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Sequential Injection Kinetic Method for the Assay of Aspirin in Drug Formulations

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Abstract

For the first time the sequential injection analysis technique was fully utilized for quantitative kinetic determination of Aspirin and comprehensive reaction rate measurement using permanganate as an oxidant in sulphuric acid media. The absorbance was monitored by the color decrease of permanganate absorbed at the wavelength 525 nm. The reaction orders with respect to Aspirin, permanganate and hydrogen ions were found to be positive one (+1), positive one (+1), and inverse one (-1) respectively. The Reaction mechanism was postulated and a fixed time kinetic method for quantitative analysis of Aspirin was adopted. A full kinetic study was executed resulted in postulating the mechanism of oxidation of Aspirin by permanganate for the validation of a newly adopted kinetic method for quantitative determination of the drug in pharmaceutical preparations. The fixed time kinetic method was applied and the following calibration equation A = -0.09980 + 0.00617 C with r = 0.999 was obtained at a the fixed time exactly 90 s with a flow rate of 25 µl/s. The linearity of the method was found to be ranged between 5 to 400 ppm for the analyte when injecting 30 µl of it and keeping constant concentrations and volumes of 30 µl 2.29 x10^{-3} mol l^{-1} potassium permanganate, 30 µl 0.05 mol l^{-1} sulphuric acid and 20 µl as spacer solution (water). The above calibration equation was employed for quantitative determination of Aspirin in drug formulations in different proprietary tablets. The results obtained proved accurate and precise for the assay of Aspirin in drug formulations without suffering interferences from other active ingredients and any of excipients usually added in tablet formulations.

1.Introduction

Aspirin is a member of a family of chemicals called salicylates. It is chemically known as acetylsalicylic acid as in Fig. 1 (abbreviated in this manuscript as ASA) commonly prescribed for its analgesic, antipyretic and antirheumatic actions. Various methods for its determination have been employed by using different techniques including spectrophotometry[1-21], chromatography[22-30], electrochemistry[31-34], flow-techniques[35-45] and electrophoresis[46] . In the BP[47] monograph Aspirin is assayed by back-titrating the excess alkali added to saponify Aspirin to salicylic acid and acetic acid with a standard acid, a method which is unsuitable for determination of
Aspirin in micro amounts and it is also time consuming. In the USP[48] monograph, Aspirin is assayed by HPLC, a time-consuming method that requires lengthy column conditioning and long extraction procedures. The previous method published by main author[10] requires the use of high concentration of ammonium meta-vanadate in 0.8 M sulphuric acid and measuring the absorbance at λ_{max} 730 nm of the reduced form of vanadium(II).

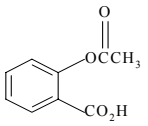

Fig. 1 Aspirin structure

2. Experimental

2.1 Apparatus

The Alitea USA/FIALab 3000 (Medina, WA USA) was used in this method. The apparatus (Fig. 2) consists of a syringe pump, a multi-position valve, a fiber optic spectrophotometer and a PC.

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.

The Multi-Position Valve has eight ports with a standard pressure of 250 psi (gas)/600 psi (liquid); zero dead volume; chemically inert; port selection is usually done using the software program.

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 Plexiglas 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 (200 cm long) and the reaction coil (100 cm Long).

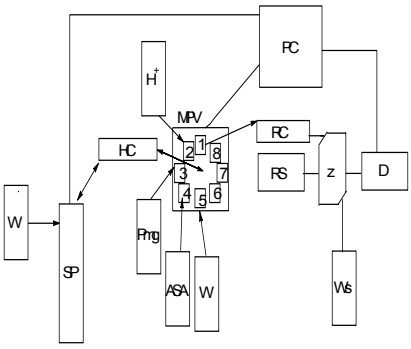

Fig. 2 SIA manifold diagram, W: water; SP: syringe pump; HC: holding coil; H^+: sulphuric acid; Pmg: potassium permanganate; ASA: acetyl salicylic acid; MPV: multi-position valve; 1-8: ports; PC: personal computer; RC: reaction coil; RS: radiation source; Z: Z-cell; D: detector; Ws: waste.

