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Comparison of soluble manganese(IV) and acidic potassium permanganate chemiluminescence detection using flow injection and sequential injection analysis for the determination of ascorbic acid in Vitamin C tablets

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Abstract

The limits of detection (3*s*) for ascorbic acid were 5×10^{-8} M with acidic potassium permanganate using both flow injection analysis (FIA) and sequential injection analysis (SIA) whereas the soluble manganese(IV) afforded 1×10^{-8} M and 5×10^{-9} M for FIA and SIA, respectively. Determinations of ascorbic acid in Vitamin C tablets were achieved with minimal sample pretreatment using a standard additions calibration and gave good agreement with those of iodimetric titration.

Keywords: Flow injection analysis; Sequential injection analysis; Vitamin C

1. Introduction

The analytical utility of acidic potassium permanganate chemiluminescence has been known since 1975 [1] and was the subject a recent review [2], however the spectrally identical emission resulting from the reaction of soluble manganese(IV) with various analytes was first reported [3] in 2001. In a review by Yebra-Biurrun [4] on the determination of ascorbic acid by flow injection methods the author cites fifteen papers (ca. 16% of those reviewed) that employed various types of chemiluminescence detection, of these, only the work of Agater and Jewsbury [5] utilised acidic potassium permanganate as a direct reagent. Tsaplev [6] first reported the chemiluminescence from the oxidation of ascorbic acid by acidic potassium permanganate in 1991, some years later Agater et al. [7] observed red light from the same reaction and Zhu et al. [8] utilised this chemistry for batch determination of the analyte. The detection limits obtained by these workers [5,8] were $<5 \times 10^{-7}$ M and 3.0×10^{-7} M, respectively, the former being an estimation as no value was quoted. Additionally, using the luminol [9] lucigenin [10] and cerium(IV)–Rhodamine B [11] reactions detection limits of 1.0×10^{-12} M, 2.0×10^{-10} M and 1.0×0^{-13} M, respectively, have been achieved for ascorbic acid. Given the relatively poor detection limits attained for ascorbic acid [5,8] with acidic potassium permanganate chemiluminescence, compared to other species [2], it offered a challenge to the efficacy and utility of soluble manganese(IV). Consequently, this paper presents a simple, rapid and accurate method for the determination of ascorbic acid in Vitamin C tablets using flow injection analysis (FIA) and sequential injection analysis (SIA) with the two manganese-based chemiluminescence detection chemistries.

2. Experimental

2.1. Instrumentation

2.1.1. Flow injection analysis (FIA)

A simple two-line FIA manifold was used for all experiments. Sample and standard solutions (30 μl) were injected

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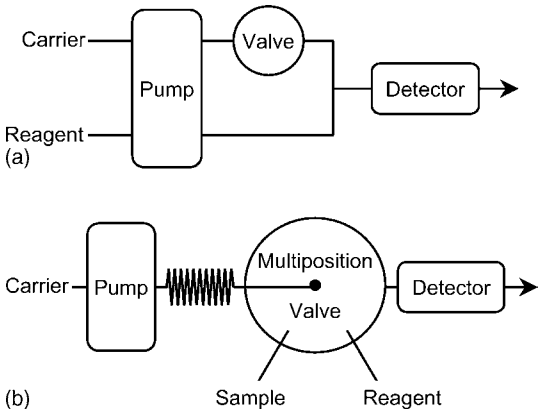

Fig. 1. Schematic diagram of (a) FIA manifold and (b) SIA manifold. For reagent and instrumental details please refer to text.

using a six-port valve (Valco Instruments, Houston, TX, USA) into a carrier stream of sodium hexametaphosphate whilst the second line delivered the chemiluminescence reagent. A Gilson Minipuls 3 peristaltic pump (John Morris Scientific, Melbourne, Australia) with PVC pump tubing (1.85 mm i.d., AI Scientific, Melbourne, Australia) propelled the two streams at equal rates (total flow 8.5 ml min^{-1}) all other tubing was PTFE (0.5 mm i.d., Chromolytic Technologies, Melbourne, Australia). The reagent and analyte streams merged at a Y-piece directly attached to a glass spiral flow cell (∼ 80 μl, 0.5 mm i.d., Embell Scientific, Murwillumbah, NSW, Australia) positioned flush against the window of a red sensitive photomultiplier tube (Thorn EMI 9798SB, Ruislip, Middlesex, UK) operated at 700 V, provided by a stable power supply (PM28BN, Thorn EMI). The flow cell and photomultiplier tube were encased in light tight housing and the output from the detector was recorded on a strip chart recorder (Omniscribe, Houston, TX, USA) with signals measured manually as peak heights (Fig. 1).

