Characterization of handmade Nepali paper as a platform for paper analytical device to determine anti-diabetic drug

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Abstract

The COVID-19 pandemic has highlighted the need of eco-friendly and locally or distributed manufacturing of diagnostic and safety products. Here, we characterized five handmade papers for their potential application to make paper analytical device (PADs). The handmade papers were made from locally available plant fiber using eco-friendly method. Thickness, grammage, and apparent density of the paper samples ranged from 198 μm to 314 μm, 49 g/m^2 to 117.8 g/m^2, and 0.23 to 0.39 g/cm^3, respectively. Moisture content, water filtration and wicking speed ranged from 5.2% to 7.1%, 35.7 to 156.7, and 0.062 to 0.124 mms^{-1}, respectively. Further, water contact angle and porosity ranged from 76˚ to 112˚ and 79% to 83%, respectively. The best paper sample one was chosen to fabricate PADs which were used for the determination of metformin. The metformin assay on PADs followed linear range from 0.0625 to 0.5 mg/mL. The assay had limit of detection and limit of quantitation of 0.05 mg/mL and 0.18 mg/mL respectively. The new method was used to test metformin samples (n=20) collected from local pharmacies. The average amount of metformin concentration in samples was 465.6 ± 15.1mg/tablet. Three samples did not meet the regulatory standards. When compared with spectrophotometric method, PADs assay correctly predicted 18 out of 20 samples. The PADs assay on handmade paper may provide a low-cost and easy-to-use system to screening the quality of drugs and other point-of-need applications.

Keywords: Drug quality, Lokta paper, Metformin, Point-of-need device, Protein assay

Introduction

Paper-based analytical devices (PADs) are a class of low-cost, portable, and easy-to-use point-of-need assay platforms. Assays on PADs need significantly smaller volume of reagents and samples thus generating lower volume of waste[1]. In recent years, various efforts have been made to improve the performance, design, and applicability of PADs by fabricating devices for multiplex assays[1], three-dimensional devices[2], fully enclosed paper devices[3], programmable diagnostic devices[4], and enzymatic biofuel cells[5].

The PADs can be integrated with various detection methods for analysis. The most commonly used methods are colorimetry, chemiluminescence, electrochemical detection, and Raman spectroscopy[6]. Among them, colorimetry is one of the highly used detection methods in PADs in which analysis is performed by adding reagent(s) to the reaction zones within the paper along with the analyte of interest[7]. Change in color in the detection or assay zone in the paper device is identified or measured visually or using a camera and scanner. This minimizes the need for expensive and sophisticated instrumentations and facilities[8]. Because of these advantages, interesting applications[7] of PADs have been demonstrated in environmental analysis, clinical diagnosis, pharmaceutical analysis[9], chemical and biological testing using colorimetric assays such as for proteins[10], glucose[11], uric acid[12], drugs[9], and biomarkers[1].

Several methods are available for the fabrication of (micro)fluidic channels in PADs[1] such as photolithography[13], plasma treatment and inkjet printing[14], wax printing[15], screen printing[16], wax dipping[17], flexographic printing[18] and laser cutting[19]. Filter paper, blotting paper, and chromatography papers are among the most widely used paper substrates for fabricating PADs[9,20]. Paper is a low-cost and ubiquitous material with a wide range of choices. Whatman Grade 1

chromatographic paper and Whatman No. 1 filter paper are made of cellulose (>98%) and have been widely applied for the development of PADs[20]. Whatman Grade 1 chromatography paper has clean surface, uniform thickness, high hygroscopic property, wicking properties, flow rate, and cost effectiveness[21]. Whatman Grade 4 chromatography paper has a pore size of 20-25 µm and has been used in the development of diagnostic devices[14]. Nitrocellulose (NC) has been used as a substrate for protein immobilization as it provides high protein-binding capacity due to charge-charge interactions and weak secondary forces[22]. In addition, other paper substrates, Grade 3 chromatography paper[23], Whatman P81[24], paper towel[21,25], and office paper[26] have been used as a suitable platform in the fabrication of paper-based sensors. Although various types of paper substrates are currently being used for the fabrication of PADs, researchers are still looking for paper substrates having unique properties or are locally made or manufactured eco-friendly. The need for locally or distributed manufacturing has been highlighted during the recent COVID-19 pandemic to overcome the global shortage of diagnostic tools and personal protective equipment[27].

In this work, we characterized five different locally made handmade papers known as *Nepali kagaj* for their potential use in fabricating PADs. *Nepali kagaj* is made from the fibrous bark of *Daphne bholua* and *D. papyracea* or other similar plant species following the traditional eco-friendly method of fiber processing and pulping[28]. Nepali handmade paper is considered to be highly resistant to germs like mildew, paper crawlers, and termites[29]. It has been used traditionally for recording government records and religious texts. However, in modern days, it is used as wrapping papers, paper lamps, restaurant menus, greeting cards, and photo frames[29]. We characterized commercially available Nepali handmade papers by measuring several physical and fluid flow characteristics. The best type of paper was chosen to make PADs for two assays. As a proof of concept, we used the PADs made from local handmade paper for colorimetric protein

assay. We then developed an assay for screening drug quality analysis and measured the active pharmaceutical ingredient (API) in metformin drugs purchased from the local market.

Experimental

Materials and reagents

Tetra bromophenol blue (TBPB), citric acid, tri-sodium citrate, sodium hydroxide, potassium dichromate, sodium nitroprusside (SNP), and sodium hypochlorite (NaOCl) were bought from Thermo Fisher Scientific India Pvt. Ltd., India. Metformin standard was bought from Accord Healthcare Pvt. Ltd., India, and was standardized according to Indian Pharmacopeia. Bovine serum albumin (BSA) – Fraction V, purchased from Himedia Laboratories Pvt. Ltd., India, was used as standard protein. All chemicals were used as received without further purification. We purchased five different Nepali handmade paper samples (hereunder named as P1, P2, P3, P4, and P5) from local handmade paper enterprises and stored them in airtight Ziplock bags until performing experiments.

