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Measurement of sodium ion concentration in undiluted urine with cation-selective polymeric membrane electrodes after the removal of interfering compounds

Feyisayo Phillips¹, Kim Kaczor¹, Neel Gandhi¹, Bradford D. Pendley^{1,2}, Robert K. Danish², Michael R. Neuman³, Blanka Toth⁴, Viola Horvath⁵, and Erno Lindner¹

¹ Department of Biomedical Engineering, The University of Memphis, Memphis, TN 38152

² Department of Pediatrics, University of Tennessee Health Science Center Memphis, TN 38103

³ Department of Biomedical Engineering, Michigan Technological University, Houghton, MI 49931

⁴ Department of Inorganic and Analytical Chemistry, H-1111 Budapest, Szt. Gellért tér 4, Hungary

⁵ Research Group of Technical Analytical Chemistry, Hungarian Academy of Sciences H-1111 Budapest, Szt. Gellért tér 4, Hungary

Abstract

The measurement of sodium ion concentration in urine can provide diagnostic information and guide therapy. Unfortunately, neutral-carrier-based ion-selective electrodes show a large positive drift and loss in selectivity in undiluted urine. The extraction of electrically neutral lipids from the urine into the sensing membrane was suggested as the main source of the drift, loss of selectivity and the consequent incorrect concentration readings.

In this work, (i) solvent-solvent extraction, (ii) membrane-immobilized solvent extraction, and (iii) solid phase extraction were used to remove interfering compounds from urine samples. The “cleaned” urine samples were subsequently analyzed using a calixarene (sodium ionophore X)-based, solid-contact, sodium-selective electrode in a flow-through manifold. The solid-contact sodium sensors had excellent stability in cleaned urine and an acceptable bias compared to commercial clinical analyzers.

INTRODUCTION

Modern medicine relies heavily on the measurement of ions (e.g., sodium, potassium) and small molecules (e.g., glucose) to provide information necessary to diagnose illnesses and monitor treatment. Many of these measurements are made in the hospital clinical laboratory, but miniaturized sensor technologies such as the iSTAT [1] and glucometers have enabled accurate measurements to be made at the patient’s bedside in real time, thus allowing assessment and therapy to occur more quickly.

While current sensor technologies measure analytes in blood, urine measurements also provide diagnostic information. A scenario commonly encountered is differentiating between causes of low urine output, which can be distinguished in part by measuring the concentration of sodium in a sample of urine. A relatively low urine sodium concentration indicates conservation

of sodium and water by the kidney (e.g., the patient is dehydrated) while a relatively high urine sodium concentration suggests acute renal failure.

Urine sodium measurements can also play a role in optimizing therapy. It is estimated that 18.2 million American have diabetes mellitus, the sixth leading cause of death in 2000 [2]. Diabetes can lead to life-threatening electrolyte and metabolic derangements, including diabetic ketoacidosis (DKA). Treatment of DKA relies heavily on monitoring the patient's plasma electrolytes and other small molecules (e.g., glucose) to ensure that the proper amounts of fluids and insulin are provided. Since patients respond to this therapy in differing fashions, treatment must be individualized to avoid complications. For example, a standard fluid therapy involves giving intravenous normal saline (e.g., 154 mM NaCl) initially followed by administration of a solution of reduced concentration of sodium chloride (e.g., half-normal saline or 77 mM NaCl) to avoid the excessive elevation of the plasma sodium levels. If the kidneys retain sodium and too much sodium chloride is given, the plasma sodium will increase undesirably. If the kidneys lose sodium excessively, the plasma sodium concentration can decrease too rapidly leading to neurologic complications. Monitoring how the kidneys are handling sodium may improve treatment management.

In this work, the feasibility of measuring urinary sodium ion concentration with a solid-contact sodium ion-selective electrode was tested. The sodium electrodes were incorporated into a flow channel, which eventually could be connected to a Foley urinary catheter. Such a device might be used to monitor rehydration therapy in patients with DKA and dehydration.

The measurement of ion concentrations in undiluted urine with neutral carrier-based ion-selective electrodes has serious challenges. The measurements result in systematically low readings [3], a large positive drift [4] and loss in selectivity [4,5]. Previously, these systematic errors were attributed to "anion interference" [6], since they could be reduced significantly by using highly hydrophobic silicone rubber-based membranes [3]. Unfortunately, most ionophores do not work adequately in silicone rubber membranes. Therefore the measurements have been commonly performed in diluted urine samples [3,7,8] when using PVC membrane-based ion-selective electrodes. Recently, the extraction of electrically neutral lipophilic compounds (natural lipids) from the urine into the sensing membrane has been suggested as the main source of drifting potentials and changing selectivity coefficients [4] because these compounds are believed to act as secondary ionophores in the membrane with poor selectivity. Interference induced by high lipid concentrations in serum were also reported [9] upon the measurement of sodium, potassium, and chloride concentrations by ion-selective electrodes. Consequently, the prevention of the extraction of these compounds into the membrane phase or their removal from the sample would allow the measurement in undiluted urine. The use of novel, fluororous membranes doped with fluorophilic tetraphenylborate salts are expected to minimize the interference related to the extraction of highly lipophilic compounds into the membrane [10,11]. In this work, the removal of the interfering compounds from urine were performed by (i) solvent-solvent extraction; (ii) membrane-immobilized solvent extraction; and (iii) solid phase extraction. These extraction protocols provided undiluted, "clean" urine in which the sodium concentrations were assessed using solid-contact, sodium-selective electrodes integrated into a flow-through manifold. The solid-contact sodium sensors showed superb stability in the "clean" urine samples and the results of the measurements correlated well with data provided by commercial clinical analyzers for "unclean" urine samples.

