Supporting Information for

Modeling physicochemical interactions affecting cellular dosimetry of engineered nanomaterials

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Zeta potential (ζ) variation with ionic strength (I) and pH

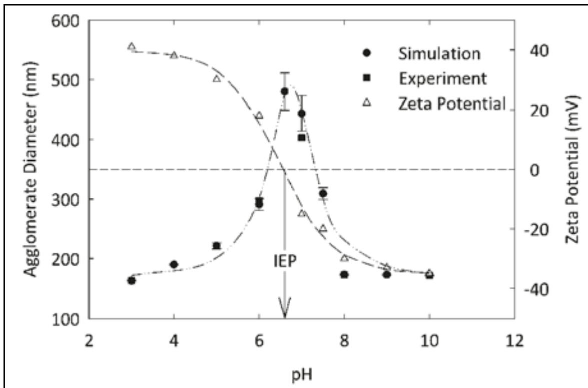

Figure S1. Logistic nature of the curves for variation of ζ with pH and the isoelectric point (IEP). Figure reprinted with permission from Liu et al.[(1)]. Copyright 2011 American Chemical Society.

Figure S1 (reproduced from Liu et al.[(1)]) shows the logistic nature of the curves for variation of ζ with pH and the isoelectric point (IEP). The IEP is the value of pH at which the surface zeta potential of particles dispersed in a medium becomes zero.

Reported zeta potential measurements (from Leo et al.[(2)]) corresponding to the *in vitro* measurements compared with the model predictions:

pH = 3	pH = 5	pH = 7
ξ = -18.2 mV	ξ = -22.5 mV	ξ = -32.5 mV

The values of ζ were used to fit a logistic curve as: $\zeta = \beta\left(\frac{1-e^{\lambda}}{1+e^{\lambda}}\right)$ where, $\lambda = pH - pI$ (S1)

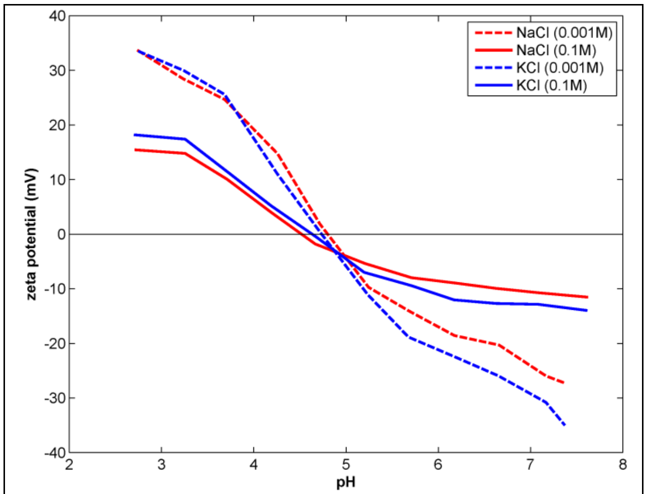

Figure S2. Data from Salgin *et al.*[3] for NaCl and KCl for 2 different ionic strengths 0.001M and 0.1M

where, pI = IEP = 1.75 and β = 37. β is the half of the range of the y-axis, i.e. the maximum value of zeta potential. Figure S2 shows data from Salgin et al.[3] for NaCl and KCl for 2 different ionic strengths 0.001M and 0.1M. Increase in ionic strength consistently reduces the range of zeta potentials, i.e. reduces the parameter β. Additional data in Salgin et al.[3] also point to the same fact. Eq. (S1) was fitted to the data in the Figure S2, and the values of β estimated are shown in Table S1.

Table S1. Data from Leo *et al.* 2013[2]

	KCl	NaCl	KCl	NaCl
I	0.001M	0.001M	0.1M	0.1M
β	42.46	35.64	20.02	16.37
$\log_{10} I$	-3	-3	-1	-1

Let the value of β at I = 0.1 be β_o. Then we can express β as:

$$\beta = -\beta_o(1+\log_{10} I)$$

The data in Table S1 (Leo et al.[2]) was fitted to Eq. (S1) to obtain, β_o = 37 (since the I for this set of experiments was close to 0.1M as shown in the next section). So we can show the final expression as:

$$\zeta = -\beta_o(1+\log_{10} I)\left(\frac{1-e^{\lambda}}{1+e^{\lambda}}\right) \quad \text{(S2)}$$

where β_o = 37 for the present set of particles and incubation medium.

