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Amperometric sensing of norepinephrine at picomolar concentrations using screen printed, high surface area mesoporous carbon

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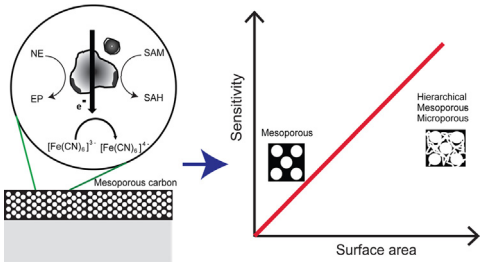
HIGHLIGHTS

- Screen printed norepinephrine sensors are prepared using high surface area carbon inks.
- The impact of surface area and pore size of the carbons to the sensitivity of the sensors has been investigated.
- Increase in surface area and pore size improves the sensitivity of the sensor.
- The detection limit of norepinephrine in whole rabbit blood is as low as 100 pg mL^{-1}.

GRAPHICAL ABSTRACT



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ABSTRACT

Norepinephrine (NE) is detected amperometrically using the enzyme Phenylethanolamine N-methyl transferase and cofactor S-(5′-Adenosyl)-L-methionine chloride dihydrochloride with disposable screen printed mesoporous carbon electrodes. The role of internal surface area and pore size of the mesoporous carbon is systematically examined using soft-templated, mesoporous silica–carbon powders with highly microporous walls obtained from etching of the silica to produce powders with surface areas ranging from 671–2339 m^2 g^{-1}. As the surface area increases, the sensitivity of the biosensor at very low NE concentrations (0–500 pg mL^{-1}) in phosphate buffered saline (PBS) increases just as the current signal increases with respect to the NE concentration of 81–1581 μA mL ng^{-1} cm^{-2} for the mesoporous carbons. The best performing electrode provides similar sensitivity in whole rabbit blood in comparison to PBS despite no membrane layer to filter the non-desired reactants; the small (<5 nm) pore size and large internal surface area acts to minimize non-specific events that decrease sensitivity.

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1. Introduction

Neurotransmitters play a crucial role of endogenous primary chemical messengers to transport information among biological cells in mammalian central nervous system and are indicative of health and disease [1]. One important catecholamine neurotransmitter is norepinephrine (NE), which is associated with stress, blood pressure regulation, heart rate, immune responses and glycogen metabolism [2,3]. Abnormal NE concentrations in blood can be indicators of diseases such as thyroid hormone deficiency, congestive heart failure, arrhythmias and idiopathic postural hypotension [4,5]. Thus, facile detection of NE could provide for advances in clinical diagnosis, study of disease pathologies, discovery of new drugs and rapid point-of-care diagnosis of stress and trauma [2,6].

Given the potential impact associated with understanding NE concentration *in vivo*, there has been significant interest in developing techniques that provide fast and accurate measures of NE concentration as existing technologies (peroxidase-based spectrophotometric method [7], high performance liquid chromatography technique [8], stable-isotope dilution gas chromatography-tandem mass spectrometry [9] and capillary electrophoresis [10]) are limited in their speed and sensitivity. Electrochemical sensors with electrodes modified for NE detection provide significant advantages over these other techniques such as quick response, simple procedure, high selectivity and direct information [4,11,12]. The electrochemical sensor electrode modification acts to promote the electrochemical reaction of NE in order to improve the sensitivity, selectivity and reproducibility of the sensor [1]. For example, gold nanoparticles deposited on an electrode exhibits potent and persistent electro-mediating behavior to detect NE [2]. Similarly, modifying activated glassy carbon with Ni and polyurethane increases the selectivity for NE [13]. However, these modification schemes add complexity and/or costly materials to the electrode design. Carbon materials are commonly utilized in disposable electrochemical sensors due to their high conductivity, chemical inertness, bio-compatibility, and low cost [14–17]. Electrodes based upon carbon nanotubes [11,12,18], carbon paste [5], Ag ion irradiated multi-walled carbon nanotube [3] and carbon fiber [19] have been used to detect NE successfully. However, the current signal is generally extremely low, leading to poor sensitivity for NE.

