Candidate primary reference measurement procedure for the amount-of-substance content of a complexing agent expressed as EDTA based on the coulometric titration with electrogenerated Hg ions

Kyungmin Jo* and Euijin Hwang¹,*

Inorganic Metrology Group, Division of Chemical and Biological Metrology, Korea Research Institute of Standards and Science (KRISS), 267 Gajeong-ro, Yuseong-gu, Daejeon, Republic of Korea

E-mail: kyungmin.jo@kriss.re.kr and hwangeuijin@gmail.com

Received 12 April 2022, revised 28 June 2022
Accepted for publication 6 July 2022
Published 19 August 2022

Abstract
The primary reference measurement procedure is a method to realize the definition of the quantity without relation to a reference standard of the same quantity. This paper presents a candidate primary reference measurement procedure for the measurement of the amount-of-substance of a complexing agent expressed as ethylenediaminetetraacetic acid (EDTA) based on coulometric titration. Herein, mercury (Hg) was used as the anode because it does not undergo spontaneous oxidation. Furthermore, a 1:1 complex is formed between the electrogenerated Hg²⁺ ions and EDTA (in the form of Y⁴⁻). Although the electrochemical oxidation of Hg may generate Hg₂²⁺ ion, Hg₂²⁺ undergoes disproportionation in the presence of EDTA, thereby forming the desired Hg²⁺ species. The equivalence point occurs at the maximum of the derivative of the theoretical titration curve. As such, the measurement results were obtained by assuming that the maximum of the derivative of the experimental titration curve was the end point. The developed procedure was employed for the measurement of the amount-of-substance content of the complexing agent in EDTA reference material certified at the Slovak Institute of Metrology. The experimental measurements agreed well with the certified value for the reference material within the measurement uncertainty. The candidate procedure will be useful in certifying EDTA reference material and establishing international equivalence of amount content of the complexing agent.

Keywords: primary reference measurement procedure, amount of substance, coulometric titration, ethylenediaminetetraacetic acid (EDTA), electrogenerated Hg ions

(Some figures may appear in colour only in the online journal)
1. Introduction

Coulometry is one of the methods that can practically realize an SI unit, the mole, based on chemical electrolysis [1]. In this method, the charge consumed or produced during the electrolysis of an analyte is measured and converted into the amount of substance using the Faraday constant as a conversion constant. This method has been successfully employed by the majority of the national measurement institutes, including the Korea Research Institute of Standards and Science (KRISS), which participated in international comparisons organized by the Electrochemical Analysis Working Group (EAWG) within the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM), in which the assay of high purity materials used as reference materials for acid-base, argentometric, and oxidation-reduction titrations was performed [2–9]. However, equivalent results from different national metrology institutes for the coulometric assay of ethylenediaminetetraacetic acid (EDTA), which is a representative substance of known high purity used as a reference for complexometric titration, have not been obtained up until the present. For CCQM-P112 entitled ‘Assay of EDTA’, two institutes performed EDTA assay using coulometric titration, but it was not possible to demonstrate the equivalence between measurement results presumably due to differences in measurement procedure and problems with mastering all possible sources of bias in measurement techniques [10]. The assay and the relevant comparisons still need to be carried out according to the most recent strategy document of the CCQM-EAWG [11]. Moreover, there is no vast literature on the establishment of a metrologically valid coulometric EDTA titration procedure that can be used as a primary reference measurement procedure.

Precise coulometric assay of EDTA has been exclusively carried out with electrolytically generated zinc(II) ion (Zn²⁺) [12, 13], although various other electrogenerated metallic ions have been shown to be used for the assay [14–16]. When Zn is utilized as an anode, it is used in the form of amalgam. Using this form, which has high hydrogen overpotential, the spontaneous oxidation of Zn caused by the hydrogen evolution reaction (HER) can be minimized [17]. However, the spontaneous dissolution of Zn may still occur due to the trace amounts of dissolved oxygen in the electrolyte [18, 19]. The effects of the spontaneous oxidation and dissolution of Zn should be considered when using Zn amalgam as the electrode for the coulometric assay of EDTA. However, the evaluation and correction of these effects are challenging because the oxidation rate depends on the type and concentration of the electrolyte as well as the mass transport phenomena in the system [20, 21].

In this study, a candidate primary reference measurement procedure for the amount-of-substance content (amount content) of complexing agent expressed as EDTA is proposed based on a coulometric titration method using electrogenerated mercury (Hg) ions. Herein, Hg is used as an anode because the spontaneous oxidation of Hg is unlikely due to its relatively high standard electrode potential and high hydrogen overpotential. When EDTA (denoted as H₂Y) is titrated with the electrochemically generated Hg ions, a Hg²⁺—Y⁴⁻ complex (HgY²⁻) is ultimately formed due to the disproportionation of the electrogenerated Hg₂⁺ ions.

2. Experimental section

2.1. Chemicals

EDTA (≥99.995%) used in all coulometric assays except for the validation experiment was purchased from Honeywell Fluka (Charlotte, NC, USA). EDTA certified reference material (CRM) used for the comparison experiment was purchased from Slovak Institute of Metrology (SMU). EDTA disodium salt dihydrate (ACS reagent grade) used for the initial titration of the coulometric assay, sodium nitrate (≥99.0%), sodium silicate solution (reagent grade), sodium hydroxide (99.99%), and agaropectin sodium acetate (99.997%) were obtained from Alfa Aesar (Ward Hill, MA, USA). Nitric acid (70%, semiconductor grade) was obtained from Chemitop (Jincheon, Republic of Korea). Platinum (Pt) foil and wire (≥99.99%) were obtained from Wollfmetal (Yongin, Republic of Korea). All solutions were prepared using Milli-Q water (Merck Millipore, Darmstadt, Germany). All materials, except the Hg and Pt electrodes, were used without further purification. Electrodes, Pt boat, and Pt wires were cleaned using 2 mol l⁻¹ nitric acid and then rinsed with copious amounts of deionized water. The electrolyte solution was composed of 1 mol l⁻¹ sodium nitrate and 0.1 mol l⁻¹ acetate buffer (pH ≈ 5.5).

