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Spectroscopic investigation and oxidation of the anticholinergic drug atropine sulfate monohydrate by hexacyanoferrate(III) in aqueous alkaline media: a mechanistic approach

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Abstract: The oxidation of the anticholinergic drug atropine sulfate monohydrate by hexacyanoferrate(III) in aqueous alkaline media was investigated spectrophotometrically by monitoring the decrease in absorbance of hexacyanoferrate(III) (HCF(III)). Oxidation products were identified. The oxidation mechanism was proposed from kinetic studies. The reaction constants involved in the different steps of the mechanism were calculated. The effects of added products, ionic strength, and dielectric constant of the reaction were investigated. The polymerization test revealed that oxidation occurred with intervention of free radicals. The activation parameters were evaluated.

Key words: Kinetics of oxidation, mechanism, hexacyanoferrate(III), atropine sulfate

1. Introduction

Hexacyanoferrate(III) has been widely used to oxidize numerous organic and inorganic compounds in alkaline media.[1,2] Many transition and nontransition metal ions in their complex form act as good oxidants in acidic, basic, or neutral media. However, oxidation capacity depends on their redox potential. It is also known that the redox potential of the couple is pH dependent. For instance, the redox potential[3] of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ in acid medium is +0.36 V and in basic medium is +0.40 V. This indicates that hexacyanoferrate(III) is a good oxidant in basic medium. It is a one-equivalent oxidant leading to its reduction to hexacyanoferrate(II), a stable product.[4] Oxidation by HCF(III) ion generally proceeds through an outer sphere electron transfer mechanism, which depends not only on the nature of the substrate but also on the medium of the reaction.[5]

Tropane alkaloid (atropine) is extracted from deadly nightshade (*Atropa belladonna*), jimsonweed (*Datura stramonium*), mandrake (*Mandragora officinarum*), and other plants of the family Solanaceaeare widely used as parasympatholytic, anticholinergic, and antiemetic drugs.[6] Atropine sulfate is (RS)-(1R,3r,5S)-3-tropoyloxytropanium sulfate monohydrate (Figure 1).[7]

Atropine sulfate, the monohydrate of (1R,3r,5S)-3-tropoyloxytropanium sulfate, can be used for suppressing unstriated muscle, controlling glandular excretion, in small doses stimulating the central nervous system, having the action of mydriasis in ophthalmology, etc.[8] However, most alkaloids have special and significant biological activity, and so caution is called for in establishing a sound method to detect atropine sulfate in a clinical assay.[9] Its degradation by microorganisms has been reported by several groups[10,11] and, in cases for

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which the mechanism has been studied, hydrolysis of the ester linkage to give the 2 separate cyclic components is the initial step.[12]

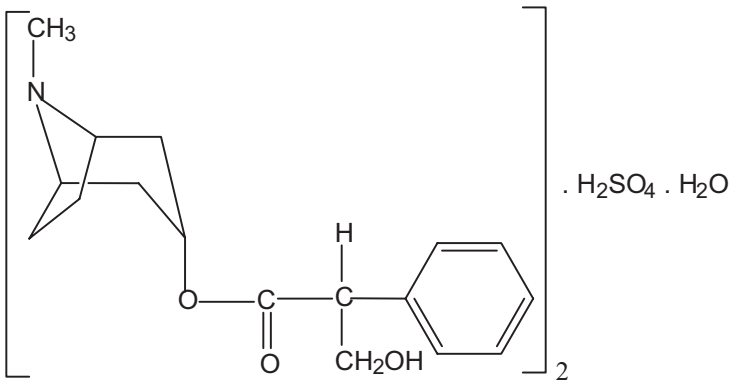


Figure 1. Chemical structure of Atropine sulfate monohydrate.

Although some work on oxidation of atropine sulfate monohydrate by various oxidants has been carried out,[12] there is a lack of literature on the oxidation of this drug by hexacyanoferrate(III). The objectives of the present study were to (i) accumulate the kinetic data, (ii) elucidate plausible mechanisms, (iii) design kinetic rate laws, (iv) ascertain the reactive species, (v) deduce thermodynamic parameters, and (vi) characterize the products.

2. Results and discussion

2.1. Reaction orders

The order with respect to [ASM] and [alkali] were found from the graph of log k_{obs} versus log(concentration) plots.

