

## **Transformation of Tetracycline Antibiotics and Fe(II)/(III) Species Induced by Their Complexation**

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Journal: *Environmental Science & Technology*

Date Prepared: November 27, 2015

Text: Notation and S1-S5

Figures: S1-S13

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**Notation for TTC and Fe(II) species:**

TTC species (+1 charge)	H <sub>3</sub> L
Parent TTC (0 charge)	H <sub>2</sub> L
TTC species (-1 charge)	HL
TTC species (-2 charge)	L
Total TTC concentration (including different TTC species)	TTC <sub>T</sub>
Uncomplexed TTC (including different uncomplexed TTC species)	TTC
Uncomplexed TTC concentration (including different uncomplexed TTC species)	[TTC]
Total Fe(II) (including complexed with organic and not complexed)	Fe(II) <sub>T</sub>
Uncomplexed Fe(II)	Fe(II)
Uncomplexed Fe(II) concentration	[Fe(II)]
Total Fe(II) and TTC complexes	Fe(II)-TTC
Fe(II)-H <sub>2</sub> L complex	Fe <sup>II</sup> -H <sub>2</sub> L
Fe(II)-HL complex	Fe <sup>II</sup> -HL

**Text S1. Chemicals and Reagents**

Ammonium iron(II) sulfate hexahydrate (Fe(NH<sub>4</sub>SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O) was obtained from Acros at 99% purity. TTC, OTC, CTC, ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid, monosodium salt hydrate), *tert*-butanol (TBA), catalase (lyophilized powder, 2,000-5,000 units/mg protein, from bovine liver) and superoxide dismutase (SOD, lyophilized powder, ≥3,000 units/mg protein, from bovine erythrocytes) were obtained from Sigma at 90-99% purity. Humic acid (HA) was obtained from MP Biomedicals (Santa Ana, CA, US). All other reagents used (e.g., buffers, acids, etc.) were obtained from Fisher Scientific (Hampton, NH, US), Acros (Geel, BEL) or Aldrich (St. Louis, MO, US) at analytical grade. All chemicals were used directly without further purification. Reagent water

was produced from a Millipore Milli-Q Ultrapure Gradient A10 purification system (Millipore, Billerica, MA, US).

TC stock solutions were prepared in reagent water at 1.6 mM, protected from light, stored at 5°C and used within 2-3 days. The 4.0 mM Fe(II) stock solutions were prepared by dissolving  $\text{Fe}(\text{NH}_4\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  in 0.2 M HCl,<sup>1</sup> stored at 5°C and used within one month. Fe(III) stock solution was made by dissolving 2.0 mM  $\text{FeCl}_3$  at pH 2.3 (adjusted by HCl) and stored at 4°C. The desired solution pH was maintained using 10 mM buffer of 2-(*N*-morpholino)ethanesulfonic acid (MES) for pH 5.5-6.5, and 4-morpholinepropanesulfonic acid (MOPS) for pH 6.5-7.5.

### **Text S2. Fe(II) determination by ferrozine (FZ)**

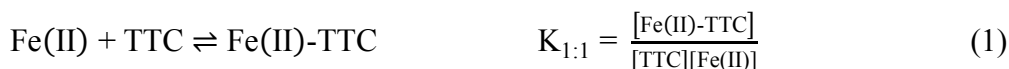
To measure Fe(II) concentration, typically 0.5 mL of sample was added into a cuvette (1.0 cm path length) that contained 0.4 mL of ferrozine (FZ) solution. Three concentrations of FZ solution (1, 10 and 100 mM) were tested to determine the sufficient FZ concentration for this study. It was found that when FZ concentration was increased from 1 to 100 mM for various samples in this study, the absorbance of the detected Fe(II) (including experiments conducted in the presence of TCs and HA) did not change. Thus, 1 mM FZ was considered sufficient for Fe(II) determination in this study. Moreover, it was found that at FZ concentration of 1 mM, all samples' initial absorbance was nearly identical regardless of varying concentrations of Fe(II), TC and/or HA, which also verified that 1 mM FZ was sufficient for Fe(II) detection in this study.

### **Text S3. Determination of the complexation constants of Fe(II)-TTC Complexes**

Experiments to study the Fe(II)-TC complexation were conducted in anoxic conditions to minimize Fe(II)'s oxidation during the measurement. Before the complexation reaction, all the stock solutions (including the Fe(II) stock, TTC stock and buffers) were purged by high purity nitrogen for 1 hour to drive the dissolved oxygen out. The complexation experiments were conducted in 10-mL centrifuge tubes with cover. The experiments were started by adding a fixed amount of Fe(II) stock to the buffers (pH 6.0, 6.5 and 7.0) containing different concentrations of TTC (varying from 10 to 160  $\mu\text{M}$ ). Nitrogen was purged throughout the

complexation reaction (<30 s). Then, the centrifuge tubes were covered until measurement. Shortly after, aliquots were taken from the covered centrifuge tubes and transferred to cuvettes to measure UV absorbance within 30 seconds. Thus, the entire process was completed in less than 1 min.

For 1:1 Fe(II)-TTC complex formation (eq 1), based on the Benesi-Hildebrand equation (eqn 1),<sup>2,3</sup> there would be an relationship between [TTC] and  $\Delta(\text{Absorbance})$  (eq 2):

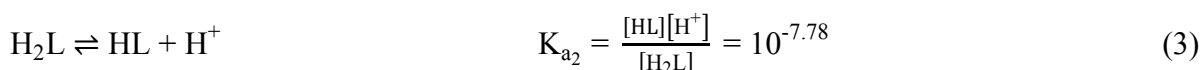


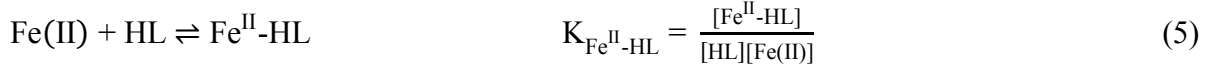
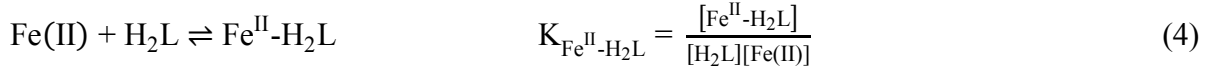
where [Fe(II)] and [TTC] are the concentrations of uncomplexed Fe(II) and TTC, respectively, and  $K_{1:1}$  is the 1:1 complexation constant.

$$\frac{1}{\Delta(\text{Absorbance})} = \frac{1}{\text{Fe(II)}_T K_{1:1} \Delta\epsilon_1} \times \frac{1}{[\text{TTC}]} + \frac{1}{\text{Fe(II)}_T \Delta\epsilon_1} \quad (2)$$

where  $\Delta(\text{Absorbance})$  was the difference in the UV absorbance of the Fe(II)-TTC complex in comparison to the UV absorbance of the uncomplexed Fe(II) plus the UV absorbance of uncomplexed TTC;  $\text{Fe(II)}_T$  was the total Fe(II) concentration; and  $\Delta\epsilon_1 = \epsilon_1 - \epsilon_{\text{Fe(II)}} - \epsilon_{\text{TTC}}$  (the molar absorptivity of Fe(II)-TTC complex minus the absorptivities of uncomplexed Fe(II) and uncomplexed TTC). By plotting  $1/[\text{TTC}]$  versus  $1/\Delta(\text{Absorbance})$ , the complexation constant  $K_{1:1}$  could be calculated from dividing the intercept by the slope. Complexometric titration experiments were conducted at a fixed  $\text{Fe(II)}_T$  (40  $\mu\text{M}$ ) with varying [TTC] (30 - 160  $\mu\text{M}$ ) (Assuming  $[\text{TTC}] \approx [\text{TTC}]_0$ , because  $[\text{Fe(II)-TTC}] \ll [\text{TTC}]$ ). As shown in Figure S5 as an example, the  $K_{1:1}$  value was determined to be 2713.5 ( $\pm 316.1$ ) for pH 6.0, 3856.0 ( $\pm 352.0$ ) for pH 6.5 and 4974.4 ( $\pm 448.9$ ) for pH 7.0.

