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We demonstrate that bisulfite can be used for reduction of a highly electrophilic anthraquinone derivitive, *N,N'*-dimethyl-2,6-diaza-9,10-anthraquinonediium (DAAQ), and subsequent autoxidation generates an equivalent of hydrogen peroxide. The mechanism for DAAQ reduction by bisulfite, DAAQ electrochemistry, and use of a simple test strip assay for H_2O_2, are described.

Since the 1940s the most widely used industrial method of production of hydrogen peroxide (H_2O_2) has been via the catalytic reduction of a 2-substituted anthraquinone, followed by autoxidation in the presence of molecular oxygen. This method, known as the Riedl-Pfleiderer[1] process, has seen several improvements[2-10] and is very high yielding in H_2O_2.[2-4, 11-14,15] There are a variety of quantitative tests for hydrogen peroxide in aqueous media: electrochemical,[16-19] spectroscopic,[20, 21] and even simple colorimetric test strips.[22] The generation of H_2O_2 has been used to quantitate other species when their presence can be used to trigger the production of H_2O_2, most commonly via horseradish peroxidase.[23] Thus, if derivatives of anthraquinone (AQ) could be used to generate H_2O_2 in aqueous media in response to an analyte, then quantitation of H_2O_2 using any of the common methods could be used as a sensor for that analyte.

Bisulfite is a common additive to foods due to its antibacterial activity,[24] and thus is used generally as a food preservative.[25-27] However, it can cause allergic reactions with some individuals, as well as impart a sulfurous taste.[26] It is a known reducing agent and a common nucleophile for carbonyl addition reactions.[28, 29] Thus, we reasoned that the use of an anthraquinone to create H_2O_2 could be combined with the nucleophilicity and reducing properties of bisulfite to generate H_2O_2, and ultimately be used as a quantitative assay for bisulfite. However, the proper anthraquinone species needed to be created.

In 2003 Morgan and coworkers published a spectroscopic study of 2,6-diazaanthracene-9,10-dione (DAAD), an anthraquinone derivative containing two endocyclic nitrogen atoms. Among other studies, they examined the redox potential of DAAD and showed that the first reduction occurs at a less negative voltage than the corresponding reductions of anthraquinone. They also noted that while this reduction was reversible, a second, irreversible reduction could take place at more negative voltages.[30]

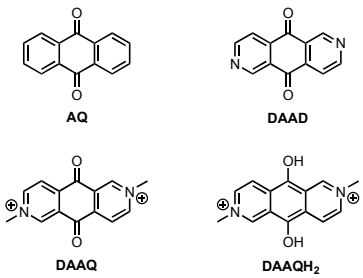

Fig. 1 Structures of relevant anthraquinone derivatives.

Ten years later, in 2013, Leventis and coworkers reported the synthesis of N,N'-dimethyl-2,6-diaza-9,10-anthraquinonediium (DAAQ) tetrafluoroborate, a bis-methylated and water soluble derivative of DAAD. In addition to obtaining a crystal structure of the compound, they undertook a comprehensive analysis of the keto-to-geminal diol equilibrium associated with the two carbonyl moieties of the central ring. They found through NMR studies that, by decreasing the pH of the aqueous solution, they could quantitatively drive this equilibrium to the quinone form of DAAQ.[31] However, their study did not include electrochemical experiments to elucidate DAAQ's redox potential or studies on its ability to generate hydrogen peroxide.

We postulated that the methylation of the nitrogens and accompanying formation of positive charges on DAAQ would

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further shift the reduction potential in the positive direction. Moreover, the water solubility of the dicationic DAAQ would eliminate the necessity to use organic solvents to generate H_2O_2 and allow reactions with water-soluble species - such as bisulfite. Herein, we report the reduction of DAAQ to the hydroquinone ($DAAQH_2$) with an equivalent of bisulfite, followed by oxidation back to the quinone in the presence of oxygen, generating an equivalent of hydrogen peroxide that can be measured with simple test strips to indirectly quantitate bisulfite.

