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On the Merits of Tetrapolar Impedance Spectroscopy for Monitoring Lithium Concentration Variations in Human Blood Plasma

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Abstract— Bipolar disorder is characterized as a manic-depressive psychiatric syndrome with life-threatening risks to the patient. Diagnosed individuals undergo long-term lithium therapy which has proven to be effective for mood stabilization. Maintaining blood lithium concentration levels within a narrow therapeutic window between 0.6-1.5 mM is vital for the patient as slightly elevated concentrations of the order of 0.1 mM can be toxic. This paper aims to evaluate the merits of tetrapolar electrical impedance spectroscopy (TEIS) as an alternative method in monitoring blood Lithium levels. Measurements were performed using a custom-made tetrapolar probe in human blood plasma with Lithium concentrations covering the therapeutic range. The results indicate a limit of detection less than 0.1 mM and a response time of less than 5 s. Prediction of Lithium concentration levels using impedance values is in good agreement with conventional standard techniques to approximately 0.05 mM. This technique provides a basis for further development of instrumentation for point of care healthcare technologies.

Index Terms—bipolar disorder, Lithium, tetrapolar, electrical impedance spectroscopy, point of care healthcare technologies

I. INTRODUCTION

BIPOLAR disorder is considered to be a psychiatric syndrome characterized by alternating manic and depressive episodes that can vary in duration and time of occurrence [1]. Episodes of acute manic condition can be potentially life-threatening as the individual's behavior can span out of control. In 1949 John Cade [2] accidentally discovered the therapeutic significance of lithium in the treatment of acute manic episodes. More than 60 years later, lithium therapy is still considered to be the most effective in controlling acute mania and preventing depressive episodes [3, 4]. However, the exact mechanism behind the action of lithium upon the neural interface responsible for mood expression is not known [5]. Long-term lithium treatment entails a considerable risk to the patient if not closely monitored, as the use of the drug must be controlled within a narrow therapeutic range. Normal levels of lithium in human blood are <0.2 mM (mmol/L) [6] and acceptable specimens for monitoring its concentration are considered to be human plasma or serum [7]. Multiple clinical studies have established that the concentration of lithium in human serum is considered to be of therapeutic value when confined within 0.6-1.5 mM with an optimal steady-state between 0.6-1.2 mM [6, 8, 9]. Slightly elevated steady-state concentrations between 0.8-1.5 mM can be indicated for treatment of acute manic episodes whereas, concentration levels beyond 1.5 mM are considered to be toxic [6]. Studies have also shown that some serious but reversible side effects can indeed occur within the therapeutic range as well [10, 11, 12]. Elevated levels beyond 2.5 mM may result in severe neurological damage and can be life threatening. Chronic toxicity can lead to renal failure, permanent nervous system damage, hypothyroidism and dementia [5, 13].

A. Conventional lithium sensing methods

Maintaining the correct therapeutic levels while avoiding the associated risks is a cumbersome process that requires regular monitoring of patients lithium levels in blood. Methods for measuring lithium concentration in serum or plasma require high sensitivity to changes of <0.1 mM as toxicity levels are in close proximity to therapeutic levels. Currently, the standard in measurement of blood lithium concentration is performed via flame emission photometry (FEP) and atomic absorption spectroscopy (AAS) [14, 15] to which several reported alternatives compare with when results are reported. FEP is generally considered to be more sensitive to lithium than AAS [14]. However, FEP is cumbersome and elaborate and thus inappropriate for applications outside a laboratory, while currently there is an increasing need for diagnostics that can be used in the clinical practice or at home where monitoring of vital signs is regularly performed by the

patient prior to any further clinical check.

B. Alternative lithium sensing methods

1) Electrochemical methods

A potential method that can be adapted towards addressing such requirements is potentiometry with the use of Ion Selective Electrodes (ISE) [16, 17, 18, 19]. ISE's exhibit a high selectivity to lithium ions relative to the much higher concentration of sodium or potassium ions present in the sample, even though these have been reported to interfere with measurements [20]. In [18] a potentiometric cell was built using lithium ISE (by applying an ion selective polymer membrane) and a reference electrode, with a limit of detection to changes in Li^+ concentration of approximately 0.11mM. A possible issue with the use of ISE's is that the presence of large proteins in a physiological sample may deter the sensitivity of the electrodes towards Lithium, thus filtration of the sample may be required [16]. Response time for an ISE recording can be within 20-40 s [18].

Another method involves the use of integrated microfluidic channels (capillaries) in which a drop of sample is deposited at the opening. Lithium is then separated from other ions with electrophoresis (due to the difference in its electrophoretic mobility). Lithium concentration is typically assessed by a two-electrode conductivity measurement [21, 22, 23, 24, 25, 26, 27, 28]. A problem typically associated with the two-electrode conductivity measurement method is the inclusion in the measurement of the relatively high and often time drifting electrode-electrolyte interface impedance. This is because the same pair of electrodes is used to inject an ac signal to the sample and to record the resulting signal across the sample. Thus, the recorded conductivity is due to the electrode-electrolyte interface conductivity together with the ionic content related conductivity of the sample. As a result recorded changes in the measured signal can sometimes be attributed to changes in the electrode-electrolyte interface conductivity and not to changes in the ionic content such as the arrival of lithium ions during electrophoresis. Also, due to the microscopic dimensions of a device using integrated microfluidic channels, the electrode-electrolyte impedance is increased due to the small electrode area [29], which can pose a challenge to the design of suitable instrumentation. In addition, a typical electrophoresis process would require the application of a high voltage across the channel in order to achieve separation of the ions and can reach values up to 4 kV [25]. Finally, capillary electrophoresis appears to be very sensitive to the loading of the sample due to the limited channel length [30].

It is evident that, overall, current electrochemical methods require sample preparation such as solution filtration for large residue removal, and either the attachment of an ionophore membrane on the sensing electrode or the use of quite high voltages.

2) Optical methods

Alternative approaches to electrochemical methods for measuring lithium have been proposed with the use of optical techniques such as spectrophotometry, spectrofluorimetry as well as techniques based on photoacoustic phenomena. Spectrofluorimetric techniques [31, 32] rely upon the reaction of lithium to a specific fluoroionophore however, to the authors' knowledge; their functionality using blood samples has not been demonstrated yet. Spectrophotometric techniques [33] make use of specific dyes which react with lithium causing a shift in light absorbance wavelengths. Finally, an in vivo study [34] performed using lithium selective nanosensors aims to present a non-invasive approach towards lithium monitoring using photoacoustic imaging.

Thus, spectrophotometric techniques require the addition of specific chromophores and fluorescent dyes that are reactive with lithium which makes the process quite elaborate. Non-invasive approaches such as the use of photoacoustic imaging require the administration of lithium reactive nanosensors. .

