Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Catechol Autoxidation: Considerations in the Design of Wet Adhesive Materials

Greg P. Maier, Christopher M. Bernt, and Alison Butler*

Department of Chemistry and Biochemistry, University of California Santa Barbara, California, USA.

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1. Synthesis, Purification, and Characterization of 5-Sulfo-2,3-DHBA

5-Sulfo-2,3-DHBA was synthesized based on previously published procedures [1], purified using prep-scale reverse phase HPLC, and characterized using ESI mass spectrometry and NMR.

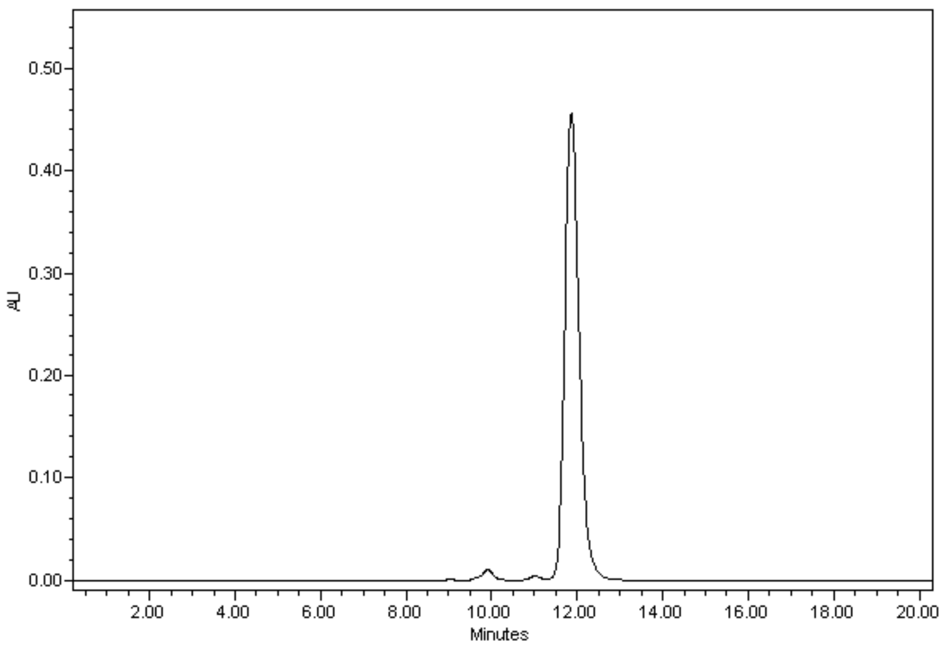


Figure S1. Reverse phase HPLC purification for 5-Sulfo-2,3-DHBA.

The eluent was monitored at 215 nm.

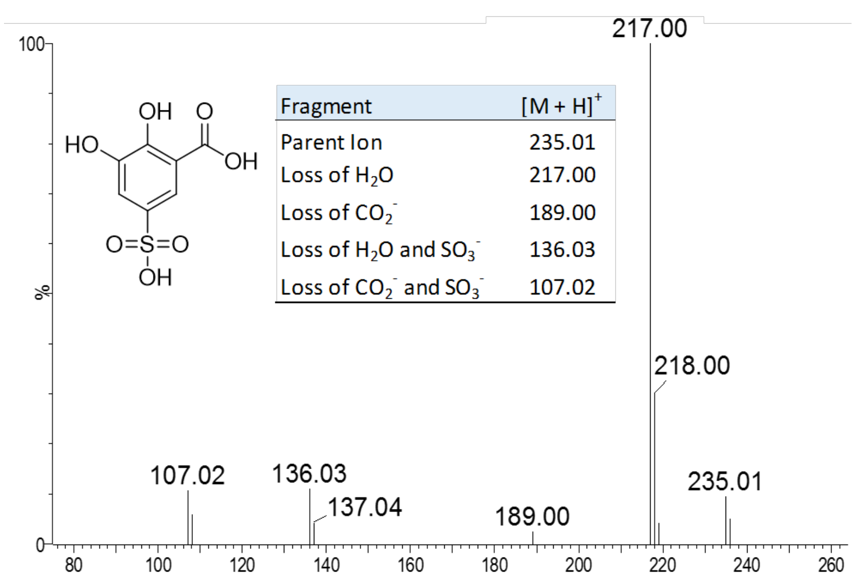
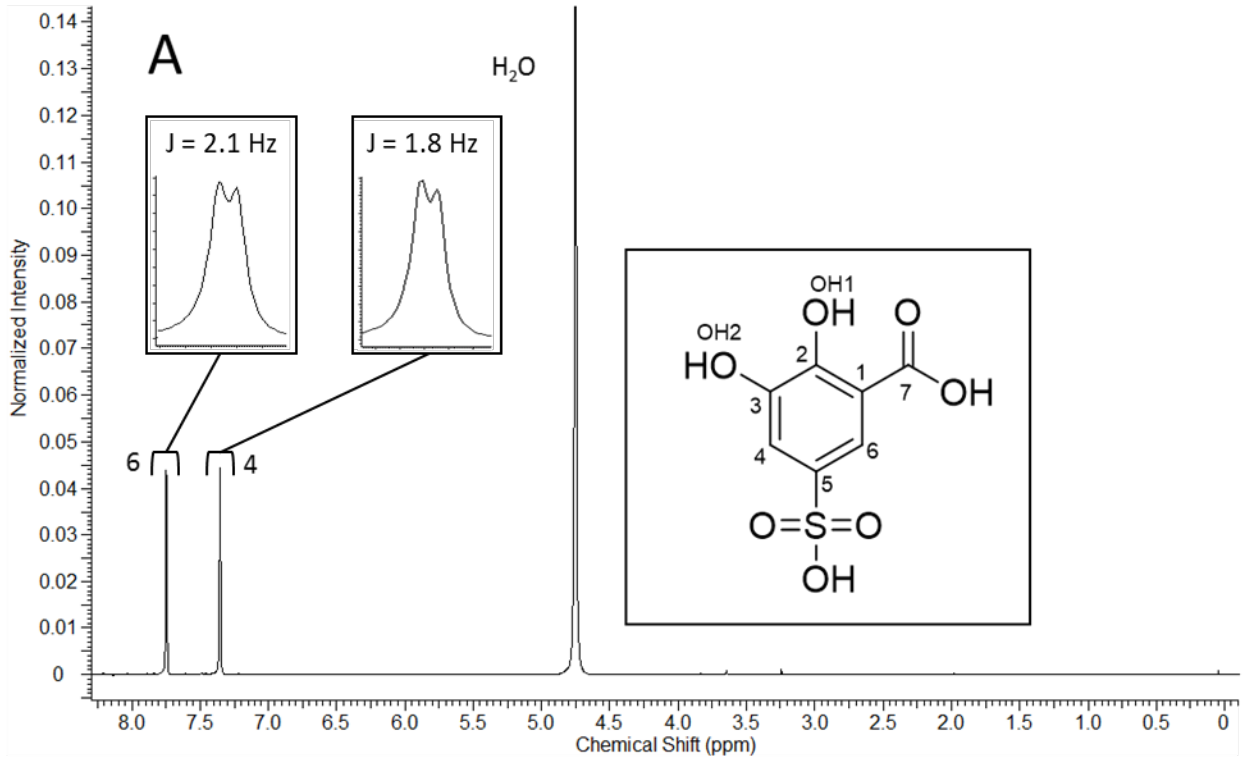


