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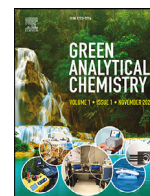
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A fully-printed electrochemical platform for assisted colorimetric detection of phosphate in saliva: Greenness and whiteness quantification by the AGREE and RGB tools

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ABSTRACT

Herein, we report the environmental impact quantification of a newly developed fully printed electrochemical device to assist a colorimetric detection of phosphate in saliva. The evaluation of the analytical procedure was performed according to the principles of Green Analytical Chemistry and White Analytical Chemistry. The standard method for phosphate detection relies on a reaction between phosphate and molybdate in presence of antimony potassium tartrate and ascorbic acid, using strong acid conditions and high volumes of reagents (100–500 mL). To deliver an eco-friendly method, we have combined a screen-printed electrode with a liquid electrolyte battery and inkjet-printed conductive paths to develop a fully printed device on a flexible polymer substrate avoiding the use of ascorbic acid and using a small amount of reagents. The printed sensor was first developed and optimized for phosphate detection in saliva, allowing for a detection limit equal to 26 μM and satisfactory repeatability (relative standard deviation value of 7.5%). Finally, the AGREE and the RGB assessment tools were applied for a quantitative evaluation of the proposed sensor and reference method, in agreement with the Green Analytical and White Analytical principles. The results demonstrated the lower environmental impact of the proposed sensor, as well as the suitability of this novel approach for phosphate detection in saliva.

1. Introduction

In 1962 Rachel Carson's *Silent Spring* became one of the most influential books, launching the environmental movement [1]. After, the environment movement imbued several disciplines, including Analytical Chemistry. In 1987, Malissa introduced the concept of the ecological paradigm in the analytical sector during the Euroanalysis VI conference [2]. In 2001, Namieśnik introduced the Green Analytical Chemistry (GAC) model [3] applying the 12 Green Chemistry principles, formulated by Anastas and Warner [4], in analytical chemistry. About 15 years later, several authors [5–7] highlighted the need to exploit the principles of Green Chemistry in the analytical sector, with the aim to boost eco-friendly analytical platforms, implementing analytical procedures

the overriding goal to combine the greenness of the method with criteria related to its efficiency, usefulness, and practical/economic evaluation.

The easiness to follow GAC or WAC rules depends on the analytical method used; for instance, chromatographic techniques require the use of organic solvents and high-energy consuming laboratory-based instrumentations [10,11].

In order to reduce the amount of chemicals and solvents, to minimize waste production, and to reduce the instrumentation size, (bio) sensors are considered well suitable to fulfill the GAC and WAC principles [11–13]. Indeed, these analytical tools require a minimum amount of sample and reagents, miniaturized equipment with low energy consumption, simple sample treatment, and they are suitable for on-line analysis. In addition, several efforts have been recently focused on the use of

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environmental-friendly substrates (i.e., paper [14–16]) and on the modification of surfaces with nanomodifiers obtained by green methodologies (i.e. using natural source reagents [17–19]) for designing greener (bio)sensors.

Despite several sensors are claimed to be “sustainable”, the mere use of an environmental-friendly substrate or the employment of a green synthesis is not enough to define the analytical methodology as “green” [20]. The application of an evaluation tool able to assess the degree of matching with the 12 GAC or WAC principles is essential to give a quantitative evaluation of the greenness or whiteness of a new analytical approach. In the last decade, several tools including Eco Scale [21], National Environmental Methods Index (NEMI) [22], and Green Analytical Procedure Index (GAPI) [23] have been developed following 12 GAC principles. The aim of these assessment metrics is to combine a score or a graphical output with a few parameters related to the greenness of the technique used. In detail, Eco-scale compares a standard ideal procedure with the newly developed, by evaluating five parameters and assigning them penalty points, giving an overall final score. NEMI delivers a circle pictogram divided into four parts, colored in green if some criteria are reached, while GAPI is based on five pictograms connected to five environmental parameters, colored in green, yellow, or red, reflecting a low, medium, or high environmental impact, respectively.

Recently, Pena-Perreira et al [24], developed a metric system called AGREE able to deliver a simple algorithm that faces the overall greenness of the method in quantitatively from 0 to 1, with an easily readable output of a pictogram that directly refers to the 12 GAC rules. Successively, several alternative algorithms have been developed to enable a global evaluation by following the WAC approach [25]; among the others, the RGB model fulfills the requirement of an easy-to-use and not complex approach for the assessment of the analytical method [26]. By applying the RGB algorithm, it is possible to calculate the quantitative parameter of whiteness, giving values to evaluate how the method fits the WAC principles.

