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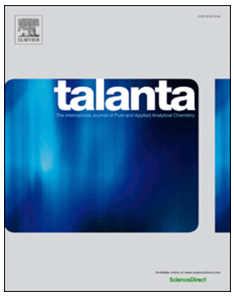

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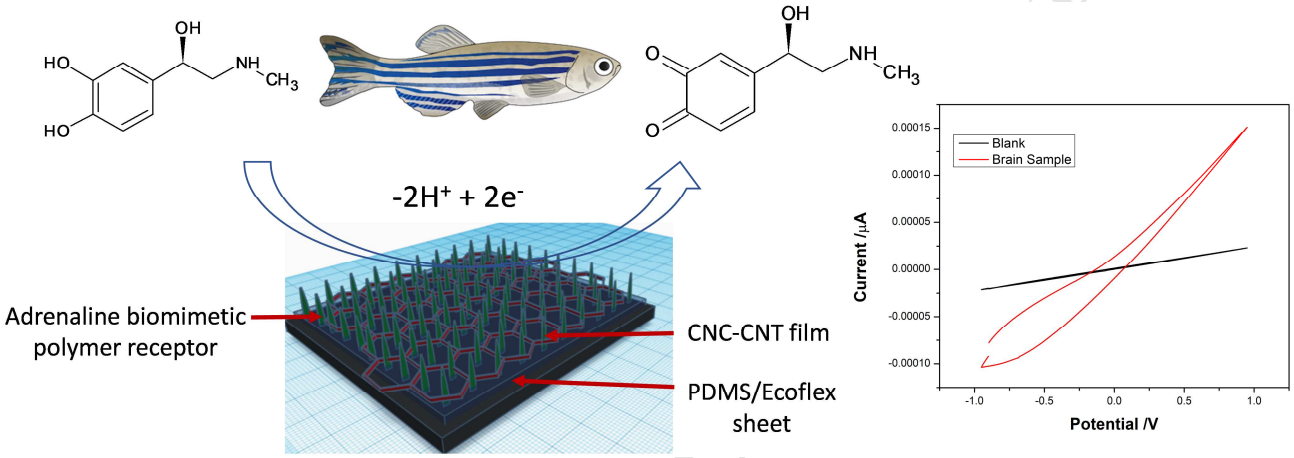
Graphical Abstract



A flexible-imprinted capacitive sensor for rapid detection of adrenaline

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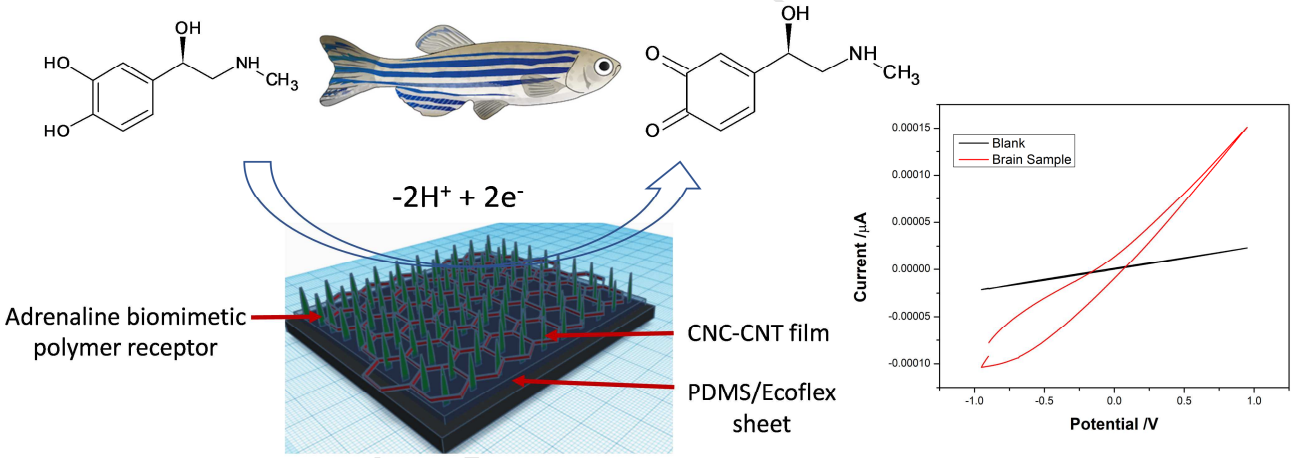
Abstract

