

Effects of Reagent Addition Sequence on the Analytical Response of the Mo(VI)-catalysed oxidation of Iodide by Hydrogen Peroxide

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A influência da ordem de adição e do processo de mistura sobre a resposta analítica (ΔA) do procedimento catalítico de determinação espectrofotométrica de Mo(VI), através da reação entre iodeto e peróxido de hidrogênio em meio ácido, foram estudadas usando o sistema de Análise em Fluxo Contínuo Monossegmentado, medindo-se a absorção do iodo em 350 nm, após um tempo de reação de 150 s. Os sinais da reação catalisada e não-catalisada foram registrados para todas as 24 permutações possíveis das linhas de reagentes. Três diferentes ordens de adição, R-I-H⁺-H₂O₂, R-I-H₂O₂-H⁺ e H⁺-H₂O₂-R-I (R= Mo(VI) ou água), mostraram maior sensibilidade e melhor reprodutibilidade. Um planejamento fatorial 2 para a ordem de adição R-I-H⁺-H₂O₂, indicou um aumento adicional de 17% na sensibilidade, por otimização das bobinas de reação. Esta configuração pode ser recomendada para trabalhos de rotina.

The extent to which the reagent confluence addition order as well as the mixing process, affects the analytical response (ΔA) of the catalytic procedure for spectrophotometric determination of Mo(VI) by oxidation of I⁻ by H₂O₂ in acidic medium, was followed by measuring the iodine absorption at 350 nm, using Monosegmented Continuous Flow Analysis, with a reaction time of 150 s. Signals were obtained for all 24 possible permutations of the reagent input lines. Three different addition orders, R-I-H⁺-H₂O₂, R-I-H₂O₂-H⁺ and H⁺-H₂O₂-R-I (R= Mo(VI) or water), have the highest sensitivities and best reproducibilities. A 2 factorial design for the addition order R-I-H⁺-H₂O₂ indicated an additional increase of about 17% in the sensitivity by optimizing the lengths of the mixing (reaction) coils. This configuration may be recommended for routine works.

Key words: Mo(VI)-catalysis; monosegmented continuous flow analysis.

Introduction

The mixing process in continuous flow injection analysis (FIA) can be accomplished in different ways, although sequential addition of confluent streams using connectors coupled to coiled reactors is the most common. Such a configuration is attractive due to its simplicity but the rates at which the microgradients are formed at the mixing points, as well as their structures, are not well established¹⁻³.

Various studies have sought to elucidate the practical and theoretical aspects of the mixing process in flow injection

methods, but only the physical aspects are usually covered. Since FIA is mainly used for fast reactions, kinetic aspects which could affect the chemical contribution to the overall dispersion coefficient, D_0 , are frequently relegated to secondary roles. However, the chemical contribution to D_0 cannot be ignored⁴. Thus, the mixing process used may seriously affect the chemical contribution to this coefficient, because sequential addition will introduce different concentrations at each confluence point, depending on the addition order. It is, therefore, of interest to acquire more experimental information about the effect of different sequential additions with

slow reaction systems, since, in principle, the analytical signal may be affected.

The oxidation of iodide by hydrogen peroxide in acidic medium^{5,6}, which in homogeneous solution follows the kinetic expression $d[I^-]/dt = (k_1^0 + k[H^+])[H_2O_2][I^-]$, is catalysed by certain elements in their higher oxidation states and is frequently used for the determination of molybdenum⁷⁻¹⁶. Although many isopolymolybdate species can be formed from molybdate ions in aqueous acid solution^{17,18}, the mechanism of the Mo(VI)-catalysed reaction apparently involves the formation of a molybdenum complex having a stoichiometric ratio of about 1:2 with hydrogen peroxide^{19,20}. Smith and Kilford²⁰ proposed a mechanism involving this molybdenum complex, consistent with their experimental rate determinations. According to their reaction mechanism, there is only one rate determining reaction step and variation of all possible binary combinations of simultaneous reagent additions was not found to affect the measured rates. They did not, however, determine the effect of sequential reagent addition order.

