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

Stephen Robert Levitt, MD, PhD*
Pain Resource Center, Inc., Hillsborough, NC 27278

Corresponding Author
* stevelevitt@earthlink.net

Keywords *lithium, saliva, spectrophotometric, Thorin, bipolar, manic depressive, spectroscopy, colorimetric, chromogen, point-of-care,*

Abstract

This study explored the feasibility of using the chromogenic dye Thorin to spectrophotometrically measure the lithium concentration in human saliva. The absorbance wavelength maximum of the Li-Thorin complex was determined to be 480 nm. Lithium concentrations were measured spectrophotometrically at 480 nm in human pooled saliva with lithium added to produce calibration standards of 0.00-5.29 mEq/L of lithium, which corresponds to a blood lithium range of 0.00-2.60 mEq/L, assuming a saliva/blood ratio of 2/1. A least-squares fit of the absorbance vs lithium concentration calibration data produced a regression equation $y = 0.128x + 1.449$ with correlation coefficient = 0.997. This regression equation was then used to predict lithium concentrations from absorbance data in prepared lithium/saliva test solutions and in hospitalized patients being treated with lithium. The results generally agreed well with those determined by atomic absorption spectroscopy. By measuring absorbance of test saliva vs reagent blank containing the same amount of saliva, interfering effects of saliva protein and electrolytes in the test samples were avoided. This study supports the continued exploration of this method as a non-invasive point-of-care testing approach for monitoring saliva lithium during lithium treatment.

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 medication 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 reduce the incidence of suicide (9).

However, lithium has the potential to be highly toxic and even deadly when its level exceeds the safe therapeutic range (10-11). The potentially toxic level, often >1.2 mEq/L, is very close to the current therapeutic range 0.4-1.2 mEq/L. (5-12). Adverse effects of lithium treatment can occur within the therapeutic range and include tremor, weight gain, polyuria/polydipsia and diarrhea (13). At higher toxic levels hyperthyroidism, neurotoxic events and other conditions can occur, with fatality a possibility at higher toxic levels (11).

Therefore, treatment with lithium must be monitored carefully and frequently, and requires the patient to undergo venipuncture to obtain a blood sample for testing. Testing often begins with 12-hour monitoring at first and then every few days to weekly testing during the initial treatment stages with medication dose

increments (10, 12). This is followed by reduced frequency monitoring, often every one- to a few-months thereafter for the duration of treatment which may continue for many years.

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 spectrometry (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, lithium monitoring non-compliance due to venipuncture, time for the blood sample to be sent off to a lab or hospital that has the expensive equipment that requires professional training, time delays for complex sample preparation and equipment readiness, and substantial overall costs.

These currently accepted methods also pose a significant risk in emergent situations, such as in the ER, involving suspected lithium toxicity and overdose where time is of-the-essence to determine if a toxic level of lithium is present requiring immediate kidney dialysis. This current prevailing approach also prevents patients from self-monitoring and making timely decisions regarding early warnings regarding changes in their lithium level. In other words, these testing methods centralize, rather than decentralize, lithium monitoring and can represent a barrier to optimum patient care.

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 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, safe and acceptably accurate measurement method 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 of its relationship to the lithium concentration in blood (6, 30-34). The lithium level in saliva has repeatedly been found to be about 2x the level in blood and this ratio often tends to remain relatively constant over time in an individual (30). This means that one or only a few venipuncture blood samples taken at essentially the same time as saliva tests at the beginning of treatment, could establish the saliva/blood ratio and allow a significant reduction in frequency of ongoing venipunctures.

The chromogenic dye Thorin 0-(2-hydroxy-3, 6-disulfo-1-naphthylazo-benzene-arsonic acid) functions as an optical ligand that combines with lithium to form a Li-Thorin complex. 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, but using visual comparisons of colors was felt 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).

A similar spectrophotometric method using Thoron to measure lithium in a biological medium, blood serum, found it necessary to both remove proteins and add a synthetic serum electrolyte to the reagent blank to compensate for serum electrolytes (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 paper reports the results of a research study conducted twenty years after this method was originally described (37), to study the feasibility of applying this spectrophotometric method using the Li/Thorin complex to measure lithium in the biological medium, human saliva, and to explore a method for removing the interfering effects of protein and electrolytes in the absorption spectra.

Methods

Saliva Collection

Three methods of saliva collection were utilized and 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 NPO without 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 produced 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 NPO without 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 was used for study.

Method C: 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.

