Determination of chlorine ions in raw milk by pulsed amperometric detection in a flow injection system

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ABSTRACT

Chloride ion concentration in milk was determined by pulsed amperometric detection in a flow injection system. Results showed that the Au electrode lost 3 electrons at 1.10 V and formed chloroaurate ions (AuCl_4^-) by combining with chloride ions, after which AuCl_4^- was partly reduced to Au at 0.6 V. Based on the electrochemical process, a triple waveform with detection potential of 1.15 V, detection time of 150 ms, oxidation potential of 1.4 V, oxidation time of 550 ms, reduction potential of 0 V, and reduction time of 400 ms was applied to the Au electrode for detecting chloride ion concentration in milk. The approach is rapid and automatic and features a detection limit of 0.005 g/L. The relative standard deviation obtained by 60 repetitive injections reached 1.48% at 2 g/L of NaCl. The method developed using the Au electrode without modification was used to analyze the chloride ion concentration in raw milk without preprocessing. The method showed good agreement with potentiometric titration.

Key words: chloride ion, pulsed amperometric detection, flow injection system, milk adulteration

INTRODUCTION

Milk is one of the high-grade products in the human diet (Mazhitova and Kulmyrzaev, 2016). Raw milk will directly affect the quality of dairy products (Andersen, 2007; Fonseca, 2012), and thus various test criteria are used for evaluation. Determination of chloride ion content in raw milk is an important quality test criteria because it can reflect the micro-contamination caused by mastitis and adulteration with sodium chloride.

Lactose and chloride ions act as osmotic regulators during lactation. Chloride ions in the blood permeate into the milk to maintain the osmotic balance of blood

and milk during the lactose decrease that results from mastitis and heavy microbial contamination. Therefore, to a certain extent, chloride ion content can reflect microbial contamination of milk. However, the excessive levels of chloride ions caused by mastitis is far less than that resulting from adulteration with sodium chloride. The simplest method of milk adulteration is adding water (Adam, 2009); however, with the use of freezing point depression testing in the acceptance of raw milk (Moreira et al., 2016), this method is no longer viable. Sodium chloride is a material that can easily increase milk density and lower the freezing point of raw milk; thus, dairy farmers sometimes add salt to raw milk to interfere with freezing point depression testing.

At present, the most common test methods for detecting chloride ions in milk are precipitation titration and potentiometric titration (Lacroix et al., 1970), both of which use silver nitrate standard solution to determine the concentration of chlorine ions, which is calculated by the consumption of the silver nitrate standard solution. The distinction between the 2 methods is the end point of titration. Potentiometric titration determines the titration end point by a spike in the potential, whereas precipitation titration determines the end point of titration by an orange color precipitation. Comparatively, potentiometric titration is more sensitive and objective than precipitation titration. In addition, many other methods are available in other fields for detection of chloride ions. For example, Noguchi et al. (2009) reported ion chromatography for simultaneous determination of fluoride ions (F^-), chloride ions (Cl^-), and bromine ions (Br^-) in cement. The limits of detection for F^- , Cl^- , and Br^- are 0.2, 0.3, and 0.5 mg/kg, respectively; however, these methods require sample pretreatment and complex procedures, reducing their suitability for automatic testing.

Milk adulteration has been a problem in the dairy industry for many years. With industry development, traditional detection methods for adulterated dairy products have been challenged, and new detection techniques have emerged (Rodrigues et al., 2012; Jawaideh et al., 2013; Gupta et al., 2014; Das et al., 2015). In gener-

Received January 5, 2018.

Accepted June 28, 2018.

