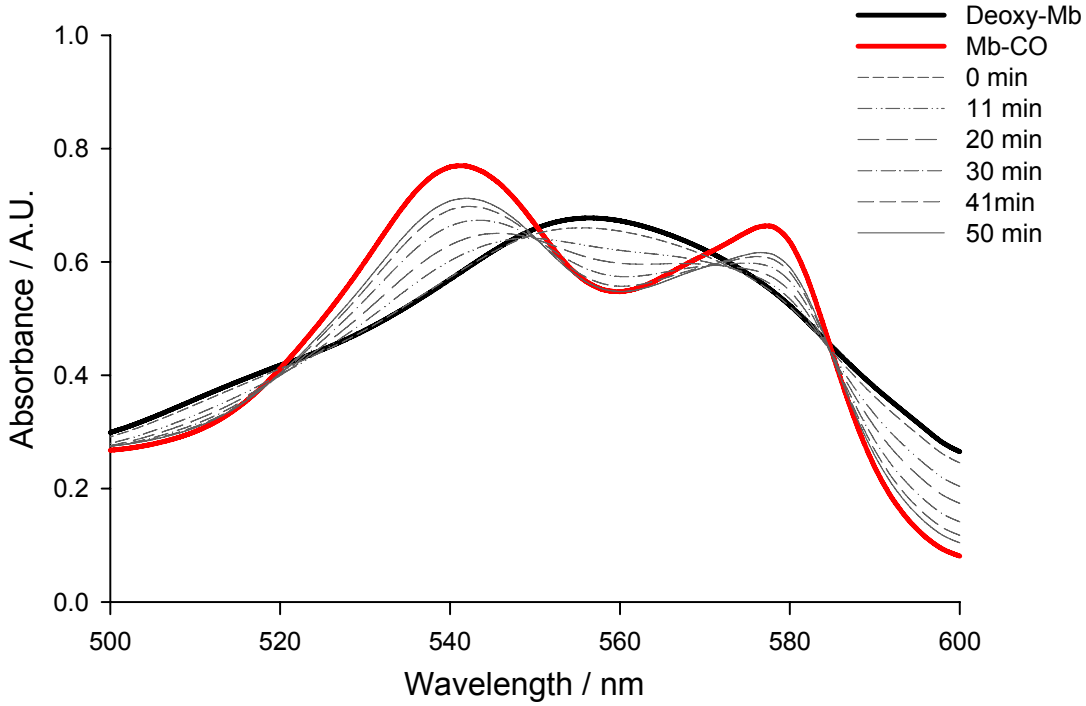
a quoted as $t_{1/4}$



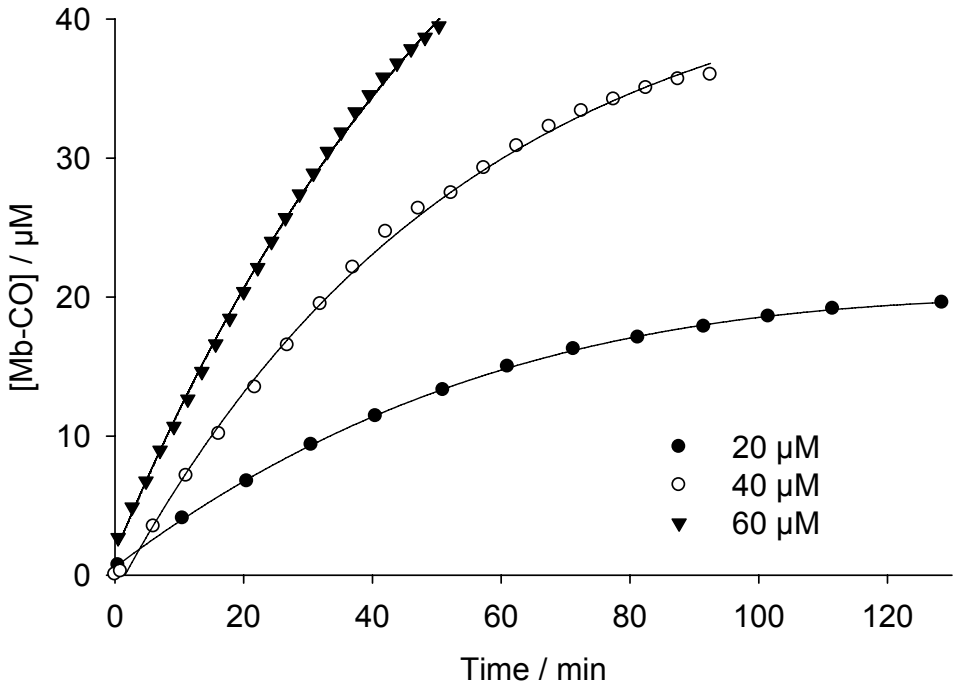
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1a** at 20 μM using DMSO as the addition solvent.



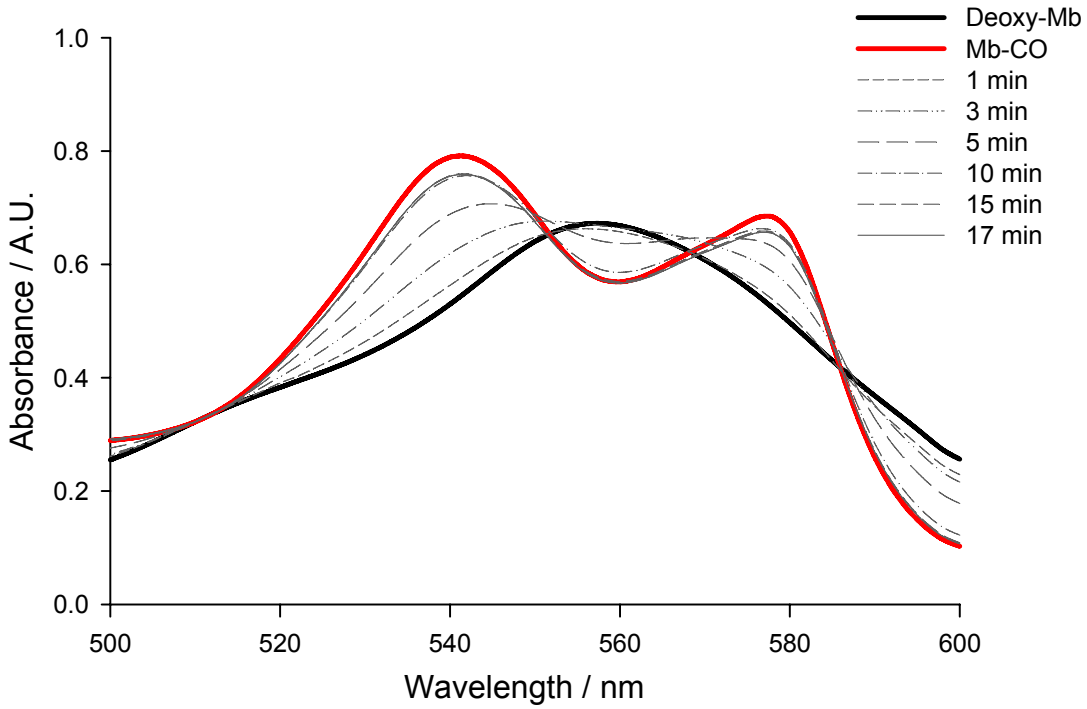
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1a** at 40 μM using DMSO as the addition solvent.



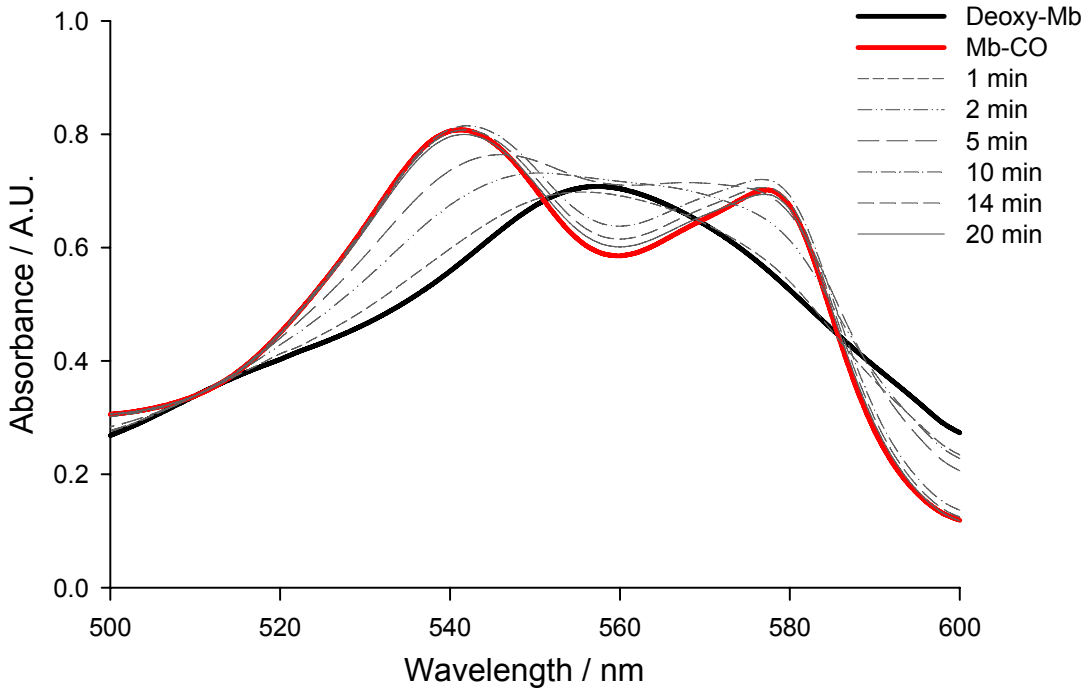
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1a** at 60 μM using DMSO as the addition solvent.



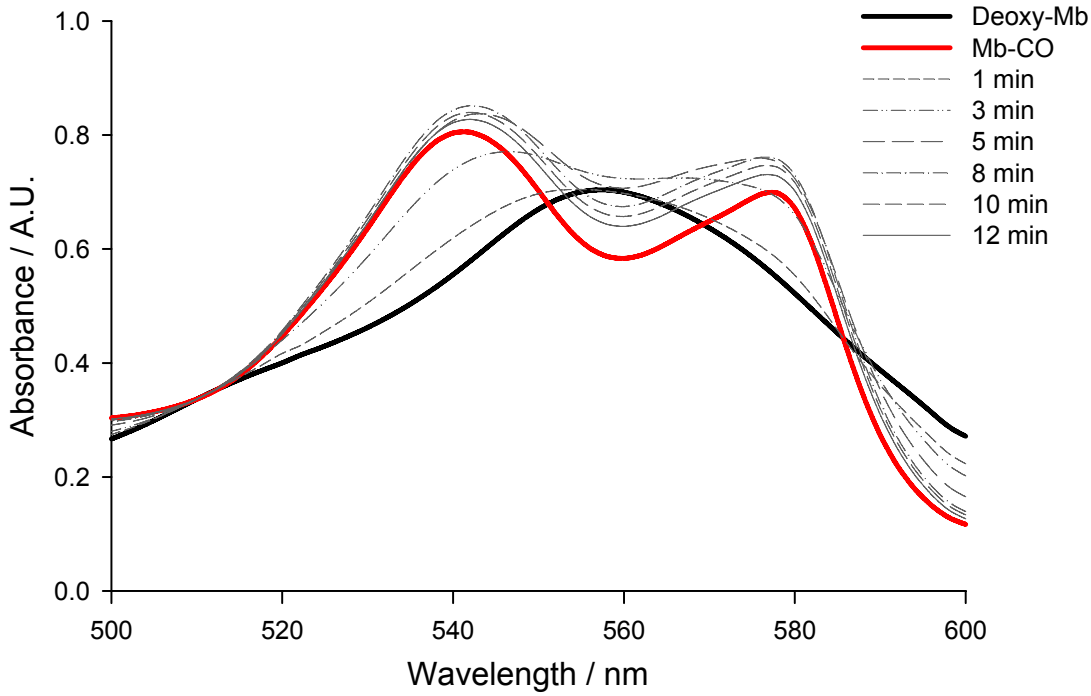
Graph of [Mb-CO] vs. time for CO-RM **1a** at 20, 40 and 60 µM.



