*Research article*

# A Microfluidic Paper-based Analytical Device for Simultaneous Measurement of Albumin, Creatinine and Uric Acid in Urine based on Standard Addition Method

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## Abstract

**Keywords**

µPAD;
albumin;
creatinine;
uric acid;
standard addition;
urine

This work describes a simple method for the simultaneous measurement of urinary albumin, creatinine and uric acid using a standard addition approach on a microfluidic paper-based analytical device (µPAD). Hydrophobic barrier of the µPAD was created by stamping indelible ink onto a filter paper. The µPAD was designed with a flower-liked configuration. After aliquoting urine to a central sample zone, the sample flowed to ten surrounding channels, namely "the inner channels", where the blank (water) or standard solutions had been added. The inner channels were connected to circular reagent zones where reagents had been immobilized. Tetrabromophenolphthalein ethyl ester, alkaline picrate, and a mixed solution of ferric chloride and ferric cyanide were used as the chromogenic reagents for the colorimetric detections of albumin, creatinine, and uric acid, respectively. Each reagent zone was linked with a circular detection zone through a second channel, namely "the outer channels". The blue-, orange- and greenish-blue colored products were observed in the detection zones for the measurement of albumin, creatinine, and uric acid, correspondingly. A digital image of the µPAD was captured with a mobile phone. The color intensities were evaluated by ImageJ$^{TM}$ and were employed for the quantitative analyses. The standard addition calibrations were found to be linear ($r^2 > 0.99$) for the spiked analyte concentrations up to 100 mg $L^{-1}$ albumin, 1000 mg $L^{-1}$ creatinine, and 50 mg $dL^{-1}$ uric acid. The paper platform provided high precision (RSD < 5 %) and good analytical recovery (91.8-109.7 %). Under paired *t*-test, the results obtained by the developed µPAD and the validating methods were not significantly different at 95 % confidence (albumin: $t_{stat} = -0.130$, $t_{cri} = 3.182$, creatinine: $t_{stat} = -0.133$, $t_{cri} = 3.182$, and uric acid: $t_{stat} = 1.119$, $t_{cri} = 3.182$).

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## 1. Introduction

Kidney plays significant roles in humans such as blood pressure regulation, hormone excretion, and toxic substance elimination. The measurement of renal dysfunction biomarkers such as albumin and creatinine in urine is an effective strategy to avoid kidney failure. A urinary albumin concentration of greater than 30 mg $L^{-1}$ is regarded as an early stage of kidney disease [1]. Creatinine is excreted from the body through glomerular filtration into urine at a relatively constant rate [2]. Its abnormal excretion levels indicate kidney malfunction. The minimum value of creatinine in real human urine has been set at 50 mg $L^{-1}$ [3]. Normally, for spot urine samples, the value of urinary albumin to creatinine ratio is utilized for clinical diagnosis of kidney disease to eliminate variation of the results due to sample dilution. Creatinine is used as the normalization factor because it is excreted at a constant rate. Therefore, the simultaneous measurement of albumin and creatine is necessary. It has also been reported that patients with hyperuricemia (high concentrations of uric acid greater than 6.8 mg $dL^{-1}$) show a high risk of renal failure [4]. Thus, the simultaneous quantitative analyses of albumin, creatinine, and uric acid in human urine are essential for clinical diagnoses of both renal dysfunction and hyperuricemia.

Many methods of quantitative analysis have been used for the determination of urinary albumin, including separation techniques [5, 6], immunology [7], spectrofluorometry [8] and spectrophotometry [9, 10]. Analytical methods for the measurement of creatinine in urine have also been reported, including the conventional Jaffé method [11], enzymatic assay [12] and HPLC [13, 14]. Various methods for the determination of uric acid are based on HPLC [15, 16], spectrophotometry [17] and electrochemistry [18]. However, the above instrumental methods are not applicable for point-of-care (POC) testing because some bulky and expensive bench-top apparatuses are required. Additionally, their analytical procedures are time-consuming and tedious.

In 2007, Whitesides and his co-workers created POC device, known as "microfluidic paper-based analytical devices" or "μPADs" [19]. The paper devices are portable, cost-effective, and easy to use. These advantages result in many applications of μPADs for the POC testing. There are several publications describing the development of μPADs for the individual determination of albumin [20, 21], creatinine [22, 23] and uric acid [24, 25]. Some researchers presented paper-based platforms for the simultaneous analysis of two renal dysfunction biomarkers, albumin and creatinine [26, 27], or creatinine and uric acid [28, 29]. To our knowledge, there has been no publication reported on the simultaneous determination of all three mentioned biomarkers in a single μPADs so far.

