



A mobile phone-based analyzer for quantitative determination of urinary albumin using self-calibration approach



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ABSTRACT

This work demonstrates use of a smart mobile phone installed with an Android application, termed 'Albumin smart test', as an analyzer for quantitative determination of urinary albumin. The reaction between albumin and tetrabromophenolphthalein ethyl ester (TBPE) in the presence of Triton X-100 was employed for detection principle. The mobile phone was exploited with the sample cassette and the test paper. One sample cassette composes of two holders for accommodation of control and test samples. The test paper was designed in order to contain standard colorimetric strip and space for situating the sample cassette. Optical images of the strip and the samples were simultaneously captured in a single shot by a digital camera of the mobile phone and were digitally processed by the developed application for quantification of the albumin concentration based on self-calibration approach. With the advantage of self-calibration, the albumin test by our mobile phone can be performed in ambient light without using any extra module integrated with lighting control device. The other advantages are portability, ease of implementation and rapid analysis (3 min) with high precision ($RSD \leq 2.5\%$) and high accuracy (Recovery = $98.7\% \pm 1.6$). The mobile device was successfully applied to diagnosis of microalbuminuria.

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1. Introduction

Chronic kidney disease (CKD) is one of a major public health problem worldwide [1,2]. In Thailand, data from the Ministry of Health reveal that the prevalence of CKD in adults is around 30% [3]. The major outcomes of CKD include kidney failure, complications of decreased kidney function and cardiovascular disease. In order to prevent or delay these adverse outcomes, early detection and treatment are very important especially for person who has high risk factors with hypertension, hyperlipidemia and diabetes [4]. Early stage of kidney dysfunction can be diagnosed using the test on urinary albumin excretion [5]. The albumin concentration in urine collected from normal people is very low ($<30 \text{ mg L}^{-1}$) [6]. In cases of early period of kidney disease, small amount of albumin can leak into urine. This leads to a condition termed as

'microalbuminuria' in which typically exhibits the urinary albumin concentration in the range of $30\text{--}300 \text{ mg L}^{-1}$ [6]. Therefore, the measurement of albumin in urine can be exploited as an initial method for diagnosis of kidney disease.

A large number of analytical methods are presented for the determination of albumin in urine with various methodologies including separation techniques [7,8], immunology [9,10], spectrofluorometry [11] and spectrophotometry [12–16]. The continuous flow-based methods using flow injection analysis (FIA) [17] and sequential injection analysis (SIA) [18–20] are also reported. Advantages of the FIA and the SIA methods are high speed of analysis and high potential for a fully automatic manipulation. However, the methods are not applicable for point-of-care testing because some bulky and costly bench-top apparatuses are required. This can limit the albumin testing to only in laboratory setting. The test paper impregnating the dye stuff is commonly used for field test with naked eye detection [21]. Nevertheless, it does not provide accurate analytical results. Development of a portable analyzer for quantitative measurement of the urinary albumin is therefore very necessary.

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Nowadays, the improvement of electronics and information technology has brought about the revolution of personal computers to smart mobile devices. With the rapid advancement of cell phones and tablets embedded with digital cameras and central processing units, simple mobile devices are reported as a portable analyzer for many applications [22–28]. However, the main limiting parameter for using the smart phones' camera as analytical instrument is that the response is mainly affected by the ambient light. In order to overcome this problem, employment of external light source and enclosure to place and keep hold the mobile phone is essential. Recently, use of a smart-phone attached with 3D-printed enclosure integrated with lighting control device for the determination of albumin in urine was demonstrated [27]. Although this analyzer was effective, requirement of the extra devices could lead the method to complicated and costly measurement.

In this work, we aim to demonstrate a simple method using a mobile phone with the installed application, namely 'Albumin smart test', as the analyzer for quantitative determination of urinary albumin. The Android application was designed for image capturing of urine sample without using any extra apparatus for illuminating control. Our mobile phone analyzer is employed with the sample cassette and the test paper assigned for self-calibration purpose. The association reaction between albumin and tetrabromophenolphthalein ethyl ester (TBPE) in the presence of Triton X-100 was chosen as detection principle. TBPE is predominantly attractive as it has a large molar absorptivity [18]. Advantage of the test paper in term of self-calibration for suppression of error from fluctuation of environmental illumination was demonstrated. Validation and application of the developed method to human urine for diagnosis of the microalbuminuria were also investigated.

