Measuring the Content of a Single Liposome via Electrocatalytic Nano-impact "Titrations"

W. Cheng and R. G Compton*

Abstract: We show the electrochemical determination of attomole glutathione contents within single individual liposomes at low overpotentials through mediated electron transfer using copper (II) as a catalyst for the oxidation of liposomal glutathione. A "titration-like" behaviour of individual liposomes was observed when impacting with the electrode to quantify the content within single liposomes. The nanoimpacts "titration" strategy allows the characterisation of nanoparticles containing redox inactive molecules at the single nanoparticle level.

Liposomes based nanocarriers have numerous applications in biomedicine [1] but their contents are generally quantified by ensemble measurements averaged over wide populations[2]. The real-time quantification of drug content at the single liposome level was impossible until recently when we showed the direct oxidation of ascorbic acid encapsulated within single liposomes when they impacted on electrodes[3]. Subsequent studies implemented a similar strategy to quantify redox molecules within liposome-like soft nanoparticles[4-6]. These studies include the quantification of nanosized vesicles from living systems containing redox active contents or directly monitoring nano-impacts of these vesicles in the intracellular environment[4], of redox active made droplets[5], and of, it is claimed, redox enzymes[6]. In the nanoimpacts method, individual nanoparticles randomly collide with an electrode held at a suitable potential by virtue of Brownian motion to induce direct oxidation or reduction of the nanoparticle, or reaction catalysed by the nanoparticle[7]. The nano-impacts method has been widely applied to characterise single "hard" and "soft" nanoparticles [8].

Hitherto, all these studies of "direct impacts" involve either electroactive nanoparticles or soft nanoparticles filled with electroactive molecules, where charge transfer occurs from direct oxidation or reduction of nanoparticles or redox molecules filled nanoparticles when they collide at electrode surfaces. This limits this otherwise very powerful strategy to studying nanoparticles of certain types. The characterisation and quantification of single nanoparticles containing redox inactive or less electroactive contents that are difficult to oxidise or reduce under normal conditions has not yet been demonstrated.

In this report, using glutathione (GSH) encapsulating liposomes (Figure 1) as a model system we show the determination of less

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electroactive contents within single individual liposomes at low overpotentials (0.1 V vs. MSE (mercury-mercurous sulfate electrode)) in neutral electrolytes through mediated electron transfer using copper (II) present in bulk aqueous solution as a catalyst for the oxidation of GSH. Specifically we observe a "titration-like" behaviour of individual liposomes when impacting with the electrode in the presence of varying increasing amounts of Cu (II) and have quantified the attomole GSH content within single liposomes. We believe this is the first application to investigate otherwise redox inactive molecules at the single liposome level by using a meditated charge transfer. This mediated charge transfer coupled with the new "titration" strategy opens a door for the detection and characterisation of non-redox active nanoparticles at the single nanoparticle level and may drive the nano-impact technique to characterise single nanoparticles of a much greater diversity.

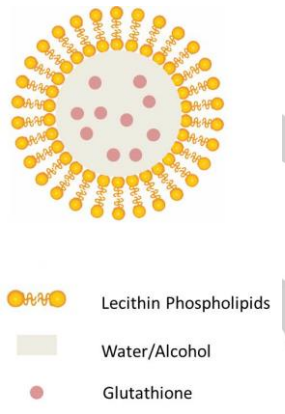


Figure 1 Schematic structure for GSH liposomes

First, the redox behaviour of an ensemble of GSH liposomes was studied by cyclic voltammetry recorded for GSH liposomes dropcast onto a macro glassy carbon electrode. There is no oxidation peak of GSH on the electrode (Figure 2) within the potential range of study, indicating that GSH is non electroactive within the potential range of -0.40 V to 0.10 V vs. MSE under the experimental conditions employed. Cyclic voltammetry was then recorded in the presence of copper (II) in the solution. There is a significant oxidation peak at -0.1 V vs. MSE (Figure 2), suggesting the oxidation of GSH is mediated by copper (II), as shown in Scheme 1, where GSSG is glutathione disulfide; Literature suggests the copper (II)-GSH complex compound ($[Cu{-}SG]^+$) was formed as an intermediator followed by oxidation of the complex[9].

$$Cu^{2+} + GSH \xrightarrow[-H^+]{} [Cu\text{-}SG]^+ \longrightarrow \tfrac{1}{2}\,GSSG + e^- + Cu^{2+}$$

