Paper card-like electrochemical platform as a smart point-of-care device for reagent-free glucose measurement in tears

*Luca Fiore, Ankita Sinha, Narjiss Seddaoui, Jessica Di Biasio, Federico Ricci, Goran M. Stojanovic, Fabiana Arduini**

Electronic Supplementary Information

1. Experimental methods

1.1 Reagents and instrumentations

Glucose, glucose oxidase, ammonium bicarbonate, potassium bicarbonate, histamine, peroxidase, IgG, sodium citrate, lysozyme, urea, sialic acid, potassium chloride, phosphoric acid, hydrogen peroxide 30% (w/w), N, N-dimethylformamide were purchased from Sigma Aldrich (St. Louis, MO, USA). Carbon Black N220 was kindly gifted by Cabot, Ravenna, Italy. Carbon black/Prussian blue nanoparticles (CB/PBNPs) powder was synthesized by using $K_3Fe(CN)_6$ and HCl obtained from Sigma and $FeCl_3$ from Fluka. A standard ISO A4 polyvinyl chloride (PVC) lamination sheets of thickness 80 µm (MBL® 80MIC Minoan Binding Laminating doo, Belgrade, Serbia) was used as a base and protective layer of the three-electrode device set-up. For the central electrode layers, gold (Au) leaf (22 carats, purity 91%, >10µm), silver (Ag) leaf (purity 99%, >32µm), and copper (Cu) foil (thickness 70 µm) were obtained from NRR, Germany. Kapton polyimide substrate (thickness 75 µm) was purchased from Minoan Binding Laminating doo, Belgrade, Serbia. PVC and conductive foils were cut using a cutter plotter (CE6000-60 PLUS, Graphtec America, Inc., USA) with a 45 °C cutting blade (CB09U) by xurography method. Bonding of different components of the device was performed using hot lamination with an A4 card laminator (FG320, Minoan Binding Laminating doo, Serbia). The Au, Ag, and Cu foils served as working (WE), reference (RE), and counter electrodes (CE), respectively, and connected externally to the electrochemical interface to operate the electrochemical measurements. Cyclic voltammetry and chronoamperometry were carried out using a portable potentiostat, PalmSens4 (PalmSens, Netherlands), in connection with a laptop. The overall design of the card-like platform was created in AutoCad (Autodesk 2021, USA). The paper card was produced with a ColorQube 8580 Xerox printer (Xerox Corporation, USA) in order to print the wax patterns.

1.2 Polyvinyl chloride electrochemical system production

The card-like platform consists of a three-electrodes system (Figure S1). To produce this device, the first step was to prepare the base and protective layers with PVC sheets. The PVC foil contains thermal glue on one of its sides, whose role was to glue two layers of foils during lamination. It was necessary to place the PVC foils so that the side on which there is no glue was in contact with the previously placed double-sided adhesive tape on the Kapton film. By using this procedure, the layer of glue on the PVC sheet was preserved, and the transparency of the sheet layers was ensured. In the next step, the conductive foils were laminated between the prepared base and protective layers of PVC sheets. The overall device set-up can be seen in Fig. S1 produced using a cutter plotter and hot card laminator under optimized parameters (Fig. S2).

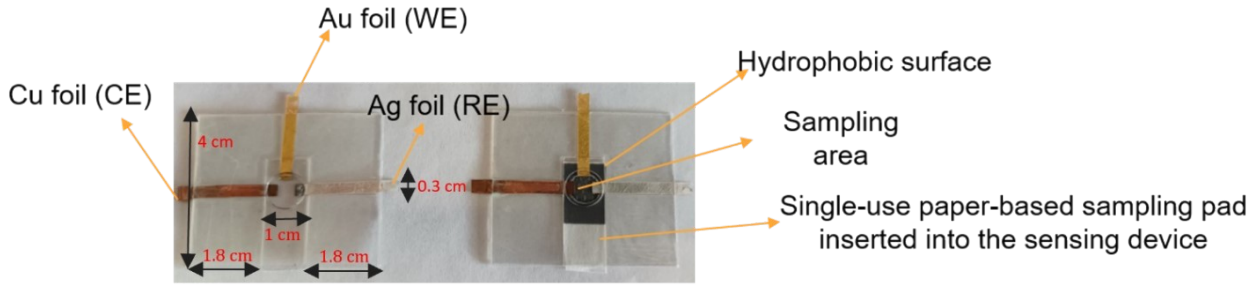

Figure S1. Photo of paper card-like pad integrated into polyvinyl chloride electrochemical system.

