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A Paper-based Colorimetric Method for Monitoring of Lithium Therapeutic Levels

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Lithium remains the "gold standard" for both acute and maintenance treatment of Bipolar Disorder (BD), a serious life-long condition characterised by recurrent episodes of depressed and manic mood states. However, lithium has a very narrow therapeutic range (0.4-1.2 mmol/L) and despite its effectiveness in preventing and reducing mood swings and suicidality, it is a potentially hazardous drug. While it is crucial to carefully monitor lithium plasma levels, the current techniques of lithium monitoring are cumbersome and require frequent blood tests with the consequent discomfort which results in patients evading treatment. Therefore, development of low-cost and facile lithium detection techniques that can be translated into point-of-care devices for personal monitoring will be a major advance in the management of BD. In the current study we present colorimetric determination of lithium therapeutic levels utilizing test paper strips, based on its reaction with the chromogenic agent Quinizarin. Exposure of Quinizarin-dipped test papers to samples of interstitial fluid (ISF) or dH_2O spiked with therapeutic concentrations of lithium, resulted in colour changes that were monitored using optical spectroscopy. The acquired spectra from the test papers show spectral variations which are related to lithium concentrations in spiked samples of dh_2O and artificial ISF with a coefficient of determination (R^2) of 0.9 and 0.8, respectively. Altogether, the spectrophotometric and colorimetric analyses demonstrated strong correlations between the observed colour changes and the concentrations of lithium present in the sample. Therefore, this study has demonstrated that Quinizarin-treated cellulose-based papers are suitable for the precise detection of changes in lithium therapeutic levels. This method is simple and very convenient and serves as a foundation for future development of a paper-based colorimetric sensor for monitoring of lithium therapeutic levels in ISF and other non-invasive biological fluids.

Introduction

Lithium, an alkali metal available for medical use as lithium carbonate or lithium citrate, has been used clinically to treat major mood disorders for 70 years and continues to be the standard and most extensively evaluated medication for Bipolar Disorder (BD) [1]. Therefore, lithium remains the "gold standard" for both acute and maintenance treatment of bipolar disorder. The current therapeutic lithium levels in serum have been recommended as 0.4- 1.2 mmol/L and lithium toxicity occurs if levels surpass 1.2 mmol/L, hence lithium levels should be checked at a regular basis [2]. In the management of bipolar disorder, avoidance of lithium intoxication has been, and remains to be, an important component in lithium treatment [3]. Currently, therapeutic monitoring of lithium levels is performed by routine flame emission photometry (FEP) and atomic absorption spectroscopy (AAS) which require frequent blood tests with the consequent discomfort that often contributes to poor treatment monitoring resulting in either lithium toxicity or treatment non-adherence [4]. Research efforts have been made towards the development of point-of-care analytical devices for lithium determination using electrochemical and optical techniques [5][6][7]. However, most of the developed technologies for lithium determination are limited by constraints associated with re-producibility and simplicity of the fabrication process.

Cellulose-based materials have received significant interest in metal-ions detection due to their low price, excellent biodegradability and biocompatibility [8]. Therefore, paper-based analytical devices can be used to detect various analytes as they have the advantages of rapid manufacturing, easy processing, low cost, high porosity, and non-toxicity [9]. Moreover, paper-based materials are easy to handle, uncomplicated, disposable, and can act as a portable analytical device by incorporating the chemical sensor with facile

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colorimetric detection methods [9]. Colorimetric methods on the other hand, are utilised to detect the presence and concentration of an analyte by evaluating the colour formation or colour change [10]. The evaluation of the colour can be performed using spectrophotometers, typically by measuring the absorbance of the sample at specific wavelengths, via direct imaging, in combination with analytical software for quantification, or using colorimeters [9]. This detection technique based on colour formation or colour change specifically using chromogenic dyes would provide accurate results at lower costs. Therefore, taken together, the development of paper-based sensors would be valuable.

