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Supporting Information containing 14 pages, 9 figures and 3 tables to accompany

manuscript titled

In vitro characterization of Reactive Oxygen Species (ROS) generation by

commercially available MesosilverTM dietary supplement

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S1: Additional experimental results

Table S1. Ag(I) speciation under various pH conditions in solutions containing 0.5 M NaCl (initial Ag(I) concentration = $20 \ \mu$ M).

Silver species	Concentration under different pH (µM)			
Silver species	pH 2	pH 4	pH 7	
Ag(OH) ²⁻	5.6×10 ⁻²⁴	5.6×10 ⁻²⁰	5.6×10 ⁻¹⁴	
Ag^+	5.8×10 ⁻⁴	5.8×10 ⁻⁴	5.8×10 ⁻⁴	
AgCl(s)	2.6×10 ⁻¹	2.6×10 ⁻¹	2.6×10 ⁻¹	
$AgCl_2^-$	11.3	11.3	11.3	
AgCl ₃ ²⁻	8.5	8.5	8.5	
AgNO ₃ (aq)	2.1×10 ⁻⁶	2.1×10 ⁻⁸	_	
AgOH(aq)	3.8×10 ⁻¹⁴	3.8×10 ⁻¹²	3.8×10-9	
% Ag as AgCl(s)	0	0	0	



Figure S1. TEM images of MesosilverTM

Table S2. Results of t	race metal analyses	of Mesosilver TM	suspensions	using ICP-MS.
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	Concentration in non-filtered	Concentration in filtered*	
Trace metal	Mesosilver TM suspension (nM)	Mesosilver TM suspension (nM)	
Cu	308.2 ± 4.3	10.7 ± 0.7	
Ca	240.8 ± 18.6	69.2 ± 48.0	
Mg	55.2 ± 10.4	49.2 ± 12.6	
As	1.8 ± 1.5	6.4 ± 1.4	

* MesosilverTM is removed by filtration by Amicon centrifugal ultrafilters No Mn, Fe and Na was detected in MesosilverTM suspension



Figure S2. Measured zeta potential of MesosilverTM suspensions as function of pH.



Figure S3. Particle size in pH 2, 4 and 7 solutions in the absence of 0.5 M NaCl.



Figure S4. Concentration of dissolved Ag(1) formed on oxidation of 20 μ 0 Mesosilver^{IM} in air saturated (open bars) and partially deoxygenated (closed bars) solutions at pH 2, 4 and 7 in the absence (panel a) and presence (panel b) of 0.5 M NaCl.



Figure S5. Absorbance spectrum of MesosilverTM in pH 2 solution in the absence of NaCl.



Figure S6. Concentration of H_2O_2 formed on oxidation of 20 μ M MesosilverTM in partially deoxygenated solutions at pH 2, 4 and 7 in the presence of 0.5 M NaCl.



Figure S7. Decrease in H_2O_2 concentration when added to 20 μ M MesosilverTM solution in the presence (blue symbols) and absence of 0.5 M NaCl (green symbols) at pH 2 (panel a), 4 (panel b) and 7 (panel c) for initial H_2O_2 concentrations of 15 μ M (circles) or 100 μ M (squares). Symbols with error bars represent experimental data. Solid lines represent model predictions.



Figure S8. Decrease in H_2O_2 concentration when 2.5 mM H_2O_2 added to 20 on MesosilverTM solutions at pH 2, 4 and 7. Symbols with error bars represent experimental data.



Figure S9. Visible spectrum of DPD^{+•} formed on oxidation of DPD by (i) 15 μ M H₂O₂ in pH 7 solution in the absence of NaCl (black line), (ii) 15 μ M H₂O₂ with measurement processed immediately after 20 μ M MesosilverTM addition to the mixture of H₂O₂, DPD and HRP in pH 7 solution in the absence of NaCl (red line) and (iii) 15 μ M H₂O₂ with measurement processed immediately on addition of H₂O₂, DPD and HRP to pH 7 solution containing 20 μ M MesosilverTM that was aggregated and oxidized for 2 h in pH 7 solution containing 0.5 M NaCl (blue line).