EXPERIMENTAL SECTION

Chemicals and reagents

In general, the chemicals used for ion-selective membrane casting and for preparing solutions were the highest analytical grade available. However, in certain solvent-solvent extraction

experiments, technical grade solvents were used. The ingredients of the sodium ion-selective membranes were Selectophore grade from Fluka Chemical Corp. (Milwaukee, WI): 4-tert-butylcalix[4]arene-tetraacetic acid tetraethylester (sodium ionophore X), bis (2-ethylhexyl) sebacate (DOS), potassium tetrakis (4-chlorophenyl) borate (KTPClPB), high molecular weight poly(vinyl chloride) (PVC). For membrane casting, tetrahydrofuran (THF) and cyclohexanone were used as solvents. Both were purchased from Fisher Scientific (Fair Lawn, NJ).

Pyrrole (Sigma Chemical Co.(St. Louis, MO) was freshly distilled before use. Sodium hexacyanoferrate (II)-decahydrate ($\text{Na}_4[\text{Fe}(\text{CN})_6] \cdot 10\text{H}_2\text{O}$) was purchased from Sigma Aldrich Laborchemikalien GmbH. (Germany), sodium chloride and magnesium chloride were obtained from Fisher Scientific (Fair Lawn, NJ). All solutions were made with 18.2 M Ω -cm resistivity deionized water (Milli-Q Gradient A10, Millipore Corp. Bedford, MA).

The response function of the sodium ion-selective electrodes were tested in: (i) constant ionic strength (0.415 M) NaCl solutions using MgCl_2 for ionic strength adjustment, (ii) Instrumentation Laboratory (Lexington, MA) standard solutions labeled as IL TEST^{TM} CAL 1 (pH 7.384, Na^+ 140 mmol/L, K^+ 5 mmol/L, Ca^{2+} 1 mmol/L and Cl^- 119 mmol/L) and IL TEST^{TM} CAL 2 (pH 6.84, Na^+ 100 mmol/L, K^+ 2 mmol/L, Ca^{2+} 3 mmol/L and Cl^- 81 mmol/L and glucose 90 mg/dL), (iii) Instrumentation Laboratory quality control standard solutions labeled as ContrIL plus Level 1- Alkalosis (pH 7.592–7.632, pCO_2 17–23 mmHg, pO_2 134–148 mmHg, Na^+ 116–122 mmol/L, K^+ 2.5–3.0 mmol/L, Ca^{2+} 0.64–0.76 mmol, Cl^- 85–93 mmol/L), Level 2- Normal (pH 7.401–7.441, pCO_2 33–39 mmHg, pO_2 84–96 mmHg, Na^+ 136–142 mmol/L, K^+ 4.3–4.7 mmol/L, Ca^{2+} 1.02–1.14 mmol, Cl^- 101–109 mmol/L) and Level 3- Acidosis (pH 7.146–7.186, pCO_2 58–68 mmHg, pO_2 51–63 mmHg, Na^+ 155–161 mmol/L, K^+ 5.9–6.7 mmol/L, Ca^{2+} 1.4–1.58 mmol, Cl^- 126–134 mmol/L and (iv) NIST Quantitative Urine Control Standards from Bio-Rad Laboratories (Irvine, CA) labeled as Level 1 Normal (Lot No 6219, Na^+ 52–64 mmol/L, K^+ 20–25 mmol/L, Ca^{2+} 4.7–5.7 mmol/L and Level 2 Abnormal (Lot No 62192, Na^+ 152–185 mmol/L, K^+ 101–124 mmol/L, Ca^{2+} 9.6–11.7 mmol/L).

Macro- and micro electrode fabrication

The conventional macro and planar microfabricated electrodes used in this study were prepared with the same sensing membrane containing 66wt% DOS (~200 mg), 33wt%, PVC (~100 mg), 0.66wt% Na^+ ionophore X (~2 mg), and 0.17 wt% KTPClPB (~0.5 mg; 50mol% compared to the ionophore). For macroelectrode preparation, the membrane components were dissolved in 2 mL THF and cast into a 30 mm diameter glass ring, fixed on a glass plate. After evaporation of the THF, an approximately 200 μm thick membrane remained on the glass plate, from which a 6.8 mm diameter disc has been incorporated into a Philips IS-560 liquid membrane electrode body (Moller glassbaserei, Zurich Switzerland). For the planar microelectrode fabrication, either two or three 7 μL microliter aliquots of the same cocktail were spread over the sensor well (see below). The macroelectrodes were prepared to test the stability of the electrodes in urine before and after the urine samples were “cleaned” by the extraction procedures. The planar sensors were assembled into a manifold in which a four- and five-site array sandwiched a zig-zag flow channel made of poly (methyl methacrylate) (PMMA) as shown in Figure 1 [12]. They were primarily used for the quantitative determination of the sodium ion content in patient urine samples.

