Determination of Copper in Fingernails by Atomic Absorption with the Graphite Furnace

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Two atomic absorption techniques, acid digestion followed by conventional flame analysis and direct analysis of solid samples with the graphite furnace, were compared for the determination of copper in fingernails. Nail samples, from 11 persons, containing between 5 and 50 μg/g of copper, were analyzed by both methods. There was good correlation between results of the two. The furnace requires less sample preparation, has good sensitivity, and solid samples may be directly analyzed.

Additional Keyphrases toxicology of copper • direct analysis of samples • nonhomogeneity of fingernails • toenails • cystic fibrosis • pediatric samples • aid to diagnosis • normal values • trace-element analysis

Analysis of fingernails as a means of monitoring trace elements in the body is attractive because the sample is easily collected without discomfort to the subject. A recent paper (1) summarizes much of the available information on fingernail analysis and describes the application of flame atomic absorption to the determination of five elements in fingernails from a group of normal subjects.

Harrison et al. (2) have indicated that the concentration of copper measured by neutron activation in the fingernails of newborns could be used to detect cystic fibrosis. According to them, the fingernails of normal infants contain 2–30 μg/g copper, those of affected infants contain 50–200 μg/g. The present work represents an effort to determine if atomic absorption can also be used to determine copper in fingernails. If it were feasible, atomic absorption analysis would have the advantages that atomic absorption spectrophotometers are far less costly and considerably easier to use than neutron activation equipment. Initial estimates showed that conventional atomic absorption analysis, in which the samples are digested in suitable acids and then atomized into a flame, might be adequate for the measurement of copper in samples from adults, but that the sensitivity would be no better than marginal for the smaller samples to be expected from infants. It was therefore decided to investigate also the use of the graphite furnace, a recently developed atomic absorption sampling device of far greater sensitivity, which might additionally be able to analyze solids directly, without prior dissolution.

Experimental Observations

The original objective of this study was to work out procedures applicable to the determination of copper in infants’ fingernails. However, since typical samples from infants weigh only 1–3 mg (3), we chose to obtain samples from older volunteers—nine adults and two teenagers—to have enough of each sample to permit analysis by both the furnace–solid sampling method and by conventional digestion–flame atomic absorption techniques. Three of the subjects provided toenail samples, six provided fingernails, and two provided both, which were treated separately, for a total of 13 samples.

The samples ranged in size from 30 to 60 mg. For the initial direct furnace work, samples no larger than 0.3 mg could be used, as shown in Table 1 and explained later in this paper. Un-
fortunately, the distribution of copper in fingernails is quite nonhomogeneous, as reported by Harrison and Clemena (4) and confirmed by us in preliminary tests. It is therefore not quite straightforward to perform the necessary task of comparing the results of direct analysis in the furnace to those of dissolution and aspiration into a flame.

When an entire sample is dissolved, the analytical result represents an average value, which may or may not agree with the result from one small chip analyzed in the graphite furnace. If very small chips are used, results from several must be averaged to obtain a value that will correlate well with a corresponding value by the flame technique.

The procedure adopted was to reduce each sample to small pieces, on the order of 0.2 to 1.0 mg, minimizing contamination by using a pair of forged-steel toenail clippers and tweezers to handle the nails. Sufficient pieces were selected at random and saved for analysis with the furnace. The remaining sample was then digested and analyzed by flame methods. If the selection process was truly random, and if both techniques were valid methods for the analysis, good correlation would be expected between the flame data and the average of a number of analyses with the furnace. From the spread of furnace values, it should then be possible to determine the degree of nonhomogeneity in the samples.

Acid Digestion–Flame Atomic Absorption Method

The dissolution method used for these experiments was adapted from that of Harrison and Tyree (7). These authors describe the problems of cleaning samples before analysis, so as to remove contamination without leaching out the trace elements. In our experiments, we instructed the donors to scrub their nails carefully with soap and water, and confined ourselves to rinsing the samples in de-ionized water followed by drying at 110°C for 1 h. About 30 mg of dried fingernail was weighed on a Cahn Gram Electrobalance (Model 1501), placed in a 10-ml volumetric flask, and 1 ml of concentrated nitric acid followed by 0.5 ml of 60% perchloric acid were added. The samples were then heated gently until a clear yellow liquid was obtained (about 1 h), cooled, and diluted to volume with de-ionized water.

