Colorimetric determinations of lithium levels in drop-volumes of human plasma for monitoring patients with bipolar mood disorder

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***Abstract*— Lithium preparations are considered the most reliable form of mood stabilizing medication for patients with Bipolar disorder. Nevertheless, lithium is a toxic element and its therapeutic range is extremely narrow, with levels of 0.6-1.0mEq considered normal, whereas levels above 1.5mEq are toxic. Thus unfortunately, many patients reach toxic levels, which leads to unnecessary complications. It is believed that personal monitoring of blood lithium levels would benefit patients. Therefore, our aim is to develop a personal lithium blood level analyzer for patients with bipolar mood disorder, and we report here, our initial results using a colorimetric-based method and testing drop-volumes of human plasma spiked with lithium. It was possible to validate results with standard flame photometry readings. Applying the Partial Least Squares (PLS) method on preprocessed spectra, therapeutic concentrations of lithium in a single drop can be predicted in a rapid manner, and furthermore, the calibration results were used to select the effective wavelengths which were employed as inputs in Multiple Linear Regression (MLR). The simplified algorithms of this would prove useful when developing a personal lithium analyzer. Overall, both calibration methods gave high correlation and small error outputs with a R^2= 0.99036 and RMSEC = 0.03778, and R^2= 0.994148 and RMSEC= 0.0294404, for PLS and MLR methods, respectively. The results show that the spectrophotometric colorimetric determination of blood lithium levels can be extended beyond laboratory application and indicate the capability of this testing principle to be employed in a personal monitoring device. Future work will now focus on the technical development of a miniaturized system for measurement of lithium levels in blood with an acceptable level of accuracy and sensitivity.**

I. Introduction

Bipolar disorder is a lifelong mood disorder characterized by episodes of hypomania/mania, almost always interspersed with episodes of depression [1, 2]. In the UK, the prevalence of bipolar disorder has been estimated at ~1% of the population [3], with a total cost of £342 million to the NHS at 2009/2010 prices, and hospitalization accounting for £207 million of that figure [1]. Various drugs are available as part of long-term treatment of bipolar disorder, designed to prevent relapse or recurrence [4]. Of these, lithium is presented as first line as recommended by NICE guidance in September 2014 [4], and is known to be more effective in reducing the risk of suicide in patients with bipolar disorder [5]. Nevertheless, lithium is potentially dangerous, and its therapeutic index is relatively narrow with normal levels being stated as 0.4-1.0mEq, whereas anything above 1.5mEq is considered toxic. As a result, many patients often surpass this threshold, which in some cases, can lead to serious consequences such as renal failure, seizures, coma and even death [6].

Moreover, toxicity can occur suddenly as a result of excessive intake or due to accumulative high levels during ongoing chronic therapy [6]. At the moment, patients are obliged to attend regular blood tests, and lithium levels are analyzed using flame photometry which is by far, the most common method for this application. However, this method is mainly suitable for laboratory analysis, which although necessary, it is becoming apparent that more is required in monitoring lithium levels. This is because high rates of treatment nonadherence exist [7], and some reasons listed by NICE include the frequency of blood tests resulting in patients defaulting from treatment. Therefore, the availability of a personal monitor would be vastly beneficial to bipolar patients by improving their sense of involvement in the management of their illness, improving adherence with effective monitoring and facilitating early detection of shifting of lithium concentrations outside the therapeutic range.

To meet this demand, we aim to develop a personal blood lithium analyzer for patients with bipolar disorder. As mentioned earlier, lithium concentrations are usually determined through flame photometry measurements of human serum/plasma. Alternative methods have been explored in the past [8]–[10] but unfortunately , a reliable device remains unavailable. We present here initial results of colorimetric determinations of lithium concentrations in drop-volumes of human plasma, based on a spectrophotometric analytical method that uses 1,4-dihydroxyanthraquinone (Quinizarin) [11]. In addition, Partial Least Squares (PLS) and Multiple Linear Regression (MLR) are performed on data in order to investigate and compare the accuracy of concentration predictions using full-range spectra and selected specific wavelengths.

II. Material and Methods

Ethics approval was granted by Senate Research Ethics committee at City University London prior to performing tests on samples of human plasma.

A. Reagents and materials

All experiments were conducted with analytical grade reagents, and aqueous solutions were prepared using distilled water.

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A standard solution of 60mEq Lithium carbonate in saline was prepared, and subsequently, this was diluted further to make a set of solutions with the following concentrations: 18, 24, 30, 36, 42, 48, 54 mEq. Additional reagents prepared were: 0.1 M of NaOH, 0.25 M of Na2CO3, 90 % (CH3)2SO and 1 mM of Quinizarin in (CH3)2SO.