The planar sensors were fabricated using conventional screen printing techniques and were assembled with polypyrrole-based solid-contact. Details on the screen printing process and the protocols for assembling the solid-contact electrodes were provided previously in connection with novel solid-contact potassium [13,14] and lead [13,14] ion sensors of excellent stability or nanomolar detection limit, respectively. In summary, a ~2mm diameter and 100 μm deep

sensing well is created with a Pt electrode on its bottom using Metech type 7600A insulating glaze (Metech Electronic Materials, Elverson, PA) as the insulating material (Figure 1 A). The sensing sites were connected through the Metech glaze insulated lead wires to the rectangular bonding pads (Figure 1A). Polypyrrole was electrochemically deposited onto the platinum electrode surfaces. The electrochemical polymerization was performed potentiostatically at an applied potential of +1V for 120 seconds in an oxygen-free 0.4 M pyrrole monomer solution containing 0.4 M $\text{Na}_4[\text{Fe}(\text{CN})_6]$. The thickness of the polypyrrole film was determined as $15 \pm 5 \mu\text{m}$ using an Alpha-Step 500 surface profiler (Tencor Instruments, Mountain View, CA). The polypyrrole-coated platinum electrodes were rinsed thoroughly to remove traces of the monomer and the $\text{Na}_4[\text{Fe}(\text{CN})_6]$ electrolyte. Finally, the electrodes were dried using a stream of N_2 gas and by dispensing a drop of THF onto the polypyrrole coated electrode surface and repeating the drying process by blowing N_2 gas over the sensor surface.

As the last step of the planar electrode preparation, the polypyrrole-coated electrode sites were coated with the plasticized PVC membrane by dispensing 7 μL of the membrane cocktail over the electrode wells and a part of the blue glaze insulating layer two to three times. The individual electrodes are arranged into four- and five-site arrays and pressed against the flow channel with silicon rubber springs placed into the inner groove of an anodized aluminum flow cell housing. As shown in Figure 1, the flow-through manifold in the flow cell was connected to the flow-through reference electrode and a multi-position valve and the peristaltic pump.

Instruments

For solution transport, a Gilson Inc. Model, Minipuls 3 (Gilson Inc., Middleton, WI) peristaltic pump was used. For the potentiometric data acquisition, a 16 channel Lawson Labs Model 201 (Malvern, PA) system was connected to a Dell computer through a 24-bit serial interface. The data acquisition system was controlled by the 3.0 version of the L-EMF DAQ software sold with the data acquisition system. For the electrochemical deposition of polypyrrole, a Model 363 (Princeton Applied Research, Oak Ridge, TN) or ECO Chemie PGSTAT 12 (Brinkmann Instruments Inc. Westbury, NY) potentiostat/galvanostat system controlled by GPES version 4.8 software was used.

In the potentiometric measurements with ion-selective macroelectrodes, a Model 90-02, double junction Ag/AgCl reference electrode (Thermo Orion, Beverly, MA) with a commercially available inner filling solution and 0.1 M lithium acetate outer filling solution was used. In the potentiometric measurements with the planar Na^+ sensor arrays in the flow-through manifold, a model 61148 flow-through Ag/AgCl reference electrode was used with saturated KCl as filling solution (Instrumentation Laboratories, Lexington, MA) downstream to the Na^+ sensors.

The sodium ion concentration of the untreated urine samples and urine samples from which the interfering components had been removed by solvent-solvent, membrane-immobilized solvent extraction or solid phase extraction protocols ("cleaned" urine samples), and the 3) NIST Quantitative Urine Control Standards were determined with a Vitros 5,1 Clinical Analyzer (Ortho-Clinical Diagnostics Inc. Rochester, NY) and/or a Synthesis 35 Analyzer (Instrumentation Laboratories, Lexington, MA). The Vitros 5,1 Clinical Analyzer is currently used in the central laboratory at LeBonheur Children's Medical Center (LBCMC, Memphis, TN) and is used for both serum and urine assays. The Vitros 5,1 analyzer utilizes *single use* Kodak slides for the determination of sodium ion concentrations in urine. These slides are equipped with two plastic membrane-based ion-selective electrodes: one for the assessment of the sodium and the other for determination of potassium ion concentration. Above 8 mmol/L K^+ concentrations, the instrument considers the interference induced by the potassium ions on the sodium sensor and a corrected Na^+ concentration is provided. The Vitros 5,1 analyzer has a dynamic range of 5 – 250 mmol/L for Na^+ in urine. The sodium ion concentrations of samples above 250 mmol/L are determined following an automated dilution.

In contrast, the sodium ion concentrations in the Synthesis 35 Analyzer are determined with a sodium ion-selective glass electrode. The measuring range of the Synthesis 35 analyzer is between 80 and 200 mmol/L sodium. Since this analyzer is not designed for urine analysis, it has no correction algorithm for considering eventual interference from potassium ions. It cannot be used for the determination of sodium ion concentrations below 80 mmol/L, and the analysis of samples with Na⁺ concentrations above 200 mmol/L must be diluted manually before analysis.

