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Single Drop Electroanalysis and Interfacial Interactions: Sensitivity versus Limit of Detection†

Charuksha Walgama^[a], Matthew Gallman^[a], and Sadagopan Krishnan^{*,[a]}

^[a] C. Walgama, M. Gallman, S. Krishnan, Department of Chemistry, Oklahoma State University, Stillwater, OK, USA 74078

Abstract

We report single drop electroanalytical measurements of pharmaceutically and biologically relevant compounds using screen printed electrodes (SPEs) modified with carboxylated multiwalled carbon nanotubes (MWCNT-COOH) as the sensor surface. Acetaminophen, nicotine, ascorbic acid, and nicotinamide adenine dinucleotide reduced form (NADH) were detected in a single drop of solution. We show that combined polar and nonpolar interactions of analytes with –COOH functional groups and large surface area of MWCNT, respectively, allow highly sensitive analyte detection with wide dynamic range. Smaller analytes can bind to a significantly greater number of sensor sites than the bulkier analytes and offer better detection sensitivity. Results suggest that sensitivity is controlled by predominant nonpolar interactions that an analyte can undergo with the MWCNT-COOH SPE sensor surface, whereas limit of detection is controlled by the extent of polar interactions between an analyte and the sensor surface, facilitating interfacial charge transport and an electrochemical signal output. Furthermore, a combination of polar and nonpolar analyte interactions with the sensor surface shows a synergistic effect on sensitivity and detection limit. This could be a likely reason for why sensitivity does not need to always correlate with lower detection limits as variations in the interfacial interactions are critical. Application of the designed single drop method to real samples was validated by estimating the amounts of acetaminophen, nicotine, ascorbic acid, and NADH in commercially available pharmaceuticals with excellent recovery.

Graphical abstract

* gopan.krishnan@okstate.edu.

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Keywords

Single drop analysis; Interfacial interactions; Sensitivity; Detection limit; Sensor surface; Real samples

1 Introduction

Chemical reactions in bulk solutions have been studied extensively, and researchers have started exploring reactions using ultra-small volumes due to advantages such as faster reaction kinetics, enhanced analytical sensitivity, and small sample requirements [1–3]. Superior biochemical characteristics of droplets are due to enhanced mixing and mass transfer and the ability to function as small reactors [4, 5]. Investigation of such properties via quantitative and qualitative analysis of the contents of droplets is only possible with sophisticated analytical techniques, including molecular spectroscopy, mass spectrometry, electrophoresis, and electrochemistry [6].

Among the various analytical techniques available, electrochemical detection of analyte droplets is favored due to its simplicity, cost-effectiveness, and the ability to modify the electrode surface to obtain good sensitivity [7, 8]. Interfacial electrochemistry of droplets placed on electrodes and fundamental insights based on mass and ion transfer mechanisms have been reported [9]. Recently, Oliveira *et al.* showed that a single drop electrochemical system consisting of two liquid phases of water and oil can diminish charge transfer resistance in the detection of 4-nitrophenol in oil [10]. Using a single drop of redox active hexachloroiridate, changes in the heterogeneous electron transfer rates with respect to number of layers of graphene and microscopic defects on the graphene surface (edge/basal planes) were electrochemically investigated [11].

Heavy metals [12], polycyclic aromatic hydrocarbons [13], microorganisms, radioactive agents, and many other environmental pollutants [14] can be monitored via single drop electrochemical analysis. Blood glucose analysis is a remarkable point-of-care biomedical application of single drop electrochemical biosensors [15–17]. Chen *et al.* developed a disposable screen printed electrode (SPE) approach for detection of uric acid in droplets of whole blood [18]. Thus, biologically and chemically relevant analytes have been detected in ultra-micro volumes.