The simple off-the-shelf dye molecule, 1,4-dihydroxy-9,10-anthraquinone or Quinizarin has been investigated for monitoring of lithium therapeutic levels as well as for detection of moisture in organic solvents (such as THF, acetone, acetonitrile and DMSO) and building materials [11][12][13]. We have previously investigated the use of this easily available and low cost dye for monitoring of lithium in the management of BD, and have reported that Quinizarin exhibits excellent sensitivity towards the changes in lithium therapeutic levels [14][15]. Moreover, we are particularly interested in monitoring lithium levels in dermal interstitial fluid (ISF), an underutilized and information-rich biofluid which can be a proxy for direct blood sampling and allow lithium drug monitoring as its lithium concentration is proportional to the concentrations in blood. Nevertheless, determination of lithium levels in extremely low volumes of ISF is only feasible by introducing a precise and facile detection technique with minimal sample volume and preparation steps. Therefore, in the current study we have explored the use of cellulose-based test papers to detect the reaction between the chromogenic agent Quinizarin and lithium ion and achieve detection of changes in lithium levels both in water and artificial ISF. Thus, we established a simple method for routine analysis, replacing solution-based analysis with paper-based colorimetric detection. The proposed method reduces the sample preparation steps by reducing the number of reagents, reduces the sample volume, and supports the future development of a facile point-of-care monitoring system for minimally invasive detection of lithium in ISF. The reaction between Li^+ and Quinizarin forms a violet colour which can be monitored spectrophotometrically or calorimetrically [16]. Therefore, we have performed both conventional spectrophotometric analysis and colorimetric analysis. This method is simple and very convenient for future development of optical sensors based on cellulose-based colorimetric lithium membranes. The results of the current study support the future development of a paper-based colorimetric sensor for monitoring of Li^+ levels in ISF and other non-invasive biological fluids.

Experimental

Chemicals and reagents

4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), sodium phosphate monobasic (NaH_2PO_4), and saccharose were obtained from Sigma Aldrich (St. Louis, MO, USA). 1,4-Dihydroxyanthraquinone 96% (Quinizarin), Methyl sulfoxide 99.9% ($(CH_3)_2SO$), and Lithium carbonate 99.9% (Li_2CO_3) were obtained from Fisher scientific (Waltham, MA, USA). All solutions were prepared using deionized water (The Deionised Water Company, Suffolk, UK).

Sample preparation

A stock solution of 20 mmol/L Li_2CO_3 was prepared by dissolving 0.3694 g Li_2CO_3 in 250 ml dH_2O. Given the fact that there are two Li^+ ions in one molecule of Li_2CO_3, the stock solution contains 40 mmol/L of Li^+ ion. Accordingly, the Li_2CO_3 solution was then further diluted to make a set of solutions with the following concentrations: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40 mmol/L. Thereafter, lithium solutions containing therapeutic concentrations of Li^+ were prepared by mixing 1 mL from each concentration of Li_2CO_3 (i.e. 4, 8, 12, 16, 20, 24, 28, 32, 36, 40 mmol/L) with 19 mL of either artificial ISF or dH_2O. Therefore, lithium solutions were prepared in 20 mL total volume and with Li^+ concentrations ranging between 0.2-2 mmol/L. The different concentrations of Li^+ ion prepared in dH_2O and samples of artificial ISF are listed in (Table 1).The artificial interstitial fluid (ISF) was prepared by mixing 2.5 mM $CaCl_2$, 10mM Hepes, 3.5 mM KCl, 0.7 mM $MgSO_4$, 123 mM NaCl, 1.5 mM NaH_2PO_4, 7.4 mM saccharose, and adjusting the solution to pH 7.5 using Thermo Scientific STAR A211 pH meter [17]. Lastly, to achieve colorimetric determination of lithium levels based on the reaction between Quinizarin and Li^+ ion, 10 mM of Quinizarin solution was prepared by dissolving 0.48 g of 1,4-dihydroxyanthaquinon in 200 ml of 90% dimethylsulfoxide $(CH_3)_2SO$.

Reference flame photometry measurements

Samples of lithium solutions which were prepared in dH_2O or artificial ISF to give Li^+ concentrations of 0.2 to 2.0 mmol/L, were first verified through FEP measurements (Table 1). A flame photometer (M410 Sherwood Scientific Ltd, Cambridge, UK) was used to perform the reference measurements. The FEP measurements showed that the range of prepared concentrations varied between the sets probably due to pipetting errors. Thus, the remainder of analysis was performed using values extrapolated from the flame photometer measurements as the standard in measuring lithium.

Table 1. FEP measurements of lithium concentrations in dH_2O and artificial ISF samples.