Lithium/Saliva Calibration Sample Preparation

Lithium Chloride, reagent grade, was added to deionized water to produce lithium chloride solutions of approximates concentrations 0, 5, 10, 20, 25, 30, 35, 40 and 50 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, .5, 1, 2, 2.5, 3, 3.5, 4 and 5 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.

Spectrophotometric Testing of Lithium/Saliva Calibration Samples

Reagents include potassium hydroxide, reagent grade; acetone, reagent grade; deionized water; and Thorin dye $C_{16}H_{11}AsN_2Na_2O_{10}S_2$, Lot No. 325002, J.T. Baker Chemical Company.

The reagent mixture used in previous findings for lithium chloride solutions (37), was modified to increase the accuracy in the present study on saliva. 0.1 ml of lithium/saliva calibration sample was pipetted into a 10 ml glass stoppered volumetric flask, to which was added 0.1 ml of 20% reagent grade potassium hydroxide solution, 1.2 ml of deionized water, 3.5 ml of reagent grade acetone, and 0.3 ml of 0.2% Thorin solution.

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 lithium-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 mEq/L lithium as measured against a water reference blank (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 $\pm$2 nm over the range 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 lithium/Thorin complex in the calibration samples containing differing concentrations of lithium was 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 prediction of the concentration of lithium in subsequent studies of patient saliva test samples.

Atomic Absorption Testing

A Perkin-Elmer Model 107 Atomic Absorption Spectrophotometer was utilized, at a frequency of 6708 A° and a slit width of 7 A°. An acetylene-air gas mixture producing an oxidizing flame was used. Harleco $LiN0_3$ 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.

Results and Discussion

The first goal was to determine at what wavelength does the absorbance maximum for the lithium/Thorin complex occur. The absorption spectra for the lithium/Thorin complex in human saliva are shown in Fig. 1, for A) 2.48 mEq/L, and B) 0 mEq/L, over the frequency range 400 to 540 nm.

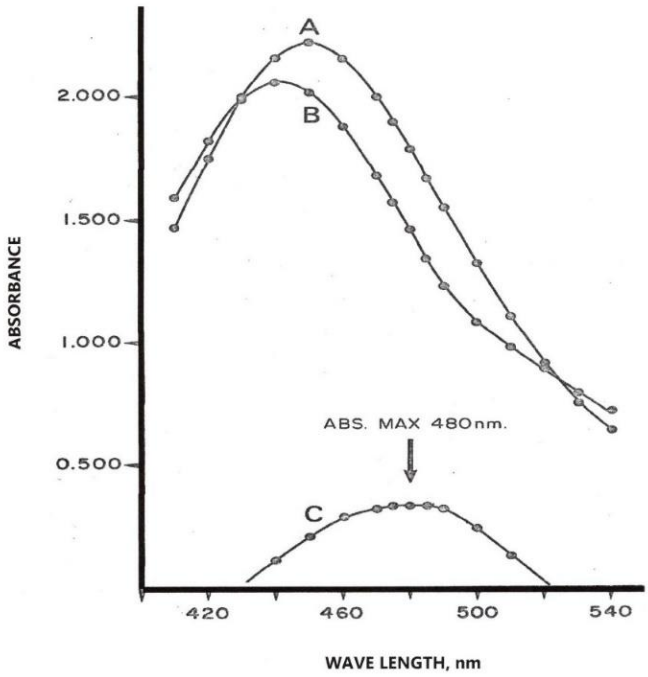

Figure 1: Absorption spectra of lithium/Thorin complex in potassium hydroxide-acetone-water medium using human saliva* containing 2.48 mEq/L lithium (Spectrum A) and 0 mEq/L lithium (Spectrum B) and measured against a water reference blank. Spectrum C is the difference spectrum between Spectrum A and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

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, measuring Spectrum A against a reference Spectrum B, 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 were made at 480 nm.

Although using 0.2 ml of 0.2% Thorin and 0.2 ml of 20% potassium hydroxide were previously used to produce the most sensitive results measuring lithium in lithium chloride solutions (37), the present study found 0.3 ml of 0.2% Thorin and 0.1 ml of 20% potassium hydroxide to produce the most accurate results in saliva.

A pooled saliva calibration standard containing 2.42 mEq/L was used to study the absorbance at 480 nm vs time, as shown in Fig. 2. As found previously (37), the reaction is essentially complete, within

experimental error, in approximately 30 minutes. Therefore, all subsequent measurements were made at 30 minutes post reaction start.

