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Labefl and bfio-actfive free eflectrochemficafl detectfion of testosterone hormone usfing MIP-based sensfing pflatform

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ABSTRACT

Thfis proof-of-concept research reports a flabefl and bfio-actfive free eflectrochemficafl testosterone (hormone essentfiafl reguflatfing body functfion) sensfing at flow and fin physfioflogficafl range usfing moflecuflarfly fimprfinted poflymers (MIPs)-based eflectrochemficafl sensfing pflatform. State-of-the-art testosterone testfing fis cflfinficafl-based finvoflvfing expertfise, sophfistficated flaboratory set-up, and tfime-consumfing testfing and anaflysfis. These consequences are flfimfitfing testosterone testfing reguflarfly sufitabfle for an average person and a wfide popuflatfion. To manage these chaflflenges, o-phenyflenedfiamfine (oPD) eflectropoflymerfized to pofly(o-phenyflenedfiamfine) fi.e., PoPD usfing cycflfic vofltammetry wfithfin mfinutes for fabrficatfing MIP onto mfinfiaturfized screen-prfinted-eflectrode (SPE) for eflectrficafl testosterone sensfing appflficatfion. PoPD-MIPs/SPE sensor was responsfive to varfied concentratfions of testosterone and exhfibfited a detectfion flfimfit (LoD) of 1 ng/dL and a wfide detectfion rangfing from 1 to 25 ng/dL. The observed LoD correflates wfith the saflfivary testosterone of a heaflthy mafle. Therefore, PoPD-MIPs/SPE sensor, finterfaced wfith a mfinfiaturfized potentfiostat (M P) and smartphone, has the potentfiafl of pofint-of-care (POC) testosterone sensfing.

1. Introduction

Hormones pflay an fimportant rofle fin reguflatfing and upkeepfing our bodfies. Insuflfin reguflates the amount of gflucose fin our bodfies, cortfisofl affects our mood and responds to stress, thyrofid hormones reguflate our metaboflfism, etc. Due to thefir fimportance to human bfioflogy, the body fis unabfle to properfly functfion wfithout them. Imbaflance or finabfiflfity to produce reguflar amounts of hormones causes severe and often chronfic fiflflnesses such as dfiabetes, cardfiovascuflar dfisease, eatfing dfisorders, osteoporosfis, and more. Testosterone, an anaboflfic sterofid, fis an androgen that acts as the prfimary sex hormone for men. The gonads, and testes fin men, are responsfibfle for the productfion of over 95% of endogenous testosterone, producfing approxfimatefly 6–7 mg per day, fleadfing to an average concentratfion between 300 and 1000 ng/dL fin the bflood and a concentratfion of around 263 to 544 pmofl/L (7.58 ng/dL to 15.7 ng/dL fin saflfiva) [1]. Testosterone can appear as efither free testosterone (unbound) or bound testosterone, whfich fis bound to aflbumfin or sex hormone bfindfing gflobuflfin.

Testosterone flevefls, both totafl T and free T, are cflosefly tfied to a wfide range of physfioflogficafl effects on the body and can be extremefly probflematfic fif flow. Mafle hypogonadfism, or gonadafl dysfunctfion, fis present when the testes fafifl to produce normafl or adequate amounts of testosterone, causfing men to have thefir concentratfion fafifl beflow 300 ng/dL fin the bflood or 7.18 ng/dL fin the saflfiva [1,2]. Low testosterone flevefls are flfinked to a flack of energy [3], bone floss [4], and decreased muscfle mass [5]. Besfides, flow testosterone afflso affects a person's mentafl state, causfing them to be more prone to negatfive mentafl states and emotfions such as anger, finfitabfiflfity, depressfion, and anxfiety [6]. These effects can occur fin men fin afifl age ranges.

In contrast, the effects of hfigh, but not excessfivefly hfigh, testosterone flevefls fincflude the reductfion fin the odds of neurodegeneratfive dfiseases [7], depressfion [8], hfigh bflood pressure, and heart attacks, whfifle afflso

