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Highly sensitive paper-based electrochemical sensor for reagent free detection of bisphenol A

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ABSTRACT

Bisphenol A is one the most relevant endocrine disruptors for its toxicity and ubiquity in the environment, being largely employed as raw material for manufacturing processes of a wide number of compounds. Furthermore, bisphenol A is released in the drinking water when plastic-based bottles are incorrectly transported under sunlight, delivering contaminated drinking water. For the health of human beings and the environment, rapid and on site detection of bisphenol A in drinking water is an important issue. Herein, we report a novel and cost-effective printed electrochemical sensor for an enzymatic-free bisphenol A detection. This sensor encompasses the entire electrochemical cell printed on filter paper and the reagents for the measurement loaded in the cellulose fiber network, for delivering a reagent-free analytical tool. The working electrode was printed using ink modified with carbon black, a cost effective nanomaterial for sensitive and sustainable bisphenol A determination. Several parameters including pH, frequency, and amplitude were optimized allowing for a detection limit of 0.03 μM with two linear ranges 0.1–0.9 μM and 1 μM–50 μM, using square wave voltammetry as electrochemical technique. The satisfactory recovery values found in river and drinking water samples demonstrated the suitability of this sensor for screening analyses in water samples. These results revealed the attractiveness of this paper-based device thanks to the synergic combination of paper and carbon black as cost-effective materials.

1. Introduction

Endocrine-disrupting compounds encompass pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, pesticides, and plasticizers and they are listed as emerging pollutants because able to affect human hormonal activities [1–3]. Among several endocrine-disrupting compounds, Bisphenol A (BPA) has attracted relevant attention by the scientific community for its toxicity and presence in the environment, being used in the industrial production of epoxy resins, polycarbonate plastics, and lacquer coating, to name a few [4–7]. Several studies demonstrated estrogens-like property of BPA, correlating the BPA exposure with diabetes, heart diseases, obesity, breast and prostate cancer, lowered sperm quality, neurotoxicity problem, and polycystic ovarian syndrome [8–11]. The detection of BPA by using rapid and sensitive sensors is thus a key issue in analytical chemistry for easily assessment of safety of water and food samples, as well as the pollution of surface waters. Several analytical methods have been developed for BPA quantification by using high performance liquid chromatography [12,13], gas chromatography–mass spectrometry [14], enzyme-linked immunosorbent assay (ELISA) [15], and molecular imprinted sensors [16]. Among these, the electrochemical ones have the advantages to be cost-effective and miniaturized, suitable for in situ analysis by unskilled personnel. In this overall scenario, several electrochemical sensors have been developed for BPA detection using glassy carbon electrodes and screen-printed electrodes [17–21].

Paper has recently taken a huge attention being an eco-friendly support to design microfluidic devices [22]. Undeniably, paper-based devices possess many unique advantages including cost-effectiveness, easiness to use, portability, sample-flow without the need of external pumps, and suitability for high throughput analyses [23–27]. Furthermore, the analyses carried out by paper-based devices required small sample volume and the final device containing the sample analysed can be safely disposed by incineration [28,29]. To supply an eco-designed device, we fabricated the paper-based sensor by combining screen-

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printing for electrochemical cell manufacturing and wax printing for designing hydrophilic regions surrounded by hydrophobic ones. In addition, we selected filter paper because it is able to load the reagent needed for the analysis exploiting its porosity for reagent-free measurement.

To improve the sensitivity, carbon black has been selected as carbon-based nanomaterial to modify the ink used to print the working electrode. Carbon black is a form of amorphous carbon characterized by high surface area to volume ratio. Up to 2010, only few examples of carbon-black based sensors were reported in literature for analyte detection in solution, while, starting from 2010, there is an increasing interest in this cost-effective nanomaterial. For instance, it showed outstanding features in terms of electrocatalytic properties towards several analytes such as NADH, thiocholine, and hydrogen peroxide [30,31]. Among different molecules, phenolic compounds namely catechol, gallic acid, caffeic acid, tyrosol, p-nitrophenol and p-cresol have been detected using carbon black-modified electrode, demonstrating a giant improved in terms of sensitivity and low applied potential [32,33].

