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A flow-based procedure with solenoid micro-pumps for the spectrophotometric determination of uric acid in urine

Diogo L. Rocha, Fábio R.P. Rocha *

Instituto de Química, Universidade de São Paulo, P.O. Box 26.077, 05599-970, São Paulo, SP, Brazil

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ABSTRACT

The determination of uric acid in urine shows clinical importance, once it can be related to human organism dysfunctions, such as gout. An analytical procedure employing a multicommuted flow system was developed for the determination of uric acid in urine samples. Cu(II) ions are reduced by uric acid to Cu(I) that can be quantified by spectrophotometry in the presence of 2,2′-biquinoline 4,4′-dicarboxylic acid (BCA). The analytical response was linear between 10 and 100 μmol L^{-1} uric acid with a detection limit of 3.0 μmol L^{-1} (99.7% confidence level). Coefficient of variation of 1.2% and sampling rate of 150 determinations per hour were achieved. Per determination, 32 μg of $CuSO_4$ and 200 μg of BCA were consumed, generating 2.0 mL of waste. Recoveries from 91 to 112% were estimated and the results for 7 urine samples agreed with those obtained by the commercially available enzymatic kit for determination of uric acid. The procedure required 100-fold dilution of urine samples, minimizing sample consumption and interfering effects. In order to avoid the manual dilution step, on-line sample dilution was achieved by a simple system reconfiguration attaining a sampling rate of 95 h^{-1}.

1. Introduction

Uric acid is the main end product of purine metabolism in human organism [1]. It is almost completely eliminated through urine [2] and its quantification has great clinical importance, because it can be related to the diagnosis of gout [3], Lesch–Nyhan Syndrome [4] and urolithiasis [5]. In addition, the urianalysis of uric acid aids on monitoring the action of chemotherapeutic drugs [2].

The procedures commonly described in literature for the determination of uric acid in biological samples are enzymatic [6–10], chromatographic [11] or based on the redox properties of the analyte [12–15]. Enzymatic procedures are based on oxidation of uric acid in the presence of uricase, generating allantoin, CO_2 and H_2O_2. The analyte consumption can be directly monitored by UV spectrophotometry [6], but the procedure requires complex sample treatment to eliminate other absorbing species. Alternatively, the concentration of uric acid can be related to the generation of H_2O_2, which reacts with 3,5-dichlorohidroxibenzenesulphonic acid and 4-aminoantipyrine, yielding a red product with absorption maximum at 505 nm [7]. This is the usual method for uric acid determination in clinical analysis, in spite of reagent instability and the need for sample heating before measurement. Electrochemical detectors can be employed to measure CO_2 generation [8] or O_2 consumption [9]. Because biological samples present a series of electro-active species, laborious separation steps are usually required [10].

Reversed phase liquid chromatography was also employed for uric acid quantification in blood and urine samples [11]. However, the employment of this technique for routine analysis in clinical laboratories is hindered by low sample throughput, high acquisition and operational costs as well as generation of toxic wastes.

Flow systems with amperometric detection employing a single line manifold [12] or multicommutation [13] were exploited for uric acid determination in urine samples. The main drawback is that purine derivatives, such as caffeine, tend to be adsorbed on the electrode surface, requiring a periodic removal of the detection flow cell for electrode activation or replacement. Uric acid can also be quantified by the reduction of phosphotungstate ion to tungsten blue, monitored by spectrophotometry at 700 nm [14]. The method lacks selectivity in view of the precipitation of proteins by phosphotungstic acid. Another spectrophotometric procedure for uric acid determination in the presence of ascorbic acid took into account the difference between signals obtained in two residence times [15]. The sample is mixed with a solution containing Fe(III) and the reduced metal ion forms a colored complex with tripyridiltriazine. The first signal corresponds to the complex formed due to the fast reaction between Fe (III) and ascorbic acid. Then, the sample zone passes through another coil, being redirected to the detector for obtaining a signal corresponding to the sum of ascorbic and uric acid concentrations. In view of the additional dispersion of the sample zone, the determination of uric acid requires complex calculations. As the final

* Corresponding author. Fax: +55 11 3815 5579.
E-mail address: frprocha@iq.usp.br (F.R.P. Rocha).

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concentration is obtained by subtraction of the signals, the result is affected by error propagation.

Most of the time of a clinical analyst is spent on tiring and repetitive sample treatment and measurement [16]. It can cause exhaustion, leading to a series of systematic errors, affecting precision and accuracy. Therefore, mechanization of procedures is necessary in view of the high demand in clinical laboratories. The consequent minimization of reagent consumption and waste generation is also highly desirable, considering the difficulties to manage and treat biological wastes. Both requirements can be achieved by flow-based procedures, which are characterized by high precision in view of controlled sample dispersion and residence time, high sampling rate, low reagent consumption and waste generation [17].

The use of computer-controlled solenoid micro-pumps for independent solution handling promotes better analytical performance and minimizes reagent consumption and waste generation. These devices actuate as fluid propellers and injectors [18] by dispensing micro-amounts of solutions for each current pulse applied to the solenoid of the micro-pump. The reproducible actuation of the solenoid micro-pumps generates a pulsed flow [19], which promotes better conditions for sample-reagent mixing and minimizes the deposition of solids into the flow cell [20]. Multicommuted flow systems with solenoid micro-pumps were employed for determination of chemical species in environmental [21,22], pharmaceutical [23,24] and food samples [25,26]. However, the potentialities of these systems were scarcely exploited for clinical assays [27].

