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Alternative method for measurement of albumin/creatinine ratio using spectrophotometric sequential injection analysis

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ABSTRACT

A simple, automatic and practical system for successive determination of albumin and creatinine has been developed by combining sequential injection analysis (SIA) and highly sensitive dye-binding assays. Albumin detection was based on the increase in the absorbance due to complex formation between albumin and eosin Y in acidic media. The absorbance of the complex was monitored at 547 nm. For the creatinine assay, the concentration of creatinine was measured by reaction with alkaline picrate to form a colored product which absorbs at 500 nm. The influences of experimental variables such as effects of pH, reagent concentration, standard/sample volume and interferences were investigated. Under optimal conditions, the automated method showed linearity up to 20 mg L^{-1} for albumin and 100 mg L^{-1} for creatinine. The 3σ detection limits were 0.6 and 3.5 mg L^{-1} for albumin and creatinine, respectively, and the relative standard deviations (n = 10) were 2.49% for 20 mg L^{-1} albumin, and 3.14% for 20 mg L^{-1} creatinine. Application of the proposed method to the direct analysis of urinary samples yielded results which agreed with those obtained from the Bradford protein assay and a creatinine enzymatic assay according to a paired t-test. The results obtained should be a step towards developing a fully automated and reliable analytical system for clinical research, which requires direct determination of albumin and creatinine and/or its ratios.

1. Introduction

Currently, high rates of mortality are the serious problem for public health worldwide [1]. Albuminuria is a marker of underlying renal disease. In a study of high-risk community, albuminuria was pervasive and indicated future risk of renal death in subjects with diabetes, cardiovascular disease and hypertension, as well as in individuals without diabetes or cardiovascular problems [2–5]. Albuminuria is a pathological condition where albumin is present in the urine. It is a type of proteinuria. At even low levels, called micro-albuminuria, the presence of albumin in urine has been recognized as a marker of many diseases [6,7]. According to the American Diabetes Association (ADA), the gold standard for the measurement of urinary albumin excretion is a 24-h urine collection [8–10]. However, this method has difficulty for assessing micro-albuminuria due to variations in protein concentration over time. For instance, a sample might show a "trace" amount of protein in concentrated urine from a disease-free individual. Conversely, a "trace" amount of protein in diluted urine is most likely to be clinically significant. To eliminate this uncertainty, a more convenient method to detect micro-albuminuria is the albumin/creatinine ratio (ACR) measured in a random urine specimen [11]. Normally, the excretion rate of creatinine in an individual is relatively constant. Thus, the ACR measurement is a more convenient test for patients and may be less prone to errors due to improper collection methods and variations in 24-h protein excretion. Presently, the National Kidney Foundation recommends the use of urine ACR to detect micro-albuminuria.

To avoid mortality, the detection of micro-albuminuria has therefore become an important screening tool to identify people who are at high risk for cardiovascular events and the progression of kidney disease, and who need more intensive therapy. The ADA and the National Kidney Foundation define micro-albuminuria as an ACR between 30 and 300 μg mg^{-1} in both men and women [10,11].

Several point-of-care testing devices for the measurement of micro-albuminuria have recently become available. New routine

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urinalysis reagent strips are now available that measure both the total protein and albumin and allow for determination of the creatinine ratio. These can be used as a first-line test to identify urine samples that should be subjected to more specific quantification. However, these methods have not been previously demonstrated to yield accurate analytical values. Therefore, simultaneous and automated albumin, creatinine and ACR determination for clinical diagnosis and/or estimation of treatment effectiveness is still required.

Dye-binding colorimetric methods are commonly employed in the clinical assay for albumin determination because of their simplicity and speed. Various dyes, for example, bromocresol green and bromocresol purple [12], bromophenol blue [13], tetrabromophenol blue [14], bromochlorophenol blue [15,16], Coomassie Brilliant Blue G [17], bis(3′,3″-diiodo-4′4″-dihydroxy-5′5″-dinitrophenyl)-3,4,5,6-tetrabromosulfo-naphthalein (DIDNTB) [18] and eosin dyes [19–21] have been utilized as color developing reagents. Among these dyes, eosin dyes are particularly interesting because they not only show the highly sensitive and stable protein-dye complexation, but can also be used to estimate a wide range of protein concentrations. Moreover, interference by commonly used laboratory reagents is minimal. Nevertheless, the methods mentioned above are batch analyses that are time-consuming and require large volumes of reagents.

