

Dual-Layered Nanomaterial-Based Molecular Patterning on Polymer Surface Biomimetic Impedimetric Sensing of a Bliss Molecule, Anandamide Neurotransmitter

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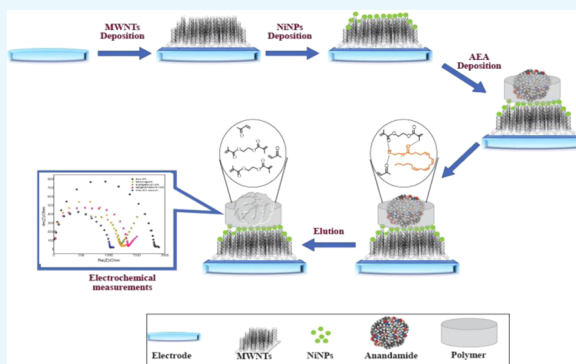


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Supporting Information

ABSTRACT: In this endeavor, a novel electrochemical biosensor was designed using multiwall carbon nanotubes (MWCNTs)- and nickel nanoparticles (NiNPs)-embedded anandamide (AEA) imprinted polymer. The NiNPs so synthesized were mortared with MWCNTs and molecularly imprinted polymer (MIP), which enhanced sensitivity and selectivity of the developed sensor, respectively. The characterization methods of AEA-based MIP included X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and Brunauer–Emmett–Teller (BET) analysis, which supported the successful synthesis of the polymer. Electrochemical studies of fabricated sensor were performed using cyclic voltammetry (CV) and electrochemical impedance spectroscopy in potentiostatic mode (PEIS). In this first phase of AEA-specific sensor development, MWCNT/NiNP/MIP@SPE was found to successfully discriminate between different concentrations of AEA. The developed sensing platform demonstrated a 100 pM–1 nM linear range with a 0.01 nM detection limit (LOD), 0.0149 mA/pM sensitivity, and 50% stability within 4 months. The sensor demonstrated selectivity toward AEA: although acetylcholine (ACh) and dopamine acted as strong interfering components because of their chemical similarity, the spiked AEA samples demonstrated ~90% recoveries. Hence, our results have passed the first step in AEA detection at home, although with a clinical setup, future advancement is still required.



1. INTRODUCTION

A worldwide medical and recreational use of *Cannabis sativa* L. (marijuana) was reported in almost every culture and society, which activates the G-protein-coupled cannabinoid 1 (CB1) receptor in the brain.^{1,2} The predominant distribution of these receptors all over the brain provoked an in depth research on the complete endocannabinoid system (eCBs) along with associated agonists and enzymes.^{3,4} In this direction, Devane and co-workers for the first time isolated anandamide (AEA; *N*-arachidonylethanolamine), an endogenous endocannabinoid ligand for CB1 receptors, from a pig brain in 1992.⁵ This endocannabinoid ligand was reported to act as a fatty acid neurotransmitter exerting a marijuana (Δ^9 -tetrahydrocannabinol)-like effect self-delivered by the brain.^{2,6} AEA is basically a Sanskrit word that means “ananda (internal bliss),” which is not only involved in physiological processes of moods and appetite^{7,8} but also an important constituent of reinforcement and reward processes in the brain.^{9,10}

Certainly, a number of studies reported an all-inclusive modulatory effect of AEA on the reward circuitry because of possessing self-delivering and addictive drug-induced reinforcing effects,^{11,12} along with stress and anxious neural disorders. Other than this, the “on demand” production of AEA endocannabinoids and rapid clearance through cellular uptake

followed by enzyme serine hydrolase fatty acid amide hydrolase (FAAH) metabolism^{6,13,14} also make it valuable in the neural system of mammals.

Healthy human blood and CSF contain ~0.7 nM¹⁵ and 0.007 ± 0.002 pmol/mL¹⁶ AEA, respectively, although this concentration varies with age, gender, type of samples, and medical conditions. Conventional methods of AEA detection reported a very low amount of concentration of the compound depending on the type of sample; for example, gas chromatography (GC)–electron ionization (EI)–mass spectrometry (MS)-based AEA quantification helped to detect 300 femtomolar (fmol) concentration of the compound,¹⁷ while Maccarrone et al. measured 20 ± 10 pmol sensitivity of AEA in liquid chromatography (LC)–MS.¹⁸ However, derivatized GC–MS-based quantification required eCB measurement to improve sensitivity and volatility, which is an intricate and time-consuming task. Therefore, several other studies analyzed

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the presence of eCBs through LC–MS and LC–MS–MS, which showed higher sensitivity compared with GC–MS and did not require additional sample preparation. In this direction, Chen and his co-workers reported 2.5 ng/mL (28.8 fmol) AEA quantification through an LC–electrospray ionization (ESI)–MS system,¹⁹ while Richardson et al. used LC–MS–MS for AEA detection at 25 fmol concentration.²⁰ Although a high signal-to-noise ratio and increased selectivity of analytical MS systems have been observed in AEA and related compounds' measurements, we still require a rapid, selective, and hands-on device for AEA detection. The conventional methods are time-consuming and expensive and have low selectivity and sensitivity toward low amounts of target samples. In this direction, Santonico and his colleagues have recently developed an innovative BIONOTE liquid sensor, which worked with the root-mean-square error in cross validation (RMSECV) method to detect 6.61 nM concentration of AEA,²¹ although prefunctionalization of the electrode is an additional step that could be eliminated using a molecularly imprinted polymer (MIP)-based detection platform.

The molecular imprinting technology/MIP technique has demonstrated cost effectiveness and selective point-of-care detection of predetermined biomolecules. This is reported as a cost-effective and improved detection platform as well as an effective surrogate for natural antibodies. This technique has overcome problems of the version of enzyme-based biosensors that suffer from low stability and selectivity along with complicated enzyme immobilization and purification procedures.^{22,23} The molecular imprinting technology uses a direct-electron-transfer shuttle-free detection strategy and creates a selective 3D space for individual target molecules in the detection system.^{24,25} The MIP technique is a polymer matrix-based approach with molecular recognition sites and target molecule removal, vacating 3D microcavities complementary to the structural and chemical properties of target molecules, which exhibit higher specificity in rebinding to the target molecule.^{26,27} The potential and effective use of MIP has been reported in sensing platform development due to its low price, high selectivity, specific adsorption capacity, elementary preparation procedures, and symmetrical distribution over the electrode.^{28,29} Recently, several researchers focused on MIP-based electrochemical and optical platforms for different neurotransmitters' detection, such as dopamine detection through a silanized magnetic graphene oxide (Si-MG)-MIP-based chemiluminescence biosensor with a detection limit of 1.5 ng/mL,³⁰ thermal wave analysis on a functional MIP interface with a detection limit of 4.7×10^{-6} M,²⁶ an MIP-fabricated quartz crystal microbalance (QCM) biosensor,³¹ an MIP-modified field-effect transistor (FET) biosensor with the detection limit of 40 nM–20 μ M,³² and polypyrrole (PPy)/ZIF-67/Nafion hybrid-based MIP-modified GCE with a detection limit of 0.0308 μ M.³³ In other studies, researchers focused on MIP-associated nanomaterials, like an MIP-coated SiO₂ nanobead core–shell-decorated electrochemical sensor,³⁴ CNT/MIP-modified GCE with a detection limit of 1.8×10^{-10} mol,³⁵ a 2D hexagonal boron nitride (2D-hBN) nanosheet-incorporated graphene quantum dot (QD)-based MIP electrochemical sensor with a detection limit of 2.0×10^{-13} M,³⁶ and an MIP-based Mn²⁺-doped ZnS QD-modified fluorescence sensor with a detection limit of 0.69 ng/mL for serotonin detection in biological samples.³⁷ Similarly, MIP-based sensors for acetylcholine,^{38–41} histamine,⁴² and glutamate⁴³ detection have also been developed previously.