In this scenario, focusing on the low energy consumption of miniaturized equipment, printing technologies have been exploited for the fabrication of numerous eco-friendly electronic devices, including electrodes [27], batteries [28], solar cells [29], and flexible displays [30], for achieving lower energy consuming miniaturized devices. In the field of energy storage, the battery technology based on zinc, manganese dioxide, and a mild zinc chloride electrolyte is among the most environmentally friendly and promising printed batteries.

Indeed, this battery configuration does not require strong acid solution, and it can supply voltage scaled in multiples of 1.5 V and a current of up to 1 mA when using a metal-free carbon-based current collector [31].

Regarding the requirement of small amount of samples and chemicals, printing techniques including screen printing, inkjet printing, and 3D printing have allowed the re-design of the electrochemical cell with the advantages of miniaturization of the electrochemical cell with requiring of few μL of sample for the analysis. Usually, the screen-printing technique has been used to massively fabricate the electrodes, while the inkjet printing technology has been employed for the production of application specific customized printed electrochemical sensors [32] and for the modification of the printed sensors with nanomaterials [33], as well.

Furthermore, the use of printed components is a useful approach, especially if the electrochemical components can assist electrochemical reaction, because it could prevent the use of oxidizing/reducing agents and therefore boost the greenness of the measurement.

Herein, we report for the first time the greenness evaluation of an entirely printed sensor device, taking the detection of phosphate in saliva as a case study. The device consists of a screen-printed battery and a screen-printed electrode connected by customized inkjet-printed conductive paths to assist colorimetric detection by reducing the chemical.

Phosphate is an essential component involved in biological reactions for maintaining the normal homeostatic control of the cells [34]. The

level of phosphate concentration in adult human serum is comprised of between 0.81 and 1.45 mM in serum [35] and 5 to 14 mM in saliva [36,37]. High phosphate levels allow for hyperphosphatemia with acute or chronic renal failure, while low levels (hypophosphatemia) have been observed in the case of e.g. hyperthyroidism [38]. Phosphate in saliva works as a buffering agent of the fluids within the oral cavity, repairing enamel/dentine and preserving the health of the teeth.

In literature, several methods have been reported for the determination of phosphate using different techniques, including chromatography, ultraviolet–visible (UV–VIS), fluorescence, and electroanalysis [39–42]. Among them, the standard method is based on colorimetric detection and remains the most employed in every field. This method developed by Murphy and Riley [43] relies on the formation of a blue-colored α -Keggin anion, the ammonium phosphomolybdate, as a result of the reaction between phosphate and molybdate, ascorbic acid, and antimony potassium tartrate in strong acid conditions. The amount of phosphate is determined spectrophotometrically because its concentration is proportional to the blue color of the analyzed sample. The drawbacks of this method include turbidity interferences, as well as the use of high strong acid conditions, and preparation of fresh ascorbic acid solution every day, failing with the purpose to deliver an analytical method with the characteristics stated by the 12 GAC principles or the more recent 12 WAC principles.

In this work, we evaluate the suitability of the entire printed device to accomplish the reduction of the phosphomolybdate and avoid the use of ascorbic acid, demonstrating that this device is suitable for a customized detection of phosphate in saliva samples.

Furthermore, we evaluate and quantify the sustainability of the new platform according to recently developed AGREE and RGB-model assessment tools [24,26].

2. Experimental section

2.1. Apparatus and reagents

Amperometric measurements were performed using a portable PalmSens Instrument (PalmSens, The Netherlands) in connection with a personal computer. The inkjet printer Dimatix Materials Printer 2831 (DMP-2831, Fujifilm Dimatix Inc., USA) was used for the printing of electrically conductive paths. A screen-printed battery (EKRA E2XL) was used to apply the voltage potential necessary for the reduction of phosphomolybdate complex. Spectrophotometric measurements were performed by a microplate reader iMark (Biorad, USA). Sulphuric acid 95–98%, ammonium heptamolybdate tetrahydrate, potassium antimonyl tartrate, potassium chloride, sodium chloride, calcium chloride, and potassium dihydrogen phosphate of reagent grade were purchased from Sigma-Aldrich.

2.2. SPE preparation

Screen-printed electrodes (SPEs) were manufactured using the procedure reported in our previous articles [44,45]. Because in this work the electrodes are only devoted to apply the reduction potential, the final configuration encompasses a working electrode and a reference electrode, as well. In detail, a graphite-based ink (Electrodag 421) from Acheson (Milan, Italy) was used for printing the working electrode, and a silver ink (Electrodag 477 SS) for the pseudo-reference electrode. A gray colored dielectric paste D2070423P5 from Gwent Electronic Materials (Pontypool, UK) was used for the insulating layer to define the surface area of the working electrode. The substrate was a 125 μm thick flexible and transparent polyester film (Autostat HT5) obtained from Autotype Italia (Milan, Italy). The electrodes were printed on the polyester films containing 48 sensors. The diameter of the circular-shaped working electrode was 0.3 cm, resulting in a geometric area of 0.07 cm^2 .