Hence, this work demonstrates use of a μPAD for the simultaneous measurement of urinary albumin, creatinine, and uric acid. The flower-liked μPAD pattern was designed to allow the performance of the simple standard addition approach on a paper platform in order to eliminate the sample matrix effect. The hydrophobic barrier of the μPAD was patterned by contact stamping of commercially available "waterproof" ink onto a laboratory filter paper. Urine was transferred onto the central zone of the μPAD. The sample then moved toward ten surrounding channels where the standard analyte solutions (or water) had been spiked. Each channel was connected to a circular-shaped reagent zone. In this work, three chromogenic reagents, i.e., tetrabromophenolphthalein ethyl ester (TBPE) [30], alkaline picrate [31] and ferric chloride in the presence of potassium ferric cyanide [32], were utilized for the simple colorimetric detection of albumin, creatinine, and uric acid, respectively. These colorimetric reactions were set as the detection principles because of their simplicity. Each reagent zone was connected to a circular detection zone via an outer (second) channel. The blue-, orange- and greenish-blue products developed were used in the measurement of albumin, creatinine, and uric acid, respectively. An optical image of the μPAD was captured and the color intensities were evaluated with ImageJ™. The developed μPAD was applied in the analysis of spiked urine samples that had been collected from normal volunteers. The results were validated against those obtained by instrumental methods. This is the first report on using a μPAD for the

simultaneous analysis of albumin, creatinine and uric acid in a urine sample based on the standard addition method.

## 2. Materials and Methods

### 2.1 Chemical and reagent preparation

All chemicals used were of analytical reagent grade. Deionized-distilled water (18 MΩ·cm) was purified using a Zeneer UP 900 unit (Human Corporation, Seoul, Korea). The standard stock solutions of 1 g $L^{-1}$ albumin and 1 g $L^{-1}$ creatinine were prepared by dissolving an accurate weight of 0.0500 g of either human serum albumin powder (Sigma-Aldrich, USA) or anhydrous creatinine (Sigma-Aldrich, USA) in 50.00 mL of water. A standard stock of 0.5 g $L^{-1}$ uric acid was prepared by dissolving an accurate weight of 0.0250 g of crystalline uric acid (Sigma-Aldrich, USA) in 1.0 mL of 1.0 mol $L^{-1}$ NaOH followed by adjustment to 50.00 mL with water. Working standard solutions were freshly prepared by appropriate dilution of the stock standard solutions with water.

A solution of 5.0 x $10^{-4}$ mol $L^{-1}$ TBPE was prepared by dissolving 0.088 g of TBPE (Aldrich, USA) in 0.05 mL of Triton X-100 (Aldrich, USA). The solution was made up with 99.99% (v/v) ethanol (Carlo Erba, Italy) to 25.0 mL. Acetate buffer solution was prepared by mixing 0.3 mol $L^{-1}$ sodium acetate (Rankem, India) in 0.3 mol $L^{-1}$ acetic acid (Carlo Erba, Italy). This solution was adjusted to obtain a final pH of 3.2. A stock picric acid solution (0.052 mol $L^{-1}$) was prepared by dissolving ~ 0.29 g picric acid (Merck, USA) in 25.0 mL water. The alkaline picrate solution (0.025 mol $L^{-1}$ picric acid in 1.0 mol $L^{-1}$ NaOH) was prepared by mixing 4.8 mL of stock picric acid with 4.0 mL of 2.5 mol $L^{-1}$ NaOH and dilution to 10.0 mL with water. The mixed solution of 0.01 mol $L^{-1}$ of ferric chloride and 0.01 mol $L^{-1}$ of ferric cyanide was prepared by dissolving of 0.016 g of $FeCl_3 \cdot 6H_2O$ and 0.033 g of $K_3Fe(CN)_6$ in 10.0 mL water.

### 2.2 Fabrication of the µPAD

Figure 1 shows the fabrication of the µPAD for the simultaneous detection of albumin, creatinine and uric acid based on the standard addition method. The µPAD was prepared according to Mathaweesansurn *et al*. [31]. Briefly, the rubber stamp was filled with indelible ink (HORSE™ No. 2, NAN MEE Co., Ltd., Thailand). The hydrophobic area was created by manually pressing the stamp onto a Whatman™ No.1 filter paper (Figure 1A). The paper was dried at ambient temperature for 5 min to remove the ink solvent. Finally, the patterned µPAD as shown in Figure 1B was obtained.