A B

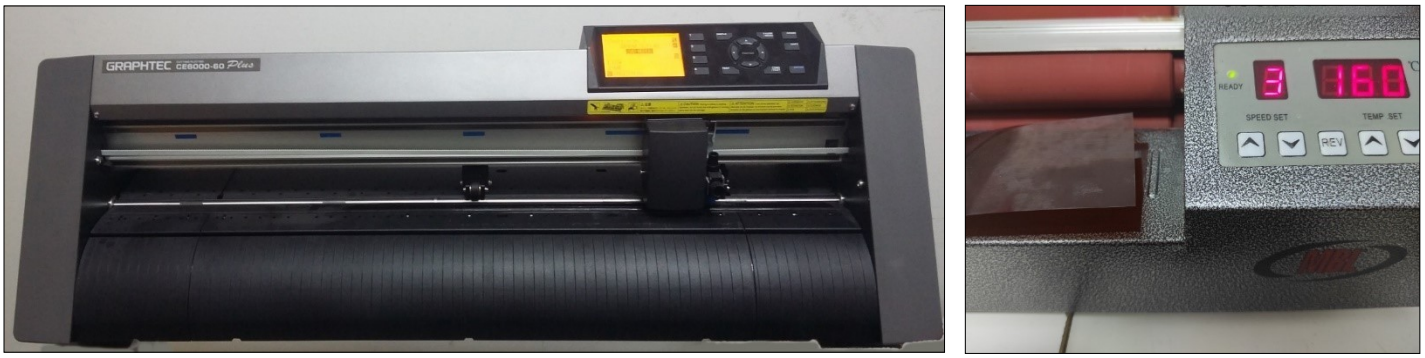

Figure S2. A) Photo of the cutter plotter (cutting speed 3 cm/s and cutting force for PVC foil 19 (out of 38 steps from 0.2 to 4.41 N). B) Display of laminator and values of speed (3 cm/min) and temperature (160 °C) parameters.

1.3 Paper card-like pad modification

The paper card-like pad is characterized by a dimension of 30 mm length × 13 mm width, while the diameter of the hydrophilic zone is equal to 7 mm. For modification via drop-casting of paper card-like pad, a 20 µL-drop of CB/PBNPs dispersion 1 mg/mL, 8 µL of glucose oxidase 15 U/mL, and 10 µL of phosphate buffer solution 10 mM, pH 7.4 were cast onto the hydrophilic zone of the paper. The CB/PBNPs nanocomposite was synthesized according to our previous work[25] and used for the preparation of a 1 mg/mL dispersion in a mixture of N,N-dimethylformamide and distilled water 1:1 (v/v) as the solvent, followed by sonication for 60 min at 59 kHz.

1.4 Glucose detection in standard solution and artificial tears

The chronoamperometric technique was used for glucose detection by connecting paper card-like device to potentiostat (PalmSens4, PalmSens, The Netherlands). Chronoamperometry measurements were performed by dropping 80 µL of standard solution at different concentrations of glucose onto the electrochemical cell and applying a potential of −0.05 V vs. Ag/AgCl pseudoreference for 60 s. The obtained current intensity was proportional to the amount of glucose in the standard solution. Between each measurement, the paper card-like pad is removed and the system is washed with 100 µL of distilled water to remove any remaining reagents. Glucose detection in artificial tears was carried out by analyzing artificial tear solutions containing different amounts of glucose.

2. Results & Discussion

2.1 Characterization of paper card-like pad integrated into polyvinyl chloride electrochemical system

To determine the electrochemical performance of different paper card-like devices, initial voltammetric measurements were carried. The polyvinyl chloride electrochemical system consists of three electrodes placed on PVC support, each produced with different materials using silver (Ag), aluminum (Al), and gold (Au) foils (Fig. S3).

