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Short communication

Conventional and planar chip sensors for potentiometric assay of uric acid in biological fluids using flow injection analysis

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Abstract

The potentiometric response properties of several PVC-based membrane sensors using phthalocyanine complexes of cobalt(II) (CoPC) and Fe(II) (FePC) as anion carriers, towards uric acid were constructed and characterized. The sensors demonstrated fast near-Nernstian response for uric acid over the concentration ranges 9.1×10^{-6} to 9.1×10^{-2} and 3.1×10^{-5} to 3.1×10^{-2} M with detection limits 0.67 and 2.85 μg mL^{-1} over pH 6.5–8 for CoPC and FePC based membrane sensors plasticized with *o*-NPOE and 1% TDMAC, respectively. A novel solid-state planar chip urate sensor was developed, characterized according to IUPAC recommendations, easily used in a single channel wall-jet flow injection system and compared with a tubular detector. The intrinsic characteristics of the detectors in a low dispersion manifold were determined and compared with data obtained under hydrodynamic mode of operation. Validation of the assay methods with the proposed sensors by measuring the lower limit, range, accuracy, precision, repeatability and between-day-variability revealed good performance characteristics confirming applicability for continuous determination of uric acid. The sensors were used for determining urate in biological fluids at an input rate of 50 samples per hour. The results compare favorably with data obtained by the standard spectrophotometry.

Keywords: Uric acid; Metal-phthalocyanines; Chip sensor; Potentiometry; Flow injection analysis; Biomedical analysis; Biological fluids

1. Introduction

Uric acid is the waste product produced from the degradation of purines. In healthy human, uric acid is filtered and removed from the blood by the kidneys and excreted into urine. Because a number of kidney diseases are known to affect uric acid levels, uric acid determination is thus important and useful in diagnosing and evaluating kidney diseases. For example, when uric acid is present in the blood at abnormally high levels, it tends to crystallize in body joints, resulting in gout, a very painful inflammatory condition. Increased levels of uric acid are also known to be associated with uremia, leukemia and pneumonia [1]. Various animal models examining hypertension/renal function have demonstrated that urate is an important mediator of endothelial dysfunction, vascular disease, inflammation and nephothelial dysfunction, vascular disease, inflammation and nephrotoxicity [2,3]. The normal uric acid levels range from 4.1 to 8.8 and 250–750 mg dL^{-1} in serum and urinary excretion, respectively [4]. Methods for rapidly and reliably monitoring urate levels in human fluids and sera in clinical diagnoses are desirable. The common method for uric acid assessment is based on its measurement directly in the UV region [5], its oxidation to allantoin and CO_2 by tungestophosphoric acid, which is reduced to a tungsten blue which can be measured between 660 and 720 nm [6,7] and rate measurements of tungsten blue formation under controlled conditions [8]. Assay methods based on chemiluminescence [9], high performance liquid chromatography (HPLC) [10,11], voltammetry [12,13] and amperometry [14,15] have been suggested. The use of uricase enzyme to catalyze the oxidation of uric acid to CO_2, allantoin and H_2O_2 and monitoring of these reaction products by using CO_2 gas sensor [16], Clark-type oxygen electrode [17], voltammetry [18], amperometry [15,19], potentiometry [20] and spectrophotometry [21,22] have been described. These methods are, however, time consuming and require expensive equipments, no reproducibility, instability, low response sensitivity, enzyme unavailability and short durability. Direct potentiometry with chemical sensors has found many applications in pharmaceutical and biomedical

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analysis [23,24]. The approach provides simple, fast, and selective technique for clinical analysis. However, as far as the available literature is concerned, very little is known about the use of this technique for uric acid determination [25]. Microfabricated-planar potentiometric sensors have been suggested to offer several advantages over the conventional electrodes particularly the small size, simple design, low cost and mass production. A screen-printed thick film [26], silicon transducer chip [27], silicon nitride base chip [28] and metal printed flexible polyimide film [29] have been developed and used. These sensors do not incorporate internal reference electrode and filling solution.

In this work, preparation, characterization and application of simple potentiometric sensors for fast determination of uric acid in biological fluids were described. These sensors are based on the use of CoPC and FePC as electroactive materials embedded in plasticized PVC matrix membranes. Tubular and planar detectors based on the use of CoPC are used in a wall-jet flow injection manifold for continuous uric acid assay. Advantages of these sensors include the simplicity in designing, small size, short measurement time, low cost, adequate precision, high accuracy, high analytical throughput, good response stability, low limit of detection and reasonable selectivity in the presence of many interferents.

