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Prussian Blue (bio)sensing device for distance-based measurements

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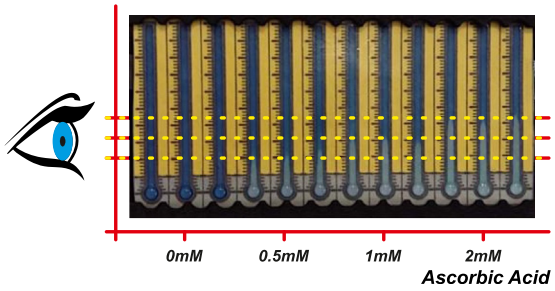
HIGHLIGHTS

- Distance-based μPADs sensitive to ascorbic acid and hydrogen peroxide are introduced.
- Prussian Blue sensing layer was used as a chemoreceptor in working area of the μPADs.
- Biomodification of systems with glucose oxidase leads to sensor for glucose.
- A successful determination of glucose and ascorbic acid in real samples are performed.
- Obtained results for quantification of analytes of interests highly correlates with reference methods.

GRAPHICAL ABSTRACT



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ABSTRACT

In this research, microfluidic paper distance-based systems for the quantification of redox species are proposed. For the preparation of the sensing zone a Prussian Blue (PB) (convertible to Prussian White (PW)) layer was deposited in the channel manufactured by wax-printing technique. According to the chemical properties of PB/PW system, it is possible to develop optical sensors sensitive to both oxidizing and reducing agents. The created systems were evaluated for the determination of ascorbic acid and hydrogen peroxide, which were chosen and used as model analytes. The final versions of the proposed systems exhibited a linear response from 0.25 mmol L^{-1} to 4.0 and 2.0 mmol L^{-1} for ascorbic acid and H_2O_2, respectively. The analytical utility of the paper systems was confirmed by measuring the levels of ascorbic acid in dietary supplements. Results correlation obtained for the described systems and the reference method was over 0.98 (Pearson's R-coefficient). All measurements were characterized by satisfactory reproducibility and acceptable uncertainty (RSD (%) < 6%). Finally, it was demonstrated that the modification of the PW-strip systems with oxidoreductase led to an enzymatic assay for glucose up to 10 mmol L^{-1} range. Practical utility of the developed bio-strips was confirmed by quantifying glucose in drinks and dietary supplement samples.

1. Introduction

The rapid growth of the number of assays performed daily forced modern analytical chemistry to look forward improvements in tests throughput and their simplicity. For this reason, the concept of microfluidic devices made of paper was introduced in the last decade [1,2]. The Whitesides' group introduced microfluidic paper-based analytical devices (μPADs) [3], as an attractive substitute to conventional microfluidics, while exhibiting similar advantages,

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namely portability, utility and cost-effectiveness. Contrary to PDMS/glass microchips [7], paper as a porous material made of the cellulose fibers [4–6], allows fluid transport without the application of any external devices. In the paper systems capillary induced transport is observed in the whole bulk mass and its attributes are strictly connected to the physical properties of the paper. For this reason, the wicking speed and μPAD utility can be regulated just with an appropriate paper selection [8].

Both PDMS and paper-based microfluidics share similar advantages: limited sample and reagents consumption, reduced analysis time, small size, and disposability [9–11]. They also provide the possibility of multi-analyte detection within a miniaturized format, with remote sample manipulation and simplification of procedures which are performed.

The bare paper sheet needs to be covered with a hydrophobic barrier to define the areas allowed for fluids and prevent the solutions to spread on whole paper volume [1,12]. Hydrophobic barriers made of wax [13–15], photosensitive resins [16,17] or ordinary office printer toner/ink [18,19] etc. were utilized as fast and reproducible fabrication methods to create microfluidic pattern.

Among all techniques suitable for the pattering of paper, wax-printing is the most widely used, due to its simplicity and only two-step fabrication [20]. This procedure utilizes solid-ink printer to fabricate the hydrophobic pattern, which is printed on the paper surface, accordingly to a computer design. In the next step pattern is heated with a laboratory dryer to melt the wax and create the impermeable barriers in the paper bulk after cooling down.

The common utilization of the paper-made devices is still limited by the insufficient development of the detection methods dedicated to μPADs. Electrochemical detection, as one of the most universal, reliable and fast techniques, requires electrodes and their modification to provide proper selectivity. Screen printed electrodes, suitable for paper-based systems, require optimization of the printing process and paste composition to provide satisfactory results [21]. Optical methods, which from the theoretical point of view, are fast and universal for the quantification of the absorbing compounds, require monochromatic light and dedicated detectors [22–24]. Specific LED's properties allowed recently the utilization of the extremely cost-effective optoelectronic PEDD detectors with paper-based devices in both photo- and fluorometric measurements [25,26]. Unfortunately, LEDs still require a low voltage power supply and simple electronic readers, and even such simple measurement setup is not attractive when the application in limited resources settings is considered.

As an alternative, a distance-based detection can be considered as a method for visual and free of any auxiliary devices signal readout. Introduced by Zuk et. all [27], it was primarily used in paper-based systems dedicated to the Point-of-Care testing and provide the possibility of healthcare monitoring with minimal user training. In contrast to naked-eye color evaluation, which is interrupted with individual attributes [28], here the analytical signal is proportional to the size of the colored or discolored band. The signal is generated as a result of a specific reaction which occurs in the working zone of the distance-based system. As the sample travels across the system, the analyte reacts with the reagent deposited in advance on the μPAD surface. To date, paper systems which utilize distance-based detection methods have been widely investigated and proposed for health monitoring [29–31] or environment pollution monitoring, for both airborne [32] as well as water impurities [33,34]. Even though distance-based detection shows promising potential to overcome economical and applicability limitations, it still requires investigations to enable mass production and a wide range of the analytes that can be determined using such methodology.

In this work, we demonstrate the distance-based optical sensors dedicated to the determination of redox species. In these investigations, the Prussian Blue (PB) layer was used as a chemosensitive compound. Ascorbic acid and hydrogen peroxide were chosen as model analytes and their quantification was performed in this work. The layer of PB can be quickly deposited almost on any material with good reproducibility in a single preparation step.

The possibility of using thin films of Prussian Blue as optically sensitive material was already proposed for the development of disposable cuvette tests [35,36]. PB insolubility and ease of transition from PB to PW and vice versa allowed for employing such thin layers deposited on polyester film as optical sensors also in flow analysis systems [37]. The ability to create organic composites during layer fabrication makes it relatively simple to chemically immobilize enzymes [38].