The electroanalytical strategies have been an excellent magnet due to their selective, rapid, cheap, and simpler use in the diagnostic area, although unmodified electrodes showed sluggish voltammetric responses along with less sensitivity toward the target. As a result, scientists are using different metallic nanoparticles to improve the sensitivity of the target within a small sample volume. MWCNT exhibited a high functionalization capability, which may help in binding the electrode surface and metallic nanoparticles.⁴⁴ Along with nickel nanoparticles, the MWCNT–NiNP system may improve the sensitivity and electric property of the developed sensing platform. Although their electrocatalytic properties may also affect the sensing platform, these catalysts lose their property over time and dissolve in the target solution. Therefore, using MIP may not only improve selectivity but also maintain the catalytic property and sensitivity of fabricated nanoparticles on the detection platform.

In this study, we attempt to detect AEA through MWCNT-conjugated NiNP and an MIP-decorated electrochemical platform, which have not been reported earlier in any study. Our proposed hypothesis is that AEA-imprinted MIP will result in an improved electrochemical response due to morphological restrictions of molecular grooves specifically for AEA. The efficient detection limit of the AEA-based sensing platform developed in this work has the potential to end up in point-of-care AEA detection in neural disease patients in hospitals as well as quick checkups at home.

2. RESULTS AND DISCUSSION

2.1. Brunauer–Emmett–Teller (BET) Analysis. The BET-based morphological analysis of nonimprinted polymer (NIP) and MIP after AEA removal is shown in Figure 1. The isotherm graph of samples demonstrated a type-1 adsorption isotherm that demonstrated restricted monolayer adsorption for a macroporous adsorbent (Figure 1A). The graph is a distorted version for monolayer adsorption where molecules were clumped and further adsorption did not occur. The surface area of MIP was also reported to be 65.522 m²/g, which is larger than that of NIP (Table 1); this supported the higher adsorption capacity of MIP in comparison with NIP.⁴⁵ The pore size distribution curve (Figure 1B) demonstrated two defined peaks at 0.0345 and 0.0331 nm for MIP, which are significantly higher than NIP-based peaks. This signifies the existence of nanocavities in the MIP matrix, although a pore diameter of <10 nm demonstrates the higher peak of NIP. This result is supported by SEM images of NIP and MIP after AEA elution along with UV–vis spectroscopy (Supporting Information).

2.2. Scanning Electron Microscopy (SEM). The SEM photographs demonstrate the restricted porous morphology of the MIP surface compared to that of NIP polymers (Figure 2A(a,b)). This may be because of selective AEA recognition cavities in the polymer that may be created during MIP synthesis.⁴⁶ Although imprinted sites mimicked the exact grooves of anandamide (data not shown) that justify the AEA-specific cavities at the nanoscale in a polymer system, there was a dense structure and no cavity was seen in the SEM image of NIP (Figure 2A(b)). The SEM images of MWCNT (Figure 2B(a)) and NiNPs (Figure 2B(b)) support nanoscale synthesis of these nanoparticles.

2.3. X-ray Diffraction (XRD). The XRD patterns of NIP and MIP before and after AEA removal are shown in Figure 3 in which each has different peaks approximately at 16.66,

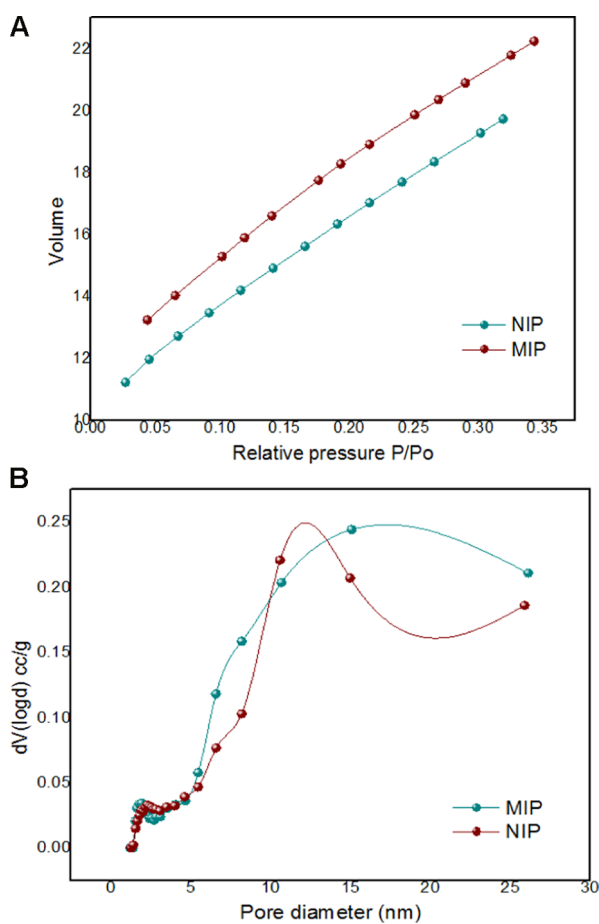


Figure 1. (A) Adsorption isotherm of anandamide-based NIP (cyan blue) and MIP (wine). (B) Pore size distribution curve of anandamide-based MIP (cyan blue) and NIP (wine).

Table 1. Surface Area, Pore Volume, and Pore Diameter of Anandamide-Based MIP and NIP

polymer	BET surface area, SBET (m ² /g)	pore volume (cm ³ /g)	pore diameter (nm)
MIP	65.522	0.172	8.192
NIP	59.781	0.150	10.574

16.03, and 17.21°, respectively. The intensity decrement before and after AEA removal demonstrated interaction between MMA and AEA, although shifting of the diffraction peak to larger 2θ before and after AEA removal indicated increased interlayer spacing along with weak interaction between AEA and the monomer.⁴⁷ In further inspections of the diffraction peak, the obtained full width at half-maximums (FWHMs) of NIP and MIP before and after AEA removal were 4.49, 6.97, and 16.99°, respectively. The smaller FWHMs suggest better mechanical properties with larger crystal size, which clearly supports NIP in our study. The diffraction pattern in Figure 3 supports both the crystalline and amorphous phases of the polymer system used. The results of the obtained 2θ values, calculated crystal size, and d -spacing are summarized in Table 2.

2.4. FTIR Spectrophotometry. FTIR results of NIP and MIP before and after template removal are depicted in Figure 4. The peaks at 1391.31 and 2958.27 cm⁻¹ denoted O–H binding vibration and O–H stretching and confirmed the presence of –COOH group during the interaction among

MMA, EGDMA, and AEA. Moreover, the peaks at 1165.4 and 1728.21 cm⁻¹ confirmed the existence of C–O and C=O stretching in the EGDMA cross-linker.⁴⁷ The variation in the peak intensity at 2958.27 cm⁻¹ (O–H stretching) supported the interaction of the O–H group of MMA with the O–H group of AEA to form H bonds, which was highly visible in the FTIR graph of MIP before AEA removal.⁴⁸ The intensity variation at the peak at 1466.35 cm⁻¹ demonstrated =C–H bending during polymerization, although the polymer–AEA binding (FTIR graph before AEA removal) lowered the peak intensity.

