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Sequential Injection Titrimetric Analysis of Vitamin C in Drug Formulation Using Potassium Permanganate

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Abstract

Sequential injection analysis technique was utilized for automatic titration of vitamin C in drug formulation. The method is based on the oxidation reaction of vitamin C with potassium permanganate in sulfuric acid media. The titration reaction was monitored by the decrease of absorbance of the permanganate at its maximum wavelength at 525 nm. The super modified simplex computer program was successfully utilized for optimizing the reaction parameters such as sulfuric acid and potassium permanganate concentration as well as determining the stoichiometry of the reaction. The optimized operating conditions were 0.418 μmol/L potassium permanganate and 7.49 μmol/L sulfuric acid with the injection of 0.25 μmol/L vitamin C thus verifying the stoichiometry of 2:5:16 for permanganate, vitamin C and sulfuric acid respectively. In addition a (3^f) factorial design was employed for studying interaction effects. The method was found to be applicable for the detection of vitamin C in the range between 100 to 350 ppm and it was found to be accurate when statistically compared with the BP standard method. The method exhibited no interferences from excepients added in drug formulation. The method was also found to be faster and more economical compared to the previous reported methods.

Keywords: Sequential injection; titrimetry; permanganate; vitamin C and spectrophotometry

1. Introduction: Since the introduction of the Sequential Injection Analysis (SIA) technique to the Analytical Chemistry circle few years ago the SIA titrimetry remains unexplored and more has to be done to validate its potential in this area. The automation, consumption of micro-quantities of reagents, accuracy and precision of the results obtained with SIA technique in general is expected to provide enormous advantages to the SIA titrimetry over the traditional FIA and conventional manual titration methods. Titration with SIA is achieved by calculating the equivalent concentration of the analyte sequentially introduced into a known excess of the reagent that has already been kept into the holding coil. Titration with flow injection (FI) is conducted by using a mixing chamber for the reagent instead of the holding coil in SIA. The SIA titration has the advantage over the conventional traditional titration method by the followings: It is significantly faster and frequency of analysis is higher. It is much more economical as micro amounts are required. It is higher precise as volumes aspirated are perfectly adjusted by the computer control of timing of the stroke. It is fully automated and could be conducted with un-attendable operations thus minimizing manpower in routine analysis. In addition, the SIA titration has the advantage over the FIA titration in two respects: Calculation of the analyte concentration relies on monitoring peak height rather than peak width at the middle of peak height as peaks in the former are very narrow compared to significantly broad peaks in the latter case due to the high pressure pump in SIA. Reagent consumption in SIA is considerably lower than that used in FIA as the latter depends on the continuous flowing stream of the carrier. The frequency and number of samples analysed with SIA is at least double that executed by the FIA titration. SIA titrimetric method for the determination of vitamin C with cerium (IV) as an oxidant has been recently reported by the same authors of this article[1]. The method is considered the first reported method for utilizing the SIA in titrimetry. However the method lacks the sensitivity as cerium(IV) was used as an oxidant and as a self indicating system and therefore inevitably using it in much higher concentrations. A FI spectrophtometric method for the assay of vitamin C using permanganate in acidic media has been reported[2] with FI disadvantages mentioned above. The standard method described by the official BP[3] monograph is a normal titration procedure involving cerium(IV) as an oxidant in sulfuric acid media. Vitamin C (l-keto-1threo-γ-lactone-2,3-enediol) has earlier been reported in literature in the medical field and later introduced in chemistry as a reducing agent and thus successfully applied as such in analytical chemistry. Several FIA and other methods for its determination have been reported with various aspects such as conductometry[2], potentiometry[4], amperometry[5,6] and coulorometry[7-9] but they are all with some complexity and subject to numerous interferences. In this paper, a SIA titrimetric method for the determination of vitamin C using permanganate as the oxidant in sulfuric acid media was described. Appropriate operating conditions were optimized and the stoichiometry of the reaction was studied. The method was applied to the determination of vitamin C in pharmaceutical products and interferences were investigated.