Herein, we present the first example of paper-based device able to detect BPA directly on river and drinking water samples without any extra-task of end-users, exploiting the porosity of filter paper to load the reagents as well as to threat the sample. The lab on a chip on paper developed requires the only addition of some μL of sample for BPA quantification. The high sensitivity was achieved exploiting the low-cost carbon black as nanomaterial for the direct BPA detection, demonstrating for the first time the effectiveness of carbon black and paper-based sensor for on-site environmental monitoring and drinking water analysis at the point-of need.

2. Experimental

2.1. Reagents and equipment

BPA was purchased from Sigma-Aldrich. 0.1 M BPA solution was freshly prepared before use by mixing acetonitrile with phosphate buffer solution 20% (v/v). Phosphate buffer solution was prepared by mixing stock solution of 0.1 M K_2HPO_4 and 0.1 KCl M and the pH adjusted with HCl or NaOH. Commercial carbon black (CB) N220 powder was kindly supplied as gift by Cabot Corporation, Italy. Graphite based ink (Electrodag 421), and Ag/AgCl ink (Electrodag 4038 SS) were purchased from Acheson, UK. Square wave voltammetry measurements were achieved using a portable EmStat Instrument (Palm-Sens, Netherlands) connected with a laptop. The substrate for paper-based sensor fabrication was Labor filter paper (67 g/m^2), purchased from Cordenons, Italy. All other reagents were of analytical grade and used as received without further purification. All measurements were carried out at room temperature.

2.2. Paper-based sensor fabrication

The fabrication procedure of the paper-based sensor is described in our previous papers [34,35]. Briefly, the working area was defined by using a wax printer Xerox ColorQube 8580 then the waxed paper is treated in the oven for 2 min at 100 °C. The step in the oven allowed the melting of wax and its diffusion through the cellulose fibers network, creating hydrophobic barriers. The three-electrode system was then screen-printed on paper using 245 DEK (UK) screen-printing machine. The pseudo-reference electrode was printed using Ag/AgCl ink for the first layer, and then the conductive graphite-based ink containing CB 5% w/w was used to print the second layer. The CB-modified graphite ink was prepared by mixing 9.5 g of graphite based ink with 0.5 g of CB. To cure the ink after the printing step, each sheet was placed in the oven at 70 °C for 20 min.

2.3. BPA detection in standard solution

Measurements of BPA were performed using square wave voltammetry technique (SWV). All voltammetric measurements were performed loading 10 μL of phosphate buffer 0.1 M + KCl 0.1 M at pH 8.0 onto the paper-based sensor and wait to become dry. After, 10 μL of BPA standard solution was added and BPA measured by SWV using a potential window comprised between 0.0 and 0.8 V, a potential step of 15 mV, frequency of 100 Hz, and amplitude of 50 mV.

2.4. BPA detection in river and drinking water sample

Samples of river water were collected from Aniene Valley (Rome, Italy), while drinking water from a commercial drinking water bottle. Before water analysis, 10 μL of phosphate buffer 0.1 M + KCl 0.1 M at pH 8.0 were loaded onto the paper-based sensor, waiting until it gets dry, to deliver a reagent free sensor. Then, the real water sample was loaded without any pre-treatment, on the backside of the printed sensor, in order to filter the sample.

3. Results and discussion

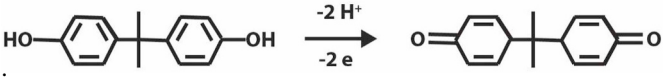
The paper-based device was conceived to detect BPA by analyzing directly the sample without any sample treatment, exploiting the filtering properties of filter paper due to the presence of pores with dimension comprised between 1 and 3 μm able to block the particulate [35,36]. To achieve this task, the sample was loaded on the backside of the printed sensor to exploit the filtration property of the paper (Fig. 1).

Previously, some μL of buffer were loaded on paper waiting for solvent evaporation. In this way, the buffer salts remained into the cellulose fiber network and the added sample allows their dissolution, delivering a working solution at the pH selected for sensitive and reproducible BPA detection. Square wave voltammetry was selected being a technique widely employed for BPA, using the conventional electrodes such as glassy carbon or carbon paste electrodes [37,38].

3.1. Cyclic voltammetric behavior of BPA at bare and CB-modified paper-based sensor

The first study aimed to evaluate the effect of CB in the direct electrochemical detection of BPA, analyzing the response by cyclic voltammetry in the potential range of 0.0–1.0 V at scan rate of 20 mV/s in phosphate buffer. As depicted in Fig. 2, a peak around 0.5 V was observed using both bare and CB-modified electrodes, due to the electrochemical reaction reported in Equation 1.

