JOURNAL OF PHYSICAL ORGANIC CHEMISTRY, VOL. 8, 175–185 (1995)

KINETICS AND MECHANISM OF REACTION OF ACRIDINE ORANGE WITH BROMATE ION AT LOW pH

P. SAWUNYAMA
Department of Chemistry, University of Zimbabwe, BOX MP 167, Mt. Pleasant, Harare, Zimbabwe
AND
S. B. JONNALAGADDA*
Department of Chemistry, University of Durban-Westville, Private Bag X 54001, Durban 4000, South Africa

The reaction between acridine orange hydrochloride [3,6-bis(dimethylamino)acridine hydrochloride] and potassium bromate in dilute sulphuric acid was studied by monitoring the absorbance change at 492 nm. The reaction exhibited complex kinetic behaviour. In excess bromate the reaction had an induction period with slow depletion of acridine orange (AC) followed by a fast depletion step. The initial reaction step was found to be first order with respect to both AC and bromate and second order with respect to H^+. The stoichiometric ratio of AC to bromate was 3 : 2. At the end of the induction period, the redox potential of the reaction mixture has shown a distinct rise, while the Br^- concentration dropped sharply. The dual role of bromide ion both as an inhibitor and autocatalyst is discussed. A 16-step reaction mechanism is proposed and the simulated curves agreed well with the kinetic curves.

INTRODUCTION

The exhibition of oscillatory behaviour in closed chemical systems has generated considerable curiosity and interest among chemists and biochemists, as evidenced by numerous publications in the last few decades. A number of chemical reactions are now known to exhibit non-linear temporal behaviour in both closed and open systems.[1,2] More often than not, reactions involving bromate in acidic media involve complex mechanisms, in which various oxy-bromo intermediates are generated in the system. In the past two decades, considerable work has been done on the elucidation of mechanisms of reaction of certain organic substrates with bromate which exhibit linear and non-linear kinetics. As a result, most of the reactive species that are generated from bromate in acidic solution have been identified.[1–7] Apart from being of academic interest, the reactions of bromate with various organic and inorganic substrates in acidic media have been successfully used for the determination of transition metal ions at levels down to parts per billion.[8–11]

Acridine orange (AC) and related acridine dyes possess mutagenic activity, and hence the nature of their physical interaction with nucleic acids is of great interest. The chemistry of various acridine compounds has been reviewed by Acheson.[12] AC, which is a 3,6-bis(dimethyl)-substituted acridine, exhibits metachromatic behaviour, i.e. the change of the colour with a change in the colour of the viewing light, and slow fluorescence.[13] AC is extensively used for staining tissue sections in fluorescence analysis and as a microscopic staining agent in biological studies.[14]

A literature survey showed that no kinetic studies have been made on the reactions of AC with any oxidizing agents in aqueous solutions. However, there has been a report on the photo-oxidation of AC in heparin–AC complexes in nitrogen and oxygen saturated aqueous solution.[15]

AC is non-toxic and non-carcinogenic in nature. It is readily soluble in water and has a sharp absorption peak in the visible region with no shift due to $[H^+]$ variation. Its absorption coefficient at λ_{max} 492 nm at pH below 2 is found to be $\varepsilon = 3{\cdot}2 \times 10^4$ dm^3 mol^{-1} cm^{-1}. the reaction between AC and bromate is studied with two objectives, first to investigate its scope as a catalytic indicator reaction with selective response to cations and

---

* Author for correspondence.

CCC 0894–3230/95/030175–11

Received 12 September 1994
Revised 28 November 1994

second to elucidate its reaction mechanism by a kinetic approach. The reaction kinetics are found to be sensitive to the presence of vanadium(V) ion. Exploiting the catalytic efficiency of the vanadium ion on the title reaction, a kinetic–catalytic method for its determination has been reported.[16] In this paper, we report the results obtained from a kinetic study of the oxidation of acridine orange hydrochloride with bromate in acidic medium and propose a plausible mechanism for the reaction.

EXPERIMENTAL

Reagents. A stock standard solution of acridine orange (10^{-3} M) was freshly prepared by dissolving acridine orange hydrochloride hydrate (Aldrich, high-purity grade free from zinc) in distilled water. All other reagents were either of AnalaR grade or high-purity chemicals.

Kinetic measurements. All the experiments were carried out at 25 °C. The reaction was monitored spectrophotometrically on a Perkin-Elmer Lambda 2 UV–visible spectrophotometer interfaced with Digital DEC PC 433dxLP computer and PECSS software package for data storage and processing. The depletion kinetics of AC were monitored by following the change in absorbance at 492 nm. In selected experiments the absorbance was monitored at 390 nm to follow the production of bromine. The reaction between aqueous bromine and acridine orange hydrochloride was followed at 492 nm on a Hi-Tech Scientific SF-61AF stopped-flow spectrophotometer. The signal from the spectrophotometer was amplified and digitized on an Omega Engineering DAS-50/1 16-bit A/D board and interfaced to a Tandon 386SX computer for storage and analysis.

Emf measurements. The overall electrochemical potential of the reaction mixture was measured by employing an Orion Research digital pH/millivolt meter (Model 611) with a chart recorder and using a platinum electrode and saturated calomel electrode as reference. The bromide ion concentration was monitored using an ion-selective electrode (Fisher Scientific) coupled with a double-junction reference electrode (Orion Model 90-02).

Determination of $(BrO_3^- + HOBr)$ *concentrations.* The total oxidizing strength of the mixture was determined iodimetrically, by adding an excess of acidified iodide to an aliquot of the reaction mixture.[3] After incubation for about 30 min, the liberated iodine was titrated with standard sodium thiosulphate using starch as an indicator.

Stoichiometry and product analysis. The stoichiometry of the reaction was determined using a 1 : 1, 1 : 2 and 2 : 1 molar ratios of AC and bromate in the 0·01 molar range of reactants and with an excess of $[H^+]$. After about 14 h of reaction, the concentration of AC was determined by measuring the absorbance at 492 nm and residual bromate was estimated iodimetrically. A correction for blank reagent was made in the titre values. The stoichiometric ratio was found to be 3 : 2 for AC : BrO_3^-, i.e. an overall four-electron oxidation of acridine orange. With a 1 : 10 AC to bromate ratio a slightly higher stoichiometric ratio was observed. The higher consumption of bromate could be due either to further bromination of acridine derivatives or to the oxidation of the reaction product, formaldehyde, to formic acid and the oxidation of bromide to bromine.

