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Design and Fabrication of a Microfluidic Chip Device for Colorimetric Quantitative Analysis of Albumin in Urine Matrix Solution

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ABSTRACT. The existence of albumin in urine indicates kidney impairment, referred to as albuminuria. Early detection of kidney dysfunction can be done through the examination of urine albumin levels. This research aims to develop a microfluidic chip device for quantitative analysis of albumin in urine matrix solution using colorimetry with a smartphone-based device. The microfluidic chip device for albumin is tested and evaluated through functional tests and analysis of the quality of measurement data generated. The functional tests of the colorimetric system include testing the hydrophobic properties of the fluid on the material surface, fluid distribution pattern, color appearance, and leakage. The quality of measurement data is evaluated based on the predictive ability of the partial least squares regression models generated by data measurement for predicting the albumin levels accurately and precisely. A prototype of the microfluidic chip device for albumin was made from transparent acrylic material, with a 3-dimensional vertical fluid flow design consisting of two main parts: the inlet section and the colorimetric system for visual fluid observation. Based on the functional tests, it is found that the acrylic material in the colorimetric system has a hydrophilic surface characteristic with a contact angle of 61.15°. The fluid can flow and distribute well within the colorimetric system without producing bubbles. The color appearance in the colorimetric system is also visualized well without fluid leakage. On the other hand, RGB HSL data, as the digital image identity of the training and test samples, can be used as predictor variables. The method validation demonstrated acceptable sensitivity, with an LOD of 3.368 mg/dL and an LOQ of 11.825 mg/dL. Precision at 16 mg/dL showed good repeatability (RSD 0.561%, recovery 99.26%;) and reproducibility (RSD 2.147%, recovery 101.42%). These results confirm the method's reliability for albumin quantification at the tested concentration level.

Keywords: Albumin, albuminuria, colorimetry, microfluidic chip, PLSR, urine analysis

INTRODUCTION

The presence of albumin compounds in urine can serve as indicators of kidney function and overall health (Ketha & Singh, 2016). Increased albumin level in urine indicate kidney dysfunction (Aitekenov et al., 2021). Albumin is a protein usually present in high concentrations in human blood serum (>30 mg/dL), but its presence in urine should not exceed the clinical threshold value of 30 mg/dL. Albuminuria is the presence of a particular concentration of albumin in the urine. Albuminuria is classified into two categories: microalbuminuria if the rate of albumin excretion into urine reaches 30-300 mg/24 hours, and macroalbuminuria if the rate of albumin excretion exceeds 300 mg/24 hours (Ketha & Singh, 2016).

Routine monitoring of albumin and creatinine levels in urine is essential for assessing kidney function (Delanghe et al., 2017), especially for high-risk individuals with a history of diabetes and cardiovascular disease (Comper & Osicka, 2005). Various instruments and methods have been developed for quantitative analysis of urinary albumin, such as spectrophotometric determination of albumin-based on the ternary complex-formation reaction (Yamaguchi et al., 2005), UPLC-MS-based analysis (Ma et al., 2020), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Ketha & Singh, 2016), and liquid chromatography-isotope dilution tandem mass spectrometry (Chen et al., 2021). While these methods provide accurate and reliable measurement results (Aitekenov et al., 2021), the primary instrumentation is typically large, heavy, non-portable, expensive, and complex. it requires skilled personnel, leading to relatively high analysis costs (Geng et al., 2023).

Overtime, on-site testing, and point-of-care diagnostics need simple, fast, accurate, and affordable analysis methods and instruments. Point-of-care diagnostics refers to the ability to perform diagnostic tests and obtain results quickly at the exact location of the patient, such as in clinics, hospitals, or direct care environments. It means there is no need to

send samples to external laboratories and wait a long time to receive results. Point-of-care diagnostic tests are designed to provide immediate and direct results, enabling prompt medical decision-making and intervention. This technology is often utilized in urgent situations, remote areas, or locations with limited access to laboratory facilities. Point-of-care diagnostics encompasses a wide range of tests, including blood, urine, or other biological sample tests, and can be conducted using portable devices or specially developed smartphone applications.

Researchers have developed various portable auxiliary devices for albumin analysis in the context of point-of-care diagnostics. Some device platforms developed, such as 3D-printed microfluidic chips (Chan et al., 2016), paper-based microfluidic chips (de Oliveira et al., 2017), paper-plastic hybrid microfluidic chips (Uddin et al., 2017), paper-based devices for visual detection (Hiraoka et al., 2020), microfluidic colorimetric detection platform (Laurenciano et al., 2021), tags for in situ sensing (Jia et al., 2022), and MIP dual electrochemical sensors for one-step albumin analysis (Wardani et al., 2023).