All of the above mentioned advantages allow for applying PB in the distance-based systems as an optical sensor. In the next step, strips sensitive to hydrogen peroxide were modified with glucose oxidase to proof the analytical performance of the proposed systems for the glucose level estimations.

2. Materials and methods

2.1. Reagents

All reagents used during the experiment were of an analytical grade. Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4), with declared activity of 135 kU g^{-1} as well as Iron (III) chloride hexahydrate (>98%) was purchased from Sigma-Aldrich (Germany), hydrogen peroxide 30% solution from Chempur (Poland), potassium hexacyanoferrate (III) (>99.8%), L (+) ascorbic acid (AA) and hydrochloric acid, 36–38% solution, from PoCh (Poland). Throughout all experiments, deionized water from Milli-Q purification system was used (conductivity below 1.4 μS cm^{-1}).

2.2. Equipment

Commercially available solid-ink printer Xerox ColorQube 8580DN was used for the fabrication of the wax pattern on the paper surface, accordingly to the computer design prepared with CorelDraw software. The heat treatment of the wax patterns was performed for 2 min using a standard laboratory dryer set to 120 °C. For one-side hot lamination, a standard office laminator and 100 μm thick foil were used. For the protection of the PB/PW layer a Scotch tape (3 M, Germany) was used. A black-colored PLA (polylactide) filament manufactured by Orbi Tech (Germany) used in FFF 3D Printer model Dreamer (Flashforge, China) obtained from Sygnis New Technologies (Poland) was used for the fabrication of paper systems holders used in experimental setup.

2.3. Paper-strips fabrication

Whatman Qualitative Paper No.1 (GE Healthcare, USA) was used in all experiments as a support material for the fabrication of the μPADs. The typical for distance-based setups pattern design, in the shape of the thermometer, was used during all investigations (presented in ESI Fig. 1). In overall, a straight flow channel (2 mm width/51 mm length), ended from one side with a circular-shaped sample introduction zone (Internal Diameter ID 5 mm). In fact, dimensions which were introduced to the designing software were slightly larger (3 mm width, 52.5 mm length) due to wax spreading process during the heat treatment. For clarity, only real dimensions (obtained after heating) will be mentioned in the further part of this contribution. A design drawing file of the system can be downloaded from the supplementary files.

Wax printing allows for fast modifications of the hydrophobic pattern in a single print out. Additionally, a scale was added to the

pattern as a part of the design improvement, allowing unaided signal readout. However, due to wax spreading, printed scale gives low resolution therefore in course of this work we always used an office ruler for the signal readout. Before the deposition of the PB strips were hot laminated from the one side and ready for further modification when this step was completed.

2.3.1. Redox sensitive paper tests fabrication

All reagent solutions or samples introduced to the systems were placed in the sample introduction zone using standard laboratory micropipette. The fluid capacity of the system was estimated to be 20–25 μL. Paper between hydrophobic barriers was modified with solution forming Prussian Blue deposited *in situ*, which contained acidic solutions (1 mol L^{-1} HCl) of the iron (III) chloride and potassium hexacyanoferrate (III), prepared and mixed just before the deposition step [35,39]. 25 μL of the mixture was introduced to the patterns and the soaked systems were left overnight for complete PB formation. After one-night deposition process, systems were washed with deionized water, introduced to 0.1 mol L^{-1} phosphate buffer pH = 7 and left to dry in the ambient conditions for about 1 h.

When the PW layer was desired, the paper strips modified with PB were treated with the solution of ascorbic acid (AA, 0.1 mol L^{-1}) prepared in KH_2PO_4/K_2HPO_4 buffer (0.1 mol L^{-1} pH = 7). The high concentration of the AA solution ensures a complete PB reduction at a high rate, whereas phosphate buffer provides the potassium ions assistance to compensate the difference in charge generated in the PB layer in the course of the reaction [40] (Fig.1). After the reduction, PW covered tests were washed firstly with deionized water, then with 0.1 mol L^{-1} phosphate buffer pH = 7 and left to dry at ambient conditions. The final stage of the strips' fabrication was to cover them with an ordinary scotch tape which protects PB/PW system from the ambient conditions during storage as well as limits the evaporation of the solutions during the measurements.

In the sampling zone, the small hole (ID 0.5 mm) was made with a drawing pin or a needle in protecting tape before its application (ESI Fig. 1). The dimensions of the hole do not limit the fluid aspiration for a relatively small hydrostatic pressure drop generated with a paper substrate. The puncture mark also plays the role of a starting point during signal measurement. To ensure fluid contact in the initial step of the analysis, single punching can be performed after drop was deposited on the system (through the drop) (see Fig.2).

2.3.2. Biosensitive paper tests fabrication

Enzyme-modified paper channels were fabricated in two modes, which are represented in Fig. 2. The first mode involves a one-step introduction of enzyme solution (5 μL of various solutions from 0.5 mg mL^{-1} to 2 mg mL^{-1}) on the flow channel (A1) followed with drying (A2) and self-adhesive tape covering (A3). The second mode involves double-step preparation, in which the same amount of enzyme solution is placed firstly on the external paper-made circles (ID 5 mm) and dried (B1). Then the enzyme-modified zones are placed (B2) and attached to the flow channel with a self-adhesive tape (B3).

2.4. Measurements

The sample/standard solution was placed in the sampling zone of the systems situated on the 3D printed support, with one wall tilted at an angle of 45°. The capillary force generated with cellulose fibers causes the sample natural wicking in the paper matrix (examples of the paper systems operation are presented in ESI video files),

Supplementary video related to this article can be found at https://doi.org/10.1016/j.aca.2020.08.037

Providing an unpowered flow. In the initial step after sample introduction to the system, 20 μL of the sample forms a droplet in the sampling zone. To avoid droplet movement along the non-covered working channel or to prevent falling the droplet off the system under the influence of the gravity, systems were hold tilted. After some time, the excess of the fluid wicks into the system and systems can be held horizontally. Incline position was not necessary in case of the glucose-sensitive strips, where the flow channel was completely covered with protective tape.

