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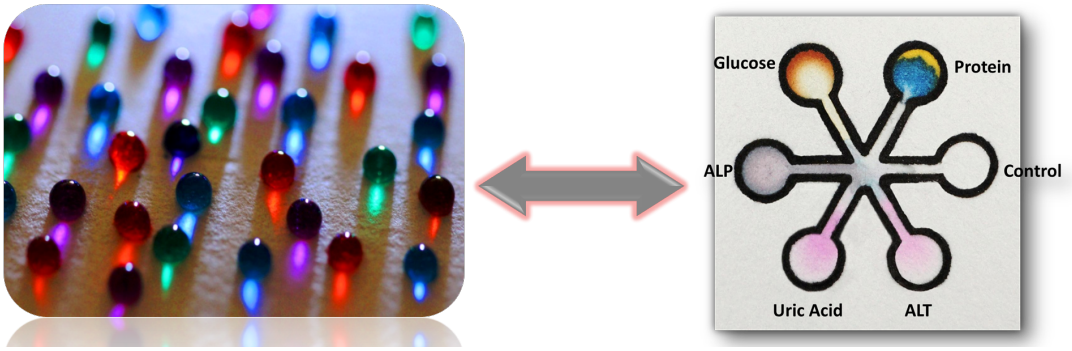
*** A simple yet versatile approach has been demonstrated for the fabrication of paper-based microfluidic platforms based on vapor-phase polymerization technique.**



Vapor-Phase Deposition of Polymers as a Simple and Versatile technique to Generate Paper-Based Microfluidic Platforms for Bioassay Applications

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Abstract

Given their simplicity and functionality, the paper-based microfluidic systems are considered to be ideal and promising bioassay platforms for use in least developed countries or in point-of-care services. Although a series of innovative techniques have been recently demonstrated for the fabrication of such platforms, development of simple, inexpensive and versatile new strategies are still needed in order to reach their full potential. In this communication, we described a simple yet facile approach to fabricate paper-based sensor platforms in desired design through a vapor-phase polymer deposition technique. We also showed that the fabricated platforms might be readily employed for the detection of varying biological target molecules including glucose, protein, ALP, ALT, and uric acid. The limit of detection for each target molecules was calculated to be 25 mg/dL for glucose, 1.04 g/L for protein, 7.81 unit/L for ALP, 1.6 nmol/L for ALT, and 0.13 mmol/L for uric acid, respectively.

Introduction

Since the pioneering work by Whitesides and co-workers,[1] the paper-based microfluidic platforms have drawn increasing attention in recent years for varying applications including clinical diagnostic,[2,3] food quality control,[4,5] and environmental monitoring [6,7] due to their ease of use, low-cost, ease of fabrication, and capability to offer quantitative or semi-quantitative information in point-of-care applications. Given its simplicity and functionality, such a promising system or device can be considered to be ideal bioassay platforms for use in least developed countries or in point-of-care services. These platforms typically consist of a channel, which allows to flow liquids in a controlled manner through capillary forces and a detection zone where the descriptive reactions take place.[8-10] To date, a number of innovative methods have been demonstrated to create hydrophilic-hydrophobic contrast on paper surface such as photolithograpy,[11] plotting with an analogue plotter,[12] ink jet etching,[13] plasma treatment,[14] paper cutting,[15] wax printing,[16] ink jet printing,[17] flexography printing,[18] screen printing,[19] and laser treatment.[20] Although they have been employed successfully in different applications, most of these methods have several drawbacks such as requiring multistep and complicated procedures for their fabrications or some restrictions for mass production.[8-10] Therefore, new strategies for the fabrication of paper-based platforms in desired pattern are still needed in order to reach their full potential.

The vapor-phase deposition of polymers is a simple, one-step, solventless, environmentally benign, and substrate independent process, and may open the doors to new opportunities for varying applications.[21-23] By utilizing this approach, non-planar substrate geometries having micro- and/or nano-scale features can be readily coated displaying uniform polymer thickness over the geometric features

present in the substrate.[24,25] Meanwhile, unwanted impurities, degradation of the underlying layer, and undesirable effects of de-wetting and surface tension related to the use of solvents can be precisely eliminated.[21-25] Recently, Gupta and co-workers have demonstrated the polymer deposition on paper substrates using initiated-Chemical vapor deposition (i-CVD) technique.[26-28] They also showed that paper substrates can be patterned in desired design for paper-based microfluidic applications through i-CVD. However, they could not succeed to create pattern using physical masking. In their method, paper substrates were patterned through photolithography before polymer deposition.[26-28]

Here we reported a simple and one-step method for the generation of paper-based microfluidic platforms in desired design based on vapor-phase polymerization of dichloro[2.2]paracyclophane. Hydrophilic channels and reaction zones on paper substrates were created with the help of metal masks that are made by a water jet cutting technique. We also demonstrated that these paper-based microfluidic platforms may be readily employed for the detection of some biological molecules such as glucose, protein, ALP, ALT, and uric acid.