The different values of  $K_{1:1}$  at different pHs indicated that the speciation of TTC needed to be taken into account in the complexation reaction. As shown in Figure S6, TTC has four possible species:  $\text{H}_3\text{L}$ ,  $\text{H}_2\text{L}$ ,  $\text{HL}$  and  $\text{L}$  (corresponding to three acid dissociation constants).<sup>4</sup> The different  $K_{1:1}$  values at different pH were due to involvement of different TTC species in the complexation. In the moderate pH range (5.5-7.5) examined in this study,  $\text{H}_2\text{L}$  and  $\text{HL}$  species were dominant (eq 3). Thus, the Fe(II)-TTC complex formation could exist as  $\text{Fe}^{\text{II}}\text{-H}_2\text{L}$  and  $\text{Fe}^{\text{II}}\text{-HL}$  accordingly (eqs 4 and 5):





Therefore,  $K_{1:1}$  represented the overall complexation formation constant for both  $\text{Fe}^{\text{II}}\text{-H}_2\text{L}$  and  $\text{Fe}^{\text{II}}\text{-HL}$  species (eq 6):

$$K_{1:1} = \frac{[\text{Fe(II)}\text{-TTC}]}{[\text{TTC}][\text{Fe(II)}]} = \frac{[\text{Fe}^{\text{II}}\text{-H}_2\text{L}] + [\text{Fe}^{\text{II}}\text{-HL}]}{([\text{H}_2\text{L}] + [\text{HL}])[\text{Fe(II)}]} \quad (6)$$

Based on eqs 3-5, eq 6 could be rewritten as eq 7:

$$K_{1:1} = \frac{[\text{Fe}^{\text{II}}\text{-H}_2\text{L}] + [\text{Fe}^{\text{II}}\text{-HL}]}{([\text{H}_2\text{L}] + [\text{HL}])[\text{Fe(II)}]} = \frac{K_{\text{Fe}^{\text{II}}\text{-H}_2\text{L}}[\text{Fe(II)}] + \frac{K_{\text{Fe}^{\text{II}}\text{-HL}}[\text{Fe(II)}]K_{a2}}{[\text{H}^+]}}{\left(1 + \frac{K_{a2}}{[\text{H}^+]}\right)[\text{Fe(II)}]} = \frac{K_{\text{Fe}^{\text{II}}\text{-H}_2\text{L}}[\text{H}^+] + K_{\text{Fe}^{\text{II}}\text{-HL}} \cdot K_{a2}}{[\text{H}^+] + K_{a2}} \quad (7)$$

Based on the measured  $K_{1:1}$  values at pH 6.0, 6.5 and 7.0, the eq 7 was solved for  $K_{\text{Fe}^{\text{II}}\text{-H}_2\text{L}}$  and  $K_{\text{Fe}^{\text{II}}\text{-HL}}$  using Matlab to be  $2.39 \times 10^3$  and  $2.05 \times 10^4$ , respectively.

Furthermore, the obtained complexation constants of  $\text{Fe}^{\text{II}}\text{-H}_2\text{L}$  and  $\text{Fe}^{\text{II}}\text{-HL}$  were used to calculate the speciation of  $\text{Fe(II)}$  (40  $\mu\text{M}$ ) in the presence of different amounts of TTC at pH 6.5 and 7.0, respectively, on the basis of the mass balances of total  $\text{Fe(II)}$  and total TTC (eqs 8 and 9).

$$\begin{aligned} \text{Fe(II)}_T &= [\text{Fe(II)}] + [\text{Fe}^{\text{II}}\text{-H}_2\text{L}] + [\text{Fe}^{\text{II}}\text{-HL}] \\ &= [\text{Fe(II)}] \left( 1 + \frac{K_{\text{Fe}^{\text{II}}\text{-H}_2\text{L}}[\text{H}^+][\text{HL}] + K_{\text{Fe}^{\text{II}}\text{-HL}}[\text{HL}]}{K_{a2}} \right) \end{aligned} \quad (8)$$

$$\begin{aligned} \text{TTC}_T &= [\text{H}_2\text{L}] + [\text{HL}] + [\text{Fe}^{\text{II}}\text{-H}_2\text{L}] + [\text{Fe}^{\text{II}}\text{-HL}] \\ &= [\text{HL}] \left( \frac{[\text{H}^+]}{K_{a2}} + 1 + \left( \frac{K_{\text{Fe}^{\text{II}}\text{-H}_2\text{L}}{K_{a2}}[\text{H}^+] + K_{\text{Fe}^{\text{II}}\text{-HL}} \right) \cdot \left( \frac{\text{Fe(II)}_T}{1 + \frac{K_{\text{Fe}^{\text{II}}\text{-H}_2\text{L}}{K_{a2}}[\text{H}^+][\text{HL}] + K_{\text{Fe}^{\text{II}}\text{-HL}}[\text{HL}]} \right) \right) \end{aligned} \quad (9)$$

As shown in Figure S7, at pH 6.5 for example, the percentage of  $\text{Fe(II)}$  complexed with TTC increased from 11% to 38% when  $\text{TTC}_T$  increased from 40  $\mu\text{M}$  to 200  $\mu\text{M}$ .