Characterization of handmade paper sheets

Handmade paper sheets were cut into rectangular shapes of different sizes to measure the grammage of the paper sample. We measured the area and weight of the paper (±0.001 g) at ~23^{o}C temperature and ~50% relative humidity. Grammage was estimated as the ratio of the weight of paper (g) to area (m^2). We measured the thickness of paper using an optical microscope (Amscope, USA) by imaging them along their thickness. The field of view of the microscope was calibrated using a linear calibration grid (grid size 10μm), and the image pixels were converted to micrometers in ImageJ software to get thickness information[30]. Apparent density was calculated by dividing grammage (g/m^2) by its thickness (μm). Five measurements were taken of each sample.

Wicking speed of the paper samples were measured using paper strips of different widths. The stripes were kept vertically in a beaker containing potassium dichromate solution and flow of colored solution was monitored my taking images every minute using a smartphone. The distance travelled by dichromate solution on paper stripe per unit time was considered as wicking speed[31].

Porosity of paper samples calculated by measuring the volume of water absorbed by rectangular shaped paper pieces of different sizes. At first, we measured dry weight of each paper sample and then they were soaked in distilled water for 2-3 minutes and the weight of wet was measured. The porosity of paper samples was calculated by dividing the absorbed weight of water by total weight of sample[32].

To measure the moisture content, 2.0 g of paper sample was oven-dried at 105±2°C for 24 hours. The sample was then cooled in a non–hygroscopic desiccator and weighed. The difference in the weight before and after drying was used to calculate the moisture content of the paper[33].

To measure the water filtration coefficient, paper samples were cut using a circular cutter into circles of different diameters. They were folded to make a 60° cone. The folded papers were then wet thoroughly with distilled water. 25 mL distilled water was then poured into the samples and the time taken by the samples to filter half its volume was noted using a stopwatch[34]. The water filtration coefficient was calculated using the following equation:

$$K = 3465/t \quad (1)$$

where, K is the water filtration coefficient. t is the time taken to filter out half volume of water

Water contact angle (WCA) was measured using a smartphone[35]. A drop of water (50 µL) was put onto the surface of paper using a micropipette and images of water drops were taken by smartphone. Images were analyzed using polynomial fitting with dropsnake plugin in ImageJ software to measure contact angle. The drop image was cropped to make the left and right interface

of the drop clearly visible. Few knots (5-10) were added on the drop contour until the spline was finalized and the plugin displayed the contact angle. WCA measurement for each type of paper surface was repeated ten times. See supplementary information for step-by-step procedure to measure the WCA using ImageJ software.

Fabrication of paper device

Paper-based devices were fabricated using a wax printing method[15]. We used Adobe Illustrator software to design assay regions as an array of circles with an inner diameter of 5.3 ± 0.2 mm with 2.7 mm line thickness. The pattern designs were transferred onto paper using wax printing method (Xerox ColorQube 8580, Japan). The wax-patterned paper was heated using heating iron for the 40s during which the wax melted and penetrated through the paper to form hydrophobic barriers across the thickness of paper. Finally, one side of the device was covered with transparent take to keep the reagents contained in the assay region.

Colorimetric assays

Protein assay was performed using TBPB method and BSA as standard protein. The testing zone of PADs was prepared by loading 3 μL of 250 mM citrate buffer (pH 2.0) and was allowed to dry at room temperature for 2 minutes. Then, 3 μL of 3 mM TBPB in 95% ethanol was added in the testing zone followed by the addition of 5 μL of protein standard. Signal of the assay was recorded after 8 minutes[10].

Metformin assay was performed by allowing 4 μL of each 0.4% (w/v) of NaOCl, 2.0 M NaOH and 4.0 0.04 M sodium nitroprusside (SNP) and 5 μL metformin react[36]. Color of the assay was recorded after 15 minutes with a smartphone. We also tested metformin samples purchased from local pharmacy stores in Kathmandu.

Image analysis

We used a Samsung Galaxy M30s smartphone to image the assay device and ImageJ image analysis software to measure the color intensity of assays. The images were converted into 8 bit and inverted. After this, the images are analyzed in three color spaces (R, G and B). Average signal value of all pixels in the assay zone was measured. Triplicate measurements were carried out for each assay. Blank assays were performed along with sample assays. The net signal of the assay was obtained by subtracting the mean signal of sample assay from the signal of blank assay. We chose green color channel because it gave a higher net intensity.[10]

A schematic of assays on paper device is outlined in figure 1.

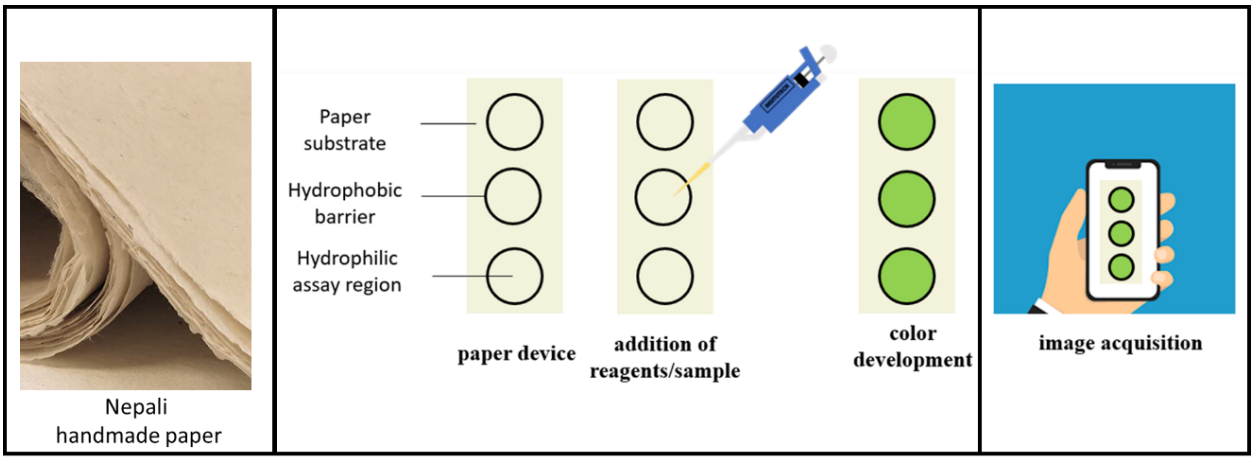

Figure 1*: A photograph of handmade paper (on left) and a general procedure for performing colorimetric assays on paper device (middle and right).*

Spectrophotometric detection of metformin

Spectrophotometric signal of metformin standard solution (5-50 µg/mL) was measured at 236 nm in a UV-Visible Spectrophotometer LVS-A20 (LABTRON, UK). Drug samples (30 µg/mL) were prepared in distilled water from 500 mg tablets. One tablet from each sample was

crushed into fine powder using pestle and was dissolved in 5mL water. Concentration of metformin in samples was estimated by using regression equation of calibration curve.

