

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

3,3',5,5'-tetramethylbenzidine as multi-colorimetric indicator of chlorine in water in line with health guideline values

Pasquale Palladino, Francesca Torrini, Simona Scarano, Maria Minunni

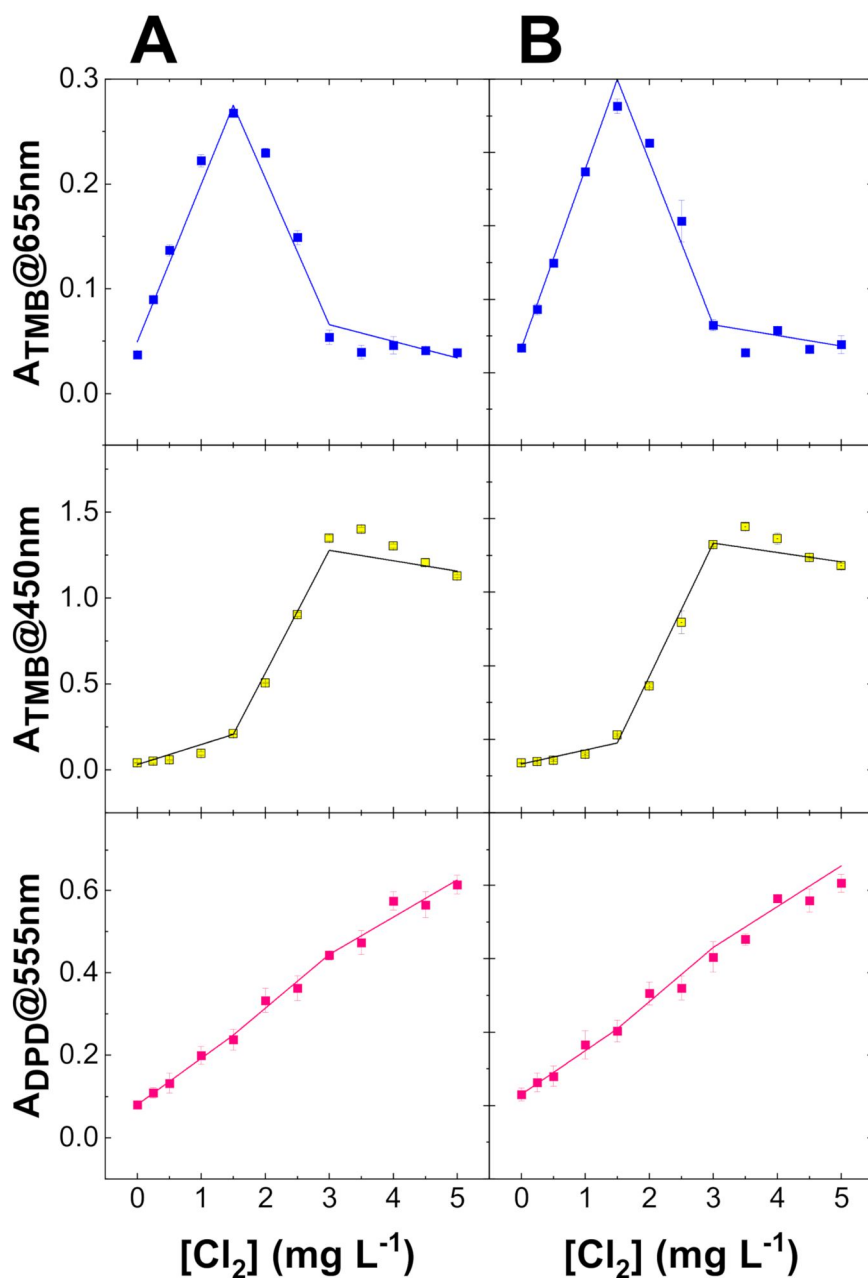


Fig. S1 Piecewise linear fitting for TMB and DPD titration experiments with chlorine in H₂O (A) and tap water (B) at $t = 0$ s. Data and fitting parameters are reported in the main text in Figure 4 and Table 3, respectively. The chlorine concentration ranges are 0.00-1.50 mg L⁻¹, 1.50-3.00 mg L⁻¹, and 3.00-5.00 mg L⁻¹