Calculation of electric potentials

The attractive van der Waals' interaction potential is given by the expression proposed by Gregory[(4)] that accounts for the electromagnetic retardation effect:

$$\phi_A(h) = -\frac{Ar_i r_j}{6h(r_i + r_j)}\left[1 - \frac{bh}{\lambda}\log\left(1 + \frac{\lambda}{bh}\right)\right] \tag{S3}$$

Here, h is the distance between the surfaces of the agglomerates, A is known as the Hamaker constant with a value of 37×10^{-21} (for silver nanoparticles) , b is a constant with a value of 5.32, and λ is the characteristic wavelength for the reaction = 100 nm.

The repulsive interaction potential between the agglomerates can be expressed via the electric double layer (EDL) interaction potential equation that was developed using the Linear Superposition Principle by Gregory[(5)] as:

$$\phi_R(h) = 128\varepsilon\pi\left(\frac{k_B T}{Ze}\right)^2\left(\frac{r_i r_j}{r_i + r_j}\right)\gamma^2 \exp(-\kappa h) \tag{S4}$$

Here, ε is the permittivity of the medium, Z is the valence of ions in the medium, e is the elementary charge, k_B is the Boltzmann's constant, κ is the Debye-Hückel parameter, and γ is the reduced surface potential which is a function of the surface zeta potential ζ of the particles and is given by:

$$\gamma = \tanh\left(\frac{Ze\zeta}{4k_B T}\right) \tag{S5}$$

The Debye-Hückel parameter, κ is expressed in terms of the ionic strength, I of the medium as:

$$\kappa = \sqrt{\frac{2N_A I e^2}{\varepsilon k_B T}} \tag{S6}$$

where, N_A is the Avogadro's number.

Citrate oxidation rate

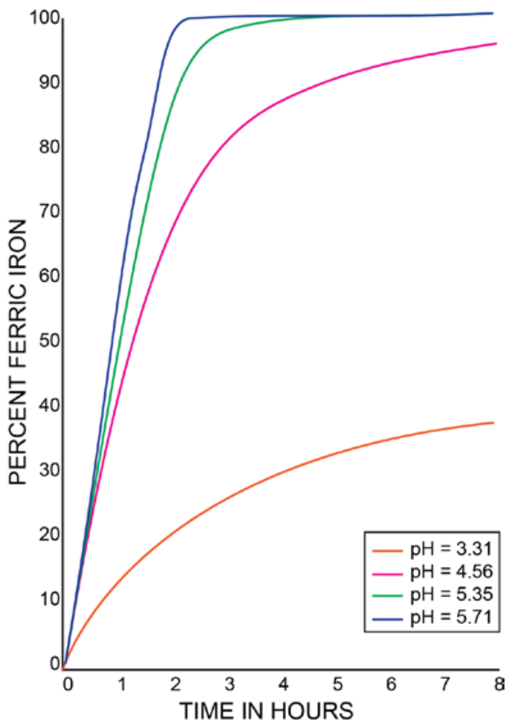

Figure S3. The effect of hydrogen-ion concentration on the rate of oxidation of solutions of ferrous citrate. Figure adapted from Bryan 1933[6] with permission from The Royal Society of Chemistry.

Figure S3 (adapted from Bryan[6]) plots rate of citrate oxidation as a function of pH.

Rate constants (% per hr) for citrate oxidation calculated as:

pH = 3.31	pH = 4.56	pH = 5.35	pH = 5.71
k = 12 h^{-1}	k = 35 h^{-1}	k = 45 h^{-1}	k = 50 h^{-1}

Ionic strength calculation

Ionic strength is affected by all the ions present in a solution and depends on the methods of preparation of a solution. Ionic strength, I, is defined as: $I = (1/2)\sum c_i z_i^2$, where c_i is the concentration of the i^{th} ionic species and z_i is the charge of that ion. Based on charge conservation, the total concentrations of positive and negative ions should be equal. The positive ions present in the solution are [H^+] and [Na^+], while the negative charges are [ClO_4^-], [OH^-], [H_2C^-], [HC^{-2}], and [C^{-3}].