2.2. Effect of [hexacyanoferrate(III)]

The concentration of hexacyanoferrate(III) was varied in the range 0.50×10^{-4}–5.0×10^{-4} mol dm^{-3} at constant [ASM], [OH^-], ionic strength, and temperature. The fairly constant k_{obs} value (Table 1) indicates that order with respect to hexacyanoferrate(III) concentration was unity. This was also confirmed by the linearity of the plot of log(absorbance) versus time up to 80% completion of the reaction.

2.3. Effect of [atropine sulfate]

The concentration of ASM was varied in the range 5.0×10^{-4}–5.0×10^{-3} mol dm^{-3} at a constant [HCF(III)], [OH^-], ionic strength, and temperature. The rate of reaction increased with the increase in [atropine sulfate] (Table 1). A plot of log k_{obs} versus log [ASM] was linear and was found to be less than unity.

2.4. Effect of [alkali]

The concentration of OH^- was varied in the range 0.10–1.0 mol dm^{-3} at constant [HCF(III)], [ASM], ionic strength, and temperature. The rate of reaction increased with the increase in [alkali] (Table 1) and the order was found to be less than unity, i.e. 0.60.

Table 1. Effect of variation in hexacyanoferrate(III), atropine sulfate, and OH^- on the oxidation of atropine sulfate by hexacyanoferrate(III) at 25 °C and I = 1.0 mol dm^{-3}.

[HCF] × 10^4 (mol dm^{-3})	[ASM] × 10^3 (mol dm^{-3})	[OH^-] (mol dm^{-3})	k_{obs} × 10^3 (s^{-1})
0.50	2.0	0.5	1.09
1.0	2.0	0.5	1.03
2.0	2.0	0.5	1.05
3.0	2.0	0.5	1.07
5.0	2.0	0.5	1.08
2.0	0.50	0.5	0.31
2.0	1.0	0.5	0.65
2.0	2.0	0.5	1.05
2.0	3.0	0.5	1.56
2.0	5.0	0.5	2.08
2.0	2.0	0.10	0.45
2.0	2.0	0.30	0.81
2.0	2.0	0.50	1.05
2.0	2.0	0.70	1.48
2.0.	2.0	1.0	1.71

2.5. Effect of ionic strength (I) and dielectric constant (D)

The effect of ionic strength was varied by varying KNO_3 concentration between 0.60, 0.80, 1.0, 1.20, and 1.40 mol dm^{-3}. The rate was found to increase with increase in ionic strength. A plot of log k_{obs} versus $I^{1/2}$ was linear with positive slope. The effect of dielectric constant was studied by varying the t-butyl alcohol-water (v/v) percentage composition from 0% to 30%. It was found that as the percentage composition of t-butyl alcohol increased in the reaction medium, the rate of reaction decreased and the plot of log k_{obs} versus 1/D was linear with negative slope.

2.6. Effect of initially added products

The initially added products, hexacyanoferrate(II), tropine, and benzaldehyde, did not have any significant effect on the rate of reaction.

