Pyrroloquinoline quinone (PQQ) is a redox cofactor found in the active sites of a number of enzymes. Most of these quinoproteins are dehydrogenases capable of catalyzing the oxidation of a broad range of substrates, such as alcohols, acids, aldehydes and sugars.\(^1\) PQQ-dependent glucose dehydrogenase, for example, is capable of oxidizing several mono- and disaccharides, including glucose, maltose, lactose, galactose, xylose and mannose.\(^1\) The uniqueness of the quinoproteins and their broad substrate specificities have led to their use in biochemical applications, including biosensors and biofuel cells.

In the absence of an enzyme, PQQ itself is capable of catalyzing redox reactions, including the oxidation of thiols,\(^2\) amino acids,\(^3\) and the redox cofactor nicotinamide adenine dinucleotide (NAD\(^+\)).\(^5\) The electrochemistry of PQQ has been studied quite extensively in both solution and immobilized forms via cyclic voltammetry (CV) and potentiometric titration studies.\(^6,7\) These studies can be challenging due to the pH dependence of the redox potentials.\(^8,9\) Using a cystamine-monolayer Au-modified electrode, Katz et al.\(^9\) studied the electrochemistry of PQQ in solution using CV and reported the change in potential with pH to be about 60 mV pH\(^{-1}\). In another study using PQQ immobilized on few-walled carbon nanotube (FWCNT)-modified glassy carbon electrodes, the change in PQQ’s redox potential was estimated to be approximately 67 mV pH\(^{-1}\), with the highest reversible reduction-oxidation observed at pH 2.\(^6\)

The nature of the modified electrode surface can also affect PQQ’s redox chemistry. The FWCNTs employed by Kanninen et al.\(^6\) were modified with carboxylic acid groups (-COOH), which will deprotonate and become more negative as the pH rises. Since PQQ also becomes more negative with pH increases due to its three carboxylic acid groups (i.e. \(-1 \leq \text{pH} \leq 4\), \(-2 \leq \text{pH} \leq 5\), and \(-3 \leq \text{pH} \leq 5\)), it makes sense that optimal activity was observed at low pH where repulsive interactions would be minimized because both the modified electrode surface and PQQ would be less negative. Similarly, in the case of the cystamine-modified Au electrode,\(^6\) the best reversible redox process of PQQ was achieved at pH \(\leq 7\), when the amino groups of the cystamine monolayer are at least partially protonated, allowing a slightly positive electrode surface to attract negatively-charged PQQ. While low pH is optimal for PQQ itself, these conditions are not likely to be compatible with proteins that utilize PQQ as a cofactor.

The standard redox potential of PQQ is \(-0.125\) V vs. SCE at pH = 7.\(^9\) However, the measured formal potentials reported in many studies deviates widely from the standard potential.\(^9\) It could be argued that the type of modified electrode, as well as the working buffer used in the studies, may play some role in the shifted potentials. Studies on PQQ electrochemistry have typically been carried out in phosphate buffers at pH 7. Phosphate can chelate many divalent metals, particularly Ca\(^{2+}\) and Mg\(^{2+}\). These divalent metal ions play important roles in the structure and catalysis of protein enzymes. They can also affect how PQQ binds to other functional biomolecules, such as aptamers, and can be responsible for maintaining or enhancing the catalytic ability of the PQQ-biomolecule complex.\(^10,13\) Thus using phosphate buffers to study the electrochemistry of protein or nucleic acid bound-PQQ could present some challenges. Using non-phosphate, physiological buffers could overcome these issues and also expand our understanding of the electrochemical behavior of PQQ in solution.