2.2. Apparatus

The general design of the constant-current coulometry system has been described elsewhere [22]. The KRISS coulometry system has three functional components: (i) charge measurement module in which the current passing through the coulometric cell is calculated from the resistance of the standard resistor and the voltage drop across the standard resistor and the time of a current flow is measured using a time interval counter, (ii) mass measurement module that enables the measurement of sample mass with a correction for air buoyancy, and (iii) end point determination module that measures the change in potential of an indicator electrode with respect to the reference electrode. The charge measurement module was composed of constant-current source (Keithley 2400, Solon, OH, USA), home-made standard resistors with precision resistor components (Vishay VHP100, Malvern, PA, USA), voltmeter (Keithley 182, Solon, OH, USA), standard cells (Guildline 9152/4, Smiths Falls, ON, Canada) as voltage standards, switch systems (Keithley 7001, Solon, OH, USA), and time interval counter (Keysight 53131A, Santa Rosa, CA, USA).

On the other hand, the mass measurement module had an ultra-microbalance (Mettler Toledo XPR2U, Columbus, OH, USA), a thermo-hygrometer (Fluke 1620A, Everett, WA, USA), and a barometer (GE Druck PACE1000, Leicester, UK). An OIML E1 class 2 g standard weight (Mettler Toledo 15910, Columbus, OH, USA) was used to calibrate the balance.
Lastly, an electrometer (Keithley 6517A, Solon, OH, USA) was used as the end point determination module. The standard resistors, voltmeter, standard cells, time interval counter, thermo-hygrometer, barometer, and standard weight were calibrated at the KRISS calibration laboratories.

Figure 1 illustrates the design of the horizontal-type coulometric cell [22, 23] with two intermediate compartments (ICs) separated by sintered glass disks. Each of the two ICs has a glass tube with a stopcock used to fill or empty the IC by applying vacuum or introducing pressurized inert gas. The anode compartment contained the Hg anode with an approximate area of 20 cm² and a saturated calomel electrode (SCE), which was used as a reference electrode. The anode was also used as an indicator electrode to measure the potential of the Hg electrode. The electric connection to the anode was made using a Pt wire sealed in a glass tube. To avoid chloride contamination from the SCE, the SCE was placed in a 1 mol l⁻¹ sodium nitrate solution in a glass tube in which one end was sealed with a glass frit and salt bridge. The salt bridge for the SCE was prepared by mixing 11 ml of a five-fold diluted sodium silicate solution and 9 ml of 1 mol l⁻¹ sodium nitrate solution in a glass tube in which one end was tilted to introduce the sample into the anode compartment. The wall of the anode and cathode compartments was separated by sintered glass disks. Each of the two ICs has a approximate area of 48 cm². The salt bridge at the cathode side was prepared by dissolving 3% (as mass fraction) agarose in the electrolyte solution. A Pt boat was used to introduce the sample. Subsequently, this boat containing a sample was hung on the Pt wires inserted into the cell cover and then placed above the electrolyte solution to ensure that air would not enter the compartment during the introduction of the sample. A polytetrafluoroethylene-coated magnetic bar was used to stir the solution.

Nitrogen gas (99.9999%, Deokyang, Ulsan, Republic of Korea) was further purified by passing it through an oxygen filter (Chromatography Research Supplies, Louisville, KY, USA). After the filtration, the concentration of oxygen was expected to be less than 0.005 ppm according to manufacturer’s instructions. The purified nitrogen gas was used to maintain an inert atmosphere above the electrolyte solution and purge dissolved oxygen from the electrolyte solution.

2.3. Procedure for the coulometric assay of EDTA

Prior to their use, the EDTA samples were dried at 110 °C for 2 h [24] without crushing and cooled at room temperature (24 °C ± 1 °C) at least overnight in a desiccator with dry silica gels.

The anode and cathode compartments of the cell were filled with approximately 90 ml of the same electrolyte solution. The purified nitrogen gas was introduced into the cell for at least 30 min through the glass frit adjacent to the anodic compartment to remove the dissolved oxygen in the electrolyte solution [12, 13].

Approximately 0.2 g of the EDTA sample was accurately weighed in a Pt boat, followed by air buoyancy corrections taking the density of EDTA as 1650 kg m⁻³ [25]. Afterward, approximately 0.4 ml of 1 mol l⁻¹ sodium hydroxide was added to the sample to reduce the pH change due to the deprotonation of EDTA during the titration. After weighing, the ICs were filled with the electrolyte solution to establish an electrical contact between the anode and cathode compartments.

Coulometric titration was carried out through an initial, a main, and a final titration stages [22]. For the initial titration, about 0.1 ml of a 0.05 mol l⁻¹ EDTA disodium solution was introduced into the anode compartment. The salt form of EDTA was used because it has higher solubility and shows less change in pH during complexation compared to the free form. A constant current of 10 mA was applied to the cell until nearly 90% of the added sample was titrated with the electro-generated Hg ions. Then, the ICs were rinsed repeatedly with the electrolyte solution. Afterward, the titration was continued by passing small increments of charge (0.01 C) by flowing a current of 10 mA for 1 s. The stabilized potential of the Hg anode was recorded after each increment. The end point was determined by fitting the derivatives of the potentiometric titration curve. Lastly, the over-titrated charge in the initial titration stage was calculated by subtracting the charge at the estimated end point from the total charge added in the initial titration and correcting the effect of rinsing the ICs as described elsewhere [22, 23].