#### Text S4. Determination of Oxidation Rate Constant of $\text{Fe(II)}$ -TTC Complex

As described in the main text, the autoxidation rate constant of  $\text{Fe(II)}$  in the presence of both  $\text{Fe(II)}$  and TTC could be expressed as:

$$k_{obs}(\text{Fe(II)}_T) = k_i[\text{Fe(II)}] + k_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}}[\text{Fe}^{\text{II}}-\text{H}_2\text{L}] + k_{\text{Fe}^{\text{II}}-\text{HL}}[\text{Fe}^{\text{II}}-\text{HL}] \quad (10)$$

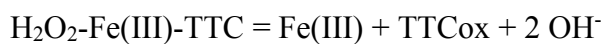
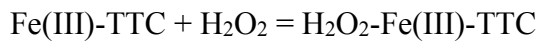
By combining with eqs 3-5 and the mass balance of total Fe(II) (eq 8), eq 10 could be expressed as eq 11.

$$\begin{aligned} k_{obs} &= \frac{k_i[\text{Fe(II)}] + k_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}}[\text{Fe}^{\text{II}}-\text{H}_2\text{L}] + k_{\text{Fe}^{\text{II}}-\text{HL}}[\text{Fe}^{\text{II}}-\text{HL}]}{[\text{Fe(II)}] + [\text{Fe}^{\text{II}}-\text{H}_2\text{L}] + [\text{Fe}^{\text{II}}-\text{HL}]} \\ &= \frac{k_i + k_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}} K_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}} [\text{H}_2\text{L}] + k_{\text{Fe}^{\text{II}}-\text{HL}} K_{\text{Fe}^{\text{II}}-\text{HL}} [\text{HL}]}{1 + K_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}} [\text{H}_2\text{L}] + K_{\text{Fe}^{\text{II}}-\text{HL}} [\text{HL}]} \\ &= \frac{k_i + k_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}} K_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}} \frac{[\text{H}^+]}{K_{a2}} [\text{HL}] + k_{\text{Fe}^{\text{II}}-\text{HL}} K_{\text{Fe}^{\text{II}}-\text{HL}} [\text{HL}]}{1 + \frac{K_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}}}{K_{a2}} [\text{H}^+] [\text{HL}] + K_{\text{Fe}^{\text{II}}-\text{HL}} [\text{HL}]} \end{aligned} \quad (11)$$

The  $k_{obs}$  was measured at a fixed initial  $[\text{Fe(II)}]_T$  (40  $\mu\text{M}$ ) but with varying initial  $[\text{TTC}]_T$  (0-200  $\mu\text{M}$ ) at pH 6.5 and 7.0, respectively. The eqs 9 and 11 were solved simultaneously with respect to the experimental data of  $k_{obs}$  for the unknown  $k_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}}$  and  $k_{\text{Fe}^{\text{II}}-\text{HL}}$  using Matlab. The  $k_{\text{Fe}^{\text{II}}-\text{H}_2\text{L}}$  and  $k_{\text{Fe}^{\text{II}}-\text{HL}}$  were calculated to be **0.269 min<sup>-1</sup>** and **1.511 min<sup>-1</sup>**, respectively.

#### Text S5. TTC's degradation in the presence of Fe(III) and H<sub>2</sub>O<sub>2</sub>

In order to test the hypothesis that H<sub>2</sub>O<sub>2</sub> complexes with Fe(III)-TTC and further oxidizes TTC (described by the two reactions below), experiments were conducted in a solution containing 40  $\mu\text{M}$  TTC, 40  $\mu\text{M}$  Fe(III), 0.1 M TBA and 3  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> at pH 6.5 (buffered by 10 mM MOPS). The concentration of Fe(III) was chosen because at most 40  $\mu\text{M}$  Fe(III) could be produced within the reaction time. Although accurate H<sub>2</sub>O<sub>2</sub> concentration was difficult to estimate, 3  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> was expected to be higher than that in the system containing 40  $\mu\text{M}$  TTC and 40  $\mu\text{M}$  Fe(II). TBA was added into the solution to scavenge ROS produced by the reaction between Fe(III) and H<sub>2</sub>O<sub>2</sub>. Results showed that less than 5% of total TTC was degraded in the H<sub>2</sub>O<sub>2</sub>-Fe(III)-TTC system within 90 min. Therefore, the degradation of TTC via the H<sub>2</sub>O<sub>2</sub>-TTC-Fe(III) complex was negligible.



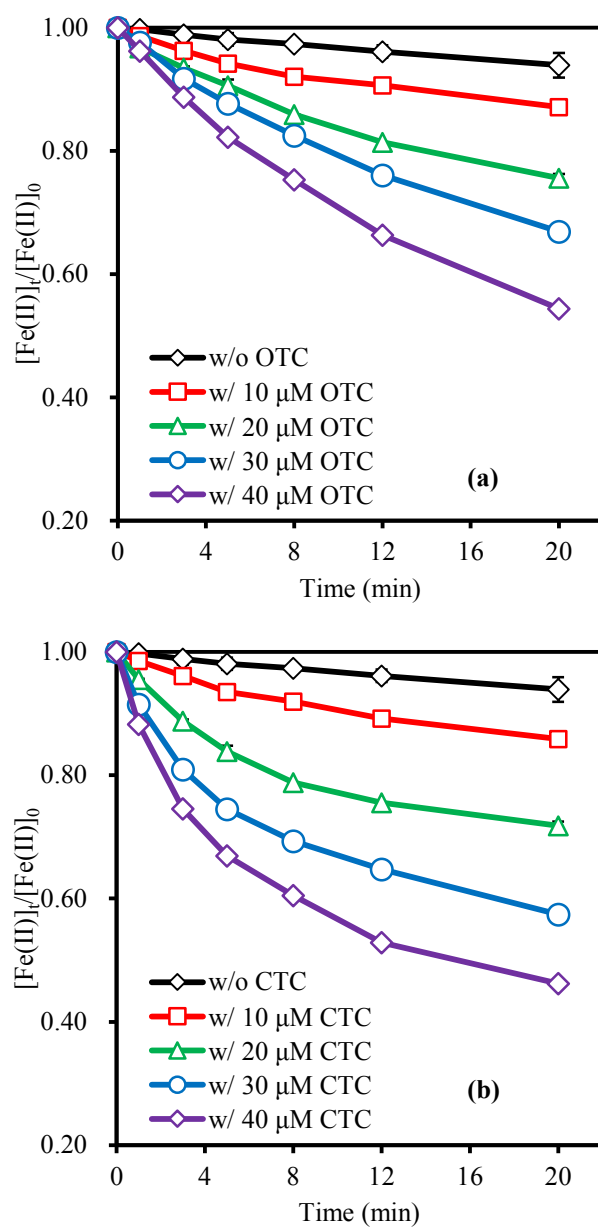


Figure S1. Oxidation of Fe(II) at different initial OTC (a) and CTC (b) concentrations in 20 min. Conditions:  $[\text{Fe(II)}]_0 = 40 \mu\text{M}$ , pH = 6.5 (buffered by 10 mM MOPS),  $T = 22^\circ\text{C}$ , with air purging.

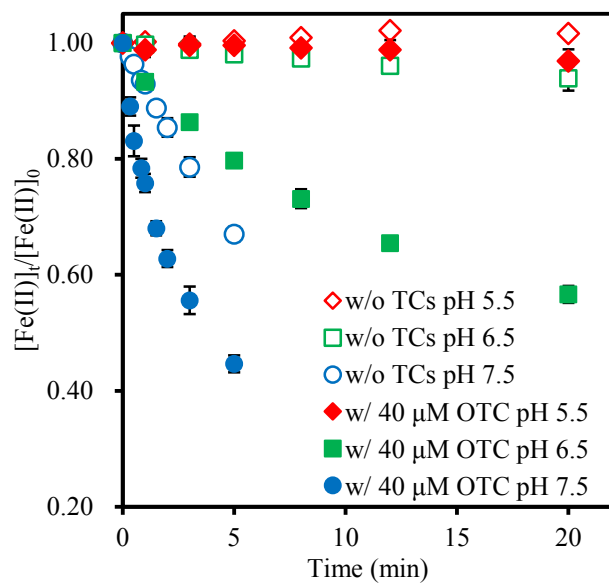


Figure S2. Fe(II)'s oxidation in the absence and presence of OTC at different pH. Conditions:  $[\text{Fe(II)}]_0 = 40 \mu\text{M}$ , buffered by 10 mM MES for pH 5.5 and 10 mM MOPS for pH 6.5 and 7.5,  $T = 22 \text{ }^\circ\text{C}$ , with air purging.



