Selective Polarographic Determination of Stannous Ion in Technetium Radiopharmaceutical Cold Kits

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The aim of this work was to develop a selective method for quantification of Sn(II) and Sn(IV) in dimercaptoisuccinic acid (DMSA), ethylcysteinate dimer (ECD), methylenediphosphonic acid (MDP), and pyrophosphate radiopharmaceutical cold kits by differential pulse polarography. **Methods:** A dripping mercury electrode 150 polarographic/striping analyzer with a conventional 3-electrode configuration was used with 3 M H2SO4 and 3 M HCl supporting electrolytes for Sn(II) and Sn(IV), respectively. The polarographic analysis was performed using a 1-s drop time, 50-mV s⁻¹ scan rate, −50-mV pulse amplitude, 40-ms pulse time, and 10-mV step amplitude. To quantify Sn(IV), oxidation of Sn(II) by H2O2 was performed. The calibration curves for Sn(II) in 3 M H2SO4 and Sn(IV) were obtained in the range of 0–10 μg·mL⁻¹. **Results:** The analytic curves for Sn(II) in 3 M H2SO4 and Sn(IV) in 3 M HCl were represented by the following equations: i (μA) = 0.098 [Sn(II)] + 0.018 (r² = 0.998) and i (μA) = 0.092 [Sn(IV)] + 0.016 (r² = 0.998), respectively. The detection limits were 0.21 μg·mL⁻¹ for Sn(II) and 0.15 μg·mL⁻¹ for Sn(IV). In DMSA, ECD, MDP, and pyrophosphate, 90.0%, 64.9%, 93.2%, and 87.5%, respectively, of the tin was present as Sn(II). In this work, selective determination of Sn(II) and Sn(IV) in DMSA, ECD, MDP, and pyrophosphate cold kits. **Key Words:** ⁹⁹mTc-radiopharmaceuticals; polarography; Sn(II); Sn(IV)

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Radiopharmaceutical cold kits for labeling with ⁹⁹mTc contain Sn(II) for reduction of technetium to lower oxidation states that are chemically reactive to specific ligands. Besides these ligands, the cold kits usually contain stannous chloride (SnCl₂) and different additives (complexing agents, antioxidants, and buffers). The amount of stannous ions varies (0.03–1.5 mg of SnCl₂), although a minimum concentration must be present to guarantee the lyophilized reagent shelf life and to allow efficient labeling with ⁹⁹mTc (I).

Depending on the medium composition, Sn(II) ions in low-concentration solutions (<2.0·10⁻⁴ M) are oxidized, and the formation of basic complexes takes place above pH 2.00 (2,3). The quantitative determination of Sn(II) in radiopharmaceutical cold kits is an important aspect of quality control, and the analytic method must be accurate even in the presence of Sn(IV) ions (4).

Such techniques as colorimetry, titrimetry, spectrophotometry, voltammetry, and polarography have been described in the literature for Sn(II) determination in cold kits (5–12).

Zimmer et al. studied the formation of a red porphyrin complex in the presence of Sn(II) ions using a colorimetric method. The time of the color fading was proportional to the Sn(II) concentration in pyrophosphate, methylenediphosphonic acid (MDP), and diethylenetriaminepentaacetic acid (DTPA) (5). Chervu et al. have used a potentiometric titration of Sn(II) in HCl and potassium iodate as the oxidizing agent to analyze DTPA, glucoheptonate, hepatoiminodiacetic acid, MDP, and pyrophosphate (6). Mudukrishna et al. developed a method based on the oxidation of Sn(II) using a known excess of iodate or iodine, and the unreacted excess of the oxidizing agent was titrated with thiosulfate in glucoheptonate, hepatoiminodiacetic acid, MDP, DTPA, human serum albumin, albumin macroaggregate, and pyrophosphate. The procedure was used to overcome the need to perform experiments in an oxygen-free atmosphere as required by the direct iodometric titration. Reducing agents such as ascorbic acid and human serum albumin interfered with this method (7).

