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Utilization of functionalized paper-based microfluidic devices for the detection and characterization of starch in milk

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We pronounce a quick cost-effective, and one-of-a-kind single-step approach for fabricating paper-based devices by using correction pens instead of expensive materials. The highlighted areas were filled with Deposits from the correction pen and the surroundings were coated with wax, showing that will have a wide range of aqueous resistances. In the transverse direction, favorable natural convection occurs during the process along the axial direction of the fabricated paper channel. Due to its cost-effectiveness, the platform is believed to be ideally suited for chemical sensing and point-of-care applications, as well as diagnostics in resource-limited settings. We show that a lab-on-a-chip method based on paper can be used for colorimetric analysis to identify both qualitative and quantitative changes in milk caused by the presence of starch impurities. The challenges of sample storage, handling, and transport to the laboratory are circumvented by combining detection technology with smartphone imagery that permits on-site data collection. Diagnostic and sensing applications in low-resource settings, such as those seen in developing nations, can now use this technology.

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1. Introduction

Both the science and the technique of creating microminiaturized devices with chambers and tunnels through which fluids flow or are constrained are known as microfluidics. Microfluidics investigates the behavior of fluids through micro-channels. In microfluidics, fluid quantities as tiny as femtoliters, or one quadrillionth of a liter, are dealt with. On a micrometric scale, fluids behave quite differently from how they do in daily life [1]; these distinctive characteristics are crucial for new scientific research and inventions [2, 3]. The Whiteside's Research Group was the first to introduce paper-based microfluidic devices in 2007. They have created a hydrophobic patterned paper device using the SU-8 2010 photoresist. After that, the paper was spun, baked, UV-light exposed with a mask, and then baked again. This resulted in the formation of hydrophobic barriers within the hydrophilic paper substrate. Martinez et al. used a colorimetric detection for glucose and protein in urine to evaluate their device. The hydrophilic character of the paper pores and the structure of the hydrophobic barrier perpendicular to the desired transport direction are key factors for the development of paper-based analytical tools [4-7].

Photolithography, flexography printing, plasma treatment, cutting, and vapor phase deposition are just a few of the pricey procedures used to create hydrophobic barriers on paper. Ink-jet printing, wax printing, screen printing, wax dipping, PDMS printing, and stamping are only a few of the low-cost techniques that have been developed. High cost, extended manufacturing time, knowledge, and the need for external weaponry such as lasers, ovens, printers, and stamps are all common downsides of the aforementioned approaches. Diagnostic or identification of diseases utilizing body-fluid-based techniques may be delayed due to a lack of access to such devices in laboratories with limited resources [8-11]. To get around this issue, you can employ free-hand drawing, which even a beginner can use to construct paper-based devices. This research presents a

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quick, cheap, and easy proof-of-concept method for creating paper-based analytical instruments by utilizing a correction pen and wax coating (Figure 1). In the past, a correction pen was used to cover up faults in printed or written material. According to the literature, it's a combination of solvents, titanium dioxide, resins, and colorants. On paper, this combination is used as a barrier patterning agent, The correction fluid is directly deposited on filter paper by hand and paraffin wax is coated to form hydrophobic barriers (Figure 1). Surprisingly, no complicated instrumentation, or trained workers are required for this procedure [12].