Equation 1. Redox reaction of BPA at the working electrode surface-



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As shed light by Fig. 2, using a bare electrode a current intensity of ca. 8 μA was observed, while the presence of CB allows for an improved sensitivity, with a peak intensity of ca. 40 μA. Furthermore, the high sensitivity in presence of CB is combined with the low background (around the same observed in case of bare electrode), succeeding in a higher signal/noise ratio when compared with the response obtained using the bare electrode. Indeed, often the modifications of the working electrode surface with (nano)materials allowed for increasing both the response but also the background current [39]. For instance, Dong et al. modified the glassy carbon electrode surface with graphite nanoparticles, observing a peak at around 0.55 V but as well a higher capacitive current [6]. In this overall scenario, the results achieved demonstrated the advantage of using CB, being able to improve the sensitivity jointed with low background current just printing the electrode with a commercial graphite ink modified with only 5% (w/w) of

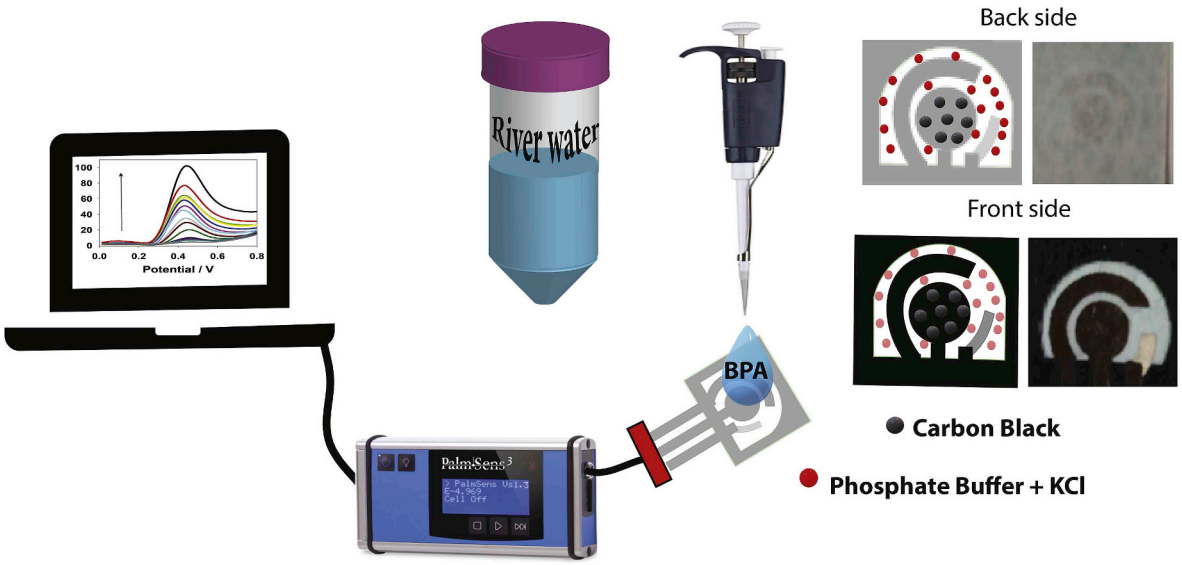

Fig. 1. Schematic representation of the configuration of paper-based sensor and measurement procedure.

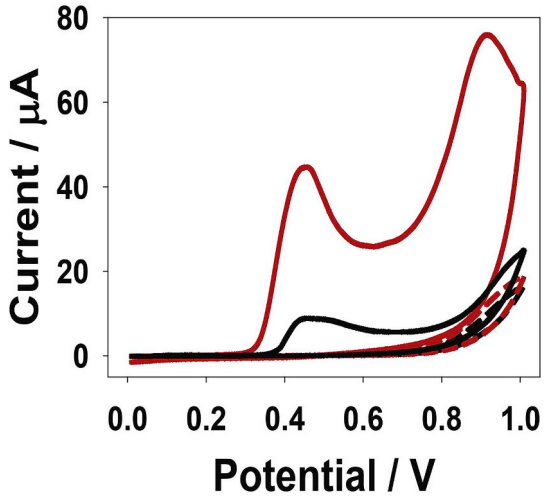

Fig. 2. Cyclic voltammetries in phosphate buffer 0.1 M + KCl 0.1 M in absence (dashed) and in presence (solid line) of BPA 1 mM, in the potential range from 0 to 1 V (vs. Ag/AgCl) with scan rate of 20 mV/s using bare (black line) and CB-modified paper-based device (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

