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Long-term Monitoring of Propofol and Fouling Effect on Pencil Graphite Electrodes

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Abstract: The evaluation of the efficacy of drugs' action is of crucial importance for preventing adverse effects due to over- or under-dosing. This is especially valuable in the case of propofol, a benchmark anesthetic, since its plasma concentration has to be continuously monitored to achieve and maintain a certain level of sedation during the surgery. To this end, long-term monitoring approaches have to be implemented. Electrochemical sensors have taken great interest for propofol monitoring. However, the problem of fouling of the propofol on the sensing electrodes, as due to compound-polymerization caused by successive measurements, makes almost impossible the

long-term drug monitoring of such a compound. For the first time by this study, we have shown that long-term monitoring of propofol can be achieved by electrochemical sensors based on *Pencil Graphite Electrode* (PGE) with a proper clay/graphite ratio. Our research has shown that a clay/graphite ratio of 0.4 prevents fouling and hence enables monitoring of propofol up to 4-hours. Surface analysis of various PGEs has been conducted via *Scanning Electron Microscopy* (SEM) and *Electrochemical Impedance Spectroscopy* (EIS) whereas detection of propofol has been investigated by *Cyclic Voltammetry* (CV).

Keywords: Propofol Long-Term Monitoring • Electrochemical detection • Electrode Fouling • Pencil Graphite Electrode

1 Introduction

One of the main challenges in intravenous general anesthesia practices is setting of a correct dosage regimen to achieve and maintain the desired level of sedation with minimal toxicity [1]. To that aim, sedative cocktail of drugs are infused in patient's veins to induce a status of amnesia, hypnosis, analgesia, and paralysis [2]. Among these drugs, the hypnotic agent is highly critical to be balanced since its action reduces the global cerebral metabolic rate and blood flow [3].

Propofol (2,6-diisopropylphenol) is the most widely used amnesic compound because of its favorable characteristics of rapid onset and fast recovery [4]. Its effective plasma concentration ranges between 0.25 and 10 $\mu\text{g/L}$ (1–60 μM).

Nowadays, *Target Controlled Infusion* (TCI) pumps have been adopted for the delivery of specific drugs and maintain their target concentration in the patient's plasma [5]. These pumps incorporate mathematical models for evaluation of the necessary drug dosage for inducing and up-keeping the desired level of sedation [6]. Despite the complexity of these mathematical models, they are not perfectly capable of reproducing the inter-patient variations in metabolism and drug tolerance [7]. This results in dosage errors, *i.e.* over- or under-dosing, that may in turn bring about the serious infusion syndrome [8] or the anesthesia awareness [9]. To overcome this limitation, there is a need for a *Therapeutic Drug Monitoring* (TDM) system able to measure the actual concentration in the patient's veins. By this way, the anesthesiologist can be informed about the change in concentration levels of a

drug thanks to a reliable feedback that allows him/her to adjust the infusion rate accordingly. As a result, a safer and more personalized anesthesia can be ensured.

To develop a TDM system for propofol detection, a reliable sensing platform is needed and on this end, electrochemical sensing has been shown to be suitable technique since the electro-active nature of propofol, which is a phenolic compound [10,11]. However, during its oxidation reaction, propofol produces free radicals that cause fouling on the sensor surface and decrease sensitivity in long-term [12,13]. Up to now, there are few studies on propofol electrochemical monitoring, and none of the studies achieved the long-term goal due to problems related to compound fouling.

In this work, we have then addressed the issue of fouling by investigating the performance of differently fabricated *Pencil Graphite Electrodes* (PGE)s as *Working Electrode* (WE). PGE is mainly composed of graphite, but it usually contains also a certain percentage of clay and wax. They are inexpensive, readily available, disposable,

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and has good conductivity [14,15]. These properties are linked with different composition of the PGE, *i. e.* the clay and the graphite content. For instance, the more the graphite the softer the PGE is [16].