Table S1 Effect of several ions and molecules on colorimetric determination of chlorine in water samples by TMB methods

Ion or Molecule added	Tolerance limit ^a [21, 22]	Interference ^b [23]	Interference ^c [24]
Sulfate	10000	+0.2 %	Negligible
Chloride	10000	-0.2 %	Negligible
Ca(II)	2000	+0.5 %	Negligible
Mg(II)	2000	-4.5 % as Mg(NO ₃) ₂	Negligible
Nitrate	2000	-2.9 % as NO ₃ ⁻	n.d.
Fe(III) ^d	50	+0.4 % for Fe(NO ₃) ₃	Negligible
Zn(II)	50	n.d.	Negligible
Cu(II)	50	-3.7 %	n.d.
Ni(II)	50	+2.3 %	n.d.
Pb(II)	50	+2.9 %	n.d.
Al(III)	50	-3.3 %	n.d.
Mn(II); Co(II); As(V); Mo(VI)	50	n.d.	n.d.
MnO ₄ ⁻	n.d.	-1.7 %	n.d.
Hg(II)	10	-2.9 %	n.d.
Se(IV)	10	n.d.	n.d.
W(VI)	10	n.d.	n.d.
MnO ₂	5	n.d.	n.d.
Cr(III)	2	n.d.	n.d.
Cr(VI)	1	+4.9 %	n.d.
V(V)	1	n.d.	n.d.
Ozone	0.2	n.d.	n.d.
Iodide; Potassium	n.d.	n.d.	Negligible
Carbonate	n.d.	-2.3 % for HCO ₃ ⁻	Negligible
H ₂ PO ₄ ^{-d}	n.d.	Buffer component	Negligible
P ₂ O ₇ ^{4-d} ; H ₂ O ₂ ^e	n.d.	n.d.	Negligible

^aInterferent/Chlorine (m/m) limit for interference. Chlorine 0.4 mg L⁻¹; pH 1.8
^bInterferent representative of the worst-case scenario. Chlorine 0.7 mg L⁻¹; pH acid
^cInterferent at 0.2 mM. Chlorine 0.02 mM (1.4 mg L⁻¹); pH 4.0.
^dPhosphate salts in buffer confer high tolerance to TMB solutions toward Fe(III)
^eTMB requires the presence of peroxidases for efficient oxidation by H₂O₂ [15].
 Percentage represents the signal interference when compared to the same standard without interfering agent. The interference is considered negligible when the signal difference is below 5 % within the error. n.d. (not determined).

Table S2.1 Coefficient of variation (%CV) for Absorbance values at 280 nm vs $[Cl_2]$ and time from Figure 2B