In general, the readout of the analytical signal was accomplished by measuring the length of the zone where the colorization/discoloration process occurs. When the strip was covered with PB and a reductant was present in the sample, an application of the sample drop into the "starting point" resulted in the color change from navy blue to turquoise. Vice versa, when the sensing zone was in the reduced form (PW), it could be oxidized when an appropriate oxidizing agent was present in the sample. As a starting point for the measurements, the puncture mark in the sampling zone was chosen (see ESI Fig. 1).

The color band, as a result of laminar flow, has a lancet-shaped head. For this reason the most distant point of the band was always selected as the end of the measured signal. To complete the analysis procedure, user needs to wait until all flow channel is fully wetted with the sample solution. The measurement of the distance should be performed immediately after the fluid front reaches the end of the channel. It was possible to complete the signal readout within 15 min after sample/standard introduction. The signal must be measured immediately after analysis is completed, because potential evaporation causes fading of the colored band.

2.5. Reference analysis

The reference analysis of the ascorbic acid content in dietary supplements obtained from a local pharmacy were performed via titration of the samples with the solution of 2,6-dichlorophenolindophenol (DCPIP) (Sigma Aldrich, Germany). This method for the determination of the ascorbic acid level in, e.g., serum or food samples, is well established and performed routinely [41,42]. A detailed description of the titration method is provided in the Electronic Supplementary Information.

As a reference measurements of glucose content in real samples a diagnostic kit from Cormay (Poland) was used. Glucose oxidase (Gox) catalyzes the oxidation of glucose to gluconate and hydrogen peroxide. The formed H_2O_2 in the presence of peroxidase (POD) causes the formation of colorful 4-(p-benzochinon-monoamino)-phenazone which is determined spectrophotometrically.

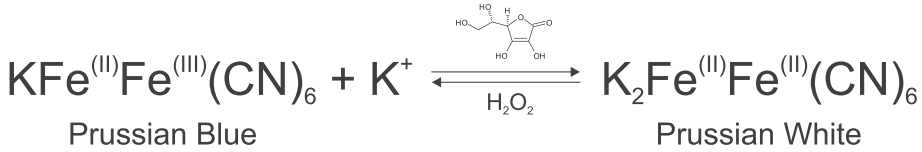

Fig. 1. Scheme of the reversible convertion of the PB into PW.

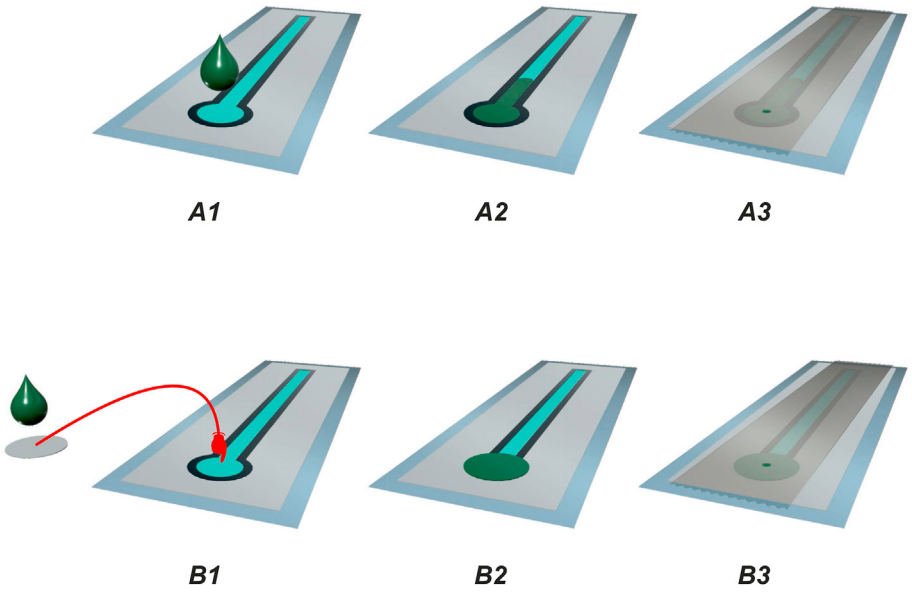

Fig. 2. A representation of the modes of enzymatic modification of the distance-based strips: A – Mode of operation when enzyme suspension is applied directly on the strip, B - Mode of operation when enzyme suspension is applied on the external circle-shaped piece of Whatman paper. Numbers indicate subsequent steps of enzymatic modification: 1 – dispensing, 2 – drying, 3 - protecting.

3. Results & discussion

3.1. Redox sensing

As the first part of the experiment, the influence of the amount of PB deposited in the channel on the analytical signal characteristic was examined. The analyte consumption is the primary effect causing the signal generation in the distance-based systems therefore the analytical parameters of the proposed systems should directly depend on the amount of PB deposited in the channel, which undergoes reduction when exposed to AA from the sample. Due to the high rate of the AA consuming in the systems characterized with high PB quantity generated signals should be lower in comparison to systems with a thin PB layer, in which the analyte consumption is prolonged.

To examine this effect, series of the systems with the same geometry were prepared using iron (III) chloride and potassium hexacyanoferrate (III) stock solutions with different concentrations (25, 20, 15, 10, and 5 mmol L^{-1} (Fig. 3)). Standards with the same molar concentration were mixed directly in equimolar amount before the dosing step. Due to the spontaneous Prussian Blue deposition, the mixture of both reagents cannot be stored for an extended time period (longer than a few hours).

The general tendency of signal generation was confirmed in such an experiment. A higher amount of PB applied in the working zone is causing low sensitivity of the tests. On the other hand, an extension of the linear range is observed. The changes of the amount of the dye do not significantly affect the measurement uncertainty; the corresponding errors expressed as a standard deviation from three replicate measurements are on the acceptable level (ESI Tab.1). Curve exhibiting a satisfactory determination coefficient above 0.99 (RSD (%) < 6%) was obtained for the test modified with solutions with the highest (25 mmol L^{-1}) concentration of the Fe^{3+} and $Fe(CN)_6^{3-}$ ions. For other solutions