No.	Reaction	Rate constant
1	$^{\circ}\mathrm{OH} + \mathrm{Cl}^{-} \rightleftharpoons \mathrm{ClOH}^{\bullet-}$	$k_1 = 4.3 \times 10^9 \mathrm{M}^{-1} \mathrm{s}^{-1}$ $k_2 = 6.1 \times 10^9 \mathrm{s}^{-1}$
2	$ClOH^{\bullet-} + H^+ \longrightarrow Cl^{\bullet} + H_2O$	$2.4 \times 10^{10} \mathrm{M}^{-1} \mathrm{s}^{-1}$
3	$\text{ClOH}^{\bullet-} + \text{Cl}^- \longrightarrow \text{Cl}_2^{\bullet-} + \text{OH}^-$	$1 \times 10^5 M^{-1} s^{-1}$
4	$Cl^{\bullet} + Cl^{-} \longrightarrow Cl_{2}^{-\bullet}$	$\begin{array}{c} 6.5{\times}10^9M^{-1}s^{-1} \\ 1.1{\times}10^5M^{-1}s^{-1} \end{array}$
5	$Cl_2 + Cl^- \Longrightarrow Cl_3^-$	$k_{5=}2 \times 10^4 \mathrm{M}^{-1} \mathrm{s}^{-1}$ $k_{-5}=1.1 \times 10^5 \mathrm{s}^{-1}$
6	$HOCl + Cl^{-} \longrightarrow Cl_2OH^{-}$	$k_{6=} 1.5 \times 10^4 \mathrm{M}^{-1} \mathrm{s}^{-1}$ $k_{-6} = 5.5 \times 10^9 \mathrm{s}^{-1}$
7	$Cl^{\bullet} + OH^{-} \longrightarrow ClOH^{\bullet-}$	$1.8 \times 10^{10} \mathrm{M}^{-1} \mathrm{s}^{-1}$
8	$Cl^{\bullet} + Cl^{\bullet} \longrightarrow Cl_{2}$	$1 \times 10^{8} M^{-1} s^{-1}$
9	$Cl^{\bullet} + H_2O \longrightarrow HClOH$	$2.5 \times 10^5 M^{-1} s^{-1}$
10	$\operatorname{Cl}_2^{-\bullet} + \operatorname{OH}^- \to \operatorname{ClOH}^{\bullet-} + \operatorname{Cl}^-$	$4.5{\times}10^7M^{-1}s^{-1}$
11	$\operatorname{Cl}_{2}^{-\bullet} + \operatorname{Cl}_{2}^{-\bullet} \rightarrow 2\operatorname{Cl}^{-} + \operatorname{Cl}_{2}$	$8.3 \times 10^8 M^{-1} s^{-1}$
12	$\mathrm{HO}^{\bullet} + \mathrm{Cl}_{2}^{-\bullet} \to \mathrm{HOCl} + \mathrm{Cl}^{-}$	$1 \times 10^9 M^{-1} s^{-1}$
13	$\mathrm{Cl}_{2}^{-\bullet} + \mathrm{H}_{2}\mathrm{O} \rightarrow \mathrm{HClOH} + \mathrm{Cl}^{-}$	$1.3 \times 10^3 M^{-1} s^{-1}$
14	$\mathrm{HClOH} \to \mathrm{H^{+}} + \mathrm{ClOH^{\bullet^{-}}}$	$1 \times 10^8 \text{s}^{-1}$
15	$\rm HClOH \rightarrow \rm Cl^{\bullet} + \rm H_2O$	$1 \times 10^2 \text{s}^{-1}$
16	$\mathrm{HClOH} + \mathrm{Cl}^- \rightarrow \mathrm{Cl}_2^{\bullet-} + \mathrm{H}_2\mathrm{O}$	$5 \times 10^9 M^{-1} s^{-1}$
17	$\mathrm{Cl_2OH^-} + \mathrm{H^+} \rightarrow \mathrm{Cl_2} + \mathrm{H_2O}$	$2 \times 10^{10} M^{-1} s^{-1}$
18	$\mathrm{HOCl} + \mathrm{HO}^{\bullet} \rightarrow \mathrm{ClO}^{\bullet} + \mathrm{H_2O}$	$2 \times 10^9 M^{-1} s^{-1}$
19	$OCl^- + HO^{\bullet} \rightarrow ClO^{\bullet} + OH^-$	$8.8 \times 10^9 M^{-1} s^{-1}$

Table S3. Reactions of chlorine-based radicals triggered by hydroxyl radical scavenging chloride ions¹

S2: Description of the kinetic model

Based on the analysis presented in the main manuscript, we propose a mathematical model to explain the dioxygen and H_2O_2 mediated MesosilverTM dissolution kinetics over a range of pH. The mathematical model described here is the same as that described previously by He and co-workers⁸ with two new features:

- (i) Catalytic decay of superoxide occur, possibly as a result of interaction with the organic moieties present in the MesosilverTM solution;
- (ii) Scavenging of hydroxyl radicals, generated on H₂O₂-mediated oxidation of MesosilverTM,
 by Cl⁻ occur under acidic conditions.²

Below we describe the key features of the kinetic model and the constraints pertaining to particular rate constants used.

S2.1 Dioxygen mediated MesosilverTM dissolution

Reaction 1 (Table 2) shows the dioxygen-mediated oxidation of MesosilverTM. The rate constant for this reaction was determined based on best-fit to the measured decrease in MesosilverTM concentration and the concentration of Ag(I) formed (Figure 2). In the absence of NaCl, the rate constant for this reaction decreases with increase in pH in accord with our experimental results. Since the presence of NaCl had no impact on the concentration of Ag(I) formed at pH 2, the rate constant for this reaction is the same in the presence and absence of Cl⁻ at pH 2 however varies significantly at pH 4 and 7.

S2.2 Decay of superoxide

The superoxide formed in Reaction 1 undergoes rapid decay either via uncatalyzed disproportionation (Reaction 2; Table 2) and/or catalytic disproportionation with the organic moieties present in the MesosilverTM solution (Reactions 3 and 4; Table 2). The rate constant for uncatalyzed disproportionation was used as reported earlier by Bielski and co-workers³ for various pH. The rate constant for Reactions 3 and 4 were determined based on the measured concentrations of dissolved Ag(I) and H₂O₂ formed on MesosilverTM oxidation (Figures 2 and 3).

