The voltammetric determination of peroxynitrite at a mercury film electrode

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This report describes the direct voltammetric detection of peroxynitrite (PN, $ONOO^-$), an important analyte in many physiological biochemical pathways, at a mercury film electrode (MFE) at alkaline pH. The voltammetric response of PN is observed as a cathodic inverted peak when the potential is swept in the oxidative direction at *ca.* −0.1 V *vs.* a Ag/AgCl/1 M KCl reference electrode. An $\overrightarrow{E}C\overleftarrow{E}$ mechanism is proposed to explain this behaviour involving the initial formation of a thin film of Hg(I) oxide on the electrode which is then oxidised by PN to form HgO in a chemical step. The HgO is then reduced back to Hg(0) at the electrode potential of interest resulting in a net cathodic current. The parameters affecting the voltammetry of PN at a MFE, including the concentration of PN (10^{-5}–10^{-4} M), the effect of varying scan rate (5–100 mV s^{-1}) and pH are investigated and the kinetics of PN decay in alkaline solutions ranging from pH 9–13 are explored electrochemically.

1. Introduction

Peroxynitrite (PN) is believed to be rapidly formed under physiological conditions from the reaction of nitric oxide, NO, with superoxide radicals, $O_2^{\bullet -}$.[1] PN is relatively stable and long-lived under alkaline conditions, but at lower pH the protonated form, ONOOH rapidly rearranges/decomposes to form nitrate, NO_3^-, nitrite, NO_2^-, protons and dioxygen, O_2, depending on the reaction conditions as shown in eqn (1).[2,3]

$$\begin{aligned} &ONOOH \rightarrow H^+ + NO_3^-;\ pH < 9 \\ &ONOOH + ONOO^- \rightarrow HNO_2 + NO_2^- + O_2;\ pH > 9 \end{aligned} \quad (1)$$

Peroxynitrite exists predominantly as the *cis* isomer in solution with a pK_a value of 6.8 (25 °C),[2–4] although this value is found to be dependent slightly on the buffer used, with Kissner *et al.* reporting pK_a values ranging from 6.5–7.5 depending on the concentration of phosphate buffer used.[5] Also there is some suggestion that certain reaction pathways at alkaline pH may involve the *trans* isomer as an intermediate which has a higher pK_a of *ca.* 8.0.[4]

The formal reduction potential for the ($ONOOH/NO_2$) couple at pH 7 is rather high at +1.4 V (25 °C)[2] and therefore peroxynitrite and its protonated form are strong, relatively long-lived oxidants (on the timescale of most cellular chemistry) with cytotoxic effects resulting in PN being linked to various pathological conditions including stroke,[6] heart disease[7] and atherosclerosis,[8] Alzheimer's, Huntington and Parkinson's diseases, AIDS and acute ischemia-reperfusion injury.[9–12]

Present methods of determining PN are predominantly based upon spectroscopic methods such as the spectrophotometric determination of PN in alkaline media by measuring the absorption due to PN at 302 nm (ε = 1670–1705 M^{-1} cm^{-1}),[13–15] photometry using iodide at pH 6–7 and measured *via* the adsorption of iodine at 355 nm,[16,17] fluorometric methods involving enzymes[18,19] and chemiluminescence studies.[20,21] The exception is the classical method of Papee *et al.* who determined PN in alkaline solutions *via* the potentiometric titration of PN with potassium permanganate.[22]

However, to date there are few reported studies involving the direct determination of PN or its protonated form using electrochemical methods although it must be noted that a significant body of research exists in the literature where PN is determined indirectly, *via* the electrochemical determination of reaction products such as NO, NO_2^- and various biochemical species, *e.g.* in coupled HPLC methodologies. The few notable literature reports of the electrochemical determination of PN are discussed below.

Iwuntze *et al.* reported that PN could be determined in simulated bodily fluid with a pH of *ca.* 7 at a bare platinum electrode at an oxidative potential of *ca.* 1.18 V *vs.* the saturated calomel electrode.[23] They determined that this process involved the two-electron oxidation of PN and reported a limit of detection of around 0.17 μM.

The standard potential for the PN couple is reported by Merenyi *et al.*[24] and Koppenol *et al.*[25] to be *ca.* 2 V but this value is calculated from spectroscopic measurements of the Gibbs energy for the one-electron oxidation of PN. Kurz *et al.*[26] studied the reduction of PN at a gold electrode in acidic media and observed several complex voltammetric waves with the pH independent reduction of PN reported to

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occur between +0.4 and −0.3 V *vs.* SCE depending on scan rate.