This article demonstrates a non-enzymatic biomimetic adrenaline capacitive sensor fabricated inexpensively by layer-by-layer (LbL) assembly. The LbL assembly consists of polydimethylsiloxane (PDMS) substrate, carbon nanotube-cellulose nanocrystals (CNC/CNT) nanofilms (first layer) with adrenaline imprinted poly (aniline/phenylboronic acid) (pANI/PBA) moieties (second layer). The flexible sensor has been effectively demonstrated for high sensitivity and selectivity in capacitive detection of adrenaline standard and adrenaline in real zebra fish brain sample. The molecularly imprinted adrenaline sensor exhibited linear response between 0.001µM - 100µM with a correlation coefficient of 0.9738 and detection limit (LOD) of 0.001µM. The developed sensor demonstrated high adrenaline selectivity and good sensor to sensor reproducibility of 15.7% for molecularly imprinted films. The sensor precision for triplicate standard runs for different adrenaline concentrations ranged from 0.5-5.0%, indicative of the overall reliability and validity of the device. The inexpensive sensor remains stable over time, responding proportionately to doses of adrenaline, and as such effective as a dosimetric sensor for near real time continuous monitoring. Frugal in sample consumption (50 µL), the

study suggested the practical utilization of the developed biosensor towards adrenaline detection in biological fluids.

Keywords: Biomimetic flexible sensors; Capacitive sensors; Adrenaline imprinted sensor, Molecular imprinting polymer (MIP)

Graphical Abstract



1. Introduction

Adrenaline, also called epinephrine is a catecholamine and adrenal cortical hormone released by renal glands (1). The main functional neurotransmitter effecter to the sympathetic nervous system, adrenaline triggers the instinctive flight, fright or fight responses, which is a useful stress physiology biomarker in mammals (2, 3). Adrenaline has also been found to be a hormone related to Parkinson's disease, heart attacks, panic attacks and other physical disorders (1, 2). Adrenaline uses the stored energy like ATP to fuel our escape from a stressful event (4). For instance, fish species that show aggressive dominant traits have shown increased level of epinephrine (~15ng/mL) compared to non-dominant species (~2ng/mL) (4). Depending on the stress level, adrenaline concentration range in human fluids is 0.015µM – 40µM (1, 5). As such, elevated adrenaline level could be a useful biomarker for anxiety, depression, and post traumatic stress disorder (PTSD) (5). These mental disorders have been increasing rapidly in society across all age groups. As such, a convenient sensor that can monitor adrenaline in non-invasive biological fluids such as saliva, sweat could be useful for personalized self-diagnosis and monitoring in both humans and animals. The convenience of production of sweat on demand using iontophoresis gives credence to further development of sweat sensors for adrenaline detection.

Traditional methods to detect adrenaline include capillary electrophoresis, liquid chromatography, flow injection analysis, spectrophotometry, fluorimetry, photoelectrochemical immunoassay, enzyme-linked immunoassay, and chemiluminescent immunoassay. Even though they have low detection limits, 0.3nM - 0.035mM, these methods are expensive, lab-localized, time consuming and unsuitable for point-of-care adrenaline detection (1, 6, 7). Electrochemical sensors are well suited for miniaturization, and for packaging into wearable point-of-care sensors

and as such desirable. In the literature, there are several designs for enzymatic based electrochemical sensors which are highly selective and sensitive (8, 9). However, enzymes are inherently unstable and lose activity over time, and as such not ideal as wearable sensors (10). To achieve good selectivity while addressing the instability of enzyme-based biosensors, there is interest in developing artificial biomimetic receptors that can be integrated for point-of-care sensor devices. In terms of other molecular receptors, aptamers are attracting significant interest (11). However, while aptamers are stable compared to enzymes, they are expensive and require specialized synthetic processes called systematic evolution of ligands by exponential enrichment (SELEX) (11). As such, aptamers are not often available for most analytical targets, and when available, their binding affinities towards the analytes are often not highly selective. More versatile artificial biomimetic receptors are molecular imprinted polymers (MIPs).Compared to other recognition systems, MIPs exhibit high selectivity for capture and preconcentration of the analyte, cost effective with excellent mechanical, thermal and chemical stability, and wide range of operating conditions (12).