For the product analysis, AC (1×10^{-3} M) and bromate (0·005 M) were mixed and after reaction overnight the mixture was neutralized by adding concentrated ammonia solution. The organic compounds were extracted into ethyl acetate. After drying, the solvent was removed under reduced pressure using a rotary evaporator. The yields of the products could not be established. The sample was analysed by mass spectrometry (Micromass 7035) and the major oxidation product of the reaction was observed to be a compound giving a parent peak at *m/z* (relative intensity, %) 267 (100), 238 (53), 237 (24), 44 (15), 28 (75) and is formulated as the *N*-demethylated-9-acridone derivative (P).[17] The mass spectral profile also indicated the formation of brominated acridones as other products. The formation of the acridone derivative is also supported by the IR spectra of the oxidized sample in Nujol (Shimadzu IR-460 IR spectrophotometer), showing a distinct carbonyl stretching at 6 μm.[18] One demethylated 9-acridone derivative (P) has been suggested as the reaction product of photo-oxidation of acridine orange by Menter *et al.*[15] Oxidation of 9-phenylacridine with perbenzoic acid or aqueous hydrogen peroxide give 10-oxides (*N*-oxides). Such acridine *N*-oxides and acridones have been reported to undergo rapid bromination at the 2- and 7-positions. Although the 9-position of the acridine ring system is usually susceptible to nucleophilic agents, it is known to undergo electrophilic attack.[19] Studies are in progress for the characterization of the products and their yields.

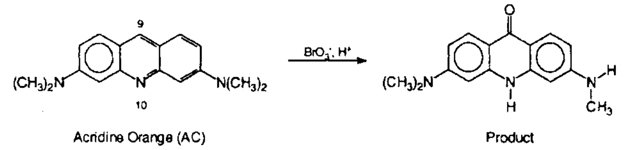
RESULTS AND DISCUSSION

The stoichiometric equation of the AC–bromate reaction is

$$3\,AC + 2\,BrO_3^- = 3\,P + 2\,Br^- + 3\,HCHO$$

With excess bromate, bromide is oxidized to bromine.

Preliminary experiments were conducted with excess initial concentrations of H^+ and bromate and low AC concentration to monitor the reaction profiles. The reaction between AC and bromate had an induction period during which the reductant was consumed



II

slowly. After the induction time, the reaction had a rapid depletion rate. The detailed kinetics of the reaction were investigated by monitoring the absorbance changes 20 s after the starting time at 492 nm. Studies were also conducted in a continuously stirred tank reactor (CSTR) to monitor the changes in redox potential and in the concentrations of bromide, acridine orange and bromine.

Effect of bromate

Figure 1 illustrates the profiles of typical absorbance versus time plots which show the effect of bromate concentration on the reaction dynamics. Considering the complexity of the reaction dynamics, the kinetic data were analysed with a three-pronged approach, i.e. by estimating the initial rates, pseudo-first-order rate constants and the induction times. The initial rates (r) were estimated taking the gradient of the data from 30 to 50 s in all the kinetic runs. The pseudo-first-order rate constants (k) were determined by an integration method with log (absorbance)–time data over a wide range and the slopes obtained had standard error coefficients of less than 1% and r^2 values not less than 0·09. Induction times (I_t) for each curve were estimated from the intersection point of the tangents drawn to enlarged segments of the sloping curves. Initial rate and pseudo-first-order rate constants were found to be directly proportional to [bromate], and both the linear graphs passed through the origin, confirming the first-order dependence of the reaction on bromate in the slow phase. The induction time was found to be inversely proportional to [bromate] (Figure 2), which suggests that the rate-limiting reaction of the autocatalytic step has a first-order dependence on [bromate]. Further, the x-intercept, when the induction period would approach infinity, indicated that for the chosen conditions of H^+ and AC, with initial [bromate] less than 0·0016 M, the kinetic curve should experience no rapid depletion. The kinetic curve with 0·001 M bromate had no rapid depletion of AC [Figure 1(a)].

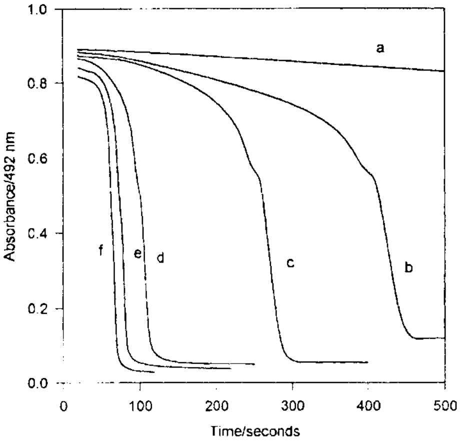


Figure 1. Effect of bromate on the AC bromate reaction: typical absorbance versus time plots at 492 nm. $[H^+]$ 2·0 M and [AC] 3×10^{-5} M. [Bromate]: (a) 0·001; (b) 0·002; (c) 0·0025; (d) 0·003; (e) 0·004; (f) 0·005 M

Effect of acid

The variation of hydrogen ion concentration had large effect on the reaction parameters. The kinetic curves shown in Figure 3 illustrate the effect of H^+ on the reaction in excess bromate. An increase in acid concentration significantly increased the r and k values, while reducing the induction period. Although the reaction was studied at a considerably high ionic strength, the effect of hydrogen ion concentration on the depletion kinetics of AC was studied at fixed ionic strength and at two different initial concentrations of bromate ion. The results obtained from two series of experiments are summarized in Table

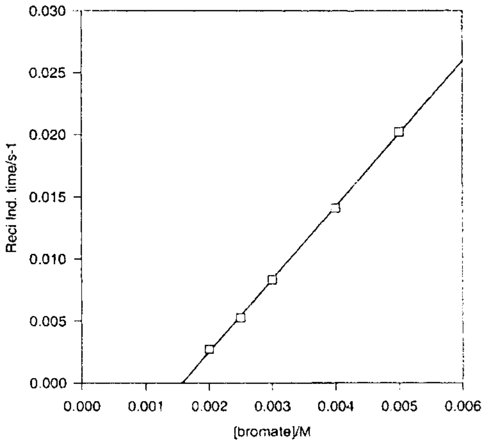


Figure 2. Reaction order with respect to bromate: reciprocal induction time versus [bromate] plot. $[H^+]$ 2·0 M and [AC] 3×10^{-5} M

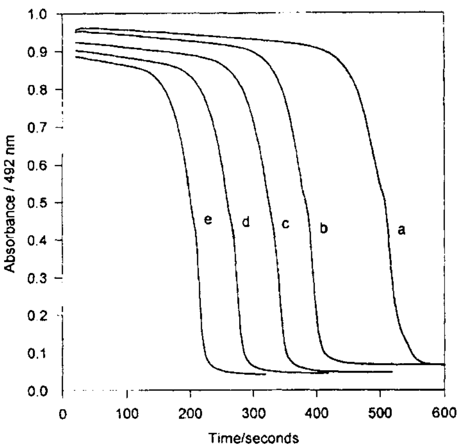


Figure 3. Effect of H^+ on the reaction. [Bromate] 0·005 M and [AC] 3×10^{-5} M. [H^+]: (a) 0·6; (b) 0·7; (c) 0·8; (d) 0·9; (e) 1·0 M