Xue *et al.* have reported an elegant indirect method of detecting PN electrochemically *in vivo* using a platinum or carbon ultramicroelectrode modified with a redox mediator, namely a poly-tetra-aminophthalocyanine complex of manganese(III/IV).[27] However, to date perhaps the most elegant example of the direct electrochemical determination of PN is that presented by Amatore and co-workers.[28] Using platinised carbon ultramicroelectrodes placed adjacent to an artificial synapse constructed using a living cell, pricked with a micropipette to simulate oxidative stress bursts, Amatore *et al.* were able to detect the direct one-electron oxidation of PN *in vitro* under simulated physiological conditions at *ca.* +0.27 *vs.* the saturated standard calomel electrode (SSCE).[28] However, due to the rapid decay of PN at pHs close to physiological conditions the work of Amatore required the use of ultramicroelectrodes (with a radius of *ca.* 5 μm) and the use of specially designed in-house potentiostats and specialist data acquisition cards which limits the wider analytical applicability of this technique. The work of Amatore *et al.* is particularly notable in that it provides the first true direct voltammetric determination of PN under physiological conditions.

In this report the facile direct detection of PN at a mercury film electrode (MFE) in alkaline solution is presented. The voltammetric signal arising from the presence of PN was observed at *ca.* −0.1 V *vs.* a Ag/AgCl/1 M KCl reference electrode, and is unusual in that it appears as an "inverted" cathodic peak in the oxidative scan direction. The various parameters affecting the determination of PN at the MFE electrode such as solution pH and scan rate are investigated. An $\vec{E}C\overleftarrow{E}$ mechanism is proposed to explain the appearance of this unusual inverted peak. This work may provide the foundations for a simple voltammetric method of determining PN concentrations.

2. Experimental

2.1 Reagents and equipment

All reagents were obtained commercially and were of analytical grade. All solutions were prepared using triply distilled water of resistivity not less than 18.2 MΩ cm (25 °C). All solutions were prepared immediately prior to use to prevent adsorption of atmospheric carbon dioxide and were purged with pure N_2 prior to any electrochemical experiment being performed to remove dissolved oxygen from the solution.

Tablets of peroxynitrite were prepared as follows:[29] 0.4 g of polycrystalline KNO_3 was pressed into tablets (diameter 16 mm) which were then irradiated using a low pressure mercury lamp at 253.7 nm. The photolysis time was 1, 2 or 3 hours. The quantum yields of $ONOO^-$ and NO_2^- within the KNO_3 tablets were 2.5×10^{-2} and 1.5×10^{-3}, respectively. The tablets of PN were then stored at 4 °C and remained stable for approximately one month. Solutions of PN were prepared by either dissolving a tablet of photolysed KNO_3 in alkaline solutions (0.01–0.2 M KOH) immediately prior to use or by photolysis of a 0.5 M KNO_3 solution in 0.2 M KOH under the mercury lamp for a period of 30 minutes. The solutions of PN were stored at 4 °C between experiments to prevent the PN from decaying. For each PN solution prepared either by dissolving PN tablets or by photolysis of KNO_3 solutions the concentration of PN in the solution was determined by three separate techniques, potentiometric titration with alkaline potassium permanganate,[22] spectrophotometrically by measuring the absorbance at 302 nm,[13–15] and finally by photometry using an excess of potassium iodide.[16,17] The concentrations of PN determined using these three methods were found to be in excellent agreement within 5% of the mean value.

Solutions of varying pH were prepared as follows: solutions with pH between 12.5 and 13.0 were prepared using 0.03–1.0 M KOH; solutions with pH ranging from 9.2 to 10.5 were prepared using 0.05 M sodium tetraborate adjusted to the appropriate pH by addition of NaOH; solutions with pH 11.0 to 11.5 were prepared using 0.05 M disodium hydrogenphosphate buffer (Na_2HPO_4) adjusted to the appropriate pH by addition of NaOH.

Spectrophotometry was performed on a Specord M-40 spectrometer with the adsorption due to PN measured at 302 nm. Photocolorimetry was performed using a КФК-2М П (Russia) instrument. An Эксперт-001 (Russia) potentiometer was used for potentiometric titration experiments and was also used in conjunction with an ЭСЛ-15-11 (Russia) ion-selective glass electrode for the measurement of solution pH.

