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Scientific paper

Spectrophotometric Measurement of Lithium in Human Saliva Using the Chromogenic Reagent Thorin

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Abstract

The feasibility of using the chromogenic dye Thorin to spectrophotometrically measure lithium concentration in human saliva was explored. Absorbance wavelength maximum of the Li-Thorin complex was found to be 480 nm. Absorbance at 480 nm was obtained for saliva calibration standards containing 0.00–5.29 mEq/L of lithium. A least-squares fit produced a regression equation y = 0.128x + 1.449, R = 0.997. This was used to predict lithium concentrations in both artificially prepared lithium/saliva test solutions and in hospitalized patients treated with lithium. Results agree well with atomic absorption spectroscopy. Using a reagent blank with an equivalent amount of saliva as the test samples eliminated protein and electrolyte absorbance interference. This study supports the continued exploration of this method as a non-invasive point-of-care testing approach for monitoring saliva lithium in bipolar disorder.

Keywords: Lithium, saliva, Thorin, spectrophotometric, bipolar, colorimetric

1. Introduction

There has been a long and increasing interest over decades in measuring lithium in human biological fluids.[1–7] This is due mainly to lithium's first line use as an important psychopharmacological medicine for the acute and maintenance phase of the psychiatric illness bipolar disorder.[8] Lithium is administered orally as a lithium salt, such as lithium carbonate/sulfate/citrate/chloride. It has been shown to effectively reduce the frequency and intensity of both the mania/hypomania and depressive cycles in bipolar illness, aid in relapse prevention, as well as decrease the incidence of suicide.[9] However, lithium has the potential to be highly toxic and even deadly when its blood level exceeds the safe therapeutic range.[10–13] Therefore, lithium must be monitored frequently which requires repeated venipunctures over long periods of time.

The qualitative and quantitative determination of lithium has presented one of the more difficult problems in analytical chemistry, due mainly to the similarities of lithium to other alkali metals and alkaline earths. Over the years, early analytical techniques for measuring lithium have included gravimetric,[14] fluorometric,[15] colorimetric,[16–18] and separation techniques.[19]

Currently, the most relied-upon methods related to clinical care measure the lithium level in blood serum, and the most commonly accepted gold-standard analytical techniques are atomic absorption spectroscopy,[20] flame emission photometry,[21] and occasionally conventional ion- selective electrodes.[10]

In spite of their accuracy, these techniques present some significant limitations. These include patient scheduling and travel inconvenience, non-compliance with monitoring due to discomfort from venipuncture, time for the blood sample to be sent off to a lab or hospital, expensive equipment that requires professional training, time delays for complex sample preparation and equipment readiness, and substantial overall costs. Current methods also prevent patient self-monitoring by centralizing rather than decentralizing lithium testing.

Point-of-care testing that avoids venipuncture by using a different body fluid than blood such as saliva, urine or sweat, combined with using low-cost and user-friendly techniques with adequate accuracy would allow physicians to test in the office in a timely, non-invasive and inexpensive manner, and allow patients to self-test at home on an as-needed basis.[22]

In spite of much progress exploring many different approaches,[23–29] no point-of-care, minimally invasive, easy-to-use, cost-effective, and acceptably accurate measurement methods combined with a suitable bodily fluid testing medium, has yet been achieved.

Research has indicated the usefulness of saliva as a biological fluid medium for measuring the lithium concentration because it is found to be about 2 times the level in blood, often remains relatively constant over time in an individual, and decreases the need for frequent venipunctures.[6,30–34]

The chromogenic dye Thorin 3-hydroxy-4-[(2-arsonophenyl)diazenyl]naphthalene-2,7-disulfonate disodium salt functions as an optical ligand that combines with lithium to form a Li-Thorin complex.[22] This causes a chelation-induced shift in the absorbance spectra.[22] This organic compound is relatively selective for lithium and forms orange-colored Li-Thorin complexes in a strongly alkaline medium.[35] Thorin was first used with visual comparisons for direct lithium detection in non-biological systems in 1948.[35] This work was refined further in 1951, however using visual comparisons of colors was observed to be limited.[36]

This was followed in 1956 by the development of a spectrophotometric method using Thoron to measure lithium in a non-biological medium.[37] An acetone-water-potassium hydroxide reagent mixture was found to be sensitive and allowed reproducible testing results. The absorbance of the Li-Thoron complex using this reagent mixture was measured at 486 nm in lithium chloride test solutions. This test method was found to have an accuracy of ± 3%. Little interference was encountered by calcium and magnesium in amounts less than 10 times the lithium concentration, or by sodium in amounts 50 times the lithium concentration. Sodium in amounts 100 times that of lithium produced a positive error of 5%.[37]