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al, pulsed amperometric detection (**PAD**) is based on a triple-potential waveform and can obtain reproducible signals over time (Guedes et al., 2015.). In this method, the electrode should be pulsed sufficiently to remove and clean adsorbed species and then return to its negative potential status to reduce the resulting oxide, inducing reactivation of the electrode surface. Thus, PAD can solve the problem of continuous measurement. Hughes and Johnson (1981) first reported the use of PAD in platinum electrodes for detection of carbohydrates following liquid chromatography separation in 1981. This approach has been developed and applied extensively as a detection technique for various substances, including carbohydrates (Rohrer et al., 2013; Nguyen et al., 2015; Jamaluddin Ahmed and Smith, 2015), cyanides (Wu et al., 2015), amines, and organo-sulfur compounds (Lee and Yeo, 2001). Flow injection (**FI**) analysis is an automated analysis method developed in recent years and is widely used in many fields (Nabi et al., 1996; Peng et al., 2015) with advantages including high efficiency and simplicity (Chetty and Surendra, 2016; Yan et al., 2016). The operating principle is that the sample solution is maintained under strictly controlled conditions and is dispersed in the current-carrying reagent. Therefore, as long as the injection method of the sample solution, rate of flow in the pipeline, temperature, and the dispersion process are unchanged, FI analysis can determine the concentration of substances according to a working curve set using the standard solution (Jolley et al., 1997). Ngamchana and Surareungchai (2004) determined formalin by PAD and applied the method to use of an Au electrode for detection in a FI system. The method showed good agreement with liquid chromatography results.

Gold shows high activity for reactions with chlorine ions (Gao et al., 1985); therefore, the use of PAD for detection of chlorine ions by an unmodified Au electrode should be examined. In this work, an automatic method for detection of chlorine ion concentration in raw milk was established through a measurement loop; in this way, we used the Au electrode as the working electrode, the platinum wire electrode as the auxiliary electrode, and a silver chloride electrode (Ag/AgCl electrode) as the reference electrode by PAD in a flow injection system (FI-PAD).

MATERIALS AND METHODS

Reagents and Samples

Potassium ferrocyanide, zinc acetate, and sodium sulfate (Na_2SO_4) were purchased from Xilong Chemical Co. Ltd. (Shantou, China). Nitric acid, urea, dextrin,

and glucose were purchased from Damao Chemical Reagent Factory (Tianjin, China). Sodium bromide and sodium chloride were purchased from Shanghai Building Industrial and Trade Co. Ltd. (Shanghai, China). All reagents were of analytical grade, and triple-distilled water was used in all experiments. The raw milk (with chloride ion content of 1.00 g/L as detected by potentiometric titration) was provided by Changshan Modern Organic Pasture (Nanchang, China).

Apparatus

All the electrodes used in the experiments were bought from the Xianren Company (Shanghai, China). A 1.0-mm-diameter Au disk was used as the working electrode, wherein the electrode surface was polished with 0.3- and 0.05- μm alumina slurries and subsequently sonicated in triple-distilled water before electrochemical measurement. A platinum wire was used as the counter electrode, whereas Ag/AgCl electrode was the reference electrode. Electrochemical measurements were performed using an electrochemical quartz crystal microbalance CHI440C, which was purchased from Chenghua Company (Shanghai, China). A LabRAM HR Laser Raman Spectrometer (excitation wavelength of 632.8 nm, spectrometer focal length of 800 nm) was purchased from Jobin Yvon Company (Longjumeau, France), with 2 measurement modes of high sensitivity and high resolution. The Hitachi S-3400N Scanning Electron Microscope was purchased from Hitachi High-Technologies Corporation (Tokyo, Japan). The computer-controlled potentiostat was purchased from Shanghai Jingke Industrial Co. Ltd. (Shanghai, China). All experiments were carried out at room temperature, and all potentials reported refer to those for the Ag/AgCl electrode. To eliminate oxygen interference with the electrochemical experiments, high-purity nitrogen was pumped into all solutions before the experiments (Ngamchana and Surareungchai, 2004).

The FI-PAD system used a 3-electrode assembly incorporated into a fabricated electrochemical flow-through cell with a volume of 1.6×10^{-3} mL. The cell was placed in the reaction chamber with the capability of shielding interference. Figure 1 shows the diagram of the FI system for detection of chlorine ions in raw milk. The 100 g/L Na_2SO_4 solution, which was used as the carrier stream, was delivered at a flow rate of 0.3 mL/s by a peristaltic pump. The injection sample solution (10 μL) was injected into the carrier with an injection valve and the sample solution was dispersed uniformly in the carrier stream when the sample solution flowed through the pipeline. Eventually, the sample solution flowed into the miniature cell for detecting chloride ions.

Because the injection valve, flow rate, and dispersion process in the pipeline were unchanged, the method could determine the concentration of chloride ions.

Electrochemical Reaction Path of Gold Electrode

To determine the optimum concentration of the carrier stream, different concentrations of sodium sulfate solution (50, 100, and 150 g/L) were detected by cyclic voltammetry (CVM). Then, 0.5 g/L of sodium chloride was added to the optimum carrier stream to study the electrochemical difference of the Au electrode before and after addition of sodium chloride. To study the effect of the potential range on the electrochemical properties of the Au electrode, the above mixed solution was detected by changing the scanning potential.