Such a study is a difficult task using conventional FIA systems due to the large contribution of physical dispersion to the overall dispersion coefficient when long mixing coils are used. However, it may be easily carried out using the Monosegmented Continuous Flow Analysis (MCFA) approach^{16,21-23}, which permits the simulation of manual reagent addition orders by sequential addition of reagents through confluence points, prior to the injection of the reactional bolus into the delay coil.

This paper reports results from a study of the behavior of the analytical signal as a function of reagent addition order using a set of 24 experiments employing the MCFA technique. All possible reagent addition sequences for the oxidation of I^- by H_2O_2 for both Mo(VI)-catalysed and non-catalysed reactions were tested. A two level factorial

design for one manifold geometry with the sequential addition order R-I-H⁺-H₂O₂ was then used to determine the influence of varying the lengths of the mixing coils on the signals, which affect the analytical response sensitivity when this procedure is used for Mo(VI) determination¹⁶

Experimental

All chemicals were of analytical grade and distilled, deionized water was used throughout. All solutions were stored in high-density polyethylene bottles.

The molybdenum standard stock solution (1000 µg/ml) was prepared by dissolving 1.5000 g of MoO₃ in 10 ml of 1+1 V/V ammonia solution and then diluting to 1 l with water. The 80 ng/ml working solutions were prepared daily by appropriate dilutions from this stock solution.

The hydrogen peroxide (4.0×10^{-3} mol/l) and the sulphuric acid (3.2×10^{-1} mol/l) solutions were also prepared daily by dilutions from concentrated solutions. The potassium iodide solution (3.0×10^{-2} mol/l) was prepared by dissolving 4.9800 g of KI in water and diluting to 1 L. The experimental setup and the reagent analytical concentrations were chosen to permit adequate readings for all possible variations in the addition sequence for both the Mo(VI)-catalysed and non-catalysed ("blank") reactions.

The experiments were carried out using the MCFA manifold shown in Fig. 1. Using this manifold, all reagents are sequentially mixed by confluence additions and 385 µl of the resulting reaction mixture is then inserted between two air bubbles upon its injection into the carrier line. The system is operated in a fixed-time mode, with a total reaction time of 150 s, measured from the first confluence point.

All solutions and the carrier were pumped using a Gilson Miniplus-2 peristaltic pump and Tygon pumping tubing. The

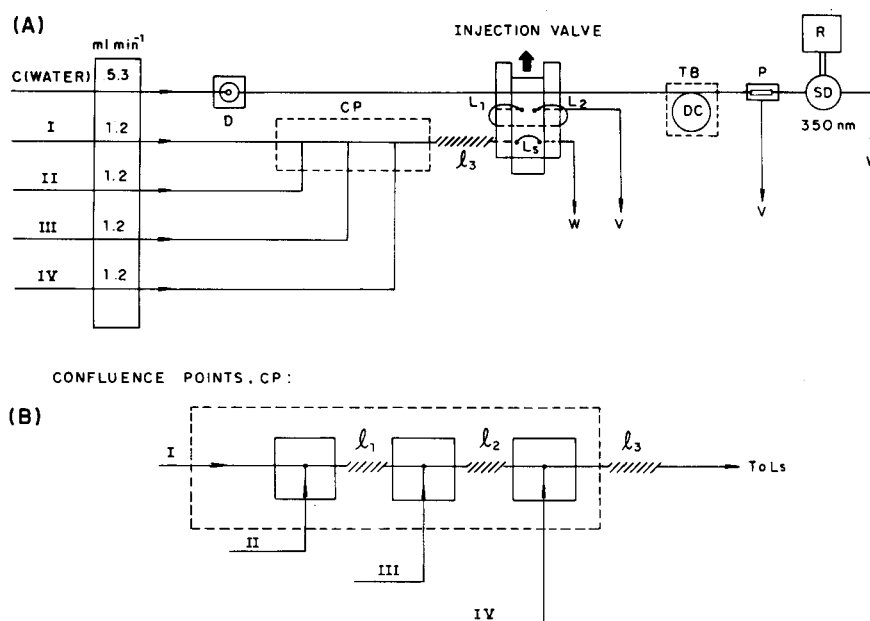


Figure 1. MCFA arrangement used to follow the oxidation of I^- by H_2O_2 . C, carrier (water); D, pulse damper, CP, confluence points; L_1 and L_2 , loops for introduction of air bubbles (200 µl); L_3 , injection loop (385 µl); TB, thermostatic bath; DC, delay coil; P, permeation cell; SD, spectrophotometric detector; R, recorder; l_1 , l_2 and l_3 , mixing (reaction) coils; V, water aspirator; W, waste. Flow-rates as indicated. I, II, III and IV are reagent input lines.