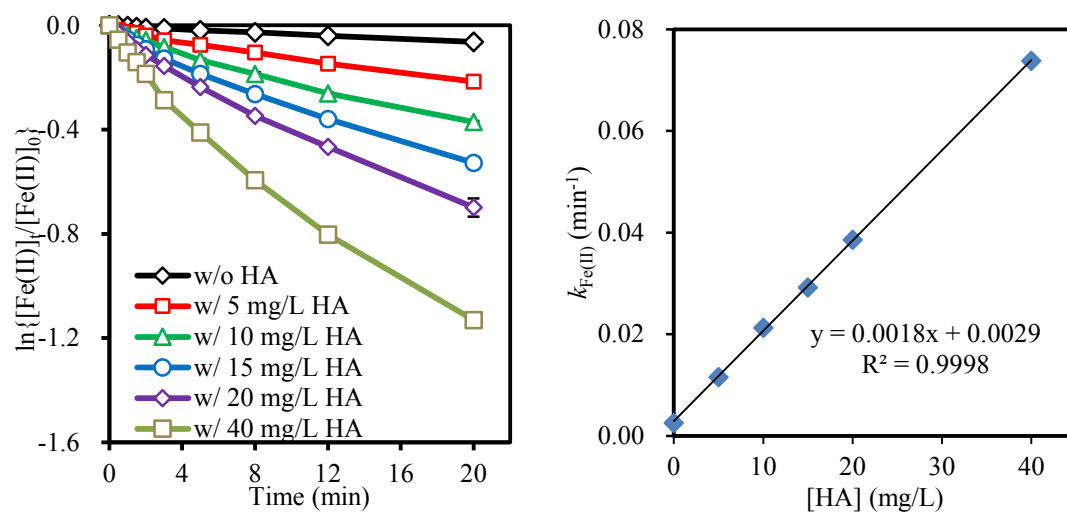


Figure S3. HA's effect on the oxidation of Fe(II). Conditions:  $[\text{Fe(II)}]_0 = 40 \mu\text{M}$ ,  $\text{pH} = 6.5$  (buffered by 10 mM MOPS),  $T = 22 \text{ }^\circ\text{C}$ , with air purging.

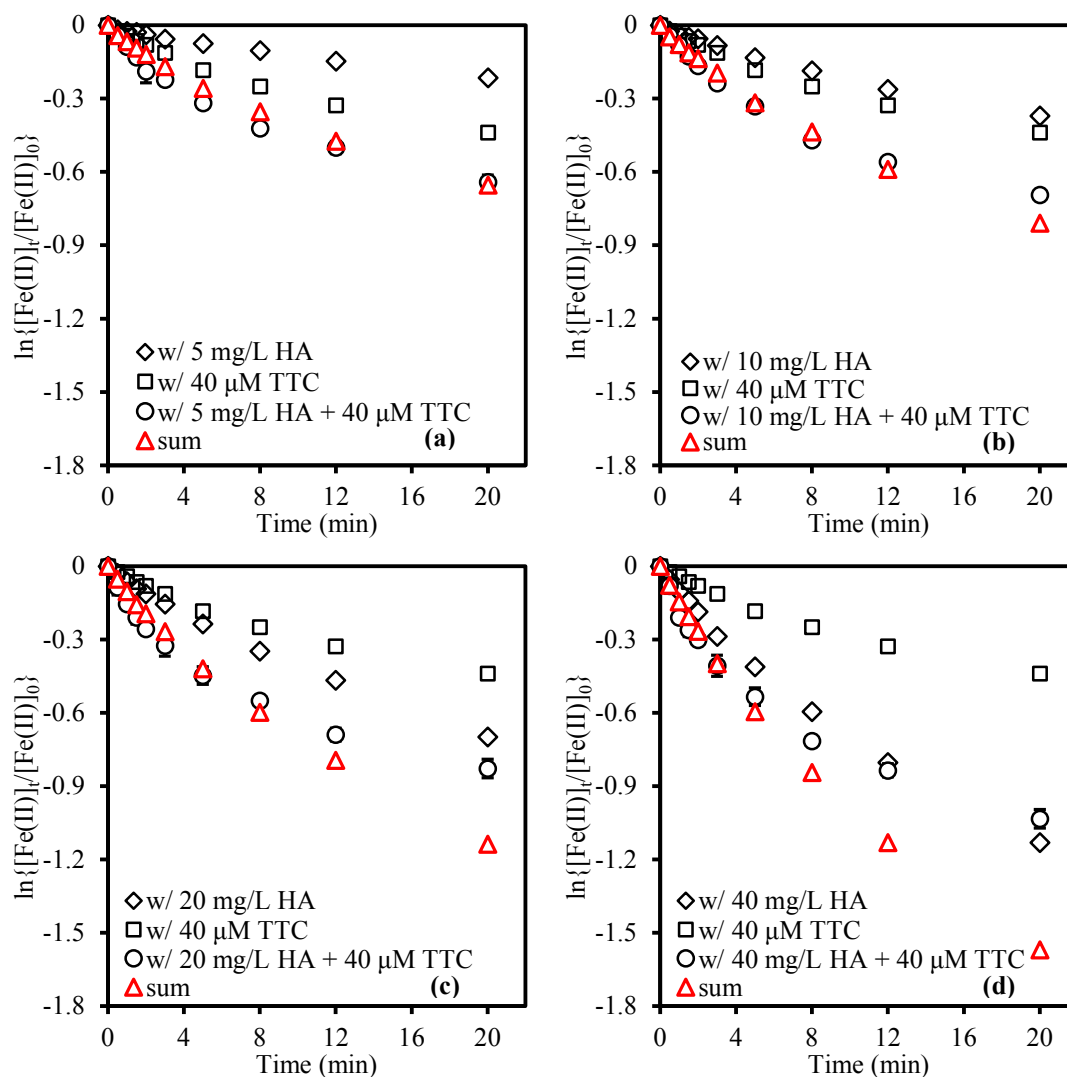


Figure S4. Oxidation of Fe(II) in the presence of TTC and HA. Condition:  $[Fe(II)]_0 = [TTC]_0 = 40 \mu M$ ,  $[HA]_0 = 5, 10, 20$  and  $40$  mg/L, pH = 6.5 (buffered by 10 mM MOPS), T = 22 °C, with air purging. Sum = the mathematic sum of the effect of HA only and the effect of 40

$\square$  M TTC only.