2.3. Manufacturing of printed battery

The screen-printed 1.5 V one-cell battery was developed on a 100 μm thick flexible and transparent polyethylene terephthalate (PET) film (Melinex 401 CW from Pütz GmbH + Co. Folien KG, Taunusstein, Germany). The current collector electrodes were deposited using the ink Electrodag PF-407C (Henkel Loctite). The zinc and manganese dioxide pastes (Sigma-Aldrich) were formulated in-house for the printing of about 6 cm^2 anode and cathode electrodes. A gelled aqueous zinc chloride (ZnCl_2) electrolyte was formulated and employed together with a fiber-based separator, keeping the battery integrity and allowing for the ionic transportation between the anode and cathode of the battery. A double-sided glue tape was applied within the lamination process to encapsulate the entire battery. The two current collectors protrude beneath the encapsulation. For manufacturing the entire stack of the printed battery, a batch-wise deposition of the functional layers was accomplished using screen-printing and the subsequent drying process using the 3D-Micromac AG microDRY unit (2 m long drying zone that was set at 110 $^\circ\text{C}$). The overall thickness of the battery after encapsulation was about 800 μm . In typical shelf storage conditions, the capacity of the battery decreases by 10–0% each year, which is similar to the round cells consisting of this material system [46].

2.4. Developing conductive paths between SPE and printed battery

To obtain the electrical connections between the printed battery and the electrodes, a silver (Ag) based nanoparticles ink (Silverjet DGP 40LT-15C from Advanced Nano Products Inc.) was also deposited on the same un-coated 100 μm thick PET substrate (Melinex 401 CW from Pütz GmbH + Co. Folien KG, Taunusstein, Germany) with the laboratory-based DMP-2831 inkjet printer. The printer typically utilizes a DMC-11,610 cartridge having 16 nozzles, that offer a nominal 10 μL drop volume from each nozzle. The special characteristic of such printer is the capability to generate printing resolutions from 100 dpi to 5080 dpi (equivalent 254–5 μm drop space), which would result in achieving the required dimension of the printed feature corresponding to an ink. In our case, the Ag ink was printed and post-treated with the following parameters: 725 dpi printing resolution (35 μm drop space), 5 inch H_2O meniscus pressure, 5 kHz jetting frequency, 35 $^\circ\text{C}$ cartridge temperature, 40 $^\circ\text{C}$ substrate temperature, 1 mm print height, 12 optimized nozzles for jetting the ink and sintering of the printed pattern in a convection oven at 130 $^\circ\text{C}$ for 30 min. The digital print pattern was systematically designed by optimizing the track length, width, and orientation of the conductive paths related to the position of the printed battery and electrodes.

2.5. Phosphate measurement using the developed analytical tool

The phosphate measurements were carried out following three steps. 80 μL of heptamolybdate 1 mM prepared in 0.1 M H_2SO_4 and phosphate at the selected concentrations were added onto the surface of the SPE, then a potential equal to -1.4 V for 3 min was applied to the solution through the printed battery. After that, the electrochemical-treated solution was added to the well of a microplate and the absorbance was measured at 831 nm.

2.6. Spectrophotometric phosphate measurement

The spectrophotometric measurements were performed following the procedure reported in the literature [47]. The stock solution of phosphate was added to the double-distilled water in order to obtain 5, 10, and 40 μM as final concentrations. 1.5 mL of the reagent mixture (previously prepared by mixing 45 mL of heptamolybdate (76.8 mM), 5 mL of potassium antimonyl tartrate (0.1 M), and 200 mL of sulphuric acid (4.5 M)) were added to 50 mL of each standard solution and then mixed for 15 min. After that, the reduction of the phosphomolybdic complex

was obtained adding the ascorbic acid solution (1.5 mL of 0.31 M aqueous solution) as a reducing agent. The absorbance was measured after a further 10 min resting time at 831 nm. For saliva detection, the relative amount of mix reagent and ascorbic acid were scaled to work with a real sample volume of 1 mL.

2.7. Phosphate detection in saliva samples

The saliva samples were obtained from volunteers, by passive drool collection into 2 mL tubes. Subsequently, 20 μL were diluted 1:5 v/v with the working solution i.e. 1 mM of heptamolybdate in 0.1 M H_2SO_4 to obtain a phosphate concentration within the linear range, considering the physiological value (c a. 5 mM). The application of the potential by the printed battery allows the reduction of the formed phosphomolybdate complex. Finally, the absorbance was measured at 831 nm.

2.8. Tool used in eco-friendly aspect evaluation

The Excel spreadsheet used for assessment according to WAC principles was based on the template developed by Novak et al [9], whereas the AGREE Python software was downloaded from the website link "https://mostwiedzy.pl/en/wojciech-wojnowski, 174,235–1/AGREE".