In 1983 a similar spectrophotometric method using Thoron was used to measure lithium in blood serum and found it necessary to reduce absorbance interference by both removing proteins and adding a synthetic serum electrolyte to the reagent blank.[29] A thorough literature search does not reveal any prior or subsequent use of this spectrophotometric method using Thoron to measure lithium in human saliva. The present research study, conducted twenty years after this method was originally described,[37] explored the feasibility of applying this spectrophotometric method using the Li-Thorin complex to measure lithium in the biological medium, human saliva. A modification of prior methods was employed to avoid the interfering effects of protein and electrolytes in the absorption spectra.

2. Methods

2. 1. Saliva Collection*

Three methods of saliva collection are designated as Method A, Method B and Method C.

Method A: Saliva was collected from subjects not taking lithium for subsequent preparation of calibration standards. Saliva was collected at various times of the day after the subjects were without eating or smoking for two hours. The subjects then stimulated saliva production by chewing on a latex rubber band for fifteen minutes, discarding saliva produced during the first five minutes, and collecting the remainder in a polyethylene bottle. The saliva was frozen until further studies were conducted, including measurement of pH and volume produced. Fifteen mL each, from five males and five females ranging in age from 30–40, were pooled to produce 150 mL of pooled human saliva to be used for calibration standards described below.

Method B: A less time-consuming method for saliva collection, used for controls and for patients treated with lithium, involved a brief rinse of the mouth with water, waiting 15 minutes during which the subject remained without eating or smoking, and then chewing on a latex rubber-band for five minutes. The saliva was collected in two small paper cups, one used for the first two minutes and the second for the remaining three minutes of collection. The saliva specimens were transferred to capped plastic tubes and frozen until needed. Only saliva collected during the latter three minutes were used for the study.

Method C: The saliva of one hospitalized patient was collected periodically during two weeks, with the saliva being collected immediately upon awakening in the morning before placing anything in the mouth, including water or smoking. The patient chewed on a latex rubber band for five minutes, depositing the first two minutes of saliva production in one paper cup and the following three minutes in a second cup which was used for testing. The samples were transferred to capped plastic tubes and frozen until further study.

2. 2. Lithium/Saliva Calibration Sample Preparation

Lithium Chloride (LiCl), reagent grade, was added to deionized water to produce lithium chloride solutions of approximate concentrations 0.0, 5.0, 10.0, 20.0, 25.0, 30.0, 35.0, 40.0 and 50.0 mEq/L. One mL aliquots of each of these solutions were added to 9 mL of the pooled human saliva collected by Method A above, to produce saliva solutions with lithium concentrations of approximately 0.0, 0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 mEq/L. In this way, the original electrolyte concentration of the pooled human saliva was reduced by approximately 10%, thereby remaining within the normal range, as later analysis revealed, Table 2. The lithium concentrations in each of these calibration standards was measured by atomic absorption spectroscopy described below.

2. 3. Spectrophotometric Testing of Lithium/Saliva Calibration and Test Samples

Reagents (Fisher Scientific) include potassium hydroxide (KOH), reagent grade; acetone, reagent grade;

* Saliva collection was performed in compliance with Department of Psychiatry, Case Western Reserve University

deionized water; and Thorin dye: C16H11AsN2Na-2O10S2, Lot No. 325002, J.T. Baker Chemical Company.**

Thorin, 3-hydroxy-4-[(2-arsonophenyl)diazenyl] naphthalene-2,7-disulfonate disodium salt, belongs to the class of compounds called naphthalenesulfonates which is used as a chromogen dye in the present study. The Thorin molecule, which is an azo compound, consists of a naphthylazo group (naphthalene rings with an azo linkage) and an arsenic acid group. The naphthylazo group contains a hydroxyl group (OH) and two sulfonic acid groups (HSO_3) attached to it. These groups can form hydrogen bonds and participate in various chemical reactions.

When a lithium cation (Li^+) interacts with the Thorin compound, it forms a coordination bond with one of the available oxygen atoms from the hydroxy groups or the arsenic atom to create a Li-Thorin complex. The exact coordination mode will depend on the reaction conditions and the molecular geometry of the complex. For example, a simple Li-Thorin complex could be formed by coordination of a single Thorin molecule with a lithium ion, resulting in a 1:1 complex.