Li+ concentrations (mmol/L) in dH2O	Li+ concentrations (mmol/L) in samples of artificial ISF
0.20	0.20
0.50	0.35
0.80	0.50
1.10	0.61
1.30	0.82
1.40	1.10
1.70	1.23
2.20	1.42
2.50	1.61
2.90	1.86

Test paper preparation

Test papers treated with Quinizarin were prepared using cellulose-based filter papers. Whatman grade 1 filter papers (Fisher scientific, Waltham, MA, USA) were cut into rectangular strips of desired dimensions (9 mm × 50 mm). Initially, the colorimetric determination of Li^+ levels in dH_2O was achieved by observing the reaction between Quinizarin and different concentrations of Li^+ in dH_2O on test papers exhibiting colour changes. Therefore, the filter paper was immersed in 5 ml solution of Quinizarin (10 mM) for 10 s. Thereafter, the paper containing Quinizarin dye was immersed in 4 ml of Li^+ in dH_2O (concentrations ranging from 0.2-2 mmol/L). The treated papers were then allowed to dry at Room Temperature (RT), and within approximately 1 h the changes in colour were visible (Fig. 1). Therefore, after 1 hour the coloured papers treated with Quinizarin and different concentrations of Li^+ were tested using optical spectroscopy (400-800 nm). Next, paper-based colorimetric detection of therapeutic concentrations of Li^+ in samples of artificial ISF was investigated. To achieve this, filter papers were immersed in 5 ml solution of Quinizarin (10 mM) for 10 s, and then 100 µl of ISF sample containing therapeutic concentrations of Li^+ was pipetted on each test paper. Similarly, the wet papers were allowed to dry at RT, and they were tested using optical spectroscopy after 1.5 h as the colour changes were less visible. All experiments were performed in four replicates, and samples of dH_2O or artificial ISF containing no lithium were included as the control.

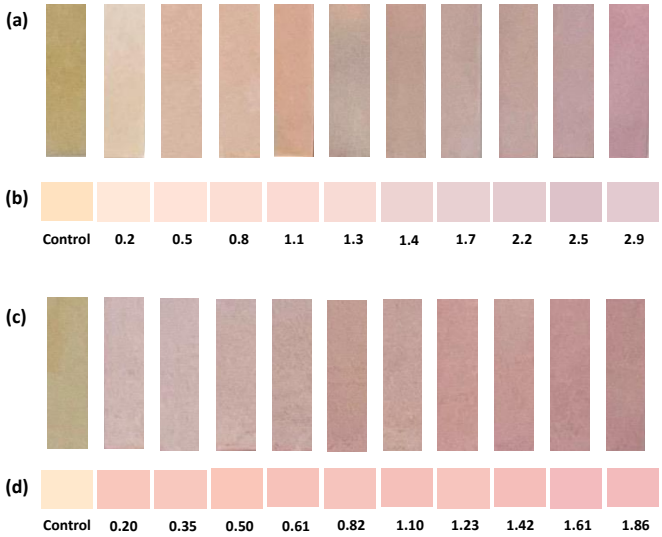

Figure 1. Colour changes of test paper strips treated with Quinizarin after being exposed to different concentrations of Li^+ in (a,b) dH_2O and (c,d) artificial ISF. (a,c) Images of Quinizarin-dipped test papers shot using smartphone camera. (b,d) colour visualisations of the reflectance spectra based on the CIELAB colour space.

Spectrophotometric measurements

To assess the observed colour changes, colorimetric analysis was performed on the prepared test papers via reflectance spectroscopy. A total reflectance spectrum of the test papers from 400− 800 nm was aquired using the LAMBDA 100 mm PMT/InGaAs Integrating Sphere detector from Perkin Elmer Corp (Waltham, MA, USA). The instrument was setup to acquire two spectra from each sample at 5 nm data interval. Reflectance spectroscopy ensures a higher sensitivity, with a better signal-to-noise ratio in the integrating sphere detector due to the multiple diffuse or scattering reflections which occur inside the white inner surface. Moreover, the spectra acquired using reflectance spectroscopy will allow us to perform spectrophotometric analysis as well as colorimetric investigations of the test papers using computational colour analysis techniques.

Analytical procedures

We have performed both conventional spectrophotometric and colorimetric analysis methods on the reflectance spectra to evaluate the colour changes observed on the test papers. For spectra collection and visualisation, UVWin Lab for LAMBDA 1050 (Perkin Elmer, Waltham, MA, USA) and Spectragryph optical spectroscopy software (Oberstdorf, Germany) were used. Furthermore, pre-processing and analysis of the spectra were performed in MATLAB R2020b, MathWorksTM (Natick, MA, USA) and the PLS-toolbox (Eigenvector Research Inc, Manson, WA, USA) Matlab® add-on. Multiple Linear Regression (MLR) analyses were performed on observed peaks relevant to lithium concentrations using the PLS-toolbox. For the colorimetric analysis, OptProp Matlab® colour properties toolbox was used as a Matlab® add-on which allowed colour analysis and visualisation of the reflectance spectra based on the CIELAB colour space. Therefore, the OptProp toolbox was

used to perform colour analysis on the reflectance measurements based on calculation and examination of colour related optical properties. Using this colorimetric method, the values of L*, a*, b* colour space (CIELAB), the most general colour space for measuring colour, were obtained.

