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All solid-state miniaturized potentiometric sensors for flunitrazepam determination in beverages

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Abstract

Flunitrazepam is one of the frequently used hypnotic drugs to incapacitate victims for sexual assault. Appropriate diagnostic tools should be available to victims regarding the growing concern about “date-rape drugs” and their adverse impact on society. Miniaturized screen-printed potentiometric sensors offer crucial point-of-care devices that alleviate this serious problem. In this study, all solid-state screen-printed potentiometric flunitrazepam sensors have been designed. The paper device was printed with silver and carbon ink. Formation of an aqueous layer in the interface between carbon-conducting material and ion-sensing membrane nevertheless poses low reproducibility in the solid-contact electrodes. Accordingly, poly(3,4-ethylenedioxythiophene) (PEDT) nano-dispersion was applied as a conducting hydrophobic polymer on the electrode surface to curb water accumulation. Conditioning of ion-sensing membrane in the vicinity of reference membrane has been considered carefully using special protocol. Electrochemical characteristics of the proposed PEDT-based sensor were calculated and compared favorably to PEDT-free one. The miniaturized device was successfully used for the determination of flunitrazepam in carbonated soft drinks, energy drink, and malt beverage. Statistical comparison between the proposed sensor and official method revealed no significant difference. Nevertheless, the proposed sensor provides simple and user-friendly diagnostic tool with less equipment for on-site determination of flunitrazepam.

Keywords Miniaturized sensor · Potentiometry · Conducting polymer · Date-rape drugs · Flunitrazepam

Introduction

Flunitrazepam (FLU) is one of the fast-acting benzodiazepines with a selective inhibitory action on the brain gamma-aminobutyric acid (GABA) receptors [1]. It is therapeutically used as a tranquilizer owing to its anxiolytic effect. FLU could also be used as a preoperative sedative or a potent hypnotic [1, 2]. Unfortunately, FLU is illicitly used for recreation with other sedative drugs [3, 4]. This misuse makes FLU as one of well-

known date-rape drugs that is secretly used in clubs or raves [5]. This club drug is spiked into drinks to intoxicate a gullible mate to incapacitate victims’ psychological and physical state [6]. Since FLU is a typical drug for sexual assault or date-rape crimes, it has attracted considerable attention of analysts to develop various analytical methods for its detection in different biological fluids and beverages [7]. In the last decade, different techniques were reported for determination of FLU, including chromatography [8–14], spectroscopy [15–17], and voltammetry [18–23]. Measures should be taken to face the terrible use of date-rape drugs in committing crimes. Point-of-care (POC) devices have beneficial outcomes since they facilitate simple and rapid testing of illegal substances at the crime scene [24]. From this perspective, fluorimetric [17] and voltammetric [18–20] sensors for FLU determination in different beverages have been previously developed.

Screen-printed electrode (SPE) is considered one of the tremendous developments of POC devices. Screen-printing technology enables the inventive designing of solid-contact ion selective electrodes using various conducting inks on paper or plastic supporting material [25]. SPE could easily fit many commercial purposes owing to their operation simplicity,

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reliability, portability, and minimal instrumentation [26, 27]. However, the probable drawback of these devices is the susceptibility to form a water layer between the applied membrane and the screen-printed solid-contact. This water layer will impair ion-to-electron transduction mechanism between sensing membrane and conducting solid interfaces. Consequently potential drifts occur upon changing sample concentration since accumulating electrolytes in this water layer need to re-equilibrate [28, 29]. Many efforts have been directed to incorporate a hydrophobic conductive layer between the membrane and the solid interface. Conducting polymers (CPs) act as hydrophobic electron-transducer layer that make water layer formation unlikely [30–33], and hence they are used with the miniaturization process of solid-contact electrodes [33, 34]. Among the electrically conducting polymers, the most popular and widely used one with quite low band gap is poly(3,4-ethylenedioxythiophene) (PEDT) [35]. PEDT has different applications including electrically conducting and antistatic coatings, electroluminescent devices, capacitors, printing wiring boards, sensors, photovoltaics [35], and bioelectronics [36]. Due to the intrinsic conjugated structure and porous nature of PEDT, it gained its superior electrochemical properties such as the ability to lower the magnitude electrochemical impedance and to improve charge injection substantially.

The aim of this work is to develop a miniaturized SPE for the potentiometric determination of FLU. This is the first developed potentiometric POC device for this date-rape drug so far. All solid-state miniaturized ion-sensing devices with reference and indicator electrodes were screen-printed on a paper substrate using silver and carbon inks, respectively. PEDT nanoparticle dispersion was applied on carbon surface. This hydrophobic intermediate ion-to-electron transducer layer between the ion-sensing membrane and the screen-printed carbon electrode significantly stabilizes the measured potentials. The potential stability was examined by water layer test and compared favorably with PEDT-free sensor. The response characteristics and response time of PEDT-based sensor was compared with that of PEDT-free one as a control experiment. Finally, the PEDT-based sensor was effectively applied for determination of FLU in a wide range of spiked carbonated and non-carbonated soft drinks. The proposed sensor succeeded to align with criteria of POC for on-site detection of FLU. The developed method showed no significant difference when statistically compared to official method.