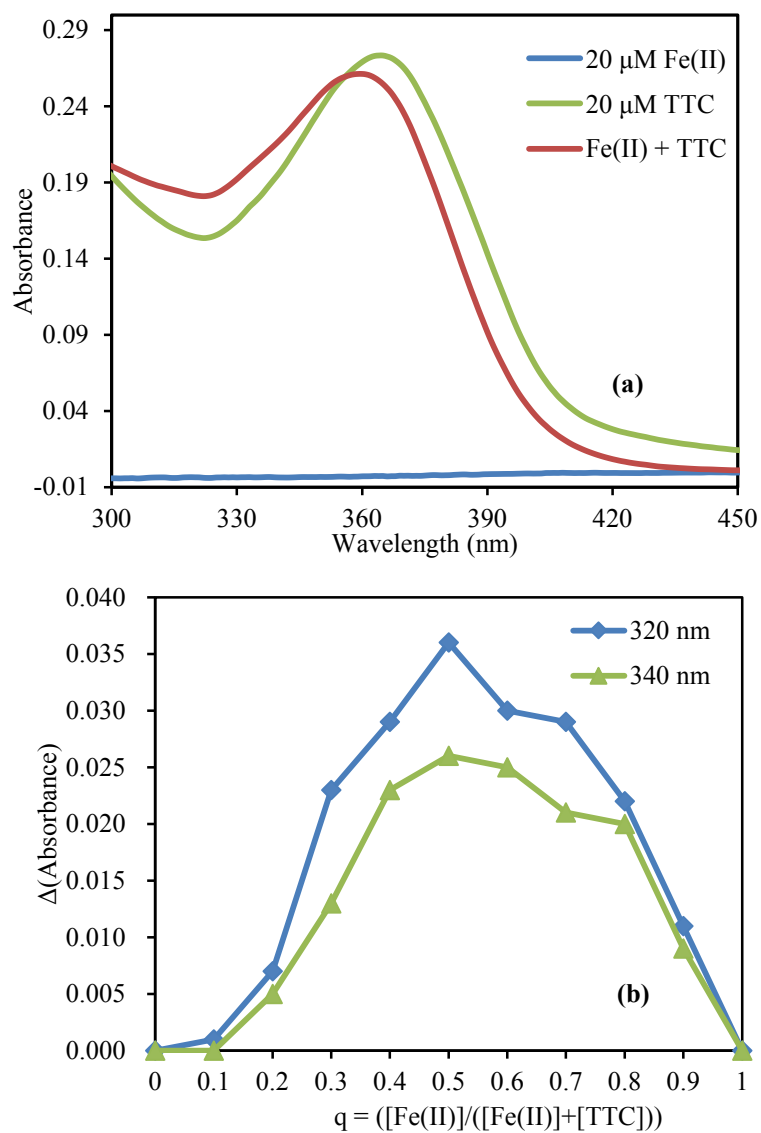


Figure S5. Complexation of Fe(II) and TTC: (a) UV spectra of solutions containing Fe(II) only, TTC only, and Fe(II) plus TTC, at pH 6.5; (b) Job's plot for the complexation of Fe(II) and TTC at pH 6.5. The total concentration of Fe(II) and TTC was held constant at 40  $\mu\text{M}$ .

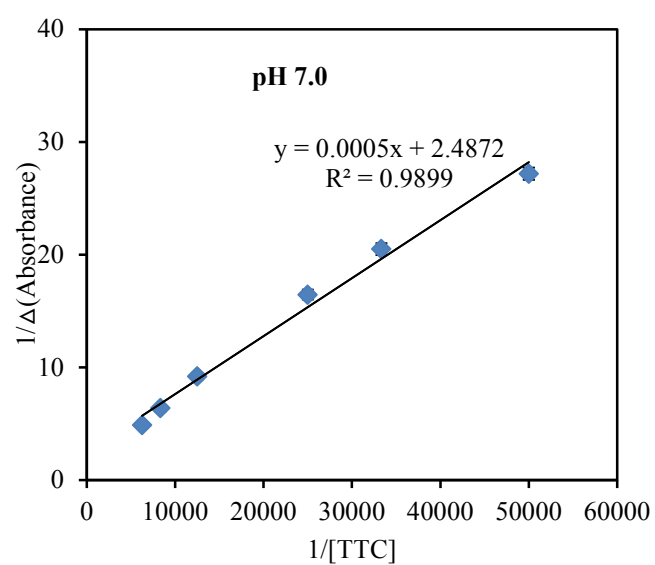


Figure S6. Linear relationship by plotting  $1/[TTC]$  versus  $1/\Delta(\text{Absorbance})$  at pH 7.0.

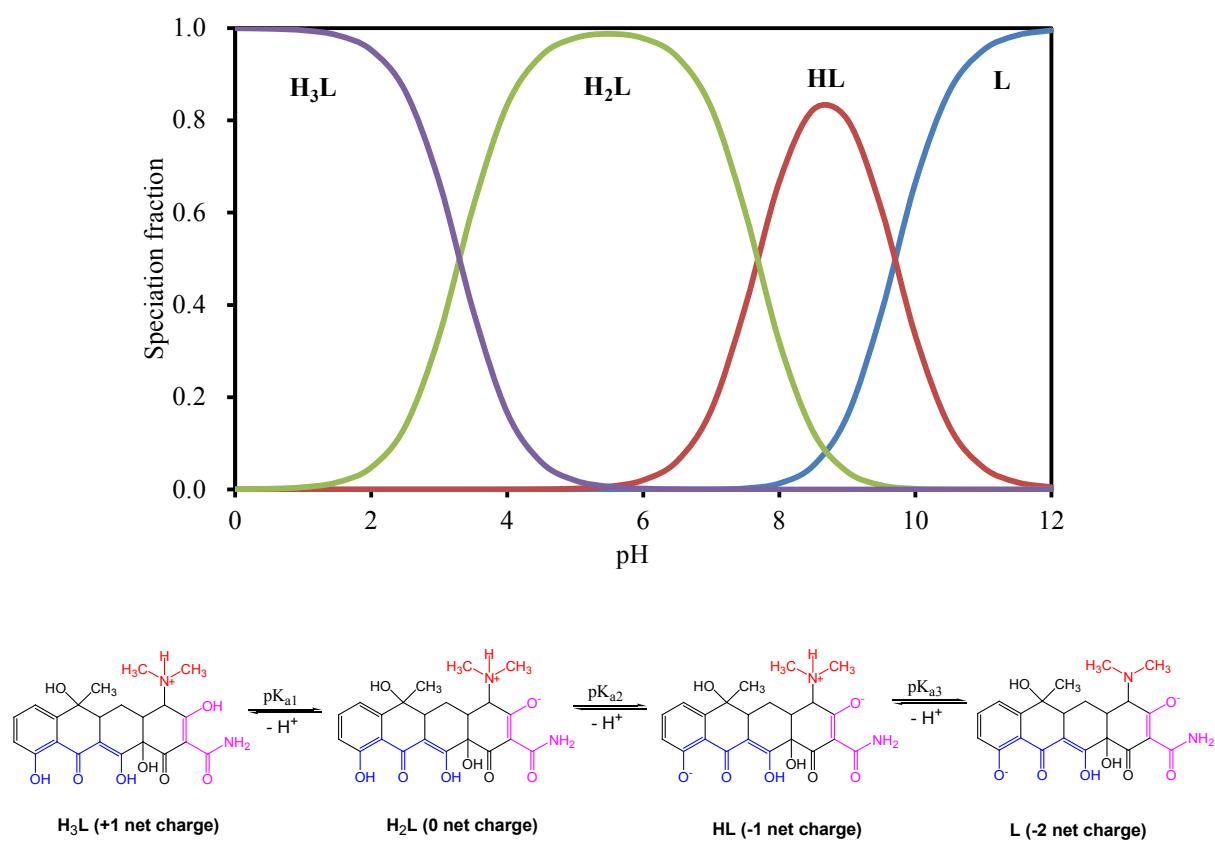


Figure S7. The speciation fraction of TC at different pH.