The Pt boat containing the sample was then lowered and tilted to introduce the sample into the anode compartment solution. For the main titration stage, a 10 mA current was applied until 98% of the sample was titrated. While the current was applied into the cell, the boat was raised and lowered repeatedly to ensure that the remaining sample in the boat was fully dissolved. After the application of the current, the electrolyte solution filled in the IC, which was next to the anode compartment, was transferred to the anode compartment to prevent the diffusional loss of the EDTA. Then, the bottom of the ICs was slightly filled with the electrolyte solution in the IC adjacent to the cathode compartment, and the titration was continued until 99.7% of the sample was titrated. Then, the ICs were rinsed successively with the electrolyte solution.

In the final titration stage, 0.02 C charge was added repeatedly by applying a 10 mA current for 2 s. Then, the ICs were rinsed with the electrolyte solution. The wall of the anode
compartment was then further rinsed by gently shaking the cell. Similar to the end point determination for the initial titration stage, the end point in this final step was estimated by fitting the first derivative of the potentiometric titration curve with a quadratic function. Finally, the amount content of the complexing agent in the EDTA sample (A) was calculated from the total charge (Q), current efficiency (f), number of electrons required to generate the Hg ions (z), Faraday constant (F), and mass of the sample (m) using the following equation

\[ A = f \frac{Q}{mzF}. \]  

In this equation, the total charge was calculated by adding the over-titrated charge in the initial stage, applied charge during the main titration step, and applied charge to the end point in the final stage. The charge in each titration stage was computed from the sum of each charge increments.

To estimate possible bias caused by chemical impurities in the electrolyte such as trace metals, complexing agents, oxidants (e.g., dissolved oxygen), and reductants, the potential of Hg anode after the completion of the initial titration was monitored over measurement time. The change in charge was estimated from the potential drift and the titration curve obtained in the initial titration. The correction was then made for the total charge using the average charge of 0.0029 C.

3. Results and discussion

3.1. Titration curve for the coulometric assay of EDTA using the electrogenerated Hg ions

Figure 2 shows the obtained titration curve wherein the potential of the Hg anode is plotted against the charge passed through the coulometric cell. After the addition of EDTA during the initial titration, the initial potential of the anode was relatively low (+0.06 V vs SCE, point A) due to the low concentration of the free Hg ions in equilibrium with the excess EDTA. The electrode potentials were not recorded during the electrochemical generation of the Hg ions until the end point determination because they are influenced by the ohmic drop when current flows through the electrolyte and Hg anode used as the indicator electrode. A typical titration curve was obtained with a distinct break near the end point during the end point determination. When EDTA was added to the electrolyte solution after the initial titration, the electrode potential decreased to +0.07 V vs SCE (point B). This is also due to the low concentration of the free Hg ions. The end point observed in the final titration stage occurred at a higher electrode potential than that observed in the initial titration because the concentration of the HgY2− complex during the final titration was much higher than that in the initial titration. As such, the concentration of the free Hg ions in equilibrium with the HgY2− complex was higher during the final titration.

The proper choice of electrode materials is critical for the coulometric assay of EDTA because the method depends on the electrochemical generation of metal ions and then on the complex formation between the generated metal ions and EDTA. In this work, Hg was used as the anode due to two primary reasons. First, the electrogenerated Hg22+ ions can be converted to Hg22+ ions by disproportionation reaction as shown in equations (2) and (3) [26]

\[ 2\text{Hg} \rightarrow \text{Hg}_{2}^{2+} + 2e^{-} (E^0 = +0.80 \text{ V vs NHE}) \]  

(2)

\[ \text{Hg}_{2}^{2+} \rightleftharpoons \text{Hg}^0 + \text{Hg}^{2+}. \]  

(3)

The overall reaction for the generation of the Hg22+ ion can be described by equation (4)

\[ \text{Hg} \rightarrow \text{Hg}^{2+} + 2e^{-}. \]  

(4)

In the presence of ligands that form stable mercuric complexes, Hg22+ rapidly disproportionate, thereby producing Hg22+ ions and elemental Hg [28, 29]. Considering these, if the conditional formation constant of the complex (Kf) at a given pH is sufficient, one can suppose that the reaction of the electrogenerated Hg ions with deprotonated EDTA (Y4+) ultimately forms a HgY2− complex through Hg22+ + Y4− \rightleftharpoons HgY2−. Thus, the overall complex formation reaction by coulometry can be described by equation (5),

\[ \text{Hg} + Y^{4−} \rightarrow \text{HgY}^{2−} + 2e^{−}. \]  

(5)

where two moles of electrons are produced per one mole of the generated Hg-EDTA complex. Interestingly, the conditional formation constants of the HgY2− complex (KfHgY) are found to be maximum and almost constant, having values close to 10^{11} \text{ mol}^{−1} \text{ L}^{−1} in the pH ranges of 3.5 to 5.5 [30]. Herein, acetate buffer (pH = 5.5) was used as the electrolyte to minimize the changes in the pH during the assay. The measured pH before and after the assay were 5.5 and 4.8, respectively. Therefore, the values of KfHgY were expected to be constant throughout the assay.
Second, the typical electrochemical side reactions, such as HER, oxygen reduction reaction (ORR), and water oxidation, are unlikely to occur on the Hg anode. The standard electrode potential for the oxidation of Hg is considerably higher than that for the HER ($E^\circ = -0.28$ V vs NHE at pH = 4.7), thereby rendering HER thermodynamically unfavorable on the Hg anode. Furthermore, HER is also suppressed due to its sluggish reaction kinetics [31]. In the case of ORR, it is unlikely to occur on the Hg anode because (i) the reversible potential for the reduction of oxygen to hydrogen peroxide (approximately $-0.1$ V vs SCE [32]) is lower than the lowest electrode potential of Hg (+0.06 V vs SCE) observed in the present study, and (ii) the concentration of the dissolved oxygen in the electrolyte is extensively reduced through nitrogen gas bubbling. Lastly, in general, the standard electrode potential for the oxidation of Hg (i.e., $E^\circ = +0.80$ V vs NHE) is lower than that for water oxidation ($E^\circ = +1.23$ V vs NHE). As such, neither water oxidation is expected to take place on the Hg anode. In fact, the Hg anode was oxidized at potentials below +0.31 V vs SCE, which is considerably lower than its standard electrode potential.

