

Determination of Free SO₂ in Wines Using a Modified Ripper Method with Potentiometric Detection a Comparative Study with an Automatic Titrator

Abstract

Sulfur dioxide (SO₂) is widely used as an additive (INS 220) in the wine industry because of its antiseptic properties and low cost regardless of unpleasant odor of free SO₂. In addition, it is believed that free radicals derived from SO₂ in aqueous solution can cause the development of respiratory diseases and even DNA damage. Hence the need to quantify the amount of free SO₂ in industrialized foods. The wine industry in Brazil uses the Ripper method for the quantification of SO₂, which is based on the titration of sulfite with iodine in the presence of starch as a visual indicator. Although this method is relatively quick and easy, some authors do not recommend it due to inaccurate end point visualization (particularly in red wine), which causes low precision in the results. In the present study, the free SO₂ content in five wine samples was determined by the Ripper method (modified) using KIO₃ as titrant with potentiometric detection. In these samples the SO₂ content was obtained using the method of multiple standard addition with Na₂S₂O₅. For comparison purposes, the same samples were analyzed with a mini automatic titrator usually used in the wine industry for specific determination of SO₂. Results for free SO₂ content obtained with these both procedures showed no statistical differences ($t = 0.636$; $p < 0.05$), therefore the potentiometric titration with KIO₃ may be a less expensive alternative to quality control laboratories that do not have specific equipment for this purpose.

Keywords: Food Additive; Sulfur Dioxide; Wine; Ripper Method; Potentiometric Analysis; Automatic Titrator

Introduction

Sulfating agents (commonly added as SO₂, SO₃²⁻, HSO₃⁻ or S₂O₅²⁻) are widely used as additives in food industry due their antiseptic properties. Sulfating agents inhibit the deterioration caused by some bacteria, molds and yeast and reactions of enzymatic and non-enzymatic browning during processing and storage as well [1,2]. Particularly in wine industry sulfur dioxide (SO₂) has been used since the Roman Empire to prevent the formation of undesirable yeasts. At that time, SO₂ was generated simply by burning natural sulfur near the barrels so that the grape juice inside them could absorb the fumes of sulfur dioxide [3]. Other compounds have been tested as

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Waila Evelyn Lima Santana and Horacio Dorigan Moya*


Faculdade de Medicina da Fundação do ABC, CEPES (Centro de Estudos, Pesquisa, Prevenção e Tratamento em Saúde) - Av. Príncipe de Gales, 821 - Príncipe de Gales - Santo André - CEP - 09060-650, Santo André, SP, Brazil

*Address for Correspondence

Horacio Dorigan Moya, Faculdade de Medicina da Fundação do ABC (FMABC) - CEPES (Centro de Estudos, Pesquisa, Prevenção e Tratamento em Saúde da FMABC) - Av. Príncipe de Gales, 821 - CEP 09060-650 - Santo André - SP - Brazil

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substitutes for sulphur dioxide in wines because of two possible situations: their intrinsic toxicity to humans (e.g. salicylic, monobromoacetic and monochloroacetic acids and ethylene oxide) or the suspicion of generating toxic compounds (e.g. diethylpyrocarbonate). Their use has been either discontinued or reproved [4]. Benzoic acid may be the most available and least expensive compound used to replace SO₂ but has not been employed because of its low yeast toxicity.

Ascorbic acid, a recognized antioxidant agent, could replace SO₂ but it does not exhibit the same inhibitory action than SO₂ in wine [4]. Sorbic acid, considered be a yeast inhibitor, is not very effective against some wine bacteria so it should be used along with SO₂ [4]. In addition, there are undesirable flavor changes because some bacteria converted sorbic acid into a geranium-like odor compound [5]. Despite the general agreement that free SO₂ odor is unpleasant it is widely used in wine industry because of its antioxidant and antimicrobial properties and its relatively low cost [4]. Currently, SO₂ (INS 220) is added during the wine processing to eliminate both the undesirable bacteria and the yeasts that would remain in the storage stage. In addition, SO₂ has the advantage of facilitating the extraction of pigments coming from the grapes. So far there seems to be no other compound as effective as sulfur dioxide (also added as metabisulfite) to replace it, since the presence of SO₂ (100 – 200) mg L⁻¹ in its free form promotes a positive antiseptic effect in wines.