Experimental

Instrumental

Jenway Digital Ion Analyzer (Model No. 3330; Essex, UK); Reference electrode (Thermo Scientific Orion double junction Ag/AgCl reference electrode No. 900200, MA, US) with 10%

KNO₃ as bridge electrolyte and 3 mol L⁻¹ KCl saturated with AgCl as reference electrolyte. Scanning Electron Microscope (Model No. Quanta 250 FEG) equipped with Schottky field emission gun and Everhart-Thornley detector. Gold Sputter Coater (Model: Edwards S150B; Manufacturer: BOC Edwards, UK).

Materials

FLU pure powder was obtained from Tocris Bioscience (Bristol, UK). Its purity was assessed and found to be 99.61% according to non-aqueous titrimetric BP method [37]. Multiwalled carbon nanotubes (MWCNTs; ≥ 98% carbon basis, O.D. x I.D. x L 10 ± 1 nm × 4.5 ± 0.5 nm × 3–6 μm), potassium tetrakis(p-chlorophenyl)borate (TpCIPB), tetradodecyl ammonium bromide (TDAB), 2-nitrophenyl octyl ether (NPOE), dibutyl sebacate (DBS), calix[6]arene (CX6), sodium dodecyl sulfate (SDS), and poly(3,4-ethylenedioxythiophene) nanoparticle dispersion in water (PEDT) were purchased from Sigma–Aldrich (Steinheim, Germany). Poly(vinyl chloride) (PVC) was obtained from Fluka Chemie GmbH (St. Louis, MO, USA). Tetrahydrofuran (THF) was obtained from BDH (Poole, England). Ultrapure water (≤ 18.3 MΩ·cm) was used. A solution of MWCNTs (3 mg/mL) was prepared in an aqueous solution of SDS (10%w/v) and used as carbon ink following the protocol of Hu et al. [38]. Britton–Robinson buffer was prepared by mixing equal volumes of acetic acid, boric acid, and phosphoric acid (0.04 mol L⁻¹ each) and adjusted to the required pH (2–5) by 0.2 mol L⁻¹ NaOH standard solution. Circuit Scribe Conductive Ink Pen (Austin, Texas, USA) was used to draw silver reference electrode. Standard and sample solutions of FLU were prepared in Robinson buffer pH 2.5 unless otherwise stated.

Procedure

Preparation of sensing membrane

Reference membrane was prepared as precisely described [39] by mixing 2×10^{-2} mmol kg⁻¹ of each of TpCIPB (9.9 mg kg⁻¹) and TDAB (15.4 mg kg⁻¹) with 68 wt% of DBS and 30 wt% PVC. A total of 600 mg of membrane solutes were dissolved in 4 mL THF and sonicated for 15 min after the addition of 0.64 g KCl and 0.24 g AgCl containing spontaneously formed silver traces. Ion selective membrane was prepared by mixing TpCIPB (5 mmol kg⁻¹) and CX6 (10 mmol kg⁻¹) with 66 wt% NPOE and 33 wt% PVC. Total membrane solutes (600 mg) were dissolved in 4 mL THF to form solutions of 15 wt% then sonicated for 10 min. A special protocol [40] that avoids traditional conditioning procedure was followed where 40 μL of aqueous FLU solution (1 × 10⁻¹ Mol L⁻¹) was sonicated with THF membrane cocktail solution (~1% v/v).

Fabrication of SPE

Laser cut black sticker was placed on a piece of glossy white paper for screen-printing of two electrodes (Fig. 1A). Each electrode consists of circular cut with 2-mm diameter with a long electrical lead of 0.6 mm width and spaced by 35 mm. This dimension fits well with 2-pin 8-mm LED strip light connector. Silver pen is used to draw the reference electrode, while carbon ink was brushed over the indicator electrode area. A couple of silver layer was enough for good conductivity, while the resistance of carbon dropped to about 400 Ω after six carbon layers. The paper strip with electrodes was cured at 130 °C (Fig. 1B). Two lamination plastic films were laser cut and used to seal paper strip. One film with two holes for circular electrode-sensing discs (Fig. 1C) and the other contain single oblong cut as a sample holder (Fig. 1D). The length covering lamination films were 2 mm shorter than the paper strip to leave electrical leads bared for connecting with 2-pin LED light connector (Fig. 1D). Ten μL aliquot of PEDT dispersion was applied once on the carbon circular disc and allowed for 10 min air drying, and then 3 μL of each of reference or indicator membrane cocktails were drop-casted three times in the corresponding circular discs (Fig. 1E). The membranes were stored in 5 μL of 3 M KCl solution for 4 h (Fig. 1F). The solutions were held by the surface tension of aqueous aliquot and kept in confined cool place.