CB. This behavior can be ascribed to several key features of CB, including nanodimensions, the onionlike carbon structure, and the high number of defect sites as reported in literature [40,41]. In addition, another relevant feature of CB relies in its low cost (around 1 for 1 Kg) and the possibility to use this nanomaterial as received by the supplier without any further physical and chemical treatment. For the higher performances observed using CB-based sensor, the nanomodified paper-based sensor was selected for the further study.

3.2. The effect of pH

As highlighted in equation (1), the pH affects the reaction and thus the detection of BPA; for this reason, pH was the first parameter investigated. To accomplish this task, we studied the voltammetric behavior over a pH range of 4.0–9.0 by using a Britton Robinson buffer and a BPA concentration of 50 µM. From pH 4 to 8, it can be seen that the peak current increased up to ca. 85 µA as the pH increased, but when pH was higher than 8, the peak current decreased (Fig. 3A) in agreement with the literature data [42]. In order to obtain a high sensitivity, pH 8 was chosen in this work. Furthermore, the effect of pH on applied potential was evaluated. As widely reported in literature, at the increase of pH, a negative potential shift is observed. A good linear relationship was obtained between the peak potential (E_p) and solution pH, described by the following equation $y = (0.96 \pm 0.01) - (0.065 \pm 0.002)$ pH (Fig. 3B). A shift of 65 mV for each pH value was observed, which is in line with the theoretical value of 59 mV per pH unit, indicating an electron transfer process convoyed by an equal number of electrons and protons, in agreement with Equation (1). Furthermore, taking into consideration the width at half height of peak around 100 mV, it can be concluded that phenol oxidation process involves the transfer of one-electron and one proton [43].

3.3. Optimization of square wave voltammetry parameters

In order to develop a highly sensitive analytical tool, SWV parameters such as frequency, amplitude, and step potential were optimized, being able to affect the height and resolution of the peak. Firstly, the effect of frequency was investigated by increasing the frequency from 10 to 100 Hz. The peak current increased with increasing of frequency (Fig. S1). With the portable instrument we used, frequencies higher than 100 Hz are not available, thus 100 Hz was selected as the frequency giving the highest sensitivity within the experimental set-up employed. The pulse amplitude is another parameter that strongly influences the peak current in SWV, thus determining the sensitivity of the measurement. The step potential was investigated in range of 1 mV–30 mV (Fig. S2), observing an increasing of peak current of BPA with the increasing of step size until to 15 mV, thus square wave step potential of 15 mV was chosen for the successive experiments. The effect of square wave amplitude on the current response was studied by varying the square wave amplitude from 10 mV to 100 mV. As expected from theory [44], the peak current increases at the variation of pulse amplitude, however combined with a worse peak resolution (Fig. 3C). For the subsequent analytical applications, a value of 50 mV was chosen as compromise between the resolution and intensity of the peak (Fig. 3C and D).

3.4. Analytical features

Once optimized the pH and SWV parameters, the sensor was tested to assess its analytical performances. The calibration curve was

