Direct and Selective Flow-Injection Method for the Simultaneous Spectrophotometric Determination of Calcium and Magnesium in Red and White Wines Using Online Dilution Based on "Zone Sampling"

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The present work reports a selective and simple flow injection method for the direct and simultaneous determination of calcium and magnesium ions in red, rose, and white wines. Both ions react with methylthymol blue (MTB) at a strongly basic medium to form colored complexes that are monitored spectrophotometrically ($\lambda_{max} = 610$ nm). The simultaneous determination is achieved by online masking of magnesium by 8-hydroxyquinoline (8-HQ). Incorporating an online dilution mode based on the "zone sampling" technique in the FI system, the determination of both analytes was achieved without any pretreatment of the samples, in the range 0–350 mg L^{-1} and 0–200 mg L^{-1} for Ca(II) and Mg(II), respectively. The 3σ detection limits were quite satisfactory (2.1 and 1.8 mg L^{-1} for Ca(II) and Mg(II) respectively), and the precision was 1.2% (at a mixture of 100.0 mg L^{-1} Ca(II) + 100.0 mg L^{-1} Mg(II), $n = 12$). A detailed study of interferences proved that the proposed method is highly selective. The application of the method to the direct analysis of red, rose, and white wines yielded excellent results compared with those obtained by using FAAS as a reference method ($e_r < 2.8\%$).

Keywords: *Calcium; magnesium; flow-injection; spectrophotometry; methylthymol blue; simultaneous determination; wine; zone sampling dilution*

INTRODUCTION

Calcium and magnesium are among the most important cations present in wines. The accurate determination of Ca(II) is important because of the problem of calcium tartrate and oxalate precipitation, which occurs very slowly and usually after the product has been bottled. On the other hand, there are some indications that the Mg(II) content may be of importance in tartrate stability and to the taste of wine. The final concentration of both metals is affected by several parameters such as the wine storage in concrete tanks, the use of ion-exchange resins, the concentration of alcohols and other constituents (tartrate, sulfate, etc.), the pH and the temperature, and time of storage. During fermentation the Ca(II) content decreases and the Mg(II)/Ca(II) ratio increases (*1*).

Flow injection (FI) is a very effective technique for the automation of chemical analysis mainly in terms of simplicity, high precision, and sampling throughput, especially when liquid samples have to be analyzed. For these reasons FI has been widely applied to the analysis of wines (*2*). Up to now four FI procedures have been reported for the determination of Ca(II) and Mg(II) in wines (*3–6*). All of them are using flame atomic absorption spectrometry (FAAS) as the detection system and different modes for the online dilution of the samples. The split-flow mode (*3*) lacks precision and robustness

(*7*), while the use of dialysis (*4, 5*) requires very frequent replacement of the clogged membranes, making this technique rather unattractive to routine analysis. On the other hand, a method that utilizes the "zone-sampling" dilution technique (*6*) uses different working conditions for the two analytes and several calibration curves are needed prior to measurement. In addition, the use of an FAAS detector increases the analysis cost, while it is not affordable for many laboratories.

An advantageous alternative technique to FI-FAAS is FI-spectrophotometry, as low cost instrumentation is required and the operational cost is lower as well. To the best of our knowledge no FI spectrophotometric methods have been reported so far for the simultaneous determination of Ca(II) and Mg(II) in wines.

In a previous work we reported a FI spectrophotometric method for the determination of Ca(II) in wines using methylthymol blue as a color forming agent (*8*). The present work exploits the possibility of the FI spectrophotometric simultaneous determination of Ca(II) and Mg(II) in red, rose, and white wines, based upon the same reaction. This method is the first-reported FI spectrophotometric procedure for the simultaneous determination of these important cations in wines and has none of the above-mentioned limitations of the FI-FAAS procedures. Ca(II) alone is determined in a first run using 8-hydroxyquinoline (8-HQ) as a masking agent for Mg(II), while Mg(II) is determined in a second run, without using 8-HQ, through multiple regression analysis (*9*). An online dilution mode based on the "zone sampling" technique (*10*) was also optimized and incor-