2.5. Preparation and Characterization of MWCNT/NiNP/MIP- or NIP-Fabricated SPE. Figure 5A showed the lowest current value (0.01 mA) with bare electrode (SPE); however, the maximum current response (0.11 mA) was observed after removal of the template (AEA). The surface of SPE manifested well-defined reversible redox peaks in an electrolyte solution (Figure 5A), which disappeared after MWCNT and NiNPs along with MIP electrodeposition. The results revealed that sequential deposition of nanoparticles improves the conductivity of fabricated electrode, but electropolymerization of AEA-based MIP impeded the electroactive molecules' diffusion to the electrode surface, which was recollected after removal of AEA from the fabricated surface (Figure 5A). Similar measurements were obtained from the PEIS Nyquist plot, in which MIP after AEA removal showed a low R_{ct} value (1 k Ω) in support of maximum conductivity than that of any another material deposited on SPE (Figure 5B). Although the NIP-based electrode did not show significant redox potential (data not shown), the reason is unknown.

2.6. Optimization Study of MWCNT/NiNP/AEA-MIP-Fabricated Electrode. Figure 6A demonstrates the Nyquist graph of different AEA (substrate) concentrations in which no sequential (nonlinear) changes in R_{ct} values were obtained in the range of 10–75 pM, although R_{ct} values gradually decreased with increasing concentration from 100 pM to 1 nM, as observed in Figure 6A. This decrement can be explained with the hypothesis of enhanced conductivity after increase in AEA concentration beyond 100 pM due to the presence of free electrons on the electrode surface, although 10 pM AEA showed the highest conductivity among all AEA concentration values and the observed linear range of the MIP-based AEA sensor was 100 pM–1 nM with a limit of detection (LOD) of 10 pM and sensitivity of 0.0149 mA/pM. PEIS measurements also helped to find the optimum pH and temperature of the MIP-fabricated electrode to be 9.0 and 70 °C (Figure 6B,D). The result of the interference study clearly indicated a significantly higher current response of AEA on a fabricated electrode, although acetylcholine and dopamine were interfering compounds in anandamide detection (Figure 6C). This may be due to the presence of –N–H and –O–H groups in AEA and dopamine; even though both AEA and ACh contain –N–H and –C=O groups, their chemical similarity may cause interference. Fifty percent activity was reported for the developed platform toward AEA after 4 months when it was stored at 4 °C. The accuracy was calculated for inter- and intrabatch coefficients of variation by adding 10 pM AEA in a gap of hours and days. The calculated accuracy was <1.51 and 2.48% for the intra- and interbatch, respectively. The results obtained from the spiked blood samples' electrochemical study also supported applicability of the developed platform in a clinical setup. The results are presented in Table 3, where initial and measured AEA

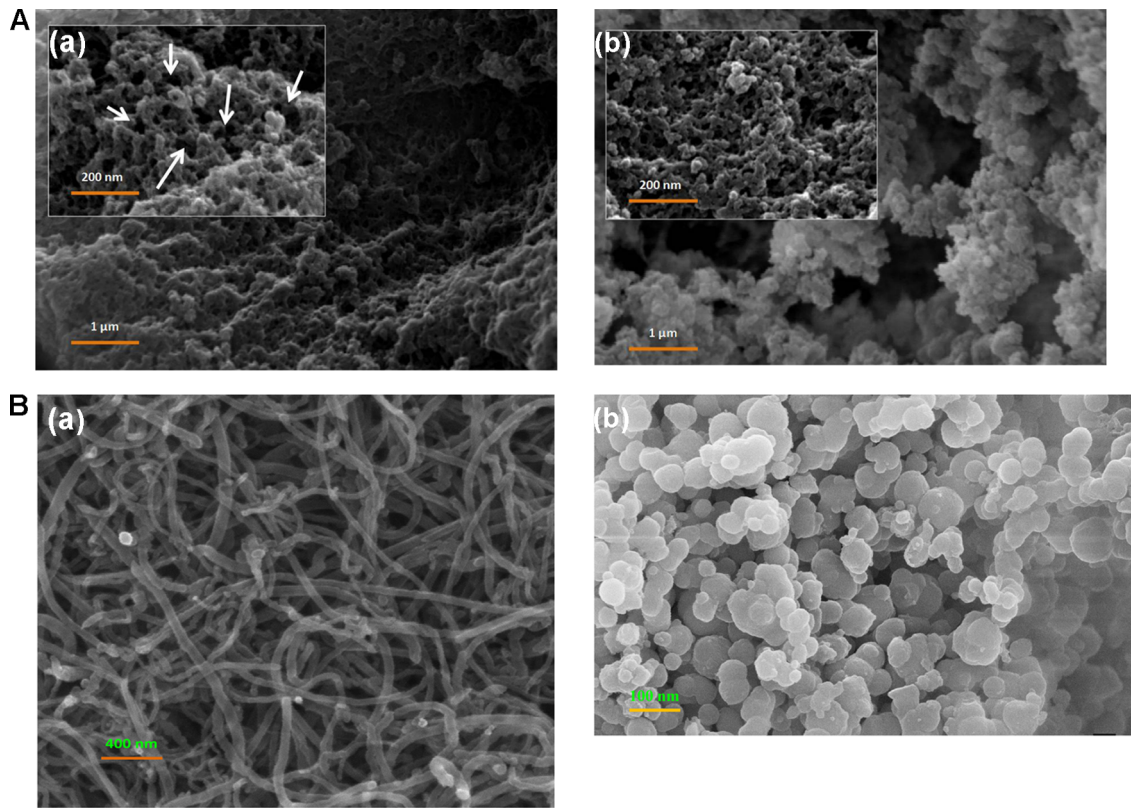


Figure 2. (A) Scanning electron microscopy (SEM) image of synthesized (a) MIP after anandamide (AEA) removal and (b) NIP. (B) Scanning electron microscopy (SEM) image of (a) MWCNTs and (b) NiNPs.

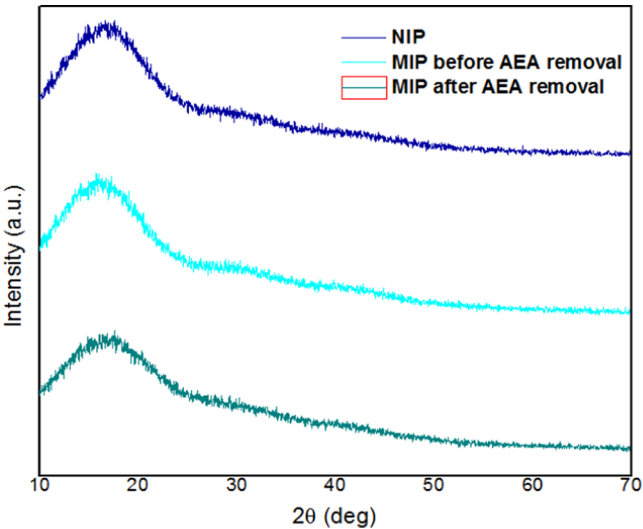


Figure 3. XRD pattern of NIP (navy blue) and MIP before (blue) and after (purple) anandamide (AEA) removal.