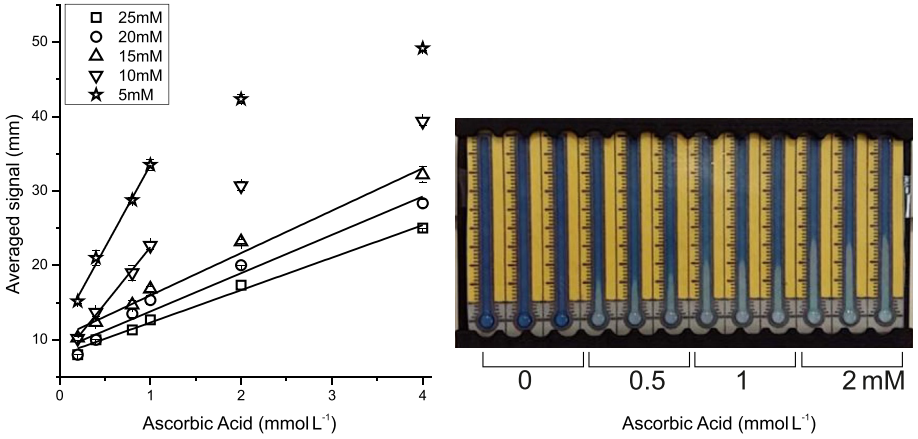

Fig. 3. Analytical signal characteristic obtained for various Prussian Blue layer thickness used in the working zone (left). Concentrations of the dye deposition solution are given in the picture. Error bars represent the reproducibility of three different strips. A photograph of the set of paper strips, modified with the mixture 15/15 mmol L^{-1} (iron (III) chloride/iron (III) hexacyanoferrate) after the color change (right). Triplicate measurements were conducted for each ascorbic acid standard. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

satisfactory linearity was observed with the determination coefficient R^2 higher than 0.96 and RSD (%) below 5%.

An analogous experiment was arranged for testing the sensitivity towards hydrogen peroxide. The systems with Prussian Blue were transformed using ascorbic acid before the measurement, as described earlier. As a result of the reduction process, a layer of Prussian White was generated along the flow channel. The storage stability of paper strips covered with PW is limited due to the spontaneous oxidizing process accelerated with oxygen from the air. To ensure the long-time stability of the systems after preparation, they were stored in the temperature 4 °C for up to 30 days. As a complementary step, application of tape to seal and protect the systems from the ambient atmosphere was used.

Nevertheless, ready-to-use systems covered with PW kept in the room temperature shows constant properties and no changes in the analytical performance were noticed during the weekly measurement cycle using one set of the strips. After this time, the systems showed slowly decreasing sensitivity towards hydrogen peroxide due to a partial oxidation of PW. In the experiment freshly reduced strips were used and for this reason it can be assumed that the instability of the dye layer is negligible and do not interfere with the results. The same methodology to check the system sensitivity was applied as previously described for AA determination. A series of strips were modified with different amounts of chemosensitive dye. The strips were later used for the determination of the hydrogen peroxide. The obtained signal characteristics and calibration curves in the linear range of the analyte concentration are presented in Fig. 4.

In comparison to the tests sensitive to ascorbic acid, these suitable for hydrogen peroxide determination are characterized by higher sensitivity but with limited linear response. Due to the quantification of the blank signal is always “zero” the calculation of the LOD/LOQ values must be estimated using parameters of the fitted calibration curve. According to the formulas: $3SD_y/S = LOD$ and $10SD_y/S = LOQ$ (SD_y is the standard deviation of y-interception, and S is calibration curve slope) higher detection and quantification limits are observed for thickly covered strips, e.g., modified with 25/25 mmol L^{-1} solutions. Linear response for hydrogen peroxide is observed in a narrower range of the analyte concentration (<2 mmol L^{-1}). However, also this time the calibration curves characterized with an acceptable determination coefficients were obtained (ESI Tab.1). A system operational examples for both ascorbic acid and hydrogen peroxide sensitive tests are included in Supplementary Video Files. Based on the result mentioned here and in ESI, for all further experiments strips covered with 25 mmol L^{-1} of PB/PW were used because they provide an objectively broad working range and the lowest variability. In these conditions, the calculated LOD and LOQ for AA were 0.44 and 1.47 mmol L^{-1} and for H_2O_2 are 0.03 and 0.124 mmol $L^{-1,}$ respectively.

3.2. Biosensing

Glucose determinations are commonly performed worldwide, especially due to clinical reasons as well as in the food industry. The determination of hydrogen peroxide has significant importance in the laboratory practice, because of its formation during many enzymatically catalyzed reactions. For the demonstration of the possibility of determining glucose content in a distance-based measurement mode, hydrogen peroxide sensitive systems with the best analytical performance were modified with glucose oxidase, which catalyzes the decomposition of β-glucose into gluconic acid and hydrogen peroxide. As mentioned in section 2.3.2, the enzyme was physically immobilized on the paper surface without an addition of any other reagents. Distance-based systems covered with PB deposited from 10 mmol L^{-1} solutions and transformed to PW system (as described above) were chosen as suitable for further biomodification, because they ensure an acceptable uncertainty and the highest sensitivity to hydrogen peroxide. H_2O_2 sensitive distance-based systems were modified in two ways: via a direct deposition of enzyme solution and with the use of an external piece of paper. The one-step procedure is the most trivial but can cause that the enzymatic zone is not well defined due to the effect of the solution spreading in the paper matrix. Using extra paper sheets soaked with the enzyme solution and dried was connected with the two-step, more complicated, and time-consuming preparation. However, the mentioned above drawbacks were compensated with a well-defined enzymatic zone and advantageous analytical parameters of the created systems.

High activity of the used lyophilized glucose oxidase specimen allows for the preparation of low concentrated solutions with high activity. Such a solution (1 mg mL^{-1}) was used for checking which way of enzyme delivery would be better for the glucose system performance. As can be seen in Fig. 5, due to different enzyme deposition methodology, calibration curves described with individual analytical parameters were obtained. Improved sensitivity and linearity can be observed when biomolecules are placed on outer paper circles. A portion of the sample contains a particular amount of the analyte that can be consumed via an enzymatic