Increasing the surface area of the electrode material provides one effective method to significantly improve the sensitivity of electrochemical sensors [20]. Thus, carbon nanotubes with their large surface area [3,21] have been explored extensively as electrodes for electrochemical sensors, but their cost is high relative to other carbons. Alternatively to obtain high surface areas, ordered mesoporous carbon can be fabricated by simple organic–organic self-assembly of inexpensive phenolic resin and commercial surfactants, followed by carbonization [22]. High surface area mesoporous carbon pastes have been demonstrated as active materials in electrochemical sensors [4,14,23–26], but the detection limit of these sensors is somewhat limited. To further increase the surface area of these mesoporous carbons, triconstituent assembly of surfactant, phenolic resin and a silica precursor can be utilized. Removal of SiO_2 after carbonization results in large increases in the surface area. The silica concentration during self-assembly controls the resultant surface area, which increases from 760 $m^2\,g^{-1}$ (no silica) to 2390 $m^2\,g^{-1}$ (46% silica) [27]. This increased surface area dramatically increases the electrochemical double layer capacitance [28–30] and enhances the adsorption capacity for bulky organics from aqueous solution [31]. The pores from the etched silica that produce this high surface area are very small (<1 nm) and included within the wall framework of the ordered mesopores. We have recently demonstrated high efficacy, amperometric glucose sensors using modest surface area, mesoporous carbon with polymer binder of poly(hydroxybutyl methacrylate). The small pores and large internal surface area provide high sensitivity and selectivity in whole rabbit blood without the use of a Nafion or other membranes [20]. However for glucose, physiological concentrations (100 mg mL^{-1}) are orders of magnitude greater than for NE (90–220 pg mL^{-1}). This low physiological concentration for NE provides a significant challenge for electrochemical sensing. Extensive efforts have focused on modifying the electrodes to decrease the detection limit of NE, including macrocyclic Ni and hydrophilic polyurethane modified glass carbon [13], carbon fiber and diamond microelectrodes [19], Ag ion irradiated nanotubes [3], and ZrO_2 nanoparticle-modified carbon paste [5]. Nevertheless, all these electrodes exhibit detection limits for NE (>10^4 pg mL^{-1}) that are at least one order of magnitude greater than physiological concentrations (1×10^3 pg mL^{-1}). As to our best knowledge, no electrode has been reported that exhibits detection limits of NE as low as 100 pg mL^{-1} as would be required for a biomedical device.

In this work, we utilize low-cost mesoporous carbon inks to screen print single-use disposable electrodes for the detection of norepinephrine amperometrically. There are numerous advantages to amperometric sensing in terms of cost, simplicity and maturity of the technologies. However, the low concentration (90–220 pg mL^{-1}) of NE in human blood provides a significant challenge to obtaining acceptable signal to noise when only the current is being measured to assess the concentration in blood. To overcome the lower concentration limits for the amperometric sensing, we increased the surface area of the electrode to enhance the current signal by use of triconstituent assembly and silica etching; surface areas from 1500 to 2300 $m^2\,g^{-1}$ can be produced depending upon the carbon–silica ratio during self-assembly. To assess the role of the small micropores, one control material without silica is also examined. The performance of these screen printed mesoporous carbon electrodes is strongly dependent upon the surface area with greatest sensitivity for the highest surface area carbon. To ensure NE can be effectively oxidized, an enzyme, phenylethanolamine N-methyl transferase, with a cofactor, S-(5′-Adenosyl)-L-methionine chloride dihydrochloride, to active the enzyme is used to catalyze the reaction. The resultant sensors can detect NE at concentrations as low as 100 pg mL^{-1} in rabbit whole blood. These results illustrate the potential of these high surface area carbons for use in electrodes for rapid and highly sensitive electrochemical detection of biological targets.

2. Experimental

2.1. Chemicals

All reagents were obtained from Sigma or Sigma–Aldrich unless otherwise specified. Phenylethanolamine N-methyl Transferase (PNMT) with an activity of 50–100 U mg^{-1} protein was obtained from Calzyme Laboratories, CA. Cofactor S-(5′-Adenosyl)-L-methionine chloride dihydrochloride (SAM) and target norepinephrine (NE) were obtained from Sigma–Aldrich. Whole rabbit blood (female, New Zealand white) was purchased from Bioreclamation. All solutions were prepared in 10 mM phosphate-buffered saline (PBS) at pH 7.4 unless otherwise specified.

2.2. Instrumentation

Amperometric detection was conducted using a 1230A CH Instruments Electrochemical Analyzer (CHI, Austin, TX, USA). X-Ray Diffraction (XRD) characterization of the mesoporous carbon materials was performed using a PANalytical X'Pert PRO (PANalytical, Netherlands). Transmission Electron Microscopy was completed

Table 1
Synthesis compositions of high surface area mesoporous carbons.