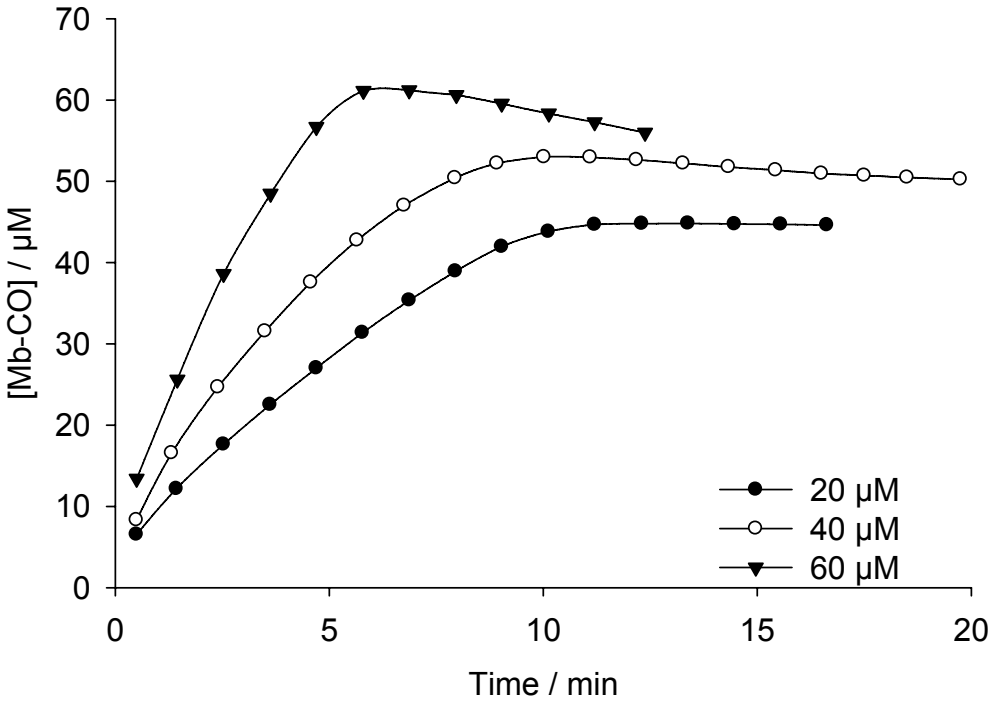
Corrected UV-Vis data for the myoglobin assay with CO-RM **1b** at 20 µM using DMSO as the addition solvent. The data is corrected by translation using the isosbestic point at 510 nm.



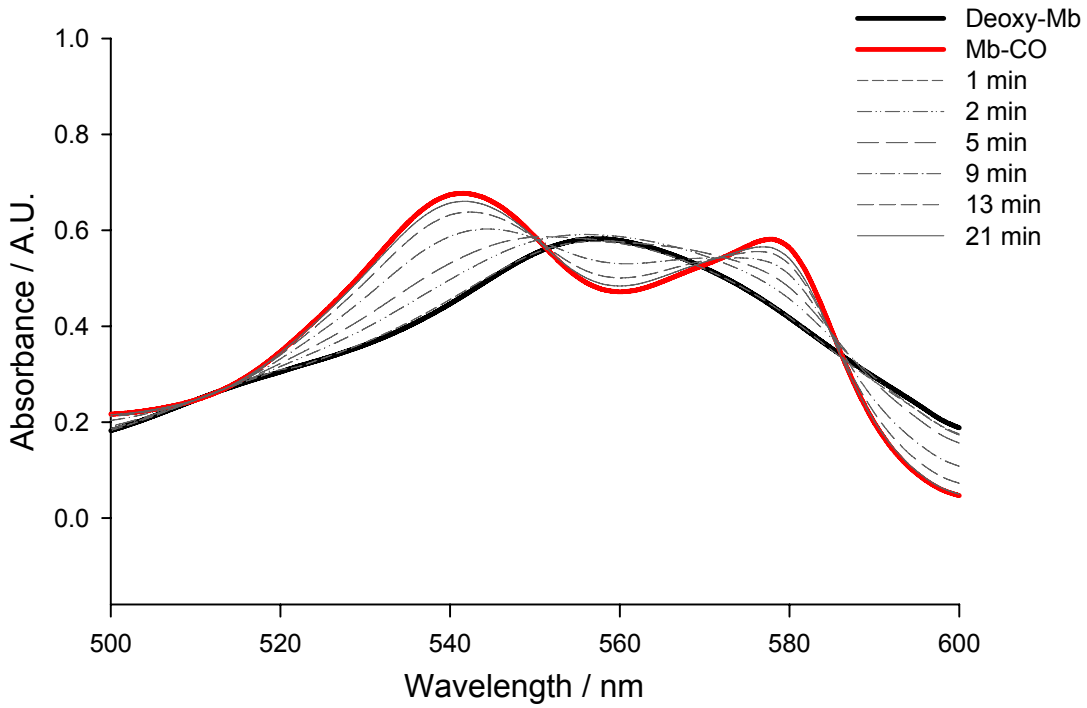
Corrected UV-Vis data for the myoglobin assay with CO-RM **1b** at 40 μM using DMSO as the addition solvent. The data is corrected by translation using the isosbestic point at 510 nm.



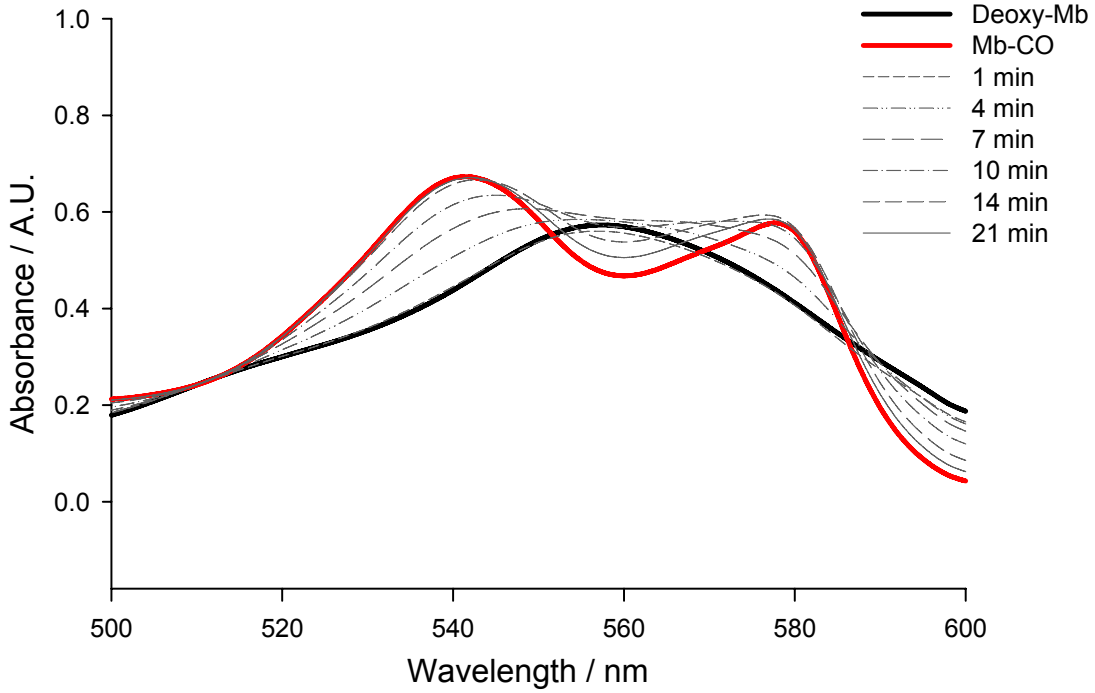
Corrected UV-Vis data for the myoglobin assay with CO-RM **1b** at 60 μM using DMSO as the addition solvent. The data is corrected by translation using the isosbestic point at 510 nm.



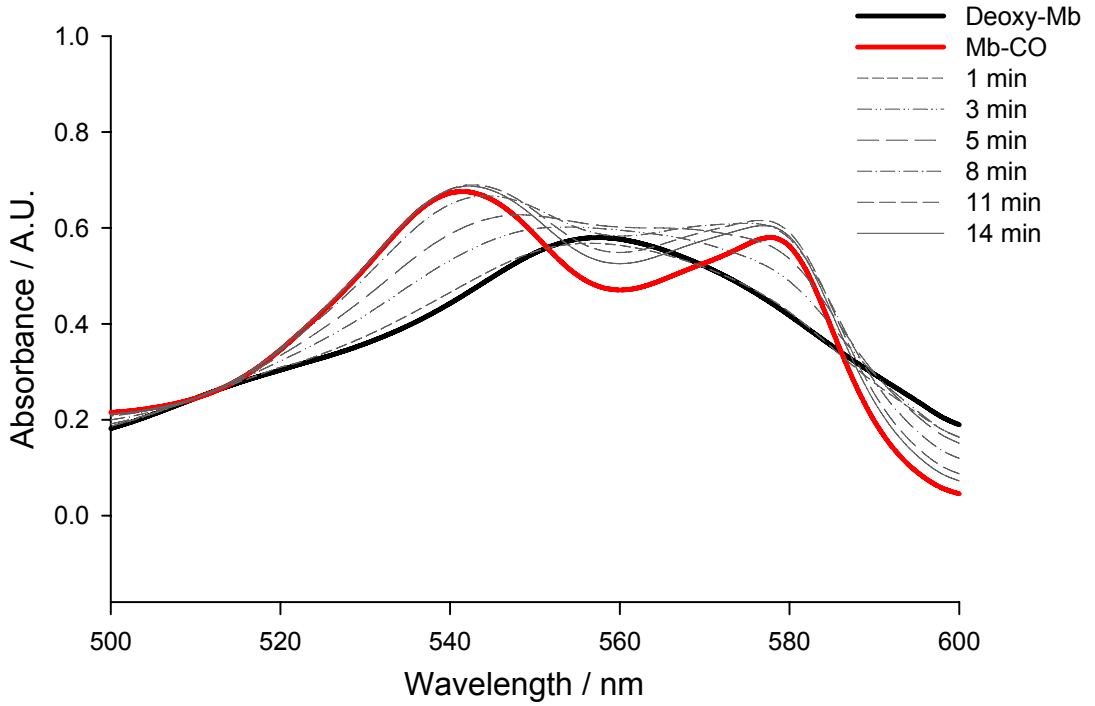
Graph of [Mb-CO] vs. time for CO-RM **1b** at 20, 40 and 60 µM. At higher concentrations the spectra are distorted due to the influence of the CO-RM, leading to apparent raised concentrations of Mb-CO which degrades over time. Further investigations point to an interaction between the CO-RM and the myoglobin, although the exact cause of this behaviour remains elusive.



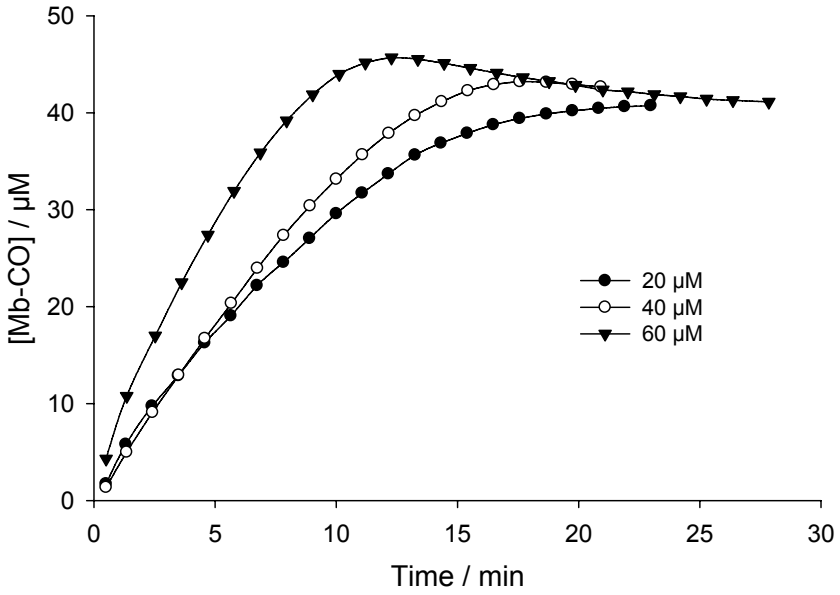
Corrected UV-Vis data for the myoglobin assay with CO-RM **1b** at 20 μM using EtOH as the addition solvent. The data is corrected by translation using the isosbestic point at 510 nm.