Another challenge encountered when studying PQQ’s reduction-oxidation properties is its strong adherence to metal surfaces, leading to irreversible redox processes on bare gold, platinum, and glassy carbon electrodes.\(^14\) This problem is overcome by the use of an interface material between PQQ and the electrode. Modifying platinum electrodes with polypyrrole led to reversible oxidation and reduction of PQQ in solution at pH 7.\(^14\) When PQQ was also entrapped in the conductive polypyrrole matrix, the reversible redox process was retained. Cystamine-modified gold electrodes show quasi-reversible reduction-oxidation of both solubilized and immobilized PQQ in neutral and acidic conditions.\(^6\)

Carbon nanotubes have also been used to modify electrode surfaces and, when functionalized, can be used to immobilize PQQ on electrode surfaces. For example, PQQ immobilized on single-walled carbon nanotube (SWNT)-modified glassy carbon electrodes could detect thiols.\(^2\) Although tethering or immobilizing PQQ on the modified electrode may ensure that PQQ is near the surface for effective direct electron transfer, the electron transfer process is not necessarily improved over that of solubilized PQQ. In fact, covalently immobilizing PQQ to an electrode could lead to different orientations.
of PQQ on the surface. Improper orientation of PQQ could affect its reversible oxidation process. Kanninen et al. reported the physical adsorption of PQQ to single-walled and few-walled carbon nanotube (FWCNT)-modified glassy carbon electrodes. Multi-walled carbon nanotubes (MWCNTs) are commercially available in different lengths and with different functionalities, which makes them useful for electrode surface modification for improved electrochemical response and for biomolecule immobilization. To date, we are unaware of any reports on the use of multi-walled carbon nanotube (MWCNT)-functionalized electrodes to study the electrochemistry of solubilized PQQ.

In the current work, we expanded upon previously reported PQQ studies to investigate the electrochemistry of solubilized PQQ using glassy carbon electrodes functionalized with two different lengths of carboxyl-modified MWCNTs in various physiological buffers. In addition to carboxyl-modified MWCNTs, we also utilized amine-modified MWCNTs to study how different functionalization influences PQQ’s redox activity. The results of these studies will provide greater flexibility in biofuel cell and biosensor development by expanding the possible buffer conditions that can be used with PQQ-utilizing biomolecules in electrochemical studies. These studies will also improve our understanding of the effect of MWCNT electrode modification on PQQ’s redox processes.

Materials and Methods

Reagents and materials.—PQQ was obtained from Berry & Associates, Inc (Dexter, MI), TRIS, MOPS, potassium phosphate monobasic, and potassium phosphate dibasic were purchased from Research Products International Corp. (Mt. Prospect, IL) and HEPES was purchased from Sigma-Aldrich (St. Louis, MO). All buffers were used as received. All electrochemical measurements were performed with a Model 810 Electrochemical Analyzer (CH Instruments, Austin, TX).

Preparation of modified electrodes.—An 8 mg/mL suspension of each functionalized-MWCNTs was made by dispersing 40 mg of the nanotubes in 5 mL of DMF and sonicating for approximately 4 h. Glassy-carbon electrodes (GCE) were polished successively with 1.0 μm, 0.05 μm, and 0.005 μm alumina slurries on microcloth and nylon polishing pads (Buehler, Lake Bluff, IL). The polished GCEs were then sonicated in Millipore water, followed by sonication in ethanol. Each sonication was for 5 min and the process was repeated before the electrodes were allowed to dry at RT. Each GCE was cast twice with 10 μL of the MWCNT suspension, allowing ample drying time prior to the second cast. The electrodes were allowed to thoroughly dry and were used immediately or kept at 4°C until use.

Electrochemical characterization.—The modified working electrodes were characterized by cyclic voltammetry (CV) using a conventional, three-electrode system. The counter electrode was platinum mesh and a saturated calomel electrode (SCE) was used as the reference electrode. All reported potential values are with reference to the SCE. MWCNT-modified GCEs were allowed to incubate in 25 mL of appropriate buffer containing 50 μM PQQ for ~3 h prior to running cyclic voltammetry experiments. For HEPES, MOPS, and Tris, buffer concentration was 100 mM and the pH was 7. These buffers also contained 200 mM KCl, 10 mM CaCl$_2$, and 50 mM MgCl$_2$, unless otherwise noted. The phosphate buffer was prepared using potassium phosphate for a final concentration of 100 mM and a pH of 7. Additional KCl was added to bring the final concentration of K$^+$ to 200 mM and divalent metal ions were omitted. Each buffer was sparged with nitrogen for at least 20 min prior to introducing the electrodes for analysis. CV measurements were recorded at a potential scan rate of 0.05 V/s and potential window of +0.3 to −0.6 V, except where noted.