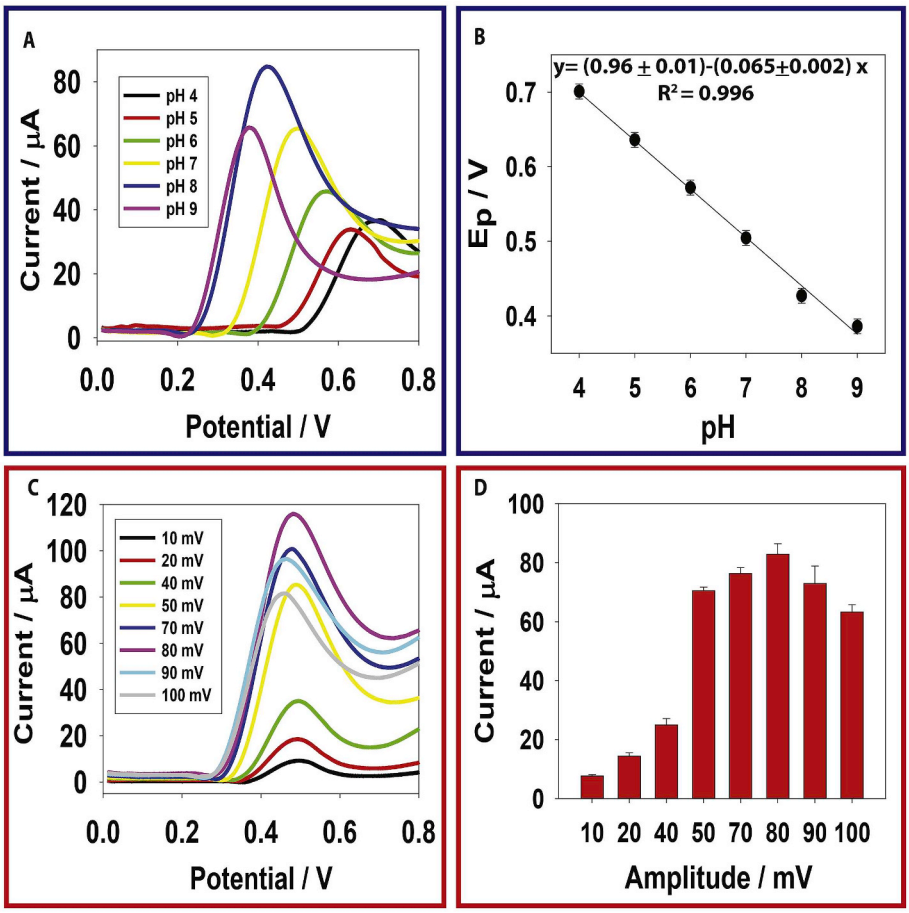

Fig. 3. Optimization of pH. (A) SWV measurements of BPA 50 µM in Britton Robinson buffer at different pH values; (B) Plot of Ep vs. pH. SWV conditions: Eampl = 50 mV, Estep = 15 mV, f = 100 Hz. (C) SWV measurements at different amplitudes of BPA 50 µM in 0.1 M phosphate buffer containing KCl 0.1 M, pH = 8; (D) Histogram of peak current vs. Amplitude. SWV conditions: Estep = 15 mV, f = 100 Hz.

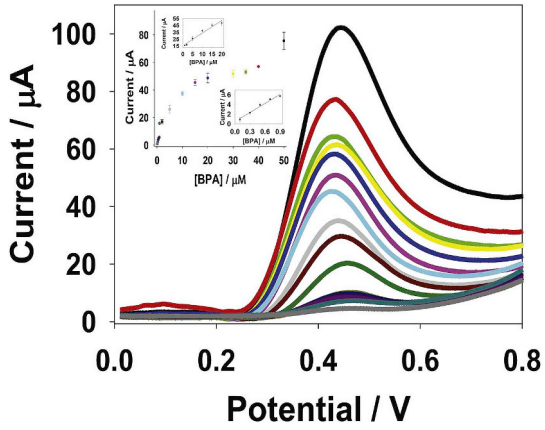

Fig. 4. SWV measurements of BPA at different concentrations up to 50 µM in 0.1 M phosphate buffer + 0.1 M KCl, pH = 8, Eampl = 50 mV, Estep = 15 mV, f = 100 Hz. Inset: calibration curves.

constructed analyzing BPA at concentrations starting from 0.1 µM until to 50 µM, observing two linear ranges (Fig. 4), described by the following equations: y= (0.5 ± 0.3) + (6.2 ± 0.5) x, in the range comprised between 0.1 and 0.9 µM, with R^2 = 0.978 and y= (15 ± 2) + (1.8 ± 0.2) x, in the range comprised between 1 and 20 µM, with R^2 = 0.961. The detection limit was calculated as 3 times the standard deviation of the blank solution divided by the slope of the calibration curve, resulting in 0.03 µM. Repeatability inter-electrodes was also assessed testing a concentration of BPA 1.5 µM, obtaining a RSD % equal to 9.5% (n = 13). When compared with paper-based electrochemical sensors reported in literature [45,46], the proposed sensor is the only one able to detect BPA at µM level with the entire electrochemical cell printed on paper. Furthermore, when compared with other electrochemical sensors reported in literature, including the electrodes modified with CB or based on screen-printed electrodes (Table 1), the obtained device shows analytic features comparable or even better, with the advantage to be the only sensor able to detect BPA in few µL of water sample without any sample treatment e.g. dilution and pH adjustment using. In this way, the porosity of the paper was exploited to i) contain the reagents needed for the measurement (KCl, phosphate salts), ii) treat the sample thanks to the structure of the 3D structure of paper used, iii) deliver a fast measurement requiring less than 30 s.