Scheme 1

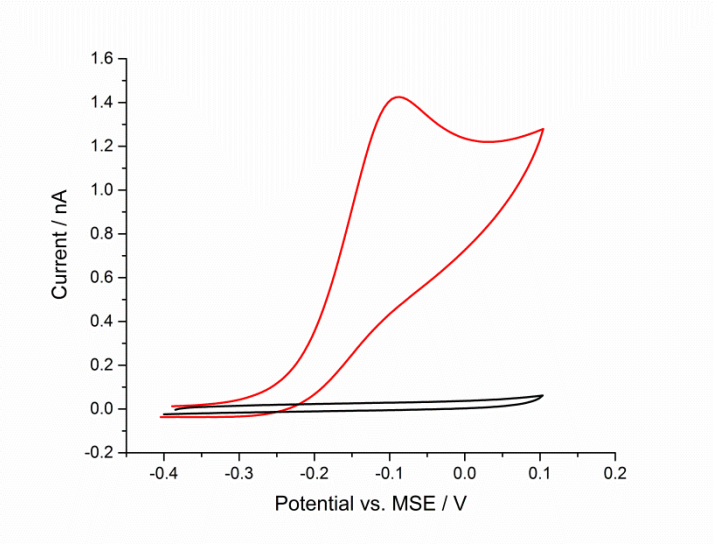
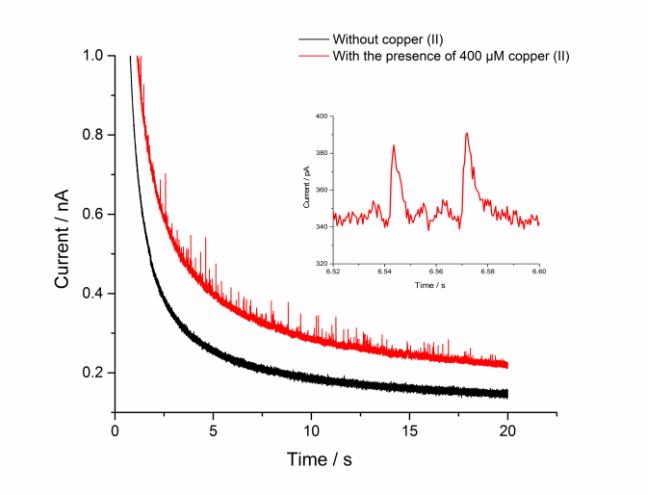


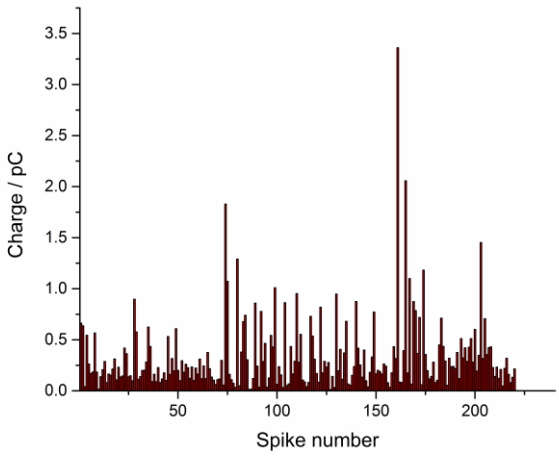
Figure 2 Cyclic voltammograms recorded for a glassy carbon macroelectrode surface modified with GSH encapsulated liposomes in 100 mM KNO_3 at 50 mV s^{-1} in the presence (red) and absence (black) of 400 µM Copper (II) nitrate.

No oxidative peak and a similar oxidative peak position were observed for GSH dissolved in the solution without and with the presence of copper (II), respectively (See Figure S2). A voltammetric method was made to determine the total amount of GSH in a liposome suspension by referring to the calibration of free GSH (see Figure S3). The observation also confirms the established character alone. No peak was observed on the backward scan and this indicates the electro-oxidation of GSH is chemically irreversible.

