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Photostabilization of Demeclocycline Hydrochloride with Reduced Glutathione

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ABSTRACT

Photodegradation of demeclocycline hydrochloride (DCL) in buffer solutions was studied in absence and presence of some potential photostabilizers under the influence of fluorescent light. Photolysis of DCL solutions followed first-order kinetics. DCL was more stable in acidic pH. Change in ionic strength of the buffer had no effect on the photolysis of DCL. Among the potential photostabilizers tested, reduced glutathione (GTH) was found to be the most effective photoprotective agent. Increase in GTH concentration decreased the photodegradation rate, but this decrease was not significant above 20 μg/ml GTH concentration. The photodegradation of DCL both in presence or absence of GTH was lowest at pH 4.5 citrate buffer, compared to acetate or phosphate buffer. A mixture of 50% (v/v) propylene glycol or 50% (v/v) PEG 400 in phosphate buffer did not demonstrate any photostabilizing effect. Aluminum foil–covered glass vials provided greater photoprotection compared to clear glass or amber glass vials.

INTRODUCTION

Demeclocycline hydrochloride (DCL) is a broad-spectrum antibiotic of the tetracycline group. The drug causes more phototoxic reactions compared to other tetracycline derivatives (1). The photodegradation products of DCL are responsible for various physiological problems that have been thoroughly investigated (2–5). UV irradiation of aerated aqueous solution of DCL caused oxidation of the compound (6). Hasan et al. (7) reported the degradation kinetics of DCL under UV light. It appears from literature reports, however, that no study has been done on the photostabilization of DCL through the use of photoprotective agents. Therefore, in view of the high photosensitivity of DCL, it is worthwhile to investigate the photodegradation of the compound and find ways to photostabilize it.

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EXPERIMENT

Materials

Demeclocycline hydrochloride (DCL) was obtained from Sigma Chemical Co. Buffers and all other chemicals were of reagent grade and were purchased from Fisher or Sigma Chemical Co. Deionized water was used to prepare all solutions.

Methods

Photodegradation: Solutions of DCL in appropriate buffers, with or without photoprotective agents, were placed in USP type I borosilicate glass vials with rubber stopper and sealed with aluminum seals. The vials were exposed to fluorescent light in the light stability cabinet (Atlas HPUV Light Exposure Cabinet, Atlas Electric Devices Co. Chicago, IL). The light intensity was maintained at 5 ± 0.1 W/m^2 and the temperature was 39° ± 1°C. Appropriate blanks were also exposed to similar light conditions. Samples were withdrawn at different time intervals and assayed for DCL content.

Assay of demeclocycline hydrochloride: Figure 1 shows a set of UV spectra of DCL solution (20 μg/ml, pH 7 phosphate buffer, ionic strength, μ = 0.1), recorded at different time intervals after exposure to fluorescent light. From the spectra it is obvious that the absorbance at 372 nm is not affected even after prolonged time of exposure. Therefore, absorbance at 372 nm was used for the quantitative determination of DCL. The photoprotective agents used were found not to interfere with DCL assay at this wavelength. Andersgaard and Pederson (8) and Asker and Habib (9) have utilized a similar method for the quantitative analysis of barbiturates and doxorubicin hydrochloride respectively. The assay was done in duplicate and the mean value was determined. The difference between the two assays was less than ±1%.

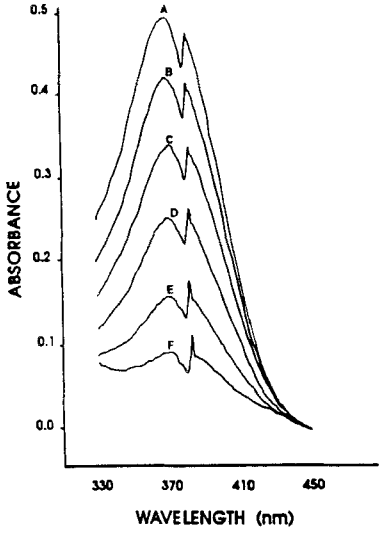

Figure 1. A set of UV spectra obtained during photodegradation of demeclocycline HCl (20 μg/ml) in pH 7 phosphate buffer (μ = 0.1) under fluorescent light of intensity 5 W/m^2. A = 0 min; B = 10 min; C = 20 min; D = 30 min; E = 40 min; F = 60 min.

Effect of pH, ionic strength, and buffer species: The influence of pH on the photodegradation of DCL was determined by using acetate buffer in the pH range of 2.45–4.5 and phosphate buffer within the pH range of 4.5–8. Photodegradation of DCL was determined at pH 6 phosphate buffer with varying ionic strength (range 0.1–0.5). The ionic strength of each solution was adjusted by adding sufficient quantity of sodium chloride to the buffer solution. The effect of phosphate, acetate, and citrate buffer (pH 4.5 and ionic strength 0.1) on photodegradation of DCL was also studied.