Results and discussion

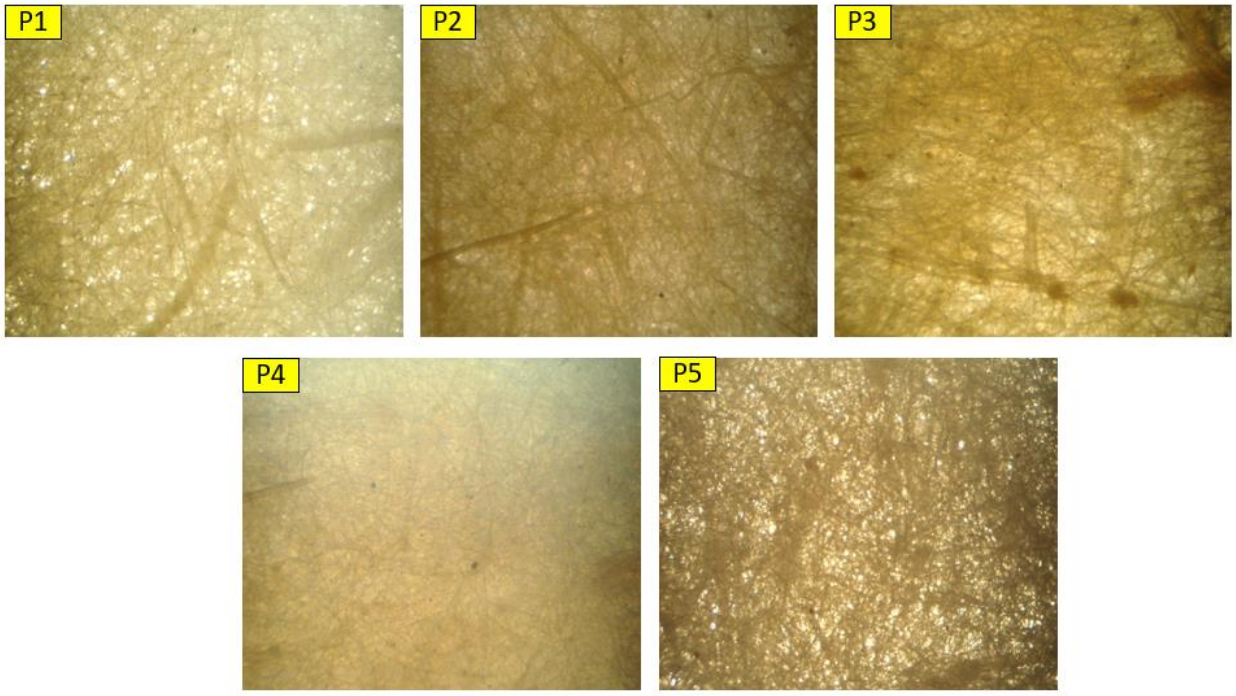

***Figure 2**: Optical microscopy images of five handmade papers*

A photograph of Nepali kagaj is shown in Figure 1. The physical parameters of all handmade papers are shown in table 1. Average thickness of the paper samples was 230.30±55.05 μm, ranged from 198 μm (P2) to 314 μm (P4). The thickness of P5 was similar to Whatman grade 1 paper (180 μm) and remaining four samples were thicker than the Whatman grade 1 paper[20]. However, P1 and P2 had similar thickness to Whatman grade 4, P5 was thinner and P3 and P4 were even thicker than Whatman grade 4 paper (205 μm)[20].

The gram per square meter (GSM) of papers, which is also known as grammage, ranged widely from 49 g/m^2 (P1) to 117.8 g/m^2 (P4). For reference, the grammage and thickness of Whatman grade 1 and 4 filter papers[20] were reported to be 88 and 96 g/m^2. A good positive

correlation (R = 0.89) between the grammage and thickness was observed. The apparent density of paper samples ranged from ~0.23 (P1) to 0.39 (P4) g/cm^3. The low apparent density of paper samples suggest that these are lightweight papers.

***Table 1**: Physical properties of Nepali handmade papers*

Paper type	Thickness (µm)	GSM (g/m^2)	Apparent density (g/cm^3)	Porosity *(ε %)*	Moisture Content (%)	Contact angle	Filtration efficiency
P1	207.40±11.08	49.07±3.52	0.23	0.83	5.92± 0.4	106.21±3.84	156.74±2.75
P2	198.59 ±7.13	75.17±2.47	0.37	0.79	6.13±0.7	102.03±3.43	132.17±2.58
P3	254.10 ±9.40	108.26±7.36	0.42	0.82	5.77±1.0	101.88±3.86	45.35±1.48
P4	314.82±9.34	117.80±8.87	0.37	0.81	7.13±0.2	112.15±4.17	35.67±1.28
P5	176.60±14.30	54.45±16.28	0.30	0.81	6.54±1.0	76.64±1.33	104.76±4.31
Grade 1*	180	88	0.40	0.68	N/A	N/A	150
Grade 4*	205	96	0.46	0.64	N/A	N/A	37

*Whatman filter paper[20]

Optical microscopy images of the handmade papers show variable fiber networking and pores (Figure 2). The pores in the images may have been impacted by light penetration through the paper which depends on the thickness of the papers. Thickness of paper substrate determines the penetration of wax while making hydrophobic barriers on paper analytical devices. Similarly optical path length, scattering, assay sensitivity, and volume of reagents for an assay is also affected by thickness in paper device[9].

Wicking speed affects contact time between sample and reagents and distribution of reagents in the reaction zone. This eventually may have an impact on the intensity and homogeneity of the color.