MIPs are synthetic receptors prepared by using template of selected analyte of interest and possess molecular cavities similar to anayte molecule (13). MIP based electrochemical sensors have been used for clinical analysis, medical diagnostics, environmental monitoring and drug sensitivity (14-17). However, MIPs are less conductive and therefore, to compensate, conductive materials such as graphene, carbon nanotubes, conductive polymers etc, are added on the surface of the film to provide high surface area, low noise and better electrical and thermal conductivity (1, 18). Adrenaline is electro-active and undergoes reduction to adrenochrome (quinonoid form) (1) and thus electrochemical detection is suitable approach for adrenaline detection.

Monitoring of adrenaline have many possible applications which includes but not limited to drug development, monitoring physiological performances of athletes during competition and training, clinical analysis, medical diagnostics, environmental monitoring and drug delivery (1, 2, 19). The present work reports a highly selective molecularly imprinted flexible sensor for rapid screening of stress biomarker adrenaline with specificity. The developed flexible sensor was successfully applied to detect the adrenaline in zebra fish brain samples. On the basis of results obtained, the developed sensor can be an advanced analytical tool for electro-screening of adrenaline in routine analytical procedures.

2. Materials and Methods

2.1 Materials

The carbon nanotube (CNT), epinephrine, ammonium persulfate, 3-aminophenylboronic acid (PBA), potassium ferricyanide, glucose, sodium hydroxide, and sulphuric acid, were obtained from Sigma Aldrich, Oakville, Ontario, Canada. The cellulose nanocrystals (CNC) was donated by Alberta Innovates, Edmonton. Whereas the aniline, urea, and ascorbic acid were procured from Fisher Scientific, Hamptons, New Hampshire, United States. The ecoflex 00-30 part A, part B and polydimethylsiloxane (PDMS) were obtained from Smooth-On Incorporation, Edmonton, Alberta, Canada. The Sylgard 184 silicone elastomer base and curing agent were obtained from Dow Corning Corporation, Midland, Michigan, United States. All aqueous solutions were prepared by using deionised water.

2.2 Sensor Fabrication

The PDMS film was prepared in two steps. Equivalent parts of silicone elastomer/curing agent (1:10) and ecoflex hardener/elastomer (1:1) were mixed and casted onto sandpaper (P60

grain size) and allowed to polymerize at 80ºC overnight to get thin layered and stretchable PDMS/ecoflex sensor base. As shown in Figure 1a, the PDMS/ecoflex patch exhibited excellent stretchability and elongation endurance ~ 100%. To fabricate the sensor, 1 mg/ml suspension of CNC/CNT (1:2) was prepared and an aliquot of 100µL of CNC/CNT solution drop casted onto PDMS base and dried at 50°C.

An imprinting mixture was prepared by mixing 700 µL of 0.1M aniline, 42.5 µL of 0.1M PBA, 157.5 µL of 2M adrenaline, with 100 µl of 0.1M APS and drop casted on to the CNC/CNT layer on PDMS and allowed to polymerize on ice packs ~ (0ºC). Non-imprinted films were prepared without adrenaline. Fig.1b illustrated the chemical structure of conductive polyaniline (pANI). After polymerization, adrenaline was removed by electrochemical cleaning by applying the potential from +0.9V to -0.9V in presence of 50 µl 0.1M phosphate buffer using cyclic voltammetry (5 CV scans at the scan rate of 0.1 V/s). The cleaning process was repeated three times to get stable and clean baselines.