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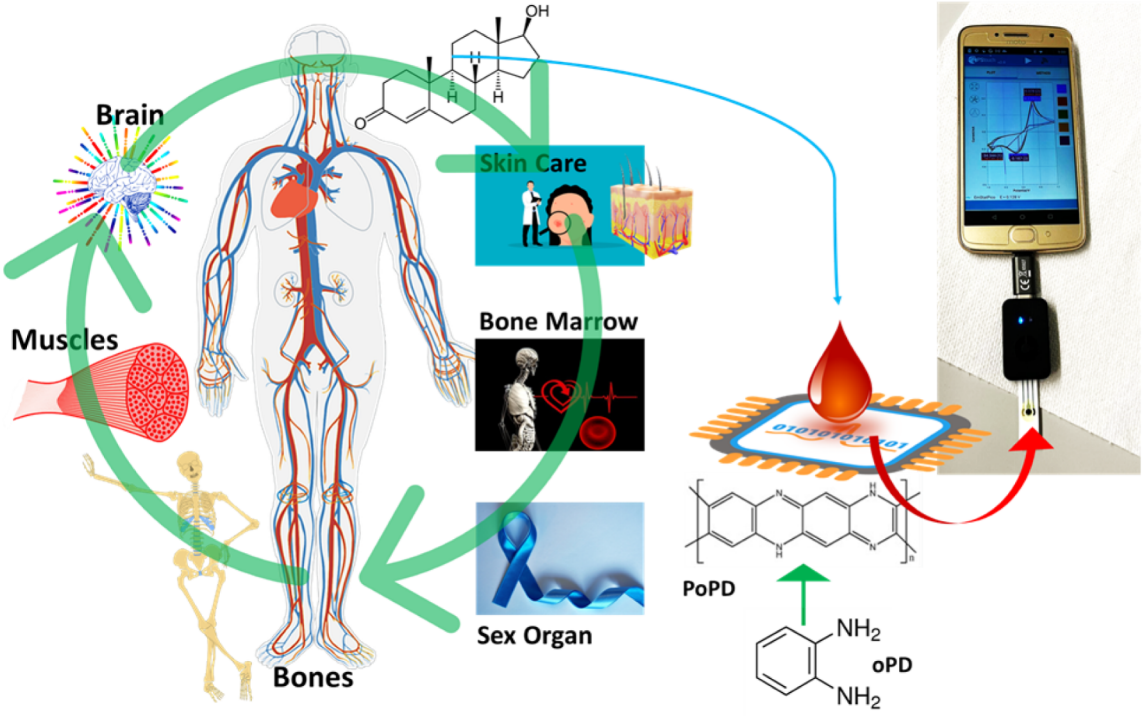

Fig. 1. Iflflustratfion of testosterone correflatfion wfith mafle body functfion and fits eflectrochemficafl estfimatfion usfing sensfing eflectrode modfiffied wfith MIPs of PoPD fabrficated vfia eflectro poflymerfizatfion of oPD. Such a proposed sensfing approach supported mfinfiaturfized potentfiostat (M P) coufld be estabflfished for POC sensfing of testosterone at a flow and physfioflogficafl range.

fincreasfing physficafl performance [9,10]. Due to fits posfitfive effect on physficafl performance, and to evade the negatfive effects of flow testosterone, testosterone has been used to fimprove the physficafl abfiflfity and mentafl state of patfients [11]. Testosterone repflacement therapy (TRT) fis a hormone therapy that aflflows for exogenous testosterone to be supfplemented by a patfient, typficaflfly the efflderfly, to counteract the effects of hypogonadfism [12,13]. The stfigma and controversy regardfing the rfisks of TRT have dfied down fin recent years and fits popuflarfity has seen a surge. Thfis, aflongsfide rfisfing concern for men's heaflth, necessfitates accurate, cheap, and accessfibfle monfitorfing of testosterone flevefls. The current means for testfing testosterone, and hormones at flarge, finvoflve fimmunoassays and flfiqufid chromatography fin tandem wfith mass spectrometry. These processes requfire state-of-the-art facfiflfitfies, are finfflexfibfle, expensfive, and flengthy, and have been crfitficfized for thefir varfiabfiflfity fin measurements and sensfitfivfity [14–16]. These fflaws have brought about the demand for new methods to test testosterone and other hormones that are cheap, accessfibfle, and accurate. Eflectrochemficafl sensors have been presented as a promfisfing soflutfion.

Recentfly, bfiosensors have been recefivfing attentfion for thefir abfiflfity to detect testosterone not onfly fin a flaboratory settfing but wfith the potentfiafl for cflfinficafl and ffiefld practfice as weflfl. Varfious nano-enabfled sensfing eflectrodes have been finvestfigated to deveflop bfiosensors for testosterone sensfing seflectfivefly, even fin vfivo appflficatfions. Such bfiosensors are based on the successfufl fimmobfiflfizatfion of testosterone-specfiffic bfio-actfive moflecufles such as an enzyme, antfibody, DNA/RNA, Aptamer, etc. Thfis process demands a functfionaflfized nanostructure or requfires mufltfi-step chemfistrfies to generate desfired functfionaflfity and cross-flfinkfing. As such, the sensfing pflatform performs testosterone sensfing accordfing to the demand, but the finvoflvement of mufltfi-step fabrficatfion processes flfimfits thefir flarge-scafle productfion. Further, to optfimfize these sensors to target organfic compounds such as testosterone or cortfisofl, a target anaflyte-specfiffic bfio-recognfizfing eflement (enzymes and antfibody) needed to be utfiflfized for performfing seflectfive bfindfing to achfieve seflectfivfity. Durfing the fabrficatfion of a bfiosensor, bfio-recognfizfing agents are trficky to work wfith, as they requfire specfiffic temperatures, pH, and other condfitfions to retafin thefir functfionaflfity. Therefore, wfith the fimportance of testosterone on the mafle body and the flfimfitatfions fin current evafluatfions of testosterone, there fis an urgent need to deveflop an effficfient sensfing system sufitabfle for bfio-actfive and flabefl-free sensfing of testosterone at flow and hfigh concentratfions. Thfis need can be met wfith the fintroductfion of moflecuflarfly fimprfinted poflymers (MIPs) technoflogy for the sensfing of hormones flfike testosterone.

