Development and Validation of Mercurimetric Titration Method for Determination of Chloride Concentration in Milks Using Novel Reagent

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ABSTRACT:

An indirect mercurimetric titration method is developed and validated for selective determination of chloride concentration in the milks using mono-thiocyanato-mercury(II) nitrate [$Hg(SCN)NO_3$] novel reagent. The method is based on standard addition of chloride in the milk sample. In the first step, the reagent $Hg(SCN)NO_3$ was prepared *in-situ* by mixing equivalent quantities of mercury(II) nitrate [$Hg(NO_3)_2$] and potassium thiocyanate [KSCN] in presence of iron(III) nitrate [$Fe(NO_3)_3$] indicator. Then, a measured amount milk sample was added into the known and excess amount of $Hg(SCN)NO_3$ reagent and the surplus $Hg(SCN)NO_3$ was measured by back titration against standard KSCN solution. Similarly, the second step was performed with another identical milk sample spiked with the measured amount of chloride. The chloride and $Hg(SCN)NO_3$ reagent were found to be reacting in the 1:1 stoichiometric ratio. So the amount chloride in the milk was determined from the amount of $Hg(SCN)NO_3$ utilized, which was then determined by performing a similar blank titration. The statistical treatment to the experimental data obtained by this method indicates that, the method is precise and accurate. The organic matter such as proteins, glucose, fructose, etc. and inorganic ions such as phosphate, sulfate, etc. present in the milk sample did not interfere with the measurement of chloride by this method. The proposed method of determination of milk chloride is simple, reliable and inexpensive. It also allows the determination of milk chloride at acidic pH with the stable and distinctly visible end point. The standard addition of chloride (amplification) also increases the sensitivity for chloride determination in milk at a trace level.

Keywords: Milk Analysis, Chloride Concentration, Mercurimetric Titration, Mono- thiocyanato-mercury(II) Nitrate, Standard Addition Method

INTRODUCTION

Milk is one of the highest-grade dairy products in the human diet because it contains all essential bioactive components for healthy growth of human body[1] (Mazhitova and Kulmyrzaev, 2016). The milk of various species differs qualitatively and raw milk directly affects the quality dairy product[2] (Andersen, 2007). The milk quality is largely affected by its adulteration, which may be done by adding many toxic chemicals and inorganic salts[3] (Nascimento et al. 2017). The simplest method of the milk adulteration is adding the water to increase the milk volume. The added water decreases density as well as the number of ions per unit volume of milk, which can be detected by measuring the electrical conductivity of the milk[4] (Das et al., 2015). Generally, for increasing the milk density, the dairy farmers are adding sodium chloride in the milk which is adulterated by adding water[5] (Moreira et al., 2016). Thus, determination of chloride concentration in the raw milk is an important quality test for detection and determination of adulteration with sodium chloride[4] (Das et al., 2015). The simple and suitable method for detection of added sodium chloride in milk is its reaction with standard silver nitrate solution containing potassium chromate. The permissible level of chloride is less than 1.42 mg/ml of milk, was tested by adding 2.0 ml of 0.1 N silver nitrate and 0.5 ml 10 % potassium chromate in 5.0 ml of milk sample[6] (FSSAI, 2018).

Recently, the most sensitive methods are available for detection and determination of chloride concentration in the milk, these includes pulse amperometric detection in flow injection system[7] (Chen, X., et al., 2018); sequential injection with automated conductimetry[8] (Silva, F. V. et al.,1999); potentiometry using silver-silver electrode[9] (Herrington and Kleyn 1960). Mono-segemented flow potentiometric titration using silver nitrate reagent titrant[10] (Vieira et al. 2003); sequential injection titration with potentiometric detection using silver-silver chloride electrode[11], (Reis Lima et al., 2004), direct titrimetric determination using acidic mercuric nitrate using a palladium-mercury calomel electrode system[12](Wenner 1960). In addition to this, the liquid chromatographic[13] (Zaky et al. 2017) and spectrophotometric methods are also found in literature for determination chloride in milk[14] (Herrero et al. 1992).