Sodium ion concentration measurements using the solid contact sodium sensors were performed in the stopped flow mode to minimize noise and sample volume. First, the individual urine samples were aspirated into the flow-through cell at 1.5 mL/min flow rate (75 s). Next the pump was stopped for two minutes. Finally the urine sample was flushed from the flow cell by a standard solution (75 s) and the pump was stopped again for two minutes. The data acquisition was continuous during the whole process but the evaluation was based on the data collected during the 2 minute periods when the pump was stopped. Indeed, the average potential in a one minute segment in this 2 minute period, with the least potential fluctuation, has been used as a characteristic potential for a certain standard or urine sample. These potential data have been determined using an automated evaluation program using Excel.

Urine Specimens

Both pooled and individual urine samples were used in our studies. IRB approval was granted to obtain urine samples from Le Bonheur Children's Medical Center (LBCMC, Memphis, TN). Thirty individual urine samples, each greater than 10 mL, were obtained from LBCMC Pathology Laboratory. The Na⁺ concentrations of these uncleaned samples were determined by the Vitros 5,1 Analyzer in the central laboratory of LBCMC and were provided to us without patient-identifying information, according to the IRB-approved protocol. The samples were transported from LBCMC to the University of Memphis on ice, where they were promptly frozen at -20°C and kept frozen until use. After removal of the interfering compounds from the samples by one of the extraction processes discussed subsequently, the sodium ion concentration in the urine was determined in our laboratory by the Synthesis 35 Clinical Analyzer and by using the planar, solid-contact Na⁺ sensors in the flow-through manifold shown in Figure 1.

In addition to the individual patient urine samples, aliquots of pooled urine samples were utilized for the determination of the stability of the solid-contact sodium sensors in "clean" urine, and for assessing the inter- and intra-assay variation of the Na⁺ concentration determinations using the solid-contact sodium sensors of this study, the Synthesis 35 and the Vitros 5,1 analyzers. Three urine samples collected from a single individual at three successive times were pooled and portioned into 4–5 mL vials, giving a total of 42 aliquots. These aliquots were frozen at -20°C and kept frozen until use.

Removal of interfering substances from urine

Solvent-solvent extraction, membrane-immobilized solvent extraction and solid phase extraction were used to remove lipophilic compounds from urine samples. Although, the chromatographic analysis of the untreated and "clean" urine samples provided qualitative information on the efficiency of the cleaning process, the components contributing to the drift of the electrodes in untreated urine could not be identified. Accordingly, to test the efficacy of the extraction process, a freshly prepared Na⁺-selective macroelectrode was placed into the urine samples and its potential drift was assessed. The extraction procedure was considered successful if the Na⁺-selective electrode did not show a positive drift when exposed to the "cleaned" urine. Once a membrane was exposed to "uncleaned" urine, it was discarded.

For solvent-solvent extraction 100 mL of urine was extracted with 6×2 mL of DOS in a separation funnel. The funnel was agitated gently on a tilt table for 30 minutes and then the funnel was placed on a ring stand for 30 minutes to let the phases separate. The urine was drained from the bottom of the funnel into a clean separatory funnel and the process was repeated with the next aliquot of fresh DOS. This protocol was designed using an “average” partition coefficient for electrically neutral lipids in an octanol-water system [4]. Using this “average” partition coefficient, we calculate that the fraction of lipophilic material in the “cleaned” urine should not exceed 4×10^{-7} of the original amount after extracting 100 mL of urine six times with 2 mL portions of DOS.

Since DOS proved to be a very effective solvent in removing the interfering compounds from urine in solvent-solvent extraction experiments, our next attempt with membrane-immobilized solvent extraction involved using highly plasticized PVC membranes with 67 wt% DOS and 33 PVC content (total volume of DOS, 40 mL) as an immobilized liquid phase extractor for the removal of interfering compounds from urine. These experiments were aimed as model experiments for the removal of the interfering compounds using highly plasticized PVC tubing. The “extractor” membranes were cast with as large a surface area to volume ratio as possible. The membranes were placed into untreated urine, which was gently agitated for 3 hours. Based on the membrane thickness and the magnitude of the diffusion coefficients of ionophore ion complexes in the membrane ($\sim 2 \times 10^{-8} \text{ cm}^2/\text{s}$) [15,16], this time was calculated to be sufficient for equilibration. The urine to DOS ratio and the number of extraction steps was the same as in the solvent-solvent extraction studies.

Solid phase extraction (SPE) cartridges, model ODS-5 Octadecyl; 18%; EC (Whatman Inc (NJ) were also tested as an extraction device. Before use, the cartridges were conditioned with 3×6 mL of methanol and rinsed with 3×6 mL deionized water. Immediately after the conditioning, 2×6 mL urine was forced through the cartridge over a time period of 2×(60–90s) by applying a mild positive pressure using a syringe. The first aliquot of urine passed through the cartridge, approximately 1 mL, was always discarded because the water present in a fully conditioned cartridge dilutes the urine samples and introduces a systematic negative error. Since there is usually plenty of urine available, this does not pose a serious limitation.