After this was concluded, 2 g/L of sodium chloride was added to the optimum carrier stream to study the effect of scanning rate, pH, and sodium chloride concentration on the detection of chloride ions. To study the effects of scanning rate (v), the above mixed solution was detected at scanning rates of 0.005 to 0.1 V/s. To determine the number of protons, the above mixed solution was detected by changing the pH (2.0, 2.5, 3.0, 3.5, 4.0). To study the effect of sodium chloride concentrations, measurements of CVM curves of the Au electrode were carried out with various sodium chloride solutions (0.5, 1.0, 1.5, 2.0, and 2.5 g/L). To study the morphology of the Au electrode before and after the detection of chloride ions, scanning electron microscopy images and surface-enhanced Raman scattering (SERS) images of the Au electrode were acquired before and after the electrochemical reaction in

the carrier stream containing 2 g/L of NaCl and 100 g/L of Na_2SO_4 .

To test whether fat and large protein molecules will interfere with the detection of chloride ions in milk, part-skim milk and UF milk permeate were prepared. First, raw milk was collected from pasture-fed cows and filtered with 4 layers of sterilized gauze to remove most impurities. Then, the milk was centrifuged at $4,150 \times g$ for 20 min after heating to 40°C in a water bath. A portion of the milk was saved for the part-skim milk, and the rest was added to the UF centrifuge tube (filtration molecular weight cut-off of 3 kDa). The milk in the UF centrifuge tube was centrifuged at $2,334 \times g$ for 10 min at 4°C . Table 1 lists the fat, protein, and chloride ion contents of raw milk, part-skim milk, and UF milk permeate.

Optimization of PAD Parameters

The values of E_{red} and E_{oxd} were optimized by CVM, whereas that of E_{det} was optimized by hydrodynamic voltammograms obtained by the application of the PAD waveform at 2 g/L of sodium chloride solution. The time periods were selected from chronoamperometry by observing the time required for double-layer charging to decay to a minimal value.

FI Determination

The regenerative capacity of electrode activity after optimizing PAD parameters was studied by 60 repetitive injections of 2 g/L of sodium chloride solution. To determine the detection limit of this method, a stan-

Figure 1. Diagram of the flow injection system for detection of chlorine ions in raw milk. PAD = pulsed amperometric detection. Color version available online.

Table 1. Concentration of fat, protein, and chloride ions of raw milk, part-skim milk, and UF milk permeate

Sample	Fat content ¹ (g/100 g)	Protein content ² (g/100 g)	Chloride ion content ³ (g/L)
Raw milk	3.11	3.13	1.00
Part-skim milk	2.51	2.67	1.04
UF milk permeate	0.18	0.23	1.05

¹Results are the average value of the triplicate study of Kjeldahl determination.

²Results are the average value of the triplicate study of Roese-Gottlieb method.

³Results are the average value of the triplicate study of potentiometric titration.

dard curve was obtained by injecting different amounts of sodium chloride.

Testing in Raw Milk Samples Spiked with Different Amounts of Chloride Ions

To obtain the recovery rates of the chloride ions in raw milk and compare the significant difference shown in the FI-PAD and the potentiometric titration method, raw milk samples spiked with different amounts of chloride ions (0.5, 1.0, 1.5, 2.0, and 4 g/L) were analyzed by using the FI-PAD and the potentiometric titration. The automatic potentiometric titrator concerned was purchased from Shanghai Leici Co. Ltd. (Shanghai, China) and it should be noted that sample

pretreatment needed to be carried out before each titration. One hundred milliliters of triple-distilled water was added to 20 mL of raw milk sample, and then the sample solution was heat-treated in a water bath at 100°C for 15 min. After the sample solution cooled to room temperature, 4 mL of 0.25 mol/L potassium ferrocyanide solution and 4 mL of 1.2 mol/L zinc acetate solution were added in turn, and the supernatant was transferred to a 200-mL volumetric flask and diluted to 200 mL with water after the reaction of 30 min (Lima et al., 2004). After the pretreatment, we added 10 mL of diluent and 5 mL of 0.5 mol/L of nitric acid solution, respectively, in a 50-mL beaker, then the chloride ions in diluent were titrated by using 0.02 mol/L of silver nitrate standard solution (Lima et al., 2004).