Most of these methods of determination of chloride in milk are time consuming and requires sophisticated analytical instrumentation with specific experimental setup. However, titrimetric analysis is the most common, convenient and favorite tool of all chemical laboratories due to its inherent reproducibility, accuracy and low costing absolute method of analysis. So, in the present study, an attempt is made to report the original approach that allows a simple and rapid titrimetric assay of milk chloride using mono-thiocyanato-mercury (II) nitrate reagent.

Materials and Methods

Materials

Double distilled water and analytical grade chemicals were used throughout the experimentation. A standard 0.05M KSCN and 0.05M NaCl solutions were prepared in distilled water. A 0.05M $Hg(NO_3)_2$ solution and a 0.2M $Fe(NO_3)_3$ indicator solution were prepared in 0.5M nitric acid (HNO_3). The molarity of KSCN was confirmed by its standardization against standard 0.05M $AgNO_3$ solution using $Fe(NO_3)_3$ indicator. The molarity of $Hg(NO_3)_2$ was adjusted exactly equal to 0.05M by its standardization against standard 0.05M KSCN solution using $Fe(NO_3)_3$ indicator. The cow and buffalo milk samples were obtained from the different dairy stations situated in Nashik city.

Preparation of 10.0ml of 0.05M $Hg(SCN)NO_3$ reagent

The fresh reagent was prepared *in situ* as it was required for the titration. A 5.0ml of standard 0.05M $Hg(NO_3)_2$ was transferred into a 250-ml titration flask containing 10.0ml of 1.0M HNO_3, 10.0ml of 0.2M $Fe(NO_3)_3$ and 100 ml of distilled water. This solution was titrated against standard 0.05M KSCN solution till faint red color was obtained. The reaction product was found soluble at acidic pH. Furthermore, 5.0ml of 0.05M $Hg(NO_3)_2$ was again added to this solution (red color disappeared). At this stage, the reaction mixture was contained 10.0ml of 0.05M $Hg(NO_3)_2$ and 10.0ml of 0.05M KSCN solutions, so it becomes 20.0ml of 0.025M [or 10.0ml of 0.05M] $Hg(SCN)NO_3$ reagent. Except $Hg(SCN)NO_3$ all other species in the mixture are not reacting with the chloride. Furthermore, the strength in normal term of $Hg(SCN)NO_3$ reagent was confirmed by its titration against standard 0.05M NaCl solution. This $Hg(SCN)NO_3$ reagent was consumed exactly 10.0ml of 0.05M NaCl solution for generation faint red color end point.

Methods

Step-I: First back titration without spiking milk by chloride

A 5.0ml of milk sample was transferred into 20.0ml of 0.025M (or 10.0ml of 0.05M) $Hg(SCN)NO_3$ reagent (prepared *in-situ* as described above). After vigorous shaking of mixture, the surplus reagent was determined by titration against standard 0.05M KCN solution till red color was obtained to the solution. This burette reading was recorded as a back titration reading (B_{K1}-ml).

Step-II: Second back titration with spiking milk by chloride

A 5.0 ml of milk sample was spiked with 0.888 g. to 1.710 g. (i.e. by adding 0.5ml to 4.0ml of standard 0.05M NaCl) of known amount of chloride and transferred into 10.0ml of 0.05M $Hg(SCN)NO_3$ reagent. After vigorous shaking of mixture, the surplus reagent was determined by titration against standard 0.05M KCN solution till faint red color was obtained to the solution. This burette reading was recorded as a back titration reading (B_{K2}-ml).

N. B. In both back titrations, the solutions obtained at the end point were simultaneously viewed against white background for identical red color intensity.

Step-III: Blank titration

A 10.0ml of 0.05M $Hg(SCN)NO_3$ reagent was prepared and titrated against standard 0.05M KCN solution till faint red color was obtained to the solution. This burette reading was recorded as a blank titration reading (B_L-ml). In this study, this reading was observed equal to 10.0 ml.