Table 2. Analysis of the XRD Pattern of NIP and MIP before and after AEA Removal

SN	sample type	2θ (deg)	d-spacing (nm)	FWHM (deg)	crystal size (nm)
1.	NIP	16.66	0.068466307	4.49	1.762955161
2.	MIP before AEA removal	16.03	0.076033581	6.97	1.127245556
3.	MIP after AEA removal	17.21	0.056308493	16.99	0.462102669

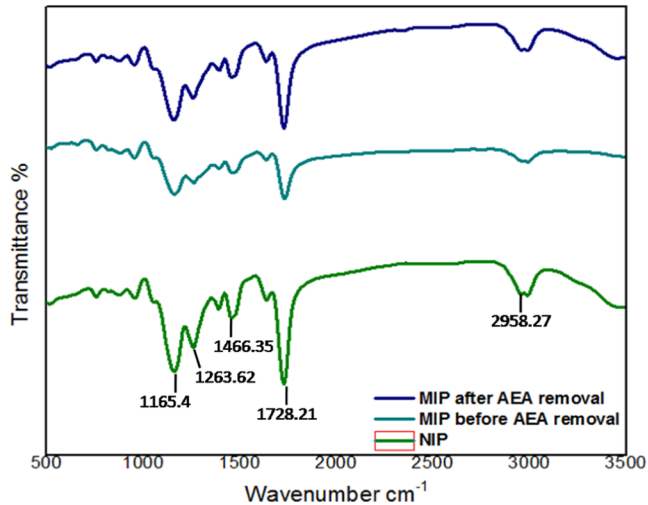


Figure 4. FTIR spectra of anandamide (AEA)-based NIP (green) and MIP before AEA removal (sky blue) and after AEA removal (cyan blue).

concentration values were used to calculate the recovery percentage. The obtained recoveries for the spiked blood samples were 93.48 and 90.08%, indicating the high accuracy of the MIP-based sensor for AEA determination in human blood samples.

3. CONCLUSIONS

A novel combined method of an electrochemical impedance spectroscopy (EIS) and MIP technology-based sensing platform for recognition of AEA was introduced in this study. The

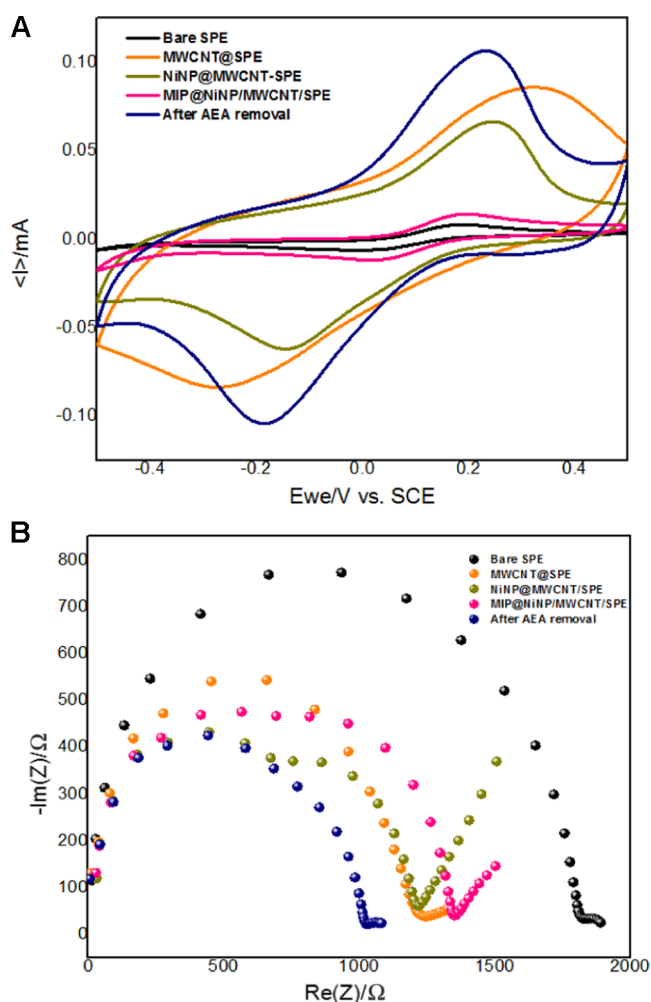


Figure 5. (A) Cyclic voltammetry (CV) (potential range of -0.5 to 0.5 V at a scan rate of 50 mV/s) in a 2.5 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ solution. (B) PEIS (frequency range of 100 Hz– 7 MHz at an amplitude of 100 mV) in a 2.5 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ solution.

proposed biosensor exhibited good selectivity and 0.0149 mA/pM sensitivity toward AEA detection, although the limit of detection (LOD) of the developed MIP sensor was reported as 10 pM, which is indeed less than that in conventional detection methods. Further modification and optimization of the electrode are under processing in our laboratory. However, at this stage, it should be noted that the developed nanosensor is simpler and stable in comparison with other immunosensors; therefore, the platform can be further modified for prognostic applications in neural disorders.

4. EXPERIMENTAL SECTION

4.1. Chemicals and Reagents. Anandamide arachidonyl ethanolamide (AEA ($\geq 97.0\%$, oil)), MWCNT, methylacrylate, ethylene glycol dimethylacrylate (EGDMA), azobisisobutyronitrile (AIBN, 98% and nickel(II) chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) obtained from Spectrochem Pvt. Ltd. Mumbai, India), were used. Other reagents like acetonitrile, acetic acid, ethanol, and methanol, including MMA and EGDMA, were purchased from Sigma-Aldrich, India. All solutions were freshly prepared in distilled water at room temperature (24 ± 3 °C). All electrochemical experiments were performed in ferro-ferric electrolyte solution (2.5 mM) at room temperature.

4.2. Apparatus and Procedures. The physical properties of AEA-based MIP and NIP were characterized using FTIR (Nicolet iS5, Thermo Scientific) and SEM. BET was performed via Quanta Chrome Novae-2200 at Ozone Scientific, Bengaluru, India. The electrochemical measurement of the AEA-based electrode was conducted using EC-Lab V11.10 software of Biopotentiostat workstation (BioLogic science Instrument, model no SP 200) equipped with a three-electrode cell. A 2.5 mM ferro-ferric electrolyte solution ($1:1$) was used for the electrochemical analysis of the MIP-based electrode system. The screen-printed electrode (SPE, ref no. C1110, Dropsens; $3.4 \times 1.0 \times 0.005$ cm³ ($l \times w \times h$)) was purchased from Dropsens, Spain.

4.3. Synthesis of Nickel Nanoparticles. The synthesis of NiNPs was carried out by El-Kemary et al. in 2013 with little modification.⁴⁹ The mixture of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.111 M) and absolute ethanol was heated until dissolved completely and used as the precursor. Then, 6.73 mL of hydrazine monohydrate solution was added drop by drop into a continuously stirred solution of nickel precursor. Potassium hydroxide was used to adjust the pH of the solution from 8.0 to 12 and allowed to stir for 2 h at 25 °C. Distilled water and acetone were used to wash the resultant solution. Finally, the obtained green nanoparticles of $\text{Ni}(\text{OH})_2 \cdot 0.5\text{H}_2\text{O}$ were forged and vacuum-dried. The thermal decomposition of the end product $\text{Ni}(\text{OH})_2 \cdot 0.5\text{H}_2\text{O}$ at 600 °C turned it into NiNPs. The light green powder was seal-packed and stored until further use under an inert condition to avoid moisture.

