New electrochemical approach for the measurement of oxidative DNA damage: Voltammetric determination of 8-oxoguanine at screen-printed graphite electrodes

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29   *Abstract*

30   Simplification and miniaturisation of analytical methods for the direct detection of DNA damage

31   is a challenging area of research and screen-printed electrodes are a promising alternative

32   approach to analytically / electroanalytically monitor the species involved. In this work we

33   demonstrate that screen-printed graphite macroelectrodes (SPEs) provide useful

34   electrochemical signatures to study the behaviour of 8-oxoguanine (8-oxoGua), which is the

35   most frequent and important marker of oxidative DNA damage and it is widely considered as a

36   biomarker, *via* differential pulse voltammetry (DPV). Under the optimum experimental

37   conditions, the proposed electrochemical sensing protocol towards 8-oxoGua using SPEs is

38   demonstrated to be possible over the concentration range of 0.1 to 12 µM. The response of the

39   SPEs is superior over routinely utilised glassy carbon electrodes in terms of sensitivity with a limit

40   of detection (3σ) found to correspond to 0.33 µM. Reproducibility and repeatability of the

41   proposed methodology at low and high concentrations were also demonstrated. Quantification

42   of 8-oxoGua in the presence of other nucleobases and different compounds of interest which

43   are present in biological fluids was successfully accomplished. Furthermore, *proof-of-concept*

44   demonstrating the potential use of the developed SPE based methodology for the detection of

45   8-oxoGua in real complex samples as demonstrated in simulated biological samples (human

46   semen).

47   *Keywords:* Screen-printed electrodes; 8-oxoguanine; DNA oxidation; Voltammetry

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1. Introduction

DNA is incessantly being damaged. In addition to known external factors, such as chemical agents, UV light or ionising radiation, a human cell must repair around 10,000 DNA lesions per cell, per day, that are due to multiple endogenous sources.[1] Oxidative stress is the most common and persistent cause of DNA destruction and it is generated through multiple processes involving production of oxygen and nitrogen species such as nitric oxide and superoxides.[2] In particular, these reactive oxygen species (ROS) have the capacity to attack cells and react with a variety of cellular components[1], including nucleotides of DNA, causing particularly hazardous damage for cells that can lead to genomic instability involved in various pathological conditions as well as mutation and cancer.[3, 4]

Guanine is the primary target of ROS in DNA[5] because it has the lowest redox potential of the four nucleobases.[6] 8-oxoguanine (8-oxoGua hereinafter) which contains an extra oxygen atom on guanine derived from the oxidation in the C8 position, is the most frequent and important oxidative DNA alteration in all aged cell types[7] and it is widely considered as a biomarker of oxidative DNA damage.[5] These modifications on the nitrogen bases abolish their ability to hydrogen-bond with the complementary bases and thus mismatch insertion or replication blockage, which can result in gene mutations.[2] In this particular case, 8-oxoGua mispairs with adenine during DNA replication that causes GC to TA transversion mutation.[3]

The most common biological samples that are examined in general for oxidative stress markers are blood, serum, urine, nasal and lung lavages, biopsied human tissue, and animal or human tissues or cells, as well as sperm, breast milk, placenta, and saliva are matrixes of interest.[8] Consequently, substantial analytical difficulties can be found in the analysis of this compound in those complex biological samples. As described in the literature, determination of 8-oxoGua has been performed by several methods such as high performance liquid chromatography with electrochemical detection (HPLC–ECD), gas chromatography coupled to mass spectrometry (GC–MS), liquid chromatography with mass spectrometry (LC–MS/MS) and enzyme linked immunosorbent assay (ELISA).[9] One of the most popular methods for the direct analysis of the released 8-oxoGua is HPLC-ECD [10-14] even though possible interference of the biological matrix and time required for the analysis of large number of samples are significant drawbacks[9]. GC-MS is also a suitable analytical technique for 8-oxoGua quantification mainly because of its sensitivity[9], but overestimation compared to HPLC-ECD, attributed to the artificial oxidation of guanine base during typical derivatisation reaction used prior GC-MS, is the main reported disadvantage.[9-11] On the other hand, specificity, sensitivity, capability to

84 quantify multiple analytes simultaneously, and compatibility with additional clean-up and

85 sample preparation methods presented by LC-MS/MS make this a promising analytical tool for

86 8-oxoGua determination and/or the overall biomarkers of DNA[8, 9, 15], however it requires

87 skilled personnel and its cost make this approach economically unfeasible for routine

88 implementation.

89 Therefore, there is a need for simple and cost-effective assays capable of measuring DNA

90 oxidation and electrochemical methods are very promising tools to perform these

91 approaches[16-18], because they are easily applied and also provide rapid and accurate results,

92 but still require improvements in terms of repeatability and reproducibility.[19] The recent

93 progress in the development of electrochemical (bio)sensors and application in detecting

94 oxidative DNA damages has been reviewed by *Fojta et al* [20]. Modification of the electrodes

95 with biochemical transducers, chemical mediators, nanoparticles or nanomaterials, among

96 other strategies, in order to improve the sensitivity of carbon-based electrodes is overviewed.