The absorption spectra of Thorin undergoes a shift in frequency and intensity upon formation of the Li-Thorin complex. The absorbance is directly proportional to the concentration of the complex, which in turn reflects the concentration of lithium ions in the test medium.

The overall structure of the complex is determined by the coordination of the lithium ion to the Thorin molecule, which influences the geometry and shape of the complex. The specific arrangement of atoms and bond angles may vary depending on the experimental conditions and the nature of the complex.

The present method applied the chemical reaction protocol of prior studies using the chromogen Thorin to form a Li-Thorin complex to spectrophotometrically determine the lithium concentration in human saliva. A reagent mixture similar to that used in previous findings for lithium chloride solutions,[37] was used in the present study for both calibration standards and patient testing. Lithium/saliva calibration sample (0.1 mL) was pipetted into a 10 mL glass stoppered volumetric flask, to which was added 0.1 mL of 20% reagent grade potassium hydroxide (KOH) solution, 1.2 mL of deionized water, 3.5 mL of reagent grade acetone, and 0.3 mL of 0.2% Thorin solution. Potassium hydroxide (KOH) was used to create the necessary alkaline solution and acetone to increase the sensitivity of the result, as established in prior studies.[29–37]

Lithium/saliva calibration standards prepared in the above manner were measured against a reference blank prepared in an identical manner, including saliva, except replacing the 0.3 mL of 0.2% Thorin solution with 0.3 mL deionized water. This was done to investigate the possibility of using a patient's own saliva for both the test sample and reagent blank, thereby cancelling out interfering effects of protein and electrolyte unique to the patient. This is discussed further in Results and Discussion.

The 10 mL flasks were kept stoppered after the addition of acetone to minimize evaporation. After addition of the Thorin dye, the flasks were carefully inverted a few times for mixing and then allowed to stand for 30 minutes. The solutions were then transferred by pipette from the stoppered volumetric flask to quartz cuvettes with a 1 cm path length and equipped with covers to minimize evaporation.

The maximum absorbance wave length of the Li-Thorin complex was first determined to be 480 nm using two saliva test samples, one with 2.48 mEq/L lithium and the other with 0.00 mEq/L lithium, as measured against a water reference blank and by measuring the difference spectrum between them (see Results and Discussion, Figure 1). All subsequent absorbance measurements of calibration standards and patient test samples were made at 480 nm.

Absorbance spectra were measured utilizing a Gilford Model 2400 automatic recording spectrophotometer set on manual mode. Wavelength scale was calibrated with Holmium oxide glass filters to an accuracy of better than ±2 nm over the range of 400 to 600 nm. Absorbance was calibrated utilizing neutral density filters at 550 nm. A spectral band width of 20 nm per nm slit width at 480 nm was used, with slit widths ranging from 0.025–0.03 nm.

The absorbance intensity of the Li-Thorin complex in the calibration samples containing differing concentrations of lithium were plotted against the actual concentration of lithium in the calibration samples as determined by atomic absorption. A least-squares fit to this data resulted in a regression equation which then allowed the prediction of the concentration of lithium in subsequent studies of patient saliva test samples.

2. 4. Atomic Absorption Testing

A Perkin-Elmer Model 107 Atomic Absorption Spectrophotometer was utilized, at a frequency of 6708 Å and a slit width of 7 Å. An acetylene-air gas mixture producing an oxidizing flame was used. Harleco $LiNO_3$ standards were used for calibration, diluting the original 1000 ppm to a series of solutions between 0.4 to 2.0 ppm. Lithium concentration was recorded in mEq/L with an accuracy of ± 0.05 mEq/L. All samples were diluted ten-fold in deionized water.

3. Results and Discussion

The first goal was to determine at what wavelength does the absorbance maximum for the Li-Thorin complex occur in human saliva. The absorption spectra for the Li-Thorin complex in human saliva are shown in Fig. 1, for A)

** Warning: Thorin is rated as dangerous, acutely toxic if swallowed or inhaled, and a health and environmental (aquatic) hazard. Take all necessary precautions when using! See Supplementary Information.

