Flexible Screen-Printed Amperometric Sensors Functionalized With Spray-Coated Carbon Nanotubes and Electrodeposited Cu Nanoclusters for Nitrate Detection

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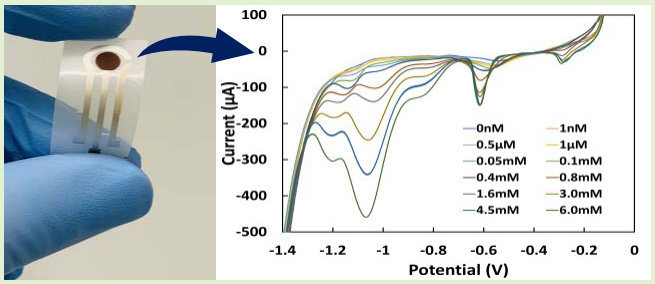
***Abstract*—In this work, we present a novel, sensitive, easy-to-fabricate, flexible amperometric sensor constituted by screen-printed silver (Ag) electrodes functionalized with a copper (Cu) film electrodeposited on top of a spray coated network of single-walled carbon nanotubes (SWCNTs). The Cu/SWCNTs/Ag electrode showed excellent catalytic activity towards the electro-reduction of nitrate ions at neutral pH with a significant increase in cathodic peak currents in comparison with the electrode without SWCNTs (Cu/Ag). The developed Cu/SWCNTs/Ag sensor showed a wide linear detection range from 0.5 μM to 6.0 mM (0.31 mg/l to 372.02 mg/l) with good sensitivity (18.39 μA/mM) and a calculated limit of detection (LOD) of 0.166 nM (10.29 μg/l). It also showed a good selectivity (maximum standard deviation (SD) was 3.25 μA) towards different interfering ions ($Fe^{2}+$, Na^{+}, $Cu^{2}+$, $SO_4^{2}-$, CH_3COO^{-}, Cl^{-}, NO_2^{-} and HCO_3^{-}), as well as good reproducibility, mechanical durability, time and temperature stability. In real sample analysis (tap and river water), the sensor exhibited good agreement with the compared outcome of high-performance liquid chromatography (HPLC) measurements, proving to be a promising analytical tool for the detection of nitrate in water.**



***Index Terms*—SWCNTs, electrochemical, nitrate sensor, flexible substrate, copper electrodeposition, screen printing.**

I. Introduction

PURE drinking water is becoming one of the major assets all over the world, especially considering the constantly growing population. A major thread to the availability of pure water is given by the industrial and agricultural sectors, which lead to water contamination [1]. Nitrate (NO_3^-) ions are among the main contaminants in water. In fact, NO_3^- is widely used not only as a food preservative to prevent poisoning from *Clostridium botulinum* in the industrial sector, but also as an additive to enhance the food color and flavor or as fertilizer in the agriculture [2], [3]. Despite these useful applications, exposure to a high level of nitrate can put humans at risk of several chronic and acute conditions such as liver disease, gastric cancer, Parkinson disease, and blue-baby syndromes [4], [5]. Indeed, NO_3^- ions can lead to the formation of various harmful nitrogenous substances, such as nitrite, nitric oxide, N-nitrosamines [6]. Considering these toxic effects, the World Health Organization (WHO) as well as the European Directives have fixed the maximum contaminant level (MCL) of NO_3^- as 50 mg per liter (ca. 0.8 mM) in pure drinking water [7]. As a result, rapid and cost-effective methods able to detect nitrate content in drinking water are extremely required. In this context, electrochemical sensors have been widely investigated in nitrate monitoring because of their high sensitivity, selectivity, quick response, portability, and miniaturization [8]–[10]. Various electrochemical sensors such

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as amperometric, potentiometric, conductometric have been exploited to detect nitrate with or without the involvement of specific bio-recognition elements, such as enzymes [11]–[14]. In fact, different types of metals such as copper (Cu), platinum (Pt), silver (Ag) and gold (Au) have been utilized as catalysts for the reduction of nitrate eliminating the need for a specific bio-recognition element which complicates the sensor fabrication and decreases the sensor shelf-life [15]–[17]. Among these electrocatalytic metals, Cu is surely the most effective metal to electro-reduce nitrate ions because of its high conductivity (58.14×10^6 S/m), improved charge transfer and cost-effectiveness [6], [18]–[20]. Recently, it has been shown that it is possible to reduce the limit of detection (LOD) of electrochemical nitrate sensors by increasing the electroactive surface area thanks to the use of nano composites made, for example, of Cu nanoparticles and carbon nanotubes or reduced graphene oxides [11], [21]. Single-walled carbon nanotubes (SWCNTs) have received considerable interest for electrochemical sensors due to their fast electron transfer, high conductivity (10,200 S/m) [22] and the ability to donate or receive electrons in a wide range of electrochemical potential, which allows them to be used as mediators in sensing platforms [23]. Moreover, because of their large area-to-volume ratio, SWCNTs allow enhancing the sensor sensitivity to the nanoscale response by increasing the electroactive surface area [24], [25].