2. Materials and methods

2.1. Equipments

All potential measurements were made at 25 ± 1 °C with an Orion (Cambridge, MA, USA) Model 720/SA pH/mV meter using urate membrane sensor in conjunction with an Orion Ag/AgCl double-junction reference electrode (Model 90-02) filled with 10% (m/v) KCl. A combination Ross glass electrode (Orion 81-02) was used for pH measurements. A computer-controlled spectrophotometer (Shimadzu, Model 1601) was used for the spectrophotometric measurements. The flow injection analysis (FIA) system manifold consisted of a two-channel Ismatech MS-REGLO model peristaltic pump. The manifold was connected with polyethylene tubing (0.71 mm i.d.) and an Omnifit injection valve (Omnifit, Cambridge, UK) with sample loop of 300 μL volume. The potential signals were recorded using a homemade high-impedance data acquisition eight-channel box connected to a PC through the interface ADC 16 (Pico Tech., UK) and PicoLog for windows (version 5.07) software.

2.2. Reagents and solutions

All solutions were prepared using Millipore Milli-Q water. All chemicals were of analytical reagent grade and were used without further purification. Sodium carbonates, uric acid, dioctylphthalate (DOP), CoPC and FePC were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Tetrahydrofurane (THF), poly(vinylchloride) (PVC) high molecular weight and dibutylsebacate (DBS) were obtained from Merck. Tridodecylmethylammonium chloride (TDMAC) and *o*-nitrophenyloctyl ether (*o*-NPOE) were obtained from Fluka. A reagent kit (DIUA-250) from BioAssay System (Hayward, CA 94545, USA) was used for spectrophotometric determination of uric acid.

A standard urate solution was prepared daily and immediately wrapped in aluminum foil to prevent photo thermal degradation. A 168.1 mg uric acid was dissolved in 100 mL 0.45% (g/v) Na_2CO_3 at 70 °C and stored at 4 °C until use. Dilute urate working standard solutions (10^{-3} to 10^{-7} M) were prepared by appropriate dilution with 10^{-2} M phosphate buffer of pH 7.2.

2.3. Sensor preparation and calibration

Three milligrams of metal-phthalocyanine ionophores were mixed with 124 mg of DBS, DOP or *o*-NPOE plasticizer, 66 mg PVC and 1 mg TDMAC in ca. 3 mL of THF in a Petri dish (3 cm diameter). The membrane solution was left to stand overnight at room temperature to allow slow evaporation of the solvent. The membrane formed was used for sensor construction as previously described [30]. A solution consisting of equal volumes of 10^{-3} M urate and 10^{-3} M KCl was used as an internal reference solution. The sensors were preconditioned by soaking overnight in a 10^{-3} M urate solution before use and were stored in distilled water between measurements. The electrochemical cell used for potential measurements was: Ag/AgCl/10^{-3} M urate, 10^{-3} M KCl//PVC membrane//test solution/Ag/AgCl double-junction reference electrode. The potential readings of the stirred 10^{-2} to 10^{-7} M urate solutions were measured at 25 ± 1 °C, and recorded after stabilization to ±0.2 mV. A calibration plot was constructed connecting logarithm concentration with electromotive force.

2.4. Planar chip sensor

A planar gold base electrode (3 mm × 5 mm) was sputtered on a (13.5 mm × 3.5 mm) flexible polyimide (Kapton®, DuPont) substrate (125 μm thick), as shown in Fig. 1; single site electrode

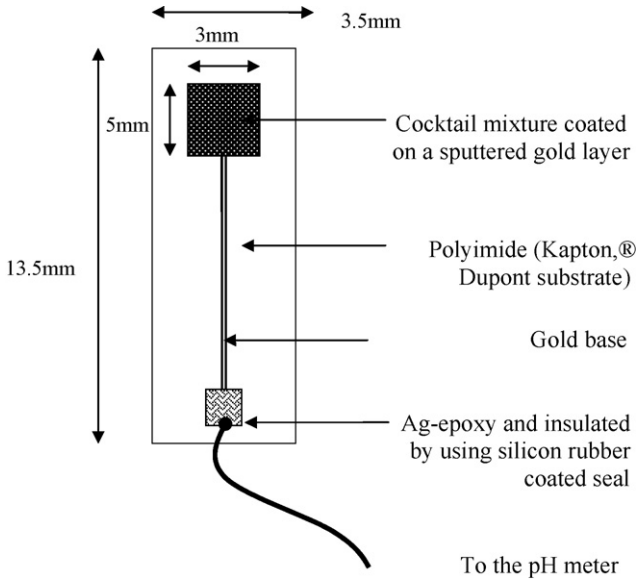

Fig. 1. Planar-chip sensor.