In this study, we performed electroanalytical measurements of four compounds in single droplets on carboxylated multiwalled carbon nanotubes (MWCNT-COOH) modified SPEs and detected their presence and concentration using cyclic voltammetry. Acetaminophen is a pain reliever and fever reducing drug; NADH is a coenzyme and a dietary supplement ; ascorbic acid is an antioxidant and also used as a dietary supplement ; and nicotine is a tobacco smoke carcinogen and present in antismoking pharmaceuticals. The focus of this study was to evaluate the detection sensitivity and limit of detection (LOD) of single drop assays relative to molecular sizes of analytes, and polar and nonpolar interfacial interactions of analytes with the MWCNT-COOH surface. To our knowledge, this study is the first to describe single drop electroanalysis for the selected compounds with insights on interfacial

interactions controlling sensitivity and LOD. Detailed electron transfer, mass transport, and electrochemical kinetics were investigated in a separate study.

Results of this study convey the importance of electroanalytical methods in measuring low levels of analytes and the significance of droplet-based biosensors utilizing desired chemically or biologically functionalized SPEs. We chose SPEs because of their user-friendly design, miniature size, portability, low cost, and disposability and because they lack interference arising from surface contamination and cleaning protocols between experiments [19, 20]. Scheme 1A illustrates the schematic of SPEs for single drop analysis of analytes based on electrochemical methods. Scheme 1B shows the structures of measured analytes in this study as single drops.

2 Experimental

2.1 Materials

SPEs (110 CNT, dimensions: length 33 mm; width 10 mm; height 0.5 mm) were purchased from Dropsens Ltd. (Llanera (Asturias) Spain). The SPE electrode geometric area was 0.16 cm², and it was modified with MWCNT-COOH. The counter electrode was made of carbon and the pseudo-reference electrode was made up of silver. L-Nicotine was purchased from ACROS Organics and all other analytical grade chemicals, including acetaminophen, L-ascorbic acid, and NADH (β -Nicotinamide adenine dinucleotide, reduced disodium salt hydrate), were purchased from Sigma. Acetaminophen, ascorbic acid, and NADH tablets, and nicotine gum were purchased from a local pharmacy.

2.2 Cyclic Voltammetric Measurements

A CH instrument 6017E electrochemical analyzer was used for cyclic voltammetry experiments under ambient atmosphere. Different concentrations of the four analytes prepared in pH 7.0 phosphate buffer were placed as 50 μ L droplets on SPEs, and voltammograms were obtained.

2.3 Sample Preparation

Acetaminophen (2 of 500 mg), ascorbic acid (1 of 1000 mg), and NADH (8 of 5 mg) tablets were finely powdered and transferred separately into 250 mL conical flasks containing 100 mL of pH 7.0 phosphate buffer. Then the contents of flasks were sonicated for 30 min and the mixtures were filtrated through Whatmann filter papers to remove insoluble materials. Filtrates were used for voltammetric analysis. The nicotine contained chewing gums (2 of 4 mg) were cut and dissolved in 20 mL of hexane. The mixture was then filtered and extracted with 100 mL pH 7.0 phosphate buffer. The buffer extract was used for voltammetric analysis [21]. Appropriate sample dilutions were made to fit in the voltammetric calibration plots and estimate the amounts in the real samples.

3 Results and Discussion

3.1 Electrochemical Behavior of Analytes

The analytes were placed as a single drop (50 μL volume) on separate MWCNT-COOH SPEs, and electrochemical signals were measured by cyclic voltammetry. Figure 1 shows the background cyclic voltammograms (CVs) for buffer droplets on the SPE electrodes (dotted lines) and that of 100 μM analyte droplets (bold lines). A small sharp spike (0–0.1 V range) was observed for bare SPEs in buffer droplets, but the spike did not interfere with the identification of analyte peaks.

Figure 2 represents the CVs of analyte droplets with increasing scan rates (at 1 mM analyte concentration). A linear relationship between oxidation peak currents and square root of scan rate was observed for all four analytes (insets in Figure 2). This indicates the diffusion-controlled electrochemical process of analytes in droplets of 50 μL volume [22]. The reported two electron oxidation reactions of acetaminophen, nicotine, ascorbic acid, and NADH are given in the Supporting Information (Figure S1).

Figure 2 also illustrates the differences in the CV peak shapes of each analyte. For example, acetaminophen CVs suggested a quasi-reversible process (significant peak separation between oxidation and reduction peaks with increase in scan rate), and nicotine, ascorbic acid and NADH displayed the feature of almost irreversible redox process. These variations in peak shapes are indicative of differences in the kinetics of direct electron transfer and mass transport of analyte molecules at the interface of the droplet and MWCNT-COOH SPE [22].