2.2 Software Packages

Alitea FIALab software was for programming, controlling the SIA system and it is also used for data acquisition.

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

2.3 Reagents and stock solutions

ASA standard solution (0.1 mol l^{-1}). A stock solution was always freshly prepared from Fluka AG (CH-9470 Buchs, Switzerland) previously dried at 50°C in vacuum oven over magnesium perchlorate. This standard solution was used to give the appropriate concentrations by dilutions.

Standard solution of $KMnO_4$ (0.02 mol l^{-1}). A standard solution was prepared by dissolving the appropriate weight 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.

Sulphuric acid Solution (1.0 mol l^{-1}). A stock solution of H_2SO_4 (95-98% w/v, Specific gravity 1.84 kg/l, Merck, UK) was prepared the usual way. Working solutions were prepared by dilutions.

3. Method and Procedure

In this method, FIALab software is utilized for controlling different SIA components as shown in Fig 2. For the sequential injection process and spectrometric measurements, 5 ml syringe pump (SP) were used to perform both aspirating and dispensing operations with the following steps:

I. All working solutions of sulphuric acid (H^+), potassium permanganate (Pmg), acetylsalicylic acid (ASA) and water (W) (for total volume adjustment and as a spacer solution for mixing process) were linked to the selector valve through ports 2, 3, 4 and 5, respectively. Tubes were loaded with their respective reagents by an aspiration run.

II. The syringe pump was filled with water as a carrier (W) by directing the two way valve to the (in-position) mode.

III. With a 150 µl/s flow rate, 500 µl were dispensed by directing the two way valve to the (out-position) mode in order to wash the holding coil (HC), the reaction coil (RC) and the "Z" photo cell (Z) and also to adjust the absorbance of the spectrophotometer to zero.

IV. With a 100 µl/s flow rate, 30 µl sulphuric acid, 30 µl potassium permanganate and 50 µl water, i.e. 30 µl equivalent to the reacted acetylsalicylic acid volume and 20 µl as a spacer solution, were sequentially aspirated into the holding coil.

V. Short reverse strokes were performed to allow all reagents to mix with a flow rate of 30 µl/s followed by continuous dispensing to the Z connected with the spectrometer, i.e. the radiation source (RS) and the detector (D), through the HC.

VI. The reference and absorbance were scanned by D and the maximum value of the monitored peak was recorded as absorbance-1 (A_1).

VII. Steps IV, V and VI were repeated with adding 30 µl ASA and reducing W to 20 µl; and absorbance-2 (A_2) was recorded after delaying the mixture in the RC for a required time to allow reaction to take place.

VIII. The absorbance equivalent to ASA reacted with Pmg was calculated as the difference between (A_1) and (A_2).

3. Results and discussion

The newly adopted SIA method was based on the oxidation reaction of ASA with potassium permanganate in sulphuric acid media. The reaction measured by monitoring the decrease of absorbance of permanganate has been found to be slower the higher the sulphuric acid concentration and accelerates at lower concentrations of the acid. Therefore, full kinetic study was found necessary to be conducted for in-depth understanding of the appropriate conditions for quantitative determination of this compound.

3.1 Reaction order with respect to [H^+]

Preliminary investigations proved that acid concentrations below 0.04 mol l^{-1} or higher than 0.09 mol l^{-1} will render the reaction inappropriate to take place, therefore, sulphuric acid concentration was adjusted in the range between 0.04 and 0.09 mol l^{-1} for the determination of the reaction order with respect to hydrogen ions. Below 0.04 mol l^{-1} the reaction was found to be too fast to follow and too slow to follow at higher than 0.09 $moll^{-1}$. Fig. 3 illustrates the absorbance-time curves at constant concentration of $1.55x10^{-3}$ mol l^{-1} potassium permanganate and of $1.14x10^{-3}$ mol l^{-1} ASA. The plot clearly indicates that the reaction takes place in two steps, one fast followed by a slow reaction step.