2.1.2. Sequential injection analysis (SIA)

All experiments were performed using purpose-built SIA instrument configured from a syringe pump (Cavro XP 300, Global FIA, Gig Harbour, WA, USA) and a ten-port multiposition valve (Valco C25Z, Scientific Glass Engineering, Melbourne, Australia) which were computer controlled with LabVIEW®-based software described previously [12]. All reagent and analyte lines were primed with solution prior to analysis. The analytical cycle began with 2.5 ml of the carrier solution being drawn into the syringe at 10 ml min^{-1} followed by 670 μl of sample or standards and 80 μl reagent aspirated sequentially into the holding coil at 1.5 ml min through separate ports on the multiposition valve. Using a third port the entire volume (3.25 ml) was propelled at 10 ml min^{-1} through the spiral glass flow cell (∼120 ml, 0.8 mm i.d., Deakin University) positioned flush against the window of the same red sensitive photomultiplier tube used in the FIA instrument. Likewise, the flow cell and photomultiplier tube were encased in light tight housing and the output from the detector was recorded on a strip chart recorder with signals measured manually as peak heights.

2.2. Reagents and samples

All solutions and samples were prepared using analytical reagent grade compounds and deionised water (Millipore, MilliQ water system, North Ryde, NSW, Australia) unless otherwise stated. Sodium hexametaphosphate, orthophosphoric acid, sulfuric acid, L-ascorbic acid, fructose, galactose, lactose, mannitol, sucrose, xylose and formaldehyde were from BDH (Poole, UK). Potassium permanganate was from Ajax (Melbourne, Australia) whilst sodium formate was purchased from Sigma (Castle Hill, NSW, Australia). Commercially available Vitamin C tablets 100 and 500 mg ascorbic acid (Cenovis Health Company, Turrella, Australia) and 100 mg ascorbic acid (Blackmores, Balgowlah, Australia) were used as samples.

The soluble manganese(IV) was prepared as described previously [3] after the method of Jáky and Zrinyi [13] via the reduction of potassium permanganate with excess sodium formate to precipitate manganese dioxide, which was collected by vacuum filtration through a glass micro fibre filter paper (GF/A, Whatman, Maidstone, England), then rinsed with water. Subsequently, an amount of the wet material (0.2 g) was dissolved in orthophosphoric acid (3 M, 500 ml) assisted by ultrasonication (30 min) and left to stand overnight. The oxidant concentration was determined by iodometric titration [14] and diluted to the required molarity with orthophosphoric acid (3 M). The sodium hexametaphosphate solution (1.0% w/v) was prepared daily and adjusted to pH 3.0 with orthophosphoric acid. The reagent was either acidic potassium permanganate (1.0×10^{-3} M in 1.0% w/v sodium hexametaphosphate) or soluble manganese(IV) (1.0×10^{-3} M in 3 M orthophosphoric acid) The standards and samples were made up in the sodium hexametaphosphate plus formaldehyde (0.4 M).

Commercial Vitamin C tablets were crushed and solubilised in deionised water (1.0% w/v, pH 3.0, 250 ml) with the aid of ultrasonication and subsequently diluted (1000-fold) with sodium hexametaphosphate plus formaldehyde (0.4 M).

3. Results and discussion

3.1. Preliminary experiments

Initial reaction conditions were chosen on the basis of our earlier determination of morphine [15,16] and employed carrier and reagent streams of sodium hexametaphosphate (1% m/v) with the latter also containing potassium permanganate (1.5×10^{-3} M at pH 3.0). These experiments revealed that the signal intensity from ascorbic acid was inferior to that observed for morphine under similar conditions [15,16] with an estimated detection limit

of approximately 1×10^{-4} M. Deftereos et al. [17] noted that the addition of formaldehyde significantly enhanced the chemiluminescence from the reaction of epinephrine with acidic potassium permanganate. Consequently, varying concentrations of formaldehyde (from 0.1 to 1.0 M) were added to an ascorbic acid standard solution (1×10^{-4} M), which resulted in a 100-fold increase in the signal intensity at 0.4 M after which the response plateaued. The presence of formaldehyde generated a measurable blank, which increased linearly with concentration. However, adding formaldehyde at 0.4 M gave the best signal to background ratio and was used for all FIA and SIA experiments. While the role of formaldehyde in this chemistry is unknown it dramatically affects the chemiluminescence from the acidic potassium permanganate and soluble manganese(IV) oxidations of ascorbic acid. However, similar experiments with morphine resulted in no significant increases in signal intensity [28], this apparently selective enhancement behaviour from formaldehyde may prove to be of some utility.

