Potentiometric Differentiation of Mono- and Macromolecular Thiol within Human Plasma at Carbon Fiber Electrodes

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Plasma thiol (PSH) concentrations have been suggested as a potential diagnostic aid through which the prognosis and treatment of various injuries and diseases could be enhanced.[1–4] It must be acknowledged, however, that much remains to be done to ascertain the biomedical value of such measurements to specific conditions. Clinical PSH values typically encompass monomolecular (cysteine, glutathione) as well as macromolecular (principally albumin) SH moieties.[5] Metabolomic strategies, however, demand a more rigorous appraisal of thiol speciation where the variation in concentration of either group can be more effectively associated with particular pathophysiological conditions. This could enhance disease identification and the early onset of complications, thereby aiding subsequent treatment. Much rests on the premise that such measurements could be easily and speedily conducted within "point of care" (POC) environments such that the diagnostic advantage is not lost. This communication describes an electromolecular approach that holds some promise for fulfilling such demands and is based on the reaction of a redox probe toward PSH and the subsequent discrete potentiometric interaction of the components at random arrays of carbon fiber electrodes.

Carbon fiber matting thermally sandwiched between the sleeves of a pre-punched commercial lamination pouch (Figure 1A) was primarily used as a facile method of preparing disposable working electrodes of defined area.[5] Mechanically drilling through a carbon fiber mat completely encapsulated within laminate, however, enables the production of carbon ring electrodes (Figure 1B). The sensing component in this instance is predominantly composed of a random array of microelectrodes[6] and represents a new route through which such electrodes can be produced within conventional laboratory environments. Cyclic voltammograms detailing the response of each electrode design to 2 mM ferrocyanide are contrasted in Figure 2A and confirm the microelectrode characteristics of the carbon-ring laminate.[6]

In this instance, however, the potentiometric response is of more immediate concern, and a naphthoquinone (NQ) indicator system has been used for the quantitation of plasma thiol. The basic reaction (detailed in Figure 1) involves a 1,4-Michael addition of the RSH to NQ (I), resulting in the corresponding reduced NQ–SR conjugate (II).[7,8] This creates a discrepancy in the relative concentrations of oxidized/reduced species within the sample, which is detected by the change in potential at the electrode using conventional potentiometric techniques.[8] The typical response of the ring electrode to glutathione (GSH) in the presence of NQ is shown in Figure 2B. The potential decreases upon the addition of thiol and then stabilizes. The magnitude of the change is dependent upon the concentration of GSH (Figure 3) and follows a logarithmic form
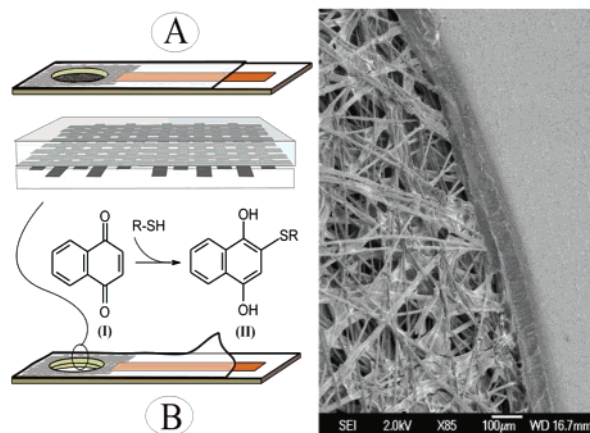
Figure 1. Electrode design and thiol detection strategy. Scanning electron micrograph examining the laminate–fiber interface.
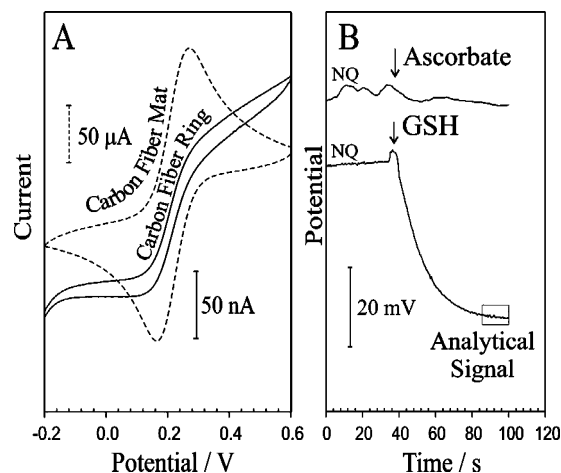
Figure 2. (A) Voltammograms detailing response to ferrocyanide (2 mM). (B) Potentiometric response of ring electrode to 100 μM ascorbate and 25 μM glutathione in the presence of NQ indicator (1 mM, pH 7).

($E = -13 \log[\text{PSH}] - 224.2$, $N = 6$, $R^2 = 0.997$). The reaction is specific for RSH as alkyl amino functionalities are protonated at physiological pH (pK_a 9–11) and hence possess little nucleophilic capability for interference.[7,8] Ascorbate and urate were also examined and not found to exert any influence on the response.