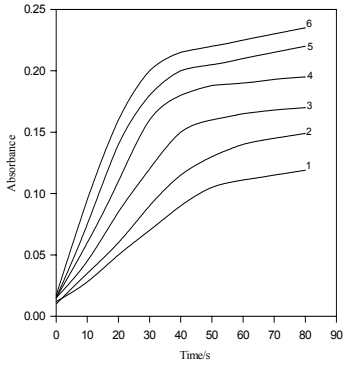

Fig. 3 Absorbance time-curves for the determination of the reaction order with respect to hydrogen ion: $[KMnO_4] = 1.55x10^{-3}$ mol l^{-1}; [ASA] = $1.14x10^{-3}$ mol l^{-1} and $[H_2SO_4]$ = 0.09 (1), 0.08 (2), 0.06 (3), 0.07 (4), 0.05 (5) and (6) 0.04 mol l^{-1}

Values for Log rate as $\Delta A/\Delta t$ versus Log$[H^+]$ were calculated from Fig. 3 and introduced in Table 1.

Table 1 Calculated rates of the reaction at 25°C for different sulphuric acid concentrations at constant concentrations of potassium permanganate ($1.55x10^{-3}$ mol l^{-1}) and ASA ($1.14x10^{-3}$ mol l^{-1}).

Exp. No.	H^+	Log$[H^+]$	Δt/s	$(\Delta A/\Delta t)\times 10^{-3}$	Log $\Delta A / \Delta t$
1	0.04	-1.396	2.04	4.90	-2.311
2	0.05	-1.301	2.31	4.32	-2.365
3	0.06	-1.220	3.02	3.31	-2.480
4	0.07	-0.155	3.51	2.69	-2.547
5	0.08	-1.095	3.98	2.51	-2.600
6	0.09	-1.044	4.47	2.24	-2.650

The following equation resulted from linear regression of Log rate as $\Delta A/\Delta t$ versus Log$[H^+]$

$$\textbf{Log}\ \text{rate} = -3.7027 - 1.0072\ \textbf{Log}\ [H^+] \quad r = 0.9941$$

The above linear equation clearly indicates that the reaction order with respect to hydrogen ion concentration is inverse one indicating that the reaction rate increases as acid decreases and that the hydrogen ion is one of the reaction products.

3.2 Reaction order with respect to ASA

Absorbance–time curves for determination of the reaction order with respect to acetylsalicylic acid were shown in Fig. 4. The concentration of sulphuric acid 0.09 and potassium permanganate $1.55x10^{-3}$ mol l^{-1} were kept constant while the concentration of ASA was varied between $1.34x10^{-3}$ to $2.10x10^{-3}$ mol l^{-1}.

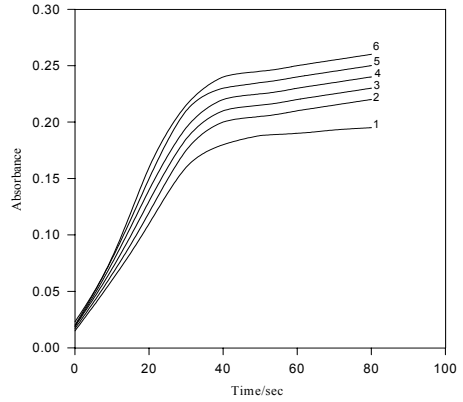

Fig. 4 Absorbance time-curves for the determination of the reaction order with respect to ASA. $[H_2SO_4]$= 0.09 mol l^{-1}; $[KMnO_4] = 1.55x10^{-3}$mol l^{-1} and [ASA]= $1.34x10^{-3}$ (1), $1.55x10^{-3}$ (2), $1.66x10^{-3}$ (3), $1.77x10^{-3}$ (4), $1.94x10^{-3}$ (5) and (6) $2.10x10^{-3}$ mol l^{-1}

Calculated values for Log rates versus Log ASA concentrations were deduced from Fig. 4 above and shown in Table 2 below:

Table 2 Data collected for the reaction rates at 25°C at fixed concentrations of sulphuric acid (0.09) and potassium permanganate ($1.55x10^{-3}$mol l^{-1}). Different concentration of ASA ranging between $1.34x10^{-3}$ and $2.10x10^{-3}$ mol l^{-1} were taken.