The usage of PGE for electrochemical sensing dates back to 1997 [17] when Bond et al. studied the detection of cadmium and lead by stripping voltammetry. Throughout years, it has been demonstrated via various studies on detection of nucleic acids [18–20], drugs [21] and organic [22–24] and inorganic [25,26] pollutants that, PGE can be used in various surface modifications and had superior performance compared to commercially available electrodes due to ease of use, price and being disposable.

Another advantage of PGE is that detection of organic compounds including phenolic ones is possible and the surface is more resistant to fouling compared to other graphite-based electrodes. In the work of Vishnu et al. [27] it was reported that detection of phenols (meta-cresol and phenol) in pH 7 *Phosphate Buffer Saline* (PBS) for diabetes monitoring can be achieved via the usage of 6B grade pencil graphite, pre-anodized at 2 V vs. Ag/AgCl as a novel electrochemical sensor providing fouling-free surface. Even if this study shows the advantage of using characteristic fouling-free surface of PGE for phenolic compound monitoring, the work does not demonstrate its usage for long-term monitoring.

In another work, Ozcan et al. [28] presented a *Molecular Imprinted Polymer* (MIP) modified PGE for the *Differential Pulse Voltammetries* (DPV) detection of paracetamol, an analgesic manufactured by phenol compounds. However, due to difficulties in the MIP fabrication process, the reproducibility of the electrodes is still limited [29].

The importance of easy- and ready-to-use electrode with high stability in time is crucial in the long-term monitoring. This is especially true for those medical treatments where difficult-to-dose-drugs are administered over a specific time-period, *e. g.* anesthesia practice. In the majority of the state-of-the-art works, the PGE is adopted only after surface modification/pretreatment while the use of unmodified pencils is uncommon [30]. Up to now, only few studies have been reported on the electrochemical characterization with potassium ferro-cyanide for different PGE compositions [31–33]. However, the performances of PGE have been never tried for the detection of the propofol, neither related to their anti-fouling character.

Therefore, main objective of our study is to investigate the different PGE compositions, in particular for the prevention of fouling effect, in order to provide the best propofol sensing performance in terms of sensitivity and course of detection time. To this aim, a variety of PGEs with different hardness from 9B (softest) to 9H (hardest), twenty different PGEs in total, have been tested for long-term monitoring (up to 4 hours) of propofol. The best performance was achieved according to two factors: (i) the sensitivity tested via electrochemical analysis and (ii) the fouling visualized via *Scanning Electrode Microscopy*

(SEM) imaging and EIS measurements. Each PGE sensor was tested over 25 voltammetric measurements, corresponding to more than 4 hours of monitoring. Therefore, for the first time, this study shows that long-term monitoring of propofol without any need for primary surface modification can be achieved by choosing the right clay/graphite ratio, determined as 0.4 for this study, in PGE composition where the graphite content is responsible for electrochemical activity while clay is responsible for shielding the surface against fouling.

2 Materials and Methods

2,6-Diisopropylphenol (propofol) was purchased from TCI chemicals and dissolved in 0.1 M NaOH to prepare the stock solution of 5.4 mM. Subsequent dilutions of propofol stock solution were done in PBS (10 mM, pH: 7.4) to obtain concentrations of: [9.9–19.6–38.5–56.6–80.5] μ M.

The 3-electrodes electrochemical cell consisted of a PGE as WE, a K0265 Ag/AgCl electrode from Ametek Scientific Instruments as *Reference Electrode* (RE) and a Pt wire as *Counter Electrode* (CE). The PGEs are made up of a composite material containing graphite, clay and a binder (typically wax, resins or high polymer) in various percentages. A Staedtler Mars Lumograph Wood Pencil Set of 20 was purchased by Cult Pens (United Kingdom) and used as WEs with different graphite-clay compositions, with wax as binder. The electrode was made by peeling the two ends of the pencil in order to expose the internal mine. One pencil extremity has been used to provide electrical contact with the potentiostat. The other has been cut so as to guarantee a flat surface before being immersed in the analyzed solution. According to the *European Letter Scale*, graphite pencils are marked with letters *H* (hardness) and *B* (blackness), as well as numbers indicating the degree of hardness or blackness, thus creating a scale spanning from 9H (the hardest) to 8B (the softest), as summarized in Figure 1. *B* type leads contain more graphite and are softer, whereas the harder *H* type leads have more clay.