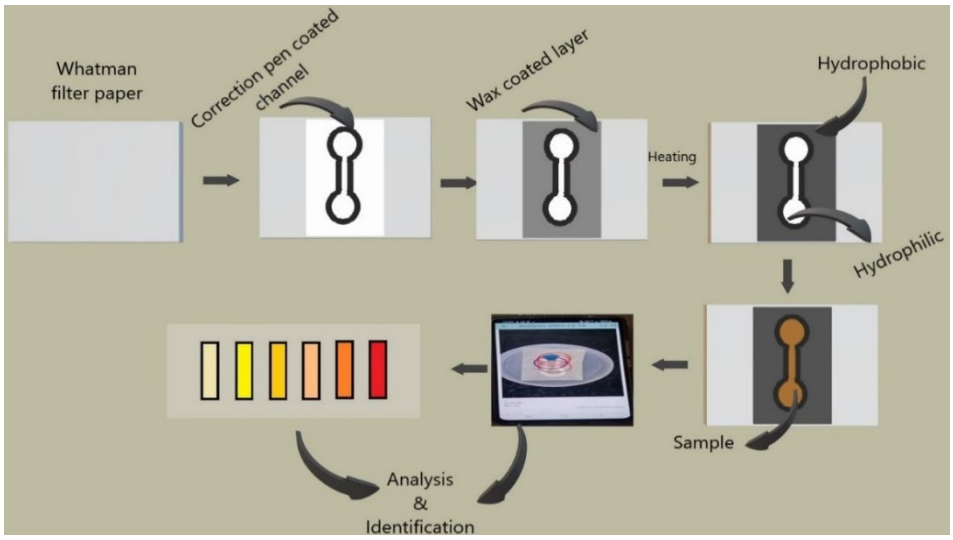
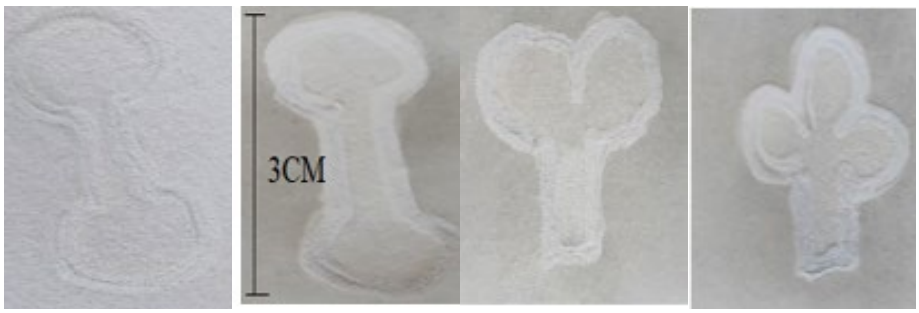


Fig. 1. Fabrication process of the device.

2. Fabrication of device

To begin, we made some different types of devices by directly patterning correction liquid from a pen on filter paper [13, 14]. After the solvent evaporated for around 10 minutes, we noticed that Titanium dioxide became lodged in the paper that covers the perforated holes, resulting in the formation of a film. After that, the paper was coated with the paraffin wax around the channel formed by a correction pen to create a hydrophobic barrier that can be seen. (Figure 2).

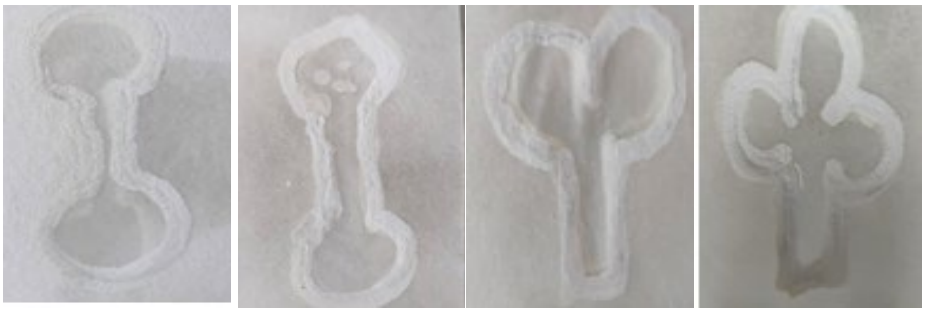


(a) *(b)* *(c)* *(d)*

Fig. 2. Fabrication of the device. (a) Channel without wax coat (b),(c) & (d) Channels with hydrophobic barrier using wax coat.

