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Evaluation of Carbon Filament Electrodes for Determination of 2,6-Diisopropylphenol (propofol) in Aqueous and Organic Solutions

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EVALUATION OF CARBON FILAMENT ELECTRODES FOR DETERMINATION OF 2,6-DIISOPROPYLPHENOL (PROPOFOL) IN AQUEOUS AND ORGANIC SOLUTIONS

by

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Abstract

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Since the common anaesthetic drug 2,6-diisopropylphenol (propofol) has a narrow physiological range, the administration of this drug could be improved through the development of a feedback-controlled delivery system. An evaluation of several carbon filaments was conducted to determine their capability to serve as the detecting element in such a system.

Planar electrochemical cells (PECs) were fabricated with working electrodes made from three carbon filaments: Goodfellow (GF) carbon fibers, 11μm diameter; Bioanalytical Systems (BAS) carbon fibers, 9μm diameter; Specialty Materials (SM) glassy carbon monofilaments, 34.5μm diameter. The cells were used to make multiple, successive determinations of propofol – via cyclic voltammetry – in both pH ~7.0 aqueous and acetonitrile solutions.

All three electrodes showed significant fouling in pH ~7.0 aqueous solutions. The SM electrodes had moderate fouling in acetonitrile solution, while both GF and BAS electrodes showed minimal fouling in acetonitrile solution.

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Key to Abbreviations

BAS = Bioanalytical Systems

CE = counter electrode

dia. = diameter

$E_{1/2}$ = half-wave potential

GF = Goodfellow

HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

ID = inner diameter

KTPFPB = potassium tetrakis (pentafluorophenyl) borate

$\log K_{ow}$ = logarithm of octanol-water partition coefficient

OD = outer diameter

oNPOE = ortho nitro phenyl octyl ether

PEC = planar electrochemical cell

pKa = negative logarithm of acid dissociation constant

PVC = poly vinyl chloride

RE = reference electrode

S/N = signal-to-noise

SHE = standard hydrogen electrode

SM = Specialty Materials

St. Dev. = standard deviation

TBACl = tetrabutyl ammonium chloride

$TBAClO_4$ = tetrabutyl ammonium perchlorate

TCIA = target-controlled infusion anaesthesia

$TPFPB^-$ = tetrakis (pentafluorophenyl) borate anion

WE = working electrode

Introduction

1. Propofol in the Body

The intravenous drug 2,6-diisopropylphenol (propofol) is commonly used for general surgery anaesthesia and long-term sedation (up to 2 weeks) [1]. Propofol is highly lipophilic, as indicated by its octanol-water partition coefficient ($\log K_{ow} = 3.7$) [2]. Due to its high lipophilicity, it preferentially partitions into fatty tissues, such as those of the nervous system [3]. Furthermore, propofol administration is characterized by rapid induction of and rapid emergence from anaesthesia – both of which contribute to the drug's broad appeal and popularity. The target steady-state concentration range of propofol in blood is between 0.25 – 4.0 μg/mL or, in molar concentrations, between 1.4 – 22.4 μM. These target values are achieved with constant infusion rates ranging between 0.3 to 3.0 mg/kg/h. After bolus injections, blood concentrations may reach 85 μM [1, 4].

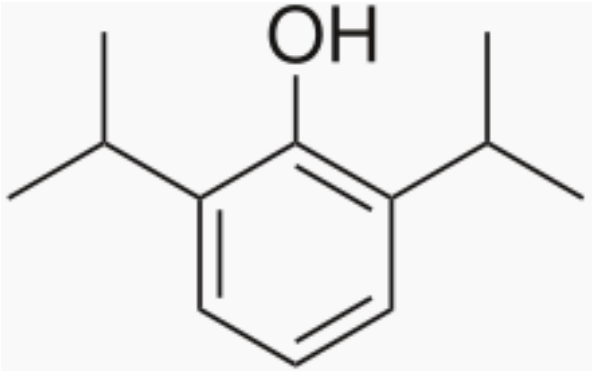

Figure 1. Structure of a 2,6-diisopropyl phenol (propofol).

2. Overall Project Goal: Propofol Detector

The research detailed in this report is part of an overall project to develop a voltammetric propofol detector for making real-time, in-vivo measurements in whole blood. With such a detector, correlations between the propofol concentration in blood

and physiological parameters used to assess the depth of anaesthesia could be studied. Information on these correlations would "enhance the safety of propofol delivery for target-controlled infusion anaesthesia (TCIA)" [5].

To perform the measurements necessary to study these correlations, the detector should meet the following requirements:

- dynamic response range between 1 and 100μM (corresponding to the clinically-relevant concentration range) [1]
- use life time up to two weeks (corresponding to the longest sedation time cited in the literature) [1]
- response time of a few seconds (corresponding to the ~ 20 s onset of anesthesia following the induction of the drug) [6]

In support of the overall project, the goal of this work was to compare the performance of several carbon filaments (Bioanalytical Systems carbon fibers, 9μm diameter (BAS); Goodfellow carbon fibers, 11μm diameter (GF); or Specialty Materials Inc. glassy carbon monofilaments, 34.5μm diameter (SM)) to serve as the working electrode in a voltammetric propofol detector. Specifically the ability of the carbon filaments to accomplish to following objectives was assessed:

- make *repeated* measurements at the upper end of the dynamic range (100μg/mL)
- make *any* measurements *at all* at the lower end of the dynamic range (1μg/mL)

For the overall detector to meet its requirements, the working electrode must meet these objectives.

3. Previous Research

There is an extensive body of literature on the electrochemistry of phenolic compounds. Such compounds can be oxidized electrochemically, but the mechanism of the oxidation depends on the experimental conditions (solvent, supporting electrolyte, presence of oxygen, etc), and the structure of the compound (number of substituents, identity of substituents) [7-10]. There is very little literature on the electrochemical oxidation of propofol specifically; however, the reaction(s) may be similar to those of other phenolic compounds. Another well documented phenomenon is the deposition of the product(s) of phenolic-compound oxidation – or products of any coupled reactions – on the working electrode surface. This is termed as 'electrode fouling'. The fouling may also be described as "polymerization" if the products are believed to be polymeric in nature [8, 9]. One possible mechanism for the electrochemical oxidation of propofol and generation of a propofol-derivative polymer is shown below (Figure 2).

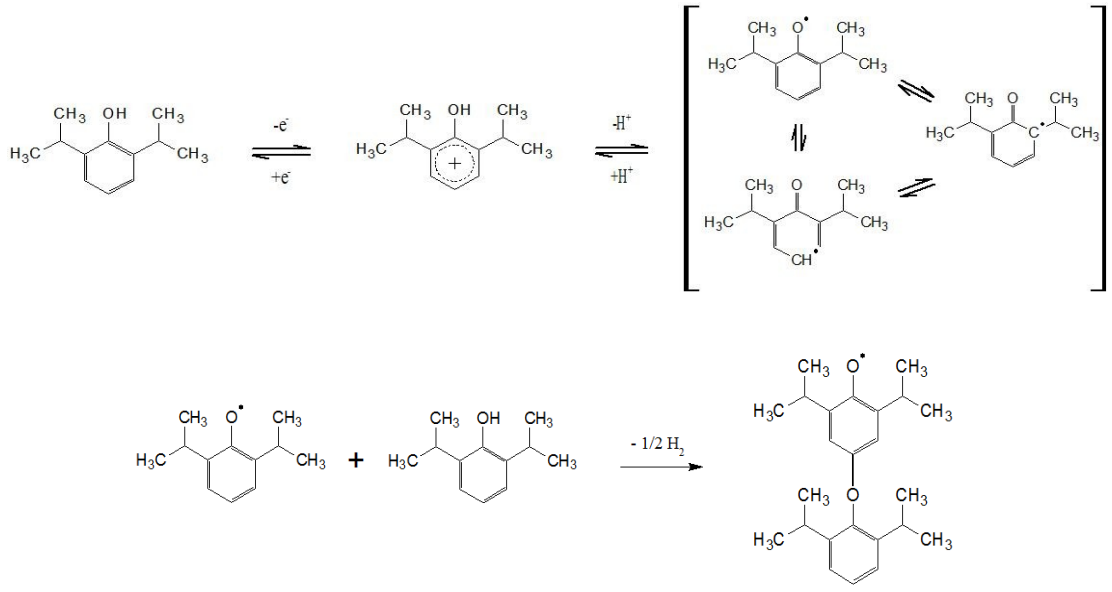

Figure 2. One possible mechanism for the electrochemical oxidation of propofol, and generation of a propofol-derivative polymer.

Langmaier et al [5] have discussed the difficulties of making electrochemical determinations of propofol in aqueous solutions. The authors showed the influence of the experimental conditions (e.g., scan rate, solution pH) and the working electrode material (platinum, gold, carbon, etc) on the shape and reproducibility of cyclic voltammograms. Using two different protocols in combination with a glassy carbon working electrode, the authors reported detection limits of 3.2 and 5.5μM [5]. In the reported protocols, the measurements were performed in highly acidic solutions and the influence of interfering compounds, commonly present in biological samples, were not discussed.

To address some of the deficiencies of the protocols used by Langmaier et al with respect of in vivo monitoring, Guo tested the performance characteristics of carbon fiber working electrodes for propofol measurements in buffered solutions around pH 7.0. Examples of the cyclic voltammograms recorded with the carbon fiber working electrodes are shown in Figure 3. As shown in the figure, the current peak around 0.4 V – whose magnitude was proportional to propofol concentration – remained constant for the first 4 scans but gradually decreased/decayed in the follow up scans (5th – 20^{th} scan) [11].

