Factors Influencing Evaporation from Sample Cups, and Assessment of Their Effect on Analytical Error

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We studied sample evaporation and its effect on analytical error. Several factors influencing evaporative loss have been identified and measured: environmental, instrumental, and operational factors, and the chemical and physical properties of the sample and its container. Such losses from several different types of sample cups have been measured, either chemically or gravimetrically, and compared with those calculated by using a model that allows evaporative loss from a cup of known geometry to be predicted under various environmental conditions. We discuss some steps that may be taken to minimize evaporative loss and give an example to demonstrate that analytical error from this source can be decreased to a routine 1–2% or less by selecting a particular cup design.

Additional Keyphrases: microliter samples • analytical error • sample-cup geometry • predicting and preventing evaporative loss • centrifugal analyzer • pediatric chemistry

Various newly developed instruments offer real and potential advantages for automated clinical analyses. Because increased sensitivity is typically a feature of these instruments, many factors that heretofore made relatively insignificant contributions to the total analytical error should now be examined and evaluated.

One such factor is evaporative loss of volatile components or liquid\(^1\) from a sample after it has been poured into its sample cup. This factor can contribute a significant analytical error, especially when small volumes are involved. Surprisingly, the clinical literature hardly discusses this problem (1–3). We have quantitatively studied the process of evaporation and how it affects analytical error in the clinical laboratory. Here, we (a) present the experimental results of this study, (b) discuss several factors that influence evaporative loss, (c) present a model that can be used to estimate the quantitative and relative evaporative loss from a given sample cup under various environmental conditions, and (a) describe preventive or corrective measures that can be taken to minimize sample evaporation during analysis.

Materials and Methods

Instrumentation

The miniature Centrifugal Fast Analyzer, the version of the centrifugal analyzer that we used in these studies, has been previously described (4, 5), as has its operation (4).

Methodology

In the quantitative studies, control and aqueous samples were analyzed for glucose by a modified hexokinase procedure (6). Reagents for the assay ("Stat-Pack" kit; Calbiochem, San Diego, Calif. 92112) were reconstituted before analysis by dissolving the contents of one vial in 2 ml of water, which was then added to and mixed with the contents of the second vial. Lyophilized control area (Monitrol I and II; Dade, Miami, Fla. 33152) were reconstituted according to the manufacturer's recommendations. Aqueous standard solutions of NBS Standard Reference Material glucose were prepared in benzoic acid-saturated distilled water.

Quantitative measurement of evaporative losses. We measured the effects of evaporation both chemi-
Table 1. Sample Cups Used in Evaporation Studies

<table>
<thead>
<tr>
<th>Code no.</th>
<th>Source</th>
<th>Diameter, cm</th>
<th>Area, cm²</th>
<th>Depth, cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Micro-Tube MT-50</td>
<td>0.357</td>
<td>0.1001</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.380</td>
<td>0.1134</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.391</td>
<td>0.1207</td>
<td>1.35</td>
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<td></td>
<td>200</td>
<td>0.422</td>
<td>0.1389</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.439</td>
<td>0.1513</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.439</td>
<td>0.1513</td>
<td>0.27</td>
</tr>
<tr>
<td>2</td>
<td>Eppendorf Tube EP-1500</td>
<td>0.91</td>
<td>0.6603</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>CentrifilChem Sample Cup (0.25 ml) UC-100</td>
<td>0.69</td>
<td>0.3739</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.69</td>
<td>0.3739</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1.00</td>
<td>0.7853</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>Nilab Sample Cup NI-1000</td>
<td>1.21</td>
<td>1.1499</td>
<td>0.72</td>
</tr>
<tr>
<td>5</td>
<td>0.5-ml AutoAnalyzer Cup TC-500</td>
<td>0.70</td>
<td>0.3848</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Technicon Corp. Tarrytown, N.Y.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5-ml SMAC Tube SM-1000</td>
<td>1.25</td>
<td>1.2271</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>1.25</td>
<td>1.2271</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>1.25</td>
<td>1.2271</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>1.25</td>
<td>1.2271</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>1.25</td>
<td>1.2271</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ABS-100 Micro-Cup AB-50</td>
<td>0.45</td>
<td>0.1590</td>
<td>0.40</td>
</tr>
</tbody>
</table>

a Cup code followed by volume (μl) tested.
b Distance from top of cup to surface of liquid.

cally and gravimetrically. In the chemical studies, the miniature Centrifugal Fast Analyzer was used to analyze either control sera or an aqueous standard solution for glucose (6) as a function of time under several different experimental conditions. In the gravimetric studies, various volumes of water or serum were dispensed into several different types of tared sample cups, and the weight loss of each was determined as a function of time by weighing the cup and its contents at 1-h intervals for as long as 8 h. We used an analytical balance that had a repeatability of ±0.1 mg and that had been calibrated against NBS certified weights. Table 1 lists the identification, source, and critical dimensions of the various cups we used.