### 3.2. Theoretical considerations on the conditional formation constant between the electrogenerated Hg ions and EDTA

In the case of the titration of EDTA with Hg$^{2+}$, there is no need to take into account the presence of Hg$_2^{2+}$ because the reverse disproportionation reaction cannot occur due to the absence of the elemental Hg. In contrast, the disproportionation reaction should be considered in the coulometric titration of EDTA using Hg anode because Hg$_2^{2+}$ and Hg$^{2+}$ ions can be generated and interconverted. In the presence of liquid Hg, the equilibrium constant of the disproportionation reaction ($K$) can be expressed as [33]

$$K = \frac{[\text{Hg}^{2+}]}{[\text{Hg}_2^{2+}]}.$$

Considering the titration of EDTA with Hg$^{2+}$, the equilibrium is established through the following reaction:

$$\text{Hg}^{2+} + Y^{4-} \rightleftharpoons \text{HgY}^{2-}. \quad (7)$$

From this reaction, $K_{f, \text{HgY}'}$ can be described as

$$K_{f, \text{HgY}'} = \frac{[\text{HgY}^{2-}]}{[\text{Hg}^{2+}][Y]} \quad (8)$$

where $[\text{Hg}^{2+}]$ and $[Y]$ are the concentrations of the Hg$^{2+}$ ions that do not react with the EDTA and of all the EDTA species that are not bound to the Hg$^{2+}$ ions, respectively [34].

Since both Hg$_2^{2+}$ and Hg$^{2+}$ species are present during the titration of EDTA, the conditional formation constant between EDTA and the electrogenerated Hg ions ($K_{f, \text{HgY}''}$) can be rewritten as

$$K_{f, \text{HgY}''} = \frac{[\text{HgY}^{2-}]}{([\text{Hg}_2^{2+}] + [\text{Hg}^{2+}])[Y]} \quad (9)$$

where $([\text{Hg}_2^{2+}] + [\text{Hg}^{2+}])$ represents the concentration of the total Hg ions that have not reacted with EDTA. Substituting the equation for $K$ (equation (6)) into equation (9) yields

$$K_{f, \text{HgY}''} = \frac{[\text{HgY}^{2-}]}{([\text{Hg}_2^{2+}])[Y]} \quad (10)$$

Since the $K$ is known to be around 0.01 [33, 35], the relationship between the conditional formation constant between EDTA and the electrogenerated Hg ions ($K_{f, \text{HgY}''}$) and that between EDTA and Hg$^{2+}$ ions ($K_{f, \text{HgY}'}$) is derived as

$$K_{f, \text{HgY}''} \approx 0.01K_{f, \text{HgY}'} \quad (11)$$

From equation (11), $K_{f, \text{HgY}''}$ (approximately $10^9$ mol$^{-1}$) is at least 100 times lower than of $K_{f}'$ (approximately $10^{11}$ mol$^{-1}$ as mentioned in section 3.1).

**Figure 3.** (a) Theoretical titration curve (black squares) and corresponding slope of the curve (red spheres) obtained considering the experimental conditions listed in table 1. The slope was calculated from the charge derivative of the electrode potential. (b) Representative experimental titration curve and corresponding slope of the curve obtained during the final titration. The red dotted curve represents quadratic fit of the slope.
3.3. Comparison between the theoretical equivalence point and experimental end point

For a complexometric coulometric titration, in which the conditions for the initial and final titration stages are different, a bias can be introduced if the end point does not coincide with the equivalence point [22]. To investigate such possibility, we (i) checked whether the derivative of the titration curve was maximum at the equivalence point and then (ii) compared the end point estimated using the experimental potentiometric data with the theoretical equivalence point. The total concentration of the unreacted Hg ions (\( [\text{Hg}_{\text{tot}}] \)), which determines the potential of the Hg electrode, was obtained from the \( K_{\text{HgY}} \) and the mass balance equations for the concentration of Hg ion and EDTA, as similarly described elsewhere [36]. The total concentration is given by the following equation (refer to the appendix for more details).

\[
[\text{Hg}_{\text{tot}}] = \frac{-(K_{\text{HgY}}n_{\text{EDTA}} + V - K_{\text{HgY}}n_{\text{Hg}}) + \sqrt{(K_{\text{HgY}}n_{\text{EDTA}} + V - K_{\text{HgY}}n_{\text{Hg}})^2 + 4VK_{\text{HgY}}n_{\text{Hg}}}}{2VK_{\text{HgY}}n_{\text{Hg}}} \tag{12}
\]

where \( V \) is the volume of the electrolyte solution, and \( n_{\text{EDTA}} \) and \( n_{\text{Hg}} \) are the amounts of EDTA and electrogenerated Hg ions, respectively. The potential of the Hg electrode (\( E \)) is given by

\[
E = E_{\text{Hg}}^{\prime} + \frac{RT}{2F} \ln[\text{Hg}^{2+}] \tag{13}
\]

where \( E_{\text{Hg}}^{\prime} \) is the formal potential of Hg in the presence of \( \text{Hg}^{2+} \), \( R \) is the gas constant, \( T \) is the temperature, \( z \) is the number of electrons consumed in the electrode reaction, and \( F \) is the Faraday constant. Since \( [\text{Hg}_{\text{tot}}] \approx [\text{Hg}^{2+}] \), the equation for the theoretical potentiometric titration curve (equation (14)) can be obtained by substituting equation (12) into equation (13).

\[
E = E_{\text{Hg}}^{\prime} + 0.0296 \ln \left[ \frac{-(K_{\text{HgY}}n_{\text{EDTA}} + V - K_{\text{HgY}}n_{\text{Hg}}) + \sqrt{(K_{\text{HgY}}n_{\text{EDTA}} + V - K_{\text{HgY}}n_{\text{Hg}})^2 + 4VK_{\text{HgY}}n_{\text{Hg}}}}{2VK_{\text{HgY}}n_{\text{Hg}}} \right] \tag{14}
\]