2. Experimental

2.1. Reagents and stock solutions

2.1.1 Vitamin C Standard Solution. A stock solution was always freshly prepared from Fluka AG (CH-9470 Buchs, Switzerland) previously dried at 50^{O}C in vacuum over magnesium perchlorate. This standard solution was used to give the appropriate concentrations by injecting different volumes .

2.1.2 Vitamin C tablet and effervescent. From the proprietary drugs ten tablets were accurately weighed, crushed and powdered. The amount of powder containing the appropriate weight to give 200 ppm vitamin C was dissolved in about 40 ml of water (effervescent tablets are left for ten minutes for all gases to seize evolving); then filtered and washed. The filtrate was made up to volume in a 100 ml volumetric flask

2.1.3 $KMnO_4$. A standard solution was prepared by dissolving exactly about 3.5 g of dried potassium permanganate (P-279 Lot 746030 Fisher scientific company, USA) in 1000 ml. The stock solution was standardized with sodium oxalate (S-356 Lot 792406 Fisher scientific company, USA). This solution was used through all the experimental processes.

2.1.4 Sulfuric acid. A stock solution of H_2SO_4 (95-98% Specific gravity 1.84 kg / l, Merck, UK) was prepared the usual way. Working solutions were prepared by dilutions.

2.2 Manifold and procedure

2.2.1 Procedure

The SIA titration method for the assay of vitamin C adopted during this work was executed following the steps described in the section below with reference to the manifold in Fig. 1: With the flow rate of 300 μL/s the syringe pump was filled by the carrier solution (water) when the valve position in. When the valve position out the following steps were carried out.

1. 1500 μL of the carrier solution (water) was dispensed with the flow rate of 350 μL/s through port 1 of the selector valve. This step was performed to adjust the absorbance of the spectrophotometer to zero.
2. 150 μL each of potassium permanganate, sulfuric acid and vitamin C solutions were aspirated with the flow rate of 150 μL/s through ports 3, 6 and 8 respectively to load the coils and the excess was discarded by dispensing 700 μL with the flow rate of 150 μL/s through port 5.
3. The corresponding volume/μL of potassium permanganate then sulfuric acid containing the appropriate amount of μmols were sequentially aspirated with the flow rate of 150 μL/s through port 3 and 6 respectively.
4. 800 μL (the mixed solution of potassium permanganate and sulfuric acid followed by water in the holding coil) were dispensed with the flow rate of 30 μL/s through port 1 and the absorbance A_2 was recorded.
5. The same volumes of potassium permanganate and sulfuric acid in step (4) in addition to vitamin C (corresponding volume/μL which contains the appropriate amount of μmols) were sequentially aspirated with the same flow rate through port 3, 6 and 8 respectively.
6. 800 μL (potassium permanganate solution after reacting with vitamin C in sulfuric acid media followed by water in the holding coil) were dispensed with the flow rate of 30 μL/s through port 1 and the absorbance A_1 was recorded.
7. Water was dispensed through port 1 to wash; and the syringe was refilled again by repeating step (1).
8. Analysis and A_2 could be obtained repeatedly by performing steps (6) and (7).

2.2.2 Apparatus

The Alitea USA/FIALab 3000 (Medina, WA USA) has been used in this method. The apparatus consists of a syringe pump, a multi-position valve, a fiber optic spectrophotometer and a PC (Figure 1). The syringe pump is a 24,000 steps syringe pump with an optical encoder feedback; 1.5 seconds to 20 minutes per stroke of 5.0 mL size. It is >99% accuracy at full stroke.