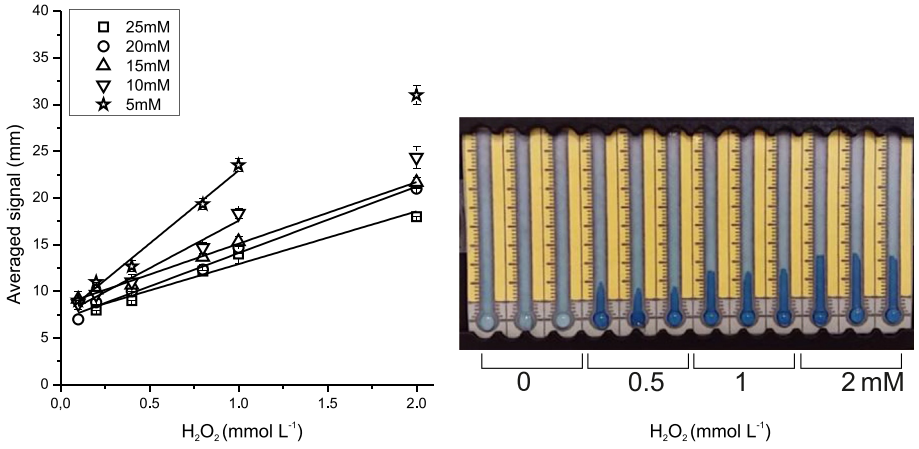

Fig. 4. Analytical signal characteristic obtained for various Prussian White layer thickness used in the working zone (left). Concentrations of the dye deposition solution are given in the picture. Error bars represent the reproducibility of three different strips. A photograph of the set of paper strips initially modified with the mixture 15/15 mmol L^{-1} (iron (III) chloride/iron (III) hexacyanoferrate) after reduction process and H_2O_2 determinations (right). Triplicate measurements were conducted for each hydrogen peroxide standard.

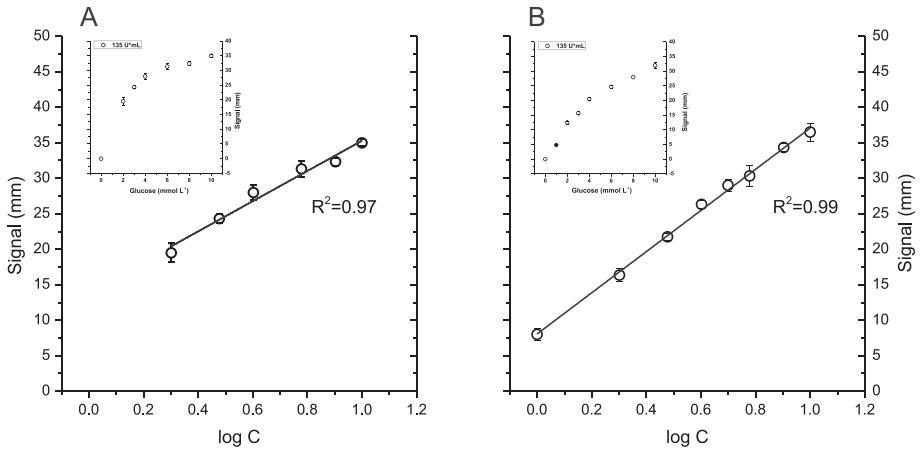

Fig. 5. Comparison between the biosensing strips performance, when an enzyme was distributed directly on the sampling zone of the flow channel (activity: 135 U mL^{-1}) (A) and when an enzyme was placed using an additional piece of paper (activity: 135 U mL^{-1}) (B).

reaction in a respected period of time. When the applied enzyme activity is high enough, all of the analyte can be converted into the detectable product instantly after the sample introduction. Due to spontaneous fluid spreading, lower "effective" enzyme activity is observed when its solution is placed directly on the flow channel. in comparison to the defined area of the paper circles. When the enzymatic zone is better defined, more hydrogen peroxide is generated right after sample introduction and then can be recorded with a distance-based system. At this point in the distant part of the flow channel only products of the enzymatic conversion are present and the unreacted analyte in coexistence with enzyme is not observed. Moreover, when the analyte consumption occurs at a high rate, it is possible to record a sharp signal cut-off because the migration of the enzyme with the unreacted analyte is limited to the early part of the flow channel.

Using the same methodology the activity of the enzyme was experimentally adjusted to provide the best results and optimize the cost-effectiveness of the (bio)tests. Four enzyme activities (67.5, 135, 205.5, and 270 U mL^{-1}) were tested and for each one a calibration curve in a logarithmic scale of the target analyte (glucose) was plotted. As a result of these experiments, an activity equal 135 U mL^{-1} delivered to the system on the external piece of paper was considered to be optimal. In such conditions a calibration curve shows satisfactory linearity in the whole tested range of the analyte concentration with calculated LOD = 1.1 mmol L^{-1}. According to ESI Tab 2. which contains parameters of the calibration graphs in more detail, worth noticing is the fact, that significant changes in the applied enzyme activity do not affect the system sensitivity and repeatability in a considerable way. A comparison of the proposed tests with other distance-based paper systems for glucose determination is presented in Table 1. The devices presented here exhibit similar analytical parameters as systems found in the literature as well as the same sample consumption.

In contrast to the examples from the literature of distance-based systems dedicated to glucose determination, our system to not require cascade enzymatic reactions. Much simpler conversion, using only a single enzyme, is adapted to transform glucose into hydrogen peroxide, which is detectable using the sensing layer of Prussian White.

3.3. Analysis of real samples

Optimized systems were used to analyze vitamin supplements and drugs containing ascorbic acid. Samples were obtained from a local pharmacy as ready-to-take pills or powder for the preparation of the oral solution. Due to the high content of ascorbic acid, some of the analyzed samples were diluted (up to 40-fold) to obtain a concentration of the analyte compatible with the linear range of the calibration curve for the optimal paper-based systems. The results obtained using both: reference (titration using DCPIP) and distance-based paper strip methods were also referred to the declared content of AA provided by the manufacturer and are presented in Table 2.

As presented, a satisfactory correlation between two methods for ascorbic acid determination was obtained. A positive correlation with the Pearson-R coefficient value of 0.999 (ESI Fig. 3) was achieved, what suggests a suitability of the distance-based systems for rapid and equipment-free quantification of ascorbic acid in real scenarios. Each of the obtained values for a particular sample represents an average of three independent measurements. Calculated RSD (%) for the paper systems does not exceed 4%. The determined concentrations of ascorbic acid were also compared with the manufacturer's information provided on the package. Manufacturer data are coherent with the obtained results using the systems proposed here. A high inconsistency between the measured and the declared value is observed for only one formulation. This error,

Table 1
The comparison of the proposed method with other literature examples of the distance-based paper systems for glucose.