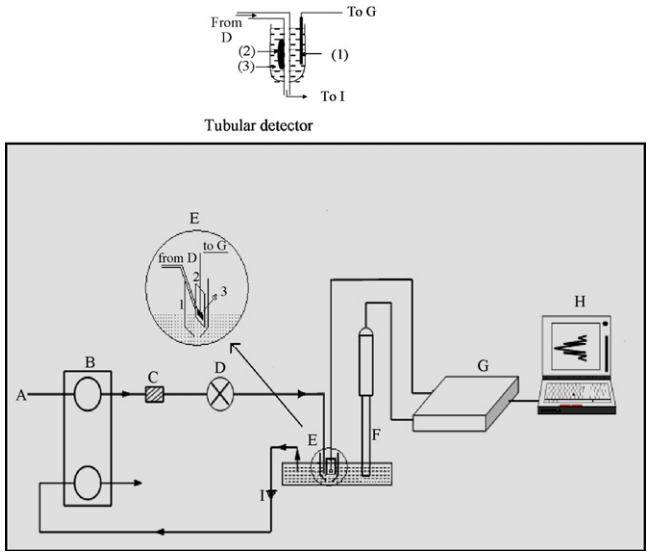

Fig. 2. FIA manifold for the evaluation of urate. (A) 10^{-2} M carrier phosphate buffer solution pH 7.2; (B) peristaltic pump; (C) pulse dumper; (D) sample injection valve; (E) flow injection detector [1: blue tip micropipette for protection of the chip; 2: planar chip; 3: the sensing membrane]; (F) reference electrode; (G) data acquisition system; (H) computer; (I) waste. (1) Ag/AgCl internal reference electrode; (2) sensing membrane; (3) internal filling solution.

(area = 0.06 cm^2) (used for all the optimization and characterization studies), and used as previously described [29]. An electrical wire was connected to the electrode by means of Ag-epoxy (Epoxy Technology). Insulation of the electrical contact was made using silicon rubber coating seal (Dow Corning 3140 RTV).

The membrane cocktail mixture was directly coated to the sputtered gold layer using microsyringe to apply few microliters of the sensing solution (typically 10 μL of membrane cocktail is dispersed), left to dry in the air for 1 min before repeating further addition (i.e. four times of the sensing solution). The coated end was protected from damage by immersing in 1 mL blue tip micropipette cut at its end to allow solutions contact as shown in Fig. 2E.

2.5. Flow cell construction

The chip was used in a single channel wall-jet flow injection system. A carrier solution consists of 10^{-2} M phosphate buffer of pH 7.2 was propelled by means of a peristaltic pump through PTEE tubing (1.13 mm i.d.) as shown in Fig. 2. The tubing was ended with a horizontally mounted 10 cm long glass capillary tube (2.6 mm o.d., 1.0 mm i.d.). The length of the tubing from the injection valve to the capillary was 35 cm. The free end of the capillary was cut at 90°. This end of tube was brought into contact with the chip surface. The small chip was mounted in a vertical position with a connection wire directed upward. The distance between the end of the capillary tube and the sensing surface of the sensor was 8 mm. The sensor with a double-junction Ag/AgCl reference electrode was place in a Petri dish where the level of solution was kept above the sensor surface (Fig. 2). The detector was conditioned by soaking in 1.0×10^{-3} M solution of urate for overnight before use and stored dry in air when is not in use.

A tubular detector was constructed as described previously [23]. The coating solution was prepared by dissolving 67 mg of PVC in 3 mL (THF) followed by the addition of 124 mg of (*o*-NPOE), 3 mg of the CoPC and 1 mg of TDMAC. This solution was deposited, using a microdropper, three to four times in a hole (3 mm wide × 5 mm length) made in the middle of a 15 cm Tygon tube (ALKEM, P/N A003494 red/red 0.071 i.d.). The tube was inserted and sealed with Araldite in 100 μL pipette tip (7 cm long, 0.4 cm diameter) (Fig. 2A). The tubular sensor was inserted into the flow injection system as schematically shown in Fig. 2.

2.6. Determination of urate in human serum

Aliquots of human blood were obtained from some patients and analyzed within 3 h of extraction. Blood was collected in tubes and then 9 mL portion of absolute ethyl alcohol was added, thoroughly mixed and left for 10 min before being centrifuged at 4000 rpm. The supernatant liquid was without removal of any particulate matter to a 20 mL beaker and then evaporated at 50 °C on a hot plate till dryness before being reconstituted in de-ionized water. A 9 mL of 10^{-2} M phosphate buffer solution of pH 7.2 was added. The extracts were transferred to 25 mL measuring flask and complete to the mark. A 10 mL aliquot of the sample solution was transferred to a 25 mL beaker. The working and reference electrode were immersed, and the potential readings were recorded after reaching the equilibrium response (10–20 s). The concentration of the uric acid, expressed as [urate], was calculated using a calibration graph.