Here, we demonstrate a novel, flexible, screen-printed amperometric electrochemical nitrate sensor, where SWCNTs are sprayed on an Ag working electrode, followed by the electro-deposition of Cu (Cu/SWCNTs/Ag sensor). With respect to the previous conference proceeding (IEEE FLEPS 2021), here we additionally presented a full characterization of the proposed sensors in terms of stability, selectivity towards different common based ions in drinking water, temperature of operation and real sample testing, which show evidence of the performance of the sensor regarding the novel aspects. The uniqueness of this proposed sensor is the combination of cost-effective and scalable techniques such as screen-printing, spray deposition of SWCNTs and electrodeposition of Cu in an easy and reliable method, if compared to previously reported papers [11], [26]. We also demonstrated that the presence of SWCNTs actively helps to increase the electroactive surface area successfully electro-reducing nitrate ions. The Cu/SWCNTs/Ag sensor is highly capable of detecting nitrate in water with a low calculated LOD (0.166 nM) and a wide linear detection range (0.5 μM to 6 mM) by using linear sweep voltammetry (LSV). The most common interferents (Fe^{2+}, Na^+, Cu^{2+}, SO_4^{2-}, CH_3COO^-, Cl^-, NO_2^- and HCO_3^-) were studied and found very negligible effect on nitrate detection. The sensor was also tested for temperature dependency, repeatability, reproducibility, and stability in time. In the end, the sensor was tested with real water samples which showed a good agreement with HPLC results.

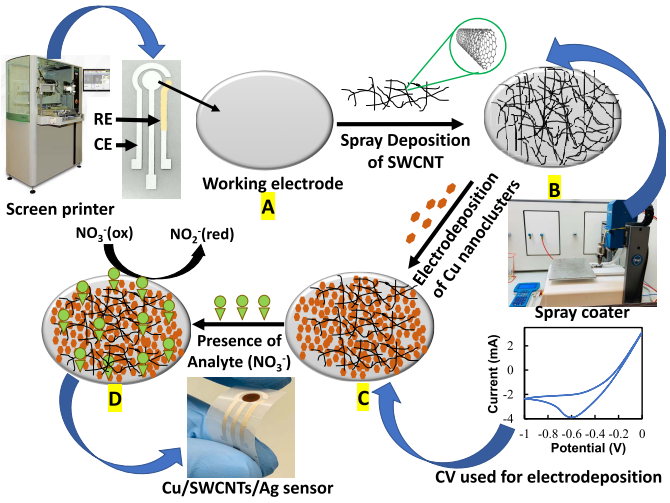

Fig. 1. Schematic illustration of the fabrication process of the proposed nitrate sensor consists of three electrodes system; working electrode (WE), counter electrode (CE) and reference electrode (RE): A) the screen-printed silver WE, B) modified with spray deposited single-walled carbon nanotubes (SWCNTs), C) further modified by electrodeposition of Cu, D) Reduction of nitrate in presence of the analyte.

II. Methods

A. Chemicals and Apparatus

All chemicals and reagents (analytical grade) were used without further purification. Double distilled water (resistivity 18.2 MΩ.cm) was used in all solutions. Ferrous sulfate ($FeSO_4$), sodium chloride (NaCl), potassium chloride (KCl), sodium sulfate (Na_2SO_4), copper sulfate ($CuSO_4 \cdot 5\,H_2O$), sulfuric acid (H_2SO_4), sodium bicarbonate ($NaHCO_3$), sodium acetate (CH_3COONa), sodium nitrate ($NaNO_3$), and sodium nitrite ($NaNO_2$) were purchased from Merck KGaA (Germany). 125 μm thick polyethylene terephthalate (PET) was used as a substrate (Rauch GmbH, Germany). To fabricate the electrodes Ag (ECI 1011) and AgCl (ECI 6038E) screen printed pastes were purchased from LOCTITE E&C (CA, USA). To electrodeposit Cu, 0.1 M $CuSO_4 \cdot 5\,H_2O$ (pH adjusted to 2.0) was used and 0.1 M KCl was used as an electrolyte in all the experiments. River water was collected from the Talvera River in Bolzano, Italy, and tap water was collected from the lab (city water supply in Bolzano, Italy). All electrochemical measurements were performed by using VersaSTAT 4 electrochemical workstation (Princeton Applied Research, USA) at room temperature. A 1525 Waters HPLC system (Waters Corporations, MA, USA) equipped with a Symmetry C18 Column (2.1 × 50 mm, 3.5 μm) and a photo-diode array detection (PDA 2998) set at 286 nm was used.

B. Sensor Fabrication

The typical three-electrode electrochemical sensor structure was realized on top of a PET substrate with the use of a semi-automatic screen-printing machine (Aurel automation S.P.A. C290, Italy). The sensor structure consists of an Ag working electrode (WE) (diameter: 4mm) (Fig. 1A), an Ag

counter electrode (CE) and an Ag/AgCl pseudo-reference electrode (RE). On top of the WE electrode, a water-based SWCNT dispersion prepared by the method of [27], was spray coated as described in [28] (Fig. 1B). Afterwards, Cu electrodeposition was performed on top of SWCNTs coated WE according to [29] (using CV at the potential range of –1.0 to 0 V with a scan rate of 0.1 Vs-1) in 0.1 M $CuSO_4 \cdot 5H_2O/H_2SO_4$ (pH = 2.0) solution at room temperature (Fig. 1C). Finally, the electrodes were slowly rinsed with double-distilled water.