All electrochemical measurements were performed using a Universal Polarograph (ПУ-1, Russia) connected to a chart recorder. A three-electrode cell configuration was used throughout. The working electrode consisted of a silver rod (diameter 0.5 mm) protruding for 7 mm from a Teflon sheath. The mercury film electrode (MFE) was prepared by immersing the entire silver working electrode into a mercury pool for *ca.* 10 s. A paraffin impregnated graphite electrode acted as the counter electrode and the cell assembly was completed by a Ag/AgCl/1.0 M KCl reference electrode. Where necessary the reference electrode compartment and the cell were connected using a 0.1 M KNO_3 salt bridge.

3. Results and discussion

3.1 The voltammetric characterisation of peroxynitrite at a mercury film electrode

The voltammetric behaviour of PN in 0.1 M KOH was first studied at a MFE using cyclic voltammetry (CV). If the potential is scanned from −0.2 V *vs.* Ag/AgCl/1.0 M KCl to −1.6 V in the absence of PN no reductive waves are observed. Upon reversing the scan direction from −1.6 V to 0.0 V no oxidative waves are seen before the anodic dissolution of the mercury film occurs at *ca.* −0.05 to −0.10 V depending on the solution pH and results in a very large anodic current. Upon subsequent cycles between 0.0 and −0.4 V a pronounced reduction peak is observed at *ca.* −0.1 V corresponding to the re-deposition of mercury onto the electrode. The addition of 0.1 M KNO_3 or 0.01 M KNO_2 did not yield any observable voltammetric waves in the region 0.0 to −1.6 V. However when PN was added to the system (in the concentration range 10^{-5}–10^{-4} M) a new "inverse" cathodic wave was observed

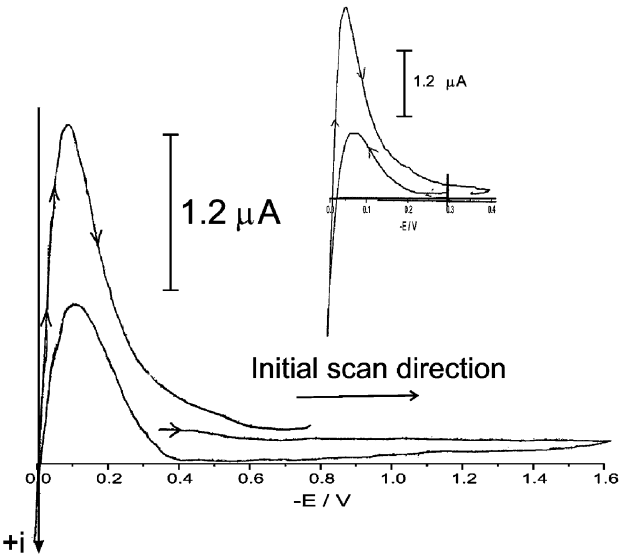

Fig. 1 The cyclic voltammetric response of a MFE in 0.1 M KOH in the presence of 10^{-4} M peroxynitrite scanning from −1.6 V to 0.0 V at 30 mV s^{-1}. Inset: The voltammetric response of 10^{-4} M peroxynitrite at a MFE in 0.1 M NaOH scanning from −0.4 V to 0.0 V at 30 mV s^{-1}.

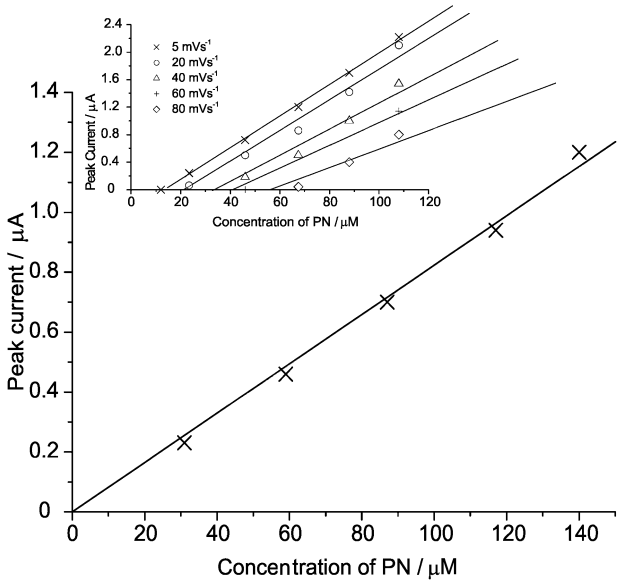

Fig. 2 A plot of peak current *vs.* PN concentration showing a linear response (scan rate 10 mV s^{-1}). Inset: the response of peak current to increasing additions of PN at different scan rates (5, 20, 40, 60 and 80 mV s^{-1}).