	9H	8H	7H	6H	5H	4H	3H	2H	H	F	HB	B	2B	3B	4B	5B	6B	7B	8B	9B
	Hard ←										→ Soft									
Graphite (%)	41	44	47	50	52	55	58	60	63	66	68	71	74	76	79	82	84	87	90	93
Clay (%)	53	50	47	45	42	39	36	34	31	28	26	23	20	18	15	12	10	7	5	2
Wax (%)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Fig. 1. The compositions of PGEs in terms of wt% of: wax, clay and graphite used in this study.

This has an influence on the chemical (*e. g.*, ion exchange) and structural properties (*e. g.*, degree of disorder and surface morphology) of the pencil graphite leads. Depending on the PGE composition, the diameter varies; therefore, constant electro-active area ($\sim 12.6 \text{ mm}^2$)

was maintained by changing the immersed height of the PGE inside the solution. Carbon (C) *Screen Printed Electrode* (SPE) from Metrohm has been used to compare the PGE performances. Measurements were performed with a Metrohm Auto-lab system (PGSTAT 302N) in conjunction with Nova 1.11 software. Data analysis and plotting were carried out in Matlab R2013a. *Cyclic Voltammetry* (CV) measurements with applied potentials in the range [+0.2, +1.4] V and scan-rate of 0.1 V/s were carried out. In particular, two main tests were evaluated to compare WE performances:

- five subsequent calibrations with the same WE to test *intra*-electrode variability with time, and fouling resistance, considered as the ability to remain unaffected by fouling agents that form gradually increasing impermeable layer on the electrode surface and therefore inhibiting the direct contact of the surface with the analyte of interest for electron transfer [34];
- three calibrations with three different WEs with the same composition to verify *interelectrode* variability.

The propofol oxidation current peak was identified as the maximum current value recorded in the voltage range [+0.7, +0.8] V. From these analysis, we have evaluated the sensor sensitivity was evaluated as the slope of the calibration line.

EIS analysis was carried out in 5 mM ferro/ferricyanide solution ($Fe(CN)_6^{3-4-}$) by using the Autolab *Frequency Response Analyzer* (FRA), with a frequency range of 0.1 Hz to 100 KHz and AC amplitude of 5 mV. The measured impedance spectra were analyzed in Nova 1.11 software with the *Electrochemical circle fit* tool.

Surface images were taken with Xlf-30 and Zeiss Gemini SEM microscopes, with accelerating voltages of 10 KV and 3 KV, respectively.

3 Results and Discussion

3.1 Electrochemical Analysis

We have compared the performances of 20 different lead compositions and a C-SPE in terms of sensitivity and fouling resistance, evaluated as the ability to perform long-term measurements. As a phenolic compound, propofol oxidation releases free radicals that, after a polymerization process, cause the undesirable phenomenon of electrode fouling, which reduces the electrode sensitivity over time [12].

To investigate the *inter*-electrode variation, three measurements of 5-points CV propofol calibration have been done for twenty types of PGE and C-SPE. From this investigation, first observed result was that it was impossible to record any data for PGEs 7B, 8B, 9B since these PGEs are not resistive enough to fouling, instead, had immediate fouling and hence no measurements could be done. Typical CV calibration curves are reported in Figure 2 for the three PGE lead compositions 9H (hardest), F (intermediate) and 6B (softest), as well as for the intermediate one which is 3H PGE. It can be noticed

