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COMMENT

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# Comment on "Electrochemical Detection of Peroxynitrite Using a Biosensor Based on a Conducting Polymer−Manganese Ion Complex"

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In a recent paper by Shim and co-workers,[1] an attempt was made to prepare an electrochemical ultramicrosensor for the detection of peroxynitrite ($ONOO^-$ so-called PON) in physiological solution (phosphate buffer solution PBS at pH = 7.4). The authors concluded that a PON biosensor has been developed. Besides the inappropriate use of "biosensor" terminology (no enzymes, proteins, living cells, or organelles were used to build the reported sensor), there are several inconsistencies and contradictions and a lack of information that make the origin of the measured electrochemical signals doubtful and thus the performance of the proposed method and the conclusions of the work. The first deficiency in the information is related to the absence of details concerning the experimental preparation of the PON stock solutions. The paper says briefly, and by citing non coherent references, that two ways of preparation of the PON solutions were used. No indications are given on which stock solution was used to obtain the data reported in Figures 1b, 4, and 5. These data are the key ones for the justification and the validation of the performances of the claimed sensor.

The major shortcoming of the results presented in these figures concerns the lifetime of the target analyte PON at neutral pH. Many studies and reviews reported in the literature discussed the very short half-life of PON at physiological pH.[2−11] In the absence of target molecules, PON reacts according to a proton-catalyzed isomerization process and decomposes through the intermediate formation of its conjugated peroxynitrous acid ONOOH ($pK_a$ = 6.8), being the main product of decomposition nitrate[2−4] with an overall rate constant 0.9 $s^{-1}$ at 37 °C.[2] This corresponds to a half-life of 0.8 s, in experimental conditions identical to those used in Shim's study. Numerous other pathways for the degradation of PON in presence of various chemicals are also well documented in the literature.[2] This makes the participation of PON itself questionable in the electroanalytical measurement involving the Mn-complex film deposited on the electrode.

Figure 4 is one of the key data of the paper. It deals with the performances and the calibration of the supposed sensor and it contains self-contradictory information. Figure 4, curve a shows amperometric decaying signals within 15 s, in line with the detection of unstable species. It is surprising, however, that in the same experimental conditions, the amperometric signals shown in the insert of Figure 4b have a plateau-like shape, without any decay as it should have been expected. These data suggest that PON remains stable at pH = 7.4 for more than 300 s which is in contradiction with all the previously reported data about the stability of PON at physiological pH. At this stage, the lack of details concerning the nature of the PON stock solution is prejudicial. Indeed, according to the experimental section, two situations should be considered: (1) If PON was synthesized from nitric oxide NO and potassium superoxide solutions, then there is a clear discrepancy and the data shown in Figure 4b cannot be related to PON. Again, synthetic PON is only stable in alkaline solution (over 2 h at room temperature), but it decomposes spontaneously (within less than 15 s) in neutral solution.[2] (2) If PON was introduced via a 0.1 mM donor solution of 3-morpholinosydnonimine (SIN-1), there is also a discrepancy between the data shown in parts a and b of Figure 4. Indeed, it is suggested in the literature that SIN-1 can be used as a donor of PON through its spontaneous decomposition in aqueous solution at neutral pH, with a rate of decomposition dependent on time. The maximal flux of PON production is obtained after ∼30 min and ranged from 1.2 to 3.6% of added SIN-1 per minute (for SIN-1 concentration of 0.1 mM).[12,13] Then, the actual concentration of PON at a given time will be dependent upon the kinetics of its rate of formation by the SIN-1 and of its rate of decomposition in a given milieu. Since the rate of formation changes with time, it is not possible to obtain a steady concentration of PON with SIN-1 as a donor, unless sequential SIN-1 injection pulses are performed.[13] Such a protocol was not developed in Shim's paper, and all these considerations are missing in the manuscript.

In addition, the characterization of the reported amperometric sensor and the interpretation of the origin of the measured signals attributed to PON relies on experiments that utilize stock solution and addition of aliquots to 0.1 M PBS (pH = 7.4) that cannot ensure the presence of authentic PON during the cyclic voltammetric measurements. Indeed, for the same reasons mentioned above, PON cannot still be available at pH 7.4 during the time scale required for the cyclic voltammetry measurements (Figure 1b[1]). Thus, there is no evidence or guarantee that the reported characterizations of the electroanalytical signals are related to PON itself. In the reported experimental conditions, $ONOO^-$ reacts with $CO_2$ to give $^{\bullet}NO_2$ and $CO_3^{-\bullet}$ radicals which are powerful oxidants[2] ($^{\bullet}NO_2$ is also a good nitrating agent), and one may consider that these species are those involved in the "supposed" electrocatalytic process occurring on the modified electrode. Also, no information was given to ensure (i) how stable were the cyclic voltammograms upon repeated scans (consecutively or not) (Figure 1b), (ii) how long after the addition of the aliquots were the measurements made and how these laps of time may influence the concentration of PON, (iii) how were the cyclic voltammograms recorded (potential scan direction, reverse scan position, etc.).[1] This lack of information

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makes the origin of the electrocatalytic signals questionable. Finally, it is also unclear whether PON is detected through its reduction or through its oxidation. The cyclic voltammogram presented in Figure 1b indicates that at the potential of 200 mV PON is reduced, a conclusion that is emphasized in the text. Thus, the current measured at this potential has a negative value. In the subsequent chronoamperometry experiments, in which results are shown in Figures 3, 4, and 5, the recorded currents are positive, which points to an oxidative process. Either the signal observed is not the reduction of PON but the oxidation of another species or of PON itself or the authors used another sign convention during the course of the paper, without letting the reader know.

Lastly, the data shown in Figure 5a related to the amperometric responses obtained in spiked rat plasma samples upon addition of PON may be suspicious if one considers that in plasma (and more generally in biological tissues and *in vivo*) SIN-1 is likely to behave more like an NO donor than a PON donor.[14] We conclude that there is no valid and accurate determination of PON.

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