

Why are Silver Nanoparticles More Toxic Than Bulk Silver? Towards Understanding the Dissolution and Toxicity of Silver Nanoparticles

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The mass-transport to a spherical particle in solution is considered in the context of the mechanism of oxygen reduction on silver nanoparticles. For small particles ($\leq 1 \mu\text{m}$) the reduction follows a two-proton, two-electron pathway due to the rapid diffusion of the intermediate hydrogen peroxide away from the interface. Above this size threshold the hydrogen peroxide is further reduced to water. This switch of mechanism is of importance when interpreting the dissolution of silver nanoparticles in aqueous media and their inherent toxicity. The release of H_2O_2 from nanoparticles but not bulk silver may explain why silver nanoparticles are thought more toxic than their macroscopic counterparts.

Keywords: Silver nanoparticles; redox dissolution; oxygen reduction; toxicity; mass-transport

1. INTRODUCTION

Concerns regarding the toxicity and environmental impact of silver nanoparticles have become prevalent.[1] This increased attention relates to the greater use of these nanomaterials in a wide range of products,[2] with many applications utilising silver as a sterilising agent. The powerful antibacterial properties of both metallic silver and the argentous salts has been recognised but for nanoparticles the specific mode of action is still of debate.[3] Questions relate to the activity of the silver nanoparticles[4]; how they act as a source of silver ions and if they exhibit greater potency due to the coupled formation of reactive oxygen species (ROS) such as hydrogen peroxide.[5,6] Although argentous ions are themselves highly toxic for biological systems[7] the concomitant release of ROS

from nano-silver may give physical insight into the origin of any enhanced toxicity relating to nanoparticulate silver.

Oxygen reduction is a highly complex redox system involving multiple coupled chemical steps including protonation and bond breaking.[8] Figure 1 gives a simplified outline of the possible routes for oxygen reduction, where the mechanism is either a direct four-electron process (k_1) or a step-wise process involving the intermediate, hydrogen peroxide (k_2). This hydrogen peroxide formed by the step-wise mechanism may be either reduced further electrochemically (k_3) or it may undergo catalytic decomposition to form water and oxygen (k_{het}).

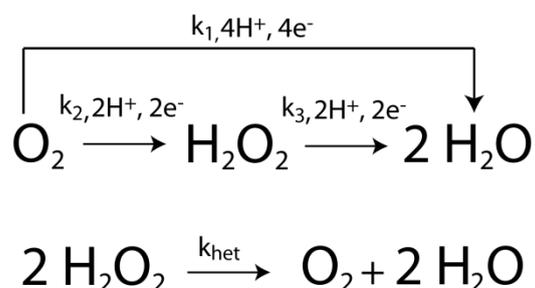


Figure 1. Schematic showing the generalised pathways for the reduction of oxygen.

Under *alkali* conditions the reduction of oxygen on a macroscopic silver electrode corresponds overall to a four-electron pathway.[9] However, at lower pHs the total number of electrons transferred to each oxygen molecule drops to $n = 3.3$ at pH 5.8 [10] and $n = 2$ at pH 1.[11] This indicates that the step-wise reduction mechanism is operative (note, even in acidic conditions near total four-electron reduction of oxygen on silver can be achieved by the application of high overpotentials [12]). At biologically relevant (near neutral) pHs this oxygen reduction process at a *macroelectrode* leads to appreciable hydrogen peroxide production on silver ($n < 4$).

It is well recognised within the electrochemical field that mass-transport can alter the observed mechanism of a multi-step interfacial reaction at a stationary electrode.[13] In analogy, in this article we consider the influence of mass-transport upon a multi-step reaction at a particle isolated in solution. Specifically, we show why more ROS can be formed at nanoparticulate as compared to macro (bulk) silver during the oxidative dissolution of the metal. Initially we focus on the mechanistic interpretation of the dissolution of Ag nanoparticles in oxygenated and non-complex (i.e. low ionic strength and non thiol containing) aqueous media. In such aqueous systems this dissolution process likely occurs due to the presence of dissolved oxygen.[14-16] Commonly, justification for this is based on the noble nature of the metal with a standard potential of +0.799 V (vs NHE) for the Ag/Ag⁺ couple,[17] consequently, the ‘only’ redox species able to oxidise the silver is oxygen. Here oxygen is assumed to undergo a four-electron reduction with a standard potential of +1.229 V (vs NHE),[17] hence the reaction is taken to be thermodynamically favourable.[14-16] However, for sufficiently small particles ($\leq 1\mu\text{m}$) it will be shown that the high rates of mass-transport means that this reaction oxygen reduction process may stop at the formation of hydrogen peroxide. The thermodynamics associated with this two-electron

reduction mechanism are also considered demonstrating that the reaction is favourable when the concentration of solution phase argentous ions is low.