For flow injection analysis (FIA), a flow stream of the carrier solution (10^{-2} M phosphate buffer of pH 7.2) was allowed to pass through the flow cell at a flow rate 3 mL min^{-1}. Successive 300 μL aliquots of standard 10^{-2} to 10^{-6} M urate and unknown test sample solutions were injected into the flowing stream. The corresponding potential change was measured and recorded versus time. A typical calibration plot was made used to determine the concentration of the unknown samples.

2.7. Determination of uric acid in human urine

A 1.0 mL aliquot of the human urine sample was diluted with 10^{-2} mL phosphate buffer of pH 7.2 in a 100 mL calibrated flask and shaken well. A 5–10 mL portion of the diluted urine solution was transferred into a 25 mL beaker. The working and reference electrode were immersed, and the potential readings were recorded after reaching the equilibrium response (10–20 s) and compared with the calibration plot.

3. Results and discussion

Potentiometric poly(vinyl chloride) matrix membrane sensors incorporated CoPC and FePC as neutral carriers were prepared. The use of these metal-phthalocyanines as neutral carriers was found to be highly responsive to urate anion. The preferential response toward urate was believed to be associated

Table 1
Response characteristics of CoPC- and FePC PVC membrane sensors in different plasticizers

Parameter	CoPC				FePC			
	DBS	DOP	*o*-NPOE	*o*-NPOE + TDMAC	DBS	DOP	*o*-NPOE	*o*-NPOE + TDMAC
Slope (mV decade^{-1})*	-21 ± 0.1	-23.2 ± 0.2	-28.4 ± 0.3	-30.4 ± 0.3	-17.5 ± 0.2	-17.9 ± 0.3	-21.3 ± 0.2	-25.7 ± 0.3
Correlation coefficients, *r*	0.9994	0.9997	0.9991	0.9994	0.9996	0.9986	0.9977	0.9990
Lower limit of linear range (M)	1.0×10^{-4}	5×10^{-5}	1.0×10^{-5}	9.1×10^{-6}	5.0×10^{-5}	2.0×10^{-5}	1.0×10^{-5}	3.1×10^{-5}
Detection limit (μg mL^{-1})*	5.44 ± 0.3	2.38 ± 0.2	0.68 ± 0.1	0.68 ± 0.1	5.27 ± 0.1	3.40 ± 0.2	0.85 ± 0.1	2.89 ± 0.2
Response time (s)	<15	<15	<15	<10	<20	<10	<20	<20
Working pH range	6.5–8	6.5–8	6.5–8	6.5–8	6.5–8	6.5–8	6.5–8	6.5–8
Standard deviation, σ_v (mV)	0.7	0.5	0.5	0.6	1.0	1.2	0.8	0.7
Recovery (%)	99.5	99.6	99.6	99.3	98.8	98.5	99.1	99.4
Repeatability, Cv_w (%)	0.5	0.8	1.1	0.9	1.1	1.5	0.9	1.1
Between-day-variability, Cv_b (%)	0.9	1.1	1.2	0.8	0.9	1.1	0.7	0.5
Life span (week)	6	6	6	6	6	6	6	6

* Average of six measurements.

with the coordination of urate with central metal ion of the carrier. It is well-known that the sensitivity and selectivity obtained for a given ionophore depends significantly on the membrane condition. Several plasticizers were used to prepare membranes with CoPC and FePC to determine the best response characteristics for the prepared sensors. The responses of these sensors towards urate anions were electrochemically evaluated when three plasticizers namely (DBS), (DOP) or (*o*-NPOE) incorporated with membranes and the results are summarized in Table 1. The membranes compositions containing 2% (w/w) CoPC or FePC neutral carriers, 34.3% (w/w) PVC and 63.7% (w/w) plasticizer reveal that membranes plasticized with *o*-NPOE elicited the best short and stable response for urate. Typical calibration plot in batch for the sensors incorporating the electroactive material responsive for urate are shown in Fig. 3. According to Fig. 3 and Table 1, it is clear that *o*-NPOE is a more effective solvent mediator than others in preparing the urate sensor. It is noteworthy that the nature of plasticizer influences both the dielectric constant of the polymeric membranes and mobility of the ionophore and its analyte complex [31]. Incorporation of lipophilic ionic sites within the membrane components like TDMAC is required to enhance selectivity, reducing the sensors response time and lowering the membrane resistance [32]. The optimum membrane composition was 1.5% CoPC or FePC, 0.5% of TDMAC, 34.3.% PVC and 63.7% *o*-NPOE. The results in Table 1, show nearly improvement in calibration slope of the sensor, in detection limit (Fig. 4) and in sensor selectivities over most anions (Table 2). Planar chip polymeric membrane sensor based on CoPC with *o*-NPOE plasticizer was prepared and tested under static mode of operation. The composition of the sensor membrane was: plasticizer 63.7%, CoPC 1.8%, and PVC 34.5%. The linear response range was 2.0×10^{-5} to 1.0×10^{-1} M and the detection limit was 8×10^{-6} M.