Exp. No.	ASA/mol l^{-1}	Log A	Δt/s	$\Delta A/\Delta t$	Log $\Delta A/\Delta t$
1	1.34×10^{-3}	-2.857	2.2	0.0045	-2.345
2	1.55×10^{-3}	2.808	2.1	0.0047	-2.326
3	1.66×10^{-3}	-2.778	1.8	0.0050	-2.301
4	1.77×10^{-3}	-2.752	1.7	0.0059	-2.229
5	1.94×10^{-3}	-2.715	1.5	0.0066	-2.180
6	2.10×10^{-3}	-2.675	1.4	0.0071	-2.147

Linear regression of Log rates versus Log ASA concentrations gave the following equation:

$$\textbf{Log}\ \text{rate} = 1.0937 + 1.2113\ \textbf{Log}\ [\text{ASA}] \quad r = 0.9688$$

From the slope of the equation above the reaction order with respect to ASA was calculated to be one, this positive result indicates that the reaction rate increases as ASA increases.

3.3 Reaction order with respect to potassium permanganate concentration

Fig. 5 shows the absorbance-time curves plotted at constant concentration of sulphuric acid 0.09 and ASA $1.66x10^{-3}$ mol l^{-1} while the concentration of potassium permanganate was varied between the range $1.01x10^{-3}$ and $1.51x10^{-3}$ mol l^{-1}

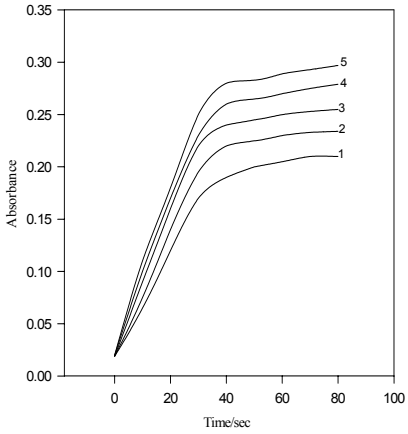

Fig. 5: Absorbance time-curves for the determination of the reaction order with respect to potassium permanganate [H_2SO_4] = 0.09; [ASA]= $1.66x10^{-3}$ mol l^{-1} and [$KMnO_4$] = $1.01x10^{-3}$ (1), $1.13x10^{-3}$ (2), $1.26x10^{-3}$ (3), $1.38x10^{-3}$ (4) and (5) $1.51x10^{-3}$ mol l^{-1}

Linear regression of Log rate versus Log permanganate concentrations (Table 3) reveals that the reaction order with respect to potassium permanganate was calculated to be one. This is clearly indicated by the value of the slope in the equation below:

Log rate = 0.7129 + 1.0318 **log** [MnO_4^-] r = 0.9986

Table 3 Calculated reaction rates at 25°C at different concentrations of potassium permanganate ranging between $1.01x10^{-3}$ to $1.51x10^{-3}$ mol l^{-1} and constant concentrations of sulphuric acid (0.09) and ASA ($1.66x10^{-3}$ mol l^{-1}).

Exp. No.	MnO_{44}/mol l^{-1}	Log A	Δt/s	ΔA/ Δt	Log ΔA/ Δt
1	1.01×10^{-3}	-2.995	2.4	0.00416	-2.380
2	1.13×10^{-3}	-2.944	2.1	0.00470	-2.322
3	1.26×10^{-3}	-2.898	1.9	0.00526	-2.278
4	1.38×10^{-3}	-2.857	1.7	0.00588	-2.230
5	1.51×10^{-3}	-2.819	1.6	0.00625	-2.200

3.4 Activation energy

The Arrhenius activation energy was computed from the Arrhenius equation below:

$$\text{Log } K' = \frac{-E_a}{2.303RT} + \text{Log } [A]$$

K' is the pseudo rate constant of the reaction
R is the gas constant (R = 8.314 J K^{-1} mol^{-1})
Ea is the activation energy.
T is the temperature in Kelvin
A is the pre-exponential factor.

Reaction rates were calculated from absorbance time curves when conducting an experiment using constant concentrations of sulphuric acid, permanganate and ASA as $1.51x10^{-3}$ mol l^{-1}, $1.51x10^{-3}$ mol l^{-1}, and 0.09 mol l^{-1} respectively while varying the temperature between 23 to 36 °C.