Detecting scheme	Linear range (mg dL^{-1})	Detection limit (mg dL^{-1})	Sample consumption per single assay	Ref.
1° *Glucose + Gox → Gluconic Acid + H_2O_2* 2° *H_2O_2 + HRP + DAB → poly-DAB*	11 ÷ 270	20.0	20 μL	[43]
1° *Glucose + Gox → Gluconic Acid + H_2O_2* 2° *H_2O_2 + HRP + DAB → poly-DAB*	12.6 ÷ 189	n.a.	20 μL	[44]
1° *Glucose + Gox → Gluconic Acid + H_2O_2* *PW + H_2O_2 → PB + H_2O + ½ O_2*	18 ÷ 180	19.8	20 μL	This Work

Table 2
A comparison of the results obtained for the determination of the ascorbic acid (AA) content in commercially available drugs and supplements.

Drugs/Supplements trade name	Declared AA	Measured AA			
	(mg/pill)	Via DCPIP titration (mg/pill)	RSD (%)	Using DB-μPAD (mg/pill)	RSD (%)
Cerutin	80	–	–	90.35 ± 3.09	3.5
Vitalsss Plus Multi	100	–	–	103.16 ± 2.33	2.8
Aspirin C	240	237.07 ± 0.40	0.2	234.62 ± 2.33	1.0
Rutinoscorbin	100	95.73 ± 1.52	1.6	84.29 ± 2.33	3.0
Gripex Hot	50	30.10 ± 0.13	0.4	33.72 ± 1.01	1.3
Ceviforte	1000	929.43 ± 6.08	0.6	903.57 ± 11.67	1.0
Witamina C Forte	1000	1129.37 ± 3.59	0.3	1105.72 ± 11.67	2.3

however, can be explained with the manufacturer inaccuracy when ascorbic acid is only a side drug component, used as a support for the active component (acetylsalicylic acid).

Systems sensitive to hydrogen peroxide and modified with glucose oxidase were used for the determination of the glucose in the samples of bottled drinks, pharmaceuticals and control human serums with the physiological and pathological range of the analyte. Depending on the sample type, they were diluted to meet the calibration curve characteristic obtained for distance-based microfluidic systems (drinks –from 50 to 100-fold, human serum 4-fold, and pharmaceuticals from 4 to 8-fold).

Results (Fig. 6, with a detailed description) and presented in Electronic Supplementary Information (ESI Tab.3) confirmed a satisfactory correlation between the results obtained using the developed distance-based paper sensors and the recommended reference method. Also, both kinds of results do not stand out from declared values. The sample dilution in the case of serum samples was necessary due to the high viscosity of serum. In these conditions capillary fluid transport in the paper matrix is disturbed and the flow parameters are not convergent with the aqueous solutions. Unfortunately, the sensitivity of the presented systems is highly influenced by sample flow parameters; thus, calibration as well as the analysis of the sample should be performed in constant conditions. For that reasons using standards exhibiting parameters comparable with samples characteristics is recommended. Further system adjustment with paper assortment and the sensing layer properties could allow for a successful serum sample analysis. However, at the same time the application of the system for other kinds of samples would be limited.

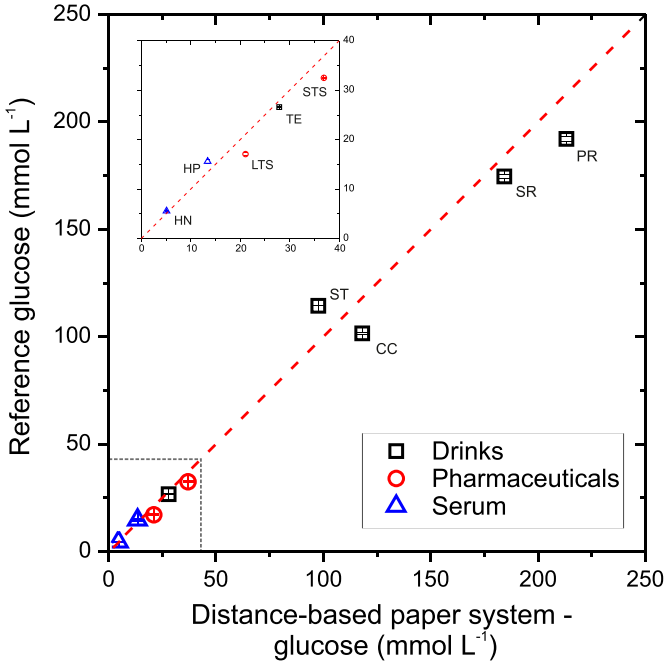

Fig. 6. Glucose determination in real samples. HP – pathological human serum standard (Cormay, declared glucose (DG): 15.1 mmol L^{-1}); HN – physiological human serum standard (Cormay, DG: 5.30 mmol L^{-1}); CC – Coca-Cola, ST – Schweppes Tonic, SR – Schweppes Russian, TE – Instant tea, PR – Powerade Lemon, LTS – Litorsal, STS – Strepsils.

4. Conclusions

This work demonstrates a paper system that utilize distance-based detection methodology with the novel approach of application of the chemosensitive dye layer in the flow channel of such a system. The development of a user-friendly, instrument-free and insusceptible for user impressions platform dedicated to the recognition of the target analytes might be a mile step towards common personalized healthcare or exposure monitoring. Test described here uses optical detection motif, which does not require any external auxillary devices for signal readout. For this reason, it is a perfect solution for fast, portable and disposable tests. Thanks to the modification with Prussian Blue or Prussian White, we were able to construct flow tests sensitive to reducing and oxidizing agents, respectively. As an analytical signal, the size of the colorized zone was measured and referred to analyte concentration.

In our research, ascorbic acid and hydrogen peroxide were used as a model analytes, used to confirm the performance of the systems. With manipulation of the dye amount deposited in the flow channel, we were able to alter the dynamic response range and systems sensitivity. Recorded calibration curves show desired linearity with excellent determination coefficient above 0.98. Moreover, single data points were described with satisfactory reproducibility and uncertainty with RSD below 6%. Tests covered with Prussian Blue were evaluated in real-life scenario of the determination of ascorbic acid in drugs and dietary supplements. Highly selective response of PB to ascorbic acid ensures results fully correlated to the manufacturer data and competitive to the reference method (Perasons' $R > 0.99$).