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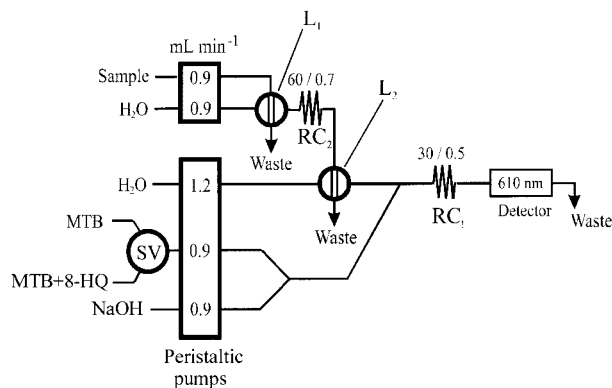
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Figure 1. Optimized FI setup for the simultaneous determination of Ca(II) and Mg(II) ions. L_1, L_2 = sample loops (40 μL); SV = selection valve; RC_1 = reaction coil; RC_2 = dilution coil; numbers above coils denote coil length (cm)/i.d. (mm) ratio.

porated in the flow system, providing sufficient dilution of the samples so that no pretreatment steps were required prior to analysis. Compared to the "cascade dilution" system used in our previous work (*8*), the "zone sampling" dilution approach offers the critical possibility of varying the dilution factor of the sample in the range 6–35 by simply adjusting the rotation time interval of the injection valve. This additional advantage is very important when samples of unknown concentrations have to be analyzed, often requiring different dilution factors. The method proved to be very selective considering the species that exist in the samples and has the significant advantage of being able to determine the analytes in both red and white wines, as no interferences were observed by the color of the samples. The results obtained from the analysis of commercially available wines by the proposed method were in excellent agreement with FAAS used as the reference method.

MATERIALS AND METHODS

Reagents. All chemicals were of analytical-reagent grade and were provided by Merck (Darmstadt, Germany), unless stated otherwise, and all solutions were made up with deionized water.

The standard stock calcium chloride [γ(Ca(II)) = 5000 mg L^{-1}] and magnesium chloride [γ(Mg(II)) = 5000 mg L^{-1}] solutions also contained 0.1 mol L^{-1} HCl in order to prevent hydrolysis of the ions.

Methylthymol blue was used without further purification. The MTB solution for the determination of both analytes (c(MTB) = 3.54 × 10^{-4} mol L^{-1}) was prepared by dissolving the required amount in water, containing 6.0 g L^{-1} polyvinylpirolidone (PVP) and 10.0 mL of 6.0 mol L^{-1} HCl, while when Ca(II) was determined 0.03 mol L^{-1} 8-hydroxyquinoline (8-HQ) was also added to mask Mg(II) ions.

A standard stock solution of 0.13 mol L^{-1} NaOH was used for pH adjustment.

The stock lanthanum solution, γ(La$^+$) = 50.0 g L^{-1}, was prepared by dissolving the appropriate amount of LaNO$_3$·6H$_2$O (Riedel de Haen, R. G.), in 600 mL deionized water.

Instrumentation. The FI system used was a Tecator 5010 analyzer (Tecator, Hõganas, Sweden) with a Tecator chemifold Type IV manifold which are shown schematically in Figure 1. The detector used was a Tecator 5023 FI star double-beam spectrophotometer, consisting of a 5032 detector controller and a 5023-011 spectrophotometer optical unit. The absorbance of the colored Ca(II)/Mg(II)−MTB complexes were monitored at 610 nm through a 1 cm path length flow cell with an 18 μL internal volume. The flow system used was 0.5 mm i.d. Teflon tubing throughout. Tygon pump tubes were used for delivering

the aqueous solutions. A Tecator V-200 double injection valve was incorporated in the system in order to perform the "zone sampling" dilution mode.

A Perkin-Elmer model 403 atomic absorption spectrometer was used for the calcium determination by the reference method.

An Orion EA940 pH-meter was employed for the pH measurements with absolute accuracy limits at the pH measurements being defined by NIST buffers.