Methods used for the identification of interfering compounds in urine

For Fourier transform infrared spectroscopy (FTIR) the 2 mL aliquots of DOS used in the solvent-solvent extraction experiments and 2 mL aliquots of DOS used for eluting the captured interfering compounds from the SPE cartridges were analyzed with a Tensor™ 27 FTIR system equipped with a biological ATR cell (Brucker, Ettlingen, Germany). We searched for changes in the recorded spectra of DOS samples collected during the successive extraction process using the FTIR-ATR spectrum of “clean” DOS as reference.

High performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC-MS-MS) was performed on urine extract components, which were separated on a Purospher RP18e reversed phase column (3×125 mm, 5 µm, Merck, Darmstadt, Germany) using a Perkin Elmer Series 200 HPLC system (Perkin Elmer Instruments, Norwalk, CT), coupled to an Applied Biosystems 4000 Q Trap triple quadrupole mass spectrometer equipped with an ESI source (TurboIonSpray, Applied Biosystems GmbH, Darmstadt, Germany). Eluents were 0.1% aqueous solution of formic acid (eluent A - puriss. p.a., Riedel-deHaën, Seelze, Germany) and 0.1% formic acid in acetonitrile (eluent B - HPLC gradient grade, Panreac, Barcelona, Spain). The urine components were eluted by gradient using a linear increment from 90% to 0% eluent A for 10 minutes then an isocratic period of 10 minutes at 0% eluent A, finally switching instantly to 90% eluent A and holding it in isocratic mode for 3 minutes. Fifteen microliter samples were injected. The urine extract components were detected by a Q1 scan (m/z 50–1000 amu) in positive (5000 V) mode with a source temperature of 400 °C.

A 7.0 mL aliquot of human urine was extracted with either 21 mL ethyl acetate (super purity solvent from Romil Ltd. Cambridge, England) or 21 mL dioctyl phthalate (DOP) (purum grade, Fluka Chemical Corp., Buchs, Switzerland), in a stoppered glass tube that was vortexed for 5 minutes. The mixture was centrifuged at 3700 rpm for 15 minutes. The upper ethyl acetate layer was aspirated into a glass tube and dried under N₂ flow at 30°C, then redissolved in 0.5 mL acetonitrile and measured in HPLC-MS-MS. The DOP extract was discarded as it cannot be treated similarly due to its high boiling point.

Unextracted urine samples and extracted urine samples were concentrated on a Chromabond C18 ec (100 mg) SPE cartridge (Macherey-Nagel, Düren, Germany). Previous experiments have shown that the suspected interfering compounds can be extracted from the urine on a C18 SPE column. The SPE cartridge was conditioned with 5.0 mL methanol, and with 1.0 mL water before the 5.0 mL urine sample was applied to it. Next, the cartridge was washed with 2.0 mL water and dried by aspirating dry air through it for 5 minutes. Finally, the sample was eluted with 0.5 mL acetonitrile and the eluate was injected into the HPLC-MS-MS instrument.

RESULTS AND DISCUSSION

Response of the sodium sensor in aqueous solutions

The long-term goal of this work is the incorporation of an ion-selective electrode into a Foley catheter (Figure 1F) for the continuous monitoring of urine sodium ion concentration during the first 24 hours of treatment of patients with DKA. The first step towards achieving this goal is the development of a miniature sodium sensor with excellent potential stability that is robust and without need of frequent calibration. Recently, we reported on planar potassium and lead sensors with a hexacyanoferrate (II)/(III) doped polypyrrole inner contact [13, 14, 17] that would fulfill these criteria. Based on these experiences, a novel solid-contact sodium ion-selective electrode was constructed using sodium ionophore X and with a Na₄[Fe(CN)₆]-doped, electrochemically deposited inner contact. These sensors were tested in a flow-through manifold shown in Figure 1. In Figure 2, we show the results of a 24-hour experiment in which the potential of 5 solid-contact electrodes was recorded continuously in the zig-zag flow channel while the electrodes were flushed with 100 mmol/L NaCl solution at 0.01 mL/min flow rate.

As shown in Figure 2, all five electrodes showed excellent potential stability with an average drift of less than 0.2 mV/day. The dip in the recorded data between 11 pm and 6 am is related to changes in the room temperature overnight. The response slopes of these electrodes in constant ionic strength (0.415 M) NaCl solutions were determined as 56.6±0.2 mV/[log Na⁺] in the concentration range of 10 to 317 mmol/L NaCl. The reproducibility of the electrode potentials in the different NaCl solutions was ± 0.2 mV. A typical potential time trace recorded with a planar sodium sensor during the calibration procedure is shown in Figure 3. The planar sodium-selective electrode with the sodium hexacyanoferrate II/III-doped, polypyrrole-based solid inner contact showed the same or even better performance characteristics as the solid-contact potassium [13] and lead [14] ion-selective electrodes of similar construction or the ISEs used in blood electrolyte analyzers.

