On-Line Dilutor for Use with Flame Photometers

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This note describes a reliable, convenient way to provide automatic, on-line dilution of samples for flame photometry. The method applies to any flame photometer that has a vertical aspirating needle.

Many clinical laboratory flame photometers used for sodium and potassium determinations in serum and urine aspirate a solution that is about a 200-fold dilution of the original sample. Manual dilution is too slow. Therefore, many laboratories speed the process by the use of a semiautomatic dilutor, which also provides the repeatability necessary for reliable data. A semiautomatic system is certainly an improvement over a completely manual one, but an even faster system for dilution is desirable since a semiautomatic system still requires much glassware handling, with the inherent possibilities of contamination, or the use of expensive disposable containers.

Instrumentation Labs., Inc., Lexington, Mass., supplies an on-line dilutor. It is a peristaltic pump which pumps sample, concentrated lithium solution, and water through separate tubes into a mixing chamber and then pumps the mixture onto the flame aspirator. We evaluated this dilutor in late 1968 and found that, in our hands, its reliability was poor. But by this time our technicians had become accustomed to the significant time savings and the eliminated need for washing glassware. Accordingly, we devised the dilutor described here, which combines the usefulness of an on-line dilutor with sufficient reliability.

Experimental

The dilutor incorporates a modified AutoAnalyzer Proportioning Pump I, developed at Duke for other purposes [1]. The modification is made by substituting a Bodine B2270-60G, 30 rpm synchronous motor for the standard drive motor (Technicon, Inc., Tarrytown, N.Y.). To do this, a special mounting plate was fabricated (Fig.1). This plate is mounted to the pump chassis with the four screws removed from the original motor. The new pump is mounted to this plate with four $1/4 \times 20 \times 1/2$ in. screws. Also a 1/2-in. shaft sprocket (Martin 41B8 with set screw) was needed for the larger 1/2-in. motor shaft, and four links (2 3/8 in.) had to be added to the drive chain to accommodate the added distance between the two drive-chain sprockets. This pump has a roller movement three times faster than a standard Proportioning Pump I.

To make an on-line dilutor from this pump, we used a five-tube manifold, with four 0.081 in. i.d. tubes to pump the LiNO$_3$ solution (15 mEq of Li$^+$ per liter) and one 0.010-in. i.d. tube to pump sample. On a standard pump each of the 0.081-in. i.d. tubes pumps 2.50 ml/min for a total of 10.0 ml of LiNO$_3$ per min and the sample tube (0.010 in. i.d.) pumps at a rate of 0.08 ml/min; the resulting dilution ratio is 200 parts of LiNO$_3$ to 1 part of sample. On the high-speed pump, each of these tubes pumps three times as fast, for a total flow rate of 30.15 ml/min, and makes the same dilution ratio. Alternatively, the manifold can be made with three 0.100-in. i.d. tubes pumping LiNO$_3$ solution, if a dilution ratio of slightly higher than

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Table 1. Effect of Pumps on Analytical Features

<table>
<thead>
<tr>
<th></th>
<th>Lag time, s</th>
<th>Time to steady output, s</th>
<th>Fluctuations in output, mEq/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard pump</td>
<td>65</td>
<td>20</td>
<td>Na 138 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>K 4.90 ± 2.0</td>
</tr>
<tr>
<td>Fast pump</td>
<td>18</td>
<td>10</td>
<td>Na 138 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>K 4.90 ± 0.1</td>
</tr>
</tbody>
</table>

200:1 is acceptable. These tubes pump 3.40 ml/min on a standard pump, which would give a 204:1 ratio with the same sample line (0.010 in. i.d.). The output of the four tubes that conduct LiNO₃ solution are connected by a KO glass fitting and then pumped through a PC 1 surge suppressor (these and other parts mentioned are from Technicon) to help minimize the effects of pressure variations.

The sample is added to the LiNO₃ stream through an A6 glass fitting and then passed through a jet mixer made from 8-mm lengths of 0.035-in. i.d. tubing and 8-mm lengths of 0.090-in. i.d. tubing. These dimensions are slightly larger than the original jet mixer described by Skeggs and Hochstrasser (2) because the flow rates used in this work are larger. The stream is then connected to an A1 glass fitting for aspiration into the flame photometer. The A1 fitting is held by tubing so that its main axis is horizontal and the capillary side arm is vertical. The aspirating needle from the flame photometer (Instrumentation Laboratory, Model 143) is pushed down into the capillary side arm of the glass fitting until its tip almost touches the bottom of the fitting. The tip should be deep in the horizontal part of the glass tube so that small bubbles, which enter the system when the sample probe is not in liquid, do not get aspirated into the flame photometer.

Results and Discussion

The pump modification was necessary because a standard-speed pump produced large fluctuations in the digital readout of the flame photometer, probably because of variations in sample addition to the stream of LiNO₃ solution. This variable sample addition is caused by surges in liquid flow resulting from pressure changes in the pump tubing when rollers leave the platen. These pressure surges are more frequent, but are smaller when the manifold is used with the modified pump. The faster pump also affords a shorter lag time (time for sample to move from probe to flame) and a faster approach to a steady photometer output for each sample, which increases the rate at which samples can be analyzed. The data in Table 1 summarize this information for serum sodium and potassium determinations.

Fifteen replicate determinations of a reconstituted commercial control serum were run to provide data on repeatability. The sodium mean was 137.9 mEq/liter with a range of 137 mEq/liter to 139 mEq/liter and a coefficient of variation of 0.38%. The potassium mean was 4.89 mEq/liter with a range of 4.85 to 4.95 and a coefficient of variation of 0.12%.

Table 2 compares data obtained on 10 samples of patient serum run in duplicate by three methods: AutoAnalyzer; I. L. Model 143 flame photometer with predilution made with an Auto Dilutor (Scientific Products) set at 0.10 ml sample and 19.9 ml LiNO₃ (15 mEq of Li⁺ per liter) and 50-ml plastic Erlenmeyer flasks, washed in our laboratory dishwasher, as containers; and the on-line dilutor described here, with the same I. L. flame photometer.

The Auto Dilutor and on-line dilutor were each run with a sequence of samples, numbers 1 to 10, then from numbers 10 to 1. The flame photometer
An Improved Method for Preparation of Feces for Bomb Calorimetry

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As a preliminary to bomb calorimetry, weighed, homogenized fecal slurries are prefrozen and lyophilized. Advantages of this method over the method of drying in a vacuum oven include: more moisture is removed from the sample initially, drying time is reduced from 48 h to 16 h, grinding is eliminated and objectionable odors are eliminated during sample processing.

The classical procedure for preparing feces for bomb calorimetry by the method of Atwater (1) has many inherent disadvantages. The temperature must be carefully controlled to avoid overheating and charring of the sample. Lengthy time periods (36 to 48 h) are required to ensure that the sample has been dried to a constant weight. Grinding is necessary to reduce the sample to a powder for pellet formation. An improved method has been developed for preparing human feces for calorimetry.

Method

Feces samples were obtained from human subjects on liquid diets over a specified time period. After the sample has been collected and weighed, distilled water, equal to the weight of the feces, is added. The feces-water mixture is homogenized in a Waring Blendor for 3 to 5 min.

Parts for modifying the pump and the pump itself cost about $1,000, which is justified by savings of personnel time and equipment. In our opinion, the cost is justified by reliability and technician satisfaction alone.

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References