Figure S2. ESI Mass Spectrometry of 5-Sulfo-2,3-DHBA.

Data was collected on a XEVO G2-XS TOF Mass Spectrometer and the parent ion and fragmentations are listed.



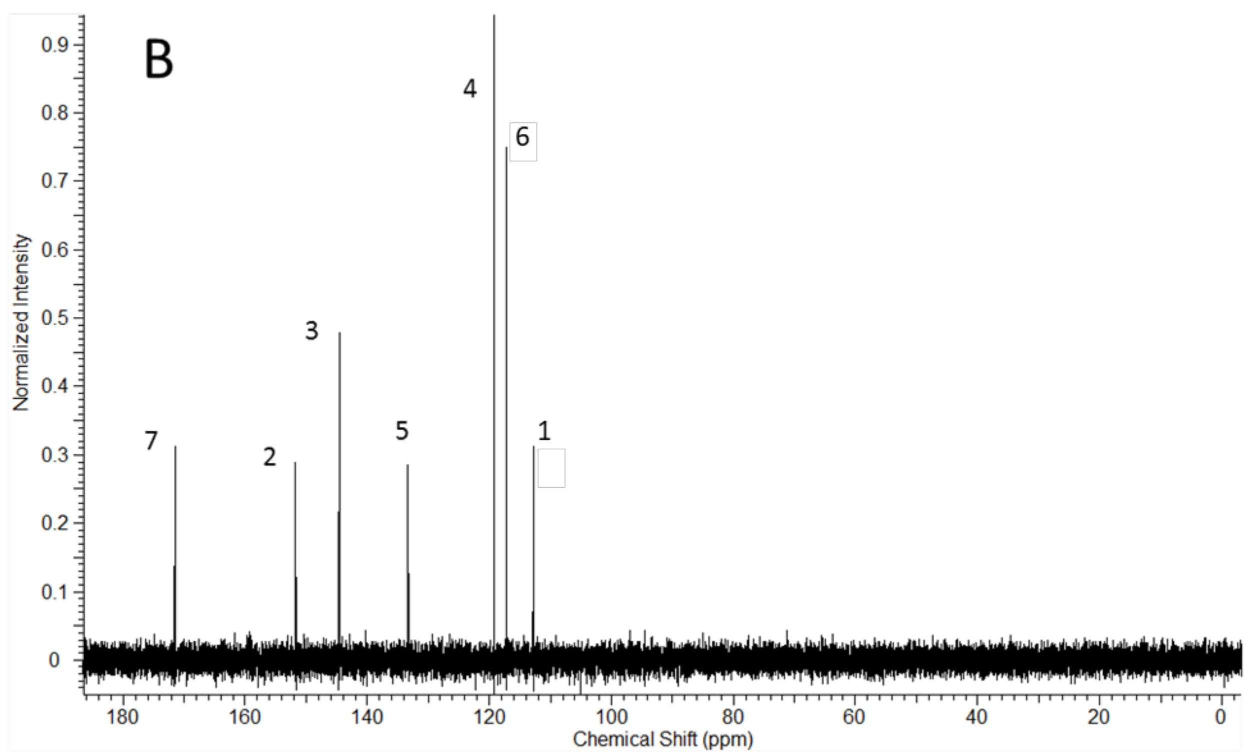


Figure S3. NMR Data to Establish Purity of 5-Sulfo-2,3-DHBA

(A) ^{1}H NMR Data for 5-sulfo-2,3-DHBA. NMR (Varian Unity Inova 600 MHz Spectrometer) in D_2O with enlarged aromatic region. (B) ^{13}C NMR Data for 5-sulfo-2,3-DHBA. NMR (Varian Unity Inova 600 MHz Spectrometer) in D_2O.

2. Oxidation of Catechol by O_2

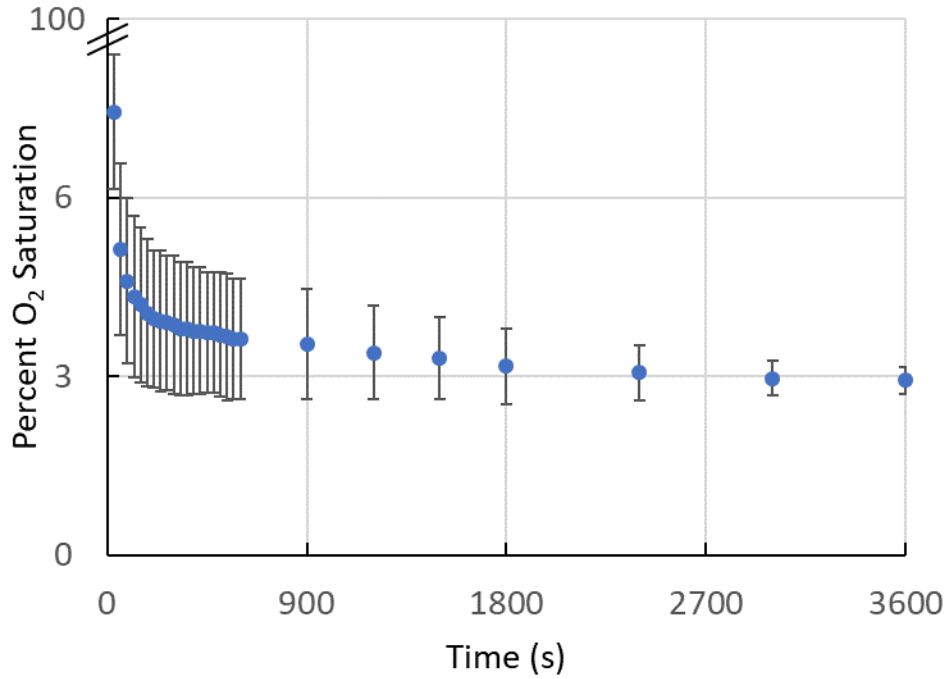


Figure S4. Minimum Percent Oxygen Saturation Measurement for Clark-type Electrode

The minimum percent O_2 saturation for the Clark-type oxygen electrode was determined by removing all dissolved oxygen from the system. Dissolved O_2 was removed via the oxidation of 16.6 mM catechol at pH 12.0 in 50 mM phosphate buffer at 29.5°C. The high pH and high concentration of catechol were chosen to promote fast oxidation and ensure complete removal of dissolved O_2. The baseline reading was determined to be 2.9% ± 0.2% O_2 saturation after all O_2 was removed from the reaction solution. All O_2 saturation measurements were adjusted based on the baseline reading of 2.9% according to the following formula: Corrected % O_2 Saturation = 100 – (% O_2 Consumed * 1.0298).

