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Spectrophotometric analysis of Lithium Carbonate used for Bipolar Disorder

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Abstract: Lithium therapy is the gold standard of treatment for patients with Bipolar Disorder. However, despite its effectiveness, it is a potentially hazardous drug requiring regular monitoring of blood levels to ensure toxic levels are not reached. This paper describes the spectrophotometric analysis of Lithium carbonate in solution as a first step in developing a portable home monitoring device for blood lithium analysis.. Using a high-end spectrophotometer, solutions of lithium carbonate (Li_2CO_3) have been optically fingerprinted. Preliminary measurements indicate that the ultraviolet region shows a strong distinction between different lithium concentrations. Utilizing second derivative absorption curves, the region of 220 nm to 230 nm demonstrated the ability to differentiate between concentrations representing those found in patients. Furthermore, the method could determine to within a 1–6% accuracy whether an unknown solution of Li_2CO_3 is either inside or outside the high-end of the therapeutic limit.
tissue diagnostics.

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1. Introduction

Bipolar disorder is a chronic condition, characterized by recurring episodes of depressed and manic mood states [1]. In its more severe forms, bipolar disorder is associated with significant impairment of personal and social functioning, and with high risk of death through suicide as well as poor physical health. It is estimated to affect up to 254 million worldwide [2], often developing between the ages of 18 and 24 years.

Lithium, prescribed in the form of a carbonate (Li_2CO_3) or citrate ($Li_3C_6H_5O_7$), is a widely used medication for treating bipolar disorder [3], reducing the frequency and intensity of mood swings and suicidal tendencies. However, despite its effectiveness, it is a potentially hazardous drug requiring regular monitoring of blood levels to ensure toxic levels are not reached.

Lithium has a very narrow therapeutic index (concentrations ranging from 0.4 to 1.0 mM) with the upper limit being uncomfortably close to toxic levels [4]. Toxic lithium levels can cause circulatory collapse, kidney failure, neurological abnormalities, seizures, coma and even death [4- 6]. For patients newly prescribed lithium medication, the dosage adjustment can take months before stability is reached and lithium is within the therapeutic range. Even when lithium concentrations are reasonably stable, they can rapidly reach toxic levels during intercurrent illness such as febrile conditions and dehydration or the addition of some drugs [7]. Therefore, lithium requires regular on-going monitoring to maintain therapeutic levels and avoid toxicity [8].

Determination of blood lithium levels involves relatively complex laboratory methods, such as flame photometry or ion-selective electrode analysis, which require withdrawal of blood samples and transport of samples to a laboratory [9]. The National Institute for Health and Clinical Excellence (NICE) guidelines recommend that lithium levels should be checked one week after starting and one week after every dose change until the levels are stable [10]. Following stability, NICE recommends that lithium levels are checked every three months. However, in a national-level audit of lithium monitoring practice in the UK, it was found that contemporary lithium monitoring falls short of the standards recommended by NICE [11]. This failure to ensure the safe use of lithium and/or to ensure adequate monitoring of established treatment may place patients at risk of avoidable drug related morbidity. Furthermore, bipolar patient adherence to lithium therapy is often “erratic” [12]. Reasons include frequent blood tests, which can contribute to patients defaulting from treatment. This is associated with unacceptably high levels of relapse.

Currently, there aren’t any non-invasive blood lithium monitors available to enable bipolar patients to check their lithium levels at home. Such a device, would put more control into the hands of the patient while also providing an early warning that lithium concentrations are drifting outside the therapeutic range.

This research explores the feasibility of optically detecting lithium concentrations, with the ultimate goal of providing a portable non-invasive sensing technology for bipolar disorder patients. This paper outlines the first steps in optically fingerprinting lithium carbonate in order to determine the optical window of interest and the capability of optical techniques to identify the concentrations of lithium found in blood.

2. Materials and Methods

Lithium concentrations are typically measured in plasma. However, as a precursor to measurements in plasma or whole blood, the optical absorption characteristics of lithium carbonate in solution were investigated in order to identify the appropriate wavelengths and optical window on which to focus measurements. Furthermore, this investigation would allow for the feasibility of optical detection of lithium to be explored. Therefore, solutions of lithium

carbonate in distilled water were analyzed in the laboratory, including solutions whose concentrations reflect those typically found in blood.