Results

Colorimetric determination of lithium levels based on its reaction with Quinizarin

We endeavoured to achieve paper-based colorimetric detection of lithium therapeutic levels based on the reaction between the chromogenic agent Quinizarin and lithium which can be monitored using spectrophotometric and colorimetric methods. It was observed that Quinizarin-dipped papers treated with different concentrations of lithium exhibited colour changes that seem to relate to lithium levels present in the sample (Fig. 1a). In order to evaluate and display the colours observed on test papers, acquired reflectance data were used to achieve accurate measurement of each colour based on the CIELAB colour space [18]. The reflectance data were used to calculate L*, a*, and b* values according to the CIELAB system, which allowed display of colour differences. Therefore, reflectance measurements provided the CIE coordinates of the colour displays and were used to observe the changes in colour intensity. Figure 1b displays the calculated colour of Quinizarin-dipped papers treated with different concentrations of lithium in dH_2O. From the colorimetric results, it can be postulated that the intensity of colour change in the test paper related to the concentration of Li^+ present in the solution. Samples containing lower concentrations of lithium displayed more yellowish colours, with the intensity of this colour increasing as the lithium concentration increases, until reaching a violet colour for higher concentrations (Fig. 1b). This is while the Quinizarin-dipped test paper containing no lithium (control) displayed a bright yellow colour.

In order to evaluate these changes, the difference between the colour representing each lithium concentration and the colour representing the control was used to calculate Delta E (ΔE). To determine ΔE, values assigned to each of the L*, a*, and b* attributes of two colours were used to determine the distance between their two placements in the CIELAB colour space. As demonstrated in Fig. 2a, the overall difference between the test paper representing each lithium concentration and the control is represented as delta E and is proportional to the concentration of lithium, with ΔE values increasing as the concentration of lithium increases. While ΔE provides a value indicating the overall difference between each concentration and the control, it does not provide any colour-related data such as the lightness, blueness, or redness of colours. To further understand how the colours representing each concentration are different, we have plotted L* values (indicating lightness) against b* values [indicating yellowness (+) or blueness (-)] (Fig. 2b). It can be postulated from Fig. 2b that, while lower concentrations are representing higher L* values and display lighter colours, lower L* values attributed to higher concentrations demonstrate how test papers are getting darker as the concentration of lithium in the sample increases. Moreover, lower b* values calculated at higher concentrations represents the “blueness” of the test papers which explains the more violet colour observed at these concentrations. Lastly, lower concentrations are representing the highest b* values, displaying the yellowish colour observed on the test papers when exposed to lower concentrations of lithium.

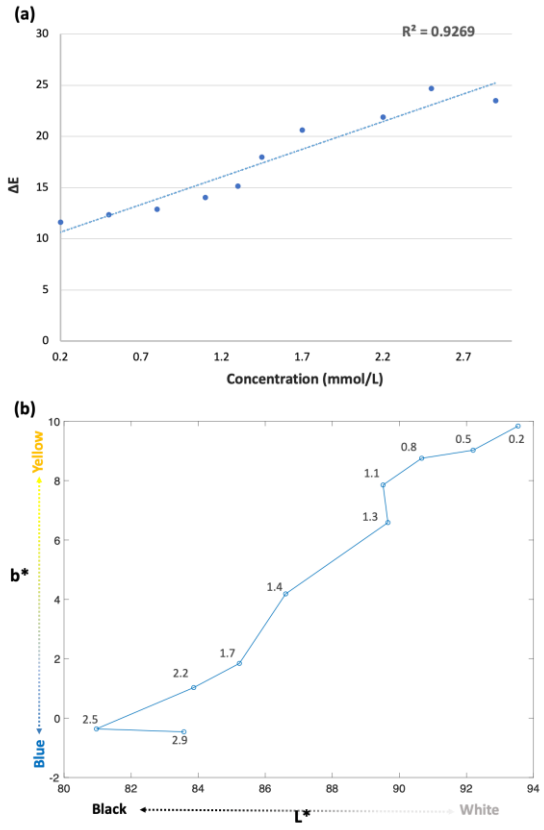

Figure 2. (a) scatter plot of ΔE values indicating the colour differences between the colour of test papers treated with therapeutic concentrations of Li^+ (0.2-2.9 mmol/L) and the control test paper. (b) the colour coordinates of each test paper displayed as L* versus b* values. While the higher b* values for lower concentrations display the yellowness of the colours, lower b* values represented by higher concentrations display the blueness of the test papers at these concentrations. Lower L* values for higher concentrations indicate the darker colours of the test papers, the higher L* values for lower concentrations indicates the lightness of the observed colours.