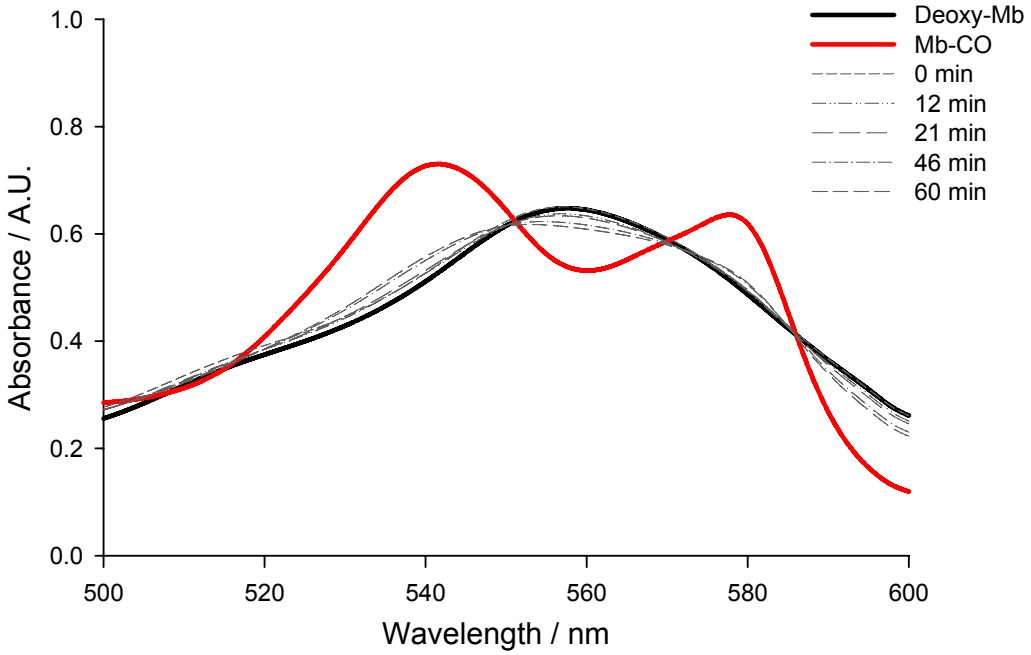
Corrected UV-Vis data for the myoglobin assay with CO-RM **1b** at 40 μM using EtOH as the addition solvent. The data is corrected by translation using the isosbestic point at 510 nm.



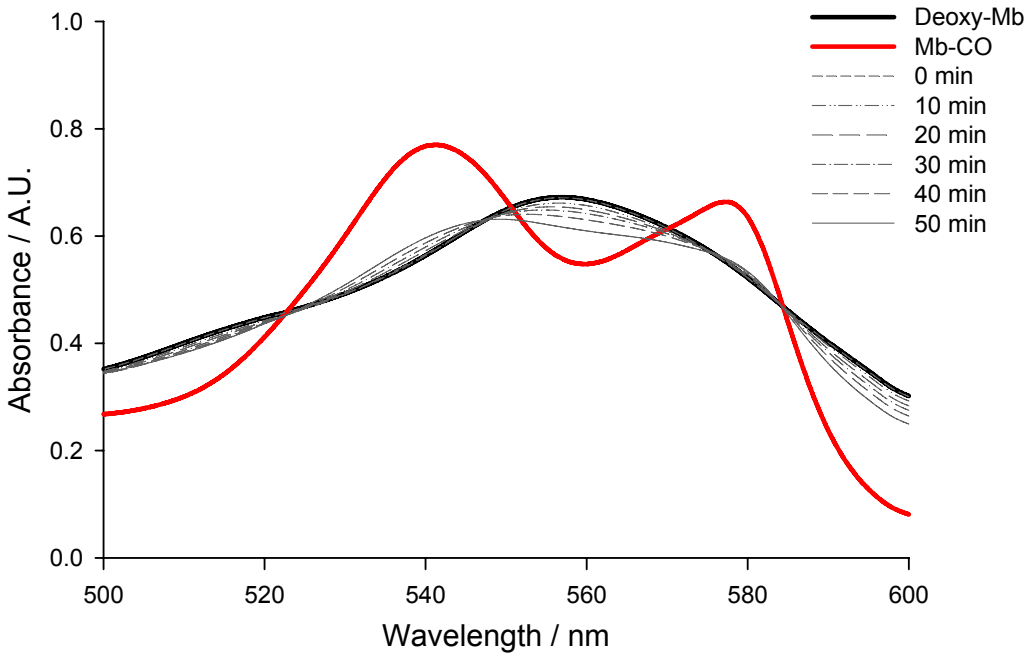
Corrected UV-Vis data for the myoglobin assay with CO-RM **1b** at 60 μM using EtOH as the addition solvent. The data is corrected by translation using the isosbestic point at 510 nm.



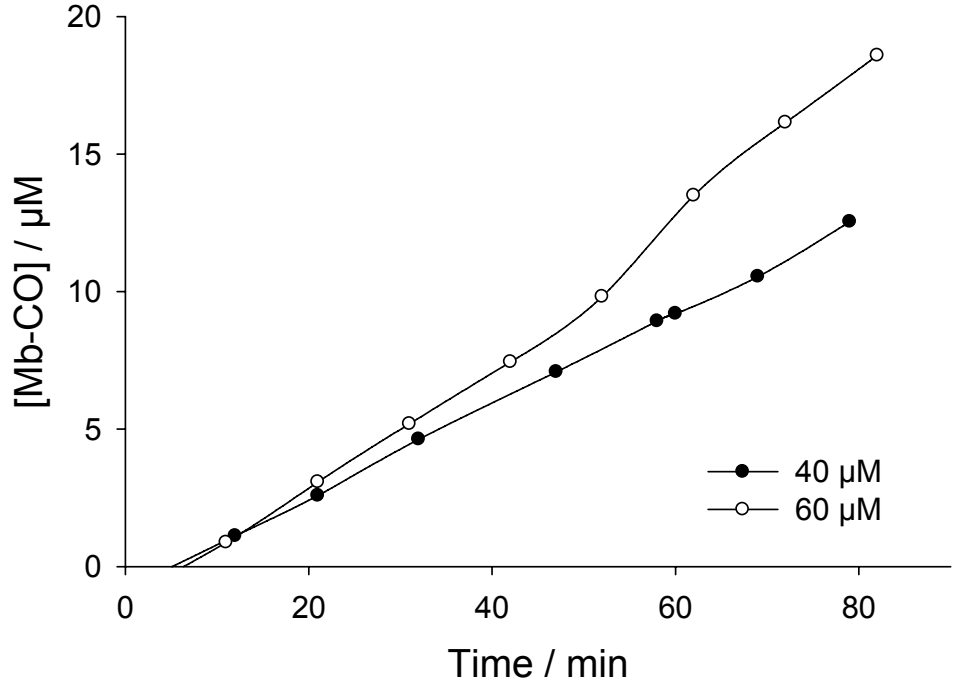
Graph of [Mb-CO] vs. time for CO-RM **1b** at 20, 40 and 60 µM. At higher concentrations the spectra are distorted due to the influence of this specific CO-RM, leading to apparent raised concentrations of Mb-CO, which degrades over time. Further investigations point to an interaction between the CO-RM and the myoglobin, although the exact cause of this behaviour remains elusive (note that solutions are homogeneous).



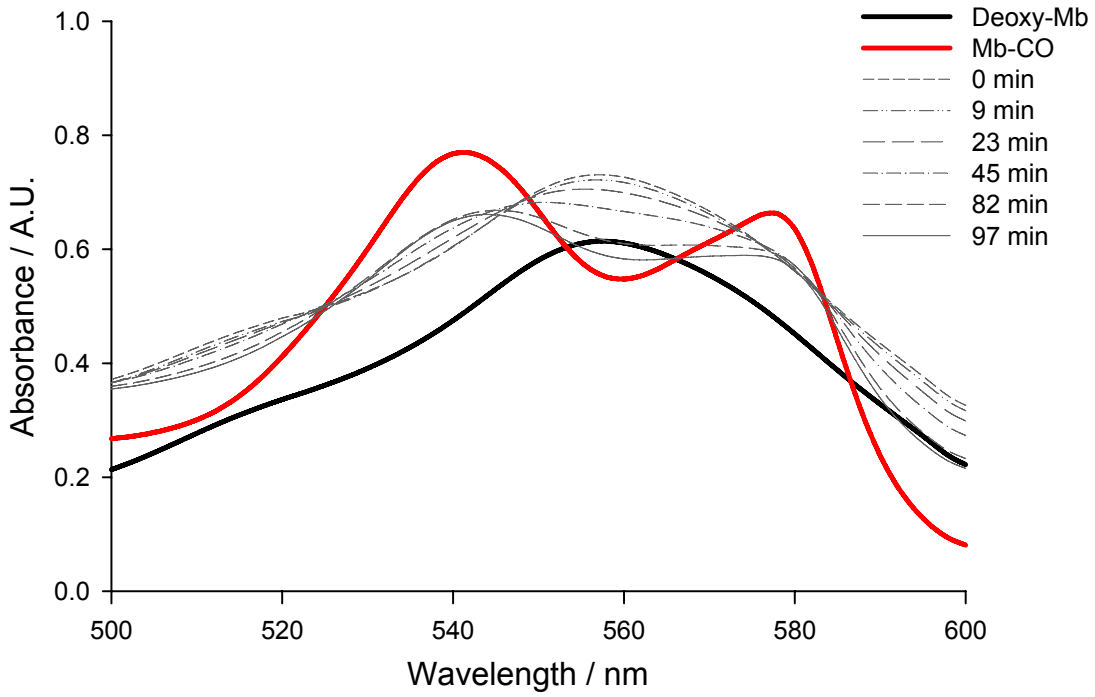
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1c** at 40 µM using DMSO as the addition solvent.



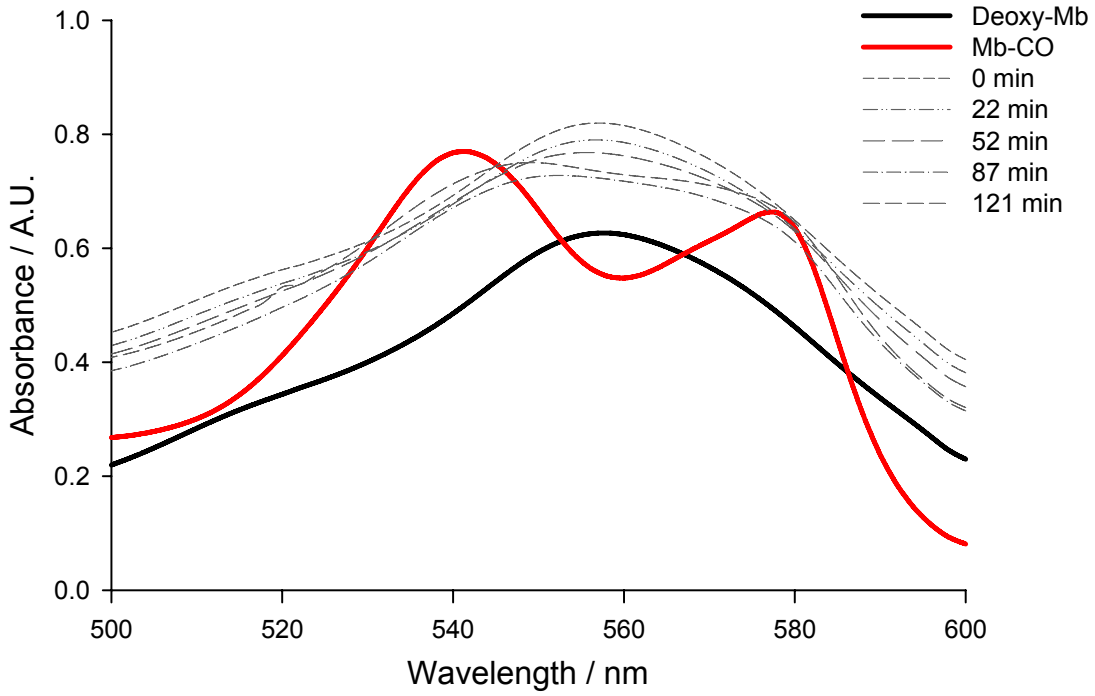
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1c** at 60 µM using DMSO as the addition solvent.