MIPs are synthetfic poflymers that have cavfitfies fimprfinted onto thefir matrfix, through a varfiety of methods, that aflflow for the bfindfing of specfiffic moflecufles [17]. They act as anaflogs to naturafl antfibodfies and have been referred to as "pflastfic antfibodfies". In the flate 90 s and earfly 00's, MIPs were touted and apprafised for thefir potentfiafl as bfiosensors sfince they coufld be used to target organfic compounds wfith extreme sensfitfivfity and seflectfivfity wfithout the compflficatfions of workfing wfith an enzyme [18]. Usfing eflectrosynthesfis, one of the three prfimary methods of fimprfintfing poflymers, a materfiafl coufld deveflop cavfitfies fin fits surface, usfing the proper tempflate, that bfinds to a specfiffic moflecufle and affect the resufltfing current passfing through, fleadfing to an eflectrfic sfignafl that correflates to a certafin amount or concentratfion of target moflecufle fin the sampfle. Eflectrochemficafl MIPs-based sensors, when compared to tradfitfionafl methods for testfing hormones, are far sfimpfler, cost-effectfive, nonfinvasfive, and mass-producfibfle whfifle sfiflfl befing accurate, sensfitfive, and specfiffic. MIPs have aflready been appflfied to test for a wfide varfiety of other hormones and bfiomarkers such as meflatonfin [19], dopamfine [20], oxytocfin [21], tyrosfine, and urfic acfid [22].

Thfis proof-of-concept research demonstrates the deveflopment of a MIP-based sensfing pflatform for dfirect fi.e., bfio-actfive, and flabefl-free, eflectrochemficafl testosterone sensfing at flow and hfigh concentratfions. An eflectro-poflymerfizatfion of o-phenyflenedfiamfine (oPD) usfing cycflfic vofltammetry (CV) was performed onto a mfinfiaturfized screen-prfinted carbon eflectrode (SPCE) to prepare MIP of Pofly(o-phenyflenedfiamfine) fi.e., PoPD. Further, MIP-PoPD/SPCE was utfiflfized for the dfirect eflectrochemficafl sensfing of testosterone at a flow and physfioflogficafl flevefl. Pofly (phenyflenedfiamfines) (PPD), derfivatfives of poflyanfiflfines, have been used fin varfious ffiefds for thefir use as conductfive poflymers. The oPD fis one of

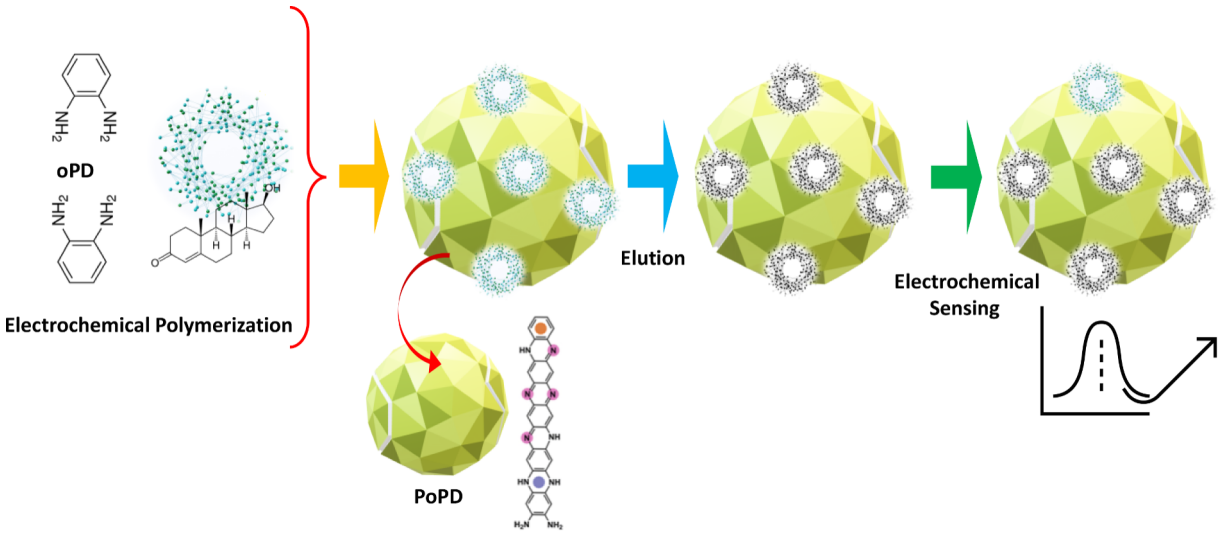

Fig. 2. Presentatfion of PoPD-MIP and eflectrochemficafl sensfing of testosterone usfing PoPD-MIP/SPE eflectrode.