3. Results and discussions

3.1. Configuration

The detection of phosphate in water sample relies on formation of the phosphomolybdate complex. In detail, the reference method uses 50 mL of the sample, 1.5 mL of the mix reagent constituted of 45 mL molybdate 0.0768 M, 200 mL sulphuric acid 4.5 M, and 5 mL tartrate 0.1 M, followed by the addition of 1.5 mL ascorbic acid 0.4 M [47]. To execute an eco-friendly analysis, several commercial kits propose the use of a microplate with reduced volumes (1–200 μL) to decrease the amount of sample as well as of the chemical reagents needed [48,49]. However, for the production of the colored phosphomolybdate complex, the addition of ascorbic acid is mandatory to provide a chemical reduction. In agreement with the principles stated by GAC [8] and WAC [9], we focused on the prevention of the waste as well as the reduction of additional reagents, with the overriding goal to reduce the employment of chemicals. Motivated by this issue, herein we report a phosphate detection replacing the chemical reduction with a chemical-free electrochemical reduction of the phosphomolybdate complex by using a printed system, still obtaining a blue compound optically monitored for the detection of phosphate in low volume of saliva samples and chemicals. This electrochemical miniaturised system encompasses a printed primary battery, a printed sensor produced by screen printing technology, and electrically conductive paths connecting the battery and sensor device printed by the inkjet printing technology. After the happened reaction between the customized low volumes of standards (or sample) and molybdate, 80 μL of the resulting solution are dropped onto the electrochemical cell. The press of the battery contacts allows for the application of a reductive potential (-1.4 V vs. Ag/AgCl) with the ascorbic acid-free production of the colored complex. Next, the absorbance of the complex was monitored using a portable microplate reader (Fig. 1).

3.2. Optimization

After the configuration of the printed device was optimised, several other parameters including applied potential and time of the applied potential were investigated.

3.2.1. Study of applied potential

To detect the blue phosphomolybdate complex, the applied potential for its reduction was investigated, studying it in the range from -1 V to -1.8 V. As shown in Fig. 2A, a low absorbance was obtained at -1 V

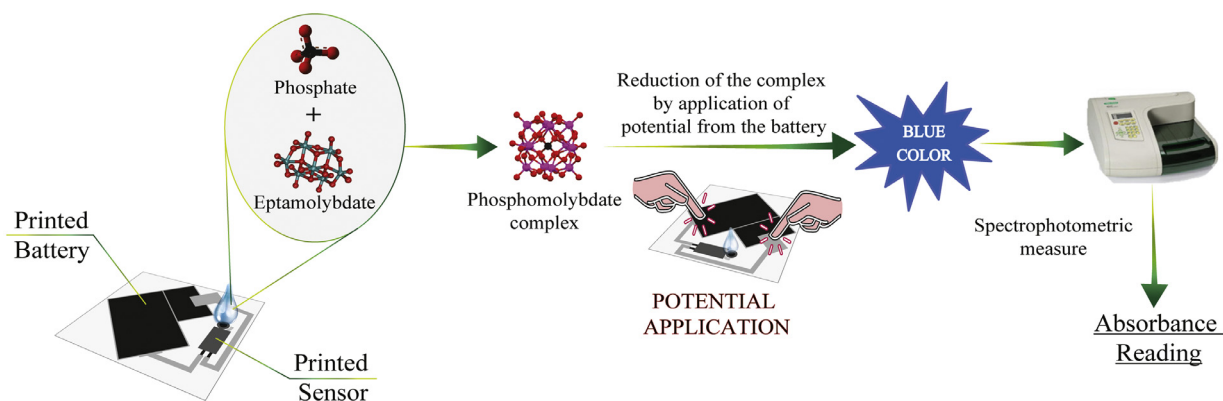


Fig. 1. Scheme of the designed platform for phosphate detection in saliva.