when the potential was scanned in the *positive* direction at *ca.* −0.1 V immediately before the onset of mercury dissolution (Fig. 1). This inverse peak was found to be reproducible in both 0.1 M KOH and 0.1 M NaOH solutions and was found to be independent of both the initial potential that the scan was started from and any effects of varying the accumulation time that the initial potential was held for prior to scanning in an anodic direction. Fig. 1 shows the resulting CV response when the potential was cycled from an initial potential of −0.4 V.

However the observed voltammetric signal due to PN in the concentration range 10^{-5}–10^{-4} M was found to decrease in the presence of an excess quantity of the following: (1) reducing anions such as SO_3^{2-}, cysteine or I^- (10^{-3} M); (2) oxidising agents such as $KMnO_4$ or O_2 gas bubbled through the solution; (3) CO_3^{2-} ions; (4) complexing agents such as ethylenediamine tetra acetic acid (EDTA). The reduction of PN signal by EDTA suggests that the Hg(II) ion is involved in the observed electrochemistry of PN at the MFE as the binding constant of Hg(I) by PN is rather small compared to that of Hg(II).[30] Also whilst the effect of pH on the voltammetric response of PN is discussed below, it is interesting to note here that at very high concentrations of hydroxide ion (0.5 M) the signal due to PN is also found to decrease. These latter two observations are important in lending support to the possible mechanism proposed in Section 3.3.

The response to increasing the concentration of PN was investigated over the concentration range of PN of 10^{-5} to 10^{-4} M. To this end, 0.2 mL aliquots of an alkaline solution containing dissolved tablets of photolysed KNO_3 (PN concentration determined as 1.25 mM) were added to the blank 0.1 M KOH solution corresponding to 31 μM additions of PN and the resulting CV response recorded. The height of the inverse peak was found to reproducibly increase in a linear fashion with increasing PN concentration as shown in Fig. 2, suggesting that this peak is indeed due to the presence of PN. The sensitivity was found to be 41 mA M^{-1} with a limit of detection based on 3σ of 12.0 μM. Furthermore experiments where PN solutions were made using photolysed KNO_3 tablets which had been irradiated for 1, 2 and 3 hours also showed an increase in peak current with increasing PN concentration.

However, the analytical utility of this approach towards the determination of PN concentration is limited, being strongly dependent on the scan rate used as shown in the inset of Fig. 2. Thus at low scan rates (5–10 mV s^{-1}) the MFE may be used for the electroanalytical detection of PN at alkaline pHs in the range of pH 12–14 subject to the absence of interferents listed above and over the PN concentration range of 10^{-5} to 10^{-4} M. At higher scan rates, the peak currents are smaller in magnitude (discussed below) and the corresponding limit of detection is drastically reduced, such that no peaks corresponding to PN were observed at a scan rate of 100 mV s^{-1} except at the very highest concentration studied. Also note that the current at low PN concentrations becomes negative at higher scan rates corresponding to the anodic dissolution of mercury (under the convention used throughout this work oxidative currents are assigned as flowing in a negative direction).

Finally the effect of varying scan rate, υ, on the voltammetric response of PN at a MFE was investigated from 10–100 mV s^{-1} in the concentration range of PN of 10^{-5} to 10^{-4} M. Fig. 3 shows the CV response of the MFE towards PN at varying scan rates with a corresponding plot of peak height *vs.* scan rate inset. Surprisingly the peak current, i_p, is found to *decrease* with increasing scan rate in a linear fashion ($y = 4.0 \times 10^5$–$2.3 \times 10^{-7}\ x$, $R^2 = 0.9937$). This suggests that the resulting electrode process is not operating under diffusion control nor is it typical of a diffusion-controlled process (note that a plot of peak current *vs.* $\upsilon^{1/2}$ shows significant deviation