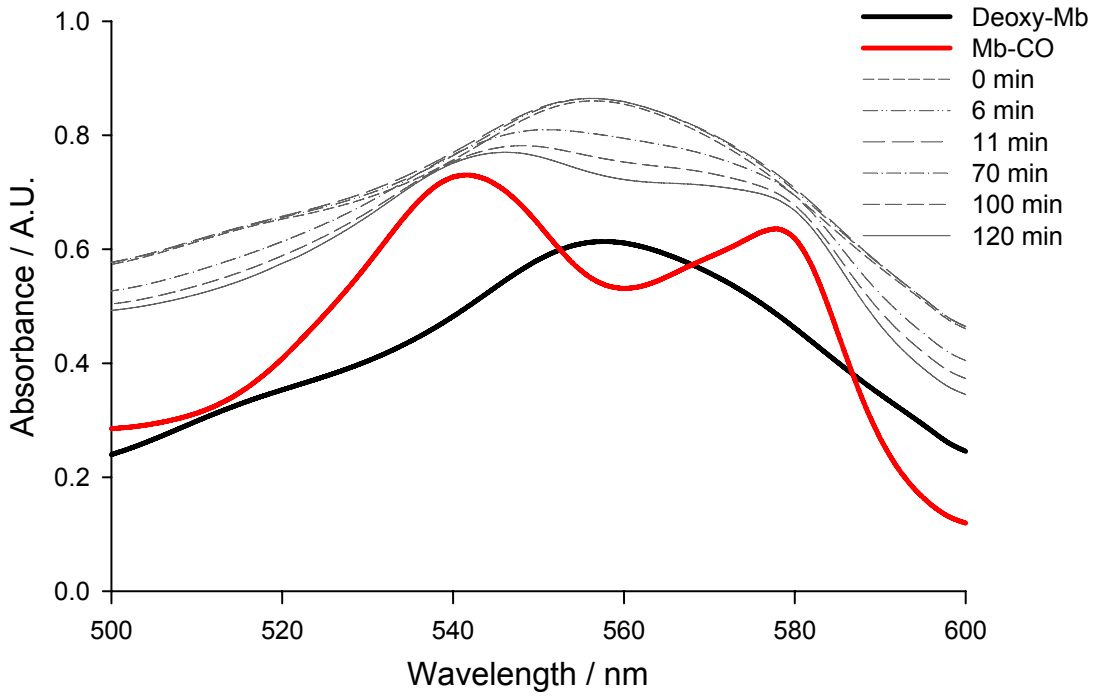
Graph of [Mb-CO] vs. time for CO-RM **1c** at 40 and 60 µM using DMSO as the addition solvent.



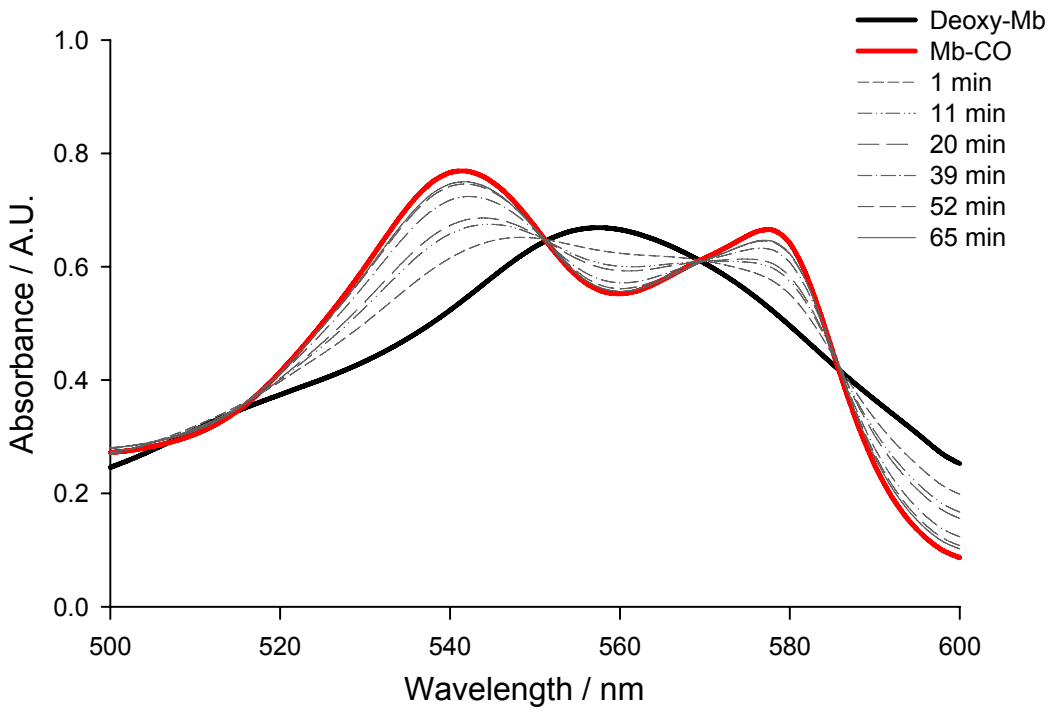
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1d** at 60 μM using DMSO as the addition solvent.*



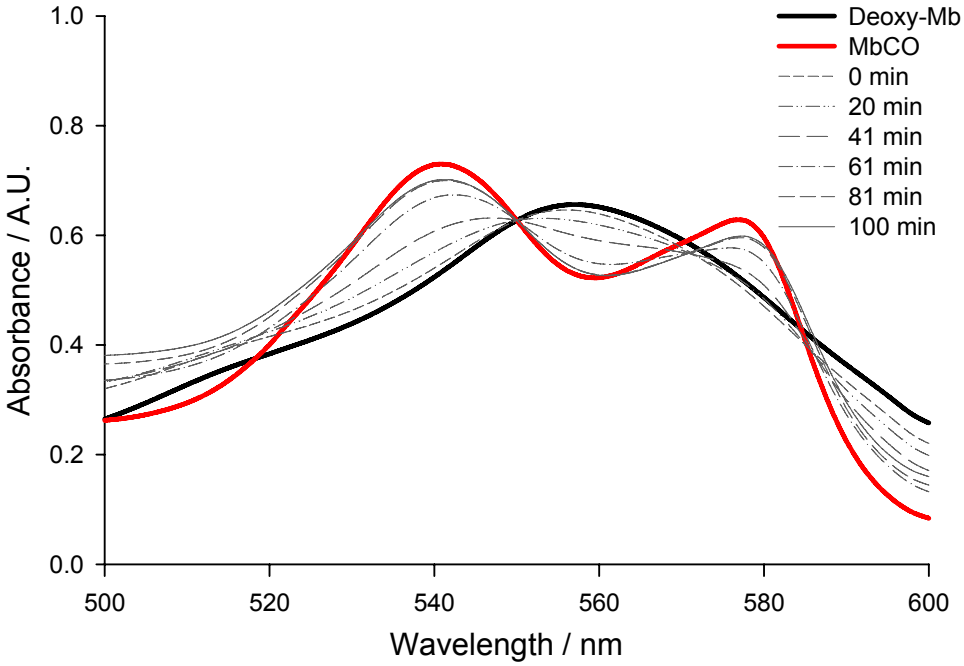
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1e** at 60 μM using DMSO as the addition solvent.*



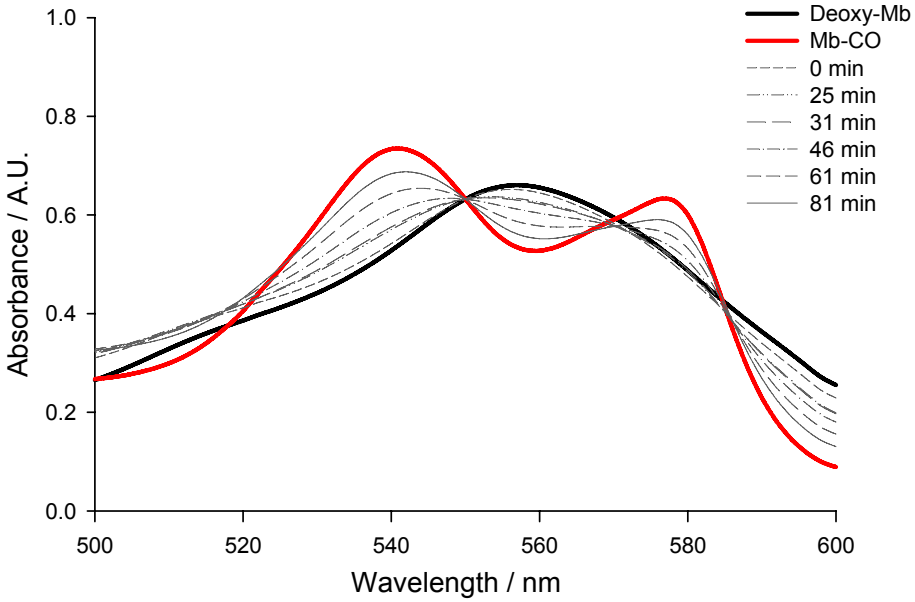
Uncorrected UV-Vis data for the myoglobin assay with CO-RM **1f** at 60 μM using DMSO as the addition solvent.*



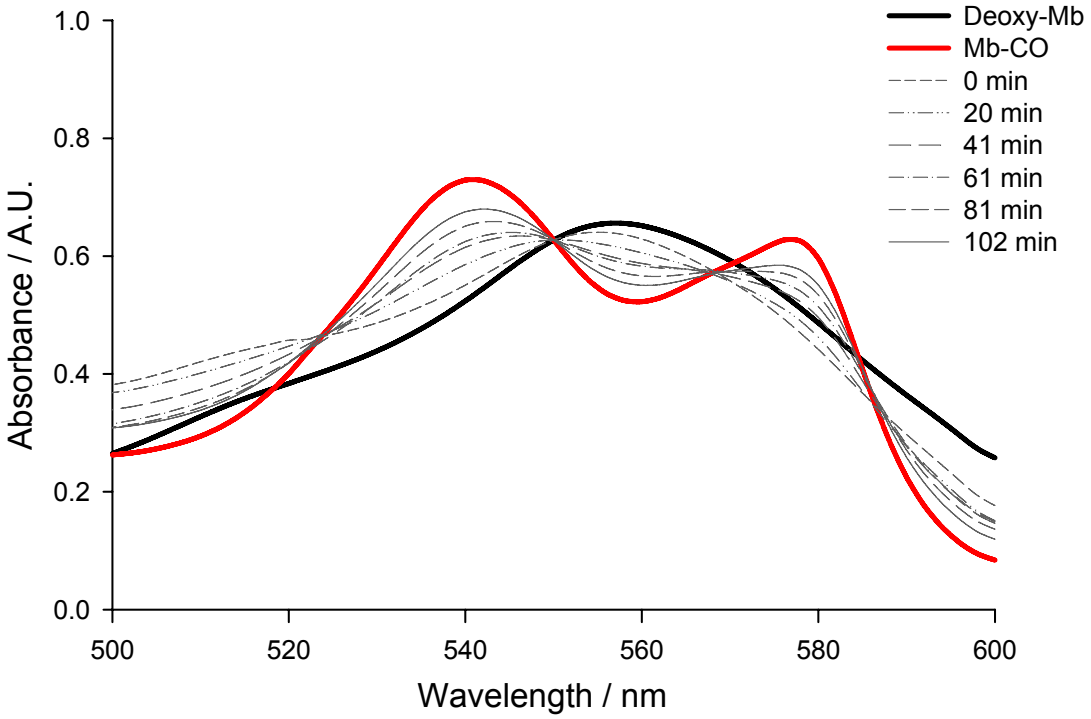
Uncorrected UV-Vis data for the myoglobin assay with $[Co(CO)_4][NEt_4]$ at 60 μM using DMSO as the addition solvent.