Calculations

Determination of milk chloride without spiking milk by chloride

The 10.0ml of 0.05M $Hg(SCN)NO_3$ reagent was used for both blank as well as back titrations, so the volume difference (B_L-B_{K1} in ml) of standard 0.05M KSCN was proportional to the amount of $Hg(SCN)NO_3$ utilized for reaction with chloride. Therefore, using the atomic weight of chlorine (35.5) the concentration ($C_{Cl\ milk}$) of chloride in mg/ml of milk sample was calculated by using following Eq. (1):

$$C_{Cl\ Milk} = \frac{M_{KSCN} \times (B_L - B_{K1}) \times 35.5}{V_{Milk}} \quad \ldots Eq.\ (1)$$

In Eq.(1), M_{KSCN} is molarity of KSCN solution and V_{Milk} is the volume of milk sample taken for titration.

Determination of milk chloride by standard addition method (spiking)

In this case, the volume difference (B_L-B_{K2} in ml) of standard 0.05M KSCN solution was proportional to the amount of $Hg(SCN)NO_3$ utilized for reaction with milk chloride and spiked chloride. Therefore, the concentration ($C_{Cl\ Milk}$) of milk chloride in mg/ml was calculated by subtracting the mg amount of spiked chloride from the total chloride as calculated by following Eq. (2):

$$C_{Cl\ Milk} = \frac{[(M_{KSCN} \times (B_L - B_{K2}) \times 35.5) - (M_{NaCl} \times V_{NaCl} \times 35.5)]}{V_{Milk}} \quad \ldots Eq.\ (2)$$

In Eq.(2), M_{NaCl} and V_{NaCl} are the molarity and volume of the NaCl which was spiked to milk sample.

Similarly, the amount of chloride spiked in milk ($C_{Cl\ Spiked}$) was calculated by subtracting the amount of chloride found in milk [determined by Eq. (1)], from the total chloride calculated by the Eq.(3):

$$C_{Cl\ Spiked} = [\text{Total chloride found}] - [\text{Milk chloride}] \quad \ldots Eq.\ (3)$$

$$C_{Cl\ Spiked} = [(M_{KSCN} \times (B_L - B_{K2}) \times 35.5)] - [(M_{KSCN} \times (B_L - B_{K1}) \times 35.5)] \quad \ldots Eq.\ (3)$$

Determination of chloride milk by double spiking method

The more reliable results were obtained with two identical milk samples spiked with two different amount of known chloride solution. In which, $[T_1]$ was the titration reading obtained (by $B_L - B_{K2}$) for solution containing an aliquot of the milk sample plus known amount of standard chloride having concentration C_1(in mg). Similarly, $[T_2]$ was the titration reading obtained (by $B_L - B_{K3}$) for a solution containing the same quantity of milk sample plus a measured amount of a standard chloride having concentration C_2 (in mg). Then, chloride concentration (C_u) in the milk, was determined by using following Eq. (4):

$$\frac{[T_2]}{[T_1]} = \frac{(C_u + C_2)}{(C_u + C_1)}$$

After simplification,

$$(T_2 x\, C_u - T_1 x\, C_2) = (T_1 x\, C_u - T_2 x\, C_1)$$

On rearranging, we get

$$C_u = \frac{[(T_1 x\, C_2) - (T_2 x\, C_1)]}{(T_2 - T_1)} \quad \ldots \text{Eq. (4)}$$

The positive (modd) value obtained by Eq. (4) is equal to mg of chloride present in the sample.

The Eq. (4) of calculation of chloride is true when the reagents concentration is identical in both the titrations; at this condition the concentration of chloride is a linear function of volume of titrant.

