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Short Communication

A flow injection method for the determination of oxalate in urine based on a promoting effect

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Introduction

The application of kinetic methods of analysis has been expanded by the use of the so-called 'modifying' effects. The rate of a given reaction can be diminished (inhibitory effect), or augmented (activating and promoting effects). Promoting effects can be exerted on catalysed and non-catalysed reactions because promoting agents can act both on catalyst and on indicator systems by accelerating the first reaction step, after which they undergo a deactivating effect through competitive reactions.

Promoting effects had not been used so far in flow injection analysis (FIA), although several FIA determinations of oxalate have been reported in response to the clinical interest of this analyte [1]. Thus, a method based on the formation of a highly fluorescent complex between zirconyl ion and flavonol in sulphuric acid [2] was developed by Gaetani *et al.* [3]. Fluorescence quenching by oxalate ions, which subtract zirconyl ions from the complex, was monitored. A more recent enzymatic method proposed by Almuaibed and Townshend [4] was based on the use of the catalyst (oxalate oxidase) on controlled pore glass and amperometric detection of the hydrogen peroxide formed. Neither method was applied to the determination of the analyte in real samples. Other, more sophisticated methods for this analyte also involve the use of oxalate oxidase in a potentiometric or voltammetric enzyme electrode [5] or disposable oxalate oxidase membrane strips [6], and the use of HPLC [7].

This paper reports a method for the determination of oxalate based on its promoting effect on the oxidation of ferroin by Cr(VI). This chemical system has been widely studied by conventional methods by Eswara and Mottola [8] and applied to the determination of oxalate in urine and serum by a kinetic method that requires previous precipitation and double extraction of the samples [9].

Experimental

Reagents

Stock solutions 0.01 M of K_2CrO_4, or 1 M H_2SO_4, of 5 g l^{-1} Cl_2Ca and of 2.5×10^{-2} M ferroin (this last was prepared from equimolar aqueous solutions of 1,10-phenanthroline and $Fe(N\text{-}H_4)_2(SO_4)_2$) were used. From these, more diluted solutions were prepared as required.

Instruments and apparatus

A Perkin–Elmer Lambda-1 spectrophotometer equipped with a Radiometer REC-80 recorder and a Hellma 178.12QS flow-cell of 18-μl inner volume was used. A Rheodyne 5041 injection valve, a Gilson Minipuls-2 peristaltic pump, a Tecator-II chemifold and a Selecta S-380 thermostatic bath were also used.

Sample pretreatment

The procedure reported by Archer *et al.* [10] was used. Thus, the pH of 5 ml of urine sample was adjusted to between 5.0 and 5.2 by adding 2 ml of $CaCl_2$ (5 g l^{-1}) and the samples were allowed to stand at room temperature for 12 h. The calcium oxalate precipitate was separated

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by centrifugation for 30 min. The supernatant fluid was decanted and the precipitate was washed with dilute ammonia. The washed precipitate was dissolved in 1 ml 1 N sulphuric acid and the solution was thus rendered ready for introduction into the loop of the FIA injection valve.

Procedure and manifold

Figure 1 shows the manifold used. The Cr(VI) and ferroin streams (flow-rate of 0.8 ml min^{-1}) were merged prior to the injection valve through which the sample was injected and then proceeded to the detector, where the transient signal was obtained. The height of the FIA peak was compared with data from the calibration curve run from standards manipulated in the same way as the sample.

Results and Discussion

Chemical reaction

Ferroin is a well-known redox indicator whose oxidation to ferroin can be made slow enough by adjusting the sulphuric acid concentration used.

Oxalic acid exerts an accelerating effect on the reaction rate of the oxidation of ferroin by Cr(VI) in a sulphuric medium. This is a transient effect appearing at the start of the reaction and then disappearing on decomposition and/or complexation with Cr(III). The reaction was monitored at 510 nm by measuring the decrease in the ferroin concentration.

The difference between the modified and non-modified reaction increased with the sulphuric acid concentration. Hydrochloric acid and Cr(VI) exert the same effect, but not phosphoric acid.

Preliminary studies

The aim of these studies was to evaluate the general effect of the chemical and FIA variables on the system. The following conclusions were arrived at:

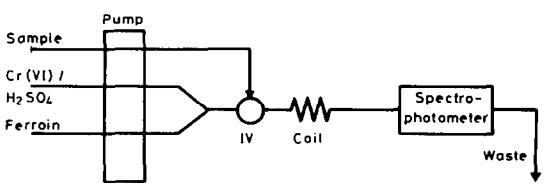

Figure 1
Manifold used for the determination of oxalate in urine. IV, injection valve.

(1) The oxidant must be used in a large excess over ferroin and on sulphuric acid;

(2) The sulphuric acid concentration must be close to 0.3 M. Long reactors must be used;

(3) The sample pH must be lower than the pK_2 of oxalic acid because the $HC_2O_4^-$ and $C_2O_4^{2-}$ have no promoting effect.

The influence of the reactor length on the height of the FIA peak, equivalent to that of the residence time of the reacting plug in the FIA system, was investigated. The height of the FIA peak increased markedly with the reactor length between 90 and 330 cm (residence times between 18 and 35 s). It remained constant between 320 and 500 cm, and then decreased at longer reactor lengths. The effect of the oxalate concentration at the start of the reaction was thus obvious. It increased with the oxalate concentration, and after 3 min, the reaction rate became almost constant. This is consistent with the results obtained in the extraction of the chemical system into tert-butyl phosphate and esterification with diazomethane prior to gas chromatography, which proved the absence of the analyte 4–5 min after mixing the reagents. The possibility of a complex being formed between the Cr(III) formed in the reaction and Cr(VI) which cannot be extracted into tert-butyl phosphate made the chromatographic results less convincing. The use of oxalic acid with ^{14}C showed that 10–15% of this was de-carboxylated during the reaction.