Each sample solution was analyzed four times with a Model 403 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp.). The standard conditions (5) for copper, the maximum scale expansion, and the “100-Average” mode were used. The results were read on the digits. Each sample analysis was bracketed by a blank and a standard containing either 50 or 100 μg of copper per liter. Acid blanks were also prepared and analyzed in the same manner.

Several samples were analyzed both by the method of additions and by direct comparison with aqueous standards. No significant difference was found between the two sets of data. We therefore decided that simple aqueous standards could be used. The usual practice—adding the same amount of acid to the standards as contained in the sample solutions—was avoided because of a significant copper contamination in the available acids. The sample solutions were found to contain between 10 and 130 μg of copper per liter after correction for an acid blank of about 10 μg of copper per liter.

Furnace–Solid Sample Method

The graphite furnace procedure was considerably simpler than the method described above. A sample of the proper size was weighed, placed in the “Solid Sampling Spoon,” and inserted into the furnace. It was then ashed for 20 s at 1000°C and atomized for 10 s at 2200°C. Aqueous copper solutions were used for standardization.

The principle of the graphite furnace has been described previously (6, 7). Basically, it is a hollow graphite cylinder about 50 mm long and 10 mm across, so placed that the sample beam passes along its axis. Liquid samples, in volumes up to 100 μl, are pipetted into it through a small hole near its center. Solid samples are inserted from the end by means of a Sampling Spoon (see Figure 1). The cylinder is heated by a current passing along its walls; it is located in a water-cooled enclosure that is continuously purged with nitrogen or argon.

The power supply we used was the Model HGA-2000 (Perkin-Elmer Corp.), which differs in some respects from previously described equipment (6, 7). Current is provided by a supply that is regulated against changes in line voltage and contact resistance. Drying, charring, and atomization temperatures can be varied from ambient temperature to 2700°C, and time is continuously vari-

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Copper for 0.5 absorbance units</th>
<th>Sample weight for a 0.5 absorbance units signal, assuming 50 μg of Cu/g of nail</th>
</tr>
</thead>
<tbody>
<tr>
<td>324.7</td>
<td>7.5</td>
<td>0.15</td>
</tr>
<tr>
<td>327.4</td>
<td>15</td>
<td>0.3</td>
</tr>
<tr>
<td>249.2</td>
<td>500</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 1. Working Ranges of Copper Lines Examined
able over appropriate intervals. Once set up, the whole sequence operates automatically. A meter on the power supply is calibrated directly in furnace temperature.

The analytical results take the form of relatively sharp absorbance peaks on a laboratory recorder, as shown in Figure 2. The peak height is a measure of the element of interest. In the system that was used, the recorder scan starts automatically 15 s before atomization begins, and terminates automatically 15 s after it ends.

For determining copper in fingernails, the furnace technique is almost too sensitive. The analyst is forced either to use very small samples, creating possible problems with nonhomogeneity, or to seek a less sensitive wavelength at which to perform the analysis. It was known from the flame analyses that our samples contained a maximum of 50 μg of copper per gram, so for our experiments, we were interested in analyses up to 50 μg/g.

We constructed analytical curves at several potentially useful copper wavelengths by analyzing aqueous standards added to the furnace with Eppendorf pipets. In all cases the calibration curves were adequately linear up to 0.5 absorbance units, but were increasingly nonlinear above that point. It was therefore reasonable, based on the above, to select the sample size so that one containing 50 μg/g of copper would show an absorbance of about 0.5.

Table 1 summarizes sample size data for three copper lines investigated as part of this study. If the objective of the analysis is to determine copper distribution within a nail sample, then either the 324.7-nm line or the 327.4-nm line should be chosen. If, however, an overall average copper analysis is desired, the line at 249.2 nm is an appropriate choice, because larger samples are less likely to exhibit variations caused by nonhomogeneity.

Because the present study was exploratory in nature, most of the work was done at the 327.4-nm line, with small samples. To overcome nonhomogeneity, the results of a number of individual analyses were averaged before comparison with the value obtained by conventional flame techniques. A few determinations were also performed at the much less sensitive 249.2-nm line. In this case, the sample size could be made large enough so that the result of one furnace analysis represented the total sample, allowing direct comparison with flame atomic absorption.