Experimental

Polymer Deposition on Paper Samples

The conformal coating of poly(chloro-p-xylene) [PPX] films on paper samples (Whatman no. 1 chromatography paper) were performed using a SCS-PDS2010 deposition system. A hydrophobic dichloro[2.2]paracyclophane molecule was used in the deposition process as a starting monomer. The polymer deposition process was started by placing proper amounts of monomer (0.01 g – 2.0 g) into evacuated sublimator chamber. These monomers were then evaporated at ~175 °C and converted to radicalic monomers by pyrolysis (~695 °C). They were subsequently deposited and polymerized onto paper samples. All process was carried out under vacuum condition (32 mtorr). The corresponding PPX thickness on paper samples was controlled through the amount of the loaded monomer. The hydrophilic channels on paper samples, which allow to transport the analyte solutions via capillary penetration, were created using a metal mask with desired pattern. The paper samples were sandwiched between metal masks and magnets. Metal masks were fabricated with the help of a water jet cutting machine (OMAX 55100, USA). In this process, desired pattern of mask designs were first drawn using the CorelDraw software and uploaded to cutting machine. An iron plate with a ~3.1 mm of thickness was used as a starting material. After placing iron plates into the machine, cutting process was carried out automatically using machine software. After polymer deposition, the morphologies of fabricated paper samples were characterized by using a Quanta 400F field-emission scanning electron microscope (FE-SEM) with an acceleration voltage of 10 kV. The thicknesses of deposited polymer films on the papers, which were measured indirectly using a silicon wafer placed next to the paper samples during deposition, were analyzed with a variable-angle spectroscopic-

phase-modulated ellipsometer (UVISEL, Jobin Yvon-Horiba). In the layer thickness analysis, a four-phase model consisting of silicon substrate/SiO_2/overlayer/air was assumed. All thickness values given in the text are the averages of at least three measurements taken at three different locations on each sample surface. A Fourier transform infrared (FTIR) spectrometer (Thermo Nicolet IR) operated under an attenuated total reflection mode was also utilized to collect spectra of the polymer deposited samples. The IR spectra were recorded with a 4 cm^{-1} resolution. The static contact angle measurements were carried out at 25 °C in ambient air using an automatic contact angle goniometer equipped with a flash camera (DSA 100 Krüss, Germany) applying the sessile drop method. The volume of the drop was set to be 5.0 μL in all measurements. The contact angles were then calculated by using the software of the instrument. All reported values are the averages of at least five measurements taken over five different locations on each sample surface. Deionized water was used for all contact angle measurements.

Glucose Detection Assay

The normal glucose level in healthy adults is between 70-100 mg/dL. For diabetics, this range should be 90-130 mg/dL. Any level outside these ranges may indicate a medical problem.[29] In our work, glucose level was determined through the enzymatic oxidation of iodide to iodine. In a typical experiment, a 5.0 μL of potassium iodide solution (0.6 M) was first dropped into the designed zones on paper. After drying the paper in ambient condition, 4 μL of horseradish peroxidase/glucose oxidase enzyme mixture (1:5) was spotted on the same zones. 10 μL of standard glucose solutions (0-500 mg/dL in pH 7.4 buffer) were finally added to pre-activated paper zones and resultant color changes were monitored. All of the reagents in experiments were purchased from Sigma–Aldrich.

Protein Detection Assay

The amount of total protein in blood normally ranges from 60 to 83 g/L. Concentrations below this reference range can indicate, for instance, liver disease, acute infection or kidney disorders.[29] As for concentrations above this range it may also reflect paraproteinamia, leukemia or any condition related to increasing immunoglobulins level.[29] To evaluate the detection of protein level in our paper based sensor platforms, we used a simple colorimetric assay based on the binding of tetrabromophenol blue (TBPB) to albumin. In this context, a 5.0 µL of 250 mM citrate buffer (pH 1.8) containing Triton X-100 was spotted two times to designed zones on paper. Afterwards, 5.0 µL of 9.0 mM tetrabromophenol blue (TBPB) in 4% ethanol in water was added to the same spots. Finally, proper amount of albumin solutions (0-266 g/L in pH 7.4 buffer solution) were pipetted on reagent impregnated spots and resultant color changes from yellow to blue were monitored. All of the reagents in experiments were also obtained from Sigma–Aldrich.