2.48 mEq/L, and B) 0.00 mEq/L, over the frequency range 400 to 540 nm. The absorbance maximum in B, in the absence of lithium, occurs at 440 nm. There is an increase in absorption and a bathochromic shift to 450 nm in the presence of lithium, Spectrum A. The difference spectrum C, obtained by measuring a saliva test sample containing 2.48 mEq/L lithium against a reference blank containing 0.00 mEq/L lithium in an identical reagent medium shows a broad absorbance maximum peaking at approximately 480 nm. These results in saliva are similar to those obtained previously for pure lithium chloride/water solutions.[37] Therefore, all subsequent absorbance measurements for determination of lithium concentration in saliva were made at 480 nm.

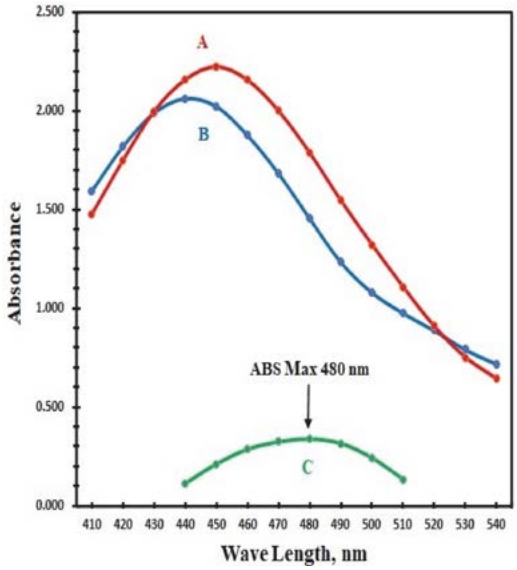

Figure 1: Absorption spectra of Li-Thorin complex in potassium hydroxide-acetone-water medium using human saliva* containing 2.48 mEq/L lithium (Spectrum A) and 0 mEq/L lithium (Spectrum B) and measured against a water reference blank. Spectrum C is the difference spectrum between Spectrum A and B.

* Saliva collected from an adult male by Method A with LiCl solution, 10% by volume, added to produce 2.48 mEq/L of lithium as determined by atomic absorption spectroscopy.

Absorbance of a pooled saliva calibration standard containing 2.42 mEq/L lithium was measured at 480 nm vs time with reference to a saliva calibration reference blank containing 0.00 mEq/L lithium. This saliva lithium concentration is equivalent to the usual maximum desired therapeutic blood level of about 1.2 mEq/L, assuming a saliva/blood ratio often found to be about 2:1.[6,30–34] Figure 2 shows that absorbance is 98% complete at 30 minutes post-reaction start. Therefore, the Li-Thorin reaction is essentially complete, within experimental error, in approximately 30 minutes agreeing with previous findings in LiCl solutions.[37] Therefore, all subsequent measurements were made at 30 minutes post reaction.

Saliva secretion rate and pH of saliva collected for the production of pooled human saliva is shown in Table 1.

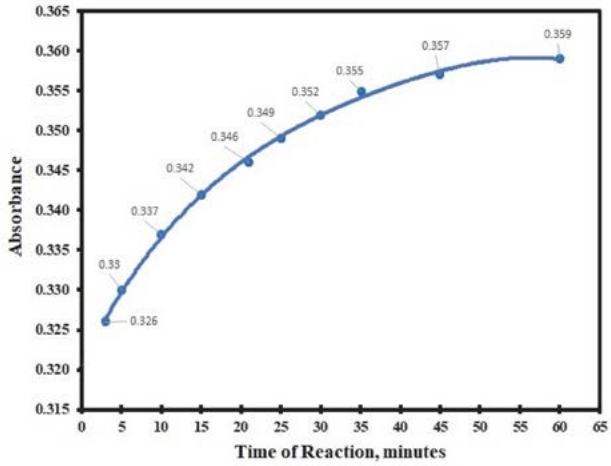

Figure 2: Absorbance vs time at 480 nm for lithium/Thorin complex using pooled saliva with 2.42 mEq/L of lithium.*

*Absorbance measured vs reference blank containing same amounts of constituents, except substituting 0.1 mL pooled human saliva containing 0 mEq/L lithium for 0.1 mL pooled saliva containing 2.42 mEq/L lithium.

