Spectrophotometric Determination of Lactose in Milk with $PdCl_2$

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The spectrophotometric method (using $PdCl_2$) for determination of hexoses was applied to samples of milk to determine the lactose content with previous estimation of the conditions for quantitative determination. The method was carried out on milk serum samples, obtained according to four different procedures, in accordance with literature data, to identify the one most suitable to application of the $PdCl_2$ method. The experiments were undertaken on milk serum samples of different pH values, temperatures, and storage times. The possibility of application of the palladometric method was ascertained by statistical treatment of these results using the reference Luff−Schoorl method. The results of the investigations show that the first procedure (using Fehling I) for obtaining milk serum was the most suitable one for applying the $PdCl_2$ spectrophotometric method and that the pH value, temperature, and time of storage did not influence the method and its ability to accurately determine the lactose content.

Keywords: *Lactose; milk serum; palladium chloride*

INTRODUCTION

Determination of lactose content in milk samples is based on the classical quantitative analytical methods for determination of carbohydrates, that is, the ability of aldoses and ketoses (free glycoside group) to reduce metal ions from their salts in an alkaline medium, most frequently using Cu(II) ions. This is obtained by complexing Cu(II) ions with tartarate in an alkaline medium. The formation of a Cu(II)−tartarate complex thus contributes to gradual and continuous reaction but does not result in a stoichiometrical and quantitative one. Therefore, it is necessary to use empirical tables to calculate the contents of carbohydrates.

The application of a recent spectrophotometric (palladometric) method, based on the reduction of $PdCl_2$ and oxidation of carbohydrates, enables the reaction between reductive sugars and Pd(II) ions to be not only quantitative but also stoichiometrical, and sugar content can be determined without the preliminary establishment of empirical tables. In this method the oxidizing agent $PdCl_2$ was used in a mixture with Na_2SO_3 and $KNaC_4H_4O_6$ in a decreased alkaline medium. The necessary reagents Na_2SO_3 and $KNaC_4H_4O_6$ were added to obtain the reaction, which enabled it to be maintained stably, and the quantitative stoichiometrical ratio between $PdCl_2$ and carbohydrates to be determined (Petrushevska-Tozi, 1987; Petrushevska-Tozi et al., 1988, 1995; Ristov, 1984).

Quantitative determination of lactose content in milk samples can be performed only in milk serum samples obtained after previous removal of fats and proteins. Fats and proteins need to be sedimented and removed from the milk medium, without influencing the carbohydrates in the milk solutions in any way. There are many known reagents used for this purpose, but the ones used most are Fehling I in weak alkaline medium, $K_4/Fe(CN)_6/$ and $Zn(CH_3COO)_2$, ethanol, and acetic acid and $Al_2(SO_4)_3$ in weak alkaline medium, in accordance with literature data (Trajkovic et al., 1983; Jankovic, 1937; Zimmermann ef Cie, S.A., 1939).

The aim of the present work was to find the most suitable milk serum preparation for applying the spectrophotometric $PdCl_2$ method under previously established conditions (different pH values, storage times, and temperatures) to determine the quantity of lactose.

MATERIALS AND METHODS

Samples. Nine samples of pasteurized and homogenized cow's milk and one sample of full-fat milk powder bought from the local markets in Macedonia were analyzed.

Procedures for Preparation of Milk Serum Samples. *First Procedure.* The sedimentation of balance substances in samples of milk was achived using weak alkaline Fehling I solution. The pH of the serum obtained was 6.00 (Trajkovic et al., 1983).

Second Procedure. $K_4/Fe(CN)_6/$ and $Zn(CH_3COO)_2$ were used for sedimentation in milk samples, and the pH was 6.00 (Trajkovic et al., 1983).

Third Procedure. Sixty-five percent ethanol with acetic acid was used for sedimentation in samples; the pH was 3.00 (Jankovic, 1937).

Fourth Procedure. Sedimentation was achived using $Al_2(SO_4)_3$ and NaOH, pH 4.50 (Zimmermann ef Cie S.A., 1939).

The filtration of the sediment, in all procedures, used blue ribbon filter paper.

Reagent and Solutions. *Reagent 1*: 5.65×10^{-2} M basic solution of $PdCl_2$, prepared with concentrated HCl.

Reagent 2: solution mixture of 5 mL of 5.65×10^{-2} M $PdCl_2$, 1.5 g of $C_4H_4KNaO_6$, 20 mL of 4.76×10^{-2} M Na_2SO_3, and 20 mL of 3.75×10^{-1} M NaOH, diluted with water to 100 mL.

Reagent 3: 6×10^{-1} M KI solution.

Reagent 4: 5×10^{-1} M HCl solution.

Standard Solutions: Various concentrations of lactose monohydrate water solutions (0.55×10^{-3} to 2.22×10^{-3} M).

All used reagents were high analytical p.a. grade chemicals, purchased from Merck (Darmstadt, Germany).

Apparatus. A Perkin-Elmer UV−vis Lambda 16 was used.