Estimation of Evaporative Losses

Results of the evaporative studies led to the development of a mathematical model that allows one to estimate the quantity of a particular liquid lost by evaporation over a specific period of time from a cup of known geometry (Figure 1). The theoretical basis for the model was diffusion through a stagnant gas film (7). The following assumptions are made: (a) water diffuses upward through a stagnant film of air; (b) the mole fraction of water at the gas/liquid interface can be calculated from the vapor pressure of water at room temperature; (c) the mole fraction of water in air at the top of the cup is the same as in the laboratory atmosphere; (d) the air/water mixture is an ideal gas; (e) the solubility of air in water is negligible; (f) the entire system is at constant temperature and pressure; and (g) that part of the cup that is above the liquid level can be approximated by a cylinder with the same diameter as the liquid interface. The applicable equation is:

\[ N_{\text{H}_2\text{O}|z=z_1} = \frac{p \cdot (D_{\text{H}_2\text{O}-\text{air}})}{R \cdot T \cdot (Z_2 - Z_1)} \ln \frac{p_{\text{air}}}{p_{\text{water}}}, \]

where

\[ N_{\text{H}_2\text{O}|z=z_1} = \text{rate of water evaporation at the interface, mol/cm}^2 \cdot \text{s} \]

\[ Z_1 = \text{gas/liquid interface, cm} \]

2 Variables and constants included in the model are given in metric units, because most current handbooks list them this way. In SI units, the following units would be substituted:

- pressure, N/m² (1 atm = 101325 N/m²)
- gas constant = 8.31433 x 10⁻⁵ cm³·atm·K⁻¹·mol⁻¹

All other units are as listed in text.
\[ Z_2 = \text{top of cup, cm; } \]
\[ p = \text{atmospheric pressure, atm; } \]
\[ D_{H_2O-air} = \text{diffusivity of water in air, cm}^2/\text{s; } \]
\[ R = \text{gas constant} = 82.05 \text{ atm} \cdot \text{cm}^3/\text{mol} \cdot \text{K}; \]
\[ T = \text{temperature, K}; \]
\[ p_{air_1} = p - p_{H_2O} (p_{H_2O} = \text{vapor pressure of water}); \]
\[ p_{air_2} = p - (\text{RH}) \cdot (p_{H_2O}/100) \] (RH, relative humidity, %).

With the incorporation of the appropriate terms, Equation 1 can be expressed in terms of the volume lost at the interface, as follows:

\[
\frac{dv}{dt} \text{ cm}^3/\text{s} = \left( N_{H_2O} \frac{g}{cm^2 \cdot s} \right) \cdot \left( \frac{M_{H_2O}}{g/mol} \right) \cdot \left( \frac{1}{\rho_{H_2O}} \frac{cm^3}{g} \right) \cdot (A_1, \text{cm}^2). \tag{2}
\]

The expression then becomes:

\[
\frac{dv}{dt} = \frac{p \cdot M_{H_2O} \cdot (D_{H_2O-air}) \cdot A_1}{\rho_{H_2O} \cdot R \cdot T \cdot h} \cdot \ln \frac{p_{air_2}}{p_{air_1}}, \tag{3}
\]

where

\[ dv/dt = \text{rate of water evaporation at the interface, } \text{cm}^3/\text{s}; \]
\[ M_{H_2O} = \text{molecular weight of water (g/mol); } \]
\[ \rho_{H_2O} = \text{density of water (g/cm}^3); \]
\[ A_1 = \text{area of sample cup at the liquid/air interface, cm}^2; \]
\[ h = Z_2 - Z_1 \text{ (cm), height of stagnant air mass (i.e., the vertical distance from the surface of the air/liquid interface to the top of the sample cup). } \]