97 Also, advances in the development of sensitive photoelectrochemical biosensor for monitoring

98 the DNA biorecognition has been reviewed by *Zhao et al* [21]. Nevertheless, screen-printing

99 technology, which emerged in the last decade as a revolutionary new concept of simplifying

100 electrochemical laboratory based protocols, opening doors to unlimited designs and

101 encouraging new applications, is becoming a very attractive alternative for the development of

102 reliable and simple methodologies for the direct application in this field. Screen-printed

103 electrodes have scales of economy and provide one-shot disposable and reproducible

104 electrochemical sensors that require no pre-treatment or electrode polishing between

105 measurements as can be the case for traditional solid electrodes.

106 Due to high sensitivity, voltammetric techniques have been demonstrated their suitability to

107 detect low concentrations of 8-oxoGua [22, 23]. In this particular case, a minimum concentration

108 value that 8-oxoGua is found in biological samples is challenging to define because it depends

109 on numerous factors (intensity of induced damage among many others). Using electrochemical

110 detection coupled to liquid chromatography, a concentration of 8-oxoGua below 100 nM was

111 reported as basal value excreted into urine due to aerobic cellular metabolism[15]. Other few

112 publications reported that levels also below 100 nM were quantified in urine samples[13, 24],

113 with the exception or another one that presented a concentration around 580 nM[25].

114 Isolated electroanalytical detection of 8-oxoGua (not coupled to other separation analytical

115 technique) is surprisingly seldom explored in the literature. The direct electrochemical

116 determination of 8-oxoGua was reported for the first time by *Brett et al* [23] utilising glassy

117   carbon electrodes. *Rebelo et al* [22] also investigated the electrochemical behaviour of this

118   compound in the presence of uric acid using glassy carbon electrodes studying exhaustively the

119   influence of pH in the simultaneous determination. *Ferantopova* [26] also compared the redox

120   transformations of 8-oxoGua, specifically as intermediate species, to study the influence of

121   electrode material in the oxidation process of guanine using polycrystalline gold electrodes.

122   Recently, *Sanjuán et al* [27] used a new three electrode configuration set-up consisting of a

123   boron doped diamond (BDD) as working electrode and a screen-printed electrode providing only

124   the counter and pseudo-reference electrodes for the analysis of 7-methylguanine, considering

125   8-oxoGua just as interference species that might compromise the analysis. Overall, the potential

126   disadvantage is that the electrode needs extensive pre-treatment, in the form of polishing,

127   between measurements and are expensive. In summary, a thorough study of 8-oxoGua at SPEs

128   has not been carried out and the advantage of using these as electrochemical sensing platforms

129   has yet to be investigated.

130   To the best of our knowledge, this paper is the first report of the electrochemical behaviour of

131   8-oxoguanine at screen-printed graphite macroelectrodes (SPEs). Differential pulse

132   voltammetry (DPV) has been used herein to develop an electroanalytical methodology which

133   has been successfully validated and applied for the determination of 8-oxoguanine in simulated

134   biological samples (human semen).

135

136   *2. Experimental*

137   *2.1. Chemicals and solutions preparation*

138   All analytical and highest analytical grade chemicals were used as received without any further

139   purification. 8-hydroxyguanine (8-oxoguanine) was received in individual 10 mg amber glass

140   vials purchased from Enzo Life Sciences (Exeter, UK) and kept in the freezer until used. After

141   accurately weighted desirable amount of product for the preparation of solutions, nitrogen was

142   gently applied into the vial to eliminate oxygen and avoid premature oxidation of 8-oxoguanine.

143   0.1 M potassium phosphate monobasic and 0.1 M potassium phosphate dibasic trihydrate were

144   used to prepare phosphate buffer solutions (PBS) at different pH throughout this work. All

145   solutions were prepared with deionised water of resistivity not less than 18.2 MΩ cm (25 °C).

146   Stock solution of 8-oxoguanine were prepared by dissolving the powder in ultrapure water and

147   to ensure total solubility of the compound, 20 μL of 8 M NaOH solution were added to give a

148   final pH value of 9-10, as verified with pH-indicator paper.[14] The solution was protected with

149 aluminium foil and constantly stirred for 24 h and kept in the fridge until analysed. The same

150 methodology was applied for the preparation of adenine (A) and guanine (G) solutions also

151 tested in this work. The final concentration of freshly prepared 8-oxoGua, A and G solutions

152 were determined via UV-vis spectrophotometry using an extinction coefficient of 7,762 cm^{-1} M^{-1}

153 ^{1} at 285 nm for 8-oxoGua[28], an extinction coefficient of 13,400 cm^{-1} M^{-1} at 261 nm for

154 adenine[29] and an extinction coefficient of 10,700 cm^{-1} M^{-1} at 243 nm for guanine.[30]

2.2. Fabrication of screen-printed graphite macroelectrodes (SPEs)

156 Screen-printed graphite macrcoelectrodes (SPEs) which have 3 mm diameter working electrode,

157 were fabricated in-house with appropriate stencil designs using a DEK 248 screen-printing

158 machine (DEK, Weymouth, UK). A previously used carbon-graphite ink formulation (product

159 code: C2000802P2; Gwent Electronic Materials Ltd, UK) was first screen-printed onto a polyester

160 flexible film (Autostat, 250 µm thickness). This layer was cured in a fan oven at 60 °C for 30

161 minutes. Next a silver/silver chloride pseudo reference electrode was included by screen-

162 printing Ag/AgCl paste (product code: C2030812P3; Gwent Electronic Materials Ltd, UK) onto

163 the polyester substrate. A dielectric paste/ink (product code: D2070423D5; Gwent Electronic

164 Materials Ltd, UK) was next printed to cover the connections. After curing again at the same

165 conditions as before, the screen-printed electrodes were ready to be used. The SPEs were then

166 precisely cut to remove the Ag/AgCl pseudo reference and carbon counter and used into a

167 standard three electrodes configuration.