Results and Discussion

Effect of buffer composition on PQQ electrochemistry.—The effect of pH on the electrochemistry of PQQ has been reported by several groups. Generally, a reversible electrochemical process is observed at pH ≤ 7, although the nature of the modified electrode surface is a key determinant of the pH effect. In addition, the redox properties are also affected by whether the PQQ is covalently immobilized on the electrode surface or free in solution and only adsorbed to the electrode surface.

Electrochemical studies with biomolecules commonly use phosphate buffers. These buffers may be problematic due to phosphate’s ability to chelate divalent metal ions, which are commonly required for enzyme activity and functional nucleic acid structure. Phosphate buffer has also been shown to decrease the redox ability of PQQ, specifically toward the oxidation of NAD(P)H. Thus, we set out to investigate alternative buffers that are compatible with enzymes and nucleic acids. We focused our efforts on HEPES, MOPS, and TRIS buffers. All buffers contained 10 mM CaCl$_2$ and 50 mM MgCl$_2$, and 200 mM KCl, except for our phosphate buffer, which did not contain the divalent metal ions. These metal ion conditions were chosen based on our recent report of DNA aptamers for PQQ, but also represent conditions that could be used with other biomolecules. Calcium is well-known to enhance the enzyme activity of several PQQ-containing dehydrogenases, and has been investigated with immobilized PQQ on gold and platinum electrodes. Ca$^{2+}$ and Mg$^{2+}$ were omitted from the phosphate buffer because of uncertain free concentrations due to potential chelation.

Figure 1 shows voltammograms of PQQ reduction-oxidation in various pH 7 buffers, using GCEs modified with the two different lengths of carboxyl-functionalized MWCNTs. Both lengths of MWCNTs-COOH were able to reversibly reduce PQQ in all four buffers, although there are slight differences in voltammograms among the buffers. These differences are more evident in the voltammograms for the long MWCNTs due to the sharper peaks (Figure 1a). For the short MWCNTs, the only key difference among the buffers is in the slight negative shift in reduction potential of PQQ in TRIS and phosphate buffers (Figure 1b). TRIS buffer also displayed overall lower peak currents with both MWCNT lengths.

The measured reduction peak currents and differences in anodic and cathodic peak potentials (ΔEp) are summarized in Table I. PQQ undergoes a 2-electron, 2-proton transfer process. If we define the best performing buffer based on the smallest deviation from Nernstian behavior (ΔEp of approximately 30 mV for n = 2), we observe similar trends for both the short and long MWCNTs. For the long MWCNTs, HEPES, MOPS, and TRIS have similar ΔEp while phosphate buffer showed a larger ΔEp. For the short MWCNTs, HEPES has the smallest ΔEp of ~100 mV. The other buffers had similar, slightly larger ΔEp. The similar results in these different buffers indicate that the buffer compounds themselves are not likely forming complexes with PQQ and causing differences in redox activity. The results also indicate that different biological buffers can be used with PQQ, which could improve the biological activity of PQQ-dependent enzymes in electrochemical applications.