RESULTS AND DISCUSSION

Traditionally, the concentration of chloride in the milk is determined by precipitation titration by means of the Volhard and Mohr's methods using silver nitrate salt[15] (Furman, 1962). Mohr's method is suitable for determination chloride at neutral pH, to which the coprecipitation of milk protein and many inorganic interfering species yields results erroneously high and the method is not considered appropriate for accurate determination of milk chloride[16] (Caldwell and Moyer, 1935). In order to avoid the coprecipitation of the interfering species with the silver salt, another modified indirect procedure called as Volhard method, is applied for determination of milk chloride at acidic pH. However, Volhard method also found suitable only by removal of silver chloride precipitate before back titration against potassium thiocyanate[16] (Caldwell and Moyer, 1935). Although the filtration of silver chloride precipitate is omitted by adding any immiscible liquid like nitrobenzene, but the method facing a problem in detection of end point. In Volhard method the end point is partially obscured and red color fading occurs when silver chloride (coated with nitrobenzene) is not removed. This is due to fact that silver chloride is more soluble than silver thiocyanate[16] (Caldwell and Moyer, 1935), therefore extra amount of thiocyanate solution is required for obtaining end point. In this study, it was again confirmed by titrating a mixture of 10.0 ml, 0.05M NaCl and 10.0 ml, 0.05M $AgNO_3$ against 0.05M KSCN in presence of HNO_3 medium containing nitrobenzene and $Fe(NO_3)_3$ indicator. In this reaction mixture $AgNO_3$ was absent but it consumed 1.2 ml of 0.05M KSCN solution for obtaining red color end point. Thus it is proves that, Volhard method of determination of chloride is suitable only with the removal of silver chloride precipitate before back titration against thiocyanate solution.

Furthermore, chloride concentration is also determined by direct titration against $Hg(NO_3)_2$ using diphenyl-carbazone indicator[17] (Day and Underwood.,1993). But the mixture of diphenyl-carbazone with bromothymol blue gives the better end point of the titration[18] (Clarke, 1950). But without precise adjustment of the pH in between 3.2–3.3, the detection of the exact end point is a difficult task in this titration[18] (Clarke, 1950). The mercurimetric determination of chloride is the well-known examples of complexometric titration[17] (Day and Underwood., 1993). When sample solution containing chloride ions is directly titrated

against mercuric nitrate solution, it results simultaneously the formation of $[Hg(Cl)]^{+1}$, $[Hg(Cl)_2]$, $[Hg(Cl)_3]^{-1}$ and $[Hg(Cl)_4]^{-2}$ complexes in which the reaction stoichiometry of the titration becomes indistinguishable. Which is explain on the basis of formation/stability constant (log K) value of these complexes; since, the reported log K value of these four complexes of Hg(II) does not differ widely[19-20] (Patnaik, 2004 and Speight, 2005). When chloride determined by direct titration against mercuric nitrate[12] (Wenner 1960) is also associated with similar type of problem. In this case the reaction stoichiometry becomes indistinguishable at higher concentration of chloride, so the method[12] (Wenner 1960) is applicable for determination of lower range chloride.

Similarly, the reported value log K_2 and log K_4 of thiocyanate complexes of Hg(II) suggest only the formation of $[Hg(SCN)_2]$ and $[Hg(SCN)_4]^{-2}$ complexes[19-20] (Patnaik, 2004 and Speight, 2005). Furthermore, the large difference in log K_2 and log K_4 of these thiocyanate complexes of Hg(II), also make it possible to titrate Hg(II) against KSCN up to the reaction product $Hg(SCN)_2$, using ferric ions indicator[12] (Furman, 1962). Accordingly, both chloride as well as thiocyanate ions forms complexes with Hg(II), but the reactivity of chloride towards the Hg(II) ion is quite more; since it displaces the thiocyanate ions from $Hg(SCN)_2$. This fact was used for spectrophotometric determination of chloride[20] (Yoshinaga and Ohta, 1990). The present study of determination of milk chloride is also complexometric titration in which $Hg(SCN)NO_3$ reagent permit us for controlling reaction stoichiometry in the 1:1 ratio.