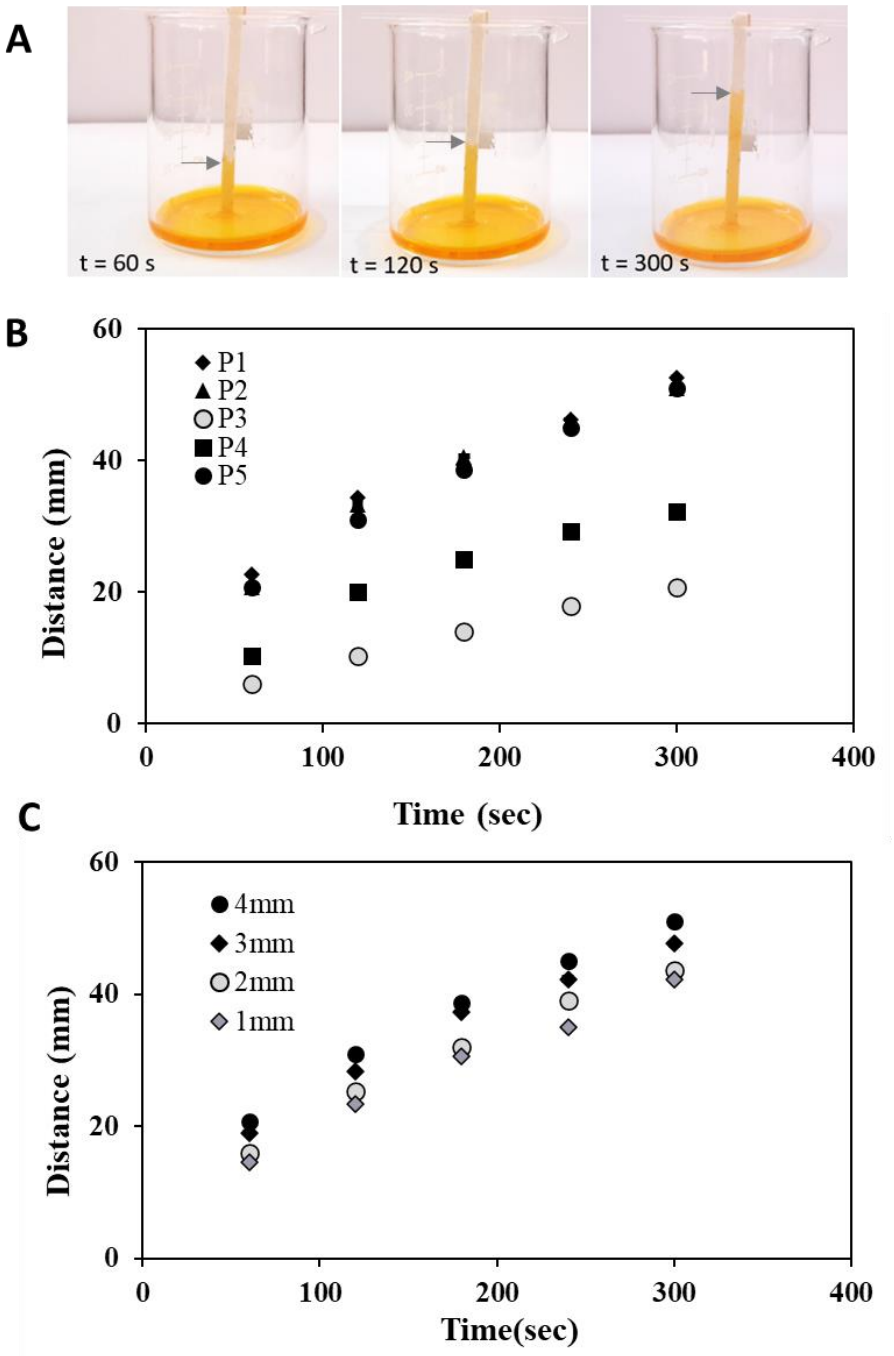

Figure 3: *(A) Demonstration of flow visualization using a color solution at different times. Fluid front is indicated with arrows; (B) variation of distance travelled by color solution in different paper samples with times; (C) variation of distance travelled by the color solution in paper strips (P5) of different width.*

We measured the wicking speed of aqueous solution on the paper stripes. Experimental set up of the measurement is given in figure 2A. The dichromate solution wicked upwards on the stripes with time. The wicking speed of P1, P2, P3, P4 and P5 were found to be 0.12, 0.123, 0.062, 0.089 and 0.124 mms^{-1} (*see* Table S1*)*. The thicker substrates (P3 and P4) transferred solution at slower rate in comparison with the thinner substrates (P1, P2 and P5). Different types of handmade Nepali papers provided variable wicking speed of aqueous (Figure 2B). The graph representing distance with time for each paper were fitted with linear function to estimate the speed. We also explored the impact of width of paper stripe on wicking speed. The wicking speed of P5 samples in the strips of width 4, 3, 2 and 1 mm were 0.124, 0.119, 0.115 and 0.112 mms^{-1} respectively (*see* Table S2). We found that wicking speed decreased in the channels of smaller width. Narrow channel provides more resistance for the fluid flow in paper and therefore slows down the flow[37]. The fluid flow also depends on the thickness of the paper stripe as thinner stripes provide lower resistance to flow leading to faster fluid flow[20].

The porosity of handmade paper samples was in the range of ~79 % (P2) to 83 % (P1). The porosity of Whatman qualitative filter papers is reported in the range of ~64 % (grade 2) to 68% (grade1)[20]. Papers with high porosity increase the absorbance of ink and help the ink to dry quickly. High porosity is caused by stiff fibers, excessive flocculation of fibers, insufficient refining, or calendaring[38]. Porosity is useful in calculating the total volume of liquid reagent required to wet the substrate[39].

The amount of water contained in the paper is expressed as a percentage of the paper's weight. Equilibrium moisture content (EMC) of the paper samples ranged from ~5.2% (P3) to 7.13 % (P4). The water filtration coefficient of handmade paper samples was found in the range of 35.67 (P4) to 156.74 (P1). The filtration efficiency is affected by the density, thickness, and the

size of water-permeable pores in the paper. Paper with high efficiency has high filtering speed and resolution.

We started the water contact angle (WCA) measurement experiments with Polytetrafluoroethylene (PTFE) substrate as a reference. The contact angle between distilled water and PTFE was found to be 106.8˚ which is close to reported value of 108˚ which was measured by atomic force microscopy[40]. The WCA of handmade paper ranged from ~76˚ (P5) to 112˚(P4) (Table 1). Most of the papers had a contact angle greater than 90˚ which indicates the hydrophobic nature of the papers while P5 is hydrophilic. The WCA values reported in our case may have some measurement errors due to surface roughness and inhomogeneity of paper surfaces making the drop not perfectly axisymmetric[35].