In addition, the relationship between the rate of evaporation, \( dv/dt \), and the rate of change in the height of the stagnant air mass can be expressed as follows:

\[
\frac{dv}{dt} = A_1 \cdot \frac{dh}{dt}. \tag{4}
\]

Substituting the expression for \( dv/dt \) from Equation 3 into Equation 4, we have:

\[
\frac{dh}{dt} = \left[ \frac{p \cdot M_{H_2O} \cdot (D_{H_2O-air})}{\rho_{H_2O} \cdot R \cdot T} \cdot \ln \frac{p_{air_2}}{p_{air_1}} \right] \cdot \frac{1}{h}. \tag{5}
\]

The term in brackets is a constant \( (C) \) for a particular set of conditions, and can be readily calculated.

Integrating Equation 5 between the initial height, \( h_1 \), and the final height after evaporation, \( h_f \), gives

\[
\frac{(h_f)^2 - (h_1)^2}{2} = C \cdot t. \tag{6}
\]

From the geometry of the cup, one may calculate the volume loss corresponding to the change in height of the stagnant air mass \( (h_f - h_1) \) for a given time, \( t \).

This model can then be used to predict evaporative loss from a given cup containing a specific volume of liquid under a selected set of environmental conditions. It should be noted, however, that losses owing to convection are not included in the model.

Results and Discussion

Analytical Effects of Evaporation

The primary effect of evaporation is on analytical accuracy, because the concentration of dissolved solutes in a sample will increase as solvent evaporation continues, as is demonstrated in Figure 2, which shows the glucose concentrations of duplicate aliquots of two control sera as a function of time. To minimize evaporation, 75 \( \mu \)l of silicone oil (Sera-Seal; Abbott Diagnostics Div., South Pasadena, Calif. 91030) was layered over one of the aliquots. The results very clearly indicated that evaporation had occurred in the uncovered samples, because their glu-
Table 2. Factors Affecting Evaporative Loss

I. Time
II. Environmental factors
   A. Ambient temperature
   B. Relative humidity
   C. Air flow
III. Sample cup properties
   A. Design
   B. Volume capacity
IV. Sample properties
   A. Nature of solvent
   B. Solutes
   C. Surface effects
V. Instrumental factors
   A. Mode of operation
   B. Sample cup environment
   C. Sample protection
VI. Operator technique

Factors Affecting Evaporative Loss

Many factors influence the magnitude of evaporative loss; several of the more obvious ones are listed in Table 2. By using either actual measurements of the evaporative loss or the experimental model to estimate it, we have attempted to quantitate the effects of the more important factors.

Time. Evaporation is a time-dependent phenomenon, and thus time is a very critical factor in determining the magnitude of evaporative loss. From the moment the sample is withdrawn from the patient until the final quantitative measurement is made, evaporation can occur throughout the analytical process and cause an appreciable analytical error. For example, the data that will be subsequently presented and discussed will demonstrate that this error can be as high as 40 to 50% over an 8-h period.

Environmental factors. As would be expected, environmental factors have a pronounced influence on the evaporative loss of a liquid from a sample cup. The mathematical model discussed earlier (Equation 3) can be used to demonstrate the effect of environmental factors on evaporative loss. Figure 3 shows evaporative loss as a function of ambient temperature and relative humidity; as may be seen, it is directly proportional to ambient temperature and inversely proportional to relative humidity. The strong influence that these two environmental factors exert on evaporation may be partially responsible for the seasonal fluctuations some laboratories experience in their quality-control data.

A third environmental factor that potentially can cause the greatest evaporative loss is the magnitude of air flow within the laboratory. As the air flow increases across the surface of a liquid/air interface, vapor is swept with it, providing a driving force for evaporation. For this reason, sample covers are provided with most instruments. However, as will be discussed later, appreciable evaporative loss can occur even when such covers are used.

Sample cup properties. Although the evaporation of liquid from a sample cup is basically a function of the interaction of several environmental factors, its magnitude is influenced by the geometry, design, and physical properties of the cup.

The effect of sample-cup geometry was determined by placing various volumes of distilled water into various types of tared sample cups and then weighing them at specified times to determine evaporative losses. Between weighings, unless otherwise specified, the sample cups were covered. The cups that were
studied and their critical dimensions and volumes are listed in Table 1. Cross-sectional views of the cups are illustrated in Figure 4.