Frozen mixed pool blood plasma from healthy individuals was obtained from a reliable provider. Samples of human plasma that have been spiked with lithium were prepared by mixing 1 mL from each reagent of lithium carbonate prepared earlier i.e. 18, 24, 30, 36, 42, 48, 54 and 60mEq with 29 mL of blood plasma, thus giving 8 samples of 30 mL, with lithium concentrations of 0.6-2.0 mEq.

B. Testing procedure

An amount of 500 µL from spiked samples of plasma were pipetted into a test tube, then were diluted with water to make up to 1 mL. For tests performed using a standard 1 mm quartz cell, a procedure similar to that described by Gracia et al [11] was followed where 100 µL from diluted plasma was 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 100 µL of 1mM of Quinizarin in (CH3)2SO.

Tests performed using drop-volumes of plasma required slight modifications to the procedure described previously. Instead, the volumes of diluted plasma and Quinizarin were reduced to 50 µL. The same volumes were maintained for the remainder of reagents. Prior to testing, all samples were kept in a thermostatic bath at 25º for 30 mins.

C. Instrumental setup

The PerkinElmer Lambda 1050 dual beam spectrophotometer (PerkinElmer Corp, Waltham, MA) was used, with two different types of cells. The first was a set of standard 1 mm quartz cells, and the second was the Traycell (Helma GmbH & Co, Mulheim, Germany), which only requires 4-5 µL of the sample to be measured. Absorbance measurements using standard quartz cells of 1 mm thickness were taken with a reagent blank prepared under identical conditions being placed in the reference compartment. When using the Traycell, the reagent blank was measured separately then subtracted from the remainder of spectra.

The instrument was setup to acquire spectra between 450-700 nm at increments of 1 nm, with a response time of 0.2 secs and two running cycles for each sample. Slit width of the detector was fixed at 2 nm, and both reference and sample attenuators were kept at 100 %. Data pre-treatment, Partial Least Squares (PLS) and Multiple Linear Regression (MLR) analysis were performed using the Matlab® software (Mathworks Inc, Novi, MI), and the PLS-toolbox (Eigenvector Research Inc, Manson, WA) Matlab® add-on. The pre-processing methods which were applied to the spectra included weighted least squares baseline correction, and Savitsky-Golay algorithms for both smoothing and second derivatives.

III. RESULTS AND DISCUSSIONS

Fig. 1 shows the original absorbance spectra and the second derivative spectra of therapeutic concentrations of lithium, recorded using a standard pair of 1 mm pathlength

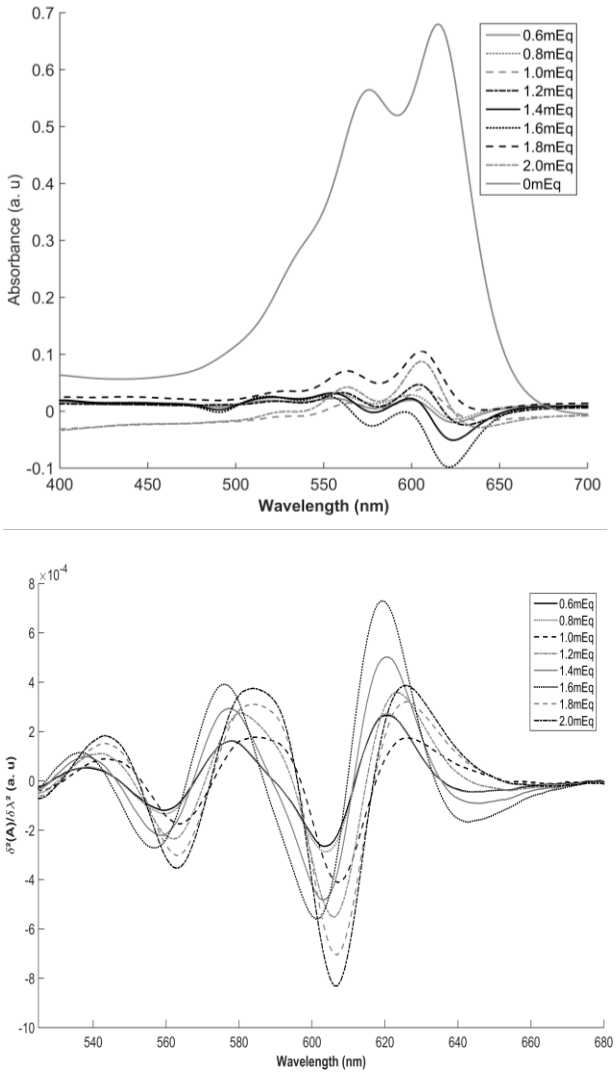

Figure 1. Raw spectra (top), and Second derivative spectra (bottom) of therapeutic concentrations of lithium in human plasma, tested using a colorimetric method with standard 1 mm quartz cells.

cells, where particularly in the latter, similar patterns are observed which showed expected variations in absorption minima between 601-607 nm regions, and which correlated with the amounts of lithium in the sample. Less significant but proportional changes with lithium concentrations were also observed in minima absorptions between 557-562 nm. Thus in both regions, spiked samples which contained the most amount of lithium expressed more intense absorptions and this decreased in accordance with lessening of lithium.