C. Tetrapolar Electrical Impedance Spectroscopy

The study presented here aims to investigate the merits of tetrapolar electrical impedance spectroscopy (TEIS) as an alternative method in the detection of lithium concentration and concentration variations in human plasma. It is aimed to be an in-vitro pilot study to assess the advantages and possible disadvantages of a technique that does not require a specialist to operate it, whilst it can be miniaturized if needed, making it attractive for future use in portable diagnostics outside the laboratory. TEIS is performed typically by applying an ac current via a pair of electrodes and recording the induced difference in potential via a separate pair. This way an impedance characteristic of the Sample Under Test (SUT) can be extracted and its frequency variation can be studied. The tetrapolar technique has a considerably lower sensitivity to the electrode-electrolyte interface impedance than its two-electrode equivalent and changes in the recorded signal can be directly attributed to changes in the conductivity of the SUT. Sample preparation such as solution filtration for large residue removal is not required, thus reducing the possibility of user error, making the process less elaborate and reducing the processing time to just a few seconds. Current or voltage signals applied via the electrodes are at very low levels, typically in the range of µA-mA or mV to a few volts depending on the measurement setup. Solutions used in this study were prepared in a controlled manner so as to alter the concentration of lithium only, thus leaving the concentration of other ions unaffected. The study however at this stage aims to report upon the merits of the proposed TEIS in terms of its sensitivity to lithium changes in human plasma (comparable to current state of the art) without the need for specific ion selective membranes or addition of lithium binding dyes. An additional merit would also be the relatively fast response time of the proposed sensor relative to the aforementioned methods. The design of a tetrapolar probe together with the preparation of saline and blood plasma solutions is described in section II. Section III presents experimental evaluation of the TEIS probe including both saline and human plasma solutions and comparison of the results with FEP. Some concluding remarks are presented in section IV.
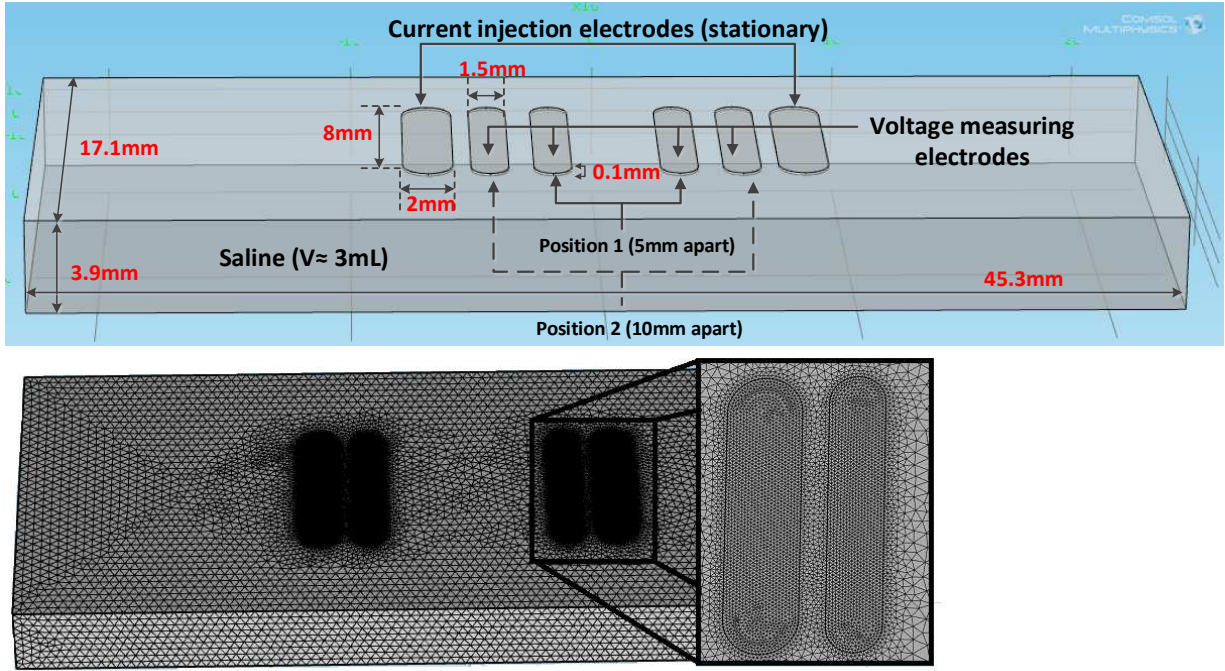
Fig. 1: (Top) Comsol geometrical model of saline solution and electrodes. Two inter-electrode positions with regards to the voltage measuring ones were studied. (Bottom) Meshing resulted in 644742 tetrahedral and 52940 triangular elements.

II. MATERIALS AND METHODS

A. Tetrapolar probe

Tetrapolar impedance measurements were performed using a custom made co-planar 4-electrode probe placed at the bottom of a rectangular container (petri dish) able to hold a volume of solution approximately 3 mL. The initial design incorporated 5 mm distance between the recording electrodes, in order to avoid small baseline impedance values associated with smaller configurations. Following initial experimentation it was deemed preferable to use Finite Element Model (FEM) simulations in order to methodically study the sensitivity field and transfer impedance characteristics of the electrode configuration, similar to [35], and to monitor these characteristics for different inner inter-electrode distance values. While used here to ensure that the setup is not affected by intuitive design, this preliminary model will be the basis for future refinement and optimization of the probe.

1) Finite Element Model (FEM) for sensitivity distribution

The impedance measured between two electrodes is specific to them and it is a transfer characteristic termed "transfer impedance". The change in transfer impedance measured due to a change in the conductivity of a particular region of the volume conductor under study is termed as the sensitivity (**S**) [36]:

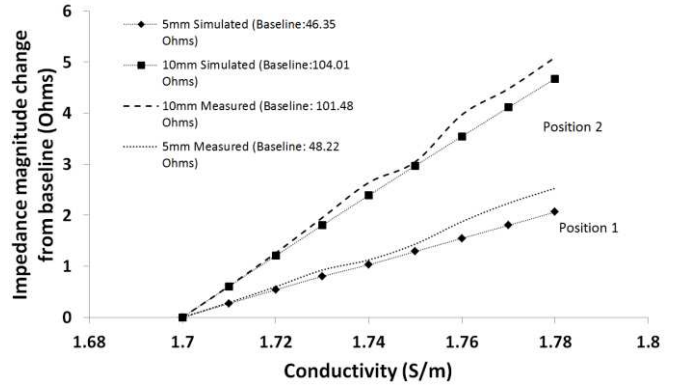$$S = \frac{J_1 \cdot J_2}{I^2} \qquad (1)$$

