Differential Pulse Polarographic Determination of 2,3-Dimercaptosuccinic Acid and Tin(II) in Radiopharmaceuticals

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Abstract □ A differential pulse polarographic procedure was developed for the assay of dimercaptosuccinic acid and tin(II), components of a commercially available pharmaceutical kit for kidney scintigraphy. The method is quantitative and qualitative for both the chelated and unchelated forms of dimercaptosuccinic acid and tin(II) in a mixture of the two.

Keyphrases □ Dimercaptosuccinic acid—analysis, differential pulse polarography, in radiopharmaceuticals, and tin □ Tin—analysis, differential pulse polarography, in radiopharmaceuticals, and dimercaptosuccinic acid □ Polarography, differential pulse—analysis, dimercaptosuccinic acid and tin in radiopharmaceuticals □ Scintigraphy, kidney—analysis of dimercaptosuccinic acid and tin(II) in commercial preparations, differential pulse polarography

Dimercaptosuccinic acid (I) possesses clinical importance as a detoxification agent for mercury, arsenic, and other heavy metals (1) and, more recently, in conjunction with tin(II) and ^{99m}technetium as a radiopharmaceutical for kidney scintigraphy (2–4). Although the chemistry of several I chelates has been studied (5) with application to biological systems (6, 7), little work has been done to develop a specific analytical procedure.

UV procedures (8, 9) depend on the I thiol reducing properties and are inappropriate in the presence of another reducing agent. Since tin(II) is present in the kidney reagent final product (3 m*M* I + 1 m*M* $SnCl_2$), an alternative means of analysis is necessary. Krejcarek *et al.* (10) employed differential pulse polarography to analyze both tin(II) and I but gave few details of their unpublished procedure. McBride *et al.* (11) employed the same technique to quantitate tin(II) in pyrophosphate and polyphosphate radiopharmaceutical kits.

This paper expands on this recent work and presents a quantitative and qualitative technique to analyze both the chelated and unchelated forms of I and tin(II) in a mixture of the two. The method was successfully applied to ampuls of final product reagent.

EXPERIMENTAL

Reagents and Chemicals—The reagents and chemicals used were *meso*-dimercaptosuccinic acid, anhydrous stannous chloride[1], and analytical grade lithium perchlorate[2], sodium phosphate·$7H_2O$[3], and citric acid[3]. The mercury[4] used was triple distilled.

Instrumentation—A polarographic analyzer[5], equipped with a drop-time assembly[6], was used in conjunction with a three-electrode system consisting of a dropping mercury electrode, a silver–silver chloride reference electrode, and a platinum counter electrode. The mercury column height was 60 cm; the automatic drop time was 0.5 sec. At this column height, the dropping mercury electrode had an open circuit drop time of 7.36 sec and a mercury flow rate of 0.93 mg/sec in the buffer.

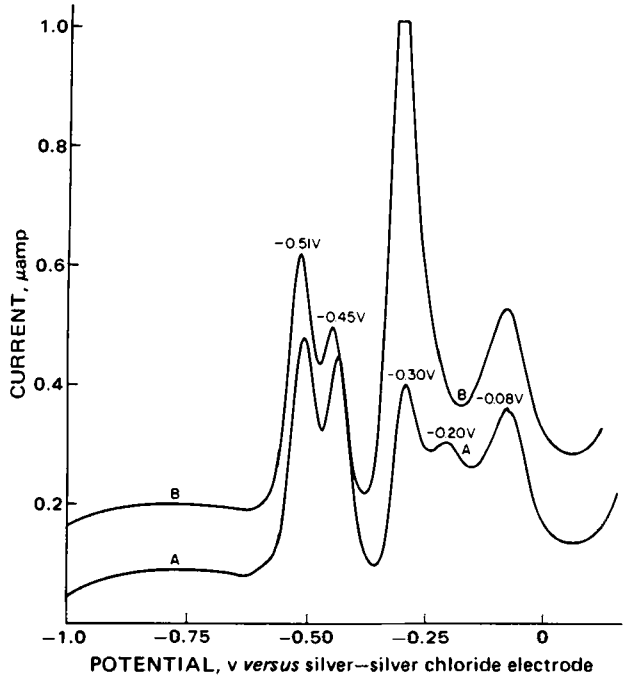

Figure 1—*Sample polarograms of ampul in supporting electrolyte (polarogram A) and with addition of excess I (polarogram B).*

A scan rate of 2 mv/sec and a pulse amplitude of 25 mv were employed. The current range was set at 1 μamp, and the scan range was 0.75 v. The samples were scanned positively from −0.70 to +0.05 v against silver–silver chloride, and the differential pulse polarograms were obtained on an *x*–*y* recorder[7].

Dissolved air was removed from solutions by bubbling prepurified nitrogen through the cell for 5 min and passing it over the solution during polarography.

Determination of I and Tin(II) in Kidney Reagent—Dilute tin(II) solutions such as those used in this procedure are susceptible to oxidation, especially when exposed to light. Consequently, it is essential to establish a technique that minimizes exposure to air and light. Dimercaptosuccinic acid slowly hydrolyzes in solution; standards and reagents containing this material should be prepared just before use.