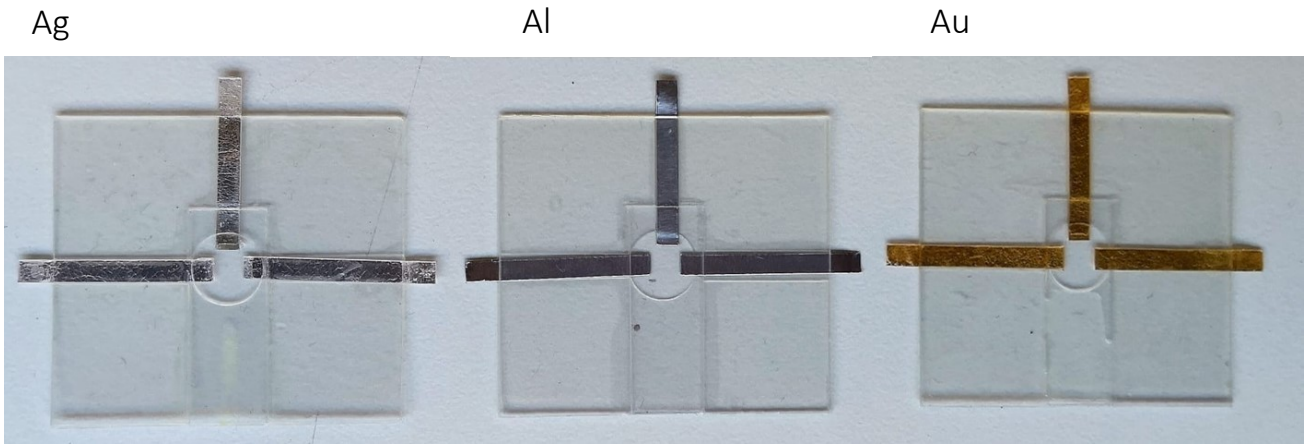

Figure S3. Three-electrode system produced with different materials.

The electrochemical technique used to carry out the measurements is cyclic voltammetry, using the following parameters:

- E begin -0.4 V
- E end 0.6 V
- E step 0.01 V
- Scan rate 0.05 V/s

- Number of scans 2

The measurements were carried out by inserting a piece of filter paper inside the three-electrode system and then wetting the paper with 140 µL of the solution to be analyzed. Two cyclic voltammograms were performed by analyzing 0.1 M KCl solution and a solution of potassium ferro-ferricyanide (5 mM) in 0.1 M KCl for each three-electrode system, as shown in Fig. S4.