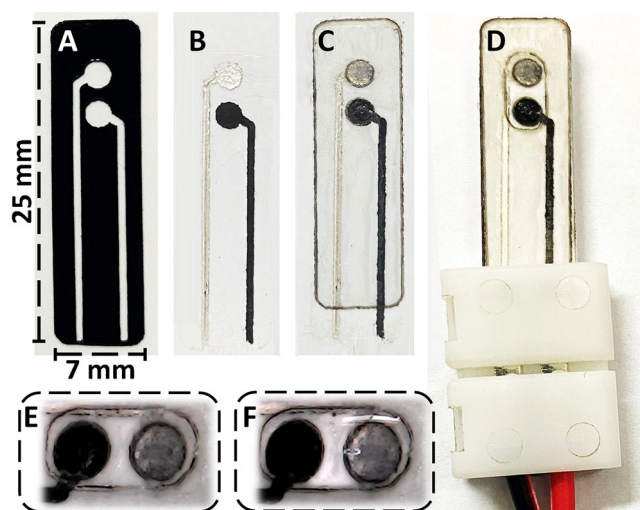


Fig. 1 Photograph of the fabrication steps of the proposed sensor **A** laser cut black screen on glossy paper; **B** printed silver and carbon inks on paper substrate; **C** covering electrical leads with lamination plastic film leaving circular discs of electrodes bared; **D** final lamination of plastic film with single oblong cut as a sample holder then plugged into 2-pin LED light connector; insets show the surface of the two electrodes after application of the corresponding membranes (**E**) and conditioning in 3 M KCl solution (**F**)

Calibration construction and response characteristics

Calibration was constructed through consecutive additions of 5 μL of FLU buffered standard solutions (pH 2.5) at different concentration levels (1×10^{-7} – 1×10^{-2} mol L⁻¹) in the defined zone. The electrode surface was wiped by filter paper between measurements. Potential readings were correlated to the logarithm of corresponding standard concentration, and the electrochemical response was characterized based on IUPAC recommendations. Detection limit was estimated at the point of intersection of calibration plot at the final low concentration level and the extrapolated linear segment of background potential. Stability of the proposed sensor was evaluated by standard deviation of slopes obtained from different calibrations constructed daily. Working pH range was also examined at two levels (1×10^{-3} and 1×10^{-4} mol L⁻¹) of FLU standard solutions prepared at different pH range (2–5).

Selectivity measurements

Separate solutions method was applied to calculate the selectivity coefficients ($K_{A,B}^{pot}$) for different interferences. Values were calculated for a diverse range of soft and alcoholic drinks' ingredients. Potentials were measured for the primary ion (A; FLU) and interfering ingredient (B) standard solutions, both having the same concentration (1×10^{-3} mol L⁻¹ in buffer pH 2.5), and then $K_{A,B}^{pot}$ was calculated [41]. Other related benzodiazepines were also examined by plotting calibration curves with the proposed sensor conditioned in the primary ion [41]. Unbiased selectivity coefficients were calculated using 1×10^{-4} mol L⁻¹ as a common concentration level of these organic cationic drugs that showed Nernstian responses.

Application on spiked beverages

Some selected beverages were individually spiked with FLU to obtain a concentration of 2.5×10^{-3} mg mL⁻¹. Aliquots of the spiked carbonated soda beverages (5 μL) were directly used for potentiometric measurement. On the other hand, 2 mL aliquot of either energy drink, malt beverage, or mineral water was transferred into 10-mL volumetric flask prior to analysis and diluted to volume with buffer pH 2.5.

Results and discussion

FLU belongs to benzodiazepines group with imine functionality (Fig. S1, supplementary information). This ionizable functional group is the core of potentiometric sensing mechanism that suggests the use of cation exchanger in indicator membrane. TpCIPB is a lipophilic negative ion that

considerably limits the extraction of co-ions along with FLU cation and hence improves the phase-boundary potential at the vicinity of membrane surface [42, 43]. On the other hand, CX6 is an uncharged ionophore that improves the ion-exchange mechanism with respect to thermodynamic process [44]. In this study, all solid-state potentiometric sensors were designed as a portable and easy-to-use POC paper device for FLU analysis. This miniaturized SPE comprised silver ink for reference electrode and carbon ink modified with hydrophobic layer of PEDT nanoparticle dispersion for indicator electrode. Stability of both reference and indicator electrodes were tested, and electrochemical characteristics were then evaluated and compared to PEDT-free sensor for reliable application on different beverages.