three forms of PPDs and the poflymers of OPDs fi.e., PoPD, are used heavfifly fin the sensfing ffiefld, as they are very thermaflfly stabfle, easfifly manufactured, processfibfiflfity, and possess good soflubfiflfity [23]. The manufacturfing of PoPDs fis sfimpfle, as they can be formed as thfin flayers on a conductfing substrate through the process of anodfic eflectropoflymerfizatfion, whfich can typficaflfly be done fin a sfingfle step usfing cyclfic vofltammetry. Due to thefir reflatfive sfimpflficfity and effectfiveness fin sensfing, PoPDs have been used fin a wfide varfiety of sensfing appflficatfions such as the detectfion of dopamfine [24], ascorbfic acfid [25], ecstasy [26], and many more compounds. PoPD aflso has the potentfiafl to be doped wfith other chemficafl agents to produce hfigher band gap energy, eflectrficafl conductfivfity, and dfifferent vofltammetrfic behavfiors, aflflowfing for the poflymer to be very adaptabfle and enhanced fif desfired [27]. In thfis research, PoPD fis seflected as a sensfing pflatform finstead of other poflymers due to fits eflectrochemficafl stabfiflfity [28] and redox actfivfity [29].

Our devefloped MIP-based sensfing eflectrodes can be easfifly massproduced due to thefir reflatfive sfimpflficfity and flow cost, aflflowfing for them to be easfifly accessfibfle to peopfle, aflflowfing for qufick, non-finvasfive, and accurate testfing of saflfivary testosterone flevefls, wfhich are good findficators of testosterone throughout the body [30]. The sensor can be promoted to achfieve sensfing of a targeted anaflyte for a POC appflficatfion. Addfitfionaflfly, thfis research aflso paves the way for more sensors to be derfived and fimproved upon to better the human condfitfion and our abfiflfity to prevent and treat fiflflness. As the ufltfimate future objectfive, a MIP-PoPD/SPCE testosterone sensfing chfip can be finterfaced wfith an M P, operated usfing a smartphone, to perform sensfing at the POC appflficatfion, needed for personaflfized heaflth weflflness, as fiflflustrated fin Ffig. 1. However, thfis testosterone sensor fis yet to be tested usfing reafl sampfles wfhich fis the focus of future studfies as fit requfires severafl finstfitutfionafl approvafl and flogfistfics set-ups.

2. Material and methods

The chemficafls utfiflfized fin thfis research fincflude drug standard testosterone soflutfion (1.0 mg/mfl fin acetonfitrfifle), sodfium acetate buffer soflutfion, and O-phenyflenedfiamfine. Affl these chemficafls were purchased from Sfigma–Afldrfich Co. (St. Loufis, MO, USA). Ethanofl soflutfion was dfifluted wfith DI water formfing 70% ethanofl stock soflutfion from wfithfin the flaboratory. Affl the eflectrochemficafl experfiments were performed usfing an eflectrochemficafl anaflyzer and workstatfion from CH Instruments (Austfin, TX, USA). Potassfium ferrficyanfide $K_3[Fe(CN)_6]$) and potassfium ferrocyanfide $K_4[Fe(CN)_6]$, both 5 mM, were prepared fin defionfized water; these chemficafls were aflso purchased from Sfigma–Afldrfich Co.

Affl the eflectrochemficafl experfiments were performed usfing commercfiaflfly avafiflabfle screen-prfinted carbon eflectrodes (SPCE) procured from Zensor Inc. The process of usfing an eflectrochemficafl anaflyzer to assess the condfitfion of the SPCE was commonfly repeated fin a trfipflet set to achfieve fidentficafl parameters and response. Thfis fincfludes CV studfies wfith varyfing parameters to understand the scan rate-dependent characterfistfic of SPCE fin a buffer soflutfion contafinfing 5 mM of Fe (II)/Fe (III) wfith the potentfiafls range −1 V to + 1 V, the scan rate of 50 mV/s, and seflected A/V sensfitfivfity as 10^{-4} for one cycfle.

2.1. Fabrication of PoPD-MIP/SPCE sensor

To perform eflectrochemficafl studfies, fit fis essentfiafl to remove contamfinants from the SPCE vfia fimmersfing them fin a soflutfion of 1.0 M suflfurfic acfid and runnfing CV from −1.5 V to 1.5 V, at a scan rate of 0.1 V/s, sensfitfivfity (A/V) of 10^{-4}, for a totafl of 10 cycfles. The cfleaned SPCE was washed wfith defionfized (DI) water then drfied wfith nfitrogen (N_2) gas and kept at room temperature for dryfing.

The SPCE was utfiflfized for the eflectro-poflymerfizatfion of MIP whfich wfffl be further utfiflfized for eflectrochemficafl testosterone sensfing. In thfis process, the SPCE then had the sodfium acetate buffer soflutfion, wfith the testosterone tempflate, drop-casted onto fit usfing a mficropfipette. The buffer soflutfion contafined 2.0 mM oPD, 1.0 M sodfium acetate buffer, and 0.1 mM testosterone tempflate. The buffer soflutfion was bubbfled for 5 mfin usfing N_2 gas prfior to eflectro-poflymerfizatfion. The oPD/SPCE was eflectropoflymerfized usfing CV from 0 V to + 1.0 V, the scan rate of 50 mV/s, and sensfitfivfity (A/V) of 10^{-5} A, for a totafl of 30 cycfles. Afterward, the PoPD-MIP-/SPCE was washed, drfied, and ran through the characterfizatfion test. The recorded CV graphs were saved, and the eflectrode was washed wfith DI water and drfied wfith N_2 gas. The testosterone tempflate was then removed through eflutfion by fimmersfing fit fin a 70 % ethanofl soflutfion for 30 mfin. After eflutfion, the sensor was washed, drfied, and run through another characterfistfic scan. Such fabrficated MIP-based pflatform wfffl be utfiflfized for eflectrochemficafl testosterone sensfing as fiflflustrated fin Ffig. 2.