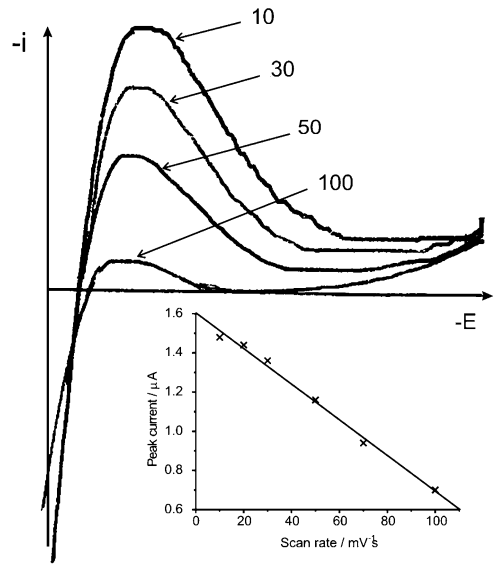

Fig. 3 The voltammetric behaviour of *ca.* 10^{-5} M peroxynitrite at varying scan rates. Inset: A plot of peak current *vs.* scan rate showing the unusual voltammetric behaviour of PN at a MFE in 0.1 M KOH.

from linearity). However this result does provide some insight into the possible mechanism by which the presence of PN results in an inverse cathodic peak being observed at a MFE when the potential is scanned in an anodic direction which is discussed in Section 3.3.

3.2 The effect of pH on the voltammetric determination of PN at a MFE and the electrochemical measurement of the kinetics of PN decay

The effect of varying the solution pH over the range 9–13 on the stability of the observed voltammetric behaviour of PN at a MFE was investigated. As discussed in the introduction, PN decays *via* its protonated form ONOOH to produce a mixture of products including NO_3^- at more acidic pH, and NO_2^- and O_2 at pH > 9 (see eqn (1)),[3] whilst homolytic cleavage of the O–O bond to produce hydroxyl radicals has been shown to be highly unlikely.[2,3] Thus as the pH is lowered towards that of the pK_a value of ONOOH the decay of PN becomes increasingly rapid.

In 0.1 M KOH or NaOH solutions the PN voltammetric signal remained stable with repetitive scans over a period of 90 minutes. However in 0.01 M KOH solutions the voltammetric signal due to PN was found to steadily decrease with repeated scans over the same period of time, and furthermore the initial height of the PN peak for solutions prepared from tablets of PN that had been irradiated for the same amount of time was also found to decrease as the pH of the solution was lowered. This is consistent with the increasingly rapid decay of PN in the solution at lower pHs. Note that the peak potential of the voltammetric wave due to PN was found to be independent of pH over the pH range studied (pH 9–13) and remained fixed at *ca.* −0.1 V. However, as discussed in Section 3.1, at much higher concentration of hydroxide (0.5 M) the observed peak potential shifted to more negative potentials and the peak current rapidly decreased. Therefore the kinetics of PN decay were investigated electrochemically from pH 9–13 by measuring the decrease in peak current of the voltammetric peak at −0.1 V due to PN with time.

To this end one tablet of photolysed KNO_3 was crushed and dissolved in 10 mL of buffer solutions of varying pH (see Section 2.1) so that the initial composition of each solution initially contained 0.4 M KNO_3, 150 μM PN and 15 μM of KNO_2 and the ionic strength of each buffer solution remained practically constant throughout. Note that sodium carbonate buffer solutions could not be used as the carbonate anion can exist in equilibrium with CO_2 in solution which is known to readily react with PN to form nitrosoperoxycarbonate ($ONO_2CO_2^-$) which then rapidly decays.[28]

Each solution of known pH was then immediately analysed voltammetrically by scanning from −0.2 V up to the potential of mercury dissolution which varied slightly with pH between +0.05 V and −0.05 V and the peak height of the PN wave was measured at regular time intervals. The peak current, i_p, is directly proportional to the concentration of PN and a plot of $\log_{10}(i_p)$ against time revealed that the decay of PN exhibited first order or pseudo-first order kinetics in the concentration of PN as shown in Fig. 4.

Table 1 lists the calculated average (taken from 4 repeat measurements) first order rate constant for the decay of PN at various pHs studied in phosphate buffer, whilst Table 2 lists the first order rate constants when the decay of PN was studied in borate buffer. Note that it was not possible to obtain data below pH 9 due to the decay of PN being so rapid on the timescale of the experiment that a rate constant could not be determined without very significant errors being introduced into the data. The data presented in Table 1 is in reasonable agreement with the values reported by Pfeiffer *et al.* using stopped-flow spectrophotometric techniques.[3] The variation of the logarithm of the measured rate constants with pH, $\frac{d(\log_{10} k)}{d(pH)}$, gave a value of 1.2 ± 0.1 which indicates that the number of protons involved in the decomposition of PN is likely equal to one, consistent with the decomposition of PN occurring through the intermediate ONOOH formed by protonation of PN.