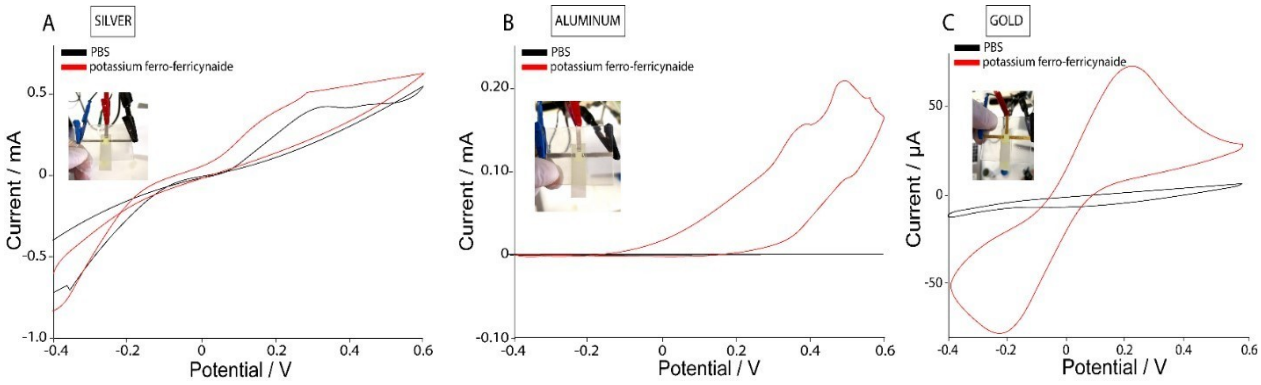

Figure S4. A) Cyclic voltammograms obtained by analyzing 140 µL of 0.1 M KCl solution (in black) and 140 µL of 5 mM potassium ferro-ferricyanide in 0.1 M KCl (in red), using silver three-electrodes system. B) Cyclic voltammograms obtained by analyzing 140 µL of 0.1 M KCl solution (in black) and 140 µL of 5 mM potassium ferro-ferricyanide in 0.1 M KCl (in red), using aluminum three- electrodes system. C) Cyclic voltammograms obtained by analyzing 140 µL of 0.1 M KCl solution (in black) and 140 µL of 5 mM potassium ferro-ferricyanide in 0.1 M KCl (in red), using gold three-electrodes system.

The results obtained show that it is possible to observe the classic oxidation and reduction peaks that are characteristic of potassium ferro-ferricyanide electrochemical probe, using only the system consisting of three gold electrodes, obtaining better electrochemical response in terms of anodic peak intensity and peak-to-peak separation. These initial measurements led to developing a low-cost paper card-like three-electrode device, consisting of one gold (working electrode), one silver (reference electrode), and one copper electrode (counter electrode) which was characterized and analyzed in this work for glucose monitoring in tear fluid.

The electrochemical behaviour of the prepared paper card-like device was evaluated in the presence of ferro-ferricyanide redox probe by applying different scan rates as shown in Figure S5A. Regarding Figure S5B, it represents the cyclic voltammogram of CB-PBNPs preloaded on the paper card-like pad by measuring phosphate buffer solution.

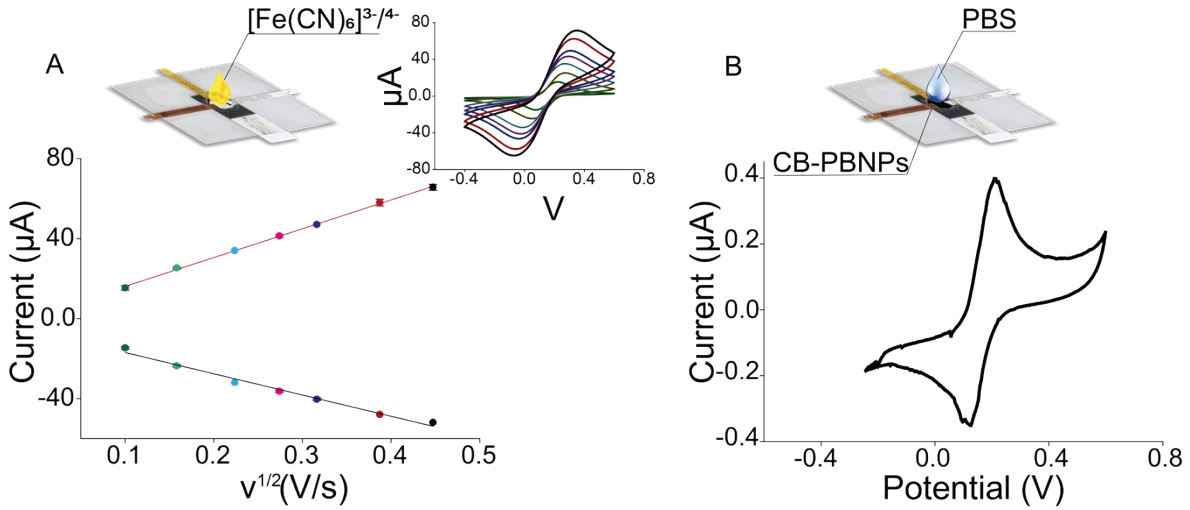

Figure S5. (A) Linear fitting of anodic (red line) and cathodic (black line) currents at different scan rates using the paper card-like pad inserted into the PVC-based electrochemical cell. The paper card-like pad was loaded

with 80 µL of 5 mM ferro/ferricyanide solution in 0.1 M KCl. Inset: Cyclic voltammograms at different scan rates. (B) Cyclic voltammograms obtained at a scan rate of 50 mV s^{-1} using the paper-card modified with carbon black-Prussian blue nanoparticles (CB-PBNPs) and inserted into the PVC-based electrochemical cell. The measurement was carried out by adding 80 µL of 0.05 M phosphate buffer solution + 0.1 M KCl, pH = 7.4.