2. Materials and methods

2.1. Standard and reagents preparation

All standard and reagents used were of analytical reagent grade. Deionized-distilled water purified by Milli-Q apparatus (18 M Ω /cm, Millipore, USA) was used throughout all the experiments. An albumin standard stock solution containing 1000 mg albumin L⁻¹ was prepared by dissolving 0.1 g of solid powder of human serum albumin (Fluka, Switzerland) in 100.0 mL of water. This standard stock solution was kept in refrigerator (4° C) and the working standard solutions (1 to 50 mg albumin L⁻¹) were freshly prepared by appropriate dilution of the standard stock solution with water. The chromogenic reagent solution (2.0 \times 10⁻⁴ mol L⁻¹ TBPE in 0.2% v/v Triton X-100) was daily prepared by dissolving 0.014 g of TBPE (Aldrich, USA) in 5.0 mL of 99.9% ethanol (Carlo Erba, Italy). Into this solution, 2.0 mL of Triton X-100 (Aldrich, USA) was added. The solution was then made up with ethanol to 100.0 mL. Acetate buffer solution (0.1 mol L⁻¹), exploited for maintaining the pH of solutions, was prepared by adding 0.1 mol L⁻¹ sodium acetate (Rankem, India) in 0.1 mol L⁻¹ acetic acid (Mallinckrodt, Thailand). This solution was adjusted to a final pH of 3.1. The concentrations for all reagents are adapted from P. Inpota et al. [29].

2.2. Apparatuses and analytical workflows

Fig. 1 illustrates key apparatuses exploited for quantitative determination of albumin in urine by our mobile phone-based analyzer. The smart mobile device in Fig. 1A is Samsung Galaxy S5 (Samsung, South Korea) embedded with Android 5.0 operation system. Its specifications are full HD (1920 \times 1080 pixel) main

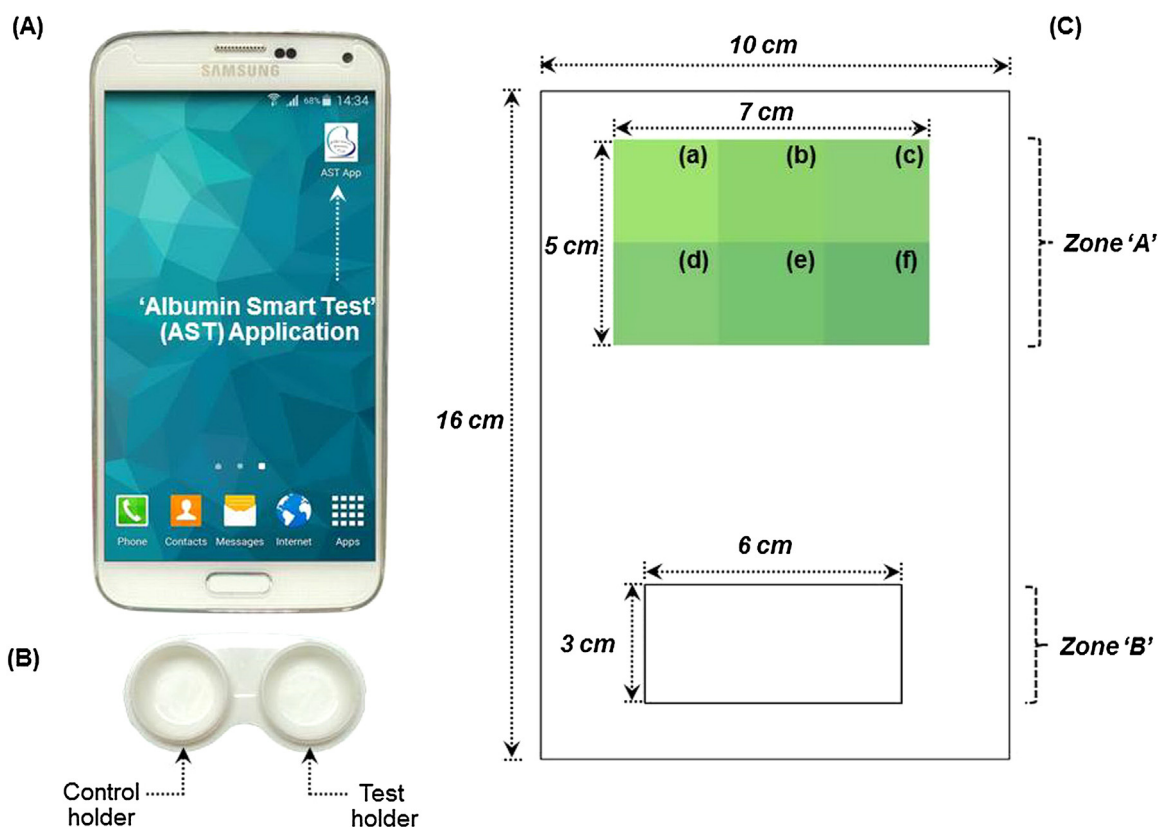


Fig. 1. Key apparatuses exploited with the mobile phone-based analyzer for quantitative determination of urinary albumin. (A) Smart mobile phone, (B) Sample cassette (shown as lids open) and (C) Test paper which contains Zone 'A' and Zone 'B'. Zone 'A': Standard colorimetric strip that composes of printed reference colors representation of (a) 1 mg L⁻¹, (b) 10 mg L⁻¹, (c) 20 mg L⁻¹, (d) 30 mg L⁻¹, (e) 40 mg L⁻¹ and (f) 50 mg L⁻¹ standard albumin. Zone 'B': Space for situating the sample cassette.

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