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Optical Determination of Lithium Levels in Artificial Interstitial Fluid for Treatment Management of Bipolar Disorder

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Abstract— **Bipolar Disorder (BD), characterized by mood fluctuating between episodes of mood elevation and depression, is a leading cause of disability worldwide. Lithium continues to be prescribed as a first-line mood stabilizer for the management of BD. However, lithium has a very narrow therapeutic index and it is crucial to carefully monitor lithium plasma levels as concentrations greater than 1.2 mmol/L are potentially toxic and can be fatal. The current techniques of lithium monitoring are cumbersome and require frequent blood tests with the consequent discomfort which results in patients evading treatment. Dermal interstitial fluid (ISF), an underutilized information-rich biofluid, can be a proxy for direct blood sampling and allow lithium drug monitoring as its lithium concentration is proportional to the concentrations in blood. Therefore, in this study we seek to investigate the measurement of lithium therapeutic concentrations in artificial ISF. Our study employs a colorimetric method, based on the reaction between chromogenic agent Quinizarin and Li^+ ion which can be detected using optical spectroscopy in the visible region (400-800 nm), to determine lithium levels in artificial ISF. The resulting spectra of our experiments show spectral variations which are related to lithium concentrations in spiked samples of artificial ISF, with a correlation coefficient (R) of 0.9. Future work will focus on investigating the feasibility of utilizing ISF for real-time and minimally-invasive lithium drug monitoring.**

I. Introduction

Bipolar disorder (BD) is a recurrent psychiatric illness characterized by mood fluctuating between episodes of mania and depression. BD is a leading cause of disability worldwide, with an unknown biological basis and unsatisfactory treatment. Lithium remains the "gold standard" for both acute and maintenance treatment of bipolar disorder, reducing the risk of relapse and suicide [1]. However, lithium has a narrow therapeutic range and lithium plasma levels greater than 1.2 mmol/L are potentially toxic and can be fatal when the levels exceed 2.0 mmol/L, hence blood levels must be frequently monitored [2][3]. Monitoring of blood levels is performed on a weekly basis upon commencement of lithium therapy, and monthly once the required therapeutic dose is established [3]. The current techniques of blood lithium measurement, namely flame emission photometry (FEP) and atomic absorption spectroscopy (AAS), are complex and expensive laboratory methods that cannot be translated into point of care devices for personal monitoring. Previous studies have reported employing electrochemical methodologies such as ion-selective electrode (ISE) and electrophoresis for lithium monitoring, which also have limitations related to interference with other ions and need for sample filtration as well as constraints associated with cost and simplicity of the instrumentation [4][5]. Furthermore, the need for regular monitoring via venepuncture is one of the main reasons that can lead to lithium toxicity or treatment nonadherence [2]. Therefore, development of a minimally-invasive monitoring method will be a major advance in the management of BD. Efforts have been made in order to develop point-of-care lithium monitoring devices utilizing matrices such as saliva and sweat which have shown limitations related to drug instability and the potential presence of contaminants [6][7].

Dermal interstitial fluid (ISF), the fluid bathing the viable tissue of skin, is an accessible and reproducible matrix that is suitable for minimally invasive detection of biomarkers and drugs with good correlation with venous blood [8]. Concentration of lithium in the ISF is suggested to be correlated with venous blood [9], hence we seek to explore the detection of lithium in this matrix. Previous studies have combined potentiometric sensors with reverse iontophoresis (RI), used for extraction of lithium across the skin, in order to achieve monitoring of lithium in ISF [10][11]. However, these methodologies have several issues including the need for quite complicated technology, prolonged preparation time, and lack of sensitivity [12]. Our group has demonstrated that optical spectroscopy techniques provide accurate detection of lithium concentrations in blood. Our aim is to further investigate this methodology in interstitial fluid as it provides a more accessible source for monitoring lithium and developing a minimally-invasive sensor. Minimally invasive, continuous monitoring of lithium in the interstitial fluid offers a promising route of personalisation of medication regimens and will allow bipolar patients to monitor the medication which will greatly reduce the risk of adverse effects during the course of their treatment [13].