Reference membrane comprises both anion (TDAB) and cation (TpCIPB) exchangers. This screen-printed silver reference electrode was evaluated by measuring the potentials of FLU standard solutions (pH 2.5) in conjunction with conventional Ag/AgCl double-junction macroelectrode (Fig. S2, supplementary information). Potential stability and low drift of the prepared miniaturized silver screen-printed reference electrode were observed and thus ascertained the use of double-salt approach for faster response [39]. The indicator membrane contains TpCIPB as cation exchanger for FLU sensing and CX6 as an ionophore that facilitates the thermodynamic equilibrium of the target drug. This indicator membrane usually conditioned in the target ion aqueous solution before use. In previous study [27], we have exploited surface tension phenomenon to hold the aqueous conditioning solution in place, whereas this procedure was not working for this sensor in miniature. Hence a special protocol [40] that circumvent this traditional procedure was applied in this study. Ion exchange equilibrium between organic membrane phase and aqueous solution took place during sonication where FLU as a primary ion exchange with potassium in membrane phase for FLU-TpCIPB ion pairing. This un-conditioning protocol facilitated not only membrane preparation but also was beneficial for such miniaturized electrode, where conditioning of indicator membrane in vicinity of reference membrane is unattainable in this confined space.

MWCNTs exhibit high thermal and chemical stability as they decrease resistance and enhance the electron transfer process between the electrode and electro active species [45]. This carbon surface of the indicator electrode was modified with PEDT nanoparticles in order to prevent the formation of water layer and improve potential reproducibility. This hydrophobic conductive polymer alleviates the setback for water layer formation in the interface between carbon and sensing membrane. Long-term stability of the indicator electrode was evaluated at different levels of applied PEDT suspension. Water layer formation test was conducted, and potential drifts were detected upon changing FLU solution ($1 \times 10^{-4} \text{ mol L}^{-1}$) to interfering ion solution (diazepam; $1 \times 10^{-2} \text{ mol L}^{-1}$) then

back again to the same FLU solution. The obtained data were then plotted and compared to results obtained from PEDT free sensor (Fig. 2). Electrodes with PEDT (10 μL) showed no significant potential drift when immersed in the primary ion after incubation in the interfering ion as well as good reversibility of the proposed sensor. It has to be mentioned that no further improvement was registered for higher levels of PEDT. On the other hand, electrodes without PEDT suffered from significant potential drift that is attributed to formation of a water layer beneath the membrane that triggered ion fluxes and subsequent changes in ionic composition [46]. Exploiting PEDT as a conducting polymer substantially curbs the formation of water layer and enhances the long-term stability of the proposed sensor. Figure 3 shows a uniform distribution of PEDT nanoparticles ($70 \pm 15 \text{ nm}$ in diameter) on the MWCNTs surface.

Performance characteristics of the proposed sensor

IUPAC standards were followed to evaluate the proposed PEDT-based sensor response characteristics in parallel with PEDT-free sensor (Table 1) [47]. Calibration plots for FLU in the range of 1.00×10^{-6} – $1.00 \times 10^{-2} \text{ mol L}^{-1}$ were constructed (Fig. 4A), and the slopes were found to 57.9 and 55.5 mV/decade for PEDT-based and PEDT-free sensors, respectively. The calculated slopes are close enough to the response of monovalent cation drug. Calibration curves of three different PEDT-based sensors were almost superimposed with SD of intercept = 0.9 mV. This insignificant offset indicates the good reproducibility of PEDT-based solid-contact system compared to PEDT-free one (Fig. S3, supplementary information). Detection limit for FLU was determined at the point of intersection of the extrapolated lines of the Nernstian response and linear segments of the background buffer [47].

