Direct Determination of Cadmium and Lead in Whole Blood by Potentiometric Stripping Analysis

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The commercially available equipment for potentiometric stripping analysis (PSA) was tested for routine lead and cadmium determination in whole-blood samples. In contrast to anodic stripping voltammetry, PSA is not subject to background interferences from organic electroactive constituents in the sample or to the presence of dissolved oxygen (i.e., oxygen removal is not necessary). To determine lead and cadmium by PSA, it is sufficient to dilute the blood sample with an appropriate supporting electrolyte (0.5 mol/L HCl). The detection limit changes with deposition time and volume of blood sample used. For 1 mL of blood and a 1-min deposition time, the detection limit is 1 μg/L for both elements. If the deposition time increases to 10 min, cadmium can be determined at its normal concentration in blood (the detection limit is improved to <0.1 μg/L). Procedures for routine determination of lead and cadmium in whole blood are presented.

Additional Keyphrases: electrochemical detection · anodic stripping voltammetry compared · potentiometry · trace elements

For routine determinations of cadmium and lead in body fluids, only a few analytical methods are suitable, given the low concentration of these elements (especially of cadmium). In interlaboratory comparisons in recent years, the analytical technique applied by the majority of the participating laboratories was electrothermal atomic absorption spectrometry (ETAAS) with various pretreatment techniques, such as chemical matrix modifiers and Zeeman background correction. Anodic stripping voltammetry (ASV), because of its sensitivity, was the only alternative method used.

Electroanalytical methods based on the stripping technique have become widely used for trace metal analysis in biological fluids. The remarkable sensitivity of stripping analysis is attributed to the preconcentration (deposition) step that takes place before the actual measurement (stripping) step. For trace metal determination in body fluids, ASV and potentiometric stripping analysis (PSA) have received attention. ASV, especially the differential pulse mode, is a very sensitive low-cost method that often allows the simultaneous determination of several metals (e.g., Cd and Pb). However, because small amounts of organic impurities in the solution may interfere with the determination by differential pulse ASV, complete digestion of the sample is necessary.

The stripping analyzer from ESA (Bedford, MA), which is widely used for clinical purposes (4–7), utilizes the staircase stripping mode. The sensitivity of this equipment is not sufficient to determine the low concentrations of cadmium ordinarily present in whole-blood samples (5). However, lead can be quantified in blood with this instrument even at concentrations <100 μg/L, but only if one has a human blood standard with lead at 20–30 μg/L (6). A polymer-coated thin mercury film electrode can be used to directly determine lead in whole blood, urine, and sweat, but the sample must be deaserated (8). PSA is more suitable for trace metal analysis in blood and serum samples (9, 10).

In this work I tested the commercially available systems of computerized PSA for routine lead and cadmium determinations in whole-blood samples after a simple pretreatment of the sample.

Materials and Methods

Instrumentation

I used a TraceLab™ and SAM20 sample station (Radiometer, Copenhagen, Denmark) in connection with a Vectra 286/12 computer (Hewlett-Packard, Palo Alto, CA). For possible automation I tested a SAC80 Sample Changer and an ABU93 Triburette, both from Radiometer. The Tap2 software package was used to carry out the analysis. For ASV after high-pressure digestion with nitric acid in a high-pressure ashing system (HPA; Hans Küerner, Rosenheim, FRG) (11), I used the Model 384B Polarographic Analyzer from EG&G (PAR, Princeton, NJ). All glassware, pipette tips, and vessels were cleaned several times with 10-fold-diluted nitric acid. All operations of sample preparation and determination were done in a laminar-flow hood with a high-efficiency particle-accumulator filter (Karl Blaymehl, Jülich, FRG).

Chemicals

A mercury plating solution with Hg(II), 800 mg/L, in 1.3 mol/L hydrochloric acid was prepared from Hg₂Cl₂ (p.A. grade; Merck, Darmstadt, FRG) and concentrated hydrochloric acid (30%, Suprapur; Merck); alternatively, I used the plating solution supplied by Radiometer (no. S2201).