Effect of buffer ionic composition on PQQ electrochemistry.—In the studies presented in Figure 1, the phosphate buffer lacked added divalent metal ions due to possible chelation. Ca$^{2+}$ in particular is commonly found in the active site of PQQ-dependent enzymes and has previously been shown to enhance the ability of PQQ-modified gold and platinum electrodes to electrocatalytically oxidize NADH and NADPH in TRIS buffer. To explore the effect of added ions on the electrochemistry of PQQ in solution, we also carried out CV experiments using MWCNTs-COOH-modified electrodes in plain HEPES buffer containing 200 mM NaCl, 10 mM CaCl$_2$, and 50 mM MgCl$_2$. Unlike the phosphate buffer, these conditions exhibited a faradic signal, with ΔEp of ~150 mV at pH 7, corresponding to a +0.7 V peak potential. This indicates that divalent metal ions are present in solution, which is consistent with our previous observations on the effect of pH on the electrochemistry of PQQ.
buffer containing 50 μM PQQ but no supporting electrolyte. HEPES was chosen because it slightly outperformed other buffers in our initial study. While our previous results using a buffer containing Ca\(^{2+}\), Mg\(^{2+}\), and K\(^{+}\) showed PQQ redox activity (Figure 1), we observed no reduction or oxidation peaks for PQQ in the plain buffer, suggesting that at least one of our added ions is necessary for PQQ’s redox activity in HEPES buffer. This raised the question as to whether Ca\(^{2+}\), Mg\(^{2+}\), or K\(^{+}\) alone or in some combination were sufficient to support the reversible reduction-oxidation of PQQ in solution. Table II details the buffers used to test the effects of ionic composition.

The Plain H buffer did not support reduction-oxidation of PQQ, likely due to no supporting electrolyte and poor adsorption of the negatively charged PQQ at the COOH-modified electrode surface. It also appears that Ca\(^{2+}\) alone is not sufficient as a supporting electrolyte for effective, reversible reduction of PQQ (Figure 2). The voltammogram for PQQ with the long MWCNTs-COOH-modified electrodes in HC buffer (Figure 2a) exhibits a quasi-reversible wave and slow electron transfer kinetics as inferred from the large peak-to-peak separation (ca. 230 mV). This slow reduction-oxidation of PQQ in HC buffer was even worse in the case of the short MWCNTs, where virtually no reduction or oxidation peak is observed (Figure 2b). These results contrast work by Katz et al. in which Ca\(^{2+}\) alone enhanced the electrochemistry of immobilized PQQ in TRIS buffer. Ca\(^{2+}\) has been shown to bind to PQQ in solution, and this binding can affect PQQ’s reactivity. In our studies, it is possible that PQQ-Ca\(^{2+}\) complex formation interferes with soluble PQQ’s ability to interact with the MWCNT-modified electrode surface, resulting in reduced activity. With the addition of either K\(^{+}\), Mg\(^{2+}\), or both, the reversible process for PQQ is drastically improved (Figure 2). For electrodes modified with the long MWCNTs-COOH, we see no significant difference in the redox potentials of PQQ when K\(^{+}\) and/or Mg\(^{2+}\) are added to the buffer (Figure 2a). The midpoint potentials (E\(\text{m} \)) of PQQ in these buffers are centered around −0.130 V, which is in excellent agreement with the reported standard reduction potential of PQQ of −0.125 V. Also, the ratio of oxidation (anodic) peak current to the reduction (cathodic) peak current (i\(_p\)/i\(_pc\)) in all the K\(^{+}\) and/or Mg\(^{2+}\) containing buffers range from 0.97 to 1.13, indicating a nearly perfect reversible process (i\(_p\)/i\(_pc\) = 1.0). Unlike Ca\(^{2+}\), improved redox process of PQQ was achieved in buffer containing either K\(^{+}\) or Mg\(^{2+}\) alone or in combination with each other and with Ca\(^{2+}\) (Figure 2). While previous studies demonstrated that Mg\(^{2+}\) could also enhance immobilized PQQ activity to a lesser degree than Ca\(^{2+}\), the enhanced activity with K\(^{+}\) was unexpected. One possible explanation is that K\(^{+}\) is better able to counter the negative charge of the MWCNTs-COOH, which allows PQQ to interact more effectively with the electrode surface. This effect appears to be more prominent in the case of the short MWCNT’s as the buffers containing K\(^{+}\) product slightly sharper peaks than those lacking K\(^{+}\). Increasing Ca\(^{2+}\) concentration in the presence of K\(^{+}\) had little effect on PQQ redox activity (Figure S1). The fact that the buffer compositions that contained K\(^{+}\) showed improved or superior reversible processes of PQQ over the divalent ion counterparts (Table III) could account for why phosphate buffers which contain only K\(^{+}\) ions are still capable of supporting reversible redox chemistry of PQQ (Figure 1). The slight differences in voltammograms for these electrolyte combinations with the short MWCNTs (Figure 2b) may be due to the differences in electron transfer kinetics at the ends of the nanotubes, as discussed below.