On the other hand, it is common knowledge that free sulfur oxide radicals (SO₃[•], SO₄^{•-} and SO₅^{•-}) are easily generated when S(IV), present in aqueous solution as SO₂, bisulfite (HSO₃⁻) or sulphite (SO₃²⁻), undergoes catalyzed autoxidation by transition metals [6,7]. Also it is believed that the development of some respiratory diseases and the manifestation of mutagenic, co-mutagenic and co-carcinogenic

effects may be related to the interaction of these sulfur oxide radicals with cell membranes, proteins and even DNA [7-9]. Hence the amount of free SO₂ used as additive in food products should be regulated and monitored. The Monier-Williams procedure is based on the conversion of sulfite to SO₂ by heating and refluxing the mixture with hydrochloric acid solution. A stream of N₂ is passed through this solution to transport the formed sulfur dioxide to a flask containing 3% H₂O₂ solution in which the SO₂ is oxidized to H₂SO₄. The formed H₂SO₄ is then determined by titration with a standardized NaOH solution [10].

This method was one of the first analytical methods developed for the determination of sulfites in food and beverages [10,11]. In 1989, the US Food and Drug Administration (FDA) proposed changes to improve the sensitivity of that method, which was then accepted by the Association of Official Analytical Chemists [12,13]. In Brazil and in other countries the wine industry uses the Ripper Method, which is based on titration of sulfite with iodine [10] (or iodate) [14,15] and starch as a visual indicator, for the quantification of free and bound SO₂. Although the Ripper Method is relatively faster and easier to perform than the Monier-Williams method some authors did not recommend it due to presence of systematic errors due to difficulty with visual perception of the end point (particularly in red wine samples) which promotes low precision in the results [16,17].

In the present study the free SO₂ level in wine samples was determined by the Ripper Method using a standard potassium iodate solution with potentiometric detection. In the analyzed samples the concentration of SO₂ was calculated by the multiple standard addition method using a freshly prepared sodium metabisulfite solution. For comparison purposes, the same samples were analyzed with an automatic titrator for specific determination of SO₂ level, which was routinely used in wine industry.

Materials and Methods

Reagents

Reverse osmosis water (Quimis Q842-210, Diadema, Brazil) was used to prepare all solutions. A 2.5×10⁻² M potassium iodate (KIO₃, FW 214.01 g mol⁻¹, 99.5 %, Reagen, Brazil) standard solution was prepared by dissolving 0.5350 g in water in a 100.0 mL volumetric flask without further standardization. A 3.4×10⁻³ M sodium metabisulfite (Na₂S₂O₅, 99%, FW 190.10 g mol⁻¹, Merck, Brazil) solution was prepared by dissolving 0.1600 g in water in a 250.0 mL volumetric flask. Potassium iodide (KI, FW 166.00 g mol⁻¹, 99 %, Merck, Brazil) was used in solid form. A 1 % starch (≥ 99 %, Sigma, Brazil) solution was prepared by dissolving 0.5 g in 50 mL of boiling water. A 2.0 M sulfuric acid (H₂SO₄, FW 98.08 g mol⁻¹, 98%, d = 1.84 g mL⁻¹, Synth, Brazil) solution was prepared by diluting 108.7 mL in water in 1.0 L volumetric flask.

Materials

A 5.0 mL micro burette (Metrohm AG Herisau, 60402100) was used in all titrations of wine samples. Potential measurements (± 0.1 mV) were carried out using a pH Meter (Metrohm, 654) with a combined Pt/saturated calomel electrode (SCE) filled with NaCl solution (Metrohm, 60402100).