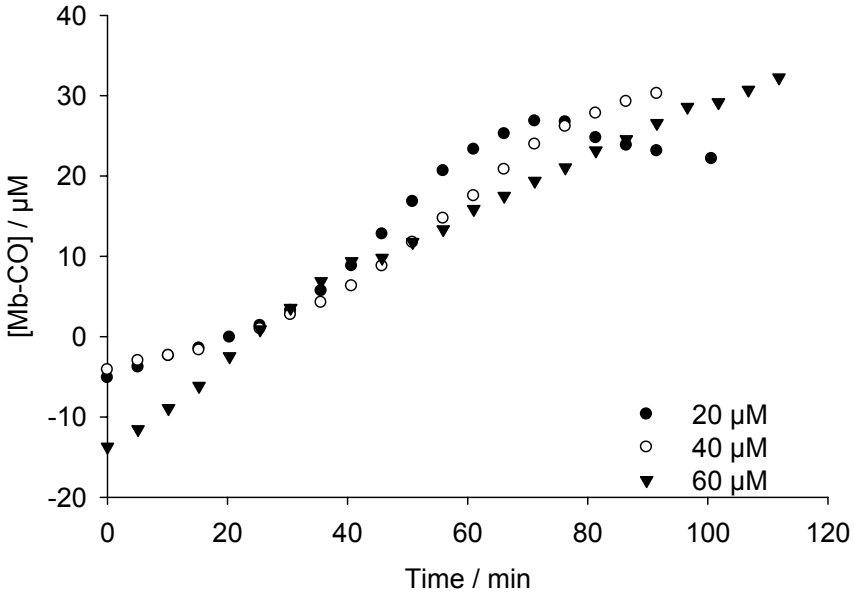
Corrected UV-Vis data for the myoglobin assay with $[Co(PPh_3)_3(CO)_2][Co(CO)_4]$ at 20 μM using DMSO as the addition solvent. The spectra are translated so that all the spectra intersect at 550 nm (an isosbestic point of the Deoxy-Mb and Mb-CO curves).



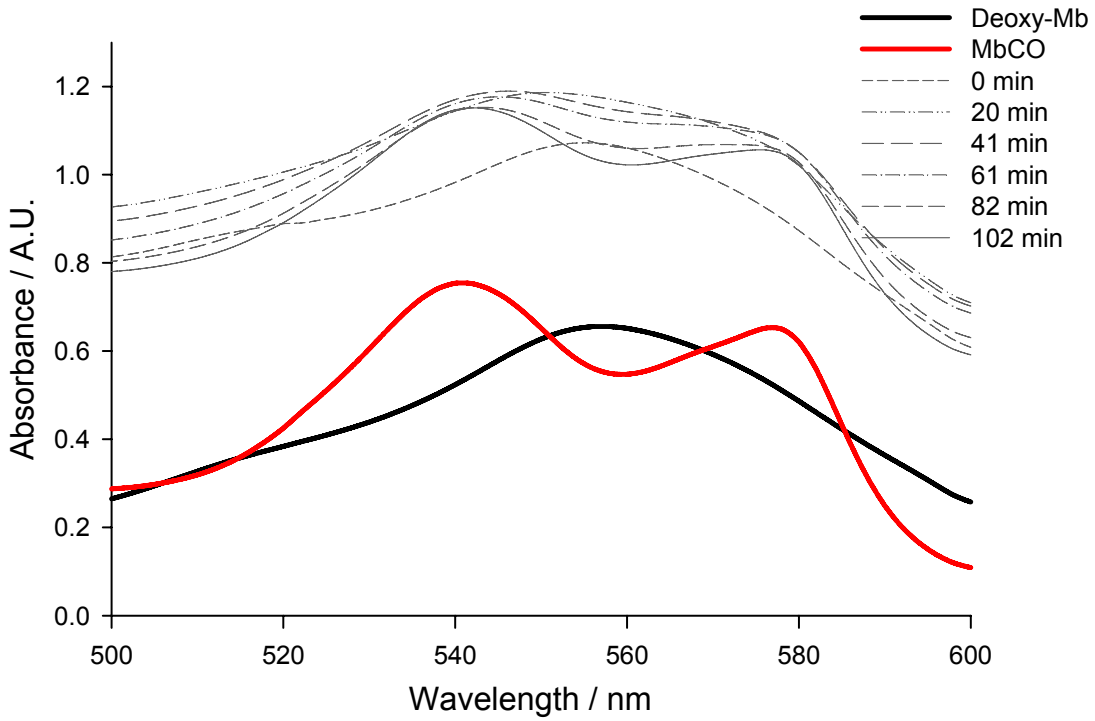
Corrected UV-Vis data for the myoglobin assay with $[Co(PPh_3)_3(CO)_2][Co(CO)_4]$ at 40 μM using DMSO as the addition solvent. The spectra are translated so that all the spectra intersect at 550 nm (an isosbestic point of the deoxy-Mb and Mb-CO curves).



Corrected UV-Vis data for the myoglobin assay with $[Co(PPh_3)_3(CO)_2][Co(CO)_4]$ at 60 µM using DMSO as the addition solvent. The spectra are translated so that all the spectra intersect at 550 nm (an isosbestic point of the deoxy-Mb and Mb-CO curves).



Graph of [Mb-CO] vs. time for $[Co(PPh_3)_3(CO)_2][Co(CO)_4]$ at 20, 40 and 60 µM. The precipitation of the complex causes shifts in the spectra; this shift is only partly counteracted by using the isosbestic point at 510 nm as a reference point. This accounts for the negative values of [Mb-CO] initially (particularly noticeable at higher concentrations of CO-RM).



Uncorrected UV-Vis data for the myoglobin assay with $[Co(PPh_3)_3(CO)_2][Co(CO)_4]$ at 60 μM using DMSO as the addition solvent. The spectra do not share any points in common with the deoxy-Mb and Mb-CO spectra. This is due to the precipitation of the complex in the assay increasing the absorbance.

* CO-RMs **1d-f** release negligible CO after 2 h; we believe that the increase in absorption upon addition of the CO-RM is due to poorer solubility in DMSO/H_2O mixtures.

3.3. Cell Based Assays

3.3.1. Cell Culture

Murine RAW264.7 monocyte macrophages (European Collection of Cell Cultures, Wiltshire, UK) were cultured in medium consisting of: Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 0.1 mg/mL streptomycin. The existing medium in each flask was removed and discarded. The cells were then washed with 5 mL warm (37 °C) 10x PBS (Gibco, Paisley, UK) which was then replaced with warm DMEM (37 °C, 8 mL) DMEM and the cells scraped using a rubber plastic Thomas policeman (Thomas Scientific, Swedesboro, NJ) to remove them from the flask surface. The cells were then pipetted (2 mL) into 4 new T-75 flasks (Sarstedt, Leicester, UK) each containing 8 mL DMEM and returned to the incubator (LEEC).

3.3.2. Subculture of Cells into 24 Wells

Cells were prepared as per section 3.3.1 up until the cells had been scraped (RAW264.7). At this stage 4 mL from the cell suspension, was added to 50 mL of complete medium in a falcon tube, mixed and then 2 mL added to each well of a 24 well plate. The plate was then incubated at 37 °C until confluent.

3.3.3. Assay for Nitrite Levels[9]

Nitrite levels were determined using the Griess assay protocol, a colourimetric assay. The medium from treated cells cultured in 24 well plates was removed and centrifuged (Beckman Avanti^{TM} 30 centrifuge) at 500 g for 5 min at 25 °C to remove any cells before it was plated out onto a 96 well plate in triplicate (50 µL per well). 50 µL of Griess reagent (sulphanilamide (125 mg), N-(1-napthyl)ethylenediamine (dihydrochloride) (Sigma, 12.5 mg) dissolved in dH_2O (5 mL) and 0.735 mL of orthophosphoric acid (85%), the total volume was made up to 25 mL using dH_2O) was added to each well to begin the reaction. The plate was then shaken for 10 min and the absorbance was read at 550 nm on a plate reader (Molecular devices VERSAmax tuneable microplate reader). Nitrite levels of each sample were determined using a calibration curve derived from standards.

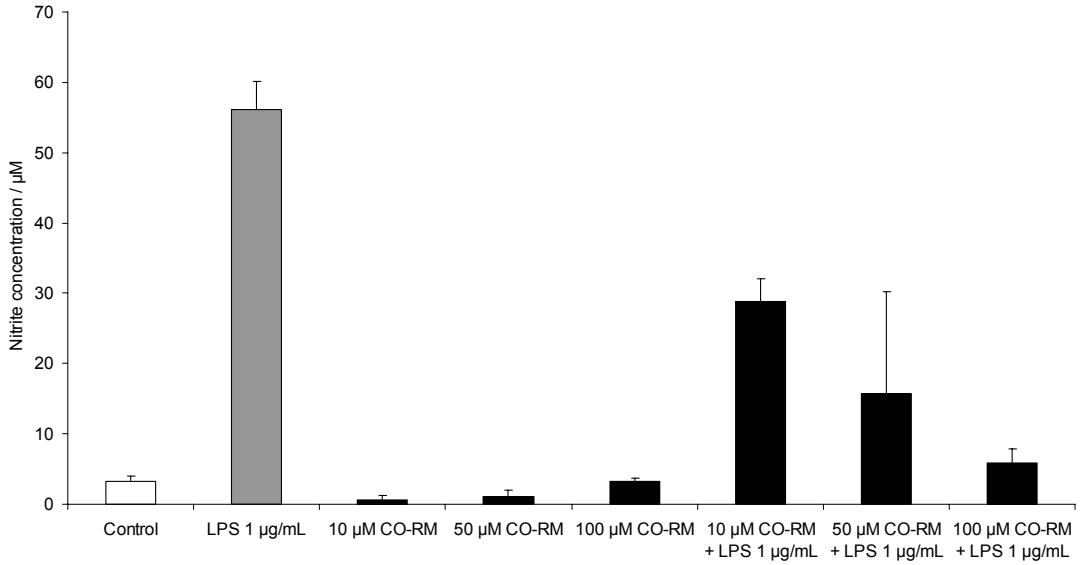
3.3.4. Alamar Blue Assay[9,10]

Cell metabolism was determined by the use of an Alamar Blue assay kit and carried out according to the manufacturer's instructions (Serotec, UK). The medium from treated wells was removed from the wells on the plate and replaced by 1 mL of a 10% AlamarBlue: 90% complete cell culture medium mixture. The plates were left to incubate for 4 h, after which 200 μL from each well was loaded onto a 96 well plate to be read on a plate reader at 570 nm (subtracting any background absorbance at 600 nm). The intensity of the red colour is proportional to the metabolism of the cells, which is calculated as the difference in absorbance between 570 and 600 nm and expressed as a percentage of the control.