Fig. 2: Simulated and experimental change in the magnitude of the measured transfer impedance from the baseline value in both cases as a function of conductivity change in saline

where the numerator represents the dot product of J_1, the current density at a particular region of the volume (voxel) and J_2, the current density at each voxel if the current was being injected by the other electrode pair. I is the injected current. Hence, the sensitivity can be either positive, negative or zero. The total measured transfer impedance would be a summation or a volume integral of the total sensitivity field multiplied by the resistivity of the volume conductor. It is important to reduce regions of negative sensitivity as they provide an opposite change to the measured transfer impedance if the conductivity changes. Careful choice of electrode dimensions and spacing can increase regions of positive sensitivity and reduce regions of negative sensitivity
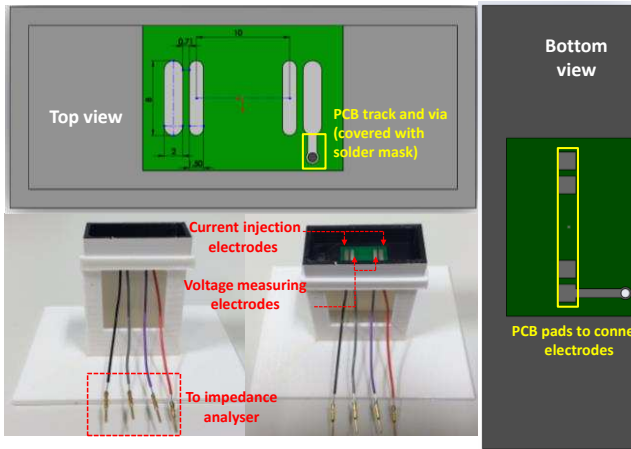
Fig. 3: Probe design. Four silver plated electrodes on a PCB fitted at the bottom of a custom made 3D-printed petri dish. Top view is showing the connection of the electrode surface to the bottom of the PCB for one electrode. However the PCB track and via were covered with solder mask insulation to avoid contact with the solution (shown for illustration purposes only). Electrical contact to the electrodes was made via 4 pads at the bottom side of the PCB.

Fig. 4: Electrical impedance measurement setup. L_{CUR} and H_{CUR} ports of the 4294A impedance analyzer connect to the current injection electrodes while L_{POT} and H_{POT} ports connect to the voltage measuring electrodes.

to changes in the conductivity. In this particular application, overall positive sensitivity to small changes in the conductivity of the medium is important.

In order to obtain the sensitivity distribution inside a 3D volume, four co-planar electrodes submerged in saline were modelled using Comsol multiphysics (Comsol, Inc., 5.1, 2015). As shown in Fig. 1 the model consists of a rectangular volume of approximately 3 mL (as saline) with 3.9 mm depth, 17.1 mm height and 45.3 mm width, similar to the container used in vitro. Four co-planar rectangular electrodes where defined with 0.1 mm thickness and smooth corners. Current injection electrodes (outer pair) were defined to be 8 mm high and 2 mm wide while voltage measuring electrodes (inner pair) were defined to be 8 mm high and 1.5 mm wide. Voltage measuring electrodes for impedance measurements methods do not necessarily need to be the same size as current injection ones. Typically current injection electrodes are wider as they need to possess relatively low electrode-electrolyte interface impedance and thus avoid further loading effects to the driving instrumentation. Voltage measuring electrodes can be of smaller dimensions as the voltage measurement instrumentation is usually chosen to exhibit very high input impedance. Two inter-electrode separation distances of 5mm and 10mm were simulated with regards to the voltage measuring ones respectively (Position 1 and 2 as shown in Fig. 1). Position 1 which refers to a 5mm separation distance was an initial design consideration, which allowed us to observe whether similar effects of increasing the inter-electrode distance take place as in [35].

Simulations were performed using the Comsol multiphysics 5.1 AC/DC module by a quasi-static solution of Maxwell's equations to solve for the electrical potential. No electrode-electrolyte interface was included in the model. The rectangular volume was assigned a conductivity value of 1.704 S/m to match the conductivity of prepared saline solution (isotropic). Electrodes were assigned silver material properties through Comsol material library with a relative
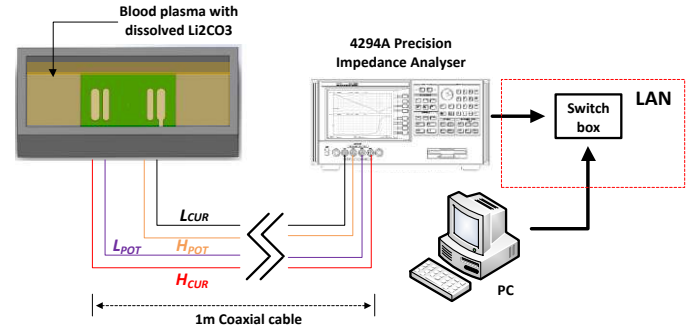
permittivity of 1. A current terminal type was assigned to one of the electrodes on each pair with a 1A current being assigned as a boundary condition. The other electrode at each pair was assigned as a ground terminal of zero potential. No current flow through the exterior boundaries of the model was assumed. This can be a valid assumption as the exterior boundaries can be considered to be the material of the container and air which both are poor conductors of electric current. The discretized geometry is shown in Fig. 1. In order to investigate the sensitivity distribution inside the constructed volume for the electrode positions, a 3D version of (1) was input into Comsol.

From the simulation results the sensitivity field was examined for the two positions as well as the change in the measured transfer impedance for 10 mS/m increments in the conductivity of the saline. Simulated conductivity increments resulted in higher impedance changes for the case of 10mm separation which was also verified experimentally (Fig. 2). The simulated transfer impedance for a baseline conductivity of 1.704 S/m was 46.35 Ω for 5 mm separation and 104.01 Ω for 10 mm separation. Transfer impedance was simulated after defining the inner electrodes as two voltage boundary probes which evaluate the average of the potential distribution over their surface. The transfer impedance is then calculated by taking differential measurement of the two probes and dividing by the injected current.

Overall, it can be concluded, that the positive sensitivity distribution is increased in the case of a higher separation distance between the voltage measuring electrodes as also concluded in [35].