The evaporation rates obtained for these cups and the environmental conditions under which they were measured are summarized in Table 3. In general, the rate of evaporation was proportional to the surface area of the liquid and inversely proportional to the vertical height of the stagnant air mass which is located between the liquid surface and the top of the sample cup.

The experiments summarized in Table 3 indicated that evaporative loss depends on the type of sample cup used. Because a gravimetric study is tiresome and time-consuming and there are literally hundreds of different types of sample cups, it was desirable to develop a model that could be used to predict evaporative loss for any cup of known geometry under specific environmental conditions. Consequently, we developed the model described in the section on Materials and Methods.

The predictive capabilities of the model were evaluated by comparing the evaporative losses measured gravimetrically for the seven cups studied with those calculated by the model (Table 3). In general, the agreement was good, indicating that the model is approximately correct. Note that the discrepancies encountered always occurred when the liquid level was near the top of the cup. Under these conditions, differences between the predicted and measured values can be attributed to convective air currents and measurement errors. When the liquid level is near the top of the cup, convective air currents would be expected to have a more important effect. Since the model is based on diffusion of a vapor through a stagnant air mass, air convection would be expected to decrease the predictive power of the model, which is consistent with the data obtained in our study. In addition, when the liquid level is near the top of the cup, it is more difficult to measure the depth of the stagnant air mass; hence the predictive value of the model is more subject to error and uncertainties under these conditions.

The results of these experiments and those pre-
dicted by the model indicated a direct relationship between the volume of liquid placed in a sample cup of a particular design and the absolute quantity of liquid lost by evaporation. For example, when the evaporation data obtained from the UC cup are plotted as a function of time and sample volume (Figure 5), the largest quantitative loss was observed with the 400-μl sample, with lesser losses observed for the 300-, 200-, and 100-μl samples. Similar results were also obtained with the various volumes placed in the SMAC and MT sample cups (Table 3).

When relating evaporative loss in terms of its effect on analytical error, it can be demonstrated that it is proportional to the relative loss of liquid from the sample. For example, the initial concentration of a given solute can be expressed as:

\[ C_0 = \frac{q}{V_0} \]  

where \( C_0 \) = initial concentration of solute; \( q \) = quantity of substance; \( V_0 \) = initial volume of sample. After a finite time (\( t \)) in which an evaporative volume loss of \( V_e \) has occurred, the concentration of the solute then becomes:

\[ C_f = \frac{q}{V_0 - V_e} \]

Relating the initial to the final concentration, it can be shown that:

\[ \frac{C_0}{C_f} = \frac{V_0}{V_0 - V_e} \]

Thus the change in concentration of the substance is proportional to the relative loss of liquid from the example.

When the data shown in Figure 5 are replotted as a relative evaporative loss vs. time and sample volume (Figure 6), the 100-μl sample is found to have the greatest percentage loss, followed by the 400-, 200-, and 300-μl samples. Thus one would expect the analytical error caused by evaporation to be greater in the 100-μl sample than in the 400-, 200-, or 300-μl samples, an expectation confirmed experimentally by analyzing various volumes of an aqueous glucose solution for glucose content as a function of time. The data obtained in this experiment (Table 4) are consistent with the above observation and indicate a greater analytical error owing to evaporation for the 100-μl sample; the smallest error was observed for the 300-μl sample. Therefore, when the problem of evaporation is considered, one should relate the quantitative evaporative loss to the starting volume, because the analytical error is proportional to the relative loss instead of the quantitative loss. Consequently, the relative evaporative losses determined for the various sample cups are also listed in Table 3.

The experiments discussed above demonstrated that evaporative loss should be expressed on a percentage or fractional basis in order to relate it to analytical error. This fractional loss can also be expressed in terms of the change in depth of the liquid sample as evaporation occurs. For example, for a cup of constant diameter (Figure 1) the fractional loss (\( F \)) can be expressed as:

\[ F = \frac{h_f - h_1}{h_0} \]

where \( F \) = fraction of liquid lost; \( h_0 \) = initial depth of liquid;\(^3 \) \( h_f \) = height of stagnant air mass after evaporation; \( h_1 \) = initial height of stagnant air mass. As shown earlier in Equation 6 of the evaporation model, the final height of the stagnant air mass is given, after rearrangement, by the equation:

\[ h_f = \sqrt{h_1^2 + 2 \cdot C \cdot t} = h_1 \sqrt{1 + \frac{2 \cdot C \cdot t}{h_1^2}} \]  

(11)

The square root term for this expression can be expanded into a power series, and for small evaporation losses (e.g., when \( 2 \cdot C \cdot t/h_1^2 \ll 1 \)) the final height of the stagnant air mass is approximately:

\[ h_f = h_1 \left( 1 + \frac{C \cdot t}{h_1^2} + \ldots \right) \]  

(12)

\(^3 \)The initial depth of the liquid (\( h_0 \)) can be measured directly or calculated from the relationship:

\[ h_0 = \frac{4 \cdot V_0}{\pi \cdot d^2} \]

where

\( V_0 \) = initial volume of sample, and

\( d \) = diameter of the cylindrical section of the cup.