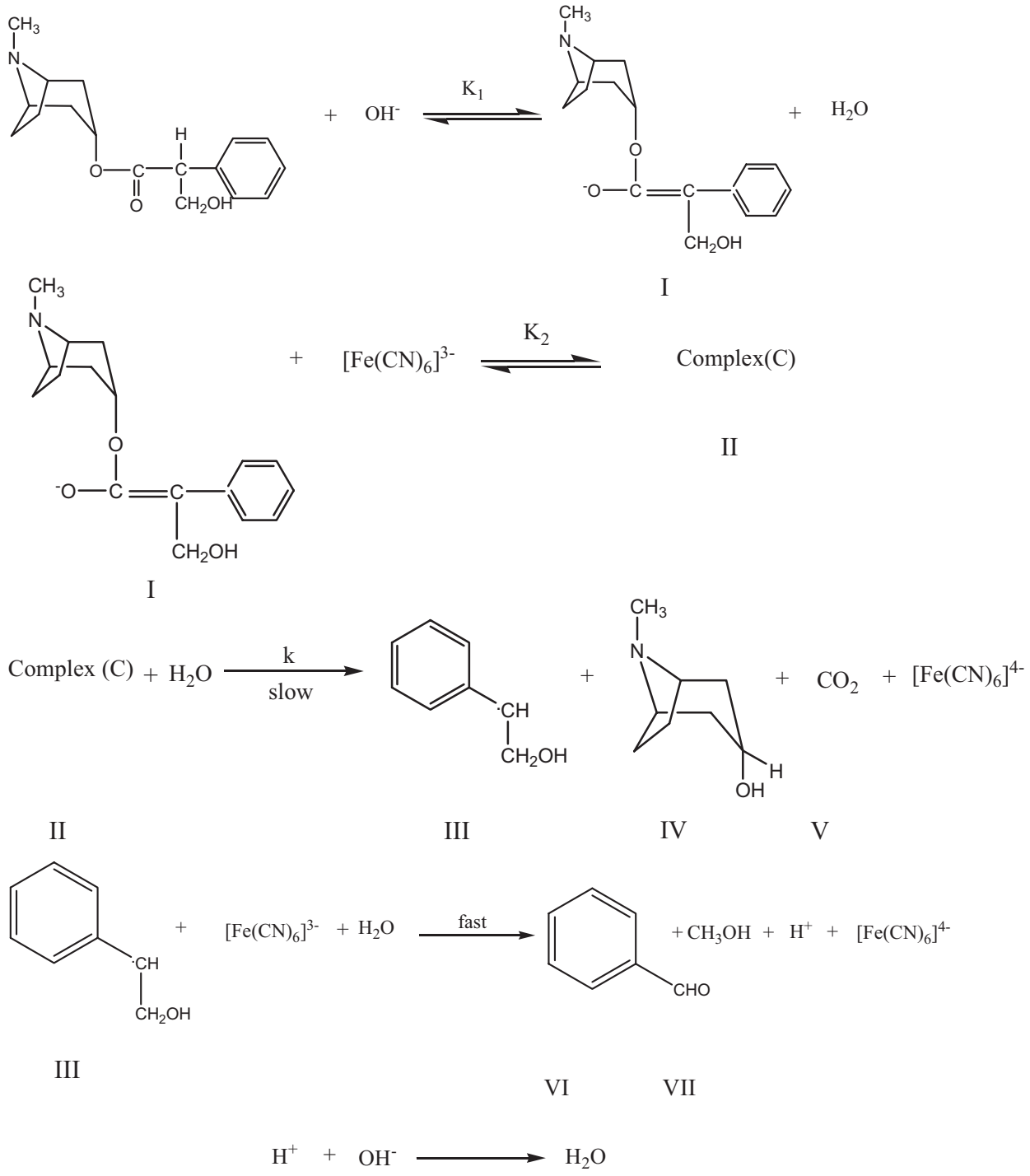
2.7. Test for free radicals (polymerization study)

The reaction mixture was mixed with a known quantity of acrylonitrile monomer and kept for 2 h under inert atmosphere. On diluting with methanol, a white precipitate of polymer was formed, indicating the intervention of free radicals in the reaction. The experiment of either $Fe(CN)_6^{3-}$ or ASM with acrylonitrile alone did not induce polymerization under conditions similar to those induced with the reaction mixture. Initially added acrylonitrile decreases the rate, also indicating the free radical intervention in the reaction.[13]

2.8. Effect of temperature

The rate of reaction was measured at different temperatures, 15, 25, 35, and 45 °C, under varying concentrations of [ASM] and [alkali], keeping the other conditions constant for the reaction. The rate constants (k) of the slow step of Scheme 1 were obtained from the intercepts of the plots of $1/k_{obs}$ versus 1/[ASM] at the 4 different temperatures. The values are given in Table 2. The energy of activation for the rate determining step was

obtained by the least square method of the plot of log k versus 1/T, and the other activation parameters were calculated and are given in Table 2.



Scheme 1. Stoichiometry of the oxidation of atropine by hexacyanoferrate(III) in alkaline medium.

Table 2. Activation parameters and thermodynamic quantities for the oxidation of atropine sulfate by hexacyanoferrate(III) in alkaline medium with respect to the slow step of Scheme 1.

(A) Effect of temperature and activation parameters

Temperature (K)	k× 10^2 (s^{-1})	Parameters	Values
288	0.27 ± 0.01	Ea (kJ mol^{-1})	53 ± 3
298	0.52 ± 0.02	$\Delta H^{\#}$ (kJ mol^{-1})	50 ± 3
308	1.04 ± 0.03	$\Delta S^{\#}$ (J K^{-1} mol^{-1})	–120 ± 10
318	2.15 ± 0.03	$\Delta G^{\#}$ (kJ mol^{-1})	85 ± 3
		log A	7.0 ± 0.1