Time (s)	$[Cl_2]$ mg L ⁻¹ in H ₂ O					
	0.00	1.00	2.00	3.00	4.00	5.00
	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}
0	0.92	1.00	6.02	14.53	1.44	6.17
12	1.00	1.04	5.90	15.54	3.42	7.01
22	0.97	1.05	5.73	15.06	5.16	5.23
32	1.00	1.02	6.01	14.96	7.94	4.89
42	1.03	1.06	6.06	14.87	10.00	1.70
52	1.06	1.05	5.77	14.68	9.85	4.10
62	1.05	1.09	5.89	11.29	10.04	4.44
72	1.03	1.36	5.96	14.70	9.22	3.64
82	1.05	1.06	6.21	14.63	9.17	7.47
92	1.09	0.78	5.79	14.84	10.60	3.84
102	1.11	1.08	5.73	14.81	10.27	3.05
112	1.06	1.10	5.24	16.72	9.43	3.46
122	1.13	1.04	5.41	14.26	0.79	2.56
132	1.13	1.09	5.56	14.20	11.19	3.24
142	1.15	0.96	6.32	13.85	11.06	3.48
152	1.16	1.62	8.65	14.26	11.54	2.02
162	1.19	1.15	6.67	14.11	9.28	3.19
172	1.18	1.22	5.31	13.97	9.18	3.22
182	1.15	1.21	5.38	14.20	9.74	3.01
192	1.17	1.14	5.47	11.55	9.63	2.62
202	1.18	1.22	5.47	13.89	10.01	2.90
212	1.19	1.16	5.40	13.55	9.50	3.34
222	1.15	1.16	5.24	13.52	10.62	3.25
232	1.20	1.09	5.29	13.85	9.68	3.16
242	1.22	1.20	6.11	13.47	9.79	1.99
252	1.22	1.21	5.06	13.58	9.02	3.84
262	1.23	1.11	5.05	13.78	9.08	3.66
272	1.30	1.01	5.04	13.62	8.55	3.95
282	1.27	1.06	5.01	13.69	8.87	3.31
292	1.25	1.06	5.02	13.94	9.12	3.10
302	1.24	0.97	5.06	14.25	8.94	2.66
%CV_{mean} (0-300 s)	1.13	1.11	5.70	14.13	8.78	3.66

Table S2.2 Coefficient of variation (%CV) for Absorbance values at 450 nm vs [Cl₂] and time from Figure 2B

Time (s)	[Cl ₂] mg L ⁻¹ in H ₂ O					
	0.00	1.00	2.00	3.00	4.00	5.00
	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}
0	4.89	9.58	2.86	3.35	1.00	0.54
12	3.07	7.66	2.86	5.16	0.99	0.34
22	4.78	15.69	2.24	2.92	0.59	0.28
32	5.44	13.35	2.02	2.92	0.16	0.26
42	5.63	14.97	1.53	2.79	0.65	0.20
52	13.07	14.92	1.42	2.79	0.90	0.19
62	10.51	14.06	1.31	2.83	1.33	0.18
72	6.58	13.61	1.31	2.75	1.27	0.18
82	2.25	8.36	1.20	2.77	1.43	0.17
92	9.88	13.77	0.70	2.77	1.41	0.10
102	5.27	15.56	1.25	2.79	1.21	0.18
112	2.86	13.05	1.26	2.77	1.28	0.18
122	9.81	12.95	1.64	2.72	1.37	0.24
132	1.63	13.16	1.26	2.80	1.36	0.18
142	7.58	13.31	1.10	2.79	1.18	0.16
152	14.42	13.99	0.73	2.75	1.32	0.11
162	10.35	9.24	0.33	2.75	1.32	0.05
172	0.27	12.19	0.61	2.71	1.34	0.09
182	8.97	12.06	1.20	2.72	1.35	0.18
192	1.56	11.62	1.18	2.78	1.40	0.18
202	2.59	12.09	1.11	2.77	1.16	0.26
212	2.71	12.23	1.11	2.73	1.30	0.39
222	3.75	12.09	1.14	2.75	1.27	0.17
232	0.55	11.76	1.18	2.67	1.24	0.18
242	12.90	12.37	1.04	2.66	1.32	0.16
252	3.72	11.54	0.66	2.72	1.35	0.10
262	4.36	11.86	1.13	2.69	1.35	0.17
272	9.40	12.66	1.18	2.67	1.29	0.18
282	9.13	13.32	1.18	2.69	1.35	0.18
292	1.56	12.93	1.16	2.67	1.27	0.33
302	1.35	13.48	1.23	2.71	1.35	0.19
%CV_{mean} (0-300 s)	5.83	12.56	1.29	2.85	1.20	0.20

Table S2.3 Coefficient of variation (%CV) for Absorbance values at 655 nm vs $[Cl_2]$ and time from Figure 2B