Table 1: Saliva Secretion Rate and pH From Non-Patient Adults

Male			Female		
Age (years)	Secretion Rate mL/minute	pH*	Age (years)	Secretion Rate mL/minute	pH*
33	2.3	8	32	1.5	7–8
30	1.5	7–8	30	1.9	7–8
38	1.9	7–8	36	1.7	7–8
39	1.7	7	38	1.5	6–7
31	1.5	6–7	31	1.5	7

* pH measured with indicator papers

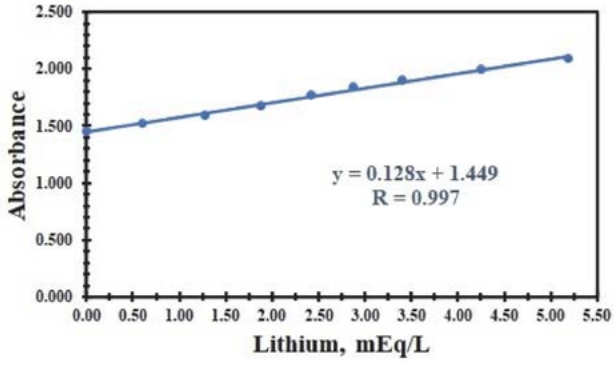

Figure 3: Absorbance at 480 nm for pooled human saliva* calibration standards containing different lithium concentrations in test solution 0.1 mL saliva, 0.1 mL 20% reagent grade KOH, 3.5 mL reagent grade acetone, 1.2 mL deionized H_2O, 0.3 mL 0.2% Thorin solution. Absorbance, 30 minutes post reaction start, measured against a reference reagent blank with same contents as the test solution except replacing 0.3 mL 2% Thorin with 0.3 mL H_2O. The Graph shows the least-squares regression equation and line calculated from absorbance means, along with slope, Y-intercept and correlation coefficient, R.

* Pooled saliva from 10 adults collected by Method A.

Table 2: Absorbance of Pooled Saliva Calibration Standards*

Li** mEg/L	Absorbance vs Reagent Blank***	Absorbance Mean	Absorbance Range	Std Deviation	Std Error of Mean
0.0	1.447				
	1.464				
	1.442				
	1.461				
	1.431	1.449	0.033	0.012	±0.005
0.61	1.524				
	1.538				
	1.514	1.525	0.024	0.010	±0.006
1.27	1.575				
	1.595				
	1.587	1.589	0.020	0.008	±0.005
1.87	1.682				
	1.688				
	1.664	1.678	0.024	0.010	±0.006
2.42	1.773				
	1.782				
	1.780	1.778	0.009	0.004	±0.002
2.88	1.865				
	1.830				
	1.844	1.846	0.035	0.014	±0.008
3.40	1.907				
	1.903				
	1.900	1.903	0.007	0.003	±0.002
4.25	1.979				
	2.008				
	2.001	1.996	0.029	0.012	±0.007
5.19	2.095				
	2.096				
	2.082	2.091	0.014	0.006	±0.004
		Average Values	0.022	0.010	±0.005

* Pooled saliva standards all contain: 16.4±1.1 mEq/L Na^+, 17.5±0.6 mEq/L K^+, 5.2±.04 mg% Ca^{++}, and 0.6±0.5 mg% Mg^{++}. Na and K determined on Corning Flame Photometer, Model 450 using propane gas and automatic dilution. Ca and Mg determined on DuPont Automatic Clinical analyzer.

** Atomic Absorption

*** Reagent blank identical to test solution except 0.3 mL of 0.2% Thorin replaced by 0.3 mL deionized H_2O

Chemical analysis of the calibration standards produced from this pooled saliva is listed at the bottom of Table 2. Three separate absorbance measurements were taken for each lithium concentration calibration standard, except for 0.00 mEq/L which had five measurements, Table 2. The average range of absorbance measurement was 0.022 with an average standard deviation of 0.010 and average standard deviation of the mean of 0.005.

The absorbance mean for each of the lithium concentrations was used in a least squares regression line calculation resulting in the calibration line plotted in Fig. 3, with slope 0.128, y-intercept 1.449 and correlation coefficient 0.997.

Measuring lithium in biological media such as saliva poses the additional problem of interference by saliva proteins and electrolytes. The protein interference can be eliminated by centrifuging the saliva test sample or precipitating out the protein. The electrolyte interference may be avoided by adding synthetic electrolytes with an average concentration of elements to the reagent blank.[29] The latter approach only approximates the electrolytes in the patient's test saliva. Furthermore, centrifuging the saliva or precipitating out the protein would add several additional steps to the measurement which would not be feasible in a point-of-care test.