II. Materials and Methods

A. Chemicals and reagents

4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), calcium chloride (CaCl2), potassium chloride (KCl), magnesium sulfate (MgSO4), sodium chloride (NaCl), sodium phosphate monobasic (NaH2PO4), and saccharose were obtained from Sigma Aldrich (St. Louis, MO, USA). 1,4-Dihydroxyanthraquinone 96% (Quinizarin), Methyl sulfoxide 99.9% ((CH3)2SO), Sodium hydroxide (NaOH), Sodium carbonate (Na2CO3), and Lithium carbonate 99.9% (Li2CO3) were obtained from Fisher scientific (Waltham, MA, USA).

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All solutions were prepared using deionized water (The Deionised Water Company, Suffolk, UK).

B. Preparation of artificial interstitial fluid

To prepare the artificial interstitial fluid (ISF), 2.5 mM CaCl2, 10mM Hepes, 3.5 mM KCl, 0.7 mM MgSO4, 123 mM NaCl, 1.5 mM NaH2PO4, 7.4 mM saccharose were mixed, and the solution was adjusted to pH 7.5 using Thermo Scientific STAR A211 pH meter [8].

C. Sample preparation

A stock solution of 60 mM Lithium was prepared by dissolving 1.1082 g Li2CO3 in 250 ml dH2O. The Li2CO3 solution was then further diluted to make a set of solutions with the following concentrations: 6, 12, 18, 24, 30, 36, 42, 48, 54 mM. Thereafter, samples of artificial ISF prepared earlier were spiked with lithium by mixing 1 mL from each concentration of Li2CO3 (i.e. 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60 mM) with 29 mL of artificial ISF. Therefore, 10 samples of 30 mL spiked ISF with lithium concentrations ranging between 0.2-2 mM were achieved.

D. Reagent preparation

The following reagents were prepared for colorimetric analysis of lithium using Quinizarin: 0.1 M of NaOH, 0.25 M of Na2CO3, 99.9 % (CH3)2SO and 1 mM of Quinizarin in (CH3)2SO. A 1 M stock solution of Sodium hydroxide was prepared by dissolving 10 g of NaOH in 250 mL dH2O. A 0.25 M stock solution of sodium carbonate was prepared by dissolving 5.299 g of Na2CO3 in 200 mL dH2O. 1 mM of Quinizarin solution was also prepared by dissolving 60 mg of 1,4-dihydroxyanthaquinon in 250 ml of dimethylsulfoxide (CH3)2SO.

E. Optical measurements of lithium in artificial ISF

An amount of 50 µl from ISF samples spiked with different concentrations of lithium were pipetted into a test tube. A sample of ISF without lithium was also included as the control. Prepared samples of ISF were then mixed with 100 µL of 0.1 M NaOH, 10 µL of 0.25 M Na2CO3, 40 µL of water, 2.15 mL of 90 % (CH3)2SO and 50 µL of 1mM of Quinizarin in (CH3)2SO. All samples were kept in a thermostatic bath (Grant InstrumentsTM TC120 Series Heated Circulating Bath) at 25o for 30 mins prior to testing.

Data was acquired using a dual beam spectrophotometer (Model: Lambda 1050, PerkinElmer Corp, Waltham, MA) using plastic cuvettes. The instrument was setup to acquire three spectra from samples in the spectral region between 400-800 nm and at increments of 1 nm. The detector system was set to use the PMT detector for up to 860.80 nm, where the slit size was kept fixed at 2 nm, and for up to 900 nm InGaAs detector was used where the slit size was kept on "servo mode". The gain settings for PMT and InGaAs detectors were set on "Auto" and 1, respectively; while the response time for both detectors was set on 0.2 seconds. The reference and sample attenuators were both set to 100%. UV Winlab Data Processor and Viewer program was used to display the spectra, carry out arithmetic and derivative processes, and export data as excel files to generate line graphs and perform regression analysis using IBM SPSS Statistics.