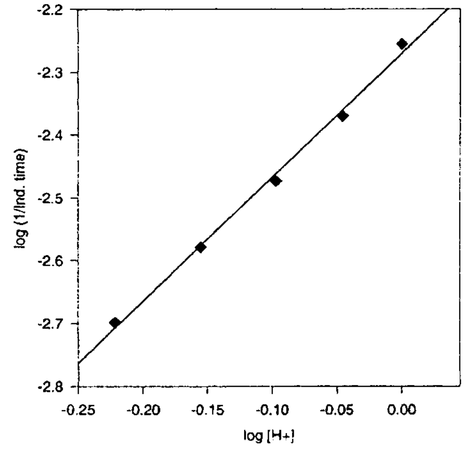


Figure 4. Dependence of reaction order on acid concentration: log (reciprocal induction period) versus log[H^+] plot. [Bromate] 0·005 M and [AC] 3×10^{-5} M

1. At constant ionic strength, over an [H^+] range of 0·6–2·0 M, the log (initial rate) versus log[H^+] plot gave a gradient of 1·98 (standard error coefficient 0·03). The log k versus log[H^+] plot was also linear with a slope of 2·01 (standard error coefficient 0·02), confirming the second-order dependence of the slow phase of the reaction on H^+ concentration. Within the limits of experimental error, the plot of log (reciprocal induction period) versus log[H^+] gave a linear graph with a gradient of 1·99 (standard error coefficient 0·06) (Figure 4). The gradient of 1·99 obtained suggests that the rate of the rapid reaction phase has a second-order dependence on the H^+ concentration.

Effect of ionic strength

The acid variation experiments at 0·005 M bromate and $3{\cdot}0 \times 10^{-5}$ M AC were also repeated with added neutral salt (sodium sulphate) to maintain the ionic strength. Under otherwise identical conditions, the kinetic runs with lower ionic strength (with no added salt) had relatively lower initial rates, lower k values and shorter induction times (data not presented). The

Table 1. Effect of hydrogen ion concentration at constant ionic strength on the initial rate (r), pseudo-first-order rate constant (k) and induction time (I_t)

Conditions	[H^+]/M	$r/10^{-8}$ M s^{-1}	$k/10^{-4}$ s^{-1}	I_t/s	k_6[c]/10^{-2} M^{-3} s^{-1}
A[a]	0·6	0·15	0·45	810	3·13
	0·8	0·25	0·82	440	3·20
	1·0	0·38	1·24	250	3·10
	1·2	0·55	1·88	180	3·26
	1·4	0·74	2·50	133	3·19
	1·6	1·03	3·15	108	3·08
	1·8	1·22	4·13	91	3·19
	2·0	1·63	5·13	71	3·21
					Mean: 3·25 ± 0·11
B[b]	0·6	1·63	0·57	499	3·14
	0·7	2·38	0·76	379	3·10
	0·8	3·03	1·02	297	3·19
	0·9	3·84	1·30	234	3·21
	1·0	4·66	1·60	180	3·20
					Mean: 3·13 ± 0·07

[a] [Bromate] 0·004 M and [AC] $3{\cdot}0 \times 10^{-5}$ M and constant ionic strength $\mu = 3{\cdot}004$.
[b] [Bromate] 0·005 M and [AC] $3{\cdot}0 \times 10^{-5}$ M and ionic strength $\mu = 3{\cdot}004$.
[c] $k_6 = k/[H^+]^2$[bromate].

positive salt effect for the initial phase of the reaction suggests that the rate-limiting step involves species of opposite charge, while the decrease in induction period with low ionic strength, i.e. the negative salt effect on the rapid phase of the reaction, indicates that the rate-controlling step in involves like-charged species.

Effect of bromide ion

Bromide ion had a profound effect on the profiles of the depletion of AC. Figure 5 illustrates selected kinetic curves in the absence and presence of added bromide ion under otherwise identical conditions of $[H^+]$ 1·0 M, [bromate] 0·005 M and [AC] $3{\cdot}0 \times 10^{-5}$ M. With the added bromide ion, the initial rates and k values of the slow phase of the reaction increased proportionally to $[Br^-]$. However, at low added bromide ($2{\cdot}0 \times 10^{-6}$–$2{\cdot}0 \times 10^{-5}$ M) the induction periods were prolonged and at high bromide ($>5{\cdot}0 \times 10^{-5}$ M) the induction times were reduced, illustrating the dual nature of bromide ion as an inhibitor and autocatalyst in the reaction, depending on its concentration. Similar observations with low and high concentrations of bromide ion were also reported previously during the oxidation of methyl orange with aqueous bromine[20] and in a number of reactions involving bromate as an oxidizing species, such as the oxidation of indigo carmine,[21] methylene blue[22] and *o*-dianisidine.[23]

Dependence of reaction dynamics on acridine orange

With excess concentrations of bromate and acid, AC followed first-order kinetics, but the variation of the initial concentration of AC over a fivefold range gave interesting insight into the reaction behaviour (Table 2 and Figure 6). The pseudo-first-order rate constants remained unaltered and the initial rate increased proportionally to [AC], with the intercept passing through the origin [Figure 7(a)]. The fairly constant k values and the increase in the initial rate proportional to [AC] confirm that the slow phase of the reaction is first order with respect to AC. However, the induction time increased with increasing concentration of acridine orange. A plot of reciprocal of induction time versus [AC] gave a good linear graph with negative slope ($R^2 = 0{\cdot}98$) [Figure 7(b)]. The increase in the induction time indicates that AC competes with bromide, the autocatalytic species, which is responsible for the rapid kinetics.