Figure 2. Optimization of different concentrations of sodium sulfate solution (Na_2SO_4). To reveal the effect of Na_2SO_4 , we obtained the cyclic voltammetry (CVM) curves of the Au electrode by electrochemical quartz crystal microbalance under different Na_2SO_4 contents: (A) 150 g/L, (B) 100 g/L, and (C) 50 g/L.

Data Analysis

All data were obtained in triplicate to prove the reproducibility of the assay. Data were analyzed for significant differences using SPSS version 19 (SPSS Inc., Chicago, IL). Results with $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Optimization of Na_2SO_4 Concentration

Figure 2 shows the CVM curves of the Au electrode with different concentrations of Na_2SO_4 solution. As shown in the figure, a small oxidation peak was observed at approximately 0.33 V, and a small reduction peak was noted at approximately 0.05 V. These 2 peaks, respectively, corresponded to the adsorption and desorption of hydroxyl ions on the Au electrode surface (Strbac et al., 1998). The oxidation curve at 1.0 V and the reduction peak at 0.58 V corresponded to the formation and reduction of Au oxide, respectively. Comparing the CVM curves of the Au electrode at different concentrations of Na_2SO_4 solution, the potentials of each peak in 100 and 50 g/L of Na_2SO_4 solution

were almost the same, and current intensity (I) slightly increased. However, no significant difference was observed in the potentials and current of each peak in the 100 g/L of Na_2SO_4 solution and 150 g/L of Na_2SO_4 solution. These results demonstrated that the electrochemical reaction stabilized when the sodium sulfate concentration reached 100 g/L. Thus, we selected 100 g/L of Na_2SO_4 solution as the carrier stream of the electrochemical reaction.

Effect of Scanning Range

The waves in Figure 3 represent the differences in the Au electrode electrochemical process before and after adding 0.5 g/L of sodium chloride to the carrier stream. After addition of chloride ions, the Au electrode produced a larger oxidation peak at 1.10 V and a reduction peak at 0.6 V. The response current intensity caused by addition of chloride ions was 30 times more than that caused by the carrier stream, indicating that addition of chloride ions produced a strong electrochemical reaction. The starting potential was fixed at 0 V, and the scan termination potential was changed. When the termination potential was less than 1.2 V, the 0.6

Figure 3. Electrochemical difference of the Au electrode before and after adding 0.5 g/L of sodium chloride (NaCl) to the carrier stream. To reveal the effect of adding chloride ions, we obtained the cyclic voltammetry curves of the Au electrode before and after adding 0.5 g/L of NaCl to the carrier stream by electrochemical quartz crystal microbalance. (A) Blank carrier solution at 100 g/L of Na_2SO_4 ; (B) adding 0.5 g/L of NaCl to 100 g/L of Na_2SO_4 .

V reduction peak resulted in no significant increase. Termination potential was then fixed at 1.4 V, and the scanning start potential was changed. When the initial potential was higher than 0.6 V, a larger oxidation peak at 1.10 V still appeared. This result showed that the reduction peak at 0.6 V was mainly formed by the oxidation product at 1.10 V.

Effect of Scan Rate and pH

Figure 4 displays the CVM curves of the Au electrode under different scanning rates. The CVM curves show that both the anode current intensity (I_{pa}) and cathode current intensity (I_{pc}) increased with increasing scan rate and featured a good linear relationship with the square of the scan rate ($I_{pa} = -1.1016v^{1/2} + 1.62529$; $R^2 = 0.9955$, $I_{pc} = 0.65394v^{1/2} - 0.72984$, $R^2 = 0.9984$; I_{pa} and I_{pc} represent the anode current intensity and cathode current intensity, respectively, whereas v represents the scan rate). These findings demonstrate that both the anode reaction at 1.10 V and the cathode reaction at 0.6 V were controlled by diffusion and the electrons transfer number of 3 was obtained according to Laviron's theory. The peak improved when the

scan rate was fixed at 0.01 V/s, and thus 0.01 V/s was selected as the scan rate.

Figure 5 illustrates the CVM curves of the Au electrode at different pH values. The figure shows that almost no movement was noted in the oxidation and reduction peaks, indicating that no protons were involved in the reaction. As shown in the figure, a small reduction peak appeared at 0.9 V at pH 2.0, corresponding to the reduction of AuO under acidic conditions.

