Dyes and Pigments **19** (1992) 51–57

Photochemical Degradation of C.I. Acid Black 1

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(Received 25 November 1991; accepted 2 January 1992)

ABSTRACT

The disazo dye Naphthalene Black (C.I. Acid Black 1; C.I. 20470), which has wide application in analytical chemistry and histochemistry as well as in the coloration of wool, undergoes photochemical degradation in aqueous solution. The reaction is accelerated in the presence of hydrogen peroxide and retarded in the presence of mannitol. In addition, the reaction rate is enhanced by the incorporation of a triplet sensitizer dye. These findings are strongly suggestive that Naphthalene Black undergoes hydroxyl radical and triplet sensitized photochemical degradation. Such effects might reduce the efficacy of the dye in its applications.

1 INTRODUCTION

Naphthalene Black 12B (C.I. Acid Black 1; C.I. 20470) is used for dyeing wool and has also been used in analytical work for the staining of protein in electrophoresis[1,2] and for the determination of the total protein content of milk.[3,4] It has also been used for the histochemical staining of a range of tissue components such as nuclei,[5] nucleolar protein and nucleoli,[6–8] mitochondria[9] and Paneth's cell granules.[10] Naphthalene Black has been used in the tannic acid–phosphomolybdic acid Amido Black (TPA) method for haemoglobin,[11–13] as well as for the demonstration of basement membranes, reticulum and collagen in picric acid mixtures of the van Gieson type[12,14,15] and for collagen and reticulum in the allochrome stain.[16]

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Dyes and Pigments 0143-7208/92/$05.00

Impurities in commercial samples of the dyes both for use in protein electrophoresis[17] and histochemical methods[6,12,13,18,19] has led to substandard staining in many instances. Thus the purified dye[20] has been used for the demonstration of the granules of eosinophilic granulocytes in tissue sections containing parasitic helminths.[21,22]

Dilute aqueous solutions of the dye are known to fade when exposed to light and the primary purpose of the present work was to quantify this photodegradation of Naphthalene Black, and to propose a mechanism for this process.

2 EXPERIMENTAL

2.1 Naphthalene Black

Samples of the dye were obtained from commercial suppliers (BDH, Aldrich Chemical Company, Sigma Chemical Company Ltd and Kodak Ltd). Samples were purified by column chromatography with propan-2-ol as the principal solvent;[20] this procedure removed possible impurities of manufacture[30] as well as 'packing agents'.[23] A dilute aqueous solution of the purified dye (0·01%, w/v) was used throughout the study; this has been shown to provide optimal histochemical staining of tissue components.[21,22]

2.2 Assay of Naphthalene Black

A series of dilutions from a stock solution of the dye in distilled water were prepared (0·001, 0·002, 0·005, 0·0075, 0·01, 0·0125 and 0·015%, 2/v). The absorbance at 618 nm was measured against a distilled-water blank and a calibration graph constructed. Linear regression analysis of the mean of three sets of readings showed a correlation coefficient of 0·994, indicating compliance with the Beer–Lambert law.

2.2.1 Photochemical degradation

A photoreactor unit[24] was used; this consisted of a battery of eight blue/black tubes (8 W each) (emission 300–400 nm) mounted into the internal surface of a metal drum which had the end-pieces removed; the device was so constructed that it provided a constant non-thermal light source with minimal short-wavelength light intensity.

2.2.2 Methodology

Aqueous solutions of the dyes were purged with dry nitrogen gas, as were samples containing dye and (1) 6% hydrogen peroxide (2 ml), (2) 6%

hydrogen peroxide (2 ml) and 6% mannitol (2 ml) and (3) 6% hydrogen peroxide and 6% 1-methyl-4-(4′-hydroxybenzylidyl)pyrrolidine-2,3-dione (2 ml) as a triplet sensitizer. In each case 250 ml of solution was used. The aqueous dye solution was irradiated in the photoreactor unit for up to 40 days; the dye in the presence of the additives was similarly treated for up to 10 days. All irradiation was performed at room temperature ($23 \pm 1°C$). After irradiation, the samples were assayed for dye content spectrophotometrically and the remaining dye content determined. The rate constants and order of reaction were calculated.