2.3. Electrochemical measurements

169 Electrochemical measurements were carried out with a Palmsens (Palm Instruments BV, The

170 Netherlands) potentiostat controlled by software PSTrace 4.7. All experiments throughout this

171 study were conducted using a three electrodes configuration electrochemical cell with SPEs,

172 platinum wire and saturated calomel electrode (SCE) as a working, counter and reference

173 electrodes, respectively. All electrochemical experiments were performed at room temperature

174 and solutions were not deaerated before analysis.

175 Screen-printed graphite macroelectrodes were electrochemically conditioned before use by

176 applying 10 scans in cyclic voltammetry (CV) between 0.0 V and +1.0 V at 50 mV s^{-1} in PBS pH

177 7.44. Instrumental parameters of differential pulse voltammetry (DPV) method were thoroughly

178 optimised based on current intensity of the oxidation of 8-oxoGua and final conditions used

179 were: step potential 5 mV, pulse amplitude 90 mV, pulse width 200 ms and scan rate 5 mV s^{-1}.

180   CV method was also separately performed for scan rate study of 8-oxoGua at SPEs using 5, 10,
181   15, 20, 30, 40 and 60 mV s^{-1}.

182   *2.4. Human semen simulant preparation*

183   Voltammetric methodology developed for the detection of 8-oxoGua was applied in synthetic
184   human semen simulant prepared in the laboratory. Following the protocol proposed by *Owen*
185   *and Katz (2005)[31]*, formulation (final volume 100 mL) consisted of: sodium phosphate
186   monobasic monohydrate (5.46 mL of 0.123 M), sodium phosphate dibasic anhydrate (49.14 mL
187   of 0.123 M), sodium citrate dehydrate (813 mg), potassium chloride (90.8 mg), potassium
188   hydroxide (88.1 mg), fructose (272 mg), glucose anhydrous (102 mg), lactic acid (62 mg), urea
189   (45 mg), bovine serum albumin (5.04 g), calcium chloride dihydrate (101 mg dissolved in 15.13
190   mL of water), magnesium chloride hexahydrate (92 mg dissolved in 15.13 mL of water) and zinc
191   chloride (34.4 mg dissolved in 15.13 mL of water). In the final solution, the pH was raised to
192   7.76 adding small amounts of sodium hydroxide, then filtered with 0.45 µm PTFE filter disc and
193   kept in the fridge until assayed.

194

195   *3. Results and discussion*

196   *3.1. Electrochemical response of 8-oxoGua at screen-printed macroelectrodes (SPEs)*

197   We first explored the voltammetric behaviour of 8-oxoGua at screen-printed graphite
198   macroelectrodes (SPEs). Cyclic voltammetry (CV) in PBS pH 7.44 was first applied for the
199   electrochemical pretreatment of the surface of SPEs after which the differential pulse
200   voltammetric (DPV) response of 8-oxoGua in PBS 7.57 was explored. A very well-defined and
201   intense anodic peak at 245 mV was observed, as depicted within Figure 1. Although it is reported
202   that the oxidation of 8-oxoGua by ROS can generate a complex mixture of products which can
203   be even electrochemically detected suggesting different electrode processes involved[23], we
204   have focused our studies on the main peak observed and its voltammetric behaviour in different
205   pH conditions. Note that it will be frequently observed throughout this work a small oxidation
206   peak prior to the electrochemical 8-oxoGua signal (around -100 or -200 mV *vs.* SCE, depending
207   on pH) probably related to the oxidation of any species on the surface of the electrode but which
208   is not influencing the detection of the target compound.

209   To further explore the electrochemical response of 8-oxoGua at SPEs, a voltammetric scan rate
210   study was carried out by applying CV in the presence of 6 µM of 8-oxoGua in PBS pH 7.07 at 5,

211 10, 15, 20, 30, 40 and 60 mV s^{-1} scan rates (Figure ESI 1A). It is clearly observed a linear

212 dependency of peak current intensity (I_p) *vs.* square root of the scan rate ($v^{1/2}$) and peak current

213 logarithm (Log I_p) *vs.* scan rate logarithm (Log v) (Figures ESI 1B and 1C). The analysis of this data

214 indicates that the oxidation of 8-oxoGua at SPEs is diffusion controlled which is also confirmed

215 by the gradient of the linear plot of Log I_p *vs.* Log v (Log I_p= 0.5046 Log v + 0.1233; R^2 = 0.9872)

216 close to the theoretical value of 0.5. This observation is in agreement with previous results

217 reported at a glassy carbon electrode[23]. Moreover, the observed linear dependency of peak

218 potential with scan rate[27] (Figure ESI 1D) denotes irreversibility of the electrode process of 8-

219 oxoGua that is not completely in agreement with a previous study performed using a glassy