Finally, having studied the thermodynamics and mechanistic aspects of this oxygen induced dissolution of silver particles in de-ionised and oxygenated solutions the discussion is extended towards the consideration of the influence of biologically relevant silver complexing groups such as chloride or thiols upon the nanoparticle dissolution process. Here the presence of these groups will make the dissolution process more thermodynamically favourable.

2. RESULTS AND DISCUSSION

Recent work by Neumann *et al.* has demonstrated that the mixed mechanism for oxygen reduction at a macro silver electrode at pH 5.8 is a result of initial electrochemical formation of hydrogen peroxide at the surface followed by a relatively slow heterogeneous decomposition reaction to form oxygen and water (k_{het}). This reformed oxygen may then participate further in the reduction process. A value of $1.3 \times 10^{-2} \text{ cm s}^{-1}$ was reported for the heterogeneous decomposition rate constant.[10] This rate constant is in good agreement with independent prior work which reported a surface decomposition rate in alkali media as $\sim 1 \times 10^{-2} \text{ cm s}^{-1}$. [18] Utilising this value of $1.3 \times 10^{-2} \text{ cm s}^{-1}$ for the silver-catalysed decomposition rate (k_{het}), the mechanism of oxygen reduction at an isolated silver particle can be considered. Analysis is predicated upon estimation of the minimum mass-transfer coefficient for hydrogen peroxide at a particle in solution. This calculation is achieved through the use of the experimentally verified work of Harriott,[19;20] and using a diffusion coefficient of $0.83 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for the hydrogen peroxide in solution¹; the results of which are shown in Figure 2. For small particles the mass-transport coefficient to and from the surface is found to be equal to D/r (black line), where D is the diffusion coefficient and r is the radius of the particle. For larger particles the mass-transport coefficient is found to deviate from this simple limit as the influence of gravity must be considered due to the sedimentation of the particle within solution (red line) leading to convective transport.[19;20]

Via comparison of this calculated mass-transport coefficient – as a function of particle size – with the known heterogeneous rate constant for the hydrogen peroxide decomposition, it is concluded that for large particles ($\geq 1 \mu\text{m}$ diameter) the mechanism of oxygen reduction is a mixed mechanism resulting in both the formation of water and hydrogen peroxide, with the proportion of water production increasing with increasing size. This mixed oxygen reduction mechanism arises due to the comparable magnitudes of the mass-transport coefficient and the heterogeneous decomposition rate. For example, for a particle with a diameter of $1.3 \mu\text{m}$, due to the greater rate of mass-transport, 90% of the formed hydrogen peroxide is predicted to diffuse away prior to catalytic decomposition upon the surface. Moreover, for particles with a sub-micron diameter the mass-transport away from the particle

¹ A range of diffusion coefficients are reported for hydrogen peroxide in the literature; however, the value of $0.83 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ [21] has been recorded over H_2O_2 concentrations of 2-30 mM and is in agreement with the values reported by Meyer.[22]

is even greater and so nearly all of the formed H_2O_2 intermediate will escape from the particles' surface prior to catalytic decomposition at the metallic interface.