Selection of the proper charring and atomization temperatures requires consideration of both the matrix and element being determined. When a charring temperature is chosen, it should be high enough to destroy the organic matrix material rapidly and completely, but low enough to avoid loss of the element of interest. A charring temperature of 1000°C for 20 s followed by 10 s of atomization at 2200°C was experimentally selected as appropriate for this analysis.

**Background Absorption**

Background absorption is a potential problem when samples are analyzed in the furnace. If the matrix is not completely destroyed in the charring step, smoke may be formed during the atomization cycle, absorb or scatter part of the radiation from the hollow cathode lamp, and thus increase the apparent copper concentration. If the amount of background absorption is not too great, it is possible to compensate for its presence with the Background Corrector (8). Since the background absorption is most commonly a continuum, and not discrete like an atomic line, it is possible to measure the amount of background independent of copper absorption by using a nonabsorbable line close to the line of interest.

We assumed that human fingernails contain little or no silver, and the absorption was measured at the 328.1-nm silver line. Any absorption at that line can be attributed to background. In a series
of tests, the average background absorption was equivalent to 1 μg of copper per gram, an insignificant value in comparison to the amounts found in the samples. It was thereupon decided that copper could be measured at the 327.4-nm line without background correction.

Background absorption at the less sensitive 249.2-nm copper line was also checked by using a palladium line at 247.6 nm. The average background signal was equivalent to 11 μg/g, a significant error. The Deuterium Background Corrector, which was found to eliminate the incorrect signal completely, was therefore used for all further work at this wavelength.

Results and Discussion

The analytical data collected at the 327.4-nm line with the furnace are summarized in Table 2, along with the flame atomic absorption data. These data demonstrate that copper is not homogeneously distributed within the fingernails of one person. These data also show that the average of five analyses of a sample with the graphite furnace correlates well with the value obtained by flame analysis.

The range of values observed for one sample with the furnace could lead one to the conclusion that the analytical technique was inadequate rather than that the sample was not homogeneous. It was not possible to obtain precision data on the samples themselves, but this type of data was compiled for the standardization runs. An aqueous standard containing either 10 or 20 ng of copper was run after every one or two fingernail samples. During the course of one working day, 26 runs of this type were made, yielding the data shown in Table 3. The solution concentrations were chosen so that a 50-μl sample could be used in each case. The precision was excellent and, because these data were obtained at various times during the day, also shows that the calibration curve is very stable and that only infrequent standardization is actually required.

Table 4 summarizes the analytical data obtained with the copper line at 249.2 nm. These data were obtained with sample left over after the other analyses had been performed, which explains the variations in sample size. In each case the sample was made up of a number of smaller pieces of nail that had been randomly selected from the original sample as discussed above. Only Sample 2 showed a significant difference between the furnace–solid sampling value and the value obtained by flame techniques. This sample also showed the greatest range among the analyses performed at the 327.4-nm line, which suggests that part of the sample may have been contaminated in some way.

Discussion

These experiments demonstrate that atomic absorption is an accurate technique for determination of copper in fingernails and toenails. Good correlation is obtained between results for the digestion–flame aspiration method and direct analysis in the graphite furnace. Flame atomic absorption is limited in sensitivity, however, and is suitable only for samples weighing more than 10 mg, which excludes pediatric work. On the other hand, the nonhomogeneity of the copper distribution in
fingernails makes it highly desirable to measure as large a portion of the total sample as possible.

There are two adequate modes of procedure. One is to digest the entire sample and, after mixing, insert an aliquot into the furnace. The drawbacks to digestion are the labor involved and the increased risks of contamination. The second procedure, which we prefer, is to take a representative sample between 5 and 10 mg and analyze it by using the secondary line at 249.2 nm. If the amount available is less than 5 mg, it is all used. The time taken per sample, including weighing, cutting up, standardizing, measurement, and computation, should take about 5 min, so that 10 samples per hour can easily be analyzed. At the 249.2-nm line, however, the use of a Deuterium Background Corrector is essential.

It is likely that slight modifications of the method may make it possible to determine other metallic elements in fingernails, and that other solid or semi-solid body materials may be analyzed directly. However, we have not yet performed such investigations.

References