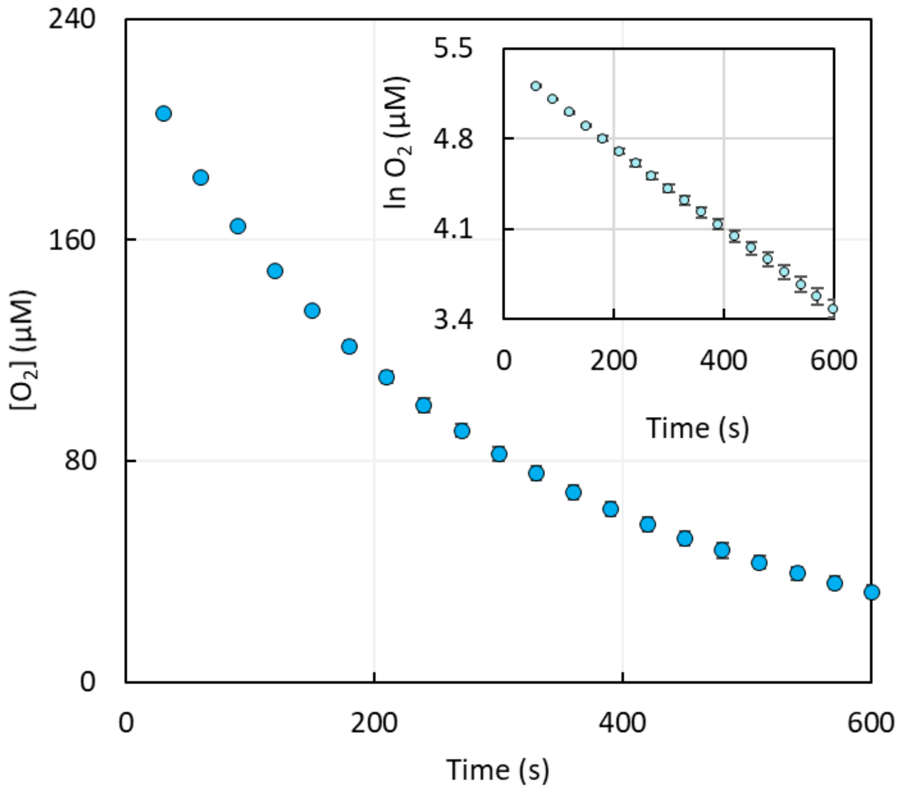


Figure S5. Consumption of Dioxygen During the Autoxidation of 6.64 mM Catechol at pH 9.0

The oxidation of 6.64 mM catechol by O_2 was carried out in triplicate under pseudo first order conditions in 50 mM CAPSO buffer pH 9.0 at 29.5°C with catechol in excess (6.64 mM) and an initial O_2 concentration of 223 μM. A Clark-type oxygen electrode was used to monitor the consumption of dissolved O_2. The initial data point was not included to account for mixing upon the addition of catechol. The inset shows the plot of ln(O_2) versus time. The pseudo 1st order rate constant was determined from the initial portion of the reaction from the [O_2] versus time plot using Dynafit software (version 4.04.087, Biokin Ltd.).

3. Oxidation of Catechol by O_2: Order with Respect to Catechol

The kinetics of oxidation of catechol by O_2, under pseudo first-order conditions, with catechol in excess of O_2, was monitored by a Clark-type oxygen electrode in buffered aqueous solution as a function of [Catechol] from 1.66 x 10^{-2} M to 4.98 x 10^{-2} M (Figure S6). A plot of ln[initial rate] from the slopes of Figure S7 versus ln[Catechol] is linear with a slope of 1.00 ± 0.06, indicating that the rate of catechol autoxidation is likely first-order in catechol. A greater range in concentration is needed to confirm that catechol autoxidation is first-order in O_2.

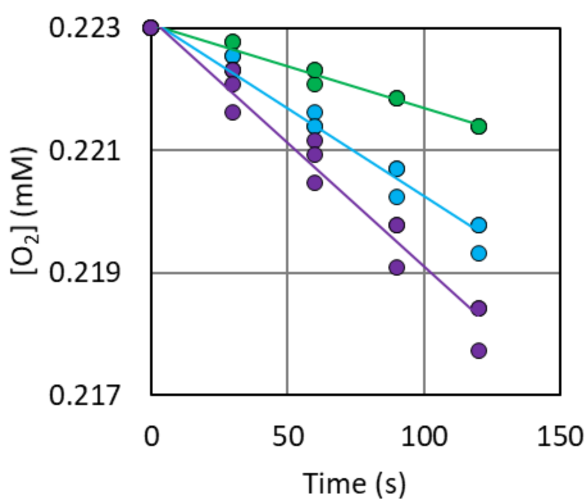


Figure S6. Dependence of Rate of Catechol Oxidation on Catechol Concentration

Reactions were carried out under pseudo first-order conditions in 50 mM phosphate buffer pH 7.0, at 29.5°C with variable catechol concentration, which is in excess of $[O_2]$. Each reaction at a defined catechol concentration was carried out in triplicate. The initial catechol concentrations were 16.6 mM, 33.2 mM, and 49.8 mM, as listed from top to bottom. The best fit lines were determined over the first 120 seconds of reaction (i.e., the first five data points), which corresponds to less than 10% consumption of $[O_2]$.

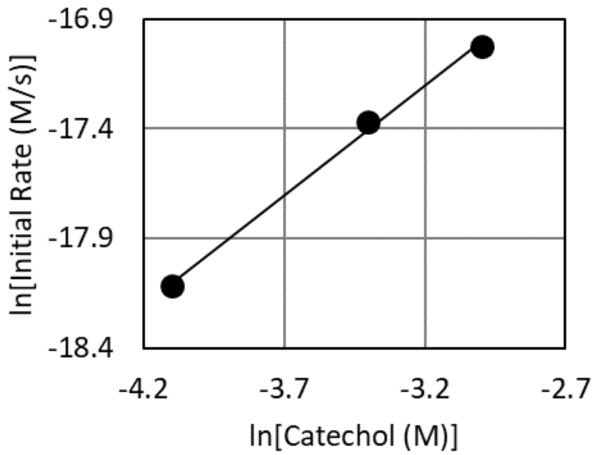


Figure S7. Catechol Autoxidation: Order with Respect to Catechol

The plot of ln(initial rate) from the slopes of Figure S17 versus ln[catechol], is linear with a slope of 1.00 ± 0.06 and an $R^2 = 0.996$, indicating that the rate of catechol autoxidation is first-order in O_2.