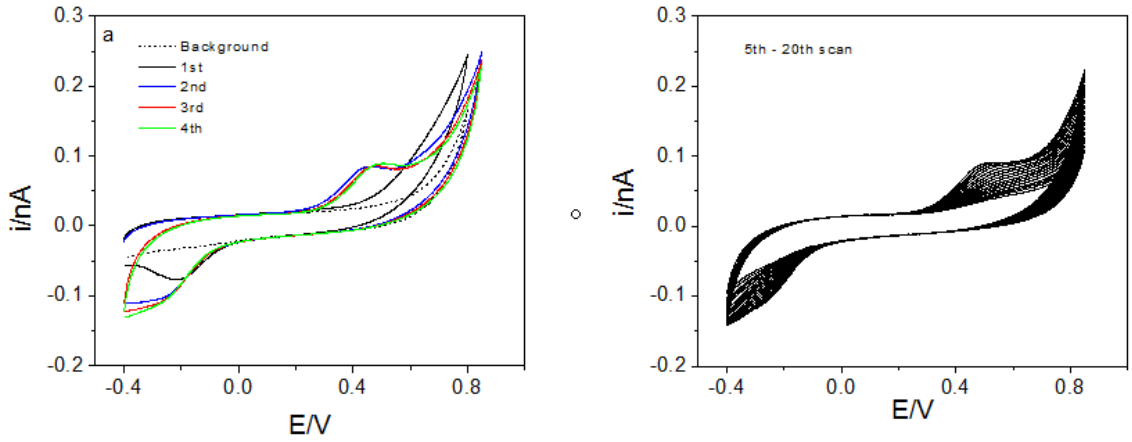

Figure 3. Cyclic voltammograms recorded with an 8µm diameter Bioanalytical Systems (BAS) carbon fiber working electrode in 100µM propofol solution of pH 7.0. Reference electrode: Ag|AgCl wire immersed into the solution, counter electrode: platinum wire. Background electrolyte: 0.01 M Na_2SO_4, 0.01 M HEPES Scan rate: 50mV/s. [11]

The decay of the peak currents recorded at 0.4 V and –0.4 V was attributed to electrode fouling. Similar experiments were also performed in acetonitrile, a more organic solvent – as indicated by its lower dielectric constant. At 25°C and 1 atm, the dielectric constant of water is 78 and the dielectric constant of acetonitrile is 36 [12]. In these experiments in acetonitrile, practically no electrode fouling was observed, as shown in Figure 4. The peak currents recorded in the first and the 18^{th} scans were almost identical [11].

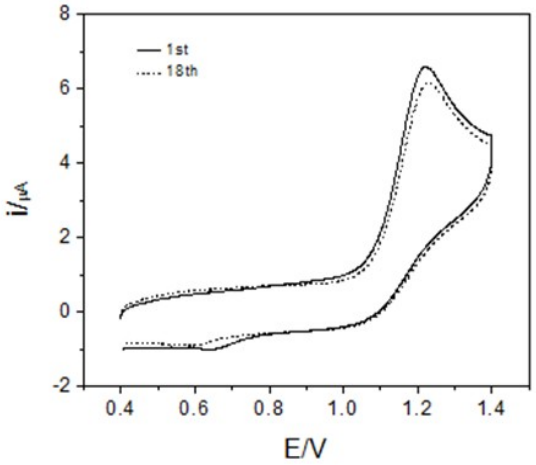

Figure 4. Cyclic voltammograms recorded with a glassy carbon macro electrode (3mm diameter) in acetonitrile solution of 100μM propofol. Reference electrode: Ag|AgCl wire immersed into the solution, counter electrode: platinum wire. Background electrolyte: 0.1M $TBAClO_4$. Scan rate 50mV/s. [11]

4. PVC Membrane Coated Detectors

Since the working electrode fouling was minimal in acetonitrile, Guo evaluated the possibility of coating the working electrode with a PVC membrane plasticized with up to 66% ortho nitro phenol octyl ether (oNPOE). oNPOE is an organic solvent (dielectric constant = 24) commonly used as the plasticizer in PVC membranes [13]. With the working electrode now coated with the organic PVC membrane, the electrochemical cell was expected to experience the following benefits (when used to make measurements of propofol in buffered pH 7 aqueous solutions):

- Reduction of electrode fouling because the electrochemical oxidation is being performed in an organic phase where the products of the electrochemical oxidation do not form a resistive film.
- Improvement of the detection limit of the voltammetric detector because propofol is lipophilic, and therefore expected to *preferentially partition* into the

organic phase (PVC membrane), i.e., its concentration is expected to be higher in the membrane than in the aqueous solution. Again, note high logarithm of the octanol/water partition coefficient for propofol, $\log K_{ow} = 3.7$ [2].

- Reduction of the level of interference by electrochemically active compounds like ascorbic acid, uric acid and p-acetamido phenol because they are hydrophilic, and therefore they will preferentially remain in the aqueous sample solution. At 25°C, the pKa of ascorbic acid is 4.1 and the pKa of uric acid is 5.4 (so in a pH 7 aqueous solution both acids will be in their dissociated, and therefore very hydrophilic, forms) [14]. And, the octanol/water partition coefficient ($\log K_{ow}$) of p-acetamido phenol is 0.51 [15].

Figure 5 shows a PVC membrane-coated working electrode schematically.

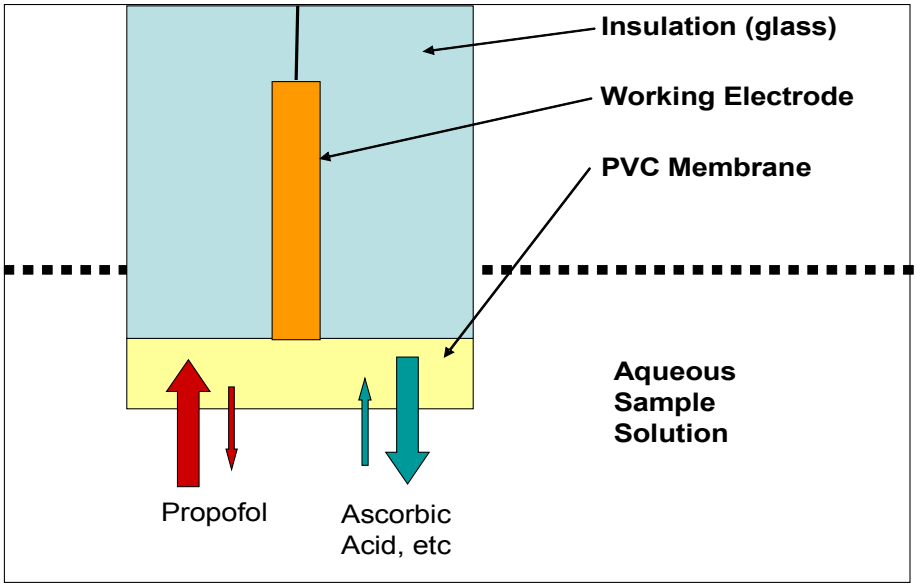

Figure 5. Diagram of the PVC Membrane-Coated Working Electrode. The arrows indicate the preferential partition of propofol into the PVC membrane compared to the interferents like ascorbic acid.

5. Electroneutrality in PVC Membranes'

When propofol is oxidized at the working electrode surface, positively charged species are generated (see Figure 2). There are several approaches balancing this positive charge and satisfying the requirement of electroneutrality in the organic membrane: (i) uptake of lipophilic anions from the aqueous sample into the membrane, (ii) release of hydrophilic cations from the membrane into the aqueous sample, or (iii) in-situ generation of negatively charged species in the membrane by coating the reference and counter electrode with the PVC membrane, as well.

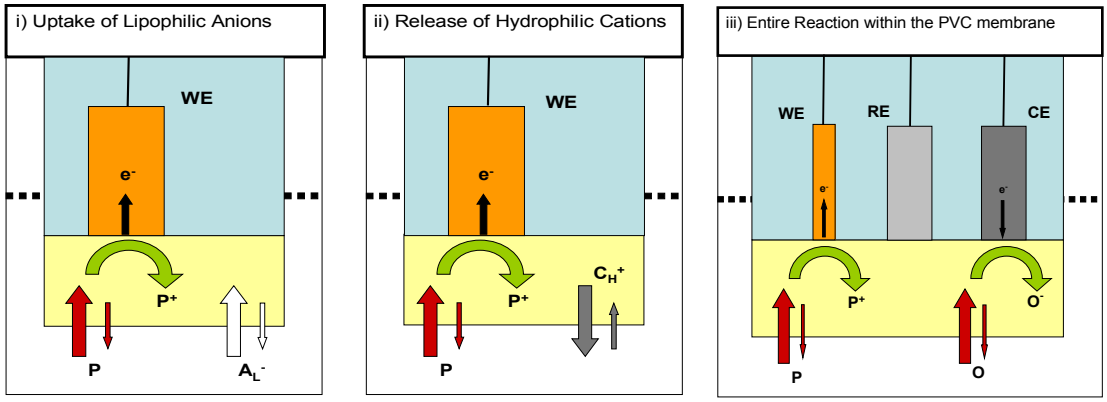

Figure 6. Diagram of the three approaches of maintaining electroneutrality in a PVC membrane during the electrochemical oxidation of propofol on the working electrode surface. WE: working electrode, RE: reference electrode, CE: counter electrode, P: propofol or propofol derivative, A_L^-: lipophilic anion, C_H^+: hydrophilic cation, O: oxidant, e^-: electron. Note: the drawings are NOT to scale.

Using the first approach (Figure 6, panel i) the sample solution is compounded with an electrolyte containing a highly lipophilic anion. Using 8mM tetrabutylammonium perchlorate ($TBAClO_4$) in the sample solution, Guo achieved a detection limit of 1μM with his PVC membrane-coated glassy carbon macro electrode

[11]. Although promising, compounding the sample with perchlorate ions can not be applied for in vivo monitoring.

