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Electroanalytical overview: The electroanalytical detection of theophylline

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ABSTRACT

In this overview, we explore the electroanalytical determination of theophylline. Theophylline finds use as a bronchodilator for treating diseases such as asthma and chronic obstructive pulmonary disease (COPD). There is a need to measure the concentration of theophylline in pharmaceuticals for QA/QC purposes as well as in plasma samples to ensure the doses of theophylline are at the correct therapeutic levels. If the concentration levels of theophylline deviate from the therapeutic levels (10–20 μg/mL for asthma), then patients can experience adverse effects. As such, there is a desire to progress from traditional laboratory based techniques to portable rapid testing. In this overview, we review the endeavours directed to the development of theophylline electroanalytical sensors, noting current and future trends.

Introduction: theophylline

Theophylline (1,3-dimethylxanthine; see Scheme 1) is a naturally occurring plant alkaloid which has been used for over 80 years to treat asthma, chronic obstructive pulmonary disease and neonatal apnoea worldwide, since it is inexpensive and widely available [1-4]. Theophylline is usually taken in tablet or capsule form and monitoring is required via blood/serum samples to ensure correct dosages are being administered, as well as its measurement for quality assurance/quality control (QA/QC) of the tablet/capsule. Theophylline has a narrow therapeutic window and is rapidly absorbed from the gastrointestinal tract when administrated in liquid form or as an uncoated table with peak concentrations occurring within 1–2 h on an empty stomach or 6–10 h after food. Theophylline has a half-life average of 6–9 h and is excreted in urine with only a small fraction unchanged, with most of the drug metabolised in the liver and urinary excretion of the metabolites 1,3 dimethyluric, 1-methyluric acid and 3-methlyxanthine. Note that aminophylline is a formulation that contains theophylline with ethylenediamine in a 2:1 ratio; the ethylenediamine improves solubility and makes it more suitable for intravenous use, but is less potent and shorter-acting than theophylline. The therapeutic window of theophylline, as sampled in serum is 10–20 μg/mL for asthma, and 6–11 μg/mL in neonatal apnoea [5]. Outside this window, below 10 μg/mL mild effects such as nausea, headache and jitteriness occur, while at concentrations above 20 μg/mL more serious side effects such as tremors, agitation, insomnia, diarrhoea, palpitations, cardiac arrhythmias and seizures; hence, it is clearly important to accurately measure theophylline levels. Electroanalytical sensing platforms have potential for this target analyte allowing progression from traditional laboratory based equipment to portable, rapid devices to improve efficiency and patient outcome. Consequently, in this overview, we explore the evolution of sensing theophylline from an electroanalytical perspective, exploring recent trends and new directions.

Detection of theophylline

As is the case for important analytes, rigorous laboratory based analytical techniques have been reported, including, for example: high performance liquid chromatography (HPLC) [6-8], radioimmunoassay [9], fluorescence polarization immunoassay [10], capillary electrophoresis [11] and capillary chromatography [12]. Electrochemical methods offer distinct advantages over these analytical methods, which include: fast, precise, portable and affordable methods/devices whilst also offering high sensitivity and selectivity towards electroactive analytes [13].

Hyphenated techniques have been utilised for the detection of theophylline [14-17] and overcome the potential disadvantage, in some cases, of electrochemical approaches, where in complex samples, selectivity might be an issue when compared to traditional laboratory based analytical techniques. Zhang et al. [17] reported theophylline and caffeine determination using a poly(dimethylsiloxane) microchannel electrophoresis with electrochemical detection using a carbon fiber microdisk electrode (diameter of 8 μm). A linear range from 6 μM to 0.6 mM was shown to be possible for both theophylline and caffeine with detection limits of 4 μM. This analytical methodology was successfully applied to determine caffeine and theophylline in rat serum and urine, which was found to agree with individual HPLC analysis. This analytical approach, as stated by the authors has advantages such as: cheapness, simplification, good resolution, and low reagent consumption [17]. Augustijns and Verbeke described the use of HPLC with elec-

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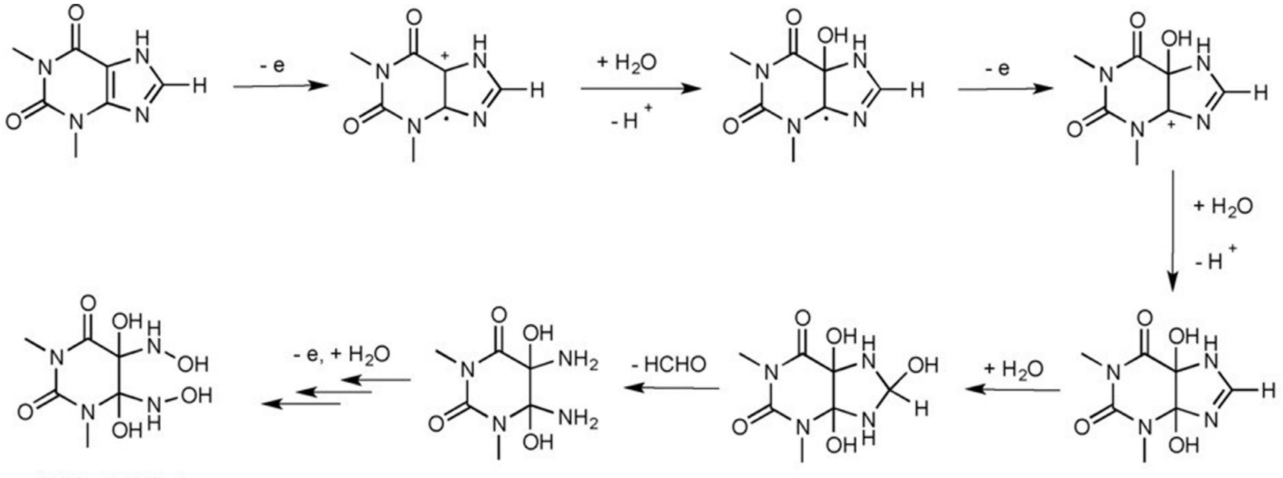

1 (M = 222 Da)

2 (M = 214 Da)

Scheme 1. Electrochemical oxidation products of theophylline in pH 7 on a platinum electrode**.** Reproduced with permission from Ref [57]. Copyright Wiley 2019.

trochemical detection (HPLC-ECD), using a double electrode configuration method for the sensing of theophylline which exhibited a linear range from 2.5 to 20 pg/mL with a limit of detection (LOD) of 0.2 pg/mL. The authors determined theophylline concentration in human plasma and compared their approach with a fluorescence-polarized immunoassay, providing good agreement between the two analytical methods. Through the use of electrochemical detection, a 5 times improvement in the sensitivity could be obtained as compared with UV detection [15]. Meyer and co-workers [16] detailed the use of HPLC-ECD with a glassy carbon (GC) electrode for the detection of theophylline with adenine, caffeine and theobromine; the authors noted that in comparison with the UV-detection the sensitivity of the amperometric detection was ~ 2–5 fold higher. Given the benefits of hyphenated techniques using electrochemical-based detectors, where improved sensitivities with inherent selectivity can be realised, it is surprising there are not more literature reports directed to pursuing this avenue of research.

Electroanalytical approaches

Table 1 overviews the current literature, at the time of writing this review, of the various endeavours directed to the sensing of theophylline; consequently, below we explore the current approaches and trends.

As expected, biosensors have been developed for theophylline detection; for example Wang et al. [18] developed an amperometric biosensor for theophylline, based on the enzyme theophylline oxidase, which is entrapped together with a ferricytochrome C cofactor, within a polymeric (Nafion) coating. The authors report, using flow-injection measurements, that the anodic detection (at +0.4 V vs Ag|AgCl) is facilitated by the addition of a redox-mediating hexacyanoferrate(iii) ion at a platinum macrodisc electrode. A detection limit of 2×10^{-6} M was shown to be possible with linearity prevailing up to 3×10^{-4} M. The rapid flow-injection measurements allows a frequency of 180 samples per hour to be potentially realised. Stredansky and co-workers [19] described the use of xanthine oxidase to fabricate amperometric enzyme electrodes for the sensing of theophylline. Fig. 1 shows the construction of the enzymatic electrode and the underlying sensing mechanism of the theophylline biosensor. The microbial xanthine oxidase in the presence of molecular oxygen oxidizes theophylline with the produced hydrogen peroxide detected by the peroxidase–ferrocene system. The reduced mediator is then simultaneously regenerated upon the electrode surface giving rise to an amperometric signal, which is directly proportional to the theophylline concentration. The detection limit of 2×10^{-7} M is 10 times lower than other work using theophylline oxidase [20] and the low working potential (0 mV vs SCE) produces a significant decrease of interfering signals arising from electroactive species present in real samples [19]. The authors show that their enzyme biosensor could determine free (unbound) and total theophylline in whole blood samples. The biosensor exhibited good operational (>6 hrs) and shelf (>3 months) stability when trehalose was used as a stabiliser of the biocatalytic layer [19].