To overcome the limitation of batch analyses, spectrophotometric flow-based analysis systems for protein determination have been reported by several research groups. For example, Zaia et al. proposed a method for total protein analysis using the Bradford method [22]. Furthermore, Sakai et al. proposed tetrabromophenolphthalein ethyl ester (TBPEH) dissolved in a micelle formed by adding Triton X-100 as a chromogenic reagent for protein-TBPE associate formation [23]. In addition, the same research group also demonstrated the availability of sequential injection analysis (SIA) for successive determination of urinary protein and glucose [24].

Creatinine is a byproduct of the creatine kinase reaction and it is formed from spontaneous cyclization of creatine after the dephosphorylation reaction and is produced in a constant ratio versus muscle mass. Many different methods have been proposed for the determination of creatinine in plasma, serum and urine, and most of these have been based on the colorimetric reaction, which takes place between creatinine and sodium picrate in an alkaline medium [25–29]. Several flow injection analysis systems for the determination of creatinine were also proposed to increase the speed and reproducibility of analytical methods [30,31].

The present study describes an automated SIA system and a highly sensitive dye-binding assay for successive determination of albumin, creatinine and their ratio in urinary samples based on eosin Y binding and Jaffe's reaction with sodium picrate, respectively. Two factors prompted us to determine the diagnostic role of measuring urinary albumin and creatinine concentrations and the albumin/creatinine ratios. First, the introduction of selection valve analyzers is widely practicable for the measurement of urinary albumin and creatinine concentrations. Hence, the albumin/creatinine ratio is increasingly used as an aid to interpret elevated urinary protein concentrations. Furthermore, there are few publications that support the use of the albumin/creatinine ratio. This accurate and precise method proposed herein should be useful as a diagnostic identification assay for diseases.

2. Experimental

2.1. Reagents

All chemicals and reagents obtained from various suppliers were analytical reagent grade and were used without further purification. The DI water used throughout the experiments was purified by an Advantec GSH-210 apparatus.

A standard solution of bovine serum albumin (BSA) (1000 mg L^{-1}) was prepared by dissolving 0.0250 g of BSA (Wako Pure Chemical Co., Japan) in 25 mL of water, the BSA was dissolved slowly. This BSA standard solution should be prepared freshly every time. Working BSA standard solutions were prepared by dilution of the standard solution.

A standard solution of creatinine (1000 mg L^{-1}) was prepared by dissolving 0.0250 g of creatinine (Wako Pure Chemical Co., Japan) in 0.1 M hydrochloric acid. Working solutions were prepared by accurate dilution of the stock solution with 0.03 M potassium dihydrogenphosphate.

Potassium dihydrogenphosphate solution (0.03 M) was prepared by dissolving 2.041 g of potassium dihydrogenphosphate (Wako Pure Chemical Co., Japan) in 500 mL of water.

The stock solution of eosin Y (0.12%) was prepared by dissolving 0.0600 g of eosin Y (sodium tetrabromofluorescin, Wako Pure Chemical Co., Japan) in 50 mL of water. The working concentrations of the dye were prepared by suitable dilution with hydrochloric acid solutions ranging from pH 2.0 to 4.5.

A stock solution of sodium picrate was prepared by dissolving 0.8073 g of picric acid (Wako Pure Chemical Co., Japan) in 50 mL of water. Working alkaline picrate solutions were prepared by adding a suitable volume of 10% NaOH solution (final concentrations ranging from 0.5 to 3.0% NaOH), and then diluting to 10 mL with water. Fresh solutions were prepared daily.