A higher increase in the measured impedance to a certain change in the conductivity of the solution would also allow for a higher sensor resolution to small changes in Lithium concentration and relax tight instrumentation resolution constraints. In the context of the pilot experiments presented in this paper this model was solely intended for indicating the appropriate distance between the injecting and measuring electrodes so as to reduce the effects of negative sensitivity. The resulting - indicative -model will form the basis for more complex designs in the future, if required.
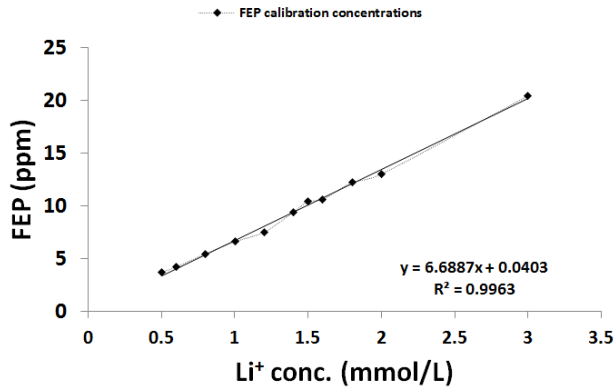
Fig. 5: Flame photometer readings of saline solutions with known Lithium concentration.

TABLE I
PLASMA LITHIUM CONCENTRATIONS

Trendline	$FEP(ppm) = 6.6887 Li^+(mmol/L) + 0.0403$						
FEP(ppm)	3.2	5.7	6.5	7.6	8.7	9.3	10
Li$^+$ (mM)	0.47	0.85	0.97	1.13	1.29	1.38	1.49

Table I: Extrapolated plasma Lithium concentrations from FEP.

2) Probe fabrication, and electrical impedance measurement setup.

Following the FEM analysis the tetrapolar probe was fabricated. Copper electrodes were designed on a printed circuit board (PCB) and silver plated in order to reduce the electrode-electrolyte interface impedance. Silver is known to possess a lower charge transfer (faradaic) resistance when electrochemical reactions take place at the electrode interface [37]. Current injection electrodes (outer pair) were 8 mm (height) by 2 mm (wide) whereas voltage measurement electrodes were 8mm (height) by 1.5mm (wide). Separation between the voltage measuring electrodes was 10 mm and the PCB was 21 mm (wide) by 15mm (height). The PCB was fitted at the bottom of a custom made 3D printed rectangular petri dish, fabricated using poly-lactic acid (PLA) material, with internal dimensions equal to the ones used for the FEM model. PLA material was in contact with the solutions used but does not cause any adverse effects.

Probe design and PCB are shown in Fig. 3. Electrical connection to the electrodes was made via four contacts at the bottom side of the PCB. Once wires were soldered onto the four contacts the bottom side of the petri dish was sealed with epoxy to avoid leakage of the solution.

Electrical impedance measurements were performed using the Agilent 4294A precision impedance analyzer. Calibration of the instrument was performed with a standard resistive load in order to minimize errors in the measurements due to the cabling. Connection to the impedance analyzer was made via four connecting wires at the bottom of the PCB (Fig. 3) and a 1m 4-BNC coaxial cable. The impedance analyzer was connected to a PC via a fast Ethernet switch (Netgear FS105) to save the measured data for processing. The electrical impedance measurement set up is shown in Fig. 4.

Measurements were performed in a frequency bandwidth from 30 kHz to 300 kHz.

At first, a volume of 3 mL saline solution (0.9%, no Li$_2$CO$_3$ added) was measured to be used as a calibration measurement against plasma solutions. Then saline solutions with dissolved Li$_2$CO$_3$ were subsequently measured. Lithium concentrations in the saline solutions were 0.6mM, 0.8 mM, 1.0 mM, 1.2mM, 1.4 mM, 1.6 mM, 1.8 mM and 2.0 mM. Each measurement was repeated three times for statistical analysis. Volumes were injected into the petri-dish using a pipette (Thermo scientific 0.5-5mL) to avoid formation of any air bubbles. Measurements were performed at room temperature conditions (~22℃).

B. Blood plasma sample preparation

Non-sterile, mixed pool human blood plasma (1L, Citrated) was used for this study (TCS biosciences Ltd, Buckingham, UK). Solid Li$_2$CO$_3$ powder (99.999% pure, ACROS Organics) was dissolved in saline (0.9%), to a total volume of 100 mL and eight stock solutions were prepared (S$_1$-S$_8$). The concentration of Li$^+$ (double the concentration of Li$_2$CO$_3$ since it consists of two Lithium ions) in the stock solutions was calculated to cover the therapeutic range and was within the range between 0.6 mM and 2 mM. A separate stock solution (S$_9$) was also prepared to a Li$^+$ concentration of 3 mM. A total of 206.5 mL of plasma was then split equally into seven glass containers (P$_1$-P$_7$) holding 29.5 mL each and an additional 0.5 mL from the each saline stock solution was added to a total volume of 30 mL and stirred for one minute. An additional 30 mL glass container holding only plasma (P$_0$) was also withdrawn from the 1 L container for baseline measurements. Solutions were then stored in a fridge at 5℃ and were taken out in order to equilibrate with room temperature conditions (~22℃).

C. Flame photometry apparatus and method

A flame photometer (M410 Sherwood Scientific Ltd, Cambridge, UK) was used to measure the concentration of dissolved lithium in the plasma samples. Measurements of concentration are displayed in parts per million (ppm, 1mmol/L of Li$^+$ is 6.93 ppm). Initially the instrument was left to warm up with the flame alight, and distilled water was aspirated for approximately 30 minutes. A standard solution provided by the manufacturer (containing 1.5 mM of Li$^+$) was at first aspirated for 20 seconds and the instrument ppm reading was set to 10.4 (corresponding to 1.5 mM). Successive measurements of known lithium concentrations from the prepared saline stock solutions were taken and the corresponding ppm readings are shown in Fig. 5. A least squares linear regression was performed (r^2:0.9998) in order to calculate the corresponding concentration of Lithium from the plasma samples (P$_1$-P$_7$). The corresponding FEP readings and extrapolated plasma Lithium concentrations are shown in Table I.
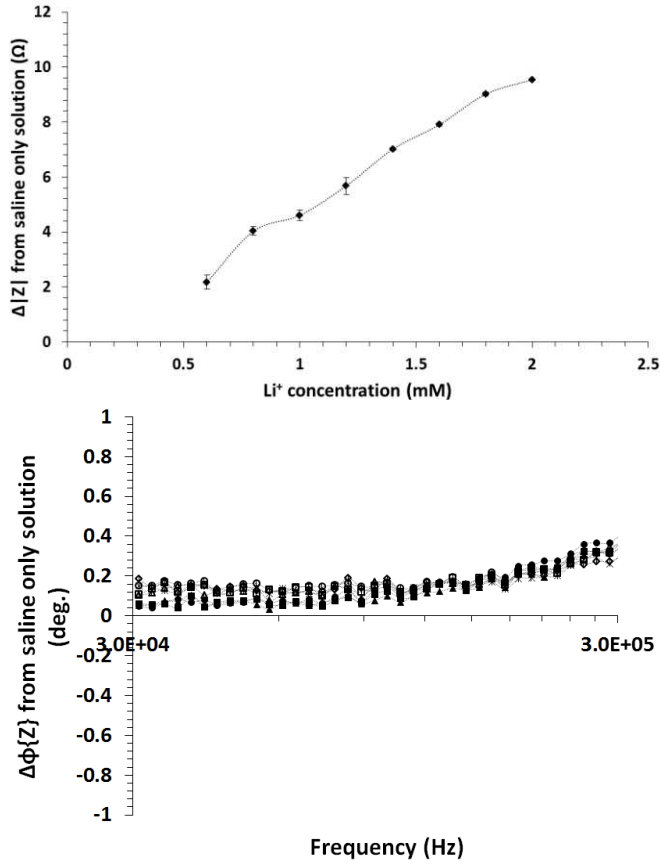
Fig. 6: (Top) Mean change in impedance magnitude from saline only solution for different Li⁺ concentrations. (Bottom) Mean change in the phase of recorded impedance from a saline only solution within the specified bandwidth. Li⁺ concentrations range from 0.6mM to 2mM which covers the entire therapeutic range.