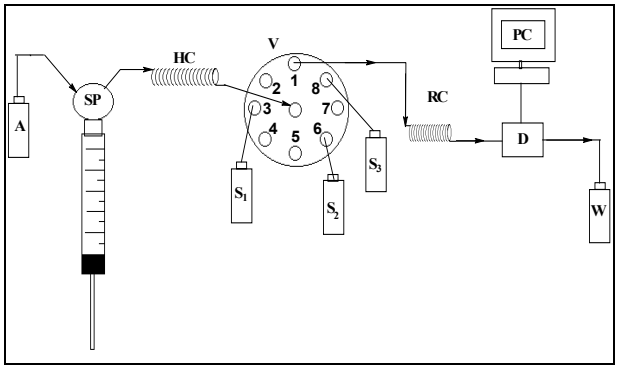

Fig. 1: SIA manifold comprised of; A. carrier (water); SP. 5 mL syringe pump; HC. holding coil; V. eight ports selector valve; RC. reaction coil; S_1. $KMnO_4$; S_2.sulfuric acid; S_3. vitamin C; RC. Reaction coil; D. spectrophotometer; PC. Computer and W. waste.

The multi-position valve has eight(8) ports with a standard pressure of 250 psi(gas)/600 psi (liquid); zero dead volume; chemically inert; port selection by manual or software control.

The spectrophotometer is the S2000 miniature fiber optic spectrometer pre-set to 200-850 nm wavelength range, UV2 detector, multi-band pass coating, 25 micron entrance slit from ocean optics, Inc. USA.

Z-Flow cell is 10 mm path length Teflon and Plexiglas or high-grade stainless steel compatible with standard SMA terminated fiber optics

Pump tubing of 0.30" ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used for connecting the different units, making the holding coil (400 cm long) and the reaction coil (100 cm Long).

2.3 Software Packages

2.3.1 Alitea FIALab software has been used for programming and controlling the SIA system.

2.3.2 Sigma plot, version 1.02 (Jandel Scientific, Erkrath, Germany) was employed for data-handling calculations, multiple regression analysis and constructing graphs.

2.3.3 COPS i.e. "Chemometric Optimization by Simplex" programme was obtained from Elsevier Scientific Co., The Netherlands, and utilized for the optimization of variables using a compatible IBM personal computer.

3. Results and discussion

3.1 Chemical System and Optimization

The present method is based on the oxidation of vitamin C to the dehydro-ascorbic acid with potassium permanganate in sulfuric acid media. Excess of the permanganate was aspirated into the holding coil followed by sequential aspiration of sulfuric acid and vitamin C. The decrease in the absorbance of the permanganate after injecting the analyte was spectrophotometrically monitored for calculating its concentration. It is important to note here that peak heights were considered rather than peak width at half peak height as usually done in FIA titrations[10]. This is mainly due to the fact that with SIA a syringe pump is used, which creates high pressure thus forcing the carrier solution with the products in a faster rate resulting in narrow peaks with a negligible peak width at base. Water was used as the carrier blank to adjust the absorbance to zero before commencing the titration.

Preliminary investigations on the permanganate absorption spectra revealed five maxima, which could be attributed to different oxidation states of the permanganate in dilute sulfuric acid. The maximum at 525 nm was found to be a prominent broad peak and the highest, therefore considered the working wavelength. Other maxima were not significant at higher acidities indicating stability of the manganese(VII) oxidation state probably due to the competing sulfate ligand. The reaction kinetics indicated that vitamin C reaction with permanganate was always faster than its reaction with cerium(IV), iron(III) and other oxidants owing to the higher redox potential of the permanganate, which is considered the most powerful and strongest oxidant. The result of the kinetic investigation showed that the reaction was fast enough in a wider range of sulfuric acid (and up to 3 $molL^{-1}$). It is interesting to compare this level with that in cerium(IV)[1] and iron(III)[11] where the reaction slows down at higher acidity and stops at about 0.5 $molL^{-1}$. In this system higher acidities favor higher redox potential value for the permanganate making it more powerful oxidant[12-14] than protonating the vitamin C and rendering it un-reactive as in the case with other oxidants thus working without fear of incompletion of the reaction.