C. Morphological and Compositional Characterization

To analyze the surface morphology of the Cu/SWCNTs/Ag sensor and the crystal structure of electrodeposited Cu, scanning electron microscopy (SEM, Quanta 600F, FEI, USA), atomic force microscope (AFM, CoreAFM, Nanosurf, Sweden), X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDS) were carried out. AFM was operated in static force mode with the 20nN of force setpoint having silicon AFM probes (ContAl-G) and a resonant frequency of 13 kHz. XRD patterns were measured by using an Ital Structures IPD3000 unit, Cu Kα source, and multilayer monochromator at an incident angle on the sample of 5°. Powder patterns were acquired by means of a Dectris Mythen detector (1280 channels) over 10-130° 2-theta range with a 0.02° angular resolution and a total acquisition time of 2400 seconds for each sample. For EDS spectra, 30 s acquisition time and 20 kV acceleration voltage were used. The thickness and roughness of the sensor were measured using a Dektak 150 Surface Profiler (Veeco, NY, USA).

D. Electrochemical and Mechanical Measurements

To evaluate the electrode reaction mechanism and to calculate the active surface area, cyclic voltammetry (CV) was applied (potential range from −0.1 V to −1.4 V) with different scan rates (25 to 500 mVs-1) using 3.0 mM of $NaNO_3$ in 0.1 M KCl electrolyte solution. For the other characterizations, LSV over CV was chosen as measuring method because LSV identifies clearer nitrate reduction peak current [30]. LSV was performed in the potential range of −0.1 to −1.4 V with a scan rate of 10 mV s-1 on different concentrations of NO_3^-. To evaluate the effect of the temperature on the behavior of the sensor, it was tested changing the electrolyte temperature from 15 to 35 °C. Additionally, a customized bending setup was used to study the mechanical stability of the proposed sensor that was cyclically bent to a radius of 5 mm up to 1000 cycles.

III. Results and Discussion

A. Surface Characterization

Fig. 2 shows the SEM image of the uniformly distributed Cu nanoclusters on top of the spray coated SWCNTs. From the image, it is easily noticeable that SWCNTs are uniformly covered by globular-shaped Cu nanoclusters. The diameter of a single Cu nanoparticle is homogeneous all over the WE in a range of 200 nm to 500 nm [31]. The SEM images from Fig. S1A show the bare Ag electrode, where the Ag flakes

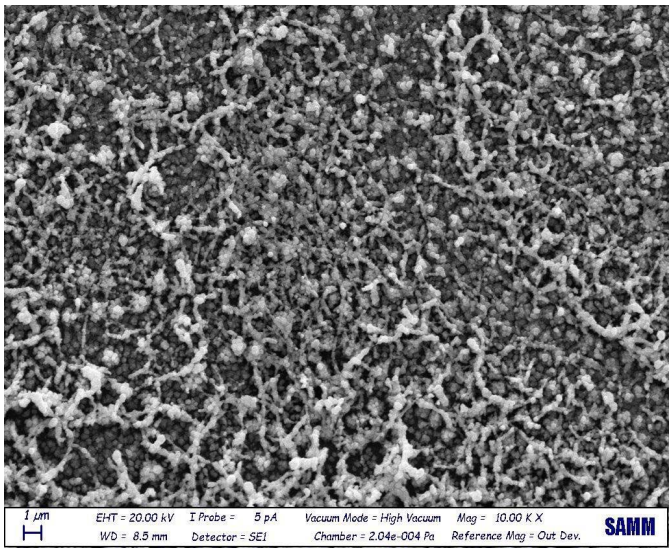

Fig. 2. Scanning electron microscopy (SEM) micrograph of electrodeposited Cu nanoclusters on top of SWCNTs coated Ag electrode.

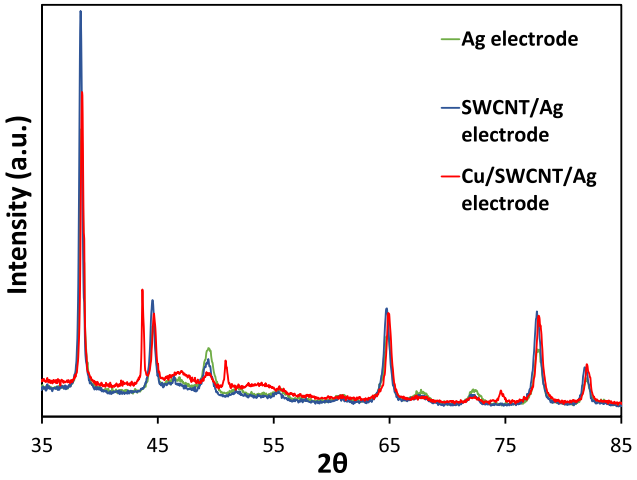

Fig. 3. A comparison of XRD patterns on different fabrication steps of the working electrode (Ag WE (green), SWCNTs/Ag WE (blue), and Cu/SWCNTs/Ag WE (red)).