It should also be noted that this expression can be used to obtain an effective liquid depth should the cup contain a conical bottom.
Substituting this expression for \( h_f \) into Equation 10, the fractional loss of liquid becomes:

\[
F \approx \frac{h_1 \left( 1 + \frac{C \cdot t}{h_1^2} \right) - h_1}{h_0},
\]

(13)

which reduces to

\[
F \approx \frac{C \cdot t}{h_1 \cdot h_0}.
\]

(14)

Note that Equation 14 predicts that the fraction of liquid loss will increase linearly with time, and for a given cup will depend on the product of the initial depth of the liquid in the cup and the height of the stagnant air mass located over it as well as the atmospheric parameters included in the constant \( C \). The height of the stagnant air mass can also be related to the depth of liquid by the expression:

\[
h = h_0 + h_1,
\]

(15)

where \( h \) = the height of the sample cup. Then the rate of fractional liquid loss, \( R \), for short times (small losses) is:

\[
R = \frac{dF}{dt} = \frac{C}{(h - h_0) \cdot h_0} \cdot p \cdot M_{H_2O} \cdot \left( D_{H_2O-air} \right) \cdot \ln \frac{P_{air2}}{P_{air1}}.
\]

(16)

In addition to predicting the evaporation rate for a given cup, Equation 16 also suggests an optimum depth for filling the cup with liquid to reduce the effects of evaporation. The rate of fractional evaporation loss, \( R \), is a function of \( h_0 \) and is a minimum when the denominator of Equation 16 is a maximum. This occurs when:

\[
\frac{d[(h-h_0) \cdot h_0]}{dh_0} = 0 = h - 2h_0;
\]

(17)

e.g., when

\[
h_0 = \frac{h}{2}.
\]

(18)

Thus, to minimize the fractional evaporative loss from a sample cup, the cup initially should be only half full. This somewhat surprising relationship has been confirmed experimentally, as shown by the data in Table 3. For example, when the fractional evaporative losses obtained from the MT sample cups were plotted as a function of cup capacity (Figure 7), the minimum loss occurred when the cups were approximately half full. Similar curves were also obtained for the SMAC and UC cups. The data also suggest that for a given sample cup a useful volumetric range to minimize evaporation would be from 25 to 75% of the cup capacity. Sample volumes below or above this range would be more severely affected by evaporation.

Sample properties. The chemical and physical properties of a sample will affect evaporative loss. For example, the properties of the solvent in which the solutes of the sample are dissolved or suspended are important in determining the magnitude of the loss. In biological samples, this solvent is water; therefore, evaporation from such samples is a function of the physical properties of water, including vapor pressure, diffusivity, density, molecular weight, etc. When a liquid other than water is used as the solvent (e.g., an organic solvent from an extractive process), its physical properties will be different and the rate of evaporation will be affected accordingly. In most cases, the organic solvent will have a greater vapor pressure than water and a greater evaporative loss.
The inter- and intramolecular forces exerted between the solutes and solvent of a liquid sample will also affect evaporative loss. For example, it has been reported that evaporation of water was more rapid from buffered solutions of ovalbumin or hemoglobin than from solutions of various surface-active agents or from buffer alone (8). In a gravimetric study of the comparative evaporative losses of water versus serum samples as a function of volume size, we found the evaporative loss from serum samples to be consistently higher than those from water samples (Figure 8).

There is also an interaction between the surface properties of the sample and the material used to fabricate the sample cup. If the surface of the cup is wet by the sample, the deeper meniscus will provide additional surface for evaporation. If the surface of the cup is nonwettable, the sample will have a shallow meniscus that will result in relatively less evaporative loss than from a similar cup fabricated from a wettable material. Thus, one means by which evaporative loss can be decreased is to use cups made of nonwettable material.