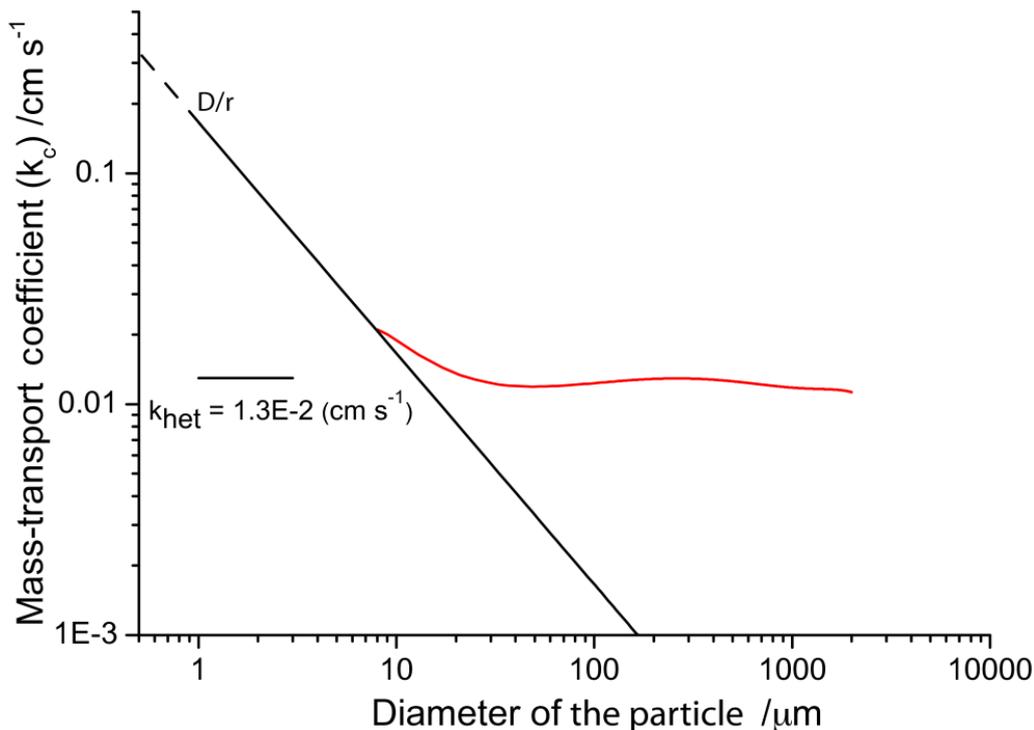
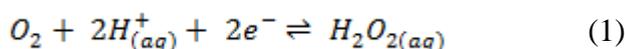


Figure 2. Calculated minimum (i.e. only considering the terminal velocity) mass-transfer coefficient of H_2O_2 to the surface of a spherical particle in solution, as compared to the heterogeneous rate constant (k_{het}) for the surface decomposition of H_2O_2 . Black line: diffusion only steady-state mass-transport to an isolated particle ($= D/r$) and red line: the corrected mass-transport coefficient accounting for sedimentation of the particle [19] under the force of gravity, note for small diameters the red line is equal to the uncorrected value. For the calculation, the difference in density of the silver and the aqueous solvent was taken as 9.5 g cm^{-3} with the density of water being 1 g cm^{-3} and the dynamic viscosity of water was taken to be 0.89 cP at 298 K . [23]

This conclusion indicates that for the aerobic dissolution of silver *nanoparticles*, the oxygen reduction mechanism is likely to follow a two-electron pathway resulting in the formation of hydrogen peroxide. Previous work in the literature has verified the formation of hydrogen peroxide during the dissolution process in de-ionised water, [14] corroborating this mechanistic interpretation. Due to this alteration to the proposed mechanism (a two- versus a four-electron reduction), the oxidative dissolution process and its thermodynamics require re-evaluation. The standard potential for the two-electron reduction of oxygen, as given by,

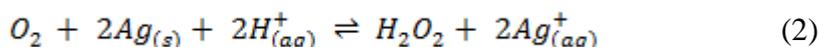


is $+0.695 \text{ V}$ (vs NHE). [17] Consequently, even in acidic media (and in the absence of Ag^+ complexing species such as chloride or thiols) the dissolution is *not* thermodynamically favourable ($E_{(Ag/Ag^+)}^\theta = +0.799 \text{ V}$ [vs NHE]). However, due to the initially extremely low Ag^+ concentration in solution and

from consideration of the Nernst equation it can be seen that the driving force towards dissolution will be significant. For example, in accordance with the Nernst equation, for even a 1 μM solution phase concentration of Ag^+ in solution the potential of the Ag/Ag^+ couple is +0.444 V (vs NHE). This analysis of the thermodynamics is corroborated by the fact that the dissolution of the silver nanoparticles does not go to completion and limits to what is ascribed to be an equilibrium value. [24]