Chemistry of titration

The $Hg(SCN)NO_3$ reagent was preparation by titrating 5.0 ml of 0.05M $Hg(NO_3)_2$ against 0.05M KSCN solution in the 1:2 stoichiometric using $Fe(NO_3)_3$ indicator[12] (Furman, 1962).

$$Hg(NO_3)_2 + 2KSCN \rightarrow Hg(SCN)_2 \text{ (soluble)} + 2KNO_3 \qquad \text{(At the equivalence)}$$

$$KSCN + Fe(NO_3)_3 \rightarrow [Fe(SCN)(NO_3)_2] + KNO_3 \qquad \text{(At the end point)}$$

When 5.0 ml of 0.05M $Hg(NO_3)_2$ was added into above solution, the disproportionation reaction of $Hg(NO_3)_2$ with $Hg(SCN)_2$ results in the formation of $Hg(SCN)(NO_3)$.

$$Hg(NO_3)_2 + Hg(SCN)_2 \rightarrow 2[Hg(SCN)(NO_3)] \qquad \text{(Disproportionation)}$$

After addition of $Hg(NO_3)_2$, the red color of titration mixture was get disappeared, because of the conversion of small amount of mono-thiocyanato-iron(III) nitrate $[Fe(SCN)(NO_3)_2]$ into colorless $Hg(SCN)(NO_3)$.

$$Hg(NO_3)_2 + [Fe(SCN)(NO_3)_2] \rightarrow Hg(SCN)(NO_3) + Fe(NO_3)_3$$

The amount of $Hg(SCN)(NO_3)$ generated by $Fe(SCN)(NO_3)_2$ does affect chloride measurement. Because during reagent preparation the total concentration of $Hg(SCN)(NO_3)$ produced was totally depends on the amount of $Hg(NO_3)_2$ (*viz.* 5.0 ml of 0.05M) added after formation red color end point. A small amount of $Hg(SCN)_2$ remains surplus in the disproportionation reaction. Thus, only $Hg(SCN)(NO_3)$ is the only reacting species reacts with chloride ions and all other chemical species including $Hg(SCN)_2$ were not reacting with chloride.

The milk chloride (say in the form of NaCl) reacts with $Hg(SCN)(NO_3)$, as shown below:

$[Hg(SCN)(NO_3)] + NaCl \rightarrow [Hg(SCN)(Cl)] + NaNO_3$ (At the equivalence)

As the titration reaction of milk chloride with $Hg(SCN)(NO_3)$ was completed in the 1:1 stoichiometric ratio, next drop of thiocyanate solution regenerates red colored $[Fe(SCN)(NO_3)_2]$ at the end point.

$KSCN + Fe(NO_3)_3 \rightarrow [Fe(SCN)(NO_3)_2] + KNO_3$ (At the end point)

Study of the effect of sulfate and phosphate on milk chloride assay

Milk contains milk protein and many inorganic ions, which forms slightly insoluble precipitate with silver salt; so yields erroneously high results of chloride determination[16] (Caldwell and Moyer, 1935). Therefore, the interfering role of phosphate and sulfate was studied by titration of $Hg(SCN)(NO_3)$ containing 5.0ml of milk sample was carried out as described by step-I and the volume of 0.05M KSCN was noted at the end. Similar titrations were performed by adding different amount of phosphate (up to 25 mg in the form of Na_2HPO_4) and sulfate (up to 25 mg in the form of Na_2SO_4) in the 5.0ml of same milk sample. In every titration the volume of 0.05M KSCN was found identical, which illustrate that the phosphate and sulfate of the milk as well as externally added does interfere in determination of milk chloride. The acidic pH (~0.85) of the reaction mixture, does not allow these interfering ions to form compounds with $Hg(SCN)(NO_3)$ reagent. In addition, glucose and fructose up to 30 mg (per 5.0ml of milk) also gave similar burette readings of 0.05M KSCN.

Accuracy and precision in determination of chloride

The five-replicate determination of milk chloride were carried out by using standard 0.005M, 0.01M, 0.025M, 0.04M and 0.05M NaCl solutions. The accuracy and precision of the measurement are calculated in the form of relative error and average deviation respectively. The results presented in table-1 indicate the proposed procedure is accurate and precise.