Samples

Certified Reference Materials (CRMs) BCR 194, BCR 195, and BCR 196 (Community Bureau of Reference, Brussels, Belgium) and two standards prepared in our
laboratories (stored frozen at -150 °C) were used for testing the analytical method. All remaining samples were analyzed as part of international comparison programs or were taken from apparently normal people in our medical institute. These blood samples were stabilized with K2 EDTA (Monovette; Sarstedt Inc., Newton, NC).

Procedures

**Mercury plating procedure.** Every morning the glassy-carbon electrode was polished with aluminum oxide powder (0.3-μm particles) and rinsed with water. After mixing 10 mL of water and 1 mL of plating solution in the 5-20-mL beaker (Radiometer, code no. 904-488), I set the potential to -3000 mV (vs a saturated standard calomel electrode) and the stirrer to "stir 5." The evolution of hydrogen bubbles on the electrode surface marks the cleaning of electrode surface by this procedure. After a few seconds I turned off the potential, rinsed the electrode with water, and then started the plating procedure. After being plated for 6 min at different potentials (starting at -300 mV, then decreasing at 1-min intervals by 100 mV, to end at -800 mV), the electrode surface is covered with a mercury film. This film can be used for several analyses (all day or for several days if the potential is maintained over an extended period). Before determining cadmium and lead by ASV, the whole-blood samples are digested with nitric acid in the high-pressure ashing system.

**Lead determination.** I mixed 9.3 mL of water with 0.1-0.25 mL of whole blood in the standard 5-20-mL beaker (no. 904-488), 0.5 mL of concentrated hydrochloric acid, and 0.2 mL of plating solution. I placed the beaker at the SAM20 sample station and started the program for lead determination. The deposition time for lead with stirring was 1 min at -800 mV and the curve was recorded after an equilibrium time of 30 s. To calibrate the sample with only one standard addition, one should have the standard addition increase the concentration of lead by fivefold. If two or more standard additions are used, then each can be approximately twice the analyte concentration in the sample solution used. To precondition a freshly prepared mercury thin-film electrode, I performed one deposition/stripping cycle in the sample before using the electrode for trace metal determination.

**Combined cadmium and lead determination.** I mixed 9.3 mL of water with 0.5 or 1 mL of blood in the standard beaker, then added 0.5 mL of concentrated hydrochloric acid and 0.2 mL of plating solution. Cadmium and lead were deposited at -1150 mV. The deposition time depended on the cadmium concentration. For cadmium concentrations <0.1 μg/L, the deposition required 10 min; for concentrations of ~1 μg/L, 1 min was sufficient. All other steps are similar to those described for the lead determination procedure.

Results and Discussion

Optimization of the Procedure

In stripping techniques three conditions are most important: deposition potential, deposition time, and sample volume. Figure 1 demonstrates the influence of the deposition potential on the peak area of cadmium and lead in diluted CRM BCR 196 at constant deposition time. The optimal deposition potentials for lead and cadmium determinations, under the described conditions, ranged from -1100 to -1300 mV (vs a saturated standard calomel electrode). At constant deposition potential (~1150 mV), the peak area changes with the acid concentration (see Figure 2), for two reasons: the liberation of cadmium and lead ions and the evolution of hydrogen. At pH <2, all cadmium and lead ions are electrochemically available. An increase of H+ concentration increases the amount that can be reduced to form H2 gas. The small hydrogen bubbles destroy the absorption layer of organic substances at the electrode, so the peak areas for cadmium and lead increase. For very high H+ concentrations or at more negative deposition potentials, the evolution of hydrogen gas on the electrode surface (large H2 bubbles) decreases the electrode surface, so the peak areas for lead and cadmium decrease also.

The lead and cadmium peak areas also change linearly with the blood volume used (Figure 3). This is the most important factor for cadmium determinations in whole-blood samples at "normal" concentrations. "Normal" lead concentration in whole-blood samples is <30 μg/L (12). For cadmium the "normal" concentration can be <1/300 of the lead concentration (13). Given these differences, it is very difficult to determine both elements at the same time. For lead determination a sample size of 50 μL is adequate; however, handling such a small sample needs an experienced technician, given the risks for contamination or volume errors when transferring the whole-blood samples (the first volume pipetted must be discarded). For cadmium quantification, ≥0.5 mL of whole blood is necessary.