4.4. Preparation of Molecularly Imprinted Polymers. The neurotransmitter-based MIP synthesis has been already standardized in our laboratory. With the ratio of $1:4:4$, AEA, methyl methacrylate (MMA), and ethylene glycol dimethacrylate (EGDMA) were used as the template/functional monomer/cross-linker for MIP bulk polymerization. Then, 10 μL of AEA (1 M; stock concentration 5 mg/ μL), 674 μL of MMA, and 6.04 mL of EGDMA were dissolved in 5 μL of porogen and sonicated for 10 min followed by N_2 purging for 15 min. Five milligrams of AIBN was added to the AEA–MMA–EGDMA solution and seal-packed in inert conditions. The solution was placed on a rotating stirrer over an oil bath at 60 °C till the mixture got solidified. The resultant end product was crushed, ground, and sieved through a motor pistol. Then, half of the AEA-based MIP powder was packed under inert conditions for further characterization and the other half of the powder was used in AEA template removal from polymeric material particles in two steps. The obtained polymeric powder was transferred into 15 mL falcon tubes and mixed into $70:30$ v/v% mixtures of methanol and water. The solution was sonicated for 10 min followed by a 24 h hold. The methanol–water-based polymeric solution was centrifuged for 10 min at 6000 rpm the next day, and this washing cycle was repeated 5 times. The supernatant was discarded, and the polymeric precipitate was mixed in a $3:1:1$ ratio of porogenic solvent, acetic acid, followed by sonication for 10 min and holding for 48 h. At the end, the mixture was centrifuged for 10 min at 6000 rpm and this was repeated three times. The end product was dried and stored at an inert condition until further characterization. Similarly, nonimprinted polymers were prepared with the same protocol and polymerization condition as in MIP synthesis, excluding the addition of AEA as the template molecule (Scheme 1).

4.5. Characterization. **4.5.1. Scanning Electron Microscopy (SEM).** The surface morphologies of MIP (after AEA

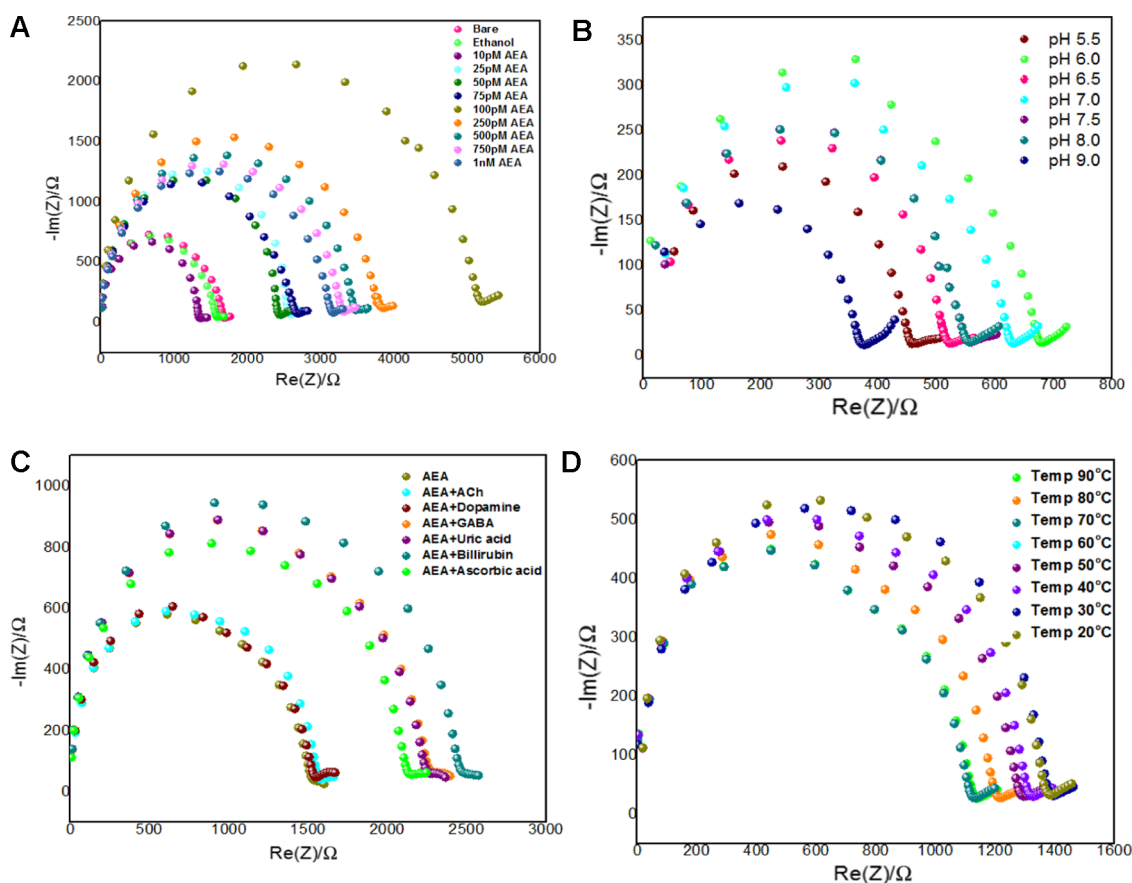


Figure 6. Nyquist graphs (a frequency range of 100 Hz–7 MHz at an amplitude of 100 mV) in a 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution: (A) At different concentrations of anandamide (AEA) ranging from 0.01 to 1 nM in 100 mM ethanol and (B) at different pH values ranging from 5.5 to 9.0, (C) Interference study in the presence of 0.1 μ L of AEA. (D) At different temperatures ranging from 20 to 90 $^{\circ}$ C.

Table 3. AEA Recoveries in Blood Samples Using an MIP-Based Sensor

SN	spiked value (nM)	measured value (nM)	recovery %
1.	0.5	0.46	93.48
2.	1	0.9	90.08

removal) and NIP along with those of MWCNT and NiNP were observed by SEM (ZEISS) at Amity University, Noida, India. MWCNTs were purchased commercially.

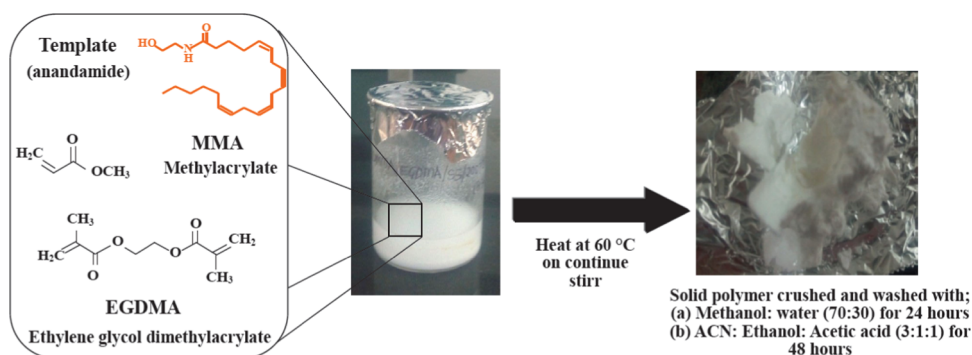
4.5.2. Brunauer–Emmett–Teller (BET) Analysis. The specific surface area, isotherm, and pore size distribution of

optimized MIP and corresponding NIP were analyzed through BET on commercial basis.