Methods

Determination of free SO₂ level by potentiometric titration

Calibration curves were obtained by transferring aliquots of 25,0; 50,0; 75,0 and 100,0 mL of a 3.4×10⁻³ M Na₂S₂O₅ solution to four 250 mL beakers in which 0.8 g of KI, 0,5 mL of 1% starch solution and 5,0 mL of 2.0 M H₂SO₄ solution were added in that order. These solutions were titrated with a 2.5×10⁻² M KIO₃ standard solution and potentiometric detection yielded a E (mV) vs. V (mL) KIO₃ 2.5×10⁻² M curve. For the analysis of the samples aliquots of 100 mL of wine (red or white) were transferred to four 250 mL beakers and the standard addition method was applied. From the second to the fourth beaker increasing aliquots (25,0; 50,0 and 75,0 mL) of the same 3.4×10⁻³ M Na₂S₂O₅ solution were added and the procedure described was performed.

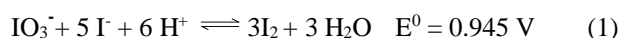
Determination of free SO₂ level using a Mini-Titrator

The procedure used in this determination is described in detail in the equipment manual [15].

Results and Discussion

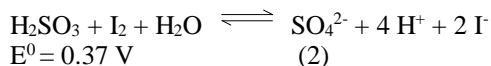
In the original Ripper Method the determination of the free SO₂ content in the wine sample is conducted by direct titration with I₂ using starch as a visual indicator [14]. The end point of this analysis is not very easy to visualize when the analyzed samples are red wines. In addition, it is recurrent the addition of H₂SO₄ solution in order to reduce the oxidation of polyphenols by I₂ [18] and under these conditions the blue characteristic color of the starch-iodine complex does not form fully [19].

In the modified Ripper Method used in the present study a potassium iodate freshly prepared solution was used as titrant instead of iodine standardized solution. Potassium iodate solution has advantages over the iodine solution since it can be prepared by simply dissolving an appropriate amount of pure KIO₃ in water without the need for further standardization. This solution is quite stable and can be stored without change in its concentration for a long time [20]. In addition, KIO₃ (E⁰ IO₃⁻/I = 1.08 V vs. NHE) [21] is a source of a known amount of I₂ (E⁰ I₂/I = 0.540 V vs. NHE) [21] after addition of iodide in acid medium (equation 1).



In the analyzed samples an excess of solid KI (in acid medium)

was added and the solution was homogenized. When the KIO_3 solution was introduced by the buret I_2 was formed ($E^0 \text{I}_2/\text{I}^- = 0.54 \text{ V vs. NHE}$) [21] (equation 1) and then reacted with the free SO_2 in acid medium ($E^0 \text{SO}_4^{2-}/\text{H}_2\text{SO}_3 = 0.17 \text{ V vs. NHE}$) [21] according to equation 2 providing the stoichiometry for the global process as $1[\text{IO}_3^-]: 3[\text{H}_2\text{SO}_3]$.



The multiple standard addition method (with $\text{Na}_2\text{S}_2\text{O}_5$ solution) was used to quantify the free SO_2 in all wine samples. $\text{Na}_2\text{S}_2\text{O}_5$ was chosen as the standard of SO_2 because the anion metabisulfite ($\text{S}_2\text{O}_5^{2-}$) hydrolyzes forming hydrogen sulfite (HSO_3^-) which in acid solution forms SO_2 (equations 3 e 4) that reacts with I_2 .

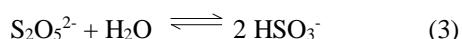


Figure 1 shows the potentiometric titration curves of a red wine sample after several additions of $3.4 \times 10^{-3} \text{ M Na}_2\text{S}_2\text{O}_5$ solution. The volume consumed of $2.5 \times 10^{-2} \text{ M KIO}_3$ standard solution to reach the end point of the reaction found potentiometrically created conditions to perform the determination of the free SO_2 concentration (mg L^{-1}) in the samples analyzed. It can be noted that the volume of the $2.5 \times 10^{-2} \text{ M KIO}_3$ solution consumed in these redox titrations increases proportionally as the addition of $3.4 \times 10^{-3} \text{ M Na}_2\text{S}_2\text{O}_5$ (Figure 1) but the potential value in the final point, (310 ± 20) mV vs SCE, does not change greatly which shows the good reproducibility of the analyzes of these samples. Considering all these redox titrations performed (calibration curves with $\text{Na}_2\text{S}_2\text{O}_5$ standard solution and samples) the potential value obtained at the end of these reactions was (314 ± 16) mV (CV 5 %) (Table 1). This suggests that under these experimental conditions only SO_2 present in the wine samples was quantified and not other compounds (e.g. polyphenols).