2.3 Preparation of Zebra fish brain samples

1.13 g of Zebra fish brain sample was homogenized with 150 µL methanol and water (1:1), ultrasonicated for 20 min and centrifuged for 5 min. The extraction procedure was repeated in triplicate and supernatant collected. The supernatant was dried under a nitrogen stream to remove methanol and reconstituted with water.

2.4 Electrochemical detection procedure

For capacitive detection, cyclic voltammetry (CV) was done using PGSTAT 101 Autolab potentiostat controlled by Nova 2.1 software. The CV measurements were performed in a potential range -0.9-0.9V at scan rate of 0.1 V/s using three-electrode system, with the MIP

sensor as the working electrode. Platinum wire and Ag/AgCl electrode were utilized as auxiliary and reference electrode, respectively. Real sample measurements were done according to standard addition method. First 25 µl of phosphate buffer was measured as blank and then 50 µL of the neat brain sample, followed by 5 µl spike increments of 100 µM adrenaline standard. Fig. 1c exhibited the systematic illustration for sensor fabrication and detection of adrenaline.

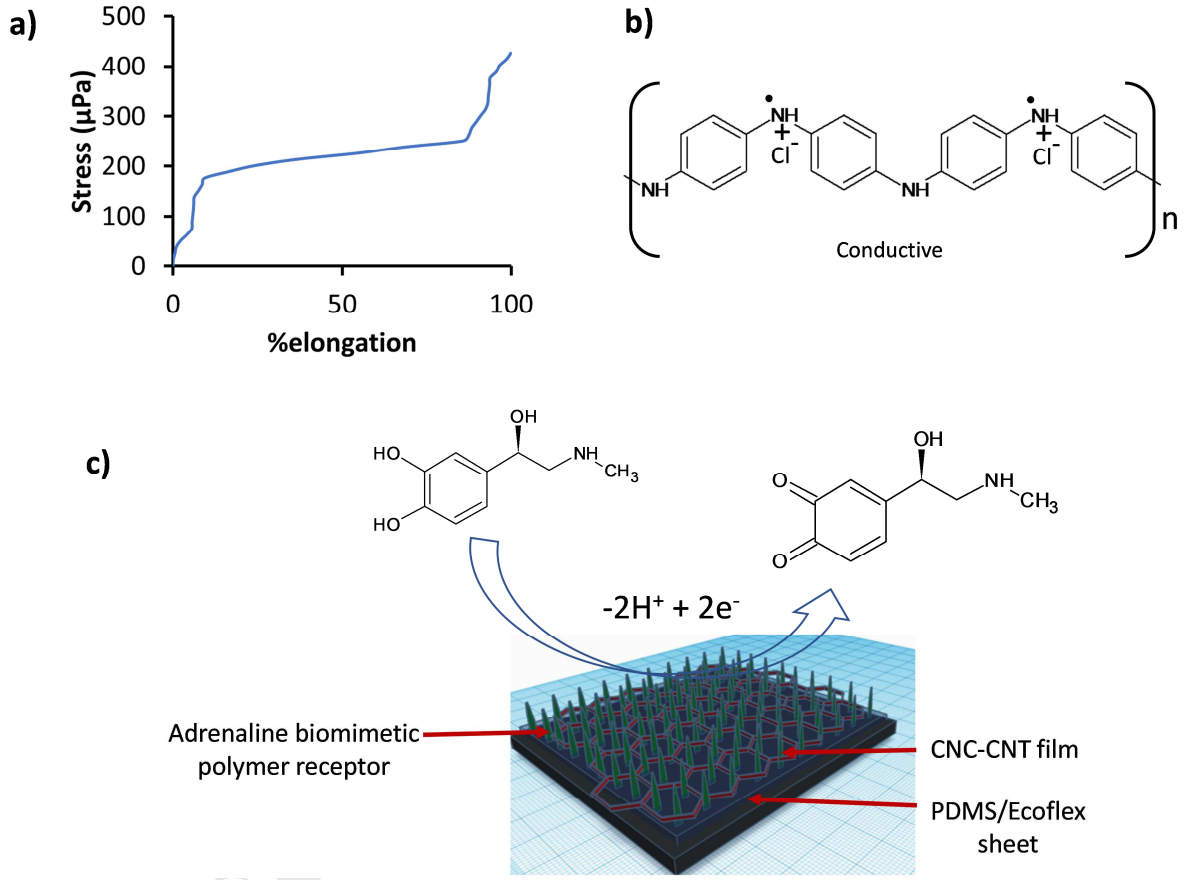


Figure 1. a) Stretchability and elongation of PDMS/ecoflex sheet; b) conductive pANI; c) schematic illustration showing detection of adrenaline on developed MIP sensor.