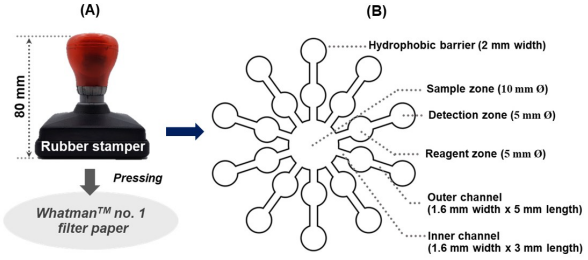


**Figure 1.** Schematic drawings represent (A): the fabrication procedure and (B): the components of the µPAD (with their dimensions) for the simultaneous detection of urinary albumin, creatinine and uric acid based on standard addition.

## 2.3 Standard addition procedure on the µPAD

The standard addition assay was started by immobilizing the chromogenic reagents onto the reagent zones of the µPAD (Figure 2A). TBPE (0.6 µL) and acetate buffer pH 3.2 (0.2 µL) were dropped onto lobes (a) to (c). Alkaline picrate solution (0.6 µL) was aliquoted onto lobes (d) to (f). Then, 0.6 µL of the mixed solution of ferric chloride and ferric cyanide were transferred onto lobes (g) to (i). The PAD was then dried using a hair dryer for 30 s. Next, spiking 1.0 µL of the blank (water) or series of the standard solutions onto the inner channels (*i* to *ix*) (see Figure 2B) as follows: water to the channels (*i*), (*iv*), and (*vii*); 50 and 100 mg $L^{-1}$ albumin to the channels (*ii*) and (*iii*); 500 and 1000 mg $L^{-1}$ creatinine to the channels (*v*) and (*vi*); 25 and 50 mg $dL^{-1}$ uric acid to the channels (*viii*) and (*ix*). Then, a 70 µL urine sample was added to the central sample zone (Figure 2B).

When the colored products had developed (Figure 2C), the PAD was transferred into a light-controlled studio (width×length×height: 400×400×400 $mm^3$). At exactly 3 min after adding the sample, a digital image of the µPAD was recorded using a smart mobile phone (iPhone™ 11 Pro Max, USA) (Figure 2D). The RGB color intensities of the detection zones were digitally processed by the ImageJ™ software. A fixed area was set for measurement of the color images in the circular detection lobes. The mean intensity values of the Red (R), Blue (B) and Green (G) intensities for each detection zone were recorded.

In this work, the ratios of the (B/R) intensity, the (G/R) intensity and the (B/R) intensity values increased in proportion to the albumin, creatinine, and uric acid concentrations. Thus, the mentioned intensity ratios were used for construction of the standard addition linear plots. It was also found that all the intensity ratio values of the reagent blanks were close to 1.0. Therefore, the value of the intensity ratio must always have values of greater than 1.0 for samples containing the target analytes. The standard addition plots were then constructed using the values of (the color intensity ratio -1) versus the concentrations of the standard analytes.

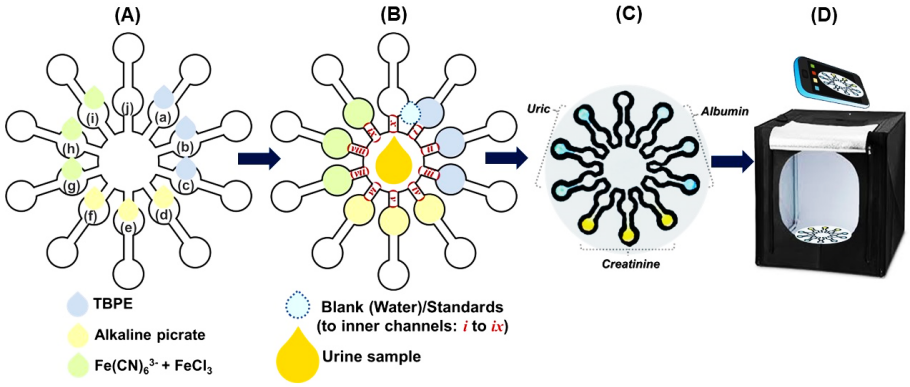


**Figure 2.** Analytical steps of the standard addition assay for the simultaneous determination of urinary albumin, creatinine, and uric acid by the developed µPAD. (A): Immobilizing the chromogenic reagents, (B): Spiking the blank (water) or series of the standard solutions and transferring the urine. (C): An example of the optical image of the µPAD showing the colored products on the detection zones and (D): Capturing the optical image of the µPAD, situated in the light-controlled studio with a mobile phone.

