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Analysis of the voltage response to identify macromolecule quantities in an electrolyte

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Abstract— **Current techniques of detecting proteins in solution can prove to be time consuming and expensive. Although low cost techniques are available, these are often slow and inaccurate. The authors propose an innovative technique for detecting the presence of proteins and other organic macromolecules in an electrolyte by analyzing the voltage waveform resulting from a biphasic, constant-current, charge-balanced electrical stimulation. Initial experiments have proven the viability of the technique. However due to the limitation of technique accuracy, further refinement of the measurement approach is required.**

I. Introduction

The aim of this paper is to investigate the protein concentration in urine samples using electrochemical techniques to provide a novel means of detection of conditions such as pre-eclampsia in pregnant women. Pre-eclampsia is a common disorder in human pregnancy that affects about 10% of pregnant women. It is a medical condition stemming from high blood pressure (hypertension) that usually occurs in the third trimester. In the early stage of pre-eclampsia, symptoms include: elevation of blood pressure associated with increasing water retention, and protein excretion from the kidneys (proteinuria). If left untreated, pre-eclampsia can develop into more serious symptoms. These include severe headaches, vision disturbances, vomiting, and abdominal pain. In the severest of cases, the condition can lead to renal failure and the death of mother and child [1].

TABLE I. Urine Dipstick Test Classifications

Multistix	Criterion
Neg	Neg
Trace	15 mg/dL
1+ (30 mg/dL)	30 mg/dL
2+ (100 mg/dL)	100 mg/dL
3+ (300 mg/dL)	≥500 mg/dL
4+ (>2000 mg/dL)	≥500 mg/dL

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Pre-eclampsia is defined by systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg after 20 weeks' pregnancy in women with previously normal blood pressure. Other definitions include urine protein content of greater than 300 mg over a period of 24-hours. This is typically assessed via a urine dipstick test from which a classification is shown in TABLE I. Urine dipstick results of 1+ are indicative of pre-eclampsia [2].

Severe pre-eclampsia is indicated by systolic blood pressure of 160 mmHg or higher and/or diastolic blood pressure of 110 mmHg or higher, more than 5 g protein in 24-hour urine collection which is associated with 3+ or greater on urine dipstick readings [3].

The innovative technique for detecting the presence of proteins and other organic macromolecules in an electrolyte by analyzing the voltage waveform resulting from a biphasic, constant-current, charge-balanced electrical stimulation are fast in response and have the potential of being developed into a diagnosis tool.

II. Materials and Methods

A. Materials

To mimic the composition of human urine, four levels of protein electrolyte solutions TABLE II were prepared at room temperature: Stock Solution, Normal Urine Solution, Proteinuria Solution and Severe Proteinuria Solution. Stock Solution contained 2.365% urea, 0.977% NaCl, 0.620% $NaH_2PO_4 \cdot H_2O$ and 0.585% KCl. Normal Urine Solution was composed of the Stock Solution, with additional 0.005% bovine serum albumin and 0.2% creatinine. Proteinuria Solution also contained the Stock Solution, but with additional 0.03% bovine serum albumin and 0.754% creatinine, consistent with protein content of urine in clinical cases of proteinuria. Finally, Severe Proteinuria Solution was comprised of the Stock Solution with 0.5% bovine serum albumin and 1.2% creatinine, consistent with protein content of urine in severe cases of proteinuria [4].

An in-house built, 20 mm diameter tank comprising platinum ball electrodes attached to a constant current, charge-balanced, biphasic stimulator with 8 bit resolution on the current control was used to stimulate the solutions. The platinum balls were nominally 400 μm in diameter and spaced 1 mm apart.