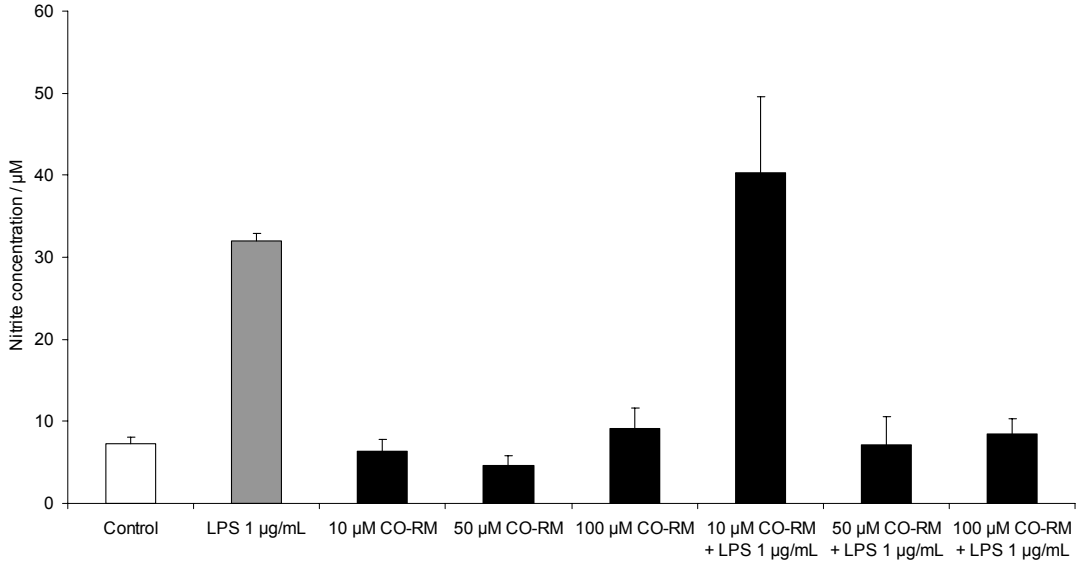
3.3.5 Lactate Dehydrogenase (LDH) Assay[9,10]

The LDH based cytotoxicity detection kit (Roche Diagnostics, UK) gives a method for the colourimetric assay for the quantification of cell death and cell lysis based on the measurement of LDH activity released from the cytosol of damaged cells into the supernatant. The assay was carried out according to the manufacturers instructions. Upon completion of the treatment the medium of each well was spun (Beckman Avanti^{TM} 30 centrifuge) at 500 g for 5 min at 25 °C to remove cellular debris. The supernatant (100 μL) was transferred to a 96 well plate to which 100 μL of reaction mixture was added. The reaction mixture consisted of a lysophilised catalyst which was reconstituted in dH_2O (1 mL) and an INT die (ratio 1:46). The plate was then incubated at 37 °C and 5% CO_2 for 15 min and protected from light. Samples were read on a plate reader at 490 nm with a reference wavelength of 690 nm and blanked against cell culture medium. Samples were run in triplicate and cytotoxicity was expressed as a percentage of a 1% Triton control.

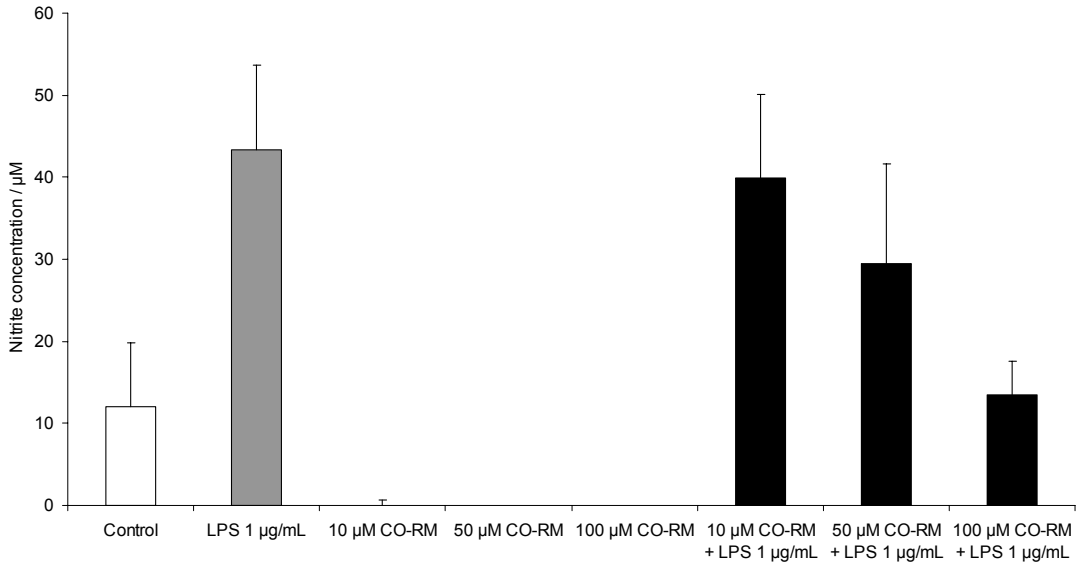
3.3.6. Cell Assay Results



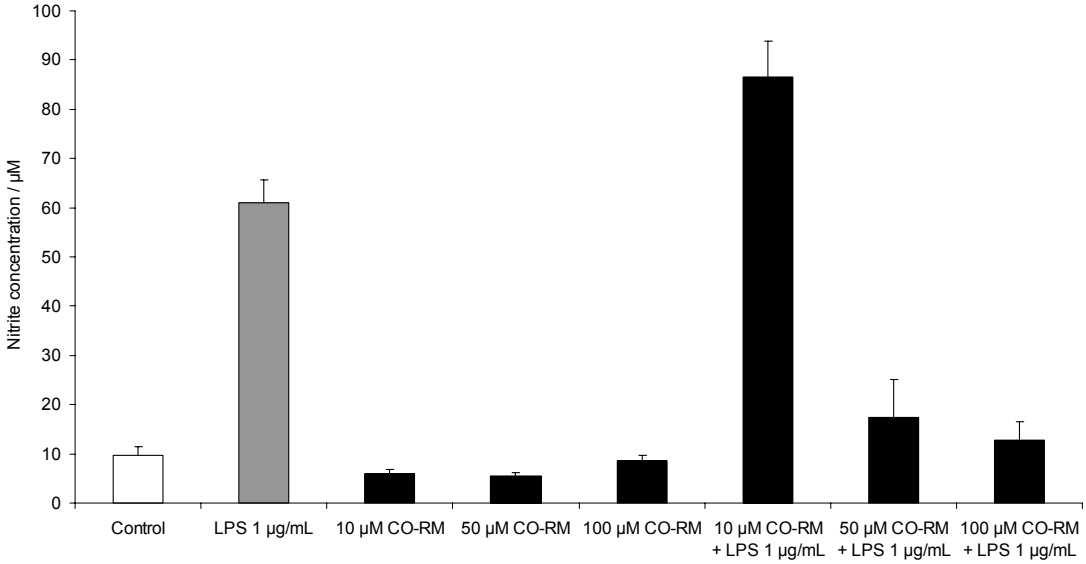
Nitrite Assay on RAW264.7 treated with LPS and CO-RM **1a** for 24 h.



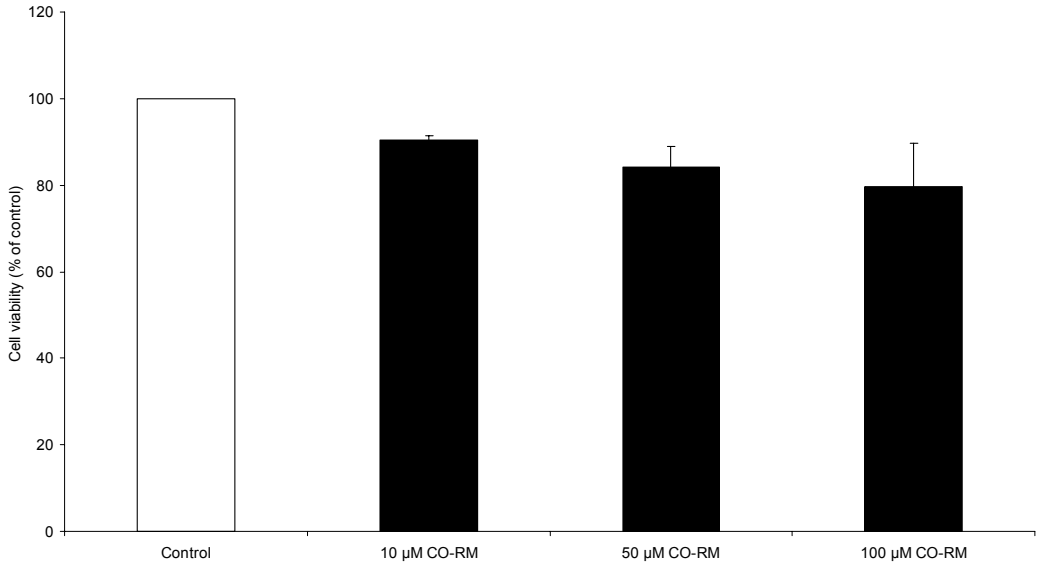
Nitrite Assay on RAW264.7 treated with LPS and CO-RM **1b** for 24 h.



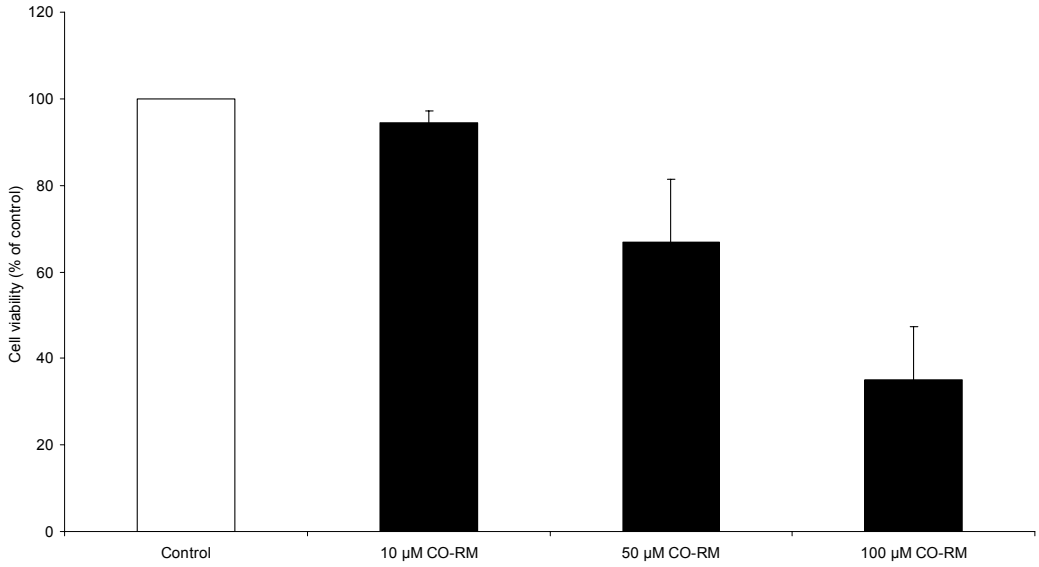
Nitrite Assay on RAW264.7 treated with LPS and CO-RM **1d** for 24 h.



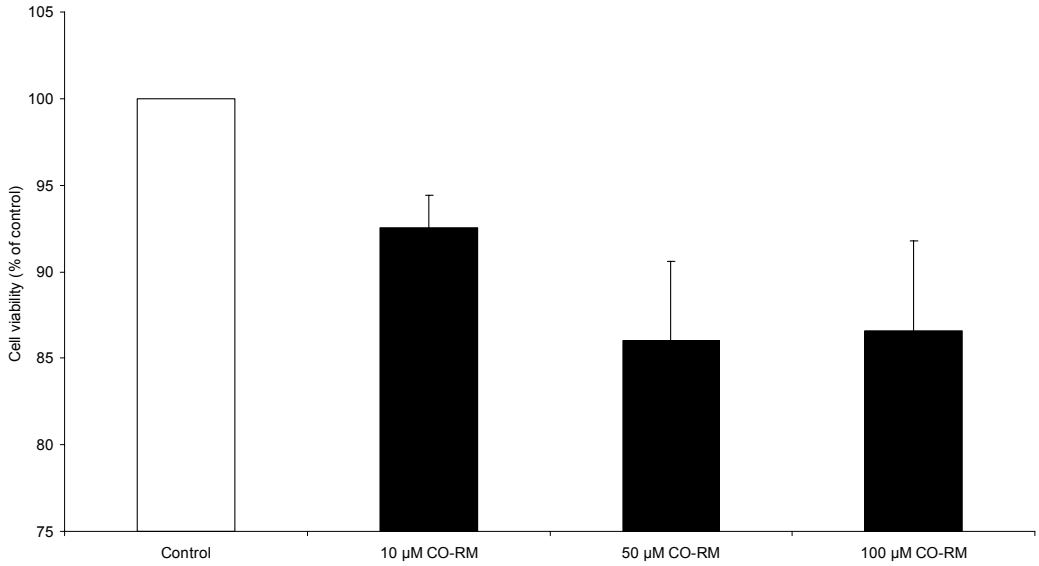
Nitrite Assay on RAW264.7 treated with LPS and CO-RM **1e** for 24 h.