### 2.4 Validating methods

The validating method for the determination of albumin was based on immunology. The analysis was carried out with the contribution by Lopburi Central Lab and Pathology Co. Ltd. HPLC was employed as the validating method for the quantitative measurement of creatinine and uric acid. The parameters of the HPLC system were: Symmetry$^{TM}$ C-18 column (3.9 mm×150 mm, 5µm) at 25°C, isocratic elution with 20 mmol $L^{-1}$ ammonium dihydrogen orthophosphate (pH 7.4, Merck, USA) as mobile phase at flow rate of 0.8 mL $min^{-1}$, 20 µL injection volume and UV-absorbance detection (231 nm). Urine samples were diluted with the mobile phase at appropriate dilution ratios and filtered through a 0.22 µm nylon membrane before injection.

## 3. Results and Discussion

### 3.1 Design of the µPAD for standard addition on paper

Figure 1B illustrates a schematic drawing of the components of the µPAD with their dimensions and terms. The µPAD was patterned in a flower-liked shape. It was composed of a central sample zone, ten reagent zones, and ten detection zones. The sample zone was surrounded by the reagent and detection zones. The sample zone was connected to the reagent zones via the inner channels and the reagent zones were linked to the detection zones by the outer channels (See Figure 1B). A blank (water) and a series of standard solutions were spiked onto the inner channels. The outer channels served as the analytical paths. After transferring the sample, it moved along the inner channels, the reagent zones, the outer channels, and the detection zones, where the colored product was monitored. In this work, the paper-based device was designed so that the standard addition was simply carried out on the paper platform. The standard addition method was selected for the quantification because it eliminated the matrix effects of the urine sample. Tedious sample preparation such as extraction or centrifugation for removal of the sample matrix effect was thus not required. For other paper-based devices [22], urine sample preparation prior to the analysis was necessary because an external calibration was employed for quantification. With this design, simple standard addition approaches for the simultaneous determination of the three analytes on the single paper platform were achieved.

### 3.2 Optimization study

#### 3.2.1 Aliquoting volumes of sample and chromogenic reagents

The effects of sample volumes were studied by pipetting different volumes of the commercially available red food coloring solution (Winner$^{TM}$, Thailand). The solution was used as produced by the manufacturer without any prior preparation. The results shown in Figure 3C and 3D imply that the red food coloring solution successfully spread over all hydrophilic areas at the volumes of 70 and 80 µL. Nevertheless, 70 µL is selected for minimizing the sample volume.

The aliquoting volume of the chromogenic reagent was fixed at 0.6 µL, which was determined in our previous work [31], for which the dimensions of the reagent reservoir were the same as in the current device. It was noted that for the albumin detection, pipetting of both TBPE (the colored-developing reagent) and the buffer solutions were necessary for the colorimetric reaction. In this work, the immobilizing volume of TBPE was kept constant at 0.6 µL as mentioned earlier while the effects of buffer volume variation were examined from 0.2 to 0.6 µL. It was observed that the color intensities of the products were not different (results are not shown). The volume of 0.2 µL was selected for reducing the buffer consumption.

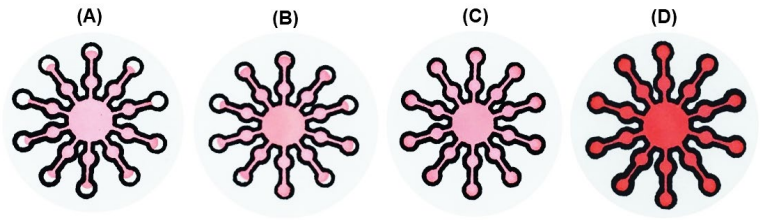


**Figure 3.** The optical images of the μPADs with different aliquoting volumes of the red food coloring solution. A: 50 μL, B: 60 μL, C: 70 μL and D: 80 μL

### 3.2.2 Concentrations of the reagents

The study of the effects of the concentration of each chromogenic reagent was carried out by the individual detection of a single analyte on the μPAD platform, as presented in Figure 1B. The experiments were performed without any step of the standard addition method. The chromogenic solution (0.6 μL) and the standard analyte solution (70 μL) were transferred to the reagent zones and the sample zone, respectively. The suitable concentrations of the reagents were assessed according to the sensitivities in terms of the slope of the external calibration plots.