(B) Effect of temperature on first and second equilibrium step of Scheme 1

Temperature (K)	K_1 (dm^3 mol^{-1})	K_2× 10^{-2} (dm^3 mol^{-1})
288	0.78 ± 0.03	4.98 ± 0.20
298	1.82 ± 0.05	3.03 ± 0.20
308	3.44 ± 0.10	2.15 ± 0.10
318	5.81 ± 0.25	0.98 ± 0.06

(C) Thermodynamic quantities with respect to K_1 and K_2

Thermodynamic quantities	Values from K_1	Values from K_2
ΔH (kJ mol^{-1})	51 ± 4	–39 ± 3
ΔS (J K^{-1} mol^{-1})	174 ± 12	–85 ± 5
ΔG_{298} (kJ mol^{-1})	–1.50 ± 0.1	–14 ± 0.8

The variation in the concentrations of the oxidant, substrate, and alkali, while keeping the others constant, showed that the reaction is first order in oxidant and less than the unit order in substrate and alkali concentrations (Table 1). The reaction between atropine sulfate and $Fe(CN)_6^{3-}$ has a stoichiometry of 1:2. Based on the experimental results, a mechanism can be proposed for which all the observed orders in each constituent such as [oxidant], [reductant], and [OH^-] may be well accommodated. Oxidation of atropine sulfate by hexacyanoferrate(III) in alkaline media is a noncomplementary reaction with 2 moles of oxidant reacting with 1 mole of substrate.

In the present study, alkali combines first with atropine sulfate to give the anionic form of atropine(I) in a prior equilibrium step, which is also supported by the observed fractional order in [OH^-] and [ASM]. The hexacyanoferrate(III) species reacts with the anionic form of atropine sulfate(I) to give a complex C(II), which decomposes in a slow step to form an intermediate 2-phenyl ethanol free radical species(III) and a final product of tropine(IV) derived from atropine sulfate and byproduct CO_2 (V). This intermediate 2-phenyl ethanol free radical species(III) further reacts with another mole of hexacyanoferrate(III) in a fast step to form final products such as benzaldehyde(VI), methyl alcohol(VII), and $Fe(CN)_6^{4-}$. All these results may be interpreted in the detailed mechanistic Scheme 1 as shown below.

Since Scheme 1 is in accordance with the generally well-accepted principle of noncomplementary oxidations taking place in the sequence of one-electron steps, the reaction between the substrate and oxidant would afford a radical intermediate. A free radical scavenging experiment revealed such a possibility. Spectroscopic evidence for complex formation between oxidant and substrate was obtained from the UV-Vis spectra of hexacyanoferrate(III) (2.0×10^{-4} mol dm^{-3}), ASM (2.0×10^{-3} mol dm^{-3}), [OH^-] (0.5 mol dm^{-3}), and a mixture of hexacyanoferrate(III), ASM, and alkali. A hypsochromic shift of about 4 nm from 263 to 259 nm in the spectra of hexacyanoferrate(III) to a mixture of hexacyanoferrate(III) and ASM was observed. The plots of

k_{obs} versus [ASM] and k_{obs} versus $[OH^-]$ were nonlinear, whereas the linearity of the Michalis–Menten plots proved the complex formation between oxidant and substrate, which also explains less than unit order in [ASM].

Scheme 1 leads to the rate law (1)

$$Rate = \frac{-d[Fe(CN)_6^{3-}}{dt} = \frac{kK_1K_2[ASM][OH^-][Fe(CN)_6^{3-}}{1 + K_1[OH^-] + K_1K_2[ASM][OH^-]} \quad (1)$$

$$k_{obs} = \frac{Rate}{[Fe(CN)_6^{3-}} = \frac{kK_1K_2[ASM][OH^-]}{1 + K_1[OH^-] + K_1K_2[ASM][OH^-]} \quad (2)$$

Eq. (2) can be rearranged to the following form, which is suitable for verification:

$$\frac{1}{k_{obs}} = \frac{1}{kK_1K_2[ASM][OH^-]} + \frac{1}{kK_2[ASM]} + \frac{1}{k} \quad (3)$$