3.2 Optimization

The chemical system discussed above indicates that the reaction is affected by variations of the permanganate and sulfuric acid concentrations; therefore a proper optimization procedure was sought. A statistical assessment for checking the interactive effects of the chemical factors namely permanganate, vitamin C and sulfuric acid was conducted by designing a three-factorial (3^f) experiment[15], where f is 3 denoting number of factors under scrutiny. Higher concentrations of the three factors were 0.6, 0.45 and 10.0 µmol/L respectively, and introduced in Table 1 as upper coded levels (+1) whereas lower concentrations were 0.3, 0.2 and 5.0 µmol/L respectively and introduced as lower coded levels (-1) in the same table. A total of 27 experiments were generated with response values as absorbance difference of the permanganate before and after adding the vitamin C. Multiple regression of the results obtained concluded that main effects of potassium permanganate concentration, vitamin C and sulfuric Acid concentration were found to be 29.75, 23.25 and 14.25 µmol/L respectively. The interaction effects of sulfuric acid concentration with permanganate was calculated to be 2.75 µmol/L, that of with sulfuric acid was 6.75 µmol/L, that of permanganate with vitamin C was 14.25 µmol/L and the interaction of all three factors was –0.5. This result indicate that the interaction effects of permanganate with sulfuric acid and with vitamin C are higher and more significant compared to the interaction of sulfuric acid with the permanganate. A response surface plot for permanganate versus sulfuric acid concentrations was generated as in Fig. 2. The 3-D plot revealed that the effect of both factors were similar showing positive effect with an increase in the response value to a certain limit and decreases slightly with continuous increase in the concentration versus the response value.

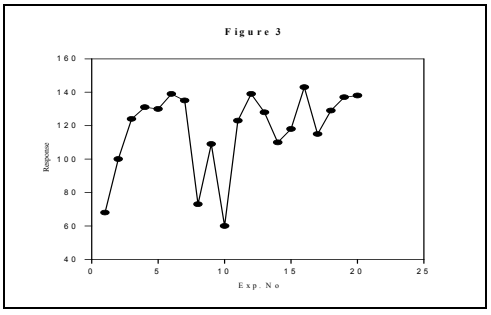

Fig. 3: Response function progress of the optimization experiments.

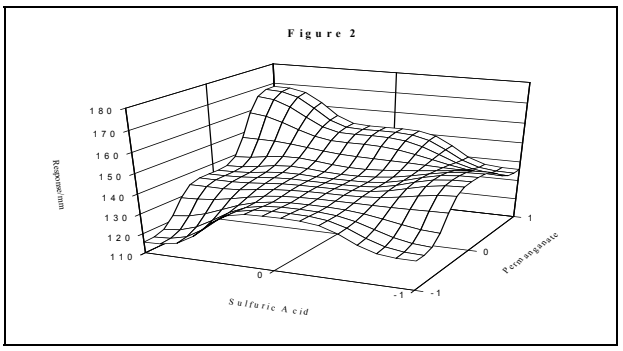

Fig. 2: Surface plot of the response in mm versus sulfuric acid and $KMnO_4$ levels.

The results obtained above, necessitates a thorough optimization of the two factors namely potassium permanganate and sulfuric acid to be carried out. The super modified simplex computer program was utilized for the optimization of the two main factors. Maximization of peak absorbance difference was considered to be the criterion for evaluating the performance of the chemical system.

The response recorded at each experiment was the difference between the absorbance of potassium permanganate before and after adding vitamin C. Before commencing the optimization procedure, the programme was fed with the information necessary to conduct the experiment such as upper and lower limit of both reagent and acid concentrations. In this respect the least concentrations were selected considering the ratio of molecularity postulated theoretically for the reaction. The least concentration taken for sulfuric acid was at least eight times the maximum concentration of permanganate. Likewise the least concentration of vitamin C was at least five times the maximum concentration of permanganate. Upper concentration limit of 0.6 and 10.0 µmol/L and lower concentration limits of 0.3 and 5.0 µmol/L were considered for permanganate and sulfuric acid respectively. Step values of 0.15 and 3.0 µmol/L were considered for both reagents respectively. All experiments were conducted by aspirating fixed concentration of 0.25 µmol/L of vitamin C. The results of the optimization is typically shown in Table 2 and graphically presented in Fig. 3 taking fixed amount of vitamin C as 0.25 µmol/L. The procedure was repeated several times taking different concentrations of vitamin C in the range 0.10 to 0.40 µmol/L.