Alkaline Phosphatase Detection Assay

A healthy adult has low concentration of alkaline phosphatase [ALP] (30−120 U/L) in blood serum. High level of ALP may reflect liver or bile ducts problems.[29] For the detection of ALP level, we first prepared a 20.0 µL of a reagent solution containing 1.8 mM nitro blue tetrazolium, 2.7 mM 5-bromo-4-chloro-3-indolyl phosphate in 5% DMSO in water. A 5.0 µL of prepared solution was then spotted to designed zones on paper sample and let the solution evaporate. This procedure was applied twice. Finally, 10 µL of ALP solutions (0-500 unit/L) were dropped onto reagent impregnated zones and resultant color changes were monitored. All of the reagents in experiments were purchased from Sigma–Aldrich.

Uric Acid and Alanine Aminotransferase (ALT) Detection Assays

In our experiments, commercial available assay kits were utilized for detection of uric acid and ALT levels and recommended protocols by supplier were applied step by step (Sigma-Aldrich, Cat No: MAK077, MAK052). Briefly, 2.0 µL of uric acid or 10 µL of ALT probe solution was spotted twice to designed zones on paper. After drying, proper amounts of uric acid (0-0.8 mmol/L) or ALT (0-20 nmol/L) solutions were dropped on activated zones and resultant color changes were monitored.

Quantitative Data Analysis

Following the descriptive color change completed for each target molecules, high-resolution photographs of the paper samples were taken using a digital SLR camera (EOS 650D, Canon, Japan) fitted with a macro lens (EF 100 mm focal length, F/2.8, Canon, Japan). In order to obtain the best image, all photographs were taken with close-up shooting mode and auto white balancing under artificial white light without flash. For the stabilization of images, camera was also mounted on a commercial available tripod. Other specifications of camera was not changed and used in standard (default) modes. After transferring the images, which were saved as jpeg files, to the computer, color intensities for each colorimetric assay were analyzed with freeware IMAGEJ image analysis program sizing a circle grid. Once each grid positioned manually over colored zones on paper was in place, intensity changes depending on target concentrations were determined for total color. For all measurements, background correction was also performed.

Results and Discussion

In this communication, we demonstrated an alternative approach to fabricate paper-based sensor platforms through a vapor-phase polymer deposition technique. The vapor-phase deposition of polymers, which is an environmental friendly, solvent-free, and material independent method, offers unique advantages over the conventional solvent-based techniques.[21-25] By this way, polymers can be conformally deposited in a controlled manner onto substrates with non-planar geometries like paper and unwanted impurities, degradation of the underlying layer, and changes in the mechanical/chemical features associated with the use of solvents can be precisely eliminated.[24,25] Figure 1a shows the schematic representation of the fabrication process of a polymer deposited paper sample. A hydrophobic dichloro[2.2]paracyclophane was selected as a starting monomer in our work. Briefly, proper amount of monomer (0.01 g – 2.0 g) was first placed in a sublimator chamber and converted to radicalic monomer via pyrolysis. Subsequently, they were deposited and polymerized on paper. Resultant polymer on sample is poly(chloro-p-xylene) [PPX]. All process is carried out under vacuum condition (32 mtorr). The thicknesses of deposited polymers can be controlled by manipulating of sublimation temperature, pyrolysis temperature or amount of monomer which was used. In order to create hydrophilic channel to transport the analyte solutions via capillary penetration on paper, we used a metal mask with desired pattern made by a water jet cutting technique. The paper samples were sandwiched between metal masks and magnets (Fig. 1b).