Following previously reported procedures,[31, 32] we synthesized DAAQ and confirmed its purity via ^{1}H-NMR spectroscopy in a mixture of D_2O and DCl (deuterium chloride). Next, based on our prediction that DAAQ would have a high electron affinity, we demonstrated its ease of reduction using bisulfite.[25-27] To do so, DAAQ was titrated with bisulfite in a pH 4.6 citrate buffer as a solvent (Fig. 2). This pH establishes the aforementioned equilibrium of DAAQ to favor the keto form and the bisulfite in its mono-anionic, nucleophilic state. A reaction was observed to take place essentially instantaneously, as evidenced by the immediate color change of the solution of DAAQ from beige to purple-pink upon addition of one equivalent of bisulfite. The titration proceeds with the occurrence of two isosbestic points at ~297 and ~322 nm, indicating a clean 1 to 1 conversion of reactant to product.

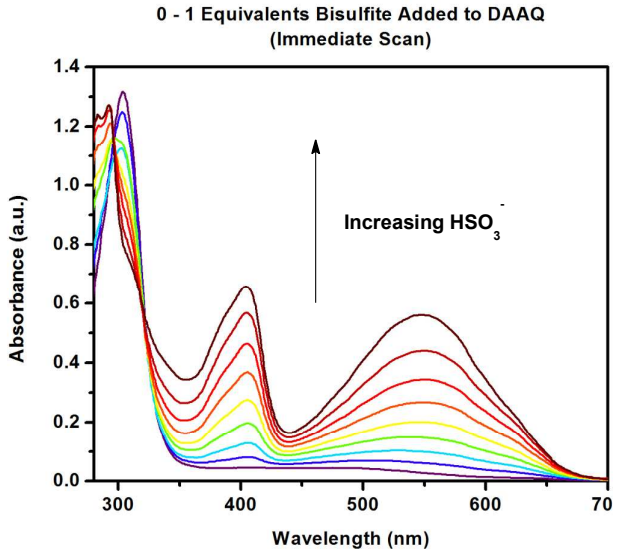


Fig. 2 UV/Vis titration of DAAQ (300 µM) reduction with 0-1 equiv. of bisulfite.

To confirm that the color change observed was associated with our postulated reduction of DAAQ to its hydroquinone form ($DAAQH_2$), the product was characterized by standard procedures (Supp. Mat.). Further, we obtained a crystal structure of the highly colored product (Fig. 3). Crystals grew as red laths from a saturated solution of equimolar amounts of DAAQ and sodium bisulfite in 1.69 M aqueous hydrochloric acid. The structure is entirely planar, with a single chloride counterion in the unit cell, coming from the hydrochloric acid present in solution. The $DAAQH_2$ lies on a crystallographic inversion center, and thus ½ of this entity and one chloride make up the unit cell.

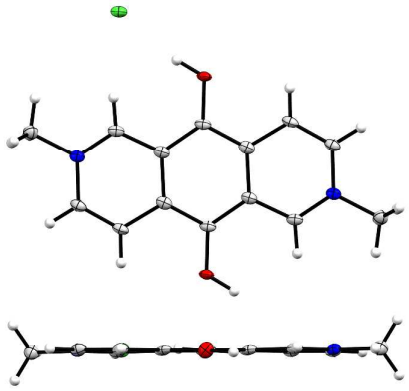
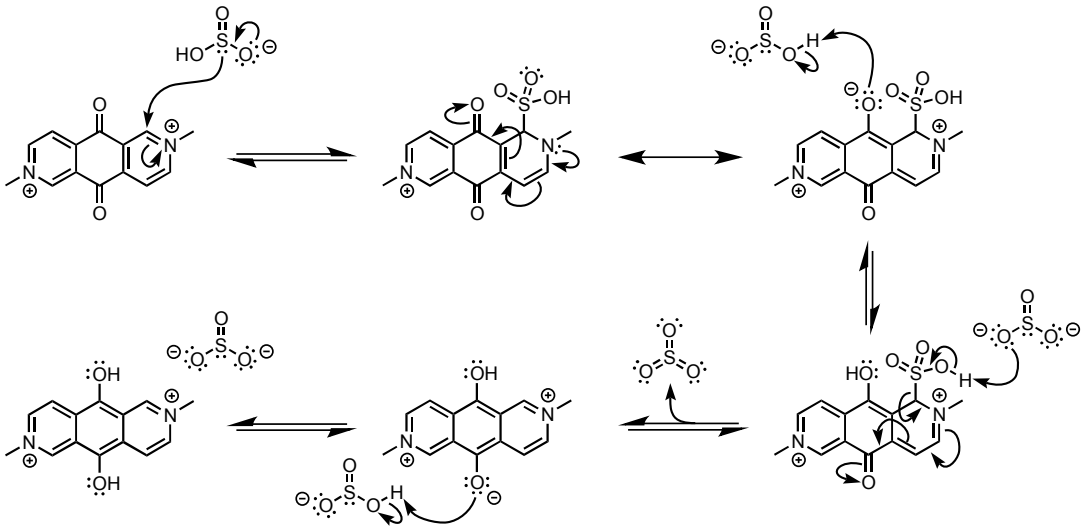