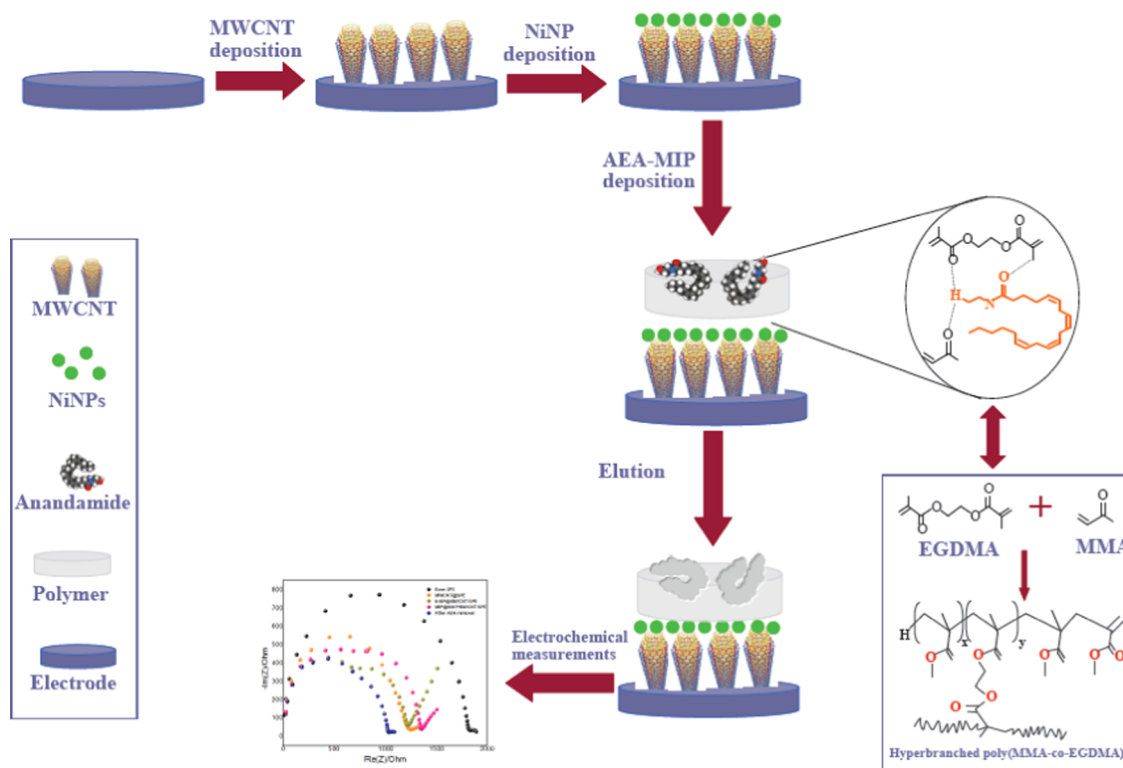
4.5.3. FTIR Spectrophotometry (FTIR). The compositions of NIP and MIP, before and after washing, were identified through FTIR spectra (Nicolet iSS, Thermo Scientific, India). The samples were settled in a sample holder and analyzed in the range of 500–3500 cm^{-1} .

4.5.4. X-ray Diffraction (XRD). The XRD patterns of NIP and MIP before and after washing were recorded at $\lambda = 1.5406$ Å and 40 kV/40 mA at Jamia Millia Islamia University, New Delhi. The d -spacing, FWHM, and crystal size were calculated from the observed data. The d -spacing was characterized by

Scheme 1. Bulk Polymerization for Anandamide-Based Molecularly Imprinted Polymer (MIP) Synthesis



Scheme 2. Fabrication and Electrochemical Study of the Working Electrode



the distance in the lattice plane of synthesized materials, which was calculated by Bragg's law

$$n\lambda = 2d \sin \theta$$

where n and λ symbolize the integer value and wavelength (1.504 nm), respectively, and θ is the Bragg angle, whereas the crystal size (D) is evaluated with the Debye–Scherrer equation, which is

$$D = K\lambda/(\beta \cos \theta)$$

where K is the Scherrer constant (0.94), θ is the Bragg angle, β is the full width at half-maximum (FWHM), and λ is the wavelength (1.504 nm).

4.6. Fabrication of MWCNT/NiNP/MIP on SPE. MWCNT, NiNP, and MIP/NIP were electrochemically deposited onto SPE in a specific sequence. At first, the SPE electrode was electrochemically washed with a 2.5 mM ferro-ferri electrolyte solution in the potential range of 1.0 to -1.0 V for 30 cycles. MWCNT was deposited onto SPE according to Rawal et al., 2011, with little modification.⁵⁰ One percent of MWCNT was electrochemically deposited onto SPE in the presence of 1 M KCl followed by sonication for 30 min. The cyclic voltammetric measurement was carried out in the potential range of 0.0–1.5 V for 30 cycles at a scan rate of 50 mV/s. In sequential NiNP deposition, CV was performed from the potential range of 0.0–1.5 V for 20 cycles at a scan rate of 20 mV/s in NiNP in a 2.5 mM ferro-ferri electrolyte solution. The electrochemical reaction that would have occurred can be presented as follows.

At the working electrode, nickel ions formed via



At the reference electrode, the reaction of nickel deposition can be given by



Then, SPE was fabricated with a fresh solution containing a 1:4:4 molar ratio of AEA, MMA, and EGDMA (prepared in porogen). The electrochemical deposition was carried out at -0.2 to 0.6 V at a scan rate of 50 mV/s for 15 cycles. The electrochemical analysis of MWCNT/NiNP/MIP-fabricated SPE was performed through cyclic voltammetry (-0.5 to 0.5 V potential range and 50 mV/s scan rate), and PEIS (a frequency range of 100 Hz–7 MHz at an amplitude of 100 mV) was performed in a 2.5 mM ferro-ferri electrolyte solution after each step of individual MWCNT, NiNP, and MIP/NIP electrodeposition. At the end, the electrode was washed with a 70:30 ratio of methanol/water (for 24 h) and a 3:1:1 ratio of porogen/ethanol/acetic acid (for 48 h) and similar electrochemical measurements CV and PEIS were performed after the washing of AEA. NIP was also electrochemically deposited on SPE at -0.2 to 0.6 V at a scan rate of 50 mV/s for 15 cycles, and CV and PEIS were carried out (Scheme 2).

4.7. Optimization and Evaluation of MIP Sensor. The analytical performance of the MWCNT/NiNP/MIP-modified electrode was explained through recognition of different concentrations of AEA on MIP surface via the R_{ct} value in the EIS technique (a frequency range of 100 Hz–7 MHz at an amplitude of 100 mV). To study the effect of different AEA concentrations, 10, 25, 50, 75, 100, 250, 500, 750 pM, and 1 nM concentrations of AEA were used in the PEIS-based study in the presence of the ferro-ferri electrolyte solution. The MIP-based sensor was optimized for pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0 phosphate buffers (each at 0.1 M) in which electrochemical measurements were performed for these buffers and the 2.5 mM ferro-ferri electrolyte solution.

Similarly, an electrochemical study was performed for the heated ferro-ferri electrolyte solution at a gradually decreasing temperature from 90 to 20 °C at a difference of 10 °C. Selectivity of the sensor was estimated by the electrochemical response of other interfering biochemicals such as acetylcholine (ACh), dopamine, GABA, uric acid, bilirubin, and ascorbic acid in the interference study of the MIP-modified electrode. For further evaluation of analytical reliability and potential application in a clinical system, the platform was used to detect AEA in spiked blood samples. Different concentrations of 0.1 mL of AEA were spiked in blood samples, and electrochemical measurements were recorded. Deidentified blood samples were collected from Biodiagnostic Lab., East Rohini, Delhi, India, and stored at −20 °C before use.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c00285>.