So we have: $$[H^+]+[Na^+]=[ClO_4^-]+[OH^-]+[C^{-3}]+[HC^{-2}]+[H_2C^{-1}] \quad (S7)$$

For the citrate-stabilized nAg incubation study (Leo et al.[2]) that has been used to evaluate the model, the incubation solutions were prepared with 0.1M NaClO4 solutions (pH = 6) and then titrated with 0.1M HClO4 solution (pH = 1) for reaching pH values of 3 and 5 and titrated with 0.1M NaOH solution (pH = 12) for reaching a pH of 7. The ions from the citrate are considered in the next section. The ionic concentrations of all medium ions are summarized in Table S2 below:

Table S2. Ionic concentrations of all medium ions

	pH = 3	pH = 5	pH = 7
H^+	1×10^{-3}	1×10^{-5}	1×10^{-7}
Na^+	0.099	0.1	0.09989
$ClO4^-$	0.1	0.1	0.099
OH^-	1×10^{-11}	1×10^{-9}	1×10^{-7}

Ionic strength calculation for media used by Tejamaya *et al.*, 2012[(7)]

Salts	**Conc. (mg/L)**	**Mol. wt.**	**Molarity (mmol/L) (c_i)**	$c_i z_i^2$	**Ionic Strength (mol/L) ($\Sigma\, c_i z_i^2$)**
CM-1					
$CaCl_2.2H_2O$	294	147	2	12	0.0178
$MgSO_4.7H_2O$	123.25	246	0.501	4.008	
$NaHCO_3$	64.75	84	0.771	1.542	
KCl	5.75	74.5	0.077	0.154	
Na_2SeO_3	2	173	0.012	0.072	
NM-1					
$Ca(NO_3)_2.4H_2O$	472.25	236	2.001	12.006	0.0178
$MgSO_4.7H_2O$	123.25	246	0.501	4.008	
$NaHCO_3$	64.75	84	0.771	1.542	
KNO_3	7.5	101	0.074	0.148	
Na_2SeO_3	2	173	0.012	0.072	
SM-1					
$CaSO_4$	271.75	136	1.998	15.984	0.0218
$MgSO_4.7H_2O$	123.25	246	0.501	4.008	
$NaHCO_3$	64.75	84	0.771	1.542	
K_2SO_4	6.75	174	0.039	0.234	
Na_2SeO_3	2	173	0.012	0.072	

CM-10, NM-10, & SM-10 were media obtained by 10-fold dilution of the above media.

Protonation of citrate ions

Protonation of citrate ions is a process controlled by ionic equilibria. The equilibrium of various citrate species is shown below:

$$C^{-3} + 3H^+ \xrightleftharpoons{pKa_3} HC^{-2} + 2H^+ \xrightleftharpoons{pKa_2} H_2C^{-1} + H^+ \xrightleftharpoons{pKa_1} H_3C$$

Here, C denotes the citrate ion and so H_3C is citric acid and H_2C and HC are types of the citrate ion at different extents of protonation. The 3 *pKa* values for citric acid are: $pKa_1 = 3.13$, $pKa_2 = 4.76$, $pKa_3 = 6.4$.