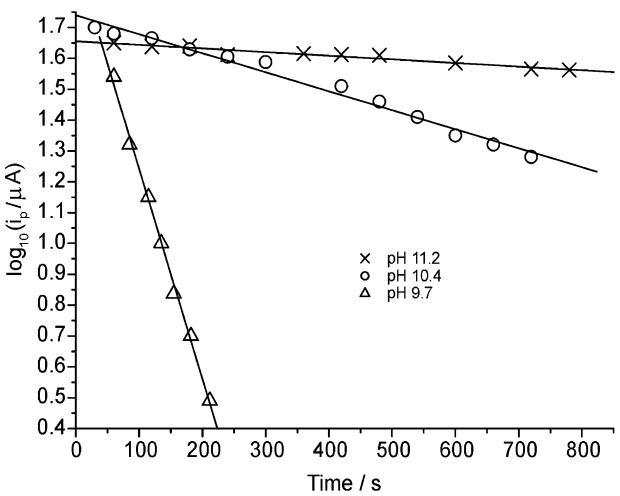

Fig. 4 Overlaid plots of $\log_{10}(i_p/\mu A)$ *vs.* time showing the first order or pseudo-first order kinetics of PN decay at various pHs.

Table 1 The average first order rate constants for the decay of PN at various pHs calculated from the voltammetric response of PN at a MFE in 0.5 M Na_2HPO_4 buffer adjusted to the appropriate pH by addition of NaOH

pH (measured)	k_{PN}/s^{-1} (average of 4 repeated experiments)
9.7	$1.4 \pm 0.3 \times 10^{-2}$
10.2	$6.2 \pm 0.2 \times 10^{-3}$
11.2	$2.3 \pm 0.6 \times 10^{-4}$

Interestingly, the rate constants measured in phosphate buffer, and particularly in borate buffer solutions, are consistently slightly larger than those reported by Pfeiffer *et al.*[3] Indeed, the rate constants presented here are more consistent with the pK_a of PN being *ca.* 8 rather than 6.8 as has been previously reported.[2–4] There are two possible explanations for this. First, as Kissner *et al.* have reported, the apparent pK_a of PN strongly depends on the nature and concentration of the buffer constituents. Thus in phosphate buffer solutions Kissner *et al.* determined the pK_a of PN to vary between 6.5 and 7.3, yet in 0.1 M borate buffer they determined the pK_a of PN to be 8.5.[5] This would seemingly explain why the rate constants obtained here in phosphate buffer are in closer agreement with Pfeiffer's reported values (where the pK_a of PN is taken to be 6.8), and why the rate constants measured in borate buffer are significantly higher, consistent with a pK_a value for PN in our systems being closer to 8.0. Second, the pK_a value of 6.8 refers to the *cis* isomer of PN. Tsai *et al.* have shown that the pK_a of the less stable *trans* isomer is closer to 8.0.[4] Whilst the *cis* isomers of both PN and ONOOH are more stable than the *trans* isomers, there is some suggestion that the decomposition mechanism of PN may pass through a *trans* transition state. This is because the electronic structure of the *trans* isomer allows the facile rearrangement of PN to form NO_3^- as a decomposition product, whilst the *cis* isomer of PN anion is unable to undergo such a rearrangement as the negative charge is delocalised over the entire molecule resulting in some partial bonding character between the two terminal oxygen atoms, the strength of this interaction being equivalent to that of a hydrogen bond.[4] The possible involvement of some *trans* character in the decomposition of PN may also account for the higher apparent pK_a value seen here.

3.3 Discussion of the possible mechanism for the voltammetric behaviour of peroxynitrite at a mercury film electrode

In order to consider the mechanism by which the unusual voltammetric behaviour of PN at a MFE occurs further cyclic voltammetric experiments were performed where the potential was first scanned from −0.2 V in the anodic direction. The scan direction was then reversed just after the inverse cathodic PN peak was observed at −0.1 V but before the onset of any significant anodic dissolution of the mercury film occurred (Fig. 5). In this case, however, an additional cathodic current was also observed at every potential where the PN inverse peak was observed corresponding to the electrodeposition of mercury onto the electrode surface (see below).