Fig. 3 Two views of the $DAAQH_2$ crystal structure.

A mechanism for the reduction is presented in Scheme 1. Due to the known nucleophilicity at sulfur, bisulfite is postulated to add at the 2-position of one of the pyridine rings, followed by a series of proton transfers from bisulfite, buffer, or solvent. This sequence of events results in the bisulfite donating two electrons to DAAQ and being oxidized to molecular sulfur trioxide, which would transform to sulfuric acid.

To support our hypothesis that DAAQ would be more easily reducible than anthraquinone (AQ), we measured the reduction potential of DAAQ via cyclic voltammetry in anhydrous acetonitrile. This solvent was chosen instead of water in the interest of being able to compare the reduction potential to that of AQ (not water-soluble). Unlike DAAD's single reversible reduction, DAAQ undergoes two distinct, reversible reductions, attributed to the two carbonyl groups on the central ring (Fig. 4). However, as the voltage is continuously scanned more negative it undergoes irreversible reductions, causing the molecule to degrade (not shown). Furthermore, when compared to the cyclic voltammogram of anthraquinone, both reductions of DAAQ (E_1° = −0.18 *V* vs −1.36 *V* for AQ, E_2° = −0.44 *V* vs −2.02 *V* for AQ) are significantly shifted in the positive direction, confirming its higher affinity for electrons.

While the voltammograms clearly illustrate the ease with which DAAQ is reduced, due to the possibility of irreversible over reduction, further investigation was required. It is evident the two redox events in the AQ voltammogram have nearly the same peak height, while in the DAAQ voltammogram there is a clear difference between the first and second redox events. We hypothesize these differences arise from two factors: (1) diffusion properties and (2) electron transfer kinetics between the different species produced at the electrode surface. While AQ starts as a neutral molecule



Scheme 1 Proposed mechanism of reduction of DAAQ with bisulfite to $DAAQH_2$.

before first being reduced to the radical anion and subsequently to the stable dianionic form, DAAQ begins in a dicationic state. After undergoing its first reduction to the radical monocation, the molecule becomes singly charged, which changes the diffusion of the radical to and from the electrode surface and inherently the observed peak height. Similar to DAAD, when narrowing the potential window the radical monocation of DAAQ is stable on the electrochemical time scale. In the case of DAAD, however, irreversible reduction of the radical anion is more favorable than reduction of the second carbonyl group.[30] This difference in stability changes the electron transfer kinetics of the second reduction. To support this claim, we looked at the peak splitting of the redox events associated with AQ, DAAQ, and DAAD. In the AQ system the first and second redox events have peak splittings of 66 and 111mV, while DAAQ has peak splittings of 50 and 156 mV, respectively. For both AQ and DAAQ the splitting of the first redox event exhibits nearly ideal reversible 1-electron transfer characteristics. The second event shows larger splitting, which can be related to increased difficulty in electron transfer or a change in transfer kinetics. Given that the splitting is larger for DAAQ than AQ and, in comparison to the 130mV splitting seen in DAAD, this lends support to our claim that the increased stability of the radical monocation affects the electron transfer kinetics.[30] Therefore, we postulate these two factors are responsible for the changes in peak heights observed in the DAAQ voltammogram.