This study investigated a different approach to this problem. Patient saliva was used in both the test sample and reagent blank. Therefore, the interference of both saliva proteins and electrolytes was avoided by measuring absorbance of each calibration or patient test sample with reference to a

Table 3: Predicted Li Concentration for Artificially Prepared Li-Saliva Solutions*

Li** mEq/L	Absorbance Li/Saliva vs reagent blank	Predicted Li *** mEq/L	Prediction Error mEq/L	Prediction Error %
0.00	1.445	–0.03	–0.30	–
2.05	1.742	2.29	0.24	11.7
2.48	1.777	2.56	0.08	3.2
2.73	1.823	2.92	0.19	7.0
3.58	1.916	3.65	0.07	2.0
4.26	1.984	4.18	–0.08	1.9
5.12	2.079	4.92	–0.20	3.9
		Mean	0.04	
		Standard Deviation	0.14	
		Standard Error of Mean	0.05	

* Saliva collected from one subject my Method A (see Methods)
** Atomic Absorption
*** Calculated from calibration regression equation y= 0.128x + 1.449, Figure 3
Absorbance reagent: 0.1 mL Li/saliva + 0.1 mL 20% KOH + 3.5 mL acetone + 1.2 mL H_2O + 0.3 mL 0.2% Thorin Reagent Blank: same as above except replace 0.3 mL 0.2% Thorin with0.3 mL H_2O

reagent blank which had the same saliva contents as the test sample. Both test sample and reference blank had an equivalent amount of saliva protein and interfering ion effects which were therefore nullified. The use of 0.1 mL of saliva allowed the lithium concentration to fall within the range satisfying Beer's law as found in previous studies.[37]

To be able to detect the lithium-Thorin complex in the calibration or patient test saliva, the 0.3 mL of 0.2% Thorin in the reagent blank was replaced by 0.3 mL water. In the absence of Thorin in the reagent blank, the lithium in the reagent blank saliva is not detected and produces no absorbance. This allows the lithium-Thorin complex in the calibration or patient test saliva to be measured. However, the absorbance of Thorin dye itself in the calibration or patient test sample will now be present, as it is not cancelled-out due to the absence of Thorin in the reagent blank. This means that the absorbance intensity of the calibration or patient saliva will be due to both the lithium-Thorin complex and due to the Thorin dye.

An absorbance value of 1.449 was found for the calibration standard containing 0.0 mEq/L lithium, Figure 3. This absorbance is due to the Thorin dye itself. Therefore, the regression line intercept at 1.449 on the y-axis represents the baseline for other absorbance measurements of calibration or patient test samples containing the lithium-Thorin complex, which will have absorbance value greater than 1.449 based on their lithium concentration.

The first attempt to utilize this calibration curve was made on a series of saliva solutions collected from one subject by saliva collection Method A, to which various

Table 4: Effect of pH on Predicted Lithium Concentration in Saliva*

pH	Absorbance vs Reagent Blank**	Predicted Li*** mEq/L	Prediction Error mEq/L	Prediction Error %
8.5	1.774	2.54	0.06	2.4
7.0	1.788	2.65	0.17	6.9
6.2	1.774	2.54	0.06	2.4
5.5	1.776	2.55	0.07	2.8
		Mean	0.09	
		Standard Deviation	0.05	
		Standard Error of Mean	0.02	

* Saliva from one subject collected by Method A with 2.48 mEq/L of lithium. Saliva Li Concentration maintained at 2.48 mEq/L for all pH values by using concentrated HCL to alter pH.
** Absorbance reagent: 0.1 mL Li/Saliva+0.1 mL 20% KOH+3.5 mL acetone+1.2 mL water+0.3 mL 0.2% Thorin. Reagent blank: same as above except replace 0.3 mL 0.2% Thorin with 0.3 mL water.
*** Calculated from calibration regression equation y = 0.128x + 1.449, Figure 3.

concentrations of lithium chloride solutions were added, Table 3. Lithium concentrations vary from 0.0 mEq/L to well into the toxic blood level range found clinically, assuming a saliva/blood lithium ratio of 2:1.[6,30–34] The mean error was found to be 0.04 mEq/L with standard deviation 0.14 mEq/L, and standard error of mean 0.05 mEq/L. Percent error in Li prediction vs atomic absorption measurements averaged less than 5%.

Using saliva from one subject collected by Method A with 2.48 mEq/L of lithium, the effect of saliva pH on accuracy of this technique is shown in Table 4. The pH was varied from 8.5 to 5.5 while maintaining a constant lithium concentration. The results demonstrate no substantial error introduced over the entire pH range found in human mixed saliva.[20] Average % error for predicted lithium was 3.6%.