3. Results and Discussion

3.1 Morphological characterization

Morphological characterization of fabricated sensors was done by scanning electron microscopy (SEM). Figure 2 illustrates the SEM images of PDMS/CNC-CNT (a) and molecularly imprinted sensor on PDMS sheet (b). A web of carbon nanotubes were embedded in CNC matrix can be easily observed in Fig. 2a. Highly ordered and stable network of CNC-CNT is due to the tendency of hydrogen bonding interaction between the surface carboxylic acid groups of CNT and hydroxyl groups of β-1,4-anhydro-d-glucopyranose units of CNC. On the other hand the Fig. 2b exhibited the nanoflakes like structure of polyaniline MIP. PBA incorporated into pANI anchored on stabilized CNC-CNT microgel layer, aids the detection of adrenaline due to cis-diol interaction (20), Fig. 2c. The composite layers also afforded good water absorbency due to the hydrogel nature of the CNC. The nanoporous electrode film and its inherent high surface area would be significant in assisting aqueous analyte mobilization (wicking effect) and trapping to the conductive film electrode surface. The insets in Fig. 2a and 2b exhibits the contact angle of CNC-CNT and final MIP sensor.

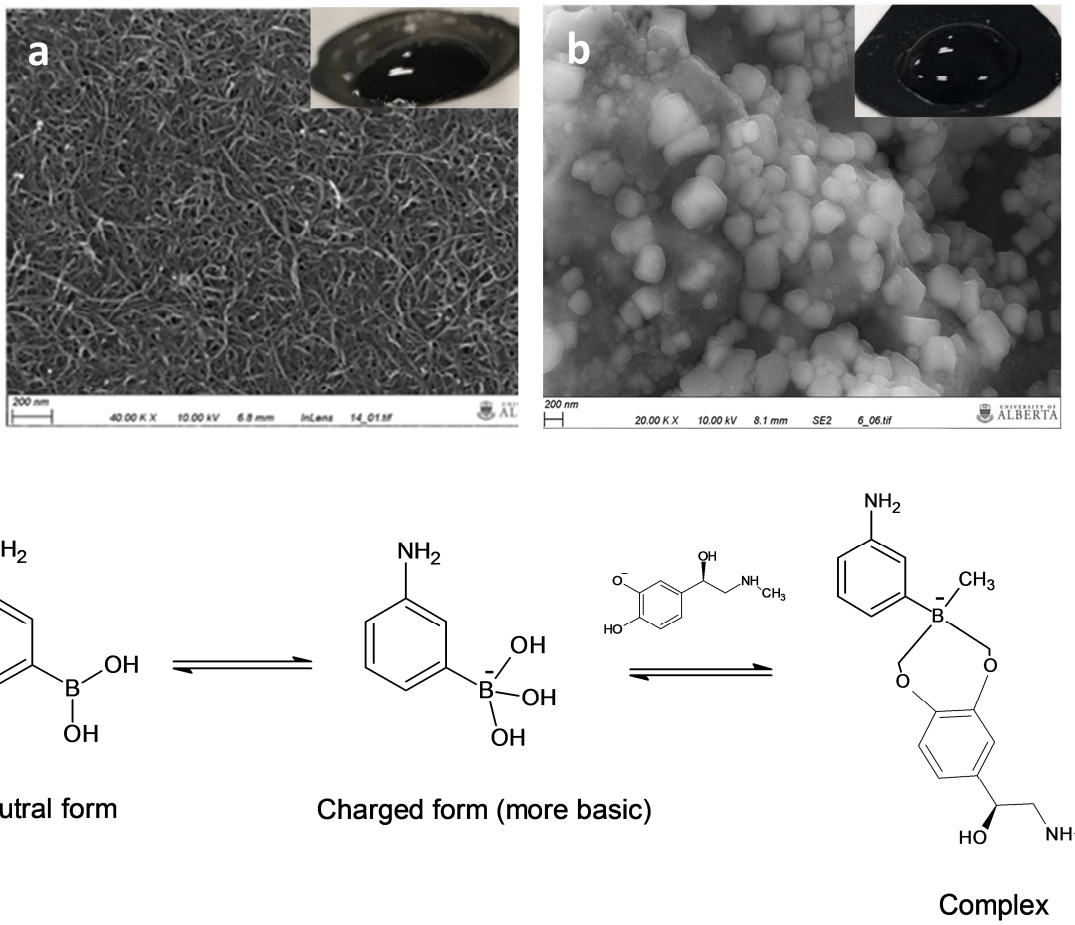


Figure 2. Scanning electron micrographs of a) PDMS/CNC-CNT; b) PDMS/CNC-CNT/PBA-pANI; and c) cis–diol interaction between PBA and adrenaline.