There are limited reports of the development of potentiometric sensors [21, 22] for theophylline determination. One approach reports the use of 2,6-bis(phenyl)-4(phenyl)3H-thiopyran (PPT) as the ionophore in a PVC membrane, exhibiting a linear range from 1.0×10^{-6} to 1.0×10^{-2} M for theophylline detection with a slope 54.5 ± 0.4 mV per decade with a limit of detection (LOD) of 5.5×10^{-7} M [21]. A large range of possible interferents were explored demonstrating the sensor to be highly selective to theophylline and was consequently applied to the determination of theophylline in tablets. Other approaches have constructed a carbon paste electrode where graphite powder is mixed with CuO nanoparticle functionalised MWCNTs and an ionophore, which exhibited a linearity from 1×10^{-2} M to 1×10^{-7} M with a LOD of 2.5×10^{-8} M and stability observed for 46 days [22]. This approach was shown to measure theophylline in a range of green tea extracts, which involved boiling each tea sample followed by filtration and dilution and modification to the pH chosen for analysis.

Molecularly imprinted polymers (MIPs) have been utilised in sensing platforms as biomimetic recognition elements for a wide range of analytical targets [23] and it is therefore no surprise that MIPs have been explored for theophylline [24-30]. For example, Kindschy and Alocilja discribed the development of a MIP that was highly selective to theophylline over caffeine but with a poor detection range (mM) [25]. Of note, Zhang et al. [30] reported a biomimetic sensor for theophylline detection, which is summarised in Fig. 2. First, a gold macroelectrode was polished, cleaned and the surface was modified via electropolymerisation with a functional monomer (thiophene-3-acetic acid, 3-TAA) and theophylline. Following this, theophylline was removed using acetonitrile and an applied potential (+0.4 V). The next step involves the addition of initiator-coupled theophylline into the electrode surface via surface initiated atom transfer radical polymerization (SI-ATRP) and was chosen since it can be performed with a variety of functional monomers at mild temperatures, and in aqueous or organic solvents. Acrylamide (AM) was next used as the growing chain units. The polymer (PAM; Polyacrylamide) was formed and then functionalized with phenothiazine sodium sulfonate (PTZ-343). Fig. 2 shows the response in the presence and absence of theophylline. Using differential pulse voltammetry (DPV), the authors demonstrated that the MIP based sensor could detect theophylline over the range 0.03 nM – 30 μM with a remarkable LOD of

Table 1
An overview of various electrochemical approaches reported towards the detection of theophylline. Note that the therapeutic window of theophylline is 10–20 μg/mL (55.5–111 μM) (see text).

Electrode	Electrode modifier	Technique	Linear range	Limit of detection	Sample medium	Reference
Carbon	Nitrocellulose/Antibodies/liposomes	Amperometric	10–20 μg/mL	5 μg/mL	Aqueous	[75]
GC	Nafion®/Pb-Ru oxide pyrochlore	SWV	0.1–100 μM	0.1 μM	Drug tablet, black tea, green tea	[76]
Graphite	Xanthine oxidase/peroxidase/ferrocene	Chronoamperometry	0.2–50 μM	0.2 μM	Human blood	[19]
BDD	NA	LSV	1–400 μM	ND	Coffee and cola	[77]
Graphite	Theophylline oxidase	Amperometric	0.2–2 mM	0.2 mM	Aqueous	[78]
Carbon Paste	Nano-CoPc	DPV	0.4–100 μM	0.14 μM	Drug tablet, green tea	[39]
ITO and Si	MIPs (MAA/EGDMA)	CV	ND	ND	Aqueous	[24]
Pyrex *w/* Ag|AgCl	PVC/DBP/PTT/OA	Potentiometric	1–10,000 μM	0.55 μM	Drug tablets	[21]
GC	MWCNT	CV	0.3–10 μM	0.05 μM	Drug tablets	[44]
Graphite	ThOx/DDAB	Chronoamperometry	0.2–8 mM	0.2 mM	ND	[79]
Au	ThOx/SAM	Chronoamperometry	2-3 mM	2 mM		
ITO	MIPs (MAA/EGDMA)	CV	2–4 mM	ND	ND	[25]
Au	MIPs (Phenol)	Capacitance	1–15 μM	1 μM	ND	[27]
GC	Cysteic acid	DPV	2.5–68 μM	1.2 μM	Drug Tablets	[80]
Au	MB/Aptamer	DPV	2–300 μM	2 μM	Serum	[32]
GC	MWCNT-Pt$_{nano}$[omim][PF$_6$]	CV	0.01–10 μM	8 nM	Drug tablets, green tea	[68]
Carbon paste	CTAB	DPV	0.08–200 μM	0.185 μM	Drug tablets, urine	[40]
GC	Nafion/MWCNT	DPV	0.08–60 μM	0.02 μM	Drug tablets	[81]
GC	AuNP/Aptamer	DPV	2–50 μM	1.2 μM	ND	[34]
GC	NA	SWV	0.1–100 μM	0.013 μM	Drug tablets	[38]
Carbon fiber microdisk electrode (8 μm)	NA	Microchannel electrophoresis	6–600 μM	4 μM	rat serum and urine	[17]
ND	NA	HPLC-EC	2.5 -20 pg/ml	0.2 pg/ml	Plasma (human)	[15]
GC	NA	HPLC-EC	50 - 10 μg ml^{-1}	1 ng	Beverages and foodstuffs	[16]
PFC	NA	HPLC-EC	2 – 100 μM	0.55 μM	Rat plasma	[14]
GC	AHNSA	DPV	1 – 100 μM	0.047 μM	Drug tablets	[82]
GC	Graphene/Nafion	DPV	0.01 – 1, 2 – 30 μM	6 nM	Drug tablets	[48]
MWCNT carbon paste	NA	DPV	2–150 μM	0.0197 μM	Drug tablets, urine	[45]
GC	CdSe microparticles	DPV	1–40, 40–700 μM	0.4 μM	Tea, cola drink, fruit juice, fermented milk, preserved fruit	[70]
GC	AuNP/L-cysteine/graphene/Nafion	DPV	0.004 – 60 μM	0.4 nM	Drug tablets, tea	[60]
GC	rGO	LSV	0.8–60 μM	0.1 μM	Green tea	[83]
GC	SWCNT-LMC/Nafion	DPV	0.3–38 μM	0.08 μM	Blood serum, urine	[46]
GC	LMC/Nafion	DPV	0.8–180 μM	0.37 μM	Blood serum, tea, cola, coffee	[84]
GC	Chitosan/NH$_2$-IL/MnO$_x$	DPV	0.05–120 μM	0.05 μM	Drug tablets	[85]
Au	Duplex DNA	CV	0–50 μM	3.2 μM	Human serum	[86]
GC	MWCNT-IL	DPV	0.5–98 μM	0.16 μM	Syrup, urine	[87]
GC	rGO	Chronoamperometry	0.05–40 μM	2.9 nM	Drug tablets	[88]
Graphene paste	1,4-BBFT/IL	SWV	0.06–700 μM	12 nM	Tea, human blood serum, urine	[71]
SPE	NA	CV	55–110 μM	10 μM	Aqueous	[36]
GC	AuNP-Chitosan-IL/rGO	DPV	0.025 – 2.1 μM	1.32 nM	Green tea, pu'er tea, energy drink, drug tablet, akafen powder	[51]
Carbon paste	MIP (MAA/EGDMA)	DPV	ND	1 μM	Aqueous	[28]
BDD	NA	DPV SWV	2–380 μM 2–380 μM	0.91 μM 1.45 μM	Drug tablets	[37]
GC	MIP/CNP-SO$_3$H	DPASV	0.05–30 μM	1.4 nM	Drug tablets	[29]
Graphite pencil	CTAB	DPV	0.1–1.3 μM	2.63 nM	Drug tablets, urine	[41]
GC	WS$_2$/AgNPs	DPV	0.05–150 μM	3 nM	Drug tablets, Green, Pu'er, Xinyan maojian and Biluochun tea	[62]
GC	MIP (H-A)	Amperometry	0.4–17 μM	0.32 μM	Drug tablets	[26]
CFE	NA	FSV	0.5–30 μM	ND	Aqueous	[89]
GC	CTAB	DPV	0.5–1000 μM	0.11 μM	Urine	[42]