Based on the Henderson-Hasselbach equation, we can write:

$$pH = pKa_1 + \log\left(\frac{[H_2C^{-1}]}{[H_3C]}\right) \tag{S8}$$

$$pH = pKa_2 + \log\left(\frac{[HC^{-2}]}{[H_2C^{-1}]}\right) \tag{S9}$$

$$pH = pKa_3 + \log\left(\frac{[C^{-3}]}{[HC^{-2}]}\right) \tag{S10}$$

The ionic concentrations of $[H^+]$, $[Na^+]$, $[ClO_4^-]$, and $[OH^-]$ are estimated in the previous section. So the total of citrate ions, [Cit], is given by:

$$[\mathrm{Cit}] = [C^{-3}] + [HC^{-2}] + [H_2C^{-1}] = [H^+] + [Na^+] - [ClO_4^-] - [OH^-]$$

So based on Henderson-Hasselbach equation, we can write:

$$\begin{aligned}\frac{[\mathrm{Cit}]}{[H_3C]} &= \frac{[C^{-3}]}{[H_3C]} + \frac{[HC^{-2}]}{[H_3C]} + \frac{[H_2C^{-1}]}{[H_3C]} \\ &= \frac{[C^{-3}]}{[HC^{-2}]}\frac{[HC^{-2}]}{[H_2C^{-1}]}\frac{[H_2C^{-1}]}{[H_3C]} + \frac{[HC^{-2}]}{[H_2C^{-1}]}\frac{[H_2C^{-1}]}{[H_3C]} + \frac{[H_2C^{-1}]}{[H_3C]} \\ &= 10^{pH-pKa_1}.(1+10^{pH-pKa_2}.(1+10^{pH-pKa_3}))\end{aligned} \tag{S11}$$

The value of [Cit] is calculated in the previous section. The value of H_3C can be calculated from Equation (S11). Then, the values of $[H_2C]$, [HC], and $[C^{-3}]$ can be calculated as:

$$[H_2C^{-1}] = [H_3C].10^{pH-pKa_1} \tag{S12}$$

$$[HC^{-2}] = [H_2C^{-1}].10^{pH-pKa_2} \tag{S13}$$

$$[C^{-3}] = [HC^{-2}].10^{pH-pKa_3} \tag{S14}$$

Thus the ionic strength, *I*, can be calculated once we have the concentrations of all ions in solution using the formula: $I = (1/2)\sum c_i z_i^2$.

Nanoparticle surface coverage

Table S3. Data from Siriwardane[8]

pH	NP diameter, d (nm)	Surface coverage, f (×10^{14}) (molecules/cm^2)
2	4	3.7
4	4	2.9
5.5	4	2.2
2	9	5.9
4	9	3.7
5.5	9	2.7
2	39	51.9
4	39	18.5
5.5	39	17.2
7.5	4	0.92

Initial fraction of NP surface area coated, f_{Ao} would be given by:

$$f_{Ao} = \frac{N_C . N_A . (\pi r^2)}{SA_T} \quad \text{(S15)}$$

where N_C is the total moles of citrate present, N_A is the Avogadro No., r is the radius of a citrate ion, and SA_T is the total surface area of the NPs.

Fraction of SA that is dynamically available for reaction can be estimated as:

$$f_A = f_{Ao} \frac{C_{Cit} / SA_T}{C_{Cit}^o / SA_T^o} = f_{Ao} \frac{C_{Cit}}{SA_T} \frac{1}{F_{coat}^o} \quad \text{(S16)}$$

where, C_{Cit} is the concentration of citrate ions in the medium and F_{coat} is the parameter quantifying the extent of surface protection as defined in the main body of the article as:

$$F_{coat} = \frac{\text{Total moles Citrate adhered to NPs}}{\text{Total SA of NPs in medium}} = \frac{n_{Cit}}{SA_T} \quad \text{(S17)}$$

The value of f is approximately linear for every value of pH

pH	Relation	Slope, m
2	f = 1.432d - 4.315	1.432
4	f = 0.4623d + 0.353	0.4623
5.5	f = 0.4477d - 0.393	0.4477