3.2 Electrochemical characterization of fabricated electrodes

Cyclic voltammetry and electrochemical impedance spectroscopy (EIS) have been commonly employed to study the interfacial characteristics of sensors. The electrochemical properties of fabricated sensors depend on the eletroactive behaviour of the transducer materials used for the fabrication. The developed flexible sensors were then characterized by CV and EIS using 5mM K_3FeCN_6 containing 0.1M KCl as standard redox probe. Fig 3a manifests the cyclic voltammograms for different layered sensors. The CV curves shows that there was an increase in redox current after integration of polyaniline (MIP/NIP) onto CNC-CNT layer. The enhanced

conductivity is attributed to fact that CNT acts as a conducting bridge between pANI ES (emeraldine salt) domains (large surface area and aspect ratio) [21, 22]. Moreover the enhanced conductivity was also attributed to synergistic effect of CNT and conductive pANI. As shown in Fig 3B, similar results were obtained by EIS confirming the enhanced electron transfer at PDMS/CNC-CNT/MIP. The electron transfer resistance obtained at different electrodes was calculated as 455Ω, 337Ω and 275Ω for PDMS/CNC-CNT, PDMS/CNC-CNT/NIP and PDMS/CNC-CNT/MIP, respectively. Lower the electron transfer resistance value greater is the conductivity of fabricated sensor due to lesser resistance to electron transfer.