Digital image colorimetry on smartphone is one of the simple, easy, and affordable analysis methods (Fan et al., 2021). Smartphone-based colorimetric sensors enable convenient and immediate on-site testing and point-of-care diagnostics, serving urgent needs beyond the confines of a laboratory (Geng et al., 2023). Smartphones are versatile analytical tools that enable data acquisition, processing, and on-device result generation (Böck et al., 2020). Several application platforms, such as ColorX (Šafranko et al., 2019), Color Analysis Pro, Albumin Tester (Coskun et al., 2013), Albumin Smart Test (Mathaweesansurn et al., 2017), and PhotoMetrix® (Böck et al., 2020), are reported for smartphone-based colorimetric analysis. Despite these advances, many of these strategies remain constrained by cumbersome sample holders, limited integration of fluidic control, and a reliance on discrete calibration strips.

In this work, we introduce an acrylic-based microfluidic chip featuring a 3D vertical-flow architecture that uniquely combines precise fluid handling with a simplified fabrication workflow. Acrylic fabrication affords robust channel definition, optical clarity for colorimetric readout, and compatibility with low-cost laser-cutting or CNC processes. Together, these features constitute a portable, accurate, and user-friendly for point-of-care monitoring of urinary albumin, advancing beyond prior paper-based and lateral-flow devices.

EXPERIMENTAL SECTION

The materials utilized in this research included the bromocresol green albumin assay kit (Sigma Aldrich, USA), Albumin standard solution 5 g/dL (Sigma Aldrich, USA), distillated water (Ikapharmindo Putramas, Indonesia), and morning spot urine samples. The equipment used comprised a 100 µL microfluidic chip device made of acrylic material, a 5-50 µL micropipette, a 25 ml volumetric pipette, a 100 ml measuring flask, a 500 µL PCR vial, a digital lux meter (Benetech), a HiView digital microscope (made in China), and a Samsung Galaxy A325F/DS smartphone equipped with a quad-camera rear system. The primary camera boasts a 64MP resolution, an 8MP ultra-wide camera, a 5MP macro camera, and a 2MP depth sensor.

Design and Fabrication of Microfluidic Devices

A prototype microfluidic chip device for albumin was developed, featuring a three-dimensional design with a vertical fluidic flow mechanism. The microfluidic chip design comprises two primary compartments: a fluid inlet channel and a colorimetric system chamber. The inlet channel is 1 mm in diameter and 5 mm in length. The colorimetric system chamber measures 9.24 mm in diameter and 1 mm in thickness and can hold 100 µL volume of fluid (Figure 1). Design and fabrication of the microfluidic chip device involved the utilization of Corel draw software and a laser cutting machine. Following fabrication, the microfluidic chip device underwent functional colorimetric tests, including assessments of fluid-material hydrophobicity, fluid distribution patterns, color visualization, and leakages.

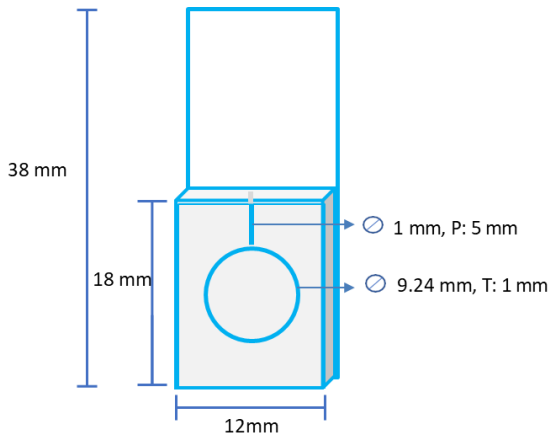

Figure 1. Microfluidic Chip Design

Preparation of Urine Solution

About 25 ml of urine was mixed with distilled water in a measuring flask and diluted until the total volume reached 100 ml. The mixture was then vigorously shaken until a uniform solution was achieved.

Preparation of Albumin in Urine Matrix Solution.

The stock solution of the albumin in urine as matrix solution was prepared by mixing 50 µL of the diluted urine fluid with a certain amount of standard albumin solution (5 g/dL) and distilled water to obtain albumin-urine solutions with various concentration variations, as detailed in **Table 1**.

Preparation of Training Sample

The training sample solutions for colorimetric analysis (CS) were prepared by reacting five microliters of albumin-urine solution from previous preparation (AU) with 120 µL of bromocresol green (BCG) reagent in Eppendorf tubes (**Table 2**) and allowed to stand at room temperature for 5 minutes.

Preparation of Testing Sample

Testing samples were prepared with an albumin of 0.4 g/dL. The procedure for preparing the testing sample was the same as the training sample, where it was made by taking 5 µL of the albumin–urine stock solution (AU-TS) and reacting it with 120 µL of the BCG reagent solution, yielding an effective concentration post-reagent addition of 16 mg/dL (**Table 3**). The testing sample was left to stand for 5 minutes before conducting colorimetric measurements.