Table 2 The average first order rate constants for the decay of PN at various pHs calculated from the voltammetric response of PN at a MFE in 0.5 M sodium tetraborate buffer adjusted to the appropriate pH by addition of NaOH

pH (measured)	k_{PN}/s^{-1} (average of 4 repeated experiments)
9.7	$4 \pm 2 \times 10^{-2}$
10.0	$2 \pm 0.8 \times 10^{-2}$
10.5	$6 \pm 1 \times 10^{-3}$
11.0	$1 \pm 0.5 \times 10^{-3}$
11.5	$2.5 \pm 0.3 \times 10^{-4}$

First, it is particularly interesting to note that in their classical polarographic studies into the electrochemical behaviour of organic alkyl peroxides at a dropping mercury electrode, Levin and Yamshikov also observed an inverted "pre-wave" just before the onset of mercury dissolution at *ca.* −0.1 V *vs.* SSCE.[31,32] This wave was only observed in alkaline solutions, typically containing 0.1 M NaOH. The peak current of this wave was always found to be less than the calculated diffusion limited value and instead indicated that the electrochemical response was characteristic of a surface adsorbed species. Levin carried out simple thermodynamic calculations showing that in 0.1 M NaOH solutions the HgO/Hg couple has a half-wave potential of *ca.* −0.08 V *vs.* SSCE, coincident with the peak potential of the observed pre-wave. Based on these observations Levin and Yamshikov suggested that the following mechanism could explain this unusual behaviour:[31,32]

$$\begin{aligned} Hg + HO_2^- &\xrightarrow{k} HgO + OH^- \\ HgO + H_2O + 2e^- &\rightarrow Hg + 2OH^- \end{aligned} \qquad (2)$$

However, in the case of the voltammetric response of PN observed here, if no significant electrochemically driven anodic dissolution of the MFE occurs at potentials just negative of

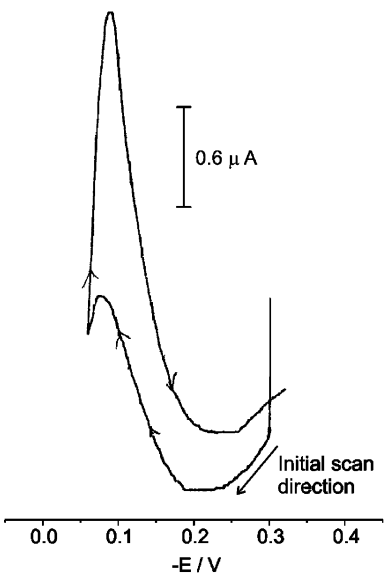

Fig. 5 The cyclic voltammetric response of PN in 0.1 M KOH solution where the scan direction is reversed before the onset of mercury anodic dissolution, showing the presence of a cathodic current due to the reduction of Hg(I) to Hg(0) and the inverse cathodic PN wave in the anodic scan at identical potentials (see text).

the onset of the inverted peak, then one must assume that the Hg(II) ions must be generated by the oxidation of Hg(0) by PN, which, as discussed in the introduction is a relatively strong oxidant. This argument allows us to propose the following CE mechanism to explain the voltammetry observed at a MFE in the presence of PN in alkaline solution, where the rate determining step is the first electron transfer:

$$\begin{array}{l} \mathrm{Hg} + \mathrm{ONOO}^- \overset{k}{\rightarrow} \mathrm{HgO} + \mathrm{NO}_2^-;\ \text{C-step} \\ \mathrm{HgO} + \mathrm{H_2O} + 2e^- \rightarrow \mathrm{Hg} + 2\mathrm{OH}^-;\ \text{E-step} \end{array} \quad (3)$$

In this mechanism the chemical step is rather unusual in that it is dependent on the electrode potential, *i.e.* it is a *chemical* redox process. The oxidation of Hg(0) to Hg(II) by PN can only occur when there is a sufficient match between the Fermi level of the electrons within the mercury film and the LUMO on the PN molecule for electron transfer to take place. Thus at more negative potentials than −0.1 V *vs.* Ag/AgCl/1.0 M KCl the Fermi level of the mercury film lies too deep in energy for oxidation by PN to occur and no voltammetry is observed until the potential is swept close to −0.1 V and sufficient match between the Fermi level of the electrode and the LUMO of PN is achieved.