The hydrophobic barrier resistance will be confirmed by wetting the paper with water and various chemicals and coloring agents [15]. The hydrophobic barriers confined the colored water without disturbance or leakage when it was introduced to the constructed device. We designed our designs using freehand drawing and double-checked their water containment ability and we also tested the paper device's wicking ability (Figure 3). This approach has advantages such as reduced manufacturing time, one-time correction fluid deposition, and wax coating, and no reliance on highly expensive materials [16, 17]. Studying the generated barrier's resistance or inertness to various reagents is important before using our technology for biosensing or point-of-care diagnostics. We measured barrier resistance and fluid disruption using several distinct structural devices per reagent. It was intriguing to observe that at least 80% of the devices successfully confined the compounds with no issues.

We deduce from barrier compatibility tests that our construction approach has a high chance of restricting the majority of routinely used water solutions and chemical agents. The length of the channel was 10mm and the width of the channel was 4mm dimensions. Based on that the velocity of the water is measured as 0.19mm/s and the flow rate is calculated as 7.6 mm^3 / s.



(a) (b) (c) (d)

Fig. 3. Compatibility of barrier with water (a) Channel without a wax coat (b),(c) &(d) Channels with hydrophobic barrier using a wax coat.

The next solution tested was a methylene blue solution. methylene blue is a salt used as a dye and as a medication. Phenothiazine has a formal derivative called methylene blue. The powder is dark green, and when dissolved in water, it turns blue. Each methylene blue molecule in the hydrated state contains 3 molecules of water. Methylene blue solution was carried via the channels to exhibit channel resolution and barrier pattern straightness. A micro-syringe was used to inject methylene blue solution into the inlet of channels (Figure 4). The methylene blue solution was then conveyed along the patterns until it reached the channel's terminus. The color change in channels was seen using a CCD microscope. According to the channel length, the velocity of the methylene blue solution is measured as 0.13mm\s and the flow rate is calculated as 5.2mm^3\s for iodine solution the velocity is 0.104mm\s and the flow rate is measured as 4.1mm^3\s.

(a) (b) (c)

Fig. 4. Compatibility of barrier with (a) Methylene blue, (b) Iodine solution, and (c) Green dye. Channels with hydrophobic barrier using correction pen along with wax coat.

Numerous studies on the chemical compatibility of microfluidic devices with solvents and other chemicals have already been published [18]. A wide range of organic solvents have been used in various test analyses. In this work, the barrier compatibility of paper-based microfluidic devices with barrier materials solvents and surfactants has been studied. The chemical compatibility of the correction fluid was also tested with an iodine solution and a green dye solution. Research into the produced barrier's resistance to or inertness to diverse compounds is crucial if our technology is to be used for biosensing or point-of-care testing (Figure 4). In this scenario, the barrier quality was measured using more than five devices per reagent. It was interesting to see that at least 80% of the equipment accurately and error-free blended the reagents. The device's compatibility with different substances is shown in Figure 4. Our production technique has a significant chance of containing the majority of frequently used aqueous solutions and organic solvents, per a barrier compatibility test.

3. Characterization

The Whatman filter paper's coated and bare portions are depicted in the figure as SEM micrographs. The difference in surface morphology supports the theory that particles from the correction stylus are clogging pores. Energy Dispersive Spectroscopy was used to analyze the fundamental structure of the coated and uncoated surfaces, and the results showed that the coated surface had an abundance of titanium and the wax coat supports for efficient clogging pores(Figure 5). This finding is directly related to the three surfaces' different morphologies. Overall, the findings make it abundantly obvious that the ingredients in correction pens and wax coats obstruct pore openings and mask paper surfaces, leading to sample confinement [19]. Using contact angle measurements, we analyzed the wetting properties of coated and uncoated filter paper with acetone.