2.3 Detection of 1-naphthylamine

Photodegradation of Naphthalene Black was confirmed by the detection of 1-naphthylamine in the reaction mixture by thin-layer chromatography followed by comparison of the IR spectra of the eluates with authentic samples of compounds including 1-naphthylamine. This amine is known to be a degradation product of the photolysis of Amaranth (C.I. 16185);[25] similar results have also been reported from the irradiation of azo dyes other than Naphthalene Black under similar experimental conditions to those described above.[26]

3 RESULTS AND DISCUSSION

Table 1 shows the results of irradiation of Naphthalene Black solution. After 40 days, 91·7% of the dye remained and the absorption maximum at 618 nm was unchanged. The correlation coefficients show that the reaction follows zero-order kinetics and that the rate constant is 0·20.

TABLE 1
Photochemical Degradation of Naphthalene Black (0·01%, w/v) in Water

Irradiation time (days)	*Dye remaining (%)*	*log [Dye remaining (%)]*	*1/[Dye remaining (%)]*
0	100·00	2·000 0	0·010 00
26	94·27	1·973 9	0·010 62
29	94·08	1·973 5	0·010 63
33	94·00	1·973 1	0·010 64
40	91·66	1·962 2	0·010 91
Correlation coefficient	0·990 3	−0·988 7	0·988
Rate constant	0·20		

TABLE 2
Photochemical Degradation of Naphthalene Black (0·01%, w/v) in Water with 6% Hydrogen Peroxide (2 ml) Added in 250 ml of Solution

Irradiation time (days)	*Dye remaining (%)*	*log [Dye remaining (%)]*	*1/[Dye remaining (%)]*
0	100·00	2·000 0	0·010 0
3	80·72	1·907 0	0·012 39
5	61·53	1·789 1	0·016 31
7	34·32	1·535 5	0·029 14
10	21·74	1·377 3	0·045 99
Correlation coefficient	−0·986 2	−0·975 0	0·938 2
Rate constant	8·34		

Table 2 shows the results after irradiation of the dye in the presence of hydrogen peroxide. After 10 days, only 21·7% of the dye remained. Examination of the correlation coefficients shows that the reaction follows zero-order kinetics and the rate constant is 8·34.

Table 3 shows the results of irradiation of the dye in the presence of both hydrogen peroxide and mannitol. After 10 days, 82·6% of the dye remained.

Comparison of the correlation coefficients shows that the reaction follows either zero- or first-order kinetics. By using a computer graphics method, it was possible to show that the reaction followed zero-order kinetics[27] and the rate constant was 1·76.

The findings from Tables 2 and 3 suggest strongly that the photochemical degradative reaction of the dye is, at least in part, hydroxyl radical mediated,

TABLE 3
Photochemical Degradation of Naphthalene Black (0·01% w/v) in Water with 6% Hydrogen Peroxide (2 ml) and 6% (w/v) Mannitol in 250 ml

Irradiation time (days)	*Dye remaining (%)*	*log [Dye remaining (%)]*	*1/[Dye remaining (%)]*
0	100·00	2·000 0	0·010 000
3	94·67	1·976 2	0·010 563
5	91·35	1·960 7	0·010 947
7	87·20	1·940 5	0·011 467
10	82·57	1·916 9	0·012 108
Correlation coefficient	−0·999 2	−0·999 2	0·998 2
Rate constant	1·76		

TABLE 4
Photochemical Degradation of Naphthalene Black (0·01%, w/v) in Water with 6% Hydrogen Peroxide (2 ml) and 0·01% 1-Methyl-4-(4′-hydroxybenzylidyl)pyrrolidine-2,3-dione in 250 ml

Irradiation time (days)	*Dye remaining (%)*	*log [Dye remaining (%)]*	*1/[Dye remaining (%)]*
0	100·00	2·000 0	0·010 0
3	88·17	1·945 3	0·011 34
5	65·89	1·818 8	0·015 17
7	33·62	1·526 6	0·029 74
10	9·74	0·988 5	0·102 66
Correlation coefficient	0·979 0	0·931 2	0·835 5
Rate constant	9·66		

since the addition of mannitol (a known hydroxyl radical scavenger) considerably reduced the breakdown of the dye in the presence of hydrogen peroxide, a hydroxyl radical generator.