220 carbon electrode[23] where authors reported the existence of a reverse peak of 8-oxoGua in

221 square wave voltammetry (SWV), but of course we are comparing two different electrode

222 surfaces/materials; however, non-observation of reduction peak in cyclic voltammetry could be

223 due to very fast hydrolysis of 8-oxoGua.[32]

224 We next investigated the effect of pH upon the electrochemical oxidation of 8-oxoGua at SPEs.

225 Considering that normal pH values of different biological samples in real conditions, pH-

226 dependence study over the range of 4.00 to 8.00 was systematically performed using different

227 phosphate buffer solutions. Different SPEs were conditioned and used for each pH assayed. As

228 shown in Figure 2, DPV signals of 2 µM of 8-oxoGua undergo a peak potential shifting to lower

229 positive potentials from increasing the solution pH. A linear dependence of the anodic peak of

230 8-oxoGua with pH is clearly observed with a slope of 61 mV pH^{-1} that is very close to the

231 theoretical Nernstian value of 59 mV pH^{-1}. This suggests that, as expected and reported

232 previously on glassy carbon electrode[23], the electrochemical reaction that takes place on the

233 surface of SPE involving the same number of electrons and protons, specifically two of them in

234 the electrochemical oxidation of 8-oxoGua. On the other hand, slight changes in the peak

235 current of 2 µM of 8-oxoGua at different pH studied were observed; nevertheless, a negligible

236 loss of 30 nA of current intensity of anodic peak of 8-oxoGua at pH 8.00 compared to pH 4.00

237 was detected. Note that it is an important advantage to consider for further applications of this

238 methodology in real conditions that possible changes in the usual pH of biological samples are

239 not going to compromise the voltammetric determination of 8-oxoGua in terms of peak current

240 intensity; this data suggest that SPEs are a suitable and feasible analytical approach for the *in-*

241 *situ* analysis of 8-oxoGua.

242

243

244 *3.2. Analytical benchmarking of the SPEs towards the sensing of 8-oxoGua*

245 The electroanalytical performance of SPEs towards the detection of 8-oxoGua was next

246 evaluated. A calibration curve was constructed using DPV and a single SPE over the entire

247 concentration range (0.10 to 12.0 µM) in 0.1 M phosphate buffer solution pH 7.00. Three

248 replicates of each concentration were performed. Experimental observations determined that

249 it was not necessary to clean the SPE surface between measurements. Consequently, any

250 conditioning potential was applied before each voltammetric response apart from the initial

251 pretreatment of the surface by applying 10 scans in CV at pH 7.44. As shown in Figure 3, there

252 was an expected increase of the peak current upon the increasing of the concentration of 8-

253 oxoGua. A straight line calibration plot was obtained (I/µA = 0.0353 µA [µM^{-1}] + 0.0049 µA; R^2 =

254 0.9947; N = 7) for the detection of 8-oxoGua over the analytical range studied (Figure 3 inset)

255 with a limit of detection (3σ) found to correspond to 0.33 µM. In comparison to the limit of

256 detection of 0.80 µM previously reported for the direct electrochemical determination of 8-

257 oxoGua using a glassy carbon electrode[23], a significant improvement was achieved with the

258 proposed methodology. The improved electrochemical performance over that of a GC is due to

259 the greater density of edge plane like- sites/defects on the SPE. [33, 34]

260 In order to demonstrate the suitability and reliability of SPEs platforms for the

261 determination of 8-oxoGua at different concentration levels likely to be found in real samples,

262 the reproducibility and repeatability of 8-oxoGua voltammetric measurements at SPEs were

263 additionally assessed at low (1 µM) and high (9µM) concentrations, respectively. DPV analyses

264 were performed in 0.1 M phosphate buffer solution pH 7.00. Five different SPEs were selected

265 for reproducibility studies and five DPV measurements were recorded for each one. Also, a

266 single SPE was used for repeatability study and thirty times DPV measurements were

267 continuously run using the same electrode. Note that thirty times measurements using one

268 single SPE were considered enough to complete the study but even so the electrode did not

269 become deteriorated. Results obtained are summarised in Table 1. In general, SPEs showed

270 adequate relative standard deviation (RSD) values in all cases. Only the value of 11.9 % for 1 µM

271 of 8-oxoGua seems to be high but still acceptable considering the low concentration analysed.

272 Likewise, a relatively high value of 8 % for repeatability of SPEs at high concentration of 8-

273 oxoGua could be related to a partial blocking of the surface because of the adsorption of the

274 compound onto the surface after 30 runs without the application of any conditioning potential

275 between measurements. Therefore, we have proved the feasibility of the developed

276 methodology to perform accurately *in-situ* calibration and direct measurements of 8-oxoGua in

277 practical applications using the same screen-printed electrode sensor.