Effect of DCL concentration: The effect of concentration of DCL (20–2000 μg/ml) on photodegradation was studied in pH 7 phosphate buffer (μ = 0.1).

Effect of various photostabilizers: The influence of various photostabilizers on the photodegradation of DCL in pH 7 phosphate buffer was also investigated. Both the concentration of DCL and photostabilizers were kept constant at 20 μg/ml. The effect of varying the concentration of reduced glutathione (5–50 μg/ml) on the photostability of DCL was also studied.

Effect of various solvents and containers: The photodegradation of DCL was investigated in a mixture of 50% (v/v) propylene glycol in pH 7 phosphate buffer and 50% (v/v) PEG 400 in pH 7 phosphate buffer. The photodegradation of DCL was also tested in amber vials and in clear glass vials covered with aluminum foil.

RESULTS AND DISCUSSION

Kinetics of photodegradation: Under fluorescent light, demeclocycline HCl (DCL) was found to undergo first-order photodegradation. A typical representative curve is shown in Figure 2. DCL is reported to undergo first-order photodegradation under UV light in buffered solutions (7). Here photodegradation of DCL includes photohydrolysis, photoreduction, and photodechlorination (10). However, no attempt has been made to isolate, identify, and quantify the various degradation prod-

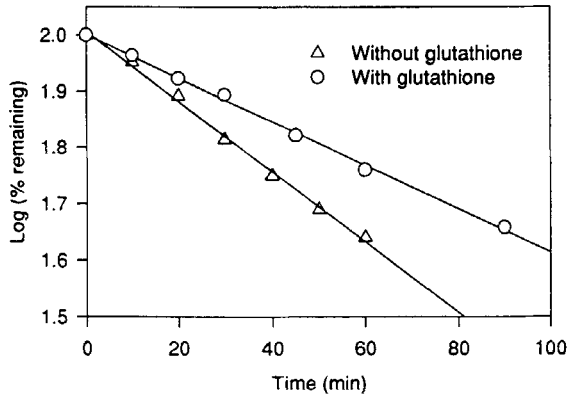

Figure 2. First-order photodegradation of DCL in absence or presence of reduced glutathione in pH 7 phosphate buffer (μ = 0.1).

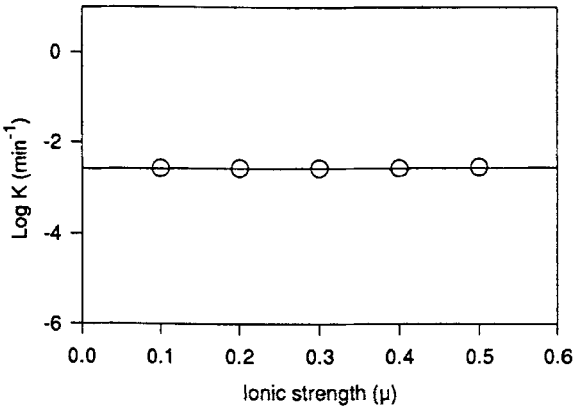

Figure 4. Effect of ionic strength of pH 6 phosphate buffer on photodegradation rate constant of DCL.

ucts, so all photodegradation rate constants reported here are the observed overall degradation rate constants.

Effect of pH: Photodegradation of DCL was studied in the pH range of 2.45–8.0. The plot of log K against pH is shown in Figure 3. The pH of the buffer solution demonstrated a profound effect on the degradation of DCL. Photodegradation rate was faster at higher pH values. Decrease in pH decreased the degradation rate and the compound was most stable at pH 2.45. The faster rate of photodegradation of DCL at higher pH can be attributed to various degradation pathways, which include not only photohydrolysis, but also photoreduction and dechlorination degradation (10).

Effect of ionic strength: The effect of ionic strength on the photodegradation of DCL under fluorescent light is shown in Figure 4. Increase in ionic strength from 0.1 to 0.5 did not produce any significant change in the photodegradation rate constant of DCL. Similar findings had been reported by Cruz et al. (11), who found that thermal degradation of furosemide was not significantly affected by the ionic strength of the buffer.

Effect of DCL concentration: Figure 5 shows the effect of DCL concentration on photodegradation. Increase in drug concentration caused a slight decrease in degradation rate constant. This is due to the fact that when concentrated solutions are exposed to intense light, a yellow color develops. This color acts as a light filter and slightly lowers the degradation rate constant. Horton and Stevens (12) reported similar observations on the photodecomposition of dacarbazine.