are clearly visible. The surface morphology is changed after SWCNT and Cu deposition (Fig. S1B). As shown in the AFM image in Fig.S2 (done by spraying 100 layers of SWCNTs on top of glass slide) the nanotubes are uniformly distributed on the substrate. XRD was measured at each step of the sensor fabrication to further investigate the outcome of the subsequent deposition processes for the different materials. Fig. 3 shows XRD patterns of the Ag WE electrode, SWCNTs/Ag WE electrode, and Cu/SWCNTs/Ag WE electrode. All the XRD patterns show peaks at Bragg reflections of crystalline Ag (111), (200), and (220). After the Cu electrodeposition process, the (111), (200) and (220) reflections of crystalline Cu, respectively observed at 43.7°, 50.9° and 74.7°, confirm the presence of crystalline Cu. This observation is in agreement to those previously found also by Chen *et al.* [2]. The presence of Cu is also indicated by an EDS and another XRD measurements (Fig. S3 and S4). The EDS results (Fig. S3) show a normalized mass of 55.23% for Cu, 32.99% for C and 11.78% for O, consistent values with the multi-layers fabrication process.

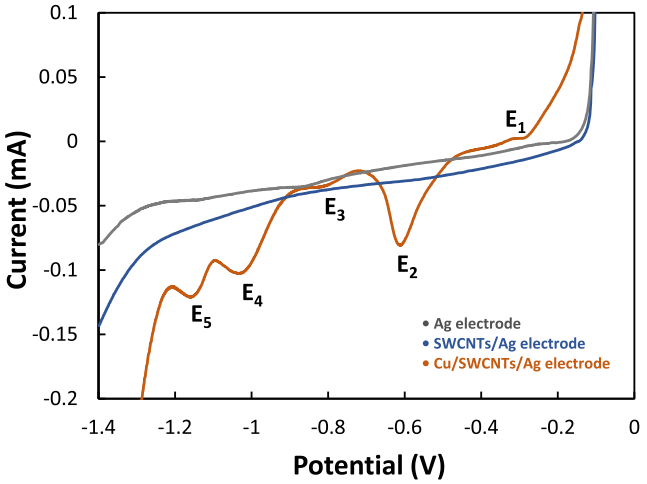

Fig. 4. Linear sweep voltammetry (LSV) analysis showing cathodic reduction peak current of Ag (ash), SWCNTs/Ag (blue) and Cu/SWCNTs/Ag (orange) electrode in the presence of 0.8 mM of NO_3^- concentration.

Whereas XRD results clearly indicate the presence of crystalline Cu after Cu electrodeposition, features of SWCNTs are not easily distinguishable on the XRD patterns of the samples where they are expected. This is likely because of the relatively small amount of SWCNTs material deposited on the surface as compared with Ag and Cu, and of the limits given by the S/N ratio. Additionally, the thickness and roughness results measured by using a surface profilometer demonstrated an increment from 2.34 ± 0.04 μm to 3.26 ± 0.03 μm and from 0.80 ± 0.01 to 1.00 ± 0.20 μm, respectively, before and after SWCNTs and Cu deposition for the Cu/SWCNTs/Ag sensors. This observation has a good agreement with the SEM images (Fig. 2), demonstrating that the surface area increased by Cu electrodeposition.

B. Electrochemical Characterization

Fig. 4 shows three different LSV performed to investigate the voltametric response of the reduction peak current of nitrate (0.8 mM in 0.1 M KCl) at each step of the sensor fabrication. The results show no response of nitrate reduction for both Ag and SWCNTs/Ag electrodes. Instead, the Cu/SWCNTs/Ag electrode (orange curve) shows 5 different cathodic reduction peaks. The peaks at -0.2 V and -0.6 V are attributed to the Cu (I) (E_1) and Cu (II) (E_2) reduction, respectively shown in Equations 1 and 2. At negative potentials, NO_3^- was reduced to NO_2^- (E_3), NO_2^- was reduced to NH_3 (E_4) and in neutral or alkaline electrolyte concentration NO_2^- can be further reduced to NH_2OH (E_5). These consecutive reaction shows three reduction peak at -0.8 V, -1 V and -1.15 V, respectively as similarly reported also in [29], [32]. Indeed, the primary product of NO_3^- reduction was NO_2^- which again is reduced to NH_3 as showed in the equation 3 and 4 and also verified by Hasnat *et al.* [33] and Zhad and Lai [34]. From this observation it can be said that despite the possibility of Cu to form Cu_2O and CuO, it does not interfere with the sensing mechanism for nitrate reduction.

