ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 995–1001

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Bioanalytical utility of sonovoltammetry

Emma L. Beckett, Nathan S. Lawrence, Yu Chen Tsai, James Davis, Richard G. Compton *

Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK

Received 26 March 2001; received in revised form 7 May 2001; accepted 20 May 2001

Abstract

Dopamine dissolved within egg homogenate was used as a model system to study the effects of electrode contamination and its subsequent reactivation through ultrasonically mediated in situ cleaning effects. The merits in conducting electroanalytical investigations under the influence of the ultrasonic field were also appraised. Maintaining the ultrasound field during oxidative measurements was found to yield hydrodynamic profiles that were linear over the concentration range 2–20 μM dopamine. The resulting sonolinear sweep voltammograms were compared with conventional rotating disk measurements, with the former found to provide significantly increased limiting currents that were attenuable through the manipulation of the field intensity. The problem of retaining selectivity in the presence of high concentrations of ascorbate was also assessed with the addition of cupric ion prior to commencing the measurements found to efficiently negate an otherwise substantive interference. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Sonoelectrochemistry; Ultrasound; Electrochemical detection; Electroanalysis; Dopamine

1. Introduction

The versatility of electrochemical techniques in aiding the quantification of organic compounds is beyond question yet their utilisation for routine analytical determinations has traditionally been eschewed in favour of chromatographic and capillary electrophoretic techniques [1–6]. This lack of acceptance can be attributed in part to the variability of the electrode response when confronted with matrices of complex composition. Irreproducible behaviour resulting from fouling of the electrode surface is a perennial problem in electroanalysis and, as such, numerous methods have arisen to counter the deleterious effects that adventitious adsorption can impart on the analytical signal. Electrode substrate modifications aimed at alleviating such problems abound within the literature [6], however, the complexities associated with the fabrication of such assemblies often serves to preclude their widespread adoption. This is particularly true when considering routine screening applications, where non-specialist users would be responsible for the implementation of the protocol. The principal aim of the current

* Corresponding author. Tel.: +44-1865-275-413; fax: +44-1865-275-410.

E-mail address: richard.compton@chemistry.ox.ac.uk (R.G. Compton).

0731-7085/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(01)00494-0

communication has been to assess the utility of employing a standard ultrasound probe within an electrochemical cell as means of automating the electrode cleaning process and hence remove the ambiguities associated with modified electrodes and mechanical polishing.

The combination of ultrasound with electroanalytical stripping protocols has been shown to confer a number of operational advantages to the quantification of metal ions, of which continued electrode activation and increased sensitivity are among the more prominent [7]. Imposing a high intensity ultrasonic field within the cell during the deposition stage has enabled the extraction of the target metals in media that would otherwise serve to passivate the electrode [7–10]. The principal advantage possessed by this approach over other convective sources tends to lie in the generation and subsequent collapse of cavitation bubbles at the surface of the electrode. These processes provide an ablative action that efficiently removes adsorbed material from the electrode surface and maintains electrode activity throughout the accumulation period [7]. The actual measurement phase, however, is almost invariably conducted under silent conditions using conventional sweep or pulse techniques [8–10].

The extrapolation of sonoelectrochemical techniques to the analysis of organic species that are incapable of accumulation at the electrode surface has yet to be fully examined and a number of questions remain to be addressed. Foremost, is the possibility that in such instances, the only beneficial action that can arise from such a combination is an in situ cleaning action. Exploitation of the hydrodynamic conditions resulting from the imposition of the ultrasound field during the actual measurement phase, however, could provide an added bonus of increased sensitivity [7]. However, the ability to reliably quantify the signal arising from the intensely turbulent conditions is a major consideration as is the retention of sufficient selectivity in the midst of other, more concentrated, electroactive interferences. The latter has confounded many hydrodynamic schemes and as a result they are usually employed as post column detection systems (wall-jet) or restricted to fundamental studies of electrode processes (rotating disk).

The oxidation of dopamine within unfiltered egg homogenate was selected as a model system with which to probe the utility of the proposed assembly. The complex composition and adhesive qualities of the egg solution ensured that electrode re-activation (whether through in situ ablation or ex situ mechanical polishing) would be required soon after immersion. Dopamine was chosen as the redox probe as its electrochemical characteristics are well established and routinely used to explore the efficacy of new strategies aimed at improving electrode response and/or selectivity [11–16]. The report details the electrode responses obtained using ultrasound purely as a means of refreshing the electrode prior to commencing the voltammetric scan, and also explores the various merits and limitations associated with sustaining the ultrasound field throughout the course of the analytical measurement.