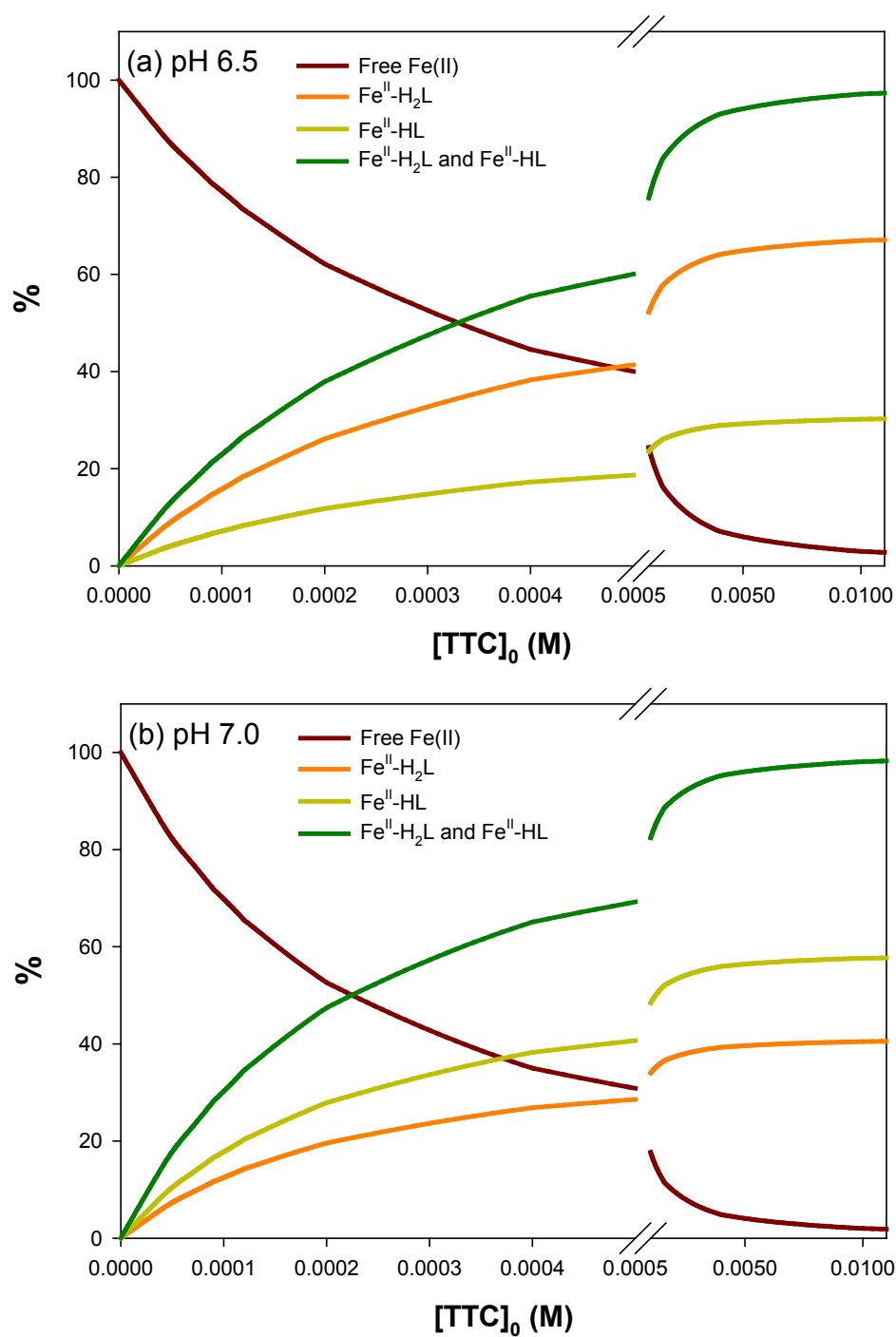


Figure S8. Fe(II)'s speciation in the presence of different amounts of TTC at pH 6.5 and 7.0.  
Condition: [Fe(II)] = 40  $\mu$ M.

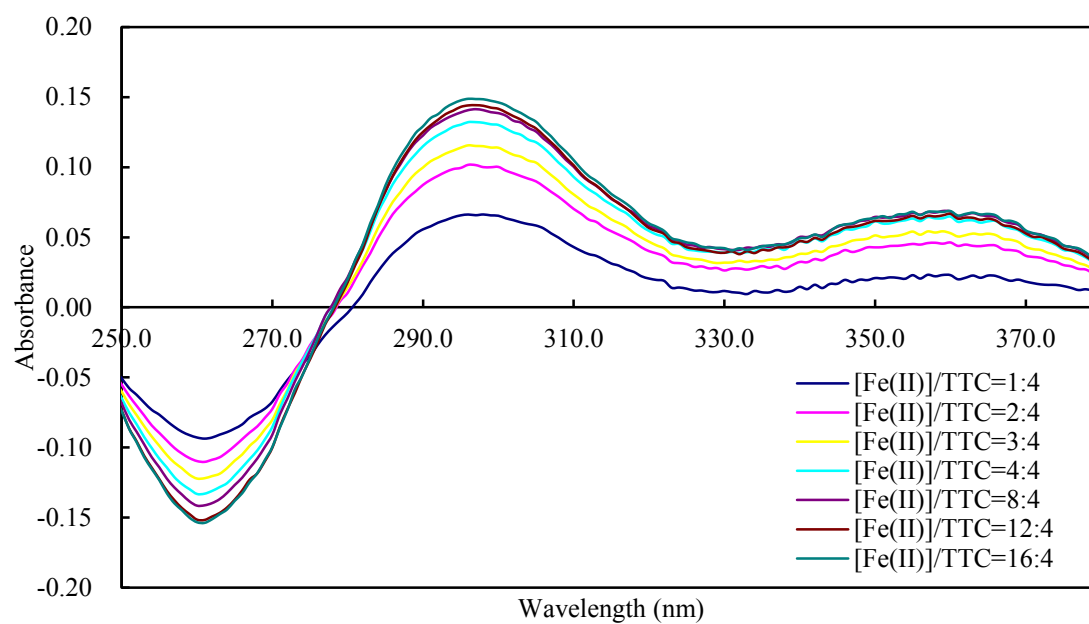


Figure S9. Changes in UV absorbance ( $\Delta$ Absorbance) under different Fe(II):TTC ratios at wavelengths 250-380 nm. Condition: [TTC] = 40  $\mu$ M, pH = 6.5 (buffered by 10 mM MOPS)