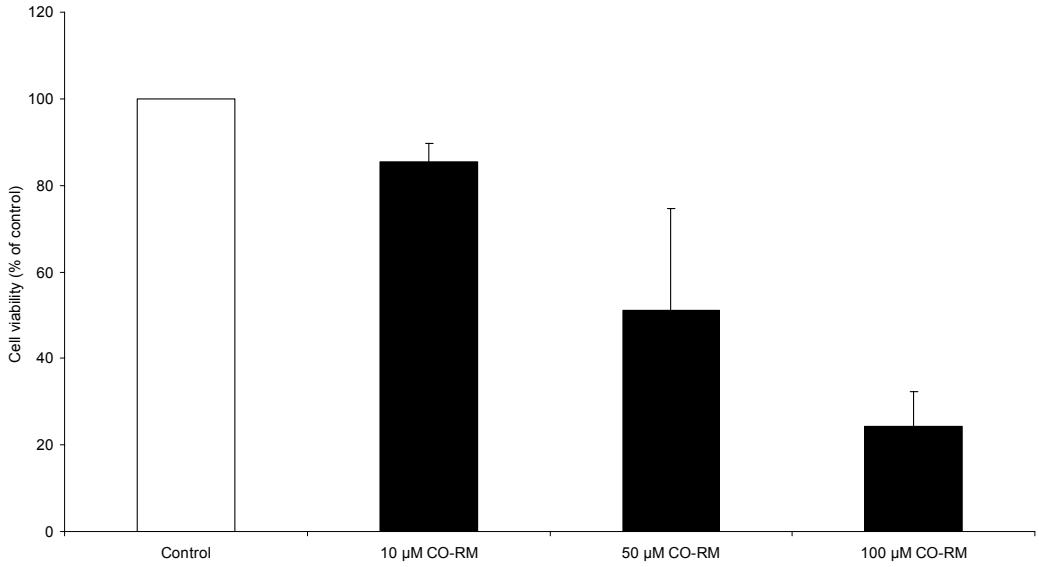
Alamar Blue Assay on RAW264.7 treated with CO-RM **1a** for 24 h



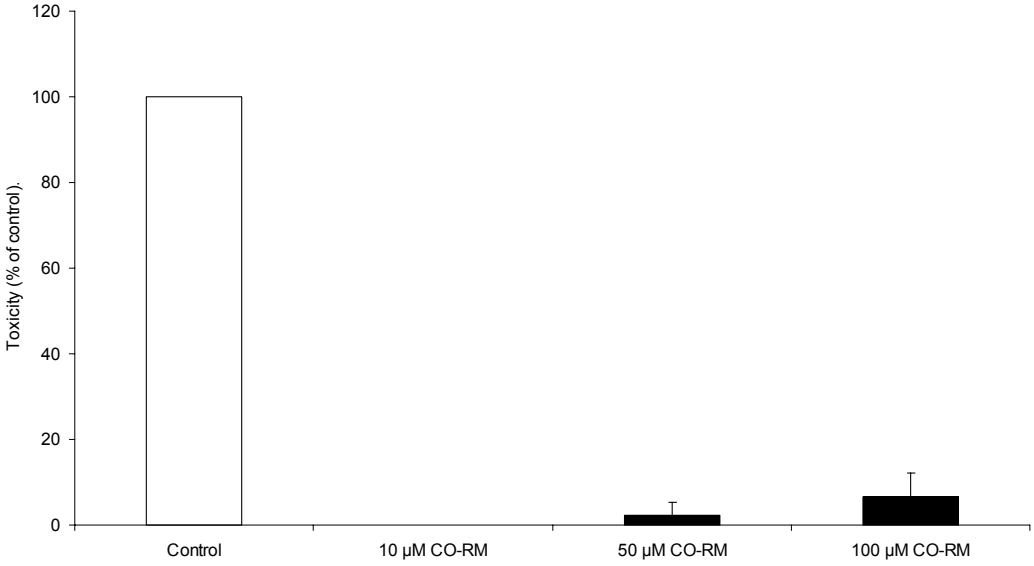
Alamar Blue Assay on RAW264.7 treated with CO-RM **1b** for 24 h



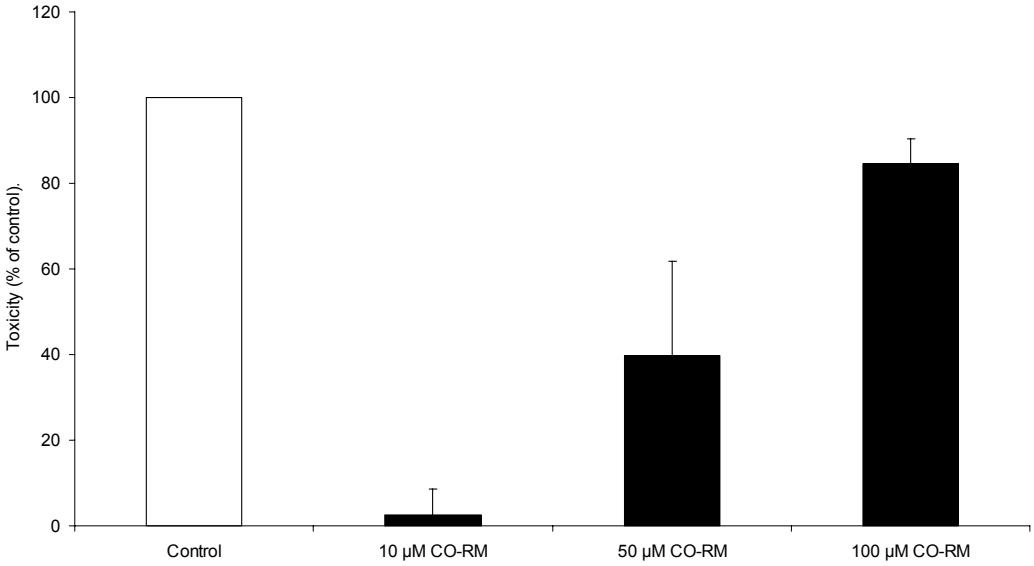
Alamar Blue Assay on RAW264.7 treated with CO-RM **1d** for 24 h.



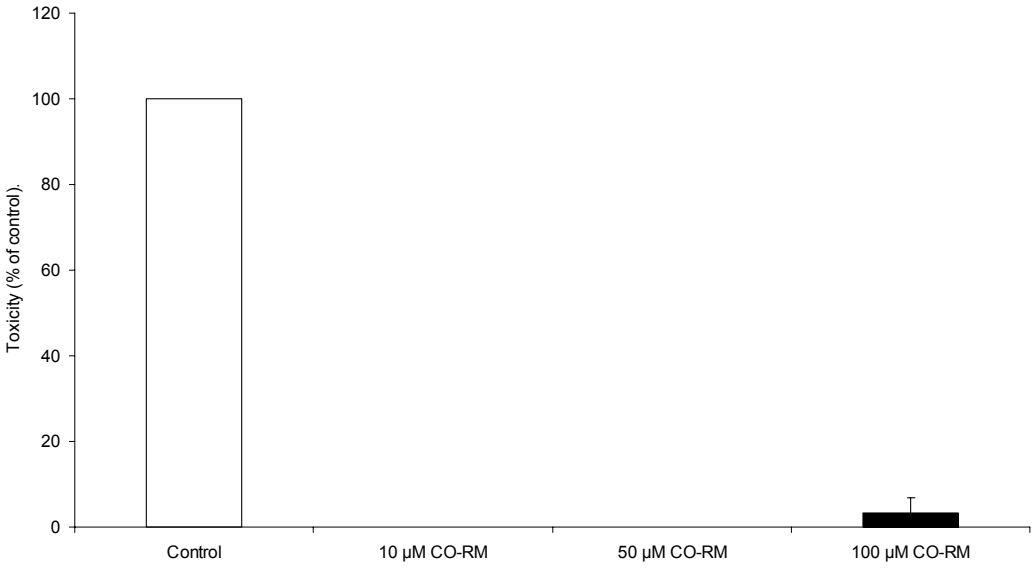
Alamar Blue Assay on RAW264.7 treated with CO-RM **1e** for 24 h.



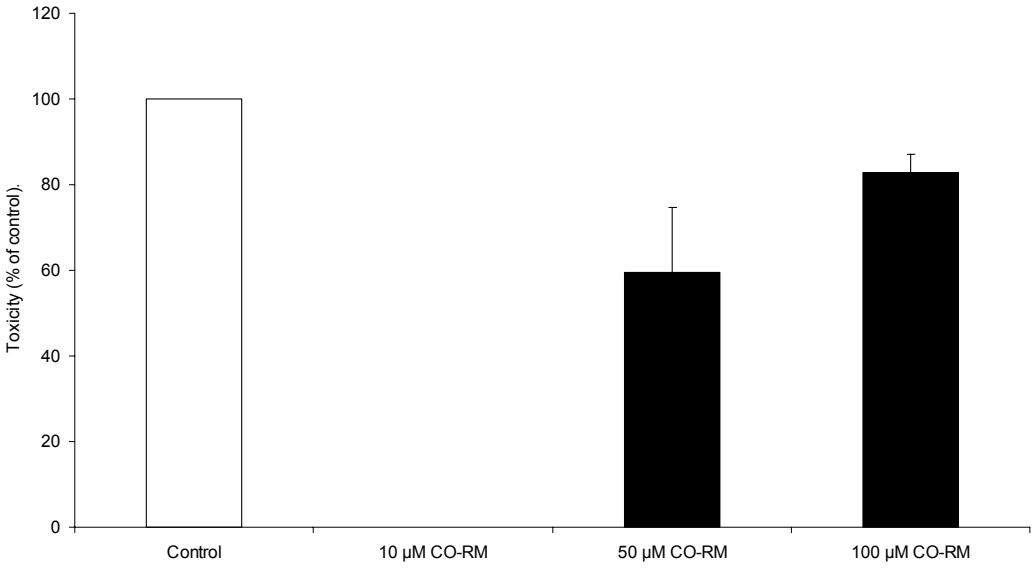
LDH Assay on RAW264.7 treated with CO-RM **1a** for 24 h.