2. Experimental details

All reagents were obtained from Aldrich and were used without further purification. Solutions were prepared using deionised water with a resistivity of not less than 18 MΩ cm (Elgastat, UK). Electrochemical measurements were recorded using a μAutolab computer controlled potentiostat with a conventional three electrode configuration. Glassy carbon (3 mm diameter, BAS Technicol, UK) served as the working electrode with a platinum wire counter electrode and saturated calomel reference electrode completing the cell. Sonoelectrochemical investigations were conducted using a 20 kHz transducer with a 3 mm stepped titanium probe placed 5 mm directly above the face of the working electrode. The ultrasound horn was operated using a transducer intensity setting of between 5 and 20%. The probe was electrically isolated from the test solution through the combined use of a PTFE spacer and connecting screw. The cell assembly used for these studies has been detailed in Fig. 1 with a typical solution volume of 15 cm^3 used throughout. Thermostating of the cell was achieved by the introduction of a glass cooling coil through which water from a constant temperature bath (20°C ±

2) was circulated. Linear sweep voltammetry was conducted with a scan rate of 50 mV s^{-1}. Rotating disk analysis was conducted using an Oxford electrode motor controller with the three electrode arrangement described above. Surface morphology comparisons were obtained using a Topometrix TMX 2010 Discoverer atomic force microscope operating in contact mode with SFM probes (type 1520-00) and a 75 μm scanner (type 5590-00).

3. Preparation of egg homogenate solution

In each set of experiments, egg (white and yolk) was homogenised in a beaker and 50 cm^3 of the resulting mixture adjusted to pH 7 through the drop-wise addition of NaOH. Supporting electrolyte (0.1 M KCl) was added to the solution before finally adjusting the volume to 100 cm^3. The egg homogenate was pipetted into the pre-assembled electrochemical cell (15 cm^3) and the appropriate measurement conducted. Standard additions of dopamine (typically 30 μl of a 0.01 M stock solution) were introduced and the solution mixed by the syringing action of a Pasteur pipette to avoid foaming. The solution was then left to stand for 5 min before commencing the electrochemical measurements.

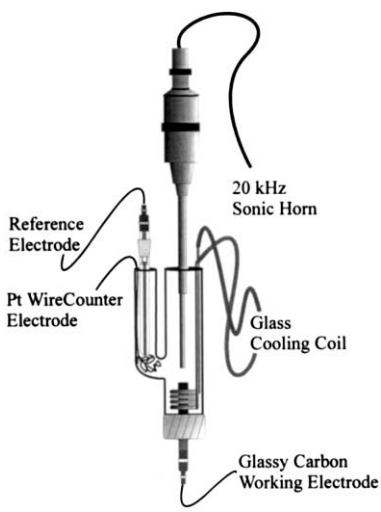

Fig. 1. Sonoelectrochemical assembly.

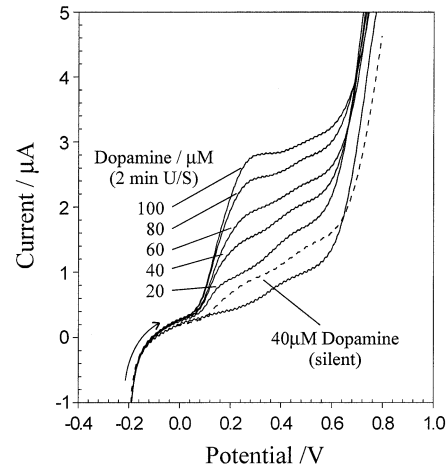

Fig. 2. Linear sweep voltammograms detailing the oxidation of dopamine (20 μM aliquots) at a glassy carbon electrode within egg homogenate (pH 7). Scan rate, 50 mV s^{-1}.

4. Results and discussion

Linear sweep voltammograms were used to determine the response of a glassy carbon electrode to increasing additions of dopamine within the egg homogenate. Two series of experiments were performed whereby the responses obtained in a solution in which no attempt was made to clean the electrode between scans were compared with those recorded after the imposition of an ultrasonic pulse. In the latter, ultrasound was applied for a period of 2 min with the subsequent linear sweep recorded under silent, quiescent, conditions. Voltammograms detailing the responses obtained to increasing additions of dopamine (20 μM aliquots) at a pre-insonated electrode are shown in Fig. 2. The response to 40 μM dopamine obtained under conventional, silent, conditions (dashed line) has been included for comparison. It can be seen that the introduction of the ultrasound cleaning step significantly improves the resolution of the oxidation wave with the magnitude of the oxidation process found to be more than double that attainable under non-insonated conditions.