Nevertheless, once this procedure was repeated using the Traycell, in order to explore the possibility of accurate determinations of lithium using only drop-volumes of blood, the resulting spectra shown in Fig. 2(a) were found. As can be seen, absorptions witnessed previously in Fig. 1 were significantly reduced, with a more complex relationship existing now between lithium levels and absorptions at the relevant bands. Hence, it was necessary to alter the ratios of NaOH, Quinizarin and spiked sample until reasonable absorptions were achieved. The resulting spectra of the modified testing procedure, described previously in the

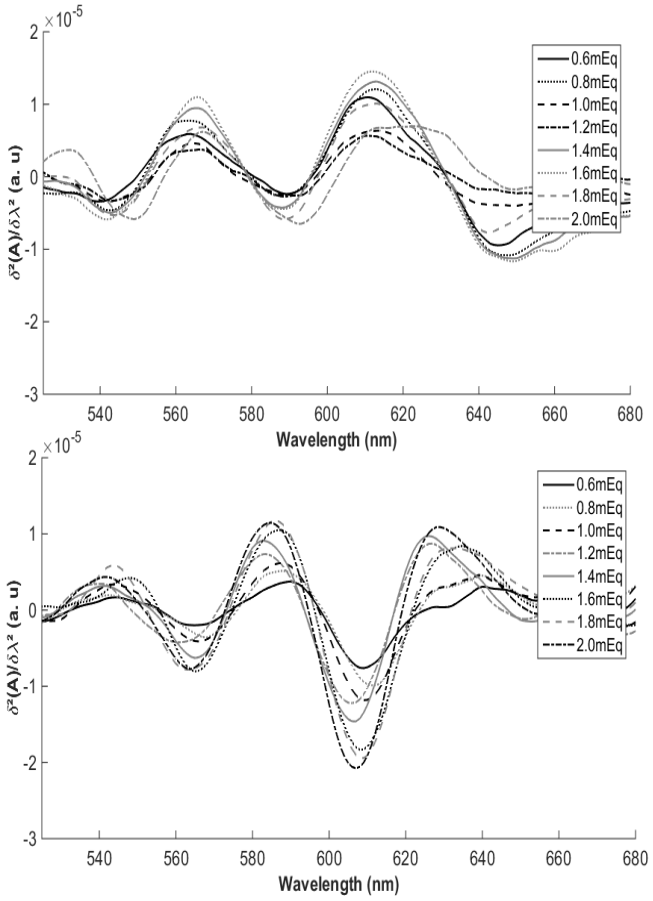

Figure 2. Second derivative spectra of therapeutic concentrations of lithium in human plasma tested using Traycell; (top) prior to, and (bottom) after optimizing the testing procedure.

methods section, are shown in Fig. 2(b), where once again, a somewhat linear relationship is evident between lithium concentrations of spiked samples and peak absorptions at the relevant bands.

Given the improved spectral outputs, PLS analysis was carried out on the data acquired and shown in Fig. 2(b) to establish a rapid method for lithium determinations using chemometrics, The spectral region selected to build the PLS calibration of the measured set was between 480-690 nm, using pre-processed data and venetian blinds cross-validation. The generated model could explain 81.80 % of spectral variations using two Latent Variables (LVs), each accounting for 57.53 % and 24.27 % of the total. In turn, this could explain 99.04 % of the variations in concentrations of lithium in spiked samples, as measured by flame photometry. The model produced a low calibration error and high correlation coefficient, as indicated by the root mean error of calibration (RMSEC) and R^2 values shown in Table. 1.