Effect of Chloride Ion Concentration

Figure 6 shows the CVM curves of the Au electrode under different chloride ion concentrations. The figure also indicates that as the concentration of chloride ions increased, both the oxidation peak at 1.10 V and the reduction peak current at 0.6 V increased significantly and featured a positive correlation with chloride ion concentration. On the other hand, the oxidation peak potential continuously shifted with increasing chloride ion concentration, and the oxidation peak disappeared when chloride ion concentration exceeded 30 g/L. Figures 4 and 6 showed that with increasing sweep rate and concentration, the symmetry of anode and cathode

Figure 4. Cyclic voltammetry (CVM) curves of the Au electrode under different scanning rates. To reveal the effect of scanning rates, we obtained the CVM curves of the Au electrode in the mixed solution containing 2 g/L of NaCl and 100 g/L of Na₂SO₄ under different scanning rates. (A) 0.005 V/s, (B) 0.01 V/s, (C) 0.025 V/s, (D) 0.075 V/s, and (E) 0.1 V/s.

peaks decreased, and the difference in peak potential also grew, indicating that reversibility became poor with increasing sweep rate and concentration of the reaction.

Reaction Process of the Gold Electrode

To sum up, the electrochemical reaction of chloride ions was initially concluded as follows. First, Au lost 3 electrons and combined with chloride ions, forming AuCl_4^- at 1.10 V ($\text{Au} + 4\text{Cl}^- - 3\text{e}^- \rightarrow \text{AuCl}_4^-$); then, a part of AuCl_4^- reverted to Au at 0.6 V; thus, the potential significantly influenced the adsorption of chloride ions. Gold dissolved due to the adsorption of chloride ions at high electrode potential, and scanning electron microscopy and SERS experiments confirmed this conclusion.

Scanning Electron Microscopy and SERS Experiments

Scanning electron microscopy images and the Raman image of the Au electrode were obtained before and

after carrying out electrochemical reactions in the carrier stream containing 2 g/L of NaCl and 100 g/L of Na_2SO_4 . As shown in Figure 7, the surface of the Au electrode after electrochemical reaction was remarkably uneven compared with the surface of the electrode before the electrochemical reaction. Figure 8 shows that the electrode before the electrochemical reaction presented no Raman response at 100 to 400 cm^{-1} , and the Raman peak of the Au electrode after electrochemical reaction appeared at 269 cm^{-1} . These results were consistent with those of a previous study on Au-Cl Raman peak position (Li et al., 2004). These results confirmed that chloride ions caused the anodic dissolution of the Au electrode in the Na_2SO_4 system.

Electrochemical Properties of the Gold Electrode in Raw Milk

Figure 9 displays the CVM curves of the Au electrode in raw milk, part-skim milk, and UF milk permeate, all with added 100 g/L of Na_2SO_4 . The curves indicate a large oxidation peak at 1.12 V and a reduction peak at 0.57 V, which was generally consistent with the

Figure 5. Cyclic voltammetry (CVM) curves of the Au electrode under different pH values. To determine the number of protons involved in the reaction, we obtained the CVM curves of the Au electrode in the mixed solution containing 2 g/L of NaCl and 100 g/L of Na_2SO_4 under different pH values. (A) pH 2.0, (B) pH 2.5, (C) pH 3.0, (D) pH 3.5, and (E) pH 4.0; insert image for local magnification of dotted box.

Figure 6. Cyclic voltammetry (CVM) curves of the Au electrode under different concentrations of chloride ions. To reveal the effect of chloride ion content, we obtained the CVM curves of the Au electrode under different chloride ion contents. (A) Blank carrier solution at 100 g/L of Na_2SO_4 , (B) the carrier solution plus 0.5 g/L of NaCl, (C) the carrier solution plus 1.0 g/L of NaCl, (D) the carrier solution plus 1.5 g/L of NaCl, (E) the carrier solution plus 2.0 g/L of NaCl, and (F) the carrier solution plus 2.5 g/L of NaCl; insert image for local magnification of dotted box.

peak of the Au electrode in Na_2SO_4 solution containing chloride ions. As presented in Table 1, fat and protein contents of the raw milk, part-skim milk, and UF milk

permeate significantly decreased, but chloride ion content remained almost unchanged. Figure 9 shows that no significant electrochemical change occurred with

Figure 7. Morphological scanning electron microscope images of the Au electrode before (A) and after (B) the detection of chloride ions by electrochemical reaction in the carrier stream containing 2 g/L of NaCl and 100 g/L of Na_2SO_4 .