B. Methods

The stimulator delivered square (constant current), biphasic waveforms of equal but opposite charge per phase

TABLE II. FOUR LEVELS OF PROTEIN ELECTROLYTE SOLUTIONS

Solution Type	Stock Solution	Normal Urine Solution	Proteinuria Solution	Severe Proteinuria Solution
Urea	2.365%	2.365%	2.365%	2.365%
NaCl	0.977%	0.977%	0.977%	0.977%
$NaH_2PO_4 \cdot H_2O$	0.620%	0.620%	0.620%	0.620%
KCl	0.585%	0.585%	0.585%	0.585%
Bovine serum albumin	n/a	0.005%	0.030%	0.500%
Creatinine	n/a	0.200%	0.754%	1.200%

with an amplitude of 200 μA and phase durations of 250 μs each which are chosen due to previous experience with similar stimulations. Waveform data was displayed and recorded using an isolated oscilloscope (Tektronics TPS2014, Beaverton, Oregon, USA) for both the delivered current and the resultant voltage waveforms across the electrodes.

Stimulation was delivered with a post stimulus shorting of the electrodes to ensure charge neutrality prior to the next pulse. Inter-stimulation interval was 2 ms to ensure the voltage returned to baseline level, and there was no inter-phase delay.

All data was processed with in-house developed software and MATLAB.

1) 2 minutes each of stimulation was repeated on Stock Solution for 20 times to establish a comparative baseline for subsequent experiments. Between any two stimulations, the electrodes were cleaned using Cleaning Method A (TABLE III).
2) 2 minutes of stimulation was then repeated 8 times, due to complexity of the experiment, on Normal Urine Solution, Proteinuria Solution and Severe Proteinuria Solution. The electrodes were cleaned using Cleaning Method B (TABLE III) between any two stimulations.

TABLE III. CLEANING METHODS

	Cleaning Method A	Cleaning Method B
Purpose	after stimulation on solutions without proteins	after stimulation on solutions with proteins
Step 1	pour solutions into waste jar	pour solutions into waste jar
Step 2	rinse with deionised water 5 times	rinse with 80% ethanol once
Step 3	soak in water for a minimum of 2 minutes	soak in 80% ethanol for a minimum of 40 minutes
Step 4	rinse with water 5 times	rinse with water 5 times
Step 5	carefully dry the tank with Q—tips, avoiding electrodes	carefully dry the tank with Q—tips avoiding electrodes
Step 6	air dry the electrodes	air dry the electrodes

III. RESULTS

The characteristics of each bi-phasic voltage waveform were identified as shown in Figure 1. These included: the important voltage points in the waveform at access voltage (A), end of phase 1 voltage (C), interphase voltage (D), reverse access voltage (E) and end of phase 2 voltage (G). From these values the slope of the voltage waveform in phase 1 and phase 2 (B and F) were calculated using (1) and (2). As well, the access voltages (A1, A2, A3, A4) at the start and end of each phase were determined. The A4 voltage transition was complicated by any residual charge left on the electrodes, as indicated by H and the A4* values.

$$B = \frac{\Delta t_{A-C}}{\Delta V_{A-C}} \quad (1)$$

$$F = \frac{\Delta t_{G-E}}{\Delta V_{G-E}} \quad (2)$$

A summary of One Way Analysis of Variance (ANOVA) followed by Tukey's Comparisons tests results for each of the points in Figure 1 is interpreted in TABLE IV. The results indicated that the capacitance on phase 1 (B), end of phase 1 voltage (C), interphase voltage (D), capacitance on phase 2 (F), end of phase 2 voltage (G), and reverse access voltage of phase 2 (A4) increased with a corresponding increase in protein concentration.

IV. DISCUSSION

A. Waveform Characteristics

The results in TABLE IV indicated that each characteristic of the voltage waveform varied in response to increased macromolecule concentration. Access voltage of phase 1 (A1) was generally taken as the voltage drop across the electrolytes; it was present as a result of the resistance of the solution between the two electrodes and their surface area and topography. Protein adsorption onto the electrode surface and the ionic composition of the protein could alter A1. Results from these experiments did not show a clear trend of change in A1 with increased protein concentration. This may be attributed to the solutions being stimulated immediately after addition of the solutions into the tank, which was consistent with the requirement of rapid testing, providing insufficient time for protein adsorption to occur.