Mushtaq et al. have proposed a method in which the amount of Sn(II) in various cold kits was determined by a
spectrophotometric method using palladium chloride. This method was not applicable to dimercaptosuccinic acid (DMSA), DTPA, ethylenecysteinyl dimer (ECD), or methoxysobutyl isonitrile because of ligand or antioxidant interference (δ). Boutakhrit et al. determined Sn(II) ions in DMSA by flow injection analysis with amperometric detection. Metal ions did not influence the signal of the Sn(II)-8-hydroxyquinoline complex and ascorbic acid, but medronic acid, 4-amínobenzoic acid, and pentetic acid increased or decreased the peak (9).

Polarographic analysis has been used as an alternative method in which the main advantage is the differentiation between Sn(II) and Sn(IV) ions. On a hanging drop of mercury, a redox process occurs, and a current proportional to the amount of the species taking part in the electro-chemical reaction is measured (13,14).

Polarographic methods for selective determination of Sn(II) in radiopharmaceutical cold kits were reported by McBride et al., Decristoforo et al., Lejeune et al. and Ross et al. (10–12,15).

McBride et al. proposed a differential pulse polarography method for Sn(II) quantification in pyrophosphate and polyphosphate, in which 1 M H2SO4 was used as the supporting electrolyte. Sn(IV) interference in the Sn(II) signal was not evaluated in 0.12 M HCl (16).

The aim of this work was to develop a selective method for the determination of Sn(II) and Sn(IV) (total tin) in DMSA, ECD, MDP, and pyrophosphate radiopharmaceutical cold kits.

**MATERIALS AND METHODS**

**Apparatus**

The polarographic analysis was performed using a dropping mercury electrode 150 polarographic/stripping analyzer (Radiometer Analytic), with a conventional 3-electrode configuration consisting of a static mercury drop electrode, an AgCl/Ag (saturated KCl) reference electrode, and a platinum wire auxiliary electrode. The experimental conditions were a 1-s drop time, 50-mV-s−1 scan rate, −50-mV pulse amplitude, 40-ms pulse time, and 10-mV step amplitude.

**Reagents and Solutions**

DMSA, ECD, MDP, and pyrophosphate radiopharmaceutical cold kits were from IPEN-CNEN/SP. The formulations were DMSA (1 mg) and SnCl2·2H2O (0.41 mg); ECD (1 mg), mannitol (24 mg), ethylenediamine tetraacetate acid (0.36 mg), and SnCl2·2H2O (0.125 mg); MDP (5 mg), ascorbic acid (0.10 mg), pyrophosphate (45 mg), and SnCl2·2H2O (1 mg); and pyrophosphate (10 mg) and SnCl2·2H2O (2 mg).

SnCl2·2H2O and a 1,000 µg·mL−1 standard solution of Sn(IV) were prepared in 1000 µL of 1 M H2SO4 and 3 M HCl, respectively. The polarograms in H2SO4 were obtained separately from those in HCl. The tin concentration in the cell was 2.0 µg·mL−1 (17.5 µM) for each 20-µL aliquot of 1,000 µg·mL−1 Sn standard solution in the cell was evaluated. The concentration range of each component was based on the tin-to-component ratio of the formulation.

Titrimetric determinations for Sn(II) in DMSA, ECD, MDP, and pyrophosphate were performed using 5.0 mM I2 (17).

**RESULTS**

Figure 1 shows a typical differential pulse polarogram of Sn(II) and Sn(IV) in 3 M H2SO4 and 3 M HCl, respectively. The polarograms in H2SO4 were obtained separately from those in HCl. The tin concentration in the cell was 2.0 µg·mL−1 (17.7 µM) for each 20-µL aliquot of 1,000 µg·mL−1 Sn standard solution, and after 5 successive additions the total tin concentration was 10.0 µg·mL−1 (88.5 µM).

The analytic curves for Sn(II) in 3 M H2SO4 and Sn(IV) in 3 M HCl are represented by Equations 1 and 2, respectively.

\[ i(\mu A) = 0.098[Sn(II)] + 0.018 \quad (r^2 = 0.998, n = 6) \quad \text{Eq. 1.} \]
\[ i(\mu A) = 0.092[Sn(IV)] + 0.036 \quad (r^2 = 0.998, n = 6) \quad \text{Eq. 2.} \]

where \( i(\mu A) \) is the current of the tin wave (peak height).