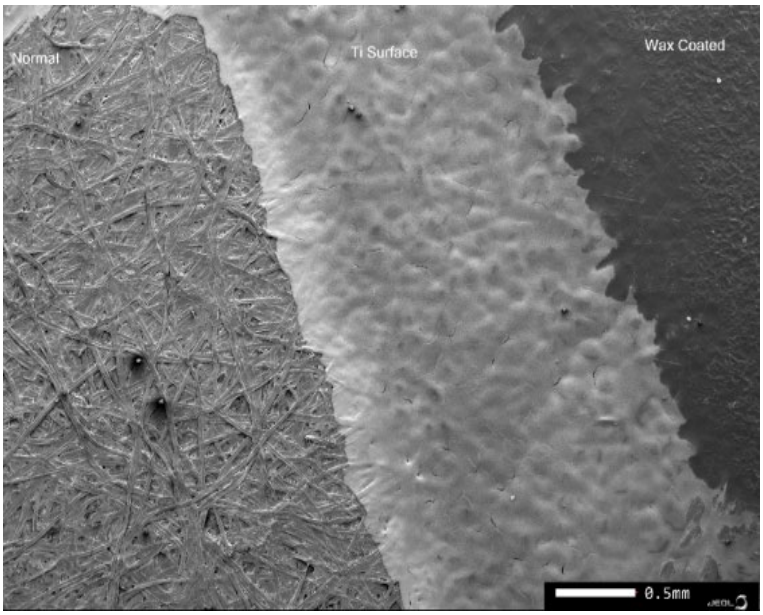


Fig. 5. SEM Image of whatman (R) cellulose filter paper; Coated and uncoated with the liquid of correction pen and wax.

The altered wettability of the surface is seen in Figure 6. The statistics show that the difference between the contact angles of coated and uncoated paper is 11° and 44°, respectively [20]. Paper with a correction pen or wax coating has a hydrophobic surface, as indicated by a high contact angle, while uncoated paper has a hydrophilic surface, as indicated by a low contact angle. Contact angle measurements for both surfaces are displayed graphically over time in Figure 8.

(a) *(b)*

Fig. 6. Contact angle measurement (a) Uncoated paper surface (b) Coated paper surface.

The surface of untreated paper is hydrophilic, but the surface of TiO2-coated and wax-coated paper is extremely hydrophobic. The organic solvents immediately evaporated after drawing on the surface, leaving just TiO2 particles securely bonded to form a compact thin layer. This coating procedure can be utilized to create intricate patterns on the bilayer's surface to induce any desired shape change in response to external stimuli. The preceding observation supports the employment of a simple titanium dioxide-based correction pen for the manufacture of hydrophobic barriers, which is compatible with our proposed method. Figure 7 shows the molecule of the Tio2 layer.

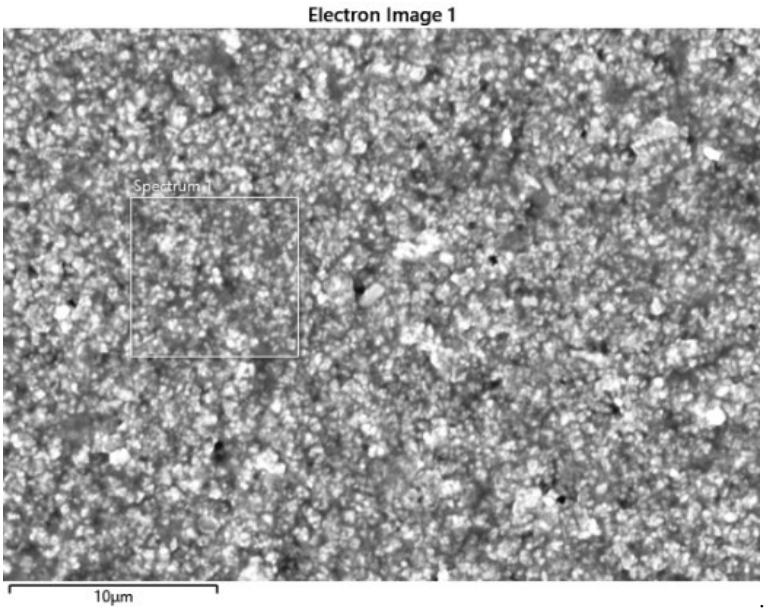


Fig. 7. Electron image of a coated layer.

