Supporting information for
Ion-Exchange Based Immobilization of Chromogenic Reagents on Microfluidic Paper Analytical Devices

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1. Instrumentation

A wax printer (Colorqube 8870, Xerox, Norwalk, CT, USA) was used to print the microfluidic patterns upon the filter papers. A Silhouette CAMEO® 3 desktop craft plotter/cutter (Silhouette America®, Lindon, Utah, USA) along with the Mars® matic 700 technical pens (STAEDTLER Mars GmbH & Co. KG, Nuernberg, Germany, line width = 0.5 mm) were used for deposition of chemical reagents on the detection zones of the distance-based μPADs. Silhouette Studio® (V4.1.479) free software was used to design the μPADs patterns. A UV lamp (Spectrolin®e®, XX-15NF, USA) was used to observe the fluorescence emission developed upon the paper.

2. Design and Fabrication of μPADs

The μPADs were fabricated according to the previous reports with a few minor changes [1-3]. Briefly, the microfluidic patterns were designed using the Silhouette Studio® and wax printed upon the paper. The distance-based microfluidic design comprised of a circular sample zone (5 mm) and a straight detection channel (30 mm). Scale bars were also printed at 1 mm intervals next to the channels for naked-eye measurement of distance signals. For distance-based μPADs used in total calcium and acid measurements, the corresponding chemical reagents were deposited over the detection channels via the Cameo plotter. Finally, the paper was placed on a hot plate (130 °C- 5 min) to melt the wax and then laminated (80 °C) from both sides.
Figure S1. SEM images of filter papers. (A, B) Standard Whatman paper, grade 1. (C, D) DE81 anion-exchange filter paper.
Figure S2. Printability of IE paper. Circles were wax printed on both standard and IE paper and then after wax melting step (on a hot plate, 130 °C- 5 min), the line width was measured (using Dino-Lite digital microscope) which was found to be almost the same (~1.5 mm) for both types of papers, indicating similar behavior of papers toward the wax printing and melting processes. The wax printed circles on the IE paper were also able to confine the loaded liquids without any leakage.
Figure S3. Comparison of the flow rate between IE (▲) and standard (♦) paper. Water was loaded (10 μL) into the sample inlets of the distance-based μPADs and the sample flow through the microfluidic channels was recorded using a digital camera. Later the solvent front was monitored to measure the flow rate. The error bars represent the standard deviations from the average values. Insets are representative photographs of μPADs used in this experiment.
Figure S4. Determination of the basicity (pKa) of the IE paper. The pH indicators (bromothymol blue (pKa=7.1), phenol red (7.9), thymol blue (8.9), phenolphthalein (9.3), nile blue A (9.5), alizarin yellow (11.2)) were prepared as 0.1% w/v in 20% v/v ethanol. The pH of each solution was adjusted (using NaOH and HCl, 100 mM) to the pKa of that particular indicator in order to provide the highest possible sensitivity to the pH changes. In other words, the pH of the indicator solution was kept exactly on the border of the acidic and basic range, ready to react to the slight changes of pH. The solutions were pipetted (1.5 μL) into the wax-printed wells, and the color change was observed (scanned after 1 min).
Figure S5. Immobilization test continued. Fluorescein spot (1 μL- 0.1 mM) on IE paper strip was kept in water for 2h. No elution was observed and the spot was absolutely intact indicating the strong interaction force between the ionized dye molecule and the IE paper.
Figure S6. (A) Immobilization test using bromothymol blue (BTB- 0.1 μL- 0.1% w/v in ethanol 20% v/v) on IE μPAD devices. 

(i) Representative photographs of BTB spots on an IE-μPAD device after spotting and drying (left), after rinsing in water (3× 10 μL) (middle) and after rinsing in aqueous NaCl (200 mM, 1× 10 μL) (right). (ii) Representative photographs of BTB spots on a conventional μPAD device after spotting and drying (left) and after rinsing with water (1× 10 μL) (right). (iii) Representative photographs of basic form (pH adjusted to 9 using NaOH) of BTB on a conventional μPAD device after spotting and drying (left) and after rinsing with water (1× 10 μL) (right).  

(IV) The ionized structure of BTB comprising two anionic functional groups anchoring the dye on the positively charged IE paper. (B) IE paper strip were spotted with BTB and kept in water for 2h, indicating strong attraction force between the dye and the paper surface, without any elution happening.
Figure S7. Immobilization test using (A) Arsensazo III (1 mM in TBE buffer (5×)) (B) Calcein (0.1 mM in water) (C) 2,6-Dichlorophenolindophenol (DCPIP) sodium salt (0.1 mM in water) (D) Thymol blue (0.1% in ethanol 20% v/v) on IE μPAD devices. (i) Representative photographs of spots on an IE-μPAD device after spotting and drying (left), after rinsing in water (3× 10 μL) (right). (ii) Representative photographs of spots on a conventional μPAD device after spotting and drying (left) and after rinsing with water (1× 10 μL) (right). (iii) The ionized structure of dyes comprising anionic functional groups anchoring them on the positively charged IE paper.
Figure S8. Mechanism (top) and representative photographs (bottom) of total acid assay performed on IE paper. (A) Wax printed IE-μPADs. (B) Deprotonated bromothymol blue (BTB) (blue) is immobilized in the detection channel via its sulfonate and phenolic groups. (C) Acid containing sample is loaded and wicked from left to right; upon reaction with BTB, the dye protonates and appears blue.
Figure S9. Effect of sample zone removal on the distance signal obtained in the total acid assay (n= 5). The distance signal for the applied tartaric acid samples with two different concentrations (5 and 20 mM) on the IE-μPADs were A) 0 and 2.31±0.53 mm, sample inlets not cut out (B) 2.37±0.63 and 7.11±0.70 mm, sample inlets cut out. Removing the extra paper (titrant) in the sample zone is basically reducing the amount of titrant available on the IE-μPADs, resulting in longer distance signals (i.e. higher sensitivity).
Figure S10. Certificate of analysis of the sheep serum sample used in this work to demonstrate the performance of the IE-μPADs for distance-based determination of calcium.
Movie S-1. Immobilization test: Flushing fluorescein spot (1 μL) with water.

Movie S-2. Immobilization test: Flushing BTB spot (1 μL) with water.

References