The increase in the rate with increasing ionic strength is contrary to a reaction between neutral and charged species of reactants, as presented in Scheme 1. This might be due to the presence of different ions and the high ionic strength of the reaction medium. The effect of solvent on the reaction rate has been described in detail in the literature.[14] For the limiting case of a zero angle approach between 2 dipoles or an anion–dipole system, Amis[15] has shown that a plot of log k_{obs} versus 1/D gives a straight line, with a negative slope for a reaction between a negative ion and a dipole or 2 dipoles, and with a positive slope for positive ion and dipole interaction. In the present study, the plot observed had a negative slope as shown in, which supports the involvement of negative ions as given in Scheme 1. The thermodynamic quantities for the different equilibrium steps in Scheme 1 can be evaluated as follows. The [ASM] and $[OH^-]$ (Table 1) were varied at 4 different temperatures. According to Eq. (3), other conditions being constant, plots of $1/k_{obs}$ versus 1/[ASM] and $1/k_{obs}$ versus $1/[OH^-]$ should be linear and are found to be so (Figures 2A and 2B). From the slopes and intercepts, the values of K_1, K_2, and k were calculated at different temperatures (Table 2). A van't Hoff plot was made for the variation in K_1 and K_2 with temperature (log K_1 versus 1/T and log K_2 versus 1/T). The values of enthalpy of reaction ΔH, entropy of reaction ΔS, and free energy of reaction ΔG were calculated for the first and second equilibrium steps of Scheme 2. These values are given in Table 2C. A comparison of the ΔH value (51 kJ mol^{-1}) from K_1 of the first step with that of $\Delta H^{\#}$ (50 kJ mol^{-1}) obtained for the rate determining step shows that the reaction before the rate determining step is fairly fast as it involves low activation energy.[16] A high negative value of $\Delta S^{\#}$ (–112 J K^{-1} mol^{-1}) suggests that the intermediate complex (C) is more ordered than the reactants.[17]

In conclusion, the oxidation of atropine sulfate monohydrate by hexacyanoferrate(III) in aqueous alkaline media was investigated. Based on the experimental observations, a mechanism was proposed via the formation of an intermediate complex between atropine sulfate and hexacyanoferrate(III). The rate constant of the slow step and other equilibrium constants involved in the mechanism were evaluated and activation parameters with respect to the slow step of the reaction were computed. The overall sequence described here is consistent with all experimental findings, including the product, and spectral, mechanistic and kinetic studies.

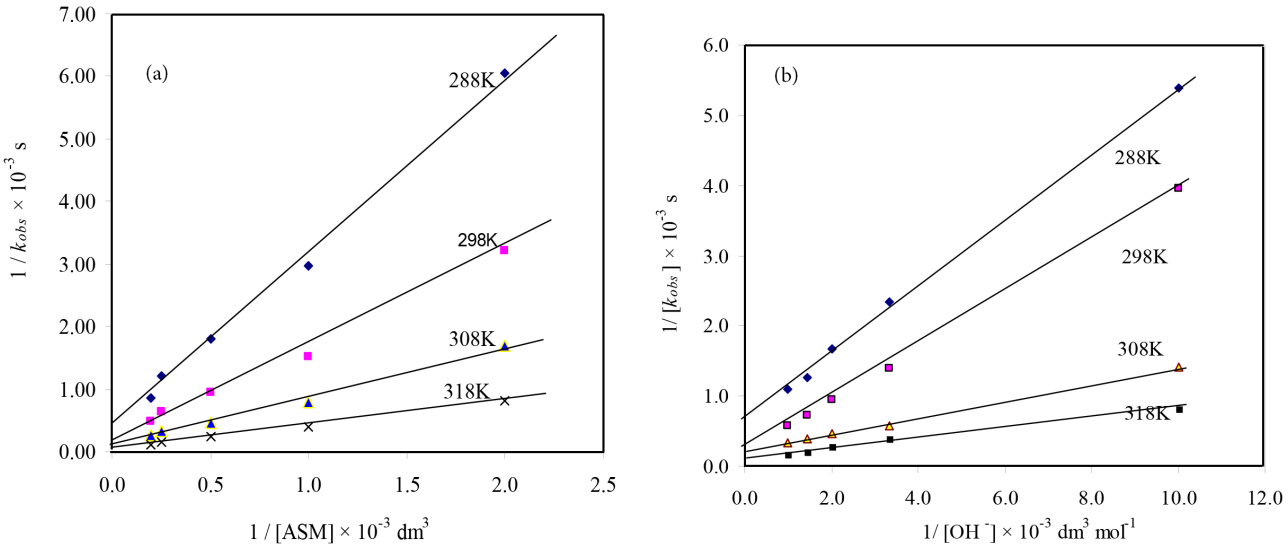
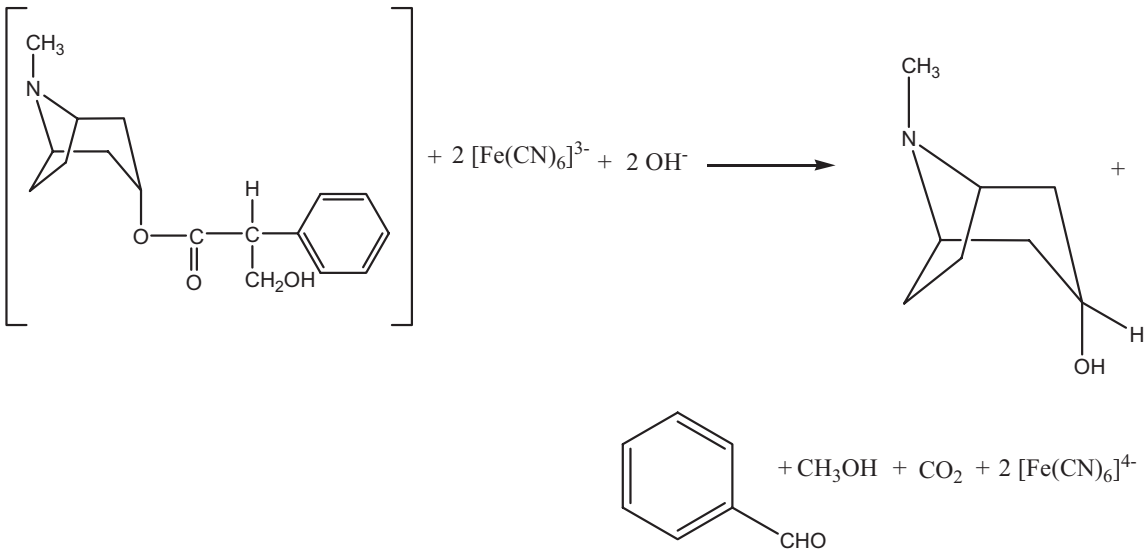