Whilst the mechanism proposed by Levin encapsulates the main features of the voltammetry of PN the requirement for an unusual potential dependent chemical step indicates that this mechanism is a little oversimplified. Instead we propose that the voltammetry of PN at a MFE can be explained by an $\vec{\mathrm{E}}\mathrm{C}\overleftarrow{\mathrm{E}}$ reaction shown in eqn (4) where the $\vec{E}$-step is likely rate determining (see below).

$$\begin{array}{l} 2\mathrm{Hg} + 2\mathrm{OH}^- \overset{\text{slow}}{\rightarrow} \mathrm{Hg_2O} + 2e^- + \mathrm{H_2O};\ \vec{\mathrm{E}}\text{-step} \\ \mathrm{Hg_2O} + \mathrm{ONO}_2^- \overset{\text{fast}}{\rightarrow} 2\mathrm{HgO} + \mathrm{NO}_2^-;\ \text{C-step} \\ \mathrm{HgO} + 2e^- + \mathrm{H_2O} \overset{\text{fast}}{\rightarrow} \mathrm{Hg} + 2\mathrm{OH}^- \quad \overleftarrow{\mathrm{E}}\text{-step} \end{array} \quad (4)$$

As the electrode potential is swept from negative potentials towards the onset of anodic dissolution a thin film a few Å thick of Hg(I) oxide begins to form on the electrode surface. The Hg(I) at the interface of this film and the electrolyte solution is then chemically oxidised by PN in solution to form Hg(II) species. As the electrode potential is still more negative of the reduction potential for HgO, the Hg(II) ions are able to diffuse across this thin oxide layer, provided it is sufficiently thin, to be reduced at the electrode back to Hg(0). The net transfer of mercury atoms is therefore equal to zero. However the voltammetry is actually the sum of two consecutive processes, a one-electron oxidation and a two-electron reduction, so that there is a net transfer of one electron to the electrode giving rise to a net cathodic current. As the potential is swept still more positive, the anodic dissolution of mercury becomes significant such that a peak is observed in the net cathodic current and then the onset of mercury dissolution dominates the voltammetry.

The observed decrease in peak current with increasing scan rate can be attributed to the lack of significant nucleation of Hg_2O film needed on the experimental timescale at faster scan rates before the onset of mercury dissolution. In solutions of high hydroxide ion concentration (>0.5 M) the voltammetric response due to PN is inhibited, possibly due to either complexes of Hg with OH and subsequent HgO formation and/or the formation of thicker Hg_2O films. The last is in agreement with the earlier work of Brainina, who considered the formation of oxide films on mercury electrodes as semi-conducting phase layers and the effect that they could have on the observed voltammetric behaviour.[33]

4. Conclusion

In this report we have shown that PN can be detected voltammetrically as an inverse cathodic peak when the potential is scanned anodically at a MFE in alkaline solutions. The voltammetric behaviour of PN in alkaline solutions at a MFE has been characterised with respect to the effect of varying the potential scan rate and the pH of the electrolyte solution. An $\vec{\mathrm{E}}\mathrm{C}\overleftarrow{\mathrm{E}}$ mechanism is proposed as a refinement to the CE mechanism proposed by Levin in his early work on the polarography of organic peroxides at mercury electrodes to explain this behaviour.

The voltammetric response of the MFE was found to vary in a linear fashion to increasing PN concentrations in the range of 1.10^{-5} to 2.10^{-4} M and thus may form the basis of a direct electroanalytical method for determining PN concentration subject to certain experimental limitations, discussed in Section 3.1. The effect of the solution pH on the stability of PN was investigated and first order rate constants for the decay of PN were determined electrochemically over the pH range 9–13. Whilst these values are in reasonable agreement with those reported previously using spectroscopic techniques, the values obtained herein are always slightly higher and are somewhat dependent on the composition of the buffer solution used. This is again consistent with previous published reports which suggest that the pK_a of PN may under similar conditions to those used here be rather higher than the cited value for the *cis* isomer of PN of 6.8; being closer to 8.0. This also raises the possibility of the *trans* isomer being implicated in the decay of PN, as the pK_a value of the *trans* isomer is estimated to be between 1 and 1.5 units higher than the *cis* isomer.

We believe that further work is required in order to optimise the electroanalytical detection methodology proffered by this initial investigation and also to explore the effect of interferents and different electrode substrates capable of operating *via* a similar mechanism. This is the subject of a follow-up report currently being prepared for publication.

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