Time (s)	$[Cl_2]$ mg L ⁻¹ in H ₂ O					
	0.00	1.00	2.00	3.00	4.00	5.00
	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}
0	1.55	8.08	1.06	1.91	3.39	10.32
12	8.72	8.23	0.99	1.79	6.24	5.58
22	1.66	8.28	0.86	1.55	6.38	4.78
32	1.77	8.12	0.78	1.40	6.06	2.69
42	2.20	7.98	0.63	1.12	6.04	2.81
52	6.13	8.24	0.67	1.21	5.88	5.88
62	3.91	8.58	0.54	0.97	7.09	5.09
72	3.21	6.83	0.50	0.89	6.94	3.02
82	9.05	8.07	0.52	0.92	7.60	1.89
92	3.88	8.70	0.69	1.23	7.77	2.49
102	2.26	8.33	0.66	1.18	8.13	7.49
112	1.96	8.08	0.53	0.94	8.98	12.79
122	4.55	8.47	0.56	0.99	8.53	6.47
132	7.39	8.26	0.53	0.94	8.58	5.38
142	3.34	8.56	0.54	0.96	8.08	12.38
152	6.49	7.31	0.42	0.75	8.55	12.34
162	4.88	7.72	0.41	0.73	9.20	7.09
172	0.13	8.28	0.56	0.99	8.57	5.89
182	4.46	8.31	0.52	0.92	9.53	5.62
192	0.76	8.01	0.43	0.75	9.40	14.25
202	1.25	8.21	0.51	0.89	10.33	5.46
212	1.31	8.56	0.55	0.96	9.78	22.33
222	1.86	7.91	0.45	0.79	9.91	9.28
232	0.27	8.34	0.35	0.62	9.88	4.24
242	9.54	8.07	0.34	0.59	10.65	3.73
252	1.83	8.74	0.45	0.79	10.09	13.20
262	2.16	8.37	0.37	0.65	10.19	4.37
272	4.61	8.67	0.63	1.11	11.70	5.12
282	9.35	8.65	0.39	0.68	11.86	12.94
292	7.62	8.85	0.43	0.76	9.84	13.81
302	7.07	8.81	0.50	0.87	10.13	9.14
%CV_{mean} (0-300 s)	4.04	8.25	0.56	1.00	8.56	7.67

Table S3.1 Coefficient of variation (%CV) for Absorbance values at 280 nm vs [Cl₂] and time from Figure 3B

Time (s)	[Cl ₂] mg L ⁻¹ in tap water					
	0.00	1.00	2.00	3.00	4.00	5.00
	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}	%CV _{280nm}
0	0.29	2.02	5.03	1.27	5.85	2.87
12	0.47	1.95	4.98	1.02	6.48	3.29
22	0.25	2.00	5.06	0.70	6.14	2.46
32	0.21	1.98	5.06	1.29	5.47	2.30
42	0.19	1.93	5.11	1.55	6.38	0.82
52	0.21	1.98	5.15	1.56	6.13	1.97
62	0.21	1.96	5.35	1.62	5.88	2.14
72	0.18	1.92	5.50	1.52	5.86	1.77
82	0.15	1.92	5.44	2.01	6.00	3.76
92	0.15	1.91	5.19	1.71	5.82	1.90
102	0.17	1.88	5.14	1.72	5.82	1.50
112	0.15	1.85	5.43	1.87	6.12	1.70
122	0.16	1.86	5.39	1.89	5.45	1.28
132	0.16	1.86	5.46	1.71	5.78	1.63
142	0.15	1.82	5.44	1.67	6.00	1.75
152	0.14	1.85	5.50	1.69	5.65	1.02
162	0.14	1.89	5.57	1.89	5.97	1.60
172	0.16	1.88	5.52	1.60	5.85	1.62
182	0.18	1.92	5.56	1.78	5.72	1.51
192	0.20	1.94	5.58	0.62	6.10	1.33
202	0.20	1.93	5.62	1.62	6.45	1.47
212	0.19	1.95	5.62	1.40	6.10	1.69
222	0.19	1.94	5.67	1.60	5.99	1.66
232	0.15	1.95	5.90	1.55	5.83	1.62
242	0.17	1.94	6.23	1.44	5.91	1.03
252	0.17	1.94	6.29	1.60	6.15	1.99
262	0.18	1.97	5.84	1.65	6.06	1.90
272	0.17	1.97	5.80	1.67	6.06	2.05
282	0.12	1.97	5.80	1.61	5.82	1.70
292	0.10	1.96	5.82	1.51	5.76	1.59
302	0.07	2.30	5.85	1.46	5.88	1.37
%CV_{mean} (0-300 s)	0.18	1.94	5.51	1.54	5.95	1.82

