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Dedicated to Niculae I. Ionescu PhD
on the occasion of his 85th anniversary

KINETIC INSIGHTS INTO MILD OXIDATION OF PHTHALOCYANINE DYES: POTENTIAL APPLICATION IN FORENSIC ANALYSIS

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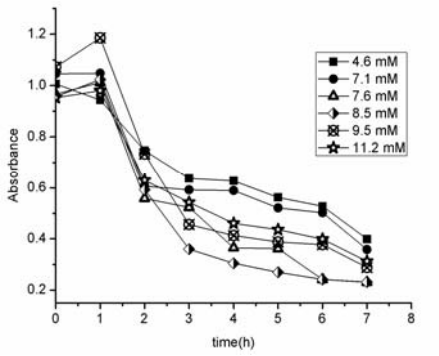
This work provides a kinetic insight into decolourization process of oxidation-resistant phthalocyanine dyes from textile materials with permanganate in alkaline environment. It was found that the overall oxidation process may be modelled as sequence comprising two apparent first-order reactions coupled by a normal intermediate. Although the kinetic model was roughly simplified, it described satisfactorily the time evolution of phthalocyanine concentration despite the complexity of the permanganate chemistry involving numerous transient species, in different oxidation states. The optimized condition provided high degradation degrees – around 95% - and the overall degradation process displayed moderate activation energy (up to 40 kJ/mol), proving once more the efficiency of permanganate assisted oxidation in alkaline conditions.



INTRODUCTION

Phthalocyanine (Pc) dyes are chemicals widely used in textile, printing, pharmaceutical, paint and coating, semiconductor and computer industries. [1,2] These very resistant dyes are also used to dye jeans outfits almost indispensable among humans worldwide.[3] It was lately found that phthalocyanines (Pcs) are able to bind single- and double-stranded DNA[4], being also tested as photosensitizers in photodynamic therapy of cancer.[5] The planar 18-π electron system of the tetrabenzo tetraazaporphyrin ring and the binding of transitional metal ions, provide Pcs with high stability to oxidative degradation,[6] therefore their removal from textile wastewaters may become a difficult task.[7] Pc's metallic complexes (mostly with copper) are used to produce blue and green shades. Soluble Pcs are obtained by attaching ionic groups at the periphery of the dinitrile, *i.e.* sulfonic acid moieties and chloride. [8] Unsubstituted Pcs along with mono- and polysulfonated copper Pcs are frequently used as the basic components of denim dyes (Reactive Blue 15, Reactive Blue 21,[9] Reactive Blue 38,[6] and C. I. Direct Blue 199[10]). Pc dyes can be partially decolourized under biological anaerobic conditions,[11]

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reduced using palladium-catalysed H_2[7] or oxidized via several advanced oxidation processes (AOP's)[10,12] or biological processes.[9,13] The oxidation is performed through hydroxyl radical; moreover, the decolourization degree can be increased up to almost 100% during wet oxidation in the presence of $CuSO_4$ at 175°C and O_2 at a pressure of 0.69 bar.[14]

Copper Pcs were reported to bind the double helix DNA[15,16] and also to inhibit Taq polymerase (polymerase from bacterium *Thermus aquaticus*) during the DNA amplification.[17] Therefore they must be removed from denim materials during DNA extraction prior the Polymerase chain reaction (PCR) amplification step.[18] The Chelex extraction is a widely used procedure for DNA extraction employing the chelator resin Chelex1-100 (Bio-Rad Laboratories, CA, USA). This resin contains styrene divinylbenzene copolymers with paired imino-diacetate ions designed to bind polyvalent transition metal ions.[19] The extraction occurs at elevated pH values (around 11) and temperatures, near to boiling point of the solution, which favours the cell breakdown and allows the chelating groups to bind to the cellular components, thus protecting the DNA from degradation.[17] The method is fast and does not require toxic organic solvents,[17,18,20] but fails to remove the inhibitors often found in blood-stained textile samples. In order to remove the potential inhibitors from biological stains, the conventional oxidation methods using strong acidic conditions are not effective since the pH of the medium should be kept at alkaline values for preserving the DNA strands. It was recently shown that Pcs removal from biological samples can be achieved through the potassium permanganate- oxidation in alkaline environment.[21] It is widely known that the permanganate anion is a powerful oxidant able to oxidize stable organic compounds, including several azo-dyes (Acid Orange 7, Acid Red 14).[22] In alkaline conditions, permanganate acts as a mild oxidant (E_0 =0.59 V *vs.* NHE[23]) being able to decolorize several Pcs and to preserve the integrity of DNA single strands. We have already demonstrated recently the efficiency of the permanganate assisted oxidation of several persistent pththalocyanine dyes: copper α-phthalocyanine Cl-αCuPc, copper β-phthalocyanine (βCuPc) and copper phthalocyanine-3,4′,4″,4‴-tetrasulfonic acid tetrasodium salt(βCuPcS4) during the Chelex extraction of DNA samples.[21] Therefore, we focused our study on the kinetic insights into the removal of tetrasulfonated copper (II) β-phthalocyanine (βCuPcS4) from aqueous solution through degradative oxidation with permanganate.