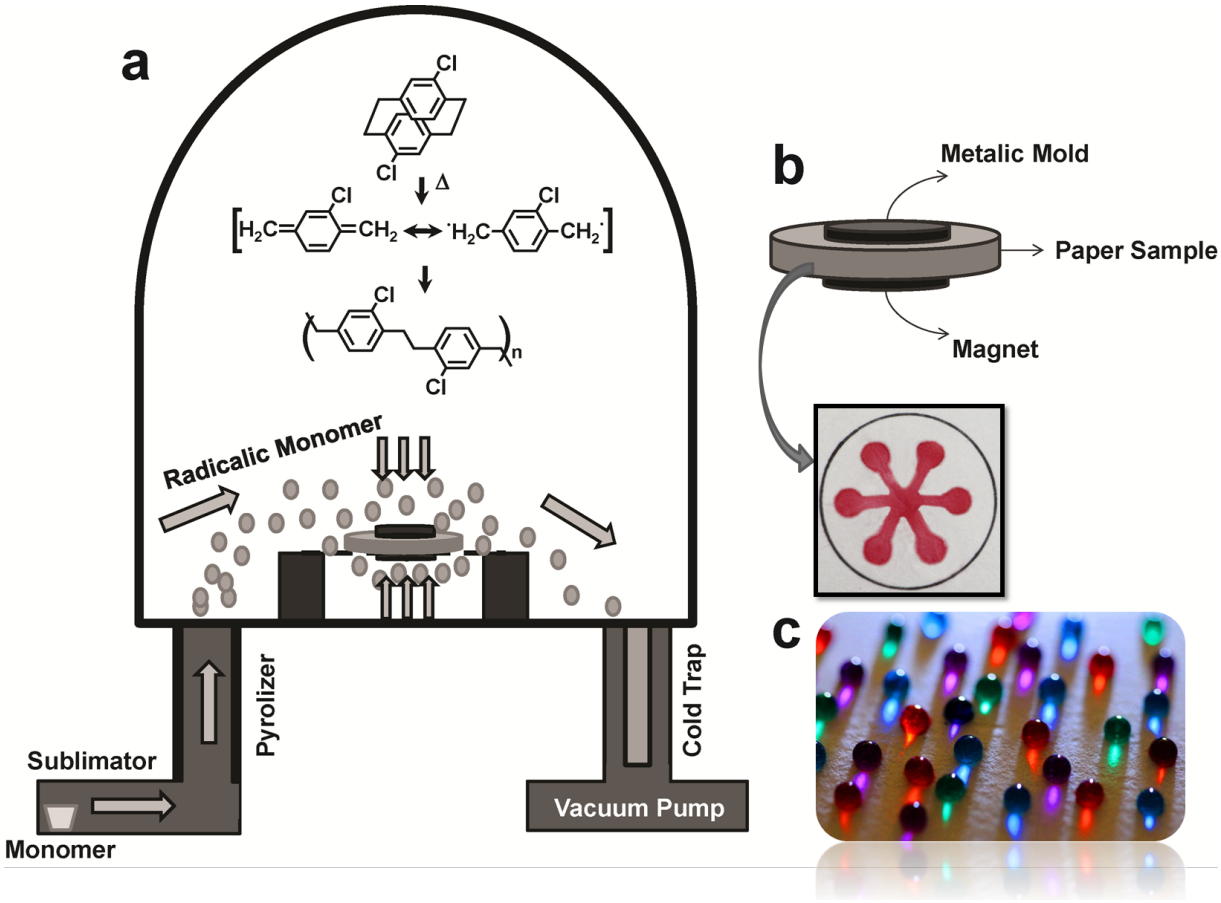

Figure 1. *Schematic representation of fabrication process of polymer deposition on paper substrate [inset polymerization mechanism of PPX] (a), sandwich array for patterning of paper (b), and colored water droplets on polymer deposited paper (c).*

Before studying the sensor performances of polymer deposited paper platforms in desired design, we characterized them using varying techniques. Changes in topographical structure of paper after polymer deposition were verified by SEM. The plain paper has almost smooth cellulose fibers having different diameters (Fig. 2a). Following the polymer coating, the resultant morphologies of randomly organized cellulose fibers were changed depending on monomer amounts that were used during deposition process. In the case of 0.01 g of monomer, slightly changed in the topographical structure of polymer deposited paper was observed compared with plain paper due to the low thickness of deposited polymer (Fig. 2b). However, in

the cases of 0.5 and 1.0 g of monomers, obvious polymer coating was distinguished (Fig. 2c and 2d).