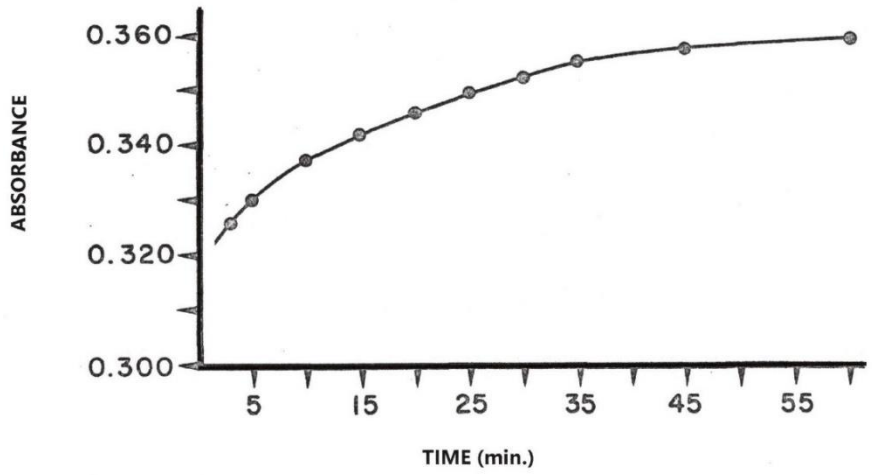

Figure 2: Absorbance vs time at 480 nm for lithium/Thorin complex using pooled saliva with 2.42 mEq/L of lithium.* *Absorbance measured vs reference blank containing same amounts of constituents, except substituting 0.1 ml pooled human saliva containing 0 mEq/L lithium for 0.1 ml pooled saliva containing 2.42 mEq/L lithium.

Saliva secretion rate and pH of saliva collected for the production of pooled human saliva is shown in Table 1. Chemical analysis of the calibration standards produced from this pooled saliva is listed at the bottom of Table 2.

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

Three separate absorbance measurements were taken for each lithium concentration standard, except for 0 mEq/L which had five measurements, Table 2.

Table 2

Absorbance of Pooled Saliva Calibration Standards*

Li** mEq/L	Absorbance vs Reagent Blank***	Absorbance Mean	Absorbance Range	Standard Deviation	Std. Error of the 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 $\pm$ 1.1 mEq/L Na^{+}, 17.5 $\pm$ 0.6 mEq/L K^{+}, 5.2 $\pm$ .04 mg% Ca^{++}, and 0.6 $\pm$ .05 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

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. A regression line was fitted to the absorbance

means for each lithium concentration based on a least squares analysis. The resulting calibration curve is plotted in Fig. 3, with slope 0.128, y-intercept 1.449 and correlation coefficient 0.997.

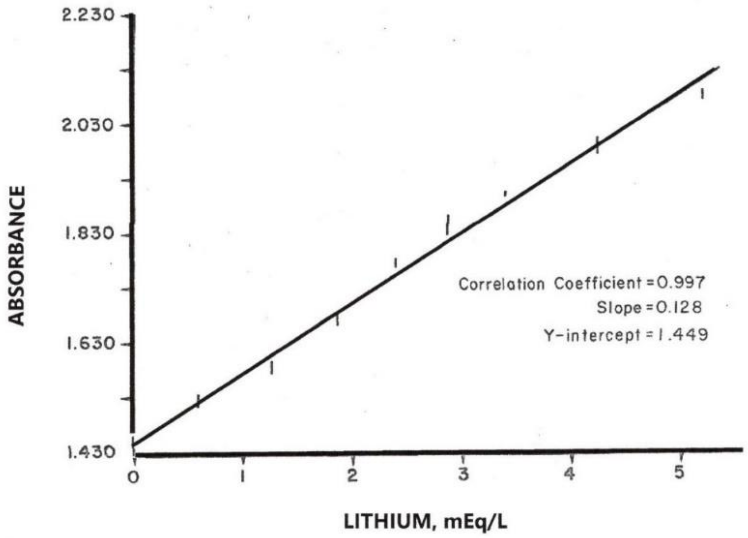

Figure 3: Absorbance at 480 nm for pooled human saliva* calibration standards containing different lithium concentrations in test solution 0.1 ml saliva, 0.1 ml 20% KOH, 3.5 ml acetone, 1.2 ml H_2O, 0.3 ml 0.2% Thorin solution. Absorbance, 30 minutes post reaction start, measured against a reference reagent blank with same contents as the test solution except replacing 0.3 ml 2% Thorin with 0.3 ml H_2O. Graph shows absorbance range bars and least-squares regression line slope and Y-intercept based on absorbance means.
*Saliva from 10 adults collected by Method A.