Fig. 7: (Top) Mean impedance magnitude change from plasma only solution for different Li⁺ concentrations. (Bottom) Mean change in the phase of recorded Electrical impedance phase plot of the same solutions. Li⁺ concentrations range from 0.47mM to 1.49mM and are measured using FEP.

TABLE II
SALINE ELECTRICAL IMPEDANCE MEASUREMENTS

Concentration (mM)*	$|Z| \pm SE$ (Ω)**	$|t|$ ***	$\varphi(Z) \pm SE$ (deg.)**
Saline only	91.04 ± 0.27	--------	-1.27 ± 0.01
0.6	88.87 ± 0.26	5.84	-1.27 ± 0.01
0.8	87.01 ± 0.15	6.14	-1.28 ± 0.02
1.0	86.43 ± 0.20	2.3	-1.25 ± 0.01
1.2	85.36 ± 0.30	2.92	-1.25 ± 0.02
1.4	84.03 ± 0.04	4.32	-1.24 ± 0.01
1.6	83.14 ± 0.05	12.91	-1.23 ± 0.01
1.8	82.02 ± 0.06	13.3	-1.24 ± 0.01
2.0	81.49 ± 0.01	8.39	-1.24 ± 0.01

*Li⁺ concentration ** Mean ***$t_{[0.05]}$=2.13 (df=N1+N2-2), N1=N2=3

Table II: Electrical impedance measurements of saline (0.9%) and saline with dissolved Li_2CO_3. Mean values for a bandwidth between 30 kHz and 300 kHz.

TABLE III
BLOOD PLASMA ELECTRICAL IMPEDANCE MEASUREMENTS

Concentration (mM)*	$|Z| \pm SE$ (Ω)**	$|t|$ ***	$\varphi(Z) \pm SE$ (deg.)**
Plasma only	152.42 ± 0.24	-------	-1.18 ± 0.01
0.47	149.70 ± 0.06	10.77	-1.15 ± 0.01
0.85	144.98 ± 0.23	17.06	-1.12 ± 0.01
0.97	144.11 ± 0.11	3.36	-1.11 ± 0.01
1.13	143.01 ± 0.12	6.27	-1.11 ± 0.01
1.29	142.33 ± 0.21	2.78	-1.09 ± 0.01
1.38	140.27 ± 0.30	5.54	-1.08 ± 0.01
1.49	139.28 ± 0.35	2.14	-1.07 ± 0.01

*Li⁺ concentration ** Mean *** $t_{[0.05]}$=2.13 (df=N1+N2-2), N1=N2=3

Table III: Electrical impedance measurements of blood plasma only and blood plasma with dissolved Li_2CO_3. Mean values for a bandwidth between 30 kHz and 300 kHz.

III. EXPERIMENTAL RESULTS AND DISCUSSION

A. Saline electrical impedance measurements

Electrical impedance measurements using saline solutions are shown in Table II and Fig. 6. Table II reports the mean impedance magnitude and phase for a bandwidth between 30 kHz and 300 kHz for s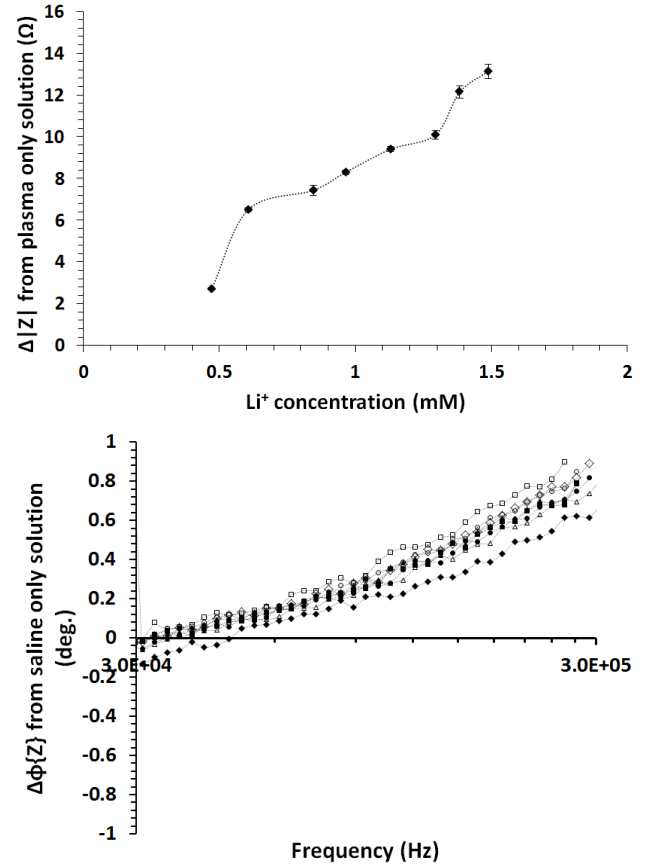aline only and saline with dissolved Li_2CO_3. Standard error was calculated from three measurements in each case. On the contrary, no significant change is observed in the mean impedance phase. This, however, is expected as saline is simply considered an ionic solution and changes to the concentration of dissolved species would be reflected to changes in the impedance magnitude
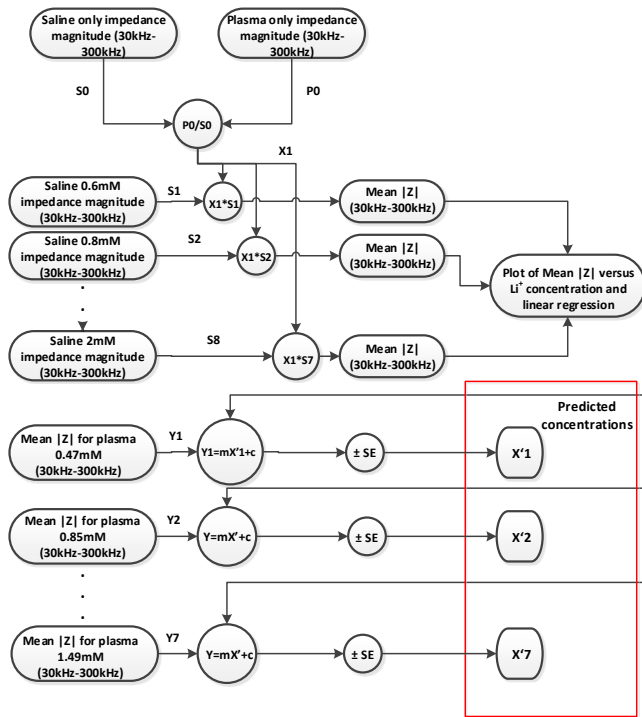
Fig. 8: Analysis steps in extracting predicted Li$^+$ concentrations from measurements to compare with the FEP readings
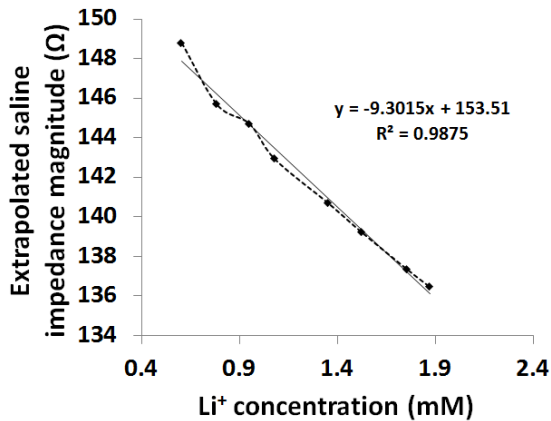