Experimental setup of UV spectroscopy to determine AEA removal from polymer matrix (PDF)

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Author Contributions

N.C. and U.J. conceived and designed the experiments; S.S. and U.J. performed the experiments; the UV–vis spectroscopy analysis was conducted at M.K.'s laboratory; S.S., U.J., and N.C. further analyzed the data; N.C. contributed reagents/materials/analysis tools; S.S. and U.J. wrote the paper, which was further corrected by U.J. and N.C.; M.K. helped with performing and examining the analytical performance of the developed sensor; and Y.P.S.B. helped with clinical aspects of AEA and its importance in neural disorders.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Lanz, C.; Mattsson, J.; Stickel, F.; Dufour, J. F.; Brenneisen, R. Determination of the Endocannabinoids Anandamide and 2-Arachidonoyl Glycerol with Gas Chromatography-Mass Spectrometry: Analytical and Preanalytical Challenges and Pitfalls. *Med. Cannabis Cannabinoids* **2018**, *1*, 9–18.
- (2) Scherma, M.; Masia, P.; Satta, V.; Fratta, W.; Fadda, P.; Tanda, G. Brain Activity of Anandamide: A Rewarding Bliss? *Acta Pharmacol. Sin.* **2019**, 309–323.
- (3) Thieme, U.; Schelling, G.; Hauer, D.; Greif, R.; Dame, T.; Laubender, R. P.; Bernhard, W.; Thieme, D.; Campolongo, P.; Theiler, L. Quantification of Anandamide and 2-Arachidonoylglycerol Plasma Levels to Examine Potential Influences of Tetrahydrocannabinol Application on the Endocannabinoid System in Humans. *Drug Test. Anal.* **2014**, *6*, 17–23.
- (4) Patel, S.; Hill, M. N.; Cheer, J. F.; Wotjak, C. T.; Holmes, A. The Endocannabinoid System as a Target for Novel Anxiolytic Drugs. *Neurosci. Biobehav. Rev.* **2017**, 56–66.
- (5) Devane, W. A.; Hanuš, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor. *Science* **1992**, *258*, 11946–11949.
- (6) Fowler, C. J. Anandamide Uptake Explained? *Trends Pharmacol. Sci.* **2012**, 181–185.
- (7) Kirkham, T. C. Endocannabinoids in the Regulation of Appetite and Body Weight. *Behav. Pharmacol.* **2005**, *16*, 297–313.
- (8) Woodhams, S. G.; Chapman, V.; Finn, D. P.; Hohmann, A. G.; Neugebauer, V. The Cannabinoid System and Pain. *Neuropharmacology* **2017**, *124*, 105–120.
- (9) Fride, E. The Endocannabinoid-CB 1 Receptor System in Pre- and Postnatal Life. *Eur. J. Pharmacol.* **2004**, 289–297.
- (10) Gardner, E. L. Endocannabinoid Signaling System and Brain Reward: Emphasis on Dopamine. *Pharmacol., Biochem. Behav.* **2005**, 263–284.
- (11) Tanda, G. Modulation of the Endocannabinoid System: Therapeutic Potential against Cocaine Dependence. *Pharmacol. Res.* **2007**, 406–417.
- (12) Mereu, M.; Tronci, V.; Chun, L. E.; Thomas, A. M.; Green, J. L.; Katz, J. L.; Tanda, G. Cocaine-Induced Endocannabinoid Release Modulates Behavioral and Neurochemical Sensitization in Mice. *Addict. Biol.* **2015**, *20*, 91–103.
- (13) Kathuria, S.; Gaetani, S.; Fegley, D.; Valiño, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La Rana, G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. Modulation of Anxiety through Blockade of Anandamide Hydrolysis. *Nat. Med.* **2003**, *9*, 76–81.
- (14) Mayo, L. M.; Asratian, A.; Lindé, J.; Holm, L.; Nätt, D.; Augier, G.; Stensson, N.; Vecchiarelli, H. A.; Balsevich, G.; Aukema, R. J.; et al. Protective Effects of Elevated Anandamide on Stress and Fear-Related Behaviors: Translational Evidence from Humans and Mice. *Mol. Psychiatry* **2018**, 1–13.
- (15) Lanz, C.; Mattsson, J.; Stickel, F.; Dufour, J. F.; Brenneisen, R. Determination of the Endocannabinoids Anandamide and 2-Arachidonoyl Glycerol with Gas Chromatography-Mass Spectrometry: Analytical and Preanalytical Challenges and Pitfalls. *Med. Cannabis Cannabinoids* **2018**, *1*, 9–18.
- (16) Giuffrida, A.; Leweke, F. M.; Gerth, C. W.; Schreiber, D.; Koethe, D.; Faulhaber, J.; Klosterkötter, J.; Piomelli, D. 2004. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* **2004**, *29*, 2108–2114.
- (17) Giuffrida, A.; Rodríguez De Fonseca, F.; Piomelli, D. Quantification of Bioactive Acylethanolamides in Rat Plasma by Electrospray Mass Spectrometry. *Anal. Biochem.* **2000**, *280*, 87–93.
- (18) Maccarrone, M.; Attinà, M.; Cartoni, A.; Bari, M.; Finazzi-Agrò, A. Gas Chromatography-Mass Spectrometry Analysis of Endogenous

Cannabinoids in Healthy and Tumoral Human Brain and Human Cells in Culture. *J. Neurochem.* **2001**, 76, 594–601.

(19) Chen, Y.; Azad, M. B.; Gibson, S. B. Superoxide Is the Major Reactive Oxygen Species Regulating Autophagy. *Cell Death Differ.* **2009**, 16, 1040–1052.

(20) Richardson, D.; Pearson, R. G.; Kurian, N.; Latif, M. L.; Garle, M. J.; Barrett, D. A.; Kendall, D. A.; Scammell, B. E.; Reeve, A. J.; Chapman, V. Characterisation of the Cannabinoid Receptor System in Synovial Tissue and Fluid in Patients with Osteoarthritis and Rheumatoid Arthritis. *Arthritis Res. Ther.* **2008**, 10, No. R43.

(21) Grasso, S.; Santonico, M.; Bisogno, T.; Pennazza, G.; Zompanti, A.; Sabatini, A.; Maccarrone, M. An Innovative Liquid Biosensor for the Detection of Lipid Molecules Involved in Diseases of the Nervous System. *Proceedings* **2018**, 2, 760.

(22) Palanisamy, S.; Cheemalapati, S.; Chen, S. M. Amperometric Glucose Biosensor Based on Glucose Oxidase Dispersed in Multi-walled Carbon Nanotubes/Graphene Oxide Hybrid Biocomposite. *Mater. Sci. Eng., C* **2014**, 34, 207–213.

(23) Zaidi, S. A.; Shin, J. H. Recent Developments in Nanostructure Based Electrochemical Glucose Sensors. *Talanta* **2016**, 30–42.

(24) Liu, B.; Xiao, B.; Cui, L.; Wang, M. Molecularly Imprinted Electrochemical Sensor for the Highly Selective and Sensitive Determination of Melamine. *Mater. Sci. Eng., C* **2015**, 55, 457–461.

(25) Betlem, K.; Down, M. P.; Foster, C. W.; Akthar, S.; Eersels, K.; van Grinsven, B.; Cleij, T. J.; Banks, C. E.; Peeters, M. Development of a Flexible MIP-Based Biosensor Platform for the Thermal Detection of Neurotransmitters. *MRS Adv.* **2018**, 3, 1569–1574.

(26) Casadio, S.; Lowdon, J. W.; Betlem, K.; Ueta, J. T.; Foster, C. W.; Cleij, T. J.; van Grinsven, B.; Sutcliffe, O. B.; Banks, C. E.; Peeters, M. Development of a Novel Flexible Polymer-Based Biosensor Platform for the Thermal Detection of Noradrenaline in Aqueous Solutions. *Chem. Eng. J.* **2017**, 315, 459–468.