TABLE IV. ANOVA AND TUKEY'S COMPARISONS TEST RESULTS FOR EACH POINTS IN BI-PHASIC VOLTAGE WAVEFORM AMONG STOCK SOLUTION (SS), NORMAL URINE SOLUTION (NUS), PROTEINURIA SOLUTION (PS) AND SEVERE PROTEINURIA SOLUTION (SPS)

	SS vs NUS	**SS vs PS**	**SS vs SPS**	**NUS vs PS**	**NUS vs SPS**	**PS vs SPS**	**Showed Trend of Increasing/Decreasing with increase of protein concentration**
Access voltage point 1 (A)			x		x	x	
Capacitance on phase 1 (B)	x	x	x		x		x
End of phase 1 voltage (C)	x	x	x				x
Interphase voltage (D)	x	x	x		x		x
Reverse access voltage (E)	x	x	x		x	x	
Capacitance on phase 2 (F)	x	x	x		x	x	x
End of phase 2 voltage (G)							x
Last access voltage (H)		x					
Access voltage of phase 1 (A1)			x		x	x	
Reverse access voltage of phase 1 (A2)		x	x		x	x	
Access voltage of phase 2 (A3)	x	x	x		x	x	
Reverse access voltage of phase 2 (A4)							x
Discharge voltage A4*							

Moreover, the ionic composition of each of the solutions did not vary to the extent that A1 was significantly changed.

The voltage remaining after recovery of the access voltage of phase 1 (A2) provided an indication of the residual charge on the electrodes after phase 1. This voltage was most significantly affected by the capacitive properties of the electrode-electrolyte interface - indicated by the slope, B, but to a lesser extent by inequalities between the magnitudes of A1 and A2.

In all the samples, A2 was smaller than A1, indicating that the resistive properties of the electrode-electrolyte interface

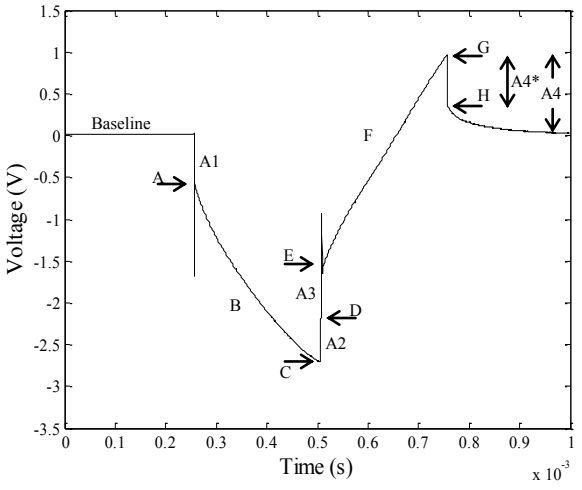

Figure 1. Elements of the voltage waveform resulting from a constant current stimulus: access voltage (A), capacitance on phase 1 (B), end of phase 1 voltage (C), interphase voltage (D), reverse access voltage (E), capacitance on phase 2 (F), end of phase 2 voltage (G), start of discharge voltage at end of stimulation (H), and access voltages (A, A2, A3, A4).

varied at the start and finish of the phase. This may be explained by the electrode-electrolyte interfaces being in a different chemical environment. At the end of the phase, there were more active ions present, having been created during the course of the phase. For instance, in the presence of the stimulation currents, some proteins were broken into smaller units (e.g. peptides or amino acids), and that these would provide ions that were more readily available to the chemical reactions present in the electrode-electrolyte interface, thus changing its characteristics.

In Stock Solution which did not contain proteins, A3 was slightly smaller than A1. However, in Normal Urine Solution, Proteinuria Solution and Severe Proteinuria Solution each of which contains proteins, A3 was slightly larger than A1.

This phenomenon indicated a change in the nature of the solution when the current reversed. For solutions that did not contain proteins, electrons would firstly neutralize with ions on the electrodes, therefore, the voltage drop would be smaller compared to the voltage drop at the beginning of phase 1.

Capacitive properties of the waveform (from B and F) revealed the gradients of phases 1 and 2 of the voltage waveform, which corresponded (approximately) to the capacitance of the bilayer formed on the electrode surface. The gradient of phase 1 for Stock Solution was much larger than the gradient of phase 1 for the other solutions that contained proteins. This indicated that the capacitance was smaller for Stock Solution than for the solutions that contain proteins. This was consistent with adsorption of different molecules onto the surfaces of the electrodes.