Optical spectroscopy was used to investigate the colour transition observed on Quinizarin-dipped test papers treated with therapeutic concentrations of lithium. The spectral variations observed in the visible region (400-800 nm) are pertinent to the reaction between Li^+ ion and Quinizarin and the resulting colour changes, and hence can be used to predict Li^+ concentrations. Therefore, the spectrophotometric results obtained from test papers displaying the colour changes were used to achieve quantitative analysis of Li^+ on these test papers. As shown in (Fig. 3a), the spectral data collected using reflectance spectroscopy were used to plot the changes in absorbance intensity against the Li^+ concentrations.

From the absorbance spectra illustrated in (Fig. 3a), 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 on the test papers increases. Therefore, we sought to assess the relationship between the acquired spectra and lithium levels through Multiple Linear Regression (MLR) using the spectral data between 550-620 nm. Spectral data were used as calibration data input whilst lithium concentrations obtained through FEP measurements were used as the dependent variable. Prior to producing the model, venetian blinds cross-validation was applied. The results of regression yielded a cross-validated R^2 value of 0.91732, hence suggesting that using the proposed paper-based colorimetric method lithium concentrations could be predicted with more than 90% accuracy. Moreover, the regression analysis demonstrated in (Fig. 3b) suggests that with the increase in concentrations of lithium from 0.2 to 2.9 mmol/L there is an increase in the absorption values (decreased reflectance) which implies the darker colours observed at these concentrations.

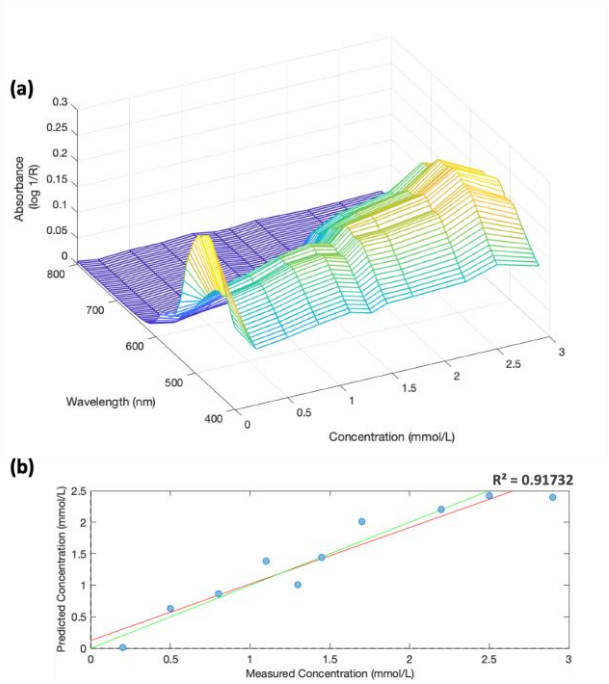

Figure 3. (a) changes in absorbance intensities of Quinizarin-dipped test papers treated with of therapeutic concentrations of lithium in dH_2O (0.2-2.9 mmol/L). (b) Measured (FEP) versus predicted concentration of lithium ion in dH_2O using feature wavelengths (550-620 nm) (Multiple linear regression (MLR) details: RMSEC: 0.127316, RMSECV: 0.24058, Bias: -4.21885e-15, CV Bias: -0.0283572, R^2 Cal: 0.976372, R^2 CV: 0.91732).

Interestingly, it can be postulated from (Fig. 4a and b) that as the concentration of Li^+ increases and with the transition towards more violet colours, the Area Under the Curve (AUC) of the spectral data acquired from the test papers decreases. In (Fig. 4b), lower reflectance values observed at higher concentrations are illustrated in colour blue. Therefore, at higher concentrations the difference between the reflectance value to the xy plane is lower which demonstrates lower AUC values. Therefore, we sought to investigate the relationship between the AUC values of the spectral data acquired and the concentrations of lithium. As illustrated in (Fig. 4c), the AUC values correlate linearly to the concentrations of lithium with R^2 value of 0.8948.