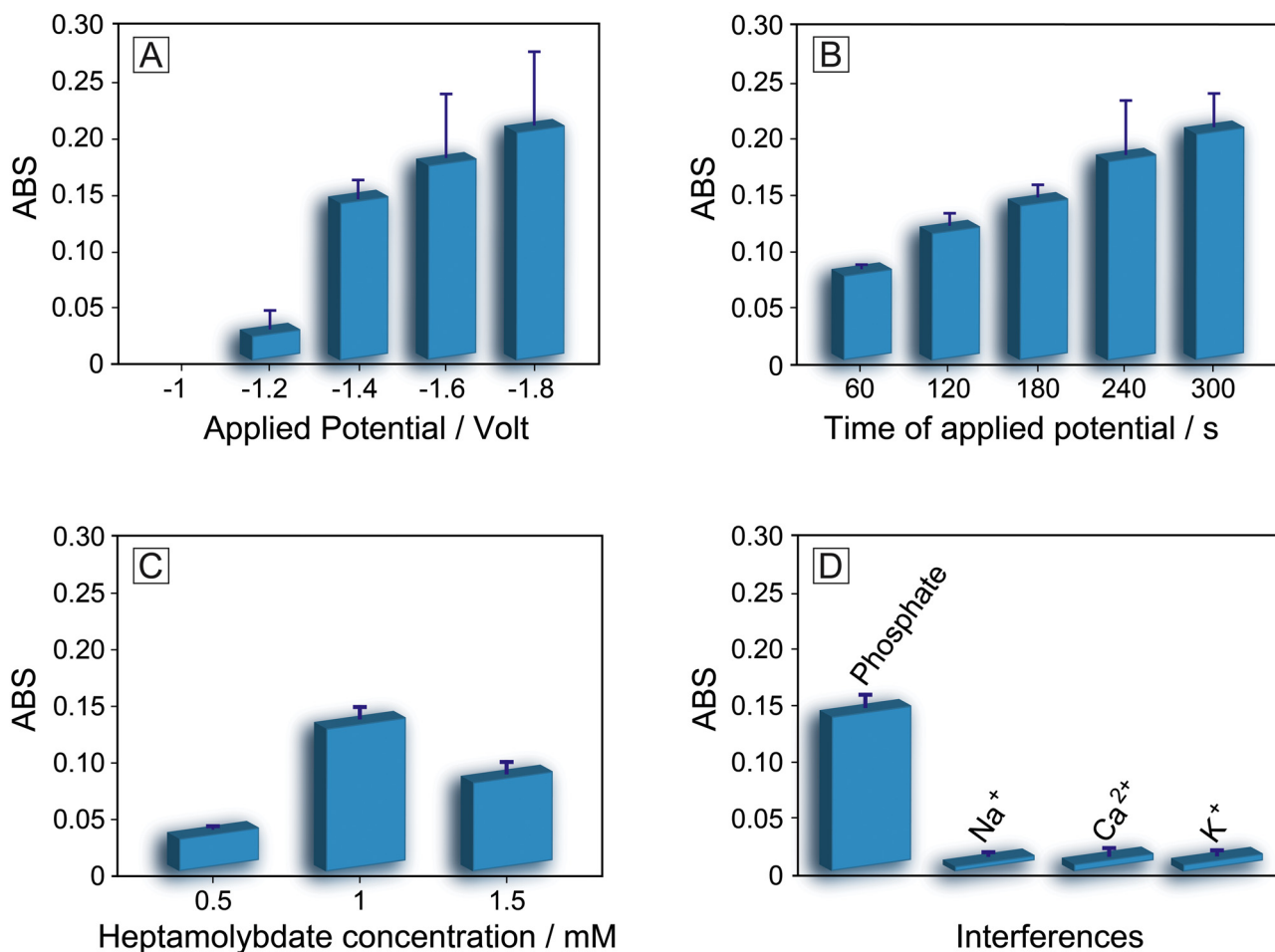


Fig. 2. Optimization of working conditions namely: Applied potential (A), Applied potential time (B), Heptamolybdate concentration (C), testing $[\text{PO}_4^{3-}] = 250 \mu\text{M}$ in H_2SO_4 0.1 M + KCl 0.1 M. (D) Study of selectivity.

and -1.2 V, while higher absorbance was recorded when the potentials increased up to -1.8 V. However, low repeatability, with a relative standard deviation (RSD%) equal to 31% and 30% for -1.6 V and -1.8 V, respectively, was obtained at these high applied potentials. Lastly, when a potential of -1.4 V was applied, an RSD% equal to 10% was obtained, underlining good repeatability, with a good absorbance signal. For this reason, this last potential was chosen for the following measurements.

3.2.2. Optimization of time of applied potential

To accomplish a sensitive measurement, the time needed for the reduction of the complex was evaluated, by applying the different potentials with the external potentiostat. As expected, at the increase of the time we observed an increase of sensitivity, until reaching a plateau. However, the increase of the sensitivity is associated with the increase of RSD % values, equal to 26% and 14%, respectively for 240 s and 300 s;

