Direct Determination of Therapeutic Concentrations of Lithium in Serum by Flow-Injection Analysis with Atomic Absorption Spectroscopic Detection

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In this flow-injection system for direct determination of lithium in serum by atomic absorption spectroscopy, the 10-μL sample is manually injected into a continuously flowing non-segmented stream of de-ionized water, which is pumped, via a dispersion tube, to the spectrometer's nebulizer. Controlled dispersion of the sample zone, before it is introduced into the nebulizer, produces the required sample dilution. Effects of varying the length of the dispersion tube, the flow rate, and the sample size were studied. Analytical readout is obtained, in the form of transient peaks, 5 s after sample injection. It is necessary to include physiological concentrations of sodium and potassium in the standards because each of these cations enhances the lithium absorbance signal. Analytical recovery (98.5 to 101%) and CV (about 2%) are good, and results compare well with those obtained by aspiration of prediluted samples (n = 121, r = 0.99).

Additional Keyphrases: monitoring therapy for mental disorders - pediatric chemistry

Therapy with lithium is now generally accepted by psychiatrists as one of the major regimens for the treatment of acute mania as well as for the prevention of relapse in recurrent bipolar mood disorders. Its use has been investigated in at least 30 other psychiatric and non-psychiatric disorders (1). Close monitoring of therapy is essential, because individual patients vary in their tolerance of and sensitivity to response to the drug. Because of possible side effects (including polyuria, ataxia, hypothyroidism, and weight gain), the concentration in serum must be controlled within narrow limits. The normal concentration of lithium in serum is about 3 μmol/L, and the therapeutic range is suggested (2) to be between 0.3 and 1.3 μmol/L (based on the standardized estimation 12 h after a dose), with 1.5 mmol/L representing the lower limit of risk for intoxication. Serum values exceeding 3.5 mmol of lithium per liter should be regarded as potentially lethal. Because concentrations near the therapeutic range can produce symptoms of toxicity, it is important to have a fast, convenient method for monitoring the lithium concentration in serum.

Flame emission photometry (3-5) and atomic absorption spectroscopy (6-8) form the basis of most of the published methods for the estimation of therapeutic concentrations of lithium in serum, and there appears to be little basis for a choice between the two techniques. Flame emission is generally more sensitive for lithium determinations than atomic absorption spectroscopy but is also the less precise of the two methods (9-13).

Using atomic absorption spectroscopy, Lehmann (6) diluted serum 20-fold with 0.1 mol/L hydrochloric acid; Price (8) precipitated the proteins with trichloroacetic acid before determination. Most other authors favor direct dilution of the serum with de-ionized water before assay.

Flow-injection analysis (14, 15) is a fast, efficient analytical technique in which a sample is "injected" into a continuously flowing non-segmented carrier solution. If the carrier solution contains a color-forming reagent, then the colored zone that results when the sample diffuses into the carrier can be measured by passing the flowing stream through a flow-through cuvet placed in a colorimeter. Alternatively, the technique may be used with various other detectors, including atomic absorption spectrometers (16-19). Conventional use of atomic absorption instrumentation relies upon sample aspiration and generation of a steady-state signal. Flow-injection analysis (FIA) differs from the traditional method in that the measurements are not made at equilibrium. The sample slug, having dispersed into the carrier solution to the required degree, is pumped into the atomic absorption nebulizer as a discrete zone, resulting in a transient signal response, which is related to the quantity of analyte in the injected sample.

Transient signal measurements have been used with atomic absorption flame techniques before FIA was introduced. For example, in the discrete-volume method of nebulization (also variously known as the "direct injection," "aliquot," or "pulse nebulization" method) a 50-200 μL sample is aspirated from a cone-shaped cup in the flame to give a transient signal (20-23); in this case the sample slug is bounded only by air as it is drawn into the nebulizer. In contrast, the FIA sample disperses into the carrier stream as it is pumped towards the nebulizer. This dispersion is highly reproducible if the flow of carrier is constant and a consistent injection technique is used. Additionally, the degree of sample dispersion can be controlled by varying flow rate, sample size, and the dimensions of the capillary tube leading to the nebulizer. By careful choice of these variables the sample dispersion may be manipulated to produce optimal detector response and high sample throughput with minimal sample consumption.