A 150-μl aliquot of the freshly opened ampul of kidney reagent final product was diluted to 10 ml with deaerated pH 3.0, 0.1 *N* $LiClO_4$–0.1 *M* citrate–phosphate buffer supporting electrolyte, and the polarogram was recorded. The peak current at −0.30 v (due to the unchelated I) was measured and compared to a standard calibration curve for unchelated I. A second peak current at about −0.51 v (due to the reduction of chelated stannous ion) was measured also and compared to a standard curve for chelated tin(II). This second value was then normalized with respect to stoichiometry [at this pH, the chelate formula is $Sn(I)_2$] and added to the unchelated I value to arrive at the total I concentration (Fig. 1A).

Another 150-μl aliquot of the ampul contents was transferred to a

[1] Purity >99%, Medi+Physics Inc., Emeryville, CA 94608.
[2] ROC/RIC.
[3] Mallinckrodt.
[4] Bethlehem Instruments.
[5] Princeton Applied Research model 174 A.
[6] Princeton Applied Research model 174/70.
[7] Hewlett-Packard model 7001 AM.

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Table I—Peak Currents of I and Tin(II) Solutions

I Concentration, m*M*	Tin(II) Concentration, m*M*	i_p (microamperes) at E = −0.51 v	E = −0.30 v	E = −0.20 v	E = −0.08 v
0.0580	—	—	1.13	—	—
0.0580	0.0108	0.28	0.68	—	0.16
0.0580	0.0161	0.40	0.49	—	0.21
0.0580	0.0215	0.52	0.20	0.05	0.26
0.0580	0.0269	0.61	Shoulder	0.12	0.28
0.0580	0.0323	0.71	—	0.25	0.31

second 10-ml volumetric flask, flooded with excess deaerated I solution (1.5 ml of 1 m*M* solution) to ensure complete chelation of the tin, and diluted to volume with deaerated supporting electrolyte. The solution was analyzed polarographically and the peak current was measured at −0.51 v. Comparison to the standard curve of chelated tin gave the total stannous content (Fig. 1B).

Standard free I and chelated tin(II) curves were prepared. Five solutions, ranging in concentration from 0.012 to 0.018 m*M* of both I in the absence of tin and tin(II) flooded by an excess of I, were diluted in supporting electrolyte. The I solutions were analyzed polarographically, and the peak currents measured at −0.30 v were plotted against the respective concentrations to obtain the free I calibration curve. Similarly, for tin(II) solutions in an excess of I, the peak currents at −0.51 v were measured and plotted against concentration to obtain the chelated tin(II) calibration curve.

RESULTS AND DISCUSSION

Tin(II) in the absence of I had two distinct polarographic peaks in pH 3.0, 0.1 *N* $LiClO_4$–0.1 *M* citrate–phosphate buffer. One, at −0.20 v, was due to the tin(II) to tin(IV) oxidation. The second, at −0.45 v, was due to the reduction to tin metal. When tin(II) was chelated by I, these peaks shifted to −0.45 and −0.51 v, respectively. The standard curve was linear with concentration, demonstrating that the method can be used to determine chelated tin(II). Tin(IV) will produce a reduction peak only in the presence of high hydrogen-ion and chloride-ion concentrations (12) and does not interfere in this electrolyte.

The I wave at −0.30 v is due to the oxidation of mercury to form the mercury mercaptide salt and not to oxidation of the sulfhydryl group itself (13–16). At 0.15–0.6 m*M* levels, the peak current appeared to be independent of concentration, remaining virtually the same as the concentration was increased fourfold. A second standard curve at much lower concentrations (0.012–0.018 m*M*), however, was linear. The mercury mercaptide product apparently coats the mercury drop, preventing further oxidation. This phenomenon could also have produced the erratic baselines encountered when the scan was initiated at 0.0 v and scanned negatively. To avoid this result, all polarograms were begun at −0.70 v and scanned positively.

Adding tin(II) to I decreased the peak at −0.30 v, while a new peak at −0.08 (chelated I) grew. Further addition of tin(II) resulted in the complete elimination of the free I peak and the emergence of a second new peak at −0.20 v [unchelated tin(II)] (Table I). Since the total I concentration was known and the remaining free I and the chelated tin(II) concentrations could be evaluated, the stoichiometry of the chelate could be determined. At this pH, tin(II) was complexed to two I molecules, confirming the previous work (10) where the same ratio was determined by difference UV spectroscopy. Electrolytically reducing an aliquot of the final product at −0.75 v *versus* silver–silver chloride produced only the free I peak at −0.30 v when analyzed polarographically. These additional data provide convincing evidence of complexation.

Table II—Final Product Kidney Results

Ampul	I, m*M*	Tin(II), m*M*
First of filling operation	3.05	1.02
Middle of filling operation	3.05	1.01
Last of filling operation	3.17	1.03
Middle of filling operation	3.13, 3.10, 3.09	1.00, 0.99, 0.97
	3.11 ± σ of 0.02	0.987 ± σ of 0.015

Three ampuls taken at various stages of a production filling operation were analyzed for I and tin(II) content. A fourth ampul was analyzed in triplicate for both components. The results (Table II) are precise and show good correlation with the quantities added.

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