Methods. A 5 mL milk serum sample solution (0.05 g of milk) with a lactose content of <1.8 g/L and 10 mL of reagent 2 were heated at 70 °C for 50 min in a water bath. The mixture was then transferred into a volumetric flask and diluted to 100 mL with water. Five milliliters of transparent liquid was transferred into a 25 mL volumetric flask, and 5 mL of 6×10^{-1} M KI solution (reagent 3) and 1 mL of 5×10^{-1} M HCl solution (reagent 4) were added and diluted to 25 mL with water. After 5 min, the absorbency was measured on $\lambda = 410$ nm wavelengths.

The lactose content was calculated according to the standard curve.

The Luff−Schoorl method was used as a reference method for determination of lactose in milk (Luff and Schoorl, 1929).

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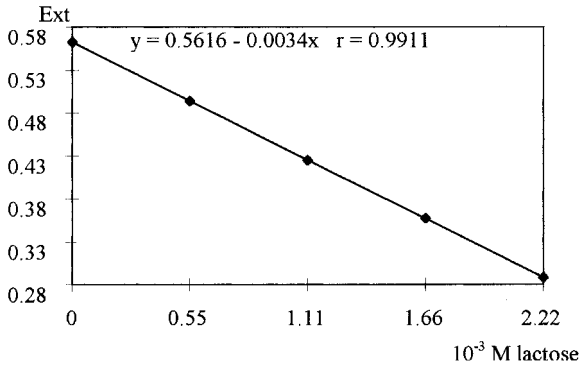

Figure 1. Standard curve for lactose determination with $PdCl_2$ method.

Table 1. Statistical Analysis of Lactose Content Determinations in Aqueous Solution[a]

applied x_1, 10^{-3} M	determined x_2, 10^{-3} M	SD	CV	recovery, %
0.55	0.52	0.0231×10^{-3}	4.43	95.57
1.11	1.04	0.0191×10^{-3}	1.84	93.69
1.66	1.67	0.0205×10^{-3}	1.23	100.50
2.22	2.24	0.0148×10^{-3}	0.66	100.90

[a] $N = 10$.

Preparation of Standard Curve. Five milliliter lactose monohydrate solutions in the concentration ranges 0.55×10^{-3}, 1.11×10^{-3}, 1.66×10^{-3}, and 2.22×10^{-3} M were used and treated according to the $PdCl_2$ method.

Statistical Calculations. Statistical data processing was carried out on STATGRAPHICS v. 4.0 software. The data represented mean values (x), standard deviations (SD), coefficients of variation (CV), and significance (t values).

RESULTS AND DISCUSSION

Lactose content was determined using a spectrophotometric $PdCl_2$ oxidoreducing method in milk serum samples, prepared according to four different procedures.

The $PdCl_2$ procedure was applied on the aqueous solution of lactose (various concentrations), and a standard curve was made (Figure 1). The results obtained from this investigation showed that the ratio between Pd(II) ions and disaccharides (lactose) was 1 mol:1 mol, as was expected. The results from the statistical analyses of lactose content showed that the $PdCl_2$ method applied for determination of lactose was accurate; the average deviation from medium values and variability of results were insignificant (Table 1).

For establishing the conditions of $PdCl_2$ determinations, the investigations were carried out on milk samples prepared by reconstituting the dried milk powder.

The $PdCl_2$ method applied on milk serum samples obtained from reconstituting milk powder was used in four different literature procedures. The lactose content varied from 1.74 to 5.28% on the basis of the procedure used (Table 2). These results were gained when the $PdCl_2$ method was applied on milk serum samples obtained by original procedures on their original pH values (between 3 and 6). Because of the known behavior of carbohydrates in alkaline or acidic medium, the $PdCl_2$ method was applied on milk serum samples on which pH values were altered to pH 7.00. The results of the lactose content in this case varied from 1.70 to 5.65%. Comparison of these values for lactose content showed that no significant difference was observed using the first, second, and third procedures,

Table 2. Lactose Content in Samples Prepared According to Four Prescribed Procedures at Various pH Values

procedure	original pH value			neutralized pH 7			original pH values/ neutralized	
	x	SD	CV	x	SD	CV	t	significance
first	4.64	0.13	2.80	4.71	0.08	1.69	0.97	$p > 0.05$
second	5.28	0.10	1.89	5.63	0.20	3.55	2.29	$p > 0.05$
third	1.74	0.48	27.59	1.70	0.48	28.23	0.08	$p > 0.05$
fourth	5.06	0.23	4.54	5.65	0.20	3.54	3.74	$p < 0.05$

but the most constant results were observed using the first and third procedures for preparing milk serum samples. The use of the fourth procedure gave high ($t = 3.74$) values and significant difference ($p < 0.05$) and so is not recommended for preparing serum for lactose determination.

Determination of lactose content of milk serum samples from reconstituted milk powder, using the $PdCl_2$ method, was carried out in various conditions by storeing the serums at different temperatures. The investigations were carried out immediately on milk samples prepared the same day (immediately) and after 17 days of storage at 20 or 0 °C. The results are shown in Table 3. The lactose content was not changed in the milk serum samples prepared according to the first and third procedures, even when they were stored at 20 °C. This is probably due to the acidity (pH 3) of serum in the third procedure and the bacteriostatic ability of $CuSO_4$ present in the first procedure.