(*continued on next page*)

Table 1 (*continued*)

Electrode	Electrode modifier	Technique	Linear range	Limit of detection	Sample medium	Reference
Carbon paste	EFTAG	SWV	0.02–1 mM	15 µM	Human blood serum, urine	[90]
GC	Poly(PABSA)	Chronoamperometry	10–100 µM	7.02 µM	Drug tablet, tea, urine, serum	[91]
GC	P(L-Asp)/MWCNTs	SWV	0.1–50 µM	0.02 µM	Green tea, blood serum, drug tablets	[92]
Carbon paste	MWCNTs	SWV	0.8–90 µM	0.194 µM	Human plasma	[47]
Carbon paste	MB	DPV	0.2–10 µM	2.25 nM	Drug tablets, urine	[93]
Au	AuNPs/aptamer	SWV	0.1–80 µM	0.07 µM	serum	[33]
GC	AuNP/MWCNT	DPV	0.5–20 µM	90 nM	Drug tablets, black tea, green tea	[67]
Carbon paste	Eriochrome black-T	SWV	0.01–100 µM	20 nM	Drug tablets, urine	[94]
Carbon paste	Fe_3O_4/SWCNT	Chronoamperometry	0.1–300 µM	ND	Green tea, fruit juice, cola drink, fish meat	[64]
SPE	GQD	DPV	1–700 µM	0.2 µM	Oral solution, urine	[95]
GC	2eOHMnPc-CNT	DPV	0.04–12 µM	6.6 nM	Green tea, cola drink, serum	[96]
Carbon paste	Kaolinite	SWV	1–5 mM	190 µM	Drug tablets, blood	[97]
Au	AgNP/Aptamer	LSV	0.5–70 µM	50 nM	Serum	[35]
GC	PLCY/N-CNT	DPV	0.1–70 µM	0.033 µM	Green tea, drug tablets, energy drink	[98]
GC	TiO_2 NPs	DPV	23–200 nM	23 nM	Drug tablets, urine	[59]
GC	AFW/Nafion	DPV	0.1–160 µM	2.8 nM	Human serum, black tea, urine	[63]
GC	CB-g-PAA/La_2O_3	DPV	0.02–888 µM	15 nM	Serum, urine	[99]
GC	WO_3/MWCNT	DPASV	0.025 – 2.6 µM	8.3 nM	Drug tablet, urine	[69]
Au	MIP/PAM/PTZ-343	DPV	0.00003–30 µM	11 pM	Aqueous	[30]
Carbon paste	MWCNT/CuO	Potentiometric	0.1 – 10,000 µM	0.025 µM	Chinese black tea, Chinese green tea, Indian black tea, Indian green tea.	[22]
GC	TiO_2 MPs	Amperometric	0.02–209.6 µM	13.26 nM	Serum, drug tablets	[56]
GC	ErGO-CP6	DPV	0.2–130.4 µM	40 nM	Drug tablets	[100]
GC	rGO/SDS/Nafion	DPV	0.01 – 0.1, 0.1 – 1, 1 – 40 µM	5 nM	Drug tablets	[43]
GC	La_2O_3/MWCNT	DPV	0.1–400 µM	0.01 µM	Human blood serum, urine	[66]
GC	ZnONPs/MWCNT/Cyt C	DPV	0.4–15 µM	1.2 nM	Pharmaceutical syrup	[65]
GC	TiO_2NRs/MWCNT	DPV	0.56–893 µM	0.56 µM	Urine, chocolate powder	[74]
SPE	RuO_x-FSWCNT	Amperometric	0.005–500 µM	0.001 µM	Drug tablets	[101]
GC	CNF/PSA	DPV	0.6–137 µM	0.2 µM	Drug tablets	[102]
GC	βH-MnO_2 nanoflower	DPV	0.01–320 µM	5.9 µM	Chocolate, black tea, coffee, drug tablet	[61]

GC: glassy carbon; SWV: square wave voltammetry; BDD: boron-doped diamond; LSV: linear sweep voltammetry; ND: not disclosed; nano-CoPc: nanoszied cobalt phthalocyanine; ITO: indium tin oxide; MIPs: molecularly imprinted polymers; CV: cyclic voltammetry; MAA: methacrylic aicd; EGDMA: ethylene glycol dimethacrylate; PVC: poly(vinyl chloride); DBP: dibutyl phthalate; PPT: 2,6-bis(phenyl)-4(phenyl3H-thiopyran; OA: oleic aicd; DPV: differential pulse voltammetry; MB: methylene blue; MWCNT: multi-walled carbon nanotubes; [omim][PF_6]: 1-octyl-3-methylimidazolium hexafluorophosphate; ThOx: theophyllineoxidase; PFC: plastic formed carbon; AHNSA: 4-amino-3-hydroxynaphthalene sulfonic acid; AuNPs: gold nanoparticles; rGO: reduced graphene oxide: SWCNT-LMC: single-walled carbon nanotubes-large mesoporous carbon; IL: ionic liquid; 1,4-BBFT: 1-(4-bromobenzyl)-4-ferrocenyl-1H-[1,2,3]-triazole; SPE: screen-printed electrode; NA: not applicable; CNP-SO_3H: carbon nanoparticles containing sulfonic acid groups; DPASV: differential pulse anodic stripping voltammetry; CTAB: cetyltrimethyl ammonium bromide; AgNPs: silver nanoparticles; H-A: 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid; CFE: carbon fiber electrode; FSV: fast scan voltammetry; EFTAG: ethyl 2-(4-ferrocenyl-[1,2,3]triazol-1-yl) acetate; PABSA: para amino benzene sulfonic acid; P(L-Asp): poly(L-aspartic acid); GQD: graphene quantum dots; 2eOHMnPc-CNT: [tetra-(5-chloroquinolin-8-yloxy) phthalocyanato] manganese(III)-carbon nanotube; PLCY: poly(L-cysteine); N-CNT: nitrogen-doped carbon nanotubes; AFW: aloe vera plant extract decorated iron tungstate nanorods; CB-g-PAA: carbon black grafted poly(acrylic acid); PAM: polyacrylamide; PTZ-343: phenothiazine sodium sulfonate; ErGO-CP6: electrochemicall reduced graphene oxide – cationic pillar[6]arene; NRs: nanorods; FSWCNTs: functionalised single-walled carbon nanotubes; CNF: carbon nanofiber; PSA: poly(sulfosalicylic acid);

11 pM [30] in model solutions. A slight drawback is that each concentration being measured takes 13 mins for binding to occur. The sensor was demonstrated to exhibit a high selectivity towards theophylline, which was attributed by the authors to the polymer film possessing robust imprinted sites/cavities that preserved precisely the memory of the shape, size, and conformation of the template molecule theophylline [30].

Another interesting approach which appears to bring electrochemical based technologies closer to being used for medical interventions, is a report by Aaryashree and co-workers [31], who developed MIPs for the detection of theophylline (and other important antibacterial drugs, vancomycin and meropenem, and antiepileptic drugs, phenobarbital). Fig. 3 shows a typical electrochemical-based sensing device, which is comprised of a ceramic based upon which platinum is used to define the

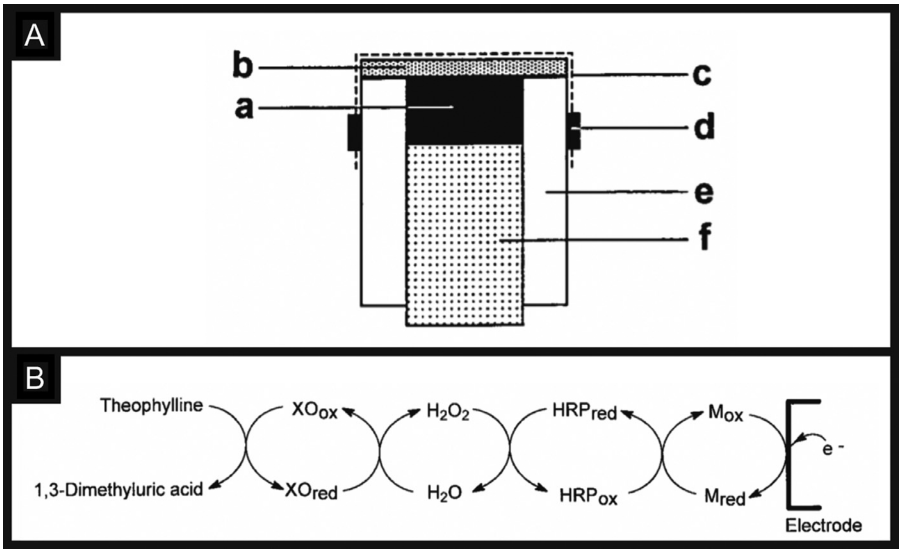

Fig. 1. **A:** Sketch of the enzyme electrode: a, graphite composite with mediator; b, enzyme layer; c, dialysis membrane; d, PVC O-ring; e, PVC-tip; f, brass rod. **B:** Reaction sequence for the theophylline biosensor based on microbial xanthine oxidase (XO), peroxidase (HRP), and ferrocene electron mediator (M). Reproduced with permission from Ref [19]. Copyright Elsevier 2000.