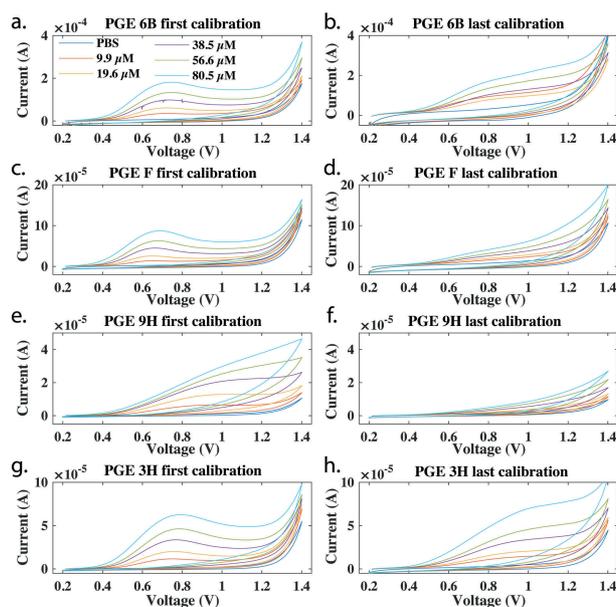


Fig. 2. 1st and 5th CV calibration curves respectively for at various PGE compositions : a.) and b.) 6B, c.) and d.) F, e.) and f.) 9H, g.) and h.) 3H.

by Figure 2.a and in Figure 2.c that the peak height of propofol oxidation current decreases by half the magnitude due to increase in clay content, that has an insulation effect on the PGE lead.

At the same time, the oxidation peak becomes more well-defined at higher graphite contents. Furthermore, we can notice that all 9H, F and 6B PGEs lost their sensitivity over time (Figure 2.b, Figure 2.d and Figure 2.f), whereas as an intermediate lead composition, 3H PGE provided good results to be considered a suitable compromise between sensitivity and fouling resistance. Indeed, even if the shape of the oxidative peak varies during successive calibrations due to modification of the capacitive current after hours immersed in the solution, the peak amplitude, which correlates to the drug concentration, does not change significantly, as shown in Figure 2.g and Figure 2.h.

Figure 3.a summarizes the calculated sensitivity values for all PGE compositions as well as C-SPE. There is an obvious increasing trend in sensitivity for higher content (wt%) of graphite in the lead composition. Despite this, C-SPE seemed to have the lowest sensitivity. Looking at the sensitivity values, it is possible to determine the range where the $S \pm \sigma$ is approximately constant, namely the range between 3B to 5H PGE leads. In addition, the high standard deviation for 5B and 6B PGEs can be justified with their soft composition. Indeed, high graphite content makes PGE very soft and fragile so that it is almost impossible to obtain the same electro-active surface area for repetitive measurements. Therefore, a higher standard deviation from these lead compositions was recorded.

Moreover, the plot where the relationship between electrode sensitivity versus percentage of clay or graphite content can be seen, Figure 3it can be deduced that .b, the