The effects of the concentrations of TBPE were investigated using the standard albumin solutions at concentrations ranging from 1 to 500 mg $L^{-1}$. The results in Figure 4A show that increasing the concentration of TBPE resulted in an increase in the sensitivity. A concentration of $5.0 \times 10^{-4}$ mol $L^{-1}$ was appropriate as it provided the highest sensitivity.

For the albumin measurement, acetate buffer was one of the key parameters that could affect the intensity of the developed color of the product [30]. Therefore, the effects of the concentration of acetate buffer were evaluated. The pH values of all the investigated concentrations of the buffers were fixed at 3.2. When the buffer concentrations were increased, sensitivities also increased (Figure 4B). However, the sensitivity decreased at higher buffer concentrations. This was because of the ionic strength effect. Therefore, a concentration of 0.3 mol $L^{-1}$ was considered suitable. The effects of varying the pH of the acetate buffer solutions were assessed over the pH range of 2.8 to 3.4, while the buffer concentration was kept constant at 0.3 mol $L^{-1}$. With increasing pH, the blue-colored product derived from the association reaction between albumin and TBPE became more intense; however, the color of the reagent blank increased gradually. Moreover, at higher pH, the color intensity of the product was not proportional to the albumin concentration. This was because the association reaction between albumin and TBPE was not stoichiometric [33]. The maximum sensitivity was attained at pH 3.2 (Figure 4C), and thus this pH value was selected.

The chromogenic reagent for the creatinine detection was alkaline picrate which was prepared by dissolving solid powder of picric acid in NaOH. In this work, we prepared a solution of 0.025 mol $L^{-1}$ picric acid in 1.0 mol $L^{-1}$ NaOH, which we had adopted in our previous work for the determination of creatinine in urine by the PAD [31].

For the detection of uric acid, a mixed solution of ferric chloride and ferric cyanide was exploited as the chromogenic reagent. It was previously reported that ferric chloride plays an important role in the development of the greenish-blue colored product [32]. In this work, the effects of varying the concentration of ferric chloride from 0.005 to 0.05 mol $L^{-1}$ was studied while the concentration of ferric cyanide was fixed at 0.01 mol $L^{-1}$. The concentrations of the standard uric acid solutions were studied over the range of 5-50 mg $dL^{-1}$. The sensitivities increased when the concentration of ferric chloride was increased and then reached a plateau (Figure 4D). The concentration of 0.01 mol $L^{-1}$ was regarded as suitable compromise between sensitivity and reagent consumption.

### 3.2.3 Reaction time

In this work, the reaction time was defined as the period that elapsed between the standard/sample solutions being transferred to the PAD and the optical image of the product being captured. When the reaction time was increased from 1 to 3 min, the sensitivity of detection of creatinine and uric acid also increased (Figure 4E). At grater reaction time, the sensitivities of detection of the above-mentioned analytes were not different. Nevertheless, in the case of the detection of an albumin, the sensitivity gradually increased when the investigated time was increased. A reaction time of 3 min was selected after considering the sensitivity of the method for all three analytes and the analysis time. By the selected time, a sample throughput of ~ 20 samples $h^{-1}$ (for the simultaneous determination of three analytes) became possible. This was very useful for real routine work.