Table S3.2 Coefficient of variation (%CV) for Absorbance values at 450 nm vs [Cl₂] and time from Figure 3B

Time (s)	[Cl ₂] mg L ⁻¹ in tap water					
	0.00	1.00	2.00	3.00	4.00	5.00
	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}
0	8.25	4.16	0.69	2.51	3.15	0.60
12	3.21	3.79	0.81	2.32	3.45	2.70
22	6.49	3.99	1.17	2.29	3.55	2.82
32	1.91	4.07	1.22	2.16	3.61	2.91
42	3.82	4.46	1.24	2.10	3.71	2.99
52	5.89	3.79	1.40	2.06	3.71	3.03
62	5.10	4.27	1.25	2.02	3.76	3.07
72	4.52	4.32	0.92	2.00	3.76	3.06
82	3.86	4.29	1.31	1.94	3.76	3.09
92	3.90	4.63	1.70	1.98	3.81	3.11
102	6.51	4.58	1.84	1.96	3.80	3.11
112	6.10	4.44	1.62	1.92	3.81	3.13
122	4.02	4.31	1.67	1.86	3.75	3.09
132	4.68	4.71	1.64	1.86	3.80	3.14
142	1.94	4.74	1.64	1.84	3.81	3.16
152	6.13	4.22	1.62	1.83	3.79	3.15
162	3.78	4.62	1.59	1.80	3.79	3.15
172	3.39	4.29	1.66	1.79	3.81	3.18
182	7.29	4.17	1.58	1.80	3.76	3.15
192	9.22	4.27	1.60	1.88	3.77	3.16
202	11.42	4.10	1.60	1.83	3.80	3.18
212	10.72	4.02	1.58	1.78	3.80	3.18
222	11.02	4.07	1.54	1.78	3.76	3.16
232	8.50	3.96	1.31	1.79	3.79	3.19
242	10.41	4.22	0.85	1.78	3.77	3.18
252	7.94	3.79	0.85	1.77	3.80	3.20
262	10.16	3.80	1.33	1.77	3.79	3.21
272	10.55	3.68	1.37	1.75	3.76	3.18
282	6.57	3.62	1.35	1.73	3.75	3.17
292	1.13	3.85	1.33	1.74	3.76	3.19
302	5.13	1.98	1.25	1.74	3.75	3.19
%CV_{mean} (0-300 s)	6.24	4.10	1.37	1.92	3.73	3.03

Table S3.3 Coefficient of variation (%CV) for Absorbance values at 655 nm vs [Cl₂] and time from Figure 3B