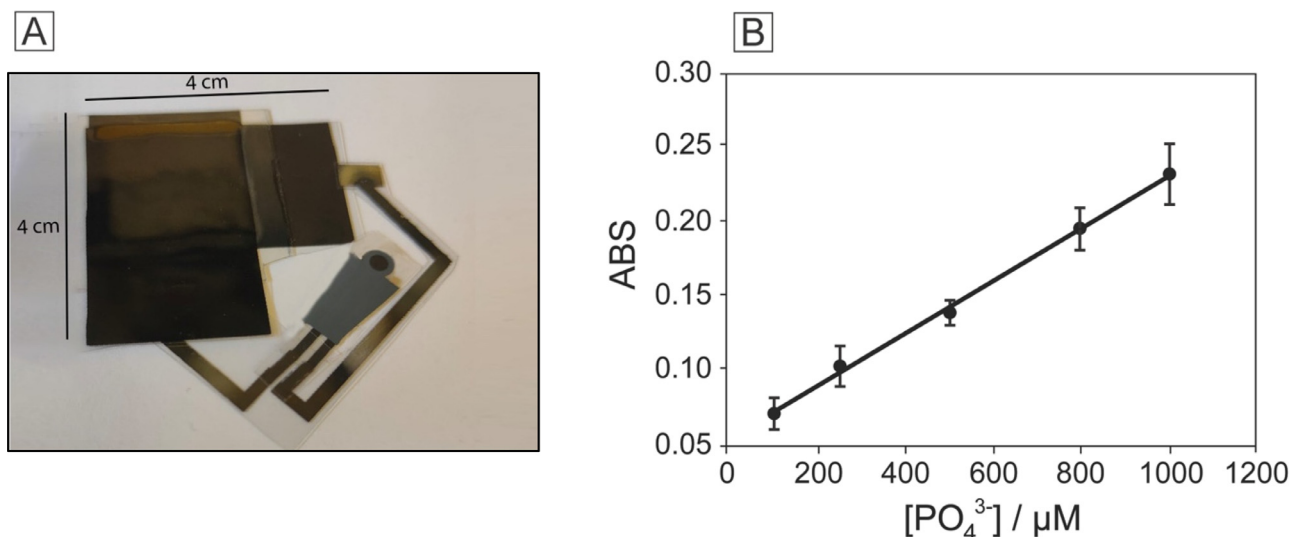


Fig. 3. (A) Picture of the developed printed electrochemical platform on flexible polymer, (B) Calibration curve obtained testing phosphate standard solutions at different concentrations.

thus, 180 s was the time selected as a compromise between sensitivity and repeatability (Fig. 2B).

3.2.3. Molybdate concentration

The concentration of molybdate plays a crucial role in the formation of the complex, which does occur only at strong acid pH. Because each mole of phosphate requires 51 mol of H^+ , the concentration of the acid needs to be largely in excess in respect to the phosphate, not to be the limiting factor. Being 1 mM the concentration of molybdate usually adopted [42], three molybdate concentrations were tested, namely 0.5 mM, 1 mM, and 1.5 mM. The highest absorbance signal with satisfactory repeatability (RSD% = 7%) was obtained using molybdate 1 mM, confirming the data of literature also in this novel configuration. Thus, 1 mM was selected to assess the interference study and analytical features in standard solution and saliva samples, as well (Fig. 2C).

3.3. Interference study

To evaluate the selectivity of the developed system, different ions including sodium, potassium, and calcium were tested at concentrations usually found in saliva [50]. In detail, the analytical tool was tested evaluating the response of sodium, potassium, and calcium ions at three different concentrations equal to 10, 15, and 20 mM (Fig. 2D). The absence of absorbance signal in presence of the ions at the different concentration levels reveals the capability of the molybdate to react selectively with phosphate in the selected optimised working conditions.

3.4. Analytical features

The analytical features were evaluated with different concentrations of phosphate standard solutions using the printed system shown in Fig. 3A. A linear correlation up to 1000 μM described by the following equation $y = (0.00020 \pm 0.00001)x + (0.107 \pm 0.006)$, ($R^2 = 0.969$) was observed as depicted in Fig. 3B. The detection limit, calculated as $3\sigma_B/m$ (where σ_B is the standard deviation of blank measurements and m is the slope of the regression line) was found equal to 26 μM . The repeatability was assessed by testing a solution of phosphate 250 μM ($n = 6$), obtaining a satisfactory RSD % value equal to 7.5%.

3.5. Phosphate detection in saliva

In the last years, efforts have been devoted to search the design of analytical tools for biomarker detection using non-invasive sample col-

Table 1

Comparison of the phosphate detection for saliva samples using the proposed and reference methods.

saliva sample	Phosphate detected (mM)		Physiological Value [50]
	Our method	Reference method	
1	4.1 ± 0.5	4.8 ± 0.3	4.8 ± 1.1
2	5.1 ± 0.6	5.3 ± 0.2	
3	6.0 ± 0.3	6.2 ± 0.4	

lection. Among several biological fluids, saliva has been recognized as an attractive diagnostic medium for the easiness of sampling and the possibility to find relevant biomarkers. For instance, in the case of cystic fibrosis, lactate dehydrogenase activity, lipids, and proteins, chlorine, sodium, calcium, and phosphate are established as useful biomarkers in saliva [51]. For this reason, the analytical tool here developed was tested in saliva, exploiting its capability to require a low sample volume and carry out the measurement on-site, being a candidate as a point of care diagnostics. The accuracy of the measurement was evaluated by measuring three different samples of saliva with the developed tool and the standard method [47] (Table 1), obtaining a good agreement using the green developed analytical tool in respect with the analysis carried out by using the reference method with high consumption of chemical reagents.