III. Results

A. Lithium can be detected in the visible region based on its reaction with the chromogenic agent Quinizarin

Lithium, like other alkali metals, shows relatively poor chemical coordination, hence it does not have any optical signature in the spectral region. However, its high charge density provides great affinity to ligands with donor oxygen, and several chromogenic organic reagents such as 1,4-dihydroxyanthraquinone (Quinizarin) have been used as complexing agents of Li^+. In dimethyl sulfoxide medium (90%) and in the presence of sodium hydroxide and sodium carbonate, the reaction of Li^+ ion with Quinizarin results in the development of a bluish-violet color which can be detected using visible spectrophotometry [14]. Therefore, we sought to investigate the colorimetric determinations of lithium levels in artificial ISF based on the mentioned methodology. In order to achieve this, artificial ISF was prepared and spiked with therapeutic concentrations of lithium from 0.2 to 2 mmol/L. Raw spectral data of lithium spiked ISF samples in the presence of Quinizarin are shown in "Fig. 1A". The original spectra in "Fig. 1A" represents two absorption bands around 560-580 nm and 600-630 nm, which represents the complementary color to the wavelengths absorbed. In order to manifest the peaks of ISF samples spiked with 0.2-2 mM of lithium, the spectrum representing the sample containing no lithium was subtracted from the others, and the difference spectra are illustrated in "Fig. 1B".

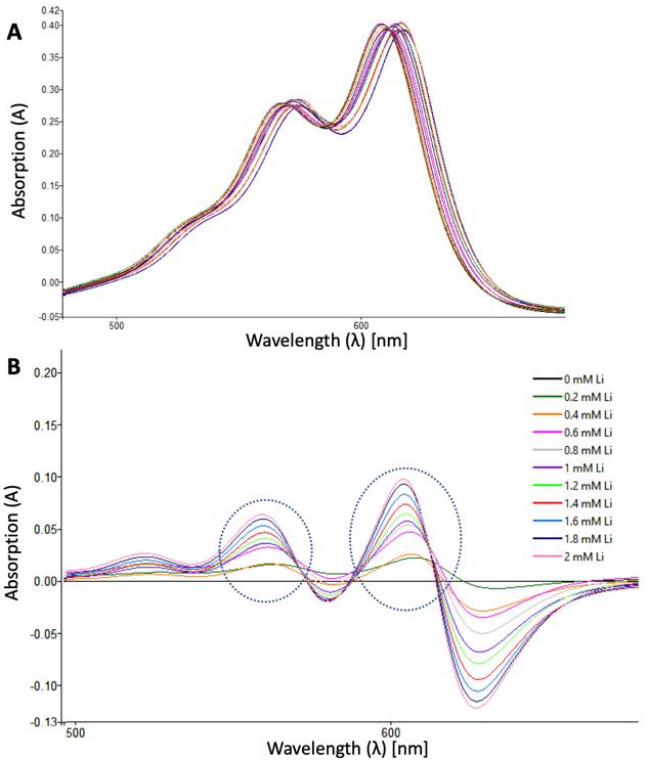

Figure 1. Raw spectra (A), and difference spectra (B) of therapeutic concentrations of lithium (0.2-2 mM/L) in artificial interstitial fluid, tested using colorimetric method based on the reaction of lithium ion with Quinizarin.

B. The variations in absorption peaks are proportional to the concentrations of lithium

From the difference spectra illustrated in "Fig. 1B", it can be determined that there are variations in absorption which seem to be proportional to the concentrations of lithium, with the absorption values increasing as the concentration of lithium in the spiked samples of artificial ISF increases. In order to further investigate these variations, a second-order derivative is applied to separate the peaks of overlapping bands and remove the spectral interferences and baseline effects which helps increase the selectivity of the assay. Second derivative spectra of the therapeutic concentrations of lithium is demonstrated in "Fig. 2". Second derivative gives negative peaks for each crest and trough in the spectrum. As depicted in "Fig. 2" there are variations in absorption minima between 620-640 nm regions, which correlate with the amounts of lithium in the sample. Less significant changes are also observed in minima absorptions between 560-580 nm. Therefore, wavelengths from these prominent regions can be used in order to detect various concentrations of lithium.