278   *3.3. Detection of 8-oxoGua in the presence of other compounds of interest*

279   Attention was next turned to exploring the voltammetric response of 8-oxoGua simultaneously

280   with other nucleobases in DNA, which are likely to be present within real biological samples and

281   can be potential interfering molecules, mainly guanine (G) and adenine (A). A mixture of 5.6 µM

282   of 8-oxoGua, 6.3 µM of G and 8.8 µM of A in 0.1 M phosphate buffer solution pH 7.74 was tested

283   using DPV and SPEs. As represented in Figure 4A, voltammetric peaks of 8-oxoGua, G and A

284   appear one after the other at peak potentials about 245 mV, 635 mV and 930 mV (*vs.* SCE),

285   respectively. There is a difference of 380 mV (*vs.* SCE) between 8-oxoGua and G peak potentials

286   and 300 mV (*vs.* SCE) between G and A peak potentials, which indicates sufficient voltammetric

287   separation to detect them simultaneously at SPEs. Even though 8-oxoGua peak is slightly shifted

288   in the presence of G and A, it can be determined that there is no cooperative effect upon the

289   voltammetric responses of each compound.[27] On the other hand, the presence of G and A in

290   solution resulted in a reduction of 33 % (46 nA) of 8-oxoGua current intensity, thus the presence

291   of those nucleobases somewhat interferes in the electrooxidation of 8-oxoGua at SPEs. Even

292   though SPEs did not show a clear fouling of the surface when these three compounds are

293   simultaneously detected, as described by *Sanjuán et al. (2016)[27]* using BDDE, the presence of

294   G and A are somehow competing by the adsorption sites of the SPE surface resulting in a partial

295   blocking and decreasing of 8-oxoGua voltammetric signal.

296   The influence in the electrochemical response of 8-oxoGua due to other co-existent compounds

297   with important implications in several biological processes and possibly found in real samples,

298   was also investigated. Therefore, dopamine and ascorbic acid were also determined

299   simultaneously with 8-oxoGua by applying the optimised DPV methodology. First, a solution

300   containing 5.6 µM of 8-oxoGua and 500 µM of dopamine in 0.1 M PBS pH 7.76 was analysed. As

301   illustrated in Figure 4B, it is possible to perform their simultaneous determination since peak

302   potentials of individual compounds are separated by 170 mV (*vs.* SCE). The presence of

303   dopamine does not affect the peak potential of 8-oxoGua, which remains unaltered, and a small

304   reduction of 20 % (28 nA) in the peak current is observed; however, a significant decrease of

305   peak current is undertaken by dopamine with a loss of 84 % of current intensity compared to

306   voltammetric signal of the individual compound. This can be attributed to the fact that 8-oxoGua

307   is more easily oxidised than dopamine and dominates the achievement of more active sites on

308   the surface of SPEs blocking significantly the electrooxidation of dopamine.

309   On the other hand, DPV determination of 5.6 µM of 8-oxoGua was additionally carried out in

310   the presence of 27 µM of ascorbic acid in 0.1 M PBS pH 7.84. Partially overlapped voltammetric

peaks are observed (Figure 4C). Hence, the simultaneous detection of ascorbic acid and 8-oxoGua is evidently possible but improvements in the voltammetric method and/or even optimisation of pH conditions would be required to achieve better separation and to evaluate appropriately this interference.

3.4. Determination of 8-oxoGua in simulated human semen sample as a proof-of-concept

The feasibility of the voltammetric detection of 8-oxoGua using SPEs was verified by applying the methodology in a human semen simulant prepared in the laboratory following the procedure described in the Experimental Section.

The complex mixture of chemicals that is part of the synthetic human semen simulation (final pH 7.76) provided a defined electrochemical signal at a potential of 575 mV (*vs.* SCE) at SPEs using DPV; typical voltammograms are shown in Figure 5A and 5B. Experiments were performed in non-diluted and diluted 1:10 semen simulant preparations and as expected a higher current intensity was obtained for the non-diluted sample. Addition of an appropriate amount of 8-oxoGua for a final concentration of 12 µM within semen simulant solutions resulted in a clear voltammetric peak at 300 mV (*vs.* SCE). The 8-oxoGua signal was significantly higher in diluted simulated semen sample, as expected, but in both cases, the DPV response was well-defined and sufficient intense for certainly quantification. Due to the complex mixture of chemicals that comprise the semen simulant a shift in the of 8-oxoGua signal / peak to more positive potentials, compared to the response / peak determined in simply phosphate buffer solution at the same pH is evident. Even though the current intensity of voltammetric peak of 8-oxoGua showed a significant reduction (about 50%) when analysed in synthetic biological solutions, the reliability of SPEs for its detection and appropriately quantification in practical conditions has been demonstrated.

4. Conclusions

This paper has investigated, for the first time, the utilisation of screen-printed graphite electrodes for the potential evaluation of DNA damage *via* the electrochemical determination of 8-oxoguanine. CV and DPV were carefully optimised for high-performance of screen-printed platforms for the direct quantification of 8-oxoGua.

According to insignificant changes in the current intensity of the target compound over the range of pH studied (4.00 to 8.00), the developed methodology demonstrated its suitability for 8-oxoGua determination at usual pH of biological samples. The proposed methodology was also validated in terms of linearity obtaining a good linear regression in the concentration range of 0.1-12 µM with a sensitivity of 0.0353 µA µM^{-1}. A very good LOD of 0.33 µM was obtained and it was significantly better than LOD reported for conventional glassy carbon electrodes (0.80 µM)[23]. Additionally, reproducibility of different screen-printed platforms and repeatability on a single sensor were performed at high and low concentrations and they provided acceptable RSD values demonstrating the reliability of SPEs for direct determination of 8-oxoGua.