In the same titrations above described 1.0 mL of 1% starch solution was added as visual indicator. As expected, the endpoint visualization was difficult due to the color of the sample and that is why the results could not be properly established.

For comparison purposes the free SO_2 concentration was also determined with the Hanna HI-84500 Mini-Titrator. Regarding the samples analyzed (Table 1) the results show that the values of free SO_2 concentration obtained by the Ripper method with the potentiometric titration and with the Mini-Titrator are strictly close. The t-test paired showed that there were no significant differences between the two procedures considering the five samples analyzed ($t = 0.636$; $p < 0.05$). Figure 2 shows both the calibration curves of the $3.4 \times 10^{-3} \text{ M Na}_2\text{S}_2\text{O}_5$ solution and of a red wine sample (after multiple addition of the same $\text{Na}_2\text{S}_2\text{O}_5$ solution) obtained with the $2.5 \times 10^{-2} \text{ M KIO}_3$ standard solution. The calibration curve, KIO_3 (V mL) vs. $\text{Na}_2\text{S}_2\text{O}_5$ (V mL), obtained with four aliquots of $3.4 \times 10^{-3} \text{ M Na}_2\text{S}_2\text{O}_5$

solution is represented by a straight line equation ($y = -0.175 + 0.0408 \times X$; $r = 0.998$). It provided a detection limit (defined as 3 times the standard deviation of the linear coefficient divided by the angular coefficient of the calibration curve) of $6.85 \text{ mg L}^{-1} \text{ SO}_2$, which allows the determination of low SO_2 levels in wine samples. The excellent linearity and parallelism observed between these two curves (5.6 %) show the sample is not under any pronounced matrix effect, which is confirmed by the very good values found in the recovery rate, ($101.8 \pm 9.6\%$), for the added aliquots of the $3.4 \times 10^{-3} \text{ M Na}_2\text{S}_2\text{O}_5$ solution (Table 2).

The observations described above show that the quantification of free SO_2 in wine samples using the Ripper method can be easily achieved using a solution of potassium iodate as titrant with potentiometric detection. Potentiometry titrations are low cost, easy to operate and the equipment used (potentiometer with electrodes) are affordable in most quality control laboratories. Finally, it should be emphasized that both assays used (manual titration and Mini-Titrator) do not differentiate SO_2 when occurring by natural means during yeast fermentation (present in young wines) from that generated by the addition of any sulfating agent commonly used in food products (Table 3).

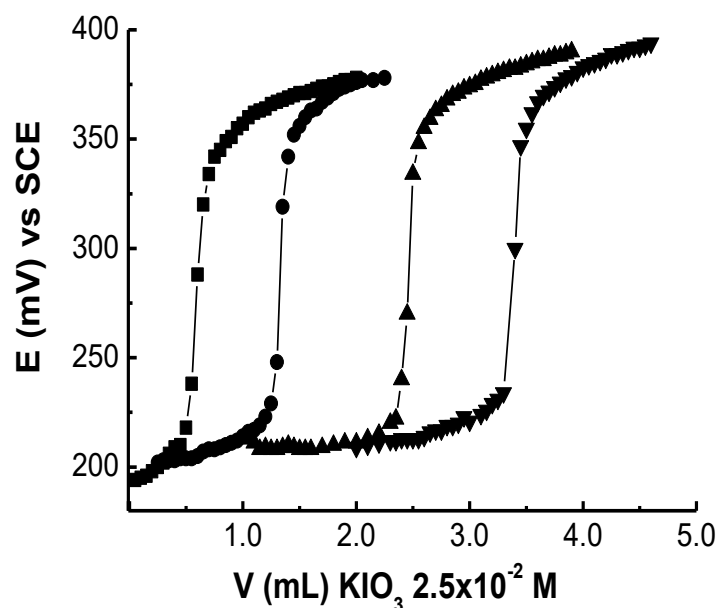


Figure 1: Potentiometric curves obtained of titration of 100 mL of a red wine sample with $\text{KIO}_3 2.5 \times 10^{-2} \text{ M}$ standard solution. Addition (mL) of $3.4 \times 10^{-3} \text{ M Na}_2\text{S}_2\text{O}_5$ solution: (■) 0; (●) 25 (▲) 50 and (▼) 75.