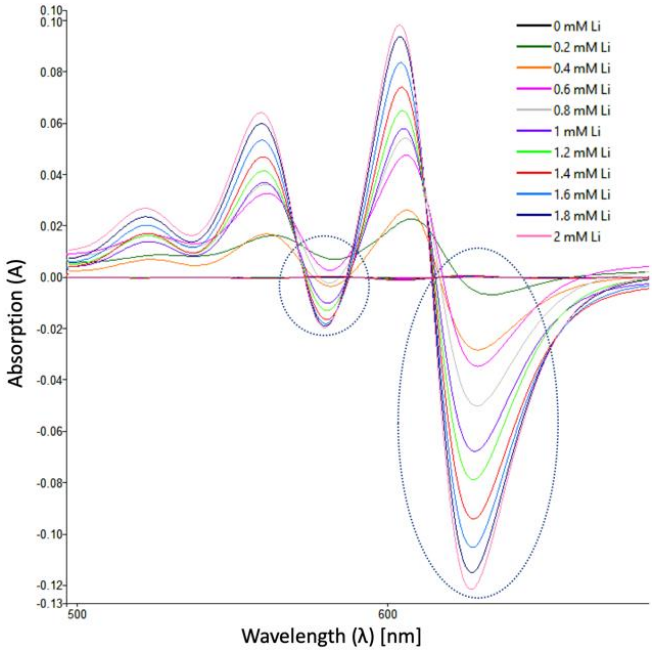

Figure 2. Second derivative spectra of therapeutic concentrations of lithium in artificial interstitial fluid in the range 400-800 nm. Prominent bands found at 630 nm and 580 nm (circled).

C. Levels of lithium in ISF can be determined using few selected wavelengths only

From the difference spectra illustrated in "Fig. 1B", it can be determined that there are variations in absorption peaks between 540-570 nm and 590-615 nm which seem to be proportional to the concentrations of lithium. In order to investigate this correlation, linear regression analysis was performed using two wavelengths from the prominent peaks "Fig. 3A and B". Higher correlation of determination (R^2) was achieved for the second peak compared to the smaller peak. In accordance with Beer-Lambert law, with the increase in concentrations of lithium from 0.2 to 2 mM there is an increase in the absorption values in the identified regions. From the Coefficient of Determination of 90% achieved from the regression analyses it can be concluded that the identified regions can be used for lithium concentration prediction in ISF with high accuracy.