Table 1: Full treatment combinations in both their original and coded levels along with the responses * The codes (–1), (0) and (+1) denote the

	$KMnO_4$	Vit C	H_2SO_4	KMnO / µm	Vit C// / µmol	H_2SO_4 // µmol	Peak height in mm se
1	-1	-1	-1	0.3	0.2	5	136
2	+1	-1	-1	0.6	0.2	5	80
3	-1	+1	-1	0.3	0.45	5	110
4	+1	+1	-1	0.6	0.45	5	164
5	-1	-1	+1	0.3	0.2	10	100
6	+1	-1	+1	0.6	0.2	10	156
7	-1	+1	+1	0.3	0.45	10	113
8	+1	+1	+1	0.6	0.45	10	178
9	0	-1	-1	0.45	0.2	5	136
10	0	+1	-1	0.45	0.45	5	160
11	0	-1	+1	0.45	0.2	10	124
12	0	+1	+1	0.45	0.45	10	153
13	-1	0	-1	0.3	0.325	5	127
14	+1	0	-1	0.6	0.325	5	164
15	-1	0	+1	0.3	0.325	10	134
16	+1	0	+1	0.6	0.325	10	171
17	-1	-1	0	0.3	0.2	7.5	114
18	+1	-1	0	0.6	0.2	7.5	137
19	-1	+1	0	0.3	0.45	7.5	152
20	+1	+1	0	0.6	0.45	7.5	165
21	0	0	+1	0.45	0.325	10	130
22	0	0	-1	0.45	0.325	5	142
23	+1	0	0	0.6	0.325	7.5	153
24	-1	0	0	0.3	0.325	7.5	144
25	0	+1	0	0.45	0.45	7.5	155
26	0	-1	0	0.45	0.2	7.5	122
27	0	0	0	0.45	0.325	7.5	136

All results were consistent and maximum difference in peak absorbance was always obtained when the molecularity of the permanganate and the sulfuric acid concentrations were 2 : 16 respectively. This ratio is not incorporating the vitamin C reactant as it is the limiting factor of the reaction. However, this ratio was found to be affected by the amount of vitamin C to the extent that absorbance value diminishes at higher vitamin concentration. Therefore all experiments were conducted with lower concentrations of vitamin C. A separate normal investigation on the reaction stoichiometry at fixed higher sulfuric acid showed that vitamin C reaction was always 2 : 5 with respect to permanganate.

This result is in quite agreement with the reported stoichiometry of oxidation reactions involving permanganate and exactly resembling the well-known permanganate-oxalate system[16-18]. Both vitamin C and oxalate are weak diprotic acids and their reactions are expected to be quite similar.

3.3 Analytical Appraisals

Different aliquots of vitamin C concentrations were prepared (in series of standard solutions) and analyzed by the method described above under the optimum operating conditions of 7.49 µmol/L sulfuric acid and 0.418 µmol/L $KMnO_4$.

Table 2 Super-simplex optimization progress

	$KMnO_4$/ µmolL^{-1}	H_2SO_4/ µmol L^{-1}	Peak height in mm
1	0.300	5.50	68.00
2	0.444	6.27	100.00
3	0.338	8.39	124.00
4	0.483	9.17	131.00 R
5	0.479	9.09	130.00
6	0.428	7.53	139.00 R
7	0.423	7.89	135.00
8	0.572	8.30	73.00 R
9	0.411	8.36	109.00
10	0.500	8.33	60.00 R
11	0.442	8.35	123.00
12	0.428	7.53	139.00 R
13	0.386	6.71	128.00
14	0.459	8.55	110.00 R
15	0.372	5.88	118.00
16	0.418	7.49	143.00 R
17	0.459	8.30	115.00
18	0.412	7.27	129.00 R
19	0.433	7.75	137.00
20	0.426	7.58	138.00