Procedure for Aqueous Solutions. The FI used is depicted in Figure 1. Forty microliters of the samples/standards were injected into an aqueous stream and passed through a 60-cm long 0.7-mm i.d. dilution coil (RC_2), to a second injection valve. A 40-μL portion of the dispersed zone was reinjected into a second aqueous carrier stream and merged with the reagents. The colored Mg(II)−MTB and Ca(II)−MTB complexes were formed on the passage of the reaction mixture through a 30-cm long 0.5-mm i.d. reaction coil (RC_1) and were detected spectrophotometrically at 610 nm. When Ca(II) was determined, the MTB/8-HQ reagent was selected through a selection valve (SV), while the MTB reagent was selected subsequently to determine Mg(II). In the first case Ca(II) was determined using an aqueous Ca(II) calibration curve. In the second case both analytes were determined through a calibration curve of the type $A = b_1[\text{Ca(II)}] + b_2[\text{Mg(II)}] + a$, which was obtained using mixtures of both analytes. The found from the first calibration curve Ca(II) mass concentration was inserted in the equation of the mixtures, and the mass concentration of Mg(II) was calculated.

The cycle time was set at 50 s (72 injections h^{-1}), while each sample/standard was run in five replicate injections.

Determination of Ca(II) and Mg(II) by the AAS Reference Method. For the determination of Ca(II) and Mg(II) by the FAAS reference method, the wine samples were diluted 25- and 250-fold, respectively, and a final La(III) mass concentration of 5000 mg L^{-1} was added in order to avoid chemical interferences. The mixed Ca(II) (0−6.0 mg L^{-1}) and Mg(II) (0−0.5 mg L^{-1}) standards also contained 5000 mg L^{-1} La(III). The resulting sample and standard solutions were aspirated directly into the air/acetylene flame.

RESULTS AND DISCUSSION

Optimization of the FI System. The effect of the chemical and FI variables of the FI system shown in Figure 1—without the online dilution mode upon the determination of Ca(II) are discussed in detail in a previous work (*8*). The same optimal results were found regarding Mg(II) ions as well. The only modification was on the sample injection volume, which was reduced from 130 μL to 40 μL in order to achieve the widest possible calibration curve for both analytes.

Optimization of the "Zone Sampling" Dilution Mode. The "zone sampling" dilution technique is a very powerful and effective process when online dilution of the samples is required prior to analysis (*10*). It is based on a double injection process. A fixed volume of the sample is injected in an aqueous carrier and is dispersed through a dilution coil to a second injection valve. Utilizing precise operation timing, a selected section from the gradient of the sample zone is reinjected into a second carrier or reagent stream and undergoes further dispersion. The main factor that affects the dilution is the timing of the second injection (residence time), which determines the section of the sample zone to be reinjected.

The optimization of the "zone sampling" technique was done using a manifold similar to that shown in Figure 1, with the difference being that bromothymol blue was used as a model sample, while all the other streams were 0.01 mol L^{-1} borax solutions. The flow rate of the first carrier stream was set at 0.9 mL min^{-1}
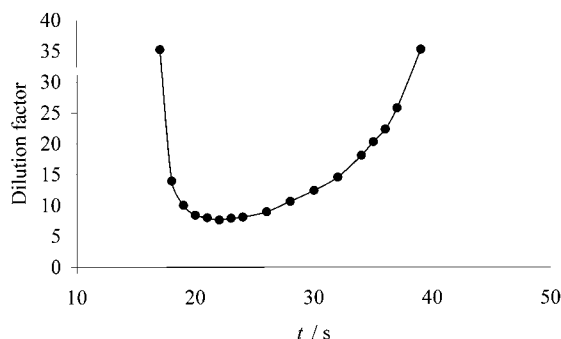
Figure 2. Effect of the second injection residence time on the dilution of the samples using the "zone sampling" approach.

to achieve acceptable sampling rate, and the dilution coil (RC_2) was fixed at 60-cm/0.7-mm i.d. to produce sufficient dispersion of the sample.