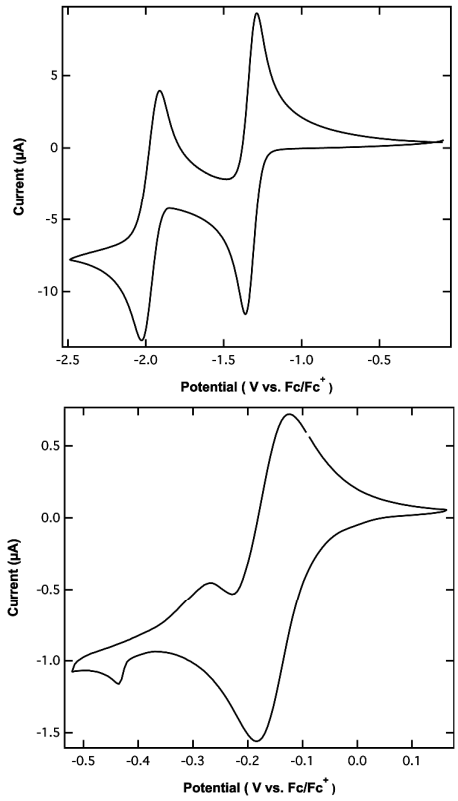


Fig. 4 Cyclic voltammograms of anthraquinone (above) and DAAQ (below) in MeCN.

After characterizing the electrochemistry, we then proceeded to check that $DAAQH_2$ would oxidize back to DAAQ readily in the presence of molecular oxygen and produce hydrogen peroxide, similar to the Riedl-Pfleiderer[1] process with AQ. A solution of $DAAQH_2$ crystals dissolved in citrate buffer, generating a 300 μM concentration, was shaken in a vial filled with air, and within minutes the color changed back to the beige color of the unreduced DAAQ. To verify the formation of H_2O_2 we used a simple aqueous-based peroxide

test strip and observed a color corresponding to a ppm level of H_2O_2 within the expected range, based on the concentrations of reagents used (Fig. 5).

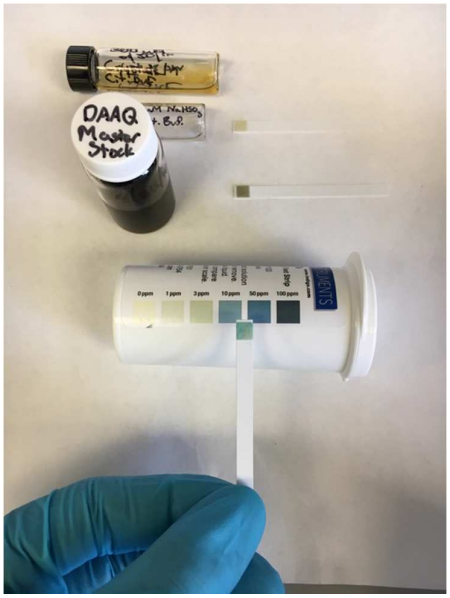


Fig. 5 Test strip dipped into the reoxidized 300 µM $DAAQH_2$ solution in the top vial, showing the presence of hydrogen peroxide in the expected amount (300 µM, i.e. 10.2 ppm). The middle vial contains a 300 µM solution of sodium bisulfite and the bottom vial contains a 1.2 mM solution of DAAQ that had not been subjected to the redox reaction.

In summary, DAAQ undergoes reaction with one equivalent of bisulfite in aqueous citrate buffer to generate $DAAQH_2$. DAAQ has a lower reduction potential than both AQ and DAAD, leading to the ability for bisulfite to act as a reductant. Autoxidation of $DAAQH_2$ back to DAAQ leads to the production of one equivalent of hydrogen peroxide, which can be easily quantitated with commercial test strips. We are currently exploring the use of this methodology a as sensor in a cyclic process to quantitate bisulfite in wines and other food products, potentially generating a simple test strip assay for bisulfite.

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Conflicts of interest

The authors declare no conflict of interest.

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