During our initial study of soluble manganese(IV) [3] we noted that the addition of formaldehyde (0.2 M) considerably improved the chemiluminescence emission intensity. Using simple univariate searches we established that for ascorbic acid a good signal to background ratio was achieved using formaldehyde at 0.4 M with the reagent (1.0×10^{-3} M in 3 M orthophosphoric acid) plus 1.0% w/v sodium hexametaphosphate. The enhancement from the presence of the sodium hexametaphosphate in the new reagent [3] is consistent with its essential and as yet unknown role in manganese-based chemiluminescent reactions [2,29].

3.2. Analytical figures of merit

A series of twelve standard solutions (from 1×10^{-10} M to 5×10^{-4} M) were used to produce calibration graphs from the four combinations of reagents and instrumentation, in common with previous reports [2] these functions were not linear over this concentration range. However, the calibrations approximated linearity within a limited range as can be seen from the analytical figures of merit summarised in Table 1. While our detection limits were not as impressive as those reported by Alwarthan [9], Perez-Ruiz et al. [10] or Ma et al. [11], they were superior to the work of both Agater and Jewsbury [5] and Zhu et al. [8] who had employed acidic potassium permanganate and they compared quite favourably with other chemiluminescence systems [18–25] (see Table 2). Also, the methodologies described herein were both chemically and instrumentally less complex than many of those listed in Table 2 and exhibited more than adequate sensitivity for the analyses performed with a sample frequency in excess of 180 per hour (h^{-1}).

Table 2
Detection limits for ascorbic acid achieved with various chemiluminescent reactions

Reaction type	Detection limits (M)	Reference
Fe(II) catalysation of luminol.	1.0×10^{-12}	[9]
Inhibition of luminol-Fe(II)-superoxide	1.0×10^{-9}	[18]
Inhibition of luminol-H_2O_2-KIO_4	6.0×10^{-8}	[19]
Luminol-KIO_4	5.0×10^{-9}	[20]
Cr(III) catalysation of luminol	7.0×10^{-9}	[21]
Cr(III) catalysation of luminol	8.0×10^{-9}	[22]
Lucigenin-photo oxidation products-toluidine blue	2.0×10^{-10}	[10]
Fe(II) catalysation of lucigenin	2.0×10^{-9}	[23]
tris(2,2-Bipyridyl)ruthenium(III)	5.0×10^{-7}	[24]
Peroxyoxalate with perylene	5.0×10^{-8}	[25]
Ce(IV) and Rhodamine B	1.0×10^{-13}	[11]
Acidic potassium permanganate	3.0×10^{-7}	[8]
Acidic potassium permanganate	$<5.0 \times 10^{-7}$	[5]

3.3. Analysis of Vitamin C tablets

Five replicate analyses were performed on each of the three commercially available Vitamin C preparations using both external standards and standard addition calibration protocols for the four reagent and instrumental combinations. Also, individual tablets (five of each type) were dissolved in sulfuric acid (0.3 M, 60 ml) and assayed for their ascorbic acid content using iodimetric titrations after the method described in Harris [26]. The mean values of these analyses are shown in Table 3 with the quoted uncertainties representing the maximum ranges of each result. Given that for each of the three tablet types, the variation in mass about the mean was less than ±1% ($n = 50$) the observed intra sample variation may reflect the difficulty in producing a homogeneous solid formulation.

Clearly, the use of simple aqueous external calibration standards proved to be inappropriate in all cases, however the observed negative interferences for sample II were unexpected on the basis of previous studies [5,7,27] which noted that carbohydrates elicited significant response with acidic potassium permanganate, interestingly sample I was marketed as a children's formulation being free from excipients such as sugars, milk products, yeast, starch, gluten

Table 1
Comparative analytical figures of merit for ascorbic acid

Oxidant	FIA	SIA
Mn(IV)	Linearity range: 1×10^{-7} M to 5×10^{-5} M; $y = 2 \times 10^{-6}x + 1.802$; $R^2 = 0.9923$; L.O.D. $= 5 \times 10^{-8}$ M	Linearity range: 5×10^{-8} M to 5×10^{-5} M; $y = 3 \times 10^{-7}x + 40.99$; $R^2 = 0.9917$; L.O.D. $= 5 \times 10^{-8}$ M
Mn(VII)	Linearity range: 1×10^{-7} M to 5×10^{-6} M; $y = 6 \times 10^{-6}x + 1.065$; $R^2 = 0.9999$; L.O.D. $= 1 \times 10^{-8}$ M	Linearity range: 5×10^{-7} M to 5×10^{-6} M; $y = 4 \times 10^{-7}x + 45.62$; $R^2 = 0.9944$; L.O.D. $= 5 \times 10^{-9}$ M