Effect of photostabilizers: In the presence of photostabilizers, DCL demonstrated first-order degradation.

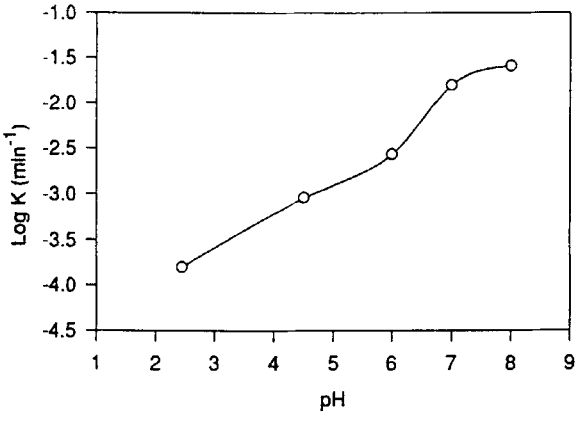

Figure 3. Log K-pH profile of photodegradation of DCL under fluorescent light.

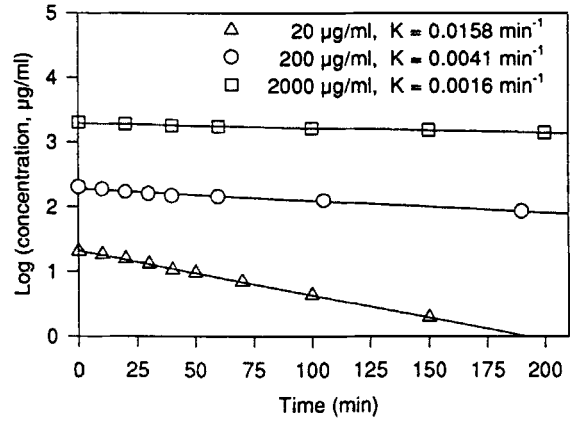

Figure 5. Effect of DCL concentration on photodegradation in pH 7 phosphate buffer (μ = 0.1) under fluorescent light.

Table 1

Effect of Various Photoprotective Agents on the Photodegradation of Demeclocycline Hydrochloride (20 μg/ml) in pH 7 Phosphate Buffer (μ = 0.1)[a]

Photoprotective Agent	Rate Constant $K_p \times 10^3$ min-1	$t_{½}$ min	% Increase in Photostability[b]
Glutathione	7.82	88.61	50.63
EDTA	11.71	59.20	26.07
Sodium thiosulfate	12.08	57.35	23.74
Sodium metabisulfite	12.49	55.51	21.15
PABA	12.97	53.42	18.12
Sodium benzoate	14.17	48.92	10.86

[a]The concentration of each photoprotective agent was 20 μ/ml.
[b]% increase in photostability = $[(K_c - K_p)/(K_c)] \times 100$.
$K_c \times 10^3$ = degradation rate constant with no photoprotective agent = 15.84 min^{-1}.

A representative curve is shown in Figure 2. Various compounds were tested to photostabilize DCL, and the results are summarized in Table 1. Among the potential photostabilizers tested, reduced glutathione (GTH) was found to be most effective, followed by EDTA, sodium thiosulfate, sodium metabisulfite, PABA, and sodium benzoate. These compounds act as photoprotective agents by virtue of their antioxidant, chelating, or light-absorbing effect. GTH decreased the photodegradation rate by 50% and proportionately the $t_{½}$ doubled compared to the photodegradation without the photoprotective agent. GTH has been reported to be an effective photostabilizer for tetracycline and dacarbazine (13,14).

Effect of GTH concentration: Reduced glutathione (GTH) was found to be the most effective photostabilizer. Therefore, the effect of GTH concentration on the photodegradation of DCL was studied. The results are shown in Figure 6. Increase in GTH concentration up to 20 μg/ml significantly lowered the observed degradation rate constant. The optimum concentration of GTH, which produced the maximum decrease in degradation rate, was equal to the DCL concentration in the buffer solution. Further increase in GTH concentration did not produce any significant decrease in degradation rate. GTH and other photoprotective agents were found to demonstrate optimum concentrations for their photostabilizing effect (13-15).

Effect of buffer species: Photostability of DCL at pH 4.5 under fluorescent light appeared to be influenced by the buffer species (Table 2). The stabilizing effect of these buffers, both in absence and presence of GTH, followed the order: citrate > acetate > phosphate. It is also apparent that the GTH offered greater photoprotection in phosphate buffer than in acetate and citrate buffers. Different buffer species in solutions of the same pH have been found to influence photostability of drugs (16).