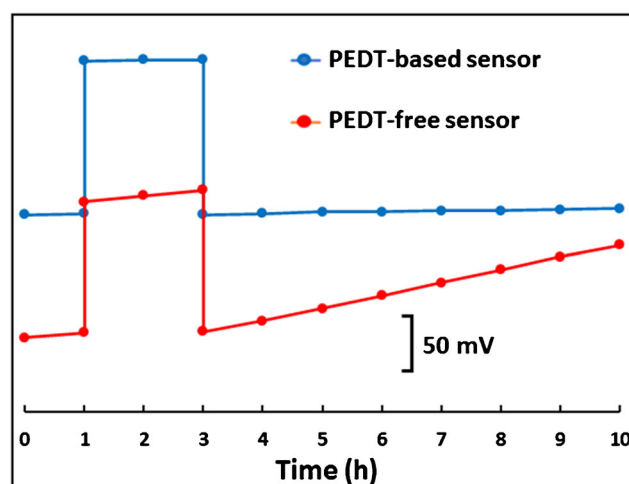
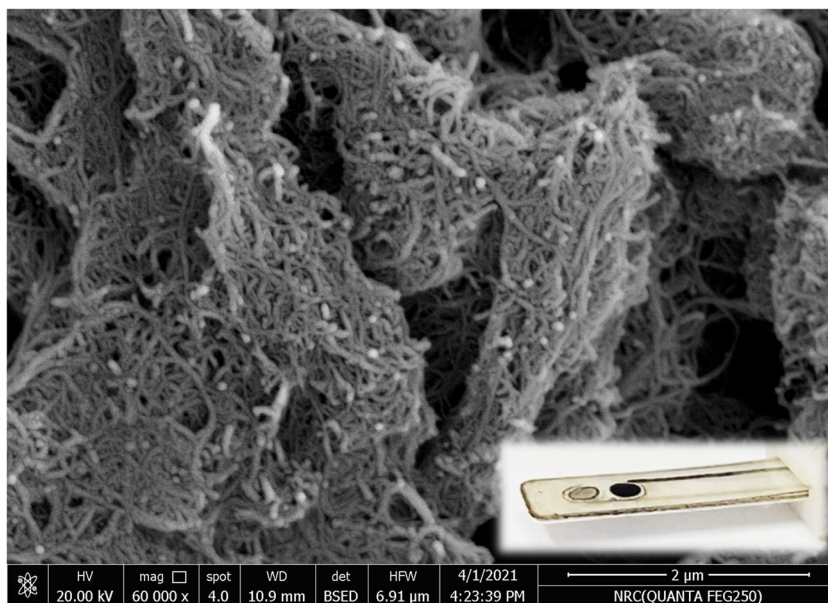


Fig. 2 Water layer formation test of PEDT-based and PEDT-free sensors showing potential drifts upon changing FLU solution ($1 \times 10^{-4} \text{ mol L}^{-1}$) to interfering ion solution (diazepam; $1 \times 10^{-2} \text{ mol L}^{-1}$) then back again to the same FLU solution

Fig. 3 Scanning electron microscopy image of PEDT nanoparticles drop casted on MWCNTs electrode



The results reveal that PEDT-based sensor can detect FLU down to 5.5×10^{-7} which is significantly below than 8.0×10^{-7} mol L⁻¹ for PEDT-free one. These results may be attributed to the absence of inner aqueous layer in which primary ion is trapped. Those ions would further leach during measurements of dilute samples [48]. The effect of PEDT on the long-term potential stability in 1×10^{-4} mol L⁻¹ FLU solution was favorably compared to PEDT-free sensor. The

potential drift in PEDT-based sensor was about 9×10^{-1} mV h⁻¹ which is significantly lower than PEDT-free sensor with 5 mV h⁻¹ potential drift (Fig. 4B). In addition, each sensor was monitored for a continuous use for 1-month period. Table S1, supplementary information shows a lower slope variation for PEDT-based sensor (± 1.4) compared to PEDT-free one (± 2.8) which proves the beneficial effect of PEDT on sensitivity. It is worth noting that the potential drift and the decreased

Table 1 Electrochemical response characteristics of the proposed PEDT-based potentiometric sensor in parallels with PEDT-free one

Parameter	PEDT-based sensor	PEDT-free sensor
Slope ^a (mV/decade)	57.9	55.5
Intercept (mV)	501.6	387.8
LOD (mol L ⁻¹)	5.5×10^{-7}	8.0×10^{-7}
Response time (s)	5	10
Working pH range	2–3	2–3
Concentration range (mol L ⁻¹)	1.0×10^{-6} – 1.0×10^{-2}	1.0×10^{-6} – 1.0×10^{-2}
Stability (days)	40	35
Correlation coefficient	0.9999	0.9997
Accuracy ^b	98.04 \pm 1.744	–
Repeatability ^c	1.767	–
Intermediate precision ^c	1.983	–
Reproducibility ^d	4.699	–
Robustness ^e	0.574	–