Fig. 9: Extrapolated saline mean impedance magnitude readings, based on the ratio between the baseline measurements of saline and plasma only solutions, vs Li$^+$ concentrations (from TableII). Least square linear regression fit of the data can provide with a function in predicitng the concentration of Li$^+$ from impedance magnitude measurements perfromed with plasma

Fig. 10: (Top) Predicted Li$^+$ concetrations based on linear function in Fig. 9, with 'y' variable being the mean impedance mangitude of plasma solutions reported in Table III, and actual concentrations measured usign FEP. (Bottom) Comparison of the values obtained from both methods and linear regression.

TABLE IV
PERFOMANCE OF TEIS

| Parameter | Work | | | | |
	[18]*	[24]**	[26]***	[22]****	This work***
Change in [Li$^+$](mM)	0.1	0.1	0.25	0.15	<0.1
Response time (s)	40	~55	120	20	<5

*Measured in artificial serum
** Not measured in blood or blood constituents
*** Measured in blood plasma
**** Measured in blood serum

Table IV: Comparison of TEIS with current state of the art in the field of ISE's and microchip capillary electrophoresis

(due to change in the ionic content) and not to the phase (due to absence of any reactive species).

The calculated t-values (Table II) reflect the difference in mean impedance magnitude between samples with a null hypothesis that there is no significant difference between them. Calculated t values are higher than the critical value at 95% confidence interval (t$_{[0.05]}$=2.13) which rejects the null hypothesis showing a significant difference between the recorded mean impedance magnitude.

Fig. 6 (top), reports the mean change in impedance magnitude for different Li$^+$ concentration from a saline only solution. The trend shows the absolute change (hence increasing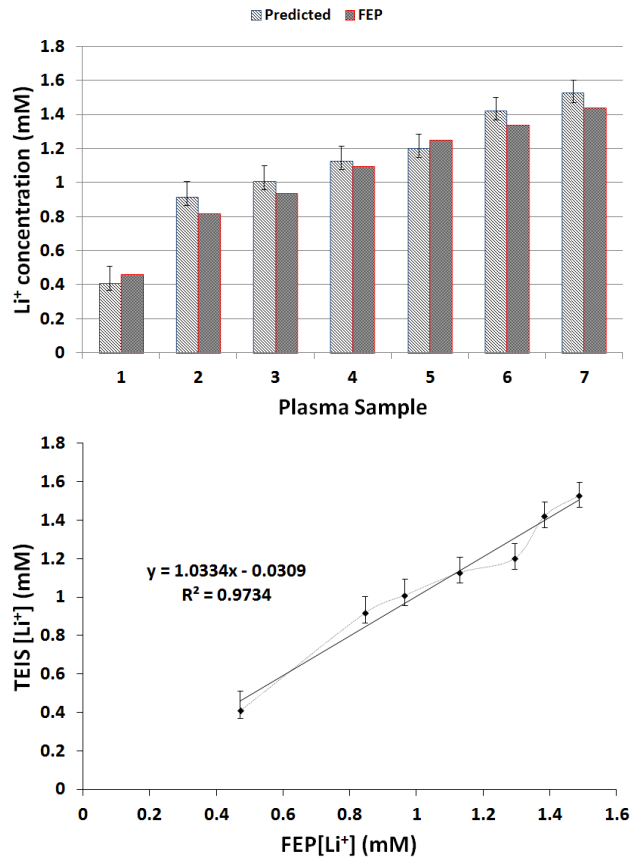); however, in reality, the mean impedance magnitude is decreasing with increased Li$^+$ concentration. Fig. 6 (bottom), reports the relative change in the recorded phase for different Li$^+$ concentrations within the specified bandwidth. An almost equal change in the phase is observed

within the recorded bandwidth in all samples with a maximum of 0.3 degrees. The deviation from a zero phase (as expected in a purely ionic solution) can be attributed to instrumentation phase delays together with the presence of small parasitic capacitances between the electrodes.

Saline measurements reported can thus clearly indicate the suitability of the method to detect changes in the concentration of Lithium in saline down to 0.2 mM. The minimum change in the recorded magnitude is 0.5 Ω (limit of the 4294A impedance analyzer is 10mΩ). Error bars show the standard error in the reading taken from three individual measurements of the same solution within the bandwidth of interest.