(27) Canfarotta, F.; Rapini, R.; Piletsky, S. Recent Advances in Electrochemical Sensors Based on Chiral and Nano-Sized Imprinted Polymers. *Curr. Opin. Electrochem.* **2018**, 146–152.

(28) Yáñez-Sedeño, P.; Campuzano, S.; Pingarrón, J. M. Electrochemical Sensors Based on Magnetic Molecularly Imprinted Polymers: A Review. *Anal. Chim. Acta* **2017**, 1–17.

(29) Ansari, S. Application of Magnetic Molecularly Imprinted Polymer as a Versatile and Highly Selective Tool in Food and Environmental Analysis: Recent Developments and Trends. *TrAC, Trends Anal. Chem.* **2017**, 89–106.

(30) Duan, H.; Li, L.; Wang, X.; Wang, Y.; Li, J.; Luo, C. A Sensitive and Selective Chemiluminescence Sensor for the Determination of Dopamine Based on Silanized Magnetic Graphene Oxide-Molecularly Imprinted Polymer. *Spectrochim. Acta, Part A* **2015**, 139, 374–379.

(31) Naklua, W.; Suedee, R.; Lieberzeit, P. A. Dopaminergic Receptor-Ligand Binding Assays Based on Molecularly Imprinted Polymers on Quartz Crystal Microbalance Sensors. *Biosens. Bioelectron.* **2016**, 81, 117–124.

(32) Kajisa, T.; Li, W.; Michinobu, T.; Sakata, T. Well-Designed Dopamine-Imprinted Polymer Interface for Selective and Quantitative Dopamine Detection among Catecholamines Using a Potentiometric Biosensor. *Biosens. Bioelectron.* **2018**, 117, 810–817.

(33) Zhang, W.; Duan, D.; Liu, S.; Zhang, Y.; Leng, L.; Li, X.; Chen, N.; Zhang, Y. Metal-Organic Framework-Based Molecularly Imprinted Polymer as a High Sensitive and Selective Hybrid for the Determination of Dopamine in Injections and Human Serum Samples. *Biosens. Bioelectron.* **2018**, 118, 129–136.

(34) Introna, B.; Malitesta, C.; Mazzotta, E.; Rella, S.; Mohammadi, R.; Ramezany, F.; Sellerger, B. In *Electrochemical Sensor for Serotonin Based on a Composite Made of Core-Shell Molecularly Imprinted Polymer Nanoparticles and Polyethylenedioxythiophene*, Proceedings of the 2015 18th AISEM Annual Conference; AISEM, 2015; pp 1–4.

(35) Wang, Z.; Xu, L.; Wu, G.; Zhu, L.; Lu, X. Development and Application of the Serotonin Voltametric Sensors Based on Molecularly Imprinting Technology. *J. Electrochem. Soc.* **2015**, 162, B201–B206.

(36) Yola, M. L.; Atar, N. A Novel Detection Approach for Serotonin by Graphene Quantum Dots/Two-Dimensional (2D) Hexagonal Boron Nitride Nanosheets with Molecularly Imprinted Polymer. *Appl. Surf. Sci.* **2018**, 458, 648–655.

(37) Chauhan, N.; Pundir, C. S. Amperometric Determination of Acetylcholine-A Neurotransmitter, by Chitosan/Gold-Coated Ferric Oxide Nanoparticles Modified Gold Electrode. *Biosens. Bioelectron.* **2014**, 61, 1–8.

(38) Chauhan, N.; Chawla, S.; Pundir, C. S.; Jain, U. An Electrochemical Sensor for Detection of Neurotransmitter-Acetylcholine Using Metal Nanoparticles, 2D Material and Conducting Polymer Modified Electrode. *Biosens. Bioelectron.* **2017**, 89, 377–383.

(39) Chauhan, N.; Tiwari, S.; Narayan, T.; Jain, U. Bionzymatic Assembly Formed @ Pt Nano Sensing Framework Detecting Acetylcholine in Aqueous Phase. *Appl. Surf. Sci.* **2019**, 474, 154–160.

(40) Kamel, A. H.; Al Hamid, F. A.; Soror, T. Y.; Galal, H. R.; El Gendy, F. A. Solid Contact Biosensor Based On Man-Tailored Polymers For Acetylcholine Detection: Application To Acetylcholinesterase Assay. *Eur. Chem. Bull.* **2015**, 5, 266–273.

(41) Sacramento, A. S.; Moreira, F. T. C.; Guerreiro, J. L.; Tavares, A. P.; Sales, M. G. F. Novel Biomimetic Composite Material for Potentiometric Screening of Acetylcholine, a Neurotransmitter in Alzheimer's Disease. *Mater. Sci. Eng., C* **2017**, 79, 541–549.

(42) Akhondian, M.; Rüter, A.; Shinde, S. Ultratrace Detection of Histamine Using a Molecularly-Imprinted Polymer-Based Voltametric Sensor. *Sensors* **2017**, 17, 265.

(43) Karthikeyan, M.; Rajkumar, R.; Kumar, M. D.; Balamurugan, M.; Muthukumar, K.; Karunakaran, C. Theoretical Screening and Selection of Functional Monomers for Molecular Imprinted Electropolymer as Sensor for Glutamate. *Sens. Lett.* **2018**, 16, 590–597.

(44) Sireesha, M.; Jagadeesh Babu, V.; Kranthi Kiran, A. S.; Ramakrishna, S. A review on carbon nanotubes in biosensor devices and their applications in medicine. *Nanocomposites* **2018**, 4, 36–57.

(45) Zhou, T.; Tao, Y.; Jin, H.; Song, B.; Jing, T.; Luo, D.; Zhou, Y.; Zhou, Y.; Lee, Y. I.; Mei, S. Fabrication of a Selective and Sensitive Sensor Based on Molecularly Imprinted Polymer/Acetylene Black for the Determination of Azithromycin in Pharmaceuticals and Biological Samples. *PLoS One* **2016**, 11, No. e0147002.

(46) Alizadeh, T.; Akhondian, M.; Movahed, Z. Indirectly Determination of Urea Using Chloride Ion Selective Electrode after Its Extraction and Preconcentration by Molecularly Imprinted Polymer. *Anal. Bioanal. Electrochem.* **2009**, 1, 246–256.

(47) Yanti, Nurhayati, T.; Royani, I.; Widayani; Khairurrijal. Synthesis and Characterization of MAA-Based Molecularly-Imprinted Polymer (MIP) with D-Glucose Template. *J. Phys.: Conf. Ser.* **2016**, No. 012143.

(48) Fauziah, S.; Soekamto, N. H.; Taba, P.; Amran, M. B. Selectivity of β -Sitosterol Imprinted Polymers as Adsorbent. *J. Phys.: Conf. Ser.* **2018**, No. 012059.

(49) El-Kemary, M.; Nagy, N.; El-Mehasseb, I. Nickel Oxide Nanoparticles: Synthesis and Spectral Studies of Interactions with Glucose. *Mater. Sci. Semicond. Process.* **2013**, 16, 1747–1752.

(50) Rawal, R.; Chawla, S.; Dahiya, T.; Pundir, C. S. Development of an Amperometric Sulfite Biosensor Based on a Gold Nanoparticles/Chitosan/Multiwalled Carbon Nanotubes/Polyaniline-Modified Gold Electrode. *Anal. Bioanal. Chem.* **2011**, 40, 2599–2608.