2.1 Sample Preparation

Solutions of lithium carbonate (Li_2CO_3) were prepared in pure distilled water by diluting a concentrated solution. The concentrated solution was prepared using a solid pure (99.999%) form of Li_2CO_3 from Acros Organics (NJ, USA). As there are two Lithium ions (Li+) in one molecule of Li_2CO_3, it was only necessary to prepare the whole solution to half the desired concentration in order to achieve the therapeutic doses of lithium. Li_2CO_3 has negative water solubility, i.e. the amount able to dissolve into water decreases with increasing temperature. Therefore, all samples were prepared and tested at ambient temperature (21°C) to ensure that no solute precipitated out of the solution during measurements.

In order to initially identify a spectral region of interest and fingerprint Li_2CO_3 in solution, a batch of solutions with high concentrations was prepared. These solutions are referred to as the "Fingerprinting concentrations" (see Table 1). Following this, solutions whose concentrations reflect those commonly found in blood were prepared and evaluated to determine the ability of optical absorption spectrophotometry to identify Li concentrations around the therapeutic window. These solutions are referred to as "Therapeutic concentrations" (see Table 1).

Table 1. Concentrations of Li_2CO_3 in solution of distilled water with the effective Li^+ concentration

Finger Printing Concentrations		Therapeutic Concentrations	
Li_2CO_2 mmol/L	Li^+ mmol/L	Li_2CO_2 mmol/L	Li^+ mmol/L
5.3	10.6	0.1	0.2
10.7	21.4	0.2	0.4
21.3	42.6	0.3	0.6
42.5	85.0	0.4	0.8
-	-	0.5	1.0
-	-	0.6	1.2

2.3 Spectrophotometry Apparatus and Methods

To obtain full spectra of the different fingering printing solutions, a Lambda 1050 dual beam UV/Visible/NIR spectrophotometer (Perkin Elmer Inc, Massachusetts, USA) was used with a spectral region of 200–3300 nm, thus covering parts of the ultraviolet region, the entire visible and NIR, as well as parts of the mid-infrared region. The increment step was set to 1 nm. For wavelengths between 200–319.20 nm, the source was a deuterium lamp, while a halogen tungsten lamp was used for the range 319.20nm to 3300nm. The detector system was set to use a photomultiplier tube detector (PMT) for the shortest wavelengths from 200 nm to 860.80 nm, an indium gallium arsenide detector (InGaAs) for wavelengths between 860.80–1800.80 nm and a lead sulphide detector (PbS) for the rest of the spectral region up to 3300 nm. Slit settings for the PMT detector were fixed at 2 nm, whilst both the InGaAs and PbS detectors were set on 'servo mode', whereby the system monitors the reference beam energy and adjusts the slits accordingly to avoid over saturation of the detectors. Gain settings for the detectors were set to 5 for the InGaAs and PbS detectors, while the PMT gain was set on auto to allow the instrument to determine the appropriate value. The response time for all detectors was set at 0.4 seconds. The front and rear attenuators were set at 100% for both the sample and reference beams. Prior to placing the samples in the spectrophotometer, baseline corrections (100 %T/0A and 0%T/blocked beam) were performed to remove 'background noise' and help negate the effects of any stray light in the system.

First a sample of distilled water was evaluated in the spectrophotometer in order to provide a reference, followed by the finger printing concentration samples from Table 1. Each sample was contained in a rectangular quartz SUPRASIL cell, made of high purity synthetic fused silica material with a light path length of 1 mm (Perkin Elmer Inc, Massachusetts, USA). Samples were then placed into the sample compartment whilst a blank optically matched cell was inserted into the reference compartment.

Once a spectral region of interest was identified, the scan settings were changed to fix the range between 200-315nm (the region where it was observed to be changing with increasing Li_2CO_3 concentrations). A second derivative of the spectrum in this region was then calculated to better identify specific absorption bands.

2.4 Data Analysis

For each sample, spectral measurements were repeated three times, and the results averaged and smoothed using a Savitzky-Golay filter in UV WinLab Data Processor and Viewer software (Perkin Elmer Inc, Massachusetts, USA). The second derivative of the curves was calculated and spectral bands of interest were identified. Utilizing UV WinLab's maths functions the peak, maximum height and area under each curve in the bands of interest was calculated and saved to a spreadsheet for further analysis.