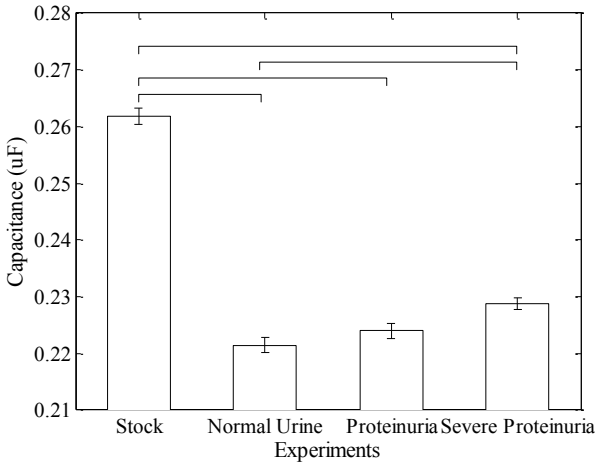

Figure 2. Capacitance on phase 1, combined with Tukey's Comparisons test results showing pairs with statistical significance and error bars showing variance of measurements.

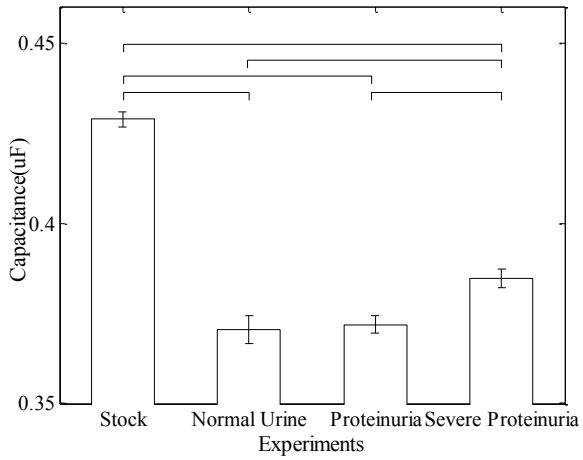

Figure 3. Capacitance on phase 2, combined with Tukey's Comparisons test results showing pairs with statistical significance and error bars showing variance of measurements.

B. Detection of Proteins

Comparison of capacitance on phase 1 or capacitance on phase 2 would be a potential test characteristic for determining the relative quantity of protein present in urine. As plotted in Figure 2 and Figure 3, an increase in protein presents as an increase in capacitance of the electrode. Capacitance on phases 1 and 2 provided more information than single point readings, such as points C, D, or G. For instance, the measure of capacitance on phase 1 comprised the voltages on points A and C as well as the pulse width.

The solutions made for testing were the minimum requirements for proteinuria and severe proteinuria. Although the differences between Normal Urine Solution and Proteinuria Solution were not sufficient to be statistically significant, the technique showed the potential of being used as a preliminary diagnostic system to warn patients about potential proteinuria by clearly distinguishing severe proteinuria from normal urine, and identifying borderline cases such that medical attention may be sought.

In the current technique, the stimulator used, imperfections in the electrodes, or the voltage acquisition system introduced instrument noise. In particular, the in-house stimulator generated noise in the range of 20-100 mV, which affected the analysis of the final waveform. Although its temporal resolution was excellent (2.5 MHz), the voltage range (from positive to negative) was only 8 bits and thus introduced digitization errors into the data.

Changes in the environment such as the ambient temperature could affect the ability of proteins to bind to the electrodes and therefore, alter the capacitance on phases 1 and 2.

The authors posit that refining the technique through improvements to the measurement system, increasing measurement quantities, compensating for the ambient temperature would result in more accurate readings.

V. CONCLUSION

Voltage waveform analysis is able to show the relative presence of proteins in electrolyte solutions from the large amount of information contained in the waveform. It is promising that with further refinement of the experiment, the voltage waveform could be analyzed and used for preliminarily diagnosis of pre-eclampsia.

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