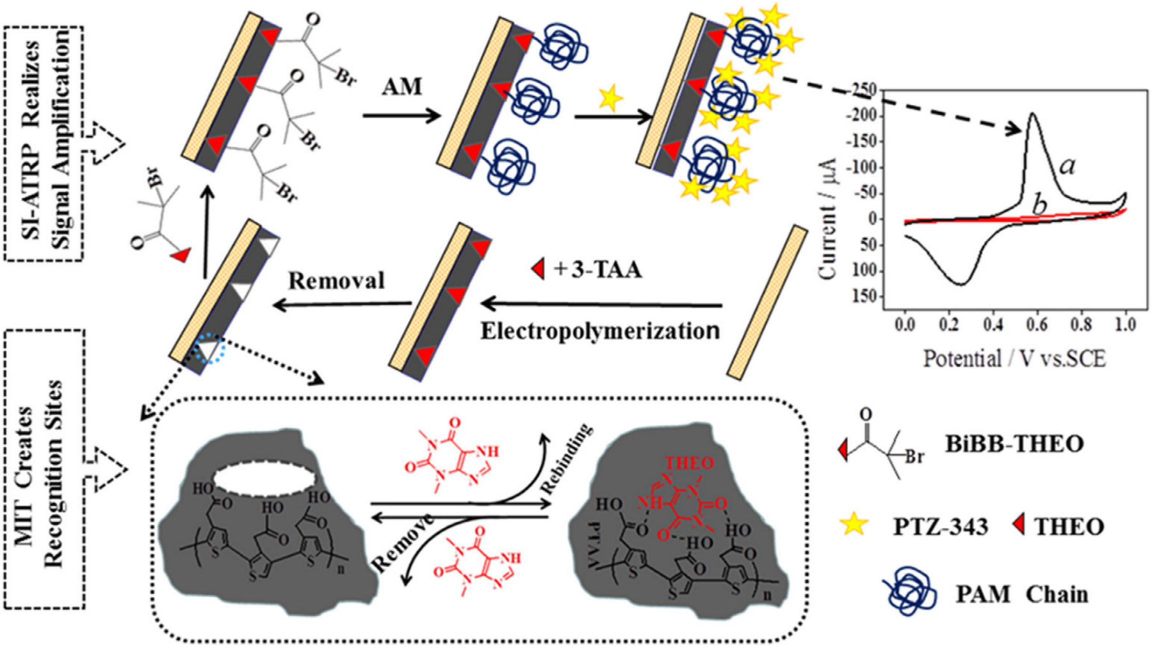

Fig. 2. Illustration of the fabrication process of a novel electrochemical biomimetic sensor based on electropolymerized molecularly imprinted polymer (E-MIP) artificial receptor and Surface initiated atom transfer radical polymerization (SI-ATRP) assisted signal amplification and finally used for detecting ultralow concentrations of theophylline. The cyclic voltammetric response, current vs. potential is shown for the case of the absence (b) and presence (a) of theophylline. Reproduced with permission from Ref [30]. Copyright Elsevier 2019.

underlying connections (see Fig. 3A). Fig. 3B show a lateral side view of the device which shows how a reservoir is produced (10 × 10 mm) with the device containing three holes for the counter electrode (diameter 2.0 mm), reference electrode (diameter 0.9 mm), and the working electrode (diameter 0.9 mm), each of depth 0.2 mm, connected with platinum wiring at the bottom. The chosen MIP is prepared as a carbon paste and added, as shown in Fig. 3D, along with the reference electrode and with platinum as the counter electrode. This exciting approach was utilised via differential pulse voltammetry and applied in buffer saline or whole bovine blood samples, the latter were modified with sodium citrate as an anticoagulant. The benefits of this approach, as stated by the authors are as follows [31]: (a) easy to use, (b) single-use or disposable, (c) measurement in whole blood, (d) only 50 μL of solution required for sensing, (e) reagentless measurement technique, (f) faster TDM than immunoassay and liquid chromatography, and (g) low cost, so that it can be used in the developing countries. That said, independent validation against current approaches (*e.g.* HPLC) still need perusing and verifying.

RNA aptamers has been also widely explored [32-35], for example Chen and co-workers [33] developed an electrochemical biosensor for theophylline through the utilisation of an RNA aptamer and gold nanoparticle (AuNP)-based amplification technique. Fig. 4A shows the overview of the electrochemical biosensor, which provides an indirect method to measure the analytical target theophylline. In this approach, a gold macroelectrode is modified with a DNA tetrahedron/RNA probe that, as shown in Fig. 4B, gives rise to negligible electrochemical signals. Next, the introduction of RNA probe b modified gold nanoparticles, which have been labelled with methylene blue, in the presence of theophylline, gives rise to the analytical signal, facilitated by the measurement of the methylene blue. Effectively this is a “chemical jigsaw”, and when all the pieces come together, an easily quantifiable signal is

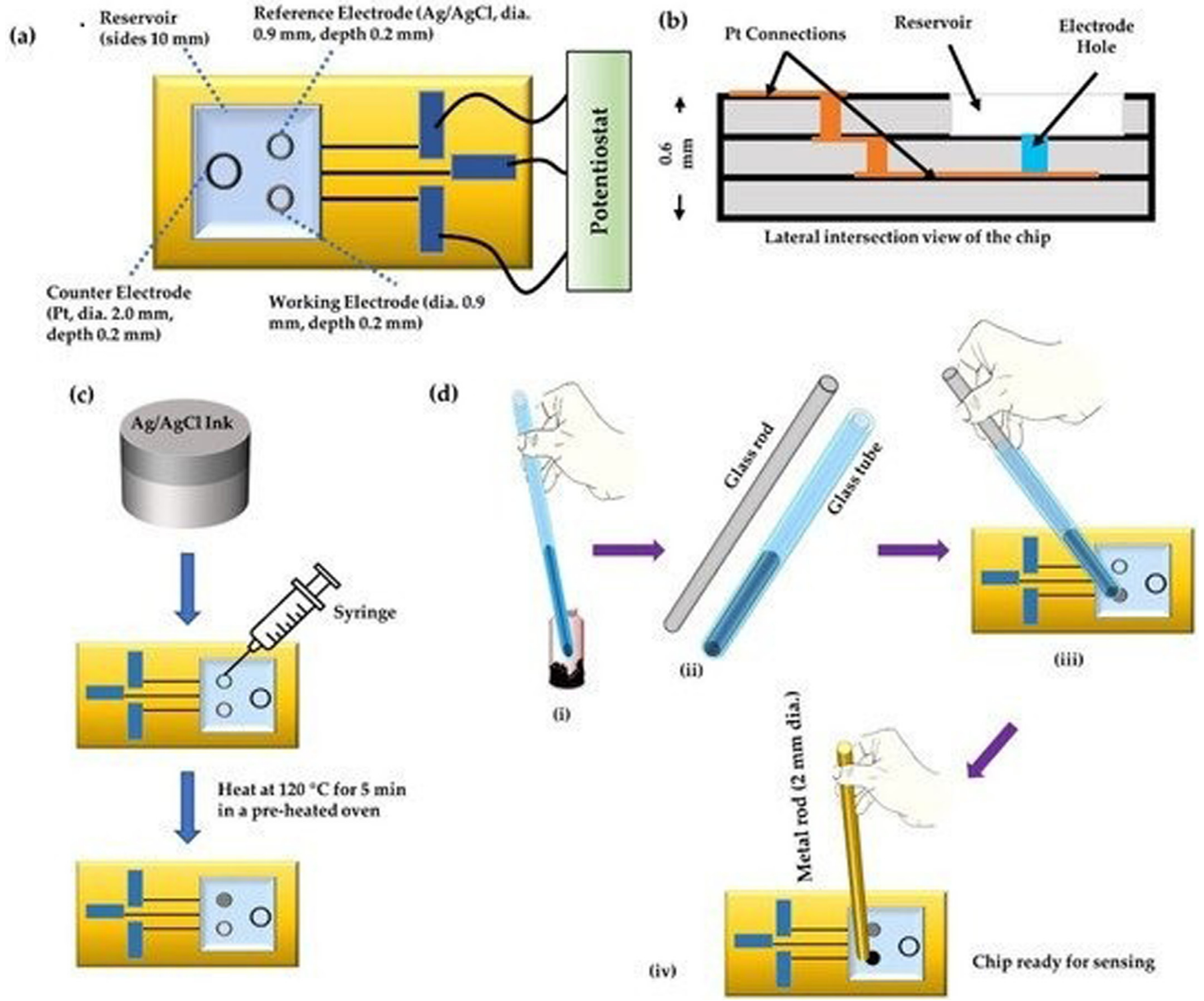

Fig. 3. **(a)** Design of an electrochemical based sensing device showing, (**b**) lateral view inside view of the device, (**c,d**) illustration of the method of filing the reference electrode and the working electrode. Reproduced with permission from Ref [31]. Copyright MDPI 2020.