4. Oxidation of Catechol by O_2: Order with Respect to O_2

The kinetics of oxidation of catechol by O_2, under pseudo first-order conditions, with catechol in excess of O_2, was monitored by a Clark-type oxygen electrode in buffered aqueous solution as a function of $[O_2]$ from 1.76×10^{-4} M to 1.06×10^{-3} M (Figure S8). Dioxygen concentrations were controlled by bubbling the buffered reaction solution with varying ratios of O_2 gas and N gas. A plot of ln(initial rate) from the slopes of Figure S9 versus $\ln([O_2])$ is linear with a slope of 0.96 ± 0.02, indicating that the rate of catechol autoxidation is first-order in O_2 (Figure S8).

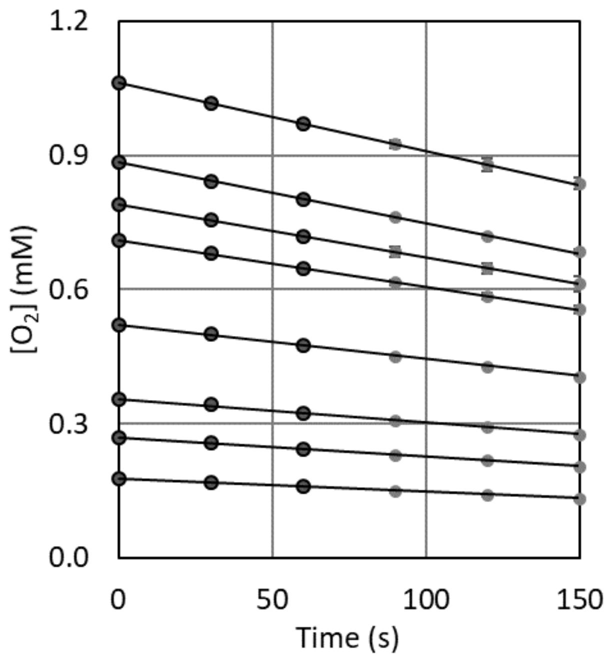


Figure S8. Dependence of Rate of Catechol Oxidation on O_2 Concentration.

Reactions were carried out under pseudo first-order conditions in 50 mM phosphate buffer pH 8.5, at 29.5°C with 13.3 mM catechol, which is in excess of $[O_2]$. Each reaction at a defined initial O_2 concentration was carried out in triplicate. The initial O_2 concentrations were 1.06 mM, 0.883 mM, 0.790 mM, 0.710 mM, 0.521 mM, 0.356 mM, 0.269 mM, and 0.176 mM, as listed from top to bottom. The best fit lines were determined over the first 60 seconds of reaction (i.e., the first three data points of each kinetic run), which corresponds to 10% consumption of $[O_2]$.

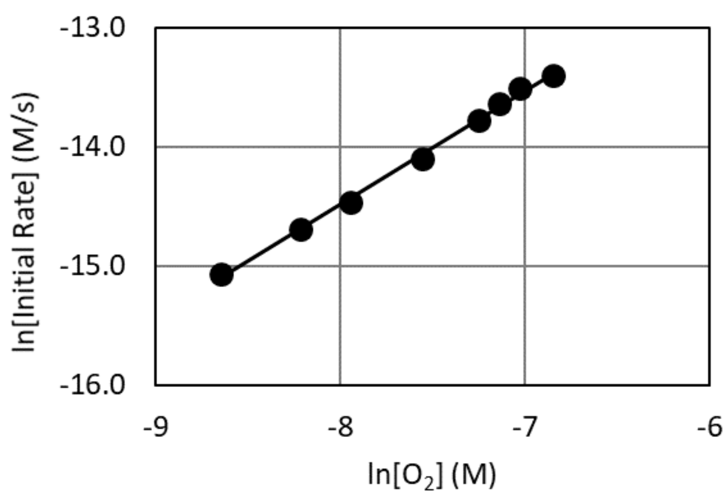


Figure S9. Catechol Autoxidation: Order with Respect to O_2.

The plot of ln(initial rate) from the slopes of Figure S15 versus $\ln([O_2])$, is linear with a slope of 0.96 ± 0.02, indicating that the rate of catechol autoxidation is first-order in O_2.