Preparation of Blank Solutions for LOD and LOQ Determination

Seven matrix blank replicates were prepared by mixing 5 μL of distilled water with 120 μL of BCG reagent and loading each mixture into the microfluidic chip. The blanks were left to stand for 5 minutes before conducting colorimetric measurements under identical lighting and with the same digital camera settings. From each image, the mean RGB and HSL values within the region of interest were extracted and submitted to the pre-trained PLSR model to yield predicted blank concentrations (Y_B). The overall blank mean ($\hat{Y}_B$) and standard deviation (S_B) were calculated across all replicates. The limits of detection (LOD) and quantitation (LOQ) were then defined as $\hat{Y}_B + (3 \times S_B)$ and $\hat{Y}_B + (10 \times S_B)$, respectively, then interpreted directly in units of mg/dL.

Table 1. Preparation of albumin in urine matrix solution (AU)

ID	Albumin stock solution 5 g/dL (ml)	Distilled Water (ml)	Urine (ml)	Total Volume (ml)	[Albumin-Urine] (g/dL)
AU-1	100	100	50	250	2
AU-2	80	120	50	250	1.6
AU-3	40	160	50	250	0.8
AU-4	12	188	50	250	0.24
AU-5	2	198	50	250	0.04
Blank	0	200	50	250	0.00
AU-TS	20	180	50	250	0.4

Table 2. Preparation of training sample solution for colorimetric analysis

ID	[Albumin-Urin] (g/dL)	Vol. [Albumin-Urin] (ml)	Vol. BCG (ml)	[Albumin-Urin]* (mg/dL)
CS-1	AU-1: 2	5	120	80
CS-2	AU-2: 1.6	5	120	64
CS-3	AU-3: 0.8	5	120	32
CS-4	AU-4: 0.24	5	120	9.6
CS-5	AU-6: 0.04	5	120	1.6
Blank	Blank: 0.00	0	120	0

**Effective concentration post-reagent addition*

Table 3. Preparation of testing sample

ID	AU-TS: 0.4 g/dL (ml)	BCG (ml)	[Albumin-Urin]* (mg/dL)
TS-TR	5	120	16
TS-PR-1	5	120	16
TS-PR-2	5	120	16
TS-PR-3	5	120	16

**Effective concentration post-reagent addition*

Colorimetric Analysis and Digital Image Processing

The colorimetric analysis was performed by injecting 100 µL of the training sample and testing sample solutions into a colorimetry system that utilizes a microvolume chip device. The color appearance observed on the microfluidic chip device was recorded as a photograph using a smartphone camera under ambient lighting conditions of 109.5 lux. The captured image was subsequently processed and analyzed using Color Analysis Pro software version 6 (Leizersoft) for extracting RGB and HSL parameter values.

To control illumination-induced variability in image-based measurements, the testing sample (TS-TR) was photographed in triplicate under identical smartphone camera and lighting conditions. These technical replicates were employed to assess repeatability of the RGB–HSL data extraction procedure. Subsequently, to evaluate the variability associated with sample preparation, three independent aliquots of the 0.40 g/dL albumin sample solution were prepared separately, and each was photographed under identical lighting conditions using the same camera device. These sample preparative replicates (TS-PR) reflect intermediate precision (within-laboratory reproducibility), capturing variability arising from pipetting, mixing, and reagent addition.

Building Predictive Models and Evaluating the Quality of Measurement Data

The predictive model for analyzing albumin levels in urine matrix solutions was performed using a chemometric approach, i.e., Partial Least Squares Regression (PLSR). This process was conducted using Minitab 18 software (Minitab Inc., USA). The obtained predictive model was evaluated for accuracy (% recovery) and precision (RSD).

RESULTS AND DISCUSSION

Designing and fabricating a microfluidic chip device

The research focused on developing a microfluidic chip device that serves as an assisting tool for colorimetric quantitative albumin analysis. The device was fabricated from acrylic materials, measures 38×12×3 mm in overall dimensions, and consists of an inlet channel and a color-visualizing chamber. The carefully designed microvolume inlet channel, with a 1 mm diameter and 5 mm length, prevents sudden liquid spillage from the microfluidic chip device. It is due to the significant atmospheric pressure difference between the external and internal compartments. On the other hand, the colorimetry visualizing chamber, with a 9.24 mm in diameter and 1 mm thickness, provides a larger and more spacious area for convenient visual color observation and image capture, even with microvolume-sized samples (see **Figure 2**).

Functional Evaluation of Colorimetry in a Microfluidic Chip

The microfluidic chip's performance was evaluated using four criteria: the surface hydrophobicity properties of the material, fluid dispersion, color appearance, and device leakage. As an assistive colorimetric analysis tool, it is desirable to have a surface material with hydrophilic characteristics that align with the properties of the albumin-urine solution. Additionally, the device should exhibit homogenous fluid dispersion without air bubbles, enable observable and easily distinguishable color appearance, and be free from leakage.