Table 1: Results obtained in determination of precision of the titration method in terms of standard deviation and relative standard deviation

Milk Sample No.	Milk Sample Volume (ml)	*Average Chloride Found (mg)	Chloride Found (mg/ml)	Average Chloride Found (mg/ml)	**Mean/ Average Deviation	**Standard Deviation	**Relative Standard Deviation
Analysis of Cow Milk							
1	4.0	5.006	1.251		0.114	0.148	0.030
	5.0	6.213	1.243		0.142	0.177	0.029
	6.0	7.491	1.249	**1.248**	0.171	0.194	0.026
2	4.0	5.290	1.322		0.114	0.149	0.028
	5.0	6.674	1.335		0.086	0.097	0.015
	6.0	7.952	1.325	**1.327**	0.114	0.149	0.019
3	4.0	5.609	1.402		0.086	0.097	0.018
	5.0	7.029	1.406		0.086	0.097	0.014
	6.0	8.414	1.402	**1.403**	0.086	0.097	0.012
Analysis of Buffalo Milk							
1	4.0	4.757	1.189		0.114	0.148	0.034
	5.0	6.000	1.200		0.114	0.148	0.025
	6.0	7.207	1.201	**1.197**	0.086	0.097	0.014
2	4.0	5.361	1.340		0.114	0.148	0.027
	5.0	6.745	1.349		0.072	0.125	0.019
	6.0	8.059	1.343	**1.344**	0.086	0.097	0.012
3	4.0	4.970	1.243		0.071	0.126	0.025
	5.0	6.248	1.250		0.114	0.148	0.023
	6.0	7.526	1.254	**1.249**	0.086	0.097	0.013

*Average value for five replicate measurements; ** Determined for five replicate measurements

Table 2: Results obtained (by analysis of COW MILK) in determination of accuracy of the method in terms of absolute errors and relative errors

Cow Milk Sample No	Standard Chloride Spiked (mg/ml)	Milk volume (ml)	Total chloride found (mg)	For milk sample				For spiked chloride			
				Expected chloride (mg)	Chloride found (mg)	Absolute Error	Relative Error (%)	Chloride spiked (mg)	Chloride found (mg)	Absolute Error	Relative Error (%)
1	5.85	4.0	10.828	4.992	4.978	-0.015	-0.290	5.850	5.836	-0.015	-0.25
1	6.75	4.0	11.893	4.992	5.143	0.151	3.015	6.750	6.901	0.151	2.23
1	4.44	5.0	10.650	6.240	6.210	-0.030	-0.481	4.440	4.410	-0.030	-0.68
1	5.32	5.0	11.715	6.240	6.395	0.155	2.484	5.320	5.475	0.155	2.91
1	2.66	6.0	10.118	7.488	7.458	-0.031	-0.407	2.660	2.630	-0.031	-1.15
1	1.77	6.0	9.053	7.488	7.283	-0.206	-2.744	1.770	1.565	-0.206	-11.61
						0.004*	**0.263***			**0.004***	**-1.423***
2	6.21	4.0	11.538	5.308	5.328	0.019	0.367	6.210	6.230	0.019	0.314
2	5.68	4.0	10.828	5.308	5.148	-0.161	-3.024	5.680	5.520	-0.161	-2.826
2	3.90	5.0	10.473	6.635	6.573	-0.063	-0.942	3.900	3.838	-0.062	-1.603
2	3.20	5.0	9.940	6.635	6.740	0.105	1.583	3.200	3.305	0.105	3.281
2	2.66	6.0	10.650	7.962	7.990	0.028	0.352	2.660	2.688	0.028	1.053
2	1.77	6.0	9.585	7.962	7.815	-0.147	-1.846	1.770	1.623	-0.147	-8.305
						-0.036*	**-0.585***			**-0.036***	**-1.348***
3	6.75	4.0	12.425	5.612	5.675	0.063	1.123	6.750	6.813	0.063	0.933
3	5.85	4.0	11.360	5.612	5.510	-0.102	-1.818	5.850	5.748	-0.102	-1.744
3	4.79	5.0	11.715	7.015	6.925	-0.090	-1.283	4.790	4.700	-0.090	-1.879
3	4.44	5.0	11.360	7.015	6.920	-0.095	-1.354	4.440	4.345	-0.095	-2.140
3	2.66	6.0	11.183	8.418	8.523	0.105	1.241	2.660	2.765	0.105	3.929
3	2.30	6.0	10.828	8.418	8.528	0.110	1.301	2.300	2.410	0.110	4.761
*Average value for six measurements						**-0.002***	**-0.132***			**-0.002***	**0.643***