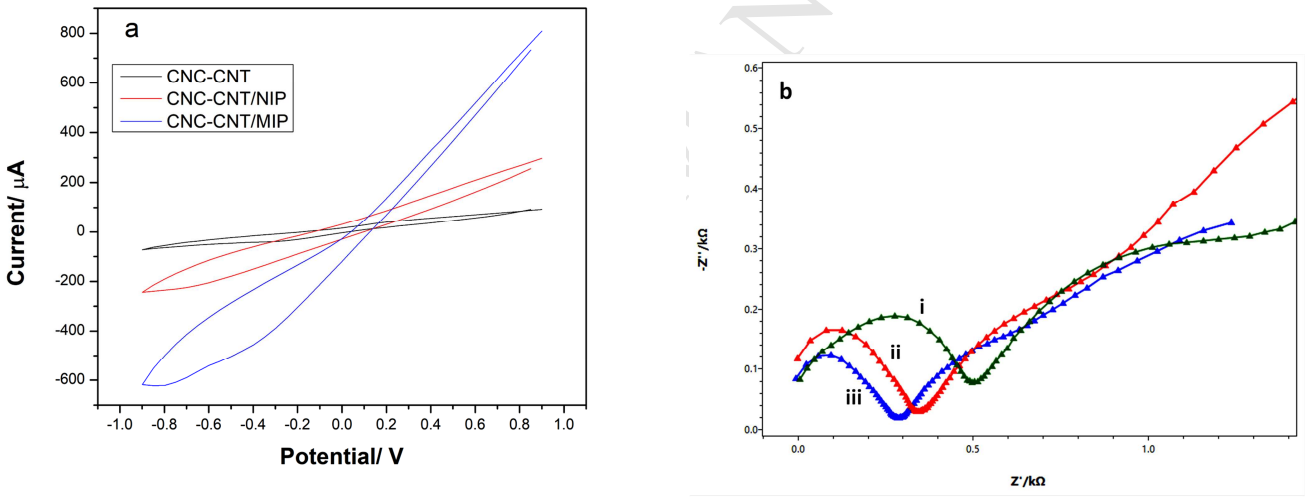


Figure 3. a) Cyclic voltammograms and b) nyquist plots obtained at i) PDMS/CNC-CNT, ii) PDMS/CNC-CNT/NIP and iii) PDMS/CNC-CNT/MIP.

3.3 Capacitive detection

Molecular imprinting creates a recognition sites as the solution dries on the film (23). Adrenaline was introduced as a functional template to the polymer film creating molecular cavities and interaction sites in the sensor coatings. This is a tailored effect complementary to the analyte by engulfing adrenaline and crosslinking the polymeric material (23). Capacitive

detection was done by using cyclic voltammetry in the potential range from -0.9 to +0.9 V. As shown in Fig. 4A CV was done after each addition of adrenaline concentration. Capacitance is calculated by dividing the resultant current by the scan rate. The calibration curves were prepared by plotting capacitance versus the concentration.

Figure 4 (b) displays the calibration curve for the different concentrations of adrenaline. The capacitance changes linearly as function of adrenaline. As shown in Fig. 4(b), the slope for MIP film is steeper as compared to the NIP film showing the high selectivity for adrenaline. The limit of detection (LOD) for MIP film is 0.001 µM as calculated by taking the inverse of signal to noise = 3. The lower the LOD, higher the sensitivity of electrode in terms of adrenaline capturing through molecular imprinting technique.

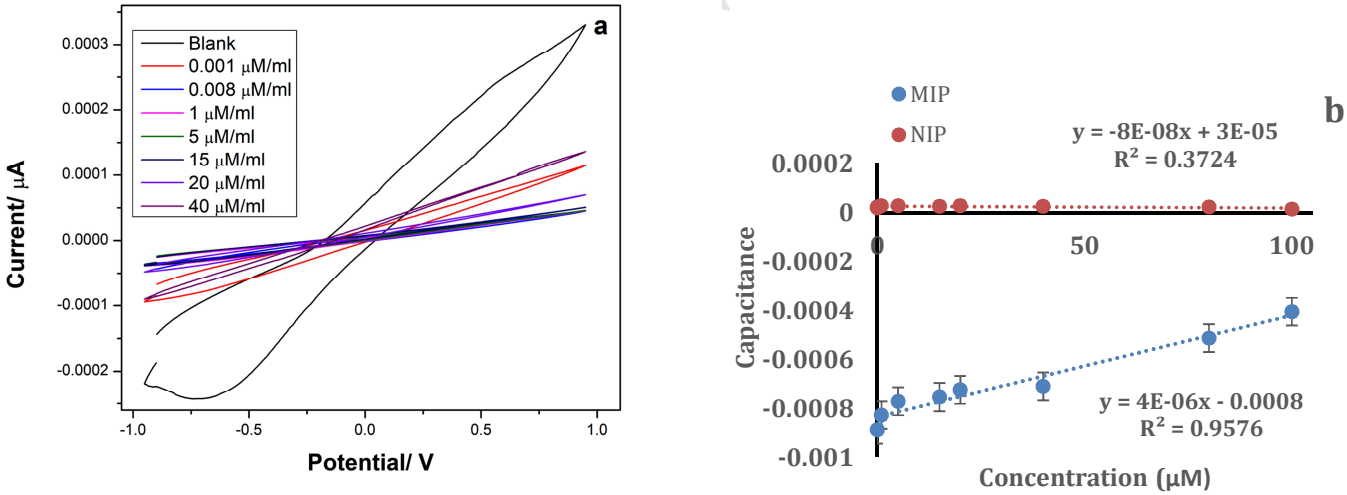


Figure 4. a) Cyclic voltammograms obtained after the successive addition of adrenaline standard solution using developed MIP sensor and b) corresponding calibration curves.