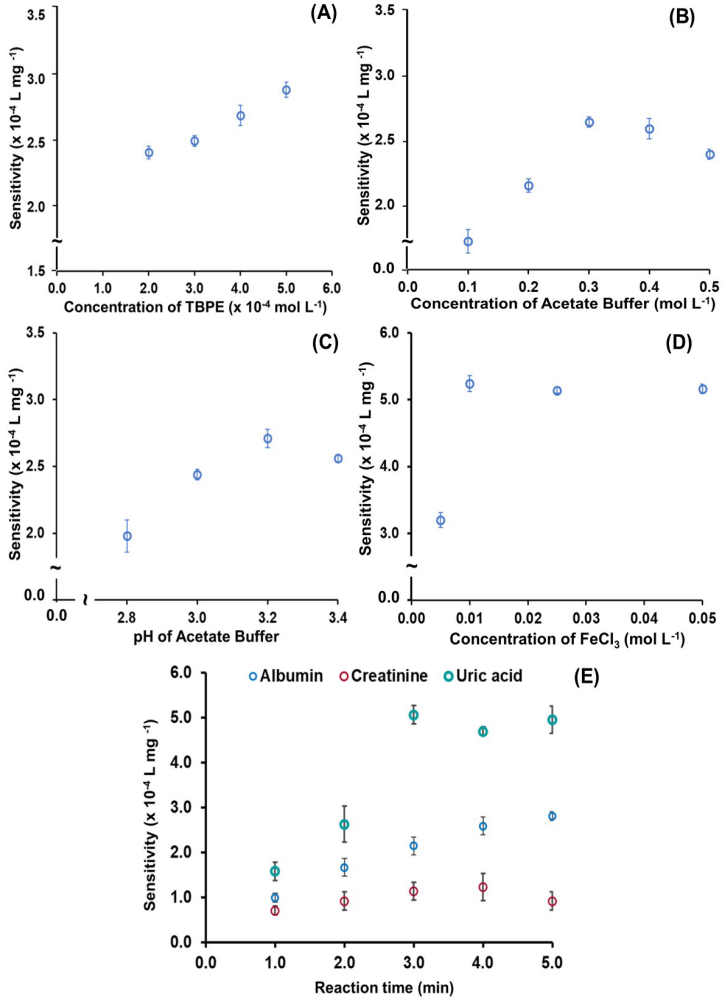


**Figure 4.** Summary of the results for the optimization studies. (A): Effect of concentration of TBPE, (B): Effect of concentration of acetate buffer, (C): Effect of pH of acetate buffer, (D): Effect of concentration of $FeCl_3$, and (E): Effect of reaction time

### 3.3 Interference effect

The interference effect of foreign ions and some organic compounds found in human urine were examined using another design of the μPAD as depicted in Figure 5. The hydrophobic zone (the black color area) was still created by contact stamping of the indelible ink. The evaluated concentrations of the interferences were much greater than their values in typical human urine. Each chromogenic reagent was added onto all the hydrophilic lobes (from A to S), excluding the control well (C′) of each μPAD (See Figure 5A to 5C). The results clearly demonstrated that the colored products developed only when the target analytes were applied to the μPADs at the standard analyte (S) lobes. These results confirmed that the proposed method was not perturbed by the interference effect and the employed chromogenic reagents were highly selective.

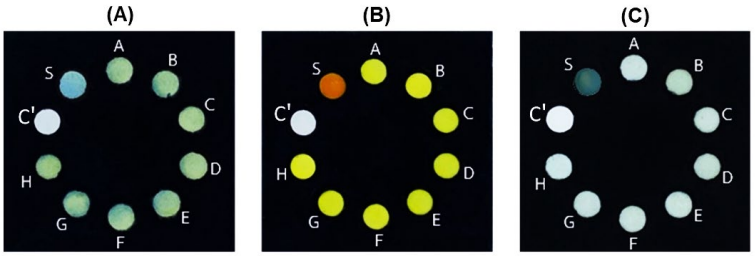


**Figure 5.** Optical images of the mPADs after spiking various interferences. Each PAD was immobilized with the chromogenic reagents by dropping, (A): TBPE ($5 \times 10^{-4}$ mol $L^{-1}$), (B): 0.025 mol $L^{-1}$ picric acid in 1.0 mol $L^{-1}$ NaOH (0.025 mol $L^{-1}$ picric acid in 1.0 mol $L^{-1}$) and (C): mixed reagent solution (0.01 mol $L^{-1}$ ferric chloride+0.01 mol $L^{-1}$ ferric cyanide) on all the hydrophilic lobs (excepting the control C' lobe). Note: Each lobe on each PAD was spiked with 0.6 μL of the blank/interference solutions as listed: A: water, B: 20 g $L^{-1}$ urea, C: 30 mg $L^{-1}$ glucose, D: 3 mg $L^{-1}$ascorbic acid, E: 3 g $L^{-1}$ NaCl, F: 3 g $L^{-1}$ $NH_4Cl$, G: 3 g $L^{-1}$ KCl, H: 3 g $L^{-1}$ $MgSO_4$ and S: Mixed standard solutions (100 mg $L^{-1}$ albumin+3 g $L^{-1}$ creatinine+50 mg $dL^{-1}$ uric acid).