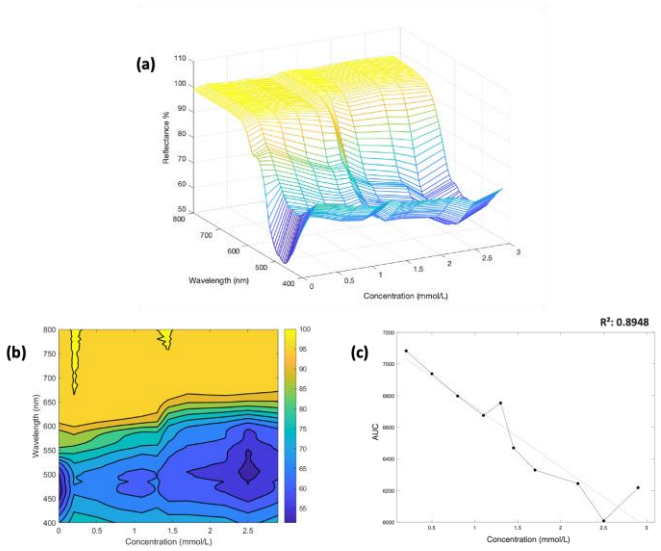

Figure 4. (a) reflectance spectra of Quinizarin-dipped test papers treated with therapeutic concentrations of Li^+ in dH_2O (0.2-2.9 mmol/L). (b) contour plot of reflectance values with lower values illustrated in blue. (c) scatter plot of Area Under the Curve (AUC) values of the spectral data acquired from the test papers, indicating the decrease in AUC values with the increase in Li^+ concentrations (R^2= 0.8948).

Colorimetric determination of lithium levels in artificial ISF

The reported paper-based colorimetric method was further explored to achieve determination of therapeutic concentrations of lithium in artificial ISF. The colour coordinates of each test paper were calculated according to the CIELAB colour system from the reflectance measurements. Figure 1c illustrates the colour changes observed on the Quinizarin-dipped test papers after exposure to ISF samples containing various concentrations of lithium. It can be seen from colorimetric results in (Fig. 1d) that as the concentration of Li^+ in samples of ISF increases, the intensity of colour on the test papers increases, with darker colours observed at higher concentrations. Figure 5a also demonstrates that with the increase in concentrations of Li^+ in samples ISF, there is an increase in ΔE values. Therefore, ΔE values, calculated from the difference between the colour displaying each lithium concentration and the colour representing the control, are proportional to Li^+ concentrations. This suggests that there is a higher intensity of colour change for higher concentrations compared to the control than for the lower concentrations.

To further investigate the pattern of colour change on test papers treated with therapeutic concentrations of Li^+ in ISF, L* values (indicating lightness) were plotted against a* values [indicating redness (+) or greenness (-)] (Fig. 5b). As depicted in (Fig. 5b), while the higher L* values acquired at lower concentrations of Li^+ represent the lightness in colour of the test papers at these concentrations, lower L* values at higher concentrations indicate how the test papers get darker as

concentration of Li^+ in the sample increases. Moreover, the higher a* values observed at higher concentrations of Li^+ representing the "redness" of the test papers suggest the increase in the intensity of the colours as the Li^+ level increases.

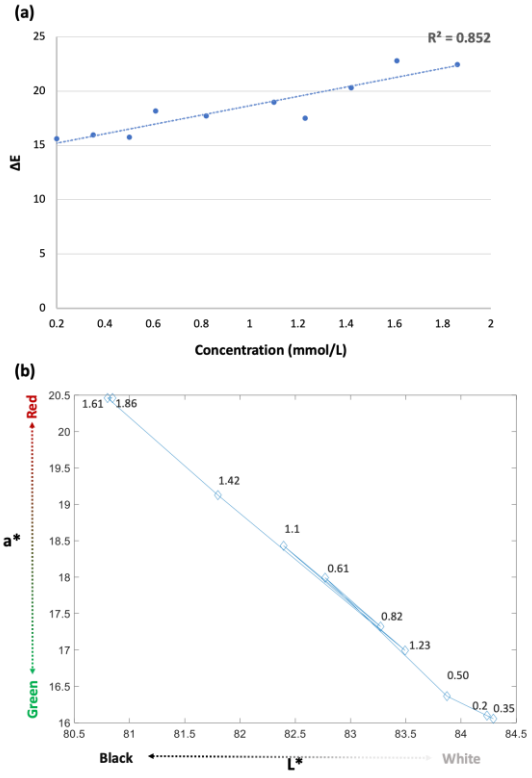

Figure 5. (a) scatter plot of ΔE values indicating the colour differences between the colour of test papers treated with therapeutic concentrations of Li^+ (0.2-2.9 mmol/L) and the control test paper. (b) the colour coordinates of each test paper displayed as L* versus a* values. The increase in a* values for higher concentrations indicates the shift towards the increase in "redness" of the colours. The shift from lower L* values for higher concentrations towards higher L* values for lower concentrations, demonstrates the darker and lighter colours observed for higher and lower concentrations, respectively.