In the second approach (Figure 6, panel ii) the PVC membrane is loaded with a salt (potassium tetrakis (pentafluorophenyl) borate, KTPFPB), consisting of a hydrophilic cation (K^+) and a lipophilic anion ($TPFPB^-$). As positively charged species are generated in the membrane, hydrophilic K^+ cations are released into solution.

In the third approach (Figure 6, panel iii) the entire electrochemical cell – consisting of a working, reference, and counter electrodes – is coated with the PVC membrane. However in Guo's research, the second and the third approaches were applied simultaneously – the membrane coated the entire cell but at the same time contained KTPFPB, which could release K^+ hydrophilic cations. The presence of KTPFPB into the membrane provided as additional benefit by reducing the membrane resistance, which is essential to minimize the ohmic potential drop during electrochemical measurements in the membrane.

6. Planar Electrochemical Cells (PECs)

Preparation of membrane-coated electrochemical cells, the "third approach", requires protocols for making planar electrochemical cells (PECs) which could accommodate a variety of electrodes (working, counter, and reference) of different materials and dimensions. The protocol must allow the fabrication of PECs with working electrodes made from different brands of carbon filaments.

Since the electrodes of the cell will be arrayed on a planar surface, it will be possible to cover them with an organic PVC membrane via spin coating. Indeed, once an optimized membrane composition and spin-coating procedure had been developed, they

can be coated with such a membrane and used to make measurements of propofol in aqueous solution. Those measurements would allow a more relevant, or realistic, comparison of the carbon filament working electrodes (since the many benefits of the PVC membrane mean the voltammetric propofol detector is very likely to employ a PVC membrane coating in it's final design).

Materials and Fabrication Methods

1. Planar Electrochemical Cell Fabrication

The working electrodes of the PECs are fabricated by incorporating carbon filaments into glass capillaries. First, long (~3cm) pieces of borosilicate glass capillary tube (OD: 1.5mm, ID: 0.75mm; length: 10cm, single barrel, Sutter Instruments) are pulled off with a capillary puller (Sutter Instruments, Model P-30) with the following settings: Heat 1, 825; Pull, 800. Next, each of the pulled ends are heated over a Bunsen Burner (set to high-heat flame) to close them off; thus creating a test tube-like structures. This protocol for making electrodes is termed the 'capillary method'.

Next, the working electrode material is inserted into one of the tubes (Figure 7, step 1). As stated previously, the working electrode is made of from one three materials: Bioanalytical Systems carbon fibers, 9μm diameter (BAS); Goodfellow carbon fibers, 11μm diameter (GF); or Specialty Materials Inc. glassy carbon monofilaments, 34.5μm diameter (SM). For the two carbon fibers, they are inserted into the capillary tube by laying the carbon fiber on a paper towel, pushing the capillary tube over one end of the fiber until the end of the fiber reaches the end of the tube, and then breaking off the excess length of carbon fiber. For the glassy carbon monofilaments, short sections can simply be dropped down the capillary tube.

After that, a vacuum is applied to the open end of the capillary tube (with the SPX Robinair Cooltech High Performance 6CFM vacuum pump and two partially-nested sections of Tygon Flexible Plastic Tubing (OD: 5mm; ID: 2.5mm, and OD: 3mm; ID: 1.5mm)) and, ***immediately after the vacuum pump is turned on***, the closed-off end of the capillary is heated over a Bunsen Burner (set to low-heat flame) until the glass collapses around the first ~1cm of the fiber (Figure 7, step 2). The heating will sometimes create 'bubbles' at the tip of the carbon filament. This is normal, and as long as they are at least a few millimeters of glass-enclosed carbon filament without bubbles, the final electrode will not be affected.

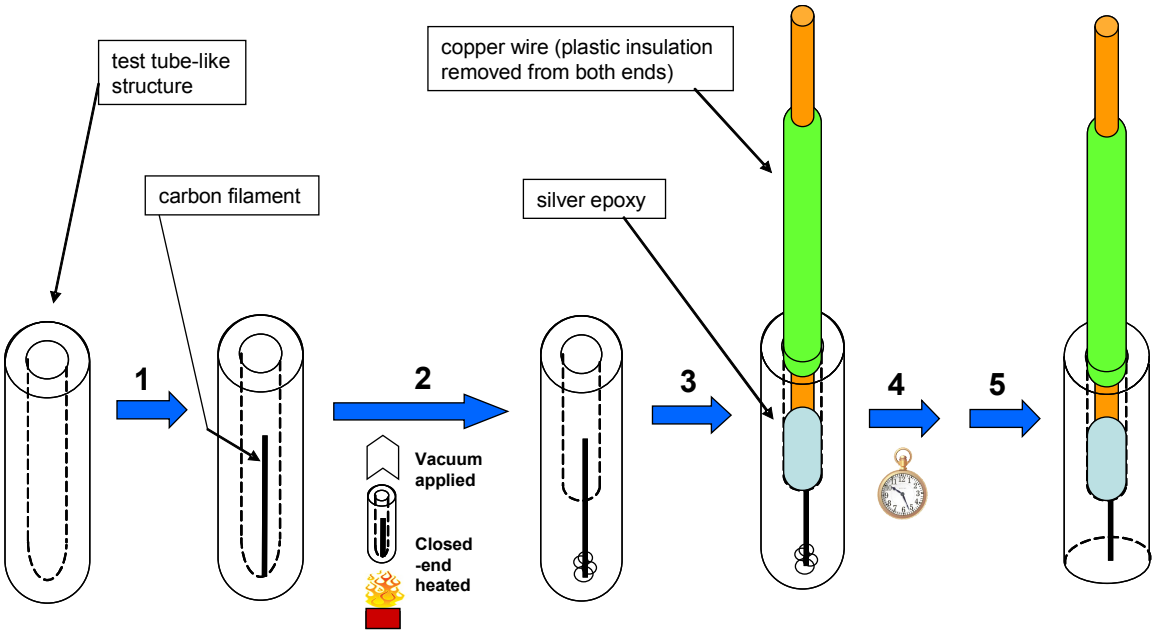

Figure 7. Diagram of the working electrode fabrication by the 'capillary method'. 1: Inserting carbon filament in glass tube, 2: Melting the glass around ~1 of carbon filament, 3-4: Preparing of electrical connection, 5: Removing of the tip of the capillary to expose the carbon electrode. Note: the drawings are NOT to scale.

An electrical connection is made between the carbon filament and a copper wire by filling silver epoxy (Epoxy Technology H20E kit – Parts A and B) from the open end

of the capillary with the help of a silver epoxy-filled syringe and a 0.15mm diameter needle and inserting the copper wire inserted into capillary till it contacts the silver epoxy. Next, the entire carbon filament/capillary/connecting wire assembly is placed into a 100ºC oven (Fisher Scientific, 10-750-14 Isotemp Programmable Forced-Air Muffle Furnace) for 2 hours to cure the silver epoxy (Figure 7 steps 3 and 4).

After that, the tip is scored with a file, broken off with pliers, and the exposed surface of glass is visually checked to ensure the carbon filament electrode has emerged. If it has not, the tip is sanded with SIA:60 grit sandpaper until it does (see Figure 7 step 5). Finally, the newly-exposed electrode surface is sanded with SIA: 60 grit, and then Sancap: 220 grit, sandpaper to remove any rough edges.

The counter and reference electrodes may be made by the same basic protocol. Though, a 0.7mm diameter (Pentel) graphite bar and a 0.5mm diameter, silver wire (99.9%, Aldrich Chemical Co.) are used as the counter and reference electrode materials, respectively. The connections between the copper wires and the counter and reference electrodes are verified with a multimeter (AW Speery Techmaster, DM-8600).

Alternatively, the counter and reference electrodes may be made by the 'tape method' in which the insulation between the wires is achieved by a piece of celluloid tape (Scotch brand, 3M). A ~2cm section of the electrode material and the stripped-end of an insulated copper wire are laid down end-to-end on the sticky side of the tape (Figure 8 frame A).

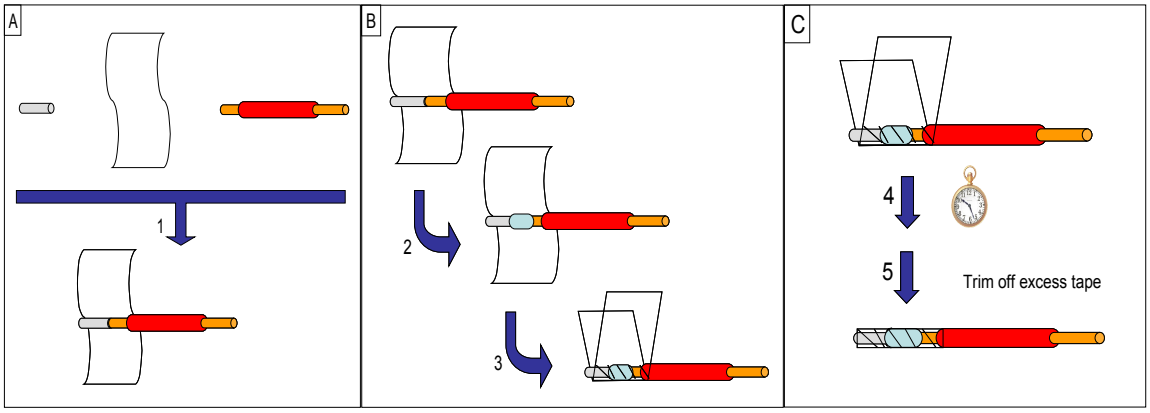

Figure 8. Diagram of the steps of the counter and reference electrode fabrication by the 'tape method'. Detailed description in the text. Note: the drawings are NOT to scale.