Reaction mechanism

The Belousov–Zhabotinskii-type temporal behaviour is exhibited by many chemical systems which involve bromate ion in strongly acidic medium. The chemistry of bromate in acidic solutions has been extensively studied and the important reaction steps involving the bromo and oxy-bromo species can be represented as follows:[4,24,25]

$$2\,H^+ + Br^- + BrO_3^- \rightleftharpoons HOBr + HBrO_2 \qquad (1)$$

$$H^+ + Br^- + HBrO_2 \rightleftharpoons 2\,HOBr \qquad (2)$$

$$2\,HBrO_2 \rightleftharpoons H^+ + HOBr + BrO_3^- \qquad (3)$$

$$H^+ + Br^- + HOBr \rightleftharpoons Br_2 + H_2O \qquad (4)$$

$$H^+ + BrO_3^- + HBrO_2 \rightleftharpoons 2\,BrO_2^{\bullet} + H_2O \qquad (5)$$

In closed systems such as continuously stirred or unstirred tank reactors, there exists an induction time for the build-up of reactive intermediates HOBr,

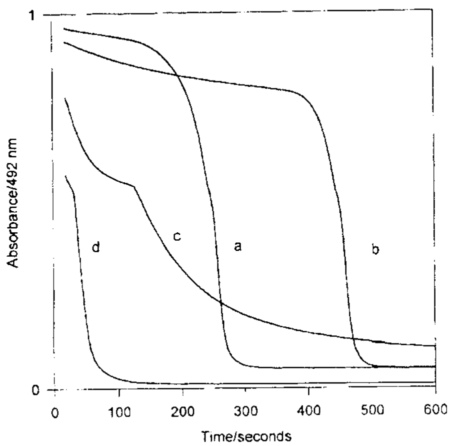


Figure 5. Kinetic curves in the absence and presence of added bromide. $[H^+]$ 1·0 M, [bromate] 0·005 M, [AC] $3{\cdot}0 \times 10^{-5}$ M and $[Br^-]$: (a) 0, (b) 5×10^{-6}, (c) 5×10^{-5} and (d) 1×10^{-4} M

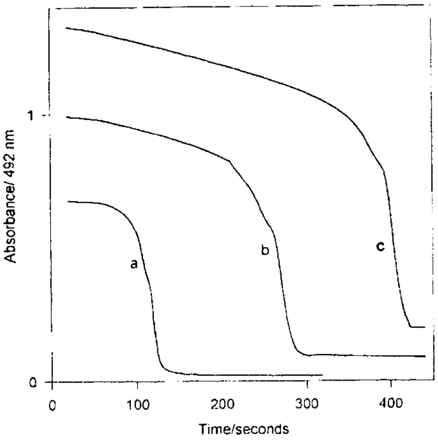


Figure 6. Effect of variation of acridine orange. $[H^+]$ 2·0 M and [bromate] 0·003 M. [AC]: (a) $2{\cdot}5 \times 10^{-5}$; (b) $4{\cdot}0 \times 10^{-5}$; (c) $5{\cdot}0 \times 10^{-5}$ M

$HBrO_2$ and Br_2. In most systems of Belousov–Zhabotinskii chemical oscillatory reactions involving different organic species, the basic feature is the role of bromide as the control intermediate which can switch between high or low concentration conditions. A switch from the high $[Br^-]$ to low $[Br^-]$ occurs when the concentration of bromide ion is driven below a critical value by reaction with $HBrO_2$ and bromate. However, in an oscillatory chemical system the reactive oxy-bromo species lead to the production of bromide which eventually switches the system back to high bromide concentration. The regeneration of bromide ion, the control intermediate and also the autocatalyst intimately depend upon the rates and nature of the reactions of the reducing substrate and its intermediates with reactive oxy-bromo and bromo species, which ultimately decide the linear or non-linear behaviour of the system.[26] When the bromide concentration is sufficiently high in the reaction system, reactions (1) and (2) are sufficiently rapid to increase the concentrations of HOBr. Further, $HBrO_2$ disproportionates to give bromate and HOBr through equation (3). The concentrations of HOBr, Br_2 and Br^- are related through equilibrium reaction (4). WHen the concentration of bromine is high and that of hypobromous acid is low, the associated bromide levels could inhibit the reaction of reducing organic substrate with bromate ion.[3]

Similarly, a high concentration of reducing substrate which competes with bromide for oxidizing species such as bromate also slows down the reaction of bromide with bromate and HOBr, thus delaying the regeneration of HOBr, leading to prolonged induction times prior to bromide-induced autocatalysed consumption of the reducing substrate by oxy-bromo and bromo species.

In the present studies, with no initial Br^- in the initial slow phase of the reaction, the first-order dependence of the reaction rate on the concentrations of AC and bromate and the second-order dependence on $[H^+]$ as reflected by the corresponding initial rates and k values suggest that the rate-limiting step involves one molecule of AC, one bromate ion and

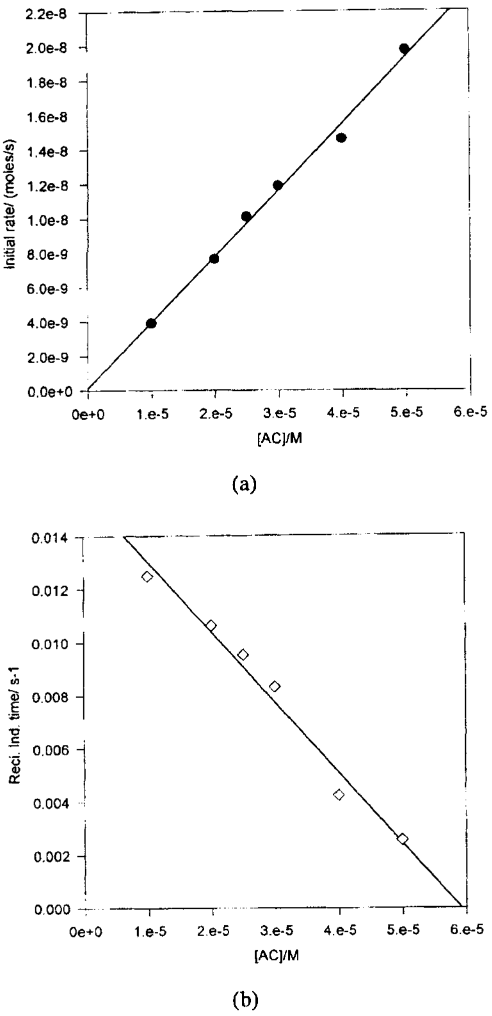


Figure 7. Effect of AC on the reaction kinetics: (a) initial rate versus [AC] plot; (b) reciprocal induction time versus [AC] plot. $[H^+]$ 2·0 M and [bromate] 0·003 M

Table 2. Effect of acid on the reaction between acridine orange and bromate with $[H^+]$ 2·0 M and [bromate] $4{\cdot}0 \times 10^{-3}$ M