absorption measurements were made using a Zeiss PM2A spectrophotometer operated at 350 nm, with a 80 μ l quartz flow cell (optical path length 10 mm). Polyethylene tubing (i.d. 0.8 mm) was used for the loops, transmission lines and mixing coils. A 2.0 mm internal diameter PTFE tubing, supported on a 5 cm diameter cylinder, was used as the delay coil. The temperature of this coil was controlled at 25.0 ± 0.2 °C by a Robersshaw model 50TR constant temperature bath. The remaining parts of the MCFA system were at ambient temperature (22 ± 2 °C).

The injection valve, a laboratory-made acrylic proportional injector, was operated by means of two solenoid valves, controlled by an electronic sequence timer programmed to unload the sample loop (L_s) within 30 s, after a loading period of 20 s. After 150 s the delay coil is completely filled and a sampling frequency of 72 h^{-1} is obtained with negligible carry-over. Hence it is possible to carry out complete sets of experiments within the same working day. This permits a more reliable internal data comparison, as non-specific interferences are reduced to a minimum.

For the four reagents, R (Mo(VI) or water), I^- , H^+ and H_2O_2 , 24 addition orders are possible. Experiments with eight replicates were made for each addition order, first for the blanks (R = water) and then for the solutions containing molybdenum (R = Mo(VI)). These experiments used short reaction coils ($l_1 = l_2 = 3.5$ cm) between the confluence points and a longer coil ($l_3 = 26.5$ cm) and between the last confluence point and the injection loop, L (Fig. 1). Optimization studies with the addition order R-I-H⁺-H₂O₂ then varied the lengths of all three reaction coils.

Results and Discussion

An analysis of variance (ANOVA)²⁴ showed that the variations in the absorbance (calculated from the peak heights) for both the blank and molybdenum-catalysed mixtures, due to changes in the addition order, are, in general, larger than the variations in absorbance when the mixture order is not changed. F-test values of 32.8 and 38.9, well above the 99% significance level, were obtained for the blank and catalysed mixtures, respectively. Differences in absorbance values for the blank and molybdenum-catalysed reactions were also subjected to an analysis of the variance. The ΔA_i values, where $i = 1, 2, \dots, 8$, were obtained by subtracting the molybdenum-catalysed and non-catalysed absorbance values in a sequential order. Variations in the ΔA_i values due to changes in the addition order were also larger than the variations in these values when the order was not changed. The F-test value for the analysis of variance for the ΔA_i values was 7.4, also significant at the 99% confidence level. However, this value is lower than the F-test values for the individual absorbances, showing that the mixing order affects the absorbances of individual blanks and molybdenum-catalysed reactions more than it affect their differences, ΔA_1 .

However, as only the ΔA values are of analytical interest, our attention was initially focused on this parameter. In Table 1, averages ($\overline{\Delta A}$) and standard deviations (SD) of the differences in absorbances for the molybdenum-catalysed and blank reactions are shown for all possible reagent addition sequences. The R-I-H⁺-H₂O₂, R-I-H₂O₂-H⁺ and H⁺-H₂O₂-R-I addition sequences (runs n° 10, 12 and 15

Table 1. Averages ($\overline{\Delta A}$) and standard deviations (SD) of the differences in absorbance of Mo (VI)-catalysed and blank reactions, for the 24 different reagent addition sequences.

