

## Application of GFAAS to the Determination of Trace Metals in Blood

### **Introduction:**

25 trace elements have been determined in blood by GFAAS. Lead received the greatest attention followed in turn by Al, Cd, Cu, Mn, Se, Zn, Cr, Ni, Au, Co, Pt, Pd, Fe, V, Be, Bi, Li, Tl, As, Sn, Ga, Mo, Sb, Si, Sr and Te. The most frequently used procedures include (a) direct injection into the graphite furnace, (b) dilution with water, Triton X-100 or dilute acid (especially nitric acid), (c) deproteinization with acid (*i.e.* nitric acid) or alkali (ammonia) or (d) matrix modification.

### **Direct Injection:**

A hospital or clinical laboratory prefers direct methods because they are simple and rapid, involve little sample pre-treatment and facilitate large sample throughput, especially with automated sample introduction. Direct methods should however be used with caution. Excessive nonspecific absorption can occur due to the matrix constituents of the blood sample. When a deuterium lamp is used for background correction, it is important to ensure that the deuterium emission is optically coincident with the hollow cathode lamp emission. Problems of foaming, frothing and splattering of the blood sample along with a buildup of carbonaceous residues in the graphite tube may also arise when direct injection is used.

### **Dilution:**

Dilution of blood with water or Triton X-100 minimizes background absorption. The extent of background reduction depends on the dilution factor, which, in turn depends on the concentration of the element of interest in the sample. For example, a dilution factor of 50 to 100 can be used for Cu, Fe and Zn. At such a high dilution, matrix effects are not significant and these elements can easily be determined. For the remaining elements, the maximum allowable dilution factors are rather marginal and therefore considerable background absorption and residual buildup can occur. This is especially true for volatile elements such as arsenic, lead, cadmium and selenium. The maximum ashing temperatures for these elements are below 500°C. At this temperature the matrix components of the blood sample can only be partially destroyed. The use of a higher ashing temperature for which the matrix species can be completely removed would result in volatile loss of the target analyte.

## **Matrix Modification:**

Various matrix modification procedures have been developed to overcome the difficulties that arise with direct injection and dilution methods. For example, addition of ammonium phosphate or ammonium phosphate plus magnesium nitrate can stabilize Cd and Pb to 650-850°C and addition of Ni or Pd can stabilize As and Se to 1100-1400°C. At these temperatures, the organic matter in blood will be completely destroyed. Inorganic matter might still be present, but its chemical interference can be minimized by isothermal atomization. When a suitable background correction is used, normal calibration methods can be used.

## **Deproteinization:**

De-proteinization of blood with nitric acid can be used for the determination of Al, As, Cd, Cr, Mn, Ni and Pb. This process virtually removes the organic matter leaving the supernatant protein free. As a result, setting ashing temperature becomes less critical, especially for As and Pb. Unfortunately, de-proteinization does not eliminate chemical interferences. Therefore, matrix matched calibration is required to achieve accurate results. Another option is to use matrix modifiers to minimize/eliminate chemical interference.

## **Optimization of Temperature Program:**

Careful optimization of the temperature program is necessary for obtaining reliable and precise results. The temperature programs published in literature can only be used as a reference. In a complex matrix like blood, the mechanisms of atomization are complicated and optimization of the temperature program requires an empirical approach. Optimal temperature programs should be designed by each individual user.

Spattering and spreading of the blood sample can occur during the drying step. Furthermore, occasional problems of frothing, foaming and fogging of the quartz windows can be encountered. These problems can be prevented by careful optimization of the drying temperature and time. It is best to use multiple drying steps and increase the drying temperature gradually through ramp programming.

The ashing temperature should be optimized to remove as much of the matrix as possible without causing loss of analyte. If this is done properly the analyte peak will be temporally separated from the background peak. For this reason, the optimization of the ashing temperature should be based on both background absorption and background-corrected analyte signal. It is recommended to always use the maximum heating rate for the atomization step.