2.2. Apparatus

A schematic diagram of the developed sequential injection system for successive determination of albumin and creatinine in urinary samples is displayed in Fig. 1. A syringe pump (FIAlab instruments, USA) and a 10-port selection valve (C25-3180EMH, Valco Instrument Co. Inc.) were used to control carrier and reagent flow. The flow lines were Teflon (0.5 mm inner diameter). The length of the tubing that connected each port with its respective solution reservoir was 15 cm. A detection unit including a light source (LS-1LL, tungsten halogen lamp, Ocean Optics, Inc., USA), a flow cell (Z cell, 10 mm path length, constructed in-house), a spectrophotometer (USB2000, Ocean Optics Inc., USA) and fiber optic cables (P200-2UV/VIS, Ocean Optics, Inc. USA), was installed at the selection valve (port #9). The length of the tubing that connected port #9 with the flow cell was 7 cm. The reagents and albumin/creatinine solution were mixed at a holding coil. The FIAlab software was used for system control and signal processing. Peak evaluation was per-

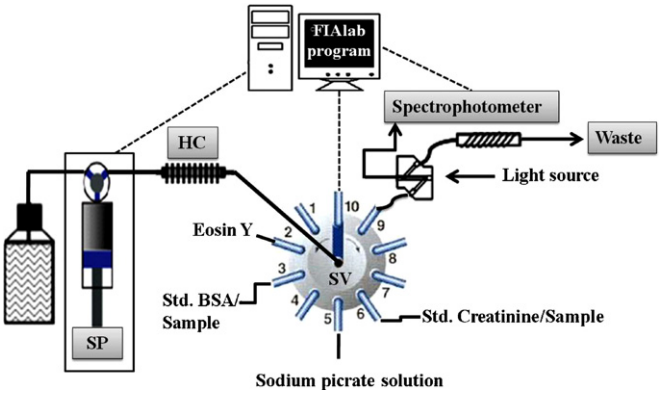

Fig. 1. Manifold of SI system for the successive determination of albumin and creatinine in urine samples. HC: holding coil, SP: syringe pump, eosin Y: 0.03%, picrate solution: 0.03 M.

Table 1
Protocol sequence for the successive determination of albumin and creatinine in urinary samples.

Step	Valve position	Flow rate (μL min^{-1})	Description
1	2	50	Aspirate of 0.03% Eosin Y
2	3	50	Aspirate of standard BSA/sample
3	9	50	Flow reversal process for mixing
4	9	50	Monitoring of product at 547 nm
5	5	50	Aspirate of 0.03 M of sodium picrate
6	6	50	Aspirate of standard creatinine/sample
7	9	50	Flow reversal process for mixing
8	9	70	Monitoring of product at 500 nm

formed using Origin software with manual measurement of peak height.

2.3. Procedure

The flow protocol and time sequence for successive determination of albumin and creatinine in urinary samples is listed in Table 1. The flow lines were initially filled with water using a syringe pump and a selection valve. Albumin measurement was started by aspirating 50 μL of eosin Y (port #2) into the system. Then, 100 μL of the standard BSA or sample solution (port #3) was introduced into the carrier stream. Mixing of the reagent and the standard/sample was promoted after three rounds of flow reversal to produce the albumin–eosin Y complex. The product produced was dispensed through port #9 for absorbance monitoring at 547 nm. After product detection, the system was cleaned using carrier water.

To start the creatinine determination process, 100 μL of alkaline picrate (port #5) was aspirated into the holding coil, followed by 150 μL of standard creatinine or sample solution (port #6). Flow reversal was performed to promote mixing of the reagents and the standard/sample via the flow cell port of the selection valve. The color product zone was produced after three flow reversals, and was then transferred to the detection unit for absorbance monitoring at 500 nm.

2.4. Calibration curve, limit of detection and precision

Calibration was performed under optimized conditions with standard solutions covering the concentration range from 0 to 100 mg L^{-1} for albumin and 0 to 200 mg L^{-1} for creatinine, respectively. For each standard solution, three replicate injections were carried out. The calibration data were evaluated by linear regression analysis using Excel software. The limit of detection was calculated via the 3σ method as the concentration of albumin or creatinine giving rise to signal exceeding three times the standard deviation of the blank signal. The precision of the peak heights was estimated by performing 10 replicate injections of two standard solutions containing 20 and 50 mg L^{-1} of albumin or creatinine.

2.5. Sample preparation

Urine samples were collected from diabetic patients and then stored at −20 °C. Before analysis, the urine sample solutions were filtered by filter paper (Whatman #1) to remove small particles. Next, the filtered solution was diluted at least 600-fold with water for albumin detection and 80-fold with 0.03 M potassium dihydrogenphosphate for creatinine detection. The sample solutions obtained had a concentration in the range of the calibration graph. Bradford protein assay [32] and the creatinine enzymatic assay [33,34] were employed for comparison purposes.