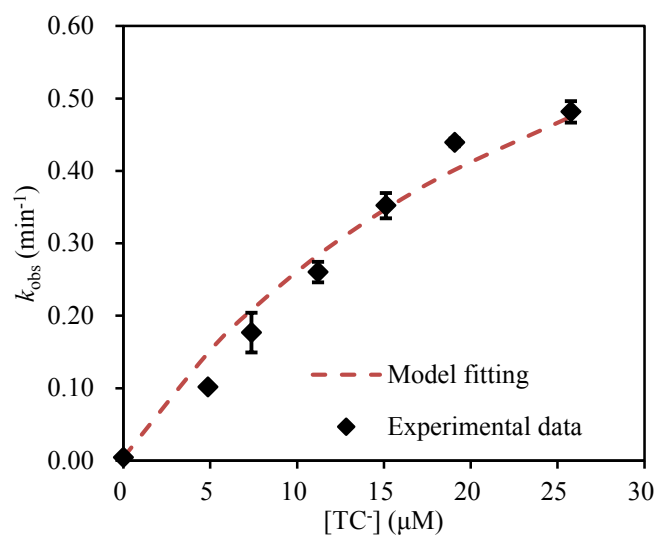


Figure S10. Experimental data and model fitting of observed Fe(II) oxidation rate constants with varying initial TTC concentrations at pH 7.0. Condition:  $[\text{Fe(II)}]_0 = 40 \mu\text{M}$ ,  $[\text{TTC}]_0 = 0$ , 40, 60, 90, 120, 150 and 200  $\mu\text{M}$ , buffered by 10 mM MOPS,  $T = 22 \text{ }^\circ\text{C}$ , with air purging.



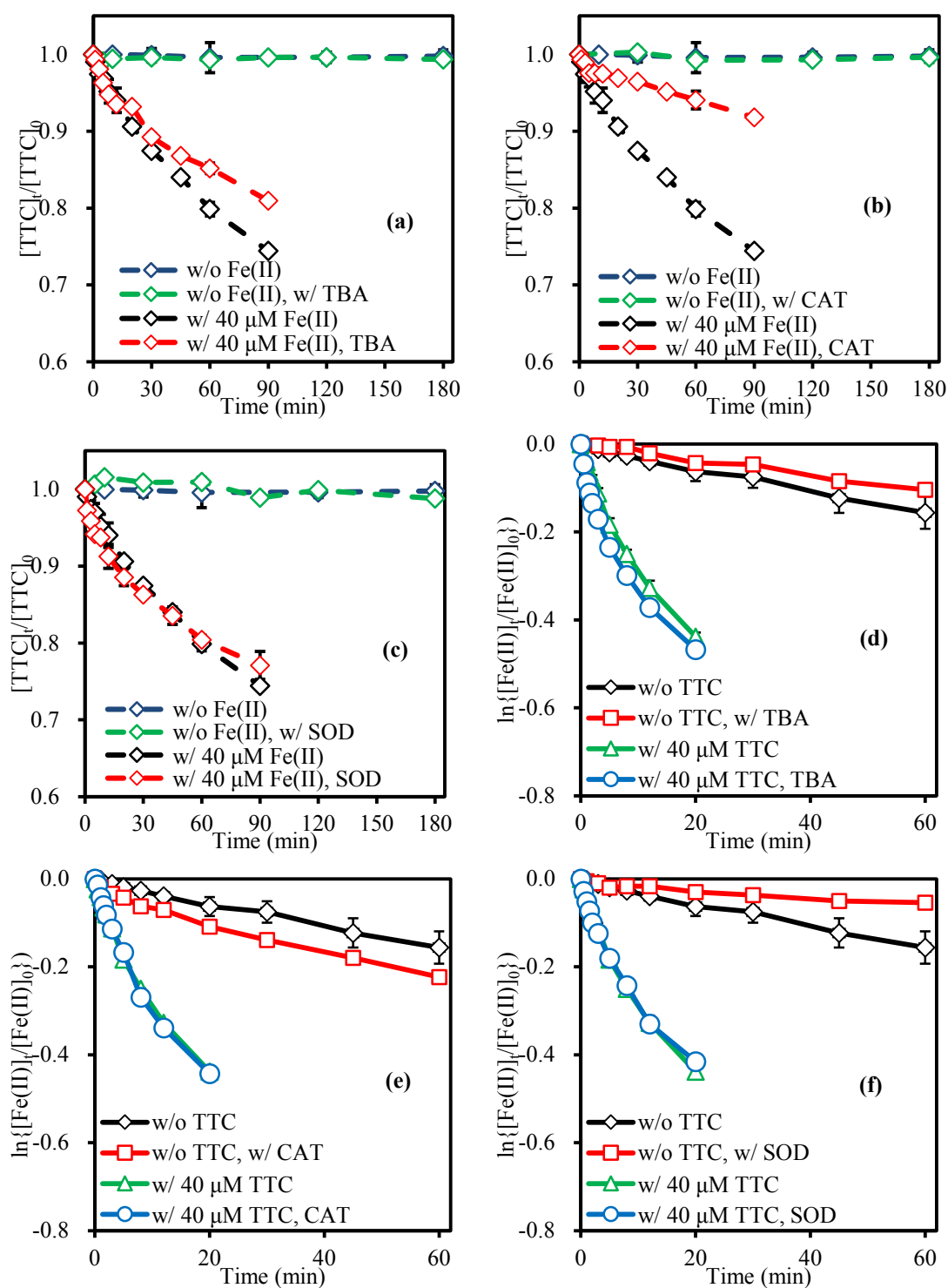


Figure S11. Effects of ROS scavengers (TBA, catalase, SOD) on TTC degradation and Fe(II) oxidation. Conditions:  $[Fe(II)]_0 = [TTC]_0 = 40 \mu\text{M}$ ,  $[TBA] = 1.0 \text{ M}$ ,  $[\text{catalase}] = 400 \text{ units}\cdot\text{mL}^{-1}$ ,  $[SOD] = 400 \text{ units}\cdot\text{mL}^{-1}$ ,  $\text{pH} = 6.5$  (buffered by 10 mM MOPS),  $T = 22 \text{ }^\circ\text{C}$ , with air purging.