Simultaneous detection of 8-oxoGua in the presence of other important nucleobases (adenine and guanine) and some co-existent compounds in biological fluids were also successfully carried out. Finally, the successful application of the developed methodology for the determination of 8-oxoGua in synthetic human semen demonstrating the feasibility of SPEs for further biomedical applications; such work is underway.

369 **Table 1.** Reproducibility and repeatability of 1 µM and 9 µM DPV responses of 8-oxoGua at SPEs

370 in 0.1 M PBS pH 7.00. N value for reproducibility corresponds to different electrodes used while

371 the N value for repeatability indicates the number of times of repetition of voltammetric

372 measurements using a single SPE.

	Concentration of 8-oxoGua	
	1 µM	**9 µM**
Reproducibility (*N=5*)	0.028±0.003 µA (RSD 11.9 %)	0.298±0.013 µA (RSD 4.5 %)
Repeatability (*N=30*)	0.023±0.001 µA (RSD 6.2 %)	0.319±0.026 µA (RSD 8.0 %)

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376 **Figure 1.** Differential pulse voltammetry of 20 μM of 8-oxoGua in 0.1 M PBS pH 7.57 (solid line)
377 using SPEs. The background (no 8-oxoguanine) was recorded and is presented as the dashed
378 line.

379
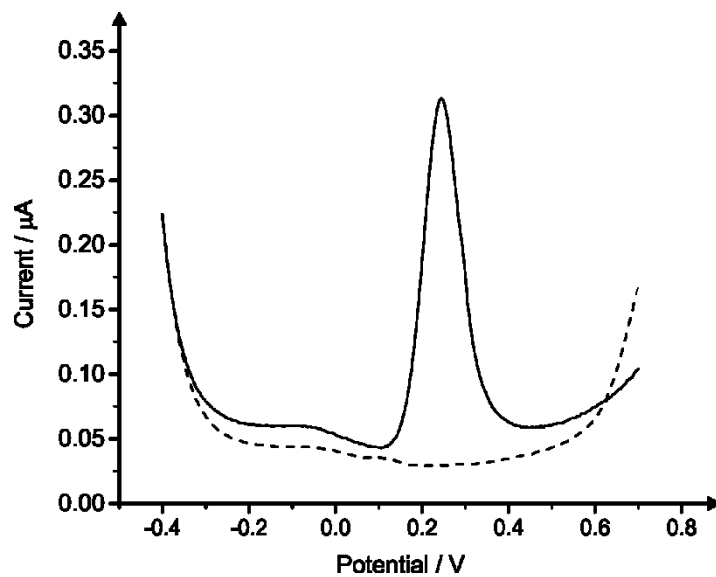
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382 **Figure 2.** Effect of pH on the DPV responses of 2 µM of 8-oxoGua in 0.1 M PBS using SPEs. Inset
383 Figure depicts the decreasing of peak potential of 8-oxoGua with changing the solution pH.

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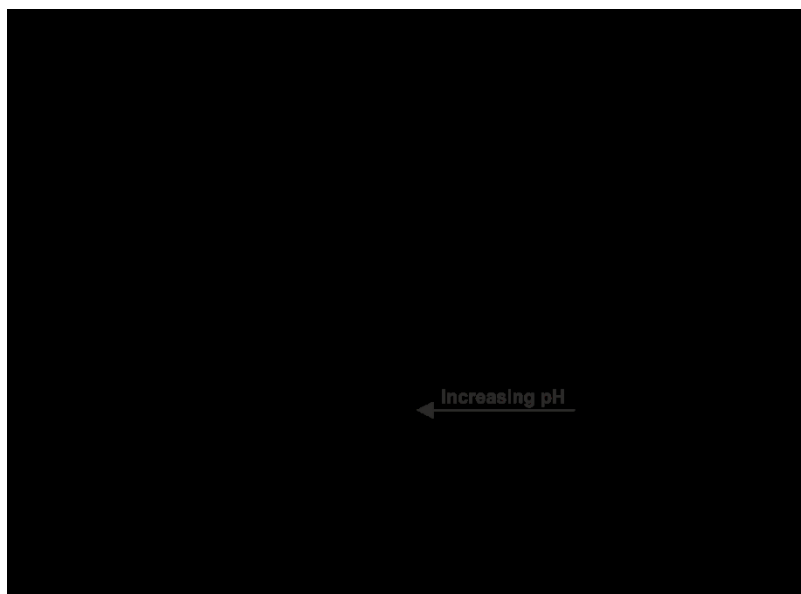
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388 **Figure 3.** DPV measurements of standard solutions of 8-oxoGua (0.1, 0.3, 1.0, 3.0, 6.0, 9.0 and
389 12.0 µM) in 0.1M PBS pH 7.44 using a *single* SPE. Figure inset shows linear a calibration plot of
390 current intensity *vs.* concentration of 8-oxoGua. The insert plot shows an average and standard
391 deviation at each concentration.