Time (s)	[Cl ₂] mg L ⁻¹ in tap water					
	0.00	1.00	2.00	3.00	4.00	5.00
	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}
0	1.06	4.72	2.69	0.05	1.39	0.30
12	14.64	4.70	2.9	0.46	2.12	1.84
22	2.25	4.83	2.97	0.46	2.46	0.34
32	2.52	4.40	3.19	0.50	2.66	0.35
42	3.67	4.64	3.22	0.39	2.51	0.48
52	9.04	4.64	3.23	0.48	2.67	1.32
62	6.84	4.70	3.37	0.56	2.66	0.97
72	5.11	4.80	3.71	0.57	2.69	0.70
82	15.28	4.70	3.47	0.72	3.16	2.03
92	6.23	4.73	3.32	0.71	2.80	0.95
102	4.37	4.80	3.50	0.71	3.21	0.61
112	3.24	4.40	3.35	0.81	3.01	0.43
122	6.75	4.80	3.59	0.68	3.02	1.03
132	11.36	4.73	3.58	0.80	2.99	1.60
142	5.36	4.89	3.79	0.89	2.76	0.81
152	10.8	4.58	3.70	0.68	2.91	1.55
162	8.42	4.79	3.73	0.73	2.96	1.15
172	0.23	4.78	3.61	1.01	2.71	0.03
182	6.97	4.40	3.70	0.89	2.72	1.01
192	1.31	4.32	3.70	1.10	2.88	0.17
202	2.31	4.71	3.64	1.35	2.63	0.3
212	2.23	4.61	3.73	1.09	2.56	0.31
222	3.1	4.66	3.86	1.35	2.73	0.44
232	0.49	4.52	3.92	1.11	2.85	0.06
242	1.75	4.73	4.38	1.24	2.36	2.26
252	3.57	4.69	4.27	1.39	2.83	0.44
262	4.13	4.69	4.00	1.25	2.80	0.51
272	8.32	4.60	3.92	1.26	2.85	1.11
282	8.87	4.68	4.03	1.34	2.70	2.19
292	14.26	4.57	4.01	1.32	2.80	1.89
302	11.03	4.28	2.69	1.31	2.86	1.58
%CV_{mean} (0-300 s)	5.98	4.65	3.57	0.88	2.72	0.93

Table S4.1 Coefficient of variation (%CV) for DPD Absorbance values at 555 nm vs $[\text{Cl}_2]$ and time from Figure 4C (H_2O)

	0 s	30 s	60 s	120 s	240 s
$[\text{Cl}_2]$ mg L⁻¹	%CV_{555nm}	%CV_{555nm}	%CV_{555nm}	%CV_{555nm}	%CV_{555nm}
0.00	5.89	5.76	4.91	4.56	4.61
0.25	11.04	14.74	15.40	15.85	13.59
0.50	18.40	17.26	17.23	16.20	15.86
1.00	11.11	8.09	6.75	4.73	3.55
1.50	10.90	10.45	8.99	7.21	6.43
2.00	8.86	7.18	4.57	2.68	3.27
2.50	8.19	6.43	5.03	4.54	4.86
3.00	2.57	2.63	2.67	2.70	3.05
3.50	6.20	6.03	5.81	5.43	4.65
4.00	3.92	2.41	1.64	1.07	1.46
4.50	5.68	4.38	4.21	3.44	4.44
5.00	3.60	2.83	1.57	2.11	2.77
%CV_{mean}	8.03	7.35	6.57	5.88	5.71

Table S4.2 Coefficient of variation (%CV) for DPD Absorbance values at 555 nm vs $[\text{Cl}_2]$ and time from Figure 4D (Tap water)

	0 s	30 s	60 s	120 s	240 s
$[\text{Cl}_2]$ mg L⁻¹	%CV_{555nm}	%CV_{555nm}	%CV_{555nm}	%CV_{555nm}	%CV_{555nm}
0.00	11.82	10.26	10.21	9.68	8.48
0.25	9.16	8.96	8.20	7.80	6.94
0.50	11.82	10.26	10.21	9.68	8.48
1.00	9.16	8.96	8.20	7.80	6.94
1.50	11.82	10.26	10.21	9.68	8.48
2.00	9.16	8.96	8.20	7.80	6.94
2.50	11.82	10.26	10.21	9.68	8.48
3.00	9.16	8.96	8.20	7.80	6.94
3.50	11.82	10.26	10.21	9.68	8.48
4.00	9.16	8.96	8.20	7.80	6.94
4.50	11.82	10.26	10.21	9.68	8.48
5.00	9.16	8.96	8.20	7.80	6.94
%CV_{mean}	9.16	8.96	8.20	7.80	6.94

Table S4.3 Coefficient of variation (%CV) for TMB Absorbance values at 450 nm vs [Cl₂] and time from Figure 4C (H₂O)