RESULTS AND DISCUSSION

The chemical degradation of βCuPcS4 was followed through the disappearance of the characteristic absorption bands at 615 and 714 nm (Fig. 1).

The solution changed colour from blue to green during the degradation experiments; finally a precipitate appeared and the reaction mixture became light yellow. The time required for complete decolourization varied between 4 and 24 hours, depending on the reactants' concentration. The decrease of the absorption peaks at 615 and/or at 715 nm in time was used to calculate the decolourization degree. In all assays a slight increase of the absorption bands was observed in the early stages of reaction, followed by a significant decrease at both characteristic wavelengths (Fig. 2).

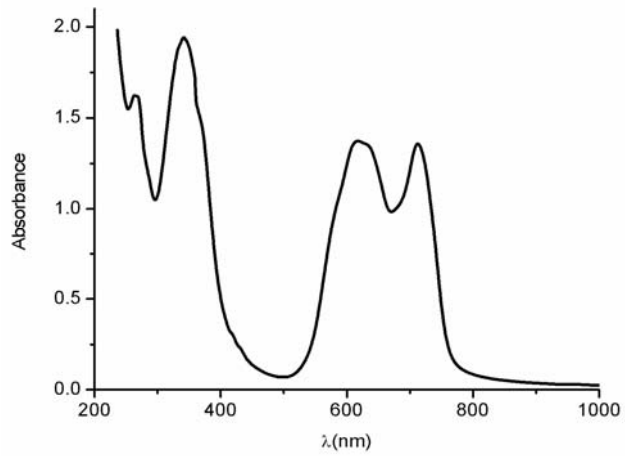

Fig. 1 – UV-VIS spectral features of βCuPcS4 in conditions similar to DNA Chelex extraction (0.008g/L phthalocyanine in 0.01% APG, pH 11 at 56°C).

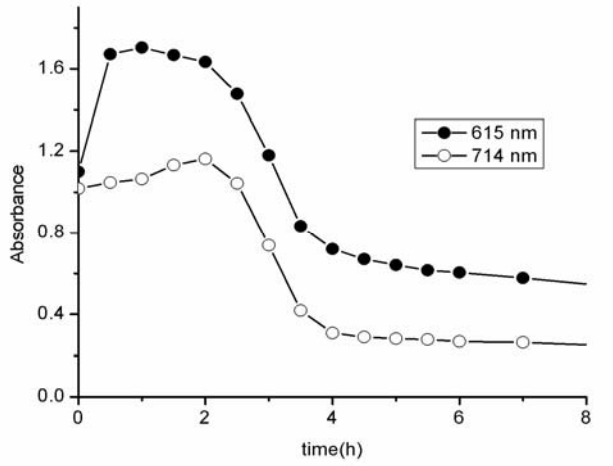

Fig. 2 – Typical absorbance changes at characteristic wavelengths during the permanganate assisted oxidation of βCuPcS4 in alkaline medium (initial concentrations: 0.008 g/L βCuPcS4, 7.6 mM potassium permanganate at pH 11 and 56°C).

While the absorption peaks display the same intensity at both studied wavelengths in alkaline solution without permanganate, the absorbance at 615 nm was higher than at 714 nm during the degradation experiments. This is probably due to the formation of manganate anion (MnO_4^{2-}) a transient species absorbing at 608 nm. [24] In order to avoid these inferences, we performed the kinetic runs by measuring the absorbance drop at 714 nm.

The increase of the peak intensity at 714 nm in the early stage of the reaction was found to depend on temperature as well as on permanganate and hydroxide amounts. During the reaction in alkaline medium, permanganate is reduced to various oxidation states - Mn(VI), Mn(V), Mn(IV) - depending on the pH of the medium. In strong alkaline conditions (pH around 12-13) the main reaction product of the reaction is the manganate anion (MnO_4^{2-}); below pH 12 the oxidation of permanganate proceeds with the formation of manganate Mn(VI), hypomanganate Mn(V) and finally Mn(IV), which slowly develops an yellow turbidity and finally precipitates as MnO_2. [25] In all experiments the pH ranged within 10 and 12; therefore, one can assume that besides permanganate, species containing manganese in all mentioned oxidation states are present. Moreover, it was noticed that absorbance increases in the presence of Mn (II) traces found in the permanganate stock solution, while the time to reach the maximum value decreased with pH and temperature.