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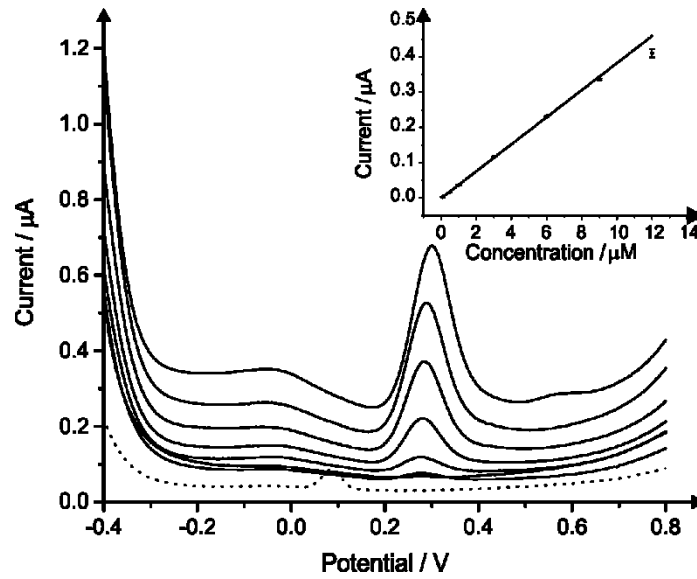
Figure 4. DPV consecutive measurements of 8-oxoGua in the presence of guanine and adenine in 0.1 M PBS pH 7.74 (A). DPV curves of the simultaneous determination of 8-oxoGua and dopamine in 0.1 M PBS pH 7.76 (B). DPV responses of a mixture of 8-oxoGua and ascorbic acid in 0.1 M PBS pH 7.84 (C).
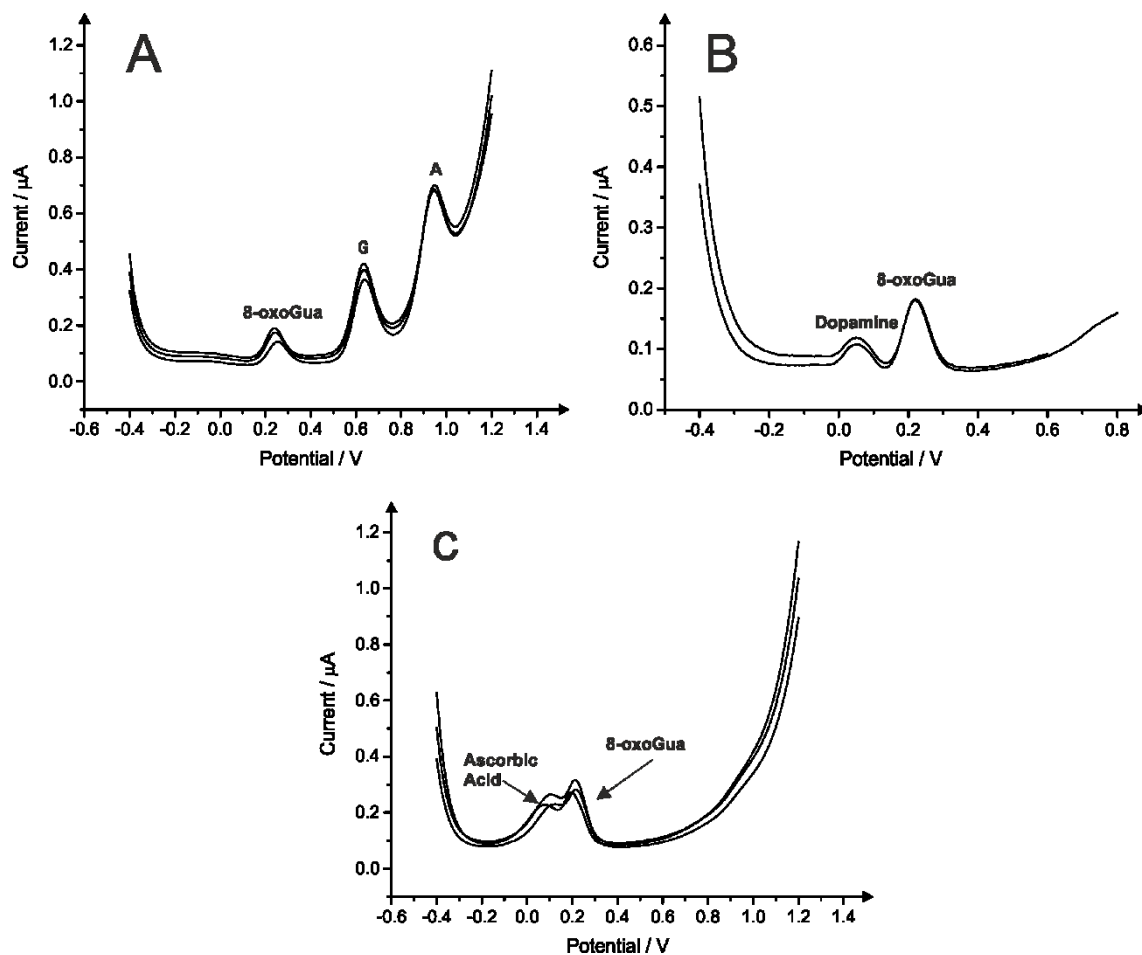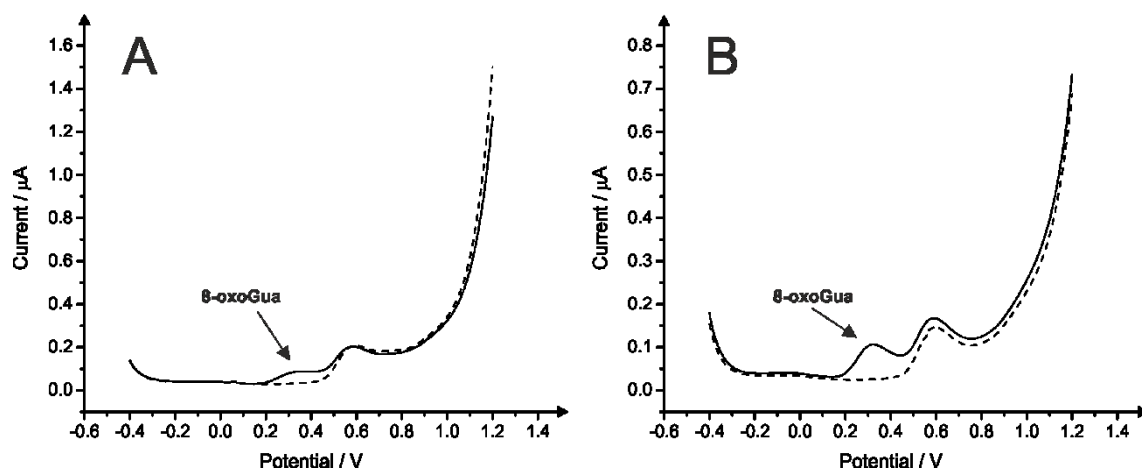
403   **Figure 5.** Voltammetric responses of 8-oxoGua in a complex human semen simulant preparation
404   without any dilution (A) and diluted 1:10 (B) with a final pH of 7.76 using SPEs.