Saliva was collected by Method B from nine hospitalized patients, ages 18 to 49, being treated with Li_2CO_3. The patients' duration of lithium therapy varied from one to two days up to five years. No restrictions were placed on time of saliva collection relative to the last lithium dose, time of day, or last meal, diet, or other medications. Predicted lithium levels based on this colorimetric technique and calculated errors from atomic absorption results are shown in Table 5.

Average error was 0.13 mEq/L with a standard deviation of 0.15 mEq/L and a standard error of the mean of 0.05 mEq/L. The overall average error was 6%. The predicted lithium error in mEq/L will result in a higher % error at lower actual concentrations of saliva lithium. The usual target therapeutic lithium level in blood is 0.4–1.2 mEq/L lithium, which roughly corresponds to 0.8–2.4 mEq/L saliva lithium assuming a saliva/blood ratio of about 2:1.[6,30–34] Within this range the average error in the present results is found to be 4%.

Finally, this method was used to monitor the lithium levels of a hospitalized patient (52 year old male) for 13 days starting on day 4 of the initial medication titration phase of treatment with Li_2CO_3. Treatment was initiated on Day 0 with Li_2CO_3 300 mg 1x/day. Saliva lithium monitoring began on Day 4. The Li_2CO_3 dose was increased to 300 mg 2x/day on Day 5, 300 mg 3x/day on Day 7 and 600

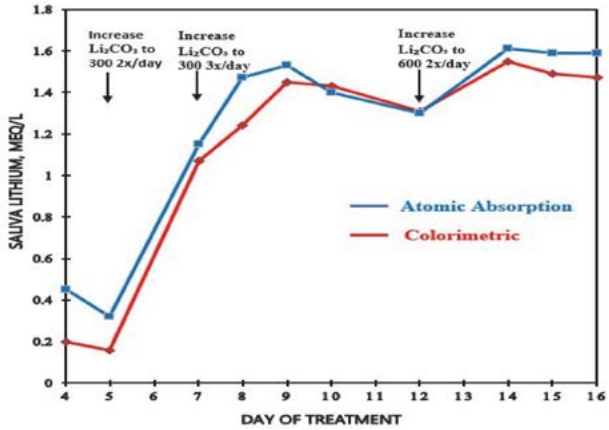

Figure 4: Comparison of Colorimetric vs Atomic Absorption Monitoring of Saliva Lithium in a Hospitalized Patient during Initial Treatment Titration with Lithium Carbonate (Li_2CO_3).*

* Male patient, age 52, admitted with diagnosis of hypomanic episode and therapy initiated with Li_2CO_3 on Day 0. Saliva levels were monitored starting on 4th day of Li therapy. Saliva collected by Method C, without any restrictions on diet or medications.

Table 5: Predicted Saliva Lithium Concentration[a] in Hospitalized Patients on Lithium Therapy[b]

Gender (M/F)	Age (yrs)	Absorbance[c] vs Reagent Blank	Predicted Li[d] (mEq/L)	Actual Li[e] (mEq/L)	Prediction Error (mEq/L)	Prediction Error (%)
M	18	1.836	3.02	3.28	–0.26	7.9
M	49	1.666	1.70	1.79	–0.09	5.0
M	30	1.594	1.13	1.18	–0.05	4.2
M	22	1.792	2.68	2.56	0.12	4.7
M	37	1.703	1.98	2.05	–0.07	3.4
M	31	1.699	1.95	1.90	0.05	2.6
F	35	1.759	2.42	2.74	–0.32	11.7
F	43	1.800	2.74	2.98	–0.24	8.1
F	33	1.811	2.83	3.10	–0.27	8.7
			Mean		0.13	
			Standard Deviation		0.15	
			Standard Error of Mean		0.05	

[a] Saliva collected by Method B
[b] Patients hospitalized at Hanna Pavilion, University Hospitals, CWRU, Cleveland Ohio and St. Lukes Hospital, Psychiatric Division, Cleveland, Ohio
[c] Absorbance test sample: 0.1 mL Saliva/Li + 0.1 mL 20% KOH + 3.5 mL acetone + 1.2 mL water +0.3 mL 0.2% Thorin. Reagent Blank: same as for Absorbance test sample except replace 0.3 mL 0.2% Thorin with 0.3 mL ionized water. Absorbance measured at 480 nm.
[d] Calculated from calibration regression equation y = 0.128x + 1.449, Figure 3.
[e] Atomic Absorption

mg 2x/day on Day 12, after which the lithium level was allowed to stabilize during Days 14–16.