$$Cu(I) + 1e^- \longrightarrow Cu \quad (1)$$

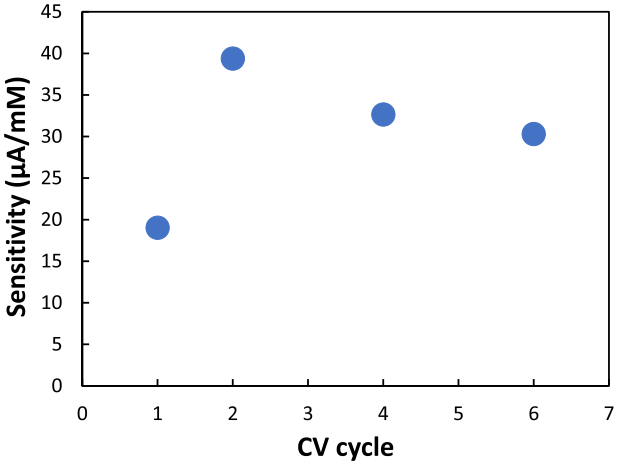

Fig. 5. Sensitivity (obtained using 4 different concentrations (0, 0.1, 0.8 and 1.6 mM of NO_3^-) for different sensors which were prepared through different number of cyclic voltammetry (CV) cycles.

$$Cu(II) + 2e^- \longrightarrow Cu \quad (2)$$

$$NO_3^- + H_2O + 2e^- \longrightarrow NO_2^- + 2\,OH^- \quad (3)$$

$$NO_2^- + 5\,H_2O + 2e^- \longrightarrow NH_3 + 7\,OH^- \quad (4)$$

$$NO_2^- + 4\,H_2O + 4e^- \longrightarrow NH_2OH + 5\,OH^- \quad (5)$$

As the fabrication process involves a procedure of electrodeposition of Cu by CV, it is relevant to investigate the influence of the performed number of CV cycles on the sensor performance [31]. Therefore, electrodes realized by using 4 different CV cycles (1, 2, 4 and 6) of Cu deposition on top of SWCNTs coated WE were measured in terms of current reduction peak at different nitrate concentrations (0, 0.1, 0.8, and 1.6 mM) (Fig. S5). The results (Fig. 5) show that the electrode realized using 2 CV cycles has the highest sensitivity and also the highest nitrate peak current reduction (Fig. S6). More than 2 CV cycles cause excess Cu nanoclusters deposition which may decrease the surface area because of reduction of the porosity by hiding the CNTs of the electrode. Therefore, 2 CV cycle was used as optimized electrodeposition of Cu to fabricate the sensor.

To investigate the nature of the electrochemical reaction of Cu/SWCNTs/Ag electrodes in presence of NO_3^-, the effect of the scan rate on the reduction peak current was studied in the set of measurements. Plotting the reduction peak current versus the square root of scan rate (v) shown in Fig. S7, a linear correlation ($R^2 = 0.969$) was observed suggesting that a diffusion-controlled process is taking place [39]. Additionally, the electrochemical active surface areas of both Cu/Ag (Cu was electrodeposited on screen printed Ag using 2 CV cycles) and Cu/SWCNTs/Ag was determined using the Randles-Sevcik equation [40] for comparison. It has been found that the effective surface areas of Cu/SWCNTs/Ag and Cu/Ag sensors were 0.082 cm^2 and 0.042 cm^2, respectively, proving that the incorporation of SWCNTs can increase the surface area by up to 95.5% [41].

C. Sensor Performance for Nitrate Detection

To investigate the sensor performance, different concentrations (0 nM, 1 nM, 0.5 μM, 1 μM, 0.05mM, 0.1 mM,

TABLE I
COMPARISON BETWEEN THE PERFORMANCE OF DIFFERENT NITRATE SENSOR WHERE CARBON NANOTUBES WERE INCORPORATED WITH OR WITHOUT THE PRESENCE OF CU NANOCLUSTERS

Electrode modification	Method	Linear range (mM)	Sensitivity ($\mu A/mM$)	LOD (nM)	Ref.
Cu/MWCNT/RGO/GCE	SWV	0.001 – 0.075	21.52×10^3	20	[11]
CNT/PPy/NR/GCE	CV	0.44 – 1.45	0.3	17	[22]
MWCNT/CuNPs/Ag-probe	LSV	0.001 - 5	80.62	0.333	[35]
CuNps/MWCNT-PEI/PPy-PSS/GCE	Am	0.1 - 5	137	3×10^4	[36]
CuO/MWCNT/GCE	DPV	0.01 - 0.7	-	-	[37]
L-MWCNT/GCE	CV	0.5 - 10	-	900	[38]
Cu/SWCNT/SPSE	LSV	5×10^{-4} - 6	18.39	0.166	This work

Cu: Copper, MWCNT: Multi-walled carbon nanotubes, RGO: Reduced graphene oxide, GCE: Glassy carbon electrode, CNT: carbon nanotubes, NR: Nitrate reductase, CuNPs: Copper nanoparticles, PEI: Polyethyleneimine, PPy: Polypyrrole, PSS: Polystyrene sulfonate, L-MWCNT: Lipophilic multi-walled carbon nanotubes, SPSE: Screen-printed silver electrode, SWV: Square wave voltammetry, CV: Cyclic voltammetry, LSV: Linear sweep voltammetry, Am: Amperometry, DPV: Differential pulse voltammetry.