Effect of various containers: Demecloclycline HCl solutions in pH 7 phosphate buffer (μ = 0.1) were kept in clear borosilicate vials, amber vials, and clear vials wrapped with aluminum foil and exposed to fluorescent light. The results are shown in Figure 7. The degradation of DCL in aluminum foil-covered clear glass vials was due to temperature only and served as a control. More than 60% of DCL was photodegraded in clear vials after 1 hour. However, in the case of amber vials and clear vials wrapped with aluminum foil, about the same percentage degraded after exposure for more than

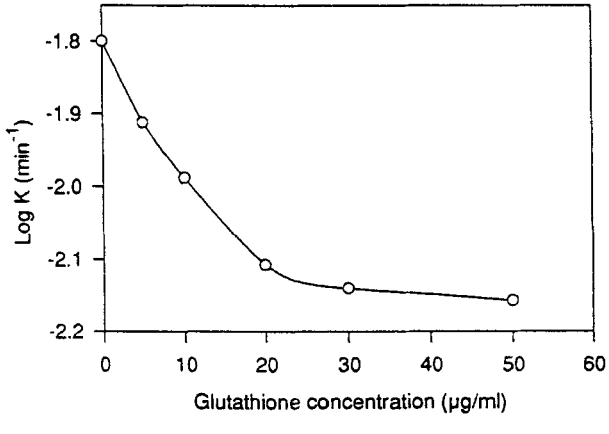

Figure 6. Effect of concentration of glutathione on photodegradation rate constant of DCL in pH 7 phosphate buffer (μ = 0.1).

Table 2

Effect of Buffer Species on the Photodegradation of DCL in Fluorescent Light[a]

Buffer Species	Rate Constant ($K \times 10^4$ min^{-1}) and $t_{1/2}$ (min) Without GTH K_c	Without GTH $t_{1/2}$	With GTH K_p	With GTH $t_{1/2}$	% Increase in Photostability $[(K_c - K_p)/(K_c)] \times 100$
Phosphate buffer	9.14	758.2	7.34	943.6	19.7
Acetate buffer	5.60	1237.3	4.96	1398.3	11.4
Citrate buffer	3.68	1885.4	3.38	2052.6	8.2

[a]The pH of each buffer was 4.5 and ionic strength was 0.1.

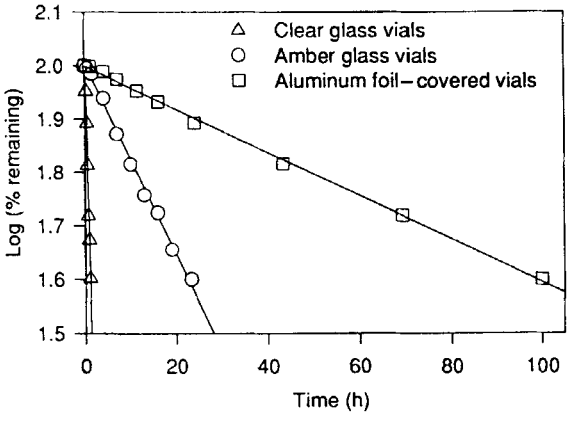

Figure 7. Effect of container on photodegradation rate of DCL in pH 7 phosphate buffer when exposed to fluorescent light.

20 and 100 hours respectively. These results indicate that amber glass is not always protective for light-sensitive drugs. This may be attributed to the heavy metal ions used to color the glass, which are leached out into the solution and thus act as catalysts in enhancing photodegradation (17).

Effect of various solvent mixtures: The effects of various solvent mixtures on the photodegradation of DCL are summarized in Table 3. A mixture of 50% (v/v) propylene glycol or 50% (v/v) PEG 400 with phosphate buffer (pH 7) increased the photodegradation rate constant by 1.8 and 2.5 times respectively compared to pH 7 phosphate buffer alone. Replacing water partially or completely with inert organic solvent is supposed to lower hydrolytic degradation. However, in this study the solvent mixtures increased the photodegradation rate of DCL. No definite conclusions can be made about the reason for this increased photodegradation. Similar findings have been reported for faster photodegradation of furosemide in a mixture of 50% (v/v) PEG 400 in pH 7 phosphate buffer (18).

Table 3

Effect of Various Solvent Mixtures on the Photostability of DCL (20 μg/ml) Under Fluorescent Light

Solvent	Rate Constant $K \times 10^2$ min^{-1}	$t_{1/2}$ min
Phosphate buffer (pH 7)	1.58	43.7
50% (v/v) Propylene glycol in pH 7 phosphate buffer	2.77	25.0
50% (v/v) PEG 400 in pH 7 phosphate buffer	3.94	17.6

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