The calibration equation was deduced from the plot of the difference in the absorbance of potassium permanganate before (A_2) and after (A_1) adding the analyte i.e. ($A_2 - A_1$) versus standard vitamin C in different concentrations. The calibration plot was found to be linear over vitamin C concentration range between 100 to 350 ppm with a correlation coefficient of (0.996). The calibration equation obtained was as follows: $A_2 - A_1 = 0.05980 + 0.00169$ C Where (C) is the concentration of vitamin C in ppm

3.4 Application

The procedure detailed in section 2 was followed for the assay wed for the assay of vitamin C in proprietary forms as incorporated in Table 3. Vitamin C concentration was calculated by directly compensating into the calibration equation stated above. The method was found to be tolerant non-affected by the excipients added to proprietary drugs such as calcium, $CaCO_3$, citric acid, vitamin B_6, vitamin D, acetylsalicylic acid, vitamin E, folic acid, vitamin B_1, vitamin B_2, niacin amide, vitamin B_{12}, pantothenic acid, copper and zinc. Paracetamol showed positive interaction with the permanganate indicating an interference effects when present together with vitamin C. The same batch of proprietary drugs were also analysed following the BP method[2]. The results obtained by the present method and those obtained by the BP method were statistically compared. The student-t-test values calculated as shown in Table 3 indicate no significant difference in the results obtained by both methods confirming the accuracy and the validity of the proposed SIA method.

4. Conclusion

The adopted titration method for the assay of vitamin C is considered a successful application for the SIA in titrimetry. The method has the advantage of being automated, economical and faster than the conventional manual titration methods and others reported for this compound. The method has the advantage of being more sensitive than the cerium(IV) SIA titration method recently documented. The use of the permanganate as a powerful oxidant renders the present method more accurate than any other method employing other oxidants as the reaction is fast and reaches completion more easily. The simplex optimization procedure was successfully applied for obtaining quick actual operating conditions and at the same time postulating the stoichiometry of the reaction. The method proved to be applicable to the determination of vitamin C in pharmaceutical preparation in tablet forms.

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Table 3: Results obtained by the BP and the SIA methods

Drug	Supplier	Contents/mg	Mean recovery ± RSD(%)*		t**
			SIA method	BP method	
Aspirin C	Bayer	acetylsalicylic acid 400 vitamin C 240	100.4± 0.9	99.2 ± 1.2	0.5
Redoxon	F.Hoffmann-La Roche AS,Bale (Suisse)	ascorbic acid 1000 effervescent tablet	99.6 ± 1.3	99.3 ± 0.4	2.1
Cebion	Merck KgaA,Darmstadt, (Germany)	vitamin C 1000 effervescent tablet	100.7± 0.7	99.7 ± 0.8	0.4
Cal-C Vita	F.Hoffmann-La Roche AS,Bale (Suisse)	ascorbic acid 1000 calcium 250 $CaCO_3$ 625 citric acid 1350 vitamin B_6 15 vitamin D	99.3 ± 0.5	100.1 ± 0.6	0.6
UPSA-	UPSA, Malmaison(France)	vitamin C 1000 effervescent tablet	100.6 ± 0.2	100.2 ± 0.8	1.2
Sedergine	UPSA, Malmaison(France)	acetylsalicylic acid 330 vitamin C 200	99.5 ± 1.1	99.4 ± 0.5	1.4
Stresstabs	Lederly(USA)	vitamin E 45 IU vitamin C folic Acid 400mcg vitamin B_1 20 vitamin $B_2$10 niacinamide 100 vitamin B_6 10 vitamin B_{12} 25 pantothenic acid 25 copper 3 zinc 23.9	99.9 ± 0.8	99.6 ± 0.7	0.7

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