Due to particle agglomeration [15] many fundamental studies on nanoparticle dissolution have been undertaken with de-ionised water or low ionic strength media. Extrapolation of this experimental evidence to more complex biological systems is neither obvious nor facile. In terms of the influence of agglomeration upon the oxygen reduction mechanism, work by Schlager *et al.* noted that for macrophages exposed to silver nanoparticles the maximum agglomerate size within the cells was found to be of the order of 2 μm . [5] Therefore, on the basis of the above discussion it would seem likely that even here in the confined cellular environment the *main* operative pathway will be a two-electron reduction mechanism for oxygen to form hydrogen peroxide. It should, however, be noted that hydrogen peroxide occurs naturally within a cellular environment and is present either as a metabolic by-product or due to its use as a signalling molecule. For healthy cells the intracellular concentration is limited to micromolar concentrations. [25] Due to the relatively high in vivo oxygen concentration, the proposed reduction mechanism for oxygen reduction at the silver nanoparticle interface will likely result in relatively high localised concentrations of H_2O_2 . Consequently, the observation of enhanced levels of oxidative stress of cells exposed to silver nanoparticles, may in part, reflect the release of H_2O_2 via aerobic dissolution of the nanoparticle.

Within biological systems the presence of both chloride and of thiol containing species is of likely major significance. First, for thiol functionalities, due to both the high formation constant of Ag^+ with thiol groups (cf. $\log K_f = 11.9$ for cysteine [26]) and the high concentration of thiols present within a cellular environment (in the mitochondria the protein surface exposed thiol groups have concentrations as high as 60-90mM [27]) it is likely that any Ag^+ released into the cell will rapidly become sequestered through thiol binding. In contrast, literature has concluded that due to the relatively high chloride concentrations in biological systems of interest (30-150mM), the release of Ag^+ will result in a supersaturated AgCl solution. [28] In light of the above comments this conclusion seems unlikely. Moreover, any free (i.e non-thiol bound) Ag^+ will likely be solubilised as $\text{AgCl}_x^{(1-x)}$ complexes [29] due to the low (non-thiol bound) Ag^+ concentration and the large chloride to Ag^+ ratio. Second, the presence of both these thiol functionalities and the relatively high chloride concentrations will have a significant effect on the thermodynamics of the oxygen reduction process. In the absence of chloride or thiols the dissolution process is,



However, in the presence of chloride and/or thiols, this equilibrium will be shifted to the right due to complexation with the formed Ag^+ . Consequently, on this basis, it is predicted that within the cellular environment the dissolution of the nanoparticles may – in contrast to the ex-situ studies – go to completion.

More generally, the example highlighted within this text is a paradigmatic example of how a multi-step redox reaction may be profoundly altered at the sub-micron/nano level not due to an inherent change in the interfacial kinetics or thermodynamics of the reaction but due to the alteration of the mass-transport to and from the interface.

4. CONCLUSION

The interplay between mass-transport and the heterogeneous rate constant for the decomposition of H_2O_2 is of significant importance for interpreting the dissolution of silver nanoparticles in aqueous oxygenated systems. The combination of the relatively slow heterogeneous rate constant for the decomposition of H_2O_2 on silver and the dependency of the mass-transport away from the particle on its dimensions, means that, for sufficiently small particles ($\leq 1\mu\text{m}$) the dissolution process will be driven by the two-electron, two-proton reduction of oxygen. This value of the $1\mu\text{m}$ is obtained through consideration of the relative rate of the mass-transport of hydrogen peroxide at an isolated particle ($\sim D/r$ for small particles) as compared to its rate of heterogeneous decomposition ($1.3 \times 10^{-2} \text{ cm s}^{-1}$) upon the metallic interface. Above this size limit the dissolution reaction is predicted to tend towards that found for macroscopic (bulk) silver, where the formation of water will become more pronounced. The presence of Ag^+ complex species such as chloride or thiol functionalities in solution can make this dissolution process more thermodynamically favourable. In summary, beyond the inherent toxicity of the formed argentous ions,[6] the concomitant release of hydrogen peroxide may help explain the enhanced toxicity of silver nanoparticles over their macroscopic counter parts.