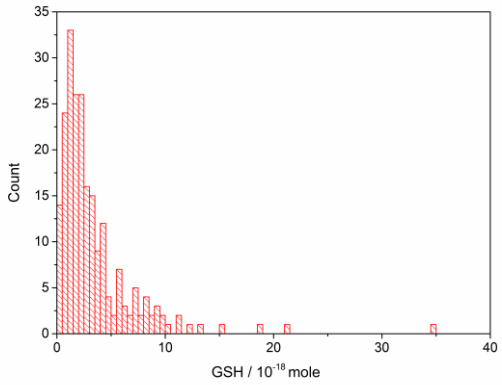
Next, a clean carbon micro electrode was placed in 100 mM potassium nitrate and a known concentration of dispersed liposomes added. In the absence of Cu^{2+}, no spikes are observed at any potential in the range of -0.40 V to 0.10 V vs. MSE, suggesting that the GSH containing liposome is not oxidised. In the presence of 400 µM copper, clear oxidative spikes were observed under potentiostatted conditions at + 0.10 V vs. MSE (Figure 3), a potential significantly higher than the oxidative potential observed for GSH (as confirmed by the data in Figure S2) to ensure the complete oxidation, while no spikes was observed at oxidation potentials lower than -0.2 V. The observation suggests the onset of these spikes was dependent on oxidation potentials and the spikes seen at + 0.10 V vs. MSE may result from the Faradaic oxidation of the GSH containing liposomes. Figure 3a shows a typical chronoamperometric profile of oxidative Faradaic spikes of individual GSH liposome at + 0.10 V vs. MSE, attributed to the oxidation of encapsulated GSH mediated by copper (II) when the liposome collides with the electrode (Figure 4).



(a)



(b)



(c)

Figure 3 (a) Chronoamperometric profiles showing oxidative Faradaic spikes of GSH encapsulated in single liposomes in the presence (red) and absence (black) of 400 µM copper (II) in 100 mM KNO_3 at 0.10 V vs. MSE; The inset shows the detailed impact spikes (b) Counting and oxidative charge from copper mediated GSH oxidation of 220 single individual liposomes (c) Counting and inferred molar content of GSH encapsulated in 220 single individual liposomes from charge per current spike from nano-impact experiments (Figure 2b) according to Equation 1.

A control experiment was conducted at the potential of 0.1 V vs. MSE with no liposomes in the solution and no spikes were detected, suggesting that the occurrence of oxidative spikes is due to the random collisions of liposome with the electrode and subsequently oxidation of released GSH catalysed by copper (II). In the presence of 400 mM copper (II), the charge transferred during oxidation of GSH within individual liposome impacts was collected from 220 Faradaic spikes, corresponding to the impacting and oxidation of 220 single individual liposomes on the electrode (Figure 3a). The average charge (Q_{v1} = 0.33 ± 0.03 pC) gives the average mole amount of GSH within single liposomes (m_1 = 3.4 ± 0.3 attomole) (Figure 3b), with a modal mole amount of 1.25 attomole GSH (Figure 3c), according to Equation 1.

$$m = \frac{QM}{nF}$$

Equation 1

where Q is measured charge, F is the Faraday constant, the parameter n is the number of electrons transferred per molecule during oxidation (n = 1 for copper (II) mediated GSH oxidation), m is the mole contents of GSH encapsulated in single liposome

This oxidation of the content of single liposomes during collision events has been observed for the first time through *mediated* electron transfer to show the possibility to measure the contents of non-electroactive molecules encapsulated within single soft nanoparticles by means of indirect electron transfer. Moreover, the observation clearly demonstrates the essential need for Cu^{2+} to be present for GSH to undergo oxidation at electrodes as no signal are seen with the absence of Cu^{2+} either for dissolved GSH or liposome contained GSH.

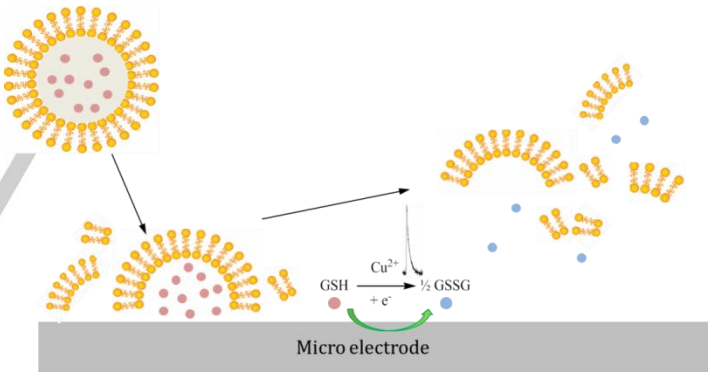


Figure 4 Schematic showing oxidation of encapsulated GSH mediated by copper (II) when the liposome collides with the electrode.