Successful biomodification of the H_2O_2-sensitive tests with glucose oxidase led to formation of effective glucose biosensors. Evaluation of the immobilization methodology and optimization of enzyme activity allowed for examination of the bio-systems in the determination of glucose levels in popular drinks, model human serum, and dietary supplements. Glucose-sensitive systems show linear response in 18–180 mg dL^{-1} range with 19.8 mg dL^{-1} LOD. These parameters confirm glucose-sensitive systems suitability for the determination of glucose in human serum/blood. Based on the results, further integration of the systems sensitive to hydrogen peroxide with enzymes will guide for the development of the full range of specified biosensors suitable to quantify more complicated analytes selectively or simultaneously via cascade enzymatic reactions.

CRediT authorship contribution statement

Mateusz Granica: Conceptualization, Methodology, Validation, Writing - original draft. **Łukasz Tymecki:** Writing - review & editing, Visualization, Supervision.

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

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References

[1] A.W. Martinez, S.T. Phillips, M.J. Butte, G.M. Whitesides, Patterned paper as a platform for inexpensive, low-volume, portable bioassays, Angew. Chemie - Int. Ed. 46 (2007) 1318–1320, https://doi.org/10.1002/anie.200603817.
[2] C. Carrell, A. Kava, M. Nguyen, R. Menger, Z. Munshi, Z. Call, M. Nussbaum, C. Henry, Beyond the lateral flow assay: a review of paper-based microfluidics, Microelectron. Eng. 206 (2019) 45–54, https://doi.org/10.1016/j.mee.2018.12.002.
[3] E. Carrilho, A.W. Martinez, G.M. Whitesides, A simple micropatterning process for paper-based microfluidics, Anal. Chem. 81 (2009) 7091–7095.
[4] K. Yamada, H. Shibata, K. Suzuki, D. Citterio, Toward practical application of paper-based microfluidics for medical diagnostics: state-of-the-art and challenges, Lab Chip 17 (2017) 1206–1249, https://doi.org/10.1039/c6lc01577h.
[5] G.G. Morbioli, T. Mazzu-Nascimento, A.M. Stockton, E. Carrilho, Technical aspects and challenges of colorimetric detection with microfluidic paper-based analytical devices (μPADs) - a review, Anal. Chim. Acta 970 (2017) 1–22, https://doi.org/10.1016/j.aca.2017.03.037.
[6] D.D. Liana, B. Raguse, J. Justin Gooding, E. Chow, Recent advances in paper-based sensors, Sensors 12 (2012) 11505–11526, https://doi.org/10.3390/s120911505.
[7] M. Trojanowicz, K. Kołacińska, Recent advances in flow injection analysis, Analyst 141 (2016) 2085–2139, https://doi.org/10.1039/c5an02522b.
[8] C.-S. Lee, K.-S. Park, S.-G. Jeong, S.H. Jin, J. Kim, Flow control in paper-based microfluidic device for automatic multistep assays: a focused minireview, Korean J. Chem. Eng. 33 (2016) 2761–2770, https://doi.org/10.1007/s11814-016-0161-z.
[9] E.K. Sackmann, A.L. Fulton, D.J. Beebe, The present and future role of microfluidics in biomedical research, Nature 507 (2014) 181–189, https://doi.org/10.1038/nature13118.
[10] E. Samiei, M. Tabrizian, M. Hoorfar, A review of digital microfluidics as portable platforms for lab-on a-chip applications, Lab Chip 16 (2016) 2376–2396, https://doi.org/10.1039/c6lc00387g.
[11] B. Ozdil, S. Onal, T. Oruc, D. Pesen Okvur, Fabrication of 3D controlled in vitro microenvironments, MethodsX 1 (2014) 60–66, https://doi.org/10.1016/j.mex.2014.06.003.
[12] P.M.D. Real-time, O. Diagnosis, A.W. Martinez, S.T. Phillips, E. Carrilho, S.W. T III, H. Sindi, G.M. Whitesides, Simple telemedicine for developing Regions : camera phones and simple telemedicine for developing Regions : camera phones and paper-based microfluidic devices for real-time , off-site diagnosis, Anal. Chem. 80 (2008) 3699–3707, https://doi.org/10.1021/ac800112r.
[13] W. Dungchai, O. Chailapakul, C.S. Henry, A low-cost, simple, and rapid fabrication method for paper-based microfluidics using wax screen-printing, Analyst 136 (2011) 77–82, https://doi.org/10.1039/c0an00406e.
[14] A.K. Yetisen, M.S. Akram, C.R. Lowe, Paper-based microfluidic point-of-care diagnostic devices, Lab Chip 13 (2013) 2210–2251, https://doi.org/10.1039/c3lc50169h.
[15] R. Gerbers, W. Foellscher, H. Chen, C. Anagnostopoulos, M. Faghri, A new paper-based platform technology for point-of-care diagnostics, Lab Chip 14 (2014) 4042–4049, https://doi.org/10.1039/c4lc00786g.
[16] Y. He, W. Bin Wu, J.Z. Fu, Rapid fabrication of paper-based microfluidic analytical devices with desktop stereolithography 3D printer, RSC Adv. 5 (2015) 2694–2701, https://doi.org/10.1039/c4ra12165a.
[17] L. Ouyang, C. Wang, F. Du, T. Zheng, H. Liang, Electrochromatographic separations of multi-component metal complexes on a microfluidic paper-based device with a simplified photolithography, RSC Adv. 4 (2014) 1093–1101, https://doi.org/10.1039/c3ra43625j.
[18] D. Barona, A. Amirfazli, Producing a superhydrophobic paper and altering its repellency through ink-jet printing, Lab Chip 11 (2011) 936–940, https://doi.org/10.1039/c0lc00335b.
[19] K.M. Schilling, A.L. Lepore, J.A. Kurian, A.W. Martinez, Fully enclosed microfluidic paper-based analytical devices, Anal. Chem. 84 (2012) 1579–1585, https://doi.org/10.1021/ac202837s.
[20] T. Akyazi, L. Basabe-Desmonts, F. Benito-Lopez, Review on microfluidic paper-based analytical devices towards commercialisation, Anal. Chim. Acta 1001 (2018) 1–17, https://doi.org/10.1016/j.aca.2017.11.010.