5. Concentration and pH-Dependent Oxidation of Catechol and Substituted Catechols by O_2

Table S1. Table of Kinetic Data

Compound	pH	Conc. (mM)	Pseudo 1st Order k_{obs} (sec^{-1})	Standard Error	R^2	2nd Order k_{obs} ($M^{-1}s^{-1}$)	Average 2nd Order k_{obs} ($M^{-1}s^{-1}$)	Standard Deviation
4-Methylcatechol (p-Me)	6.0	13.3	8.79E-05	7.6E-07	0.955	6.61E-03	5.54E-03	9.51E-04
		26.6	1.39E-04	8.8E-07	0.984	5.23E-03		
		39.9	1.91E-04	1.9E-06	0.986	4.79E-03		
	7.0	13.3	7.06E-04	6.0E-06	0.996	5.31E-02	4.78E-02	4.75E-03
		19.9	9.24E-04	7.6E-06	0.991	4.64E-02		
		26.6	1.17E-03	1.0E-05	0.995	4.39E-02		
	7.5	6.64	2.74E-04	2.4E-06	0.965	4.13E-02	8.27E-02	3.59E-02
		9.96	1.03E-03	6.3E-06	0.996	1.03E-01		
		13.3	1.38E-03	9.2E-06	0.996	1.04E-01		
	8.0	3.32	7.61E-04	3.7E-06	0.992	2.29E-01	2.52E-01	2.54E-02
		6.64	1.65E-03	6.6E-06	0.997	2.48E-01		
		13.3	3.72E-03	3.0E-05	0.995	2.80E-01		
	9.0	3.32	1.68E-02	1.4E-04	0.997	5.05E+00	5.05E+00	
4-Ethylcatechol (p-Et)	6.5	16.6	1.52E-04	1.1E-06	0.966	9.16E-03	7.52E-03	1.52E-03
		33.2	2.41E-04	4.4E-06	0.980	7.26E-03		
		49.8	3.06E-04	1.9E-06	0.967	6.14E-03		
	7.0	16.6	3.65E-04	2.0E-06	0.988	2.20E-02	1.79E-02	3.89E-03
		33.2	5.75E-04	3.0E-06	0.988	1.73E-02		
		49.8	7.10E-04	3.6E-06	0.984	1.43E-02		
	7.5	9.96	5.59E-04	2.3E-06	0.993	5.61E-02	4.99E-02	8.42E-03
		16.6	8.83E-04	3.7E-06	0.996	5.32E-02		
		33.2	1.34E-03	4.5E-06	0.985	4.03E-02		
	8.0	3.32	4.24E-04	1.8E-06	0.986	1.28E-01	1.10E-01	1.56E-02
		9.96	1.05E-03	2.3E-06	0.999	1.06E-01		
		16.6	1.62E-03	8.2E-06	0.998	9.75E-02		
	9.0	3.32	8.35E-03	5.3E-05	0.997	2.52E+00	2.52E+00	
Catechol (p-H)	7.0	16.6	6.05E-05	5.50E-07	0.975	3.64E-03	3.96E-03	2.83E-04
		33.2	1.39E-04	1.60E-06	0.965	4.19E-03		
		49.8	2.02E-04	1.70E-06	0.963	4.06E-03		
	7.5	9.96	1.69E-04	1.60E-06	0.940	1.69E-02	1.49E-02	2.00E-03
		23.3	3.44E-04	2.50E-06	0.986	1.48E-02		
		33.2	4.30E-04	3.50E-06	0.995	1.29E-02		
	8.0	6.64	4.44E-04	1.70E-06	0.998	6.68E-02	6.59E-02	1.99E-03
		13.3	8.96E-04	5.20E-06	0.993	6.73E-02		
		19.9	1.27E-03	5.30E-06	0.998	6.37E-02		
	8.5	3.32	7.34E-04	5.80E-06	0.985	2.21E-01	2.17E-01	4.32E-03
		6.64	1.44E-03	1.60E-05	0.963	2.16E-01		
		9.96	2.12E-03	1.30E-05	0.987	2.13E-01		
	9.0	3.32	2.11E-03	5.30E-06	0.983	6.36E-01	5.79E-01	6.71E-02
		3.32	2.12E-03	5.30E-06	0.994	6.39E-01		
		4.98	2.63E-03	5.20E-06	0.999	5.28E-01		
		6.64	3.42E-03	1.40E-05	0.996	5.15E-01		