The third variable that influences peak area is the deposition time. As expected, peak areas of cadmium and lead change linearly with the deposition time (Fig-
Figure 3. Change of peak area with sample volume (BCR 196) and deposition time.
Deposition potential -1150 mV; deposition time 3 min. Conditions as in Fig. 1.

figure 3). The deposition time determines the total analysis time. If the total analysis time for both elements with two standard additions is to be <10 min, then ≥1-mL samples of whole blood must be used for the cadmium determination at normal concentrations. If only very small sample sizes are available for the determination, a longer deposition time is needed (especially for cadmium). Moreover, because of the very low cadmium concentration in whole blood, all operations with this sample must be done (if possible) under clean-bench conditions.

Figure 4 demonstrates the influence of the sample volume and the deposition time on the reproducibility of the peak area. If the peak area is ~5 s/V, the CV varies between 5% and 10%. At normal cadmium concentrations in blood, sufficiently good results can be obtained by using 1 mL of sample and a 1-min deposition time (or 0.1 mL of sample and a 10-min deposition time). For normal lead concentrations, 0.1 mL of sample and a deposition time of 1 min are adequate.

The mercury(II) concentration in the solution influences the sensitivity of the determinations. If the Hg²⁺ concentration in the solution is increased, the time needed for oxidation of lead and cadmium from the amalgam is reduced and the sensitivity decreases. To minimize environmental pollution by mercury (waste water and the laboratory environment), the concentration of Hg²⁺ in the solution should be kept low. I found that for low concentrations of Hg²⁺ (<4 mg/L) the reproducibility of the peak area is poor (CV 10–15%). For Hg²⁺ concentrations >40 mg/L, the CV of the peak area is ~2%. I chose an Hg²⁺ concentration between 8 and 20 mg/L as a compromise between peak reproducibility and environmental pollution.

The concentration of hydrochloric acid is also important in these lead and cadmium determinations (Figure 2). At greater pH values (pH >3), the complexing agents in the blood sample (EDTA, proteins, etc.) form complexes with lead and cadmium, so that no oxidation peaks for these elements appear. At low pH values (pH <0.5), the proteins in the sample coagulate; however, this does not substantially influence the results obtained.

As Figure 2 shows, the cadmium and lead peak areas depend very strongly on the pH of the whole-blood sample. At pH <0.6, the cadmium peak area changed with time, probably because of an exchange between free and bound cadmium ions. At pH 1.5, after 1 h only ~80% of the cadmium ions are electrochemically active at the deposition potential of ~1150 mV. For lead, the exchange between bound and free ions is very quick and
in all cases (pH < 2) the total amount of lead is determined (see data in Table 1). From these experimental data it can be concluded that "speciation" of elements could also be carried out by PSA.

The observed change of the peak area with pH affects the sensitivity of the determination for lead. The peak area for lead at pH 0.6 is ~75% greater than at pH 1.5. For cadmium this change is even more significant. The probable explanation for this phenomenon is the self-cleaning of the electrode surface, which removes adsorbed surfactants by hydrogen evolution.

Jagner et al. (10) used high concentrations of Triton X-100 in the supporting electrolyte for PSA determination of lead in blood. They developed a new type of electrode for very small sample volumes without a stirrer. In my system, stirring is a condition that can be used to increase the sensitivity. At greater rotational speeds, the amount of reduced elements on the electrode surface increases (the peak area is larger). The optimal stirrer rotation speed for a volume of 10 mL, determined experimentally, is 5–7. At higher stirrer rotations the solution becomes turbulent.

Figure 5 demonstrates the influence of Triton X-100 on the peak areas for lead and cadmium determinations in blood. For lead, increasing the Triton X-100 concentration makes no substantial differences in the peak area. For cadmium, a very low concentration of Triton X-100 greatly reduces the peak area. At higher Triton X-100 concentrations (>10 mL/L), the stirred solution is saturated with air bubbles, which can be adsorbed on the electrode surface. For cadmium determinations in stirred blood samples, the addition of Triton X-100 is not useful.