Figure 8. Raman response image of the Au electrode before (A) and after (B) the detection of chloride ions by electrochemical reaction in the carrier stream containing 2 g/L of NaCl and 100 g/L of Na₂SO₄.

changes in fat and protein contents, indicating that the electrochemical process is not related to fat and protein contents. Therefore, large protein molecules in milk caused no interference with the detection of chloride ions, and milk samples to be tested can be directly added to the device without special preparations.

PAD Optimization

Pulsed amperometric detection parameters were optimized based on CVM. The potentials for E_{oxd} and E_{red} were kept constant at 0 and 1.4 V, respectively, and were determined according to the starting and termination potentials of CVM. The E_{red} and E_{oxd} were kept constant, and the E_{det} value, whose optimization index was current intensity, was optimized at 0.95 to 1.2 V. As shown in Figure 10, the Faradaic current, which is produced by the reaction of the Au electrode with chloride ions, increased with the increase in potential from the beginning. However, when the potential reached 1.15 V, the current value decreased, thus 1.15 V was selected as the optimal parameter of E_{det} . Notably, the Au electrode in 100 g/L of Na₂SO₄ solu-

Figure 9. Cyclic voltammetry curves of the Au electrode under (A) raw milk, (B) part-skim milk, or (C) UF milk permeate (UF milk permeate) added with 100 g/L of Na₂SO₄. Inset images show local magnification of dotted boxes.

Figure 10. Optimization parameter of detection potential by the flow injection hydrodynamic voltammogram of the Au electrode before and after adding 2 g/L of NaCl to the carrier stream. The solution containing 100 g/L of Na_2SO_4 (▼) and the mixed solution containing 2 g/L of NaCl and 100 g/L of Na_2SO_4 (●) are shown. Error bars indicate SD of the 5 replicates.

tion showed no PAD response, confirming that the carrier solution caused almost no effect on measurement. Optimal timing parameters for PAD cannot be deduced from the CVM response, thus the time periods were selected through chronoamperometry by observing the time required for double-layer charging to decay to a minimal value. Chronoamperometric curves following step 0 to 1.15 V showed that the current from double-layer charging decayed to 0 after 100 ms. Therefore,

Figure 11. Pulsed amperometric detection response of 60 repetitive injections of 2 g/L of NaCl in the flow injection system under a carrier solution of 100 g/L of Na_2SO_4 at a flow rate of 0.30 mL/s and an injection volume of 10 μL .

any value for $t_{\text{det}} > 100$ ms was satisfactory. For t_{oxd} and t_{red} , the same process was followed. From the step 1.15 to 1.2 V, the current from double-layer charging decayed to 0 after 500 ms. From the step 1.2 to 0 V, the current from double-layer charging decayed to 0 after 370 ms. Hence, t_{det} , t_{oxd} , and t_{red} were finally selected as 150, 550, and 400 ms, respectively.

Flow Injection Determination Characteristics

The PAD response of 2 g/L of NaCl was obtained in the carrier solution of 100 g/L of Na_2SO_4 at a flow rate of 0.30 mL/s and an injection volume of 10 μL . The result of 60 repetitive injections is shown in Figure 11, of which the average current intensity of 2 g/L of NaCl was 2.187×10^{-4} A, with a standard deviation of 3.242×10^{-6} ($n = 60$). No apparent change with repeated measurement was observed; however, the measurement result was continuously increasing, which might be caused by the constant corrosion of the Au electrode after multiple measurements, therefore, the Au electrode needed to be regenerated after 60 measurements. In addition, a good linear relationship was found between the PAD current intensity and the chloride ion concentration (Figure 12), in which the linear equation was $I (10^{-4} \text{ A}) = -1.508 \times 10^{-4} - 3.3259 \times 10^{-5} C (\text{g/L})$, $R^2 = 0.9992$ (I represents current intensity, and C represents chloride ion concentration). The detection limit of 0.005 g/L was calculated by the definition in accordance with the International Union of Pure and Applied Chemistry recommendation. The anti-interference test results are shown in Table 2, of which urea, dextrin, and glucose did not interfere in the detection,

Figure 12. Establishment of standard curves for chloride ions by pulsed amperometric detection in a flow injection system. Error bars indicate SD of triplicate studies of this assay.