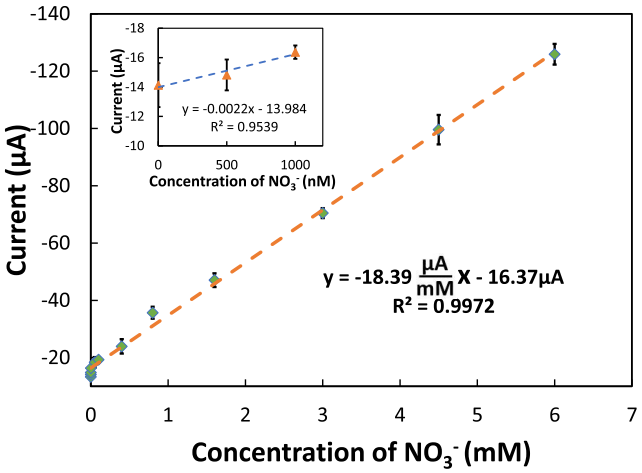

Fig. 6. Calibration curve of Cu/SWCNTs/Ag sensor for nitrate detection (NO_3^- reduction peak current versus NO_3^- concentration). Inset shows the lowest concentration (1nM, 500nM and 1000 nM). Each point is the average peak current performed by 3 electrodes, where the standard deviation with error bars is shown.

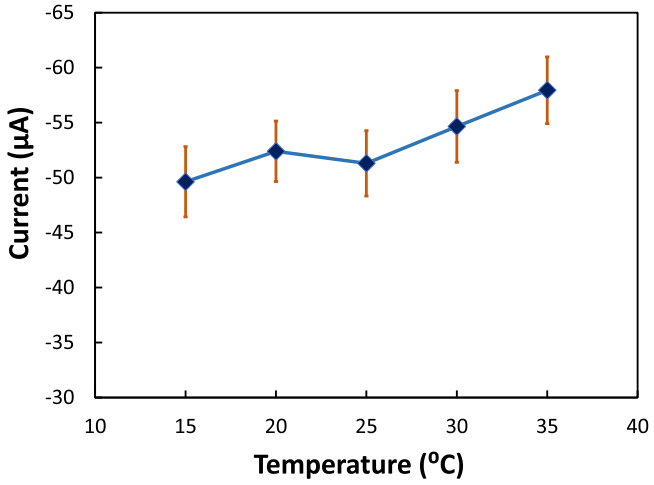

Fig. 7. The effect of temperature analysis in 5 different temperature (15, 20, 25, 30, and 35 °C) using 0.8 mM of NO_3^-.

0.4 mM, 0.8 mM, 1.6 mM, 3.0 mM, 4.5 mM, and 6.0 mM) of NO_3^- were tested by LSV at optimized experimental conditions for Cu/SWCNTs/Ag sensor (Fig. S8). The calibration curve (Fig. 6) plotted by averaging the nitrate reduction peak current of 3 samples for each concentration, shows a wide linear range from 0.5 μM to 6.0 mM (1 nM was not considered due to the high SD value), with good repeatability and reproducibility (SD ranges from 0.91 to 5.14 μA). As expected, the Cu/SWCNTs/Ag sensor showed excellent sensitivity (18.39 μA/mM) with a high coefficient of determination (99.72%). This sensor showed higher sensitivity comparing to Cu/Ag sensor (sensitivity (12.19 μA/mM with $R^2 = 0.98$). To calculate the detection limit, the following equation was used:

$$LOD = (3.3\ STDEV\ I_0)/m \tag{6}$$

where I_0 is the generated peak current at 0 mM NO_3^- and m is the slope of the linear response curve.

The LOD was found to be 0.166 nM for Cu/SWCNTs/Ag sensor comparing to Cu/Ag sensor (LOD 0.381 nM) [41], which indicates that the incorporation of SWCNTs increased the electron transfer kinetics, possibly thanks to the increase of the electroactive surface area. While comparing the calculated LOD as well as the linear detection range of this sensor with other nitrate sensors where carbon nanotubes were incorporated with or without various forms of Cu (Table I), it can be found that the obtained LOD was lower, with a wider range of detection. This is probably due to the covering of SWCNTs by electrodeposited Cu, providing straight conducting pathways for electron transfer and higher surface area with outstanding adsorption property [32]. Moreover, these results were obtained using a neutral electrolyte solution (0.1 M KCl) showing the ability to measure nitrate ions in real water sample without the need of pH control, whereas for most of the proposed sensors an acidic medium (Na_2SO_4, pH 2.0) was used as electrolyte [11], [26], [42].

D. Effect of Temperature

The effect of temperature on the sensor performance is one relevant factor that has to be evaluated as it can change the performance of the sensors and the kinetics of the electrochemical reaction. The temperature effect was investigated between 15 to 35°C by testing the Cu/SWCNTs/Ag electrode

at 0.8 mM of NO_3^- using 3 sensors for each tested temperature. The results (Fig.7) show that the reduction peak current is stable till 25°C and later it starts to increase with temperature showing a 6.5% and 13% variation at 30 and 35°C, respectively, if compared to 25°C. This behaviour can be ascribed to the increase of the reaction rate with increasing temperature as reported by [43] and it can be compensated with the integration of a temperature sensor.