This paper describes a combination of FIA and atomic absorption spectroscopy (AAS) for the direct analysis of lithium in 10-μL samples of serum. The FIA method is compared with a conventional aspiration AAS method, and the significance of the results is discussed. We believe that this is the first application of the combination of these two techniques to clinical chemistry.

Materials and Methods

Round-bottom plastic test tubes (75 × 12 mm, cat. no. RT30) were obtained from Sterlin Ltd., Teddington, Middlesex, U.K.

Reagents

1. Viscosity-adjusted (0.18 Pa·s, or 1.8 centipoise) flame photometer standard containing 140 mmol of NaCl and 5 mmol of KCl per liter (Instrumentation Laboratory Ltd., Warrington, Cheshire, U.K.).
2. Lithium stock standard containing 100 mmol of lithium chloride per liter (BDH Chemicals, Poole, Dorset, U.K.).
3. Working standards containing 0, 0.5, 1.0, 1.5, and 2.0 mmol of lithium per liter were prepared by diluting reagent 2 with reagent 1.
4. Wellcomtrol One and Two (lot nos. K9518 and K9040; Wellcome Reagents Ltd., Beckenham, Kent, U.K.) control
sera were reconstituted according to the manufacturer's instructions.

5. Sodium and potassium chloride (analytical grade) were used to prepare the aqueous solutions used for the interference studies. Solutions containing proteins were made up from pooled "lithium-free" serum; the viscosity-adjusted solutions were made up in reagent 1.

**Instrumentation**

Atomic absorption measurements were made with an Atomspek H1550 (Rank Hilger, Margate, Kent, U.K.). The atomic absorption operating conditions are shown in Table 1. Peaks were recorded with a Servoscribe RE 541-20 potentiometric recorder with a response time of 0.5 s (Smiths Industries Ltd., Crichtlewood, London, U.K.). The pump was an LC750 constant-flow pump (Applied Chromatography Systems Ltd., Luton, Bedfordshire, U.K.). Chromatography syringes of 10- and 100-μL capacity were manufactured by Hamilton, Bonaduz, Switzerland, and the septum injector was supplied by Omnifit Ltd., Cambridge, U.K.

**Procedures**

Blood was sampled from patients being treated with lithium carbonate preparations for bipolar mood disorders, 5 mL being collected into plain glass tubes and the serum separated within 2 to 4 h of collection.

Two different procedures were used. The samples were first assayed by our established method (procedure 1). This method, based on the work of Pybus and Bowers (10), has been used routinely in our laboratory for several years. The same samples were then assayed by the FIA method (procedure 2).

"Established" method. Standards, samples, and controls (200 μL) were added to test tubes containing 1.8 mL of de-ionized water, vortex-mixed, and aspirated into the Atomspek. The diluted standard solutions were used to calibrate the instrument in concentration units, and the results of the sample analysis were printed out automatically after the steady-state signal had been integrated for 5 s.

**FIA method.** Figure 1 shows a diagram of the flow system. De-ionized water is pumped at a constant rate (4 mL/min) through the septum injector and the dispersion or mixing tube [25 cm of 0.5 mm i.d. poly(tetrafluoroethylene) tubing] into the Atomspek nebulizer. Samples of standards or serum (10 μL) are directly injected into the continuously flowing stream of water, and the resulting transient peaks are recorded. The Atomspek has a peak-area integration facility and, when required, the integration period was set at 5 s and triggered manually immediately before injection. The peak areas were automatically printed at the end of the integration period. Standards containing 0–4 mmol of lithium per liter gave a linear calibration plot with the origin at zero. In practice, samples with lithium concentrations greater than 1.5 mmol/L are seldom encountered, and standards between 0 and 2 mmol/L were routinely used to calibrate the instrument, (Figure 2). The peak heights, or peak areas, of the standards were used to construct a calibration graph, from which the tests and controls were read.

**Results**

When a sample is injected into a solution flowing in a laminar fashion through a thin tube of uniform diameter, the height, width, and shape of the recorded peaks will be affected by three interrelated factors: tube length, flow rate, and sample volume.