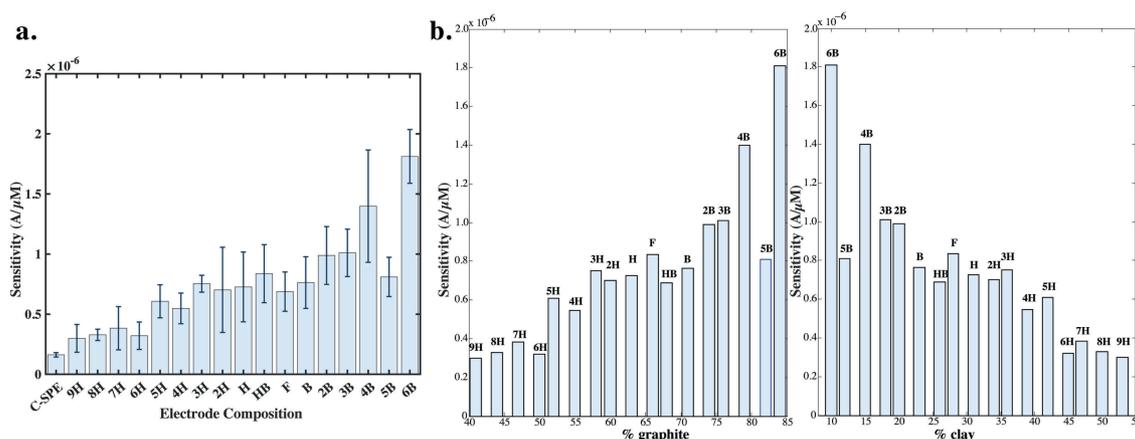


Fig. 3. Different representation of electrode sensitivity. a) Diagram of the *inter*-electrode sensitivity for different PGE compositions and C-SPE. b) Comparison in sensitivity for different wt% of graphite and clay in lead compositions.

sensitivity increases with higher wt% of graphite on the contrary, decreases with higher wt% of clay due to conductive and insulating nature of those materials respectively.

Therefore, while the graphite-content of the electrode is key for the electrochemical activity, the contained clay could provide a sort of *protective shield* against fouling.

In order to support these results, we have evaluated the fouling-resistant properties of the electrodes by performing five subsequent calibrations ($n=5$) with the same WE. Figure 4 shows the change in sensitivity upon five calibrations for various PGE leads and C-SPE.

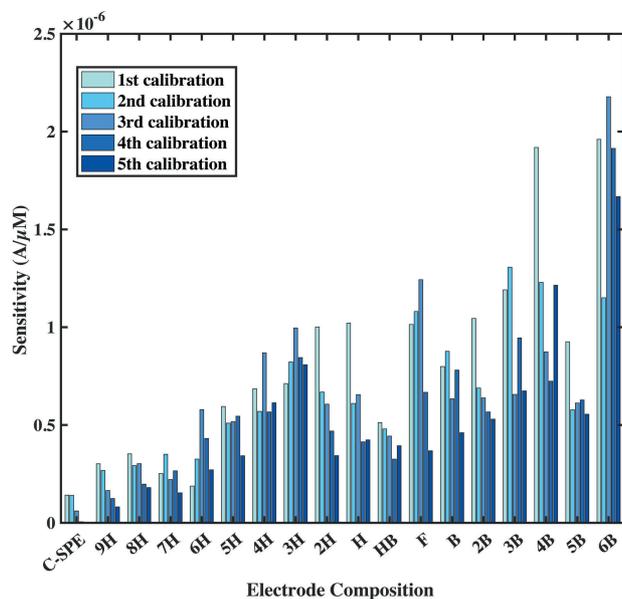


Fig. 4. Shows the *intra*-electrode sensitivities obtained by five subsequent propofol calibrations performed on each PGE. Trend for all the different lead compositions are reported.

First thing to draw attention from Figure 4 is that C-SPE electrode undergoes a strong fouling effect, which prevents to perform subsequent calibrations after the third one. This phenomenon is less pronounced in other PGEs that have a decreased sensitivity over time but still they could provide a signal. When the graphite (wt%) is reduced and the clay (wt%) is increased in PGE lead, it is possible to identify a *switching-point* in clay-to-graphite ratio for which the fouling effect is less evident in the electrochemical analyses. As already mentioned, the graphite content facilitates the electrochemical activity, while the clay content provides a protection against fouling. Therefore, it can be said that, with a certain clay content, pre-treatment or regeneration of electrodes are not needed due to the fact that fouling does not occur, and thanks to this property these PGEs can be used for electrochemical detection of phenolic compounds, such as propofol. The identified optimum point of clay/graphite ratio is set for a value of 0.4, which corresponds to the lead composition having 66% w/w in graphite and 28% w/w in clay (assuming a fixed amount of wax at around 5% w/w). In other words, for the reliable long-monitoring of propofol, clay/graphite ratio should be above 0.4 and can go until 1.5 as in the case of the range of PGE leads *F* to *9H*.