Surface hydrophobicity properties of the microfluidic chip material

The inclusion of carbonyl groups in the structure of polymethyl methacrylate allows the acrylic material utilized in microfluidic chip fabrication to possess hydrophilic surface properties. The surface hydrophobicity testing results confirm it (see **Figure 3**). The acrylic material has a hydrophilic surface characteristic with a contact angle of 61.15°. This characteristic offers an advantage in which sample solution droplets placed in the color visualization compartment are securely held within the microvolume space, preventing undesired spills or leaks from the compartment area, even when the inlet channel is positioned horizontally or upside down (see **Figure 6**).

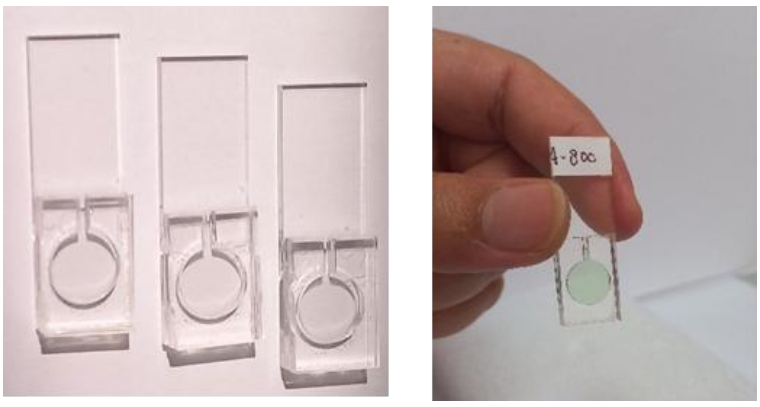

Figure 2: Prototype of a microfluidic chip device

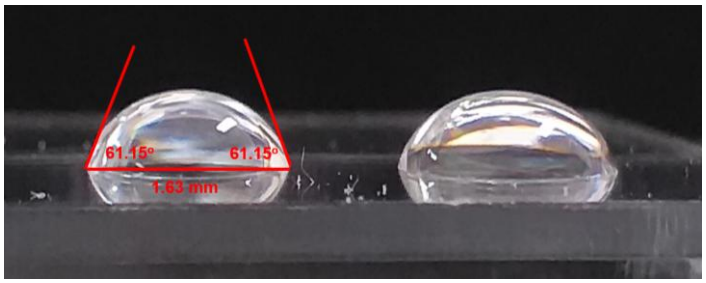

Figure 3. Prototype Surface hydrophobicity properties of the acrylic

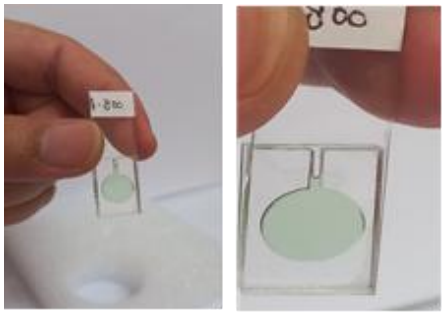

Figure 4. Dispersion of the fluid in a microfluidic chip

Dispersion of the fluid in a microfluidic chip

Achieving a homogeneous fluid dispersion within the microvolume space is crucial for obtaining an even and consistent color appearance. Trapped air bubbles within the color visualization chamber typically cause non-uniform fluid dispersion. Removing these air bubbles becomes challenging once they are formed and trapped within the compartment because of the fluid surface tension and the high atmospheric pressure. Based on the observed results of the fluid dispersion testing conducted in the microfluidic chip device, it is evident that the device effectively retains and evenly disperses the fluid, resulting in a homogeneous and bubble-free fluid distribution (**Figure 4**).

Color appearance of the fluid in a microfluidic chip

The color appearance of the sample solution in the colorimetry visualization chamber is crucial and influential for achieving successful colorimetric analysis. The BCG reagent exhibits an initial yellow-orange color, transitioning to a yellowish-green and ultimately a bluish green (cyan) shade with increasing albumin concentration (**Figure 6**). These color variations serve as a visual indicator of the albumin concentration in the analyzed sample solution. Despite the small volume capacity, the color differences in the sample fluid within the microfluidic chip device can be easily observed.

Leakage testing of the microfluidic chip device

After assembling and connecting the acrylic chip pieces, a leakage test is conducted to ensure there is no fluid leakage outside the fluid flow system. Leakage in microfluidic chip devices can cause changes in volume and color stability. The leakage test involves injecting a colored solution into the color visualization chamber in a microfluidic chip device and allowing it to remain for 12 hours for observation. There is no leakage observed during the 12-hour observation period. Additionally, the fluid does not easily spill even when the chip is placed horizontally (**Figure 6**).

Colorimetric Analysis

Image processing

The colorimetric analysis involves capturing digital images of both the calibration and test samples using a smartphone. These digital images are then processed using specialized software called Color Analysis Pro. The software extracts various data, including color group information, percentage of color groups, specific color names, RGB color intensity, and HSL values. An example of this analysis can be seen in **Figure 8**. **Table 4** presents the comprehensive digital-image analysis results for the test samples, including technical replicate test samples (to assess repeatability) and preparative replicate test samples (to assess reproducibility).