Run	Sequence of additions(a)				$\overline{\Delta A}^{(b)}$	SD ($\times 10^3$)
	I	II	III	IV		
1	I ⁻	H ⁺	H ₂ O ₂	R ^(c)	0.216	12.9
2	H ⁺	I ⁻	H ₂ O ₂	R	0.232	21.8
3	I ⁻	H ⁺	R	H ₂ O ₂	0.214	10.8
4	H ⁺	I ⁻	R	H ₂ O ₂	0.244	7.6
5	I ⁻	H ₂ O ₂	H ⁺	R	0.233	8.8
6	H ₂ O ₂	I ⁻	H ⁺	R	0.231	9.1
7	I ⁻	H ₂ O ₂	R	H ⁺	0.225	11.1
8	H ₂ O ₂	I ⁻	R	H ⁺	0.256	11.4
9	I ⁻	R	H ⁺	H ₂ O ₂	0.229	7.4
10	R	I ⁻	H ⁺	H ₂ O ₂	0.249	5.0
11	I ⁻	R	H ₂ O ₂	H ⁺	0.234	7.7
12	R	I ⁻	H ₂ O ₂	H ⁺	0.247	5.6
13	H ⁺	H ₂ O ₂	I ⁻	R	0.246	12.8
14	H ₂ O ₂	H ⁺	I ⁻	R	0.232	11.6
15	H ⁺	H ₂ O ₂	R	I ⁻	0.250	6.3
16	H ₂ O ₂	H ⁺	R	I ⁻	0.233	6.1
17	H ⁺	R	I ⁻	H ₂ O ₂	0.244	8.1
18	R	H ⁺	I ⁻	H ₂ O ₂	0.232	11.3
19	H ⁺	R	H ₂ O ₂	I ⁻	0.246	13.5
20	R	H ⁺	H ₂ O ₂	I ⁻	0.237	11.2
21	H ₂ O ₂	R	I ⁻	H ⁺	0.239	13.8
22	R	H ₂ O ₂	I ⁻	H ⁺	0.241	13.1
23	H ₂ O ₂	R	H ⁺	I ⁻	0.233	10.3
24	R	H ₂ O ₂	H ⁺	I ⁻	0.244	11.0

(a) I, II, III and IV refer to the relative positions of the reagent input lines at the confluence points (see Fig. 1).

(b) $\overline{\Delta A} = \Sigma \Delta A_i / 8$ ($i = 1, 2, \dots, 8$). The ΔA_i values were obtained by subtracting the molybdenum-catalysed and blank absorbances in a sequential order (see text).

(c) R is either a 80 ng ml⁻¹ Mo (VI) solution (molybdenum-catalysed reaction) or water (blank reaction).

respectively) provide the largest differences in absorbance (0.249, 0.247 and 0.250) and the smallest standard deviations of these differences (5.0×10^{-3} , 5.6×10^{-3} and 6.3×10^{-3}), thus corresponding to the addition sequences with the highest sensitivities and best reproducibilities.

The reagent addition order in these experiments was changed by moving the relative positions of the input lines (I, II, III and IV) at the confluence points (Fig. 1B). Thus the remarkable variations in absorbance observed for both the molybdenum-catalysed and blank reactions resulting in the variations in the average absorbance differences ($\overline{\Delta A}_1$, Table 1) are not simply a dilution problem since line interchange was performed at the confluence points. Hence, possible differences in flow-rates cannot be used to explain the changes in the ΔA values. However, as changes would not be expected on reversing the input lines at equivalent confluence points, the results are tentatively explained as being due to effects caused by inhomogeneous mixing processes at these points.

An optimization study was then performed, considering the effects of the lengths of the reaction coils (l_1 , l_2 and l_3 , in Fig. 1) on the analytical response and its standard deviation. For this optimization work the R-I-H⁺-H₂O₂ reagent addition

Table 2. 2^4 factorial design for optimizing coil lengths.

Variables				Levels	
				-	+
(1) Coil length 1, l_1 (cm)				26.5	51.5
(2) Coil length 2, l_2 (cm)				26.5	51.5
(3) Coil length 3, l_3 (cm)				26.5	51.5
(4) Mo (VI) concentration (ng ml ⁻¹)				0	80

(1)	(2)	(3)	(4)	Mean Absorbance ^(a)	SD ($\times 10^3$) ^(a)
-	-	-	-	0.380	2.0
+	-	-	-	0.397	11.6
-	+	-	-	0.404	9.1
+	+	-	-	0.403	7.2
-	-	+	-	0.387	4.7
+	-	+	-	0.411	5.9
-	+	+	-	0.389	4.3
+	+	+	-	0.399	4.4
-	-	-	+	0.637	13.1
+	-	-	+	0.630	5.7
-	+	-	+	0.686	6.9
+	+	-	+	0.664	12.3
-	-	+	+	0.652	2.2
+	-	+	+	0.687	7.0
-	+	+	+	0.656	7.8
+	+	+	+	0.653	21.8

(a) Average and standard deviation (SD) values for 8 replicate determinations.