Figure 2. (a) Plots of k_{obs} versus [ASM] and $1/k_{obs}$ versus 1/[ASM] and (b) k_{obs} versus [OH^-] and $1/k_{obs}$ versus 1/[OH^-] at 4 different temperatures (conditions as in Table 1).



Scheme 2. Detailed scheme for the oxidation of atropine sulfate by alkaline hexacyanoferrate(III).

3. Experimental

3.1. Materials and reagents

All materials employed in the present work were of reagent grade. Atropine sulfate was obtained from s.d. fine-Chem Ltd and $K_3Fe(CN)_6$ were purchased from SISCO CHEM. The stock solution of atropine sulfate was prepared by dissolving known amounts of samples in distilled water. Solutions of atropine sulfate were always freshly prepared before use. A stock solution of the oxidant, hexacyanoferrate(III), was prepared by dissolving $K_3Fe(CN)_6$ in distilled water and the solution was standardized iodometrically.[18] Hexacyanoferrate(II) solution

was prepared by dissolving a known amount of $K_4Fe(CN)_6$ (s.d. fine-Chem) in water. The required alkalinity and ionic strength were maintained with KOH (Fisher Scientific) and KNO_3 (Fisher Scientific), respectively, in the reaction solutions. t-Butyl alcohol (SPECTROCHEM) was used to vary the dielectric constant of the media.

3.2. Kinetic measurements

Reactions were carried out under pseudo-first-order conditions with known excess of [substrate] over [oxidant] at constant temperature. The reaction was initiated by mixing required quantities hexacyanoferrate(III) with the ASM solution, which also contained the required concentrations of KNO_3 and KOH. The progress of the reaction was monitored spectrophotometrically at 420 nm by measuring the decrease in absorbance of hexacyanoferrate(III) ($\varepsilon = 1070 \pm 10$ dm^3 mol^{-1} cm^{-1}).

The spectral changes during the chemical reaction for the standard condition at 25 °C are shown in Figure 3. It is evident from the figure that the concentration of HCF decreases at 420 nm. It was verified that there was almost no interference from other species in the reaction mixture at this wavelength (420 nm). The first order rate constants (k_{obs}) were obtained from the plots of log(absorbance) versus time plots. The rate constants were reproducible within ±5%. In view of the modest concentrations of alkali used in the reaction medium, attention was also given to the effect of the surface of the reaction vessels on the kinetics. Use of polythene/acrylic ware and quartz or polyacrylate cells gave the same results as glass vessels and cells, indicating that the surfaces play no important role in the rate of reaction.