Effect of exposure to urine

The exposure of cation-selective polymeric membrane electrodes to undiluted urine induces a positive potential drift in the measured potential that is coupled to the deterioration of the selectivity coefficients of the electrode [4]. The magnitude of the drift depends both on the composition of the membrane and the particular urine sample. An example of this drift is shown in Figure 4A. The extraction of natural, electrically neutral lipids into the sensing membrane has been suggested to be the source of this drift [4]. The removal of lipophilic compounds from

the urine sample eliminates the drift (Figure 4B). Although the specific cause of the drift remains unknown, our results are consistent with the importance of lipophilic compounds in inducing the drift. Despite numerous attempts to identify the compounds via HPLC-MS-MS and FTIR, we were unable to ascertain which compounds removed via extraction caused this potential drift.

In this study, solvent-solvent extraction, membrane-immobilized solvent extraction and solid phase extraction (SPE) were used to remove the lipophilic compounds from urine samples. In the first two processes, DOS served as extraction phase. In the SPE process, a 1.0 cm long column filled with C-18-coated hydrophobic particles served as the extraction phase. To assess the efficacy of removal of potentially interfering compounds by the extraction process, a sodium-selective macroelectrode was placed in the urine sample cleaned with one of the extraction processes. The macroelectrodes used for this screening test were only exposed to aqueous NaCl solutions before the test, i.e., they were not in contact with urine samples prior to this experiment. Although the extraction processes were different, our results were the same. A typical example is shown in Figure 4B. The stability of the macroelectrodes in urine samples “cleaned” with the different extraction procedures was the same as in a NaCl solution of the same concentration. This result is in sharp contrast to when the macroelectrodes with identical membranes were placed in the untreated urine samples (Figure 4A).

Analysis of “cleaned” urine samples

In evaluating the different extraction procedures, we showed that the sodium concentration of the urine samples does not change during these processes by determining the sodium ion concentrations of pooled urine samples before and after they were processed by solvent-solvent or solid phase extraction using the Vitros 5,1 and the Synthesis 35 commercial analyzers. The results were unambiguous: the sodium concentration did not change. Once it was shown that the applied extraction processes did not change the Na^+ concentration of urine samples, the reproducibility of the sodium ion concentration measurements in urine was determined (inter-assay variability). The sodium ion concentration was determined in separate aliquots of the same pooled urine sample during a one-week period using all three instruments: the Vitros 5,1 and Synthesis 35 analyzers and the solid-contact electrode in the flow-through manifold. There was no statistically significant difference in the inter-assay coefficients of variation, which ranged between 1.3% and 1.5%.

Finally, the agreement between the Na^+ concentrations provided for 30 individual patient samples by LBCMC Pathology Laboratory using the Vitros 5,1 analyzer and measured in our laboratory using the Synthesis 35 analyzer and the solid-contact sodium sensor-based flow-through manifold was evaluated. The sodium concentration values measured in our laboratory reflect the concentrations of the same samples after they were treated by SPE.

A representative section of the potential time traces recorded during the determination of the Na^+ ion concentration of individual urine samples is shown in Figure 5. The Na^+ concentration of the individual samples was determined using a two-point calibration performed at the beginning of the assay (not shown) and a single point calibration performed after the measurement of each sample. After a certain number of samples (generally eight) the two-point calibration was repeated to confirm that the slope and the E° value of the calibration curves of the solid-contact sodium sensors did not change significantly during these assays.

In Figure 6 we show two different representations of the correlation between the data provided for us by LBCMC Pathology Laboratory, based on measurements of untreated urine samples with the Vitros 5,1 analyzer and the data measured in our laboratory with SPE-cleaned urine samples using the solid-contact sodium sensors in the flow-through manifold. Figure 6A shows the conventional representation of the correlation in which a line is fitted to the data and a

correlation coefficient is used to quantify the quality of the fit (regression analysis). In this plot, besides the best-fit line the upper and lower 95% confidence limits of the data points are also shown. This kind of regression analysis and the use of the correlation coefficient has been criticized by Bland and Altman [18,19] who appropriately emphasized that the correlation coefficient measures the strength of the correlation between the two data sets and not the agreement between them, arguing that it is more appropriate to plot the differences between the two methods against the mean of the two methods as shown in Figure 6B. If the data in such a plot fall along a horizontal line, then a constant error or bias exists between the two methods that can be assessed by their analysis by calculating the mean (5.5) and standard deviation (SD = 8.1) of the differences. The horizontal lines in the figure represent the mean and the mean $\pm 2 \times$ SD values (21.7 and -10.7). If the lack of agreement can be represented by a constant bias, the slope in a plot like that shown in Figure 6A is unity and the fitted line has some non-zero intercept.

However, the data in Figure 6B apparently have concentration dependence illustrated by the slanted line in the figure. The line is plotted with its 95% confidence interval to prove that the slope is significantly different from the zero slope. Under such circumstances, a logarithmic transformation of the data, as shown in Figure 7, is advocated by Bland and Altman. The mean value of the logarithmic differences (0.018) and the 95% limits of agreement (0.08 and -0.04, respectively) are depicted as parallel lines in the figure. The antilog values of the mean of the logarithmic differences (1.04) indicate that the sodium ion concentration data measured with the Vitros 5,1 analyzer are expected to be 1.04 times larger than the value measured with the flow through manifold. This is the same conclusion one would draw from the slope of the best-fit data in Figure 6A in which the slope of the correlation plot Na^+ (Vitros) vs. Na^+ (solid

contact ISE) is $0.096(\frac{1}{0.96} = 1.04)$.