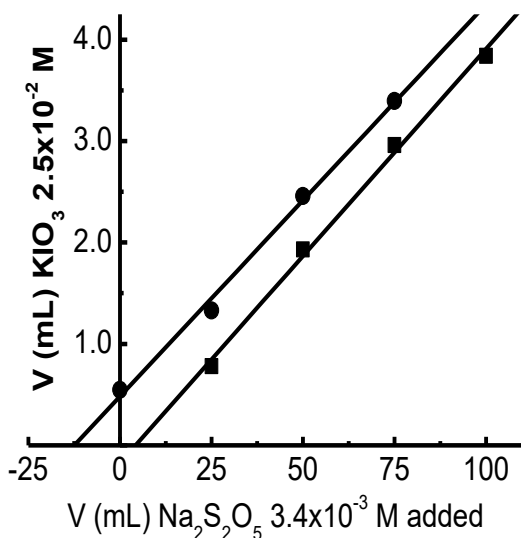


Figure 2: Calibration curves: (■) aliquots of 3.4×10^{-3} M $\text{Na}_2\text{S}_2\text{O}_5$ solution ($Y = -0.175 + 0.0408 \times X$; $r = 0.998$) e (●) addition of aliquots of 3.4×10^{-3} M $\text{Na}_2\text{S}_2\text{O}_5$ solution in a red wine sample ($Y = 0.482 + 0.0387 \times X$; $r = 0.998$).

n	Type	E (mV)	Free SO_2 (mg L^{-1})	
			Present work	Mini-Titrator Hanna ³
1	Red	310 ± 20	22.9 ± 0.7	17.6 ± 0.4
2	Red	323 ± 10	20.0 ± 0.4	18.9 ± 0.2
3	Red	324 ± 19	26.3 ± 0.2	28.4 ± 0.5
4	Red	328 ± 10	11.2 ± 0.5	10.1 ± 0.7
5	White	287 ± 12	12.0 ± 0.7	13.3 ± 0.2

Table 1: Free SO_2 level in the wine samples analyzed.

All values are mean and standard deviation of at least three titrations.

n	Type	3.4×10^{-3} M $\text{Na}_2\text{S}_2\text{O}_5$ (mL)		
		Added	Found	Recovery (%)
1	Red	25.0	23.4	95.5
		50.0	51.1	93.4
		75.0	74.0	89.4
2	Red	25.0	29.4	118
		50.0	54.3	108
		75.0	75.0	100
3	Red	25.0	21.8	87.4
		50.0	48.2	96.4
		75.0	70.1	93.5
4	Red	25.0	24.7	98.9
		50.0	46.4	92.9
		75.0	72.0	96.1
5	White	25.0	29.2	117
		50.0	54.9	110
		75.0	77.6	103

Table 2: Recovery rates of the addition of three aliquots of 3.4×10^{-3} M $\text{Na}_2\text{S}_2\text{O}_5$ solution in wine samples.

INS	220	221	222	223	224	225	226	227	228
Compound	SO_2	Na_2SO_3	NaHSO_3	$\text{Na}_2\text{S}_2\text{O}_5$	$\text{K}_2\text{S}_2\text{O}_5$	K_2SO_3	CaSO_3	$\text{Ca}(\text{HSO}_3)$	KHSO_3

Table 3: Some sulfating agents added as food additives.

Conclusion

The Ripper method performed with standard solution of potassium iodate and potentiometry detection proved to be efficient and reproducible for the determination of free SO_2 content in wine samples. This may be a less expensive alternative to quality control laboratories that do not have specific equipment for this purpose.

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