The detection limits for Sn(II) and Sn(IV) were 0.21 µg mL−1 (18.6 µM) and 0.15 µg mL−1 (13.3 µM), respectively.
The wave shapes, peak heights, and potentials of the maximum current for Sn(II) or Sn(IV) were compared in the absence and in the presence of increasing concentrations of each component of the formulation. Figure 2 shows the influence of MDP components (MDP, ascorbic acid, and pyrophosphate) on the peak height of 2.0–6.0 μg·mL⁻¹ (0.2–0.6 mg) Sn(II) in 3 M H₂SO₄ and 2.0–4.0 μg·mL⁻¹ (0.2–0.4 mg) Sn(IV) in 3 M HCl. The abscissa was represented in a logarithmic scale. Because the SnCl₂·2H₂O content in MDP was 1 mg (i.e., 0.52 mg of Sn(II)), 1:10 to 1:50 dilution factors were used to calculate the mass range of the reagents and to perform the Sn(II) and Sn(IV) analyses using the analytic curve working range.

Table 1 presents the Sn(II) and Sn(IV) concentrations in DMSA, ECD, MDP, and pyrophosphate considering the dilutions.

**DISCUSSION**

Differential pulse polarography was used for selective determination of Sn(II) and Sn(IV) in DMSA, ECD, MDP, and pyrophosphate. The SnCl₂·2H₂O mass ranging from 0.125 to 2 mg covered most of the lyophilized reagent for labeling with ⁹⁹ᵐTc used in nuclear medicine.

For a specific and selective determination of Sn(II) and Sn(IV), 3 M H₂SO₄ was considered the most appropriate medium for Sn(II) because only Sn(II) produced a polarographic wave with the maximum current in -370 mV (Fig. 1A). Under the same conditions, no current could be determined for Sn(IV). In 3 M HCl, Sn(II) and Sn(IV) were electroactive and the potential of the 2 polarographic waves appeared in -250 and -470 mV (Fig. 1B).

Lingane et al. reported that Sn(IV) is an air-stable form and does not produce polarographic waves either in HNO₃ or H₂SO₄ because Sn(IV) ions are extensively hydrolyzed and precipitate as basic salts even in a highly acidic medium (4,18). On the other hand, HCl or bromide complexes can stabilize high-order halide complexes and produce 2 reduction waves for Sn(IV) (19).

An investigation of the polarographic behavior of Sn(IV) in fairly concentrated H₂SO₄, HClO₄, HNO₃, or HF showed no wave in any of these media. After the addition of HCl, polarographic waves were observed for Sn(IV) and Sn(II) in a Sn(IV) solution, with an increase in the wave height without altering the half-wave potential (19).

The adequate establishment of the supporting electrolyte allows selective determination of the ion. The polarography study of tin in various supporting electrolytes was described by Lingane et al., and the Sn(II) waves in HCl, HNO₃, and H₂SO₄ had a sigmoidal shape and were considered well defined for analytic use. The choice of the supporting electrolyte and the presence of stabilizing agents are the main factors that affect the reduction potential (18). In most media, Sn(II) ions produce 2 polarographic waves. In the cathodic wave, tin is reversibly reduced to Sn(amalgam), and in the anodic wave, Sn(II) is oxidized to Sn(IV), and the degree of reversibility and suitability for analytic application is dependent on the particular medium (19).