E. Selectivity and Reproducibility

Since the objective of this proposed sensor is to detect nitrate in real water, it is important to evaluate if the anions and cations (Fe^{2+}, Na^+, Cu^{2+}, SO_4^{2-}, CH_3COO^-, Cl^-, NO_2^- and HCO_3^-), commonly found in water, can interfere with the sensor performance [11], [15], [18], [42], [44]. Thus, 0.8 mM concentration of the above-mentioned interfering agents were prepared and tested for nitrate reduction peak current (at −0.85 V) and compared with the blank solution (0 mM of NO_3^- in 0.1 M KCl). The results are summarized in Fig. S9. The amplitudes of the current at −0.85 V for all the tested ions are similar to the blank concentration with a small variation which can be ascribed to experimental error, demonstrating the high selectivity of the proposed Cu/SWCNTs/Ag sensor. Additionally, to check if these interfering agents co-interfere with nitrate, all these chemicals were prepared at 0.8 mM with the presence of also 0.8 mM of NO_3^-. Results are summarized in Fig. 8, where the variation of the reduction peaks of NO_3^- at −0.85 V are shown. Cl^- ions showed the highest reduction peak current if compared to the other interferents. This is commonly reported also in the literature [45], [46] as there is the possibility of the formation of $CuCl_2$ on top of the WE in presence of Cl^- ions which increase the reduction current [47]. In general, the effects of the interferents are minor and thus the proposed sensor can be selectively used in real water containing these possible interferents.

Reproducibility of the proposed sensors is another important aspect to evaluate. Therefore, 5 different concentrations of nitrate (0.1, 0.8, 1.6, 3.0, and 6.0 mM) were tested using three sensors from different batches. Standard deviation (SD) was calculated and found to be 0.73 μA, 1.87 μA, 2.28 μA, 3.23 μA and 4.99 μA, respectively. The results proved that the sensors, which go through different fabrication steps such as screen-printing, SWCNT spray deposition and Cu electrodeposition, maintained a good reproducibility.

F. Regeneration and Stability

To evaluate the regeneration of the sensor, a repeatability analysis was performed. The same sensor was examined consecutively 9 times with 0.8 mM of NO_3^- and reduction peak current was measured (Fig. S10). Each time after the measurement, the electrode was washed with DI water, dried with compressed air to make ready for the next. 3 electrodes were used for this test and results are shown in figure with error bars (SD). The results indicate that the same sensor is usable only two times, as the current decreases by 4.7% at the 2nd measurement and by 17.6% in the 3rd. The possible explanation for this change of current is the delamination

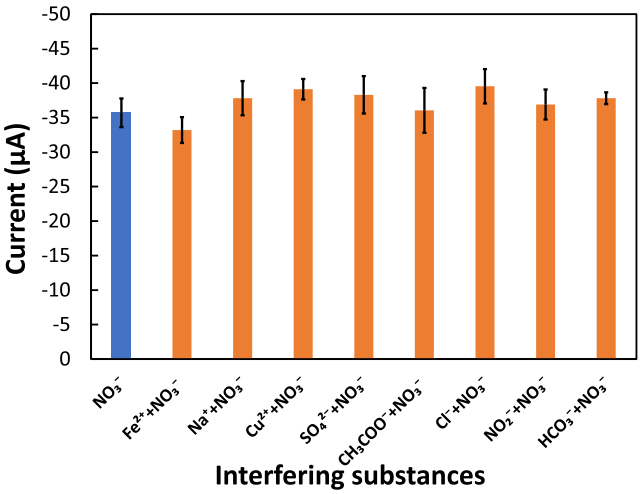

Fig. 8. Interference analysis using reduction peak current in the presence of 0.8 mM NO_3^- and 0.8 mM of Fe^{2+}, Na^+,Cu^{2+}, SO_4^{2-}, CH_3COO^-, Cl^-, NO_2^-, and HCO_3^- respectively.

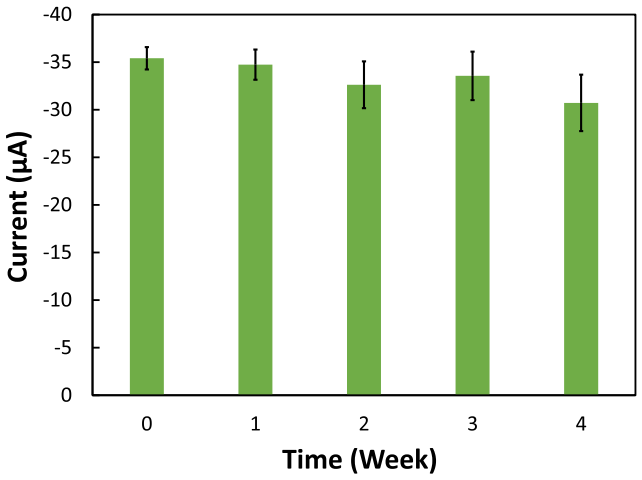

Fig. 9. Stability test for a month for Cu/SWCNTs/Ag sensor shows the reduction peak (average of 3 samples) of 0.8 mM NO_3^- repeated each week until 1 month.

of carbon nanotubes while rinsing the sensor with DI water and also the continuous applied potential that can degenerate the pseudo-reference electrode [48]. Nonetheless, this is acceptable finding because the proposed device is meant to be one-time usable and disposable.