3.5. Interference study

To assess the selectivity of the sensors, several compounds including pesticides such as 2,4 D, paraoxon, atrazine and heavy metals such as lead and cadmium were tested using the concentration of 10 ppb because their EU legal limits in water samples are at ppb level. A slight response (lower than 15%) was observed only in the case of cadmium ions. To test the selectivity towards other phenolic compounds, 10 µM of hydroquinone was tested without a measurable response, demonstrating a satisfactory selectivity towards different types of pollutants.

3.6. Water sample analysis

In order to evaluate the performance of paper-based sensor for practical analytical applications, BPA was determined in river and drinking water samples without any sample treatment. Fig. 5 reported the analyses achieved by using the standard addition method, analyzing three samples fortified with three different concentrations of BPA 10,

Table 1
Comparison of analytical performances of the proposed sensor for BPA detection with other sensors reported in literature, MIPs = molecularly imprinted polymers, MNPs = magnetic nanoparticles, CTAB = cetyltrimethyl ammonium bromide, CPE = carbon paste electrode, NH2-MIL-125/RGO = amine-functionalized metal-organic framework/reduced graphene oxide, GCE = glassy carbon electrode, AuNPs = gold nanoparticles, NPG = nanoporous gold, MIPPy-GQDs = molecularly imprinted polypyrrole/graphene quantum dots, Fe_3O_4NPs = ferroferric oxide nanoparticles CB = carbon black, CNT = carbon nanotubes, CAS = casein, RGO = reduced graphene oxide; CV = Cyclic voltammetry, DPV = differential pulse voltammetry, LS = linear scan, CA = chronoamperometry.

Type of sensor	Technique	Linear range (μM)	LOD (μM)	Sample treatment	Ref.
MIPs MNPs/CTAB/CPE	CV	0.6–100	0.1	Filtration, concentration and dilution with buffer solution	[47]
NH2-MIL-125/RGO/GCE	DPV	2–200	0.7966	Not reported	[48]
MIP–AuNPs/GCE	Amperometry	8–6 × 10^4	0.138	Filtration and dilution	[49]
NPG/GCE	DPV	0.1–50	0.0121	Addition of phosphate salts to adjust pH value at 7.5	[50]
MIPPy-GQDs/GCE	DPV	0.1–50	0.04	Filtration/Centrifugation	[51]
Fe_3O_4NPs-CB/GCE	DPV	0.1 × 10^{-3} - 50	3.1 × 10^{-5}	–	[52]
CAS-CB/GCE	LS	0.49–24	0.25	Dilution, addition of tween 20 and phosphate solution	[53]
CB/CPE	SWV	1–16	0.3	–	[54]
CB/GCE	DPV	0.01–3	0.0034	Dilution with supporting electrolyte	[55]
AuNPs/CB/SPE	DPV	0.07–10	0.0088	Dilution with phosphate buffer	[56]
RGO/CNT/AuNPs-SPE	DPV	0.00145–1.49	0.00008	–	[57]
dendritic platinum nanoparticles/SPE	CA	0.01–1.0 1.0–300	0.00663	Dilution with phosphate buffer	[58]
CB/μPAD	SWV	0.1–0.9 1–20	0.03	None	This work

20 and 30 μM, in triplicate. The satisfactory recovery values obtained (Table 2) demonstrated the effectiveness of this cost-effective and easy to use paper-based sensor to detect BPA in water samples.

Table 2
Recovery study.