The influence of pH on the response of urate membrane sensors was checked by recording the emf displayed by 1×10^{-3} and 1×10^{-4} M solutions at various pH values. It is apparent from the potential–pH profiles that the responses were fairly constant over the pH range 6.5–8. Under these alkaline solutions, uric acid is completely ionized, dissociated and sensed as a divalent anion. The potential readings over this range were constant within ± 0.2 mV. The sensors displayed, however, a significant response from at high pH values (>8.5) probably originating from the ability of hydroxide ions to compete favorably for axial coordination site of the central atom so that, all subsequent measurements in batch were measured in phosphate buffer at pH 7.2.

3.1. Potential stability and sensor selectivity

The time required to achieve a steady potential response within ± 1 mV using the proposed sensors in 10^{-6} to 10^{-2} M urate solutions with a rapid 10-fold increase in concentration was <10 s. Replicate calibrations for each sensor over a period of 7 weeks indicated low potential drift, long-term stability and negligible change in the response of the sensors. During this period, the sensors were stored and conditioned in 10^{-3} M urate solution of pH 7.2. With all sensors examined, the detection limits, response times, linear ranges and calibration slopes were reproducible to within $\pm 3\%$ of their original values over a period of at least 7 weeks.

The basic parameter characterizing the analytical properties of each new sensor is its selectivity coefficient. Therefore, the coefficients of selectivity should be determined in a standardized way by a theoretical justified method which ensures the analytical usefulness of the values determined enables the possibility of comparison of selectivity of different sensors. Potentiometric selectivity coefficients of the sensors towards different organic and cationic inorganic species commonly associated in biological samples with urate were evaluated using the mixed solution methods, namely two-solution method (TSM) [33]. Potentiometric selectivities of both sensors are related to the preferential interaction of CoPC and FePC ionophores with urate anion in alkaline medium over many inorganic anions. Selectivity data over many anions are shown in Table 2.

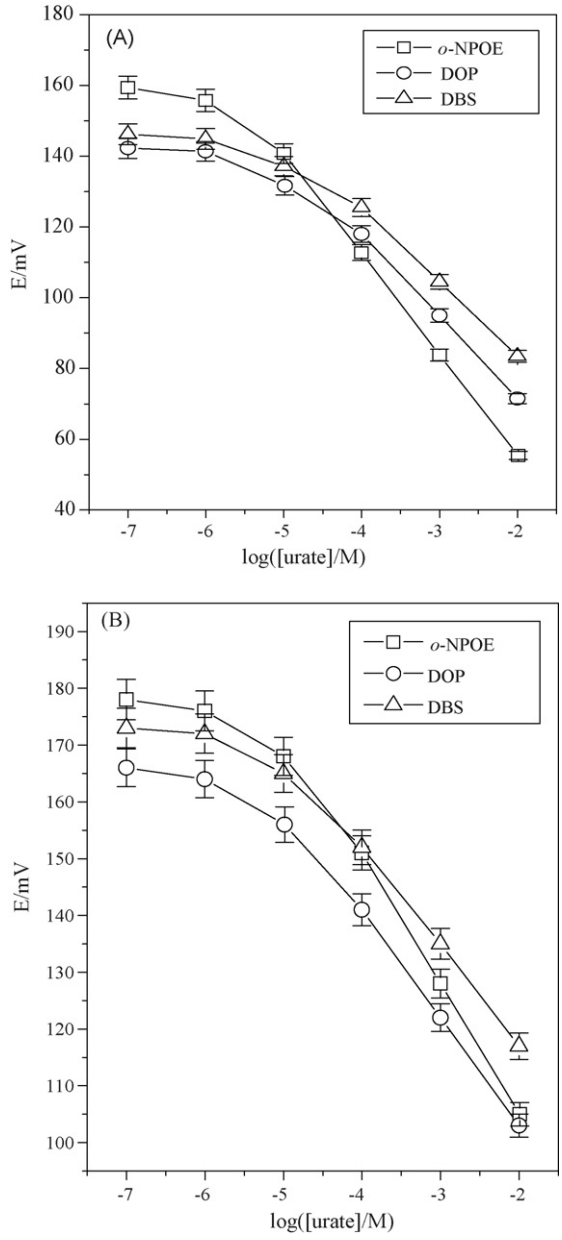

Fig. 3. Potentiometric response of urate based PVC membrane sensor in different plasticizers: (A) CoPC and (B) FePC.