Optimization of variables

The simplex method [11] was used to optimize interdependent variables influencing the system (chemical and FIA variables), whereas independent variables were optimized by the univariate method.

The optimal conditions established by the simplex method applied to the chemical variables consistent with literature recommendations [7] were as follows: sulphuric acid concentration between 0.25 and 0.75 M and chromate concentration between 1×10^{-5} and 1×10^{-3} M; the optimal ferroin concentration was that providing an absorbance within the limits of minimal errors (0.2 and 0.8 absorbance units, AU), namely, between 1.4×10^{-4} and 2.5×10^{-4} M. The sulphuric acid concentration in the samples was 0.375 M and that providing a pH slightly lower than the pK of oxalic acid. The optimal values of these variables were obtained as the average of the best

Table 1
Optimum values of the variables

Method	Variable	Optimum value
Simplex	$\lvert CrO_4^{2-}\rvert$ (M)	1.4×10^{-3}
	$\lvert H_2SO_4\rvert$ (M)	0.53
	|Ferroin| (M)	3.3×10^{-4}
	Flow-rate (ml min^{-1})	0.8
	Reactor length (cm)	330
Univariate	Injected volume (μl)	130
	Temperature (°C)	20

points of the last simplex which satisfied the preset convergence criterion and are listed in Table 1.

The three-variable simplex used for the optimization of the FIA variables (flow-rate, injected volume and reactor length) was reduced to a two-variable simplex after checking that the analytical signal increased with the sample volume, so a compromise between sensitivity and sample consumption led us to select a volume of 130 μl. The optimal flow-rate and reactor length are also given in Table 1.

The temperature and the ionic strength were optimized by the univariate method.

A temperature of 25°C gave rise to the discolouration of ferroin even in the absence of Cr(VI); thus, the maximum difference between the blank and sample signals was obtained at 20°C.

The ionic strength, adjusted between 0.05 and 0.5 M by adding to the samples different amounts of KNO_3, showed no influence on the analytical signal.

Features of the method

We studied the linear range of the calibration curve, reproducibility, sampling rate and potential interferences from foreign species.

The calibration curve was run under the optimum conditions stated above. It was linear between 0.8 and 18.0 μg ml^{-1} of oxalate, its equation and regression coefficient being:

$$AU = 0.0118 + 0.0207\,[\text{oxalate}] \qquad r^2 = 0.994.$$

The precision of the method was studied on 11 samples of 10 μg ml^{-1}, injected in triplicate. The RSD was ±2.81%. The sampling frequency was 40 h^{-1}.

The study of interferences on the method was limited to species usually occurring in urine. As the concentration of these components depends on the volume of excreted urine, the values listed in Table 2 were established by considering an average volume between 600 and 1000 ml per day and the highest possible level in healthy individuals. As can be seen from Table 2, some species exerted a strong interference. Thus, a prior separation of the analyte from the sample matrix was required for the determination of oxalate.

Table 2
Study of interferences

Foreign species	Tolerated foreign species/analyte ratio
NO_3^-	550
Na(I)	400
K(I)	330
PO_4^{3-}	220
NH_4^+	210
HCO_3^-	100
SO_4^{2-}	70
Ca(II)	50
Mg(II)	35
Hystidine	120
Glycine	90
Creatinine	10
Cisteine	2

Application of the proposed method to urine samples

The method was applied to the determination of oxalate in urine by using the pretreatment and procedure described in the Experimental section, and the results found are listed in Table 3. The concentration of the analyte in eight samples was determined (second column in Table 3) and the recovery was calculated after adding 4 and 8 μg ml^{-1} of a standard solution of the analyte (5th column in Table 3). For samples 7 and 8, urine from children, the amounts of oxalate standard added for recovery were 5 and 10 μg ml^{-1}, respectively. The real concentration of analyte in each sample is also listed in Table 3; all the values are within the normal range for healthy individuals.

Conclusion

A simple, inexpensive and convenient method for the determination of oxalate in urine was developed. It provided a wide linear determination range and acceptable precision and recovery. Its sampling rate makes it suitable for small and medium-sized laboratories.

Table 3
Determination of oxalate in urine

Sample	Concentration* ($\mu g\ ml^{-1}$)	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	Recovery (%)
1	56.95		4.55	
		4.00	8.12	89.02
		8.00	11.79	90.40
2	45.94		3.67	
		4.00	7.35	91.77
		8.00	10.80	89.02
3	10.15		0.81	
		4.00	5.03	105.53
		8.00	10.03	115.10
4	53.74		4.30	
		4.00	7.68	84.43
		8.00	11.31	87.64
5	12.75		1.02	
		4.00	5.31	107.20
		8.00	9.25	102.80
6	44.12		3.53	
		4.00	7.72	104.70
		8.00	11.42	98.60
7	12.08		4.80	
		5.00	9.47	93.32
		10.00	13.66	88.60
8	7.27		2.90	
		5.00	7.17	85.21
		10.00	12.93	100.20

*Before dilution.

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