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 electrolyte 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 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 .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 values 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 concentrations of lithium chloride solutions were added, Table 3. 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%.

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

Li** mEq/L	Predicted Li Concentration*** mEq/L	Li Prediction Error mEq/L	Li Prediction Error %
0	-0.03	-0.03	-----
2.05	2.29	0.24	11.7
2.48	2.56	0.08	3.2
2.73	2.92	0.19	7.0
3.58	3.65	0.07	2.0
4.26	4.18	-0.08	1.9
5.12	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
***Based on Absorbance vs Reagent Blank without Thorin dye

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%.

Table 4
Effect of pH on Predicted Lithium Concentration

pH	Predicted Li Concentration* mEq/L	Error of Li Prediction mEq/L	Error of Li Prediction %
8.5	2.54	0.06	2.4
7.3	2.65	0.17	6.9
6.2	2.54	0.06	2.4
5.5	2.55	0.07	2.8
	Mean	0.09	
	Standard Deviation	0.05	
	Standard Error of Mean	0.02	

*Saliva Li Concentration maintained at 2.48 mEq/L for all pH values by using concentrated HCL to alter pH.

Saliva was collected by Method B from nine hospitalized patients, ages 18 to 49, being treated with $LiC0_3$. 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 standard deviation of 0.15 mEq/L and standard error of the mean of 0.05 mEq/L. The overall average error was 6%.

Table 5
Predicted Saliva Lithium Concentration in Hospitalized Patients on Lithium Therapy*

Gender (M/F)	Age (yrs)	Saliva Excretion Rate** (ml/minute)	pH	Predicted Li*** (mEq/L)	Actual Li**** (mEq/L)	Li Error (mEq/L)	Error (%)
M	18	1.0	5.4	3.02	3.28	-0.26	7.9
M	49	2.0	7.1	1.70	1.79	-0.09	5.0
M	30	2.8	6.9	1.13	1.18	-0.05	4.2
M	22	1.3	6.3	2.68	2.56	0.12	4.7
M	37	1.3	6.2	1.98	2.05	-0.07	3.4
M	31	1.3	5.0	1.95	1.90	0.05	2.6
F	35	1.7	7.6	2.42	2.74	-0.32	11.7
F	43	3.0	7.0	2.74	2.98	-0.24	8.1
F	33	0.3	6.2	2.83	3.10	-0.27	8.7
				Mean		0.13	
				Standard Deviation		0.15	
				Standard Error of Mean		0.05	

*Patients hospitalized at Hanna Pavilion, University Hospitals, CWRU, Cleveland Ohio and St. Lukes Hospital, Psychiatric Division, Cleveland, Ohio
**Saliva collected by Method B
***Based on Absorbance vs Reagent Blank without Thorin dye at 480 nm
****Atomic Absorption

Finally, this method was used to monitor the lithium levels of a hospitalized patient for 13 days during the initial treatment and medication titration phase with $LiCO_3$. 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. Aside from a few observed differences between predicted lithium concentrations and atomic absorption, the overall results indicate the ability of this method to monitor saliva levels over time with adequate accuracy. The saliva lithium levels clearly show the expected changes in response to $LiC0_3$ dosage adjustments.

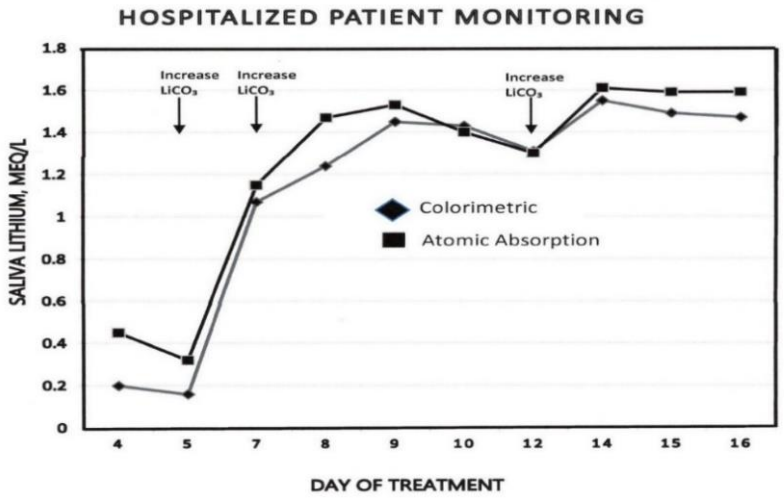

Figure 4: Predicted Saliva Lithium* For a Hospitalized Patient During Initial Treatment Titration**
*Based on Absorbance vs Reagent Blank without Thorin dye at 480 nm
**Male patient, age 52, admitted with diagnosis of hypomanic episode and therapy with $LiCO_3$ initiated. Saliva levels were monitored starting on 4th day of Li therapy. Saliva collected by Method C, without any restrictions of diet or medications.