To determine the shelf life, sensor stability was tested by measuring nitrate reduction peak current every week up to one month. A total of 15 sensors were prepared in the same batch and kept at room temperature (around 22°C). Every week, three sensors were tested in the previously mentioned 0.8 mM nitrate solution by measuring the nitrate reduction peak current. The average results with error bars from SD values are plotted in Fig. 9. The reduction peak currents did not show a sharp change but reduced gradually after every week with a slow increment of the SD value. Since the SD value of reduction peak current at 0.8 mM of NO_3^- concentration in the calibration curve showed 2.08 μA of deviation and in the 2nd week of stability test the SD value

TABLE II
NITRATE DETECTION IN DIFFERENT WATER SAMPLES

Parameters	Tap water	River water
Added NO_3^- (mM)	0.8	0.8
Detected by sensor (mM)	0.855 ± 0.037	0.883 ± 0.007
Relative recovery (%)	106.9	110
Detected by HPLC (mM)	0.858 ± 0.040	0.903 ± 0.028

showed 2.45 μA of deviation, it can be evinced that the sensor performance is stable for the first two weeks only. The reason for this fluctuation of the reduction peak current could be related to the formation of oxidative layers on top of the Cu layer in the working electrode, which could be minimized by preparing the sensor in a nitrogen atmosphere and by keeping it in airtight environment.

G. Mechanical Stability Test

The sensors were bent to a radius of 5 mm to apply tensile strain in both the electrode interconnect and sensing part for 500 and 1000 bending cycles with the help of a customized bending setup (Fig. S11A) and the nitrate reduction peak current after each bending test was measured. As shown in Fig. S11B, the current generation was unchanged after such repetitive bending cycles, demonstrating the stability of the sensors to mechanical deformation [41]. Nevertheless, the SD for both the electrode and sensor bending conditions increased from ±2.07 to ±6.07 μA and ±0.58 to ±2.48 μA respectively, after 1000 bending cycles, suggesting the possible formation of permanent nano-cracks in the electrode or other structural defects.

H. Real Sample Analysis

To validate the proposed sensor, it is fundamental to evaluate its performance using real water samples. Standard addition method (SAM) was applied here to get the accuracy in practical analysis with tap and river water [49] and finally compared the result with HPLC. By using this technique, no extra preparation or purification is needed and also it does not need the calibration lines as the matrix effect is taken into account [50]. The real sample was mixed with 0.8 mM of nitrate and experiments were performed in triplicate under identical conditions. The nitrate concentration of tap water and river water showing in Table II are 0.855 ± 0.037 and 0.883 ± 0.007, respectively using the standard addition method with a relative recovery (RR) of 106.9% and 110% with coefficient of variance of 4.36% and 0.79%, respectively. Previously, HPLC was calibrated ($R^2 = 0.9988$) with a wide range of nitrate concentration (0.1 mM to 6 mM) using double distilled water. The real water sample consists of a solution with river or tap water as solvent and a known solution of nitrate (0.8 mM). The final nitrate concentrations was measured by HPLC, which allows this to be used as a standard for the validation of devices in different studies. The HPLC of this real-water reference solutions are reported in table II. The results of nitrate content from the tap and river water on HPLC showed a good correlation with the results from the proposed sensor. Hence, it can be said that the proposed Cu/SWCNT/Ag sensor has high accuracy and reliability for nitrate detection from real environment.

IV. CONCLUSION

In this paper, a flexible, cost-effective, easy-to-fabricate Cu/SWCNTs/Ag screen-printed sensor was developed for nitrate detection in water. The incorporation of SWCNTs showed increased electron transfer kinetics by providing larger electroactive surface area (95.5% larger compared to Cu/Ag sensor). In an optimized condition, this proposed sensor can detect the nitrate from 0.5 μM to 6.0 mM with a LOD of 0.166 nM. Also, the sensor showed high reproducibility and low repeatability allowing to use same sensor only twice, which is normal for a disposable device. The stability test conducted over a month demonstrated that the sensor showed minimal change in performance within a 2-week shelf storage at ambient temperature and humidity. On the other hand, mechanical stability tests showed the sensor can easily handle at least 500 bending cycles with minimal performance alteration. Finally, to investigate if the sensor can measure the analyte of interest in a real water sample, the tests using river and tap water showed reliable performance. In future, the sensor can be improved by introducing different transducing platform, such as electrolyte-gated field-effect transistor (EGFET) to improve the sensitivity and by implementing it into a portable device.

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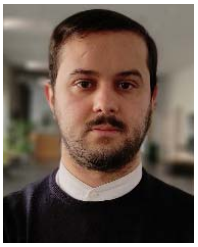

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