$[AC]/10^{-5}$ M	$r/10^{-8}$ M s^{-1}	$k/10^{-4}$ s^{-1}	I_t/s	$k^6/10^{-2}$ M^{-3} s^{-1}
1·0	0·39	3·72	80	3·25
2·0	0·77	3·82	94	3·19
2·5	1·01	3·75	105	3·35
3·0	1·19	3·81	120	3·32
4·0	1·46	3·80	235	3·05
5·0	1·97	3·85	390	3·28
				Mean: 3·24 ± 0·10

two H^+ ions, i.e. AC acts as the reducing substrate. Considering the profile of the kinetic curve of AC, one can safely speculate that the rate-limiting step involves either the disruption of the conjugate double bond or the formation of an intermediate which absorbs at the same wavelength as the AC. Based on the literature[19] and a preliminary analysis of the oxidation products, the intermediate is possibly the 9-acridone derivative (I):

$$2\,H^+ + AC + BrO_3^- \xrightarrow{\text{slow}} IH^+ + HBrO_2 \qquad (6)$$

The reaction step (6) $\{k_6 = k/([\text{bromate}][H^+]^2)\}$ is the rate-controlling step in the slow phase of the reaction and the intermediate would be cationic owing to the low pH conditions. With no bromide present in the initial stages of the reaction, $HBrO_2$ will disproportionate rapidly [equation (3), $k_3 = 4{\cdot}0 \times 10^3\ \text{M}^{-1}\,\text{s}^{-1}$] to give HOBr, which would compete with bromate for AC in a fast reaction step to give IH^+ or through the reaction of AC with bromine generated through reaction (4). The other possibility one can speculate is the reaction of AC with $BrO_2^{\bullet}$ radical generated in equation (5), in sequence, and the attack of HOBr on IH^+, possibly resulting in demethylation. The reaction product remains protonated owing to the low pH condition.

$$AC + HOBr \rightarrow IH^+ + Br^- \qquad (7)$$

$$IH^+ + HOBr \rightarrow PH^+ + Br^- + HCHO + H^+ \qquad (8)$$

Bromine generated in the system could possibly attack the reducing substrate, AC and the intermediate in competitive reactions. Various possibilities exist and the steps could either be single electron abstractions or two-electron oxidation steps as shown in the following equations:

$$AC + Br_2 + H_2O \rightarrow IH^+ + 2\,Br^- + H^+ \qquad (9)$$

or

$$AC + Br_2 + H_2O \rightarrow ACOH + Br^- + H^+ + Br^{\bullet} \qquad (9a)$$

$$ACOH + Br^{\bullet} \rightarrow IH^+ + Br^- \qquad (9b)$$

$$IH^+ + Br_2 + H_2O \rightarrow PH^+ + HCHO + 2\,Br^- + H^+ \qquad (10)$$

It is reasonable to assume that equations (7)–(10), involving reactions of AC and other intermediates with the highly reactive oxidative intermediates, hypobromous acid and bromine would be faster than step (6) involving bromate ion. Other equations to consider are the reactions of $BrO_2^{\bullet}$ radical, which would react in sequence with the reductant yielding the intermediate (I), and also the direct reaction between bromate and the one-electron abstracted organic species.

$$AC + BrO_2^{\bullet} + H_2O \rightarrow ACOH + HBrO_2 \qquad (11)$$

$$ACOH + BrO_2^{\bullet} \rightarrow I + HBrO_2 \qquad (12)$$

$$ACOH + BrO_3^- + H^+ \rightarrow I + BrO_2^{\bullet} + H_2O \qquad (13)$$

$$I + H^+ \rightarrow IH^+ \qquad (14)$$

Acridones are also known to be brominated in the presence of bromine,[19] so the further possible reaction products include the mono- and dibrominated (at the 2- and 7-positions) acridone derivatives:

$$IH^+ + Br_2 \rightarrow IHBr^+ + Br^- + H^+ \qquad (15)$$

$$IHBr^+ + Br_2 \rightarrow IHBr_2^+ + Br^- + H^+ \qquad (16)$$

Experiments were conducted monitoring the absorbance changes at 492 and 390 nm corresponding to the absorption maxima of AC and Br_2, respectively (Figure 8) in different runs under otherwise similar conditions. A perusal of the kinetic curves clearly indicates that there is a slow build-up of bromine in the slow phase and bromine forms rapidly in the later stages, synchronizing with the induction time, i.e. the fast depletion of AC.

Hence the reaction between bromine and AC was

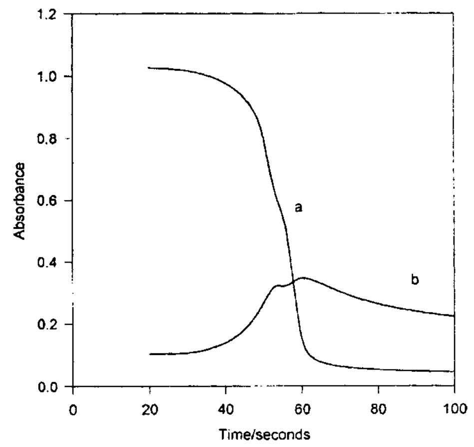
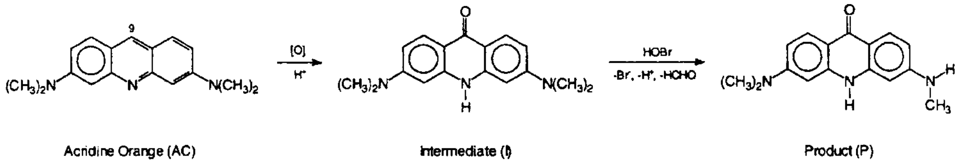


Figure 8. Kinetic curves: absorbance changes at (a) 492 nm (AC) and (b) 390 nm (Br_2). $[H^+]$ 2·0 M, [bromate] 0·005 M and [AC] $4{\cdot}0 \times 10^{-5}$ M



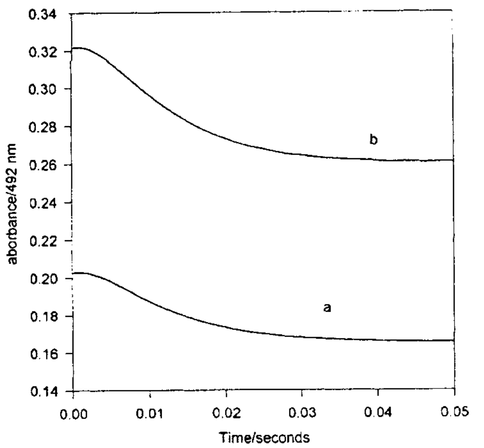


Figure 9. Reaction between aqueous bromine and AC. $[Br_2]$ $3{\cdot}8 \times 10^{-3}$ M. [AC]: (a) 2×10^{-5} and (b) 3×10^{-5} M

studied under neutral conditions, using excess aqueous bromine and monitoring the AC depletion kinetics and employing the stopped-flow technique. Figure 9 illustrates the typical kinetic curves with excess of bromine ($3{\cdot}8 \times 10^{-3}$ M) and at different initial concentration of AC (2×10^{-5} and 3×10^{-5} M). The kinetic curves were analysed from 0·005 to 0·02 s and were observed to follow pseudo-first-order kinetics. The rate coefficients had less than a 0·1% standard error and the mean value of the pseudo-first-order rate constant was 78 ± 2 s^{-1} [Figure 9(b)]. The pseudo-first-order rate constant increased proportionally with the increase in initial aqueous bromine concentration, indicating that the reaction is first order with respect to AC and bromine. From the experiments, we estimated the rate constant for the reaction under neutral conditions to be 2×10^4 M^{-1} s^{-1}. In acidic solutions the reactions were observed to be much faster and could not be monitored by the stopped-flow technique. Attempts to study the reaction with an excess of AC and monitoring the bromine concentration at 390 nm were not fruitful owing to the formation of Br_3^- which absorbs at the same wavelength as bromine but with a higher absorption coefficient. These kinetic runs confirm the role of bromine as the active oxidizing species in the reaction between AC and bromate.