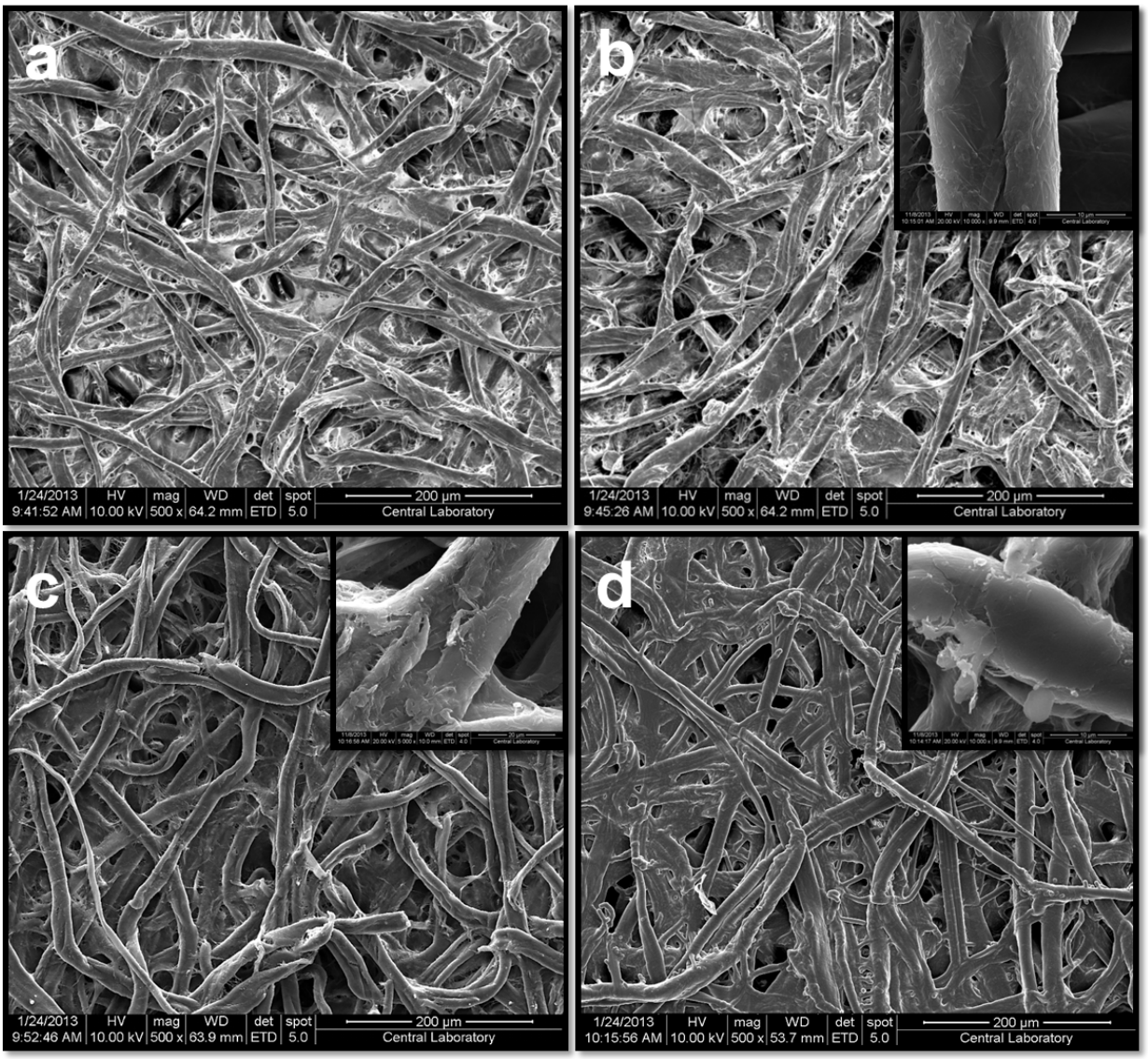

Figure 2. *SEM images of plain (a), and polymer deposited papers using 0.01 g (b), 0.5 g (c), and 1.0 g (d) of monomer.*

The thicknesses of deposited polymer films on paper samples were measured indirectly using a silicon wafer placed next to the paper samples during deposition. Figure 3a shows the changing of polymer thickness depending on monomer amounts used in deposition. As expected, thicknesses of deposited polymers increased when

the amount of monomer was increased. The polymer thicknesses were found 213 ± 78 nm for 0.01 g, 740 ± 241 nm for 0.5 g, 1480 ± 310 nm for 1.0 g, 2140 ± 370 nm for 1.5 g, and 3190 ± 480 nm for 2.0 g of monomer, respectively. Parallel to thickness measurements, the hydrophobicity of the polymer deposited paper samples was also changed upon starting monomer amount. The water contact angle values were observed in the range of 69°-121° (Fig. 3b). No substantial changes in hydrophobicity were observed for the polymer deposited samples when the amount of monomer was increased from 1.0 g to 2.0 g under the constant polymerization time (~10 min). If the amount of monomer, which was used in deposition, exceeded 1.0 g, however, the monomer vapor penetrated through the metallic mask on the paper sample and resulted in a distortion in desired pattern. Therefore, 1.0 g of monomer was selected as the optimized condition for the fabrication of paper based sensor platforms.