	0 s	30 s	60 s	120 s	240 s
[Cl ₂] mg L ⁻¹	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}
0.00	5.29	3.82	5.14	3.83	2.94
0.25	17.91	23.43	3.46	4.10	4.34
0.50	6.05	11.39	1.55	18.36	16.04
1.00	10.66	6.49	6.85	6.58	7.08
1.50	0.48	1.54	2.35	2.95	4.19
2.00	0.81	1.23	1.62	1.74	2.01
2.50	1.28	1.42	1.31	1.39	1.51
3.00	1.25	1.18	1.44	1.40	1.56
3.50	0.93	0.98	0.98	1.06	1.02
4.00	0.97	0.82	0.78	0.86	0.91
4.50	1.81	1.63	1.40	1.68	1.99
5.00	0.66	1.05	1.40	1.30	2.23
%CV_{mean}	4.01	4.58	2.36	3.77	3.82

Table S4.4 Coefficient of variation (%CV) for TMB Absorbance values at 450 nm vs [Cl₂] and time from Figure 4D (Tap water)

	0 s	30 s	60 s	120 s	240 s
[Cl ₂] mg L ⁻¹	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}	%CV _{450nm}
0.00	1.38	1.15	0.39	0.53	0.56
0.25	0.64	0.33	0.90	1.13	0.94
0.50	2.44	2.16	1.60	1.49	2.40
1.00	1.38	0.84	1.11	1.24	1.35
1.50	0.75	0.85	0.75	0.78	0.83
2.00	7.62	1.11	8.14	8.09	8.20
2.50	2.21	0.12	1.56	1.59	1.82
3.00	3.77	2.74	3.56	4.11	4.78
3.50	9.43	3.80	6.20	6.02	7.04
4.00	9.79	3.45	10.71	12.32	15.20
4.50	17.48	12.41	17.16	17.82	3.64
5.00	7.64	3.06	3.02	3.79	3.85
%CV_{mean}	5.38	2.67	4.59	4.91	4.22

Table S4.5 Coefficient of variation (%CV) for TMB Absorbance values at 655 nm vs [Cl₂] and time from Figure 4C (H₂O)

	0 s	30 s	60 s	120 s	240 s
[Cl ₂] mg L ⁻¹	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}
0.00	10.26	16.75	13.96	12.73	3.48
0.25	4.88	10.78	3.21	17.86	16.91
0.50	18.02	18.86	14.49	19.57	20.56
1.00	16.61	15.06	15.16	14.3	13.71
1.50	13.10	14.26	13.57	15.47	14.69
2.00	4.58	4.54	3.68	3.34	3.32
2.50	1.83	1.38	2.18	2.59	2.50
3.00	0.71	1.78	1.90	2.80	2.70
3.50	2.66	2.42	2.42	2.52	2.61
4.00	4.07	5.50	8.04	9.06	7.66
4.50	1.11	1.14	2.25	1.14	1.14
5.00	5.41	9.76	3.92	3.62	2.60
%CV_{mean}	6.94	8.52	7.07	8.75	7.66

Table S4.6 Coefficient of variation (%CV) for TMB Absorbance values at 655 nm vs [Cl₂] and time from Figure 4D (Tap water)

	0 s	30 s	60 s	120 s	240 s
[Cl ₂] mg L ⁻¹	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}	%CV _{655nm}
0.00	2.56	2.64	2.83	4.14	3.56
0.25	6.54	7.70	8.87	8.45	10.79
0.50	1.78	2.73	4.63	5.15	6.84
1.00	1.57	2.16	2.16	2.41	2.96
1.50	2.53	2.28	3.38	3.75	4.35
2.00	1.45	1.29	2.18	2.6	2.54
2.50	11.96	6.26	11.13	10.03	9.09
3.00	8.25	3.92	2.83	0.73	2.91
3.50	9.59	9.11	16.33	13.03	10.94
4.00	29.21	3.20	2.34	2.14	2.63
4.50	2.92	13.00	12.7	10.2	7.62
5.00	17.93	16.88	15.95	17.11	16.11
%CV_{mean}	8.02	5.93	7.11	6.65	6.70