realised; Fig. 4C shows the calibration plots and electrochemical signals, which exhibit a linear range from 0.1 to 80 µM with a LOD of 0.07 µM feasible. This approach, despite having multiple steps, provides a highly selective sensing methodology, as shown in Fig. 4D. The authors demonstrated their approach to be successfully viable for the sensing of theophylline in serum samples (details of pre-treatment missing).

A key theme in electrochemistry/electroanalysis is the use of carbon-based electrodes. To this end, Wang *et al.* explored the direct oxidation of theophylline utilising screen-printed electrodes (SPEs) [36]. The authors compared the response of SPEs with a range of other commercially available electrodes, namely: edge plane and basal plane pyrolytic graphite, gold, glassy carbon, boron-doped diamond electrodes as well as single-wall carbon nanotube SPEs finding that in terms of current density (A per cm^{-2}), the bare SPE gave rise to largest response. Proof-of-concept was shown with the electroanalytical detection of theophylline and was shown to be viable at physiological pH with an analytical range that encompassed medically relevant and toxic concentrations. Furthermore, the sensors exhibited % RSD values of no more than 5% [36]. Boron-doped diamond electrodes have been explored, which have been applied to sensing theophylline in urine samples and pharmaceutical tablets [37]. Câmpean and co-workers [38] interestingly use the electrochemical activation of a GC electrode, which involves an anodic oxidation at +1.8 V *vs* Ag/AgCl for 300 seconds in pH 5, followed by the electrode being cycled between 0 and +0.8 V until a stable current potential curve was obtained (~ 10 cycles). Note that the activation procedure has to be repeated after each determination before the electrode can be used again, after continuous sweeping for four cycles in potential range from 0 to + 1.0 V at pH 7.4. The sensing of aminophylline was shown to be viable by SWV and DPV over the range 10^{-7} to 10^{-4} M with LODs of 25 nM and 92 nM respectively, while the detection of theophylline via SWV and DPV over the range 10^{-7} to 10^{-4} M with LODs of 13 nM and 86 nM respectively. This activated electrode was applied to the sensing of theophylline and aminophylline in pharmaceutical formulation and the latter in urine samples.

A common theme in electroanalysis is to utilise an electrocatalyst, for example, as shown by Yang and co-workers who utilised nanosized cobalt phthalocyanine particles incorporated into a carbon paste electrode for the electrocatalytic determination of theophylline with a linear range of 0.4–100 µM and a LOD of 0.14 µM. Cobalt phthalocyanine of course is a well-known electrocatalyst to a wide variety of analyt-

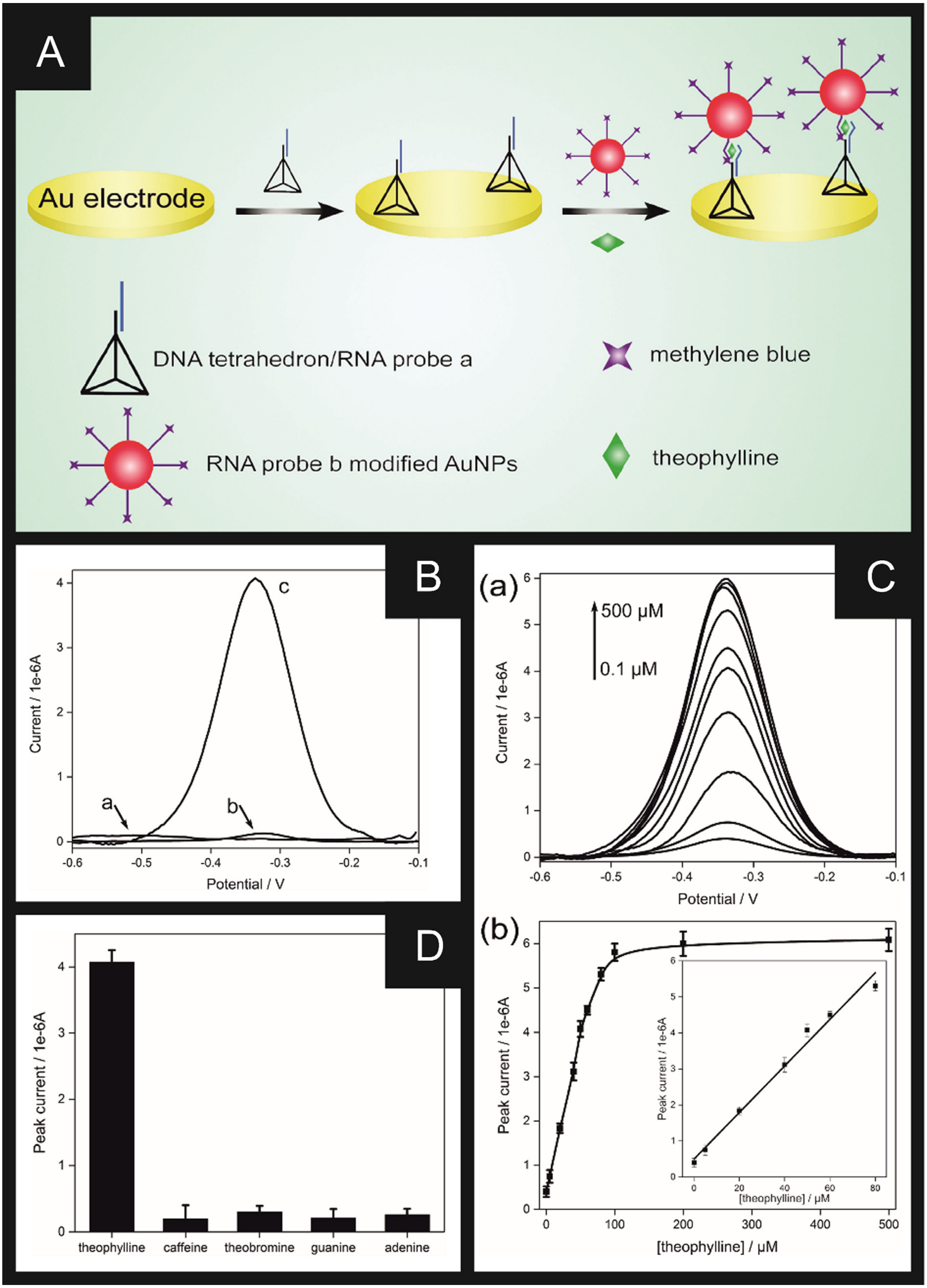

Fig. 4. **A:** Illustration of the AuNPs-based electrochemical aptasensor for theophylline detection. **B:** Square wave voltammograms of (a) DNA tetrahedron/RNA probe a modified electrode, after (b) incubation with RNA probe b modified AuNPs in the (b) absence and (c) presence of theophylline (50 μM). **C:** Square wave voltammograms for the detection theophylline with the concentrations of 0.1, 5, 20, 40, 50, 60, 80, 100, 200, 500 μM. (b) The calibration curve of peak current versus the concentration of theophylline. Inset shows the linear range. **D:** The effect of interferents: Peak current of square wave voltammograms for the detection of theophylline, caffeine, theobromine, guanine and adenine with the concentration of 50 μM. Reproduced with permission from Ref [33]. Copyright Elsevier 2018.