Figure 3(a) shows the theoretical potentiometric titration curve near the equivalence point obtained during final titration. For this theoretical plot, (i) the amount of EDTA is considered to be equal to that of the electrogenerated Hg ions at the equivalence point, and (ii) the amount of the electrogenerated Hg ions near the equivalence point is assumed to be almost identical to that of the electrogenerated \( \text{Hg}^{2+} \). Considering the experimental conditions (table 1) employed in this study, the amount of the electrogenerated Hg ions was calculated by dividing the charge by \( z \) and \( F \). The maximum derivative of the curve (0.13 V C\(^{-1}\)) was observed at the equivalence point and corresponded well with the results of the theoretical calculations [36]. For comparison, figure 3(b) shows an experimentally obtained final titration curve with the corresponding slopes. The end point as the equivalence point was determined by fitting the slopes with a quadratic function and then finding the maximum of the fitting curve. The average value with the standard deviation of the maximum slopes at the end point of the final titration was 0.10 V C\(^{-1}\) ± 0.01 V C\(^{-1}\) (\( n = 10 \)). The experimental value was close to the theoretical one. Therefore, the rationale of the proposed procedure is theoretically sound, and the bias caused by the difference between the equivalence and end points is expected to be negligible. The possible variations in end point determination are reflected in the estimated uncertainty due to random effects. On the other hand, the experimental maximum slope (2.99 V C\(^{-1}\)) for initial titration was approximately two times larger than the theoretical maximum slope (1.32 V C\(^{-1}\)) possibly because the experimental conditional formation constant was higher than the theoretical value.

3.4. Coulometric assay of EDTA

Table 2 shows the results of the coulometric titration of EDTA for ten measurements. The average amount content was 3.42105 mol kg\(^{-1}\) with a relative standard deviation (RSD) of 0.0068%. The standard uncertainty of the average value was estimated by separately evaluating the standard uncertainty arising from the random effects (\( u_{\text{rand}} \)) and that arising from the systematic effects (\( u_{\text{sys}} \)) and by combining them [37, 38]. The value of \( u_{\text{rand}} \) due to random effects, such as the repeatability/reproducibility of the measurements and homogeneity of the sample, was calculated by dividing the standard deviation of the multiple measurements by the square root of the number of measurements (\( n = 10 \)), yielding the standard deviation of the mean. Considering the different sources of uncertainty in table 3, the value of \( u_{\text{sys}} \) due to systematic effects was evaluated based on the bottom-up approach shown in table 4. In this approach, the standard uncertainty of the output quantity is calculated by combining the standard uncertainties of the input quantities as in equation (15) [39].

\[
u^2_2(y) = \sum_{i=1}^{N} \left( \frac{\partial f}{\partial x} \right)^2 u_2^2(x_i) = \sum_{i=1}^{N} c_i^2 u_2^2(x_i). \tag{15}\]

The current efficiency was assumed to be 99.9999% and its standard uncertainty was estimated to be 0.0001% because
Table 1. Summary of the parameters used to derive a theoretical potentiometric titration curve.

| Parameter                                      | Value               |
|------------------------------------------------|---------------------|
| Volume of the electrolyte (V)                  | 90 ml               |
| Conditional formation constant (K_f^″)         | 1 × 10^9 l/mol      |
| Amount of EDTA (n_{EDTA})^a                    | 7.08 × 10^{-4} mol  |
| Gas constant (R)                               | 8.314 463 J/(K mol) |
| Temperature (T)                                | 296.15 K            |
| Number of electrons consumed in the electrode reaction (z) | 2                  |
| Faraday constant (F^b)                         | 96 485.332 C/mol    |
| Charge increment in the final titration (∆Q)   | 0.02 C              |

^a The amount was calculated from the sample mass (0.206933 g) and the molar mass of EDTA (292.243 g/mol).

^b The exact value of the Faraday constant is 96485.3321233100184 C/mol because it is the product of the Avogadro constant (N_A) and elementary charge (e).

Table 2. Summary of the measurement results for the coulometric assay of EDTA.

| Sample number | Amount content/(mol/kg) | u_{sys}/(mol/kg) | Degrees of freedom (DoF) |
|---------------|-------------------------|------------------|--------------------------|
| 1             | 3.42142                 | 0.00007          | ∞                         |
| 2             | 3.42082                 | 0.00007          | ∞                         |
| 3             | 3.42112                 | 0.00007          | ∞                         |
| 4             | 3.42117                 | 0.00007          | ∞                         |
| 5             | 3.42072                 | 0.00007          | ∞                         |
| 6             | 3.42064                 | 0.00007          | ∞                         |
| 7             | 3.42107                 | 0.00007          | ∞                         |
| 8             | 3.42081                 | 0.00007          | ∞                         |
| 9             | 3.42112                 | 0.00007          | ∞                         |
| 10            | 3.42098                 | 0.00007          | ∞                         |
| Mean          | 3.42097                 | 0.00007^a        |                           |
| SD (RSD)      | 0.00023 (0.0069%)       |                  |                          |
| u_{rand}      | 0.00007                 |                  | 9                         |
| u_{sys}       | 0.00007                 |                  | ∞                         |
| u_{combined}  | 0.00011                 |                  | 36                        |
| U             | 0.00021 (k = 2.03)      |                  |                          |

^a This value is the estimated pooled standard uncertainty.