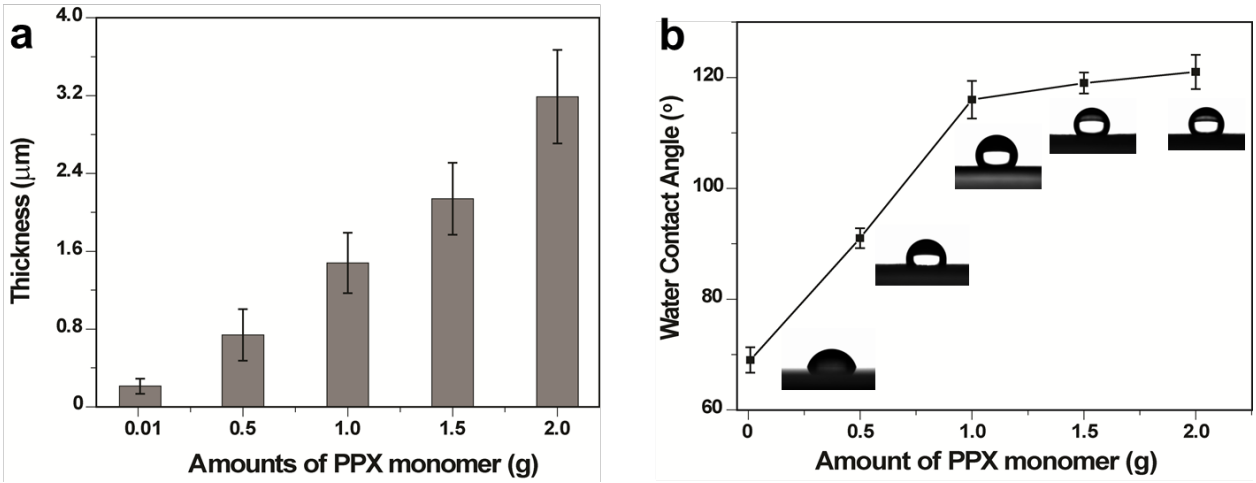

Figure 3. *Polymer thicknesses (a) and water contact angles (b) as a function of starting monomer amount in deposition process.*

Both plain and polymer deposited paper samples were also characterized by infrared (IR) spectroscopy (Fig. 4). The prominent bands for plain paper were in the ranges of 3300-3400 cm^{-1} for O-H stretching, 2924-2938 cm^{-1} for C-H stretching,

1628-1636 cm^{-1} for bound H_2O, 1420-1424 cm^{-1} for C-H bending, and 1020-1065 cm^{-1} for C-O-C bending, respectively (Fig. 4a). For all polymer deposited paper samples, the IR spectroscopy over the 500 to 3500 cm^{-1} frequency range reveals similar peaks for C-H stretching (2800–3000 cm-1), aromatic C-H stretching (3026 cm^{-1}), C-H deformation (1340 cm^{-1}), C-deformation (1401 cm^{-1}), and benzene breathing (950 cm^{-1}) (Fig. 4b-e). All collected spectra are consistent with most of the reports for PPX polymer.[23,30]