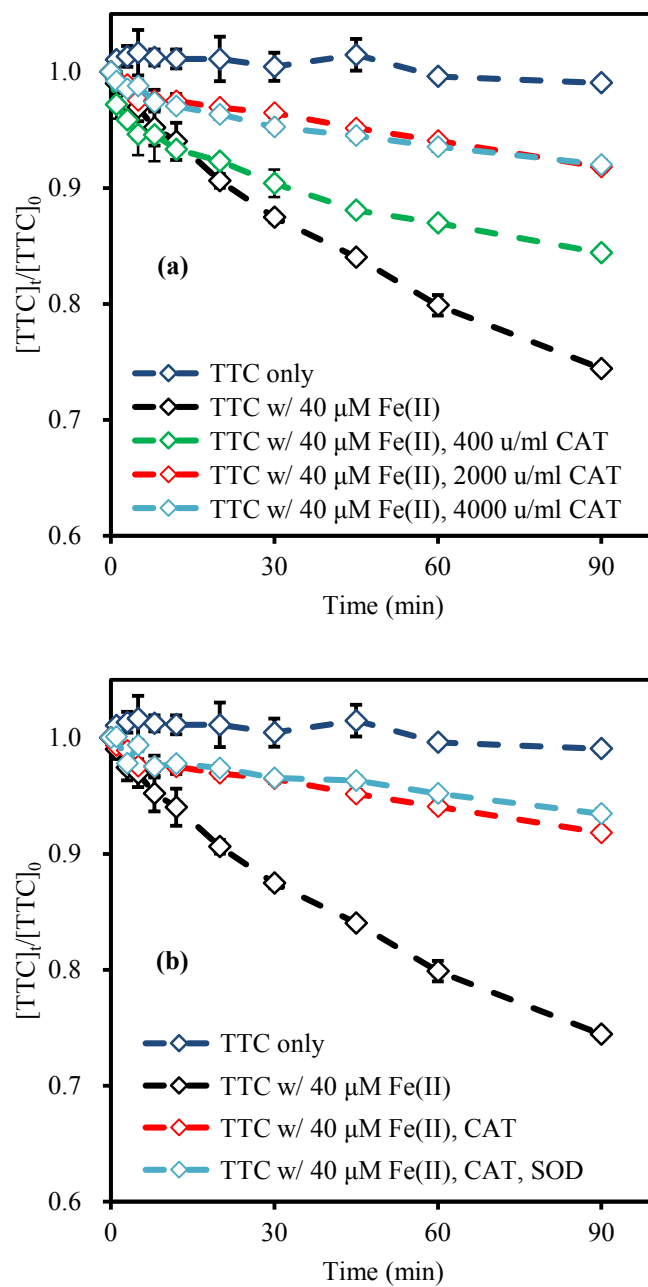


Figure S12. (a) Effect of catalase on TTC's degradation in the Fe(II)-TTC system. [catalase] = 400, 2000, 4000 units/mL; (b) The influence of co-existence of catalase and SOD on TTC's degradation in the Fe(II)-TTC system, [catalase] = [SOD] = 400 units/mL. Conditions:  $[\text{Fe(II)}]_0 = [\text{TTC}]_0 = 40 \mu\text{M}$ , pH = 6.5 (buffered by 10 mM MOPS),  $T = 22^\circ\text{C}$ , with air purging

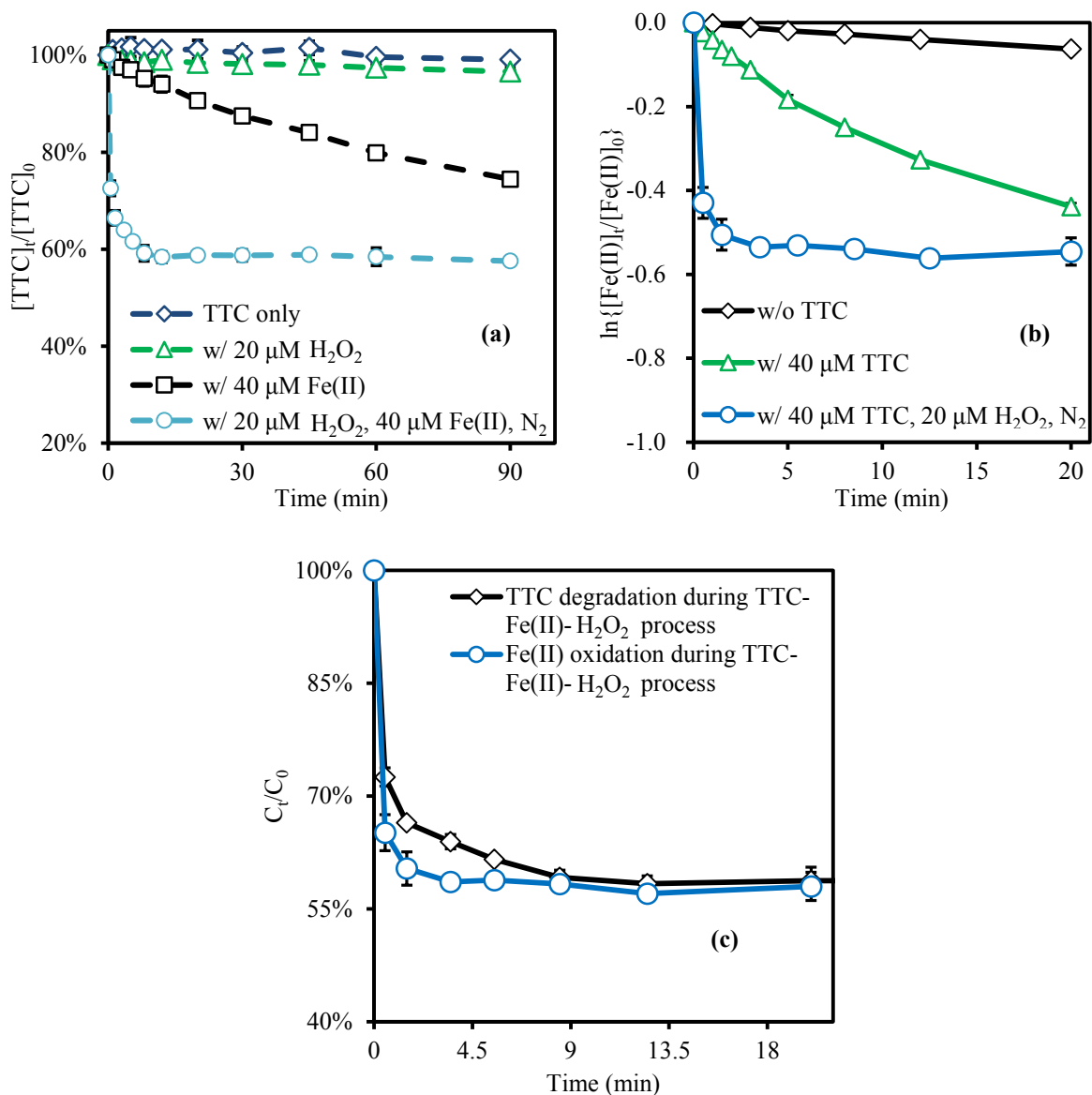


Figure S13. (a) Effect of  $\text{H}_2\text{O}_2$  on TTC's degradation; (b) Effect of  $\text{H}_2\text{O}_2$  on Fe(II)'s oxidation; (c) The consumption of TTC and Fe(II) during the reaction. Condition:  $[\text{Fe(II)}]_0 = [\text{TC}]_0 = 40 \mu\text{M}$ ,  $[\text{H}_2\text{O}_2] = 20 \mu\text{M}$ , pH = 6.5 (buffered by 10 mM MOPS), with nitrogen purging.

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