3.4 Interference studies and reproducibility

The common compounds found in human sweat or interstitial fluids are ascorbic acid, glucose and urea. Interference from these species was measured on developed sensor. The obtained responses are very low as compared to the target analyte (adrenaline) indicating that the developed sensor is not responsive towards the interference species and is highly selective towards the adrenaline. Reproducibility of the sensor was calculated for separately fabricated three sensors (n=3). The developed sensor exhibited acceptable reproducibility, % RSD calculated to be 4.03 %.

3.5 Real sample analysis

The developed MIP sensor was successfully applied for detection of adrenaline in brain samples of zebra fish. Fig. 5 illustrated the cyclic voltammograms obtained with blank and unknown brain sample. The unknown amount of adrenaline in zebra fish brain samples is found to be 120µM. Molecularly imprinted films selectively capture and detect adrenalin even in body fluids without any matrix effect indicating high selectivity of MIP film for adrenaline. However, as expected NIP films do not show high response for adrenaline in zebra fish brain samples as compared to MIP showing its non-selective nature for target adrenaline.

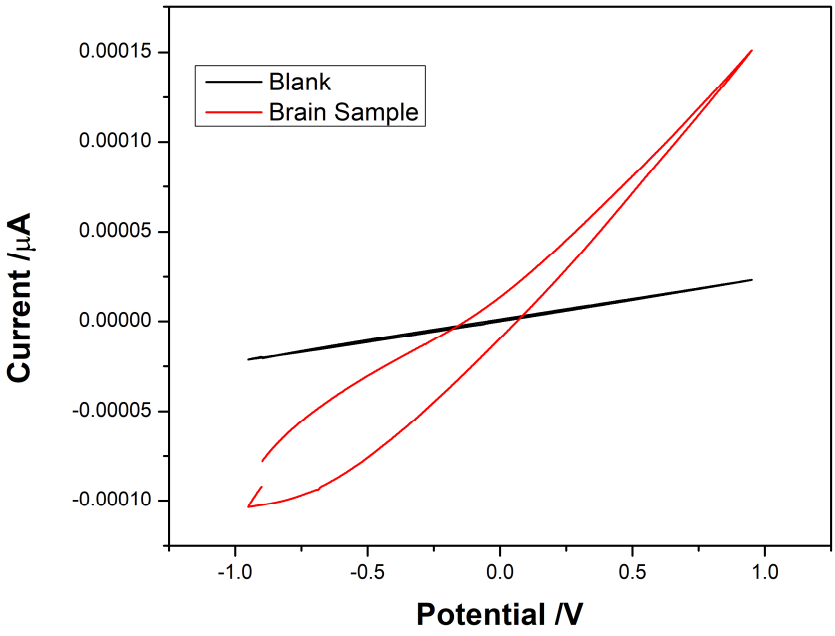


Figure 5. Cyclic voltammograms obtained at MIP sensor for blank and after the addition of neat brain sample.

4.0 Conclusion

In conclusion, a flexible, sensitive and disposable chemical sensor has been developed for the capacitive detection of adrenaline. The sensor was fabricated by simply drop-casting using layer-by-layer approach. The PDMS base provided flexibility and pANI bearing molecular cavities for adrenaline along with CNC-CNT worked as electronic transducer to convert the capturing of adrenaline into sensor signals. The fabricated sensor demonstrated high sensitivity and selectivity towards adrenaline compared to the conventional methods of adrenaline detection. The developed MIP sensor was successfully applied for rapid screening of adrenaline

in zebra fish brain samples. This sensor could further be extended for the detection of adrenaline in sports medicine usage.

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Note: Authors declare no competing interests

5. References

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Highlights

- A flexible molecularly imprinted sensor was fabricated for capacitive detection of stress biomarker adrenaline.
- The sensor was fabricated using layer-by-layer (LbL) assembly.
- Electrochemical performance of the fabricated sensor was exploited for adrenaline detection in zebra fish brain sample.