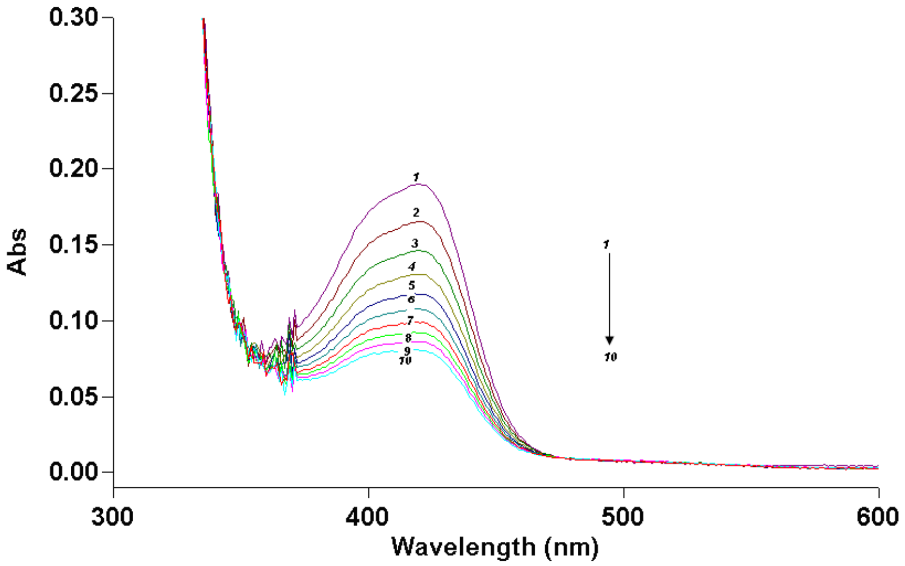


Figure 3. UV-Vis spectral changes during the oxidation of atropine sulfate by alkaline hexacyanoferrate(III) at 25 °C; $[Fe(CN)_6^{3-}] = 2.0 \times 10^{-4}$; $[ASM] = 2.0 \times 10^{-3}$; $[OH^-] = 0.5$; and I = 1.0 mol dm^{-3} with scanning time interval of 1.0 min.

3.3. Instruments used

(i) For kinetic measurements, a Peltier Accessory (temperature control) attached Varian CARY 50 Bio UV-Vis spectrophotometer (Varian, Victoria-3170, Australia) was used. (ii) For product analysis, a QP-2010S Shimadzu

gas chromatograph mass spectrometer, Nicolet 5700-FT-IR spectrometer (Thermo, USA), and 300 MHz ^{1}H NMR spectrophotometer (Bruker, Switzerland) were used.

3.4. Stoichiometry and product analysis

Different sets of reaction mixtures with different concentrations of reactants were kept for 6 h at 25 °C under nitrogen atmosphere in a closed vessel. The remaining concentration of hexacyanoferrate(III) was assayed spectrophotometrically by measuring the absorbance at 420 nm. The results indicated that 2 moles of hexacyanoferrate(III) reacted with 1 mole of atropine as given in Eq. (4):

The product was extracted with ether and purified. The ethereal layer was subjected to column chromatography using hexane and ethyl acetate in 8:2 (v/v) and the fractions were subjected to spectral investigations. The main reaction products were identified as tropine and benzaldehyde. The products were characterized by spectral studies as given below.

The IR spectrum of tropine showed a broad band at 3245 cm^{-1} assigned to –OH stretching. The ^{1}H NMR spectral analysis of tropine exhibited a broad singlet for –OH at δ 12.34 ppm (D_2O exchangeable), and a triplet in the range 3.89–3.95 ppm was observed for the protons present on the carbon carrying OH group. A multiplet due to 2 methine protons (C–H) adjacent to nitrogen and 8 methylene protons appeared at around 3.54–3.66 ppm. Methyl protons attached to nitrogen appeared as a singlet at δ 3.36 (Figure 4). The GC-MS