[21] J. Adkins, K. Boehle, C. Henry, Electrochemical paper-based microfluidic devices, Electrophoresis 36 (2015) 1811–1824, https://doi.org/10.1002/elps.201500084.
[22] H. Gai, Y. Li, E.S. Yeung, Optical detection systems on microfluidic chips, in: B. Lin (Ed.), Microfluid. Technol. Appl., Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, pp. 171–201, https://doi.org/10.1007/128_2011_144.
[23] K. Choi, J.M. Mudrik, A.R. Wheeler, A guiding light: spectroscopy on digital microfluidic devices using in-plane optical fibre waveguides, Anal. Bioanal. Chem. 407 (2015) 7467–7475, https://doi.org/10.1007/s00216-015-8913-x.
[24] K.B. Mogensen, J.P. Kutter, Optical detection in microfluidic systems, Electrophoresis 30 (2009) 92–100, https://doi.org/10.1002/elps.200900101.
[25] M. Fiedoruk-Pogrebniak, M. Granica, R. Koncki, Compact detectors made of paired LEDs for photometric and fluorometric measurements on paper, Talanta 178 (2018) 31–36, https://doi.org/10.1016/j.talanta.2017.08.091.
[26] M. Granica, M. Fiedoruk-Pogrebniak, R. Koncki, Ł. Tymecki, Flow injection analysis in lab-on-paper format, Sensor. Actuator. B Chem. 257 (2018), https://doi.org/10.1016/j.snb.2017.10.088.
[27] R.F. Zuk, V.K. Ginsberg, T. Houts, J. Rabbie, H. Merrick, E.F. Ullman, M.M. Fischer, C.C. Sizto, S.N. Stiso, D.J. Litman, Enzyme immunochromatography–a quantitative immunoassay requiring no instrumentation, Clin. Chem. 31 (1985) 1144–1150.
[28] J. Il Hong, B.Y. Chang, Development of the smartphone-based colorimetry for multi-analyte sensing arrays, Lab Chip 14 (2014) 1725–1732, https://doi.org/10.1039/c3lc51451j.
[29] K. Yamada, T.G. Henares, K. Suzuki, D. Citterio, Distance-based tear lactoferrin assay on microfluidic paper device using interfacial interactions on surface-modified cellulose, ACS Appl. Mater. Interfaces 7 (2015) 24864–24875, https://doi.org/10.1021/acsami.5b08124.
[30] M. Rahbar, B. Paull, M. Macka, Instrument-free argentometric determination of chloride via trapezoidal distance-based microfluidic paper devices, Anal. Chim. Acta 1063 (2019) 1–8, https://doi.org/10.1016/J.ACA.2019.02.048. https://www.sciencedirect.com/science/article/abs/pii/S0003267019302375?via%3Dihub.
[31] Y. Chen, W. Chu, W. Liu, X. Guo, Distance-based carcinoembryonic antigen assay on microfluidic paper immunodevice, Sensor. Actuator. B Chem. 260 (2018) 452–459, https://doi.org/10.1016/j.snb.2017.12.197.
[32] D.M. Cate, S.D. Noblitt, J. Volckens, C.S. Henry, Multiplexed paper analytical device for quantification of metals using distance-based detection, Lab Chip 15 (2015) 2808–2818, https://doi.org/10.1039/c5lc00364d.
[33] H. Shibata, Y. Hiruta, D. Citterio, Fully inkjet-printed distance-based paper microfluidic devices for colorimetric calcium determination using ion-selective optodes, Analyst (2018) 1178–1186, https://doi.org/10.1039/c8an02146e.
[34] J.C. Hofstetter, J.B. Wydallis, G. Neymark, T.H. Reilly, J. Harrington, C.S. Henry, Quantitative colorimetric paper analytical devices based on radial distance measurements for aqueous metal determination, Analyst 143 (2018) 3085–3090, https://doi.org/10.1039/c8an00632f.
[35] R. Koncki, O.S. Wolfbeis, Optical chemical sensing based on thin films of Prussian Blue, Sensor. Actuator. B Chem. 51 (1998) 355–358, https://doi.org/10.1016/S0925-4005(98)00287-1.
[36] R. Koncki, T. Lenarczuk, S. Głab, Disposable integrated cuvette test for quantitative determination of vitamin C in pharmaceutical products, Anal. Chim. Acta 379 (1999) 69–74, https://doi.org/10.1016/S0003-2670(98)00653-9.
[37] R. Koncki, T. Lenarczuk, S. Głab, Optical sensing schemes for Prussian Blue/Prussian White film system, Anal. Chim. Acta 424 (2000) 27–35, https://doi.org/10.1016/S0003-2670(00)01144-2.
[38] T. Lenarczuk, D. Wencel, S. Głab, R. Koncki, Prussian blue-based optical glucose biosensor in flow-injection analysis, Anal. Chim. Acta 447 (2001) 23–32, https://doi.org/10.1016/S0003-2670(01)01289-2.
[39] R. Koncki, S. Wolfbeis, Otto, composite films of prussian blue and N-substituted Polypyrroles : fabrication and, scan, Electron. Microsc. 70 (1998) 2544–2550.
[40] M. Pokrzywnicka, D.J. Cocovi-Solberg, M. Miró, V. Cerdà, R. Koncki, Ł. Tymecki, Miniaturized optical chemosensor for flow-based assays, Anal. Bioanal. Chem. 399 (2011) 1381–1387, https://doi.org/10.1007/s00216-010-4384-2.
[41] S.A. Margolis, D.L. Duewer, Measurement of ascorbic acid in human plasma and serum: stability, intralaboratory repeatability, and interlaboratory reproducibility, Clin. Chem. 42 (1996) 1257–1262.
[42] A.F. Danet, A.M. Pisoschi, S. Kalinowski, Ascorbic acid determination in commercial fruit juice samples by cyclic voltammetry, J. Autom. Methods Manag. Chem. 2008 (2008), https://doi.org/10.1155/2008/937651.

[43] D.M. Cate, W. Dungchai, J.C. Cunningham, J. Volckens, C.S. Henry, Simple, distance-based measurement for paper analytical devices, Lab Chip 13 (2013) 2397–2404, https://doi.org/10.1039/c3lc50072a.
[44] X. Wei, T. Tian, S. Jia, Z. Zhu, Y. Ma, J. Sun, Z. Lin, C.J. Yang, Microfluidic distance readout sweet hydrogel integrated paper-based analytical device (μDiSH-PAD) for visual quantitative point-of-care testing, Anal. Chem. 88 (2016) 2345–2352, https://doi.org/10.1021/acs.analchem.5b04294.