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Some effects of metal ions on the photogeneration of hydroxyl radicals

A.H.L. Ho, S.C. Popat, J.K. Sugden *

Department of Pharmacy, School of Applied Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK

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Abstract

The effect of common transition metal ions on the photochemical degradation of a model compound, dequalinium chloride in aqueous solution, irradiated with simulated sunlight at 38 ± 1°C has been investigated. The reactions follow first order kinetics; the addition of a standard volume of hydrogen peroxide markedly enhances the reaction rate constants of these reactions, suggesting that the reactions are hydroxyl radical-mediated in a similar manner to that of the Fenton process. In order to evaluate the contribution of the hydroperoxy radical to photochemical degradation of pharmaceutical formulations as aqueous solutions, the effect of light-generated hydroperoxy radicals was investigated with the same group of metals. The reactions in this case followed second order kinetics and were very much slower than those mediated by hydroxyl radicals; only zinc ions showed a notable increase in the reaction rate constant as compared to that of the control system.

Key words: 2,2′-Azobis(amidinopropane) dihydrochloride; Cupric sulfate; Dequalinium chloride ferrous sulfate; Hydroxyl radical; Hydroperoxy radical; Zinc sulfate

1. Introduction

Hydroxyl radicals can be generated by chemical and physical means, the most extensively studied of the chemical methods being the Fenton process (Fenton, 1894) which was subsequently shown to be hydroxyl radical mediated (Haber and Weiss, 1934; Merz and Waters, 1947, 1949). The formation of hydroxyl radicals by the Fenton process has been depicted as:

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^{\cdot} + OH^-$$

Subsequent work with a series of metals has allowed the metals tested to be arranged in an order:

$Mn^{2+} < Cu^+ < Co^{2+} < Fe^{2+}$

(Cier et al., 1959; Czapski, 1971)

However, in the presence of ascorbic acid the activity of the system was increased and the order changed to:

$Mn^{2+} < Fe^{2+} < Co^{2+} < Cu^+$ (Cier et al., 1959)

Hydroxyl radical formation can occur from water under anaerobic conditions in the presence of light and suitable compounds, such as *p*-benzoquinone (Beck and Brus, 1982). Hydroxyl radicals

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* Corresponding author.

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also can be produced via the superoxide anion in the presence of water, the superoxide anion subsequently reacting with protons to give hydrogen peroxide which can further react to give hydroxyl radicals. The objective of the present work was to determine if the presence of metal ions, commonly associated with pharmaceutical manufacturing equipment, has an effect on the photochemical degradation of dequalinium chloride in aqueous solution used as a model drug system.

2. Materials and methods

2.1. Chemicals

The following chemicals were obtained from the indicated sources: ABAP (2,2′-azobis(2-amidinopropane) dihydrochloride, Polysciences, U.S.A. (batch no. 423764); copper(II) sulphate anhydrous, Hopkin & Williams (batch no. 597202080/1); copper(I) chloride, May and Baker Ltd (batch no. CHo/18/86); dequalinium chloride, Sigma Chemical Co. Ltd (batch no. 124 F-0608); EDTA, Tristar Laboratory Supplies Ltd (batch no. 25720; hydrogen peroxide, Tristar Laboratory Supplies (batch no. 9165430 D); iron(II) sulphate heptahydrate, BDH Chemicals Ltd (batch no. 5038110 H); D-mannitol, Hopkin & Williams Ltd (batch no. SH30); and zinc(II) sulphate heptahydrate, BDH Chemicals Ltd (batch no. 2386720).

2.2. Apparatus

A Beckman DU 70 spectrophotometer and Apple Macintosh LC computer were used.

2.3. Preparation of solutions

A stock solution of dequalinium chloride in deionised water (300 μg/ml) was prepared and the container wrapped in aluminium foil to exclude light. A dilution of the stock solution was prepared containing 6 μg/ml of dequalinium chloride (50 ml), and a second solution was prepared in which hydrogen peroxide (5 ml 30% v/v) was incorporated. Additional solutions were prepared in which the following materials were added individually as shown in Table 1.

Table 1
Irradiated dequalinium chloride solutions with additives

Dequalinium chloride (6 μg/ml) solution	Metal ion solution (20 ppm; 25 ml)	Hydrogen peroxide (5 ml)	Blank used (10 ppm in water [b])
25 ml	Cu^{2+}	√	Cu^{2+}
25 ml	Fe^{2+}	–	Fe^{2+}
25 ml	Fe^{2+}	√	Fe^{2+}
25 ml	Zn^{2+}	√	Zn^{2+}
25 ml	pinene	–	water [b]
25 ml	Cu^{2+} EDTA [a]	–	Cu^{2+}
25 ml	Fe^{2+} EDTA [a]	–	Fe^{2+}
25 ml	Zn^{2+} EDTA [a]	–	Zn^{2+}

[a] Added in stoichiometric amounts.
[b] Deionised water.