A comparison of lithium concentrations predicted by this colorimetric technique with atomic absorption results is shown graphically in Fig. 4. The mean error was –0.10 mEq/L with standard deviation of 0.09 mEq/L and standard error of the mean of 0.03 mEq/L. The colorimetric results clearly show the expected increases and decreases in saliva lithium levels in response to Li_2CO_3 dosage adjustments. The overall results indicate the ability of this method to monitor saliva levels over time with reasonable accuracy.

This study suggests that several issues need further investigation to improve results. The time it takes for the Li-Thorin reaction to be completed, approximately 30 minutes, does significantly increase the time needed for test results of this method to be obtained and used. This reduces the effectiveness of this method in emergent situations, such as in an emergency room or hospital, where rapid determination of lithium toxicity is critical. Therefore, further studies to explore ways of speeding up the Li-Thorin reaction time would be helpful, such as studying the effect of temperature on the reaction time to completion

Another issue is the volatility of acetone used in the reagent mixture, which might alter test results and present difficulties in handling. The use of closed and sealed cuvettes may help. This needs further investigation for a safe and practical procedure to be developed.

The chromogen dye Thorin contains the element arsenic, and therefore is considered acutely toxic, a health and environmental risk, and a potential hazard. For this method to become practical, safe handling of Thorin containing reagents and safe ways of disposing those substances would have to be developed.

Studies of changes in saliva lithium concentration that occur with variations in how saliva production is stimulated, and with saliva pH and secretion rates, would need further investigation. How these factors might affect the saliva/blood lithium ratio would also need clarification.

Finally, the accuracy and reliability of the results found in the present study would need further improvement for clinical applications. Efforts to further refine and control sample handling and testing techniques would be helpful. Also, much larger studies in multiple venues and by different investigators would be needed to establish the general validity, reliability, and confidence in this testing method, along with developing a practical and convenient method of implementation for point-of-care use.

4. Conclusion

This study finds that a spectrophotometric method employing the chromogenic dye Thorin, in a potassium hydroxide-acetone-water reagent mixture, can be used to measure the concentration of lithium in human saliva. A regression equation for absorbance vs lithium concentration in calibration standards produced reasonably accurate predictions of lithium concentration compared to atomic absorption in both artificially prepared saliva test samples and in saliva from patients treated with lithium. Both saliva protein and electrolyte interfering effects were avoided by using an equivalent amount saliva in both the tested samples and the reagent blank. This non-invasive method of measuring lithium using saliva, instead of blood, may reduce the frequency of needed venipunctures during lithium treatment and be adaptable for point-of-care monitoring. Further refinements in technique to improve accuracy and larger scale studies to reproduce, validate and extend the present findings may now be explored, including adaptability and applicability to different clinical settings including patient self-monitoring.

Data Availability

All data are made available in the main manuscript along with additional background data supporting the reported findings uploaded as Supplementary Materials.

Author Contributions

SRL: conceived the project idea, background literature search, designed and conducted the experiments, saliva collection, calibration and test sample preparation, infrared spectroscopy, data analysis, and writing (original draft, editing and final manuscript).

Conflicts of Interest

There are no conflicts to declare.

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Povzetek

Raziskana je bila izvedljivost uporabe kromogenega barvila Thorin za spektrofotometrično merjenje koncentracije litija v človeški slini. Ugotovili smo, da se absorpcijski maksimum kompleksa Li-Thorin nahaja pri 480 nm. Absorbanco pri 480 nm smo pomerili za umeritvene standarde sline, ki so vsebovali 0,00–5,29 mEq/L litija. Prileganje po metodi najmanjših kvadratov je dalo regresijsko enačbo y = 0,128x + 1,449, R = 0,997. To smo uporabili za napovedovanje koncentracij litija tako v umetno pripravljenih testnih raztopinah litija/sline kot pri hospitaliziranih bolnikih, zdravljenih z litijem. Rezultati se dobro ujemajo z rezultati pridobljenimi z atomsko absorpcijsko spektroskopijo. Uporaba slepega reagenta z enako količino sline kot v testnih vzorcih je odpravila motnje absorbance beljakovin in elektrolitov. Ta študija podpira nadaljnje raziskovanje te metode kot neinvazivnega pristopa testiranja na točki oskrbe za spremljanje litija v slini pri osebah z bipolarno motnjo.