Colorimetric assays on paper device

Among five different types of Nepali *Kagaj*, sample P5 had contact angle less than 90˚ making it hydrophilic in nature. We selected P5 sample as an appropriate platform to fabricate paper device considering its thickness, wicking speed, and color uniformity.

As a proof of concept, we performed protein assay on the paper device. The quantification of BSA is based on its ability to interact with TBPB indicator dye through a combination of electrostatic and hydrophobic interactions to form concentration dependent bluish-green complex (Fig. 4A). To demonstrate the viability of the method, levels of protein in the BSA standard were quantified. The results showed that the net intensity increased the concentration of BSA, and the signal was proportional to the logarithmic value of BSA concentration in the range from 0.5 to 50 mg/mL (Fig. 4B). The linear range was found to be 0.5 to 6 mg/mL (inset in Fig. 4B). The limit of detection (LoD) and limit of quantification (LoQ) of this assay were 1.33 mg/mL and 4.91 mg/mL

respectively which are similar to literature reported values of 0.9 mg/mL and 2.9 mg/mL respectively[10].

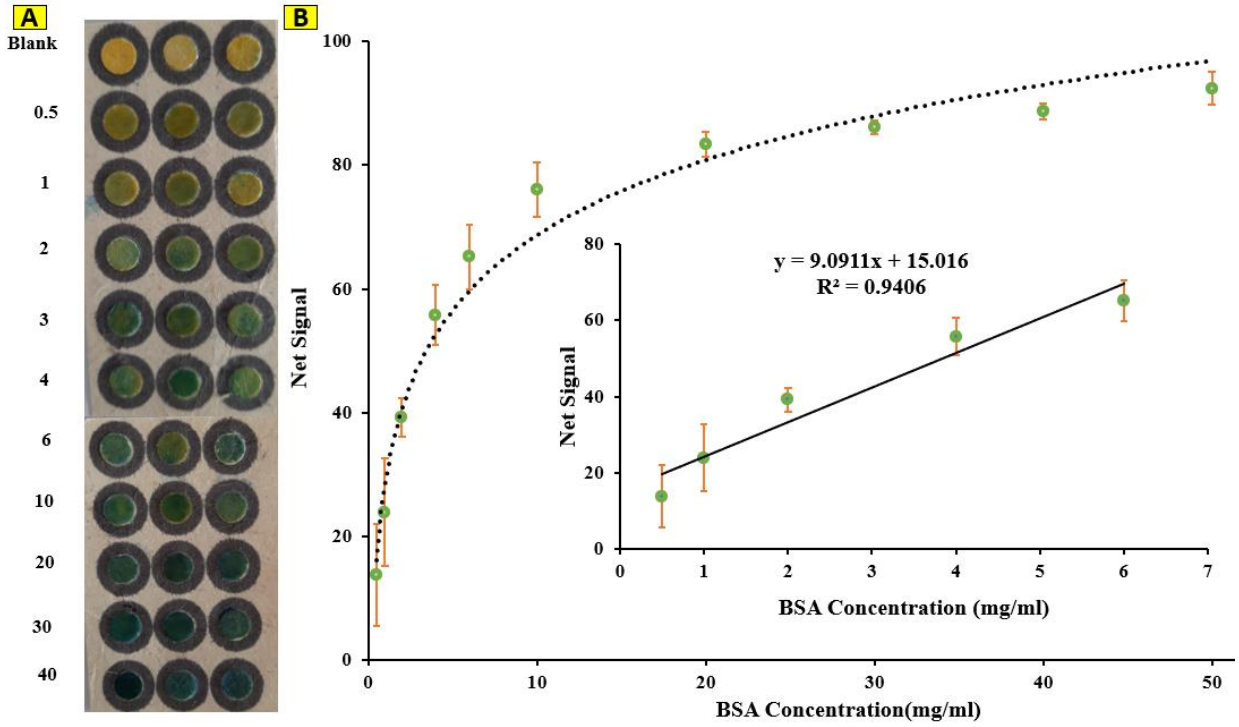

Figure 4: *(A) Images of PADs showing color development in protein assay. Triplicate images for each BSA concentration are shown. The first row shows triplicate images for blank. Numbers indicate concentration of standard protein (BSA); (B) Response curve of BSA on PADs at different concentrations (0 to 50 mg/mL). Insert shows a linear region of the response curve.*

We developed a more useful assay to expand the applicability of handmade papers. This assay determined the concentration of metformin in tablet. Drug quality is a serious issue as low-quality drugs pose social, economic and health burden to society. Recent reports have suggested that as high as 10.5% drugs worldwide are either substandard or falsified. The problem is more adverse in low- and middle-income countries (13.6%), especially in Africa (18.7%) and Asia

(13.7%)[41]. Having a low-cost, easy-to-use point-of-need drug quality screening technology such as PADs would contribute towards solving the widespread prevalence of low-quality drugs.

The metformin assay relied on the addition of sodium hypochlorite solution to an alkaline solution of metformin hydrochloride that produced ß-diketone. The ß-diketone is an oxidized product of metformin. Sodium nitroprusside (SNP) in an alkaline medium reacts with ß-diketone to give a green-colored product[36]. Photographs of assay zones after color development are shown in Figure 5A. Triplicate experiments were performed for each concentration. Assay signal responded to concentration of metformin tested from 0.0625 to 40 mg/mL in logarithmic fitting (Fig. 5B). The linear range obtained for metformin in our system is 0.0625 to 0.5 mg/mL (Figure 5B inset). The LoD and LoQ of this assay were 0.05 mg/mL and 0.18 mg/mL respectively.