In addition, changes in absorption intensity after the exposure of Quinizarin-dipped test papers to samples of ISF were plotted against the Li^+ concentrations measured using reference flame photometry. Figure 6a shows that with the increase in concentrations of Li^+ in samples ISF there is an increase in absorption values acquired from the test papers. To investigate these correlations, MLR was performed using spectra between 540-595 nm as the calibration input and the lithium concentrations obtained through FEP measurements as the dependent variable (Fig. 6b). The results of the regression suggest that the absorption values corresponding to the wavelengths of interest are linearly correlated at a high level of statistical significance to the concentration of lithium present in the sample. Therefore, the variations in absorbance values gave an understanding of the linear correlations between the observed peak and concentration changes of lithium. These results show that using the wavelengths pertinent to the reaction between Li^+ ion and Quinizarin in the visible spectral region and presented as colour changes on the test papers, the concentration of lithium in ISF can be predicted.

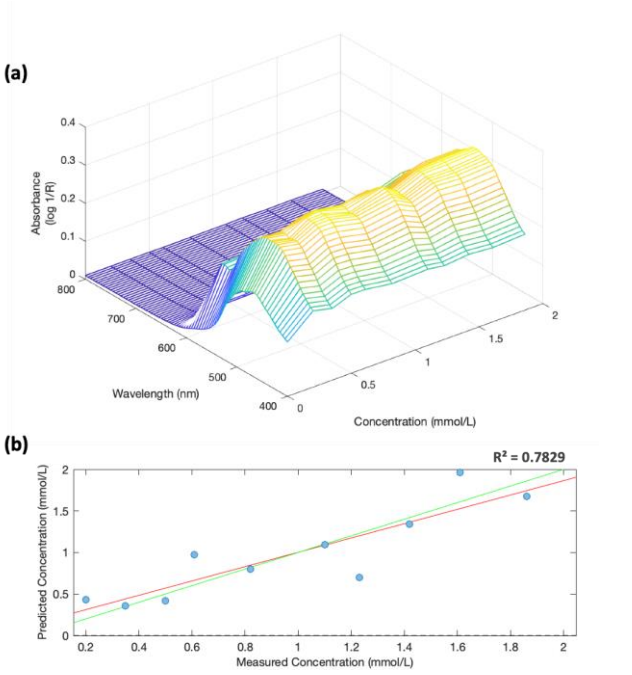

Figure 6. (a) changes in absorbance intensities of Quinizarin-dipped test papers treated with therapeutic concentrations of lithium in artificial ISF (0.2-1.86 mmol/L). (b) Measured (FEP) versus predicted concentration of lithium ion in artificial ISF using feature wavelengths (540-595 nm) (Multiple linear regression (MLR) details: RMSEC: 0.201625, RMSECV: 0.252952, Bias: 3.33067e-16, CV Bias: 0.00574088, R^2 Cal: 0.856807, R^2 CV: 0.782918).

Discussion

Development of easy-to-use devices for decentralized monitoring of lithium therapeutic levels will be a major advance in the management of bipolar disorder. However, point-of-care and low-cost monitoring of lithium therapeutic levels in bipolar patients remains a challenge. Paper-based colorimetric sensors that change in colour and the optical signal upon interaction with a trace amount of analyte have great potential for development of an inexpensive method for bulk production and widespread use. We have previously reported that the off-the-shelf commercial dye 1,4-dihydroxy-9,10-anthraquinone or Quinizarin is easily available, cheap and exhibits excellent sensitivity towards the changes in lithium therapeutic levels in both blood and ISF [14][15]. In our previous studies the reported results gave us a precise understanding of the interaction between lithium ion and the Quinizarin dye in the optical spectrum region, which was used for quantitative analysis of Li^+ levels in solution and in the presence of suitable reagents [14][15]. In the current study, we sought to replace the solution-based analysis with paper-based colorimetric detection which allowed us to monitor the reaction between Li^+ and Quinizarin without the need for sample preparation or multistep synthesis. Therefore, determination of lithium therapeutic levels using paper-based colorimetric methods was

explored to investigate the feasibility of developing cellulose-based lithium sensors using Quinizarin membranes. Utilizing test papers, the reaction between Quinizarin and various concentrations of lithium were detected via visual assessment, and spectrophotometric and colorimetric methods.