^a Average of three determinations

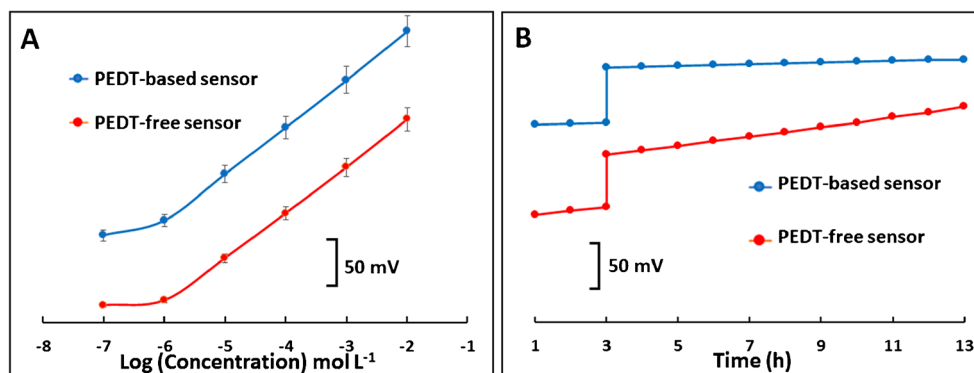
^b Mean \pm SD of recoveries for six determinations

^c RSD% of recoveries for the determination of two concentrations (1×10^{-3} and 1×10^{-4} M), repeated three times within the day for repeatability ($n = 6$) and repeated in three successive days for intermediate precision ($n = 6$)

^d RSD% of recoveries for the determination of two concentrations (10^{-3} and 10^{-4} M) measured using six different SPEs ($n = 6$)

^e RSD% of recoveries for the determination of two concentrations (1×10^{-3} and 1×10^{-4} M) measured at three different buffer pH values 2.2, 2.5, and 2.8 ($n = 6$)

Fig. 4 **A** Potential profile versus Log concentrations of FLU (1×10^{-7} – 1×10^{-2} mol L⁻¹) obtained for PEDT-based and PEDT-free sensors; **B** long-term potential stability of PEDT-based and PEDT-free sensor in 1×10^{-4} mol L⁻¹ FLU standard solution



reproducibility of the standard potential with time are attributed to the formation of a water layer between the conducting solid and the membrane phase [49–51]. These results came in accordance with the water layer test. The proposed PEDT-based SPE showed a stable potential from pH 2.0 to 3.0 (Fig. 5A), likely attributed to FLU pKa value of 1.7. The dynamic response time was also monitored through measuring the time required for each concentration within the linearity range to reach a steady potential reading (± 1 mV). Potential-time plot of the proposed PEDT-based sensor (Fig. 5B) showed rapid response time (~ 5 s.). All these positive findings proof the feasibility of the proposed PEDT-based sensor for the determination of FLU concentration.

Sensor selectivity

The effect of common interfering ingredients of beverages on the performance of PEDT-based sensor was studied by separate solutions method, and selectivity coefficients ($K_{A,B}^{pot}$) were calculated using the following equation [41]:

$$\log K_{A,B}^{pot} = \frac{E_B - E_A}{S}$$

where S is the slope of the calibration plot of the primary ion. E_A and E_B are the measured potentials for primary and

interfering ions, respectively. Table 2 shows the calculated selectivity of the proposed sensor towards the ionic FLU in presence of common soft and alcoholic drinks' ingredients. Lower values were obtained for most of sweeteners, acid regulators, preservative, and acidic or phenolic compounds; however, considerable values were obtained for amino organic compounds in fermented beverages. These biogenic amines and nitrogenous compound are hardly interfered due to their limited relative concentrations in such drinks [52]. Selectivity of PEDT-based sensor was also investigated in the presence of some structurally related drugs (Fig. S1, supplementary information). Responses of benzodiazepines like clonazepam, diazepam, and alprazolam were examined separately at different concentration levels (1×10^{-5} – 1×10^{-3} mol L⁻¹). Figure 6 shows slopes close to Nernstian for ionic benzodiazepines, and thus unbiased selectivity coefficients were calculated based on separate solutions method (Table 2) [41]. Substantial selectivity coefficient values for congeners may be attributed to their similar functionality to the target ion as well as their large log P values (~ 2.9). It is worth noting that a non-Nernstian response was obtained for some examined inorganic ions (Na^+ , K^+ , and Ca^{2+} as chlorides). The lipophilic nature of the ion selective membrane hinders the ion exchange of inorganic ions. In addition, CX6 was expected to behave as a receptor that selectively binds iminium ions. This was evidenced by higher selectivity coefficient values obtained for

Fig. 5 **A** Effect of pH on the proposed PEDT-based sensor response; **B** plot of potential as a function of time in dynamic response plot