**Table I. Performance of buffers toward reversible reduction of PQQ.**

| Buffer  | \(i_p\) (μA) | \(\Delta E_p\) (mV) | \(i_p\) (μA) | \(\Delta E_p\) (mV) |
|---------|--------------|-----------------|--------------|-----------------|
| HEPES   | 257 ± 4 (0.23) | 83 ± 1 | 210 ± 8 (0.27) | 100 ± 2 |
| MOPS    | 233 ± 13 (0.18) | 88 ± 7 | 195 ± 7 (0.14) | 113 ± 2 |
| TRIS    | 194 ± 5 (0.20) | 91 ± 2 | 127 ± 1 (0.14) | 115 ± 3 |
| Phosphate | 297 ± 5 (0.18) | 123 ± 4 | 118 ± 12 (0.18) | 117 ± 4 |

**Effect of length and functionalization of MWCNTs on PQQ electrochemistry.**—The two different lengths of MWCNTs used in the above experiments were functionalized with carboxyl groups. Generally, much attention has been focused on how the alignment or orientation of CNTs on electrodes affects the rate of electron transfer for electroactive species. CNTs aligned normal to the electrode surface has been shown to exhibit faster electrode kinetics than randomly dispersed nanotubes for redox active species in solution. However, casting CNTs on an electrode surface, which leads to randomly dispersed orientations, has been shown to be effective and is widely used in biosensor applications. Unlike the orientation, the influence of nanotube length on the rate of electron transfer is not very well understood or straightforward, as demonstrated by different
Table II. Ionic compositions for PQQ electrochemical studies in HEPES buffer.

| Component | Plain H | HC | HM | HK | HMC | HKC | HMK | HMKC |
|-----------|---------|----|----|----|-----|-----|-----|------|
| 100 mM HEPES pH7 | +       | +  | +  | +  | +   | +   | +   | +    |
| 10 mM Ca^{2+}   | –       | +  | –  | –  | +   | +   | –   | +    |
| 50 mM Mg^{2+}   | –       | –  | +  | +  | –   | +   | –   | +    |
| 200 mM K^{+}    | –       | –  | –  | +  | +   | +   | +   | +    |

*a*+ means buffer component included; –means buffer component not included.

Figure 2. Cyclic voltammograms of PQQ with (a) long MWCNTs-COOH and (b) short MWCNTs-COOH in pH 7 HEPES buffer (H) containing either no additional ions (Plain) or various combinations of Mg^{2+} (M), K^{+} (K), and Ca^{2+} (C), as supporting electrolyte(s).

Figure 3. Cyclic voltammograms of PQQ with (a) long MWCNTs-COOH and (b) short MWCNTs-COOH in pH 7 HEPES buffer (H) containing either no additional ions (Plain) or various combinations of Mg^{2+} (M), K^{+} (K), and Ca^{2+} (C), as supporting electrolyte(s).

Table III. Effect of ionic composition on reversible reduction of PQQ.

| Buffer | Long MWCNTs-COOH | Short MWCNTs-COOH |
|--------|------------------|-------------------|
|        | \(i_{pc} (\mu A)\) | \(\Delta E_p (mV)\) | \(i_{pc} (\mu A)\) | \(\Delta E_p (mV)\) |
| HKMC   | 325              | 121               | 308              | 147               |
| HK     | 238              | 81                | 281              | 198               |
| HM     | 187              | 137               | 273              | 202               |

We set out to determine if changing the length of MWCNTs-COOH and if changing the functionalization to NH_{2} affected PQQ’s redox activity.