2.2 Optimization of paper card-like integrated into polyvinyl chloride electrochemical system

The optimization of the amount of CB-PBNPs to be loaded on the paper card was investigated by dropping different volumes of the dispersion, i.e. 10, 15, 20, 25 µL, as shown in Fig. S6. Once loaded with CB-PBNPs, the paper card was dried at room temperature for 0, 2, and 30 min. The results obtained with different incubation times are depicted in Fig. S7. Furthermore, the concentration of GOx to be immobilized on the paper card-like pad was optimized as well, as reported in Fig. S8.

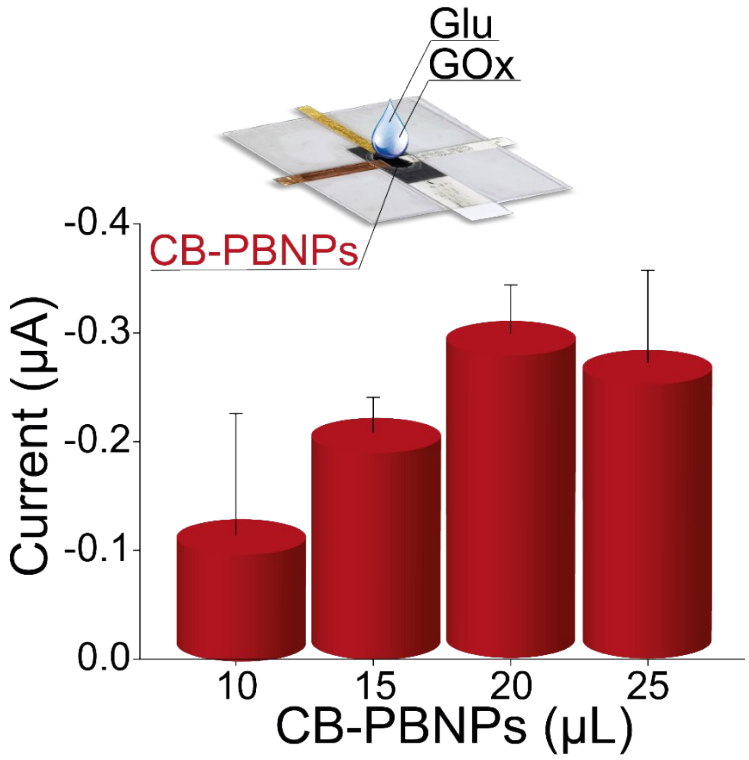

Figure S6. Optimization of the amount of CB-PBNPs to be loaded onto the paper card-like pad. The measurement was carried out at an applied potential of −0.05 V vs. Ag pseudoreference by adding 80 µL of PBS containing glucose at a concentration of 5 mM and GOx enzyme at a concentration of 15 U mL^{-1}.

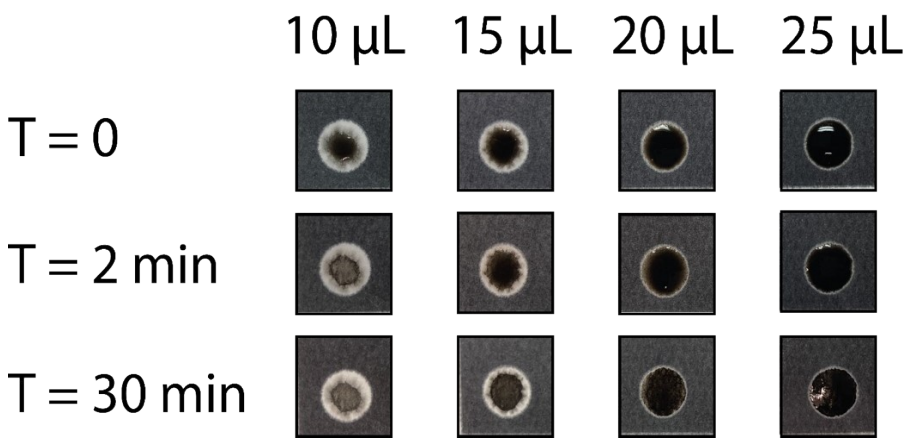

Figure S7. Photos of the paper card-like pad loaded with several volumes of CB-PBNPs.