3.6. Method assessment

3.6.1. AGREE metric system

AGREE is a recently introduced green metric system based on the conversion of method parameters correlated with each of the 12 principles of GAC into greenness scores [24]. For each principle, the algorithm evaluates the performance of the proposed analytical method by assigning a score. The scores are pre-defined by an inflexible model and are uniquely generated, ranging from 0 to 1 depending on the accordance of the method with the principle. Which, however, depends on the estimation of the input parameters to insert in AGREE software.

By taking into account this algorithm, an evaluation of the proposed sensor for each GAC principle gave the results summarized in Fig. 4, where the overall score is depicted by the classical AGREE clock-like graph with intuitive red-yellow-green color representation.

The final score of the AGREE evaluation highlights the higher greenness obtained by the proposed sensor (Fig. 4B), compared to the one

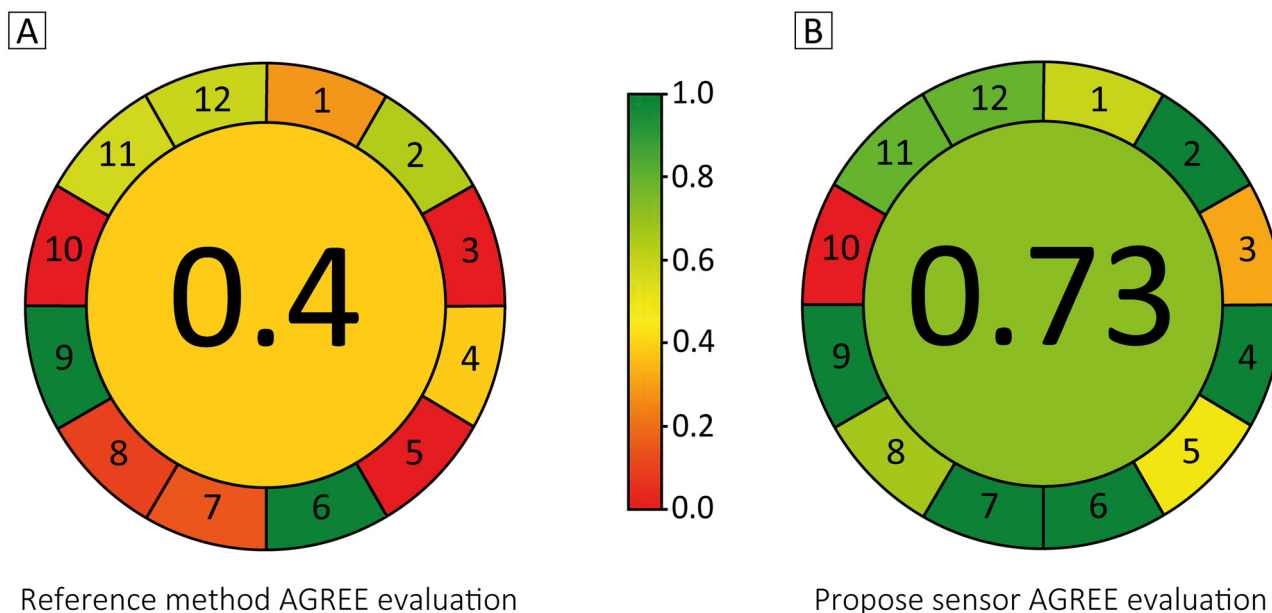


Fig. 4. Evaluation of the AGREE performances by a clock-like graph representing the overall score in the middle section. The performance of the method in each of the 12 GAC principles is depicted in the external crown, explained with a red-yellow-green color scale. (A) Reference method, (B) proposed printed sensor.

Table 2

Scoring values obtained by the reference method and the proposed sensor, for each one of the 12 principles of the GAC, according to the AGREE assessment approach.

Green Analytical Principle	Reference method	Score	Sensor method	Score
1. Direct techniques	Batch analysis few step	0.3	Analysis At Line	0.6
2. Minimal sample size (ml)	1	0.09	0.02	1.0
3. <i>In situ</i> measurement	Off line	0.0	At Line	0.33
4. Step numbers	6 step	0.4	Less than 3	1.0
5. Automated and miniaturized	Manual, not miniaturized	0.0	Manual, miniaturized	0.5
6. Derivatization	Not present	1.0	Not present	1.0
7. Waste generation (ml)	53	0.16	0.1	1.0
8. Multianalyte (number of analytes in 1 h)	2 (30 min for 1 sample)	0.12	20 (3 min for 1 sample)	0.68
9. Energy minimized	< 0.1 kWh	1.0	< 0.1 kWh	1.0
10. Renewable reagents	No	0.0	No	0.0
11. Toxic reagents (g)	0.528 g Sulphuric acid 0.002 g Antimonyl tartrate	0.0	0.784 mg Sulphuric acid	0.8
12. Safety for operator	Corrosive Toxic for aquatic life	0.6	Corrosive	0.8