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.

Another issue is the volatility of acetone used in the reagent mixture, which might alter test results and present difficulties in handling. 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 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 variability in results found in the present study would need further improvement for clinical applications. Some of this may be due to sample handling and testing technique that might be refined and better controlled. Also, much larger studies in multiple venues and by different investigators would be needed to establish the general validity and reliability of this testing method, along with issues of consistency and reproducibility of its results.

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 can now be explored.

Acknowledgments

Thank you to Douglas D. Bond, MD, former Chairman and Full Professor, Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio for approving the proposal for this research project. My sincere appreciation to Robert L. Martin, MD, Department of Pathology, for providing laboratory equipment, supplies and support, and John B. Sawyer, MD, David G. Logan, MD and Gottfried K. Spring, MD, Department of Psychiatry for facilitating patient participation in this research. Thank you to Ronald W. Chen, PhD, VA Medical Center, Cleveland, Ohio for atomic absorption measurements. My grateful appreciation to the patients and non-patient control subjects, whose cooperation made this study possible.

References

1. Lehman, V., Direct determination of lithium in serum by atomic absorption spectroscopy. Clin. Chim. Acta **1960,** 20, 523-525.
2. Little, B.R.; Platman, S.R.; Fieve, R.R., The measurement of lithium in biologic samples by atomic absorption spectrophotometry. Clin. Chem. **1968,** 14(12), 1211.
3. Robetson, R.; Fritze, K.; Grof, P., On the determination of lithium in blood and urine. Clin. Chim. Acta **1973,** 45(1), 25-31.
4. Grime, J.K.; Vickers, T.J., Determination of lithium in microliter samples of blood serum using flame atomic emission spectrometry with a tantalum filament vaporizer, Anal. Chem. **1975,** 47, 432-435.
5. Neu, C.; DiMascio, A.; Williams, D., Saliva levels: Clinical Applications. Am. J. Psychiatry **1975,** 132,66-68.
6. Ravenscroft, P.; Vozeh, S.; Weinstein, M.; Sheiner, L.B., Saliva lithium concentrations in the management of lithium therapy. Arch. Gen. Psychiatry **1978,** 35, 1123-1127.
7. Evrard, J.L.; Bauman, P.; Pera, R.; Peters-Haefeli, L., Lithium concentrations in saliva, plasma and red blood cells of patients given lithium acetate. Acta. Psychiatry Scand. **1978,** 58, 67-79.