Figures 3A–D show the CVs for various concentrations of acetaminophen, nicotine, ascorbic acid, and NADH [(i) is for lower concentration range and (ii) is for higher concentration range of analyte droplets].

Figure 4A shows the increase in oxidation peak currents with increasing concentration of each analyte. We estimated the sensitivity of the method from the slopes of this plot. The LOD of each analyte was calculated to be $3 S_b/m$, where S_b is the standard deviation of the blank signal and m is the slope of the calibration plot (Table 1) [23, 24]. The observed wide dynamic range of detection (from the LOD to 1 mM) is comparable to or better than that of many reported carbon-based SPE sensors for similar analytes (Table S1) [7, 25–29]. Additionally, non SPE – based reported electrochemical sensors are also summarized in Table S1.

The oxidation peak potential of each analyte shifted to more positive values with increasing concentration (Figure 4B). The peak shifts were more prominent for ascorbic acid and NADH compared to nictotine acetaminophen. This observation suggests greater overpotential requirements at higher concentrations of relatively polar compounds due to possible charge repulsions with the surface (related to energetics). Once the required overpotential barrier is overcome, analytical detection features from interfacial charge transfer can be achieved (i.e., current signals) [30].

The differences in the observed LODs and sensitivity among the four analytes can be explained by the nature of interfacial secondary interactions of analytes with the MWCNT-COOH-modified SPE surface. A MWCNT is made of concentric cylindrical layers of graphene. MWCNT-COOH contains both polar –COOH groups and nonpolar sp^2 carbons of MWCNT. The sidewall basal planes of MWCNT can undergo pi-pi stacking as well as nonpolar interactions with pi-bonds and nonpolar groups of analytes [31]. The edge planes and defective sites of MWCNT are mostly polar. The –COOH groups of MWCNT offer electrostatic and hydrogen bond interactions with the polar groups of analytes.

Our data show that higher sensitivity is not always correlated with lower LOD in electroanalysis (Table 1). In this study, the decreasing order of detection sensitivity was acetaminophen > nicotine > ascorbic acid > NADH (Figure 4A). The highest sensitivity observed for acetaminophen can be explained by its combined pi-pi and nonpolar interactions with MWCNT along with hydrogen bonding and electrostatic interactions with surface –COOH groups (via –OH and NH groups) (Scheme 2). Ascorbic acid is relatively more polar than acetaminophen and thus can interact predominantly via dipole-dipole and hydrogen bonding interactions with the MWCNT-COOH surface. This major polar interaction (i.e., less nonpolar) might be the reason for this analyte's lower sensitivity compared to acetaminophen. Similarly, although NADH is polar, the relatively larger size of this compound compared to ascorbic acid can cause sterically hindered interaction and thus explain its low detection sensitivity on the MWCNT-COOH surface.

Nicotine is the least polar of the four compounds and it has predominantly nonpolar (e.g., dispersion, pi-pi) interactions, which further supports the influence of nonpolar interactions in providing better sensitivity than polar ascorbic acid and NADH molecules.

The following trend of increasing magnitudes of LODs was observed: acetaminophen < ascorbic acid < NADH < nicotine. This observation suggests that polar compounds (acetaminophen, ascorbic acid, and NADH) favor better charge transport at the interface of an analyte droplet and the MWCNT-COOH SPE surface than moderately polar/nonpolar compounds (e.g., nicotine). The lowest LOD of acetaminophen is attributed to the synergistic combination of polar and nonpolar interactions with the MWCNT surface and –COOH functional groups, as described above.

Taken together, our results suggest that detection sensitivity of a redox analyte is controlled by the extent of predominant nonpolar interactions of the analyte with the MWCNT-COOH SPE sensor surface. However, LOD is controlled by the extent of charge transfer occurring at the interface of analyte molecules and the electrode and is favored by polar interfacial interactions. Thus, higher sensitivity does not always have to correlate with lower detection limits in an electroanalytical method.