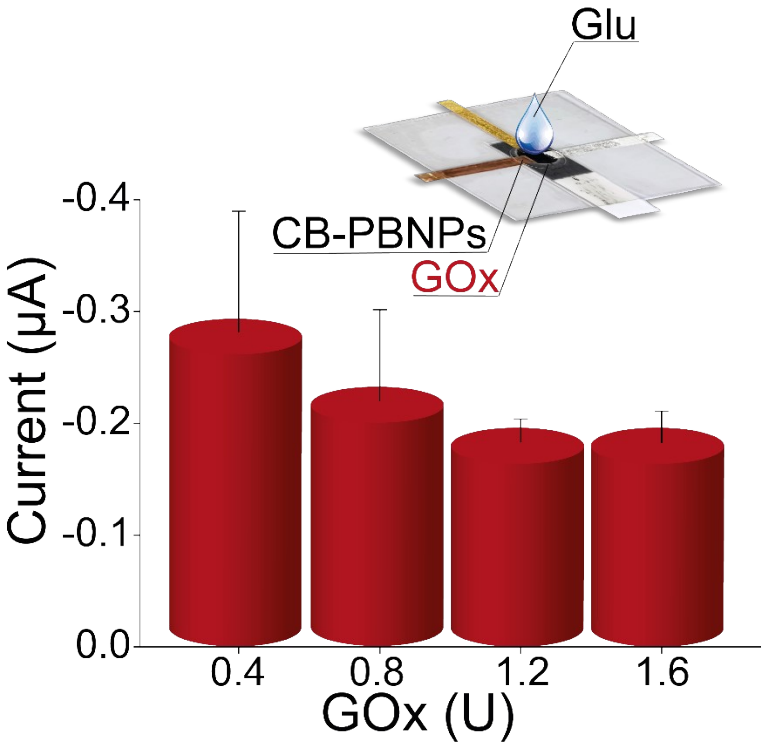

Figure S8. Optimization of the GOx enzyme concentration to immobilize on the paper card-like pad by testing 80 µL of glucose solution at a concentration of 5 mM.

2.3 Storage stability study

As observed from Fig. S9, the prepared GOx modified paper card-like pad retained more than 77% of its initial response after 7 days of storage at room temperature.