In this work, a multi-pumping flow system was employed for the determination of uric acid in urine samples, based on the redox reaction between the analyte and Cu(II). The Cu(I) ions were quantified by spectrophotometry in the presence of 2,2′-biquinoline 4,4′-dicarboxylic acid (BCA), yielding a purple complex with absorption maxima at 354 and 562 nm [28]. Analogous reactions were previously employed for determination of total protein in biological samples [29] and tannin in beverages [30].

2. Experimental

2.1. Apparatus

The multi-pumping flow system was constructed with polyethylene tubes (0.8 mm *i.d.*) and solenoid micro-pumps (Biochem Valve Inc., Boonton, NJ, USA) of 10 μL (P_1, P_2 and P_3) and 60 μL (P_4) nominal volumes. These devices were operated at 5 Hz for the flow manifold in Fig. 1a and at 3 or 5 Hz in the flow manifold in Fig. 1b. The solution flow rates for P_1, P_2 and P_3 (operated at 5 Hz) were estimated as 54, 61, 65 μL s^{-1}, respectively. The micro-pump P_4 was operated at 5 or 3 Hz, which corresponds to 169 or 150 μL s^{-1}, respectively. The micro-pumps were controlled by an AMD Athlon micro-computer through a parallel interface, employing a current drive based on the integrated circuit ULN2003. Control software was developed in Visual Basic 6.0 (Microsoft).

Measurements were carried out with a multichannel CCD spectrophotometer (Ocean Optics, Dunedin, FL, USA; model USB2000). Optical fibers were employed to transport the radiation from a tungsten–halogen lamp (Ocean Optics, model LS-1) to a 1.0 cm optical path flow cell (Hellma, Plainview, NY, USA; 80 μL internal volume) and from the cell to the detector array. The software furnished by the fabricant of the multichannel spectrophotometer was employed for data acquisition. A pH Meter (Metrohm, Herisau, Switzerland; model 654) was used for pH measurements.

2.2. Reagents and solutions

All solutions were prepared with deionized water and analytical grade chemicals. The R_1 reagent was a 2.0 mmol L^{-1} $CuSO_4$ solution containing 0.4 mol L^{-1} ammonium acetate (NH_4Ac). A 5.0 mmol L^{-1} BCA solution (R_2) was prepared by dissolving the 2,2′-biquinoline 4,4′-dicarboxylate disodium salt (Sigma, St. Louis, MO, USA) in water. Daily prepared 20 mmol L^{-1} NH_4OH (Merck, Darmstadt, Germany) solutions were employed as carrier. Stock solutions of uric acid (Sigma) were prepared by dissolution in water at 50 °C or in NaOH solution.

2.3. Proposed procedure

For the determination of uric acid, the flow manifold showed in Fig. 1a was operated according to the routine described in Table 1. The binary sampling approach [31] was exploited for solutions handling with four sampling cycles (steps 1–3). The sample zone was carried to detection at 562 nm (step 4) by the actuation of the micro-pump P_4. After sample replacement, it was necessary to guarantee the filling of the path from its reservoir until the confluence point X. In this way, micro-pump P_1 and valve V were simultaneously actuated (step 5a), changing flow direction to waste. The sample volume situated between confluence point X and valve V was then discarded by actuation of micro-pump P_4 (step 5b).

The influence of sample treatment before dilution was evaluated. The same urine sample was analyzed without treatment (1) and after addition of NaOH until pH = 11 (2), warming at 50 °C (3) and combination of procedures 2 and 3 (4). The sample was diluted 15 min after preparation. Recovery tests were carried out by spiking 25 or 35 μmol L^{-1} uric acid to 4 urine samples.

Interference studies were carried out by considering concomitant concentrations at least 5 times higher than the maximum expected in urine samples. Accuracy was evaluated comparing the results obtained by the developed procedure with those achieved by a commercially available enzymatic method for uric acid determination in urine and blood samples [7].

A flow manifold (Fig. 1b) was developed to perform on-line sample dilutions by zone sampling [27] employing two 100-cm reaction coils (B_1 and B_2), for sample dispersion and reaction development, respectively. The active devices were actuated according to the routine in Table 2. By switching valve V_2 on, 1 pulse of micro-pump P_1 and 15 pulses of micro-pump P_4 promoted the dispersion of the sample zone in B_1. Afterwards, a fraction of the dispersed sample zone was injected into coil B_2. Valve V_2 was switched off and micro-pump P_4 was actuated (2 pulses per cycle) followed by P_2 and P_3 (5 pulses of both). The process was repeated 3-times (3 sampling cycles). Before transportation of the sample zone fraction to B_2, valve V_2 was switched on and 40 pulses of micro-pump P_4 were used to remove the discarded sample from coil B_1. Then, valve V_2 was switched off and 60 pulses of micro-pump P_4 transported the sample zone in B_2 to detection. Other parameters employed were the same as previously optimized.

2.4. Reference procedure

An enzymatic test based on a commercially available kit for uric acid determination was employed as reference procedure [7]. Sample was alkalinized with NaOH until pH 7–9. A 125 μL volume of a 10-fold diluted sample was added to 1000 μL of the working reagent, prepared from 20 mL of 4 mmol L^{-1} 3,5-dichlorohidroxibenzenesulphonic and 1.0 mL of 2 mmol L^{-1} 4-aminoantipyrine in 7.7 mmol L^{-1} sodium azide, containing uricase (≥3000 U/L) and peroxidase (≥18,000 U/L) solutions, both in 100 mmol L^{-1} phosphate buffer (pH = 7.5). The mixture was immersed in a 37 ° C water bath for 5 min and the formed quinoneimine derivative was measured by spectrophotometry at 505 nm, employing a reduced volume cell.

3. Results and discussion

In the presence of uric acid, Cu(II) ions are reduced to Cu(I) (Reaction 1), which generates a purple complex with the anion of 2,2′-