In a second series of experiments a stock solution of dequalinium chloride in deionised water (60 μg /ml) was prepared. A solution of 2,2′-azobis(2-amidinopropane) hydrochloride (ABAP), (1% w/v) in deionised water was prepared. Solutions (20 ppm with respect to the cation) of the following metal ions were prepared in deionised water: copper(I) from cuprous chloride; copper(II) from cupric sulphate pentahydrate; iron(II) from ferrous sulphate heptahydrate; and zinc from zinc sulphate.

Solutions for irradiation were prepared as shown in Table 2.

2.4. Irradiation procedure

A series of calibration curves were constructed by measuring the absorbance at 326 nm for a

Table 2
Irradiated dequalinium chloride solutions with ABAP

Dequalinium chloride (60 μg/ml) solution	Metal ion (20 ppm; 25 ml)	ABAP (1% w/v) (5 ml)	Blank used (10 ppm in water [a])
25 ml	nil water [a]	√	water [a]
25 ml	Cu^{2+}	√	Cu^{2+}
25 ml	Fe^{2+}	√	Fe^{2+}
25 ml	Zn^{2+}	√	Zn^{2+}
25 ml	Cu^{+}	√	Cu^{+}

[a] Deionised water.

Table 3
Photodegradation of dequalinium chloride (DC; 3 μg/ml) in deionised water

Time of irradiation (h)	Absorbance	% DC remaining	Log % DC remaining	1/% DC remaining
0	0.1378	100.00	2.0000	0.0100
1	0.1234	89.55	1.9520	0.0116
2	0.1139	86.57	1.9374	0.0115
3	0.1153	83.67	1.9225	0.0119
4	0.1077	78.16	1.8929	0.0128
5	0.1064	77.21	1.8876	0.0129
Regression coefficient		−0.9608	−0.9696	0.9355
Slope			0.0215	
Rate constant			4.95×10^{-2}	
Order of reaction:			First	

range of concentrations (0.3, 0.6, 0.75, 1.5, 3.0, 6.0 μg/ml and 8, 16, 24, 32, 40, 48, μg/ml) by the method of Patel and Sugden (1992). The mean absorbances plotted against concentration showed linear regression coefficients of 0.9972 and 0.9994, respectively. Similar calibration curves were constructed for the solutions containing copper(II) ions, iron(II) ions and zinc (II) ions which gave linear regression correlation coefficients of 0.9981, 0.9974 and 0.9968, respectively.

The solutions were irradiated according to the method of Evans et al. (1975) and samples assayed by measurement of the absorbance at 326 nm at time intervals of every 60 min up to a maximum of 5 h from time zero. The data recorded in the tables represent the mean of three experiments.

Table 4
Photolysis results of dequalinium chloride (DC) with metals and additives

Additives (10 ppm)	Reaction order	Rate constant
DC	first	4.95×10^{-2}
DC + H_2O_2	first	70.0×10^{-2}
DC + Cu^{2+}	first	47.9×10^{-2}
DC + Cu^{2+} + H_2O_2	first	67.9×10^{-2}
DC + Fe^{2+}	first	79.2×10^{-2}
DC + Fe^{2+} + H_2O_2	first	97.6×10^{-2}
DC + Zn^{2+}	first	83.2×10^{-2}
DC + EDTA	first	57.5×10^{-2}
DC + Cu^{2+} + EDTA	first	43.7×10^{-2}
DC + Fe^{2+} + EDTA	first	59.4×10^{-2}
DC + Zn^{2+} + EDTA	first	12.1×10^{-2}

2.5. *Treatment of results*

The reaction order and rate constant were determined by carrying out a linear regressions analysis of plots of the percentage dequalinium chloride remaining vs time, the log percentage dequalinium chloride remaining vs time and the reciprocal percentage dequalinium chloride remaining vs time. The plot yielding the best straight line as determined by the method of Patel and Sugden (1992) was deemed to represent the order of the reaction. The rate constants were calculated from the slopes of the graphs.