The effect of the ultrasound pulse on the electrode solution interface was investigated by comparing the substrate morphology before and after insonation. A freshly polished electrode was

placed within the egg homogenate and allowed to sit undisturbed for a period of 2 h. The electrode was removed, rinsed in fresh phosphate buffer (pH 7) and the surface profile examined using atomic force microscopy (AFM). The resulting micrograph is detailed in Fig. 3A with the electrode surface found to be thoroughly contaminated by the egg constituents. Upon replacing the electrode within the sono assembly, detailed in Fig. 1, a 2-min pulse of ultrasound was applied and the surface re-characterised. The subsequent AFM micrograph is detailed in Fig. 3B (recorded at the same resolution in order to retain the integrity of the comparison) with the lack of any notable features, bar some original polishing marks, consistent with that expected for a clean electrode. The degree of electrode fouling experienced through sitting in the homogenate for 2 h is clearly an extreme case but it does serve to highlight the surface activation properties of the ultrasound probe.

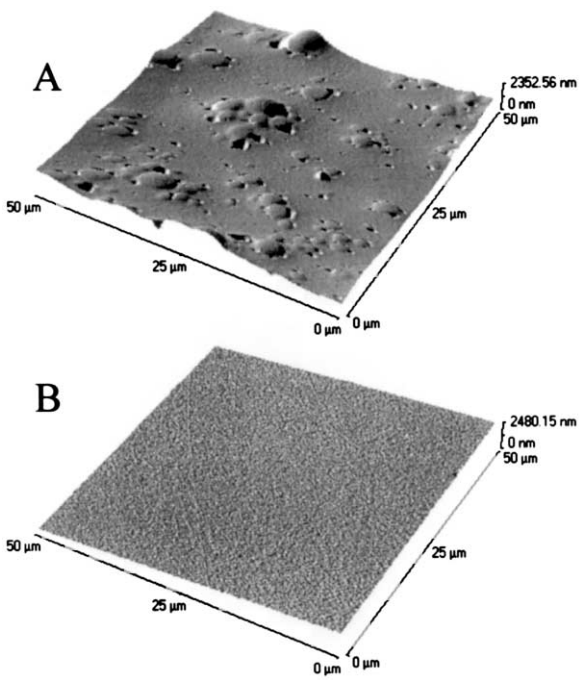

Fig. 3. Atomic force micrographs of the electrode surface after exposure to the egg homogenate for a period of 2 h (A); and after in situ ultrasonic cleaning (B).

Maintaining the ultrasound field during the actual measurement phase induces hydrodynamic behaviour with the increased mass transport to the electrode providing a steady state current profile upon commencing oxidation rather than the peaked response observed in Fig. 2. The resulting 'sonolinear' sweep voltammograms detailing the oxidation of 20 μM dopamine are compared with the responses obtained at a rotating disk electrode (RDE) under analogous conditions. While both systems exhibit hydrodynamic behaviour, two contrasting features are apparent. First, the magnitude of the limiting currents recorded under the influence of ultrasound are substantially greater than those observed at the RDE even at high rotation rates. Second, the limiting current plateau is notably noisier in the insonation profiles. Transient spikes in the current are observed once oxidation commences and is attributed to the generation and subsequent collapse of cavitation bubbles at the electrode-solution interface. Asymmetric collapse of bubbles at or near the electrode results in the release of high velocity microjets of liquid at the electrode surface. These jets invariably carry fresh, un-oxidised, material and hence the subsequent increase in the oxidation current manifests itself as a sharp spike. While this may appear to induce a degree of ambiguity in extracting the limiting current from such data, it is found that signal averaging over the plateau region provides reproducible data (%R.S.D. = 3.5, for 20 μM dopamine, $N = 14$). The response to increasing additions of dopamine was found to provide a linear increase in the limiting current, shown in the inset diagram within Fig. 4, covering the range 2–20 μM dopamine (regression data: I_{lim} (μA) = 0.204 [dopamine (μM)] − 0.07, $N = 11$, $R^2 = 0.998$). The limit of detection was found to be 0.85 μM (based on $3s_b$), which compares favourably with most direct electrochemical approaches to the determination of dopamine and those using some form of surface modification [12]. More importantly, the ability to reliably perform the measurement within complex media without any form of sample pretreatment and using a conventional, bare electrode is clearly a significant advantage.