Table 3
Determination of the ascorbic acid content of three Vitamin C tablets

		Mass of ascorbic acid (mg)		
		I Cenovis (100 mg)	II Cenovis (500 mg)	III Blackmores (100 mg)
External standardisation				
FIA	Mn(IV)	122 ± 4	345 ± 3	116 ± 4
	Mn(VII)	140 ± 3	350 ± 6	110 ± 2
SIA	Mn(IV)	118 ± 2	342 ± 4	122 ± 3
	Mn(VII)	125 ± 4	370 ± 5	124 ± 1
Standard additions				
FIA	Mn(IV)	114 ± 2	496 ± 5	105 ± 3
	Mn(VII)	110 ± 3	490 ± 8	100 ± 2
SIA	Mn(IV)	112 ± 4	502 ± 4	92 ± 4
	Mn(VII)	110 ± 3	490 ± 3	94 ± 3
Iodimetric titration [26]				
		112 ± 1	507 ± 4	102 ± 1

and preservatives, whereas sample II contained both glucose and saccharin in unspecified amounts. The exact nature of the excipients used in samples I and II was not attainable from the manufacturers and as such any postulations on the causes of the interferences were impossible. We did however investigate the potential for interference from several commonly used carbohydrate excipients by monitoring the chemiluminescence from standard solutions of fructose, galactose, lactose, mannitol, sucrose and xylose (1×10^{-4} M) using the conditions described above and they all gave responses which were less than 3% of the blank signal for both chemistries.

This lack of response from acidic potassium permanganate may reflect the respective chemiluminescence kinetics under the present reaction conditions. This is consistent with the study by Agater et al. [27], who employed Mn(II) as a catalyst to speed up the oxidations of the same carbohydrates to obtain chemiluminescence signals of analytical utility. Conversely, the chemiluminescent kinetics for ascorbic acid, using the present chemistries, appeared to be quite rapid and in order to achieve the maximum signal intensity flow rates of 8.5 and 10 ml min^{-1} (for FIA and SIA, respectively) were required to propel the reacting reagent and sample through the detector flow cell. In the FIA manifold the distance from the confluence point of the two streams to the detector was approximately 3 mm and at the above flow rate through 0.5 mm i.d. tubing this translates to an elapsed time of 4.5 ms for the reacting zone to reach the detector. Even for SIA, where the distance from the of the holding coil to the flow cell was considerably longer at around 150 mm and at 10 ml min^{-1} the time taken to reach the detector was still quite short at 180 ms. The increased time interval prior to detection for SIA was compensated for by the sample to reagent volume ratio of greater than eight to one. Interestingly, Agater et al. [27] made their measurements within 2 s after mixing, thus even with Mn(II) present as a catalyst the generation of chemiluminescence from the reaction of acidic potassium permanganate with these carbohydrates is relatively slow compared to ascorbic acid. With regard to the soluble manganese(IV) we reported previously that fructose and sucrose did not produce a measurable response with this reagent [3].

Sample III contained matrix constituents, which included iron(II) fumarate (153 mg) folic acid (167 mg), Vitamin B12 (50 mg) and common nettle powder (100 mg). Deftereos et al. [17] reported that folic acid elicited useful chemiluminescence with acidic potassium permanganate. The FIA responses from a standard solution of folic acid (1×10^{-4} M) were identical in magnitude to that of the blank signals, which again may reflect differential kinetics between the analyte and this excipient under the present conditions. Also, the folic acid content of each tablet resulted in a concentration of approximately 1.5×10^{-6} M in the sample stock solutions, which were further diluted one thousand times prior to analysis and as such would give no measurable response compared to that from the analyte. Likewise, the iron(II) fumarate would have been expected [3] to give a response with the soluble manganese(IV) as its concentration in the sample solution would have been 3.6×10^{-7} M this may have accounted for the high results in Table 3. Generally, calibration via the method of standard additions afforded good agreement with the iodimetric titration [26] figures for most samples, with the SIA results for sample III giving the worst correlations at eight and eleven percent low.

4. Conclusions

The results obtained with the four methodologies revealed that SIA afforded better detection power (for ascorbic acid) than FIA for both chemiluminescence reagents, which probably reflected the inherently quieter characteristics of the former system [12]. While the determination of ascorbic acid required standard addition calibrations the methodologies are simple, rapid and robust with high analytical throughput (>180 h^{-1}) and would be ideally suited to formulation degradation studies, quality assurance and process monitoring after a detailed investigation into the exact nature of the observed interferences.

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