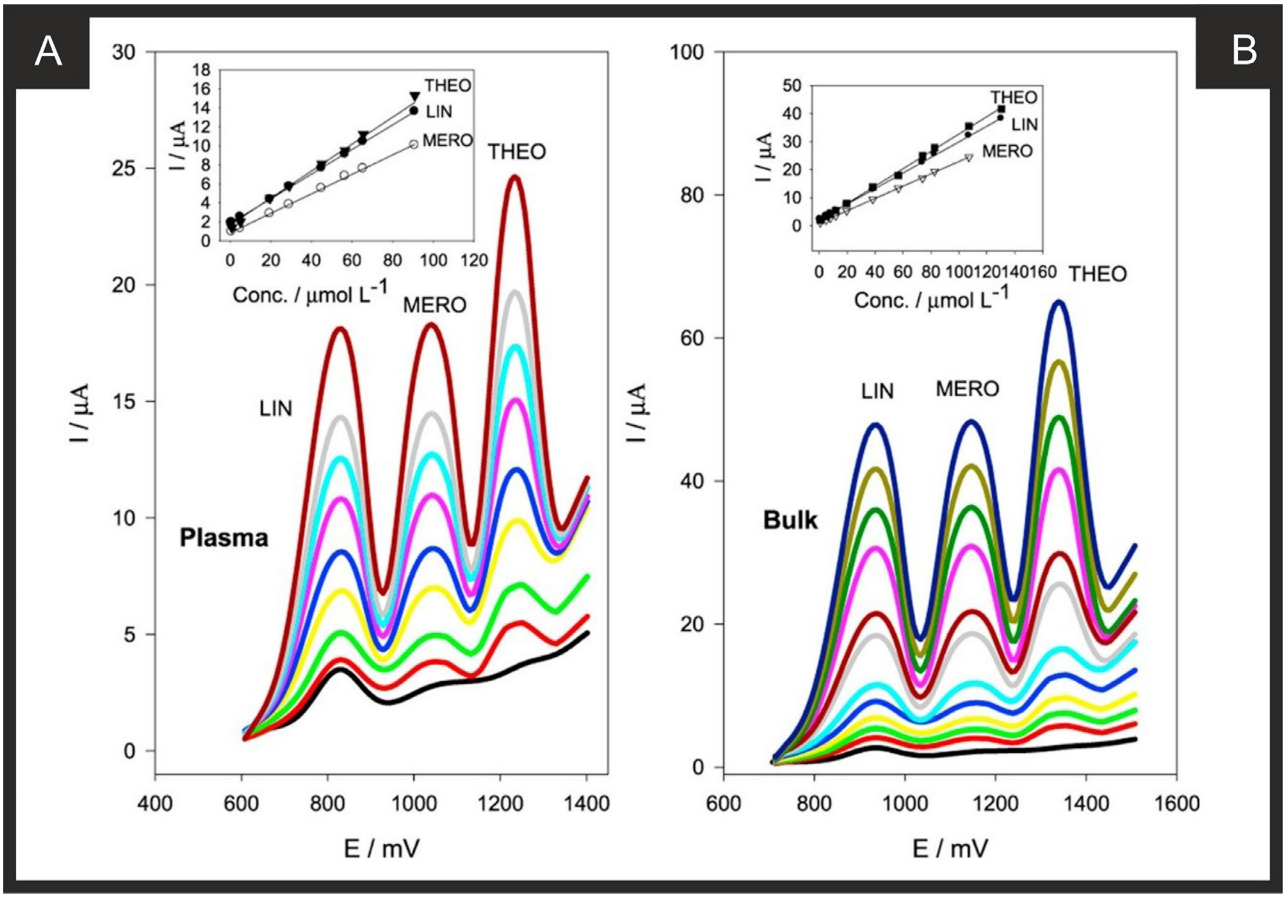

Fig. 5. **A:** Square wave voltammograms of linezolid (LIN), meropenem (MERO) and theophylline (THEO) at MWCNTCPE in BR buffer of pH 3.0 in spiked plasma, LIN: (4.0×10^{-7}-9.0×10^{-5} mol L^{-1}), MERO: (8.0×10^{-7}-9.0×10^{-5} mol L^{-1}) and THEO: (8.0×10^{-7}-9.0×10^{-5} mol L^{-1}); B: bulk (referred to as a pharmaceutical tablet in their paper [47], LIN: (4.0×10^{-7}-1.3×10^{-4} mol L^{-1}), MERO: (8.0×10^{-7}-1.0×10^{-4} mol L^{-1}) and THEO: (8.0×10^{-7}-1.3×10^{-5} mol L^{-1}). In both cases, scan rate: 50 mV s^{-1}. Insets: linear calibration curves of LIN, MERO and THEO in spiked plasma and bulk (pharmaceutical tablet). Reproduced with permission from Ref [47]. Copyright Elsevier 2018.

ical targets so its selectivity is questionable in complex samples. That said, the authors were able to determine theophylline in green tea (following being boiled, filtered and diluted and modified to an optimised and determined pH) and pharmaceutical drug samples (following being ground in a mortar, dissolved in water and modified to a determined optimised pH) of aminophylline with the latter agreeing well with an independent pharmacopoeia method (titration) [39]. This concept has been extended to manganese phthalocyanine for the simultaneous determination of theophylline and caffeine with LODs of 8.1 and 300 nM respectively, which was applied for the sensing of theophylline in serum. Given that SPEs are readily adaptable with electrocatalysts, this might new avenue of research to follow to realise electroanalytical clinical interventions.

Other approaches have used “enhancing agents”, such as surfactants. For example Hedge et al. [40] utilised a carbon paste electrode using cetyltrimethyl ammonium bromide (CTAB) with DPV, which was shown to exhibit a linear range from 0.08 to 200 µM with a LOD of 0.185 µM. The favourable enhancement of CTAB with theophylline was applied for the analysis of pharmaceuticals and spike urine [40], and the observed enhancement with CTAB attributed to the latter forming a hydrophilic film which enhances the accumulation step/process. This approach has been extended to sensing theophylline in conjugation with CTAB at graphite pencil and GC electrodes [41, 42] and SDS at reduced graphene oxide/graphene [43].

The sensing of theophylline has been naturally extended to enhance the electroanalytical sensor through the utilisation of single walled and multi walled carbon nanotubes (SW/MWCNTS). This started with basic electrode modification of GC [44] and carbon paste electrodes [45] with MWCNTs then progressing onto developing SWCNTs – large mesoporous carbon/Nafion modified GC electrodes, where using utilising this configuration, a large surface area results, which has been successfully applied for theophylline in serum and urine samples [46]. Attia and co-workers [47] detailed the simultaneous determination of linezolid, meropenem and theophylline using MWCNT modified carbon paste electrodes. Prior to this work, these target analytes had not been simultaneously measured. The motivation for their work [47] is that the treatment of healthcare associated Pneumonia caused by Methicillin-resistant Staphylococcus aureus (MRSA) requires therapeutic protocols formed of linezolid either alone or in combination with meropenem and theophylline. The inter-individual pharmacokinetic variations require the development of reliable therapeutic drug monitoring tools, especially in immunocompromised patients. Hence, the authors developed an electrochemical based sensor to facilitate this, where linezolid, meropenem and theophylline were feasibly measured in plasma over concentration ranges of 4.0×10^{-7}- 9.0×10^{-5}, 8.0×10^{-7}- 9.0×10^{-5} and 8.0×10^{-7} - 9.0×10^{-5} M, respectively - see Fig. 5. The performance of the proposed sensor was validated and the applicability for therapeutic drug monitoring was demonstrated in the plasma of healthy volunteers and pharmaceutical tablets.