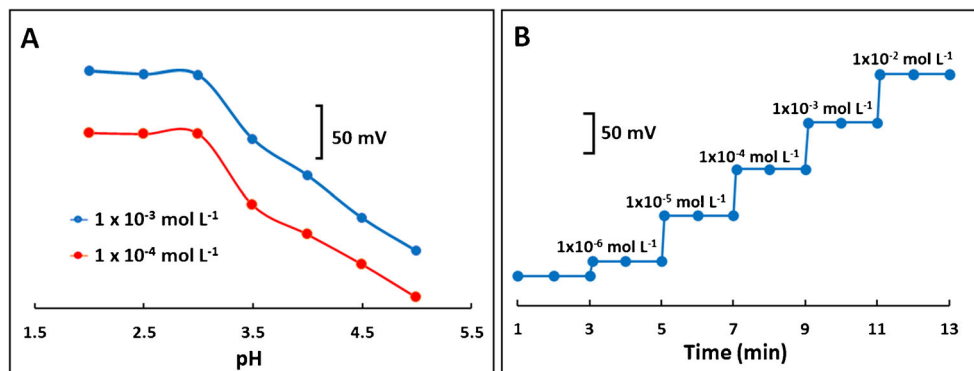


Table 2 Potentiometric selectivity coefficient ^a ($K_{A,B}^{pot}$) for the proposed PEDT-based sensor

Natural sweeteners						
Sucrose	Glucose	Fructose	Sorbitol	Sucralose	Saccharin	Aspartame
6.12×10^{-3}	4.93×10^{-3}	4.95×10^{-3}	5.41×10^{-3}	3.97×10^{-3}	5.21×10^{-3}	6.17×10^{-3}
Acidity regulators						
Citric acid	Malic acid	Succinic acid	Phosphoric acid	Potassium sorbate	Sodium benzoate	Dimethyl dicarbonate
3.09×10^{-3}	3.14×10^{-3}	2.86×10^{-3}	2.12×10^{-3}	3.87×10^{-2}	4.39×10^{-2}	1.93×10^{-3}
Ingredients in energy drinks						
Taurine	Caffeine		B vitamins ^b	Antioxidant	Nitrogenous compounds in beer	
5.87×10^{-2}	8.74×10^{-2}		5.26×10^{-2}	Ascorbic acid	Tyramine	Histamine
Aromatic acids in beer						
Salicylic acid	Phthalic acid		Cinnamic acid	Other acids in wine	Pyrazine	
3.28×10^{-3}	4.15×10^{-3}		4.22×10^{-3}	Lactic acid	5.79×10^{-1}	5.66×10^{-1}
Other nitrogenous compounds in wine						
2-Methylbutylamine	4-Methylbutylamine		Phenethylamine	Spermine	Spermidine	Ethanolamine
2.83×10^{-1}	2.55×10^{-1}		4.83×10^{-1}	4.21×10^{-1}	3.73×10^{-1}	2.99×10^{-1}
Phenolic compounds in wine						
Phenol	Cresols	Guaiacol	Acetovanillone	Delphinidin chloride	Malvidin chloride	Peonidin chloride
3.46×10^{-3}	4.42×10^{-3}	3.95×10^{-3}	4.74×10^{-3}	4.29×10^{-2}	3.89×10^{-2}	4.22×10^{-2}
Related drugs						
Clonazepam	Diazepam	Alprazolam				
6.69×10^{-2}	3.16×10^{-1}	1.74×10^{-1}				

^a Average of three determinations

^b Mixture of thiamin, niacin and cyanocobalamin

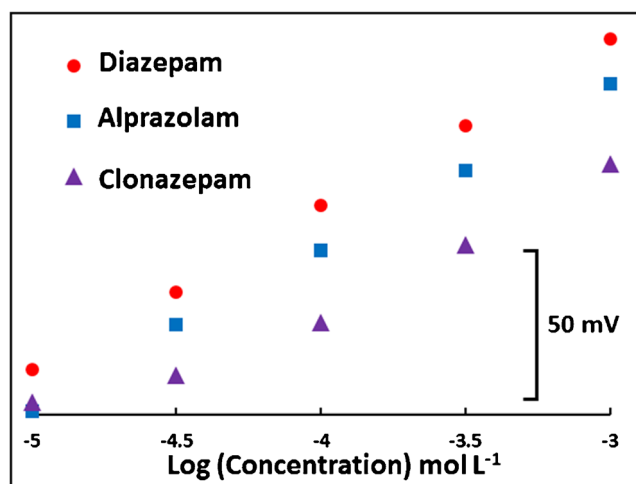


Fig. 6 The proposed PEDT-based sensor response as a function of Log concentration for flunitrazepam structurally related drugs (clonazepam, diazepam, and alprazolam) in selectivity measurements

CX6 free sensor. About 0.5–1 order of magnitudes increase were encountered for interfering organic ions.