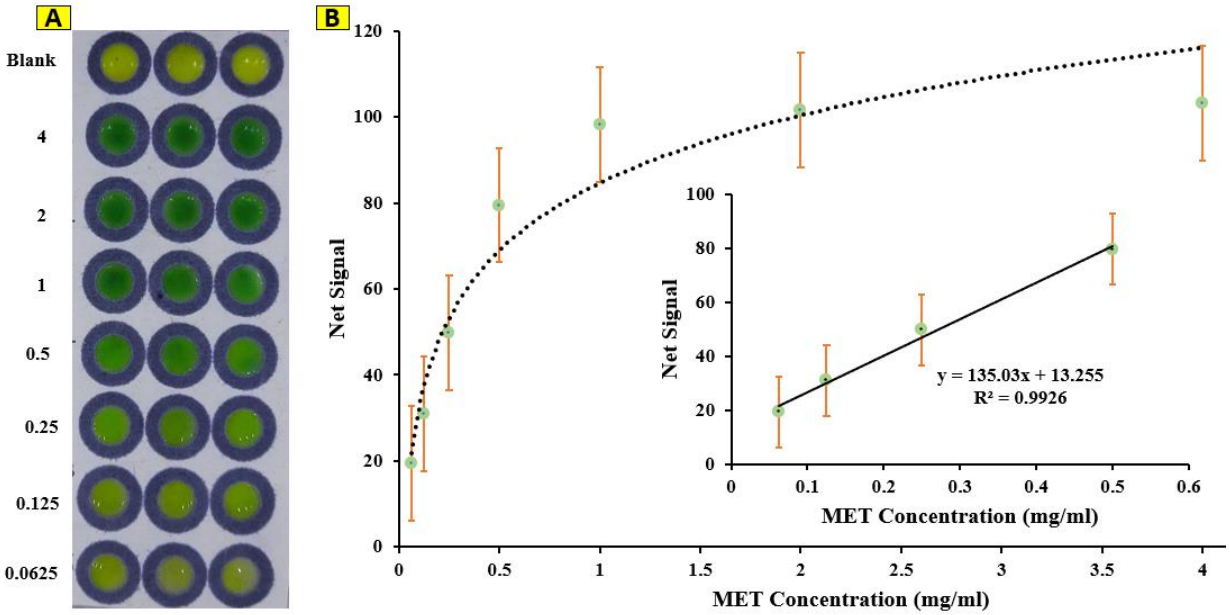

Figure 5: *(A) Images of PADs to showing green color development metformin standard. Triplicate images for each metformin concentration are shown. The first row shows triplicate images of the blank. Numbers indicate concentration of metformin; (B) Response curve of metformin analysis using PADs. The inset shows linear region of the response curve.*

Quality of metformin samples

After developing the PADs method for determining the amount of metformin, we collected metformin samples (n=20) from local pharmacies and tested them using PADs and spectrophotometric methods. The average amount of metformin concentration in samples was 465.6 ± 15.1 mg (range: 429.3 – 482.39 mg, *see* Table 2). The label claim of these samples was 500 mg. PADs determined value of metformin samples was slightly lower than the label claim. According to Indian pharmacopoeia[42], acceptable range for metformin hydrochloride tablets is 450 to 550 mg[42]. PADs assay found three samples (S2, S10 and S16) did not meet the regulatory standards, all slightly lower than 450mg.

Table 2*: Concentration of metformin in locally collected samples*

Sample ID	Metformin (mg)
S1	470.3
S2	*444.3*
S3	473.8
S4	466.2
S5	480.2
S6	480.3
S7	475.5
S8	468.1
S9	482.4
S10	*429.3*
S11	475.5
S12	452.9
S13	458.5
S14	482.3
S15	479.7
S16	*445.6*
S17	477.8
S18	463.5
S19	451.1
S20	455.9
Ave.	465.6
Stand. Dev.	15.1

To compare the performance of PADs method for the determination of metformin in tablet forms, we also tested same samples using a spectrophotometric method. Calibration curve of metformin determination using spectrophotometric method is given in Figure S1. The LoD and LoQ of spectrophotometric method were 0.75 µg/mL and 2.45 µg/mL respectively[43]. Amount of metformin in each tablets determined using spectrophotometric method was 483.8 ± 21.1 mg (range: 419.0 – 516.0 mg, *see* Figure 6). Based on spectrophotometric measurement, only one sample was found to be not within the acceptable range suggested by pharmacopeia (Table S3). Paper device underestimated the concentration of metformin samples by 18.2 ± 23.6 mg; $p < 0.001$ when compared with spectrophotometric method. Additionally, paper device correctly predicted 18 out of 20 samples. In figure 6b, we plotted the difference between two methods against mean of two methods which shows that 19 out of 20 samples were within the 10% acceptable range indicating good agreement of paper device with spectrophotometric method.

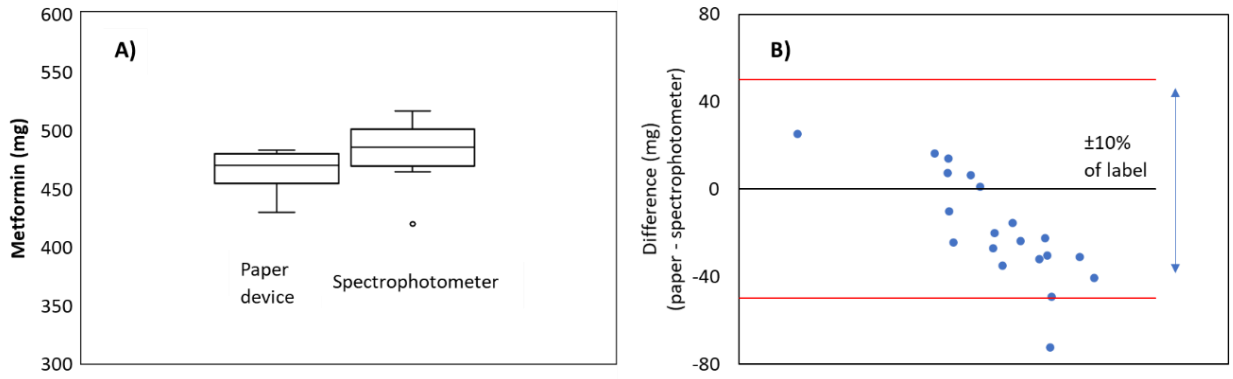

Figure 6: *Box plot of determination of metformin using two different methods (A). A comparative plot is shown in B.*

Conclusions

In this paper, we reported the characterization of five Nepali *kagaj* for their potential in fabricating PADs. The handmade *kagaj* showed wide range of properties such as thickness, grammage, wicking speed, contact angle etc. The best sample was chosen to make PADs for two different applications. At first, we demonstrated a protein assay on the PADs fabricated on handmade paper. Then the PADs were used to develop an assay to determine the amount of metformin in tablets. Both assays performed satisfactorily. We showed that the PADs fabricated on handmade Nepali *kagaj* can be a low-cost and easy to implement sensor for screening the quality of metformin, an anti-diabetic drug. Similar assay can be developed, with proper choice of colorimetric reaction, to detect and quantify other types of analytes of interests including counterfeit drugs. Our future work is looking into combining PADs made from Nepali *kagaj* and smartphone application for data reading, analyzing, and reporting which could be beneficial to users, policy makers and regulating agencies.