Log rates versus 1/T as shown in Table 4, reveals that the activation energy is calculated to be 43.3 K J mol^{-1}

Table 4 Calculated rate constant values for the reaction mixture containing [H_2S0_4] = 0.09 mol l^{-1} and [ASA] = $1.51x10^{-3}$ mol l^{-1}

T/°C	(1/T) $\times10^{-3}$	K' $\times10^{-3}$	Log K'
23	3.378	0.102	-0.991
27	3.333	0.140	-0.854
31	3.289	0.231	-0.636
36	3.236	0.316	-0.500

3.5 Suggested mechanism

The reverse order with respect to hydrogen concentration indicates that hydrogen ions are generated in the product side and this proves without doubt that the acetyl salycilate anion is the reactive species for permanganate reduction. Acetyl salicylic acid is considered as a week acid and hence one proton is released in the first step and immediately upon dissolution in slightly acidic solution as a primary reaction step as follows:

$ASAH \leftrightarrows ASA^- + H^+$ (1)

The acetyl salycilate anion reacts with permanganate in a slow rate determining step producing the acetyl salycilate anion radical and manganese(II):

$ASA^- + Mn^{7+} + 5e^- \leftrightarrows ASA^{\cdot-} + Mn^{2+}$ (2)

The manganese(II) quickly diamerises with manganese(VII) to produce manganese(III) which in turn reacts fastly with the acetyl salycilate radical to final products of manganese(II) and acetyl salycilate as in the following steps:

$ASA^{\cdot-} + Mn^{3+} + 2e^- \leftrightarrows ASA^{2-} + Mn^{2+}$ (3)

3.6 Analytical appraisals

Since the reaction is a slow one, the fixed time kinetic method would be appropriate for quantitative determination of Aspirin. Table 5 below shows the calibration plots obtained by regression of absorbance versus Aspirin concentration at different fixed times in the range between 50 to 150 s. From the values of the intercepts and the correlation coefficients, the calibration at the fixed time at 90 s was found to be the suitable time for quantitative determination of this compound.

Table 5 Calibration equations obtained at different fixed times measuring variable ASA concentrations ranging between 5 to 400 ppm at constant concentrations of potassium permanganate of ($2.29\ x10^{-3}$ mol l^{-1}) and sulphuric acid(0.05 mol l^{-1})

t (s)	Calibration equation	r^2
50	A = -0.05314 + 0.03173 C	0.98882
90	A = -0.09980 + 0.00617 C	0.99789
110	A = -0.24219 + 0.01830 C	0.99384
150	A = -0.42368 + 0.04266 C	0.99456

Table 6 Results obtained by the SIA kinetic method and the BP method[17]

Drug	Supplier	Content (mg)	Mean rrecovery ± RSD(%)*		t**
			SIA method	BP method	
Aspirine-C	Bayer, Germany	Aspirin 400 Vitamin C 240	100.3 ± 5.3	100.7 ± 0.3	1.2
Sedergine	UPSA, France	Aspirin 300 Vitamin C 200	99.8± 2.1	101.3± 1.9	1.8
Anacin	Whitehall Labs Corp., USA	Aspirin 400 Caffeine 32	102 ± 1.3	99.8 ± 3.0	2.3
Aspro	Nicholas, UK	Aspirin 320 Caffeine 32.4 Chlorpheniram-ine maleate 2.0 Phenylphrine base 10.0	102 ± 2.0	99.9 ± 2.7	1.7
Coricidin-D	Schering, USA	Aspirin 388.8 Caffeine 32.0	100.9 ± 1.4	102.2 ± 2.6	1.6

*relative standard deviation for 5 replicates
** student t-test values

3.7 Application

The newly adopted fixed time kinetic method was applied to the determination of Aspirin in proprietary drugs locally available in dispensaries (Table 6). The results obtained were statistically compared with the results of analyses of the same batch of proprietary drugs employing the BP[47] official method. The results obtained proved that the newly adopted method is accurate and excipients usually added to pharmaceutical preparations has no effect on the method. Other active ingredients such as Vitamin C, Caffeine and Chlorpheniramine maleate and phenylphrine base have no effect on Aspirin determination.

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