Selected experiments were repeated in CSTR systems by simultaneously monitoring the absorbance at 492 nm and the reaction potential using platinum and reference calomel electrodes [Figure 10(a) and (b)]. The stirred systems had shorter induction times than the unstirred systems, which is obviously a result of agitation of the solution. With $[H^+]$ 2·0 M, [bromate] $2{\cdot}5 \times 10^{-3}$ M and [AC] $2{\cdot}0 \times 10^{-5}$ M, the stirred system had $I_t = 155 \pm 10$ s, compared with 190 s in the absence of stirring. The redox potential increased continuously with the progress of the reaction and a sudden increase in emf was observed synchronizing with the fast consumption of the AC, indicating the formation of bromine and oxidation of the residual bromide.

In a separate experiment, under identical conditions, the change in the concentration of Br^- was also monitored using an ion-selective electrode [Figure 10(c)]. The results show that bromide experiences an initial drop in concentration, followed by a slow increase, reaching a maximum. It then depletes rapidly, synchronizing with the rapid depletion of AC. The continuous increase in redox potential in the initial stages could be due to the increasing levels of Br_2 and or HOBr. The redox potential of $BrO_3^-/HOBr$ is $-1{\cdot}58$ V.[27] The boost in the emf matching the rapid depletion of AC is obviously due to the formation of bromine, which is also observed experimentally.[28] The suggested mechanism is also supported by evidence from photo-oxidation studies of AC, in which the cationic form of AC undergoes bimolecular electron transfer, unlike the neutral AC, which is readily demethylated.[15]

Rate expression

The rate of consumption of AC can be represented by the following equation:

$$\text{rate} = (-k_6[H^+]^2[BrO_3^-] - k_8[Br_2])[AC]$$

Under initial reaction conditions, with $[Br_2] = [HOBr] = 0$, the above equation reduces to

$$\text{rate} = -k_6[H^+]^2[BrO_3^-][AC]$$

When the concentration of acid and bromate are in excess, then the equation reduces to

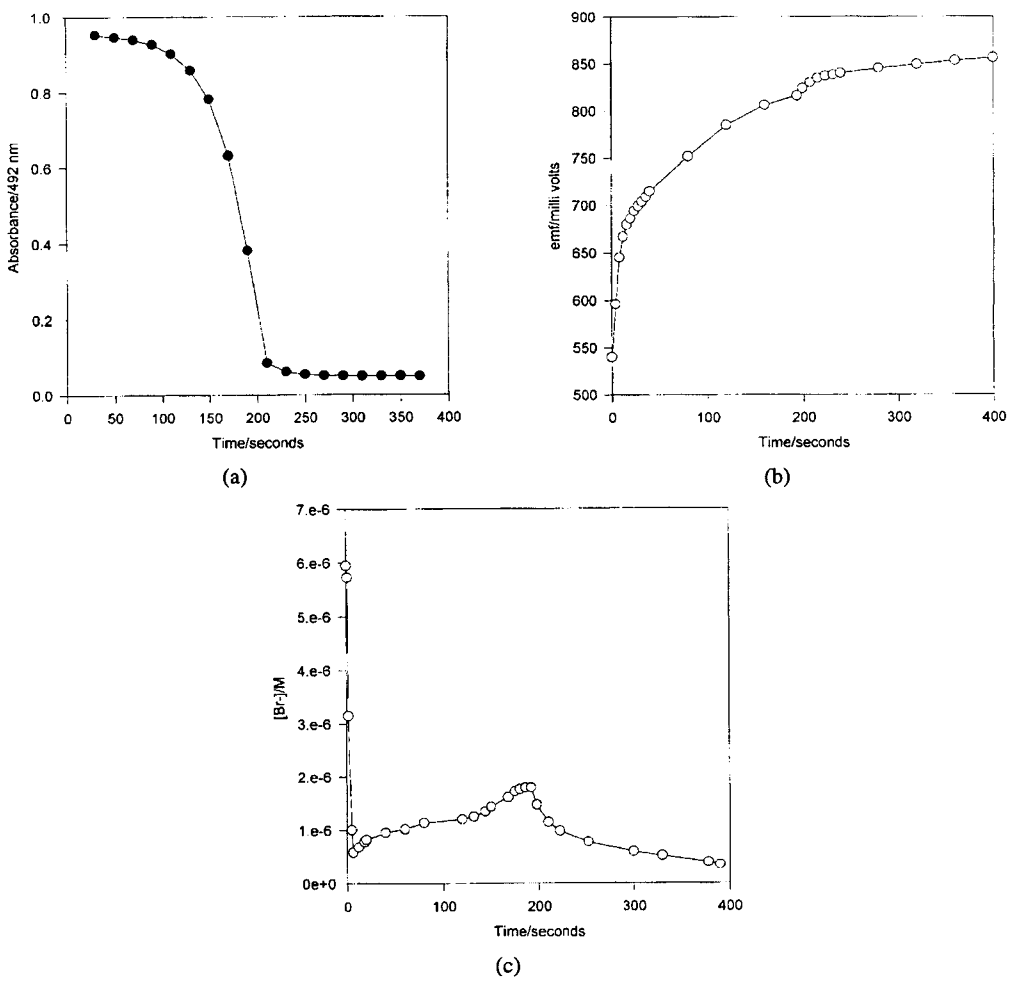
$$\text{rate} = -k[AC]$$

where the pseudo-first-order rate constant $k = k_6[H^+]^2[BrO_3^-]$.

The above rate expression agrees well with the reaction orders estimated using both initial rate and pseudo-first-order rate constants. With the progress of the reaction and with increase in the concentrations of IH^+ and $HBrO_2$, reactive intermediates, HOBr and Br_2 slowly build up. When the bromide ion concentration increases beyond a certain limit, the concentrations of bromine and possibly HOBr increase exponentially, resulting in rapid depletion of AC, which in turn generates more bromide through equations (9) and (10).