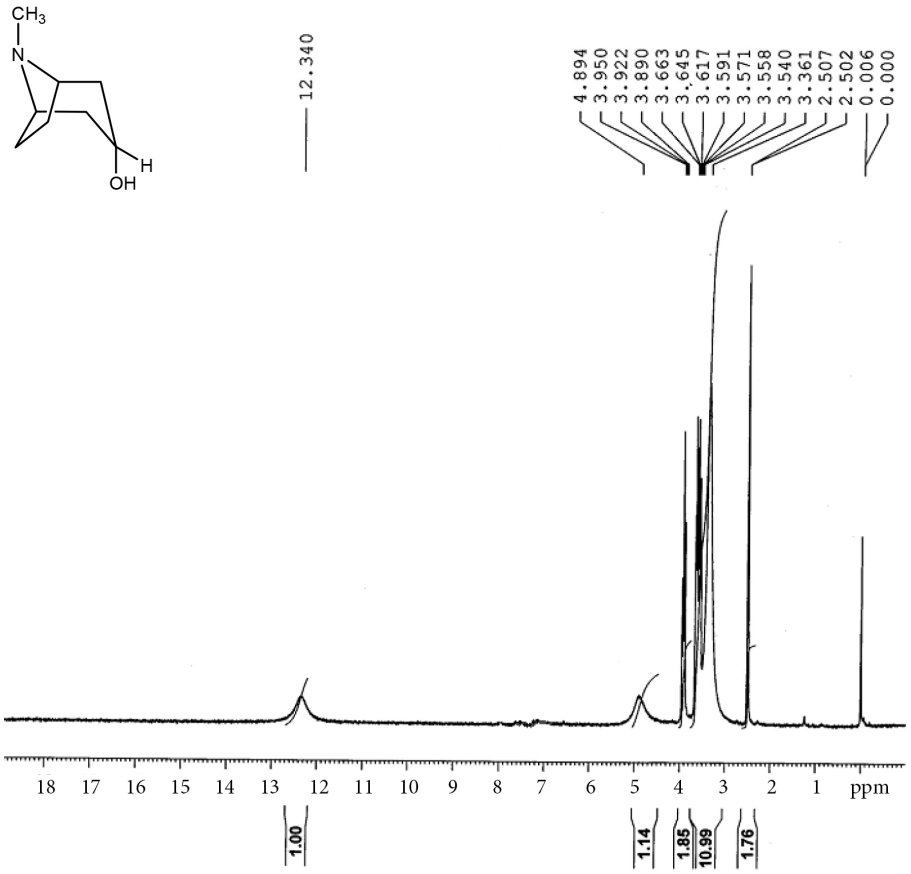


Figure 4. ^{1}H NMR spectra of tropine, the oxidation product of atropine sulfate by hexacyanoferrate(III) in alkaline medium.

mass spectrum of tropine showed a base peak at 124 amu and a molecular ion peak at 141 amu (Figure 5). Another product, benzaldehyde, was confirmed by its GC-MS spectrum, which showed a molecular ion peak at 106 amu and was also confirmed by its hydrazone derivative.[19]

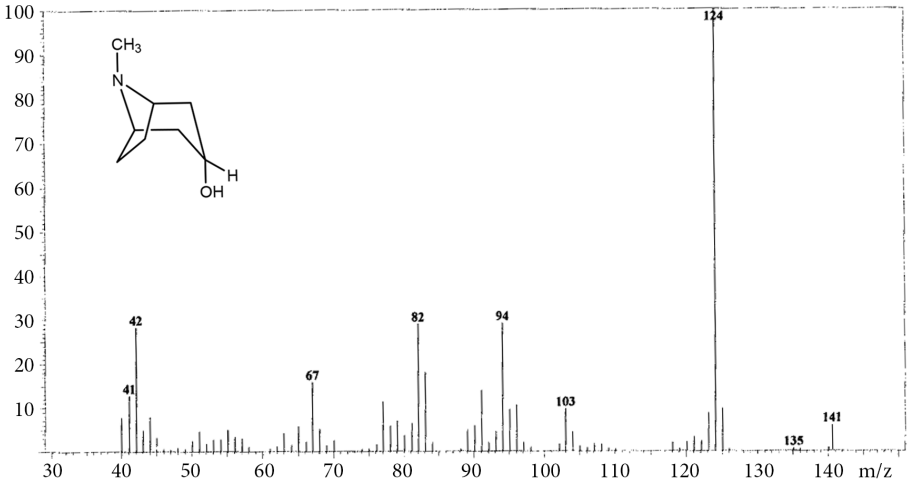


Figure 5. GC-MS spectra of tropine showed molecular ion peak at m/z 141 amu and base peak at m/z 124 amu.

The byproducts were identified as methyl alcohol, which was confirmed by sodium test,[14] and CO_2 was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through a tube containing limewater. The reaction products did not undergo further oxidation under the present kinetic conditions.

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