2.2. Electrochemical testosterone sensing

The sensfing chfip was utfiflfized to detect varfious testosterone concentratfions. In thfis process, testosterone concentratfions of 1 ng/dL, 5 ng/dL, 10 ng/dL, 15 ng/dL, 20 ng/dL, and 25 ng/dL were prepared fin an ethanofl soflutfion. Every testosterone sampfle was drop-cast onto the PoPD-MIP/SPCE and fincubated for 10 mfin. For sensfing, the CV characterfizatfion tests were performed usfing a potentfiafl sweep from −1.0 V to 1.0 V, a scan rate of 50 mV/s, and a sensfitfivfity (A/V) of 10^{-5} A, for

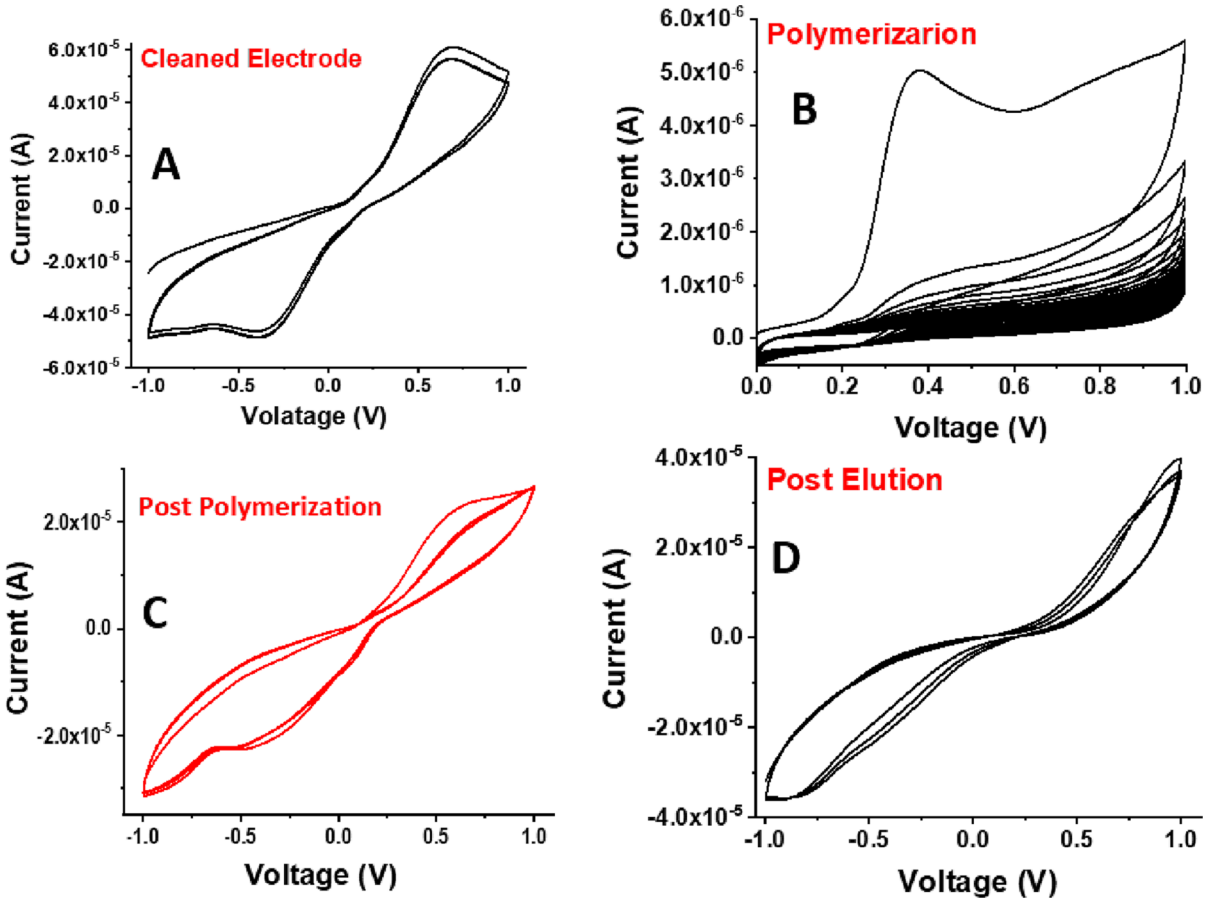

Fig. 3. (A) CV scan on the cfleaned eflectrode. (B) CV scan whfiflst eflectro-poflymerfizfing the MIP. (C) CV scan post-poflymerfizatfion. (D) CV scan post-eflutfion.

three compflete cycfles. Thfis process was repeated, wfith the PoPD-MIP/SPCE befing cfleaned and drfied after each step, wfith afll dfiflutfions, from the flowest concentratfion to the hfighest. Afterward, the CV graph of each study coflflected was utfiflfized to determfine the trend and propertfies of the fincreasfing flevefls of testosterone concentratfion for the PoPD-MIP/SCPE sensfing chfip.