Table 2. Pulsed amperometric detection response of 2 g/L of sodium chloride with or without other high-density additives or bromide ions

Additive	Current intensity ¹ (10 ⁻⁴ A)	RSD ² (%)
2 g/L of sodium chloride	-2.184	0.87
2 g/L of sodium chloride + 20 g/L of urea	-2.190	1.28
2 g/L of sodium chloride + 20 g/L of dextrin	-2.182	1.10
2 g/L of sodium chloride + 20 g/L of glucose	-2.179	1.36
2 g/L of sodium chloride + 20 g/L of sodium bromide	-11.818	1.48

¹Results are the average value obtained by 3 determinations.²RSD = relative standard deviation.

but Br⁻ seriously interfered with the measurement results. This result may be attributed to the relative similarity of Br⁻ to Cl⁻.

Sample Analysis

To obtain the recovery rates of chloride ions in raw milk, we tested milk samples spiked with different concentrations of chloride ions by using the FI-PAD method. As shown in Table 3, the recovery rate of chloride ions ranged between 93 and 109.5% and stability improved. This result indicates promising feasibility of the proposed method for rapid detection of chloride ions in raw milk. In addition, the significant difference in FI-PAD and potentiometric titration was obtained by the paired *t*-test. The average value and standard deviation of the difference between the 2 methods were -0.060 and 0.1393, respectively, and the absolute *t* was 0.096. Therefore, the calculated *t* value obtained was obviously smaller than the critical *t* value of 2.776, indicating no significant difference between the results of FI-PAD and potentiometric titration analysis for determination of chloride ion concentration in raw milk.

CONCLUSIONS

This work presented a rapid, sensitive, reproducible, and highly automated method to detect chloride ion concentration in milk. Gold lost 3 electrons at 1.10 V

and formed AuCl₄⁻ by combining with chloride ions. The AuCl₄⁻ was partly reduced to Au at around 0.6 V. Based on this study, we determined the optimal parameters of the PAD method by CVM and chronoamperometry. We used FI-PAD at a flow rate 0.30 mL/s and an injection volume of 10 µL to detect chloride ions at different concentrations. The linear equation was $I (10^{-4} \text{ A}) = -1.508 \times 10^{-4} - 3.3259 \times 10^{-5} C$ (g/L), $R^2 = 0.9992$ (*I* represents current intensity, and *C* represents chloride ion concentration). The detection limit of 0.005 g/L was calculated in accordance with the International Union of Pure and Applied Chemistry recommendation. We used the FI-PAD method and potentiometric titration to test milk containing various concentrations of chloride ions and found no significant difference with potentiometric titration. In future studies, we will try to promote the measurement of chloride ions with FI-PAD in other fields, such as industrial wastewater or organic fertilizers.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (project number 31360387). We express our heartfelt gratitude to Hou Peng of Jiangxi Sunshine Dairy in China. We also thank Li Yayun and Yang Jun of Nanchang University (Jiangxi, China) for their help with the experiment.

Table 3. Recovery rates of chloride ions (Cl⁻) and comparison results of flow injection-pulsed amperometric detection (FI-PAD) and potentiometric titration obtained by detecting raw milk spiked with different amounts of chloride ions¹

Sample	Cl ⁻ found by FI-PAD (g/L)	Added Cl ⁻ (g/L)	Cl ⁻ total found by FI-PAD (g/L)	Recovery (%)	RSD (%)	Cl ⁻ found by potentiometric titration (g/L)	RSD (%)
Milk 1	1.08	0.50	1.61	106.00	4.37	1.57	2.30
Milk 2	1.00	1.00	1.93	93.00	2.36	2.16	2.63
Milk 3	0.96	1.50	2.43	98.00	3.40	2.47	3.10
Milk 4	0.93	2.00	3.12	109.50	3.12	3.05	3.34
Milk 5	1.03	4.00	5.11	102.00	4.17	4.98	2.64

¹All samples are raw milk, which is not adulterated with sodium chloride. Results are the average value obtained by 6 determinations. Relative standard deviation (RSD) was obtained by 6 determinations.

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