Table 4 shows the results obtained from irradiation of the dye in the presence of hydrogen peroxide and the triplet sensitizer 1-methyl-4-(4′-hydroxybenzylidyl)pyrrolidine-2,3-dione. The use of this latter compound as a triplet sensitizer was suggested since the pyrrolidine-2,3-dione acts as a pH indicator dye[28,29] and could reasonably be expected to act in this manner also. The enhanced rate of photochemical degradation of Naphthalene Black when solutions are irradiated with both hydrogen peroxide and the pyrrolidine-2,3-dione dye could be attributed to a combination of two photochemical effects, viz. triplet sensitization and hydroxyl radical generation. This situation is realistic where wool or other proteinaceous material is bleached with hydrogen peroxide and a mixture of dyes is used in subsequent processing.

Examination of the regression coefficients shows that the reaction follows zero-order kinetics. However, the correlations are not as good as was observed in the other tables; this could be due to the existence of two competing reactions. The rate constant was 9·66. The UV spectrum of the dye exhibited a diminution in the intensity of the peak at 618 nm.

3.1 Chromatography

After irradiation of the reaction mixture from the photolysis of the dye in distilled water was complete, 10-ml samples of the solution were individually extracted with diethyl ether (3 × 50 ml), the extracts combined, dried over

anhydrous Na_2SO_4 and evaporated under reduced pressure. The residues were dissolved in a minimal volume of ethanol and subjected to TLC on a Merck silica gel 60 chromatographic sheet. The eluting solvent was a mixture of chloroform and hexane (7:3, v/v). The products were detected using both UV light and iodine vapour to demonstrate the presence of nitrogen-containing materials. There were several spots on the chromatogram, one of which (R_f 0·48) was identified as 1-naphthylamine. Confirmatory evidence was obtained by scraping the silica from the plate, extracting with diethyl ether and running the IR spectrum of the evaporated extract in nujol; this spectrum was superimposible on that of an authentic sample of 1-naphthylamine as a nujol mull.

The chromatographic and spectral evidence suggests that the photochemical degradation of Naphthalene Black in distilled water could follow a similar pathway to that taken by Amaranth.[25] The formation of 1-naphthylamine could be explained by the sequence shown in Fig. 1.

The findings show that Naphthalene Black can degrade much faster in the presence of an oxidising agent than in distilled water and that such breakdown alters the UV/visible spectrum by reducing the intensity of the absorption at 618 nm. This could explain the aberrant staining recorded from biochemical,[17] histochemical[6,12,13,18,19] and analytical usage[23] of the dye when it is in an unpurified state.

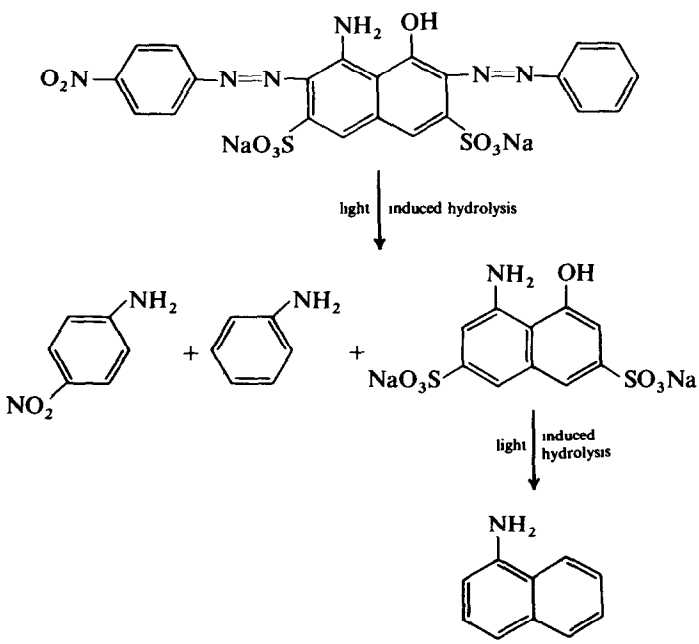

Fig. 1. Photodegradation of Naphthalene Black.

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