Table 3. Main and interaction effects on the mean and standard deviation of the absorbance upon varying coil lengths and Mo (VI) concentration.

Variable	Mean Absorbance Effects	Standard Deviation Effects
Coil length 1, l_1	0.007 \pm 0.002	0.003 \pm 0.002
Coil length 2, l_2	0.009 \pm 0.002	0.003 \pm 0.002
Coil length 3, l_3	0.004 \pm 0.002	0.001 \pm 0.002
Mo (VI) concentration, Mo	0.262 \pm 0.002	0.004 \pm 0.002
$l_1 \times l_2$	-0.011 \pm 0.002	0.001 \pm 0.002
$l_1 \times l_3$	0.010 \pm 0.002	0.001 \pm 0.002
$l_1 \times \text{Mo}$	-0.006 \pm 0.002	0.001 \pm 0.002
$l_2 \times l_3$	-0.019 \pm 0.002	0.001 \pm 0.002
$l_2 \times \text{Mo}$	0.004 \pm 0.002	0.001 \pm 0.002
$l_3 \times \text{Mo}$	0.004 \pm 0.002	0.001 \pm 0.002
$l_1 \times l_2 \times l_3$	-0.002 \pm 0.002	0.001 \pm 0.002
$l_1 \times l_2 \times \text{Mo}$	-0.003 \pm 0.002	0.002 \pm 0.002
$l_1 \times l_3 \times \text{Mo}$	0.005 \pm 0.002	0.002 \pm 0.002
$l_2 \times l_3 \times \text{Mo}$	-0.009 \pm 0.002	0.002 \pm 0.002
$l_1 \times l_1 \times l_3 \times \text{Mo}$	-0.003 \pm 0.002	-0.001 \pm 0.002

order (run n° 10) was chosen. This order has a difference in absorbance values 15% higher and a standard deviation 2.5 times smaller than those obtained using the original addition order of Yatsimirskii^{7,8} (run n° 1). The 2^4 factorial design presented in Table 2 was carried out using the lengths of the three reaction coils and the Mo (VI) concentration as the variables. The main and interaction effects are presented in Table 3.

The large principal and interaction effects of the coil lengths on the mean values of the absorbances contrast with the almost negligible effects on the standard deviations of the replicate runs. These results indicate that coil lengths may be varied, resulting in larger peak heights, without sacrificing the small standard deviations obtained for the replicate experiments. All three first order effects of coil lengths on the absorbance are positive. However, large two-variable interaction effects are found for the three coil lengths. The $l_1 \times l_2$ and $l_2 \times l_3$ interactions are large and negative whereas the $l_1 \times l_3$ effect is large and positive. Thus, an increase in absorbance can be expected upon increasing the first and third coil lengths, while holding the second constant.

In other tests, increases of about 11% and 9% for the molybdenum-catalysed and non-catalysed absorbance values were observed when l_1 and l_3 were increased to 76.5 cm, while l_2 was kept constant at 3.5 cm. Since the molybdenum-catalysed absorbances are larger than the blank ones, these percentage changes result in about a 17% increase in the absorbance difference.

On the other hand, use of a mixing chamber^{16,23} in place of the optimized sequential addition manifold results in higher absorbance values for the molybdenum-catalysed (~13%) and blank (~20%) reactions, indicating a direct effect of the reaction mechanism on the individual signals. However, in spite of these variations in the individual signals, the absorbance difference, ΔA , changed only by 2%. Since ΔA is the response of analytical interest, either approach may be employed, even though mechanistic factors may be different. The use of an optimized sequential addition of confluent streams, as described in this paper, is an attractive approach for MCFA routine analysis.

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