Preliminary experiments suggested that a carrier flow rate of about 4 mL/min and a sample size of 10 μL would be suitable starting points for investigating the FIA variables.

**Dispersion tube length.** The length of capillary tubing between the septum injector and the nebulizer was varied and the peak-height response recorded when 10 μL of a 1 mmol/L lithium standard was injected into the system at a carrier flow rate of 4 mL/min. As expected, the dispersion of the sample into the carrier stream increases with increasing tube length, resulting in lower peaks. Over the range of tube length from 25 to 85 cm, the peak height produced by the injected sample is linearly and inversely related to dispersion tube length.

We did not investigate the effect of varying the internal diameter of the dispersion tube, because tubes wider than that used (0.5 mm i.d.) could not be fitted to the nebulizer.

**Flow rate.** The tube length used was 25 cm, and 10 μL of a 1 mmol/L standard was injected. Increasing the carrier flow

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**Table 1. Atomic Absorption Spectrometer (Atomspek H1550) Settings for Analysis of Lithium in Serum**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>670.8 nm, lithium hollow-cathode lamp</td>
</tr>
<tr>
<td>Lamp current</td>
<td>5 mA</td>
</tr>
<tr>
<td>Silt width</td>
<td>50 μm</td>
</tr>
<tr>
<td>Burner</td>
<td>Single slot type (H1564)</td>
</tr>
<tr>
<td>Flame</td>
<td>Air/acetylene</td>
</tr>
<tr>
<td>Air</td>
<td>Rotameter reading 7 cm (8.5 L/min)</td>
</tr>
<tr>
<td>Acetylene</td>
<td>Rotameter reading 2.5 cm (1 L/min)</td>
</tr>
<tr>
<td>Burner height</td>
<td>2 mm</td>
</tr>
<tr>
<td>Response</td>
<td>1 s (time constant)</td>
</tr>
<tr>
<td>Recorder range</td>
<td>100 mV full-scale deflection</td>
</tr>
</tbody>
</table>

A warm-up time of 20 min was allowed before analysis. In procedure 1 the diluted samples are aspirated into the flame (the aspiration rate was 4.8 mL/min through 25 cm of capillary tubing). In procedure 2 the capillary tubing was connected to the outlet of the septum injector through which the carrier solution was pumped; samples were directly injected into the flowing stream.
rate through the nebulizer increased the recorded peak height up to a rate of 2.5 mL/min; flow rates >2.5 mL/min decreased the peak height. The latter may be attributable to one or more of the following: (a) The dispersion of the sample in the carrier stream will increase as the flow rate is increased (15). (b) The size distribution of droplets entering the spray chamber may increase at high flow rates; larger droplets will be prevented from entering the burner and hence will not be fully volatilized. Visual inspection of the spray from the nebulizer at higher flow rates confirmed the presence of a high proportion of larger droplets. (c) At high flow rates the response of the instrument may not be fast enough to collect the entire pulse generated by the flow-injection burner system. The signals also decreased when the time constant of the amplifier was increased. (Increasing the time constant from 1 s to 5 s and 20 s resulted in decreases in peak heights of 36% and 66%, respectively).

Sensitivity was maximum at a flow rate of 2.5 mL/min, but at this flow rate the measurements were rather erratic and a flow rate of 4 mL/min was preferable. (Under normal conditions, with use of similar tubing, the aspiration rate of the nebulizer is 4.8 mL/min.)

Sample size. Volumes ranging from 5 to 100 μL of a 1 mmol/L lithium standard solution were injected into the system. Peak heights increased with sample volume injected until eventually a "steady-state" signal is reached. Peak width also increases with increasing sampling volume, and this reduces sample throughput. With a 25-cm dispersion tube and a carrier flow rate of 4 mL/min, 10 μL of a 1 mmol/L lithium standard gave an absorbance reading of 0.25 A. A similar absorbance reading was obtained when the standard was diluted 12-fold with water and pumped continuously into the nebulizer to give a steady reading. Hence, the dispersion (15), D, of the injected sample in the FIA system is 12; i.e., the injected solution has been diluted 12-fold by the carrier stream by the time it reaches the nebulizer.