Silver epoxy is applied to the joint between the electrode material and the copper wire, and the tape is fold over at the joint (Figure 8 frame B). The electrode is heated in the oven at 100°C for 2 hours to cure the silver epoxy, and the excess tape is trim off (Figure 8, frame C). Electrodes can be made in less time by this "tape method" but they are more fragile.

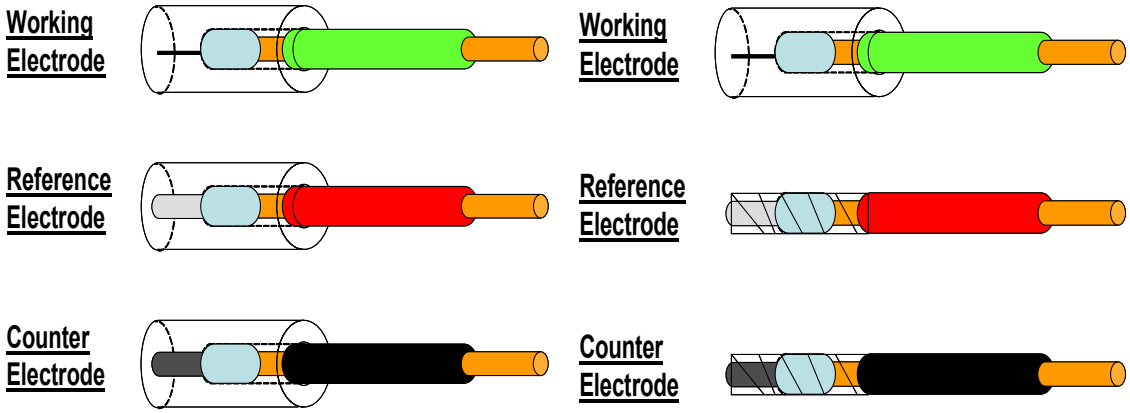

Figure 9. Diagram of the working, reference, and counter electrodes. Reference and counter electrodes may be made by either the capillary method (left panel) or the tape method (right panel). Reference electrode: silver wire, 0.5mm diameter. Counter Electrode: graphite, 0.7mm diameter. Note: the drawings are NOT to scale.

After the preparation of individual working, counter, and reference electrodes, a ~50mm long section glass tubing (borosilicate glass, OD: 6mm, ID: 4mm or OD: 4.6mm, ID: 2.5mm) is obtained and one end is closed-off by wrapping it in celluloid tape – creating another test tube-like structure. This glass tube is filled with a 1mL bolus of preheated (60 - 70 ºC) Epon 282 resin (Hexion) containing 14% m-phenyladiamine (Acros Organics), using a 5mL syringe with a 16 gauge needle (Becton Dickson). A diagram of these steps is shown in Figure 10.

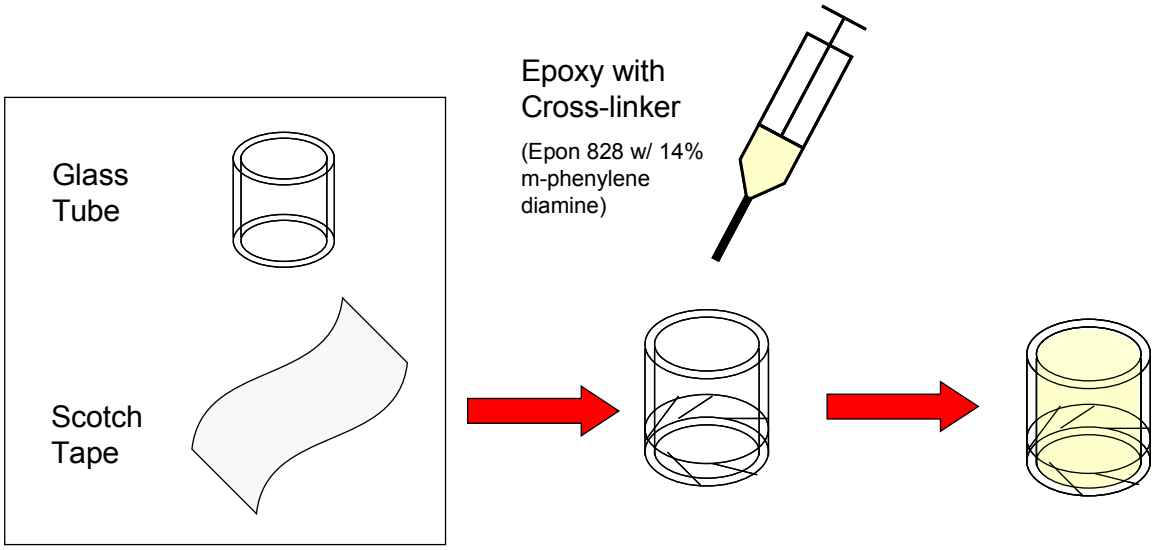

Figure 10. Diagram of assembling the resin-filled outer glass tube. See text for details. Note: the drawings are NOT to scale.

The working, counter, and reference electrodes are inserted into the resin-filled glass tubes and pushed all the way to the bottom. The planar electrochemical cells are cooled to room temperature overnight, and then placed into a 100ºC oven for 2 hours (in order to cure the Epon resin). Diagram of these steps are shown below in Figure 11.

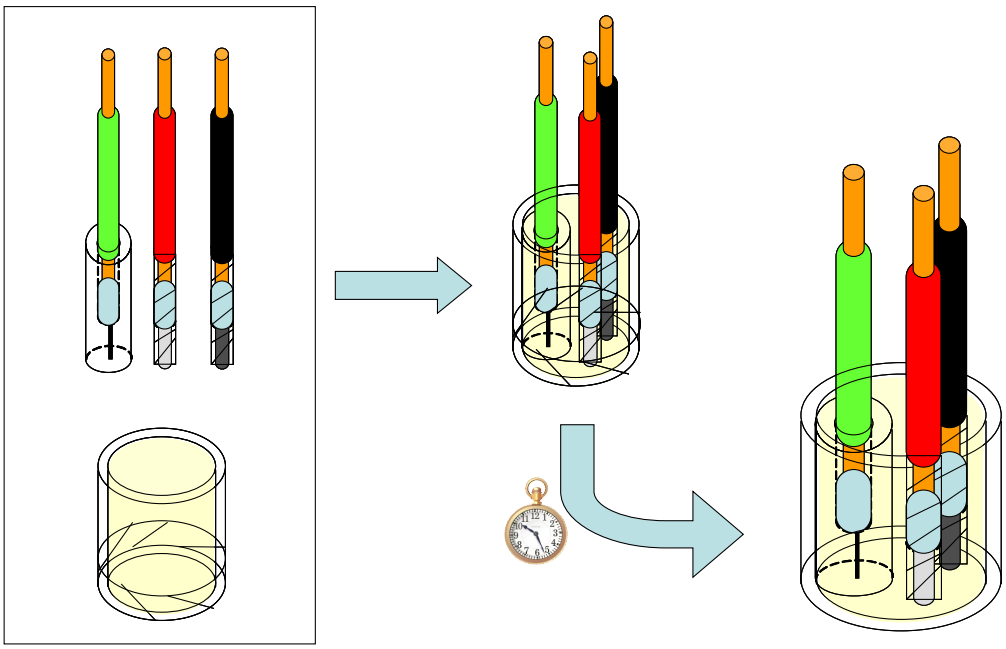

Figure 11. Diagram of the PEC assembly from individual electrodes. In this depiction, working electrode is made by the capillary method while reference and counter electrodes made by the tape method. Note: the drawings are NOT to scale.

After the epoxy is cured, the celluloid tape is removed and the electrode end of the assembly is sanded with progressively finer sandpaper (SIA: 60 grit, the Sancap: 220 grit), and polished on a polishing wheel (Shinko, ST-706B) with microcloth (for 8" wheel, Buehler, Catalog# 40-7208) with progressively finer alumina slurry (Buehler: 1.0 micron Alpha Micropolish II, then Buehler: 0.3 micron Alpha Micro Micropolish II). The final product of the process is a completed planar electrochemical cell (PEC) with three, disc-shaped electrodes at the end of an epoxy-filled cylindrical glass tube. Pictures of these PECs are shown in Figure 12.

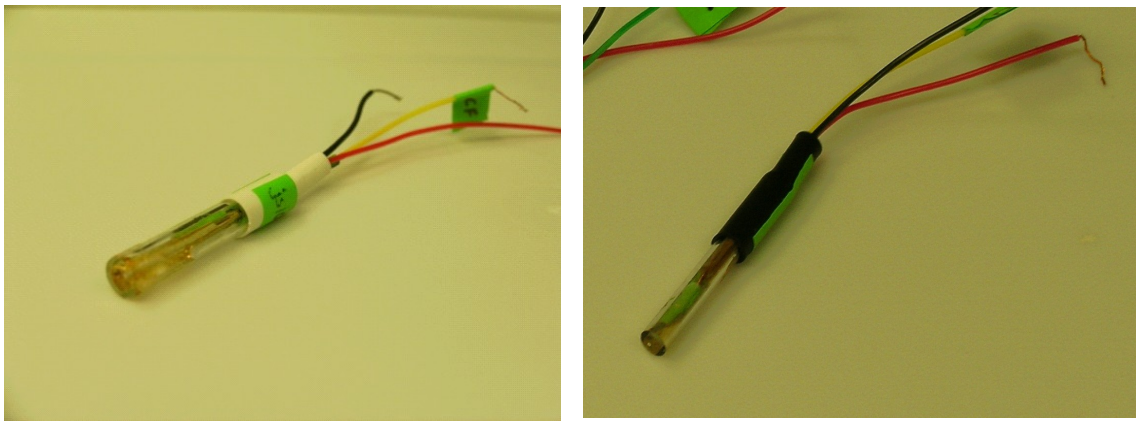

Figure 12. Photographs of the PECs. Right: planar electrochemical cell made with OD: 6mm, ID: 4mm outer glass tube. Left: planar electrochemical cell made with OD: 4.6mm, ID: 2.5mm outer glass tube.