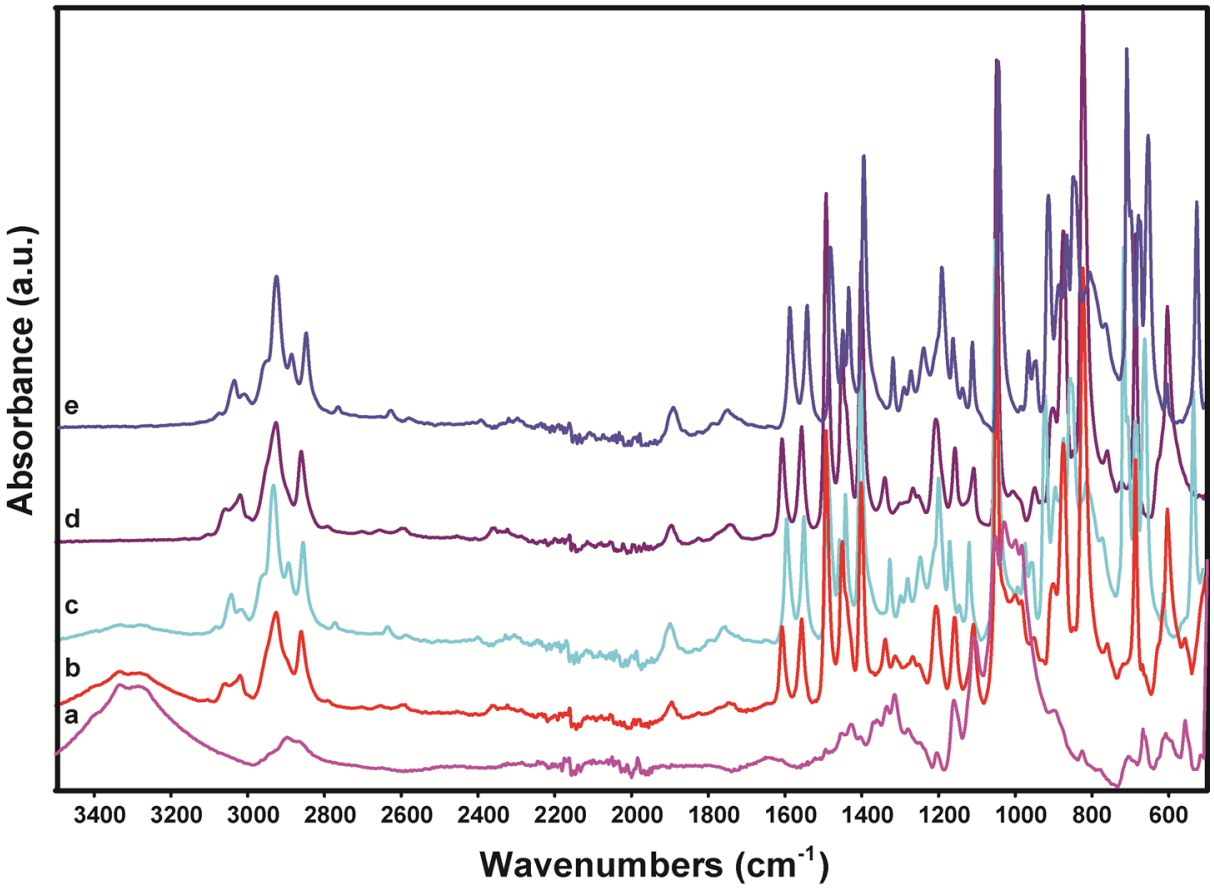

Figure 4. *FTIR spectra of plain (a), and polymer deposited papers using 0.01 g (b), 0.5 g (c), 1.0 g (d), and 2.0 g (d) of monomer.*

To evaluate the use of polymer deposited paper samples in desired design for the bio-detection of target molecules (i.e., glucose, protein, uric acid, ALT, and ALP), we first fabricated circular test zones having hydrophilic inside and hydrophobic outside features. These target molecules were selected due to their assays are well-known colorimetric assays based on enzymatic reactions or small dye molecules and most of the routine analyzes in hospitals related to them.[31-33] In this context, proper amount of particular reagents for each target molecules were spotted on circular zones as described in experimental section, and then dropped 10-30 µL of solution of each target molecules having varying concentrations into the same zones. The color changes in the zones indicate the chemical or biochemical reactions between the target molecules and specific reagents. Variation in the intensity of the resultant colors allows a quantitative evaluation for these compounds. Color intensities were analyzed using freeware IMAGEJ program. We successfully detected broad concentration of targets in the ranges 0-500 mg/dL for glucose, 0-266 g/L for protein, 0-500 unit/L for ALP, 0-20 nmol/L for ALT, and 0-0.8 mmol/L for uric acid in the assays. It is clearly found that the intensity of the resultant color in each test zone is proportional to the concentration of target molecules. Relationships between the relative color intensities and the concentrations of target molecules were demonstrated in Figure 5. The limit of detections was calculated to be 25 mg/dL for glucose, 1.04 g/L for protein, 7.81 unit/L for ALP, 1.6 nmol/L for ALT, and 0.13 mmol/L for uric acid, respectively. At lower concentrations of target molecules, calculated intensity values takes place on the corresponding test zones were illusory due to the similar color intensities were observed from the control zone.