Two main analysis have been conducted to identify the optimum PGE lead composition for the selected region of *F-9H* PGEs by looking at the prevention of fouling effect and the stability properties of the electrodes. First, we drew the bar graph with the normalized standard deviations from the *inter*-electrode sensitivity analysis (Figure 3.a) with respect to sensitivity (Standard deviation/S), as shown in Figure 5.a. Second, the bar graph was drawn with the normalized fouling standard deviation with respect to intra-electrode sensitivity (Fouling standard deviation/intra-electrode S) based on the analysis of successive calibration curves and Figure 4. Both figures, Figure 5.a and Figure 5.b show the trends of PGEs for resistance to fouling affect and stability. It can be deduced

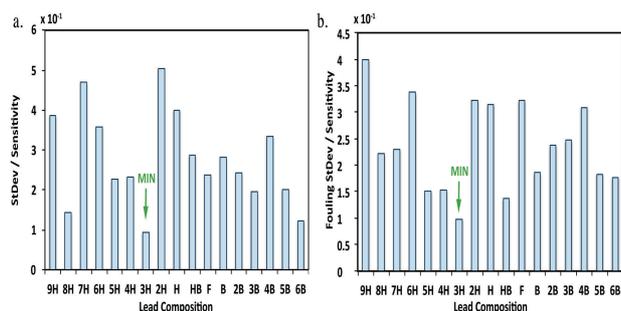


Fig. 5. Optimization study for determination of best PGE composition with respect to: a.) ratio Standard Deviation (StDev)/Sensitivity (S) in terms of high S and small *inter*-electrode variation; b.) variability in S for subsequent propofol calibrations on the same electrode respect to the average S of the electrode itself (Fouling StDev/Sensitivity).

from these figures that, $3H$ PGE composition (58% graphite, 36% clay and 5% wax, with clay-graphite ratio of 0.62, calculated as 36%/58%, and pointed by the green arrow in the figures) shows the best performance by providing the minimum normalized standard deviation values.

The best performance of $3H$ PGE in terms of stability and current amplitudes is also evident by Figure 6 where different PGEs and C-SPE were compared for long-term propofol monitoring.

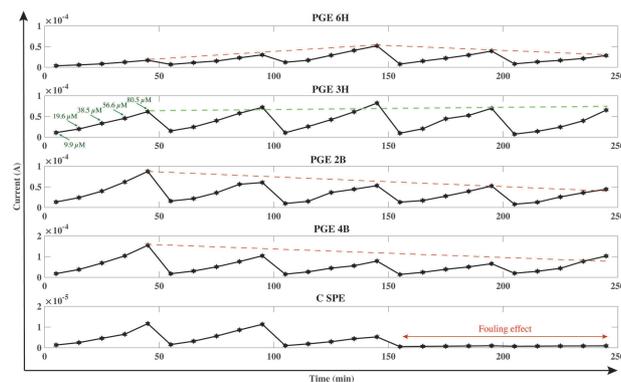


Fig. 6. Continuous monitoring experiments with four different kinds of PGE ($6H$, $3H$, $2B$ and $4B$). Five calibrations (with five increasing propofol concentrations each) were carried out resulting in 25 subsequent measurements, for a total of 4 hours, without any intermediate cleaning.

It is seen that C-SPE was affected by fouling so much that, after the third calibration (*Fouling effect zone* in the figure), no further detection could be done. Moreover, despite its higher current amplitude values, $4B$ PGE lead had a strong fouling that prevents long-term monitoring. Even if $2B$ PGE seemed to have a better performance than $4B$ PGE, it also had fouling. Although $6H$ PGE didn't show fouling since it has higher clay content, it has the lowest current values and less reproducibility. There-

fore, $3H$ composition results to be the best trade-off between sensitivity and fouling resistance properties. Moreover, the *Limit Of Detection* (LOD) of the optimum PGE, calculated as 3 times the standard deviation of the blank measurement ($StDev_{blk}$) over the sensitivity (S) ($3 \cdot StDev_{blk}/S$), was evaluated to be $7.79 \cdot 10^{-07} \mu\text{M}$, which is lower than lowest concentration in the physiological range of propofol (1–60 μM).