the purity of Hg was >99.9999%. The standard uncertainties of Q and i were calculated considering the equations Q = it and i = V_{SR}/R_{SR}, respectively. To determine the uncertainty of t, the calibration uncertainty of the time interval counter and the time delay between current switching and time switching were considered. The uncertainty of bias correction on the total charge was estimated by assuming a rectangular distribution with upper and lower bounds of 0.0029 C and 0 C. The sample mass was measured based on the balance reading (m_r), balance calibration factor (f_{cal}), and air buoyancy correction factor (f_b) using the following equation,

\[ m_s = m_f f_{cal} f_b = m_t \left( \frac{m_w}{m_{t,w}} \right) \left( \frac{1 - d_a/d_w}{1 - d_a/d_s} \right) \quad (16) \]

where \( m_w \) is the mass of standard weight, \( m_{t,w} \) is the balance reading of the standard weight, \( d_a \) is the air density, \( d_w \) is the density of standard weight, and \( d_s \) is the sample density [40]. Using equation (16), the standard uncertainty of the \( m_t \) was assessed considering the uncertainty contributions of \( m_t, f_{cal}, \) and \( f_b \). The standard uncertainty of \( m_t \) was obtained considering the linearity of the balance. On the other hand, to estimate the standard uncertainty of \( f_{cal} \), the standard uncertainties of \( m_w \) and \( m_{t,w} \) were taken into account. The uncertainties of the values of \( d_a, d_w, \) and \( d_s \) were considered to evaluate the standard uncertainty of \( f_b \). The standard uncertainty of the measured amount content was calculated considering the equation (1) and was mainly determined by the uncertainty of the current (table 4), indicating that it can be improved by applying a higher current within a shorter time interval.

In summary, the measured amount content of the complexing agent expressed as EDTA from ten measurements was \((3.42097 ± 0.00021) \text{ mol kg}^{-1} (k = 2.03)\) with 95% level of confidence. The relative expanded uncertainty of the measurement procedure was 0.0062%, which came from equal contributions of the random and systematic effects (table 2). The uncertainty of the measurement can be improved by optimizing the experimental parameters, such as current density, sample mass, and electrolyte composition, and using a different end point detection method, such as amperometry.
Table 3. Sources of uncertainty and their typical values for the estimation of the standard uncertainty due to systematic effects.

| Symbol | Description of source | Value               |
|--------|-----------------------|---------------------|
| A      | Amount content        | 3.421 06 mol/kg     |
| f      | Current efficiency    | 99.999%             |
| Q      | Total charge          | 136.610 C           |
| Qbias  | Bias due to chemical impurities | 0.0029 C     |
| i      | 10 mA current         | 0.010178 A          |
| RSR    | Resistance of 100 Ω standard resistor (SR) | 99.998 30 Ω         |
| VSR    | Voltage drop across the 100 Ω SR | 1.018 197 V         |
| t      | Time of current flow  | 13423 s             |
| m_s    | Sample mass           | 0.000 206 933 kg    |
| m_t    | Balance reading of sample | 0.000 206 818       |
| f_cal  | Calibration factor of balance | 0.999 997 g         |
| f_b    | Air buoyancy correction factor | 1.000 563         |
| d_a    | Density of air        | 1.170 kg/m³         |
| d_w    | Density of standard weight | 8000 kg/m³       |
| d_s    | Density of sample     | 1650 kg/m³          |
| F      | Faraday constant      | 96485.332 C/mol     |

Table 4. Evaluation of the uncertainty in an individual measurement due to systematic effects.

| Uncertainty components u(x_i) | Value of u(x_i) | Sensitivity coefficient C_i | |C_i| u(x_i) | DoF | Relative contribution |
|-----------------------------|-----------------|------------------------------|--|---|----------------------|-----|----------------------|
| u(A)                        | 7.5 × 10⁻⁵ mol/kg |                              |   |   |                      |     |                      |
| u(f)                        | 0.0001%          | 3.4 mol/kg                   | 3.4 × 10⁻⁶ mol/kg | ∞   | 0.21%                |     |                      |
| u(Q)                        | 0.0030 C         | 2.5 × 10⁻² mol/kg C          | 7.6 × 10⁻⁵ mol/kg | ∞   | 96.19%               |     |                      |
| u(i)                        | 2.1 × 10⁻⁷ A     | 1.3 × 10⁴ s                  | 2.8 × 10⁻³ C       | ∞   | 88.27%               |     |                      |
| u(RSR)                      | 2.0 × 10⁻⁵ Ω     | −1.0 × 10⁻² A/Ω             | 2.1 × 10⁻⁷ A       | ∞   | 88.11%               |     |                      |
| u(VSR)                      | 8.9 × 10⁻⁷ V     | 1.0 × 10⁻² A/V              | 8.9 × 10⁻⁹ A       | ∞   | 0.16%                |     |                      |
| u(t)                        | 7.5 × 10⁻⁴ s     | 1.0 × 10⁻² A                | 7.6 × 10⁻⁶ C       | ∞   | 0.00%                |     |                      |
| u(Qbias)                    | 0.0008 C         | 1                            | 0.0008 C           | 3   | 7.92%                |     |                      |
| u(m_s)                      | 8.6 × 10⁻¹⁰ kg   | −1.7 × 10⁴ mol/kg²           | 1.4 × 10⁻⁵ mol/kg  | ∞   | 3.60%                |     |                      |
| u(m_t)                      | 8.2 × 10⁻¹⁰      | 1.0 kg                       | 8.2 × 10⁻¹⁰ kg     | ∞   | 3.29%                |     |                      |
| u(f_cal)                    | 1.0 × 10⁻⁶ kg    | 2.1 × 10⁻⁴                  | 2.1 × 10⁻¹⁰ kg     | ∞   | 0.21%                |     |                      |
| u(f_b)                      | 6.7 × 10⁻⁷       | 2.1 × 10⁻⁴ kg               | 1.4 × 10⁻¹⁰ kg     | ∞   | 0.09%                |     |                      |
| u(d_a)                      | 1.1 × 10⁻³ kg/m³ | 4.8 × 10⁻⁴ m³/kg            | 5.3 × 10⁻⁷         | ∞   | 0.06%                |     |                      |
| u(d_w)                      | 17.3 kg/m³       | 1.8 × 10⁻⁴ m³/kg            | 3.2 × 10⁻⁷         | ∞   | 0.02%                |     |                      |
| u(d_s)                      | 0.6 kg/m³        | −4.3 × 10⁻⁷ m³/kg           | 2.5 × 10⁻⁷         | ∞   | 0.01%                |     |                      |
| u(F)                        | 0 C/mol          |                              | 0                | 0%  |                      |     |                      |