The data reveal that the selectivity order for sensors incorporating membrane plasticized with *o*-NPOE in presence of TDMAC is much better than other sensor without TDMAC. Generally, a sensor based on CoPC is less affected by urea, thiourea, ascorbate and iodide than that of FePC membrane based sensor. A sensor based on FePC has good selectivity for urate and is less affected by NO_3^-, NO_2^-, SO_3^{2-} and SCN^- than sensor based on CoPC.

This high preference in response to urate for the proposed sensors over highly lipophilic anions such as SCN^-, I^-, ClO_4^- exhibits a clear deviation from Hofmeister selectivity pattern and emphasizes the direct interaction of urate as an axial ligand with the metal center of the ionophore.

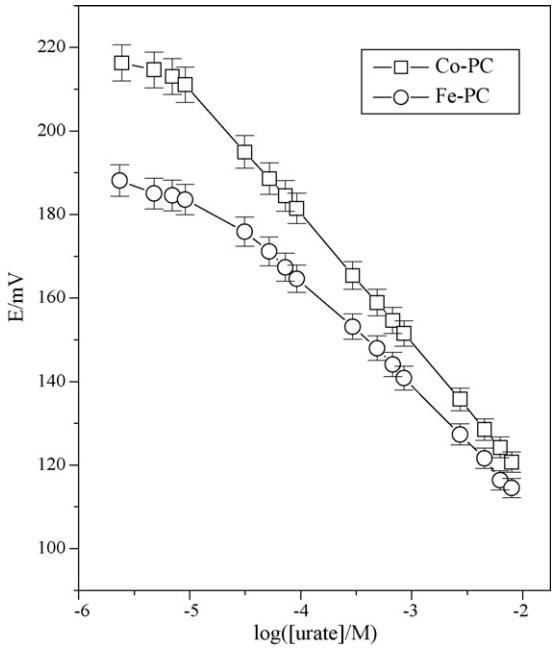

Fig. 4. Potentiometric response of urate PVC membrane sensors based on CoPC and FePC plasticized in *o*-NPOE and 0.5% (w/w) TDMAC as a cationic excluder.

3.2. Flow injection potentiometry

Flow injection analysis (FIA) is a simple and versatile analytical technology for automating wet chemical analysis, based on the physical and chemical manipulation of a dispersed sample zone formed from the injection of the sample into a flowing

Table 2
Potentiometric selectivity coefficients ($\log k^{pot}_{urate,B}$) of CoPC and FePC PVC membrane sensors

Interferent	$\log k^{pot}_{urate,B}$			
	CoPC	CoPC + TDMAC	FePC	FePC + TDMAC
Formate	−3.21	−3.25	−2.28	−2.31
Acetate	−2.90	−2.95	−2.81	−2.85
Oxalate	−3.05	−3.10	−3.10	−3.12
Tartrate	−2.43	−2.51	−2.46	−2.31
Citrate	−3.61	−3.65	−2.76	−2.81
Ascorbate	−1.95	−2.16	−1.82	−1.81
Succinate	−2.75	−2.81	−2.50	−2.54
Urea[a]	−3.61	−3.69	−3.54	−3.61
Thiourea[a]	−3.71	−3.77	−3.12	−3.18
Glycinate	−2.54	−2.65	−2.34	−2.44
Creatinine	−2.45	−2.51	−1.96	−1.98
F^-	−3.26	−3.41	−2.50	−2.54
Cl^-	−3.1	−2.86	−2.81	−2.76
Br^-	−3.26	−3.31	−2.76	−2.75
I^-	−1.21	−1.14	−1.32	−1.27
SCN^-	−1.10	−1.05	−1.23	−1.05
NO_3^-	−2.81	−2.87	−2.92	−2.90
NO_2^-	−1.82	−1.85	−1.91	−1.87
SO_4^{2-}	−4.23	−4.24	−4.15	−4.15
SO_3^{2-}	−0.98	−1.10	−1.10	−1.23
$S_2O_3^{2-}$	−3.94	−3.96	−3.58	−3.61
PO_4^{3-}	−3.91	−3.95	−4.01	−4.10
ClO_4^-	−2.61	−2.69	−2.39	−2.52

[a] Selectivity value is calculated by matched potential method (MPM).

carrier stream and detection downstream. The flow cell used for detection of urate was designed to accommodate small size of the sensor to avoid large dispersion of the sample in the cell and give good response and recovery times, with a constant geometry and a minimum "dead" space. With short tubing (10 cm between injector and cell), low total volume between injector and sensor (500 μL) and relatively large injection volume (300 μL) dispersion in this system is kept to be minimum.