Table 3: Results obtained (by analysis of BUFFALO MILK) in determination of accuracy of the method in terms of absolute errors and relative errors

Cow Milk Sample No	Standard Chloride Spiked (mg/ml)	Milk volume (ml)	Total chloride found (mg)	For milk sample				For spiked chloride			
				Expected chloride (mg)	Chloride found (mg)	Absolute Error	Relative Error (%)	Chloride spiked (mg)	Chloride found (mg)	Absolute Error	Relative Error (%)
1	6.75	4.0	11.538	4.788	4.788	0.000	0.000	6.750	6.750	0.000	0.000
1	5.85	4.0	10.650	4.788	4.800	0.012	0.251	5.850	5.862	0.012	0.205
1	5.15	5.0	11.183	5.985	6.033	0.047	0.794	5.150	5.198	0.047	0.922
1	4.44	5.0	10.295	5.985	5.855	-0.130	-2.172	4.440	4.310	-0.130	-2.928
1	3.20	6.0	10.295	7.182	7.095	-0.087	-1.211	3.200	3.113	-0.087	-2.719
1	2.30	6.0	9.585	7.182	7.285	0.103	1.434	2.300	2.403	0.103	4.478
						-0.009*	**-0.153***			**-0.009***	**-0.008***
2	6.21	4.0	11.715	5.376	5.505	0.129	2.400	6.210	6.339	0.129	2.077
2	5.68	4.0	11.005	5.376	5.325	-0.051	-0.949	5.680	5.629	-0.051	-0.898
2	5.15	5.0	11.715	6.720	6.565	-0.155	-2.307	5.150	4.995	-0.155	-3.010
2	4.26	5.0	11.005	6.720	6.745	0.025	0.372	4.260	4.285	0.025	0.587
2	2.66	6.0	10.650	8.064	7.990	-0.074	-0.918	2.660	2.586	-0.074	-2.782
2	2.13	6.0	10.295	8.064	8.165	0.101	1.252	2.130	2.231	0.101	4.742
						-0.004*	**-0.025***			**-0.004***	**0.119***
3	6.75	4.0	11.715	4.996	4.965	-0.031	-0.620	6.750	6.719	-0.031	-0.459
3	5.85	4.0	11.005	4.996	5.155	0.159	3.183	5.850	6.009	0.159	2.718
3	4.44	5.0	10.828	6.245	6.388	0.142	2.282	4.440	4.583	0.142	3.209
3	3.55	5.0	9.763	6.245	6.213	-0.033	-0.520	3.550	3.518	-0.033	-0.915
3	2.31	6.0	9.763	7.494	7.453	-0.042	-0.554	2.310	2.269	-0.042	-1.797
3	1.77	6.0	9.408	7.494	7.638	0.143	1.915	1.770	1.914	0.143	8.107
*Average value for six measurements						**0.057***	**0.947***			**0.057***	**1.811***

Table 4: Results obtained in quantitative determination of chloride in COW MILK samples by application of standard addition method