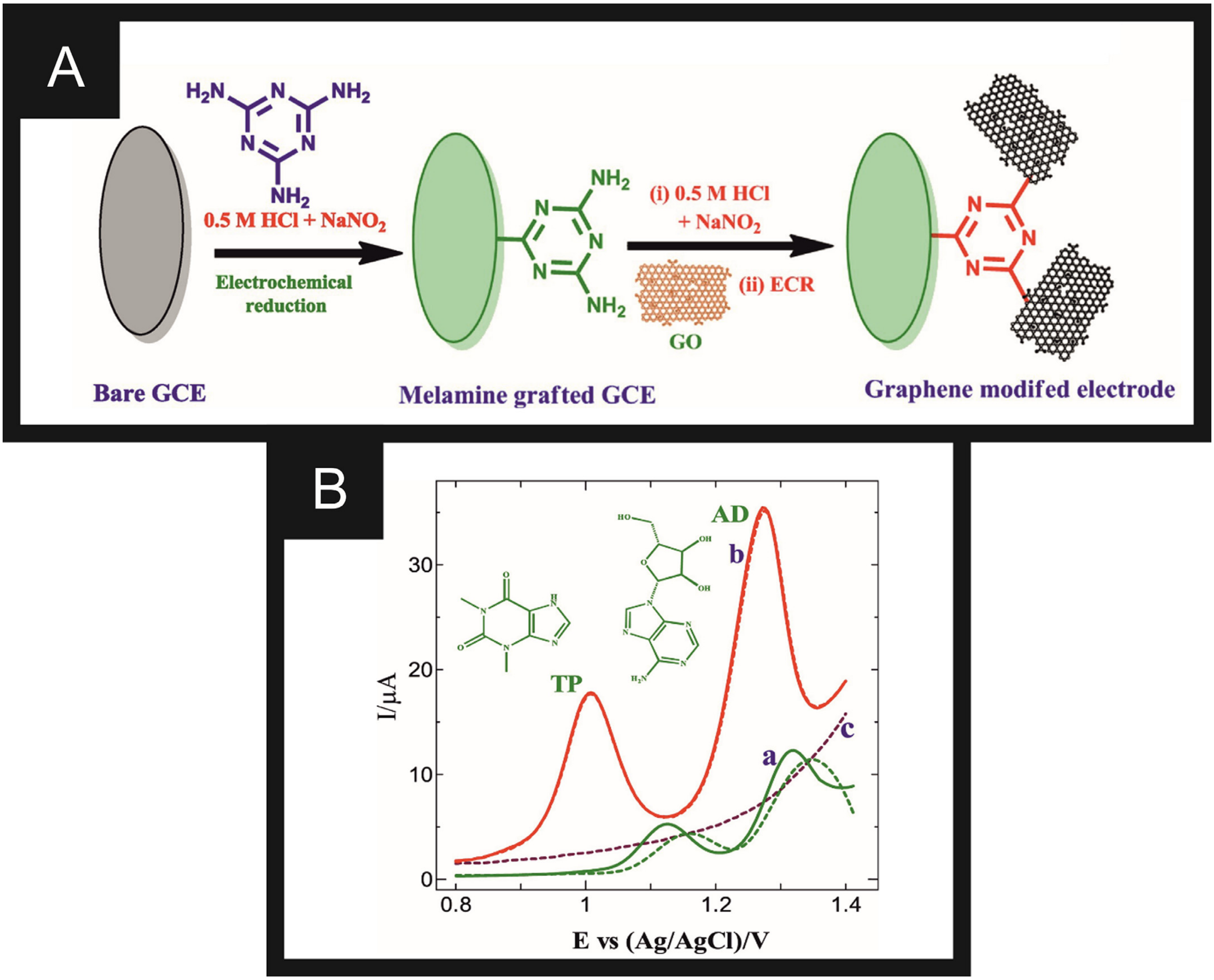

Fig. 6. **A:** Scheme showing the modification procedure of graphene upon a GCE. **B:** DPVs obtained for 0.5 mM of TP (theophylline) and 0.5 mM AD (adenosine) at (a) bare and (b) graphene modified GC electrodes in 0.2 M PBS (pH 6.0) (solid line: 1st scan; dotted line: 5th scan). (c) Graphene modified electrode GC electrode in the absence of TP and AD. Reproduced with permission from Ref [49]. Copyright Elsevier 2019.

Graphene, reported to exhibit potentially useful properties over other carbon based nanomaterials, has motivated many authors to explore this 2D material towards the sensing of theophylline. For example Li et al. [48] used graphene-Nafion suspensions drop cast onto GC electrodes with nM detection levels possible using DPV with a 120 s accumulation time. Nafion is utilised to not only help dispersion, keeping the graphene from agglomerating with a high surface area, but also contributed to the adsorption of theophylline. The graphene/Nafion sensor was applied to theophylline determination in a pharmaceutical product and aminophylline injection sample [48]. Kesavan and co-workers [49] reported a graphene modified electrode for the selective sensing of theophylline in the presence of inhibitor neurotransmitter antagonist, adenosine. The graphene modified electrode has chemical linkers between a GC electrode and graphene nanosheets. In this approach, as shown in Fig. 6, the GC surface is modified via the diazotization of melamine via electrochemistry, producing a melamine modified electrode. The next step involves chemically linking GO via the simultaneous electrochemical reduction of diazonium cations and GO, which produces chemically attached graphene to the GC electrode surface. This approach has benefits that the graphene is physically attached to the electrode surface which should provide a reproducible and stable sensor but capitalises upon exposing the edges of graphene, effectively making a vertically aligned graphene electrode [50]. This exposes more active edges of graphene than if the graphene was parallel, as fabricated via drop-casting. The simultaneous sensing of theophylline and adenosine was explored by the authors, [49] as shown in Fig. 6 which gives rise to well resolved electrochemical oxidation peaks, which are more stable at the graphene modified GC electrode over that of a bare/unmodified GC following electrode cycling; a 2.1 fold increase in the current is observed for theophylline. We infer that while adsorption likely occurs in both cases, due to the higher number of active sites for the graphene modified electrode, there are still substantially more active sites left available over that of the bare GC electrode where the graphene modified electrode gives rise to an optimal response. Furthermore, the graphene modified electrode was shown to be successfully utilised for the selective determination of theophylline in the presence of 34-fold excess of adenosine over the linear range 15 to 195 μM using DPV. To increase the sensitivity further, via amperometry, the sensing of theophylline was

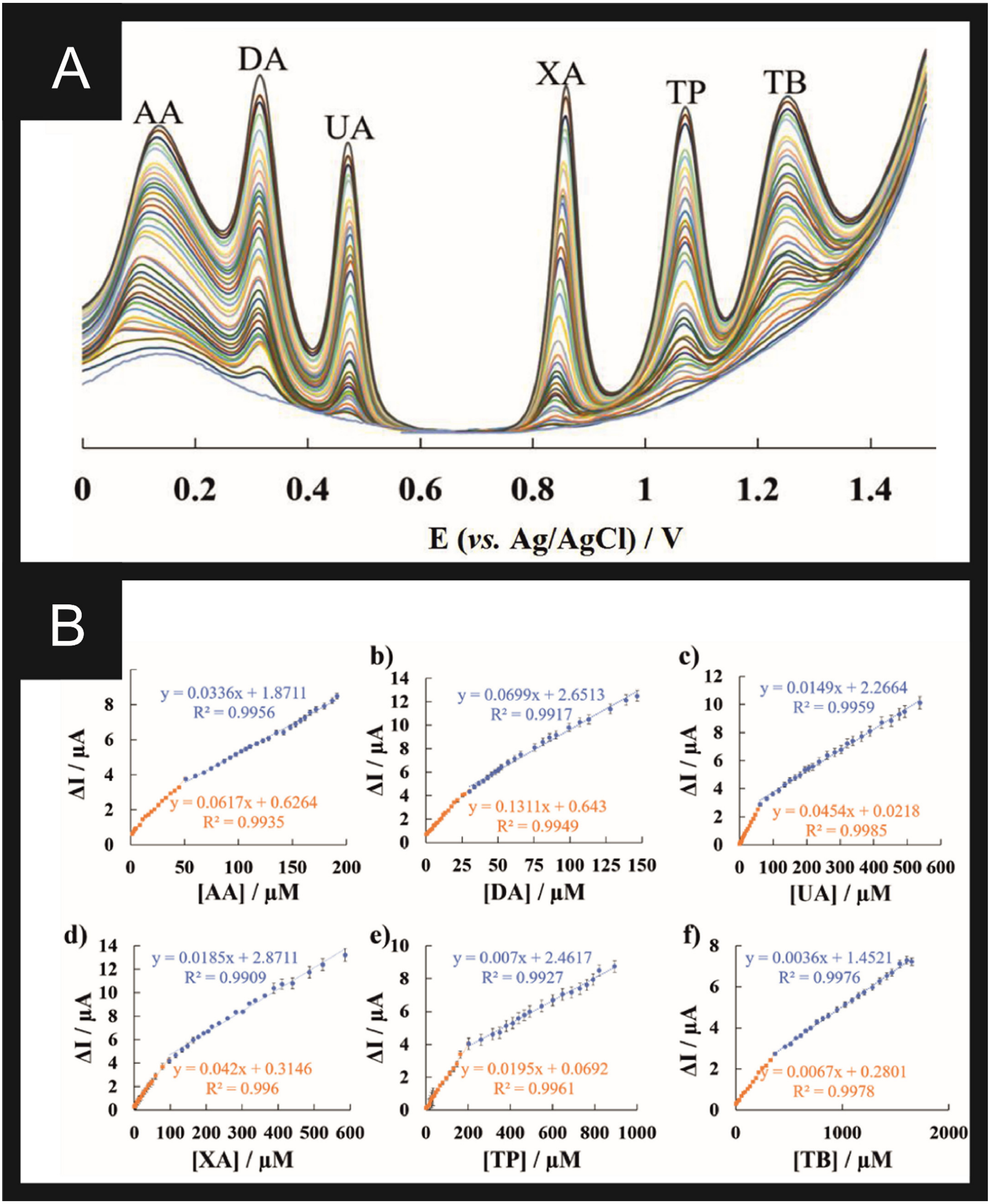

Fig. 7. **A:** DPV measurements for the simultaneous detection of AA, DA, UA, XA, TP, and TB at varying concentrations using the TiO_2NRs-MWCNTs/GCE in 0.2 M PBS (pH 4.0). **B**: The plots for the concentration dependence of increasing anodic peak current signals for (a) AA, (b) DA, (c) UA, (d) XA, (e) TP, and (f) TB with the linear range marked in blue and orange. Reproduced with permission from Ref [74].Copyright Elsevier 2020.