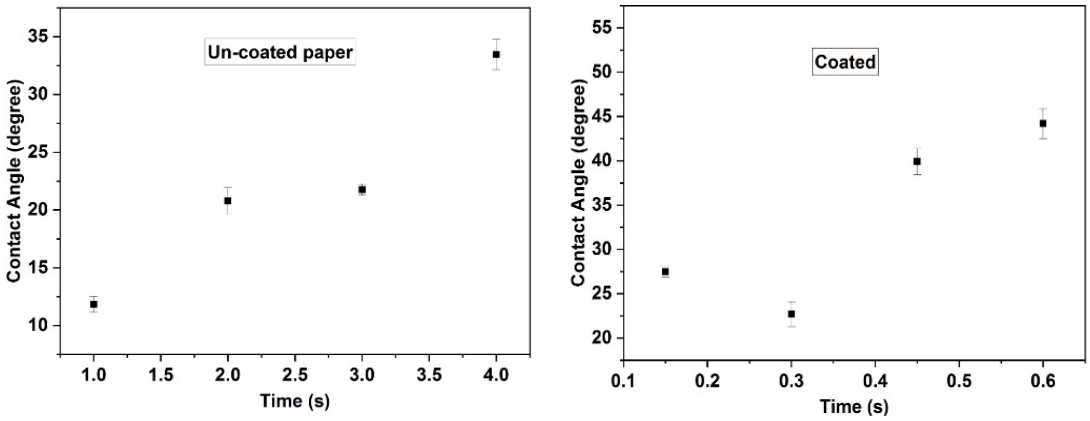


Fig. 8. Coated and uncoated paper contact angle tests were conducted. Contact angles were measured across a range of samples, and the average and standard deviation were determined.

4. Biochemical assays

Food safety authorities pay close attention to food adulteration since it endangers people's health and is a serious global issue. One of the most contaminated foods is milk, which is produced in developing nations like India, China, and Denmark, which account for around half of the world's total milk output. Milk is widely consumed since milk is a low-cost nutritious food high in protein, fat, carbs, vitamins, minerals, and so on Profitability in the dairy industry is ensured by the use of adulterants, which help to equalize supply and demand. Many factors, including fat content, Solid Not Fat value, protein concentration, and pH, contribute to a milk's overall quality. Adulterants are frequently added to milk to increase these properties. Milk's volume can be increased by adding water, and its density can be increased by adding sugar or starch. Milk with excessive levels of starch is said to cause diarrhea because it goes undigested. Additionally, increased quantities of starch buildup in the body could be dangerous for people with diabetes.

We chose an easy method of starch measurement using an iodine reagent. By varying the sample quantity and reagent concentration range, we studied the change in color intensityLong-term storage in low-resource areas always requires careful consideration of the durability of paper-based devices. The stored device showed no signs of leakage or barrier disruption. This suggests that the composition of the barrier prevents surface interaction, making it ideal for studies involving enzymes or chemicals. We also evaluated the platform's feasibility for developing a quantitative analysis using food samples [21].

In starch analysis, Store-bought milk served as the control sample [22]. For testing reasons, a second contaminated milk sample was created synthetically by liquifying a predetermined quantity of starch in milk. one side of the device was exposed to starch-based milk and the other end was exposed to an iodine solution, slowly we observed a color change based on a chemical reaction in the starch-based synthetic milk which turned dark [23, 24]. When iodine is added to amylose in starch, a dark blue hue is produced. Iodine is incorporated into the amylose molecule. Due to its low solubility in water, iodine reagent is made by dissolving iodine in water with potassium iodide, equation (1). This results in a strong blue-black color due to the formation of a soluble linear triiodide ion complex that glides into the coil of the starch. The color will remain orange or yellow if there is no amylose in the starch.