**Instrumental factors.** Several instrumental factors, for example, the type of instrument used and the method of operation, influence the analytical error caused by evaporation. In a continuous-flow or discrete-type analyzer that is electronically calibrated against the chemical responses obtained from reference standards, evaporative loss may not be a problem, especially if the reference and patients' samples are treated exactly the same. Under these conditions, as evaporation occurs, a similar volume of liquid is lost from each; thus the analytical error caused by evaporation would be masked because of the ratioing mode in which the machine is calibrated. However, one should be aware that, because evaporation will occur at different rates for serum and aqueous samples (see Figure 8), evaporation may cause analytical problems when aqueous solutions of standards are used for calibration and sera obtained from patients are the samples being analyzed. In addition, to minimize the effects of evaporation, similar volumes of standards and patient samples should be placed into the sample cups.

The instruments in which evaporation can potentially cause the greatest analytical error are the discrete-type analyzers that are operated in the batch mode, offer microvolume capabilities, and use stochiometric factors instead of chemical calibration factors. For example, with these instruments, small volumes of samples (usually 500 µl or less) are poured into cups that are subsequently placed into a carousel. During the course of the day, aliquots of sample are repetitively sampled from these cups as the technologist performs several different chemical analyses on them, sequentially and batchwise. Hence, the samples may remain in the cups for as long as several hours. Under these conditions, progressive evaporation of solvent from the small volumes involved will significantly increase the concentration of
the dissolved analytes, especially if preventive measures are not taken.

Previously we have discussed the role that environmental factors have on the magnitude of evaporative loss. The manner in which an instrument maintains temperature control can also influence evaporative loss if it changes the immediate or proximal environment of the samples. For example, evaporative loss will be increased in a machine that maintains the samples at an elevated temperature such as 30 or 37 °C. In addition, if an open water bath is used to maintain temperature, it may increase the relative humidity and thus decrease evaporative loss.

A fourth factor is governing the evaporative loss that might be expected from a particular instrument is the technique or means used to prevent or minimize evaporation. In most instruments, this consists of a cover that fits over the sample carousel and thereby protects the surface of the sample. However, one should be aware that appreciable evaporative loss can occur even if the sample cup is covered and convective air currents are eliminated. This loss is predicted by the evaporation model and was confirmed experimentally by measuring the evaporative loss from both covered and uncovered UC cups that contained 300-μl aliquots of water. In this study, the covered and uncovered sample cups had evaporative losses of 2.8% and 4.9% per hour, respectively (Table 3). Thus evaporative loss was retarded by covering the sample, but the loss even from the covered cup was appreciable.

Technique. Another factor that influences the magnitude of evaporative loss is operator technique. For the most part, technique involves the use of common sense and good laboratory practice. For example, if the system requires sample covers, the operator should make a conscientious effort to use them, even though it may delay his work schedule slightly. In addition, he should not place a loaded sample carousel in an area where there is a draft or in a sunny location near a window. In general, evaporative loss attributable to technique can be minimized if the operator is aware of the problem and exercises care in his work.

Minimizing Evaporative Loss

These studies show that evaporative loss is a serious analytical problem and must be minimized. Several solutions to achieve this goal are possible:

1. Control and maintenance of the laboratory environment. The three factors that may be controlled to minimize evaporative loss are temperature, relative humidity, and air flow. As in most situations, a compromise must be made, since the environmental conditions that would result in decreased evaporative loss (i.e., low ambient temperature, high relative humidity, and decreased air flow) would be very uncomfortable for the operator and might also result in operating problems with the system. It becomes obvious, then, that a compromise must be made between comfort and minimized evaporative loss, which, under these conditions might mean an air-conditioned laboratory having an ambient temperature of 21 °C (70 °F) and 50% relative humidity.

If fluctuations in the laboratory environment are a recognized problem, one possible solution⁴ is the use of a chamber in which the samples are maintained at a reduced temperature and at an increased relative humidity during the time they are not actively in the loading process. However, care should be taken that condensate collecting on the walls and ceiling of the chamber does not fall into the sample cups and dilute their contents. A simple alternative to the use of an environmental chamber would be to cover and place the sample carousel in a refrigerator between loadings.