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407

409   **Figure ESI 1.** CV responses of 6 µM 8-oxoGua in 0.1 M PBS pH 7.07 as a function of scan rate (A)
410   at SPEs. Plots of peak current (I_p) *vs.* square root of the scan rate ($v^{1/2}$) (B), peak current logarithm
411   (Log I_p) *vs.* scan rate logarithm (Log v) (C) and peak potential (E_p) *vs.* scan rate (v).

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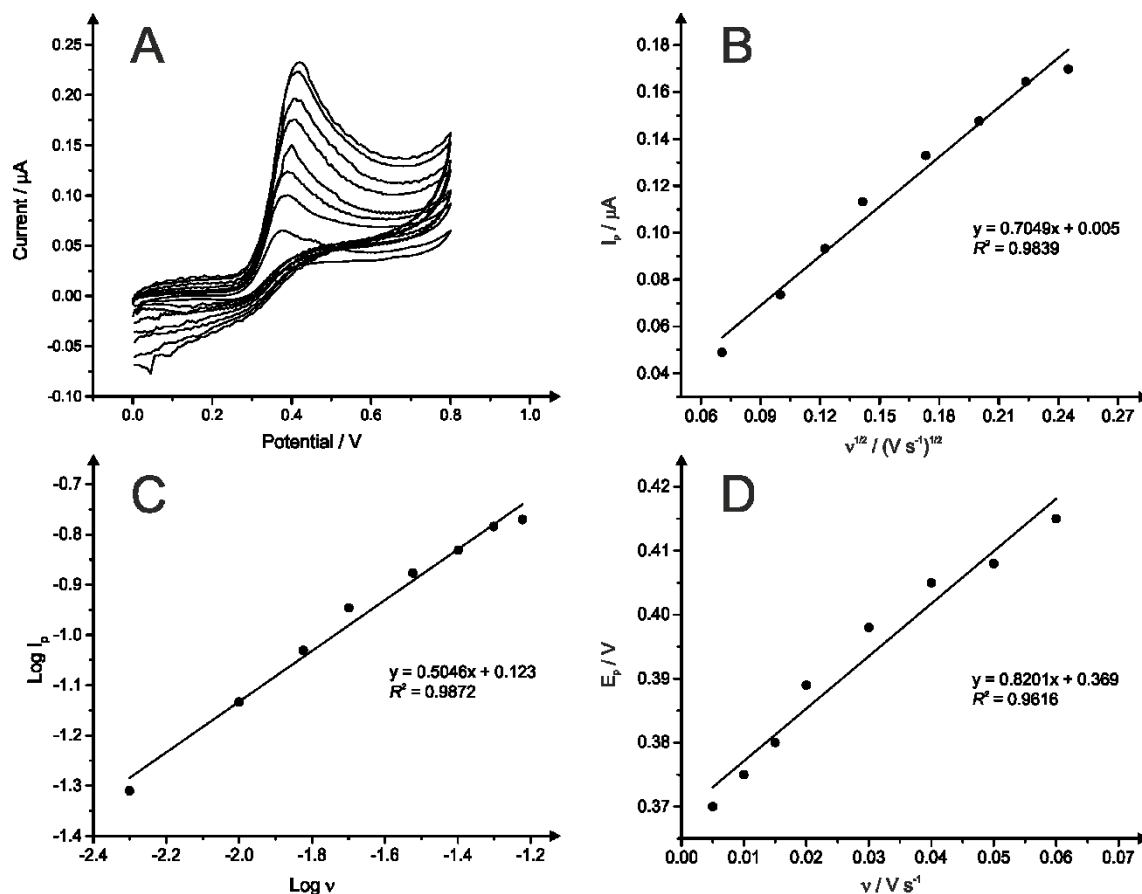
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