So the variation of slope, m with pH (Figure S6) is:

$$m = 63.53 \exp(-2.083 pH) + 0.447 \quad \text{(S18)}$$

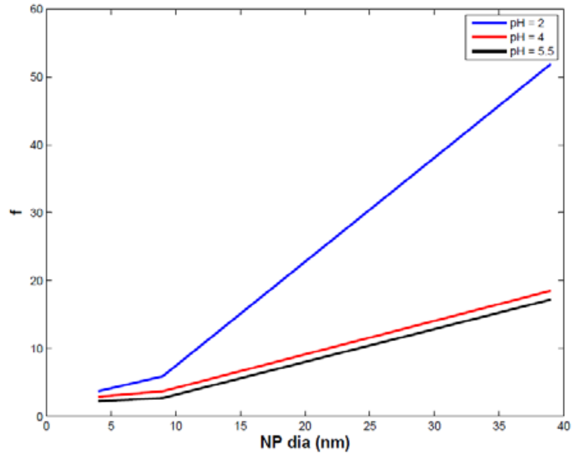

Figure S4. Variation of *f* with NP diameter for various values of pH

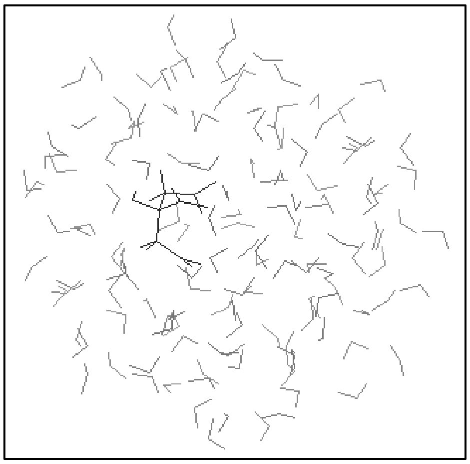

Figure S5. Citrate ion surrounded by a water ball of 10 A^{o}. Figure reproduced with permission from Parikh[9].

Accordingly, the effective ionic diameter of the citrate ion was assumed to be ~3 A^{o}

The intercept is very close to zero (Figure S4) for higher values of pH.

So for pH = 7, we can select the intercept as zero.

So $f = 0.447d$.

So the values of *f* for the citrate stabilized nAg can be estimated as:

NP	Mean dia.(nm)	*f*
Ag20	20	8.94
Ag50	50	22.35
C20	20	8.94
C110	110	49.17

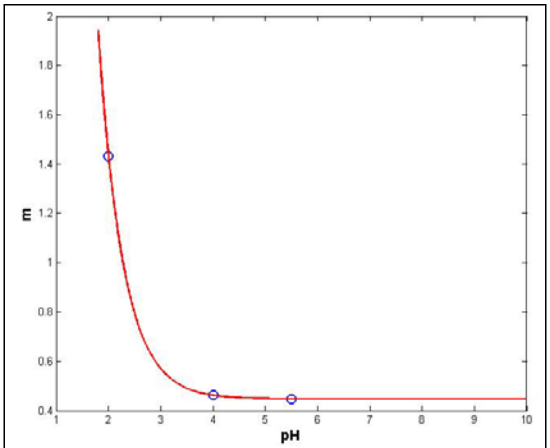

Figure S6. Variation of slope, *m* with pH

Nanoparticle surface coverage (PVP)

For PVP coated nAg, we have the PVP/Ag mass ratio from nanoComposix (www.nanoComposix.com)

NP	NP density (g/cm^3)+	PVP/Ag mass ratio*	PVP MW	Particle conc.** (NP/mL)	f ($\times 10^{14}$) ($molecules/cm^2$)
P20	10.87	20:1	10 kD	2.1×10^{13}	43.58
P110	10.49	10:1	40 kD	1.9×10^{11}	28.96

* www.nanocomposix.com/products/silver/dried
** Particle Spec Sheet - nanoComposix
\+ Ag-Au nanoComposix-13Nov2012.pdf

Calculation for P20:

Material conc. = $2.1\times10^{13} \times (\pi/6)(20)^3 \times 10^{-21} \times 10.87$ g/mL = 0.956 mg/mL

PVP conc. = $0.956 \times 20 = 19.12$ mg/mL

No. of PVP molecules per mL = $(19.12\times10^{-3}/10000) \times 6.023\times10^{23} = 1.15\times10^{18}$

PVP molecules per NP = $(1.15\times10^{18})/(2.1\times10^{13}) = 5.48\times10^{4}$

PVP molecules per cm^2 = $(5.48\times10^{4})/(400\pi\times10^{-14}) = 43.58\times10^{14}$

References

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