2. Covering the sample. Theoretically, evaporation can be minimized by keeping the specimen stoppered and the sample covered at all times and by minimizing the volume of the stagnant air mass located between the liquid level of the sample and the cover. However, since aliquots of the specimen must be sampled for analysis, this is often not a practical solution unless a means of sampling through the cover is available. Two options are available for this: (a) the cover would be of a rigid, semirigid, or permeable membrane that is punctured by the sampling probe; or (b) the cover could be an immiscible liquid such as a silicone fluid layered over the top of the sample and through which the probe can easily pass. Both of these options have disadvantages; the first requires a strong and inflexible probe, the availability of which is limited, especially for 1- to 10-μl volumes of sample, while the second risks contaminating the sample probe with the silicone fluid. Also, as shown in Figure 9, care must be taken to ensure that an adequate volume of silicone fluid is used; otherwise part of the surface of the sample will be exposed and evaporative loss will still occur.

3. Sample cup selection. As demonstrated earlier (Table 3), evaporative loss occurs at different rates from cups having different cross-sectional designs. In general, evaporative loss is less for tall cups (cups 1, 2, and 6) and greater for short cups (cups 3, 4, 5, and 7). Thus evaporative loss can be minimized by the use of a particular cup design having a relatively large height-to-diameter ratio. This condition is fulfilled by the 0.2-ml Microtube (cup 1, Figure 4). The validity of this selection is predicted by the mathematical model and has also been confirmed experimentally (Figure 7, Table 3).

The minimal evaporative loss from the Microtube led us to consider using this type of cup in the sample/reagent loader (5) that has been developed for use with the miniature Centrifugal Fast Analyzer. When selecting a sample cup, one has to consider both theoretical and practical aspects. For example, our experimental results, as well as the mathematical

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⁴ Dr. James Fenton, Institute for Health Research, San Francisco, California; personal communication.
model, indicated that evaporative loss can be minimized by decreasing the diameter of a cup relative to its height. However, there is a practical limitation to the benefit gained by this approach, because filling the cup with sample becomes an increasing problem as the diameter of the cup is decreased. In addition, the sampling probe that is inserted into the cup may also impose a practical limitation on the minimum diameter of the cup. These problems were considered specifically with regard to the dimensions of the Microtube cup. It was found that samples could be very easily introduced into this cup via a disposable pipette or a similar type of pipetting device. In addition, we found that it was possible, with the assistance of a probe guide, to position a sampling probe over the cup and then direct the probe into its contents for sampling. Consequently, the turntable of the sample/reagent loader was modified to accept a carousel that holds 17 of the Microtubes (Figure 10).

To demonstrate that the use of Microtubes will minimize the analytical effects of evaporation, we conducted an experiment in which various volumes of an aqueous standard solution of glucose were placed into Microtubes and their glucose content was measured as a function of time. The volumes studied were 50, 100, and 200 μl, with four replicate samples being assayed for each volume. For reference purposes, four tubes were filled with 200 μl each and capped between loadings. The other tubes remained open during the entire experiment. The results (summarized in Table 5) indicate that very little evaporation occurred. For example, the 8-h loss was only 3.5% for the 50-μl sample, while the losses for the 100- and 200-μl samples were hardly detectable. The samples were also assayed 24 h after the start of the experiment. At this time, evaporation had begun to have a noticeable effect on the glucose concentration of the uncapped samples; however, this is merely illustrative and would not present a problem in routine analyses, because samples are not usually allowed to remain in their cups that long.

This study with the Microtubes pointed out an additional advantage that was gained by using these cups, i.e., sample volumes as small as 50 μl could be used and processed. In fact, by positioning the sample probe to sample at the bottom of the tube, a 10-μl aliquot can be obtained from a total sample volume as small as 20 μl. This low-volume capability should prove very useful in pediatric and small-animal clinical studies.
In summary, current studies have demonstrated that the loss of liquid from a sample caused by evaporation can be a serious problem, especially when microliter volumes are involved. The magnitude of this loss is determined by the interrelationship of a complex set of variables that include environmental, instrumental, and operational factors as well as the chemical and physical properties of the sample and its container. Because the error caused by evaporation can, in some instances, be as high as 25 to 50%, steps must be undertaken to minimize its effect. Evaporative loss can be minimized by controlling the laboratory environment, selecting a sample cup of a favorable design, filling the cup only half full of sample, using effective sample covers, and following good laboratory practice. In this way, the analytical error, even with microliter volumes of sample, can be held to 1 to 2% or less.

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