Computer simulations

Computer simulations using the proposed mechanism were done using 16 equations representing the reaction steps (1)–(16). The semi-implicate Runge–Kutta method in PASCAL language (TURBO PASCAL 7.0 version), which solves autonomous ordinary differential equation systems, devised by Kaps and Rentdrop[29] was implemented. Tyson–Field–Foersterling's set of data[25] were used for the 10 rate coefficients of equations



(a)

(b)

(c)

Figure 10. Continuously stirred tank reactor (CSTR) system: (a) kinetic curve for depletion of AC (492 nm); (b) redox potential versus time (platinum and reference calomel electrode); (c) bromide concentration versus time plot. $[H^+]$ 2·0 M, [bromate] $2{\cdot}5 \times 10^{-3}$ M and [AC] 2×10^{-5} M

(1)–(5). The rate constants evaluated in the present studies was used for equations (6) (k_6) and (9) (k_9). Reactions (6)–(16) were made essentially irreversible. Estimated values were used for equations (7)–(8) and (10)–(16). Table 3 summarizes the equations and rate coefficients used in the simulations.

The simulations were found to be very sensitive to the values of k_6, k_7 and k_9. The values of k_8 and k_{10} were always kept greater than k_7 and k_9 respectively. After setting the experimental value for k_6, the values of k_7 and k_9 were varied. With low values of k_9, simulations showed an exponential decay and had no induction time for the reaction. The values of k_7 and k_9 were adjusted until the simulated curves had the same profiles and reaction duration as the experimental curves. The simulations were less sensitive to the values of k_{11}, k_{12} and k_{13}. The protonation of the intermediate, k_{14}, was made a very rapid reaction. The rate constants k_{15} and k_{16} influenced the reaction behaviour owing to their effect on the rate of regeneration of bromide ion. The profiles of the simulated curves were in good agreement with the experimental curves. Figure 11(a) shows the profiles of the experimental kinetic curve for AC and the simulated curves for AC, IH^+ and PH^+. The profiles of the simulated curves for bromine, hypobromus acid and bromide for the same conditions of H^+, bromate and acridine orange are shown in Figure 11(b). The effect of H^+ and bromate concentrations in the simulated curves were similar to the experimental results. The simulations were insensitive to the presence of

Table 3. Simulations: rate equations and constants

(1) $2\,H^+ + Br^- + BrO_3^- \rightleftharpoons HOBr + HBrO_2$
$k_1 = 2{\cdot}5\ \text{M}^{-3}\ s^{-1};\ k_{-1} = 3{\cdot}2\ \text{M}^{-1}\ s^{-1}$

(2) $H^+ + Br^- + HBrO_2 \rightleftharpoons 2\,HOBr$
$k_2 = 3{\cdot}0 \times 10^6\ \text{M}^{-2}\ s^{-1};\ k_{-2} = 2{\cdot}5 \times 10^{-5}\ \text{M}^{-1}\ s^{-1}$

(3) $2\,HBrO_2 \rightleftharpoons H^+ + HOBr + BrO_3^-$
$k_3 = 3{\cdot}0 \times 10^3\ \text{M}^{-1}\ s^{-1};\ k_{-3} = 1{\cdot}0 \times 10^{-8}\ \text{M}^{-2}\ s^{-1}$

(4) $H^+ + Br^- + HOBr \rightleftharpoons Br_2 + H_2O$
$k_4 = 8{\cdot}0 \times 10^9\ \text{M}^{-2}\ s^{-1};\ k_{-4} = 1{\cdot}1 \times 10^2\ s^{-1}$

(5) $H^+ + BrO_3^- + HBrO_2 \rightleftharpoons 2\,BrO_2^* + H_2O$
$k_5 = 4{\cdot}2 \times 10^1\ \text{M}^{-2}\ s^{-1};\ k_{-5} = 4{\cdot}2 \times 10^7\ \text{M}^{-1}\ s^{-1}$

(6) $2H^+ + AC + BrO_3^- \rightarrow IH^+ + HBrO_2$
$k_6 = 3{\cdot}2 \times 10^{-2}\ \text{M}^{-3}\ s^{-1}$

(7) $AC + HOBr \rightarrow IH^+ + Br^-$
$k_7 = 2{\cdot}0 \times 10^2\ \text{M}^{-1}\ s^{-1}$

(8) $IH^+ + HOBr \rightarrow PH^+ + Br^- + HCHO + H^+$
$k_8 = 2{\cdot}0 \times 10^3\ \text{M}^{-1}\ s^{-1}$

(9) $AC + Br_2 + H_2O \rightarrow IH^+ + 2\,Br^- + H^+$
$k_9 = 2{\cdot}0 \times 10^4\ \text{M}^{-1}\ s^{-1}$

(10) $IH^+ + Br_2 + H_2O \rightarrow PH^+ + HCHO + 2\,Br^- + H^+$
$k_{10} = 9{\cdot}0 \times 10^6\ \text{M}^{-1}\ s^{-1}$

(11) $AC + BrO_2^* + H_2O \rightarrow ACOH + HBrO_2$
$k_{11} = 1{\cdot}0 \times 10^4\ \text{M}^{-1}\ s^{-1}$

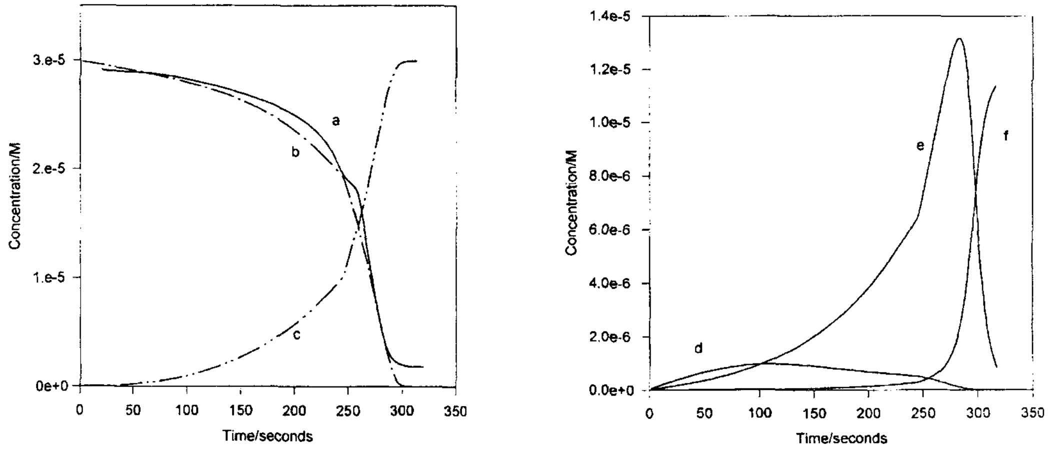
(12) $ACOH + BrO_2^* \rightarrow I + HBrO_2$
$k_{12} = 2{\cdot}0 \times 10^4\ \text{M}^{-1}\ s^{-1}$