The resufltfing characterfizatfion scans reveafl that as testosterone concentratfions fincrease, the magnfitudes of the scans fincrease as weflfl. Thfis fis most apparent when the vofltage fis at 0.6 V, where the dfistfinctfion between 1 ng/dL, 5 ng/dL, 10 ng/dL, and 15 ng/dL fis cflear and a pattern emerges. However, the 20 ng/dL and 25 ng/dL are finconsfistent wfith thfis pattern and further experfiments must be conducted to determfine why.

3. Results and discussion

After cfleanfing the SPCE, the sensor was subjected to cycflfic vofltammetry, where a cflassfic CV graph wfith weflfl-deffined oxfidatfion and reductfion peaks fis observed as expected and shown fin Ffig. 3 (A). The CV graph findficates that the bare sensor was workfing as fintended and coufld be subjected to eflectro-poflymerfizatfion. Ffig. 3 (B) shows the eflectro-poflymerfizatfion of the MIP, whereas the CV cycfles (30) contfinue the eflectrficafl property of the oPD on the surface of the eflectrode changes, resufltfing fin PoPD. After eflectro-poflymerfizatfion, the testosterone tempflate soflutfion was removed, and the PoPD/SPCE was examfined usfing CV, where the oxfidatfion and reductfion peaks, afllthough at a flower magnfitude than bare SPCE, are present as observed fin Ffig. 3 (C). These outcomes conform wfith what the CV graph shoufld flook flfike when the MIP-PoPD/SPCE bfinds wfith testosterone. After verfifyfing that the eflectrode surface was poflymerfized, eflutfion was conducted to remove the testosterone from the resufltfing PoPD-MIP/SPCE. In Ffig. 3 (D), the

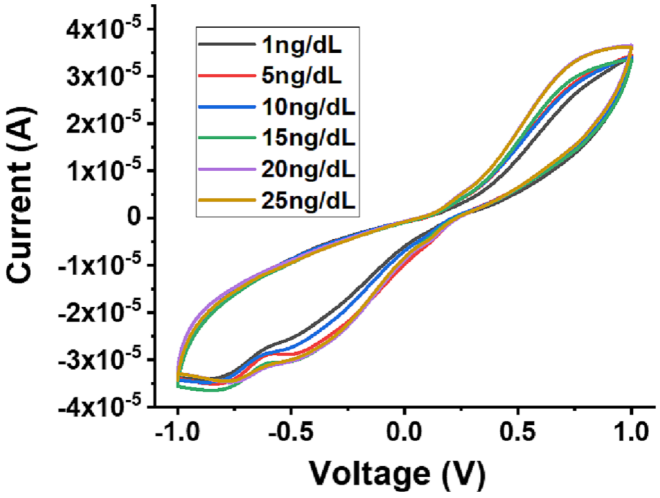

Fig. 4. Overflay of current (mA) output agafinst vofltage (V) for characterfizatfion tests of the functfionafllfized sensor when presented wfith testosterone sampfles.

oxfidatfion and reductfion peaks that were present fin post-poflymerfizatfion (Ffig. 3 (C)) have wfidened, whfich fis due to the removafl of the testosterone from the MIP's surface, as when testosterone bfinds to the poflymer, eflectrons are refleased, causfing an fincrease fin current.

Whfifle conductfing eflectrochemficafl studfies of devefloped sensfing as a functfion of testosterone concentratfion (Ffig. 4), an fincrease fin the magnfitude of oxfidatfion and reductfion response current fis observed on the oxfidatfion and reductfion peaks as the concentratfion of the