2.6. Data analysis

Standards and samples were analyzed, and absorbances were integrated. Standard curves were obtained by plotting the net absorbance against the analyte concentration and fitting to a linear equation. For comparing two measurement systems that are supposed to be equivalent, results were tested by paired *t*-test and calibration plot. The methods for this have been described in detail elsewhere [35,36].

3. Results and discussion

In this work, two specific reactions were used separately to determine albumin and creatinine levels. First, eosin Y was employed to form a color complex with albumin. The concentration of this complex could be monitored by absorption at 547 nm. At a pH lower than 3, the absorbance of unbound eosin Y is greatly reduced. After binding with albumin, a shift of the absorption occurs, along with an increase in sensitivity. This increase is, to a certain extent, proportional to the concentration of albumin. Second, creatinine levels were determined by Jaffe's reaction, where creatinine quantitatively produces an orange color with sodium picrate in alkaline medium. After allowing mixing at room temperature for color development, the absorbance was measured at 500 nm. These two detection systems form the basis of the proposed spectrophotometric SIA system for successive determination of albumin and creatinine and/or their ratio in urinary samples.

3.1. Optimization of variables in the determination of albumin

3.1.1. Effect of solution pH

The influence of solution pH on the absorbance (as peak height) of eosin Y–albumin complexes is important because formation of the complex and the blank signal were significantly affected by pH. The optimal pH of hydrochloric acid for the formation of eosin Y (0.006%) and albumin (50 mg L^{-1}) was investigated from pH 2.0 to 4.5. At pH lower than 2.0, eosin Y precipitates. As shown in Fig. 2A, signal of BSA (■) and blank (●) increased over the examined pH range. These results indicated that formation of the colored complex in acidic medium is preferable when compared to neutral or alkaline medium. It was also found that the color complex in pH 2.5 media provided the highest net signal (▲). Therefore, a hydrochloric medium at pH 2.5 was chosen as the optimal solution for albumin detection.

3.1.2. Effect of eosin Y concentration

The optimal eosin Y concentration for albumin detection was investigated using 50 mg L^{-1} of albumin at pH 2.5. The dye reagent with concentrations ranging from 0.001 to 0.1% was employed. The absorbance was measured at the specified wavelength and plotted against the eosin Y concentration (Fig. 2B). Signals increased around 95% from the initial concentration (0.001%), however, the blank signal also increased. Thus, the net signals were considered. It was found that the highest net signal was observed at 0.03% eosin Y. Therefore, 0.03% was selected as the most suitable eosin Y concentration for albumin detection.

3.1.3. Effects of reagent and standard/sample volume

The effect of eosin Y volume in the SIA operating sequence was examined over the range from 50 to 200 μL at intervals of 25 μL using 0.03% eosin Y in solution at pH 2.5. The relationship between the average signal for 50 mg L^{-1} albumin and the reagent volume is shown in Fig. 2C. It can be seen that the net signals decrease with increasing reagent volume. Therefore, a reagent volume of 50 μL was selected for subsequent work. In addition, the effect of standard/sample volume was examined over the range 25–150 μL at the