Using these results, calibration functions were developed in MATLAB® (The MathWorks, USA). This was done by fitting the results obtained from UV WinLab to linear and cubic functions for all three scans in each therapeutic concentration, and for pure water, which was given a lithium concentration value of 0.0 mmol/L for referencing purposes and zero point calibration. Each function was given an R^2 correlation score (a value greater than 0.95 for the linear fit was established as the correlation limit at which a function was rejected) to help determine which function worked best in the $[Li^+]$ range of interest. Lastly the "Therapeutic concentrations" samples were re-run in the spectrophotometer and their concentrations calculated using the cubic calibration functions with $R^2 > 0.97$.

3. Results

The resulting absorption spectra of distilled water and the Fingerprinting Li_2CO_3 solutions are shown in Figure 1, after undergoing smoothing to reduce the level of noise. It was easily observed that the primary water absorption bands in the infrared regions dominate; however the UV band of approximately 200 – 240 nm demonstrated a characteristic slope, increasing as the concentrations got higher.

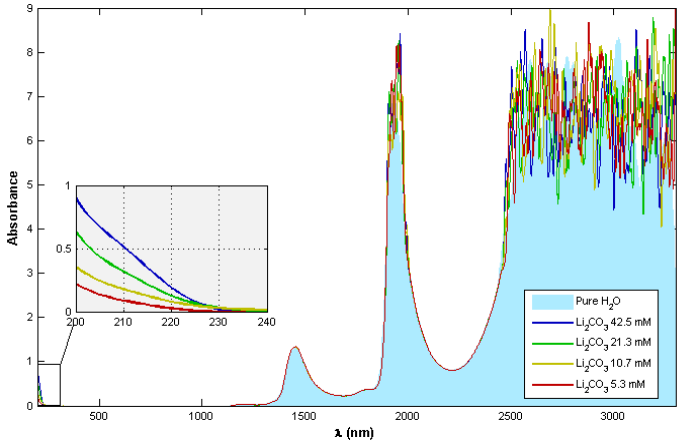

Fig. 1. Full pure water spectrum with various fingerprinting concentrations of Li_2CO_3

In the UV region of interest the 2nd derivative of batch 1 revealed two distinct absorption bands, a narrow bandwidth of approximately 205 – 209 nm (γ_{207}) and a second broader band with a bandwidth of approximately 220 - 230 nm (γ_{225}), shown in figure 2

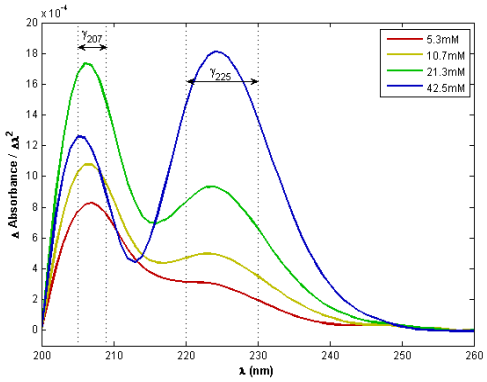

Fig. 2. Second derivative absorption curves (200 – 260 nm), fingerprinting concentrations.

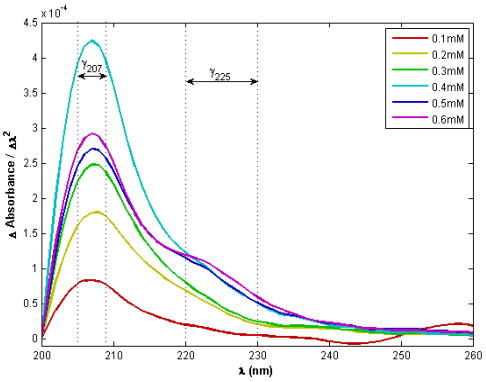

Fig. 3. Second derivative absorption curves (200 – 260 nm), therapeutic concentrations.

The second derivative of the absorption curves for the therapeutic concentrations were observed to have less of a distinct peak in the γ_{225} band, but the general trend of the absorbance at the peak value was still observed to increase with increasing concentrations, see figure 3. The calculated R^2 value for the linear (L) and cubic (C) fits are summarised in the table below, table 2.

Table 2. R^2 values for the linear (L) and cubic (C) therapeutic concentration curves for each of the identified bands (γ_{207} and γ_{225}) using the peak, maximum height and area under the curve methods.