Similarly, we analyzed the correlations between the sodium ion concentrations measured by the solid-contact ISE and the Synthesis 35 analyzer as well as between the two commercial analyzers (Synthesis 35 and Vitros 5,1) using the method of regression analysis or the graphical techniques of Bland and Altman[18,19]. As can be seen in Figure 8A and B, the best-fit lines in these correlation plots show both a constant bias and a concentration-dependent deviation from the line of equality. An overview of the results of our data analysis is summarized in Table 1. Unfortunately, in cases when both a constant bias and a proportional error exist between the compared methods, the mechanistic application of the Bland and Altman analysis may lead to incorrect conclusions. The Bland-Altman analysis of the differences (Figure 6B) quantifies the level of disagreement adequately when the disagreement can be represented by a constant bias. The logarithmic transformation of the data (Figure 7) provides adequate answer when the disagreement can be represented by proportional error (non-zero slope in the scatter plot). Consequently, for the data sets of this study (Figure 8 and the last two columns of Table 1) the scatter plot in combination with a regression analysis (plots such as shown in Figs. 6A and 8) provide the best means of assessing the agreement between the two methods.

Analysis of NIST standards

In clinical laboratories, regular testing of the instruments with quality-controlled standards is required. In this work, Liquichek Urine Chemistry Control Standards (Bio-Rad Laboratories) were used for this purpose. Similar to the individual patient samples, these quality control standard samples were also “cleaned” by solid-state extraction before their analysis with the solid-contact ISEs in the flow-through array, but were analyzed without any sample treatment using the Vitros 5,1 and the Synthesis 35 analyzers. The conclusions of the correlation analysis using individual patient data are in agreement with those based on the analysis of these quality control standards. As can be seen in Table 2, the measured values in all cases fall within the

acceptable manufacturer assigned ranges. But, while the Na⁺ concentrations for both Level 1 and Level 2 were around the upper limit of these ranges when measured with the Vitros 5,1 analyzer, they were around the lower limit of the assigned concentration ranges when measured with the solid-contact ISEs in the flow-through array.

CONCLUSIONS

Upon the exposure of liquid membrane ion-selective electrodes to undiluted urine, the measured potential values drift and the electrodes lose their selectivity. These phenomena constitute serious hurdles in monitoring ion activities in undiluted urine. The drifting potentials and deteriorating selectivity coefficients are believed to be the consequence of the extraction of lipophilic, electrically neutral molecules from the urine into the sensing membrane. In this study, these interfering compounds were removed from the urine before analysis using solvent-solvent, membrane-immobilized solvent, and solid phase extraction. The solid-contact sodium sensors showed excellent stability in the undiluted “clean” urine samples without the need for frequent calibration, i.e. using a single, two-point calibration before a 24-hour measurement period.

The agreement between the sodium ion concentration data measured in undiluted “clean” urine samples of 30 individual patients using the solid-contact ISE in a flow-through manifold and two commercial clinical analyzers was analyzed by using regression analysis and the graphical methods of Bland and Altman. The agreement between the data sets was satisfactory.

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Figure 1.

(A) Screen printed electrode chip with bonding pad, lead wire, sensing area and semicircle-shaped indentations for alignment; (B) The same electrode chip with an additional Metech 7600A type insulating glaze layer utilized for shaping a 2 mm diameter, 100 µm deep sensor well with a Pt metal electrode on its bottom, and insulating the lead wire; (C) four- and five-site electrode arrays assembled from chips shown in B with sensing membranes cast over the sensing areas; (D) The sandwiched arrangement of the four- and five-site arrays with the PMMA zig-zag flow channel; (E) Flow analytical manifold used for testing the planar sensor arrays: (1) Sample containers, (2) multi-position valve, (3) peristaltic pump, (4) zig-zag flow channel sandwiched between the four- and five-site sensor arrays, (5) data acquisition unit, (6) computer/display, (7) flow-through reference electrode, and (8) waste container; (F) Utilization of the flow-through manifold for continuous bed side analysis of urine: (9) Foley catheter, (10) urine collection bag, (11) solid phase extraction (SPE) cartridge. The fraction of urine “cleaned” with the SPE cartridge is transported to the zig-zag flow channel (4).

Figure 2. Potential stability of solid-contact Na^+ sensors. The potential time traces were recorded in the flow-through manifold shown in Figure 1 when 100 mM NaCl was continuously pumped through the flow channel at ~ 0.01 mL/min flow rate. The two vertical lines labeled as 11 pm and 6 am indicate the actual time at two time instances. The drift values indicated above the potential time traces were calculated for the time frames between 1.8 and 5.8 hours and between 17.3 and 21.5 hours.

Figure 3.