The overall oxidative process can be divided in two consecutive apparent first order reactions coupled by a stable intermediate with similar VIS features as βCuPcS4. For the following sequence:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1)$$

the time evolution of the reactant (βCuPcS4) and the unknown intermediate concentrations (c_A and c_B) are given by:

$$\begin{aligned} c_A &= c_A^0 \, \mathrm{e}^{-k_1 t} \\ c_B &= \frac{k_1 c_A^0}{k_2 - k_1} (\mathrm{e}^{-k_1 t} - \mathrm{e}^{-k_2 t}) \end{aligned} \qquad (2)$$

where k_1 and k_2 are the apparent first order rate constants, taking into account the large excess of permanganate and hydroxide with respect to βCuPcS4 and intermediate concentrations.

Since both βCuPcS4 (A) and intermediate (B) absorb at same specific wavelength (714 nm) having the individual absorbances A_A and A_B respectively, the overall measured absorbance (A) is according to Beer's law:

$$A = A_A + A_B = \varepsilon_A c_A l + \varepsilon_B c_B l \qquad (3)$$

where: l is the optical path length and $\varepsilon_A, \varepsilon_B$ are the molar absorptivities of A and B

The equation depicting the time evolution of the system's absorbance is obtained by combining eqs. (2) and (3):

$$A = A_A^0 \, \mathrm{e}^{-k_1 t} + \frac{\varepsilon_B l c_A^0 k_1}{k_2 - k_1} (\mathrm{e}^{-k_1 t} - \mathrm{e}^{-k_2 t}) \qquad (4)$$

This kinetic equation was further used to estimate the rate constants k_1 and k_2 and the molar absorptivity of the intermediate ε_B through non-linear regression analysis. The results obtained for oxidation of βCuPcS4 in excess permanganate and hydroxide were consistent with the proposed

kinetic model and the assumption that a normal intermediate has a significant absorbance at the same wavelength as βCuPcS4. The goodness of fit was not significant for all data sets, but this can be primarily attributed to the fact that complex oxidation processes involving several stable manganese species and coloured degradation products of phthalocyanine are roughly described by two apparent first order consecutive reactions. In order to obtain better estimates of the parameters from eq. (4) we used first the decreasing portion of the kinetic curve absorbance *vs.* time to estimate the rate constant k_2 by fitting on it an exponential decay equation.

$$A = A_0 \, e^{-k_2 t} \tag{5}$$

where A_0 represents a cumulated initial absorbance corresponding to a mixture intermediate/ phthalocyanine. The calculated k_2 values were used further in the equation (4) to estimate k_1 and ε_B. The results for temperature of 56°C and initial concentration of βCuPcS4 0.078g/L are presented in Table 1.

Taking into account the values of ε_B from Table 1, the average of the intermediate's molar absorptivity at 714 nm was found (28540±3590) $M^{-1}cm^{-1}$, almost three times higher than βCuPcS4's. Since for DNA extraction experiments it is of interest only the decay of coloured mixture (phthalocyanine and high molecular weight intermediates), which is corroborated with the decolourization process, the kinetic analysis was run out on the decreasing part of the extended curve absorbance *vs*. time.

Effect of permanganate concentration on the decolourization rate of βCuPcS4

The effect of permanganate concentration on the degradation rate was studied in a range of 4.6 to 11.2 mM, at settled concentrations of βCuPcS4 (0.018 g/L) and NaOH (0.066 M, corresponding to a pH = 11). It was noticed that the maximum absorbance at fixed wavelength was reached almost at the same time, irrespective of the permanganate concentration (Fig. 3). The intensity of the maximum absorption increases with the permanganate concentration; this can be explained by the increasing amounts of Mn^{2+} contained in the permanganate solution, favouring the formation of the intermediate. Additional experiments performed on mixtures of βCuPcS4/$MnCl_2$ in the presence of NaOH without permanganate confirmed the formation of a coloured intermediate absorbing at 714 nm.

The degree of decolourization (%) was calculated as:

$$D(\%) = \frac{(A_{\max} - A_{\infty})}{A_{\max}} \cdot 100 \tag{6}$$

where A_{max} and A_{∞} represent the maximum and final absorbance (after 7h). The decolourization degree displayed a peak–shaped dependence on the permanganate concentration reaching a maximum value 7.6 mM (Fig. 4) suggesting a complex overall oxidation process.