3.2.1. Optimization of the FIA set-up

Preliminary studies were carried out to establish the best flow injection parameters. The design of the flow cell used in this work is a laboratory made which depends on a tubular sensing electrode with a sensing area 7 mm × 2 mm and a planar chip membrane sensor which is attached to the flow tubing and fixed with PVC. Its sensing area is about 5 mm × 2 mm. The design of the flow cell used shows a number of advantages. These are the simple fabrication, full membrane liquid-contact and ease of accommodation of a commercial reference electrode in the cell block. These characteristics maintain the most of the general features of the traditional ion selective membrane ISE in terms of homogenous thickness and fixed area. It was found that the

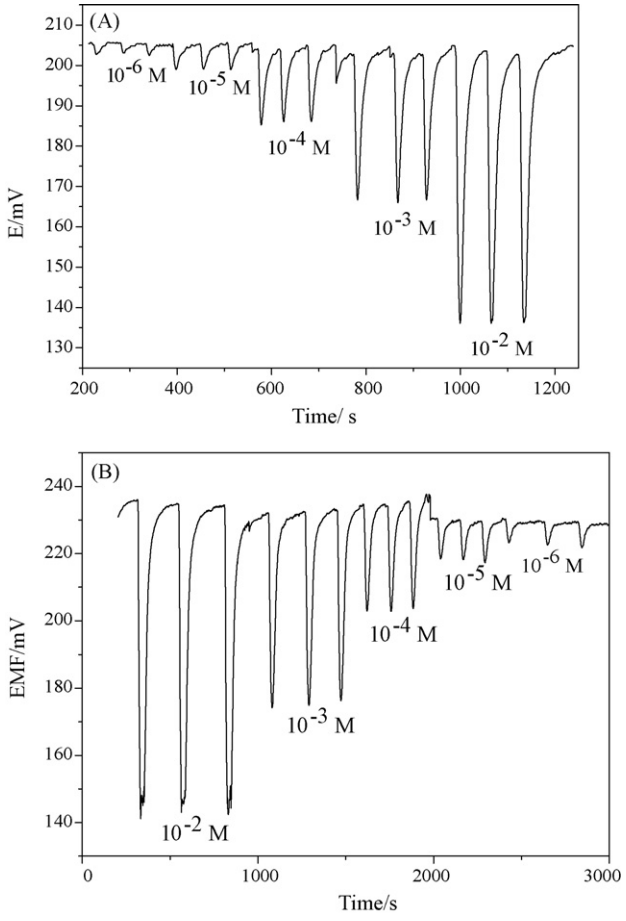

Fig. 5. Transient potentiometric signals obtained in triplicate for CoPC membrane based sensor. Conditions: carrier solution, 10^{-2} M phosphate buffer pH 7.2; flow rate, 3.5 mL min^{-1}; injection valve, 300 μL. Inset: calibration graph obtained. (A) FI signals obtained by using the planar detector. (B) FI signals obtained by using the tubular detector.

Table 3
Response characteristics of CoPC in presence of TDMAC and plasticized in *o*-NPOE under FI operation

Parameter	CoPC	
	Planar detector	Tubular detector
Slope (mV decade^{-1})*	−24.1 ± 0.6	−30.6 ± 0.3
Correlation coefficient, *r*	0.9997	0.9998
Lower limit of detection (μg mL^{-1})*	5.31 ± 0.2	6.70 ± 0.3
Limit of linear range (M)	5.6×10^{-5}	8.0×10^{-5}
Optimum flow rate (mL min^{-1})	3	3
Life span (week)	8	8
Response time (s)	5	5
Out put, sample (h^{-1})	∼50	∼20

* Average of six measurements.