Using all four procedures for preparing milk serum samples, stored for 17 days at 0 °C showed that the storage time at this temperature did not influence lactose content, according t values.

The possibility of using the $PdCl_2$ method for lactose determination in reconstituted milk powder was examined by statistical elaboration and comparison of the results obtained according to this method against the reference Luff−Schoorl method. The values for lactose content presented in Table 4 showed that the $PdCl_2$ method was correct only for determinations when milk serum was prepared according to the first procedure. The average deviations were significant [$p < 0.05$; $t = 7.33$ (second), $t = 4.88$ (third), $t = 6.14$ (fourth)] for the other three procedures used for preparing milk serums.

The possibility of appliying the $PdCl_2$ method for lactose determination was proved by using this method on samples of pasteurized and homogenized milk. The serums were obtained using the first procedure as it was shown to be the most suitable one in preliminary estimations.

The possibility of applying the $PdCl_2$ method for determination of lactose content in pasteurized milk samples was estimated on milk serum samples from nine suppliers in Macedonia. Milk serum samples were prepared according to the first procedure for sedimentation and removal of balance substances (fat and proteins). Both the $PdCl_2$ method and the Luff−Schoorl method were carried out on prepared milk serum samples obtained the same day the milk was purchased. Lactose content values obtained by $PdCl_2$ method varied from 3.04 to 4.25% and by Luff−Schoorl method from 2.86 to 3.99% (Table 5). The obtained lactose content values were within the limits proposed by the regulations for milk chemical composition.

The statistical t-test compression of the lactose values obtained according to the two methods showed that the differences between the values were not significant. The

Table 3. Lactose Contents Determined with the Palladometric Method for Various Conditions of Storage and Various Temperatures

	immediately			after 17 days						immediately/after 17 days at 20 °C		immediately/after 17 days at 0 °C	
				at 20 °C			at 0 °C						
procedure	*x*	SD	CV	*x*	SD	CV	*x*	SD	CV	*t*	significance	*t*	significance
first	4.64	0.13	2.80	4.83	0.02	0.41	4.79	0.08	1.67	3.05	$p > 0.05$	1.95	$p > 0.05$
second	5.28	0.10	1.89	5.46	0.07	1.28	5.24	0.14	2.67	2.95	$p < 0.05$	0.42	$p > 0.05$
third	1.74	0.48	27.59	2.22	0.32	14.41	1.70	0.06	3.53	1.18	$p > 0.05$	0.08	$p > 0.05$
fourth	5.06	0.23	4.54	3.91	0.16	4.09	5.35	0.16	2.99	8.23	$p < 0.05$	2.07	$p > 0.05$

Table 4. Statistical Student *t*-Test Comparison of the Lactose Content Results, Determined with the $PdCl_2$ and Luff−Schoorl Methods, for Four Procedures for Obtaining Milk Serum Samples

	palladometric method			Luff−Schoorl method			palladometric/Luff−Schoorl methods	
procedure	*x*	SD	CV	*x*	SD	CV	*t*	significance
first	4.64	0.13	2.80	4.55	0.03	0.66	1.43	$p > 0.05$
second	5.28	0.10	1.89	4.73	0.10	2.11	7.33	$p < 0.05$
third	1.74	0.48	27.59	3.26	0.06	1.84	4.88	$p < 0.05$
fourth	5.06	0.23	4.54	4.42	0.05	1.13	6.14	$p < 0.05$

Table 5. Comparison of the Lactose Content Results, Determined with the Palladometric and Luff−Schoorl Methods

pasteurized milks from	palladometric method			Luff−Schoorl method			palladometric/Luff−Schoorl method	
	x	SD	CV	*x*	SD	CV	*t*	significance
Singelik	4.11	0.30	7.29	3.74	0.10	2.67	1.65	$p > 0.05$
Trubarevo	3.49	0.02	0.57	3.39	0.08	2.36	0.34	$p > 0.05$
Tetovo I	3.89	0.09	2.31	3.70	0.10	2.70	1.99	$p > 0.05$
Skopje	3.46	0.16	4.62	3.25	0.04	1.23	1.80	$p > 0.05$
Kumanovo	4.07	0.22	5.40	3.99	0.15	3.76	0.42	$p > 0.05$
Tetovo II	3.77	0.18	4.77	3.62	0.02	0.55	1.28	$p > 0.05$
Gostivar	3.78	0.10	2.64	3.73	0.26	6.97	0.25	$p > 0.05$
Veles	3.04	0.10	3.29	2.86	0.02	0.69	2.75	$p > 0.05$
Bitola	4.25	0.08	1.88	3.94	0.01	0.25	0.37	$p > 0.05$

value for *t* coefficient ($t = 1.20$ average value) was lower than the theoretical value for $p < 0.05$, which makes the difference between the values for lactose content obtained according to both methods insignificant.

The results obtained suggest that the first procedure was the most suitable for application of the $PdCl_2$ method. The pH value, storage time, and temperature of a particular serum did not influence the determination. The same procedure was accurate in regard to the reference Luff−Schoorl method for the lactose content determination in pasteurized milks and milk powder.

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