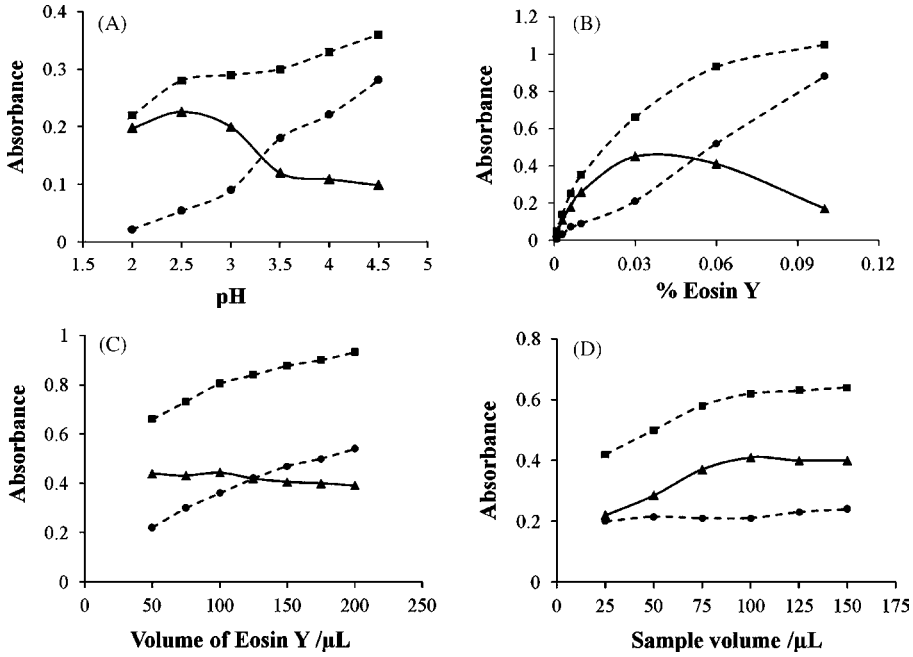

Fig. 2. Optimization parameters for albumin detection. (A) Effect of solution pH of hydrochloric acid using 0.006% eosin Y (50 μL), 50 mg L^{-1} BSA (100 μL). (B) Effect of eosin Y concentration using solution pH 2.5, reagent volume (50 μL), 50 mg L^{-1} BSA (100 μL). (C) Effect of eosin Y volume using 0.03% eosin Y in solution pH 2.5, 50 mg L^{-1} BSA (100 μL). (D) Effect of standard/sample volume using 0.03% eosin Y in solution pH 2.5 (50 μL), 50 mg L^{-1} BSA, (–●–) blank signal, (–■–) 50 mg L^{-1} BSA signal, and (-▲-) net signal.

same intervals as reagent volume. As shown in Fig. 2D, the net signal increased with increasing volume of standard/sample up to 100 μL. After that, the net signal plateaued. Hence, a standard/sample volume of 100 μL was used in the present work.

3.2. Optimization of variables in the determination of creatinine

3.2.1. Influence of sodium picrate concentration

Initially, the volume of sodium picrate solution was set at 200 μL. A 2% sodium hydroxide concentration was adopted based on a study in a previous work [30]. The optimal concentration of sodium picrate solution was studied over the range from 0.01 to 0.035 M at a creatinine concentration of 100 mg L^{-1} (100 μL). A concentration of 0.03 M was chosen as the optimal concentration (Fig. 3A) because higher concentrations did not improve the sensitivity. Moreover, sodium picrate solution at concentrations higher than 0.35 M led to precipitation of sodium picrate. Similar results were obtained at a creatinine concentration of 50 mg L^{-1} (data not shown).

3.2.2. Influence of sodium hydroxide

The influence of sodium hydroxide for the proposed assay was assessed using the same concentration of creatinine as mentioned in Section 3.2.1. Apparently, the presence of sodium hydroxide was essential for the formation of the products. Hence, sodium picrate solution was treated with several concentration of sodium hydroxide (final concentration ranged from 0.5 to 3.0%). Sodium hydroxide higher than 2.5% resulted in the precipitation of sodium picrate as well. Fig. 3B indicates that the absorbance increases with increasing sodium hydroxide concentration from 0.5 to 2.0%. In this work, 2% sodium hydroxide was used in order to maximize sensitivity.

3.2.3. Influences of reagent and standard/sample volume

To minimize the consumption of reagent volumes while maintaining the highest sensitivity (peak height) and precision, these parameters were optimized. The volumes of reagent solution and standard/sample were studied systematically. When varying the volume of solution of interest, another volume was kept constant at 0.03 M sodium picrate solution + 2% sodium hydroxide. The results are given in Fig. 3C and D. The influences of the picrate and standard/sample volumes were examined between 50 and 300 μL at 50 μL intervals. It can be observed that the maximal response was obtained at a volume of 200 μL for reagent volume. This volume also yielded the best precision (0.13% R.S.D.). For standard/sample volume, it was found that the absorbance increased up to 150 μL and remained almost constant afterwards. A volume of 100 μL was selected as an optimal standard/sample volume for subsequent measurements because this volume gave a smooth baseline in SIA grams and the best precision (2.27% R.S.D.).

3.3. Number of flow reversals

The mixing of inline reagents within the SIA system was important for the production of an albumin or a creatinine complex. The effect of the number of flow reversals was investigated. It was found that absorbance increased slightly when the number of flow reversals was increased to three rounds. After that, the absorbance gradually decreased due to dilution. Hence, three rounds of flow reversal were used in the proposed method (data not shown).