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Supporting information

Following additional information is available in the supporting information file.

A) Step-by-step procedure to measure contact angle using a smartphone and ImageJ software.

Table S1: Parameters used to determine the wicking speed of each paper. Fitting parameters corresponds to: y = wicking distance, x = wicking time, c = linear constant, m = wicking speed (mms^{-1})

Table S2: Parameters used to determine the wicking speed of P5 in strips of different widths. Fitting parameters corresponds to: y = wicking distance, x = wicking time, c = linear constant, m = wicking speed (mms^{-1})

Figure S7: Calibration curve of metformin standard using spectrophotometric measurement

Table S3: Concentration shown in metformin samples by spectrophotometer

References

(1) Ozer, T.; McMahon, C.; Henry, C. S. Advances in Paper-Based Analytical Devices. *Annual Review of Analytical Chemistry* **2020**, *13* (1), 85–109. https://doi.org/10.1146/annurev-anchem-061318-114845.

(2) Phillips, S. T.; Thom, N. K. Three-Dimensional, Paper-Based Microfluidic Devices Containing Internal Timers for Running Time-Based Diagnostic Assays. In *Microfluidic Diagnostics*; Springer, 2013; pp 185–196.

(3) Schilling, K. M.; Lepore, A. L.; Kurian, J. A.; Martinez, A. W. Fully Enclosed Microfluidic Paper-Based Analytical Devices. *Analytical chemistry* **2012**, *84* (3), 1579–1585.

(4) Martinez, A. W.; Phillips, S. T.; Nie, Z.; Cheng, C.-M.; Carrilho, E.; Wiley, B. J.; Whitesides, G. M. Programmable Diagnostic Devices Made from Paper and Tape. *Lab on a Chip* **2010**, *10* (19), 2499–2504.

(5) Wu, X. E.; Guo, Y. Z.; Chen, M. Y.; Chen, X. D. Fabrication of Flexible and Disposable Enzymatic Biofuel Cells. *Electrochimica Acta* **2013**, *98*, 20–24.

(6) Fu, L.-M.; Wang, Y.-N. Detection Methods and Applications of Microfluidic Paper-Based Analytical Devices. *TrAC Trends in Analytical Chemistry* **2018**, *107*, 196–211. https://doi.org/10.1016/j.trac.2018.08.018.

(7) Morbioli, G. G.; Mazzu-Nascimento, T.; Stockton, A. M.; Carrilho, E. Technical Aspects and Challenges of Colorimetric Detection with Microfluidic Paper-Based Analytical Devices (MPADs) - A Review. *Analytica Chimica Acta* **2017**, *970*, 1–22. https://doi.org/10.1016/j.aca.2017.03.037.

(8) Yetisen, A. K.; Akram, M. S.; Lowe, C. R. Based Microfluidic Point-of-Care Diagnostic Devices. *Lab on a Chip* **2013**, *13* (12), 2210–2251.

(9) Sharma, N.; Barstis, T.; Giri, B. Advances in Paper-Analytical Methods for Pharmaceutical Analysis. *European Journal of Pharmaceutical Sciences* **2018**, *111*, 46–56. https://doi.org/10.1016/j.ejps.2017.09.031.

(10) Pokhrel, P.; Jha, S.; Giri, B. Selection of Appropriate Protein Assay Method for a Paper Microfluidics Platform. *Practical laboratory medicine* **2020**, *21*, e00166.

(11) Huang, X.; Zhou, Y.; Liu, C.; Zhang, R.; Zhang, L.; Du, S.; Liu, B.; Han, M.-Y.; Zhang, Z. A Single Dual-Emissive Nanofluorophore Test Paper for Highly Sensitive Colorimetry-Based Quantification of Blood Glucose. *Biosensors and Bioelectronics* **2016**, *86*, 530–535.

(12) Kumar, A.; Hens, A.; Arun, R. K.; Chatterjee, M.; Mahato, K.; Layek, K.; Chanda, N. A Paper Based Microfluidic Device for Easy Detection of Uric Acid Using Positively Charged Gold Nanoparticles. *Analyst* **2015**, *140* (6), 1817–1821.

(13) Yu, L.; Shi, Z. Z. Microfluidic Paper-Based Analytical Devices Fabricated by Low-Cost Photolithography and Embossing of Parafilm®. *Lab on a Chip* **2015**, *15* (7), 1642–1645.

(14) Li, X.; Tian, J.; Garnier, G.; Shen, W. Fabrication of Paper-Based Microfluidic Sensors by Printing. *Colloids and surfaces B: Biointerfaces* **2010**, *76* (2), 564–570.

(15) Carrilho, E.; Martinez, A. W.; Whitesides, G. M. Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics. *Anal. Chem.* **2009**, *81* (16), 7091–7095. https://doi.org/10.1021/ac901071p.

(16) Sameenoi, Y.; Nongkai, P. N.; Nouanthavong, S.; Henry, C. S.; Nacapricha, D. One-Step Polymer Screen-Printing for Microfluidic Paper-Based Analytical Device (MPAD) Fabrication. *Analyst* **2014**, *139* (24), 6580–6588.

(17) Songjaroen, T.; Dungchai, W.; Chailapakul, O.; Laiwattanapaisal, W. Novel, Simple and Low-Cost Alternative Method for Fabrication of Paper-Based Microfluidics by Wax Dipping. *Talanta* **2011**, *85* (5), 2587–2593.

(18) Olkkonen, J.; Lehtinen, K.; Erho, T. Flexographically Printed Fluidic Structures in Paper. *Analytical chemistry* **2010**, *82* (24), 10246–10250.

(19) Nie, J.; Liang, Y.; Zhang, Y.; Le, S.; Li, D.; Zhang, S. One-Step Patterning of Hollow Microstructures in Paper by Laser Cutting to Create Microfluidic Analytical Devices. *Analyst* **2013**, *138* (2), 671–676.