Table S1 Continued. Table of Kinetic Data, Continued

Compound	pH	Conc. (mM)	Pseudo 1st Order k_{obs} (sec^{-1})	Standard Error	R^2	2nd Order k_{obs} ($M^{-1}s^{-1}$)	Average 2nd Order k_{obs} ($M^{-1}s^{-1}$)	Standard Deviation
4-Chlorocatechol	7.0	16.6	3.13E-04	8.5E-07	0.997	1.89E-02	1.78E-02	1.10E-03
(p-Cl)		23.3	3.89E-04	1.7E-06	0.978	1.67E-02		
		33.2	5.95E-04	5.3E-06	0.986	1.79E-02		
	7.5	9.96	4.80E-04	3.6E-06	0.968	4.82E-02	4.70E-02	1.63E-03
		16.6	7.92E-04	4.6E-06	0.995	4.77E-02		
		23.3	1.05E-03	5.0E-06	0.999	4.51E-02		
	8.0	9.96	1.00E-03	2.6E-06	0.999	1.00E-01	9.49E-02	5.40E-03
		16.6	1.57E-03	6.4E-06	0.997	9.46E-02		
		23.3	2.09E-03	1.2E-05	0.996	8.96E-02		
	8.5	3.32	9.57E-04	4.4E-06	0.995	2.88E-01	2.91E-01	5.18E-03
		6.64	1.91E-03	5.5E-06	0.999	2.88E-01		
		9.96	2.96E-03	8.3E-06	0.999	2.97E-01		
	9.0	3.32	3.01E-03	1.1E-04	0.976	9.07E-01	9.07E-01	
3,4-DHBA	9.0	3.32	9.67E-05	3.8E-07	0.995	2.91E-02	2.94E-02	3.59E-03
(p-COOH)		4.98	1.29E-04	7.7E-07	0.930	2.60E-02		
		6.64	2.20E-04	3.2E-06	0.925	3.31E-02		
	9.5	3.32	6.19E-04	1.9E-06	0.995	1.86E-01	1.54E-01	3.11E-02
		4.98	7.48E-04	5.7E-06	0.971	1.50E-01		
		6.64	8.26E-04	2.7E-06	0.995	1.24E-01		
	10.0	3.32	5.84E-04	1.7E-05	0.996	1.76E-01	1.55E-01	1.96E-02
		4.98	6.85E-04	2.3E-06	0.989	1.38E-01		
		6.64	9.97E-04	1.6E-05	0.939	1.50E-01		
	10.5	3.32	2.30E-03	3.4E-05	0.946	6.93E-01	6.20E-01	8.07E-02
		4.98	3.15E-03	3.2E-05	0.994	6.33E-01		
		6.64	3.54E-03	4.1E-05	0.995	5.33E-01		
2,3-DHBA	10.0	6.64	7.64E-05	1.5E-06	0.915	1.15E-02	1.14E-02	3.32E-04
		9.96	1.17E-04	1.2E-06	0.966	1.17E-02		
		13.3	1.48E-04	2.9E-06	0.935	1.11E-02		
	11.0	3.32	3.00E-04	5.5E-06	0.912	9.04E-02	8.68E-02	7.00E-03
		4.98	4.55E-04	7.3E-06	0.909	9.14E-02		
		6.64	5.23E-04	6.7E-06	0.952	7.88E-02		
	11.5	3.32	3.29E-03	3.0E-05	0.989	9.91E-01	7.76E-01	1.88E-01
		4.98	3.47E-03	3.0E-05	0.967	6.97E-01		
		6.64	4.26E-03	6.0E-05	0.928	6.42E-01		
	12.0	3.32	5.10E-03	9.0E-05	0.977	1.54E+00	1.54E+00	2.31E-02
		4.15	6.51E-03	9.5E-05	0.974	1.57E+00		
		4.98	7.59E-03	1.1E-04	0.991	1.52E+00		
5-Sulfo-2,3-DHBA	12.0	3.32	7.76E-05	4.9E-06	0.996	2.34E-02	1.18E-02	1.02E-02
		13.3	9.79E-05	3.2E-06	0.999	7.36E-03		
		26.6	1.21E-04	3.5E-06	0.999	4.55E-03		