B. Blood plasma electrical impedance measurements: comparison with FEP

Electrical impedance measurements of blood plasma only and blood plasma with dissolved Li_2CO_3 are shown in Table III and Fig. 7. A reduction in the mean impedance magnitude is again observed from the plasma only solution when Li_2CO_3 is added, and a subsequent reduction with increasing concentration (Table III). The calculated t-values reflect the difference in mean impedance magnitude between samples with the null hypothesis that there is no significant difference between them. Calculated t values are higher than the critical value at 95% confidence interval ($t_{[0.05]}$=2.13) which rejects the null hypothesis showing a significant difference between the recorded mean impedance magnitude. The change in the observed impedance phase is similar to that for the saline. As blood plasma contains only protein molecules, water and ions, the change in phase is expected to be similar to that of saline since there are no cells present. Cell membranes behave like tiny imperfect capacitors due to their bilayer structure. Hence, no change or difference in phase is observed and changes to the concentration of dissolved species would be reflected to changes in the impedance magnitude. Fig. 7 shows a plot of the change in the mean impedance magnitude and phase with increasing Li^+ concentrations in a similar fashion to the saline solutions. Particularly in Fig. 7(bottom) the relative change of the impedance phase, of blood plasma, with respect to the saline solutions is shown, thus indicating that plasma solutions behave in a resistive manner. Measured results indicate the suitability of the method to detect changes in the concentration of Lithium in blood plasma down to 0.09 mM. Error bars show the standard error in the reading taken from three individual measurements of the same solution.

Baseline measurements of saline (Table II) and plasma (Table III) were used in order to obtain a prediction model based on the mean impedance magnitude of the plasma solutions with dissolved Li_2CO_3 shown in table III. A ratio of the baseline impedance magnitude, between saline and plasma only solutions, was obtained at a bandwidth between 30 kHz and 300 kHz. This ratio was then used to multiply the corresponding impedance magnitude readings of the saline solutions with dissolved Li_2CO_3 and thus extrapolate their impedance magnitude readings. The average values of the extrapolated saline impedance magnitude readings for different lithium concentrations were then plotted and a least squares linear regression was performed (r^2:0.9875). The

associated function was used to obtain the predicted Lithium concentrations by inputting the mean impedance magnitude measurements from plasma solutions (with dissolved Li_2CO_3), shown in Table III. Fig. 8 shows the processing steps. A plot of the extrapolated saline impedance magnitude readings and the linear fit is shown in Fig.9. A plot of the predicted Lithium concentration from the model and the measured concentration using FEP is shown in Fig. 10. The results from Fig. 10 (top) show an average difference of 0.05 $\pm$ 0.07 mM between the predicted Lithium concentrations compared to FEP readings. However, such difference in estimating the concentration is not considered to be significant when distinguishing between toxic and non-toxic Lithium blood levels. Also, the predicted Li^+ concentration difference is well within the therapeutic window. Fig. 10 (bottom) shows a comparison of the predicted values using TEIS and the measured values using the FEP method. Least squares linear regression results in a slope factor of 1.0334 with a regression coefficient r^2:0.9734, and a negative offset concentration of 0.0309 mM. The results indicate that changes in the recorded electrical impedance magnitude using human blood plasma can reflect changes using FEP to a good accuracy.

Comparison of the TEIS technique to other potentiometric and ISE techniques is shown in Table IV. Comparison is made upon the minimum detectable change in Lithium concentration (mM) in plasma or other blood constituents, and response time. Measurements using the Agilent 4294A precision impedance analyzer in the bandwidth between 30 kHz and 300 kHz can take less than 5 seconds an dthe detected change in the concentration of Lithium can be comparable or smaller to other listed techniques.

IV. CONCLUSION

This paper has presented a new method for the detection of Lithium in blood using an alternative technique based on TEIS. Measurements in saline solution and human blood plasma with Lithium concentrations covering the bipolar therapeutic window showed a good agreement with standard techniques such as FEP. Measured results in both saline and plasma solutions indicate a resistive behavior (as the change in the impedance phase is negligible), therefore it is worth noting that AC measurements throughout a specific frequency band may be obsolete. Still, a future implementation will involve whole blood, where the cellular structure will contribute to a phase characteristic which is worth studying across a frequency spectrum. The proposed method is simple, low cost and does not require any sample preparation or generation of high voltage signals. Results can be obtained fast and the instrumentation can be packaged into a portable and compact design suitable for home diagnostics. FEM modelling has also proven to be advantageous for the design of the probe in this case in order to increase its sensitivity to changes in the concentration of Lithium. The current custom made probe is capable of distinguishing changes in Lithium concentration using real human blood plasma samples to the level suggested by many current state of the art techniques.

Whilst the study presented here was positively conclusive with regards to the capability of TEIS to accurately detect lithium variations in human blood plasma, a shortcoming of the applied method is the lack of selectivity (or specificity) to lithium. This is a very interesting research challenge and our future work will be focused on this aspect of the presented method, as well as in carrying out tests in blood and developing dedicated instrumentation. As per the aim of this study, the ability of TEIS to provide a higher degree of sensitivity (LOD) to changes in Lithium concentration was demonstrated, whilst avoiding the use of ion selective membranes and lithium binding agents.

REFERENCES

[1] R. H. Belmaker, "Bipolar Disorder," The New England Journal of Medicine, vol. 351, pp. 476-486, 2004.

[2] J. F. Cade, "Lithium salts in the treatment of psychotic excitement," Med. J. Aust., vol. 2, no. 10, pp. 349-352, 1949.

[3] R. W. Licht, "Lithium: still amajor option in the management of bipolar disorder," CNS Neurosci. Ther., vol. 18, no. 3, pp. 219-226, 2012.

[4] J. R. Geddes, S. Burgess, K. Hawton, K. Jamison and G. M. Goodwin, "Long-term lithium therapy for bipolar disorder: systematic review and meta-analysis of randomised control trials," Am. J. Psychiatry, vol. 161, no. 2, pp. 217-222, 2004.

[5] N. Stafford, Lithium for Bipolar Disorder: A Guide for Patients, Four Oaks, UK: My Mind Books, 2011.

[6] R. T. Timmer and J. M. Sands, "Lithium Intoxication," J. Am. Soc. Nephrol., vol. 10, pp. 666-674, 1999.

[7] M. W. Linder and P. E. Keck Jr., "Standards of laboratory practise: antidepresant drug monitoring," Clinical Chemistry, vol. 44, no. 5, pp. 1073-1084, 1998.

[8] M. D. Okusa and L. J. Crystal, "Clinical manifestations and management of acute lithium intoxication," Am. J. Med., vol. 97, no. 4, pp. 383-389, 1994.

[9] G. D. Christian, "Reagents for Lithium Electrodes and Sensors for Blood Serum Analysis," Sensors , vol. 2, pp. 432-435, 2002.

[10] G. Hay and N. Simpson, "Neurotoxicity associated with therapeutic serum lithium levels," Lancet, vol. 17, no. 2, pp. 160-161, 1982.