	TEOS (g)	Resol (g)	F127 (g)
MP-C-36	2.08	0.5	1.0
MP-C-46	2.08	1.0	1.6
MP-C-61	2.08	2.0	2.3

on the mesoporous carbon materials using a, JEOL 2010F microscope (JEOL, Tokyo, Japan). The Brunauer–Emmett–Teller (BET) method was performed to obtain Nitrogen adsorption–desorption isotherms to characterize the mesopore size using a Tristar II 3020 Micromeritics (Micromeritics, Atlanta, Georgia, USA).

2.3. Mesoporous carbon synthesis procedures

Highly ordered mesoporous carbon powders were synthesized by the evaporation-induced triconstituent co-assembly method using a triblock copolymer surfactant Pluronic F127 (PEO106-PPO70-PEO106, BASF), a low molecular-weight, water soluble phenolic resin (resol) and tetraethyl orthosilicate (TEOS) as the silica template [27]. Resol was prepared by sodium hydroxide (NaOH, Aldrich) catalyzed polymerization of phenol and formaldehyde (37 wt% in H_2O) as described previously [22]. Hydrolysis of TEOS was performed at fixed mass ratio of TEOS/EtOH/HCl/H_2O at $2.08{:}12{:}7.3 \times 10^{-3}{:}1.0$. The morphology of the mesoporous carbon was controlled through the resol and TEOS ratio following procedure of Zhao and co-workers [27] with a phenol/formaldehyde/NaOH/F127 = 1:2:0.1:0.006 for the carbon synthesized without TEOS. In a typical preparation, 1.6 g F127 was initially dissolved in 8.0 g ethanol at 40 °C. 0.2 g 2 M HCl was added to this surfactant solution. A second solution of 5.0 g of 20 wt% resol in ethanol and 2.08 g TEOS were added to the acidic ethanoic surfactant solution. The mixture was subsequently stirred at 40 °C for 2 h. The preparation conditions for synthesis of the other samples are shown in Table 1. After reacting for 2 h, the solution was spread into several watch glasses and allowed to dry overnight. After evaporation of the solvent, the phenolic resin was thermally crosslinked at 100 °C for 24 h. Carbonization was performed in a tubular furnace using a nitrogen atmosphere at a flow rate of 140 cm^3 min^{-1} at 900 °C for 2 h with heating rates of 1 °C min^{-1} below 600 °C, and 5 °C min^{-1} above 600 °C. After carbonization, the mesoporous carbon powder was ground with a marble mortar and pestle to obtain a fine powder.

The silica (when present) was removed after carbonization by soaking the carbonized powders in 1 M NaOH Ethanol-H_2O (volume ratio 1:1) for 3 days [32], followed by washing with distilled water three times. After overnight drying at 100 °C, the powder was ready to use for screen printed electrodes.

2.4. Mesoporous carbon norepinephrine biosensors preparation procedures

The inks for the screen printing were fabricated by physically mixing the mesoporous carbon powder with 3 wt% poly (hydroxybutyl methacrylate) (PHBMA) in ethanol to form a consistent paste. The PHBMA functioned as the polymer binder as described previously [20] and was 15 wt% of the solids in the electrode. The paste was deposited on the working area on a screen printed electrode (CHI, Austin, TX) by a small spoon and allowed to dry (weight of ink in each electrode is less than 0.1 mg after dried). A bake at 80 °C for 1 h was utilized to enhance the binder to ensure stable mesoporous carbon electrodes.

2.5. Characterization of mesoporous carbons procedures

The ordered mesostructure of the porous carbons was elucidated by XRD in $\theta/2\theta$ geometry using Cu K_α ($\lambda = 0.154$ nm) source and parallel plate collimator in combination with an incident beam optical module to minimize beam divergence. The angle of incidence, θ, was varied from 0.25 to 1.5 degrees. For direct visualization of the pores, transmission electron microscopy at 200 kV was used. Surface area and pore size information was determined using Nitrogen adsorption–desorption isotherms by application of the BET method in a relative pressure range of $P P^{-1}{}_0 = 0.05$–0.25 and Barrett–Joyner–Halenda (BJH) model using the adsorption branch of the isotherm, respectively. The samples were degassed at 300 °C for at least 1 h prior to the gas sorption measurements.