[BPA] μM added	% recovery in river water	% recovery in drinking water
10	90 ± 2	100 ± 20
20	88 ± 6	101 ± 2
30	94 ± 5	92 ± 6

4. Conclusions

Water stress, in terms of pressure on the quantity and quality of water resources, is one of the most serious problem at worldwide level. The detection of emerging pollutants *on site* is a need to address

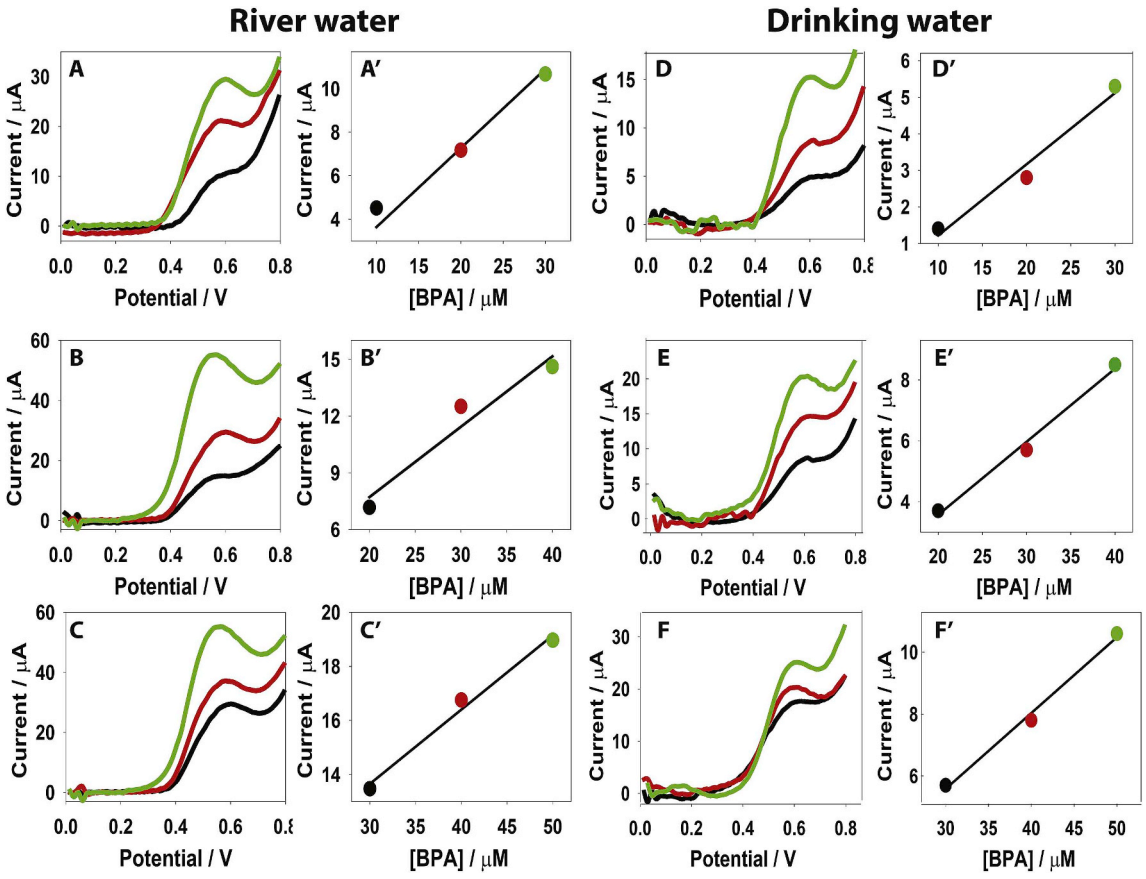

Fig. 5. SWV measurements and calibration curves obtained by using the standard addition method for water samples fortified with 10, 20 and 30 μM of BPA. Measurement conditions as reported in Fig. 4.

sustainable monitoring of water quality. In this context, the electrochemical paper-based devices are acquiring a prominent role for their cost-effectiveness, capability to be used by unskilled personnel, the requirement of minimal amount of reagents, and the suitability to be applied in complex matrices. Herein, we reported the electrochemical device able to detect BPA by requiring only the addition of the sample, thanks to the use of the filter paper as support to print the electrodes, to store the reagents, and to treat the sample. Moreover, the combination of the cheap filter paper with the cost-effective nanomaterial carbon black, allows the construction of a very inexpensive but at the same time highly sensitive sensor for BPA quantification at μM level in river and drinking water samples.

CRediT authorship contribution statement

Dhouha Jemmeli: Investigation, Data curation, Writing - original draft. **Eleonora Marcoccio:** Investigation, Data curation. **Danila Moscone:** Writing - review & editing, Resources. **Cherif Dridi:** Conceptualization, Writing - review & editing, Funding acquisition. **Fabiana Arduini:** Conceptualization, Methodology, Writing - review & editing, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.120924.

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