We have then performed an electrochemical characterization of 20 PGE lead compositions and a C-SPE electrode for propofol sensing in terms of high sensitivity and stability over long-term monitoring. According to sensitivity values, the lead range between $3B$ and $5H$ has been found to be a good compromise between immediate fouling effect due to high % of graphite in the composition and low sensitivity due to high % of clay. Among these compositions, $3H$ has been selected as optimum one due to its high stability in time, measured current amplitude and fouling resistance properties.

3.2 Electrode Surface Characterization

After having characterized the different lead compositions through electrochemical analysis, further investigation was done with SEM imaging and EIS to compare electrodes' surfaces before and after propofol monitoring. SEM images were taken for all types of electrodes (not shown), and we report herein the ones of PGE leads from F to $3H$. F PGE has been identified as the critical point for fouling and sensitivity, while $3H$ as optimum lead composition for propofol monitoring. Figure 7 compares the images of the clean PGE surface before performing propofol sensing (*Not-used PGE*) with the used one after the measurement (*Used PGE (after calibration)*). From Figure 7, it can be seen that there is a fouling layer on the surface of HB and F PGE leads after their usage. In addition to that, this layer seems to be reduced by increased clay content. Indeed, lead compositions of H , $2H$ and $3H$ does not present evidence of this undesirable effect. Moreover, since the hardness of the PGE lead increases, it becomes easier to cut the PGE so that a flatter and more homogeneous surface can be created.

As shown in Figure 8, the effect of fouling is even more evident in the case of C-SPE. Indeed, the image of the used electrode is characterized by a strong charging effect due to the presence of a polymerized film on the surface.

We further analyzed the electrodes surface by performing EIS. We have compared the charge-transfer resistance (R_{ct}) values of $3H$ and $3B$ PGE compositions before and after propofol monitoring. We have chosen these two PGE leads since $3H$ has been identified as the optimum composition and $3B$ is its complementar with higher graphite content. As it can be seen from Figure 9, the R_{ct} value of not-used $3B$ PGE is lower than the $3H$ one since it contains less clay. It is also evident that, propofol sensing resulted in fouling and hence increased R_{ct} value for $3B$, however, surface seems almost not affected for

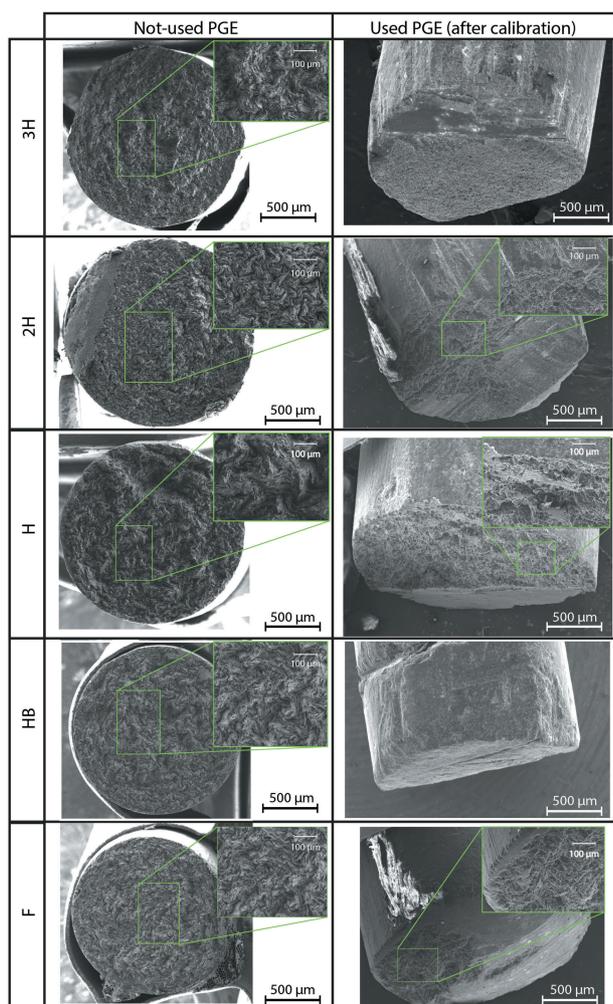


Fig. 7. SEM images of 3H, 2H, H, HB, F PGE lead compositions before and after 5-points calibration measurement.