For MWCNTs-COOH, nanotube length appears to make a difference in the voltammetric response toward the reduction of PQQ in solution (Figure S2). The difference in the voltammograms may be indicative of kinetic processes occurring at the electrode surface, as evidenced in the broader separation of the anodic and cathodic waves for the short-MWCNTs compared to that of the long-MWCNTs. The long MWCNTs-COOH also display nearly ideal reversible reduction of PQQ, while the reduction is quasi-reversible for the short MWCNTs-COOH (Figure S2). Shortening of nanotubes involves acid treatment, which eventually leaves the ends of the tubes functionalized with oxygenated carbon species, especially carboxylic acid moieties, and longer treatment leads to shorter nanotubes and more oxygenated species.\(^{22,26}\) Thus the long-MWCNTs utilized in this study would have fewer carboxy-terminating ends than the short MWCNTs. Strong evidence exists that the electrochemistry of CNTs is dominated by the end group functionalities\(^{25,27,28}\) and the presence of oxygenated species at the ends are very important for favorable electrochemical processes.\(^{21}\) Thus we considered the electron transfer kinetics for the long and short MWCNTs.

The rate constant (\(k^0\)) for the heterogeneous electron transfer process of PQQ’s electrochemistry on the long and short MWCNTs was estimated using the Kochi’s method\(^{29}\) according to the equation below:

\[
k^0 = 2.18 \left\{ \frac{\alpha n F D_o}{RT} \right\}^{1/2} \exp \left\{ -\frac{\alpha^2 n F \Delta E_p}{RT} \right\}
\]

where \(\alpha\) is the electron transfer coefficient; \(n\), the number of electrons involved in the redox process (in this case, \(n = 2\)); \(D_o\) is the diffusion coefficient; \(F\) is Faraday’s constant; \(\nu\) is the scan rate (0.05 \text{Vs}^{-1}); \(R\) is the gas constant; and \(T\) is temperature in K. Since our experiments were carried out at RT, we approximate \(T = 298\) K. With increasing scan rates, we observe that the reduction and oxidation peaks were symmetrically shifted in the negative and positive directions respectively (Figure 3), implying that the electron transfer coefficient, \(\alpha\), is equal to 0.5.\(^{30}\) Substituting the appropriate parameter values into the above equation yields electron transfer rates of \(1.64 \times 10^{-3} \text{cms}^{-1}\) and \(6.56 \times 10^{-4} \text{cms}^{-1}\), respectively for the long- and short-MWCNTs, clearly indicating a faster, direct electron transfer of PQQ at the long over the short-MWCNT modified GCE.

As shown in Figure 3, the reduction (\(E_{pc}\)) and oxidation (\(E_{pa}\)) potentials of PQQ on the two different MWCNTs-COOH-modified GCEs were found to be dependent on the scan rate, symmetrically shifting to the negative and positive directions respectively. The peak

Table III. Effect of ionic composition on reversible reduction of PQQ.

| Buffer | Long MWCNTs-COOH | Short MWCNTs-COOH |
|--------|------------------|-------------------|
|        | \(i_{pc} (\mu A)\) | \(\Delta E_p (mV)\) | \(i_{pc} (\mu A)\) | \(\Delta E_p (mV)\) |
| HKMC   | 325              | 121               | 308              | 147               |
| HK     | 238              | 81                | 281              | 198               |
| HM     | 187              | 137               | 273              | 202               |
Figure 3. Effect of scan rate on redox cycling of PQQ using different length MWCNTs-COOH. Cyclic voltammograms of 50 μM PQQ using GCEs modified with (a) long MWCNTs-COOH and (b) short MWCNTs-COOH in pH 7 HEPES buffer containing 50 mM Mg$^{2+}$, 200 mM K$^+$, and 10 mM Ca$^{2+}$ at various scan rates. Insets: Randles-Sevcik plots demonstrating the relationship of anodic and cathodic peak currents with square root of the scan rates.

Figure 4. Comparison of MWCNT modification on PQQ redox cycling. Cyclic voltammograms of 50 μM PQQ using GCEs modified with long MWCNTs-COOH (black) and MWCNTs-NH$_2$ (blue) in pH 7 HEPES buffer containing 50 mM Mg$^{2+}$, 200 mM K$^+$, and 10 mM Ca$^{2+}$. Scan rate: 0.05 V/s.

trodes was surface controlled and that PQQ freely diffused to adsorb onto the electrode surfaces (Figure 3).