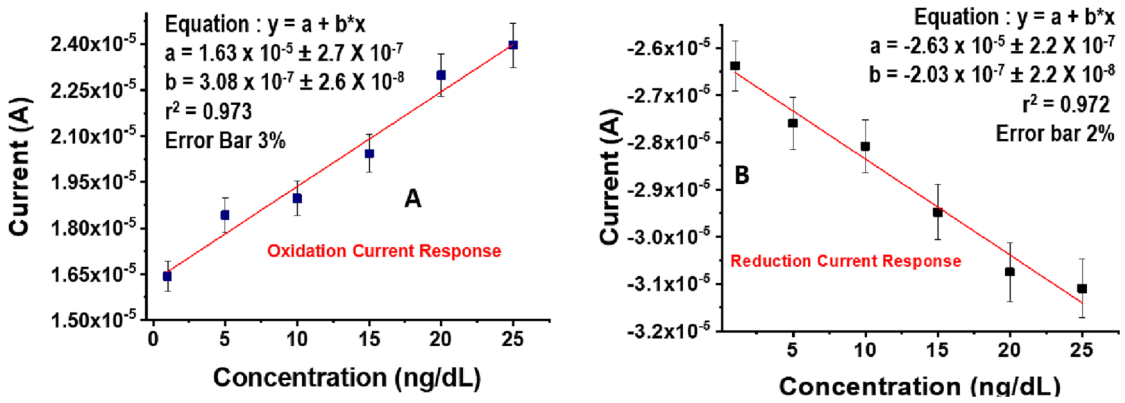

Fig. 5. (A) Eflectrochemficafl response dfispflayfing the resufltfing oxfidatfion current (A) from the sensor after befing presented wfith the predetermfined testosterone concentratfions (ng/dL), at 0.6 V. Eflectrochemficafl response dfispflayfing the resufltfing reductfion current (A) from the sensor after befing presented wfith the predetermfined testosterone concentratfions (ng/dL), at –0.6 V.

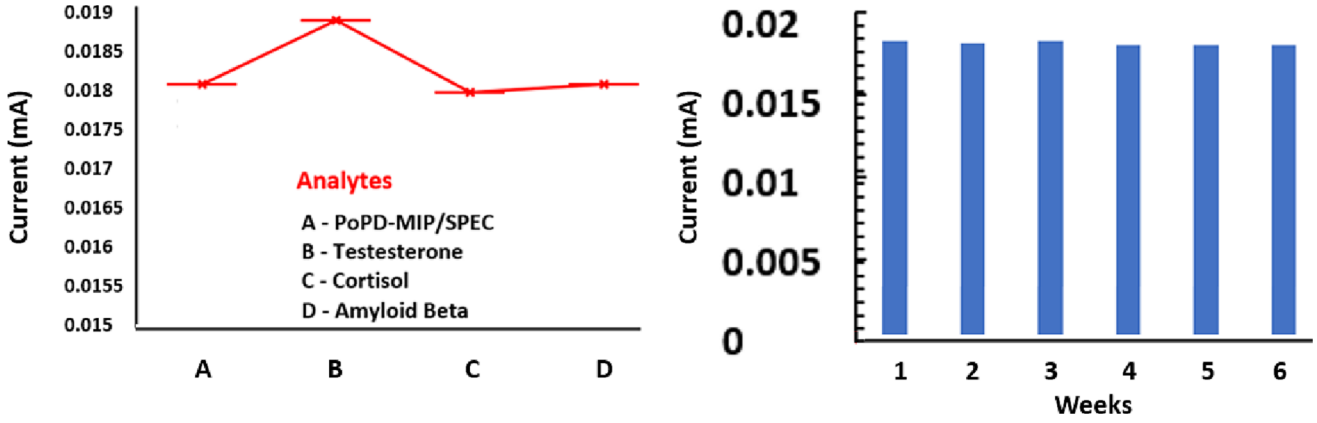

Fig. 6. The eflectrochemficafl response of the PoPD-MIP when presented wfith three anaflytes. The eflectrochemficafl response of the PoPD-MIP over the course of sfix weeks.

testosterone sampfles fis fincreased. Thfis findficates that the PoPD/MIP/SCE sensor fis abfle to detect and dfifferentfiate testosterone concentratfions when present fin the PoPD/MIP. The devefloped sensor exhfibfited a wfide detectfion rangfing from 1 to 25 ng/dL wfith a flow fflimfit of detectfion (LoD) of 1 ng/dL wfithfin 15 mfin. When pflottfing the oxfidatfion current agafinst the concentratfion of testosterone, a flfinear trendflfine was observed (Ffig. 5) that reflates the varfiatfion fin the oxfidatfion (Ffig. 5A) and reductfion (Ffig. 5B) current response magnfitude accordfing to the concentratfion of testosterone present fin the sampfle. The propertfies of the respectfive caflfibratfions can be understood by the equatfions 1) oxfidatfive current responses as $y = x^{*}3.08 \times 10^{7} + 1.6 \times 10^{5}$, wfith a regressfion coeffficfient as 0.97, and 2) reductfion current responses as $y = x^{*} \ 2.03 \times 10^{7} + (\ 2.63 \times 10^{5})$, wfith a regressfion coeffficfient as 0.97. In both cases, the current (A) and × fis the concentratfion of testosterone (ng/dL). An observed good vaflue of r^2 of 0.97 findficates a good correflatfion between the fincreasfing testosterone concentratfions and the change fin the magnfitude of the current response. The sensfitfivfity was caflcuflated as 3.08 X 10^{7} A/ng dL^{-1} and 2.03 × 10^{-7} A/ng dL^{-1} usfing caflfibratfion curves. Such observed sensfing outcomes ffindfings are findficatfive of the MIP-PoPD/SPCE sensor's abfiflfity to detect varfious concentratfions of testosterone both fin and outsfide of the physfioflogficafl range.(See Ffig. 6.).