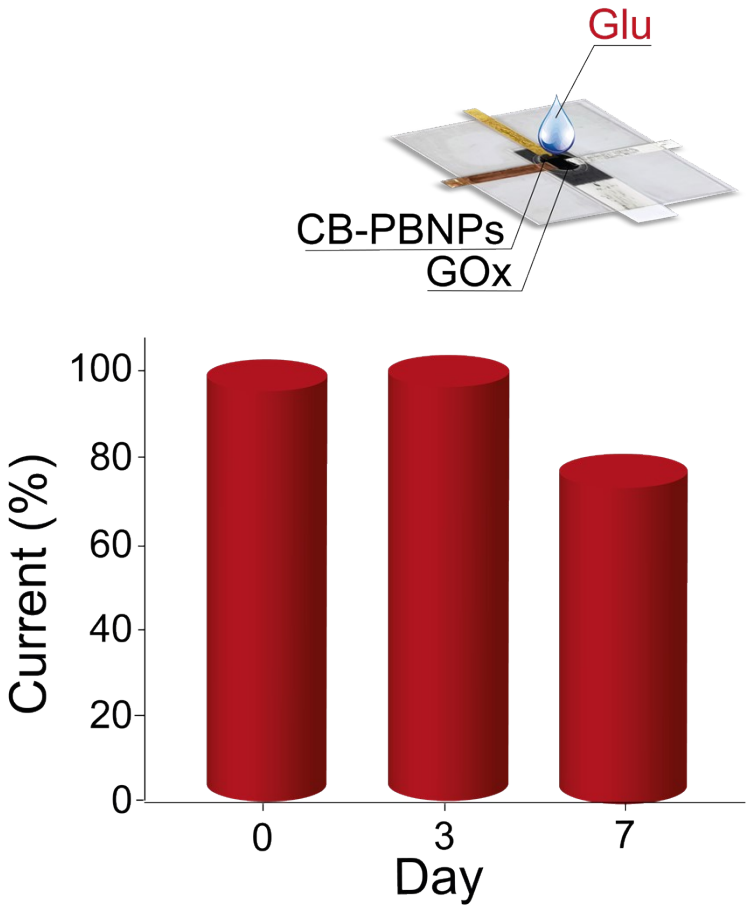

Figure S9. Storage stability of CB-PBNPs paper card-like pad modified with GOx and kept at room temperature for 7 days.

Table S1. Comparative table of different paper-based devices reported for glucose detection in tears.

Transduction	Configuration	Reagents	Enzymes immobilization	Layers number	sample volume (µL)	Assay time (sec)	Linear range	LOD	Application	Paper stability	Pros	Cons	Ref
Colorimetric	Microfluidic	TMB HRP GOx	Drop casting	6	~20 µL	30	0.025–2.5 mM	7 µM (Artificial tear samples)	Tear samples from adult volunteers	25 days (at -4°C)	-Wereable eye patch -Multiplex detection of four parameters	-Paper modification with oxidants and fixatives to reduce the interference with glucose detection -Cumbersome elaboration	[1]
Colorimetric	Microfluidic	4-AAP/DHBS/TMB HRP GOx	Chitosan	1	70 µL	900	4-AAP/DHBS: 1-10 mM TMB:1–5 mM	4-AAP/DHBS: 23 µM TMB: 57 µM (Glucose solutions)	Tear samples from nondiabetic subjects	Paper modified with chitosan :10 days (at 25 4°C)	-The presence of chitosan at the surface of the paper promotes the color intensity	-Assay not suitable for rapid on-site detection	[2]
Colorimetric	Microfluidic	TMB HRP GOx	Chitosan	2	5 µL	900	0.1–1 mM	50 µM (Glucose solutions)	Tear samples from nondiabetic subjects	Not studied	-Simple design -Easy fabrication technique	-Assay not suitable for rapid on-site detection	[3]
Colorimetric	Microfluidic	TMB HRP GOx	PVP	1	10 µL	300	0.1–1.2 mM	100 µM (Glucose solutions)	Tears range	Not studied	-Simple design -Easy fabrication technique	-Not tested in artificial tears nor in real tears	[4]
Colorimetric	Microfluidic	KI HRP GOx	PVA/ starch	1	25 µL	120	0.1–5 mM	100 µM (Glucose solutions)	Artificial tear	Not studied	-PVA can easily form a hydrogel that stabilizes	-The assay is sensitive to ambient air because of the	[5]

											enzymes and traps reagents	easy oxidation of iodine products	
Colorimetric	Schirmer strip	4-AAP+HBA HRP GOx	Drop casting	1	6 µL	300	0.1–2 mM	--	Tear samples from diabetic subjects	Not studied	Preconcentration of tears glucose	-Invasive method	[6]
Colorimetric	Microzone	TMB HRP GOx	Chitosan	2	3 µL	600	0.02–4 mM	14 µM (Glucose solutions)	Human tears	Not studied	-Chitosan offers better support for enzymes and reagents immobilization	- Assay not suitable for rapid on-site detection	[7]
Plasmonic colorimetric	Microzone	Gold nanoparticles (in- situ reduction) NaOH NaCl	--	1	5 µL	1200	--	290 µM (Glucose solutions)	Artificial tear	--	-Simple detection principle	-Other reducing molecules can interfere with gold nanoparticles reduction in the presence of glucose	[8]
Fluorescence	--	GQDs/PBA PVA Chitosan	--	3	400 µL	120	0.05–20 mM	2.1 µM (Glucose solutions)	Artificial tear	Not studied	-Highly sensitive	-Required specific instrumentation -Requires large volume of sample	[9]
Electrochemical	Microfluidic	Graphite Chitosan Polyethylene glycol Multiwalled carbon nanotubes,	Drop casting	2	5 µL	120	0.01–30 mM	5 µM (Glucose solutions)	Human tear samples from healthy adults	Not studied	-Highly sensitive -Requires less volume of tears to run	-Single use of the entire device -Cumbersome preparation	[10]