Analytical Characteristics

Choice of standard. In the amounts present in normal serum, calcium, magnesium, chloride, bicarbonate, or phosphate do not interfere with the AAS determination of lithium, but sodium and potassium in physiological concentrations reportedly cause an apparent decrease in the lithium content of serum if the standards are not adjusted for viscosity (11–13).

At a lithium concentration of 1 mmol/L an added 140 mmol of sodium chloride per liter enhanced the lithium signal by 12%. Addition of 4 mmol of potassium chloride per liter caused a 6% enhancement in apparent lithium. The joint presence of sodium and potassium in physiological concentrations caused a 12% enhancement of the lithium standard signal, and clinically encountered variations in concentrations of either cation from its normal value produced little difference. If sodium and potassium are present in about normal serum concentrations, viscosity and the presence of serum proteins do not have a significant effect.

Therefore, physiological concentrations of sodium and potassium must be included in lithium standards for analysis by this method, but viscosity-adjusted standards are not essential.

Analytical recovery. Recovery of lithium was determined by adding known amounts of stock lithium standard to two different batches of pooled "lithium-free" serum and by adding a lithium standard to six different serum samples from patients being treated with lithium preparations. Recoveries ranged from 98.5 to 101%.

Precision. The within-batch data for the various lithium assay procedures are given in Table 2. Table 2 shows the results for 10 replicate samples of Wellcomtol Two by our established conventional lithium AAS method (procedure 1), and for preliminary experiments with the injection technique in which the nebulizer was used to draw carrier through the septum injector port; i.e., the pump was not used. The precision of the injection method was improved by using a pump to propel the carrier through the system (2.9% vs 3.2%). The reproducibility of the FIA method (procedure 2) was better when using automatic peak area integration (values listed in parentheses) than when using manual measurement of peak heights.

As expected, the precision of the conventional AAS method, where the steady-state signal is averaged for 5 s, is better (CV = 1.5%) than that for the FIA method (CV = 1.8%), where a transient signal is integrated.

The between-batch precision was determined by daily analysis for two work weeks of two samples stored frozen, in 0.5-mL aliquots (Table 2). Mean between-batch CVs are about 3% for the FIA method, and 2.2% for the conventional method.

Correlation of results. During a one-month period all samples sent to our laboratory for serum lithium determination were assayed daily by both the conventional AAS procedure (x) and by the direct FIA method (y). These samples included the usual small proportion of hemolyzed and lipemic samples. We found a good correlation between results by the two methods (r = 0.996, y = 0.99x + 0.008, standard error of estimate = 0.024, SD of slope = 0.008 mmol/L, SD of intercept = 0.006 mmol/L).

Sample throughput. With the injection technique, the maximum peak was recorded about 2 s after sample injection. By 5 s after sample injection, the recorder trace had completely returned to baseline and another sample could then be injected. Replicate samples injected at 5-s intervals showed no signs of carryover. This gives a possible throughput of at least 720 samples per hour. In practice, however, when a batch of samples was being analyzed, the syringe was rinsed twice with sample, before the sample was finally injected into the system. This effectively decreases analytical throughput to two or three samples per minute, the exact time varying with the analyst.

Most hospital laboratories are requested to analyze only small batches of serum samples for lithium, usually on a daily basis, so the manual injection technique is most appropriate. However, the syringe may be replaced by a liquid-chromatographic valve; if large batches of samples are to be determined,
then the injection and sampling process may be automated (24, 25).

Discussion

The advantages of the FIA method are as follows:
1. It is a direct method. The serum sample requires no pretreatment. Controlled, on-stream dispersion of the injected sample zone produces the desired sample dilution.
2. It is fast. Detector warm-up time is the limiting factor.
3. It is a micromethod and should be suitable for the analysis of samples collected with capillary tubes from finger pricks. This is important because lithium is increasingly used to treat behavior disorders in childhood (1). Another possible application is the monitoring of lithium in infants who are being breast fed by a mother undergoing therapy with lithium.
4. The method shows good reproducibility, and the results are comparable with those obtained by a conventional method.
5. With the FIA method much less sample is introduced into the flame than in a conventional method; consequently, burner clogging is much less of a problem.

We have also used FIA and AAS to determine calcium, magnesium, copper, and zinc in serum samples. This will form the subject of a subsequent report.

References