* u(RSR) was estimated by considering the calibration uncertainty, temperature coefficient of resistance, and temperatures of the standard resistor at the time of calibration and measurements.
* u(Qbias) was estimated by assuming a rectangular distribution with a half-width of 0.0015 C.
* u(f_cal) was calculated from the calibration uncertainties of the barometer and thermo-hygrometer.
* u(d_s) was determined by assuming a rectangular distribution of the possible density values of the standard weight.
* To estimate u(d_s), the possible range of sample densities was assumed to be a rectangular distribution.

The method developed in this study is proposed as a primary reference measurement procedure for EDTA assay. Herein, the amount content was not related to any measurement standard for a quantity of the same kind. Instead, it was determined using different quantities such as current and time. Therefore, the proposed method conforms to the definition of the primary reference measurement procedure described in the International Vocabulary of Metrology (VIM) [41].

3.5. Measurement of the amount content of the complexing agent in the SMU CRM through the proposed procedure

The proposed measurement procedure was applied for the coulometric assay of the SMU EDTA CRM. Although this CRM was developed for internal use, it was used in this study because we could not find any other commercially available EDTA CRM. The certified value of the CRM was obtained using an independent measurement procedure [42, 43] wherein...
a Zn amalgam was used as the anode for the coulometric titration of EDTA. The bias from the oxidation of Zn due to the presence of dissolved oxygen was considered. The sample was taken before each experiment and used without further drying as instructed in the certificate. The amount of the complexing agent expressed as EDTA was obtained from multiple measurements \((n = 5)\). As shown in figure 4, the measured value is \((3.41867 \pm 0.00075) \text{ mol kg}^{-1} (k = 2.8)\) and it can be considered as equivalent to the certified value of the CRM \((3.41861 \pm 0.00093) \text{ mol kg}^{-1} (k = 2)\) within the measurement uncertainty. The variations in the results due to sample homogeneity were not reflected in the uncertainty of the measured value.

4. Conclusion

In this study, a candidate primary reference measurement procedure for the amount content of the complexing agent expressed as EDTA was developed based on complexometric coulometric titration using electrogenerated Hg ions. In this procedure, Hg anode was used because HER and ORR are not kinetically favored on its surface, which prevents the unwanted oxidation of the anode. Although \(\text{Hg}^{2+}\) may form due to the oxidation of the Hg anode, its disproportionation into \(\text{Hg}^{2+}\) in the presence of EDTA selectively formed the \(\text{HgY}^{2-}\) complex. For such conditions, the conditional formation constant can be calculated using the equilibrium constant of the disproportionation reaction and the conditional formation constant between EDTA (in the form of \(\text{Y}^{2-}\)) and \(\text{Hg}^{2+}\). The experimental maximum slope was not significantly different from that obtained from the theoretical titration curve. The developed procedure was used for the measurement of the amount content of the complexing agent in an EDTA CRM provided by the SMU. The measured value agreed well with the certified value of the CRM within the measurement uncertainty. We believe that the measurement procedure developed herein can be used to ensure international equivalence in the measurement of the amount content of the complexing agent through cooperation with EAWG members. In future work, we will examine the effect of sample mass and current density on the measurement results for further improvement of the measurement procedure.

Acknowledgments

This work was supported by the Korea Research Institute of Standards and Science (KRISS) under the project, Establishing the basis for inorganic analysis, with Grant No. 21011058. The authors would like to thank Michal Máríássy for supplying the SMU EDTA CRM that was originally developed for internal use.

Appendix

For the titration of EDTA with electrogenerated Hg ions, i.e., \(\text{Hg}^{2+}\) and \(\text{Hg}^{2+}\), the conditional formation constant between EDTA and the electrogenerated Hg ions is given by

\[
K_{i,HgY}'' = \frac{[\text{HgY}^{2-}]}{[[\text{Hg}^{2+}] + [\text{Hg}^{2+}]] [\text{Y}].}
\]

When the total concentration of the Hg ions that have not reacted with EDTA is denoted as \([\text{Hg}_{\text{tot}}]\), the following relationship was obtained

\[
[\text{HgY}^{2-}] = K_{i,HgY}''[\text{Hg}_{\text{tot}}][\text{Y}].
\]

The mass balance equations for the electrogenerated Hg ions and EDTA are

\[
[\text{Hg}_{\text{tot}}] + [\text{HgY}^{2-}] = \frac{n_{\text{Hg}}}{V},
\]

\[
[\text{Y}] + [\text{HgY}^{2-}] = \frac{n_{\text{EDTA}}}{V}.
\]

Substituting \(K_{i,HgY}''[\text{Hg}_{\text{tot}}][\text{Y}]\) for \([\text{HgY}^{2-}]\) in the mass balance equations yield

\[
[\text{Hg}_{\text{tot}}] + K_{i,HgY}''[\text{Hg}_{\text{tot}}][\text{Y}] = \frac{n_{\text{Hg}}}{V},
\]

\[
[\text{Y}] + K_{i,HgY}''[\text{Hg}_{\text{tot}}][\text{Y}] = [\text{Y}](1 + K_{i,HgY}''[\text{Hg}_{\text{tot}}]) = \frac{n_{\text{EDTA}}}{V}.
\]

Substituting the expression for \([\text{Y}]\) into the mass balance equations for the electrogenerated Hg ion yield

\[
[\text{Hg}_{\text{tot}}] + K_{i,HgY}''[\text{Hg}_{\text{tot}}]\frac{n_{\text{EDTA}}}{V(1 + K_{i,HgY}''[\text{Hg}_{\text{tot}}])} = \frac{n_{\text{Hg}}}{V}.
\]