Determinations in CRMs

Lead and cadmium concentrations are greater in CRMs than in normal blood samples. Thus the determination of lead in CRMs by PSA is a very rapid and easily performed analytical procedure. The total time needed
for one determination is ~5 min (with two standard additions). For lead concentrations >0.5 μmol/L (100 μg/L), the shorter deposition times can be used, which shortens the determination time considerably. Cadmium concentrations in CRMs are 3- to 100-fold greater than those found in normal blood. Thus the cadmium determination in these samples is easier and less time consuming than in real whole-blood samples. Table 1 shows lead and cadmium results obtained by PSA in some CRMs. The agreement between certified values and measured values is very good.

Determinations in Fresh Blood Samples

Lead and cadmium concentrations in fresh blood samples (not frozen, but analyzed as quickly as possible after sample collection) are lower than have been observed in the available CRMs. The lead values in these fresh samples varied between 40 and 60 μg/L, similar to the concentrations found by Novak et al. (12). The corresponding cadmium value was <1 μg/L; in only a few samples were higher values found. All fresh blood samples are deep red after dilution with water. Adding hydrochloric acid to these solutions changes the color to brown or black (depending on the proportion of blood in the diluted sample) and eliminates the differences between fresh samples and CRMs. If the fresh blood samples are stored by freezing at -20°C, the color of the stored samples changes with time to brown or black (similar to the CRMs). Figure 6 demonstrates the effect of storage time on the cadmium and lead determinations in three blood samples. The increased cadmium concentration in these samples could indicate that the blood was taken from smokers or was contaminated during sampling. Given the lack of effect of the time from the collection of the sample to its analysis, lead and cadmium can be determined in fresh whole-blood samples directly after sampling.

Method Evaluation

Results of interlaboratory comparison. Part of the evaluation for the described method involved participation in interlaboratory comparison studies. Table 2 presents the results of an external quality-control program organized by the Danish National Institute of Occupational Health. In only one case was the cadmium concentration measured lower than the values found by the reference laboratories. The second round of the interlaboratory comparison was started in spring 1991 for the determination of lead only. The results of this external quality assessment are reported elsewhere (1).

Internal quality control. To test the quality of the lead and cadmium determinations by PSA in whole-blood samples, I compared this method with other analytical methods: ETAAS, the method most widely used for trace element determination in whole-blood samples, and stripping voltammetry, the latter requiring the digestion of blood samples by high-pressure ashing (14). As shown in Table 3, in all concentration ranges the values found by PSA agree well with values found by other analytical methods.

Day-to-day precision. Table 4 presents the results of day-to-day precision studies for lead and cadmium determinations in four different blood samples. The precision depended strongly on the quality of sample preparation and standard addition. Preliminary experiments with the autosampler have demonstrated a significantly better precision for some samples if automated standard addition was used (data not shown).

Advantages of PSA for Cadmium and Lead Analysis

PSA, used here for direct determination of lead and cadmium in whole blood, may also be suitable for the direct determination of these elements in serum (9) and urine (15). In comparison with ASV, PSA has several advantages. Sample preparation for PSA consists of simple dilution with an appropriate supporting electrolyte. The contamination or loss of elements by sample digestion before an ASV determination is eliminated. Sample deoxygenation also is not necessary in PSA. The determination sensitivities of PSA and ASV are very similar, but the time needed to determine lead and cadmium in whole-blood samples by PSA is considerably less than by ASV (also without digestion). The ESA Model 3010A stripping analyzer and the TraceLab analyzer determine lead within similar times, but the TraceLab can also determine cadmium.
Table 2. Interlaboratory Survey Results of Cadmium and Lead Determinations