		Fe_3O_4 NPs GOx											
Electrochemical	Microzone	CB/PB GOx	Drop casting	1	80 µL	120	0.2–2 mM	50 µM (Artificial tears)	Basal and reflex tears	7 days (at room temperature)	-The PVC system can protect the preloaded paper from folding during the assay -The PVC system can be reused -The use of PBNPs instead of HRP reduced the cost of the assay	-80 µL of sample is required to cover the electrochemical cell	**Present work**

TMB: 3,3′,5,5′-tetramethylbenzidine; HRP: horseradish peroxidase; GOx: glucose oxidase; AAP: 4-aminoantipyrine; DHBS: sodium 3,5-dichloro-2-hydroxy-benzenesulfonate; PVP: Polyvinylpyrrolidone; PVA: Polyvinyl alcohol; KI: Potassium iodide; NaOH: Sodium hydroxide; NaCl: Sodium chloride; 4-AAP: 4-Aminoantipyrine; HBA: 4-Hydroxybenzoic acid; Fe3O4 NPs: Iron oxide nanoparticles; CB: Carbon black; PB: Prussian blue; GQDs: graphene quantum dots; PBA: phenyl boric acid.

References

[1] Xu, J., Tao, X., Liu, X., & Yang, L. (2022). Wearable eye patch biosensor for noninvasive and simultaneous detection of multiple biomarkers in human tears. Analytical Chemistry, 94(24), 8659-8667.

[2] Gabriel, E. F., Garcia, P. T., Cardoso, T. M., Lopes, F. M., Martins, F. T., & Coltro, W. K. (2016). Highly sensitive colorimetric detection of glucose and uric acid in biological fluids using chitosan-modified paper microfluidic devices. Analyst, 141(15), 4749-4756.

[3] Gabriel, E. F. M., Garcia, P. T., Lopes, F. M., & Coltro, W. K. T. (2017). Based colorimetric biosensor for tear glucose measurements. Micromachines, 8(4), 104.

[4] Allameh, S., & Rabbani, M. (2022). A distance-based microfluidic paper-based biosensor for glucose measurements in tear range. Applied Biochemistry and Biotechnology, 194(5), 2077-2092.

[5] Punnoy, P., Preechakasedkit, P., Aumnate, C., Rodthongkum, N., Potiyaraj, P., & Ruecha, N. (2021). Polyvinyl alcohol/starch modified cotton thread surface as a novel colorimetric glucose sensor. Materials Letters, 299, 130076.

[6] Kang, B. H., Park, M., & Jeong, K. H. (2017). Colorimetric Schirmer strip for tear glucose detection. BioChip Journal, 11, 294-299.

[7] Wang, X., Li, F., Cai, Z., Liu, K., Li, J., Zhang, B., & He, J. (2018). Sensitive colorimetric assay for uric acid and glucose detection based on multilayer-modified paper with smartphone as signal readout. Analytical and bioanalytical chemistry, 410, 2647-2655.

[8] Kim, H. J., Hyung, J., & Noh, H. (2020). Rationalization of in-situ synthesized plasmonic paper for colorimetric detection of glucose in ocular fluids. Chemosensors, 8(3), 81.

[9] Van Tam, T., Hur, S. H., Chung, J. S., & Choi, W. M. (2021). Novel paper-and fiber optic-based fluorescent sensor for glucose detection using aniline-functionalized graphene quantum dots. Sensors and Actuators B: Chemical, 329, 129250.

[10] Manbohi, A., & Ahmadi, S. H. (2020). Chitosan–Fe 3 O 4 nanoparticle enzymatic electrodes on paper as an efficient assay for glucose and uric acid detection in biological fluids. Chemical Papers, 74, 2675-2687.