found to be possible from 30 nM to 100 μM with a LOD of 5.4 nM. Last, the authors validated their graphene modified electrode towards the sensing of theophylline and adenosine in serum and urine (centrifuged and diluted with buffer solution to a chosen pH) independently with HPLC, which provided excellent agreement between the two analytical methods. These results provide convincing evidence that the graphene modified electrode has potential as a platform for the determination of theophylline (and adenosine) in real samples and possibly for clinical invention. Other approaches have utilised gold nanoparticle – chitosan – ionic liquid/graphene modified electrodes for theophylline and caffeine determination, which was applied into the sensing of both analytes in tea, energy drink and pharmaceutics and was directly compared with HPLC giving excellent agreement between the two [51].

Related to graphene is of course graphene oxide. This is an often overlooked material [52-54] which can give rise to beneficial responses to target analytes and itself is a useful nanomaterial in electrochemistry due to its rich oxygenated surface. Shetti and co-workers [55] utilised graphene oxide with nanoclays to realise a paste electrode for the sensing of theophylline with improved results observed using this electrode configuration over that of carbon paste electrode. A linear range from 0.01 to 0.2 μM was shown to be possible with a LOD found to correspond to 1.8 nM. The authors demonstrated their sensor to successfully measure theophylline in pharmaceuticals and spiked urine samples [55]. Other approaches have developed titanium dioxide microsphere decorated graphene oxide composites for theophylline sensing which exhibits a linear range from 0.02 to 210 μM with an LOD of 13 nM and was applied in (diluted and spiked) serum, and drug tablets [56].

In order to understand the electrochemical mechanism for the sensing of theophylline, Chiarotto et al. [57] have determined the electrochemical oxidation of theophylline in aqueous and non-aqueous solutions. Using UV–vis spectrophotometry (ex-situ), and the final electrolyzed solution, analysed by tandem mass spectrometry after chromatographic separation with an high-performance liquid chromatography-photo diode array-electrospray ionization-tandem mass spectrometry system [57]. Scheme 1 shows the mechanism of oxidation products in aqueous solutions (pH 7, Pt electrode), which shows that theophylline undergoes a 2 proton and 2 electron process forming product 2 (see Scheme 1); this has been suggested in the literature many time but no definitive evidence give until the report by Chiarotto et al. [57]. In terms of Scheme 1, in aqueous media, product 2 hydrolysis and subsequent oxidation can yield product 1. *How can the electroanalyst determine which mechanism, as proposed in Scheme 1, is the underlying case in their electrochemical sensing approach of theophylline*? Typically, the voltammetric current (oxidation peak, E_p) is plotted as a function of pH. This response is well-known to follow the Nernst equation:

$$E_p = E^0_{formal} - \frac{2.303RTm}{nF}pH$$

where E_p [V] is the peak potential, E^0_{formal} [V] is the formal potential of the redox couple, R [J K^{-1}] is the universal gas constant, T [K] is the temperature and m and n are the number of protons and electrons involved in the redox process. In this approach, a linear response will be observed up to the pK_a of theophylline, 8.77 (at 25 °C) [58]. That is, beyond a pH of 8.77, deviation from linearity will be observed. This linear response (the gradient) will yield $2.303RT$m/nF, which will correspond to 59.1 mV (at 298K) and relates to an equal number of protons and electrons transferred in the electrochemical mechanism, while for a process involving 2 protons and 3 electrons the gradient observed is 39.4 mV (at 298K). In terms of Scheme 1, if the electrochemical process realises 59.1 mV, the resulting process yields the reaction product 2, while if 39.4 mV is found, the process goes all the way to product 1. Such an approach is useful for future electroanalysts reporting their new electrode configurations.

There is a substantial body of work devoted to exploring the use of nanoparticles, which have the aim of providing enhancements in electron transfer and/or increases in electrochemically active surface area to help develop sensitive and selective electrochemical-based sensing platforms for theophylline. Various nanoparticle derived sensors have been utilised, such as: TiO_2 [59], Au [60], MnO_2 [61] and WS_2 nano-flowers/silver nanoparticle composites [62]. Another approach has utilised an aloe vera plant extract decorated with iron tungstate nanorod immobilised Nafion modified GC electrodes, which have been applied for the sensing of theophylline in spiked human serum, black tea and urine samples [63]. Utilising SW/MWCNTs, various metallic nanoparticles have been prepared and explored to theophylline sensing such as Fe_3O_4/SWCNTs [64], ZnO/MWCNTs [65], La_2O_3/MWCNTs [66], Au/MWCNTs [67] and Pt/MWCNT [68]. To increase the sensitivity of carbon nanotube modified electrodes, Rezvani and Soleymanpour [69] applied WO_3 nanoparticles (32 nm diameter), fabricated via a precipitation method in acidic media, where these were incorporated with MWCNTs and drop cast upon a GC electrode. The authors reported that the WO_3/MWCNTs gave rise to an electrochemical area 2.5 times larger than that of the underlying GC electrode. In addition to utilising the benefit of nanomaterials that have large surface areas and useful electron transfer properties, the authors further enhanced the sensors performance through using adsorptive stripping voltammetry, with a 210 second accumulation time, producing a linear range from 0.025 to 2.6 μM, with a LOD of 8 nM. In comparison to other work [51, 70-73], the authors noted an enhanced sensitivity (4.5 μA μM^{-1}) and demonstrated their approach to successfully determine theophylline in pharmaceutical samples (grinded in a mortar and dissolved in water, filtered and then dissolved into a chosen pH) and spiked urine (diluted).

Last, of note, Patel et al. [74] developed a nanocomposite of TiO_2 nanorods with MWCNTs surface modified onto a GC with Nafion, demonstrating the simultaneous determination of ascorbic acid, uric acid, dopamine, xanthine, theophylline and theobromine using DPV, Fig. 7. In the case of theophylline, two linear ranges were observed from 1 to 203 and 203 to 893 μM with a LOD of 0.56 μM; the nanocomposite was applied to the determination of ascorbic acid, uric acid, dopamine, xanthine, theophylline and theobromine in chocolate powder (made up in distilled water and diluted to a chosen pH) and urine (diluted with buffer to an optimal pH).

Conclusions and outlook

In this review, we have overviewed the various electrochemical approaches to determining theophylline, which range from hyphenated techniques through to direct electrochemical determination. We have seen how hyphenated techniques, *e.g.* HPLC-ECD are not being widely explored, despite their reported advantages over that of standard laboratory analytical instruments *e.g.* HPLC-UV and direct electrochemical techniques; the former has been shown to not only improve the analytical sensitivity, but also the selectivity, which can limit electrochemical methods, especially when applied to biological samples. That said, there appears to be direct electrochemical approaches that have been successfully applied to the determination of theophylline in real samples and validated against independent analytical methods. We also can observe that MIPs are a large area being explored due to their ability to provide sensitive and selective measurements of theophylline in real samples, such as plasma. There is scope to develop more sensors that bridge the gap between academia and clinical intervention, but these still need independent validation, such as with HPLC (or titration), which is rarely done. There are also many reports of modified electrodes, using combinations of metallic nanomaterials and carbon nanomaterials and there is clearly scope to move away from electrodes that require pretreatment prior to modification such as GC to the development and use of surface modified and bulk modified SPEs, and other related electrochemical platforms, that will move electroanalytical based sensors to realise true clinical intervention.

Declaration of Competing Interest

None.

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