(13) $ACOH + BrO_3^- + H^+ \rightarrow I + BrO_2^* + H_2O$
$k_{13} = 1{\cdot}0 \times 10^4\ \text{M}^{-2}\ s^{-1}$

(14) $I + H^+ \rightarrow IH^+$
$k_{14} = 1{\cdot}0 \times 10^9\ \text{M}^{-1}\ s^{-1}$

(15) $IH^+ + Br_2 \rightarrow IHBr^+ + Br^- + H^+$
$k_{15} = 1{\cdot}0 \times 10^1\ \text{M}^{-1}\ s^{-1}$

(16) $IHBr^+ + Br_2 \rightarrow IHBr_2 + Br^- + H^+$
$k_{16} = 1{\cdot}0 \times 10^2\ \text{M}^{-1}\ s^{-1}$



(a) (b)

Figure 11. Simulations: (a) curve a, experimental curve for AC; curve b, AC (simulation); curve c, PH^+ (simulation); (b) simulated concentration profiles of (curve d) hypobromus acid, (curve e) bromide (curve f) bromine. $[H^+]$ 2·0 M, [bromate] 0·004 M and [AC] 3×10^{-5} M

bromide at very low [Br^-], i.e. below 10^{-7} M. No inhibitory effect could be observed, but with [Br^-] $\geq 10^{-5}$ M an autocatalytic effect was observed as in the experimental kinetic curves.

The fair agreement between the simulated and experimental curves is a necessary condition to be satisfied for the plausibility of the proposed mechanism, although it may not be sufficient evidence that the proposed scheme is correct. Considering the large number of equations, to achieve good accuracy in the magnitudes of all the estimated coefficients is difficult and similar agreements between the experimental and simulated curves could also be obtained using the same mechanistic scheme and a different set of estimated rate constants. Therefore, although attempts were made to estimate the rate constants based on sound and plausible assumptions, there is still great scope for improvement in the mechanism and the simulated curves, either by further refining the values of the estimated rate coefficients or by including the reactions of $HBrO_2$ with organic species. However, the consistency in the characteristics of the experimental and simulated curves for acridine orange, bromine and bromide further support the proposed mechanism as a plausible scheme for the reaction between acridine orange and bromate ion at low pH.

ACKNOWLEDGEMENTS

The authors are grateful to Dr R. H. Simoyi, Department of Chemistry, West Virginia University, Morgonstown, WV, USA, for providing laboratory facilities for carrying out the kinetic and potentiometric measurements and Dr B. Mathiasch, Department of Analytical and Inorganic Chemistry, University of Mainz, Mainz, Germany, for the mass spectrometric analysis of the reaction samples.

REFERENCES

1. R. M. Noyes, R. J. Field and E. Koros, *J. Am. Chem. Soc.* **94**, 1394 (1972).
2. D. Edelson, R. J. Field and R. M. Noyes, *Int. J. Chem. Kinet.* 7, 417 (1975).
3. J. C. Sullivan and R. C. Thompson, *Inorg. Chem.* **18**, 2375 (1979).
4. P. Herbine and R. J. Field. *J. Phys. Chem.* **84**, 1330 (1980).
5. S. B. Jonnalagadda and K. Srinivasulu, *Z. Phys. Chem. (Leipzig)* **259**, 1191 (1978).
6. P. J. Ruoff, *Phys. Chem.* **88**, 2851(1984).
7. M. Orban, F. DeKepper and I. R. Epstein, *J. Am. Chem. Soc.* **104**, 2657 (1982).
8. S. B. Jonnalagadda, *Anal. Chem.* **55**, 2253 (1983).
9. A. Mattola, D. Perez-Bendito and H. B. Mark, Jr, *Anal. Chem. Fundam. Rev.* **60**, 181R (1988).
10. A. S. Cabeza, J. M. Eschriche and F. B. Reig, *Analyst* **109**, 1559 (1984).
11. T. Fukasawa, S. Kawakubo and T. Yamanouchi, *Anal. Chim. Acta* **177**, 325 (1985).
12. R. M. Acheson (Ed.), *Acridines*, 2nd ed. Interscience, New York (1973).
13. B. L. Van Dauren, *Chem. Rev.* **63**, 325 (1963).
14. J. P. Martin and N. Logsdon, *Photochem. Photobiol.* **46**, 45 (1987).
15. J. M. Menter, R. E. Hurst and S. S. West, *Photochem. Photobiol.* **29**, 473 (1979).
16. S. B. Jonnalagadda and P. Sawunyama, *Fresenius' J. Anal. Chem.* **340**, 173 (1991).
17. *Atlas of Spectral Data and Physical Constants for Organic Compounds*, 2nd ed. CRC Press, Cleveland, OH (1975).
18. C. J. Pouchest, *The Aldrich Library of Infrared Spectra*, 2nd ed. Aldrich Chemical, Milwaukee, WI (1973).
19. R. M. Acheson, B. Adcock, G. M. Glover and L. E. Sutton, *J. Chem. Soc.* 3367 (1960).
20. R. A. Hasty, E. J. Lima and J. M. Ottaway, *Analyst (London)* **106**, 76 (1981).
21. S. B. Jonnalagadda, R. H. Simoyi and G. K. Muthakia, *J. Chem. Soc., Perkin Trans. 2* 1111 (1988).
22. G. K. Muthakia and S. B. Jonnalagadda, *J. Phys. Chem.* **93**, 4751 (1989).
23. S. B. Jonnalagadda, N. M. Munkombwe, P. Hensman and T. Mushinga, *Int. J. Chem. Kinet.* **23**, 113 (1991).
24. L. Gyorgyi, M. Varga, E. Koros, R. J. Field and P. Ruoff, *J. Phys. Chem.* **93**, 2836 (1989).
25. K. Bar-Eli and R. J. Field, *J. Phys. Chem.* **94**, 3660 (1990).
26. Y. Zhang and R. J. Field, *J. Phys. Chem.* **94**, 7154 (1990).
27. S. B. Jonnalagadda, *Int. J. Chem. Kinet.* **16**, 1287 (1984).
28. C. R. Chinake, R. H. Simoyi and S. B. Jonnalagadda, *J. Phys. Chem.* **98**, 545 (1994).
29. P. Kaps and P. Rentdrop, *Numer. Math.* **33**, 55 (1979).