Figure 5. Color appearance of the fluid in a microfluidic chip

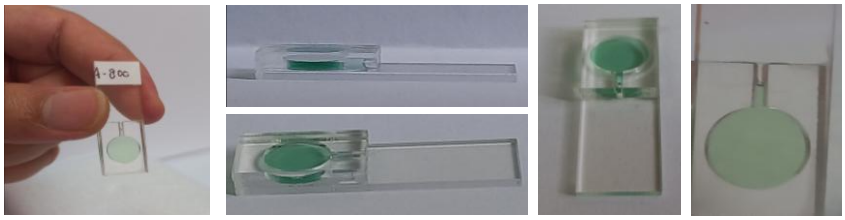

Figure 6. Leakage test of the microfluidic chip device

Predictive model development and colorimetric albumin measurement

A Partial Least Squares Regression (PLSR) model was constructed to predict albumin concentration based on six colorimetric variables extracted from digital images: Red (R), Green (G), Blue (B), Hue (H), Saturation (S), and Lightness (L). The results of the PLSR analysis are summarized in **Table 5**. The model utilized leave-one-out cross-validation for component selection. Among four components evaluated, the two-component model was selected as optimal, as it provided the lowest predicted residual sum of squares (PRESS = 197.29) and the highest predicted R-squared value (R^2_{pred} = 0.9658). The cumulative variance explained by the two components in the X matrix was 97.42%, indicating that the model effectively captured the underlying variation in the predictors. The selected PLSR model demonstrated excellent performance with a high coefficient of determination for the response variable albumin (R^2 = 0.9935), reflecting a strong linear relationship between the predictors and the measured albumin concentrations. Analysis of variance (ANOVA) supported the statistical validity of the model, with an F-value of 229.31 and a highly significant p-value (p = 0.001), confirming that the regression model significantly explained the variation in albumin levels compared to the residual error.

Standardized regression coefficients revealed the relative influence of each color parameter. Blue (B) and Hue (H) contributed positively to the albumin prediction, with standardized coefficients of 0.171 and 0.233, respectively. In contrast, Lightness (L) and Green (G) exhibited strong negative contributions (standardized coefficients of -0.213 and -0.237, respectively). The variable Saturation (S) showed a negligible effect. These results suggest that changes in blue and hue intensities are associated with higher albumin levels, while increases in green and lightness reduce the predicted concentration. Based on these PLSR-derived coefficients, the full linear regression equation is as follows:

$$[Albumin] = 119.859 + (-0.240 \times R) + (-0.602 \times G) + (0.667 \times B) + (0.163 \times H) + (-0.054 \times S) + (-1.492 \times L)$$

The model's predictive performance was further evaluated through residual analysis. The predicted albumin values closely matched the actual measurements across all samples, with small residuals and standardized residuals within acceptable limits. The PLS response plot for albumin, constructed from the leave-one-out cross-validated "$Fits_{(pred)}$" values (calculated response) versus the observed albumin concentrations (actual response), provides a direct visual assessment of the two-component PLSR model's predictive performance. In this scatter plot, each point represents one of the six urine samples, plotted with its true concentration on the x-axis and its cross-validated prediction on the y-axis (**Figure 9**).

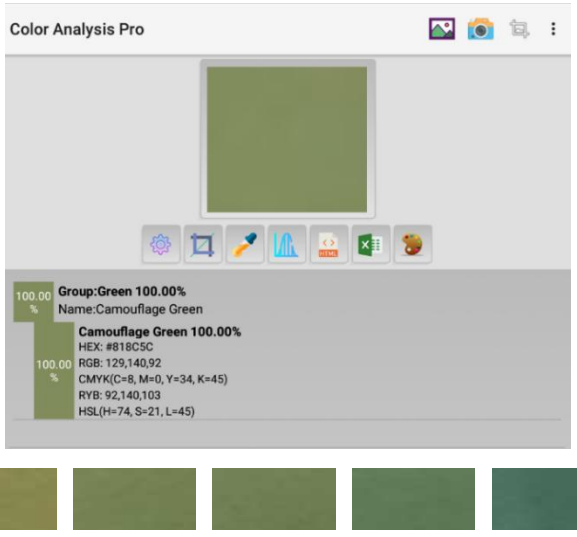

Figure 8: Digital image processing and measurement of RGB color intensity and HSL values using color analysis pro software.