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References

1. B. Nowack, *Science*, 330 (2010) 1054–1055
2. S. A. Blaser, M. Scheringer, M. MacLeod and K. Hungerbühler, *Sci. Total Environ.*, 390 (2008) 396–409
3. S. Chernousova and M. Epple, *Angew. Chem. Int. Ed.*, 52 (2013) 1636–1653
4. E. Bolea, J. Jimenez-Lamana, F. Laborda, I. Abad-Alvaro, C. Blade, L. Arolab and J. R. Castillo, *Analyst*, (2013) DOI:10.1039/c3an01443f
5. C. Carlson, S. M. Hussein, A. M. Schrand, L. K. Braydich-Stolle, K. L. Hess, R. L. Jones and J. J. Schlager, *J. Phys. Chem. B*, 112 (2008) 13608–13619
6. Z. M. Xiu, J. Ma and P. J. J. Alvarez, *Environ. Sci. Technol.*, 45 (2011) 9003–9008
7. C. Greulich, D. Braun, A. Peetsch, J. Diendorf, B. Siebers, M. Epple and M. Koller, *RSC Adv.*, 2 (2012) 6981–6987
8. J.-M. Savéant, *Chem. Rev.*, 108 (2008) 2348–2378
9. B. B. Blizanac, P. N. Ross and N. M. Markovic, *J. Phys. Chem. B*, 110 (2006) 4735–4741

10. C. C. M. Neumann, E. Laborda, K. Tschulik, K. R. Ward and R. G. Compton, *Nano Res.*, 6 (2013) 511–524
11. B. B. Blizanac, P. N. Ross and N. M. Markovic, *Electrochim. Acta*, 52 (2007) 2264–2271
12. C. M. Sánchez-Sánchez and A. J. Bard, *Anal. Chem.*, 81 (2009) 8094–8100
13. J. Heinze, *Angew. Chem. Int. Ed.*, 32 (1993) 1268–1288
14. J. Liu and R. H. Hurt, *Environ. Sci. Technol.*, 44 (2010) 2169–2175
15. W. Zhang, Y. Yao, N. Sullivan and Y. Chen, *Environ. Sci. Technol.*, 45 (2011) 4422–4428
16. A. Mittelman, A. Taghavy, Y. Wang, L. Abriola and K. Pennell, *J. Nanopart. Res.*, 15 (2013) 1–13
17. A. J. Bard, R. Parsons and J. Jordan, *Standard Potentials in Aqueous Solutions*, Internat. Union of Pure and Applied Chemistry, (1985)
18. N. D. Merkulova, G. V. Zhutaeva, N. A. Shumilova and V. S. Bagotzky, *Electrochim. Acta*, 18 (1973) 169–174
19. P. Harriott, *AIChE J.*, 8 (1962) 93 – 98
20. R. G. Compton, K. L. Pritchard and P. R. Unwin, *Freshwater Biol.*, 22 (1989) 285–288
21. S. B. Hall, E. A. Khudaish and A. L. Hart, *Electrochim. Acta*, 44 (1999) 2455–2462
22. R. Meyer, *Gmelins Handbuch der anorganischen Chemie*, Verlag Chemie, (1966)
23. D. Lide, *CRC Handbook of Chemistry and Physics.: A Ready-Reference Book of Chemical and Physical Data.*, CRC Press, (2004)
24. S. Kittler, C. Greulich, J. Diendorf, M. Köller and M. Epple, *Chem. Mater.*, 22 (2010) 4548–4554
25. M. Gioglio, M. Trinei, E. Migliaccio and P. G. Pelicci, *Nat. Rev. Mol. Cell Bio.*, 8 (2007) 722–728
26. N. W. H. Adams and J. R. Kramer, *Aquat. Geochem.*, 5 (1999) 1–11.
27. R. Requejo, T. R. Hurd, N. J. Costa and M. P. Murphy, *FEBS J.*, 277 (2010) 1465–1480
28. J. Liu, D. A. Sonshine, S. Shervani and R. H. Hurt, *ACS Nano*, 4 (2010) 6903–6913
29. J. Haworth Jonte and D. S. Martin Jr., *J. Am. Chem. Soc.*, 74 (1952) 2052–2054