### 3.4 Analytical performances

Under the optimal conditions for the colorimetric detections of the target analytes which were achieved by the optimization study and were concluded in Section 3.2, the analytical performance of the μPAD for the simultaneous determination of albumin, creatinine and uric acid is summarized in Table 1. Examples of the optical images of the μPAD and the corresponded standard addition plots of the analytes are represented in Figure 6. The x-axes are the concentrations of the spiked standards while the y-axes are the color intensity ratios. The ratio of blue to red (B/R) intensity values was calculated in the determination of albumin and uric acid while the ratio of red to green (R/G) values was calculated for the measurement of creatinine. When these ratios were plotted, the standard addition curves were found to be in good linearity at the concentrations of the analytes ($r^2$ > 0.99). The evaluation of the reproducibility was carried out by ten replicate measurements of the color intensities obtained when the standard solutions of 10 mg $L^{-1}$ albumin, 100 mg $L^{-1}$ creatinine, and 25 mg $dL^{-1}$ uric acid were investigated. The relative standard deviation (RSD) values were below 5.56 %. This indicated that the proposed μPAD offered high precision. The minimum detection level (MDL) values were sensitive enough for clinical diagnoses of kidney disease and gout. Note that the concentration of albumin at an earlier stage of kidney disorder is 30 mg $L^{-1}$ [1]. The minimum urinary creatinine content in human is 50 mg $L^{-1}$ [3], and the amount of uric acid greater than 6.8 mg $dL^{-1}$ is indicative of a high risk of hyperuricemia and kidney disease [4].

**Table 1.** The analytical performances of the μPAD for the simultaneous measurement of albumin, creatinine, and uric acid

| Analytical Performances | Kinds of Analytes | | |
|---|---|---|---|
| | **Albumin** | **Creatinine** | **Uric Acid** |
| Linear working ranges | 0-100 mg $L^{-1}$ | 0-1000 mg $L^{-1}$ | 0-50 mg $dL^{-1}$ |
| RSD (n = 10) | 2.31 % | 1.24 % | 5.56 % |
| MDL* | 10 mg $L^{-1}$ | 50 mg $L^{-1}$ | 5 mg $dL^{-1}$ |

Note: *MDL is defined as the lowest concentration of a standard analyte that can be detected on the paper device.

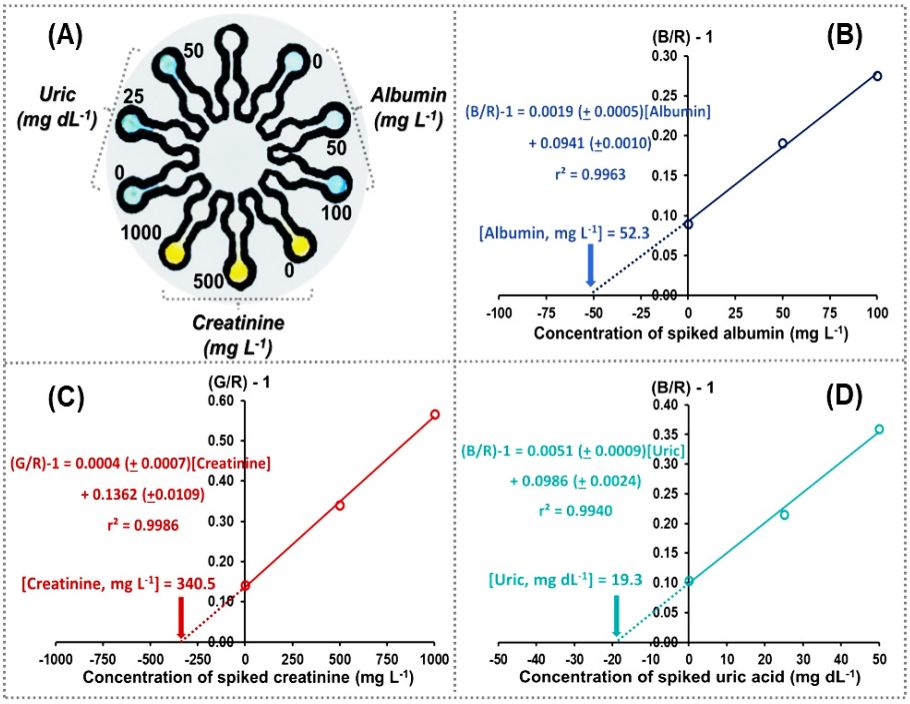


**Figure 6.** Examples of (A): Optical image of the μPAD for the simultaneous determination of urinary albumin, creatinine, and uric acid, based on the standard addition method. (B), (C) and (D): The corresponding standard addition curves for the determination of albumin, creatinine, and uric acid in urine sample, respectively.

## 3.5 Application to urine samples and validation

The developed μPAD was applied to four urine samples of normal volunteers. All samples were filtered through a 0.22 μm nylon membrane and the filtrates were two-fold diluted with water before analysis to decolorize the original urine color. Recovery was first investigated. The results in Table 2 reveal that the recoveries ranged from 91.8-109.7%. These results imply that the method was not affected by the urine matrix.