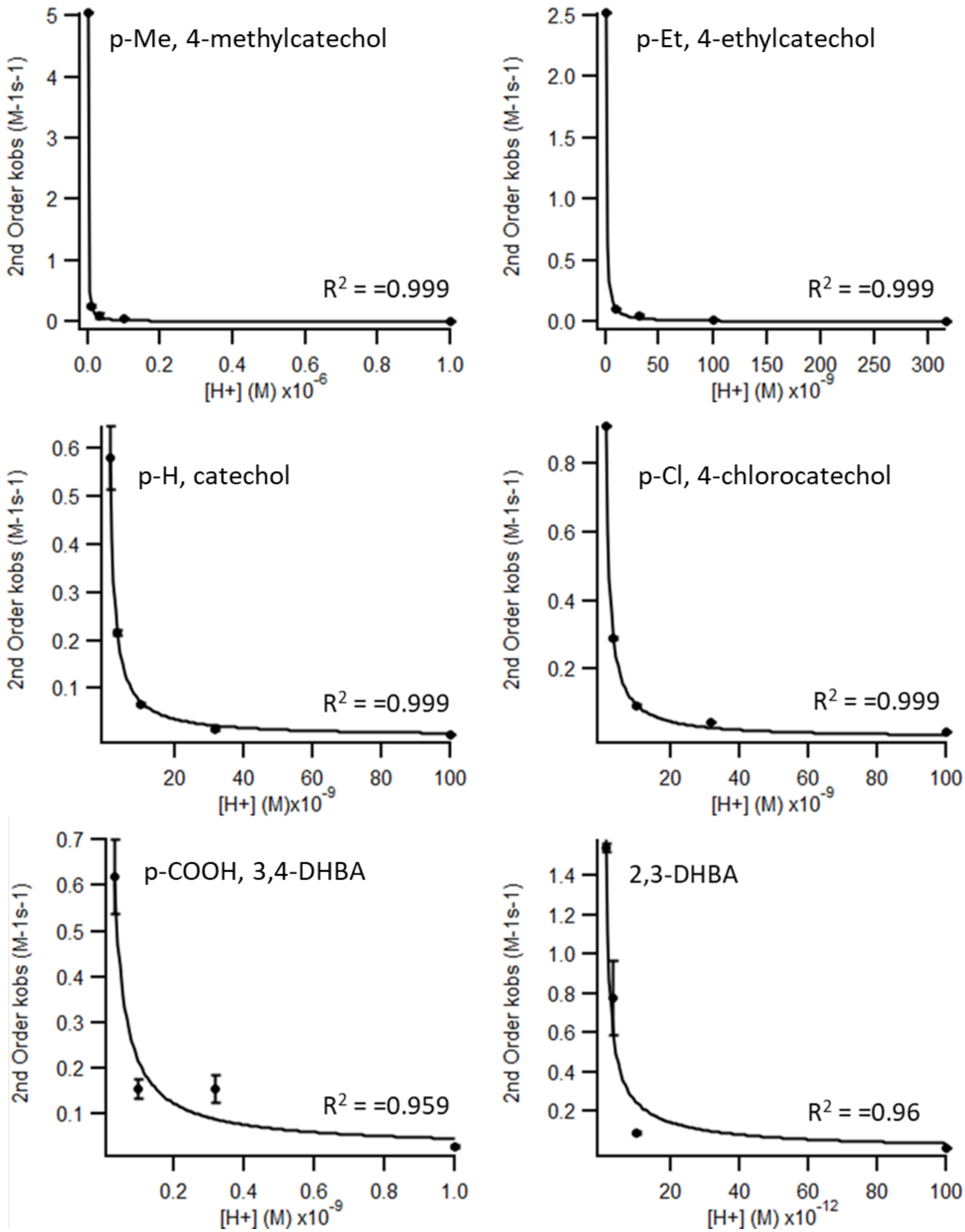


Figure S10. Plots of the Observed 2nd Order Rate Constants versus [H^+] for the Oxidation of Catechol and Substituted Catechol by O_2

Igor Pro 7 (Wavemetrics Inc.) was used to fit Eqn. (1) to the plot of observed second-order rate constant for the oxidation of catechol or substituted catechol by O_2 versus [H^+] using non-linear least squares analysis. This analysis provides the fit pK_{a1}, fit pK_{a2}, k_1, and k_2 values in Table 1.

6. Potentiometric Titration of 4-Ethylcatechol

Potentiometric titrations were performed to determine the pK_{a1} and pK_{a2} of 4-ethylcatechol using a Hanna Instruments HI2002-01 pH meter. The electrode was calibrated as previously described [2-3]. Titrations were carried out in jacketed three-necked titration vessels at 25.0 ± 0.1 °C. Solutions were degassed with Ascarite-scrubbed argon and kept under a positive pressure of argon to prevent autoxidation. Standardized NaOH (0.0982 M) was incrementally added to a solution of 10 mM 4-ethylcatechol in 0.1 M NaCl background