2. Voltammetric Measurements

All solutions were prepared with 18.2 MΩ resistivity deionized water (Milli-Q Gradient A-10, Millipore). All chemicals were analytical grade and used as received. Solutions were prepared with the following chemicals: ferrocene methanol (Aldrich Chemical), ferrocene (Eastman Kodak), 2,6-diisopropyl phenol (Sigma-Aldrich), potassium chloride (JT Baker Chemical), sodium sulfate decahydrate (Sigma-Aldrich), 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (also known as: HEPES) (Aldrich Chemical), tetrabutyl ammonium perchlorate (Eastman Fine Chemicals), tetrabutyl ammonium chloride (Aldrich), acetonitrile (Fisher Chemical). Phosphate buffer solutions were prepared from potassium dihydrogen phosphate (Sigma Chemical) and sodium monohydrogen phosphate (Fisher Chemical).

The carbon filament electrodes in the PECs and a 3mm dia. glassy carbon electrode (Bioanalytical Systems) served as the working electrodes in all experiments in this study. The graphite counter electrodes in the PECs served as the counter electrode in all experiments. Either the Ag electrodes from the PECs, an external Ag|AgCl reference

electrode (CHI Instruments) – with either 3.5M KCl or a 0.1M TBACl in acetonitrile filling solution – served as the reference electrode in all experiments. The PECs and 3mm glassy carbon electrode were polished before each experiment with 0.05 micron alumina slurry (Alpha Micro Micropolish II) on a microcloth (Buehler). All experiments were conducted with an Autolab / PGSTAT12 potentiostat and General Purpose Electrochemical System, Version 4.9 (Eco Chimie BV) software.

Results

1. Verification of Working Electrode Size

The goal of this work was to compare the electrochemical properties of the three carbon filament electrodes for the quantitative assessment of propofol. A series of five to six PECs was created out of each of the three types of carbon filaments - creating an overall set of seventeen cells (see Appendix I for a full list with details of construction).

As the first step of the characterization, we compared the nominal geometrical surface areas of the carbon filaments to the experimentally-determined electrochemical surface areas of the carbon filament electrodes in the PECs. Cyclic voltammograms were recorded with each of the PECs (each cell having three electrodes) – both in a background solution (0.1M KCl, 0.1M phosphate buffer at pH ~7.2) and in an analyte solution containing the electrolytes of the background solution (in the same concentrations, and at the same pH) and 0.5mM ferrocene methanol. By subtracting the background cyclic voltammogram from the analyte cyclic voltammogram a sigmoidal-shaped 'difference' curve was obtained (see Figure 13). The carbon filament of each PEC served as the working electrode, the graphite electrode of each PEC served as the counter electrode, and the silver electrode of each PEC served as the reference electrode. The

cyclic voltammograms were recorded between -0.1V and 0.4V at a 100mV/s scan rate. An example of these curves is shown in Fig. 12.

The cyclic voltammograms of each electrode were recorded five times. Between the individual measurements, the electrodes were removed from the solutions and polished to renew their surfaces. In summary, the study produced 85 limiting current measurements.

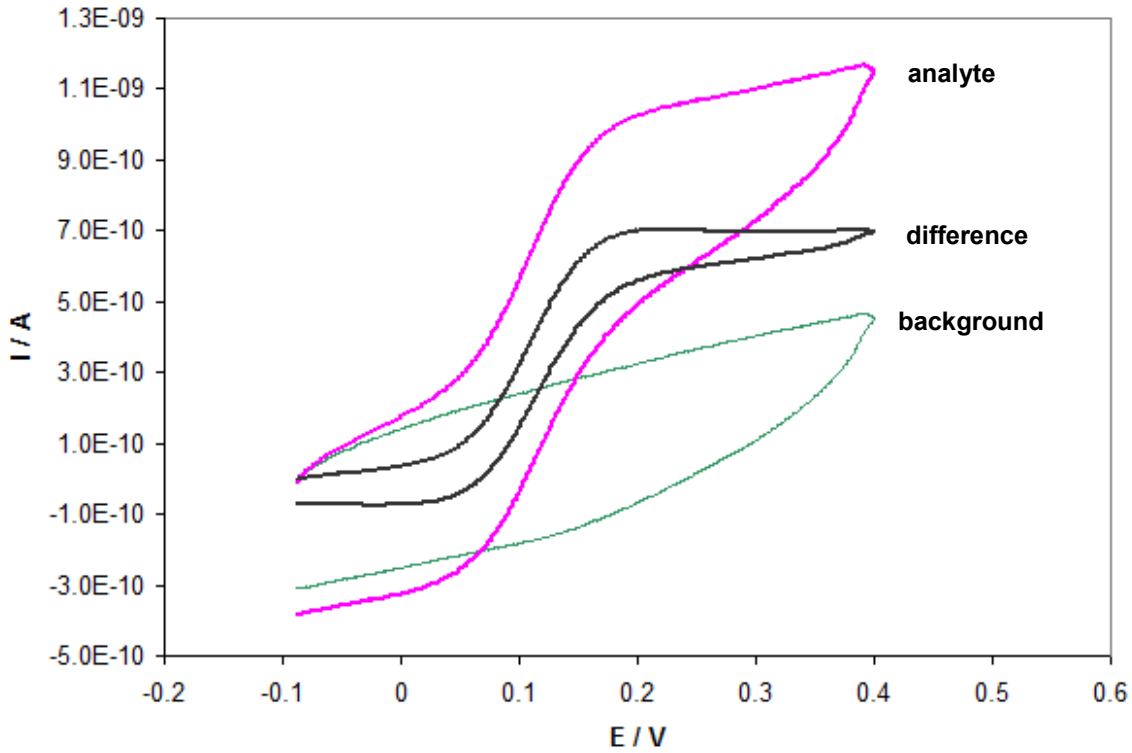

Figure 13. Typical cyclic voltammograms recorded with a carbon filament working electrode (9µm diameter) and silver reference electrode (500µm diameter). Green: recorded in 'background' solution (0.1M KCl, 0.1M phosphate buffer at pH 7.0). Magenta: recorded in 'analyte' solution (0.5mM ferrocene methanol, 0.1M KCl, 0.1M phosphate buffer at pH 7.0). Black: difference = analyte – background. Scan rate, 100mV/s. The voltammogram data has been filtered by a 21-point simple moving average to reduce noise.

Only PECs with sigmoidal responses were used for this analysis. To eliminate outliers in the recorded limiting current values, a Dixon's Q-Test was performed on each

group of data containing the 5 limiting current measurements with a single electrode. For data sets with 5 points, a critical Q-value of 0.710 will include 95% of all possible measurements. Among the 85 data points collected, only one fell outside this critical value, so it was excluded from further calculations.

Next, the mean value of each set of 5 limiting current values was calculated. This provided the *average* limiting current for each electrode. These were then separated into groups based on the brand of their carbon filament (BAS, GF or SM). To remove outliers from among electrodes of the same brand, a Dixon Q-test was performed on the average limiting currents of each group. However, no electrodes had an average limiting current above the critical value relevant to its group, so all remaining data were used for the subsequent electrode size analysis [16].

To calculate the effective electrochemical diameter of the working electrodes, the steady-state current value (i.e. the limiting current) of the sigmoidal-shape curves were used in combination with Equation 1,

$$I_l = 4nFDcr = kd \qquad (1)$$

where I_l is the limiting current; n is the number of electrons released by oxidation of analyte, ferrocene methanol (1); F is Faraday's constant (96485 C/mol); D is the diffusion coefficient of analyte, ferrocene methanol (7.5×10^{-6} cm^2/s)[17]; r is the radius of working electrode; c is the concentration of analyte, ferrocene methanol; k is the constant (7.24×10^{-7} A/cm); and d is diameter of working electrode. According to Bard, equation 1 only applies to disc electrodes with a diameter "smaller than ~25μm". Since all three carbon filaments had a circular cross-section, all working electrodes were disc-

shaped; this was verified (visually) with a digital microscope (Keyence Digital Microscope, VHX-1000). Furthermore, BAS and GF filaments had nominal diameters of 9 and 11μm, respectfully – so GF and BAS electrodes met all the criteria for using this equation 1. SM filaments, however, have a nominal diameter of 34.5μm – so equation 1 can only provide an estimation of the diameter of the SM electrodes [18]. Table 1 below show the statistics for all the measurements arranged by the carbon filament brand.

Table 1. Statistical Analysis of Effective Diameter Measurements. *

	Effective Diameter				Nominal Diameter	
Carbon Filament Type	Mean (μm)	Standard Deviation (μm)	Rel. Standard Deviation	Sample Size	Mean (μm)	Deviation (μm)
BAS	9.07	0.77	0.09	20	11	2
GF	9.05	0.72	0.08	29	8	2
SM	35.96	1.13	0.03	24	34.5	2.5

* The Effective Diameter values were experimentally measured. The Nominal Diameter values are those claimed by the filament manufacturers.

Using the data in Table 1, we tested the null hypothesis that the effective working electrode diameter was equal to the nominal carbon filament diameter: i.e. $d_{electrochemical} = d_{nominal}$. In statistical analysis, the null hypothesis is rejected if the probability of the calculated statistics is less than 5 % ($\alpha < 0.05$); or for this analysis, if the absolute value of the T-test statistic ($|T|$) is greater than the critical T statistic corresponding to $\alpha = 0.05$ ($T_{crit}(\alpha = 0.05)$). The results of our statistical analysis are summarized in Table 2 and Fig. 13. According to Table 2, the T-test statistic is less than the critical T statistic, so the null hypothesis cannot be rejected. The small differences in the nominal and measured electrode diameters are statistically not significant; they are assumed to be the results of random sampling.