8. Shorter, E., The history of lithium therapy. Bipolar Disord. **2009,** 11, 4-9.
9. deMendiola, X.P.; Hidalgo-Mazzei, D.; Vieta, E.; Gozalez-Pinto, A., Overview of lithium use: A nationwide survey. Int. J. Bipolar Disord. **2021,** 9, 1-8.
10. Gitlin, M., Lithium side effects and toxicity: Prevalence and management strategies. Int. J. Bipolar Disord. **2016,** 4, 1-10.
11. Camus, M.; Baron, G.; Peytavin,G.; Massias, L,; Farinotti, R., Comparison of lithium concentrations in red blood cells and plasma in samples collected for TDM, acute toxicity, or acute-on-chronic toxicity. Eur. J. Clin. Pharmacol. **2003,** 59, 583-587.
12. Couffignal, C.; Chevillard, L.; El Balkhi, S.; Cisterino, S.; Decleves, X., The pharmacokinetics of lithium. in The Science of Practice of Lithium Therapy; Malhi, G.S., Masson, M., Bellivier, F., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 25-53.
13. Ohlund, L.; Ott, M.; Oja, S.; Bergqvist, M.; Lundqvist, R.; Sandlund, M.; Renberg, E.S.; Werneke, U., Reasons for lithium discontinuation in men and women with bipolar disorder: A retrospective cohort study, BMC Psychiatry **2018,** 18, 1-10.
14. Fletcher, M.H., Determination of lithium in rocks by distillation. Anal. Chem. **1949,** 21(1), 173-175.
15. White, C.; Fletcher, M.; Parks, J., Determination of lithium in rock. Anal. Chem. **1951,** 23(3), 478-481.
16. Caley, E.R., The detection and estimation of small amounts of lithium. J. Amer. Chem. Soc. **1930,** 52(7), 2754-2758.
17. McMaster, L., A further study of the preparation and properties of the ammonium salts of organic acids. J. Amer. Chem. Soc. **1914,** 36(9),1916-1925.
18. Nazarenko, V.A.; Filatova, V.Ya., A rapid method of semimicro-colorimetric determination of lithium in minerals and ores. Zhur. Anal. Khim. **1950,** 5, 234-238.
19. Hering, H., Quantitative separation of lithium traces from calcium. No. CEA-R-136. Commissariat a l'energie atomique et aux Energies-Alternatives-CEA, 1952.
20. Bowman, J., The application of resonance monochromators to the determination of lithium in blood serum by atomic absorption spectrophotometry. Anal. Chem. Acta **1967,** 37, 465-471.
21. Doku, G.N.; Gadzekpo, V.P., Simultaneous determination of lithium, sodium and potassium in blood serum by flame photometric flow-injection analysis. Talanta **1996,** 43, 735-739.
22. Sheikh, M.; Qassem, M.; Triantis, I.; Kyriacou, P.A., Advances in the therapeutic monitoring of lithium in the management of bipolar disorder. Sensors **2022,** 22(3), 736.
23. Bertholf, R.L., et. al., Lithium determined in serum with an ion-selective electrode. Clin. Chem.**1988,** 34, 1500-1502.
24. Vroouwe, E.X.; Luttge, R.; Berg, A.V.D., Direct measurement of lithium in whole blood using microchip capillary electrophoresis with integrated conductivity detection. Electrophoresis **2004,** 25, 1660-1667.
25. Manfro, I.D. et.al., Determination of lithium in dried blood spots and dried plasma spots by graphite furnace atomic absorption spectrometry: method development, validation and clinical application. Talanta **2020,** 216 120907.
26. Eltayib, E. et. al., Hydrogel-forming microneedle arrays: potential for use in minimally-invasive lithium monitoring. Eur. J. Pharm. Biopharm. **2016,** 102 13-131.
27. Obare, S.O.; Hollowell, A.R.E.; Murphy, C.J., Sensing strategy for lithium ion based on gold nanoparticles. Langmuir **2002,** 18, 10407-10410.
28. Obare, S.O.; Murphy, C.J.A., A two -color fluorescent lithium-ion sensor. Inorg. Chem. **2001,** 40, 6080-6082.
29. Trautman, J.K.; Gadzekpo, V.Y P.; Christian, G.D., Spectrophotometric determination of lithium in blood serum with thoron. Talanta **1983,** 8, 587-591.
30. Shopsin, B.; Gershon, S.; Pinckney, L., The Secretion of lithium in Human Mixed Saliva: Effects of ingested lithium on electrolyte distribution in saliva and serum. Int. Pharmacopsychiatry **1969,** 2 148-169.
31. Khare, C.B.; Sankaranarayanan, A.; Goel, A.; Khandelwal, S.K.; Srinivasa, M.R., Saliva lithium levels for monitoring lithium prophylaxis of manic-depressive psychosis. Int. J. Clin. Pharmacol Ther. Toxicol. **1983,** 21(9), 451.
32. Ben-Aryeh, H.; Naon, H;, Szargel, R.; Gutman, D.; Hefetz, A., Salivary lithium concentration-a tool for monitoring psychiatric patients. Oral Surg. Oral Med. Oral Pathol. **1980,** 50(2), 127-129.

33. Bowden C.L.; Houston, J.P.; Shulman, R.S.; Clothier, J.M., Clinical Utility of salivary lithium concentration. Int. Pharmacopsychiatry **1982,** 17(2), 104.
34. Verghese, A.; Indrani, N.; Kuruvilla, K.; Hill, P.G., Usefulness of saliva lithium estimation. Br. J. Psychiatry **1977,** 130, 148-150.
35. Kuznetsov, V.I., Color reaction for lithium. Zhur. Anal. Khim. **1948,** 3 295-302.
36. Nikolaev, A.V.; Sorokina, A.A., Colorimetric determination of lithium. Doklady Akad. Nauk. S.S.S.R. **1951,** 17, 427-428.
37. Thomason, P.F. Spectrophotometric determinations of lithium. Anal. Chem.**1956,** 28, 1527-1530.