Cow Milk Sample No.	Sample Volume (ml)	(BL-BK) (ml)	Chloride Spiked (mg)	Amount Chloride Eq.(1) (mg)	Amount Chloride Eq.(4) (mg)	Difference (mg)
1	4.0	3.2	0.00	5.680		
1	4.0	6.1	5.15			
1	4.0	6.8	6.39		5.656	-0.024
1	5.0	4.0	0.00	7.100		
1	5.0	5.5	2.66			
1	5.0	6.0	3.55		7.130	0.030
1	6.0	4.8	0.00	8.520		
1	6.0	5.8	1.77			
1	6.0	6.8	3.55		8.554	0.034
2	4.0	2.8	0.00	4.970		
2	4.0	6.3	6.22			
2	4.0	6.8	7.10		4.868	-0.102
2	5.0	3.5	0.00	6.213		
2	5.0	5.9	4.26			
2	5.0	6.3	4.97		6.213	0.000
2	6.0	4.2	0.00	7.455		
2	6.0	5.4	2.13			
2	6.0	5.8	2.84		7.455	0.000
3	4.0	2.4	0.00	4.260		
3	4.0	5.2	4.97			
3	4.0	6.0	6.39		4.260	0.000
3	5.0	3.0	0.00	5.325		
3	5.0	5.6	4.62			
3	5.0	6.2	5.68		5.273	-0.052
3	6.0	3.6	0.00	6.390		
3	6.0	5.6	3.55			
3	6.0	5.8	3.91		6.530	0.140

Table 5: Results obtained in quantitative determination of chloride in BUFFALO MILK samples by application standard addition method

Buffalo Milk Sample No.	Sample Volume (ml)	(BL-BK) (ml)	Chloride Spiked (mg)	Amount Chloride Eq.(1) (mg)	Amount Chloride Eq.(4) (mg)	Difference (mg)
1	4.0	2.0	0.00	3.550		
1	4.0	5.5	6.22			
1	4.0	6.0	7.10		3.460	-0.090
1	5.0	2.5	0.00	4.438		
1	5.0	4.9	4.26			
1	5.0	5.4	5.15		4.462	0.025
1	6.0	3.0	0.00	5.325		
1	6.0	4.5	2.66			
1	6.0	5.5	4.44		5.350	0.025
2	4.0	2.4	0.00	4.260		
2	4.0	5.2	4.97			
2	4.0	5.6	5.68		4.260	0.000
2	5.0	3.0	0.00	5.325		
2	5.0	4.5	2.66			
2	5.0	5.4	4.26		5.340	0.015
2	6.0	3.6	0.00	6.390		
2	6.0	4.8	2.13			
2	6.0	5.6	3.55		6.390	0.000
3	4.0	2.8	0.00	4.970		
3	4.0	5.6	4.97			
3	4.0	5.9	5.51		5.110	0.140
3	5.0	3.5	0.00	6.213		
3	5.0	5.9	4.26			
3	5.0	6.7	5.68		6.213	0.000
3	6.0	4.2	0.00	7.455		
3	6.0	5.4	2.13			
3	6.0	6.6	4.26		7.455	0.000

CONCLUSION

In conclusion, the proposed indirect mercurimetric titration method of determination of chloride in milk is much more simple, reliable and inexpensive than other reported methods. The novel $Hg(SCN)NO_3$ reagent employed in this titration gives distinguishable reaction stoichiometry with the stable and distinctly visible end point for determination of milk chloride at acidic pH. The statistical treatment to the experimental data obtained in terms of standard deviation and relative standard deviation (Table-1) illustrate that the method have good precision for the different replicate measurements. Similarly, the results obtained in determination of absolute errors and relative errors (Table-2 and Table-3) also demonstrates that this method have good accuracy in determination milk chloride. The method is highly selective for determination of chloride as proved by the interference study as well as recovery experiment of spiking known chloride

(Table-2 and Table-3). The standard addition of chloride (amplification) increases the sensitivity of the method for chloride determination in milk at a trace level. In addition, the results (Table-4 and Table-5) obtained by conventional titrimetry were excellently matched with those obtained by standard addition method. The titration of milk chloride was carried in acidic medium without precise adjustment of the pH for detection of the end point and the homogenous nature of solution eliminates titration errors those are commonly encountered by coprecipitation in argentometric titration of chloride. Finally, the developed method is superior and excellent in comparison to argentometric methods employed for determination of milk chloride.

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