Table 2. Statistical Comparison of Effective and Nominal Electrode Diameters.

Carbon Filament Type	Degrees of Freedom	T_{crit} ($\alpha = 0.05$)	|T|	P-Value
BAS	19	2.093	0.560	0.58
GF	28	2.048	0.272	0.79
SM	23	2.069	0.263	0.79

* P-value is probability that the effective and nominal electrode diameters are from same population.

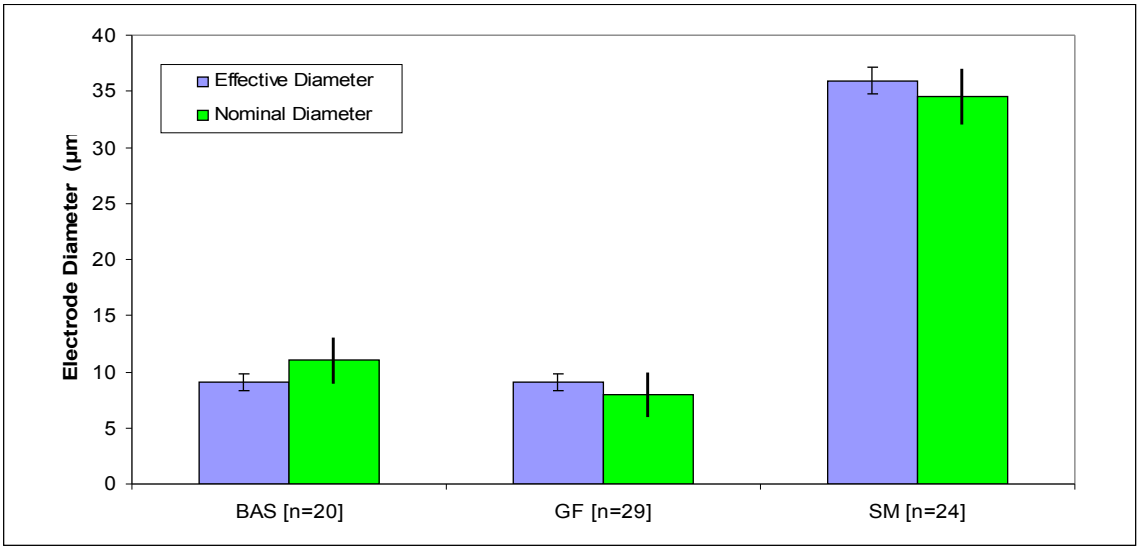

Figure 14. The Mean Values of the Electrochemical and Nominal Diameters of Bioanalytical Systems (BAS), Goodfellow (GF), and Specialty Materials (SM) carbon filaments. Note: the brackets at the top of the effective diameter bars represent standard deviation, while the brackets on the nominal diameter bars represent some undefined measure of variability provided by the manufacturer.

2. Assessment of Signal/Noise Ratio

The signal/noise (S/N) ratio is one of the most important metric in the characterization of working electrodes used in voltammetric analysis. It is one of the metrics used to calculate the detection limit and dynamic range of a detector. The exact value of the S/N ratio for any working electrode is affected by the electrode construction, the composition of the solution, the potential at which the 'signal' is defined, etc.; but if

all those variables are held constant, then differences may be attributed to the working electrode material directly.

To for this reason then, the above-described 85 cyclic voltammograms in 0.5mM ferrocene methanol were assessed to determine if there was any statistically significant difference in S/N ratios of the PEC carbon filament working electrodes. For this analysis, the S/N ratio was calculated by dividing the current of the analyte curves (at 0.4V) from the current of the background curves (at 0.4V): refer to Figure 13. As with the assessment of working electrode size, and a Dixon's Q-Test was performed on each group of data containing the 5 S/N ratio measurements with a single electrode. Among the 85 data points collected, four fell outside the critical Q-value for their group, so those four data points were excluded from further calculations. Next, the mean S/N ratio of each set of 5 S/N ratio values was calculated. This provided the average S/N ratio for a single electrode in the 0.5M ferrocene methanol. As with the limiting current measurements, no PEC working electrode had a Q-value above the critical Q-value of its group, so all remaining data were used for the subsequent electrode size analysis [16]. Table 3 below show the statistics for all the measurements arranged by the carbon filament brand.

Table 3. S/N Ratios Statistics for each Carbon Filament Brand. *

	S/N Ratio		
Carbon Filament Type	Mean	Standard Deviation	Sample Size
BAS [n=17]	9	9	17
GF [n=29]	5	3	29
SM [n=23]	33	37	23

*S/N ratio values are unit-less.

Using the data in Table 3, we used an F-test to test the null hypothesis that all the S/N ratio measurements belonged to the same population. As stated above, the null hypothesis is rejected if it has a probability of less than 5 % ($\alpha < 0.05$) – and in this analysis, that was indeed the case. The null hypothesis could not be rejected, and therefore, the S/N ratio differences between the three brands of carbon filament are assumed to be the results of random sampling. In the experiments that followed this analysis, only PECs with the highest available S/N ratio were used.

3. Cyclic voltammograms in aqueous propofol solutions

To select the best among the three carbon filaments electrodes for propofol measurements, twenty successive cyclic voltammograms were recorded in 100μM buffered propofol solutions (pH 7.14, 0.01M HEPES, 0.01M Na_2SO_4). The potential of the working electrode was scanned between -0.4 and 0.8V at 50mV/s scan rate. The results of these experiments are summarized in Figures 15-17. They are in agreement with the results reported by Guo (Figure 3). In summary, the oxidation and reduction peaks on these voltammograms decayed almost completely over the course of 20 successive scans.

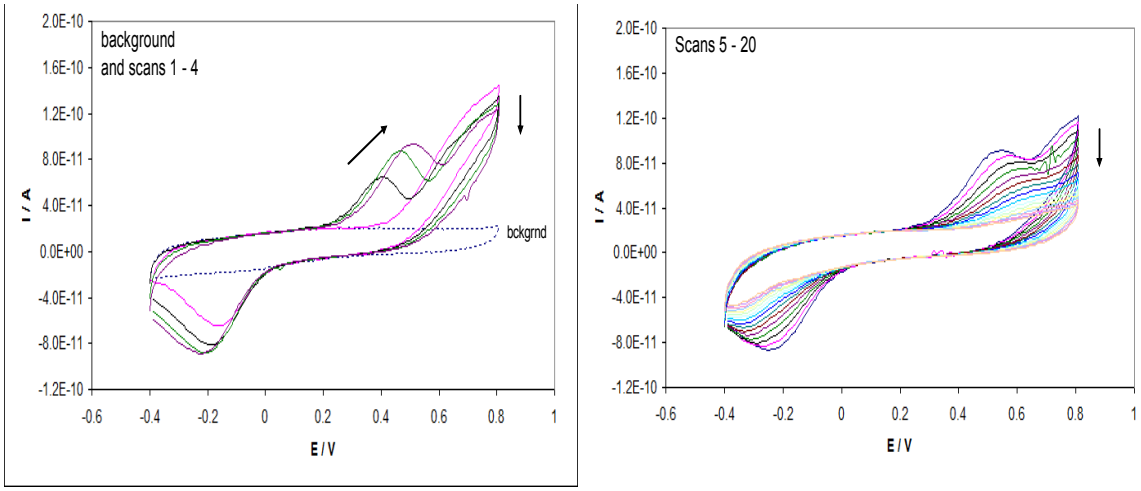

Figure 15. Successive cyclic voltammograms recorded with a 9µm diameter BAS carbon fiber working electrode in 100µM propofol solution of pH 7.0. Reference electrode: Ag|AgCl|3.5M KCl; Counter electrode: 0.5mm diameter graphite disc; Background electrolyte: pH 7.0 0.01M HEPES buffer + 0.01M Na_2SO_4; Scan rate: 50mV/s.

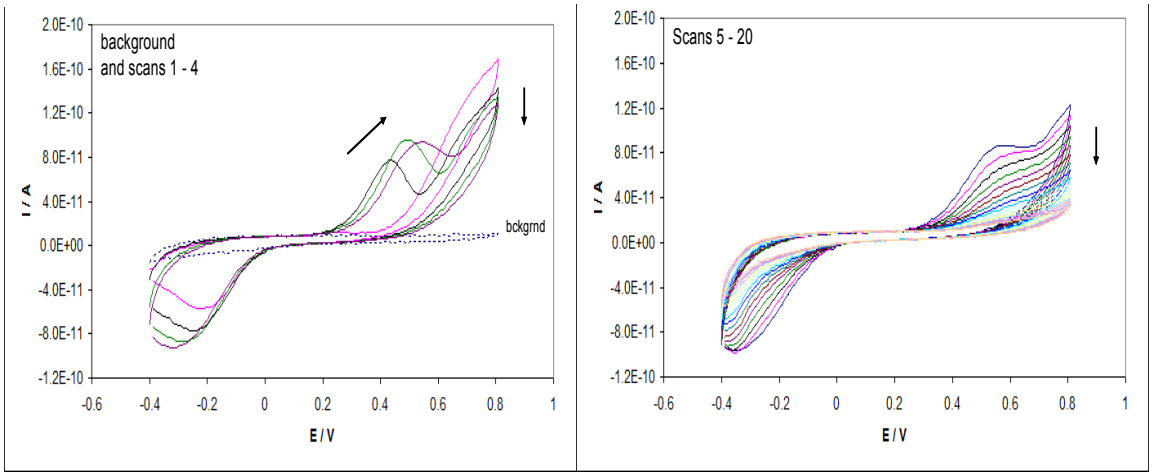

Figure 16. Successive cyclic voltammograms recorded with a 9µm diameter GF carbon fiber working electrode in 100µM propofol solution of pH 7.0. Reference electrode: Ag|AgCl|3.5M KCl; Counter electrode: 0.5mm diameter graphite disc; Background electrolyte: pH 7.0 0.01M HEPES buffer + 0.01M Na_2SO_4; Scan rate: 50mV/s.