(20) Evans, E.; Gabriel, E. F. M.; Coltro, W. K. T.; Garcia, C. D. Rational Selection of Substrates to Improve Color Intensity and Uniformity on Microfluidic Paper-Based Analytical Devices. *Analyst* **2014**, *139* (9), 2127–2132.

(21) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. ACS Publications 2010.

(22) Arrastia, M.; Avoundjian, A.; Ehrlich, P. S.; Eropkin, M.; Levine, L.; Gomez, F. A. Development of a Microfluidic-Based Assay on a Novel Nitrocellulose Platform. *Electrophoresis* **2015**, *36* (6), 884–888.

(23) Wang, W.; Wu, W.-Y.; Zhu, J.-J. Tree-Shaped Paper Strip for Semiquantitative Colorimetric Detection of Protein with Self-Calibration. *Journal of Chromatography A* **2010**, *1217* (24), 3896–3899.

(24) Shiroma, L. Y.; Santhiago, M.; Gobbi, A. L.; Kubota, L. T. Separation and Electrochemical Detection of Paracetamol and 4-Aminophenol in a Paper-Based Microfluidic Device. *Analytica chimica acta* **2012**, *725*, 44–50.

(25) Ellerbee, A. K.; Phillips, S. T.; Siegel, A. C.; Mirica, K. A.; Martinez, A. W.; Striehl, P.; Jain, N.; Prentiss, M.; Whitesides, G. M. Quantifying Colorimetric Assays in Paper-Based Microfluidic Devices by Measuring the Transmission of Light through Paper. *Analytical chemistry* **2009**, *81* (20), 8447–8452.

(26) Cioffi, A.; Mancini, M.; Gioia, V.; Cinti, S. Office Paper-Based Electrochemical Strips for Organophosphorus Pesticide Monitoring in Agricultural Soil. *Environmental Science & Technology* **2021**, *55* (13), 8859–8865.

(27) Molina, A.; Vyas, P.; Khlystov, N.; Kumar, S.; Kothari, A.; Deriso, D.; Liu, Z.; Banavar, S.; Flaum, E.; Prakash, M. Project 1000 x 1000: Centrifugal Melt Spinning for Distributed Manufacturing of N95 Filtering Facepiece Respirators. *arXiv:2004.13494 [cond-mat, physics:physics]* **2020**.

(28) Biggs, S.; Messerschmidt, D. Social Responsibility in the Growing Handmade Paper Industry of Nepal. *World Development* **2005**, *33* (11), 1821–1843.

(29) Banjara, G. B. Handmade Paper in Nepal: Upgrading with Value Chain Approach. *German Technical Cooperation/Private Sector Promotion-Rural Finance. Lalitpur, Nepal* **2007**.

(30) Giurlani, W.; Berretti, E.; Innocenti, M.; Lavacchi, A. Measuring the Thickness of Metal Coatings: A Review of the Methods. *Coatings* **2020**, *10* (12), 1211.

(31) Kasetsirikul, S.; Shiddiky, M. J.; Nguyen, N.-T. Wicking in Paper Strips under Consideration of Liquid Absorption Capacity. *Chemosensors* **2020**, *8* (3), 65.

(32) Astuti, N. H.; Wibowo, N. A.; Ayub, M. The Porosity Calculation of Various Types of Paper Using Image Analysis. *Jurnal Pendidikan Fisika Indonesia* **2018**, *14* (1), 46–51.

(33) Jindal, V. K.; Siebenmorgen, T. J. Effects of Oven Drying Temperature and Drying Time on Rough Rice Moisture Content Determination. *Transactions of the ASAE* **1987**, *30* (4), 1185–1192.

(34) Bogaty, H.; Carson, F. T. Measurement of Rate of Flow of Water through Filter Paper. *J Res Natl Bureau Stand* **1944**, *33* (5), 353–362.

(35) Chen, H.; Muros-Cobos, J. L.; Amirfazli, A. Contact Angle Measurement with a Smartphone. *Review of Scientific Instruments* **2018**, *89* (3), 035117.

(36) LABHADE, S. R.; LABHADE, K. R.; GAIKWAD, V. B. A Novel Method for the Selective Identification of Metformin in Bulk and Pharmaceutical Formulations Using Sodium

Nitroprusside as a Chromogenic Reagent by Spectrophotometry. *Chemical Science* **2014**, *3* (4), 1254–1263.

(37) Godwin, L. A.; Deal, K. S.; Hoepfner, L. D.; Jackson, L. A.; Easley, C. J. Measurement of Microchannel Fluidic Resistance with a Standard Voltage Meter. *Analytica chimica acta* **2013**, *758*, 101–107.

(38) Bajpai, P. Brief Description of the Pulp and Papermaking Process. In *Biotechnology for pulp and paper processing*; Springer, 2018; pp 9–26.

(39) Mark, D.; Haeberle, S.; Roth, G.; Von Stetten, F.; Zengerle, R. Microfluidic Lab-on-a-Chip Platforms: Requirements, Characteristics and Applications. *Microfluidics based microsystems* **2010**, 305–376.

(40) Wloch, J.; Terzyk, A. P.; Wiśniewski, M.; Kowalczyk, P. Nanoscale Water Contact Angle on Polytetrafluoroethylene Surfaces Characterized by Molecular Dynamics–Atomic Force Microscopy Imaging. *Langmuir* **2018**, *34* (15), 4526–4534.

(41) Ozawa, S.; Evans, D. R.; Bessias, S.; Haynie, D. G.; Yemeke, T. T.; Laing, S. K.; Herrington, J. E. Prevalence and Estimated Economic Burden of Substandard and Falsified Medicines in Low-and Middle-Income Countries: A Systematic Review and Meta-Analysis. *JAMA network open* **2018**, *1* (4), e181662–e181662.

(42) IPC. *Indian Pharmacopoeia 2018*; IPC, 2018.

(43) Mali, A. D.; Mali, S.; Tamboli, A.; Bathe, R. Simultaneous UV Spectrophotometric Methods for Estimation of Metformin HCl and Glimepiride in Bulk and Tablet Dosage Form. *International journal of advances in pharmaceutics* **2015**, *4* (6), 117–124.