3.4. Analytical performances

Using the optimized parameters listed above, the SIA system was evaluated for its response to different concentrations of standard albumin and creatinine solutions. Absorbance peak heights of albumin and creatinine standards and a typical calibration curve are displayed in Fig. 4. Under the optimal conditions, the calibration curve for albumin was linear between 0 and 20 mg L^{-1}, with the following calibration equation: $y = 0.0182x$, with a correlation coefficient (R^2) of 0.9984. For creatinine measurement, the calibration was linear up to 100 mg L^{-1} with the calibration equation: $y = 0.0044x$, with a correlation coefficient (R^2) of 0.9981, where Y and X represent the SIA signal as peak height, and albumin or creatinine concentrations in mg L^{-1}, respectively. The detection limits (S/N = 3σ, σ is the standard deviation of the blank (n = 10)) were

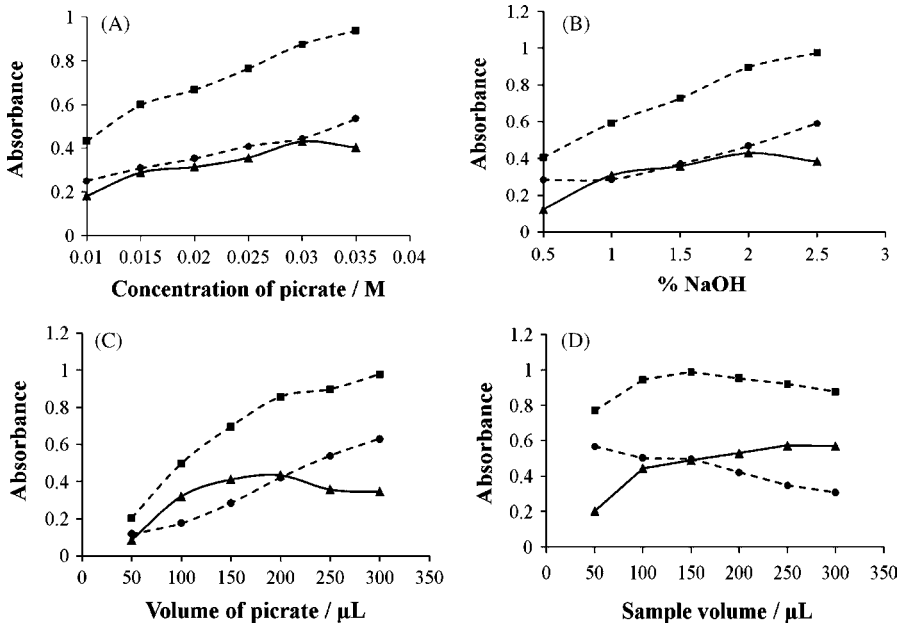

Fig. 3. Optimization of parameters for creatinine detection. (A) Effect of sodium picrate solution in 2% NaOH, reagent volume (200 µL), 100 mg L^{-1} creatinine (100 µL). (B) Effect of sodium hydroxide using 0.03 M sodium picrate (200 µL), 100 mg L^{-1} creatinine (100 µL). (C) Effect of sodium picrate volume using 0.03 M sodium picrate in 2% NaOH, 100 mg L^{-1} creatinine (100 µL). (D) Effect of standard/sample volume using 0.03 M sodium picrate in 2% NaOH (200 µL), 100 mg L^{-1} creatinine, (–●–) blank signal, (–■–) 100 mg L^{-1} creatinine signal, and (-▲-) net signal.

0.6 and 3.5 mg L^{-1} for albumin and creatinine, respectively. The quantification limits (S/N = 10σ) were 2.0 mg L^{-1} for albumin and 11.7 mg L^{-1} for creatinine. The detection limit of albumin obtained is better than both the detection limit of 10 mg L^{-1} obtained from a method based on DIDNTB [18] and the detection limit of 3.0 mg L^{-1} obtained from a spectrophotometric SIA using tetrabromophenolphthalein ethyl ester [24]. The repeatability of the proposed methodology was checked using 20 and 50 mg L^{-1} standard solutions of both analytes. The %R.S.D. values of 2.5 and 1.5 for albumin and 3.1 and 2.8 for creatinine, respectively, were registered (n = 10 measurements in each case). The sample throughput of successive determination was 18 h^{-1}.