Moreover, the loadings plot of the PLS calibration is shown in Fig 3. This was used to determine the bands which contributed mostly to the calibration, and which would of significance to predicting lithium concentrations. Hence looking at the plot in Fig. 3, it can be seen that two ranges were mainly of significance to explaining the variations in spectral data, and these were around 560-570 and 605-

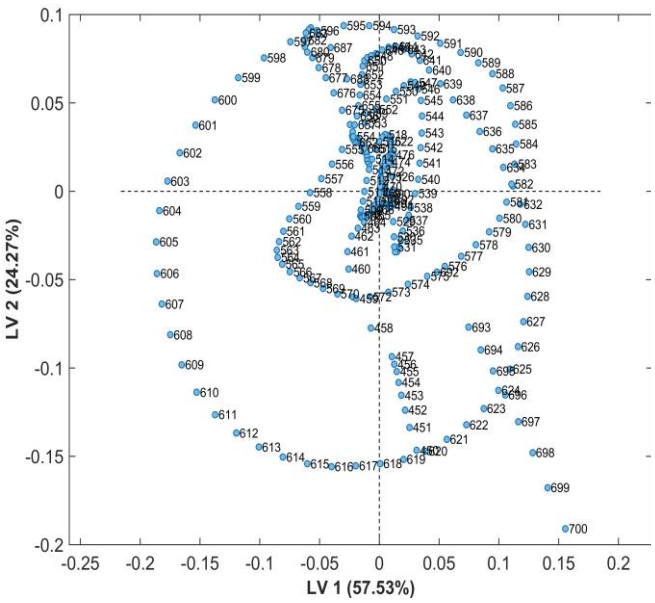

Figure 3. Loadings points from PLS regression results based on two explanatory variables.

615 nm, which again, is in correspondence with the absorptions bands observed earlier in Fig. 2(b). Using this information, spectral band values around 560 and 605 nm, prior to averaging, were extracted in order to be used in a the linear regression calibration. A third region around 660 nm was also included in the analysis. The linear regression analysis was carried out in order to establish a direct and simple combination of three selected wavelengths in relation to flame photometer readings, which in turn, would be used in developing the necessary algorithms in a miniaturized instrument that would employ a few selected wavelengths without the need of acquisition and analysis of full range spectra.

Similar to PLS results, MLR analysis also yielded a calibration model of high correlation (R^2) and low RMSEC. Table. 1 shows the values for these for both calibration and cross-validation processes. Therefore, lithium concentrations can be measured accurately using a few selected wavelengths, which in turn, can allow appropriate algorithms to be produced for a miniaturized system. The prediction ability

Table 1. Calibration and cross-validation results of PLS and MLR analysis for determination of lithium levels in blood.

	Calibration method	
	PLS	**MLR**
RMSEC	0.0377837	0.0294404
RMSECV	0.0559696	0.0689933
Bias	2.22E-16	0
CV Bias	0.0193917	0.00833188
R^2 Cal	0.99036	0.994148
R^2 CV	0.982965	0.968488

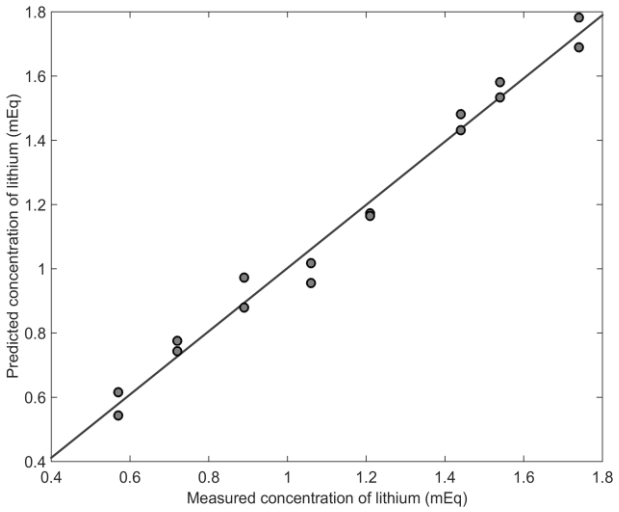

Figure 4. Predicted versus measured using only three wavelengths as variables.

of this process is apparent in Fig. 4, which shows a plot of the measured concentration of lithium against that predicted by linear regression.

IV. CONCLUSIONS

A colorimetric method that uses the chromogenic agent Quinizarin was employed and modified to allow determinations of lithium levels in drop-volumes of blood. The resulting spectra of the modified testing procedure showed spectral variations which could be related to lithium concentration in spiked samples of human plasma. Moreover, these were used to conduct PLS calibration, which yielded a model with high correlation coefficient and low root mean error. Based on this, it was also possible to reduce the number of required wavelengths and hence support future development of a miniaturized, multi-wavelength system, by conducting linear regression on three selected wavelengths only. This too produced reliable calibration of the measured set. In conclusion, these results show that the colorimetric method can be modified to allow accurate and rapid determinations of lithium in drop-volumes of blood of roughly 4-5 µL, and only using a few effective wavelengths to do this. Future work will now focus on improving the predication ability of the calibration by testing against more unknown samples, as well as building and evaluating the miniaturized system which would employ this measuring principle to provide readings of lithium levels in micro volumes of blood.

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