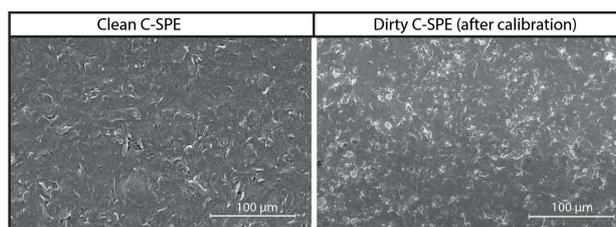


Fig. 8. SEM images for C-SPE before and after 5-points calibration measurement.

3H. Bar graph in Figure 9.c shows normalized changes in R_{ct} values (% , evaluated by the formula: $\frac{R_{ct,used} - R_{ct,notused}}{R_{ct,notused}} \cdot 100$) which is significantly higher for 3B PGE compared to 3H.

4 Conclusions

In this work, we have investigated twenty different PGE compositions to provide the best propofol sensing performance in terms of fouling-resistance to achieve long-

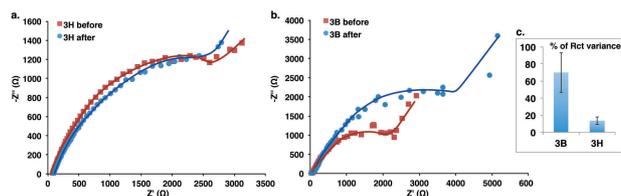


Fig. 9. Electrochemical impedance spectroscopy analysis to compare used and not-used PGEs: Nyquist plots of a) 3H and b) 3B, c) bar graph with error bars representing standard error ($n=3$) for R_{ct} variance (%).

term propofol monitoring. C-SPE has been considered as a reference to compare the performances with respect to PGE, as they are more widely used in many electrochemical platforms and also belong to the carbon-based electrode family so as PGE. Propofol has been used as a benchmark drug since it is widely used in anesthesia practices where longterm monitoring is required, while it usually presents great monitoring challenges in terms of electrochemical sensing since it is a phenolic compound that causes the undesirable fouling effect on the carbon-based sensing electrodes. The evaluation of the best PGE in this context was done by considering both the electrode sensitivity and the fouling resistance as characterized by electrochemical analysis, EIS measurements and SEM imaging. By electrochemical analysis, it has been noted that the higher is the graphite content (wt%), the higher the sensitivity, whereas the higher the clay content (wt%), the smaller the fouling effect. This is also supported by EIS results where higher charge-transfer resistance values recorded for PGEs that have higher clay content. A significant value for clay-to-graphite ratio was identified as 0.4, for which the fouling effect is not significant in time-series of measurements. Indeed, this corresponds to the best compromise between (i) the graphite-content of the electrode that supports the electrochemical detection by electron-transfer, and (ii) the clay-content that provides a protective shield against fouling effect. The identified best value of clay-to-graphite ratio is then set for a value that corresponds to the F lead composition, having about 66% w/w in graphite content and about 28% w/w in clay content (assuming a fixed amount of wax around 5% w/w). Surface characterization of the electrodes by SEM imaging and by EIS confirmed the negligible fouling of this kind of composition. Therefore, we conclude that long-term monitoring of propofol is possible if a PGE having a suitable range of the clay-to-graphite ratio (from 0.4 to 1.5 – from F to 9H) is chosen, without any need for primary surface modification such as membranes deposition. Within this range of ratios, the 3H lead (58% graphite, 36% clay and 5% wax, with clay-graphite ratio of 0.62) is identified by our results as the optimum material in terms of prevention of the fouling effect, electrode stability and detection sensitivity, for measurements of propofol over a period of time of 4 hours-long of continuous monitoring.

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