2.6. Electrochemical detection procedures

The time-based amperometric behavior was examined under applied potential of −0.2 V for all the samples. A ferricyanide-mediated system based on 0.25 mg mL^{-1} of enzyme PNMT, 0.375 mg mL^{-1} cofactor SAM, 25 mM potassium hexacyanoferrate and 25 mM potassium ferricyanide in PBS solution were used for all measurements. In a standard measurement, the contacts of the sensor were wetted by 90 μL of enzyme/cofactor/mediator solution with the entire contact area for each electrode (working, reference and counter electrode) covered by the solution. Subsequently, 10 μL sample solution (NE in PBS or blood) was injected into the center of the enzyme/mediator solution and allowed to settle for 5 s. For each set of amperometric i–t assays, a NE-in-PBS concentration gradient was used with NE concentrations of 0, 100, 200, 300, 400 and 500 pg mL^{-1} ($n = 3$), respectively. For the detection of NE level in blood, a known amount of NE was added into the whole blood to yield 1000 mg mL^{-1}. This stock solution was subsequently diluted to 100, 200, 300, 400 and 500 pg mL^{-1} for the detection measurements. The amperometric i–t response under applied potential of −0.2 V was then measured for 20 s for each concentration. Each NE concentration gradient sensing measurement was repeated at least three times to obtain the average sensitivity and error range. Every sensor was washed with fresh PBS three times and gently dried between measurements to prevent contamination from prior measurement.

3. Results and discussion

3.1. Characterization of mesoporous carbons

A well-defined small-angle diffraction peak is observed for all the carbons, indicative of an ordered mesostructure (Fig. 1). The XRD profile lacks higher order reflections and thus precludes space group identification from the diffraction pattern. However, the position of the primary diffraction peak is consistent with prior reports for similar materials [27] and shifts to lower angles as the silica content is increased (MP-C-36 to MP-C-61). The d-spacing of the framework is from 8.4 nm, 8.9 nm, 9.6 nm and 10 nm for FDU-16, MP-C-36, MP-C-61 and MP-C-46, respectively, from application of Bragg's law. It should be noted that the contraction during carbonization is significantly hindered by addition of TEOS whereby the silica network supports the framework during carbonization. This structural reinforcement by the silica leads to the shifts in the diffraction peaks. The ordered mesostructures of these carbons are confirmed by TEM (Fig. 2) with a well ordered mesoporous structure that is consistent with the global average information from XRD patterns.

From the XRD and TEM, the difference between the mesoporous carbon materials appears minor, but the etched silica yields

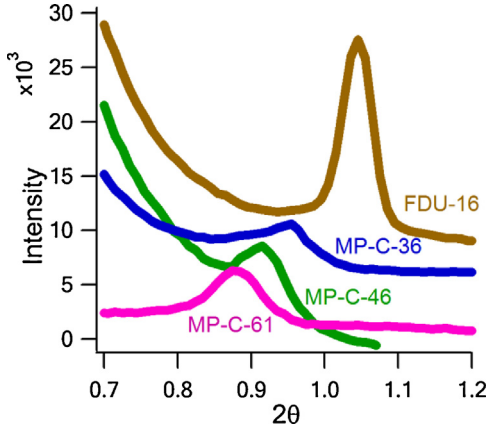

Fig. 1. Low-angle powder XRD patterns for MP-C-36, MP-C-46 and MP-C-61.

significant porosity in the mesostructure framework that can be readily assessed using N_2 sorption. Fig. 3A illustrates the N_2 adsorption/desorption isotherms for the mesoporous carbons, which are typical type IV isotherm curves for mesoporous materials. These isotherms qualitatively can provide significant insight into the pore structure of the powders. First, the volume of adsorbed N_2 for the isotherms scales with the pore volume of the mesoporous materials with MP-C-46 obviously exhibiting the largest pore volume. Additionally, the slope of the isotherm at low $P P^{-1}{}_0$ (0.05–0.25) provides insight into the surface area with MP-C-46 exhibiting the largest slope and hence the greatest surface area of the mesoporous carbons examined. From analysis of the adsorption isotherms, the pore size distribution (Fig. 3B) is calculated based upon the BJH model.

Table 2
Structural properties of mesoporous powders from B.E.T. N_2 sorption.