$$I_2 + I^- + amido \rightarrow amido - I^-_{3(aq)} \quad (1)$$

We have analyzed by varying the concentration of the maize starch with milk. We chose a wide range of concentrations of maize starch (10mg to 100mg) for this. A total of 20µl of the liquid sample was pipetted and carefully put into the detecting region's center, and any color differences were documented. From Figure 9(a) we can perceive the reaction of 20µl pure milk with 40 µl iodine solutions, before and after the reaction the color of the iodine solution remains the same. Then we

mixed various concentrations of mazine starch in 1ml milk to make synthetic milk. In Figure 9(c) we can observe the slide dark brown color result when the 40 µl iodine solution was mixed with 20 µl starch added to milk (10mg) and respectively we observed the color variations from Figure 9(d,e,f) with 25mg,60mg and 100mg concentration of maize starch. Images were taken with a smartphone and processed as indicated for additional quantitative analysis. In good lighting, the experiment photographs were taken using a mobile phone camera without a flash. The photographs were taken and processed inside a lab. The recorded images were loaded into ImageJ software on a computer, where they were analyzed afterward [25, 26]. Using built-in tools, the average color was determined, and a color picker tool was used to determine the standard red, green, and blue (RGB) values and recorded as a histogram.

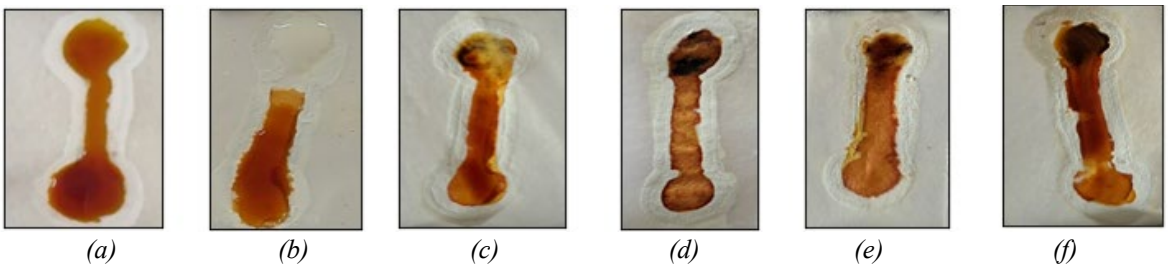


Fig. 9. Colorimetric assay (a) Pure milk with iodine solution (b) Iodine solution with starch mixed milk (c),(d),(e),(f) Iodine solution reactions with different concentration level starch mixed milk.

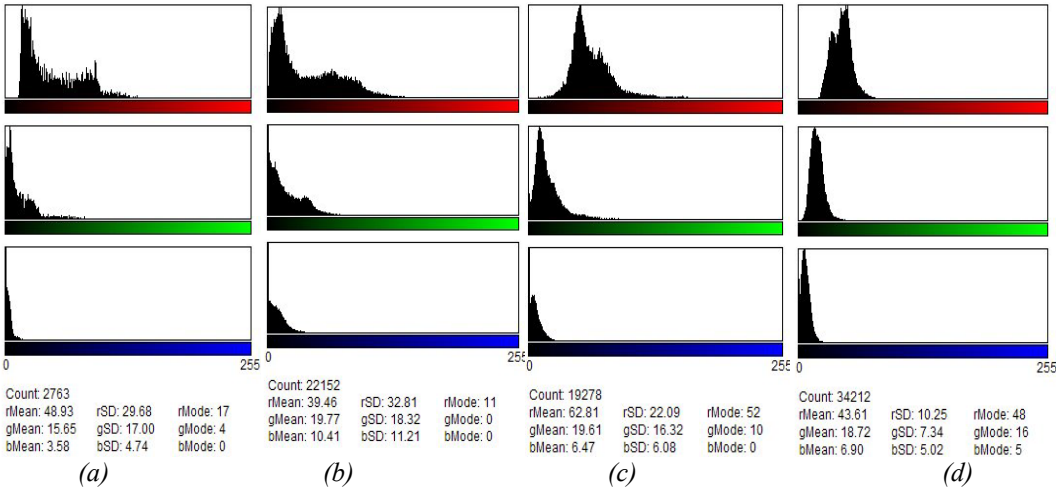


Fig. 10. (a-d): Color Histogram done by Image J software to show the different concentration levels of starch mixed in the milk.