Based on the above results and the idea that the short MWCNTs likely have more negative surface charges from the larger number of carboxylic acid moieties, it may be argued that the improved reversible processes of PQQ on the long MWCNTs are not due to the longer lengths, but rather fewer negative surface charges. A less negative surface could allow for better adsorption of PQQ onto the electrode surface. To further investigate the effect of electrode surface modification on PQQ’s electrochemistry, we also utilized amine-functionalized MWCNTs. At pH 7, the amines on the MWCNT-modified GCE will be at least partially protonated. With this positively charged surface, we reason that PQQ can adsorb more strongly to this electrode and result in better, reversible reduction-oxidation reactions. Interestingly, the performance of this amine-modified MWCNT-GCE in HKMC buffer (Table II) showed slight improvement in the reversible reduction of PQQ (Figure 4), with much sharper and distinct peaks. These results support the argument that the surface charges may play a more important role than MWCNT length for the electrochemistry of PQQ in solution, although the length dependency may need to be explored further.

The long-term stability of both COOH- and NH$_2$-MWCNT electrodes was also studied in HKMC buffer. 20 voltammetric scans were obtained once each day for three days. After each day’s experiment, the electrodes are removed from the soaking buffer, gently rinsed in plain buffer and stored dry at 4°C. The voltammograms obtained over the 3-day period were very stable and consistent, with only about 0.4% loss in current after 72 hours (data not shown).

Conclusions

The electrochemistry of soluble PQQ has been successfully studied using two different lengths of MWCNTs-COOH-modified glassy carbon electrode in four different pH 7 buffers: HEPES, MOPS, TRIS, and phosphate. The best reversible process of PQQ was supported by the HEPES buffer, although all tested buffers supported reversible processes. Despite the importance of calcium for PQQ-dependent enzymes, HEPES buffer containing Ca$^{2+}$ alone was not sufficiently effective at supporting the reversible reduction of PQQ. The reversible
PQQ activity was only achieved in HEPES buffer containing K⁺ and supplemented with Mg²⁺ and/or Ca²⁺.

The redox behavior of PQQ was also affected by the length of MWCNTs-COOH, with relatively long MWCNTs demonstrating near ideal reversible reduction-oxidation of PQQ and short MWCNTs only demonstrating quasi-reversible processes with very broad reduction and oxidation waves. To test the effect of surface charge, MWCNTs-NH₂ were employed. A slight improvement in the PQQ reversible assistance. PQQ-dependent enzymes and functional nucleic acids. that enhance the activity of biosensors and biofuel cells relying on ability to use a variety of buffers will allow for alternative conditions buffers other than phosphate support reversible PQQ activity. The PQQ at MWCNT-modified GCEs and demonstrate that biological these studies improve our understanding of the redox properties of for electrodes in these applications, so understanding how PQQ re- acts with these functionalized surfaces is important. The results of these studies improve our understanding of the redox properties of PQQ at MWCNT-modified GCEs and demonstrate that biological buffers other than phosphate support reversible PQQ activity. The ability to use a variety of buffers will allow for alternative conditions that enhance the activity of biosensors and biofuel cells relying on PQQ-dependent enzymes and functional nucleic acids.

Acknowledgments
The authors thank members of the Baum lab for technical assistance.