The effect of the residence time prior to the second injection was studied in the range 17–40 s using injection volumes of 40 μL for both injection valves. The experimental results are shown in Figure 2. As can be seen in Figure 2, the dilution factor decreased in the range 17–22 s, while it increased thereafter. The time interval 17–22 s corresponds to the head of the sample zone, with the maximum concentration (minimum dilution) being at 22 s. The 23–40 time interval corresponds to the tail of the sample zone. A residence time of 35 s was chosen as optimal, as it provided a ca. 20-fold dilution of the sample, yielding sufficiently wide calibration curves for the direct determination of Ca(II) and Mg(II) in the wine samples without any pretreatment. However, as can be seen in Figure 2, by simply adjusting the time interval at an appropriate value, dilution factors in the range 6–35 can be achieved, making possible the rapid analysis of samples of varying concentrations with no pretreatment.

The effect of the first sample injection volume was studied in the 40–200 μL range by varying the length of the loop in the first injection valve, while keeping the second injection volume at 40 μL. The experiments showed that the volume of the first injection did not affect significantly the dilution of the sample. However, volumes larger than 40 μL produced less reproducible results. For this reason the volume of 40 μL was chosen as optimal, which was the smaller possible injection volume provided by the instrument.

Study of the Masking Efficiency of 8-HQ. The masking efficiency of 8-HQ was studied using different amount concentrations of the masking reagent in the MTB solution. The experiments proved that 0.3 mol L^{-1} 8-HQ was capable of masking online the Mg(II) effectively, without affecting significantly the Ca(II) signals, as an only ca. 8.0% decrease in Ca(II) sensitivity was observed due to partial reaction of Ca(II) with 8-HQ. However, the decrease in Ca(II) sensitivity did not affect the accuracy of the quantitative results, as two calibration curves were used for Ca(II), that is, with and without the use of 8-HQ. The experimental results are shown in Table 1. As can be seen in Table 1 the recoveries of Ca(II) from Ca(II)/Mg(II) mixtures were quantitative even at an extreme Mg(II)/Ca(II) ratio of 3, while the highest possible ratio in the wine samples is 2. The tolerance ratio could be further increased using higher amount concentrations of 8-HQ, but above 0.3 mol L^{-1} the precision fell due to poor stability of the baseline.

Table 1. Determination of Ca(II) in the Presence of Mg(II) in Aqueous Solutions

Ca(II) mass concn, mg L^{-1}	Mg(II) added, mg L^{-1}	Ca(II) found,[a] mg L^{-1}	recovery, %
50.0	50.0	50.0	100.0
50.0	100.0	50.4	100.8
50.0	150.0	50.7	101.4
100.0	50.0	99.7	99.7
100.0	100.0	100.0	100.0
100.0	150.0	100.6	100.6
150.0	50.0	150.0	100.0
150.0	100.0	150.0	100.0
150.0	150.0	149.2	99.5

[a] Mean of five results.

Table 2. Study of Interferences

species added[a]	tolerated mass concn/mg L^{-1}
K$^+$, Na$^+$, Cl$^-$, I$^-$, SO$_4^{2-}$, SO$_3^{2-}$, CH$_3$COO$^-$	100 000
PO$_4^{3-}$, BO$_3^{3-}$, citrates	50 000
NH$_4^+$, tartrates, lactates	25 000
succinates	18 000
malates	15 000
ascorbates	12 000
F$^-$, Fe(II)	6000
Ni(II), Fe(III), Co(II)	1200
Mn(II)	700
Cu(II)	500
Al(III)	200
Pb(II), Ba(II), Zn(II)	50

[a] These species in the stated ratio caused a relative error of less than $\pm$2%.