electrolyte. Acid dissociation constants were determined from the nonlinear refinement of the potentiometric titration data using Hyperquad2008 software (Figure S11) [4]. The pK_{a1} and pK_{a2} of 4-ethylcatechol are listed in Table 1.

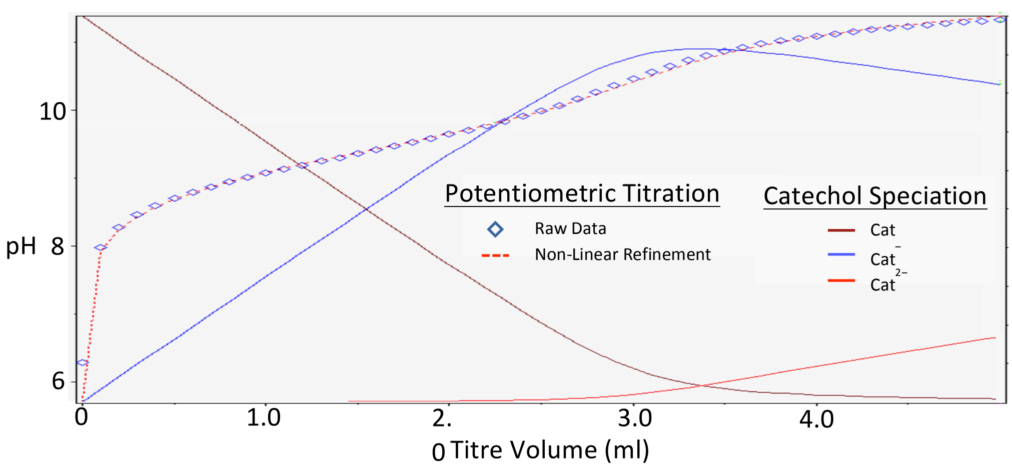


Figure S11. Potentiometric Titration and Speciation Diagram for 4-Ethylcatechol.

The dashed red line corresponds to the Hyperquad2008 calculated non-linear fit. The relative concentrations of Cat, Cat^{-}, and Cat^{2-} were calculated by Hyperquad2008. The potentiometric titration of 4-ethylcatechol was done in triplicate. The average and standard deviation for the three experiments are shown in Table 1.

7. References

1. Sundeen, J.; Zahler, R.; Jendrzejewski, S. Monobactam Hydrazides Containing Catechol Sulfonic Acid Groups. J. Sundeen, R. Zahler, S. Jendrzejewski. US Pat., 5 030 724A, 1991.
2. Zane, H. K.; Butler, A., *J Nat Prod* **2013,** *76* (4), 648-54.
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4. Gans, P.; Sabatini, A.; Vacca, A., *Talanta* **1996,** *43*, 1739-1753.