References
1. G. E. Cozier, R. A. Salleh, and C. Anthony, Biochem. J., 340(5 Pt 3), 639 (1999).
2. H. Han and H. Tachikawa, Front. Biosci., 10, 931 (2005).
3. S. Itoh, N. Kato, M. Mure, and Y. Ohshiro, Bull. Chem. Soc. Jpn., 60(1), 420 (1987).
4. M. G. van Kleef, J. Jongejan, and J. Duine, in PQQ and Quinoproteins, J. A. Jongejan and J. A. Duine, eds., p. 217, Springer Netherlands, (1989).
5. S. Itoh, M. Kinugawa, N. Mita, and Y. Ohshiro, J. Chem. Soc., Chem. Commun., (11), 694 (1989).
6. E. Katz, D. D. Schlereth, and H.-L. Schmidt, J. Electroanal. Chem., 367(1-2), 59 (1994).
7. E. Katz, D. D. Schlereth, H.-L. Schmidt, and A. J. J. Olsthoorn, J. Electroanal. Chem., 368(1), 165 (1994).
8. P. Kamineni, V. Ruiz, T. Kallio, I. V. Anoshkin, E. I. Kauppinnen, and K. Kontturi, Electrochem. Commun., 12(9), 1257 (2010).
9. H. J. Tao, P.-Y. Tsai, and C. M. Wang, J. Electroanal. Chem., 606(2), 141 (2007).
10. I. Emahi, I. M. Mulvihill, and D. A. Baum, RSC Adv., 5(10), 7450 (2015).
11. A. R. Dewanti and J. A. Duine, Biochemistry, 39(31), 9384 (2000).
12. A. Casini, A. Finazzi-Agro, S. Sabatini, E. S. El-Sherbini, S. Tortorella, and L. Scipione, Arch. Biochem. Biophys., 368(2), 385 (1999).
13. M. Ameyama, M. Nonobe, M. Hayashi, E. Shinagawa, K. Matsushita, and O. Adachi, Agr. Biol. Chem. Tokyo, 49(4), 1227 (1985).
14. H. Shinozaka, G. F. Khan, Y. Ikariyama, and M. Aizawa, Journal of Electroanalytical Chemistry and Interfacial Electrochemistry, 3041–2, 75 (1991).
15. E. Katz, M. Lion-Dagan, and I. Willner, J. Electroanal. Chem., 408(1), 107 (1996).
16. T. Inoue and J. R. Kirchhoff, Analytical Chemistry, 72(23), 5755 (2000).
17. K. Kano, K. Mori, B. Uno, T. Kubota, T. Ikeda, and M. Senda, Journal of Electroanalitical Chemistry and Interfacial Electrochemistry, 299(2), 193 (1990).
18. K. Kano, K. Mori, B. Uno, T. Kubota, T. Ikeda, and M. Senda, Journal of Electroan-alytical Chemistry and Interfacial Electrochemistry, 298(3), 227 (1990).
19. V. Luramavicius, J. Ruzumiene, A. Ramanavicius, and A. D. Ryabov, Biosens. Bioelectro., 20(6), 1217 (2004).
20. E. Katz, T. Lotzbeyster, D. D. Schlereth, W. Schuhmann, and H.-L. Schmidt, J. Elec-troanal. Chem., 373(1), 189 (1994).
21. A. Chou, T. Bocking, N. K. Singh, and J. J. Gooding, Chem. Commun., (7), 842 (2005).
22. J. J. Gooding, A. Chou, J. Liu, D. Losic, J. G. Shapter, and D. B. Hibbert, Electrochem. Commun., 9(7), 1677 (2007).
23. J. Wang and Y. Lin, J. Phys. Chem., 109(A), 2113 (2004).
24. J. J. Gooding, R. Wibowo, J. Liu, W. Yang, D. Losic, S. Orbons, F. J. Mears, J. G. Shapter, and D. B. Hibbert, J. Am. Chem. Soc., 125(30), 9006 (2003).
25. J. Liu, A. G. Rinzler, H. Dai, J. H. Hafner, R. K. Bradley, P. J. Boul, A. Lu, T. Iverson, K. Shelimov, and C. B. Huffman, Science, 208(3), 1253 (1998).
26. C. E. Banks, R. R. Moore, T. J. Davies, and R. G. Compton, Chem. Commun., (16), 1804 (2004).
27. J. Liu, A. Chou, W. Rahmat, M. N. Paddon-Row, and J. J. Gooding, Electroanal., 17(1), 38 (2005).
28. R. Klingler and J. Kochi, J. Phys. Chem., 85(12), 1731 (1981).
29. A. J. Bard and L. R. Faulkner, Electrochemical methods: fundamentals and applica-tions, Wiley New York (1980).