Table 1

Estimated rate constants k_1 and k_2 together with the molar absorptivity ε_B of the coloured intermediate at 714 nm for βCuPcS4 oxidation with permanganate in alkaline medium

$[KMnO_4]_0$ (mM)	$[NaOH]_0$(M)	ε_B ($M^{-1}cm^{-1}$)	k_2 (h^{-1})	k_1 (h^{-1})	r^2
4.9	0.066	25400	0.171	0.092	0.986
7.1	0.066	26400	0.350	0.135	0.963
7.6	0.0095	32400	0.040	0.135	0.936
7.6	0.028	29700	0.437	0.375	0.985
7.6	0.047	27200	0.807	0.254	0.979
7.6	0.066	31300	0.868	0.254	0.956
8.5	0.066	31400	0.822	0.389	0.987
9.3	0.066	27900	0.812	0.298	0.953
11.4	0.066	21300	0.799	0.036	0.942

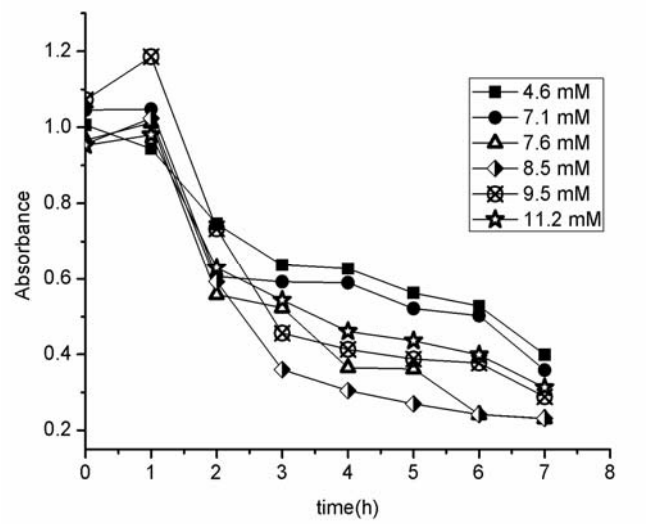

Fig. – 3 Time-course changes in the absorbance of the reaction mixtures for experiments ran out at several concentrations of permanganate (initial conditions: 0.008 g/L βCuPcS4, at pH 11 and 56°C).

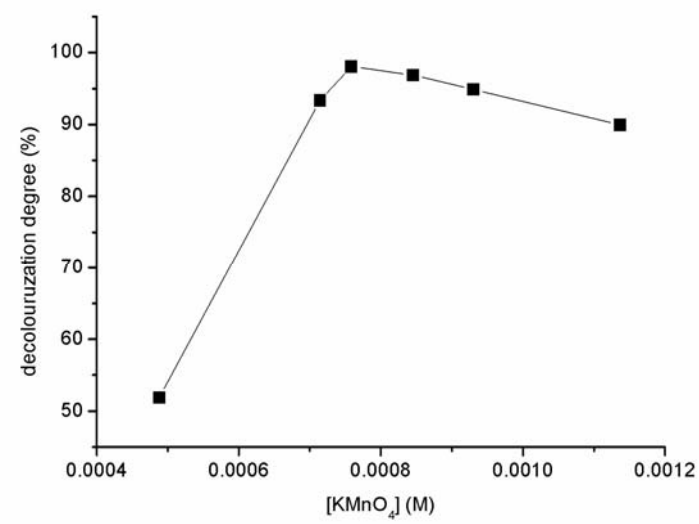

Fig. 4 – Inhibitory effect of permanganate for concentrations above 7.6 mM
(Initial conditions: 0.008 g/L βCuPcS4, pH 11 and 56°C).