<table>
<thead>
<tr>
<th>Sample</th>
<th>PSA*</th>
<th>Other labs*</th>
<th>PSA*</th>
<th>Other labs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEQAS1/1</td>
<td>33 ± 2</td>
<td>32.6 ± 5.5 (19)</td>
<td>0.172 ± 0.009</td>
<td>0.173 ± 0.029 (24)</td>
</tr>
<tr>
<td>DEQAS1/2</td>
<td>28.7 ± 1.4</td>
<td>28.2 ± 4.8 (20)</td>
<td>0.356 ± 0.017</td>
<td>0.372 ± 0.050 (25)</td>
</tr>
<tr>
<td>DEQAS1/3</td>
<td>16.5 ± 3.5</td>
<td>23.2 ± 4.7 (20)</td>
<td>0.705 ± 0.026</td>
<td>0.707 ± 0.091 (26)</td>
</tr>
<tr>
<td>DEQAS1/4</td>
<td>12.1 ± 1.1</td>
<td>12.6 ± 2.5 (17)</td>
<td>1.038 ± 0.053</td>
<td>1.228 ± 0.134 (25)</td>
</tr>
<tr>
<td>DEQAS1/5</td>
<td>9.0 ± 0.7</td>
<td>8.5 ± 1.6 (16)</td>
<td>1.606 ± 0.067</td>
<td>1.745 ± 0.150 (25)</td>
</tr>
</tbody>
</table>

* Five determinations of each sample.
*23 participants (for n < 23, not all laboratories presented usable results).
*29 participants (for n < 29, not all laboratories presented usable results).
* Listed in parentheses is n = results used for mean calculation.

Table 3. Cadmium and Lead Concentrations (μg/L) in Whole-Blood Samples Measured by Various Methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cadmium</th>
<th>Lead</th>
<th>Cadmium</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>ETAAS</td>
<td>PSA</td>
<td>ETAAS</td>
<td></td>
</tr>
<tr>
<td>Blood 1</td>
<td>3.41</td>
<td>3.74</td>
<td>30.1</td>
<td>32.0</td>
</tr>
<tr>
<td>Blood 2</td>
<td>3.03</td>
<td>3.11</td>
<td>80.2</td>
<td>75.0</td>
</tr>
<tr>
<td>Blood 3</td>
<td>2.71</td>
<td>2.78</td>
<td>139</td>
<td>134</td>
</tr>
<tr>
<td>Blood 4</td>
<td>1.88</td>
<td>1.44</td>
<td>229</td>
<td>221</td>
</tr>
<tr>
<td>Blood 5</td>
<td>0.85</td>
<td>0.90</td>
<td>322</td>
<td>300</td>
</tr>
<tr>
<td>SM-A</td>
<td>0.71</td>
<td>—</td>
<td>49.2</td>
<td>49.5</td>
</tr>
<tr>
<td>SM-B</td>
<td>0.77</td>
<td>0.84</td>
<td>90.1</td>
<td>90.6</td>
</tr>
</tbody>
</table>

SM-A and SM-B: our internal control materials, prepared in German Environmental Specimen Bank. HPA, high-pressure ashing. DPASV, differential pulse ASV.

Table 4. Day-to-Day Precision of Lead and Cadmium Determinations in Blood by PSA

<table>
<thead>
<tr>
<th>Day</th>
<th>Lead, μg/L</th>
<th>Cadmium, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1a</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4a</td>
<td></td>
</tr>
</tbody>
</table>

The main advantage of PSA over atomic absorption techniques is that it allows cadmium and lead to be determined simultaneously. The PSA instrumentation is small and does not need any servicing or special installation requirements (e.g., cooling, gas supply). For cadmium and lead determination, ETAAS needs sample volumes of whole blood similar to those in PSA.

An important advantage of ETAAS over PSA, however, is automation, although it may also be possible to automate PSA (16). A preliminary test with the sampler changer (SAC80) and automated standard addition with ABU93 (from Radiometer) demonstrated that the sample volume needed for automated PSA determination must be larger [only standard beaker 12-45 mL (code no. 904-677) can be used with the minimum solution volume of 20 mL]. The sensitivity attributable to constant stirring speed is lower than that observed with the manual system. My experience indicates that this automated system works very well for the determination of lead. In this case the total volume of whole blood needed for the determination is 0.1 mL (or lower, depending on the concentration in the sample or the deposition time used). For the cadmium determination the smallest volume that can be used is 0.5 mL. The longer deposition time needed leads to a longer analysis time. If a large number of samples are to be analyzed, the determination time can be reduced by the use of a calibration plot instead of the standard addition technique. The cost of this automated system for PSA, based on commercially available equipment, is about half that of an ETAAS system with comparable sensitivity.

The future development of an automated PSA system...
for determination of elements in body fluids can, like the use of flow cells with microelectrodes, reduce the required sample volume and determination time (17).

References