	Peak		Max Height		Area	
Band	**L**	**C**	**L**	**C**	**L**	**C**
γ_{207}	0.706	0.898	0.706	0.897	0.706	0.897
γ_{225}	0.956	0.975	0.906	0.921	0.958	0.976

The best linear fit of concentration verses either peak value, maximum value or area under the curve was found to be in the γ_{225} band ($R^2 > 0.95$). The cubic fit equations from the area and peak values (1 and 2 respectively) were then selected as the calibration functions to be used.

$$\text{BY AREA}$$
$$\left[Li_2CO_3\right] = 0.071z^3 + 0.051z^2 + 0.107z + 0.266 \quad (1)$$
$$\text{where}$$
$$z = \left\{\left(x - 0.00019\right)/\left(0.00012\right)\right\}$$

$$\text{BY PEAK}$$
$$\left[Li_2CO_3\right] = 0.069z^3 + 0.048z^2 + 0.11z + 0.266 \quad (2)$$
$$\text{where}$$
$$z = \left\{\left(x - 0.00002\right)/\left(0.000014\right)\right\}$$

and where x is the area under the curve of the second derivative or the peak value of the spectrum at each concentration of Li_2CO_3 in the γ_{225} band of the therapeutic concentration absorption curves.

The therapeutic concentration samples were re-run, and concentration calculation results are shown in table 3. The ability of the spectroscopic method in conjunction with the calibration

curve to determine the lithium carbonate concentration of solutions decreases with decreasing concentration. However, it can sufficiently identify the range of concentration.

Table 3: Li_2CO_3 concentration calculation from derived calibration functions using the γ_{225} band.

Specified [Li_2CO_3] (mM)	Area under 2nd Derivative	Calculated [Li_2CO_3] (mM) (eqn 1)	% Difference	Peak value of 2nd Derivative	Calculated [Li_2CO_3] (mM) (eqn 2)	% Difference
0.1	0.00004	0.08	23	0.000005	0.11	11
0.2	0.00015	0.23	15	0.000016	0.24	18
0.3	0.00018	0.25	15	0.000019	0.26	14
0.4	0.00029	0.44	11	0.000032	0.49	9
0.5	0.0003	0.47	6	0.000033	0.46	8
0.6	0.00033	0.59	1	0.000036	0.57	5

4. Discussion and Conclusion

It has been determined that the addition of Li_2CO_3 to pure water, in concentrations below the saturation limit at 25°C, does have a measureable contribution in the deep-UV absorption spectra. By observing the second derivative spectra of the Fingerprinting Concentrations, two distinct bands were identified: band γ_{207} (205-209nm) and band γ_{225} (220 - 230 nm). These bands were also identifiable in solutions whose concentrations reflect those typically found in blood.

Using measurements of the peak, maximum height and area under each curve from the second derivatives of the bands of interest, linear and cubic fits were applied. From this it was determined that in the therapeutic range the γ_{225}-band more accurately represents Li_2CO_3 concentrations, as the two calibration functions constructed had a very high correlation coefficient ($R^2 > 0.97$). Using these calibration functions to calculate the concentrations on a re-run of therapeutic concentrations it was revealed that the higher concentrations could be calculated more reliably, with higher accuracy. It has to be taken into account that a [Li_2CO_3] of 0.5 mM is equal to a therapeutic dose of 1.0 mM, due to the double-lithium ion in the Li_2CO_3 molecule.

Whilst lithium carbonate in saturation concentrations above about 160 mM at 25 degrees C (approximately 1.2g per 100g of water) is known as a wavelength blocking agent when measuring stray light we are working at concentrations far below this (0.1 - 0.6 mM, anything above these minute levels is highly toxic), and the effect on the absorption curve we believe is quantifiable with the provided calibration function and proven with a good level of accuracy in our re-run of the sample, especially at higher therapeutic concentrations where blood toxicity starts to become an issue.

It is therefore reasonable to conclude that with the method of measuring Li_2CO_3 reported here it can be determined to within a 1 – 6% accuracy whether an unknown solution of Li_2CO_3 is either inside or outside the high-end of the therapeutic limit.

Further investigations are planned to make an attempt at measuring other common medicinal lithium compounds in solution to help identify any characteristic in the absorption spectra that is solely unique to the lithium ions. Different mixtures with other common salts that appear in blood and other bodily fluids, such as saliva or urine, will also be conducted to investigate the effect that these have on the observed absorption curves.

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