LDH Assay on RAW264.7 treated with CO-RM **1b** for 24 h



LDH Assay on RAW264.7 treated with CO-RM **1d** for 24 h

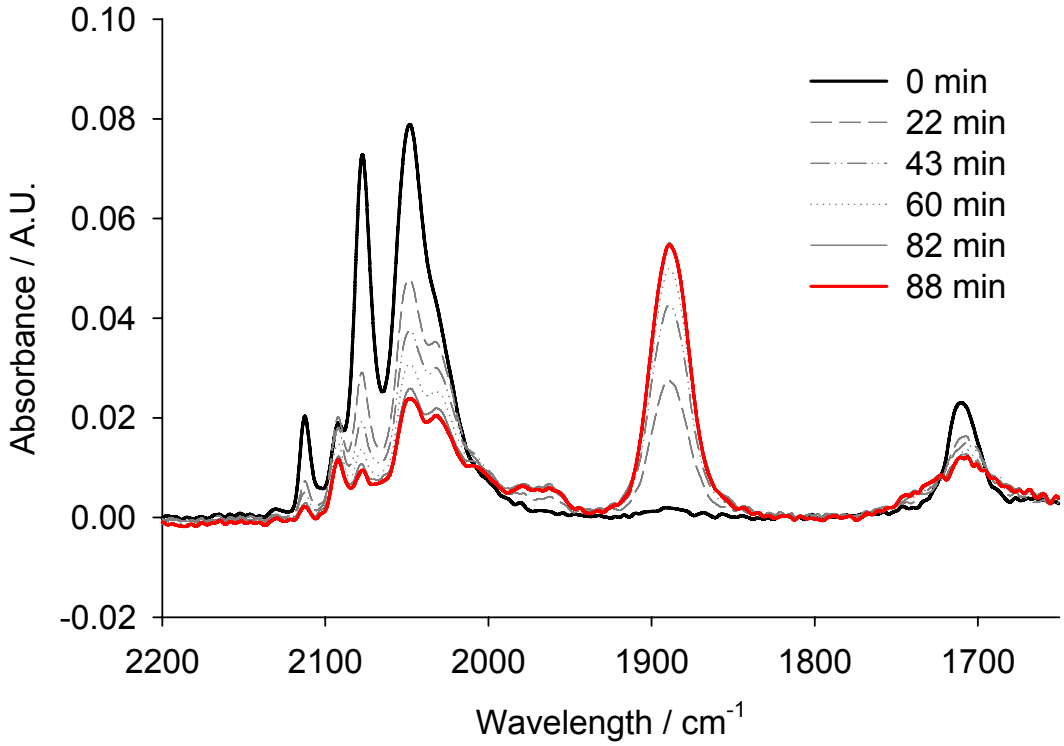


LDH Assay on RAW264.7 treated with CO-RM **1e** for 24 h

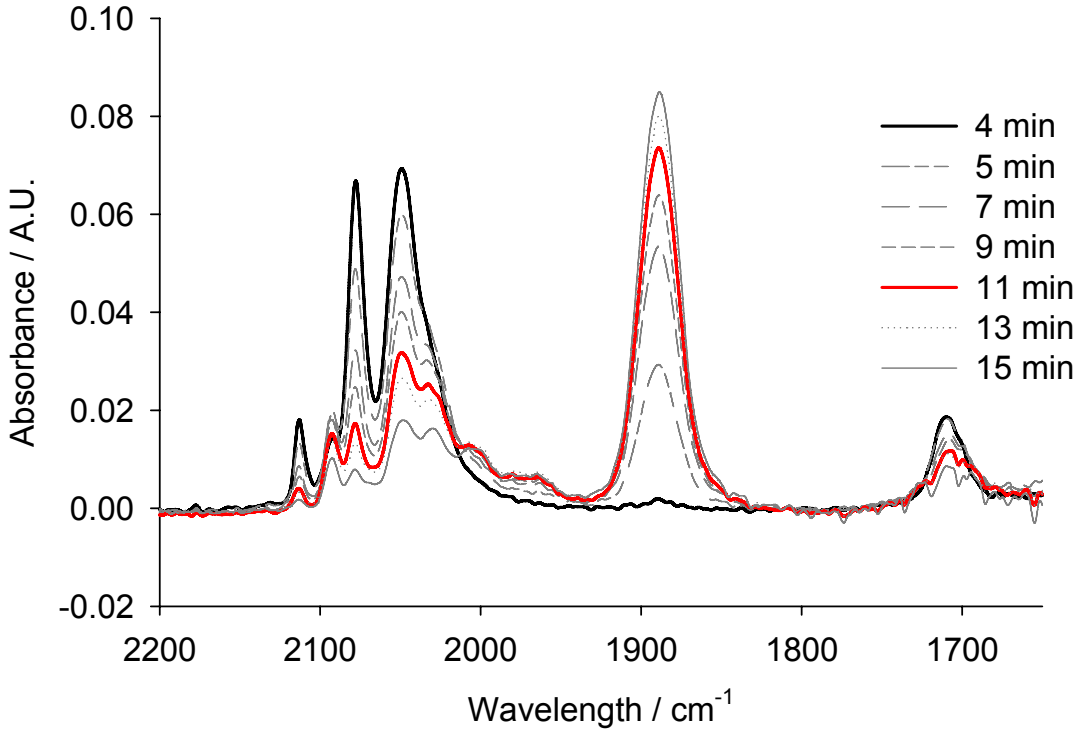
4. ReactIR™ Experiments

Acknowledgement: We are very grateful to Mettler Toledo for the loan of a ReactIR™ ic10 with K6 Conduit, SiComp (silicon) probe and MCT detector. We also thank Dr. Jon Goode for technical expertise (Reaction Analysis, Technology & Application Consultant, MT AutoChem UK). The following parameters were used: resolution = 4 cm^{-1}; range = 4000-650 cm^{-1}; gain adjustment = 1×.

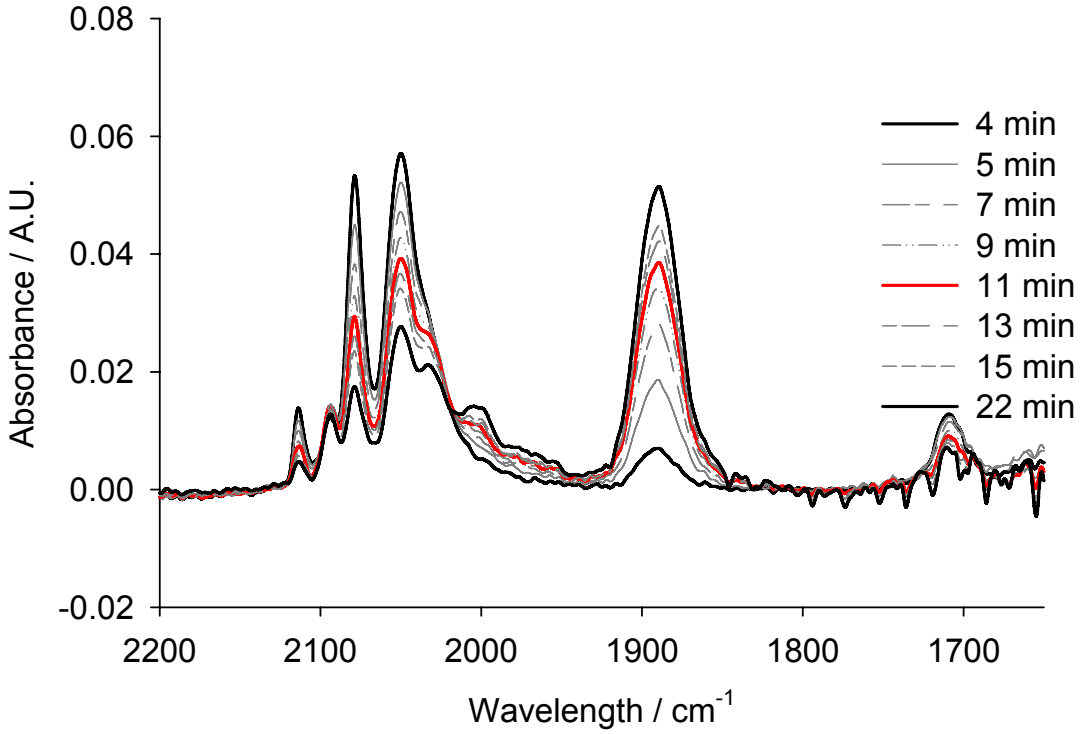
The solvent system (DMSO, EtOH, H_2O) was prepared in a small sample vial equipped with a magnetic stirrer bar and thermostated at 37 ˚C. The probe was lowered into the solution and a background spectrum was acquired. To the solvent mixture was added the CO-RM and the data acquisition started.



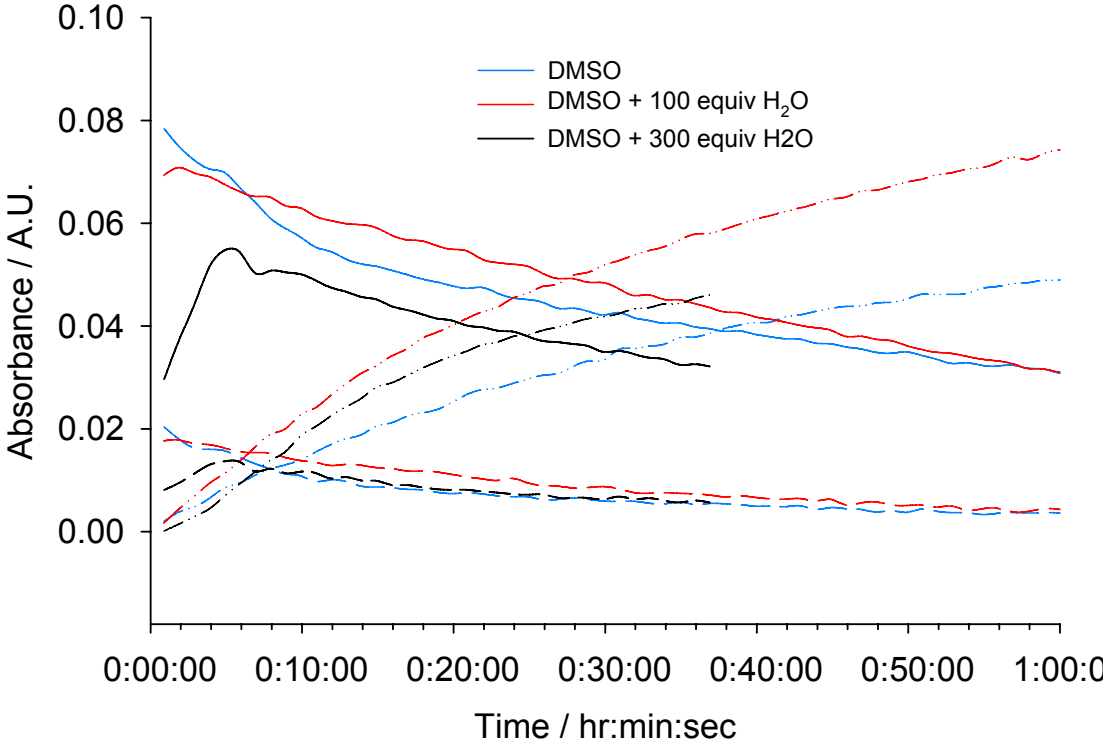
ReactIR™ spectra of **1b** in DMSO at 37 ˚C



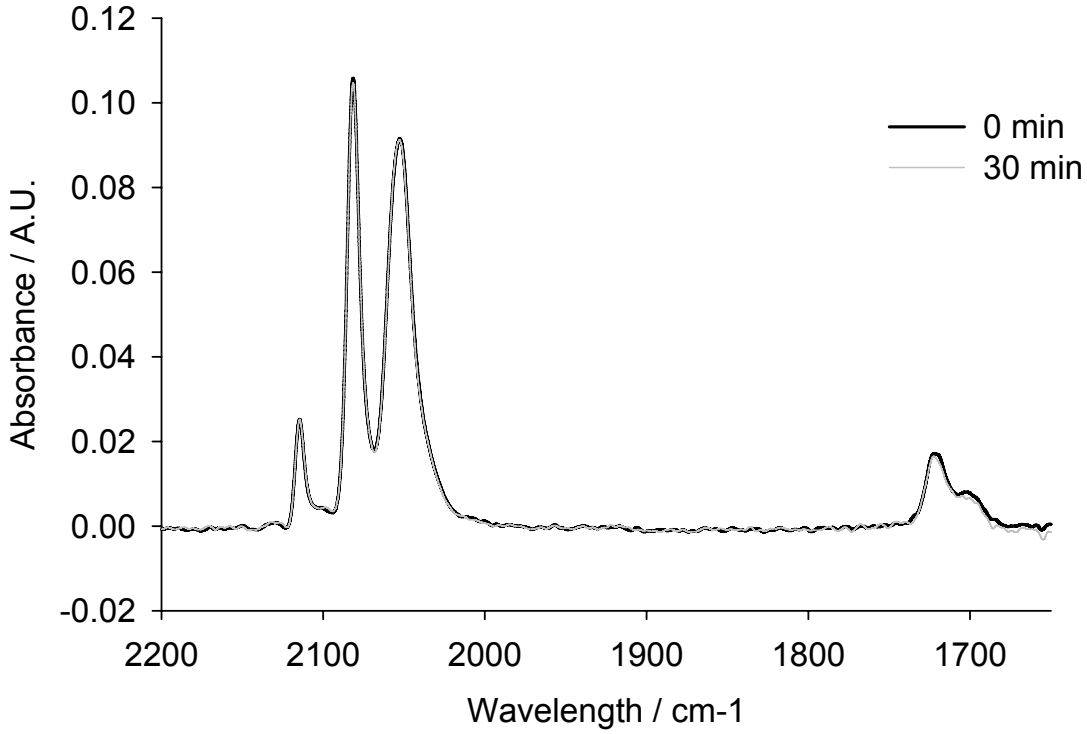
ReactIR™ spectra of **1b** in DMSO at 37 ˚C with 100 equivalents of water (relative to CO-RM)



ReactIR™ spectra of **1b** in DMSO at 37 ˚C with 300 equivalents of water (relative to CO-RM).



Graph showing the degradation of **1b** and the formation of $[Co(CO)4]^{-}$ in DMSO with 0, 100 and 300 equivalents of water.



Graph showing the stability of **1b** in EtOH over time.

5. References

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