planar chip detector is more favored than the tubular detector in the flow cell. The CoPC planar and tubular membrane based sensors give slopes −24.1 ± 0.6 and −30.6 ± 0.3 mV decade^{-1} with a detection limit 5.31 and 6.7 μg mL^{-1} over linear concentration range 5.6×10^{-5} and 8×10^{-5} M, respectively (Fig. 5). Response characteristics of both types of detectors were shown in Table 3. It showed that the detector with planar sensor is much better than that equipped with a tubular sensor for flow injection determination of urate. This can be attributed to the geometry of the tubular one and the use of a reference electrode close to the sensing membrane which reduces the signal noise, stabilizes the baseline, and decreases the drift [34]. The effect of varying sample loop length from 20 to 100 cm (100–500 μL) for urate solution ranging from 10^{-5} to 10^{-2} M on the potentiometric response (slope in mV decade^{-1}) at pH 7.2 was initially evaluated. The potentiometric response increased with the increase of sample volumes from 100 to 300 μL and was maintained constant in sample volume higher than 300 μL. Therefore, a sample volume of 300 μL was selected for further experiments.

3.2.2. Effect of flow rate

The effect of carrier buffer flow rate was examined over a range of flow rates from 1.5 to 6 mL min^{-1} for urate solutions ranging from 10^{-5} to 10^{-2} M using the small planar detector. The potentiometric response (slope in mV decade^{-1}) was recorded against the flow rate. The optimal flow rate was chosen to be 3 mL min^{-1}. In flow rates lower than 3 mL min^{-1}, the small planar detector showed a slight memory effect, long washing times, and low analytical frequency. At flow rates higher than 5 mL min^{-1}, the detector response decreases and the peak width become narrow because high flow rates decrease the residence time of the sample. The formation of the potentiometric signal is based, in general, on dynamic equilibrium reactions, thus the magnitude of the signal is independent of the rate of the transport process. Accordingly, the flow rate affects only the transient signal produced at rapid concentration changes [35]. A similar behavior for the effect of flow rate on the detector response was also reported previously [36,37]. A dynamic response with the planar chip detector was studied at a settled flow rate at 3 mL min^{-1}. Under these conditions, the relative standard deviation of the FIA signals was ±1.5% for 10–100 μg mL^{-1} urate. The sampling rate was approximately 50 runs per hour.

Table 4
Determination of urate in some biological samples using CoPC membrane based sensor under static and hydrodynamic modes of operations and spectrophotometry

Sample	Urate ($\mu g\,mL^{-1}$)[a]				
	Spectrophotometry	Direct potentiometry	Difference	FIA	Difference
Urine	176.7 ± 0.2	173.2 ± 0.6	3.5	174.1 ± 0.8	2.6
	211.2 ± 0.3	209.3 ± 0.9	1.9	210.6 ± 0.4	0.6
	131.4 ± 0.1	128.2 ± 0.7	3.2	130.3 ± 0.5	1.1
Serum	35.7 ± 0.9	33.3 ± 0.6	2.4	34.1 ± 0.9	1.6
	55.9 ± 0.8	53.7 ± 0.3	2.2	52.6 ± 0.1	3.3
	95.5 ± 0.5	96.2 ± 0.4	0.7	93.3 ± 0.2	2.2

[a] Average of six measurements.

3.3. Determination of uric acid in biological human fluids

In order to establish the usefulness of the proposed method, the determination of uric acid was performed in human serum and urine samples. These samples were stored at −20 °C until required for analysis. The same samples were analyzed by using the commercial spectrophotometric kit at 25 °C for comparison with the present potentiometric procedure under static and hydrodynamic modes of operation and the results were given in Table 4. A *t*-test reveals that there is no significant difference between the means and variances of static and hydrodynamic potentiometric sets of results. Validation of the proposed potentiometric methods for determining urate was made by measuring the range (R), precision (σ), repeatability (Cv_w), between-day-variability (Cv_b), linearity (correlation coefficient) and sensitivity (slope). Results obtained on six batches (six determinations each) using the quality assurance standards [38] were shown in Table 1. Statistical comparison between the data obtained by the proposed potentiometric method and that obtained by the spectrophotometric method show no significant difference.

4. Conclusions

The determination of urate by using a flow injection system with potentiometric detection proved to be an advantageous method over other methods, since determinations within a wide concentration range, regardless of the samples colors and turbidity could be accomplished. The potentiometric detection system of increased sensitivity provides improved precision, high sampling rates and better reproducibility. This system facilitated the determination of urate with high sampling rates, and a low consumption of sample volume. The results obtained in this work enable to conclude that this methodology can be applied to the analysis of urate in biological fluids rapidly and accurately. Tubular and planar chip urate membrane sensors are incorporated in flow-through cells and used as detectors for flow injection analysis (FIA) of urate. The sensors are used for determination of urate in human biological fluids at an input rate of 50 samples per hour. No interferences are caused by most anions that normally present in biological fluids. The results favorably compare with data obtained using the standard spectrophotometric method.

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