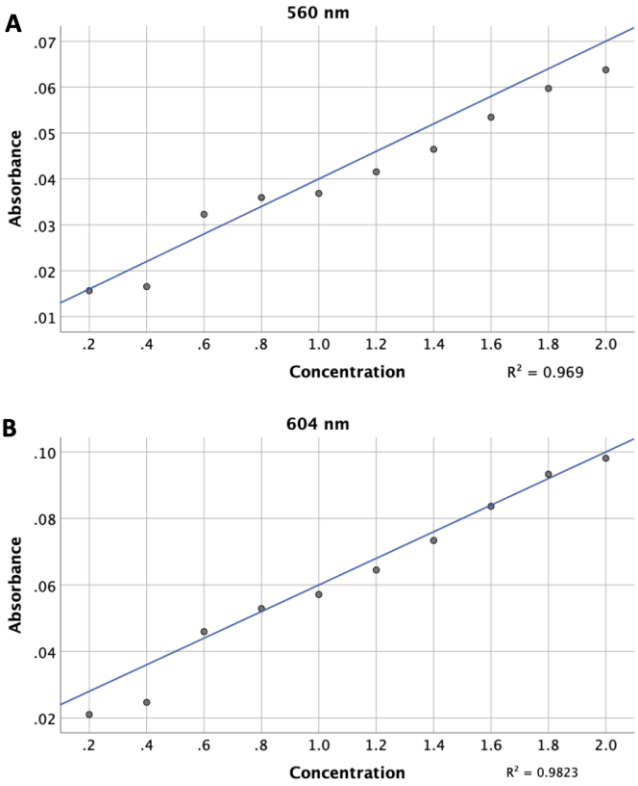

Figure 3. Linear regression analysis of absorption variations of different lithium concentrations in the region between 540-570 nm and 590-615 nm of the difference spectra. (A) Absorption variations at 560 nm representing the first peak. (B) Absorption variations at 604 nm representing the second peak. (P-value < 0.05)

IV. Discussion and Conclusions

Side effects and toxicity burden associated with lithium medication along with the crucial need for regular monitoring are the main reasons for lithium discontinuation and treatment nonadherence. Using the blood as the matrix for analytical detection of an analyte has the obvious drawback of having to do repeated blood tests. Consequently, facile monitoring of analytes of interest in a more accessible biofluid will be the main focus of next generation of devices for personal healthcare monitoring.

The accessible location of dermal ISF and the correlations found between blood and ISF levels of lithium resulted in our research effort for determining lithium therapeutic concentrations in ISF. This study was set out with the aim of detecting various concentrations of lithium in artificial ISF using a colorimetric method based on the reaction between Li^+

ion and Quinizarin. The resulting spectra of the experiments showed spectral variations which could be related to lithium concentration in spiked samples of ISF. Furthermore, linear regression analysis was conducted which showed a high correlation coefficient, meaning that the absorbance values at the wavelengths of interest were linear and correlated to the lithium concentrations. Therefore, the undertaken studies have demonstrated the feasibility of the proposed colorimetric method for determinations of lithium levels in artificial ISF using few selected wavelengths only.

So far, our results demonstrate the clear relationship between acquired spectra and lithium levels in artificial ISF samples. In our future studies we will also perform reference flame photometry measurements on ISF samples containing unknown levels of lithium to further evaluate our measurement technique. Moreover, biological ISF samples will be used to validate the results reported in this study using artificial ISF against a reference. In addition to quantitative determinations of blood lithium levels, the degree of specificity to lithium using the proposed method should also be investigated by including sodium in the samples as it is usually present at high concentrations in biological fluids.

Ultimately, we will investigate whether this method can be employed into a minimally invasive sensor for extraction and on device measurement of lithium in dermal ISF. The investigated techniques for lithium monitoring such as ISEs are associated with limitations including reduced accuracy and sensitivity as well as biocompatibility issues. However, optical methods are biocompatible, cost effective, and allow the rapid detection of lithium levels. Spectrophotometric measurement of therapeutic levels of lithium in interstitial fluid can provide the monitoring of this medication with an enhanced degree of specificity. The reported results give us a precise understanding of the light-lithium ion interaction in the optical spectrum region and its potential for development of a minimally-invasive lithium sensor. Therefore, the proposed methodology can be employed into a microneedle-based sensor to achieve facile extraction of ISF and rapid determination of lithium concentrations. In conclusion, determinations of lithium in ISF, an information-rich and accessible fluid, can support future development of a point-of-care device for continuous monitoring of lithium medication.

REFERENCES

[1] I. Grande, M. Berk, B. Birmaher, and E. Vieta, ‘Bipolar disorder’, *The Lancet*, vol. 387, no. 10027, pp. 1561–1572, Apr. 2016, doi: 10.1016/S0140-6736(15)00241-X.

[2] M. Gitlin, ‘Lithium side effects and toxicity: prevalence and management strategies’, *Int. J. Bipolar Disord.*, vol. 4, no. 1, p. 27, Dec. 2016, doi: 10.1186/s40345-016-0068-y.

[3] L. Tondo *et al.*, ‘Clinical use of lithium salts: guide for users and prescribers’, *Int. J. Bipolar Disord.*, vol. 7, no. 1, p. 16, Dec. 2019, doi: 10.1186/s40345-019-0151-2.