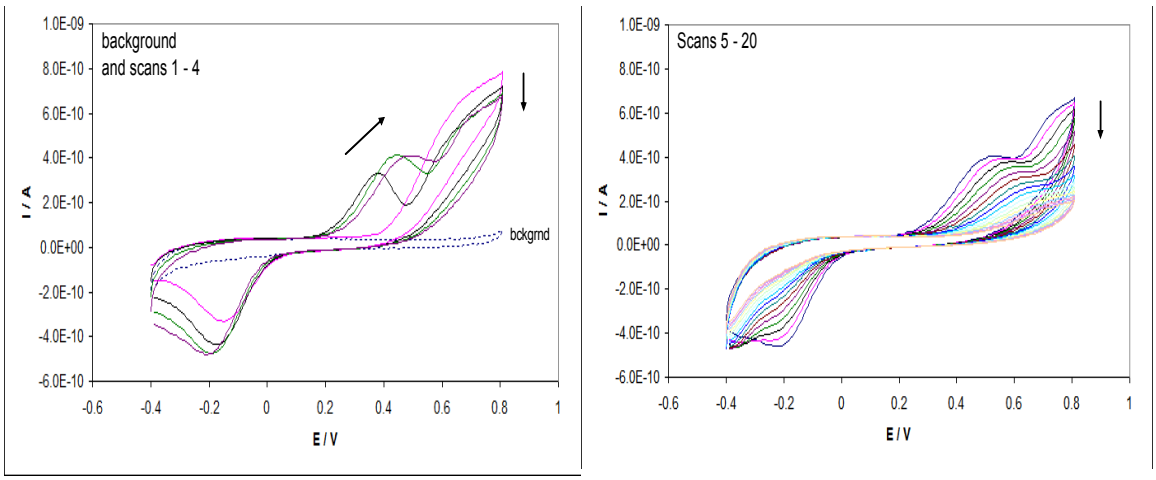

Figure 17. Successive cyclic voltammograms recorded with a 36µm diameter SM carbon fiber working electrode in 100µM propofol solution of pH 7.0. Reference electrode: Ag|AgCl|3.5M KCl; Counter electrode: 0.5mm diameter graphite disc; Background electrolyte: pH 7.0 0.01M HEPES buffer + 0.01M Na_2SO_4; Scan rate: 50mV/s.

These results show that working electrodes made from BAS, GF, and SM carbon filaments all foul after only a few measurement recorded in aqueous solution. Without a PVC membrane, none of them would be appropriate to use as the sensing element in a propofol detector.

4. Cyclic voltammograms of propofol in acetonitrile

The idea of organic film coated working electrode is traced back to the cyclic voltammetric experiments which Guo performed in acetonitrile in the presence of 100µM propofol (Figure 4). In those experiments, practically no electrode fouling was seen after 18 consecutive cyclic voltammograms using a glassy carbon macro electrode. In my experiments, I could repeat the experiments of Guo [11]. However, using a Ag|AgCl| reference electrode with a 0.1M TBACl in acetonitrile filling solution, the peak of propofol oxidation emerged at a potential almost 500 mV higher than that found by Guo (compare Fig. 18 and 4). Again, since the only difference between my experiment and Guo's experiment was the choice of reference electrode, this difference in propofol

oxidation potential is directly attributable to the potential difference between our two reference electrodes.

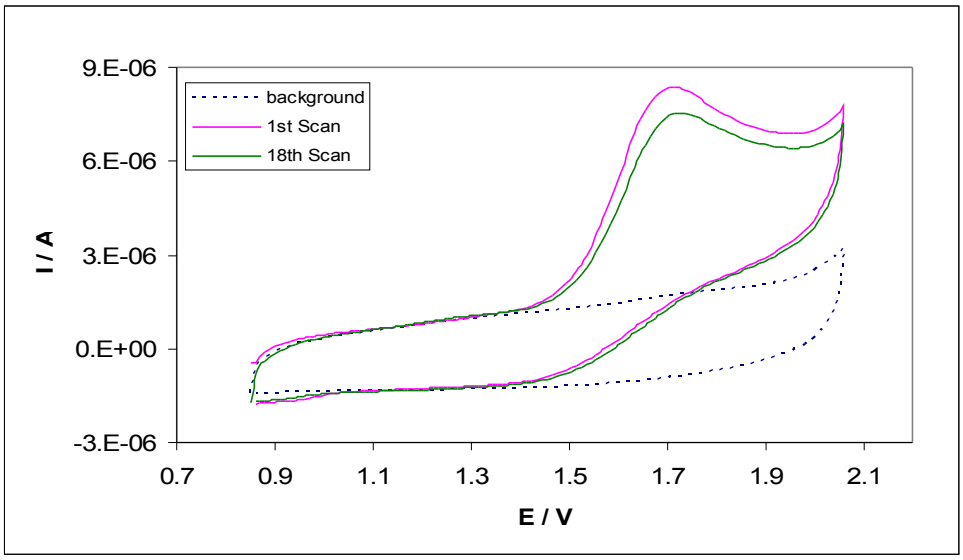

Figure 18. Successive cyclic voltammograms recorded with a 3mm dia. glassy carbon working electrode in an acetonitrile solution of 100µM propofol. WE (3mm-dia. glassy carbon), CE (Platinum wire), RE (Ag|AgCl| 0.1M TBACl in acetonitrile). Solution: background (0.1M $TBAClO_4$ in acetonitrile), analyte (100µM propofol in background). Voltammetry: cyclic voltammetry, scan rate (50mV/s), step potential (10mV), preconditioning (1s at initial potential).

Next, cyclic voltammograms (not shown) were recorded in 5mM ferrocene, 0.05M $TBAClO_4$ in acetonitrile solution with a carbon fiber working electrode and a Ag|AgCl|0.1M TBACl in acetonitrile reference electrode. The half-wave potential ($E_{1/2}$) of the ferrocene/ferrocenium couple was approximately 0.72V. According to Pavlishchuk and Addison, the $E_{1/2}$ of the ferrocene/ferrocenium couple is 0.624V (vs SHE) in all solvents [19]. Based on this, the potential of a Ag|AgCl|0.1M TBACl in acetonitrile electrode (vs SHE) is approximately -0.10V. The preceding analysis shows how the potential of the Ag|AgCl|0.1M TBACl in acetonitrile electrode was determined. This electrode was used as the reference in all the following experiments in acetonitrile.

After that, successive cyclic voltammograms were collected by scanning from 0.85 to 2.05V at 50mV/s with working and counter electrodes from the PECs (and the Ag|AgCl|0.1M TBACl in acetonitrile reference electrode) in a solution of 100µM propofol, 0.1M $TBAClO_4$ in acetonitrile. The BAS and GF carbon fiber working electrodes had minimal signal decay – 15±5 and 14±4 percent, respectively – similar to the behavior of the 3mm dia. glassy carbon electrode (see Figure 19). The glassy carbon monofilament (SM), however, had greater signal decay – averaging 33±5 percent.

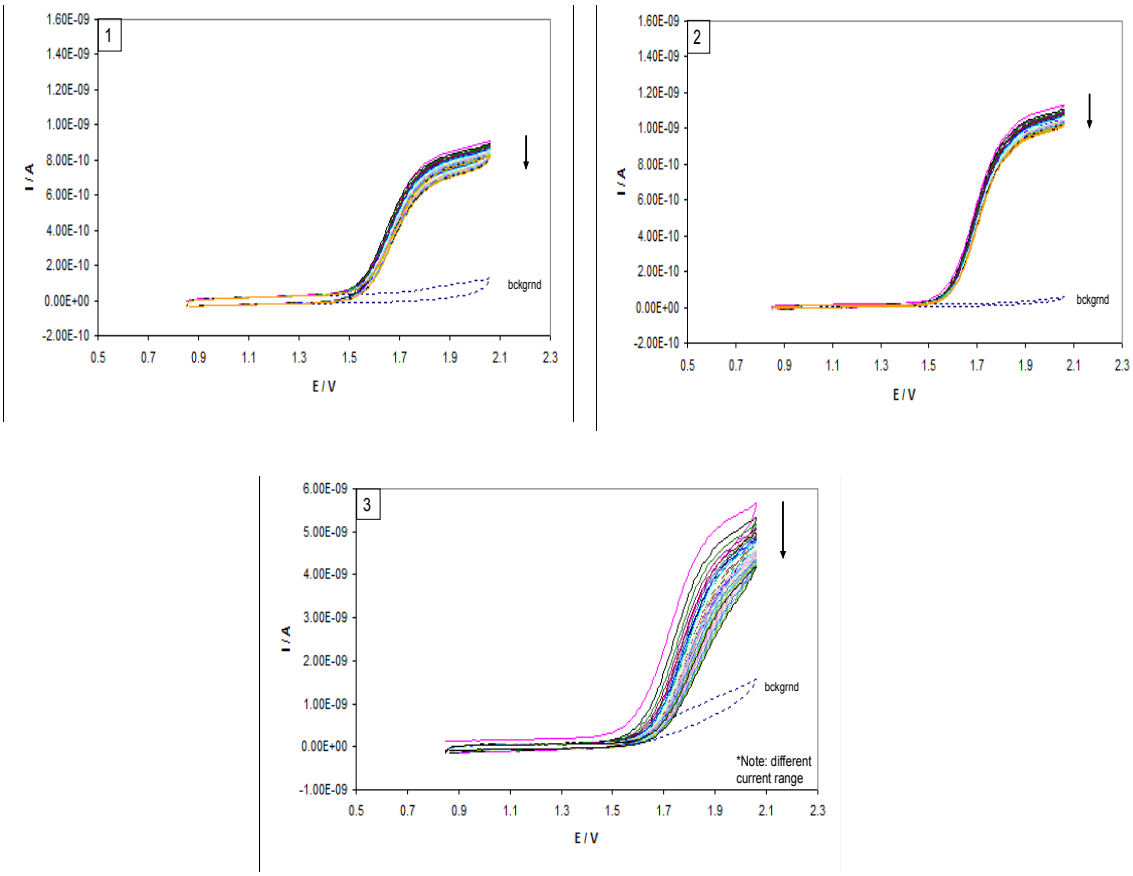

Figure 19. Successive cyclic voltammograms recorded with carbon filament working electrodes in an acetonitrile solution of 100µM propofol. Sensors: WE (BAS (plot 1), Goodfellow (plot 2), and Specialty Materials (plot 3)), CE (graphite), RE (Ag|AgCl|0.1M TBACl in acetonitrile). Solution: background (0.1M $TBAClO_4$ in acetonitrile), analyte (100µM propofol in background). Voltammetry: cyclic voltammetry, 20 scans, scan rate (50mV/s), step potential (10mV), preconditioning (1s at initial potential).