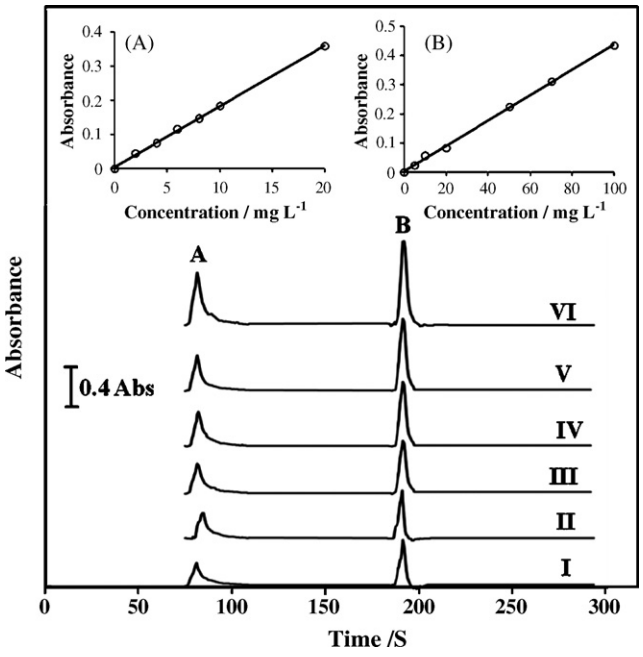

Fig. 4. Typical calibration curve detailing the response to successive determination of albumin and creatinine. The solution contains increasing concentrations of albumin [A; 2, 4, 6, 8, 10, 20 mg L^{-1} (I–VI)] and creatinine [B; 5, 10, 20, 50, 70, 100 mg L^{-1} (I–VI)]. Insets show the corresponding calibration plots. Other conditions are as in Fig. 2 for albumin and Fig. 3 for creatinine.

3.5. Interferences study

The effects of various foreign compounds on the successive determination of 50 mg L^{-1} albumin and 100 mg L^{-1} creatinine by the proposed method were tested. The results are summarized in Table 2. The tolerance limit was defined as the interference that yielded a relative error less than or equal to 5% when compared to the response obtained from the standard concentration. In normal urine, the chloride and urea content are important. The average concentration of chloride ions and urea in a normal person's urine are 4751 and 18,200 mg L^{-1}, respectively [37]. It was verified that there were no significant interferences from either chloride or urea at these concentrations. Other foreign species normally present at low concentrations in real samples also did not appear to interfere in the SIA assay. This allows utilization of the proposed method for successive determination of albumin and creatine and ACR in urine samples.

Table 2
Tolerance limits to foreign compounds for the successive determination of albumin and creatinine.

Foreign compounds	Tolerance limit (mg dL^{-1}) (Maximum concentration cause a deviation of ±5%)	
	BSA detection	Creatinine detection
Sodium chloride	100	400
Potassium chloride	100	400
Ammonium chloride	100	250
Sodium sulfate	200	400
Magnesium chloride	150	80
Urea	1000	2000
Glucose	700	1500
BSA	–	1000
Creatinine	10	–
Ascorbic acid	700	700

Table 3
The recoveries of the successive determination of 5 mg L^{-1} albumin and 50 mg L^{-1} creatinine in a real urinary sample using different dilution factors.

BSA assay		Creatinine assay	
Dilution factor	% Recovery	Dilution factor	% Recovery
80	149.6	20	114.2
100	138.3	30	111.0
200	118.1	50	107.5
300	111.4	60	103.4
450	103.7	80	100.0
600	100.0	100	100.1

3.6. Dilution effect

The influence of dilution effects was studied to ensure accurate quantitation. Urine samples may be diluted with a different solution (e.g., water, buffer) in order to get the concentration within the examined range. Recovery tests were carried out to evaluate this effect. In this experiment, we selected water and potassium dihydrogenphosphate as the diluent solutions for albumin and creatinine detection, respectively. The results (Table 3) indicate that 600-fold dilution would eliminate interferences during albumin determination, and 80-fold dilution would do the same for creatinine. This finding suggests that there was no interference and no dilution effects on the urine samples from water or potassium dihydrogenphosphate during albumin and creatinine determination. We can therefore use water or potassium dihydrogenphosphate to dilute samples before analysis.