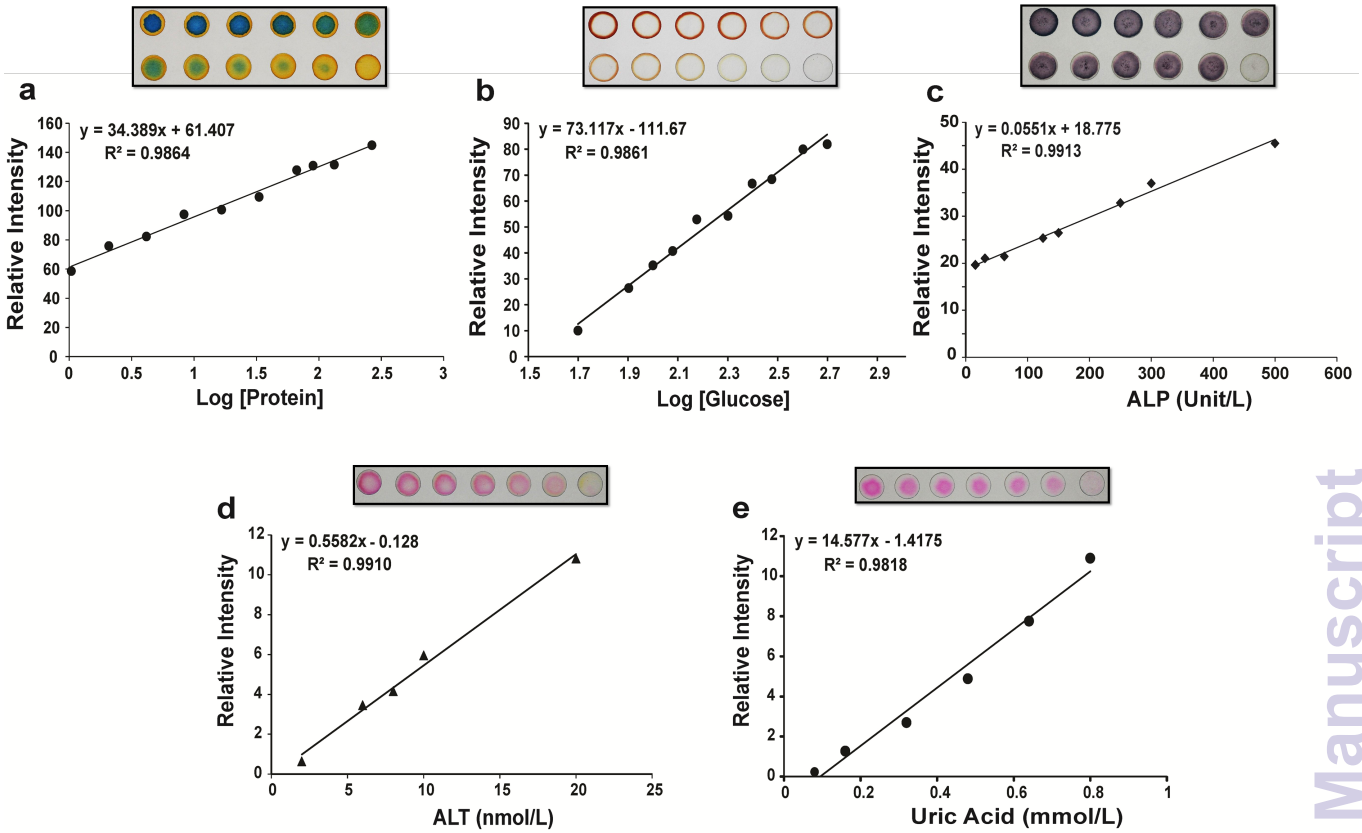

Figure 5. *Color change and related relative intensity values as a function of amount of target molecules; (a) Glucose, (b) Protein, (c) ALP, (d) ALT, and (e) Uric acid.*

After these experiments, we fabricated a flower like paper platform that performs multiple assays in parallel at the same time. Similar to circular zone assays, particular reagents for each target molecules first spotted into designed zones and dried. An artificial mixture having proper amounts of each target molecules were then freshly prepared and pipetted (~100 µL) into the center zone of design. Following the descriptive reactions completed in the zones, we did not observe any cross-contaminations between the different channels. These results obviously indicate that our proposed approach may hold great promise as a viable alternative tool for the fabrication of paper-based sensor platforms.

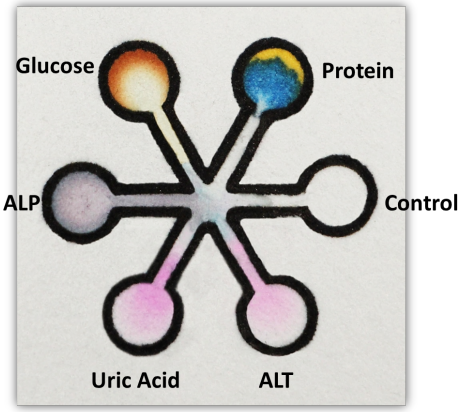

Figure 6. *Flower like paper-based sensor platform for multi-analyte detection (Channels and zones were painted with ballpoint pen for clarity after polymer deposition).*

Conclusions

We have demonstrated a technique for fabrication of paper-based sensor platforms through vapor phase polymer deposition approach. The fabricated paper platforms were successfully utilized for the detection of varying biological target molecules including glucose, protein, ALP, ALT, and uric acid. Given its environmental friendly, solvent-free, and material independent nature of vapor phase polymerization method may offer new possibilities in the field of biosensor applications.

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