Table 4. Repeatability of Percent Signal Decay across 20 scans (n=5). *

Carbon Filament Type	Trial 1 (%)	Trial 2 (%)	Trial 3 (%)	Trial 4 (%)	Trial 5 (%)	Average (%)
BAS	12	11	17	10	20	14
GF	10	16	10	19	20	15
SM	36	26	31	39	35	33

*For this analysis, the 'signal' is considered to be the background-subtracted current of the oxidation wave at 2.05V. Percent Signal decay = 100 x (sig_scan1 – sig_scan20) / sig_scan1

Statistical analysis of the 'percent signal decay' from those tests is shown in Table 5 above. The signal decay of the SM glassy carbon monofilament electrode was significantly greater that the signal decay of the BAS and GF carbon fiber electrodes. This indicate that BAS and GF electrodes have much more promise than the SM electrodes to be used as the working electrode of a voltammetric propofol detector.

Finally, the relation of the limiting current with propofol concentration was examined. A series of cyclic voltammograms were collected by scanning from 0.85 to 2.05V at 50mV/s in 0.1M $TBAClO_4$ acetonitrile solution with a GF carbon fiber working electrodes and the Ag|AgCl|0.1M TBACl in acetonitrile reference electrode. The concentration of propofol in these solutions was increased by standard addition from 1μM to 100μM. The cyclic voltammograms are shown below in Figure 20.

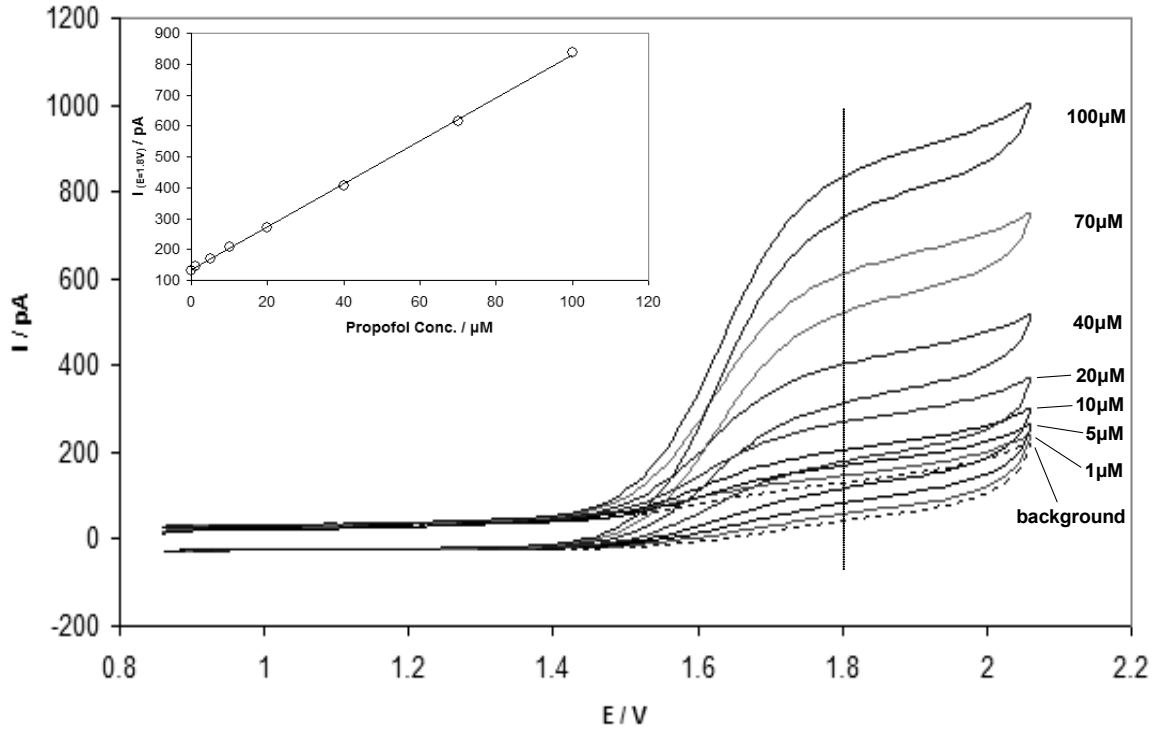

Figure 20. Cyclic voltammograms recorded with a Goodfellow carbon fiber working electrode in acetonitrile solutions of propofol with different concentrations. Inset: calibration curve constructed from the steady state current values measured at 1.8V. Sensors: WE (Goodfellow), CE (graphite), RE (Ag|AgCl|0.1M TBACl in acetonitrile). Solution: background (0.1M $TBAClO_4$ in acetonitrile), analyte (various concentrations of propofol in background). Voltammetry: cyclic voltammetry, scan rate (50mV/s), step potential (10mV), preconditioning (1s at initial potential).

The signal (current of the oxidation wave at 1.8V) increases linearly with propofol concentration across the entirety of the tested concentration range. The fitted regression line has a residual mean standard deviation (RMSD) of 5.26 [pA] and a slope (S) of 6.97 [pA/µM]. These values give the method a detection limit of 2.3µM (based on the following equation, detection limit = 3xRMSD/S).

Conclusions

Three sets of planar electrochemical cells (PECs) were constructed. The working electrodes of these PECs consisted of different types of carbon microelectrodes: Bioanalytical Systems carbon fiber, Goodfellow carbon fiber, and Specialty Materials glassy carbon monofilament. The electrochemical diameters of these working electrodes were compared to the nominal values provided by the manufacturers. The electrochemically determined diameter values were found to be the same as the nominal value for all three brands.

Next, the capabilities of the working electrodes in the PECs to determine propofol in buffered pH neutral aqueous solution were assessed. All the cells tested showed significant current decay over the course of 20 cyclic voltammetry scans. This signal decay was attributed electrode fouling.

Finally, the capability of the PECs to determine propofol in acetonitrile solution was assessed. Over the course of 20 scans, cells with the BAS and GF carbon fiber working electrodes experienced signal decays of only 14±4 and 15±5 percent, respectively; while cells with SM working electrodes showed signal decay of 33±5 percent. This indicates that electrodes made from either BAS of GF are more promising than electrodes made from the SM filaments for the development of a voltammetric propofol detector. A standard-addition curve of propofol in acetonitrile solution was collected by performing cyclic voltammograms with one of the carbon fiber cells. The detection limit was 2.3μM which is within the therapeutic range for steady-state propofol concentration.

In summary, considering that carbon fiber working electrodes experience much less fouling during determination of propofol in organic (acetonitrile) solution than during the determination of propofol in aqueous solution, PECs with carbon fiber working electrodes are a promising test-bed for the future development of voltammetric propofol detectors covered with an organic (PVC) membrane coating.

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Appendix

Item I. Inventory of Planar Electrochemical Cells

PEC Identity Code	WE Material	RE Construction Method	CE Construction Method	Size of Outer Glass Tube
js100727A	Bioanalytical Systems	tape	tape	OD: 4.6mm, ID: 2.5mm
js100727B	Bioanalytical Systems	tape	tape	OD: 4.6mm, ID: 2.5mm
js100727C	Bioanalytical Systems	tape	tape	OD: 4.6mm, ID: 2.5mm
js100727D	Bioanalytical Systems	tape	tape	OD: 4.6mm, ID: 2.5mm
js100727E	Bioanalytical Systems	tape	tape	OD: 4.6mm, ID: 2.5mm
js100719A	Goodfellow	capillary	tape	OD: 6mm, ID: 4mm
js100719B	Goodfellow	capillary	tape	OD: 6mm, ID: 4mm
js100719C	Goodfellow	capillary	tape	OD: 6mm, ID: 4mm
js100719D	Goodfellow	capillary	tape	OD: 6mm, ID: 4mm
js100719E	Goodfellow	capillary	tape	OD: 6mm, ID: 4mm
js100719F	Goodfellow	tape	tape	OD: 6mm, ID: 4mm
js100726A	Specialty Materials Inc	tape	tape	OD: 4.6mm, ID: 2.5mm
js100726B	Specialty Materials Inc	tape	tape	OD: 4.6mm, ID: 2.5mm
js100726C	Specialty Materials Inc	tape	tape	OD: 4.6mm, ID: 2.5mm
js100726D	Specialty Materials Inc	tape	tape	OD: 4.6mm, ID: 2.5mm
js100726E	Specialty Materials Inc	tape	tape	OD: 4.6mm, ID: 2.5mm
js100726F	Specialty Materials Inc	tape	tape	OD: 4.6mm, ID: 2.5mm