As reported in the previous studies the reaction of Li^+ ion with Quinizarin results in the development of a bluish-violet colour [12][11][19]. In the current study we have demonstrated that as the concentration of Li^+ in dH_2O increases this colour development becomes more prominent (Fig. 1 and 2). However, it should be noted that when the Quinizarin-dipped test papers were treated with therapeutic concentrations of Li^+ in samples ISF, the development of a bluish-violet colour was not as visible (Fig. 1d). This is potentially the result of other analytes also present in samples of ISF, which make the reaction between Li^+ ion and the dye less visible. Nonetheless, when the test papers were exposed to therapeutic concentrations of lithium, the colour change towards darker tones could clearly show an increase in the concentrations of Li^+ present in samples of ISF. Therefore, although the colour changes were not as prominent for ISF samples, they could still provide an understanding of the amount of Li^+ present in the sample using both colorimetric and spectrophotometric methods (Fig. 5 and 6). The changes in colour which could also be detected visually, were monitored by acquiring the reflectance spectra of the test papers. The acquired spectra were then used to perform colour and spectrophotometric analyses, using the CIELAB colour space [20][18]. Simultaneously, the changes in reflectance/absorption spectra investigated through regression models, indicated the changes in lithium levels. Taken together, we could demonstrate that with the increase in concentrations of Li^+ in the sample, there is an increase in absorption and ΔE values. Moreover, we have reported that as the concentration of Li^+ increases, the L* value, indicating the lightness of the developed colours, decreases. Overall, the linear changes in different parameters reported in the current study as a result of changes in lithium concentration are an important necessity for the pragmatic use of any chemical sensor and suggest the potential of the proposed method for future development of a sensor. It should be noted that while a rather simple alternative to the spectrophotometric method might be accessible by using a simple colorimeter or a colorimeter app on a smartphone, acquiring the spectral data ensures precise results. Therefore, the combination of spectrophotometric and colorimetric methods can provide accurate monitoring of lithium medication.

We have particularly investigated the feasibility of our proposed method for determination of Li^+ levels in samples of ISF as an information-rich biofluid which can be proxy to blood sampling and can support the future development of a minimally-invasive lithium sensor. Our results from both spectrophotometric and colorimetric analyses show strong correlations for determining Li^+ levels in samples of artificial ISF. Therefore, our quantitative analyses on samples of ISF suggest that Quinizarin treated cellulose-based papers might be suitable to determine the extent of unknown lithium content in a non-invasive biological fluid by simply a colour change. The pattern of colour change can be used as a standard to determine the unknown concentrations of lithium as the colour transition observed on the test papers can be used as a readout signal. It should be noted that the colour formation on the test papers took approximately about an hour which might suggest a relatively long response time. Nevertheless, the proposed methodology does not include long sample preparation steps required in the majority of chemical sensing techniques, which still makes the current method facile and time-efficient. Lastly, while existing lithium measurement techniques result in high costs and exposure of patients to an invasive procedure, the proposed detection technique based on colour formation using chromogenic dyes would provide accurate results at lower costs. It should be noted that in addition to high establishment cost and constant maintenance of common laboratory methods used for monitoring blood lithium levels, the costs associated with collecting, transporting, and processing of blood samples are also significant. However, development of a point-of-care colorimetric device for minimally-invasive monitoring of Li^+ therapeutic levels, can be an alternative to frequent blood tests and also reduce the costs associated with lithium toxicity or treatment non-adherence.

Conclusions

In conclusion, the current study provides a proof of concept for colorimetric monitoring of lithium levels using Quinizarin treated cellulose-based papers. Paper-based materials are easy to handle and can be incorporated in a potable analytical device to achieve monitoring of lithium levels with an excellent degree of biodegradability and biocompatibility. Therefore, we have successfully demonstrated that the current paper-based colorimetric methodology can be used for bulk production of Quinizarin membranes and their incorporation in lithium analytical devices. The proposed methodology offers many advantages such as easy availability, high sensitivity, and facile procedure for point-of-care lithium therapeutic monitoring. Lastly, colorimetric detection is simple and convenient and offers a low-cost method for development of a highly sensitive portable device for minimally-invasive monitoring of Li^+ therapeutic levels.

Author Contributions

Mahsa Sheikh (M.S.): Writing—original draft, visualization, conceptualization, data curation, investigation, validation, methodology. Mahsa Sheikh (M.S.) and Meha Qassem (M.Q.): Conceptualization and methodology. Meha Qassem (M.Q.) and Panayiotis A. Kyriacou (P.A.K.): Validation, investigation, and supervision.

Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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