3.7. Application to real samples

The proposed method was applied to successively evaluate albumin and creatinine in urinary samples taken from diabetic individuals. The results obtained by the proposed method for the determination of albumin and creatinine in 39 clinical urinary samples were compared with those obtained by the Bradford protein assay and the creatinine enzymatic assay. For comparison purposes, linear relationships were established either to albumin or creatinine as could be seen in Fig. 5A and B. The results demonstrated that the proposed SIA successive determination system was highly correlated with the conventional methods ($y = 0.939x + 10.01$, $R^2 = 0.989$ for albumin and $y = 1.017x - 0.57$, $R^2 = 0.976$ for creatinine, $n = 39$, respectively). From equation of regression analysis, some evidence of systematic error between the two sets of results obtained from the proposed method versus the comparison procedures was found, but the error was not significant and in the allowable range. This could be summarized that the proposed SIA system and the conventional method were well correlated.

In addition, a paired *t*-test with 38 degrees of freedom was performed on the data obtained. The experimental *t*-values between the two pairs of methods were 0.3406 for albumin and 0.5876 for creatinine. Statistical analysis revealed that the *t*-value for 38 degrees of freedom at the 95% confidence interval (2.060) was significantly higher than the above-mentioned experimental *t*-values. These illustrated the absence of statistical differences for the results obtained by the two methodologies for successive determination of albumin and creatinine. This indicates that the measurements are reliable and acceptable.

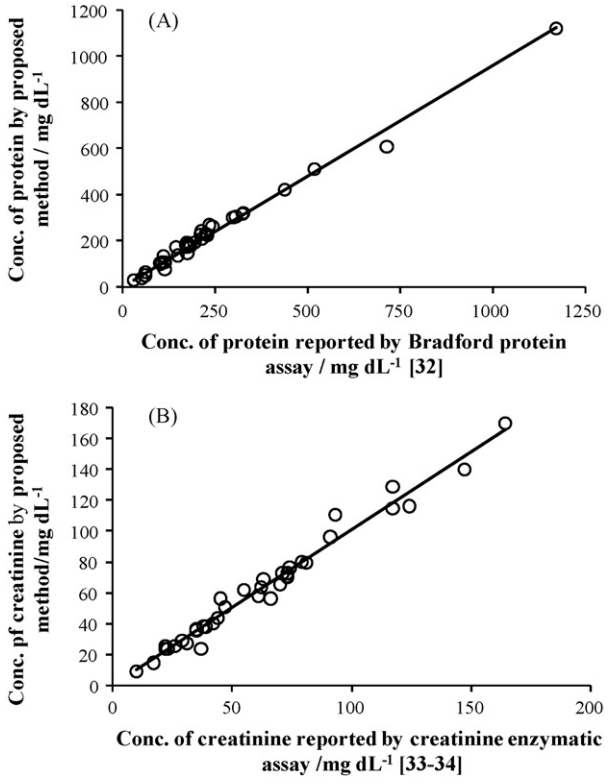

Fig. 5. Comparisons of two measurement methods, successive determination of albumin and creatinine using SIA system and Bradford protein assay (A) and creatinine enzymatic assay (B). Regression analysis gives $y = 0.939x + 10.01$, $R^2 = 0.989$ for albumin and $y = 1.017x - 0.57$, $R^2 = 0.976$ for creatinine, $n = 39$.

4. Conclusions

A simple, automatic and sensitive sequential injection system coupled with spectrophotometry was developed for the successive determination of albumin and creatinine. It was applied to the successive determination of albumin and creatinine in urinary samples. Besides the direct measurement of albumin and creatinine, the method allows the albumin/creatinine ratios to be determined. Moreover, the proposed methodology is less laborious, and is inexpensive, reliable, and more rapid compared to the conventional methods. With these satisfying results, the developed system could be preferable for routine assessment of early diabetic disease and other life-threatening diseases. Furthermore, SIA integrates the ability to perform quality-assurance measures in an automated manner. This leads to analytical systems that are more portable than comparable systems, which make these systems especially suitable for on-site analysis. Its future use as an automatic tool for screening and determination of various predictors of health status or disease risk in humans can thus be proposed.

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