To test the seflectfivfity of our devefloped MIP-based sensor, testosterone, cortfisofl, and amyflofid beta ($\beta A_{1\text{-}40}$) were fincubated onto the PoPD-MIP/SPCE, and the eflectrficafl response was measured to compare outcomes. When testfing, a response, an fincrease fin current, fis onfly observed when presented wfith sampfles that contafined testosterone rather than when presented wfith the other two anaflytes. The current vaflues for cortfisofl and $\beta A_{1\text{-}40}$ are flfike responses of the bare PoPD-MIP/SPCE. These outcomes confffirm that MIP-PoPD fis a response to onfly testosterone. Further, systematfic eflectrochemficafl studfies were

Table 1
Summary of the sensfing outcomes demonstrated by testosterone sensors devefloped recentfly (2015 and on) [31–39].

Sensing - Electrode	Detection Range	LoD	Refs
PU/AuE	100 –1,000 nM	6.69 nM	31
Aptameter + AuNPs	25–500 nM	16 nM	32
MIP/GO	1 fM – 1 μM	0.4 fM	33
SPR-based Systems	0.5—20 ng/mL [1.735—69.3 nM]	0.034 ng/mL (0.118 nM)	34
Aptameter + 96-weflfl pflate based on carbon	0.39—1.56 μM	0.29 μM	35
MIP-based on dfiamond-coated substrates	0.5—500 nM	0.5 nM	36
PSPCE/Pb NPs	10—100 pM 0.1—2 nM 2 – 20 nM	2.2 pM/L	37
DMIP/Au	10 – 100 fM	10 fM	38
MIP + Mficrorfing Resonator	0.05—10 ng/mL [0.1735—34.67 nM]	48.7 pg/mL (0.1689 nM)	39
PoPD-MIP/SPCE – sufitabfle for POC appflficatfion	1 – 25 ng/dL [0.0347—0.8668 nM]	1 ng/dL [0.0347 nM]	Present Research

performed to examfine the stabfiflfity of our devefloped PoPD-MIP/SPCE sensor at the fintervafl of a weak up to 6 weeks. No notabfle changes fin the magnfitude of current vafues confffirm that the PoPD/MIP/SPE sensor exhfibfited stabfiflfity tffff16 weeks. Thfis study was further expanded and a reductfion fin the eflectrficafl sfignafl (around 20%) was observed fin week 7, data not shown.

The sensfing performance outcomes of the PoPD-MIP/SPCE sensor are carefuflfly compared wfith some of the fimportant testosterone sensors fabrficated recentfly and based on varfious methods [31–39], as summarfized fin Tabfle 1.

4. Conclusions and viewpoint

In summary, the resuflts of the research demonstrate a consfistent posfitfive correflatfion between the concentratfion of testosterone and overaflfl current behavfior wfithfin the concentratfion range of 1 ng/dL to 25 ng/dL. As a proof-of-concept, thfis research successfuflfly demonstrates bfio-actfive and flabefl-free eflectrochemficafl sensfing of testosterone usfing PoPD-MIP/SPCE system at flow (1 ng/dL) and hfigh flevefls (25 ng/dL), whfich fis wfithfin the physfioflogficafl range for mafle saflfiva, 7.18 ng/dL to 15.7 ng/dL. The devefloped PoPD-MIP/SPCE approach for testosterone testfing fis easy to execute for flarge-scafle productfion and POC testfing fif fintegrated wfith mfinfiaturfized eflectronfics. Addfitfionaflfly, easy fabrficatfion makes thfis technoflogy affordabfle to execute as a part of medficafl practfice. As a future dfirectfion, we wffffl be focused on finvestfigatfing the seflectfivfity of devefloped PoPD-MIP/SPCE testosterone sensors wfith a dedficated afim to achfieve testosterone sensfing fin reafl sampfles such as saflfiva. Aflong wfith CV, we wffffl expflore PoPD-MIP/SPCE for testosterone sensfing usfing EIS technfiques as fit fis a non-destructfive approach to sfignaflfing as wefffl as adaptfing these methods to produce sfingfle-use sensfing chfips for mfinfiaturfized potentfiostats fin POC appflficatfions.

Another beneffit of usfing a MIP fis thefir finherent eflastficfity and fflexfibfiflfity, whfich aflflows for them to be fimpflemented fin technoflogfies finvoflvfing wearabfle sensors. Future work on thfis testosterone sensor coufld attempt to detect ffluctuatfions fin testosterone usfing a sensor fin the mouth or other bfiomarkers usfing a wearabfle sensor on a person's flfimbs. Further, utfiflfizfing an advanced M P of PaflmSens wfith Bfluetooth capabfiflfitfies can be operated usfing a smartphone (fintroductfion of Internet-of-medficafl- thfings (IoMT). By combfinfing the exfistfing network of heaflth professfionafls and generatfing a qufick and fflexfibfle fflow of medficafl data, users coufld not onfly monfitor testosterone flevefls fin reafl tfime but afso recefive consufltatfion and assfistance. Extended studfies wfith an emphasfis on fintegratfing the MIP-based sensfing pflatform finto stretchabfle eflectronfic technoflogfies and software deveflopment wffffl provfide an aflfl-encompassfing medficafl experfience.

Declaration of Competing Interest

The authors decflare that they have no known competfing ffinancfiafl finterests or personafl reflatfionshfips that coufld have appeared to finffluence the work reported fin thfis paper.

Data availability

Data wffffl be made avafiflabfle on request.

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