![Differential pulse polarograms for tin determination](image-url)
Based on the results of Lingane et al. (18), some studies described the selection of a supporting electrolyte for selective determination of Sn(II) in radiopharmaceuticals. McBride et al. observed that in 1 M H₂SO₄, the reduction potential of Sn(II) in pyrophosphate and polyphosphate was −440 mV (16), but they did not evaluate either lyophilized reagent of lower SnCl₂ mass content or the presence of complexing reagents such as DMSA and ethylenediamine tetraacetic acid, which produce interference in some reported analytic methods (8, 9). In our work, 3 M H₂SO₄ was used instead of 1 M to overcome any interference problem. Although Stefan et al. reported that only Sn(II) was electroactive in 0.1 M HClO₄ at −420 mV (2), Ross et al. observed waves for noncomplexed DMSA and tin complex in 0.1 mM perchlorate, pH 3.0, in the Sn(II) determination of DMSA (15). In our work, a DMSA wave was not observed at −200 to −800 mV potential range. In a more complex medium such as 0.7 M KF/1.8 M KNO₃, pH 7.7, or 0.7 M KF/0.1 M NaNO₃, pH 7.5, proposed by Lejeune et al., Sn(II) was not affected by Sn(IV) although it was necessary to use a buffered medium and another complexing reagent such as ZnCl₂ to suppress the MDP effect (12). Decristoforo et al. proposed a simpler medium composed of methanol in 35 mM HClO₄, and they focused their work on the evaluation of the stability of fractionated cold kits (11). The authors observed an overlapping between Sn(II) and ECD or DMSA waves. The potential range of the peak associated to the reduction of stannous ions to tin metal was −350 and −400 mV, and the reduction potential of Sn(IV) to Sn(II) was between −150 and −190 mV.

Before the quantification of Sn(II), the tin redox reaction taking place in the mercury drop was assessed by the presence of the components of the cold kit formulation other than the tin species. No variation was observed in the peak height, shape, and reduction potential of Sn(II) and Sn(IV) in the concentration range studied (Figs. 2A and 2B). Because the conclusions for DMSA, ECD, and pyrophosphate were the same, only the MDP data were presented.

The analytic curves for Sn(II) and Sn(IV) (Eqs. 1 and 2) were linear in the concentration range of 0–10 μg·mL⁻¹, and the correlation coefficients (r²) were greater than 0.997.

The results presented in Table 1 show that in ECD, only 65% of the total tin is present as Sn(II), indicating oxidation to Sn(IV) in the production process, which can yield a low ⁹⁹mTc labeling and poor radiochemical purity. In DMSA, MDP, and pyrophosphate, more than 87% of the total tin is in the Sn(II) oxidation state.

Even using a millimolar titrant concentration to quantify Sn(II), by the titrimetric conventional method, the titrant volumes and the Sn(II) mass in the cold kits were 0.12, 1.12, and 1.75 mL, resulting in 0.060 ± 0.017, 0.607 ± 0.040, and 0.953 ± 0.025 mg in ECD, MDP, and pyrophosphate, respectively. Higher SD and mass results for Sn(II) by the titrimetric analysis than by the polarographic method (Table 1) can be attributed to the difficulty of visual detection of the endpoint.

### CONCLUSION

The developed polarographic method was selective and adequate to determine Sn(II) even in the presence of Sn(IV) and can be used as a quality control to quantify Sn(II) and Sn(IV) in DMSA, ECD, MDP, and pyrophosphate radiopharmaceutical cold kits.

### ACKNOWLEDGMENTS

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### REFERENCES


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**TABLE 1**

Determination of Sn(II) and Sn(IV) Concentrations in Radiopharmaceutical Cold Kits by Differential Pulse Polarography

<table>
<thead>
<tr>
<th>Cold kit</th>
<th>mg total tin</th>
<th>H₂SO₄ 3 M</th>
<th>HCl 3 M</th>
<th>Sn(II)</th>
<th>Sn(IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSA</td>
<td>0.213</td>
<td>−0.202</td>
<td>−0.208</td>
<td>0.192 ± 0.003</td>
<td>0.211 ± 0.004</td>
</tr>
<tr>
<td>ECD</td>
<td>0.065</td>
<td>−0.152</td>
<td>−0.204</td>
<td>0.042 ± 0.001</td>
<td>0.625 ± 0.002</td>
</tr>
<tr>
<td>MDP</td>
<td>0.519</td>
<td>−0.201</td>
<td>−0.202</td>
<td>0.483 ± 0.013</td>
<td>0.499 ± 0.018</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>1.038</td>
<td>−0.190</td>
<td>−0.203</td>
<td>0.908 ± 0.023</td>
<td>1.004 ± 0.032</td>
</tr>
</tbody>
</table>

*i* (μA)  
mg tin (% Sn(II) or Sn(IV)/total tin)  

**n** = 9 (triplicate of 3 vials). Data in parentheses are percentages.


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