While a similar set of responses were observed at the bulk fiber mat electrode, the introduction of human plasma, our target sample, led to a significant discrepancy in the response characteristics between the two types of electrode. The profiles obtained are detailed in Figure 4A. The fiber mat electrode profile is analogous to that observed with GSH, whereas the carbon ring did not respond even after substantial additions of plasma (100 μL).
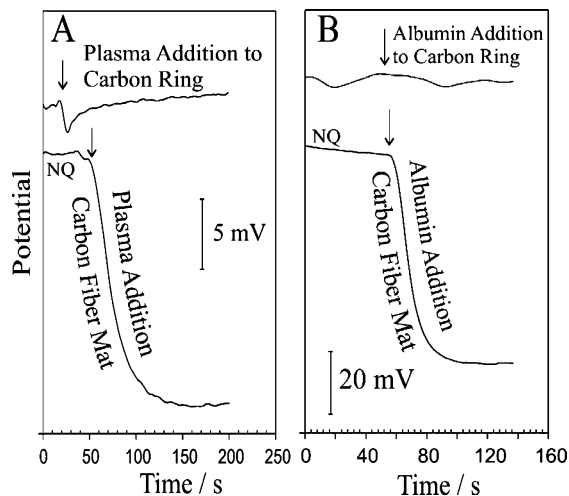
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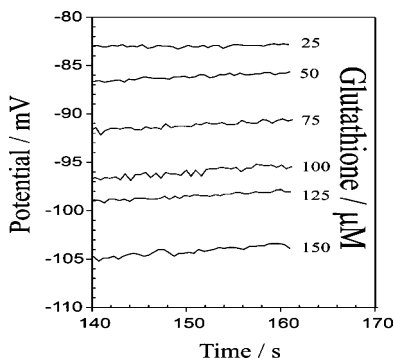
Figure 3. Steady-state response of carbon ring electrode to increasing concentration of GSH in the presence of NQ indicator (1 mM, pH 7).


Figure 4. Influence of (A) human plasma and (B) bovine serum albumin (1.2 mg/L) on the steady-state responses of carbon ring and carbon mat electrodes in the presence of NQ indicator (1 mM, pH 7).

The interaction of the electrodes to albumin-SH (AlbSH), the major component of plasma thiol (typically $400-500 \mu$M),[1-4] was further investigated, and the responses were compared in Figure 4B. Again, the carbon ring fails to respond, whereas the fiber mat produces a profile analogous to that observed with the plasma addition. To ensure that the response observed is, in fact, analytically relevant, the carbon mat electrode was used to quantitatively evaluate the concentration of plasma thiol within the human plasma of diabetic and nondiabetic control subjects. The PSH measurement within the former group has been proffered as a possible indicator in assessing their susceptibility to complications.[1-4] Plasma samples were added to the NQ indicator, and the potentiometric response was recorded. The concentration of PSH was evaluated from the calibration data previously determined at the carbon mat electrode with the results tabulated opposite. These were validated against the conventional Ellman's spectroscopic procedure and show an excellent correlation that is consistent with typical human PSH levels[3] and highlights the potential efficacy of the electrode format to this important area.The carbon mat electrode effectively detects both monomolecular (i.e., GSH) and macromolecular (AlbSH) species, whereas the ring electrode responds principally to the former. This discrepancy could be attributed to the AlbSH response being primarily a surface effect and is tentatively supported by the saturation of the electrode response at high concentrations of the

Table 1. Preliminary Comparison of Electrochemical Assay in Human Plasma Samples ($N = 3$, RSD $< 10\%$)

diabetic patients	PSH/mM	
	1	2
carbon mat electrode	0.52	0.48
Ellman's assay	0.51	0.34

nondiabetic controls	PSH/mM	
	1	2
carbon mat electrode	0.55	0.71
Ellman's assay	0.49	0.65
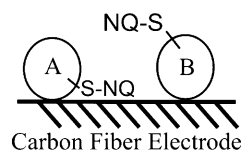
Figure 5. Possible interaction of AlbSH at carbon fiber electrode.

former. It could be anticipated that the protein adsorbs to the electrode surface with the NQ redox entity located either at the electrode interface (Figure 5A) or orientated toward the bulk solution (Figure 5B).

This will occur at both electrode designs, but the massively increased area of the mat electrode ensures a greater proportion of the interfacial NQ being present and hence leading to a visible response. The ring RAM electrode retains a response to GSH despite the adsorption of AlbSH as the GS$-$NQ conjugate is able to penetrate through the protein layer. This is not observed with the current plasma sample as the concentration of the monomolecular species is far lower (typically micromolar) than AlbSH[1-4] and is below the detection limit for the present system. It could be envisaged that by using a combination of both electrodes, information on total PSH and albumin could be obtained, whereas by subtraction, monomolecular PSH could be obtained. The simple design and detection strategy provides a versatile option for biomedical scientists to explore the diagnostic value of PSH directly within POC environments.

Supporting Information Available: Electrode fabrication, cell operation, and clinical trial data. This material is available free of charge via the Internet at http://pubs.acs.org.

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