[11] A. J. Bell, A. Cole, D. Eccleston and I. N. Ferrier, "Lithium neurotoxicity at normal therapeutic levels," Br. J. Psychiatry, vol. 162, pp. 689-692, 1993.

[12] O. Edokpolo and M. Fyyaz, "Lithium Toxicity and Neurologic Effects:Probable Neuroleptic Malignant Syndrome Resulting from Lithium Toxicity," Case Reports in Psychiatry, vol. 2012, pp. 1-3, 2012.

[13] C. Livingstone and H. Rampes, "Lihtium: a review of its metabolic adverse effects," J. of Psychopharmacology, vol. 20, no. 3, pp. 347-365, 2006.

[14] B. F. Rocks, R. A. Sherwood and C. Riley, "Direct Determination of Therapeutic Concentrations of Lithium in Serum by Flow-Injection Analysis with Atomic Absorption Spectroscopic Detection," Clin. Chem., vol. 28, no. 3, pp. 440-443, 1982.

[15] J. K. Grime and T. J. Vickers, "Determination of Lithium in Microliter Samples of Blood Serum Using Flame Atomic Emission Spectrometry with a Tanatalum Filament Vaporiser," Analytical Chemistry , vol. 47, no. 3, pp. 432-435, 1975.

[16] F. Coldur and M. Andac, "A flow-injection potentiometric system for selective and sensitive determination of serum lithium level," Electroanalysis, vol. 25, no. 3, pp. 732-740, 2013.

[17] S. Merzouk, M. T. Janan, M. Agouzoul, J. Bausells, N. Jafrezic-Renault, A. Errachid and N. Zine, "Electrochemical impedance study of Li-uISE based on PPy[LiClO4] as a solid contact," Sens. and Transd., vol. 27, pp. 264-268, 2014.

[18] M. Novell, T. Guinovart, P. Blondeau, F. X. Rius and F. J. Andrate, "A paper-based potentiomtric cell for decentralised monitoring of Li levels in whole blood," Lab Chip, vol. 14, no. 7, pp. 1308-1314, 2014.

[19] V. Gupta, S. Chandra, S. Agarwal and H. Lang, "Lithium-selective potentiometric sensor based on a second generation carbosiloxane dendrimer," Sensors and Actuators B, vol. 107, pp. 762-767, 2005.

[20] G. Dimenski, T. Badrick and A. S. John, "Ion Selective Electrodes (ISEs) and interferences - A review," Clin. Chim. Acta, vol. 411, pp. 309-317, 2010.

[21] E. X. Vrouwe, R. Luttge, W. Olthuis and A. van den Berg, "Microchip analysis of lithium in blood using moving boundary electrophoresis and zone electrophoresis," Electrophoresis, vol. 26, pp. 3032-3042, 2005.

[22] E. X. Vrouwe, R. Luttge, I. Vermes and A. van den Berg, "Microchip capillary electrophoresis for point-of-care analysis of Lithium," Clin. Chem., vol. 53, no. 1, pp. 117-123, 2007.

[23] E. Vrouwe, R. Luttge and A. van den Berg, "Direct measurement of lithium in whole blood using microchip capillary electrophoresis with integrated conductivity detection," Electrophoresis, vol. 25, pp. 1660-1667, 2004.

[24] K. Petkovic-Duran, Y. Zhu, A. Swallow, N. Noui-Mehidi, P. Leech and F. Glenn, "Detection of inorganic ions on a capillary electrophoresis microchip using a conductivity technique," in Biomedical Applications of Micro- and Nanoengineering III, Adelaide,Australia, 2006.

[25] P. Kuban and P. C. Hauser, "Evaluation of microchip capillary electrophoresis with external contactless conductivity detection for the determination of major inorganic ions and lithium in serum and urine samples," Lab Chip, vol. 8, pp. 1829-1836, 2008.

[26] R. Sewart, C. Gartner, R. Klemm, S. Schattschneider and

H. Becker, "Microfluidic device for fast on-site biomedical diagnostic on the example of lithium analysis in blood," Biomed. Tech., vol. 57, pp. 729-732, 2012.

[27] A. van den Berg, "Labs on chips for biomedical applications," in MEMS, Taiwan, 2013.

[28] A. Floris, S. Staal, S. Lenk, E. Staijen, D. Kohlheyer, J. Eijkel and A. van den Berg, "A prefilled, ready-to-use electrophoresis based lab-on-a-chip device for monitoring lithium in blood," Lab Chip, vol. 10, pp. 1799-1806, 2010.

[29] W. Franks, I. Schenker, P. Schmutz and A. Hierlemann, "Impedance characterization and modeling of electrodes for biomedical applications," IEEE Trans. Biomed. Eng., vol. 52, no. 7, pp. 1295-1302, 2005.

[30] D. Figeys and D. Pinto, "Proteomics on a chip: Promising developments," Electrophoresis, vol. 22, no. 2, pp. 208-216, 2001.

[31] D. Citterio, J. Takeda, M. Kosugi, H. Hisamoto, S. Sasaki, H. Komatsu and K. Suzuki, "pH-Independent Fluorescent Chemosensor for Highly Selsctive Lithium Ion Sensing," Anal. Chem., vol. 79, pp. 1237-1242, 2007.

[32] J. H. Kim, D. Diamond and K. T. Lau, "Optical Device for Non-invasive Monitoring of Lithium in Bipolar Disorder Patients," in 5th European IFMBE conference, Hungary, 2011.

[33] R. H. Christenson, J. J. Mandichak, S. H. Duh, J. M. Augustyn and J. C. Thompson, "Clinical performance characteristics of a new photometric lithium assay: a multicenter study," Clin. Chim Acta, vol. 327, pp. 157-164, 2003.

[34] K. J. Cash, C. Li, J. Xia, L. V. Wang and H. A. Clark, "Optical Drug Monitoring: Photoacoustic Imaginf of Nanosensors to Monitor Therapeutic Lithium in Vivo," ACS nano, vol. 9, no. 2, pp. 1692-1698, 2015.

[35] P. Kassanos, A. Demosthenous and R. H. Bayford, "Towards an optimised design for tetrapolar affinity-based impedimetric immunosensors for lab-on-a-chip applications," in IEEE BioCAS, Baltimore, 2008.

[36] S. Grimnes and O. Martinsen, "Sources of error in tetrapolar impedance measurements on biomaterials and other ionic conductors," J. of Physics D : Applied Physics, vol. 40, pp. 9-14, 2007.

[37] L. A. Geddes and R. Roeder, "Measurement of the direct-current (Faradaic) resistance of the electrode-electrolyte interface for commonly used electrode materials," Annals of Biomed. Eng., vol. 29, pp. 181-186, 2001.