To investigate whether the oxidation of GSH contents of single liposomes is total or partial, analogous nano-impacts experiments were conducted over a wide range of copper amounts (from 10 µM up to 800 µM). Clear oxidative

spikes were observed under potentiostatted conditions at + 0.10 V vs. MSE in the presence of varying [Cu^{2+}], with spikes of lower amplitudes seen in the presence of lower [Cu^{2+}] (Figure S4, S5). The average charge resulting from oxidation of individual GSH containing liposomes at varying [Cu^{2+}] was calculated by integrating the area of each spike and plotted against [Cu^{2+}], as shown in Figure 5. A "titration-like" quantification process was observed with a plateau reached for [Cu^{2+}] >320 µM, suggesting under these conditions the oxidation of liposome contents becomes quantitative. At low concentrations, the low levels of Cu^{2+} (<320 µM) lead to a partial oxidation of GSH molecules, while at high concentrations, the titration curve is flat above the equivalence point (EP), because excess titrant is present (Cu^{2+} > 320 µM) and all the GSH molecules are oxidised. Note that Fig 5 is essentially formed of two linear sections which cross at the EP. The linearity results from the 1:1 consumption of the Cu^{2+} and GSH. The charge transferred per GSH molecule increases with the increasing amounts of titrant until complete oxidation of GSH molecules within single liposomes arise when there is sufficient amount of titrant to catalyse the oxidation of the total content of GSH containing within the liposome.

We believe the single liposome "titration" strategy offers new opportunities to electrochemically "titrate" the attomole content of molecules within individual liposomes or other liposome-like soft nanoparticles where direct reduction or oxidation of encapsulated molecules is not possible.

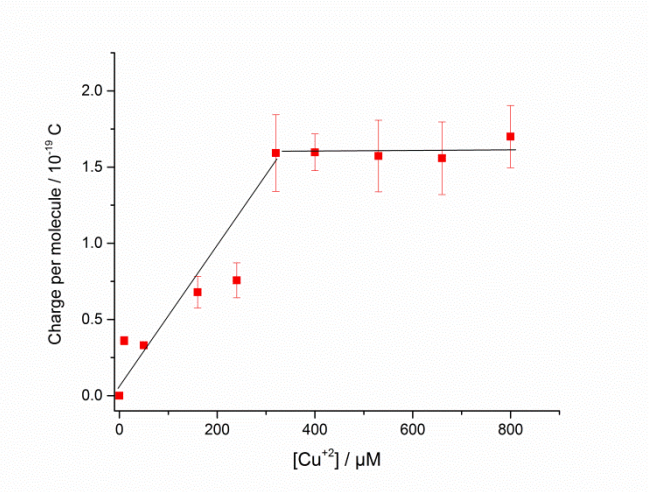


Figure 5 The average charge transferred per molecule during oxidation of GSH mediated by varying amounts of Cu^{2+} when single GSH liposome impact the electrode under potentiostatted conditions at + 0.10 V vs. MSE.

To conclude, using GSH liposomes as a model system, we show liposomes containing non-electroactive contents can be quantified in real time at the single individual liposome level by nanoimpact "titrations". To our knowledge, this is the first time otherwise redox inactive contents filled nanoparticles have been quantified by indirect oxidation through catalyst mediated charge transfer. The nanoimpacts "titration" will allow characterisation of nanoparticles containing redox inactive molecules at single nanoparticle level and will attract yet further significant applications of nanoimpacts to study single nanoparticles.

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Keywords: Liposome • Electrochemistry • Nanoimpacts • Redox Inactive • Nanoparticle •

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COMMUNICATION

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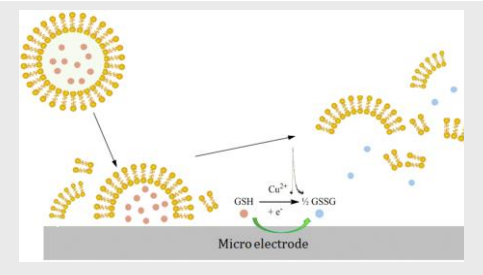

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