	Surface area ($m^2\ g^{-1}$)	Pore diameter (nm)	Pore Volume ($cm^3\ g^{-1}$)
FDU-16	671	2.9	0.14
MP-C-36	1734	3.0	0.91
MP-C-46	2339	4.0	1.68
MP-C-61	1501	4.3	1.23

The average pore diameter is between 6 and 9 nm for all mesoporous carbon materials examined as shown in Table 2. These small differences in pore size are consistent with the previous reports for analogous mesoporous carbons [27]. When TEOS is not utilized in the synthesis of the mesoporous carbon (FDU-16), the pore diameter is 5.8 nm, which is not far removed from the 6 nm diameter for MP-C-36. However, the surface area nearly triples from 671 $m^2\ g^{-1}$ using FDU-16 to 1734 $m^2\ g^{-1}$ using MP-C-36 due to the presence of micropores in the carbon framework from the etched silica. The surface area increases significantly again for MP-C-46 (2339 $m^2\ g^{-1}$), but then subsequently decreases when additional TEOS is added for MP-C-61 (1501 $m^2\ g^{-1}$). These pore characteristics are consistent with previous reports [27].

3.2. Electrochemical detection of NE

Phenylethanolamine N-methyl transferase (PNMT) is mainly localized in the adrenal medulla, which can physiologically catalyze norepinephrine into epinephrine (EP) [33,34]. During amperometric i–t measurement in the presence of PNMT and the SAM co-factor, NE is methylated into EP [2]. Meanwhile the reduction/oxidation of the mediator (ferricyanide) enables the charge transfer during the

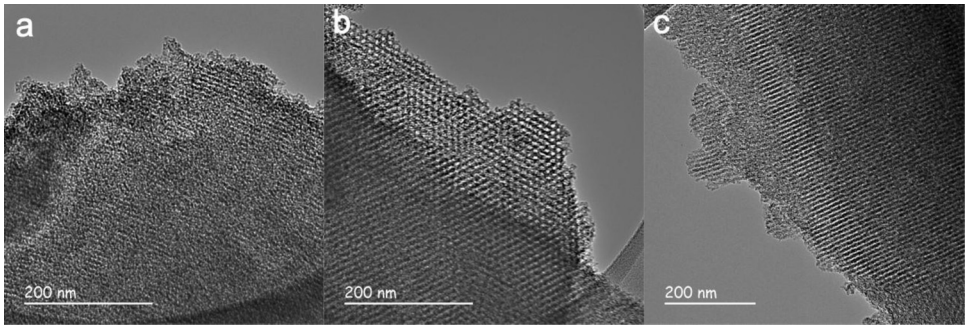

Fig. 2. TEM micrographs of mesoporous carbons: (a) MP-C-36, (b) MP-C-46 and (c) MP-C-61. A well-defined ordered structure is found for all materials.

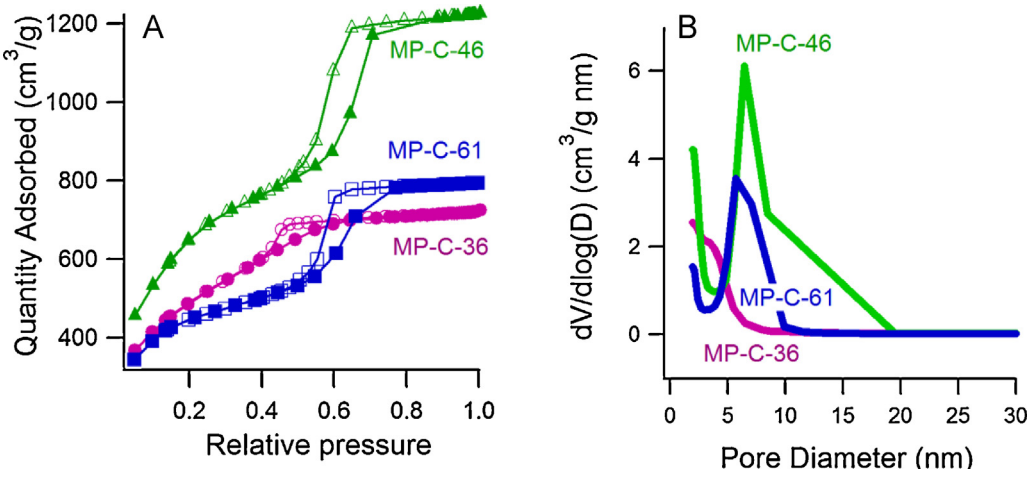

Fig. 3. (A) Adsorption (solid marker)/desorption (empty marker) isotherms and (B) pore size distribution of MP-CS-36, MP-CS-46 and MP-CS-61.