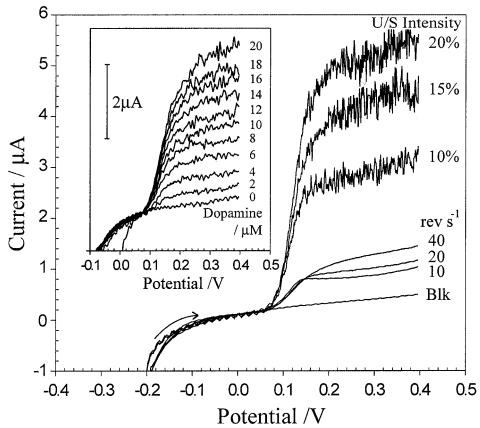

Fig. 4. Comparison between sonoelectrochemically enhanced oxidation of 20 μM dopamine and the response obtained with a rotating disk electrode. Inset, sonolinear sweep voltammograms detailing the oxidation of dopamine (2 μM aliquots). Scan rate, 50 mV s^{-1}.

Although the increased sensitivity can prove valuable for the quantification of low concentrations of analyte, caution must be exercised when dealing with complex media within which other electroactive species reside. The oxidation of these compounds will be similarly amplified by the increased mass transport and, if present in massive excess could completely obscure the true analytical signal. A disadvantage in attempting electroanalytical determinations under hydrodynamic conditions is the lack of specificity that can, in principle, be achieved through the use of pulse techniques (i.e. square wave or differential pulse voltammetry). This is highlighted in Fig. 5, where the sonoelectrochemically enhanced oxidation of 20 μM dopamine was assessed in the presence of ascorbate (0–300 μM). The oxidation of ascorbate (+0.27 V) was found to occur at potentials slightly less positive than that of the dopamine (+0.14 V) and, as such, the introduction of the interferent leads to a cumulative increase in the oxidation current. The close proximity of both processes effectively precludes discrimination between the two signals with a single limiting current plateau observed.

Efficient and indeed effective use of sonolinear sweep voltammetry clearly depends on judicious choice of application. The interference posed by ascorbate is the classic example and its ubiquity has meant that numerous strategies have evolved to counter its effect. In this instance, we have found that the addition of excess cupric ion to the analysis medium prior to commencing the analysis can negate the influence of ascorbate. The chemical oxidation of ascorbate by cupric ion is well documented but the majority of the studies conducted thus far have concentrated on the elucidation of the kinetics associated with the reaction [17,18]. The exploitation of the reaction as a means of selectively removing ascorbate within analytical contexts has yet to be appraised.

The influence exerted by the copper was assessed by comparing the change in the dopamine (20 μM) oxidation wave upon the addition of ascorbate (0–300 μM) in the presence and absence of excess cupric ion. A large excess of the cation (typically 0.5 mM) is employed to minimise possible losses due to complexation with matrix constituents. The results are detailed in Fig. 6. It can be seen that the oxidative action of the cupric ion selectively removes ascorbate through its conversion to the electro-inactive dehydroascorbate leaving the principal dopamine wave unchanged. Removal of ascorbic acid in this way was also achieved without penalising the clarity of the dopamine voltammetric profile. This is highlighted in the inset diagram within Fig. 6, where 11 sonolinear sweep voltammograms detailing the

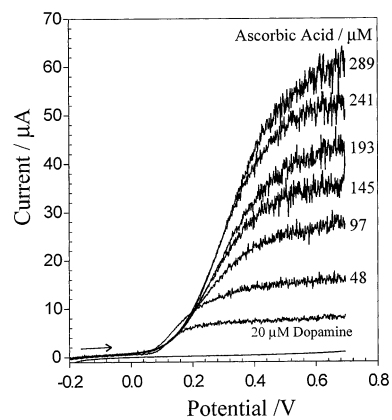

Fig. 5. Sonolinear sweep voltammograms detailing the electrode response to 20 μM dopamine in the presence of 0–300 μM ascorbic acid in pH 7 solution. Scan rate, 50 mV s^{-1}.

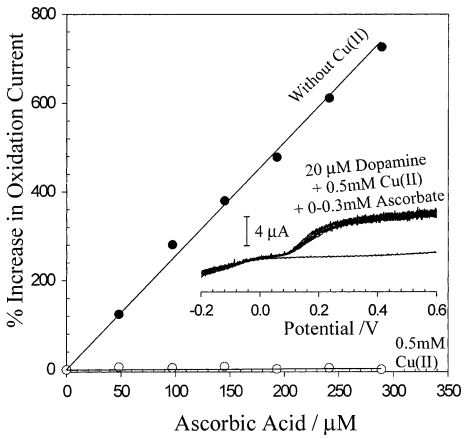

Fig. 6. Influence of cupric ion on the height of the oxidation peak. Inset, sonolinear sweep voltammograms detailing the oxidation of 20 μM dopamine in the presence of 0.5 mM Cu(II) and 0–300 μM ascorbic acid in pH 7 solution. Scan rate, 50 mV s^{-1}.