3. Results and discussion

Some metal ions undergo one electron oxidation and may participate in the Fenton process; a general formula has been postulated for this reaction (Czapski, 1972):

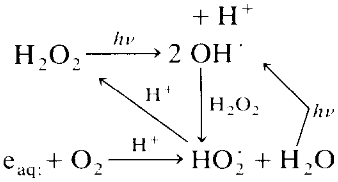
$$M^{n+} + H_2O_2 = M^{n+1} + OH^- + OH^{\cdot}$$

Table 5
Photolysis results of dequalinium chloride solutions (30 μg/ml) with metals and ABAP (1% w/v)

Additives	Reaction order	Rate constant
DC + ABAP + water	second	0.0157×10^{-2}
DC + ABAP + Cu^+	second	0.0156×10^{-2}
DC + ABAP + Cu^{2+}	second	0.0115×10^{-2}
DC + ABAP + Zn^{2+}	second	0.0202×10^{-2}
DC + ABAP + Fe^{2+}	second	no reaction

Copper(II) ions are also considered to be mild oxidants (Walling and Weil, 1974) and facilitate decomposition of water into hydroxyl radicals. Examination of Table 4 shows that the incorporation of copper(II) ions into a solution of dequalinium chloride results in an increase in rate constant for photochemical decomposition of almost an order of magnitude, indicating that they may be acting either as a direct oxidant or by facilitating the formation of hydroxyl radicals. The addition of hydrogen peroxide results in a further increase in rate constant from 47.9×10^{-2} to 67.9×10^{-2}. The addition of iron(II) ions to an aqueous solution of dequalinium chloride results in a rise in the photochemical decomposition rate constant from 4.95×10^{-2} to 79.2×10^{-2}, the incorporation of hydrogen peroxide as well as iron(II) ions giving a Fenton process and results in the rate constant for the photolysis of dequalinium chloride rising to 97.6×10^{-2}. This experimental result gives good indication that the photochemical degradation of the model compound is affected by the presence of hydroxyl radicals as proposed by Patel and Sugden (1992). Hydrogen peroxide on irradiation is known to yield hydroxyl radicals (Kochi, 1973) as in the Fenton process, so there is reason to believe that the photolysis of the dequalinium chloride solution, under these experimental conditions, is hydroxyl radical mediated to some extent. It would be reasonable to expect that the chelation of the metal ions which can participate in a Fenton process would reduce the rate constant of the photolysis of dequalinium chloride. The addition of EDTA to the model compound solution, containing copper(II) ions results in a very small reduction in the rate constant. However, in the case of iron(II) ions the reduction in rate constant is more substantial. The rate constant for the photolysis reaction in which zinc ions are incorporated is 83.2×10^{-2} whereas this value falls to 12.1×10^{-2} on the addition of a stoichiometric quantity of EDTA. Thus, there is a difference between 'd-block' metal chelates in their ability to influence the rate constant of the photolysis of dequalinium chloride solutions. It is worthy of note that in the cases of both copper(II) and iron(II) ions the addition of EDTA does not totally negate the degradative effects of these metal ions.

Niki et al. (1988) described ABAP as being a water soluble compound which was a source of hydroperoxyl radicals. Examination of Table 5 shows that ABAP promotes the photochemical degradation of dequalinium chloride, but these reactions follow second order kinetics and of the metals tested, only zinc had a marked effect in increasing the rate constant as compared to that of the control by a factor of 1.2. It was interesting to note that it was not possible to monitor the reaction between dequalinium chloride and the hydroperoxyl radical in the presence of iron(II) since ABAP appears to react with the iron(II) sulphate to form an inactive compound. One of the main reasons for investigating the effect of the hydroperoxyl radical is its relationship to the hydroxyl radical:



Any aqueous system containing oxygen has the capability of forming hydroperoxyl (HO_2) radicals and giving rise to the reactions depicted above. Such a definition would encompass most aqueous pharmaceutical formulations and many food and consumer products. It has been demonstrated with dequalinium chloride that the action of hydroxyl radicals is a significant factor in photochemical degradation.

The results of this work indicate that in pharmaceutical manufacture it is important not to have 'stray' metal ions present as they can facilitate photochemical degradation of other materials. These metal ions can be derived from the metal components of the manufacturing equipment which is often constructed from stainless steels and other metals. Clearly, the properties of stainless steels from which equipment is constructed can be of paramount importance in the light stability of the products manufactured therein. In addition, there are potential hazards in using storage vessels and pipework which allow products to come into contact with bare metal surfaces. Such components are quite readily attacked by acids leading to metallic ion contamination of the product. It is imprudent to rely on the addition of chelating agents to remove stray metal ions as the chelating agents may not be all that effective in this way or the chelates formed may have an enhancing effect on photochemical degradation reactions. Although the effect of the hydroperoxyl radical is much less than that of the hydroxyl radical, is does exert a degradative effect on dequalinium chloride, especially in the presence of zinc ions.

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