obtained by the reference method (Fig. 4A). As shown in Table 2, this is mainly a result of the lower volume of generated (toxic) waste (i.e., point 7), minimal sample size (i.e., point 2), step number (i.e., point 4), miniaturization (i.e., point 5), and toxic compound used (i.e., point 11). Furthermore, both methods avoid the use of renewable materials or reagents (i.e., point 10), derivatization (i.e., point 6), and have low consumption of energy (i.e., point 9).

3.6.2. WAC evaluation model

The concept of WAC can be considered as an extension of the green metric approach and takes into account other criteria in addition to the greenness [9]. By applying these principles, the evaluation based on the WAC approach allows for a deeper analysis in terms of analytical performances and practical benefits of a proposed method.

The evaluation of this new approach can be performed by a Red-Green-Blue (RGB) model, based on the classification of the 12 new principles divided into three areas (i.e. red, green, and blue) [26].

In detail, starting from four parameters grouped in the color “green”, related to the greenness, the model adds four criteria grouped under the “red” area, related to analytical performances, and four under the “blue” area, related to practical benefits (Table S3). By applying the RGB

model, an algorithm sums up the scores obtained by the method for each of the three areas/colors, giving the final average number of “whiteness” that quantifies how much the method fits the WAC principles.

Evaluator that uses this method is encouraged to be flexible and to adjust the assessment for an objective evaluation. According to the considerations of the authors [26], we attributed different scores for each of the 12 parameters, reported in Tables S1 and S2, to evaluate the parameters affecting the environmental impact and the practical benefits. In detail, we evaluated the analytical performances of the sensor by the red color area of the RGB model taking into account that the analytical performances of the reference method were considered as 100 score.

Therefore, the scores values of the sensor-based method have been defined following a criterion of proportionality e.g. in the case of half analytical performance 50 was reported as score.

In the case of the greenness, 100 is established for a method characterized by zero waste production or energy consumption, while in the case of blue area, 100 is attributed to a method with the requirement of a hand-held instrument or an easiness to use. In detail, tables reporting these scores allow for easily method assessments [52]; in fact, as shown in Table S3, the developed method is characterized by a lower

Fig. 5. Visualization of the evaluation results according to the RGB method for the reference method (A) and proposed sensor (B).

A

Method: Reference method					
R1: Scope of application	100.0	G1: Toxicity of reagents	90.0	B1: Cost-efficiency	75.0
R2: LOD and LOQ	100.0	G2: Amount of reagents and waste	25.0	B2: Time-efficiency	50.0
R3: Precision	100.0	G3: Energy and other media	75.0	B3: Requirements	37.5
R4: Accuracy	100.0	G4: Direct impacts	91.7	B4: Operational simplicity	33.3
100,0		70,4		49,0	
73,1					

B

Method: proposed sensor					
R1: Scope of application	9.1	G1: Toxicity of reagents	95.0	B1: Cost-efficiency	100.0
R2: LOD and LOQ	143.0	G2: Amount of reagents and waste	85.0	B2: Time-efficiency	75.0
R3: Precision	67.0	G3: Energy and other media	85.0	B3: Requirements	100.0
R4: Accuracy	100.0	G4: Direct impacts	93.3	B4: Operational simplicity	91.7
79,8		89,6		91,7	
87,0					

number of hazardous pictograms and waste amount. Finally, focusing on the blue area of practical benefits, the developed sensor gives better performances in terms of cost, time efficiency, and portability. Taking into account all the parameters, the application of the WAC approach assigns an average score to the proposed sensor equal to 87, which is higher than the reference method's score equal to 73.1 (Fig. 5).

4. Conclusions

In this work, a cost-effective and miniaturized fully printed electrochemical device has been produced to assist the colorimetric detection of phosphate, with the aim to fulfill the principles stated by the GAC and WAC. AGREE and RGB model assessment tools have been applied

to evaluate the greenness and the whiteness of the proposed sensor compared to the reference method. The combination of a screen-printed battery and electrodes, inkjet-printed conductive paths, and the concept of amperometry allows for an ascorbic acid-free detection of phosphate in saliva samples, lowering the amount of used reagents. These features have been evaluated by the assessment tools, giving a final quantification of the improved environmental and sustainability aspect compared to the reference methods, paving the way to develop printed electrochemical devices for green measurements.

Declaration of Competing Interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.greeac.2022.100006.

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