Table 4: Results of digital image analysis for the training sample (CS), test sample (TS) and blank

ID	[Albumin] mg/dL	R	G	B	H	S	L
CS-1	80	72	112	103	167	22	36
CS-2	64	74	115	99	157	22	37
CS-3	32	103	132	101	116	13	46
CS-4	9.6	129	141	95	76	19	46
CS-5	1.6	137	137	79	60	27	42
Blank	0	146	143	92	57	23	47
TS-TR-1	16	121	138	94	83	19	45
TS-TR-2	16	120	137	93	83	19	45
TS-TR-3	16	121	138	96	84	18	46
TS-PR-1	16	122	137	96	82	18	46
TS-PR-2	16	120	135	92	81	19	45
TS-PR-3	16	119	135	92	82	19	45
Blank-1	0	146	143	92	57	23	47
Blank-2	0	140	140	80	60	27	43
Blank-3	0	145	143	94	58	21	47
Blank-4	0	141	138	87	57	24	45
Blank-5	0	143	141	86	58	25	45
Blank-6	0	142	138	82	56	27	44
Blank-7	0	144	139	90	54	23	46

Table 5: Predictive model building using PLSR analysis

PLS Regression: [Albumin] versus R, G, B, H, S, L method

Cross-validation	Leave-one-out
Components to evaluate	Set
Number of components evaluated	4
Number of components selected	2

Analysis of variance for [Albumin]

Source	DF	SS	MS	F	P
Regression	2	5736.56	2868.28	229.31	0.001
Residual Error	3	37.52	12.51		
Total	5	5774.08			

Model selection and validation for [Albumin]

Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.703848	45.8624	0.992057	289.011	0.949947
2	0.974209	37.5241	0.993501	197.288	0.965832
3		23.8945	0.995862	816.146	0.858653
4		0.5889	0.999898	946.507	0.836077

Coefficients

	[Albumin]	[Albumin] standardized
Constant	119.859	0.000000
R	-0.240	-0.227581
G	-0.602	-0.236713
B	0.667	0.171239
H	0.163	0.233092
S	-0.054	-0.007512
L	-1.492	-0.212632

Fits and residuals for [Albumin]

Row	[Albumin]	Fits	Res	S_{Res}	Fits (pred)	Res (pred)
1	80.0	76.0654	3.93455	1.65848	70.5529	9.44709
2	64.0	67.9912	-3.99123	-1.49585	71.0917	-7.09165
3	32.0	32.5055	-0.50551	-0.26881	33.8828	-1.88282
4	9.6	9.9959	-0.39595	-0.13422	10.1353	-0.53526
5	1.6	2.7399	-1.13990	-0.53219	-4.8930	6.49300
6	0.0	-2.0980	2.09803	0.74409	-3.4291	3.42911

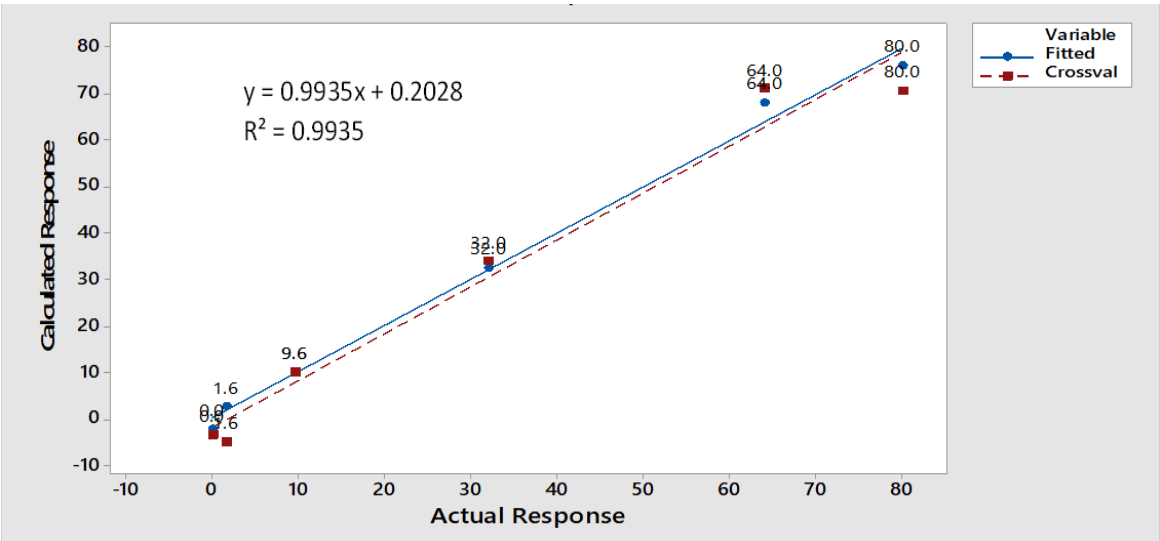

Figure 9. Scatterplot of observed versus predicted albumin concentrations

Table 6. Analytical sensitivity: LOD and LOQ

ID	Calculated Blank Concentration mg/dL	Mean ($\hat{Y}_B$) mg/dL	Stdev (S_B)	LOD $\hat{Y}_B + (3 \times S_B)$	LOQ $\hat{Y}_B + (10 \times S_B)$
Blank-1	-1.978				
Blank-2	-0.495				
Blank-3	-0.133				
Blank-4	1.827	-0.257	1.208	3.368 mg/dL	11.825 mg/dL
Blank-5	-1.017				
Blank-6	-0.581				
Blank-7	0.579				