Potential time traces recorded with three planar sodium sensors during a calibration process using the flow-through manifold. Constant ionic strength (0.415 M) NaCl standard solutions were pumped through the flow cell at 1.5 mL/min flow rate. For ionic strength adjustment, MgCl_2 was used. The sodium ion concentrations of the standards are marked on the curves in mmol/L units.

Figure 4.

The stability of sodium-selective macroelectrodes in urine (A) before and (B) after the removal of lipophilic interfering compounds from the urine sample by SPE. The three potential time traces represent the response of three individual electrodes, with identical membrane compositions, in the same urine sample.

Figure 5. Potential time traces of three planar sodium-selective electrodes during the assay of eight urine samples. The individual samples are labeled with numbers. Between the individual urine sample measurements a single point calibration was performed.

Figure 6.

Two different representation of the correlation between the urine concentrations of 30 individual patient samples measured by a Vitros 5,1 analyzer and the solid-contact sodium sensors in the flow-through manifold. A: Conventional representation of the data by using a best-fit line and a correlation coefficient. Above and below the best-fit line are the upper and lower 95% confidence limits for predicting a single response at a particular x axis value. B: Bland-Altman assessment of agreement: the differences of the Na^+ concentrations against the mean of the Na^+ concentrations measured with two methods. The open circles represent urine samples that were diluted 1:5 because they were beyond the dynamic range of the Vitros analyzer. The horizontal lines represent the mean value of the differences and the mean value $\pm$ twice the standard deviation of the differences. The slanted line with its 95% confidence bands represents the best-fit line to the data points showing the concentration dependence of the differences.

Figure 7.

Logarithmic transformation of the data shown in Figure 6B according to Bland and Altman [19]. The horizontal lines represent the mean value of the logarithmic differences and the mean value $\pm$ twice the standard deviation of the logarithmic differences. The open circles represent urine samples that were diluted 1:5 because they were beyond the dynamic range of the Vitros 5,1 analyzer.

Figure 8.

Correlation plots of urine concentrations of 30 individual patient samples measured by (A) the Synthesis 35 analyzer and the solid-contact sodium sensors in the flow array, and (B) the Vitros 5,1 analyzer and the Synthesis 35 analyzer. The equations in the figures represent the best-fit lines to the data points. The parameters of the equations (slope and offset) are provided with their standard deviation.

Table 1

Summary of the correlation analysis of data collected during the measurement of the sodium ion content of 30 individual patient urine samples with three different instruments: (i) Vitros 5,1 analyzer, (ii) Synthesis 35 analyzer and (iii) solid-contact ISE-based flow-through assembly.

	Vitros 5,1 vs. solid-contact ISE	Synthesis 35 vs. solid-contact ISE	Vitros 5,1 vs. Synthesis 35
Linear regression with zero intercept			
Slope of the fitted line $\pm$ standard deviation	0.96 $\pm$ 0.01	[1.03 $\pm$ 0.01]	[0.92 $\pm$ 0.01]
Correlation coefficient (R^2)	0.991	[0.978]	[0.962]
Residual standard deviation [†]	7.0	[9.6]	[11.3]
Linear regression with intercept			
Slope of the fitted line $\pm$ standard deviation	0.95 $\pm$ 0.02	1.11 $\pm$ 0.03	0.83 $\pm$ 0.03
Intercept $\pm$ standard deviation	2.5 $\pm$ 2.7	-13.8 $\pm$ 4.7	18.0 $\pm$ 4.5
Correlation coefficient (R^2)	0.991	0.983	0.977
Residual standard deviation [†]	7.0	8.4	8.9
Bland-Altman analysis of the differences			
Mean of the differences (bias) $\pm$ standard error	[5.5 $\pm$ 1.5]	[-3.4 $\pm$ 2.1]	[9.5 $\pm$ 2.9]
Standard deviation of differences	[8.1]	[10.5]	[14.7]
95% confidence interval of the bias	[21.6-(-10.7)]	[17.6-(-24.5)]	[38.8-(-19.8)]
Bland-Altman analysis of the logarithmic differences			
Mean differences on log scale	0.0184	[-0.0038]	[0.0193]
95% confidence interval of log differences	0.0816-(-0.0449)	[0.0589-(-0.0664)]	[0.0913-(-0.0528)]
Antilog mean $\pm$ 95% limits of agreement	1.04 $\pm$ 0.16	[0.99 $\pm$ 0.15]	[1.05 $\pm$ 0.17]

[†] Data in [bracketss] indicate circumstances when the use of the selected statistical analysis is inappropriate.

Table 2

Assigned and measured sodium ion concentrations in Liquichek Urine Chemistry Control Standards (Level 1 and 2)

Assigned [Na ⁺] (mM)	Method	Measured [Na ⁺] (mM)		
		Vitros 5,1	Synthesis 35	Flow-array
Level-1				
48–59	Vitros 5,1 analyzer	59	†	
52–64	Ion-selective electrode meas.			53.3 ± 0.8
Level-2				
144–176	Vitros 5,1 analyzer	176		
152–185	Ion-selective electrode meas.		165	154.0 ± 2

† The Synthesis 35 analyzer was designed for whole blood electrolyte analysis. It provides an error message below a certain sodium concentration value.