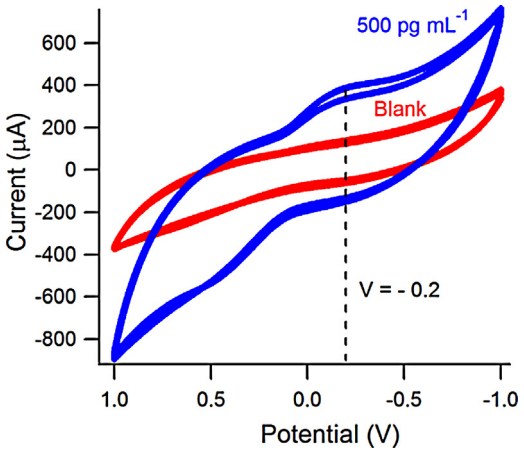

Fig. 4. Cyclic voltammetry profile using MP-C-36 electrode containing PNMT, SAM and potassium ferri/ferro-cyanide with comparison of testing a blank (0 pg mL^{-1} NE) and 500 pg mL^{-1} NE sample.

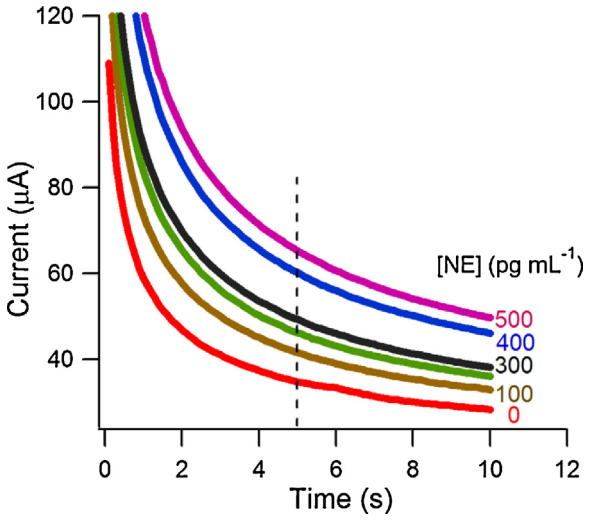

Fig. 5. Steady-state stability current measurements of MP-C-36 electrode with different concentrations of NE. From bottom to top: 0, 100, 200, 300, 400, 500 pg mL^{-1}, respectively.

reaction to be measured at the electrode, which is directly proportional to the oxidized amount of NE.

In order to effectively determine concentrations from amperometric i–t measurements, cyclic voltammograms (CV) are obtained to select the potential for i–t tests. Fig. 4 illustrates a representative CV curve using MP-C-36; the current obtained from 500 pg mL^{-1} NE solution is significantly greater than the blank solution. Moreover, the reduction/oxidation of ferricyanide/ferrocyanide from the oxidation of NE is clearly observed with a peak at −0.2 V that is present only for 500 pg mL^{-1} NE solution, but not for the blank without NE. As this peak at −0.2 V appears directly related to the oxidation and detection of NE, this potential is selected for all subsequent amperometric i–t measurements.

Calibration curves are obtained on the basis of this current-concentration relationship with a steady-state response provided by redox mediator during amperometric i–t measurements. Fig. 5 demonstrates a typical current response of i–t measurement using a MP-C-36 carbon electrode where the steady state current increases gradually as NE concentration increases. As shown in Fig. 5, the current initially drops from a very high value because of the flow of electrons in the bulk solution before NE oxidation occurs. After a few seconds, NE starts to oxidize at the potential being applied. We take data from the plateau (5 s) because most oxidation has occurred at this point and the solution is stabilized at the applied potential. In all the calibration curves, the current at 5 s is utilized to show the response of the sensor.

3.3. Impact of surface area/pore size on sensor performance

It is well established that surface area of the electrode materials is crucial for high sensitivity of electrochemical sensors. In addition to surface area, the pore size is also important to facilitate transport of the reactants for the bulk solution to the internal pores for collection of the electrons associated with the redox reaction and thus increasing the current that potentially could improve the sensitivity of the sensor [35,36]. However for these self-assembled porous materials, increasing pore size generally leads to a decrease in surface area [36,37]. Typically it is desired to use the minimum pore diameter that still allows enzyme infiltration to obtain maximum sensitivity for the sensor [36,37]. The size of SAM is small enough to neglect for size exclusion (~0.2 nm), but the hydrodynamic radius of PNMT is calculated to be ~5 nm [38], which is similar to the pore sizes. However, this size for PNMT is dominated by three flexible arms and thus it can easily enter into the smaller pores examined here with the mode peak size from BJH analysis (Fig. 3B) being 3.8 nm (MP-C-36), 6.5 nm (MP-C-46), 5.8 nm (MP-C-61). The calculated average pore size is smaller due to large number of micropores produced by removing SiO_2 from the carbon framework. These small micropores are too small to accommodate PNMT, but are accessible to the potassium ferri-/ferro-cyanide. As a result, these carbon powders have a hierarchical structure with large pores accessible to the enzyme PNMT