Table 7. Percent recovery and percent RSD of repeatability

ID	[Albumin] Actual	[Albumin] Calculated	mean	Stdev	% Recovery	RSD
TS-TR-1	16 mg/dL	15.804 mg/dL				
TS-TR-2	16 mg/dL	15.979 mg/dL	15.882 mg/dL	0.089	99.26%	0.561%
TS-TR-3	16 mg/dL	15.863 mg/dL				

Table 8 Percent recovery and percent RSD of reproducibility

ID	[Albumin] Actual	[Albumin] Calculated	mean	Stdev	% Recovery	RSD
TS-PR-1	16 mg/dL	15.889 mg/dL	16.227 mg/dL	0.0349	101.42%	2.147%
TS-PR-2	16 mg/dL	16.19 mg/dL				
TS-PR-3	16 mg/dL	16.593 mg/dL				

Method validation was carried out based on the guidelines established by AOAC International (2016), focusing on the evaluation of sensitivity, precision, and accuracy. Sensitivity was assessed by analyzing seven blank replicates (**Table 6**). The calculated mean blank concentration was –0.257 mg/dL, with a standard deviation of 1.208 mg/dL. From these values, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3.368 mg/dL and 11.825 mg/dL, respectively. These results indicate that the method possesses sufficient sensitivity for detecting albumin within the expected working range.

Method validation

Precision parameters, repeatability and reproducibility, were evaluated at a single concentration level of 16 mg/dL. Repeatability (**Table 7**) was determined using three independently captured measurements of the same sample, resulting in a mean calculated concentration of 15.882 mg/dL, a relative standard deviation (RSD) of 0.561%, and a recovery of 99.26%. These values are within the generally accepted criteria for repeatability, namely RSD ≤3.7% and recovery within 95–105%. Reproducibility (**Table 8**) was evaluated using three independently prepared samples of the same concentration. This yielded a mean calculated concentration of 16.227 mg/dL, an RSD of 2.147%, and a recovery of 101.42%, meeting the AOAC criterion of RSD ≤6% for reproducibility (Latimer Jr. & Latimer Jr., 2023).

Accuracy, expressed as percent recovery, was examined only at the 16 mg/dL level. While the recovery results at this concentration are acceptable, the limited concentration range assessed restricts the generalizability of the accuracy evaluation. Therefore, additional validation at multiple concentration levels is recommended in future work to ensure method trueness across the full analytical range.

CONCLUSIONS

In conclusion, the developed acrylic-based microfluidic chip featuring three-dimensional vertical fluid flow through dedicated inlet channels and colorimetric compartments demonstrated excellent performance in terms of surface hydrophobicity, fluid distribution, colorimetric response, and leak resistance. The proposed microfluidic colorimetric assay demonstrates robust analytical performance in accordance with AOAC International (2016) validation criteria. The method exhibits adequate sensitivity, with an LOD of 3.368 mg/dL and an LOQ of 11.825 mg/dL, ensuring reliable detection and quantitation of albumin within the target range. Precision testing at 16 mg/dL yielded excellent repeatability (RSD 0.561%, recovery 99.26%) and reproducibility (RSD 2.147%, recovery 101.42%), well within the AOAC-specified limits. These results confirm that the assay is both accurate and precise under the evaluated conditions, supporting its suitability for routine quantitative analysis. Future work should extend recovery evaluation across multiple concentration levels to fully characterize trueness throughout the method's dynamic range.

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REFFERENCE

Aitekenov, S., Gaipov, A., & Bukasov, R. (2021). Review: Detection and quantification of proteins in human urine. *Talanta*, *223*, 121718. https://doi.org/10.1016/j.talanta.2020.121718

Böck, F. C., Helfer, G. A., da Costa, A. Ben, Dessuy, M. B., & Ferrão, M. F. (2020). PhotoMetrix and colorimetric image analysis using smartphones. *Journal of Chemometrics*, *34*(12), 1–19. https://doi.org/10.1002/cem.3251

Chan, H. N., Shu, Y., Xiong, B., Chen, Y., Chen, Y., Tian, Q., Michael, S. A., Shen, B., & Wu, H. (2016). Simple, cost-effective 3D printed microfluidic components for disposable, point-of-care colorimetric analysis. *ACS Sensors*, *1*(3), 227–234. https://doi.org/10.1021/acssensors.5b00100

Chen, Y., Liu, H., Loh, T. P., Liu, Q., Teo, T. L., Lee, T. K., & Sethi, S. K. (2021). Measurement of urine albumin by liquid chromatography-isotope dilution tandem mass spectrometry and its application to value assignment of external quality assessment samples and certification of reference materials. *Clinical Chemistry and Laboratory Medicine*, *59*(4), 711–720.

https://doi.org/10.1515/cclm-2020-0969

Comper, W. D., & Osicka, T. M. (2005). Detection of urinary albumin. *Advances in Chronic Kidney Disease*, *12*(2), 170–176. https://doi.org/10.1053/j.ackd.2005.01.012