Features of the Proposed Method. Using the FI setup shown in Figure 1 and under the above-mentioned optimal conditions, two linear calibration curves were recorded. The first calibration graph (MTB+8-HQ reagent) corresponds to the Ca(II) mass concentration in the samples, which is linear over the range 0–350 mg L^{-1} Ca(II) and is described by the equation

$$A = (2.1 \pm 3.2) \times 10^{-3} + (248.3 \pm 5.1) \times 10^{-5}\gamma[\text{Ca(II)}]$$

where A is the absorbance as measured by the detector, and $\gamma[\text{Ca(II)}]$ is the mass concentration of the analyte in the aqueous solution with a relative standard deviation of $s_r = 0.6\%$ (100.0 mg L^{-1} Ca(II), $n = 10$), a correlation coefficient of $r = 0.9991$, and a 3σ detection limit of 2.1 mg L^{-1} ($n = 10$).

The second calibration curve (MTB reagent without 8-HQ) was constructed using multiple regression analysis by measuring the absorbance of various mixtures of Ca(II) and Mg(II) including the analytes alone as well ($n = 12$), at mass concentrations covering the whole linear ranges for both analytes. The obtained calibration curve was linear over the range 0–350 and 0–200 mg L^{-1} for Ca(II) and Mg(II), respectively, and was described by the equation

$$A = (4.1 \pm 3.5) \times 10^{-3} + (268.1 \pm 6.5) \times 10^{-5}\gamma[\text{Ca(II)}] + (272.7 \pm 5.3) \times 10^{-5}\gamma[\text{Mg(II)}]$$

with a relative standard deviation of $s_r = 1.2\%$ (at a mixture of 100.0 mg L^{-1} Mg(II) + 100.0 mg L^{-1} Ca(II), $n = 12$), a correlation coefficient of $r = 0.998$, and a Mg(II) 3σ detection limit of 1.8 mg L^{-1} ($n = 10$). Mg(II) was determined from the multiple regression calibration curve after subtraction of the Ca(II) mass concentration

Table 3. Determination of Ca(II) and Mg(II) in Wines

sample	added, mg L^{-1}	found,[a] mg L^{-1}	recovery, %	reference method[b]	e_r,[c] %
Determination of Ca(II)					
I (white)		90.3		91.2	−1.0
	100.0	191.5	101.2		
II (white)		81.5		80.0	+1.9
	100.0	180.3	98.8		
III (rose)		96.8		95.5	+1.4
	100.0	195.2	98.4		
IV (rose)		105.6		107.6	−1.8
	100.0	204.7	99.1		
V (red)		80.4		81.2	−1.0
	100.0	180.8	100.4		
VI (red)		133.1		131.2	+1.5
	100.0	235.7	102.6		
Determination of Mg(II)					
I (white)		65.1		66.2	−1.7
	50.0	116.4	102.6		
II (white)		84.2		86.4	−2.5
	50.0	135.3	102.2		
III (rose)		96.0		93.9	+2.2
	50.0	146.9	101.8		
IV (rose)		102.9		100.1	+2.8
	50.0	153.9	102.0		
V (red)		105.0		103.1	+1.8
	50.0	154.6	99.2		
VI (red)		110.6		112.6	−1.8
	50.0	159.5	97.8		

[a] Mean of five results. [b] FAAS reference method. [c] Relative error.

previously calculated from the first calibration curve. All standards were run in five replicate injections.

Study of Interferences. The interference effects of many species, that often accompany the analytes in the wine samples, on the determination was examined using the FI system shown in Figure 1. The results are summarized in Table 2. A volume fraction of ethanol of 30% did not interfere (the average volume fraction of ethanol in wines is 12%). The criterion for interference was fixed at an e_r of less than ±2% of the average absorbance signal taken for a Ca(II)−Mg(II) mixture corresponding to a mass concentration of 100 mg L^{-1} of each analyte.

Determination of Ca(II) and Mg(II) in Real Samples. The applicability of the proposed FI method was checked by the analysis of red, rose, and white commercial wines. The results are summarized in Table 3. The recoveries of fixed amounts of Ca(II) and Mg(II) were excellent, ranging between 97.8 and 102.6%. Table 3 also contains a comparison of the results with those obtained by FAAS. This comparison verified the accuracy of the proposed FI method, with the relative errors, e_r, being ≤2.8% in all cases.

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