S2.3 H_2O_2 mediated oxidation of MesosilverTM

Reactions 5-7 (Table 2) represent the oxidation of MesosilverTM by H₂O₂ via the mechanism reported earlier for citrate-stabilized AgNPs.⁴ As discussed earlier, the rate constant for these reactions is dependent on pH. The rate constant for reaction 5 was determined based on the best-fit to the measured decrease in the MesosilverTM concentration and the concentration of Ag(I) formed in the presence of low (micromolar) H₂O₂ concentrations (Figure 4). The rate constants for reaction 6 and 7 were determined based on the measured decrease in H₂O₂ concentration (Figure S7) and the concentration of Phth-OH formed (Figure 5) on oxidation of MesosilverTM. Note that any value of the rate constants for reaction 6 and 7 will fit our experimental results as long as these reactions are fast (i.e. $k_6 > 1 \times 10^5$ M⁻¹s⁻¹) and the ratio k_6/k_7 is maintained as described in Table 2.

S2.4 Scavenging of hydroxyl radicals formed on H_2O_2 mediated oxidation of MesosilverTM

Reactions 8 and 9 (Table 2) represent the scavenging of hydroxyl radical, formed on MesosilverTM oxidation, by Phth and H_2O_2 respectively. The rate constants for these reactions were used as reported earlier.⁵

S2.5. Aggregation of MesosilverTM

Reaction 10 (Table 2) describes the aggregation of MesosilverTM. In the absence of Cl⁻, the aggregation of MesosilverTM was observed to be unimportant at pH 2 and 4 however was observed to occur at pH 7 as supported by a small shift in the SPR peak. In the presence of Cl⁻, MesosilverTM undergoes rapid aggregation under all pH conditions investigated here however the aggregation rate constant of MesosilverTM cannot be constrained by our experimental results with suitable adjustments to rate constants for Reaction 1 and 5 for any increase in the aggregation rate constant resulting in the same overall fit to our experimental

results. Furthermore, the impact of aggregation of MesosilverTM on the overall Ag(I) formation and ROS generation was not important at pH 2 and 4 with similar fits obtained even in if this reaction is not included. Thus, the aggregation rate constant of MesosilverTM cannot be determined at pH 2 and 4.

S2.6 Formation of new AgNPs via charging-discharging mechanism

Reactions 11-13 (Table 2) represent the formation of new AgNPs via the superoxidemediated charging-discharging mechanism described in earlier studies.^{4, 6} As indicated by Henglein and Lilie,⁷ the charging process is diffusion controlled and takes place within milliseconds. Thus, a value of 1×10^{10} M⁻¹s⁻¹ was used as the second order rate constant for charging of MesosilverTM by superoxide. The ratio of the rate constants for reaction 12 and 13 determines the rate of re-formation of AgNPs, following oxidation by dioxygen or H₂O₂. To simplify the modelling, Reaction 12 was assumed to be independent of pH and the same as that reported in earlier work^{4, 6} with fitting to the experimental results achieved by varying the rate constant for Reaction 13.

S2.7 Oxidation of in situ formed AgNPs by dioxygen and H_2O_2

Reaction 14 (Table 2) represents the oxidation of in situ formed AgNPs by dioxygen. The rate constant for this reaction was determined based on the best-fit to our experimental results. Reactions 15-17 (Table 2) represent the reaction of in-situ formed AgNPs and H_2O_2 . In the presence of high concentrations of H_2O_2 , the degradation of H_2O_2 results in the re-formation of AgNPs. As such, the reactivity of in-situ formed AgNPs controls the catalytic decay rate of H_2O_2 . The rate constant for Reaction 15 was determined based on the best fit to the measured decay of H_2O_2 and the concentration of Ag(I) formed in the presence of high H_2O_2 concentrations (Figure 4). Furthermore, since the competition between AgNPs oxidation by

dioxygen and H_2O_2 will impact the hydroxyl radical generation rate, the ratio of the rate constants for Reactions 14 and 15 was further constrained by the measured hydroxyl radical generation (Figure 5). Furthermore, to explain the increase in hydroxyl radical generation with increase in temperature at pH 4 and 7, the value of the rate constant for reaction 15 at 37 °C was nearly 2-times higher than the rate constant used to fit the data at 22 °C

S2.8 Aggregation of in situ formed AgNPs

Reaction 18 represents the aggregation of in-situ formed AgNPs, the rate constant for which was determined based on best-fit to our experimental results. The aggregation of in-situ formed AgNPs was unimportant at pH 2 and 4 with the fits to overall Ag(I) formation and ROS generation the same even when this reaction was not included. We would like to highlight that this reaction may occur at pH 2 and 4 especially in the presence of NaCl; however the rate constant for this reaction cannot be determined based on our experimental results with similar fits obtained to our experimental results by suitably adjusting the rate constant for reaction 14 and 15 for any changes in the aggregation rate constant.

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