[4] M. Novell, T. Guinovart, P. Blondeau, F. X. Rius, and F. J. Andrade, ‘A paper-based potentiometric cell for decentralized monitoring of Li levels in whole blood’, *Lab. Chip*, vol. 14, no. 7, p. 1308, 2014, doi: 10.1039/c3lc51098k.

[5] M. Qassem, L. Constantinou, IasonasI. F. Triantis, M. Hickey, E. Palazidou, and P. A. Kyriacou, ‘A Method for Rapid, Reliable, and Low-Volume Measurement of Lithium in Blood for Use in Bipolar Disorder Treatment Management’, *IEEE Trans. Biomed. Eng.*, vol. 66, no. 1, pp. 130–137, Jan. 2019, doi: 10.1109/TBME.2018.2836148.

[6] J. H. Kim, D. Diamond, and K. T. Lau, ‘Development of non-invasive biochemical device for monitoring the lithium level from saliva for bipolar disorder patients’, in *2011 IEEE SENSORS Proceedings*, Limerick, Ireland, Oct. 2011, pp. 1744–1747, doi: 10.1109/ICSENS.2011.6126991.

[7] F. Criscuolo, F. Cantu, I. Taurino, S. Carrara, and G. D. Micheli, ‘Flexible sweat sensors for non-invasive optimization of lithium dose in psychiatric disorders’, in *2019 IEEE SENSORS*, Montreal, QC, Canada, Oct. 2019, pp. 1–4, doi: 10.1109/SENSORS43011.2019.8956598.

[8] P. Bollella, S. Sharma, A. E. G. Cass, and R. Antiochia, ‘Microneedle-based biosensor for minimally-invasive lactate detection’, *Biosens. Bioelectron.*, vol. 123, pp. 152–159, Jan. 2019, doi: 10.1016/j.bios.2018.08.010.

[9] B. Leboulanger, M. Fathi, R. H. Guy, and M. B. Delgado-Charro, ‘Reverse Iontophoresis as a Noninvasive Tool for Lithium Monitoring and Pharmacokinetic Profiling’, *Pharm. Res.*, vol. 21, no. 7, pp. 1214–1222, Jul. 2004, doi: 10.1023/B:PHAM.0000033008.64915.c8.

[10] M. N. Sweilam *et al.*, ‘Textile-based non-invasive lithium drug monitoring: A proof-of-concept study for wearable sensing’, *Biosens. Bioelectron.*, vol. 150, p. 111897, Feb. 2020, doi: 10.1016/j.bios.2019.111897.

[11] B. Leboulanger, J.-M. Aubry, G. Bondolfi, R. H. Guy, and M. B. Delgado-Charro, ‘Lithium Monitoring by Reverse Iontophoresis in Vivo’, *Clin. Chem.*, vol. 50, no. 11, pp. 2091–2100, Nov. 2004, doi: 10.1373/clinchem.2004.034249.

[12] E. Caffarel-Salvador *et al.*, ‘Hydrogel-Forming Microneedle Arrays Allow Detection of Drugs and Glucose In Vivo: Potential for Use in Diagnosis and Therapeutic Drug Monitoring’, *PLoS ONE*, vol. 10, no. 12, Dec. 2015, doi: 10.1371/journal.pone.0145644.

[13] S. Sharma, A. Saeed, C. Johnson, N. Gadegaard, and A. E. Cass, ‘Rapid, low cost prototyping of transdermal devices for personal healthcare monitoring’, *Sens. Bio-Sens. Res.*, vol. 13, pp. 104–108, Apr. 2017, doi: 10.1016/j.sbsr.2016.10.004.

[14] L. Gracia, ‘Spectrophotometric determination of lithium with Quinizarin in drugs and serum’, *Talanta*, vol. 44, no. 1, pp. 75–83, Jan. 1997, doi: 10.1016/S0039-9140(96)02018-8.