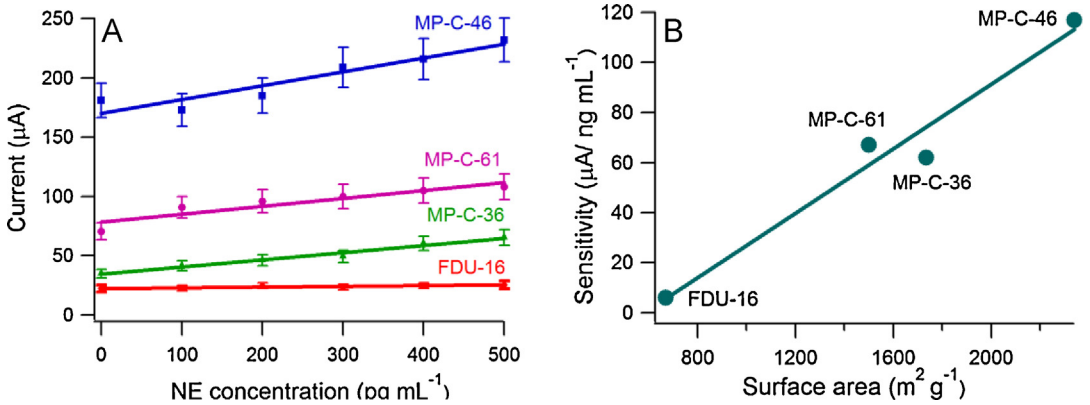

Fig. 6. (A) NE concentration gradient calibration curves in PBS of MP-C-36 (▲), MP-C-46 (■), MP-C-61 (●) and (D) FDU-16 (♦) as reference; (B) Relationship between normalized sensitivity and surface area.

and micropores that enable charge transfer from the mediator due to very large accessible surface area. To illustrate the importance of the high surface area micropores, the detection ability of these hierarchical porous carbons is compared with a control sample with similar mesopore size, but lacking micropores (FDU-16). As shown in Fig. 6A, the current from the FDU-16 is nearly invariant over the range of relevant NE concentrations (0–500 pg mL^{-1}); conversely, using MP-C-36, which exhibits a very similar pore size, as the active electrode material produces a clear linear increase in current as the NE concentration is increased over the same range. This enhanced sensitivity is despite an order of magnitude greater FDU-16 (~1 mg) than MP-C-36 (~0.1 mg) used in the electrodes for this case. Although FDU-16 exhibits excellent performance when used as electrode materials for glucose sensing [20], it lacks surface area to enable high sensitivity for low concentration analytes such as NE (6 μA mL ng^{-1} cm^{-2}). Of the mesoporous carbons examined, MP-C-46 shows the highest current signal (181–232 μA, <0.1 mg electrode); this result might be expected as MP-C-46 has both the highest surface area (2339 m^2 g^{-1}) and largest pore size (4.0 nm) of the mesoporous carbons. However as the amount of mesoporous powder varies somewhat between electrodes, the absolute current is not an appropriate measure for the sensitivity. Rather, the change in current with increasing NE concentration provides a normalized route to assess the sensitivity (the slope of the calibration lines for each sensor in Fig. 6A) of the mesoporous carbon-based sensors. And the slope of each line is further divided by the physical contact area of carbon to the solution (0.074 cm^2) to normalize the sensitivity, as shown in Fig. 6B. The most sensitive electrode is fabricated using MP-C-46 with sensitivity of 1581 (μA mL ng^{-1} cm^{-2}) this result is not surprising as this mesoporous carbon exhibits the largest pore size and surface area, both of which are important to detection of the PNMT as described previously. To further investigate the relative importance of pore size and surface area for the mesoporous carbons examined here, the detection sensitivity of NE using MP-C-36 (1734 m^2 g^{-1}, 3 nm) and MP-C-61 (1501 m^2 g^{-1}, 4.3 nm) are compared; despite the smaller pore size in MP-C-36, it has similar sensitivity (837 μA mL ng^{-1} cm^{-2}) as MP-C-61 (905 μA mL ng^{-1} cm^{-2}) because of the similar surface area. Interestingly, this sensitivity appears to be linearly correlated with surface area, as shown in Fig. 6B. Thus, the sensitivity of these mesoporous carbon electrodes is directly proportional to the specific surface area of mesoporous carbon.