oxidation of 20 μM dopamine in the presence of increasing ascorbate (0–300 μM, 48 μM increments) are presented. The superimposability of the scans stand as testament to the robustness of the approach. The generic applicability of cupric ion was briefly assessed through comparing the sonoelectrochemically enhanced oxidation of catechol and the anti-inflammatory drug 5-aminosalicylic acid in the presence of excess ascorbate. In both cases, the addition of cupric ion was found to reduce the interfering effects of the antioxidant. The limiting currents initially recorded for the oxidation of 20 μM analyte were found to increase by less than 8% in the presence of 300 μM ascorbate when 0.5 mM cupric ion was added and compared with 725% when the metal ion was omitted.

5. Conclusion

The coupling of a standard ultrasound probe with conventional electrochemical techniques as a means of providing a robust system that could operate within highly fouling media has been demonstrated. The merits and limitations of harnessing the hydrodynamic properties of the resulting field for sono-analytical purposes have been assessed and a strategy for improving selectivity in the presence of ascorbate has been presented. This has particular relevance given the multitude of compounds whose oxidative determination would traditionally have been plagued by the presence of this common antioxidant. The low micro-molar detection range and electrode activation properties afforded by the sonoelectrochemical technique clearly provides the analyst with a significant tool for investigating a range of physiologically relevant species. The ability to clean the electrode in situ hence remove the ambiguities associated with mechanical polishing combined with a potentially fast response time will also prove attractive for routine screening applications within the context of pharmaceutical analysis. The applicability of the sonoelectrochemical approach is further strengthened by the use of purely commercial equipment whose combination effectively minimises or removes the need for extensive sample clean up and electrode manipulation and, as such, offers a degree of procedural simplicity that is absent with most forms of conventional electroanalysis.

References

[1] H.H. Bauer, J.E. O'Reilly, in: G.D. Christian, J.E. O'Reilly (Eds.), Instrumental Analysis, Allyn and Bacon, Newton, MA, 1986, pp. 1–13.
[2] D. Perret, S.R. Rudge, J. Pharm. Biomed. Anal. 3 (1985) 3–27.
[3] J.W. Jorgenson, J.D. Wit, in: H.H. Hill, D.G. McMinn (Eds.), Detectors for Capillary Chromatography, vol. 121, Wiley, New York, 1992, pp. 395–425.
[4] B.R. Bryant, F.D. Swanek, A.G. Ewing, in: M.G. Khaledi (Ed.), High Performance Capillary Electrophoresis, vol. 146, Wiley, New York, 1998, pp. 355–374.
[5] T. Kappes, P.C. Hauser, Electroanalysis 12 (2000) 165–170.
[6] K. Stulik, Electroanalysis 11 (1999) 1001–1004.
[7] R.G. Compton, J.C. Eklund, F. Marken, Electroanalysis 9 (1997) 509–522.
[8] R.P. Akkermans, J.C. Ball, T.O. Rebbitt, F. Marken, R.G. Compton, Electrochim. Acta 43 (1998) 3443–3449.
[9] A.J. Saterlay, J.S. Foord, R.G. Compton, Analyst 124 (1999) 1791–1796.
[10] C. Agra-Gutierrez, J.L. Hardcastle, J.C. Ball, R.G. Compton, Analyst 124 (1999) 1053–1057.
[11] Q. Wang, N. Jiang, N.Q. Li, Microchem. J. 68 (2001) 77–85.

[12] M. Marazuela, L. Agui, A. Gonzalez-Cortes, P. Yanez-Sedeno, J. Pingarron, Electroanalysis 11 (1999) 1333–1339.
[13] Z.M. Liu, O. Niwa, R. Kurita, T. Horiuchi, Anal. Chem. 72 (2000) 1315–1321.
[14] R.C. Matos, L. Angnes, M.C.U. Araujo, T.C.B. Saldanha, Analyst 125 (2000) 2011–2015.
[15] S.M. Strawbridge, S.J. Green, J.H.R. Tucker, J. Chem. Soc. Chem. Commun. (2000) 2393–2394.
[16] A.V. Pereira, O. Fatibello-Filho, Anal. Chim. Acta 366 (1998) 55–62.
[17] J. Xu, R.B. Jordan, Inorg. Chem. 29 (1990) 2933–2936.
[18] M.J. Sisley, R.B. Jordan, J. Chem. Soc. Dalton Trans. 20 (1997) 3883–3888.