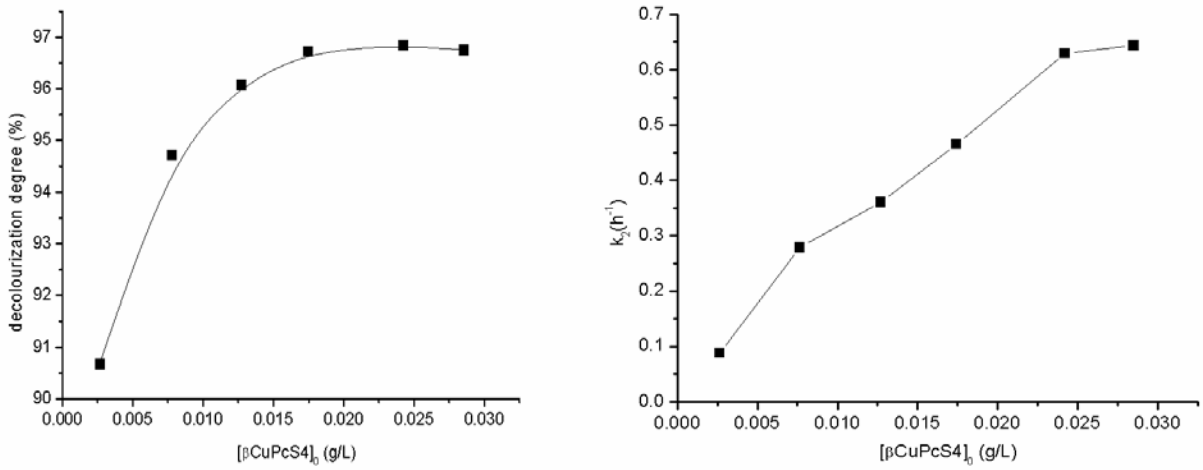

Fig. 5 – Increase of the decolourization degree (left) and degradation rate (right) over the initial concentration βCuPcS4
(Initial conditions:7.6 mM permanganate, pH 11 and 56°C).

A similar pattern of variation was observed for the apparent first-order constant k_2, which reached a maximum value for a concentration of permanganate of 7.6 mM.

Effect of phthalocyanine concentration on the efficiency of the degradation process

The reaction was followed at constant initial concentrations of KMnO4 (7.6mM) and NaOH (0.066M) and Pc between 0.005 and 0.0285 g/L. It was noticed that the degree of decolourization increased monotonically towards βCuPcS4, as well as the rate constant k_2 (Fig. 5).

Effect of NaOH concentration

The concentration of NaOH was adjusted to match the pH required for the Chelex extraction of DNA and ensuring a large excess towards βCuPcS4, varying from 9.5 to 66 mM. In this range of concentration with the pH of the reaction mixture ranging from 10 to 12, a linear increase of k_2 was noticed along with an almost complete decolourization at pH 12.

Effect of temperature

The optimized degradation conditions 0.018g/L βCuPcS4, 7.6 mM KMnO4 and 0.066M NaOH were used further to improve the degradation degree and to calculate the overall activation energy of the decolourization process. The degree of decolourization reached a peak value around 95% at 70°C (data not shown). The overall activation energy was estimated from the Arrhenius plot ln k_2 *vs* 1/T, providing the best fit value E_a = (38.4±2.5) kJ/mol (with r^2 = 0.9834). This relatively low value of the activation energy proves once more the efficiency of the oxidant system towards the degradation of phthalocyanine and its coloured by-products.

EXPERIMENTAL

Materials

Copper phthalocyanine-3,4′,4″,4‴-tetrasulfonic acid tetrasodium salt with 85% dye content (βCuPcS4), $KMnO_4$ (purity ≥99 %, ACS reagent), NaOH (purity ≥97 % ACS reagent) were purchased from Sigma. The exact concentration for $KMnO_4$ was calculated by titration with 0.05 M oxalic acid from Sigma. APG (alkyl polyglucoside, non-ionic surfactant with C8–C14, ≤ 1% C16, as 51% solution (w/w) in water) was purchased from DOW Chemical Company. βCuPcS4 stock solution (0.26 g/L) was prepared by dissolving the solid compound in 0.01% (w/w) APG solution. In all assays the concentration of APG was below the critical micellar concentration (cmc) of 0.056 % (w/w). [26]

Degradation experiments

βCuPcS4 (0.018–0.03 g/L) in excess permanganate (0.4 mM to 12 mM) and NaOH (30 mM to 100 mM), was allowed to react at least for 7 hours at temperatures between 40 and 70°C, keeping the reaction mixture at a pH ranging from 10 to 12. The βCuPcS4 decolourization was monitored by scanning the ultraviolet visible (UV-VIS) spectra between 200-900 nm at different times with a Jasco V-530 spectrophotometer with a Peltier cell for temperature and stirring control. The time required for the complete decolourization ranged between 4 and 24 hours, depending on the concentration of reactants.

CONCLUSIONS

While the permanganate anion was able to decompose oxidation-resistant phthalocyanine dyes in conditions compatible with the preservation of the DNA strands, the kinetic study of the oxidation process revealed a complex mechanism with the formation of a relatively stable intermediate (favoured in the presence of Mn^{2+}); at the same time it provided a useful tool to optimize and to economically address the issue of the degradation of PCR inhibitors from DNA stained textile materials with application in forensic analysis.

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