This equation can be rearranged as a quadratic equation in \([\text{Hg}_{\text{tot}}]\)
Lastly, the equation for $[H_{g(tot)}]$ was obtained by solving the formulated quadratic equation and getting its positive real root

$$[H_{g(tot)}] = -\frac{(K_{f,HgY}^{n,EDTA} + V - K_{f,HgY}^{n,Hg})}{2K_{f,HgY}^{n,Hg}}$$

References

[1] BIPM 2019 Mise en pratique for the definition of the mole SI Brochure 9th edn (Paris: BIPM) https://bipm.org
[2] Máriássy M et al 2006 CCQM-K34 final report: assay of potassium hydrogen phthalate Metrologia 43 08008
[3] Liandi M et al 2019 Assay of potassium hydrogen phthalate (CCQM-K34.2016) Metrologia 56 08004
[4] Pratt K W et al 2013 Final report on key comparison CCQM-K73: amount content of $H^+$ in hydrochloric acid (0.1 mol kg$^{-1}$) Metrologia 50 08001
[5] Bastkowi F et al 2021 Key comparison CCQM-K73.2018 amount content $H^+$ in hydrochloric acid (0.1 mol kg$^{-1}$) Metrologia 58 08002
[6] Liandi M, Mariassy M and Pratt K W 2010 Final report on the key comparison CCQM-K48: assay of potassium chloride Metrologia 47 08009
[7] Liandi M A et al 2016 CCQM-K48.2014: assay of potassium chloride Metrologia 53 08012
[8] Máriássy M et al 2016 Final report on key comparison CCQM-K96: determination of amount of dichromate Metrologia 50 08012
[9] Hwang E, Lim Y, Ma L and Wu B 2015 Final report of CCQM-K96.1 ‘determination of amount content of dichromate’ Metrologia 52 08016
[10] Máriássy M 2011 Final report on CCQM-P112: assay of EDTA BIPM
[11] CCQM 2021 Strategy of the CCQM Working Group on Electrochemical Analysis and Classical Chemical Methods (EAWG) 2021–2030 BIPM
[12] Marienko G and Foley R T 1975 Absolute determination of the electrochemical equivalent and the atomic weight of zinc: I. Method, apparatus, and preliminary experiments J. Res. Natl. Bur. Stand. A 79A 737–45
[13] Vetter K J 1967 Electrochemical Kinetics: Theoretical and Experimental Aspects 1st edn (New York: Academic) pp 736–9
[14] Máriássy M, Pratt K W and Spitzer P 2009 Major applications of electrochemical techniques at national metrology institutes Metrologia 46 199–213
[15] Taylor K and Smith S W 1959 Precise coulometric titration of acids and bases J. Res. Natl. Bur. Stand. A 63A 153–59
[16] Kolthoff I M et al 1969 Quantitative Chemical Analysis 4th edn (London: Macmillan) p 804
[17] Ladd M F C and Povey D C 1973 Crystallographic and spectroscopic studies on ethylenediaminetetraacetic acid (EDTA): II. Comparison of two structure determinations of $\beta$-EDTA Acta Crystallogr. B 29 2973–5
[18] Moser H C and Voigt A F 1957 Dismutation of the mercurous ion in hydrochloric acid (0.1 mol kg$^{-1}$) Metrologia 47 08009
[19] Sanemasa I 1976 Kinetics of the disproportionation reaction of mercury(I) with hydrogen cyanide in acidic solutions Inorg. Chem. 15 1973–6
[20] Hingle D N, Kirkbright G F and West T S 1967 Some observations on the determination of mercury by atomic-absorption spectroscopy in an air-acetylene flame Analyst 92 759–62
[21] Ringbom A 1963 Complexation in Analytical Chemistry: A Guide for the Critical Selection of Analytical Methods Based on Complexation Reactions (New York: Wiley) p 52
[22] Anderson J A and García M F 2011 Supported Metals in Catalysis (London: Imperial College Press) p 412
[23] Kolthoff I M and Miller C S 1941 The reduction of oxygen at the dropping mercury electrode J. Am. Chem. Soc. 737–45
[24] Sanemasa I and Hirata T 1977 The disproportionation constants of mercury(I) in dilute solutions Bull. Chem. Soc. Japan 50 3255–8
[25] Laitinen H A and Harris W E 1975 Chemical Analysis 2nd edn (Tokyo: McGraw-Hill) p 194
[26] Kolthoff I M and Miller C S 1941 Anodic waves involving electrooxidation of mercury at the dropping mercury electrode J. Am. Chem. Soc. 63 1405–11
[27] Tianlin W, Fengling L and Liming F 1993 Theoretical analysis of one–one complexometric titration curves J. Chem. Educ. 70 796–8
[28] Kim J I, Kim B and Hwang E 2014 An approach for the uncertainty evaluation of the overall result from replications of
measurement: separately combining individual uncertainty components according to their “systematic” and “random” effects Bull. Korean Chem. Soc. 35 1057–60

[38] Taylor B N and Kuyatt C E 1994 Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results NIST Technical Note 1297 National Institute of Standards and Technology (NIST) (https://doi.org/10.6028/NIST.TN.1297-1993)

[39] BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML 2008 JCGM 100:2008 Evaluation of Measurement Data—Guide to the Expression of Uncertainty in Measurement 1st edn (JCGM)

[40] González A G, Herrador M Á and Asuero A G 2005 Estimation of the uncertainty of mass measurements from in-house calibrated analytical balances Accred. Qual. Assur. 10 386–91

[41] BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML 2012 JCGM 200:2012 International Vocabulary of Metrology - Basic and General Concept and Associated Terms (VIM) 3rd edn (JCGM)

[42] Kipphardt H et al 2017 Primary standards for challenging elements EMRP-SIB09 EURAMET

[43] Private communication with the person responsible for the certification of the CRM.