**Table 2.** Summary of the results obtained by the recovery study. The determination of the analytes was carried out in triplicate.

| Sample Code | Albumin Content (mg $L^{-1}$, mean±SD) | | Albumin Recovery (%) | Creatinine Content (mg $L^{-1}$, mean±SD) | | Creatinine Recovery (%) | Uric Content (mg $dL^{-1}$, mean±SD) | | Uric Recovery (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Added | Found | | Added | Found | | Added | Found | |
| M1 | 50.0 | 52.8±0.97 | 105.6 | 200 | 535.2±13.1 | 94.16 | 20.0 | 21.0±1.79 | 105.6 |
| M2 | 50.0 | 50.7±0.62 | 101.4 | 200 | 460.7±10.8 | 103.4 | 20.0 | 18.4±0.75 | 91.80 |
| W1 | 50.0 | 48.7±1.35 | 97.33 | 200 | 611.3±8.15 | 109.7 | 20.0 | 19.4±1.46 | 96.87 |
| W2 | 50.0 | 46.9±0.78 | 93.85 | 200 | 488.0±14.5 | 95.57 | 20.0 | 18.8±0.42 | 94.00 |

The concentrations of the analytes as determined by the developed μPAD and the validating methods were compared (Figure 7A to 7C). By means of the statistical paired *t*-test [34], the results did not show significant differences at 95% confidence (albumin: $t_{stat}$ = -0.130, $t_{cri}$ = 3.182, creatinine: $t_{stat}$ = -0.133, $t_{cri}$ = 3.182, and uric acid: $t_{stat}$ = 1.119, $t_{cri}$ =: 3.182). The comparison confirmed that the developed μPAD provided reliable results.

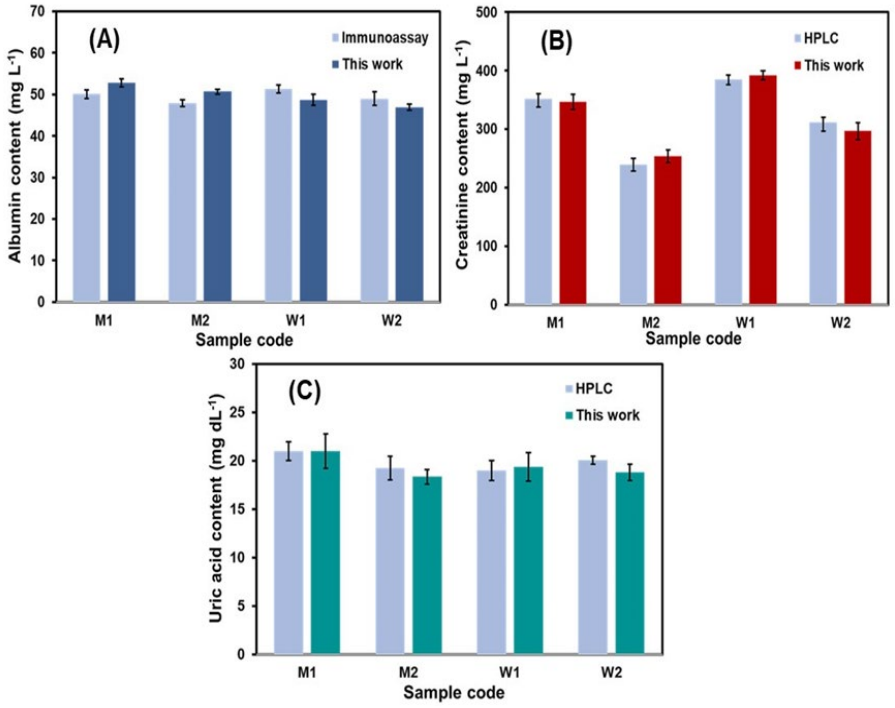


**Figure 7.** Comparison of the concentrations of (A): Albumin, (B): Creatinine, and (C): Uric acid, determined by the validating methods and by this work.

## 4. Conclusions

This work was the first report that the μPAD for the simultaneous determination of urinary albumin, creatinine, and uric acid was successfully developed. The hydrophobic area of the μPAD was easily patterned using cost-effective contact stamping of indelable ink onto filter paper. The flower-liked

μPAD configuration facilitated the simple process of standard addition on the paper platform. The μPAD offered high precision and high accurracy, and allowed rapid simultaneous analysis of the three analytes (~ 3 min / sample). Therefore, it can be concluded that the developed μPAD is one of an effective devices for the clinical diagnoses of renal dysfunction and gout.

## 5. Acknowledgements

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