Application on selected beverages

The amnesic effect of FLU is dose related, and it can impair an individual for 8–12 h after ingestion of 1 mg dose within 30 min [5]. Commercial beverage volumes are ranging from 8 (~236 mL) to 20 fl oz. (~591 mL). Accordingly, beverages should be spiked with FLU to have a concentration of 2.5×10^{-3} mg mL⁻¹ ($\sim 8 \times 10^{-6}$ mol L⁻¹) which is the threshold for sensible application. Since the pH values of Coca-cola®, Fanta®, and Sprite® (2.8 ± 0.4) are in the working pH range, 5 μ L aliquots of the spiked samples were directly applied on the specified measurement zones, whereas further fivefold dilutions with Robinson buffer (pH 2.5) were applied prior to analysis of spiked Red Bull® (pH 3.4), Barbican® (pH 4.5), and Nestlé® purelife® water (pH 6.2). These dilutions were necessary to get samples into the working pH range provided that the final concentration ($\sim 1.6 \times 10^{-6}$ mol L⁻¹) is still in the linear Nernstian response. The electromotive forces were recorded, and concentrations were then calculated using the corresponding regression equation (Table 3).

Table 3 Analysis of FLU-spiked beverages by the proposed PEDT-based sensor

Spiked carbonated soft drinks	Recovery% \pm SD ^a	Spiked other beverages	Recovery% \pm SD ^a
Coca-cola®	102.25 \pm 8.633	Red Bull®	105.25 \pm 10.88
Fanta®	100.75 \pm 6.034	Barbican®	102.62 \pm 9.23
Sprite®	101.75 \pm 5.420	Nestlé® Pure Life® water	93.12 \pm 5.59

^a Average of five determinations

Statistical analysis

The obtained results from the analysis of FLU pure powder by the proposed PEDT-based sensor ($98.04\% \pm 1.74$; $n = 6$) were compared to those of non-aqueous titrimetric British Pharmacopeia [37] method ($99.61\% \pm 0.80$; $n = 6$). Student's *t* and *F* values were then calculated (Table S2, supplementary information). There is no significant difference between the proposed method and official one regarding accuracy and precision. It should be emphasized that the proposed sensor is easier to use or to apply using less equipment than the official titration procedure besides the proposed sensor saves analysis time where about 2 min are enough to complete the analysis rather than 20 min on average for the titration method. Table 4 draws parallels between the proposed potentiometric FLU sensor and other reported voltammetric ones. Our sensor achieved comparable detection limit among the point-of-care electrochemical sensors. Besides the good selectivity obtained in a wide range of soft drinks that is attributed to insignificant potentiometric interference of common oxidizable or reducible drink additives.

Conclusion

This paper-based sensor provides a viable tool for the detection and determination of FLU in beverages. Unconditioning protocol facilitates the preparation of such miniaturized membrane sensor. PEDT proof its ability to circumvent the formation of water layer that improved the reproducibility and decreased the potential drifts during measurements by the proposed sensor. Sensitivity was slightly improved by about two-tenths order of magnitudes using PEDT sensor. A variety of beverage ingredients like sweeteners, preservatives, nitrogenous, and phenolic compounds has been tested. The sensor showed high degree of selectivity, albeit some structurally related hypnotics interfere. Special connector was used to facilitate the device operation for end-users. Both the design and the application are important to devise point-of-care test in future work. Based on the framework of point-of-care devices, the proposed novel potentiometric FLU sensor is affordable, sensitive, specific, user-friendly, rapid, and robust device utilizing less equipment and deliverable to

Table 4 An overview on recently reported electrochemical methods for the determination of flunitrazepam

Ref. No.	Electrochemical technique (miniaturization)	Nanomaterial applied	Linearity range	LOD	Application
18	Voltammetry (yes)	NA	10–200 ng/mL	6 ng/mL	Alcoholic beverage (Gordon's Gin & Tonic)
19	Voltammetry (yes)	NA	1–95.24 µg/mL	0.47 µg/mL	Coca-cola and the alcopop WKD
20	Voltammetry (yes)	Fe _x O _y	0.05–10 µM	0.015 µM	Pepsi-cola and alcoholic beverages
21	Voltammetry (yes)	TiO ₂ /CuO-N-rGO	0.001–50.0 µM	0.30 nM	Pepsi Cola, Tourtel Malt, human serum
22	Voltammetry (no)	Eβ-CD/B-GO ^a	2.0 nM–0.5 µM 0.5–20.0 µM	0.6 nM	Human serum
23	Voltammetry (no)	MnFe ₂ O ₄ /AuNPs	0.1–100 µM	0.33 nM	Human plasma
This work	Potentiometry (yes)	PEDT/MWCNTs	1 × 10 ⁻⁶ –1 × 10 ⁻² M	5.5 × 10 ⁻⁷ M	Coca-cola, Fanta, Sprite, Red Bull, Barbian, and Nestle purelife water

^a β-cyclodextrin and Boron-doped reduced graphene oxide

end users. Further studies to improve sensitivity and selectivity based on modeling a specific chemical receptor or combination of multi-detection modes would be conducted in future research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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