3.2 Application to Commercial Pharmaceuticals

The practical application of the SPEs for single drop electroanalysis of acetaminophen, nicotine, ascorbic acid, and NADH was validated using real pharmaceutical samples. Sample preparation is described under Experimental. The results are summarized in Table 2. Excellent recovery percentages (> 97 %) were obtained in the estimation of acetaminophen,

ascorbic acid, and NADH in commercially available tablets. The relatively lower percentage recovery of nicotine in chewing gum (~ 92 %) is attributed to the extraction efficiency of the procedure followed rather than the detection aspect.

4 Conclusions

In summary, we described single drop analysis of four pharmaceutically and biologically relevant compounds using MWCNT-COOH-modified SPEs. The favorable large surface area and multiple interactions offered by the electrode surface provided highly sensitive detection of analytes with a wide dynamic range, and interfacial charge transport facilitated by polar interactions offered lower detection limits. This droplet electroanalytical method and our fundamental insights regarding the molecular interactions with the sensor surface should allow design of sensors with high sensitivity and low detection limits via appropriate control of surface properties to induce desired interfacial interactions based on analyte structures confined in a droplet. Successful demonstration on real samples suggests negligible interferences from the matrix and applicability of the method for pharmaceutical quality assurance (QA) purposes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

CVs of 100 μM droplets (50 μL volume) of (A) acetaminophen, (B) nicotine, (C) ascorbic acid, and (D) NADH placed on MWCNT-COOH SPEs at 25 $^{\circ}\text{C}$, scan rate 25 mV s^{-1} (solid lines). Dashed lines are the CVs of a buffer drop with no added analyte.

Fig. 2.

Scan rate dependent CVs of 1 mM droplets (50 μL volume) of (A) acetaminophen, (B) nicotine, (C) ascorbic acid, and (D) NADH placed on MWCNT-COOH SPEs. (scan rates inner to outer : 5 to 1000 mV s⁻¹). Insets show the plots of peak current vs. square root of scan rate.

Fig. 3. CVs of 50 μL droplets of varying concentrations of analytes placed on MWCNT-COOH SPEs at 25 $^{\circ}\text{C}$, scan rate 25 mV s^{-1} : (A) acetaminophen, (B) nicotine (inset shows the enlarged view of voltammograms), (C) ascorbic acid, and (D) NADH: (i) CVs at lower concentration range and (ii) CVs at higher concentration range.

Fig. 4.

(A) Oxidation peak currents versus concentration of a single drop of analyte placed on MWCNT-COOH SPEs at 25 °C and measured by cyclic voltammetry at a scan rate of 25 mV s^{-1} . (B) Effect of varying concentration of analytes on the oxidation peak potential.

Scheme 1.

(A) Schematic representation of single droplet analysis using SPE made of a carbon counter electrode (CE), an MWCNT-COOH coated working electrode (WE), and a silver pseudo-reference electrode (RE). (B) a–d. Chemical structures of measured analytes as single drops. a. acetaminophen, b. nicotine, c. ascorbic acid, and d. NADH.

Scheme 2.

Possible interfacial interactions of analytes with the MWCNT surface and -COOH groups; these interactions may explain the observed variations in sensitivity and LODs of the measured analytes.

Table 1

Electroanalytical measurements of single drops of acetaminophen, nicotine, ascorbic acid, and NADH.

Analyte	Oxidation Peak Potential/mV (at 100 μ M)	LOD (μ M)	Sensitivity (nA μ M ⁻¹)
Acetaminophen	327 $\pm$ 7	0.13	36
Nicotine	1006 $\pm$ 4	4.8	25
Ascorbic acid	38 $\pm$ 3	0.83	18

Table 2

Determination of acetaminophen, nicotine, ascorbic acid, and NADH in commercially available pharmaceuticals.

Sample	Label amount (mg)	Determined (mg)	Recovery (%)
Acetaminophen tablet	500	489±10	97.8
Nicotine chewing gum	4	3.7±0.1	92.2
Ascorbic acid tablet	1000	985±20	98.5
NADH tablet	5	5.0±0.2	99.4