Coskun, A. F., Nagi, R., Sadeghi, K., Phillips, S., & Ozcan, A. (2013). Albumin testing in urine using a smart-phone. *Lab on a Chip*, *13*(21), 4231–4238. https://doi.org/10.1039/c3lc50785h

de Oliveira, R. A. G., Camargo, F., Pesquero, N. C., & Faria, R. C. (2017). A simple method to produce 2D and 3D microfluidic paper-based analytical devices for clinical analysis. *Analytica Chimica Acta*, *957*, 40–46. https://doi.org/10.1016/j.aca.2017.01.002

Delanghe, J. R., Himpe, J., De Cock, N., Delanghe, S., De Herde, K., Stove, V., & Speeckaert, M. M. (2017). Sensitive albuminuria analysis using dye-binding based test strips. *Clinica Chimica Acta*, *471*(March), 107–112. https://doi.org/10.1016/j.cca.2017.05.032

Fan, Y., Li, J., Guo, Y., Xie, L., & Zhang, G. (2021). Digital image colorimetry on smartphone for chemical analysis: A review. *Measurement: Journal of the International Measurement Confederation*, *171*(December 2020), 1088 29. https://doi.org/10.1016/j.measurement.2020.108829

Geng, Z., Miao, Y., Zhang, G., Liang, X. (2023). Colorimetric biosensor based on smartphone: State-of-art. *Sensors and Actuators A: Physical*, *349*(1 January 2023 114056). https://doi.org/https://doi.org/10.1016/j.sna.2022.114056

Hiraoka, R., Kuwahara, K., Wen, Y. C., Yen, T. H., Hiruta, Y., Cheng, C. M., & Citterio, D. (2020). Paper-based device for naked eye urinary albumin/creatinine ratio evaluation. *ACS Sensors*, *5*(4), 1110–1118. https://doi.org/10.1021/acssensors.0c00050

Jia, Y., Liu, G., Xu, G., Li, X., Shi, Z., Cheng, C., Xu, D., Lu, Y., Liu, Q. (2022). Battery-free and wireless tag for in situ sensing of urinary albumin/creatinine ratio (ACR) for the assessment of albuminuria. *Sensors and Actuators B: Chemical*, *367*(15 September 2022), 132050. https://doi.org/https://doi.org/10.1016/j.snb.2022.132050

Ketha, H., & Singh, R. J. (2016). Quantitation of albumin in urine by liquid chromatography tandem mass spectrometry. *Methods in Molecular Biology*, *1378*, 31–36. https://doi.org/10.1007/978-1-4939-3182-8_4

Latimer Jr., G. W., & Latimer Jr., G. W. (Eds.). (2023). AF-1Guidelines for standard method performance requirements. In *Official Methods of Analysis of AOAC International* (p. 0). Oxford University Press. https://doi.org/10.1093/9780197610145.005.006

Laurenciano, C. J. D., Tseng, C. C., Chen, S. J., Lu, S. Y., Tayo, L. L., & Fu, L. M. (2021). Microfluidic colorimetric detection platform with sliding hybrid PMMA/paper microchip for human urine and blood sample analysis. *Talanta*, *231*(March), 122362. https://doi.org/10.1016/j.talanta.2021.122362

Ma, T., Liu, T., Xie, P., Jiang, S., Yi, W., Dai, P., & Guo, X. (2020). UPLC-MS-based urine nontargeted metabolic profiling identifies dysregulation of pantothenate and CoA biosynthesis pathway in diabetic kidney disease. *Life Sciences*, *258*(6), 118160. https://doi.org/10.1016/j.lfs.2020.118160

Mathaweesansurn, A., Maneerat, N., & Choengchan, N. (2017). A mobile phone-based analyzer for quantitative determination of urinary albumin using self-calibration approach. *Sensors and Actuators, B: Chemical*, *242*, 476–483. https://doi.org/10.1016/j.snb.2016.11.057

Šafranko, S., Živković, P., Stanković, A., Medvidović-Kosanović, M., Széchenyi, A., & Jokić, S. (2019). Designing ColorX, image processing software for colorimetric determination of concentration, to facilitate students' investigation of analytical chemistry concepts using digital imaging technology. *Journal of Chemical Education*, *96*(9), 1928–1937. https://doi.org/10.1021/acs.jchemed.8b00920

Uddin, M. J., Jin, G. J., & Shim, J. S. (2017). Paper-plastic hybrid microfluidic device for smartphone-based colorimetric analysis of urine. *Analytical Chemistry*, *89*(24), 13160–13166. https://doi.org/10.1021/acs.analchem.7b02612

Yamaguchi, T., Amano, E., Kamino, S., Umehara, S., Yanaihara, C., & Fujita, Y. (2005). Spectrophotometric determination of urinary protein with o-sulfophenylfluorone-metal complex. *Analytical Sciences*, *21*(10), 1237–1240. https://doi.org/10.2116/analsci.21.1237