We have previously illustrated for glucose sensing that mesopores in FDU-16 provide efficient blocking of non-specific interactions to enable sensing without a Nafion membrane in whole blood [20]. However, the concentration of NE is significantly less than glucose at physiological conditions; thus, it is not clear if these mesoporous materials will be effective without use of a membrane to exclude other blood components from the active electrode. Additionally, due to the similarities between the carbon materials of diabetes test strips and the mesoporous carbons explored in this work, it is expected that the disposable sensor's shelf life would closely match the 6 month shelf life of the diabetes test strips [39], for more accurate modeling, thermal stability over extended periods of time can be predicted [40,41].

As MP-C-46 mesoporous carbon provides the best performance in electrochemical detection of NE, this electrode is further investigated for its efficacy in detecting NE in whole rabbit blood. Fig. 7 illustrates the amperometric response of this electrode to NE in whole rabbit blood in comparison to Zensor (unmodified commercial screen printed electrode). Obviously, the mesoporous carbon modified Zensor electrode sensor shows significantly larger current signal and sensitivity than the blank Zensor. A linear response is clearly present in the current detected by the sensor with respect to the NE concentration in the blood. The lower detection limit of this sensor without a membrane appears to be approximately

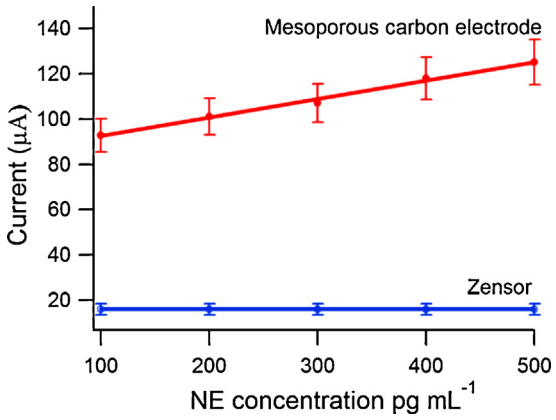

Fig. 7. NE concentration measurements in blood by MP-C-46 modified Zensor screen printed electrode and by an un-modified Zensor electrode.

100 pg mL^{-1} as the current does not deviate significantly from the control (PBS without NE) at this lowest concentration, but the current at 100 pg mL^{-1} appears to be consistent with the linear trend from higher concentrations. This result suggests that the high surface area coupled with small pores of ordered mesoporous carbon can be utilized to easily and rapidly detect electrochemically active components in blood *via* mediated enzymatic reaction using simple amperometric sensing protocols. In comparison to the clinical standard technique of catecholamine determination in patient samples, High Liquid Performance Chromatography (HPLC), the reported LLD for NE are approximately 9.36 ng mL^{-1} [8] and 60–80 ng mL^{-1} [42] in purified solutions as compared to the 100 pg mL^{-1} detection using the mesoporous carbon electrodes in blood solutions.

4. Conclusions

Low-cost disposable screen printed sensors based on high surface area mesoporous carbon electrodes and PHBMA binder can detect NE at low concentrations that are physiologically relevant. The sensitivity of these electrodes for NE is strongly dependent upon the surface area; to obtain sufficient surface area and pores compatible with PNMT, a hierarchical templating approach is utilized with mesopores (3–5 nm) to enable transport of PNMT templated by non-ionic surfactant and micropores (<1 nm) in the wall to provide high surface area templated by silica. Mesoporous carbon without the micropore template lacks sensitivity to accurately detect NE in the range of 100–500 pg mL^{-1}. A mesoporous carbon with similar pore size by significantly larger surface area as a result of the micropores provides sufficient sensitivity to NE over this range. The best-performing sensor also provides similar performance when detecting NE in both PBS and whole rabbit blood. The lower detection limit without an exclusion membrane such as Nafion is approximately 100 pg mL^{-1} in whole rabbit blood; this high sensitivity without a membrane is attributed to the molecular sieving capabilities of the mesopores and the large internal surface area of the mesoporous carbons.

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