The figure depicts the processed photos matching the experiment described above. Greyscale color intensity is computed by plugging in measured values for red, blue, and green into an equation (2).

$$GSI = 0.299 * R + 0.587 * G + 0.114 * B \quad (2)$$

GSI is the intensity of the grey scale, whereas R, G, and B are the intensities of the red, green, and blue color channels, respectively. Since white milk is a good starting point for estimating average grey levels [27]. Iodine solution causes milk to turn yellow and exhibit a slight increase in color intensity, whereas milk containing starch exhibits a notable increase in grey intensity as a result of the bluish-brown hue. This vital information can be used to determine the starch content. All of

the pictures that were taken throughout the calibration experiments underwent a similar processing. As can be observed in Figure 9, as concentration rose, the color intensity in the detecting wells rose as well. The RGB intensity levels as well as the average grey intensity values are shown in Figures 10(a-d). Whether due to artificial or natural sources, food contamination poses a major risk to the public's health. The most frequently reported milk impurity is starch, which needs to be controlled to avoid harmful health effects. Starch can be detected using a well-known test that involves the use of iodine. However, due to reagent requirements, handling, and safety considerations, it is normally performed in a chemical, biological, or food laboratory. The sample of milk being analyzed must be obtained in the field, transported in a controlled manner, and then analyzed in a laboratory setting. The time it takes to collect a sample and analyze it could cause the sample to undergo chemical changes that distort the results. The detection test method proposed here readily sidesteps such constraints on paper-based lab-on-a-chip devices. The platform's straightforward design makes it ideal for rapid construction and immediate deployment, with no special preparation or training required.

5. Methods and materials

Materials: We requested Grade 1 Whatman(R) cellulose filter paper from GE Life Sciences in India for our purifying processes. At an office supply store, we purchased correction pens from different brands, namely Camlin, Faber Castle, and Doms, each featuring distinct tip sizes. A variety of milk products were acquired from a nearby commercial dairy. Acetone, methylene blue, paraffin wax, iodine solution, and other chemical reagents were procured from Merck, a reputable supplier based in India.

Methods: The correction pen was delicately applied to release fluid onto the filter paper, with the recommendation of depositing it on both sides of the paper. The outer layer of the paper was treated with paraffin wax. The artificial apparatus was subjected to a curing process for a brief duration of time under ambient conditions. The analysis of the data was conducted using the Joel, SEMcenter software. The investigation of elemental composition was conducted using Dispersive Spectroscopy for Energy Dispersive Spectroscopy with Aztec software. The oscillator goniometer was utilized to ascertain the contact angles of the liquid.

5. Conclusions

Because of its advantages, including being affordable, compact, conventional, biodegradable, and chemically easy to modify, paper is progressively being used as a substrate for diagnostic assays. This research shows how to make hydrophilic channels and hydrophobic barriers in paper in a quick, low-cost, and repeatable manner. The described technology allows practically any research test center to manufacture microfluidic devices in paper for tests and analysis by depending upon simple tools and affordable materials for patterning. The technology paves the way for the creation of simple, low-cost, and portable diagnostic equipment that can be used in remote locations, health centers, and, in particular, less-developed countries where basic tests are critical for health monitoring and disease detection. It offers a low-cost, portable alternative to conventional diagnostic and detection technologies. Using lab-on-a-chip technology, a proof-of-concept application for detecting starch contamination in milk is presented. Other chemical and biomolecular entities can be detected using similar approaches.

Acknowledgments

We confirm that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the details and its interpretation, and agree to its submission.

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